SYNTHESIS AND CHARACTERIZATION OF DIETARY SUPPLEMENTS FOR TREATMENT OF UREA CYCLE DISORDERS

A Thesis
Presented in Partial Fulfillment of the Requirements for the degree Master of Science in the Graduate School of The Ohio State University

by

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ABSTRACT

Strynylacetac acid triglyceride has been suggested by Dr. Brusilow at Johns Hopkins Medical Center as a potential dietary supplement for treatment of urea cycle disorders. It is hoped that this compound will be metabolized to phenylacetate and will not have the unpleasant taste of phenylacetate, phenylbutyrate, and triglyceride of phenylbutyric acid, currently used or under consideration for use in treatment of urea cycle disorders.

This thesis reports the different approaches to characterization and preparation of strynylacetac acid triglyceride, which highlight the success with sulfonated charcoal as the catalyst. The analysis and synthesis of the impurities in the reaction mixture were also included, as well as the preliminary results of synthesis of other alternative dietary supplements.
TO MY PARENTS
ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. David J. Hart, for his support, encouragement and guidance throughout my graduate work at the Ohio State University. His enthusiasm and carefulness towards chemistry gave me a lot of motivation. His nice attitude about life and towards students helped to make my stay enjoyable and unforgettable.

Next, I would like to thank Dr. Soul W. Bruslow at Johns Hopkins University Medical Center for suggesting this interesting problem.

I also would like to thank the great technical support staff. Special thanks to Dr. David Chang for mass spectra analyses.

Furthermore, I am very grateful to all my friends in Columbus, especially past and present Hart group members who taught me a lot of America and American life. Special thanks to Jane Dzung and Ying for their companionship, Gilles for his chemistry and driving-learning help, Scott for his smile and computer help and Doug.

Finally, I’d like to express my sincere gratitude to my parents and my boyfriend, Jiang Wu, for their emotional and financial support during my school years.
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FIELD OF STUDY

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Studies in Organic Chemistry
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CHAPTER I

STATEMENT OF THE PROBLEM

A. Introduction.

This thesis describes the synthesis and characterization of sterylacetic acid triglycerides as potential dietary supplements for the treatment of urea cycle disorders. The results of my research will be presented in six chapters. Chapter I will describe the origin of this family disease. In Chapter II, some current therapies and problems associated with these therapies will be presented. The specific synthetic objectives of this study will also be defined. Chapter III will present model studies performed to help with characterization of synthetic targets and Chapter IV will describe the development of a process for preparing our sterylacetic acid triglycerides. Finally, identification of impurities produced in the synthesis, preliminary biological results, and other potential targets as dietary supplements will be discussed in Chapters V and VI.
B. Urea Cycle.

Animals have to consume a certain amount of protein every day. This protein serves as a source of nitrogen needed to sustain life. Except when used for growth, there is no normal mechanism for storing nitrogen in the body. This is unlike carbon, hydrogen and to a much lesser extent, oxygen atoms of food which can be stored as fat and carbohydrate in enormous amounts. Thus, nitrogen concentrations have to be carefully balanced, and in animals whose dietary intake of protein exceeds the need for protein synthesis and other biosynthetic processes, the surplus protein, in the form of free amino acids, must be used as metabolic fuel.

One pathway for $\alpha$-amino acid metabolism involves conversion to citric acid cycle intermediates and ammonia. Although ammonia, is a universal participant in amino acid synthesis and degradation, its accumulation in organisms to an abnormal degree may have toxic consequences. In some extremely serious cases, it will cause death.

Throughout the evolutionary scale, organisms have developed a number of biosynthetic pathways to secrete the ammonia or convert ammonia into waste nitrogen products that can be excreted. For example, fish can take in and pass unlimited amounts of water to dissolve and excrete ammonia through their gills. Birds and reptiles convert most ammonia into uric acid for excretion, whereas, human beings and some other advanced terrestrial vertebrates have
evolved other pathways, for example excretion of ammonia as urea. A general description of the fate of protein in humans is outlined in Figure 1.

Figure 1: The Fate of Dietary Protein

The pathway for excretion of waste nitrogen as urea, for human beings and some mammals, is called the urea cycle (Figure 2). This pathway is also known as the Krebs-Henseleit urea cycle. In this pathway, one of the nitrogen atoms of urea comes from aspartic acid. Dietary aspartic acid is not the only
Figure 2. Urea Cycle
source of this nitrogen, as aspartate is also biosynthesized from ammonia an α-ketoglutarate via glutamic acid. The other nitrogen atom and the carbon atom are derived from ammonia (as ammonium ion) and carbon dioxide. Ornithine which is the carrier of these carbon and nitrogen atoms, reacts with carbamoyl phosphate to give citrulline, which reacts with aspartate to give argininosuccinate. Finally the specific enzyme, argininosuccinase, cleaves argininosuccinate to fumarate and arginine. Arginine, the immediate precursor of urea is hydrolyzed into ornithine and urea, the waste nitrogen product.

A specific enzyme is responsible for each step in the urea cycle. A malfunction of one of these specific enzymes, results in failure of the urea cycle and inability of the organism to remove ammonia from the system. Deficiencies or malfunctions of the enzymes ornithine transcarbamoylase (which catalyzes the reaction between ornithine and carbamoyl phosphate), argininosuccinic acid synthetase (which catalyzes the transformation of citrulline to argininosuccinate), carbamoyl phosphate synthetase (which catalyzes the transformation of ammonium ion and carbon dioxide into carbamoyl phosphate), argininosuccinase (which catalyzes the cleavage of argininosuccinate into arginine and fumarate), arginase (which catalyzes the hydrolysis of arginine into ornithine and urea) are all known and result in a family of diseases known as urea cycle disorders.2

The common condition for this kind of disease is an abnormally elevated level of ammonia (ammonium ions) in the blood. This condition is also referred
to as hyperammonemia. This abnormal elevation of ammonia concentration in the blood will poison the liver and other organs. Partial deficiencies or malfunctioning of urea cycle enzymes may cause mental retardation, lethargy and episodic vomiting. Nearly total deficiencies or malfunctioning of these enzymes results in coma and eventually death.3

Urea cycle disorders are not common. As mentioned above, these disorders are due to a variety of enzyme malfunctions (Table 1), but failure of ornithine transcarbamylase and argininosuccinic acid synthetase seem to be the major causes of these disorders.3

<table>
<thead>
<tr>
<th>Enzyme Deficiency</th>
<th>Number of Cases Referred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamoyl phosphate synthetase</td>
<td>69</td>
</tr>
<tr>
<td>Ornithine transcarbamoylase</td>
<td>334</td>
</tr>
<tr>
<td>Argininosuccinic acid synthetase</td>
<td>74</td>
</tr>
<tr>
<td>Arginase</td>
<td>11</td>
</tr>
<tr>
<td>Argininosuccinase</td>
<td>57</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>545</strong></td>
</tr>
</tbody>
</table>

Table 1. A Variety of Enzyme Malfunctions
Even though the incidence of urea cycle disorders is low, treatments for handling these largely inherited diseases have been developed and used with some success. The details of these treatments will be presented in the next chapter.
CHAPTER II

THE POSSIBLE SOLUTIONS

A. Some Existing Therapies for Urea Cycle Disorders.

A number of methods have been used clinically to treat the urea cycle disorders. One therapy that has been used to promote near normal growth and development in patients involves providing them a diet that limits protein intake to the minimum dietary requirement. While this diet controls protein intake, it must do so in a manner that provides the patient with essential amino acids. This widely applied therapy can lead to lower ammonia levels in the blood and clinically improve the milder forms of these inherited diseases. But a consequence of this therapy is that it can lead to other problems, such as diseases possibly caused by a deficiency of certain essential amino acids.

To minimize hyperammonemia, encephalopathy and other treatment side effects, and at the same time correct patients’ higher than normal plasma ammonium and glutamine levels, another approach to treating urea cycle disorders has been developed. This involves activation of new pathways for waste nitrogen excretion.
It has been shown that dietary supplements of benzoate (sodium benzoate) will stimulate ammonia excretion in the form of hippurate (Figure 3).^3

![Chemical diagram of hippurate formation from benzoate and glycine]

**Figure 3. Pathway of Benzoate Stimulation of Ammonia Excretion.**
Alternatively, dietary supplements of phenylacetate or γ-phenylbutyrate (as sodium salts) can result in ammonia excretion as N-phenylacetylglutamine (Figure 4).
While phenylacetate can be regarded as a true drug, the \( \gamma \)-phenylbutyrate is really a pro-drug which is metabolized via normal fatty acid degradation pathways to provide phenylacetate (Scheme I).

\[
\begin{align*}
&\text{FAD} \quad \text{FADH}_2 \\
&\text{Dehydrogenation} \\
&\text{Hydration} \quad \text{H}_2\text{O} \\
&\text{Dehydrogenation} \\
&\text{CoA-SH} \\
\end{align*}
\]

Scheme I. \( \gamma \)-Phenylbutyrate Degradation Pathways.
Of these therapies, the latter is more effective in that two moles of ammonia are excreted per mole of dietary supplement, whereas benzoate therapy leads to excretion of one mole of ammonia per mole of dietary supplement.

Two problems are associated with the phenylacetate-phenylbutyrate therapies. One is that large quantities of supplement has to be taken orally (about 20 g of sodium phenylacetate per day). A second problem associated with these dietary supplements is their foul taste. For example, phenylacetic acid and its homolog are responsible for the stench of the stinkpot turtle. Although encapsulation of the dietary supplement can take care of some of these problems, it is still not an ideal way to administer the drug due to the large quantities involved. It would be preferable to administer the supplement in the form of a good-tasting "milkshake". This requires that: (1) the dietary supplement have a palatable taste and (2) have physical properties amenable to "milkshake preparation".

One idea that has been explored is the preparation of the triglyceride of \( \gamma \)-phenylbutyrate. This material is an oil that could potentially be converted to \( \gamma \)-phenylbutyrate by esterases. Unfortunately, this substance has a foul after taste that does not offer a real improvement over existing therapies. Based on the knowledge that introducing unsaturation adjacent to a fatty acid can improve "sensory properties" (for example, \( \beta \)-phenylpropionate has a foul taste but cinnamic acid is palatable), Dr. Saul Brusilow suggested that unsaturated
triglycerides of unsaturated carboxylic acids, such as 4-phenyl-3-butenoic acid (1) or 4-phenyl-2-butenoic acid (2), might have palatable tastes. Because of the commercial availability of styrylacetic acid, triglyceride 3 was selected as the initial target for synthesis. Of course, for 3 to be useful as a dietary supplement, it would have to be metabolized (Scheme II) into phenylacetate via

Scheme II. Pathway of Metabolism of Styrylacetic Acid Triglyceride
hydrolysis (esterases) followed by degradation (fatty acid metabolism).

Preliminary studies in the Bruisolow laboratories suggested this should be the case (vide infra).
CHAPTER III

trans-STYRYLACETIC ACID TRIGLYCERIDE: PREPARATION OF MODEL COMPOUNDS

Although symmetrical triglyceride synthesis is usually a simple process, the synthesis of triglyceride 3 poses some problems. For example, it was anticipated that esterification of glycerol with trans-styrylacetic acid would give both α,β- and β,γ-unsaturated esters due to acid-catalyzed isomerization of the double bond.

3

4

15
Although AM1 semi-empirical models show that 3 is 5.12 kcal/mol more stable than 4 in the gas phase, it was expected that isomerization might still be a problem in solution. Furthermore, problems with olefin geometrical (cis-trans) isomers were anticipated. If one considers random esterification of glycerol (three times) with a selection of carboxylic acids, it can be shown that the total number of triglycerides possible (excluding enantiomers) is given by the following equation:

\[
N = \frac{n^2 (n+1)}{2}
\]

where \(N\) is the number of triglycerides possible and \(n\) is the number of different carboxylic acid used. Thus, in the current case (\(n = 4\)), it was anticipated that up to 40 isomeric triglycerides could be formed. To help with this potential analytical problem, it was decided to prepare model compounds 5-8 to help with analysis of the NMR spectra as well as with full characterization of the target triglyceride 3. Compounds 5 and 7 were to mimic the primary esters in 3 and compounds 5 and 8 the secondary esters.
The syntheses of 5 and 6 were accomplished as shown in Scheme III.

Scheme III. Synthesis of \( \beta,\gamma \)- Unsaturated Model Compounds.
Treatment of trans-styrlyacetic acid with thionyl chloride gave acid chloride 9. Treatment of 9 with ethanol and isopropanol gave esters 5 and 6 in 63% and 67% yields, respectively. In the reaction to give 5, a small amount (6%) of α,β-unsaturated ester 7 was also produced.

Samples of 7 and 8 were prepared using Wittig reactions as shown in Scheme IV.

Scheme IV. Synthesis of α,β-unsaturated Model Compounds.

\[
\begin{align*}
\text{Br} & \text{CO}_2\text{H} & \xrightarrow{1, \text{-PrOH, TsOH (67%)}} & \xrightarrow{2, \text{Ph}_3\text{P, NaOH (98%)}} \text{Ph}_3\text{P} & \text{CO}_2\text{R} \\
10 & & & 11 \quad R = \text{Pr} & 12 \quad R = \text{Et} \\
\begin{array}{c}
\text{O} \quad \text{O} \\
\text{CH}_3 \\
\text{Et}
\end{array} & \\
13 & & \\
\begin{array}{c}
\text{O} \quad \text{Et} \\
\text{CH}_3 \\
\text{Et}
\end{array} & \\
14 & & \\
\text{Ph} & \xrightarrow{\text{PhCH}_3\text{CCHC}} & \\
\begin{array}{c}
\text{O} \quad \text{CO}_2\text{R} \\
\text{Et}
\end{array} & \\
7 & R = \text{Et} & 8 & R = \text{Pr} (43\%) & (69\%)
\end{align*}
\]
Treatment of phenylacetaldehyde with the known stabilized Wittig reagent 12 gave 7 in 43% yield. This material was contaminated with about 5% of β,γ-unsaturated ester 5, but also contaminated with about 5% of cis-α,β-unsaturated ester 14. This provided yet another model compound whose NMR chemical shifts would help analysis of spectra of triglyceride 3. Finally, phosphorane 11 was prepared in three steps from α-bromoacetic acid (Scheme IV) and reacted with phenylacetaldehyde to afford ester 8 in 69% yield. This material was contaminated by about 5% of the corresponding cis-isomer 13.

Diagnostic signals for the six model compounds described above (5-8, 13, 14) are listed in Table 2.
<table>
<thead>
<tr>
<th>Compound</th>
<th>CH₃CH=</th>
<th>OCH₃</th>
<th>OCH</th>
<th>CH₃CH=</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.25</td>
<td>4.19</td>
<td></td>
<td>6.50</td>
</tr>
<tr>
<td></td>
<td>(d, J = 6.6 Hz)</td>
<td>(q, J = 8.0 Hz)</td>
<td>(septet, J = 7.5 Hz)</td>
<td>(dt, J = 15 Hz)</td>
</tr>
<tr>
<td>6</td>
<td>5.60</td>
<td></td>
<td></td>
<td>6.50</td>
</tr>
<tr>
<td></td>
<td>(septet, J = 7.5 Hz)</td>
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<td>(dt, J = 15 Hz)</td>
<td>(dt, J = 15 Hz)</td>
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<tr>
<td>7</td>
<td>6.33</td>
<td></td>
<td></td>
<td>7.08</td>
</tr>
<tr>
<td></td>
<td>(dt, J = 15.7 Hz)</td>
<td></td>
<td>(dt, J = 15 Hz)</td>
<td>(dt, J = 15 Hz)</td>
</tr>
<tr>
<td>8</td>
<td>5.82</td>
<td></td>
<td></td>
<td>7.08</td>
</tr>
<tr>
<td></td>
<td>(dt, J = 15 Hz)</td>
<td></td>
<td>(dt, J = 15 Hz)</td>
<td>(dt, J = 15 Hz)</td>
</tr>
<tr>
<td>cis-7</td>
<td>5.07</td>
<td></td>
<td></td>
<td>5.07</td>
</tr>
<tr>
<td></td>
<td>(septet, J = 15 Hz)</td>
<td></td>
<td>(septet, J = 15 Hz)</td>
<td>(septet, J = 15 Hz)</td>
</tr>
<tr>
<td>cis-8</td>
<td>5.10</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(septet, J = 15 Hz)</td>
<td></td>
<td>(septet, J = 5 Hz)</td>
<td>(septet, J = 5 Hz)</td>
</tr>
</tbody>
</table>

Table 2. Chemical Shifts of Model Compounds
From these data, it was clear that each type of ester would most likely have certain "signature" signals in the $^1$H NMR spectrum of triglyceride 3. For example, whether an ester was $\alpha,\beta$- or $\beta,\gamma$-unsaturated could be determined by analysis of the allylic methylene region ($\delta$ 3.20-4.05) or perhaps by the appearance of olefinic (CH=CH) resonances. Whether an ester was at the primary or secondary site of glycerol could be determined by examining the $\delta$ 4.20-5.10 region of the spectrum ($\text{OCH}_2$ and $\text{OCH}_3$). Finally, it was hoped that cis-$\alpha,\beta$-unsaturated esters might be detected by signals due to the allylic methylene ($\delta$ 4.0-4.05) and perhaps by the appearance of olefinic resonances. As will be seen, the model compounds prepared here were very helpful with characterization of the synthetic target. Only models for cis-$\beta,\gamma$-unsaturated esters were not prepared. With this information in hand, the synthesis of triglyceride 3 was examined as described in Chapter IV.
CHAPTER IV

PREPARATION OF STYRYLACETIC ACID TRIGLYCERIDE

Monoglycerides of fatty acids are of wide interest and have been used in paints and other industrially important materials. Diglycerides are important components of cell membranes and triglycerides are also well known. For example, tristearin is a major component of nutmeg and its isolation and purification is frequently used to illustrate natural product isolation in undergraduate organic laboratory courses. This chapter describes our approach to the synthesis of triglyceride 3.

A. Some Failures

The initial approach to triglyceride 3 involved Fischer esterification of glycerol with trans-styrylacetic acid (1) with p-toluenesulfonic acid as catalyst (Scheme V). Treatment of glycerol with three equivalents of trans-styrylacetic acid (1) in the presence of a catalytic amount (5% by mass balance) of p-
toluenesulfonic acid using a Dean-Stark apparatus to remove water gave only 38-46% of 3 (Scheme V).

Scheme V. Attempted Esterification Using p-TsOH as the Catalyst.

\[
\begin{align*}
15 & \quad + \quad 3 \quad \xrightarrow{p\text{-TsOH}} \quad 1 \\
18 & \quad 17
\end{align*}
\]

3 (38-46%)

16

The reaction yields were unexpectedly low and capricious. To find out the reason for the low yields, the reaction was carefully monitored by TLC and then, the resulting mixture was separated by chromatography and the fractions were analyzed using 'H NMR spectroscopy. First, it was observed that the reaction
stopped before reaching completion. When more acid catalyst was added, the reaction began but again stopped before reaching completion. Second, from the $^1$H NMR analysis results it seemed that in addition to 3, significant amounts of 1,2- and 1,3-diglycerides (16 and 17) and diglyceride derived monosoylates 18 [based on $^1$H NMR signals that included a singlet at δ 2.4 (ArCH$_2$) and a doublet at δ 7.8 (ArH ortho to SO$_3$R)] were also formed. As expected, about 6% (based on $^1$H NMR) of cis isomers and α,β-unsaturated esters were present in the reaction mixture. The appearance of these reaction by-products and the observation that the reaction stopped after a while indicated that the esterification was not going to completion due to catalyst (p-TsOH) consumption. This also explains why the yields of the products were variable and largely depended on the amount of catalyst used each time. Unfortunately, thin-layer chromatography showed that the diglyceride monosoylate contaminants were very difficult to separate from styrylsalicylic acid triglyceride 3. These inseparable impurities also affected the accuracy of the combustion analysis of 3, which always showed a percentage of carbon lower than the theoretical value, although the hydrogen was correct.

Although problems existed for preparation of triglyceride 3 using this method, the NMR spectra of the product mixture showed that most of the unsaturation in the product involved β,γ-unsaturated esters (96:4 mixture of β,γ- and α,β-unsaturated esters). For example, the $^1$H NMR spectrum (Figure 5)
showed signals at δ 3.29 (d, J = 8.2 Hz, 6H, CH₃), 4.3 (dd, J = 11.6 Hz, 2H, OCH₂), 4.45 (dd, J = 11.6 Hz, 2H, OCH₂), 5.42 (septet, J = 6 Hz, OCH), 6.20-6.35 (m, 3H, CH=), 6.5 (broad d, J = 15.8 Hz, 3H, =C=Ar), 7.20-7.46 (m, 15H, ArH) that matched the structure of β,γ-unsaturated esters. Diagnostic signals due to α,β-unsaturated esters appeared at δ 3.42-3.50 (CH₃) and 6.28-6.40 (CH=). Diagnostic signals due to cis-unsaturated esters appeared at δ 3.38-3.40 and 7.08-7.20.
Figure 5. $^1$H NMR Spectrum of Triglyceride 3
Attention was next shifted to some sterically hindered sulfonic acid catalysts (the hope being that they would catalyze esterification, but would not be consumed via sulfonate formation) and a variety of Lewis acids. Three sterically hindered catalysts were used in Figure 6: 1,3,5-trimethylbenzenesulfonic acid (19), 1,3,5-trispropylbenzenesulfonic acid (20) and camphorsulfonic acid (21).

**Figure 6. Three Sterically Hindered Catalysts**

![Chemical structures](image)

However, no reaction or only a trace amount of products were obtained using these acids. Lewis acids, such as ZnCl₂, BF₃ and Ti(O-Pr)₄ have been widely applied in esterification reactions.⁹⁻¹⁰ Our attempts using several of these Lewis acids as catalysts were also not encouraging. Our attempts to accomplish the synthesis of 3 under these conditions are shown in Scheme VI.

At this point, a reasonable alternative was to find other catalysts that would be more suitable for triglyceride synthesis. It had been reported that
Scheme VI. Attempted Esterification by Using Different Catalysts

![Chemical Structure]

<table>
<thead>
<tr>
<th>Reaction Conditions</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-TsOH, Toluene, Dean-Stark</td>
<td>39-46%</td>
</tr>
<tr>
<td>19, Toluene, Dean-Stark</td>
<td>No Reaction</td>
</tr>
<tr>
<td>20, Toluene, Dean-Stark</td>
<td>No Reaction</td>
</tr>
<tr>
<td>21, Toluene, Dean-Stark</td>
<td>No reaction</td>
</tr>
<tr>
<td>ZnCl₂, Reflux</td>
<td>8%</td>
</tr>
<tr>
<td>BF₃, Reflux</td>
<td>Trace Amount</td>
</tr>
<tr>
<td>Ti(O-i-Pr)₄, Reflux</td>
<td>Trace Amount</td>
</tr>
<tr>
<td>Amberlyst-15, Cyclohexane, Reflux</td>
<td>Trace Amount</td>
</tr>
<tr>
<td>Dowex-50, Cyclohexane, Reflux</td>
<td>Trace Amount</td>
</tr>
<tr>
<td>Sulfonated Charcoal</td>
<td>71%</td>
</tr>
</tbody>
</table>
sulfonated resins were useful as the catalysts for synthesis of saturated alkyl triglycerides, such as glycceryl triacotate and glycceryl tripropionate. The yields were over 99% and 90%, respectively. However, application of the sulfonated resins Amberlyst-15 and Dowex-50 to the synthesis of 3 was not successful.

Our next approach was to prepare 3 by coupling styrylacetyl chloride (9) and glycerol. Surprisingly, the reaction of styrylacetyl chloride and glycerol in the presence of triethylamine, gave 3 in only 8% yield. From the reaction mass balance (which became lower after product purification) and thin-layer chromatographic analysis, we can suggest that (1) the reaction did not go to completion and significant amounts of 1,2- and 1,3- diglycerides were formed along with 3 and (2) the side-products were polymeric based on the physical properties of the crude reaction mixture. A possible mechanism for formation of side-products via the bis-electrophile 22 is shown in Scheme VII.

The coupling of glycerol and 1 using DCC was also tried, but this reaction only afforded 10-15% yields of 3. A more efficient method with a simple operational procedure was needed for triglyceride synthesis at this stage of the project.
Scheme VII. Possible Mechanism for Formation of By-products.

\[ \text{5} \xrightarrow{\text{EtN}} \text{Polymer} \]

\* = electrophilic site

B. Some Success.

Use of sulfonated charcoal as the catalyst finally provide 3 in 71% yield after purification by column chromatography (Scheme VIII). The sulfonated charcoal was prepared by heating a mixture of active charcoal and sulfuric acid at 240° for 12 hours, followed by filtration to afford the acid catalyst as a black powder (see experimental section). Previous work had shown that this catalyst was suitable for the esterification ethane-1,2-diol, propane-1,2-diol, butane-1,4-diol, propane-1,2,3-triol and glycerol with saturated aliphatic carboxylic acids in near quantitative yields.¹³
Scheme VIII. Esterification Using Sulfonated Charcoal as Catalyst

This Fischer esterification was carried out at the reflux temperature with azeotropic removal of water in the presence of 0.02 equivalent of sulfonated charcoal and small amount of solvent [usually around 10 mL of the solvent with a large scale reaction (80 mmol), and at the same time the Dean-Stark trap was filled with solvent as well]. We believe that reaction occurs on the solid phase. The catalyst’s activity results not only from its acidic nature, thereby activating the carboxylic acid, but also because of its strong adsorption of carboxylic acids. It seems therefore most probable that a very reactive species is formed from the acid at the surface of the sulfonated charcoal. It can be assumed that the sulfonated charcoal plays two combined roles: that of an acid catalyst, by activating the carboxylic acid, and that of a dehydrating agent which shifts the equilibrium to completion, in this case, towards ester formation.
This method gave acceptable yields (60-71%) and eventually allowed the preparation of 30 g of reasonable pure 3. Still, there were some problems: (1) a small percentage (around 6%) of the esters in the product were α,β-unsaturated, (2) the product required column chromatography for purification, which is not suitable for industrial scale-up and (3) inseparable impurities (trace amounts) were present along with the trans-styrilacetic acid triglycerides in the product mixture.

C. Evidence for the Structure of 3

Even though the combustion analysis results (percentage of carbon lower than the theoretical value) and the discovery of impurities in the reaction mixtures were not encouraging, we found some evidence that supported the structure of the product mixture and suggested that the purity of 3 was quite high. All of the 40 isomeric triglycerides that could have been formed in the preparation of 3 should give the same triglyceride upon exhaustive hydrogenation. Thus, triglyceride 3 (and isomers) was hydrogenated over 5% palladium on activated carbon in ethanol as the solvent (Scheme IX).
Scheme IX. Hydrogenation of Styrylacetic Acid Triglyceride 3

\[
\text{3} \quad \xrightarrow{\text{H}_2, \text{Pd/C}} \quad \text{EtOH} \quad \text{2,2 (100%)}
\]

The crude product, triglyceride 2,2, displayed a very clean \(^1\)H NMR spectrum with signals at \(\delta\) at 1.96-2.03 (quintet, \(J = 7.0\) Hz) due to the CH\(_2\) \(\beta\) to carbonyl, 2.40 (t, \(J = 7.0\) Hz) due to the CH\(_3\) \(\gamma\) to the carbonyl and 2.70 (t, \(J = 7.0\) Hz) due to the CH\(_3\) \(\gamma\) to the carbonyl. Without column chromatographic purification, 2,2 also gave a satisfactory combustion analysis. This result suggests that the esterification product is a styrylacetic acid triglyceride 3 isomer mixture with \(\beta,\gamma\)-unsaturated esters as the major components. At the same time, it was also clear that this material contained traces amount of inseparable impurities. The isolation and characterization of these impurities will be discussed in the next chapter.
CHAPTER V

ANALYSIS AND SYNTHESIS OF IMPURITIES

In drug production, the function and properties of the desired compound are of most concern to the chemists. However, determining the functions and properties of impurities produced by the process is also important. The process of identifying and confirming the structures of impurities formed during the preparation of triglyceride 3 will be discussed in this chapter.

A. Analysis

The crude product resulting from a large scale preparation of triglyceride 3 using sulfonated charcoal as the catalyst was chromatographed over a large amount of silica gel (80 g of silica gel per 1 g of crude product). The first fractions, which were diffuse spots by TLC analysis, were collected and analyzed by \textsuperscript{1}H NMR spectroscopy. Diagnostic signals at $\delta$ 6.65 (t, $J = 10.5$ Hz), 5.65 (s), 2.90 (dd, $J = 19$, 7 Hz) and 2.62 (dd, $J = 19$, 2 Hz) revealed that there
were trace amounts of inseparable impurities contaminating the triglyceride product 3.

These early fractions were combined and further purified by medium pressure liquid chromatography (MPLC). Individual fractions were carefully monitored by TLC analysis and analyzed by $^1$H NMR spectroscopy. Initially it was deduced that the patterns of chemical shifts in the upfield region at δ 2.90 and 2.62 corresponded to diastereotropic hydrogens α to the carbonyl group in a lactone structure. The downfield diagnostic signal at δ 6.65 was probably caused by vinylic methines and the δ 5.65 signal was suspected to be due to a methine adjacent to the lactone oxygen. To further confirm whether or not this impurity contained an unsaturated double bond and lactone structure at the same time, a portion of this mixture which was enriched in the impurity was hydrogenated and the resulting product was purified over MPLC. After comparison of $^1$H NMR spectra taken before and after the hydrogenation, it was revealed that all of the peaks in the double bond region disappeared after hydrogenation. Moreover, the rest of the peaks did not change [diagnostic signal at δ 6.65 disappeared and the rest of peaks (5.65, 2.90, 2.62) did not change]. From the peak ratios, it was revealed that the lactone was attached to styrylacetic acid and phenyl groups (the broad singlet at δ 5.65 was evidence that the phenyl group was at the γ-position of the lactone). After determining
and assigning each chemical shift and coupling constant of the impurity, it was suggested that it had structure 24 (stereochemistry unknown).

Another impurity appeared in later fractions and was fortunately able to be isolated in pure form from the product mixture. The $^1$H NMR spectrum [δ 2.07-2.31 (m, 1H, CH$_2$), 2.57-2.74 (m, 3H, CH$_3$), 5.50-5.54 (t, $J$ = 6.4 Hz, 1H, OCH$_2$), 7.28-7.41 (m, 5H, ArH)] showed that it was a lactone with a phenyl group attached and its structure was deduced to be that of lactone 25.

![Structural formulas](image)

To finally establish their structures, impurities 24 and 25 were synthesized, as described in the following section.
B. Syntheses of Lactones 24 and 25

Lactone 25 was prepared in low yield (5%) simply by refluxing trans-styrylacetic acid (1) and toluene with 5% (by mass) of sulfonated charcoal catalyst (Scheme X). Most important is that the spectra of the resulting pure product exactly matched all of the chemical shifts appearing in spectra of fractions containing 25 produced during the preparation of 3.

Scheme X. The Synthesis of Lactone 25.

$$\text{H}_2\text{SO}_4 - \text{charcoal} \quad \text{PhCH}_3, \text{reflux}$$

It is clear that the formation of 25 in the synthesis of triglyceride 3 was due to protonation of trans-styrylacetic acid followed by cyclization into the five-membered ring lactone.

To determine the structure of 24, it was decided to prepare lactones 27 and 30 and then esterify them using styrylacetic acid. Treatment of trans-styrylacetic acid (1) with osmium tetroxide and trimethylamine N-oxide gave
3,4-dihydroxy-4-phenylbutanoic acid (26) in 57% yield. Cyclization of this diol with sulfonated charcoal as the catalyst gave exclusively the five-membered lactone 27 in 43% yield. Attempts to esterify 27 using Mitsunobu conditions (1, Ph₃P, DEAD) or DCC-DMAP coupling conditions, however, met with failure (Scheme XI).

Scheme XI. Attempted Syntheses of Impurity 24.
Furthermore, the aliphatic region of the ¹H NMR spectrum of 27 did not match well with the same region of the spectrum of 24. Thus, we turned to the synthesis of lactone 30.

Treatment of trans-styrlyacetic acid (1) with two equivalents of hydrogen peroxide and a catalytic amount of hexafluoroacetone trihydrate gave a presumed epoxide intermediate which immediately cyclized into five-membered lactone 30 with the hydroxyl and phenyl groups in a trans relationship.¹⁹

Scheme XII. Synthesis of Ester 29.
The aliphatic region of the $^1$H NMR spectrum of 30 did match well with the same region of the spectrum of 24. Subsequently, lactone 30 was esterified by means of the DCC-DMAP coupling conditions to afford ester 29. Although this material was contaminated with other compounds [one pure compound ($^1$H NMR signals at $\delta$ 3.42 (d, $J$ = 5 Hz, 1H), 5.78 (t, $J$ = 5 Hz, 2H), 7.3-7.6 (m, 5H)] has been isolated and its structure is being determined]. This material also contained small amounts of the $\alpha,\beta$-unsaturated isomer ($^1$H NMR signals at $\delta$ 3.53 (d, $J$ = 7 Hz), 5.87 (t), and other upfield signals due to aliphatic protons]. The signature of the coupling patterns in the $\delta$ 2.00-3.00 region [2.62 (d, $J$ = 18 Hz) and 2.90 (dd, $J$ = 10.5, 5 Hz)] and the singlet at $\delta$ 5.50 proved that the exact structure of this impurity (24) was that of lactone 29.

It was initially assumed that the formation of 29 involved oxidation of trans-styrylacetic acid (1) by oxygen in the air or possibly in the solvent (toluene) during the high temperature reflux conditions used in the esterification to form 3. Eventually though, we found that lactone 30 was a trace impurity in the starting trans-styrylacetic acid (1) and was responsible for the appearance of impurity 29. The evidence for the presence of 30 in 1 came from its $^1$H NMR spectrum (especially in the region $\delta$ 2.00-3.00) which showed signals that matched those in the spectrum of lactone 30.

The identification of these two possible impurities (25 and 29) concluded our studies on the preparation of triglycerides 3. At this stage of the project, we
scaled up the styrylacetic acid triglyceride preparation to prepare enough material for taste and biological tests. At the same time, we directed our efforts towards the synthesis of monoglycerides of several other compounds that might fall along the metabolic pathway between ɣ-phenylbutyrate and phenylacetate. Preliminary taste and biological data as well as additional synthetic studies will be discussed in the next chapter.
CHAPTER VI

RESULTS AND OTHER POTENTIAL TARGETS

A. Biological results

The good news is that triglyceride 3 has a palatable taste. It has none of the after taste of triglyceride 22 and even tastes a bit sweet. The bad news is that triglyceride 3 does not appear to undergo metabolism in a manner that will render it useful as a food supplement for treatment of urea cycle disorders. In other words, triglyceride 3 does not afford N-phenylacetylglutamine as a metabolic product in a appropriate animal model. This was a surprise since it had been shown by Dr. Brusilow that styrlyacetic acid (1) and triglyceride 22 both lead to excretion of N-phenylacetylglutamine in the same model system. Thus, it is probable that 3 is not hydrolyzed readily by proper esterases, or suffers a metabolic fate (as yet unknown) that takes place faster than ester hydrolysis.
B. Other Alternative Dietary Supplements

As stated before, monoglycerides of acids that fall along the presumed metabolic pathway from γ-phenylbutyrate to phenylacetate are under consideration as possible dietary supplements. Possible targets for synthesis include monoglycerides $31$-$34$. 

![Chemical structures](image-url)
Not much work has been done toward these targets, but preliminary studies toward 31 have been completed (Scheme XIII).

Scheme XIII. Approach to Monoglyceride 31.
Treatment of commercially available solketal (35) with $\text{Li}$ in the presence of DCC-DMAP gave 36 in 78% yield. This material was contaminated with about 3% of the corresponding $\alpha\beta$-unsaturated ester. Hydrolysis of 36 with boric acid in aqueous tetrahydrofuran at reflux for 3 days gave 31 (63%) contaminated with substantial amounts of $\alpha\beta$-unsaturated ester 37 and what is believed to be monoglyceride 38. It is clear that these hydrolysis conditions are too harsh and improvements to this route to 31 are being sought. Due to the impurity of 31, we have yet to be able to determine its physical properties (oil vs solid) and palatability (taste). We hope that styrlyacetic acid monoglyceride 31 can simplify the analysis (only 4 isomers are possible), at the same time, it might provide good taste and be metabolized into phenylacetate. The other important fact is that styrlyacetic acid monoglyceride 31 is actually almost as efficient as triglyceride 3, in terms of dosage, as a dietary supplement. For example, 20 g of triglyceride 3 and 27 g of monoglyceride 31 would (in theory) deliver the needed daily dose of phenylacetate.

C. Conclusion

In conclusion, this thesis has described the synthesis and characterization of triglyceride 3 as a potential dietary supplement for treatment of urea cycle disorders. While 3 meets the taste requirements of a good supplement, it is not metabolized in a useful manner and there are serious
problems associated with its synthesis. Studies directed toward simpler potential supplements are underway.
Experimental Section

General Experimental. All melting points were taken with a Thomas Hoover capillary melting point apparatus and are uncorrected as are all boiling points. Proton magnetic resonance spectra (\(^1\)H NMR) were recorded on Brucker AC-200, Brucker AM-250, Brucker AM-300 spectrometers and are recorded in parts per million from external tetramethylsilane on the δ scale. The \(^1\)H NMR spectra are reported as follow: Chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants in Hz, integration, interpretation]. Carbon-13 nuclear magnetic resonance spectra (\(^{13}\)C NMR) were obtained with a Brucker-300 spectrometer and were recorded in parts per million from external tetramethylsilane. The \(^{13}\)C NMR are reported as follows: chemical shift (multiplicity). Multiplicities were determined by DEPT experiments. Infrared spectra were taken with Perkin Elmer 1600 (FT-IR) instruments. Mass spectra were obtained using Kratos MS-30 or Kratos VG 70-250s instruments at an ionization energy of 70 eV. Compounds for which an exact mass is reported exhibited no significant peak at \(m/z\) ratios greater than that of the parent. Combustion analyses were performed by Atlantic Microlab,
Norcross, GA. Gas chromatographic (GC) analyses were performed using a Hewlett Packard 5890A gas chromatograph.

Solvents and reagents were dried and purified prior to use when deemed necessary. Toluene, benzene, THF were distilled from sodium-benzophenone. Reactions requiring an inert atmosphere were run under argon. Analytical thin-layer chromatography was conducted using EM Laboratories 0.25mm thick precoated silica gel 60 F254 plates. Medium pressure liquid chromatography (MPLC) was performed using EM Laboratories Lobar prepacked silica gel columns. High pressure liquid chromatography (HPLC) was performed using an Isco Model 2350 instrument.

\[
\text{trans-4-Phenyl-3-butenoyl Chloride (9).}^{20}
\]

To 250 mg (1.54 mmol) of trans-styrllacetic acid cooled to 0°C was added 0.27 mL (440 mg, 3.70 mmol) of thionyl chloride dropwise over a period of 5 min. The mixture was stirred at 0°C for 15 min, followed by heating under reflux for 1.5 h. The solution was cooled to room temperature and excess thionyl chloride was removed in vacuo to give 255 mg (97%) of acid chloride 9 as dark brown oil. This material
was used in subsequent reactions without purification: $^1$H NMR (CDCl$_3$, 200 MHz) δ 3.8 (d, J = 7 Hz, 2H, CH$_3$), 6.25 (dt, J = 15, 6 Hz, 1H, CH$_2$CH=), 6.58 (d, J = 15 Hz, 1H, =CHAr), 7.21-7.42 (m, 5H, ArH).

![Chemical structure](image)

5

**Ethyl 4-Phenyl-3-butenoate (5).**

To a suspension of 255 mg (1.54 mmol) of acid chloride 9 in 1 mL of CH$_2$Cl$_2$ at 0°C was slowly added a solution of 0.09 mL (71 mg, 1.54 mmol) of ethyl alcohol and 0.2 mL (156 mg, 1.54 mmol) of Et$_3$N in 1 mL of CH$_2$Cl$_2$. The resulting dark brown solution was allowed to stir at 0°C for 15 min, was warmed to room temperature and stirred for 1.5 h. The resulting mixture was diluted with 20 mL of ether and washed with 20 mL of H$_2$O. The ethereal layer was dried (MgSO$_4$) and concentrated in vacuo. The residue was chromatographed over 25 g of silica gel (eluted with ethyl acetate-hexane, 1:4) to give 158 mg (54%) of ester 5 as brown oil: IR (neat) 1734 cm$^{-1}$;

$^1$H NMR (CDCl$_3$, 300 MHz) δ 1.28 (t, J = 7.5 Hz, 3H, CH$_3$), 3.25 (d, J = 6.0 Hz, 2H, CH$_2$), 4.19 (q, J = 8.0 Hz, 2H, OCH$_3$), 6.25-6.40 (dt, J = 15, 6 Hz, 1H, CH$_2$CH=), 6.50 (d, J = 15 Hz, 1H, =CHAr), 7.04-7.48 (m, 5H, ArH) [diagnostic signals due to the α,β-unsaturated ester (12%) appeared at δ 3.48-3.52 (d, J = 6 Hz, CH$_3$).
Isopropyl 4-Phenyl-3-butenoate (6)  To a suspension of 255 mg (1.54 mmol) of acid chloride 9 in 1 mL of CH₂Cl₂ at 0°C was slowly added solution of 0.12 mL (93 mg, 1.54 mmol) of isopropanol and 0.2 mL (156 mg, 1.54 mmol) in Et₂N in 1 mL of CH₂Cl₂. The resulting dark brown solution was allowed to stir at 0°C for 15 min, warmed to room temperature, and stirred for 2 h. The mixture was diluted with 20 mL of ether and washed with 20 mL of H₂O. The ethereal layer was dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed over 20 g of silica gel (eluted with ethyl acetate-hexane, 1:4) to give 211 mg (67%) of ester 6 as brown oil: IR (neat) 1729 cm⁻¹; ¹H NMR
(CDCl₃, 300 MHz) δ 1.27 (d, 6H, J = 7.5 Hz, CH₃), 3.23 (d, J = 7.5 Hz, 2H, CH₂), 5.36 (heptet, J = 7.5 Hz, 1H, OCH), 6.33 (dt, J = 15.7, 7.5 Hz, 1H, CH₂CH=), 6.50 (d, J = 15 Hz, 1H, =CHPh), 7.20-7.41 (m, 5H, ArH); ¹³C NMR (CDCl₃, 300 MHz) δ 21.7 (q), 39.7 (t), 68.0 (d), 122.0 (d), 126.2 (d), 127.4 (d), 128.4 (d), 133.1 (d), 136.8 (s), 171.0 (s); mass spectrum, m/z (relative intensity) 204 (M⁺), 117 (100), 91 (18), 43 (94); exact mass calcd. for C₁₅H₁₄O₂ m/z 204.1151, found m/z 204.1152.

![Chemical Structure](image)

Ethyl 4-Phenyl-2-butenoate (7). To a stirred solution of 0.24 mL (250 mg, 2.08 mmol) phenylacetaldehyde in 30 mL of CH₂Cl₂ cooled to 0°C was added 1.45 g (4.16 mmol) of (carbethoxymethylidene)triphenylphosphorane. The mixture was stirred at 0°C for 10 min, warmed to room temperature, and stirred for 24 h. The resulting solution was concentrated in vacuo to give yellow solid. The residue was dissolved in 1 mL of CH₂Cl₂ and purified by chromatography over 25 g of silica gel (eluted with ethyl acetate-hexane, 1:4) to give 440 mg (96%) of ethyl ester 7 as a yellow oil. IR (neat) 1714 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 1.29 (t, J = 5.0 Hz, 3H, CH₃), 3.50 (d, J = 6.6 Hz, 2H, CH₂), 51
4.20 (q, J = 6.0 Hz, 2H, OCH₂), 5.82 (dt, J = 15.0, 7.0 Hz, 1H, CH=), 7.08-7.41 (m, 6H, =CHCH₂ and ArH) [Diagnostic signals due to β,γ-unsaturated ester 5 (about 5%) appeared at δ 3.23-3.29 and 6.30-6.58. Diagnostic signals due to cis-α,β-unsaturated esters (about 5%) appeared at δ 4.02-4.10 (d, J = 6 Hz, CH₂)]. ¹³C NMR (CDCl₃, 300 MHz) δ 38.3 (q), 38.4 (t), 62.7 (t), 121.4 (d), 126.6 (d), 128.0 (d), 128.9 (d), 134.1 (d), 171.3 (s) (trace signals due to the minor isomers were also observed); mass spectrum, m/z (relative intensity) 190 (M⁺), 145 (33), 117 (100), 91 (33); exact mass calcld. for C₁₄H₁₄O₂ m/z 190.0998, found m/z 190.0998.

\[
\begin{align*}
\text{BrCH₂} & \quad \text{O} \quad \text{CH(CH₃)₂}
\end{align*}
\]

Isopropyl α-Bromoacetate (39).²⁴ To 1.5 g (10 mmol) of α-bromoacetic acid in 15 mL of toluene was added 620 mg (10 mmol) of isopropyl alcohol and 150 mg of p-toluenesulfonic acid monohydrate. The mixture was heated under reflux for 3 h with water removal using Dean-Stark apparatus. The resulting solution was diluted with 30 mL of ether and washed with two 25 mL portions of aqueous saturated NaHCO₃. The organic layer was dried (MgSO₄) and concentrated in vacuo to give 1.22 g (97%) ester 13 as a colorless oil. This
material was used in subsequent reactions without purification: $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 0.89 (d, $J = 6.0$ Hz, 6H, CH$_3$), 3.75 (s, 2H, CH$_2$), 5.00 (septet, $J = 6.0$ Hz, 1H, CH).

\[ \text{Ph}_3\text{P} = \text{O} \quad \text{O} \quad \text{CH}_3 \]

(ISOproxycarbonylimethylidene)triphenylphosphorane (11). To a stirred solution of 1.21 g (6.7 mmol) of bromide 39 and 30 mL of toluene was added 2.62 g (10 mmol) of triphenylphosphine. The mixture was heated under reflux for 1.5 h and then cooled to 0°C. The resulting phosphonium salt was collected and rinsed with two 5 mL portions of ether to give 2.40 g (98%) of phosphonium salt 40 as a white powder: mp 196-196° C; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 0.93 (d, $J = 7$ Hz, 6H, CH$_3$), 4.7 (septet, $J = 7$ Hz, 1H, CH), 5.49 (d, $J = 15$ Hz, CH$_2$), 7.61-7.89 (m, 15H, ArH). A 0.6 g (1.65 mmol) sample of this phosphonium salt was dissolved in 30 mL of H$_2$O at room temperature and washed with 20 mL of ether. To resulting aqueous portion was added 1 drop of 2% of alcoholic phenolphthalein, followed by cooling 0°C and slow addition of 20 mL of 0.1 N NaOH until a pink end point was reached (adjust the pH value to 7). The resulting white powdery phosphorane was collected by suction filtration.

53
and washed with two 30 mL portions of cold H₂O. The solid was dried in vacuo to afford 0.41 g (70%) of Wittig reagent 11 as a white powder. This material was used in subsequent reactions without purification. mp 102-105°C; ¹H NMR (CDCl₃, 200MHz) δ 1.00 (br d, 6H, CH₃), 1.7 (s, 1H, CH), 4.9 (septet, J = 7 Hz, 1H, CH), 7.40-7.68 (m, 15H, ArH).

Isopropyl 4-phenyl-2-butenoate (8). To a stirred solution of 0.24 mL (300 mg, 2.9 mmol) of phenylacetaldehyde in 30 mL of dry CH₂Cl₂ cooled to 0°C was added 1.80 g (4.98 mmol) of (isopropylcarbonylmethylene)triphénylphosphorane. The mixture was stirred at 0°C for 10 min, warmed to room temperature, and stirred for 16 h. Solvent was removed to give a colorless oil which was purified by chromatography over 25 g of silica gel (eluted with ethyl acetate-hexane, 1:4) to give 49 mg (43%) of ester 8 as a light yellow oil: IR (neat) 1719 cm⁻¹; ¹H NMR (CDCl₃, 200 MHz) δ 1.23 (d, J = 6.0 Hz, 6H, CH₃), 3.51 (d, J = 5.5 Hz, 2H, CH₂), 5.07 (septet, J = 5 Hz, 1H, CH), 5.75 (dt, J = 15.5, 5 Hz, 1H, CH₃), 7.08 (dt, J = 15.5, 5 Hz, 1H, CHCH₂), 7.10-7.40 (m, 5H, ArH); ¹³C NMR (CDCl₃, 300MHz) δ 21.7 (q), 21.8 (q), 38.3 (t), 67.5 (d), 122.7 (d), 126.5
(d), 128.5 (d), 128.6 (d), 128.7 (d), 128.8 (d), 146.8 (s), 165.9 (s). This material was free of the β,γ-unsaturated isomers (no CH₂ at δ 3.2), but contaminated some cis-α,β-unsaturated isomer (5%); based on the following ¹H NMR signals:

δ 1.32 (d, CH₃), 4.0 (d, J = 5.5 Hz, CH₂), 5.10 (septet, J = 5 Hz, OCH), 5.85 and 6.25 (m, CH=CH). This material was also contaminated with trace amounts of other materials (including triphenylphosphine oxide) by ¹H NMR and ¹³C NMR; mass spectrum, m/z (relative intensity) 204 (M⁺), 162 (68), 145 (63), 117 (100), 91 (52), 43 (34); exact mass calcd. for C₁₅H₁₄O₂ m/z 204.1151, found m/z 204.1150.

![Molecule structure](image)

Glyceryl tri-4-Phenyl-2-butenoate (3). A. General procedure for preparation of 3 using p-toluenesulfonic acid as the catalyst: A mixture of 250 mg (1.54 mmol) of trans-styrilacetic acid, 48.6 mg (0.54 mmol) of
glycerol and 17 mg of p-toluenesulfonic acid in 15 mL of dry toluene was heated under reflux for 26 h with water removal using a Dean-Stark apparatus. The solution was cooled to room temperature, diluted with 30 mL of ether, and washed with two 25 mL portions of aqueous saturated NaHCO₃. The organic layer was dried (MgSO₄) and concentrated in vacuo to give a residual yellow oil. This material was chromatographed over 25 g of silica gel (eluted with ethyl acetate-hexane, 1:4) to afford 113 mg (47%) of triglyceride 3 as 96:4 mixture of \( \beta,\gamma- \) and \( \alpha,\beta- \)unsaturated isomers (based on \(^1\)H NMR) as a pale yellow oil: IR (neat) 1744 cm\(^{-1}\); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.29 (d, \( J = 8.25 \) Hz, 6H, CH₃), 4.3 (dd, \( J = 11, 6 \) Hz, 2H, OCH₂), 4.45 (dd, \( J = 11, 6 \) Hz, 2H, OCH₂), 5.42 (septet, \( J = 6 \) Hz, 1H, OCH), 6.20-6.35 (m, 3H, CH₂Cl+Me), 6.5 (broad d, \( J = 15.8 \) Hz, 3H, =CHR), 7.20-7.46 (m, 15H, ArH). Diagnostic signals due to \( \alpha,\beta- \)unsaturated esters appeared at \( \delta \) 3.42-3.50 and 6.28-6.40. \(^{13}\)C NMR (CDCl₃, 300 MHz) \( \delta \) 37.9 (t), 38.0 (t), 62.4 (t), 69.3 (d), 121.0 (d), 121.1 (d), 126.3 (d), 127.3 (d), 128.5 (d), 133.7 (d), 133.7 (d), 136.7 (s), 170.6 (s), 170.9 (s). FAB-MS \( m/z \) (relative intensity) 117 (100), 144 (52), 363 (84); exact mass calcld for \( \text{C}_{29}\text{H}_{32}\text{O}_4 \) \( m/z \) 524.2278, found \( m/z \) 524.37. Anal calcld. for \( \text{C}_{29}\text{H}_{32}\text{O}_4 \) C, 75.52; H, 6.10. Found: C, 74.50; H, 6.18.
B. Preparation and characterization of 3 and other reaction byproducts using p-toluenesulfonic acid as the catalyst: To 950 mg (10.3 mmol) of glycerol in 100 mL of dry toluene was added 5.22 g (35 mmol) of trans-styrylacetic acid and 92 mg of p-toluenesulfonic acid. The mixture was stirred at room temperature for 17 h. TLC analysis indicated no reaction had occurred. The mixture was then warmed under reflux in a Dean-Stark apparatus. TLC analysis over silica gel (developed in ethyl acetate-hexane, 1:4 eluent) after 5 h of reflux indicated that a small amount of reaction had occurred.

To the reaction mixture was added another 98 mg portion of p-toluenesulfonic acid and reflux was continued for total 16 h. TLC analysis indicated only a small amount of change between 5 h and 16 h of reflux. A 3 mL portion of the mixture was stirred with a spatula tipful of Na₂CO₃. This sample also showed no change after 15 min by TLC analysis. To this sample was added 3 mL of H₂O followed by stirring another 5 min. TLC analysis of organic layer indicated most of the unreacted trans-styrylacetic acid had been removed. The aqueous phase
was removed by pipette. The organic phase was washed with 3 mL of H₂O, dried (MgSO₄) and concentrated in vacuo to give a pale yellow oil. This residue was subjected to radical disk chromatography (eluted with ethyl acetate-hexane, 30:70 at the rate of 5 mL min⁻¹) to give 10 mg of styrylacetic acid triglyceride as a pale yellow oil (DJH-3028A). Continued elution gave 8 mg of a mixture of materials that appeared contain some diglyceride monotosylates (based on ¹H NMR signals that included a singlet at δ 2.4 (ArCH₃) and a doublet at δ 7.8 (ArH ortho to SO₃R). Continued elution gave 20 mg of diglyceride 17 as white solid (DJH-3028C): ¹H NMR (CDCl₃, 200 MHz) δ 2.5 (s, 1H, OH), 3.30 (d, J = 7 Hz, 4H, CH₂), 4.05-4.40 (m, 5H, CH₂O and OCH), 6.30 (dt, J = 15, 7 Hz, 2H, CH₂CH=), 6.5 (d, J = 15 Hz, 2H, =CHAr), 7.10-7.46 (m, 10H, ArH). This material was mainly β,γ-unsaturated, but contained a trace of α,β-unsaturated isomers based on ¹H NMR signals at δ 3.5 (d, J = 5 Hz, CH₃) and 5.8 (d, J = 15.5 Hz, =CH). Continued elution gave 20 mg of somewhat impure diglyceride 16 as a pale yellow oil (DJH-3028D). This material contained mainly β,γ-unsaturated isomers (δ 3.30 (d, J = 7 Hz, CH₃), 6.30 (dt, J = 15, 7 Hz, 2H, CH₂CH=)) contained a 2-O-acyl group (δ 5.2 (q, J = 5 Hz, OCH₃)) contained some α,β-unsaturated material (δ 5.8 (d, J = 15.5 Hz, =CH)) and also contained some tosylates (δ 2.4 (s, ArCH₃), 7.8 (d, J = 6 Hz, Ar ortho to SO₃R)).

C. Preparation of 3 using sulfonated charcoal as the catalyst.¹³

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(a) Preparation of sulfonated charcoal catalyst: A mixture of 5 g of active charcoal and 15 g of 98% concentrated sulfuric acid was heated at 245-250°C for 28 h. The resulting black mixture was cooled and the sulfonated charcoal was vacuo filtered through an ACE Buchner funnel with a fibrous glass frit over 2 h. The resulting black powder was dried in vacuo and stored for use.

(b) Preparation of 3 using sulfonated charcoal as the catalyst: To 460 mg (5 mmol) of glycerol in 5 mL of toluene was added 3.24 g (20 mmol) of trans-styrylacetic acid (1) and 2 mg of sulfonated charcoal. The mixture was heated under reflux for 8 h with removal water using a Dean-Stark apparatus which was filled up with toluene. After cooling to room temperature, the thick brown oily residue was diluted with 25 mL of CH₂Cl₂ and filtered. The filtrate was washed with two 25 mL portions of aqueous saturated NaHCO₃. The aqueous layers were extracted with 25 mL of CH₂Cl₂. The combined dichloromethane layers were dried (MgSO₄) and concentrated in vacuo to give crude product as a brown oil. This residue was chromatographed over 120 g of silica gel (eluted with ethyl acetate-hexane, 1: 4) to give 1.86 g (71%) of 3 as a pale yellow oil. ¹H NMR analysis indicated this material was a 94:6 mixture of β,γ- and α,β-unsaturated isomers, respectively (XZ-I-114).
Glyceryl tri-4-Phenylibutyrate (22) A solution of 527 mg (1 mmol) of triglyceride 3 in 10 mL of ethyl alcohol containing 10 mg of 5% palladium on activated carbon was stirred under 1 atmosphere of hydrogen for 72 h. The reaction mixture was filtered through Celite and concentrated in vacuo to afford 533 mg (100%) of analytical pure 22 as colorless oil: IR (neat) 1714 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 1.96-2.03 (quintet, J = 7.0 Hz, 6H, CH₂), 2.40 (t, J = 7.0 Hz, 6H, CH₂CO), 2.70 (t, J = 7.0 Hz, 6H, CH₂Ar), 4.20 (dd, J = 14, 7 Hz, 2H, CH₂O), 4.40 (dd, J = 7.0, 4.9 Hz, 2H, CH₂O), 5.34-5.38 (m, 1H, OCH), 7.22-7.37 (m, 15H, ArH); ¹³C NMR (CDCl₃, 300 MHz) δ 26.3 (t), 26.4 (t), 33.2 (t), 33.4 (t), 34.9 (t), 35.0 (t), 62.1 (t), 69.0 (d), 126.0 (d), 128.4 (d), 128.4 (d), 141.2 (s), 172.5 (s), 172.8 (s); mass spectrum, m/z (relative intensity) 147 (91), 91 (100); exact mass calcd for C₃₃H₅₆O₆: m/z 530.2670, found m/z 530.2663. Anal. calcd. for C₃₃H₅₆O₆: C, 74.68; H, 7.16. found: C, 74.48; H, 7.29.
The General Procedure for Determination and Characterization of Impurities from Syntheses of Compound 3: The crude material (one 7 g sample and one 6 g sample), resulting from large scale preparations of triglyceride 3 using sulfonated charcoal as the catalyst, were individually chromatographed over 400 g of silica gel (eluted with ethyl acetate-hexane, 1:7, 1:5). After around 1500 mL of elution solvent was voided, early fractions (125 mL/fraction) between 1-10 (which were diffuse spots by TLC analysis) were collected and concentrated in vacuo to give 105 mg and 410 mg of product mixtures as a pale yellow oil (XZ-I-123-PreA and XZ-I-127-PreA). 1H NMR analysis of this material indicated that there were trace amounts of an inseparable impurity in this sample, which was at this point mainly 3. Diagnostic signals at δ 6.65 (t, J = 10.5 Hz), 5.65 (s), 2.90 (dd, J = 10.5, 5 Hz) and 2.62 (d, J = 18 Hz) corresponded to this impurity. To further identify the structure of this impurity, these residues were combined (total 515 mg), and further purified by medium pressure liquid chromatography (MPLC), eluting with ethyl acetate-hexanes, 1:10, 1:7, 1:5, 1:3. Individual fractions (8 mL/fraction) were monitored by TLC analysis and analyzed by 1H NMR spectroscopy. It was deduced from the peak ratios and their appearance in the different fractions (XZ-I-132A-F) that the peaks listed before only showed together and thus, corresponded to one compound. It was also deduced from the patterns of chemical shifts in the upfield region at δ 2.90 and 2.62 that these chemical shifts were caused by diastereotopic hydrogens α to the carbonyl group in a lactone.
structure. The downfield diagnostic signal at δ 6.65 was probably caused by the vinlyc methines and the δ 5.65 signal was suspected to be due to a methine adjacent to the lactone oxygen. To further confirm whether or not this impurity contain a double bond and lactone structure at the same time, a portion (about 80 mg) of these mixtures which was enriched in the impurity was hydrogenated and the resulting product was purified over MPLC (eluted with ethyl acetate-hexane, 1:10). After comparing 1H NMR spectra taken before and after the hydrogenation, it was revealed that all peaks in the double bond region disappeared after the hydrogenation. Moreover, the rest of the peaks (5.65, 2.90, 2.62) did not change. From the peak ratios, it was shown that the lactone was attached to styrylacetic acid and phenyl group (the broad singlet at δ 5.65 was the evidence that the phenyl group was at γ-position of the lactone). After determining each chemical shifts and coupling constants of the impurity, it was suggested that it had structure 24 (stereochemistry unknown). The other impurities appeared as diffuse spots after elution of triglyceride 3. These later fractions were combined and concentrated in vacuo to give a mixture as pale yellow oil. This residue (around 250 mg) was further chromatographed over 30 g of silica gel, eluted with ethyl acetate-hexanes, 1:7, 1:6 and 1:5. The middle fractions, which were clean spots by TLC analysis, were combined and concentrated in vacuo to give 35 mg of pure impurity 25 as a pale yellow oil. The 1H NMR spectrum (δ 2.07-2.31 (m, 1H, CH₂), 2.57-2.74 (m, 3H, CH₃), 5.50-
5.54 (t, J = 6.4 Hz, 1H, OCH) and 7.28-7.41 (m, 5H, ArH)] showed that it was a lactone structure with a phenyl group in the γ-position. This material was assigned structure 25 after a spectroscopy study. To finally establish their structures, impurities 24 and 25 were synthesized, as described below.

![Structure 25](image)

5-Phenyltetrahydrofuran-2-one (25). A mixture of 100 mg (0.6 mmol) of trans-styrylacetic acid, 5 mg of sulfonated charcoal and 10 mL of dry toluene was heated under reflux for 10 h. The resulting solution was cooled to room temperature, diluted with 20 mL of ethyl acetate, and filtered. The filtrate was washed with 20 mL of 10% aqueous NaOH and the aqueous wash was extracted with two 20 mL portions of ethyl acetate. The combined organic layers were dried (MgSO₄) and concentrated in vacuo to give 5 mg (5%) of lactone 25 as a colorless oil: IR (neat) 2924, 1770 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 2.67-2.31 (m, 1H, CH₃), 2.57-2.74 (m, 3H, CH₃), 5.50-5.54 (t, J = 6.4 Hz, 1H, OCH), 7.28-7.41 (m, 5H, ArH); ¹³C NMR (CDCl₃, 300 MHz) δ 28.9 (t), 30.9 (t), 81.2 (d), 125.2 (d), 128.4 (t), 128.7 (t), 139.3 (s) 176.8 (s); mass spectrum, m/z (relative
intensity) 162 (M⁺), 117 (57), 107 (78), 77 (54), 56 (75); exact mass calcd for \( \text{C}_{14}\text{H}_{19}\text{O}_{5} \) \( m/z \) 162.0681, found \( m/z \) 162.0679.

\[ \text{OH} \quad \text{COOH} \]

\[ \text{26} \]

**rel-(3R,4R)-Dihydroxy-4-phenylbutanoic acid (26)** To a stirred solution of 4.02 g (25 mmol) of trans-styrylactic acid (1) in 50 mL of t- BuOH and 15 mL of \( \text{H}_2\text{O} \) was added 2.78 g (25 mmol) of trimethylamine N-oxide and 10 mL (25 mmol) of 0.004 M aqueous osmium tetroxide. The mixture was stirred at 60°C for 6 h during which four additional 0.35 g portions of trimethylamine N-oxide (12.5 mmol total) were added at 1.5 h intervals. The resulting solution was cooled to room temperature and stirred for 15 h. The resulting dark brown solution was further cooled to 0°C and 20 mL of saturated NaHSO₃ was added. The solution was stirred for 10 min, acidified with 20 mL of concentrated HCl, and extracted with three 150-mL portions of ethyl acetate. The organic extracts were washed with 150 mL of saturated NaCl, dried (MgSO₄) and concentrated in vacuo to give crude diol 26 as light yellow solid. This material was recrystallized from 40 mL of ethyl acetate to afford 2.8 g (57%) of diol 26 as white powder: mp 118-120°C; \( \text{IR} \) (neat) 3284, 2896, 1708 cm\(^{-1} \); \( ^{1}H \text{NMR} \)
(DMSO-d$_6$, 250 MHz) δ 2.0 (dd, J = 14, 10 Hz, 1H, CH$_3$CO$_2$H), 2.31 (dd, J = 15.5, 5 Hz, 1H, CH$_2$CO$_2$H), 3.3 (s, 1H, OH), 3.96-4.03 (m, 1H, CH$_2$OH), 4.50 (d, J = 4 Hz, 1H, CHAr), 5.31 (s, 1H, OH), 7.22-7.37 (m, 5H, ArH), 8.7 (s, 1H, COOH).

$^{13}$C NMR (DMSO-d$_6$, 300 MHz) δ 38.2 (t), 71.8 (d), 75.3 (d), 127.0 (d), 127.1 (d), 127.8 (d), 142.7 (s), 173.2 (s); mass spectrum, m/z (relative intensity) 196 (M$^+$), 108 (46), 107 (103), 79 (65); exact mass calcd. for C$_8$H$_{12}$O$_4$: m/z 196.0736, found m/z 196.0731. Anal calcd. for C$_8$H$_{12}$O$_4$: C, 61.20; H, 6.17. Found: C, 61.15; H, 6.15.

![Chemical structure](image)

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cis-β-Hydroxy-γ-phenyl-γ-butyrolactone (27). A mixture of 495 mg (2.5 mmol) of diol 26, 10 mg of sulfonated charcoal and 10 mL of dry toluene was heated under reflux for 1 h. The resulting solution was diluted with 10 mL of ethyl acetate, filtered and concentrated in vacuo. The residued yellow oil was chromatographed over 20 g of silica gel (eluted with ethyl acetate-hexane, 1:7, 1:5, 1:3 and then pure ethyl acetate) to give 190 mg (42%) of lactone 27 as a
yellow oil: IR (neat) 3429, 1747 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 1.63 (s, 1H, OH), 2.70 (d, J = 18 Hz, 1H, CH₂CO), 2.87 (dd, J = 18, 7 Hz, 1H, CH₂CO), 4.60 (s, 1H, CHOH), 4.98 (d, J = 7 Hz, 1H, CHAr), 7.35-7.46 (m, 5H, ArH). ¹³C NMR (CDCl₃, 300 MHz) δ 38.4 (t), 70.1 (d), 85.0 (d), 126.2 (d), 128.8 (d), 128.9 (d), 132.9 (s), 175.4 (s); mass spectrum, m/z (relative intensity) 179 (M⁺1), 107 (100), 79 (43); exact mass calcd. for C₁₀H₁₀O₃ m/z 178.0630, found m/z 178.0631.

trans-β-Hydroxy-γ-phenyl-γ-butyrolactone (30). To a stirred solution of 2 mL (170 mg, 20 mmol) of 30% hydrogen peroxide in 10 mL of CH₂Cl₂ was added 0.25 mL (330 mg, 1.5 mmol) of hexafluoroacetone trihydrate. The mixture was stirred at 0°C for 5 min. Then 1.62 g (10 mmol) of trans-styrylacetic acid (1) in 20 mL of CH₂Cl₂ was added dropwise over 45 min period. The resulting pale yellow solution was kept at 0°C for 2 h and warmed to room temperature where it was kept for another 10 h. The resulting solution was diluted with 25 mL of CH₂Cl₂ and washed with two 30 mL portions of aqueous
NaHCO₃. The organic layer was dried (MgSO₄) and concentrated in vacuo to afford 210 mg of yellow solid. This residue was chromatographed over 25 g of silica gel (eluted with ethyl acetate-hexanes, 1:7, 1.5, 1:3) to afford 180 mg (8%) of lactone 30 as a yellow solid: mp 88-90°C; IR (neat) 3437, 1746 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 2.60 (dd, J = 18, 7 Hz, CH₂CO), 2.85 (dd, J = 18, 7 Hz, 1H, CH₂CO), 4.50 (dt), 5.38 (d, J = 7 Hz, 1H, CHAr), 7.25-7.40 (m, 5H, ArH); ¹³C NMR (CDCl₃, 300 MHz) δ 36.9 (t), 74.3 (d), 87.9 (d), 125.0 (d), 128.6 (d), 128.8 (d), 136.6 (s), 175.3 (s); mass spectrum, m/z (relative intensity) 178 (M⁺), 107 (100), 79 (68), 77 (46), 51 (30), 43 (33); exact mass calcld. for C₁₇H₁₄O₃, m/z 178.0630, found m/z 178.0627.

trans-β-(4-Phenyl-2-butenoic)-γ-phenyl-γ-butyrolactone (29). To a stirred mixture of lactone 30 (100 mg, 0.56 mmol) and trans-styrylacetic acid (1) (91.2 mg, 0.56 mmol) in 7 mL of dry CH₂Cl₂ was added 115 mg (0.56 mmol) of dicyclohexylcarbodiimide (DCC) and 67 mg (0.56 mmol) of 4-
(dimethylamino)pyridine (DMAP). The resulting orange solution was stirred at room temperature for 14 h. This residue was filtered through Celite and the filter cake was washed with 15 mL of CH₂Cl₂. The organic layer was washed with two 35 mL portions of saturated aqueous NaHCO₃, and one 25 mL portion of 1N HCl solution. The organic layer was dried (MgSO₄) and concentrated in vacuo to give 121 mg of crude product as an orange oil. This material was chromatographed over 40 g of silica gel (eluted with ethyl acetate-hexanes, 1:10) to give 70 mg of lactone 29 as a colorless oil: ¹H NMR (CDCl₃, 300 MHz) δ 2.6 (dd, J = 18, 2 Hz, 1H, CH₃), 2.9 (dd, J = 18, 7 Hz, 1H, CH₂), 3.37 (d, J = 7 Hz, 2H, CH₂CH=), 5.35 (dt, J = 7, 2 Hz, 1H, CH), 5.6 (s, 1H, CH), 6.25 (dt, J = 20, 7 Hz, 1H, CH=), 6.55 (d, J = 20 Hz, 1H, =CHAr), 7.20-7.51 (m, 10 H, Ar). This material was contaminated by some of the corresponding α,β-unsaturated ester [3.54 (d, J = 7 Hz) and 5.78 (t)] and an additional as yet unidentified compound that has the following ¹H NMR spectrum: δ 3.4 (d, J = 5 Hz, 1H). 5.8 (t, J = 5 Hz, 2H), 7.3-7.6 (m, 5H).
(2,3-O-Isopropylidene)glyceryl-trans-4-phenyl buenoate (36). To a mixture of 132 mg (1 mmol) of solketal and 162 mg (1 mmol) of trans-styrylactic acid (1) in 7 mL of CH₂Cl₂ was added 206 mg (1 mmol) of dicyclohexylcarbodiimide (DCC) and 120 mg (1 mmol) of 4-(N,N-dimethylanino)pyridine (DMAP) in sequence. The resulting yellow solution was stirred at room temperature for 13 h. The mixture was diluted with 25 mL of ether and filtered through Celite. The yellow ethereal filtrate was washed in sequence with two 25 mL portions of 1N HCl, one 25 mL portion of saturated NaHCO₃, and two portions of 25 mL of saturated aqueous NaCl. The organic layer was dried (MgSO₄) and concentrated in vacuo. The residue oil was chromatographed over 25 g of silica gel (eluted with ethyl acetate-hexane, 1:10) to give 215 mg (78%) of 36 as a yellow oil: IR (neat) 1736 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 1.40 (d, J = 20 Hz, 6H, CH₃), 3.30 (d, J = 7.5 Hz, 2H, CH₂CO), 3.72 (dd, J = 8, 6 Hz, 1H, OCH), 4.02-4.28 (m, 3H, OCH₃), 4.29-4.36 (m, 1H, OCH₂), 6.28 (dt, J = 15, 8 Hz, 1H, =CH), 6.5 (d, J = 15 Hz, 1H, ArCH=), 69
7.10-7.40 (m, 5H, Ar-H); 13C NMR (CDCl₃, 300 MHz) δ 25.3 (q), 25.6 (q), 38.0 (t), 64.9 (t), 66.1 (t), 73.5 (t), 109.7 (s), 121.3 (d), 126.2 (d), 127.5 (d), 128.4 (d), 128.6 (d), 133.6 (d), 136.6 (d), 171.2 (s); mass spectrum. m/z (relative intensity) 261 (M-CH₃), 218 (25), 214 (15), 144 (15), 117 (69), 115 (24), 105 (29), 101 (39), 91 (30), 77 (25), 59 (20), 57 (18), 43 (100); exact mass calc. for C₁₁H₁₀O₆
m/z 276.1362, found m/z 277.1073. Trace amounts of what is most likely the α,β-unsaturated isomer was detectable by 13C NMR and 1H NMR [δ 5.8 (d, J = 15 Hz), =CH].

![Structural diagram](image)

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1-O-Glyceryl trans-4-phenyl-3-butenoate (31). To 555 mg (2 mmol) of ester 36 in 15 mL of THF-H₂O (4:1) was added 992 mg (16 mmol) of boric acid. The mixture was heated under reflux for 72 h. The mixture was cooled to room temperature and extracted with three 20 mL portions of CH₂Cl₂. The combined dichloromethane layers were dried (MgSO₄) and concentrated in vacuo. This residue was chromatographed over 30 g of silica gel (eluted with ethyl acetate-hexanes, 1:10, 1:7, 1:5, 1:3 and pure ethyl acetate) to give 275 mg
(58%) of 31 as a yellow material: 1R (neat) 1730 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 2.31 (s, 2H, OH), 3.31 (d, J = 8 Hz, 2H, CH₂), 3.59-3.72 (m, 2H, CH₂O), 3.91-3.98 (m, 1H, OCH), 4.12-4.26 (m, 2H, CH₂O), 6.25 (dt, J = 15, 7 Hz, 1H, =CH), 6.50 (d, J = 15 Hz, 1H, =CHAr), 7.13-7.38 (m, 5H, ArH); ¹³C NMR (CDCl₃, 300 MHz) δ 38.1 (t), 63.2 (t), 65.5 (t), 70.0 (d), 121.0 (d), 126.2 (d), 127.6 (d), 128.5 (d), 133.8 (d), 138.5 (s), 172.0 (s); mass spectrum, m/z (relative intensity) 145 (35), 144 (90), 117 (100), 115 (52), 91 (35); exact mass calcld. for C₁₅H₂₀O₄, m/z 236.1049; found m/z 236.1051. This material was contaminated with small amounts of the α,β-unsaturated isomer by ¹³C NMR and ¹H NMR [δ 5.8 (d, J = 15 Hz, =CH)] and perhaps a small amounts of the 2-acylated glycerol [δ 3.85 (d, J = 8 Hz, CH₂OH), 5.0 (quintet, J = 8 Hz, OH)].
LIST OF REFERENCES


7. We thank Rose Kaplan (OSU Newark Math Lab) for deriving this equation.


24. Purchased from Aldrich Chemical Co.
APPENDIX A: $^1$H NMR and $^{13}$C NMR Spectra of Selected Compounds
XZ-I-013 (CDCl₃, 200 MHz)
DZH-3028D (CDCl₃, 200 MHz)
XZ-I-203A (CDCl₃, 300 MHz)