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THE SYNTHESIS OF CHIRAL GLYCERIDES STARTING FROM D- AND L-SERINE

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A method for synthesizing chiral glycerides starting from L- or D-serine is described. Optically-active serine (both enantiomers are commercially available) was transformed into glyceric acid by stereospecific diazotization. The configuration at carbon atom 2 was maintained during the reaction. The glyceric acid was then converted into optically pure isopropylideneglycerol – which is an important intermediate in the synthesis of mono-, di- and triglyderides – by esterification followed by acetalization with acetone and reduction with lithium aluminium hydride.

Reaction of this intermediate with triphenylphosphine in tetrachloromethane followed by acid-catalysed hydrolysis and dehydrohalogenation provided optically-active glycidol (2,3-epoxy-1-propanol). The epoxy ring of an ester of glycidol and a fatty acid was then opened stereospecifically with retention of configuration by heating the glycidol ester in the presence of a second fatty acid and a catalyst. This yielded a chiral 1,3-diglyceride which could be converted into a chiral triglyceride.

I. Introduction

The physical, chemical and biochemical properties of one enantiomer of a chiral (optically-active) glyceride may differ markedly from those of a racemic mixture. In order to study the behaviour of natural fats, which are often composed of chiral triglycerides, it may be necessary to obtain the main components of a fat in a pure state. As isolation of chiral glycerides from natural sources is almost impossible, it is necessary to synthesize these compounds. The most successful stereospecific synthesis available now is the method of Baer and Fischer [1,2] which has been improved later on [3,4]. D-mannitol was converted to its 1,2,5,6-diisopropylidene derivative, which was oxidized into two molecules of isopropylidene-D-glyceraldehyde. Reduction of this compound with Raney nickel gave 1,2-O-isopropylidene-*sn*-glycerol. This compound was then converted into chiral glycerides with the aid of protecting groups. However, the L-enantiomer of mannitol can only be prepared by a tedious and lengthy series of reactions starting from L-arabinose [5].

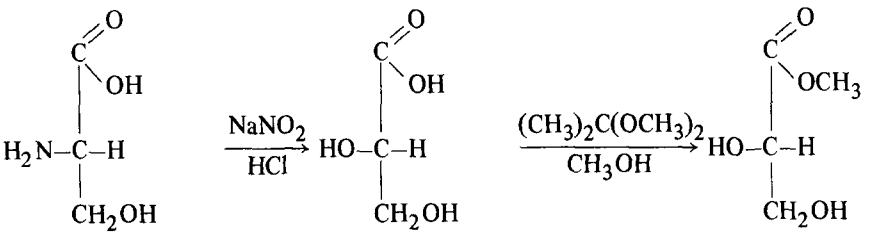
This paper describes a synthesis of chiral glycerides and their precursors from optically-active glycidol (2,3-epoxy-1-propanol) prepared from serine. Since L- and

D-enantiomers of serine are commercially available, this method has the advantage of providing *both* enantiomers of a glyceride in an equally facile way by the same reaction route. This is especially important for the synthesis of chiral mono- and 1,2-diglycerides. Although the enantiomers of a triglyceride or 1,3-diglyceride can be obtained by starting from the same isopropylidene-glyceraldehyde or serine enantiomer and interchanging the fatty acids at postitions 1 and 3 of glycerol during the reaction sequence, this strategy cannot be used for the synthesis of enantiomeric monoglycerides or 1,2-diglycerides from a common starting material.

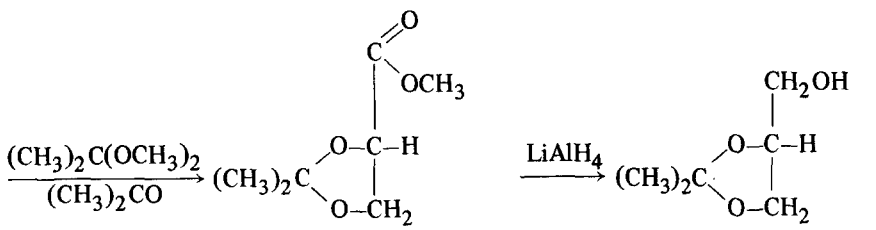
II. Results

The synthesis of chiral tri-acid triglycerides starting from L-serine will be described.

The first reaction step involved diazotization of L-serine (**I**) with sodium nitrite in dilute hydrochloric acid solution. The amino group is transformed into a hydroxyl with a net retention of configuration, presumably because of formation of a 1,2-lactone [6] accompanied by one inversion, followed by reaction of the 1,2-lactone with water to yield L-glyceric acid (**II**), with a second inversion. Without isolation or purification, **II** was esterified with a mixture of 2,2-dimethoxypropane and methanol. A small fraction of the ester **III** was spontaneously converted to methyl 2,3-0-isopropylidene-L-glycerate (**IV**). To complete this acetalization, the residue was reacted with a mixture of acetone and 2,2-dimethoxypropane. Compound **IV** was obtained by distillation in an overall yield of 57% after three steps. Subsequent reduction of **IV** with lithium aluminium hydride gave 2,3-0-isopropylidene-*sn*-glycerol (**V**) in 80% yield and in optically pure form.

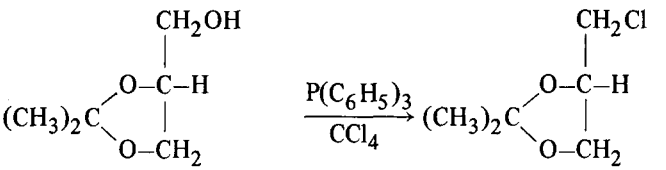


I **II** **III**

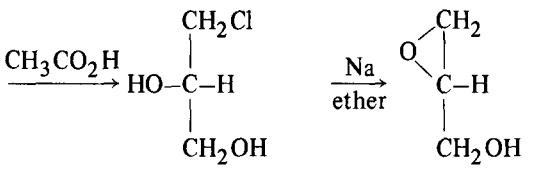


IV **V**

This compound is a useful intermediate in the synthesis of mono, di- and triglycerides as well as for phospholipids. For the synthesis of triacid triglycerides it was transformed into (−)-2,2-dimethyl-4-chloromethyl-1,3-dioxolane (**VI**) in 85% yield by heating under reflux with triphenylphosphine in excess anhydrous tetrachloromethane. Hydrolysis in 10% acetic acid (93% yield) followed by dehydrohalogenation (86% yield) provided (−)-D-glycidol (**VIII**) with a specific rotation of $[\alpha]_D^{22} - 14.3°$ (neat). Sowden and Fischer [7] report +15° for (+)-L-glycidol prepared by reacting 3-(*p*-toluenesulphonyl)-*sn*-glycerol with sodium methoxide.



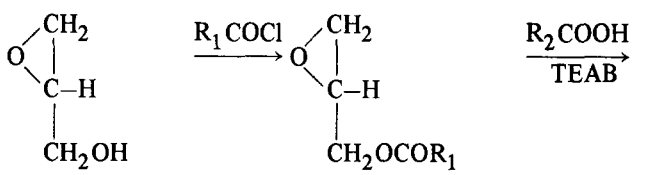
V **VI**



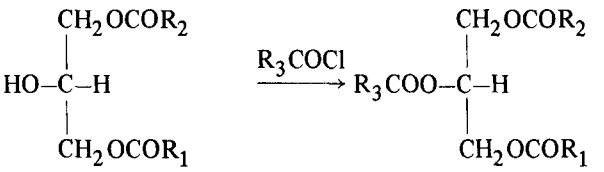
VII **VIII**

Reaction of **VIII** with an acid chloride at −10°C in the presence of pyridine produced a D-glycidol ester **IX** (72–82% yield).

Stereospecific ring opening with retention of configuration occured when glycidol ester **IX** was heated with a second fatty acid in the presence of tetraethylammonium bromide (TEAB) [8]. Solid-state isomerization [9] of the reaction mixture gave a 1,3-diglyceride (**X**) which, after purification, could be esterified by the chloride of a third fatty acid, yielding an optically active triglyceride (**XI**). Retention of configuration in **X** is in agreement with the known stereospecificity of ring opening in epoxides (see, for example, ref. [10]) and with intramolecular acyl group rearrangement during the solid-phase isomerisation of the diglyceride [8,9].



VIII **IX**



X XI

Very few of the general methods used for establishing the optical purity of organic compounds can be applied to the analysis of chiral triglycerides. For example, the asymmetry of chiral triglycerides only containing stearic and palmitic acids is so small that their rotation of polarized light cannot be measured by the present techniques. However, it was possible to distinguish the two enantiomers of several chiral triglycerides and their precursors by ^{1}H-NMR spectroscopy using chiral shift reagents. The ^{1}H-NMR spectrum of D-glycidol acetate to which a chiral shift reagent has been added did not show the presence of any L-enantiomer (less than 6% is not detectable). The same technique showed that 2,3-diacetyl-1-stearoyl-*sn*-glycerol derived from this compound, before crystallization probably contained 6% of its enantiomer and that the optical purity of 3-propionyl-1,2-distearoyl-*sn*-glycerol was at least 86%. In this case, the presence of less than 7% of its enantiomer is not detectable by this technique. The determination of the optical purity of the triglycerides by ^{1}H-NMR spectroscopy will be dealt with in more detail in a following paper [11]. 1-Butyryl-2,3-distearoyl-*sn*-glycerol and 1-palmitoyl-2,3-distearoyl-*sn*-glycerol were also prepared via the monoglyceride route of Baer and Fischer [12]. These compounds had the same melting behaviour and X-ray diffraction patterns as their enantiomers prepared for the present work.

Experimental

A. (−) Methyl 0-isopropylidene-L-glycerate (IV)

To a solution of 52.5 g (−)-L-serine (Merck) and 75 ml conc. HCl in 3 l water, 30 g $NaNO_2$ was added in portions while stirring at 0°C. Stirring was continued for 24 hr at this temperature. Then an additional 10 g $NaNO_2$ was added, and after 24 hr at 0°C and 16 hr at room temperature, the water was evaporated at reduced pressure. To the residue, 150 ml methanol, 15 ml conc. HCl and 400 ml 2,2-dimethoxypropane were added and the mixture obtained was stirred for 2 hr. The ester was not isolated but converted to its acetone acetal by filtering, evaporating the solvents under vacuum, and mixing the residue with 400 ml acetone, 100 ml 2,2-dimethoxypropane and 0.5 g *p*-toluenesulphonic acid. The mixture was stirred for 8 h at room temperature. After filtering and evaporating, the residual oil was distilled under vacuum, yielding 46 g methyl 0-isopropylidene-L-glycerate (57%), b.p. 77–80°C/10

mmHg, n_D^{21} 1.4270, $[\alpha]_D^{22}$ – 10.1° (neat), Rf 0.65 (toluene/acetone 7 : 3). IR (film): 1760, 1740, 1440, 1250, 1210, 1110 and 1028 cm^{-1} (methyl ester); 1150 and 1070 cm^{-1} (dioxolane); 1385, 1372 and 838 cm^{-1} (dimethyl substituent). ^{1}H-NMR ($CDCl_3$, Varian HR-220; δ-values in ppm, J in Hz): δ 1.39 (s, 3H, CH_3); δ 1.48 (s, 3H, CH_3); δ 3.77 (s, 3H, OCH_3); δ 4.09 (d of d, 1H of CH_2, J = 8.5 and 5.3); δ 4.24 (d of d, 1H of CH_2, J = 8.5 and 7.0); δ 4.60 (d of d, 1H, C–H, J = 7.0 and 5.3).

B (–)-2,3-0-isopropylidene-sn-*glycerol* (V)

A solution of 20 g methyl 0-isopropylidene-L-glycerate in 100 ml dry diethyl ether was slowly added to a mixture of 4 g lithium aluminium hydride and 75 ml dry ether at a rate sufficient to maintain gentle boiling. After the addition, the mixture was refluxed for 0.5 hr. Excess reagent was destroyed by adding a mixture of ethyl acetate and ether. A voluminous gelatinous precipitate formed, which was converted into a granular precipitate by adding a small amount of 1 : 1 mixture of water and ethanol. After filtration, the precipitate was washed with diethyl ether. The combined organic layers were dried over $MgSO_4$ and after filtration, evaporated under vacuum. Distillation of the residue yielded 13.2 g (80%) (–)-2,3-0-isopropylidene-*sn*-glycerol, b.p. 75.5–76.0°C/10 mmHg; n_D^{25} 1.4345 (lit. [1] 1.4345); $[\alpha]_D^{22}$ – 13.2° (neat) (lit. [5] $[\alpha]_D$ – 13.4°). IR and ^{1}H-NMR spectra were identical to those of authentic 0-isopropylideneglycerol [1]. The rotation of the enantiomeric (+)-1,2-0-isopropylidene-*sn*-glycerol prepared from D-serine was found to be of equal magnitude ($[\alpha]_D^{22}$ + 13.1°, lit. [1] $[\alpha]_D$ + 12.6°).

C. (–)-2,2-dimethyl-4-chloromethyl-1,3-dioxolane (VI)

Compound VI was prepared according to the method of Lee and Nolan [13] for the racemic compound by reaction of 2,3-0-isopropylidene-*sn*-glycerol with triphenylphosphine in excess tetrachloromethane. Yield 85%, b.p. 48–51°C/13 mm Hg. $[\alpha]_D^{22}$ – 37.5° (neat), n_D^{22} 1.4348, density 1.1079 g/ml, the IR spectrum was identical to that of the racemic compound. ^{1}H-NMR ($CDCl_3$, HR-220; δ-values in ppm, J in Hz) δ 1.36 (s, 3H, CH_3); δ 1.44 (s, 3H, CH_3); δ 3.46 (d of d, J = 10.9 and 7.5, 1H of CH_2Cl); δ 3.58 (d of d, J = 10.8 and 4.8, 1H of CH_2Cl); δ 3.87 (d of d, J = 8.6 and 5.2, 1H of CH_2O); δ 4.11 (d of d, J = 8.6 and 6.0, 1H of CH_2O); δ 4.30 (C, 1H, H-4).

D. (+)-3-chloro-1,2-propanediol (VII)

A mixture of 49.5 g VI and 350 ml 10% aqueous acetic acid was heated with vigorous stirring at 60°C for 2.5 hr. After evaporation of the resulting clear solution at reduced pressure, the residue was mixed with 50 ml water, and evaporated again to remove last traces of acetic acid. Then the product was dried by addition of 100 ml benzene, followed by evaporation. The residue was purified by distillation, yielding 33.9 g (93%) (+)-3-chloro-1,2-propanediol, b.p. 122.5–124°C/19 mmHg;

$[\alpha]_D^{22}$ + 0.96° (neat). The IR spectrum was identical to a model spectrum of the racemic compound.

E. (−)-D-glycidol (**VIII**)

Compound **VIII** was obtained by the method of Kester et al. [14] for the racemic compound. A mixture of 27.8 g (0.25 mol) (+)-3-chloro-1,2-propanediol and 150 ml anhydrous diethyl ether was cooled to 10°C in a 250 ml three-necked flask equipped with stirrer and reflux condenser. An amount of 5.63 g (0.245 mol) sodium shavings was added and the mixture stirred vigorously for 6 h at 10°C and 10 h at room temperature. At the end of this period, all the sodium had been consumed and a white precipitate of NaCl had formed. The product was filtered and distilled, yielding 15.9 g (86%) (−)-D-glycidol, b.p. 66–67°C/19 mmHg, $[\alpha]_D^{22}$ − 14.3° (neat). Sowden and Fischer [7] report $[\alpha]_D^{21}$ + 15° (neat) for (+)-L-glycidol prepared by reacting (−)-3-(*p*-toluenesulfonyl)-*sn*-glycerol with sodium methoxide.

F. (−)-D-glycidol esters (**IX**)

General procedure: To prevent formation of undesired by-products, the reaction was carried out at −15°C. An amount of 0.12 mol dry pyridine was added at −15°C to a mixture of 0.10 mol (−)-D-glycidol and 30 ml anhydrous diethyl ether in a 250 ml three-necked flask equipped with a mechanical stirrer, reflux condenser and dropping funnel. Then a solution of 0.10 mol fatty acid chloride in 30 ml anhydrous diethyl ether was slowly added to the mixture with vigorous mechanical stirring for 0.5 hr. Stirring was continued for an additional 0.5 hr at −15°C and at room temperature till the reaction was complete (1–2 hr for the shorter chain fatty acids (C_2–C_6) and overnight for palmitic acid). Washing three times with 30 ml water removed most of the pyridinium salt. The solution was dried over $MgSO_4$ and evaporated. The shorter chain glycidol esters (C_2–C_6) were purified by distillation at reduced pressure; glycidol palmitate was chromatographed on a column of SiO_2, 10% H_2O. Yields varied between 72 and 82%.

The ^{1}H-NMR and IR spectra were identical to those of the racemic compounds. The physical properties are presented in table 1.

G. 1,3-Diglycerides

A mixture of equimolar amounts of D-glycidol ester and fatty acid was heated and stirred in the presence of 0.02 mol/mol TEAB at 100°C for 2 hr or till all glycidol ester had been consumed (TLC). In the synthesis of diglycerides containing a short-chain fatty acid, the glycidol ester of the lower fatty acid was the preferred starting material. After cooling, the reaction mixture was dissolved in $CHCl_3$, washed with water to remove catalyst, dried over $MgSO_4$, filtered and evaporated. When possible, the product was isomerized in the solid state at temperatures just below the

Table 1
Physical properties of some D-glycidol esters of fatty acids.

Fatty acid	R_f [a]	m.p. (°C)	b.p. (°C)	$[\alpha]_D^{22}$ (neat) (°)
Acetic	0.38		70–71 (22 mmHg)	−29.9
Propionic	0.47		80–81 (16 mmHg)	−27.7
Butyric	0.51		90 (19 mmHg)	−28.4
Hexanoic	0.56		117 (17 mmHg)	−23.2
Palmitic	0.69	49–50		−14.4 [b]

[a] in toluene/ether, 85 : 15 (v/v)
[b] Concentration glycidol ester in $CHCl_3$ 195 mg/ml

melting point of the mixture. Recrystallization from acetone yielded in most cases a pure 1,3-diglyceride. Yields varied between 45% (low-melting diglycerides) and 85% (high-melting diglycerides). Examples: 3-butyryl-1-stearoyl-*sn*-glycerol, m.p. 55–56°C; 3-palmitoyl-1-stearoyl-*sn*-glycerol, m.p. 74–75°C and 1-oleoyl-3-palmitoyl-*sn*-glycerol, m.p. 45–46°C.

H. Triglycerides

An amount of 0.12 mol acid chloride in an equal volume of hexane was added slowly to a suspension of 0.10 mol 1,3-diglyceride in 250 ml dry hexane containing 0.13 mol pyridine. After 1 hr, the mixture was slowly heated to reflux temperature and kept at that temperature for 1 hr or till the reaction was complete (TLC). After the addition of a few ml water to destroy unreacted acid chloride, the mixture was cooled, washed with a few ml water and several times with 50 ml NH_4OH (0.5 mol/l) in methanol/water, 3 : 1 (v/v) to remove free fatty acid, and subsequently with methanol/water mixture only. The organic layer was dried over $MgSO_4$. The product was purified by column chromatography and/or crystallization from acetone or benzene/ethanol, 1 : 1 (high-melting triglycerides).

The following optically-active glycerides were synthesized: 3-acetyl-1,2-distearoyl-*sn*-glycerol m.p. 49°C, 3-propionyl-1,2-distearoyl-*sn*-glycerol m.p. 48°C, 3-butyryl-1,2-distearoyl-*sn*-glycerol m.p. 49°C, 3-palmitoyl-1,2-distearoyl-*sn*-glycerol m.p. 61°C, 2,3-dipalmitoyl-1-stearoyl-*sn*-glycerol m.p. 58°C, 1-oleoyl-2,3-dipalmitoyl-*sn*-glycerol m.p. 37°C, 2,3-diacetyl-1-stearoyl-*sn*-glycerol m.p. 47°C, 2-butyryl-3-palmitoyl-1-stearoyl-*sn*-glycerol m.p. 49°C, 3-butyryl-2-palmitoyl-1-stearoyl-*sn*-glycerol m.p. 49°C. Analysis of the triglycerides with pancreatic lipase, which does not discriminate between the 1 and 3 positions [15], showed only traces of aberrant fatty acids at the 2 and the combined 1 and 3 positions. For example: 1-oleoyl-2,3-dipalmitoyl-*sn*-glycerol, positions 1 and 3, 50.1% oleic acid and 49.5% palmitic acid; position 2, 99.5%

palmitic acid and 0.5% oleic acid; 3-palmitoyl-1,2-distearoyl-*sn*-glycerol, positions 1 and 3, 50.7% stearic acid and 49.3% palmitic acid; position 2, 98.5% stearic acid and 1.5% palmitic acid.

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References

[1] E. Baer and H.O.L. Fischer, J. Biol. Chem. 128 (1939) 463
[2] H.O.L. Fischer and E. Baer, Chem. Rev. 29, (1941) 287
[3] G. Kohan and G. Just, Synthesis 192 (1974)
[4] J. LeCocq and C.E. Ballou, Biochemistry 3 (1964) 976
[5] E. Baer and H.O.L. Fischer, J. Amer. Chem. Soc. 61 (1939) 761
[6] A. Streitwieser, Jr., J. Org. Chem. 22 (1957) 861
[7] J.C. Sowden and H.O.L. Fischer, J. Amer. Chem. Soc. 64 (1942) 1291
[8] A.P.J. Mank, J.P. Ward and D.A. van Dorp, Chem. Phys. Lipids 16 (1976) this issue
[9] W.Th.M. de Groot, Lipids 7 (1972) 626
[10] R.E. Parker and N.S. Isaacs, Chem. Rev. 59 (1959) 737
[11] J. Bus, C.M. Lok and A. Groenewegen, Chem. Phys. Lipids 16 (1976) this issue
[12] E. Baer and H.O.L. Fischer, J. Amer. Chem. Soc. 67 (1945) 2031
[13] J.B. Lee and T.J. Nolan, Can. J. Chem. 44 (1966) 1331
[14] E.B. Kester, C.J. Gaiser and M.E. Lazar, J. Org. Chem. 8 (1943) 550
[15] F. Paltauf, F. Esfandi and A. Holasek, FEBS Letters 40 (1974) 119