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SYNTHESES AND CHIROPTICAL PROPERTIES OF SOME DERIVATIVES OF l-THIOGLYCEROL

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A modified synthesis of 3-thio-*sn*-glycerol which leads to a product of high optical purity is described. The purity was demonstrated by the use of a chiral shift reagent. 3-S-Acetyl- and 3-S-oleyl-3-thio-*sn*-glycerol as well as 3-acyl derivatives of 3-thio-*sn*-glycerol have been synthesized. The ORD and CD curves of these compounds as well as of some other derivatives of 3-thio-*sn*-glycerol were analyzed and discussed. The CD curves of the triacyl derivatives show a positive effect at 260 nm and a negative effect at 230–250 nm.

I. Introduction

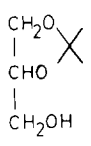
In previous papers we have studied the chiroptical properties of synthetic optically active glycerides [1,2]. Optical rotations and CD effects could be observed in triglycerides with very small differences in chain length, and the sign of the Cotton effect of the $n \rightarrow \pi^*$ transition of the ester chromophore at 230 nm could be used for determination of configuration. The effect of branching in the acyl groups on the chiroptical properties has also been investigated [2]. The optically active compounds have also been used for the study of the stereospecificity of hepatic lipases [3] and for the stereochemistry of fat digestion and absorption (Åkesson et al., unpublished results). Van Deenen et al. [4] have recently demonstrated the usefulness of acylthio ester analogues of substrates for phospholipases, lysophospholipases and lipases in continuous spectrophotometric assays for lipolytic enzymes. They found for example that lipase from pig pancrease effected hydrolysis of the acetylthio ester bond in 2-hexadecanoylthio ethanol. Lipase also hydrolyzed the tributryl ester of l-thioglycerol (3-mercapto-1,2-propanediol) and no great rate-difference in the hydrolysis of acyl- and acylthio derivatives was observed.

We therefore hoped that triglycerides derived from l-thioglycerol should be of interest in connection with our investigations on the stereospecificity of different lipases. Due to the expected higher rotations of such l-thioglycerol derivatives, we hoped that the stereospecificity could be observed, by measuring the chiroptical properties of the remaining triacylthioglycerol. This was found to be the case (Åkesson et al., unpublished results).

Also for our continuing investigation of the chiroptical properties of lipid derivatives a study of derivatives of thioglycerol was of interest as more marked effects are expected.

II. Syntheses

Starting from 1,2-O-isopropylidene-*sn*-glycerol (*1*), 1,2-O-isopropylidene-3-(p-toluenesulphonyl)-*sn*-glycerol (*2*) was prepared according to Sowden and Fischer [5]. Reaction of *2* with potassium thioacetate according to the general method described by Chapman and Owen [6] gave 1,2-O-isopropylidene-3-S-acetyl-3-thio-*sn*-glycerol (*3*) [7] in 63% yield. It is interesting to note that *3* has opposite directions of rotation in ethanol ($[\alpha]_D^{25} = +14.1°$, C 1.026) and in chloroform ($[\alpha]_D^{25} = -12.15°$, C 1.83). Anisyzzaman and Owen [7] reported $[\alpha]_D^{19} = -7.4°$C 14) in chloroform for this compound. Alkaline hydrolysis of *3* in methanol under nitrogen yielded 1,2-O-isopropylidene-3-thio-*sn*-glycerol (*4*) [7] in 72% yield. Acid hydrolysis of *3* under milder conditions (acetic acid under nitrogen) gave higher yield (82%) and higher optical



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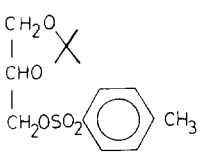

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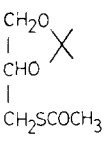

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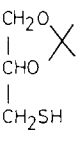

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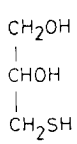

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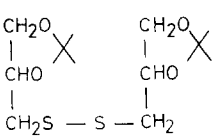

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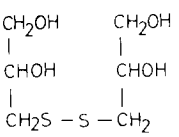

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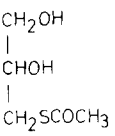

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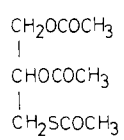

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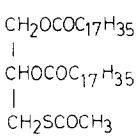

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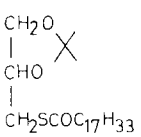

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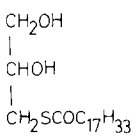

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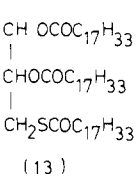


(13)

purity ($[\alpha]_D^{25} = -16.35°$, C 7.6 in ethanol) of 3-thio-*sn*-glycerol (*5*) than previously obtained [7] through hydrolysis with hydrochloric acid (42%, $[\alpha]_D^{25} = -8°$, C 11 in ethanol). In order to ascertain that our higher rotation did not depend upon the presence of disulphide, bis-1,2-0-diisopropylidene-3-thio-*sn*-glycerol (*6*) was prepared by iodine oxidation of *4*. Hydrolysis of *6* gave the disulphide of 3-mercapto-1,2-propanediol (*7*), which could not be detected by GLC in our preparation of *5*.

In order to determine the optical purity of *5*, the chiral shift reagent Tris-(3-heptafluoro)-propyl-(hydroxymethylene)-*d*-(camphorato)-europium was used. The shift reagent was first used on *4* but no effect was observed probably due to the fact that the free thiol group replaces a ligand in the chiral shift reagent. Consequently, the chiral chemical shift reagent was applied to *3*, which was prepared by reacetylation of *4*. Almost the same specific rotation was obtained as in the direct preparation of *3* from *2* showing that no racemization has occurred during the hydrolysis of *3* to *4*. The CH_3 resonance of *3* and its racemic form occurs at δ 2.35 p.p.m. Upon addition of shift reagent, a downfield shift to 3.69 p.p.m. is observed, which is split into two peaks at 3.68 and 3.70 p.p.m. in racemic *3*, while in active *3* only one peak was observed. This peak was analyzed by computer-simulated Lorentzian curves and the results indicated that the optical purity of *3* was higher than 99%. From this it follows that also *4* has at least this optical purity. The hydrolysis of *4* to *5* is carried out at such mild conditions that any racemization of *5* seems unlikely. It is therefore probable that almost maximum rotation is observed for *5*.

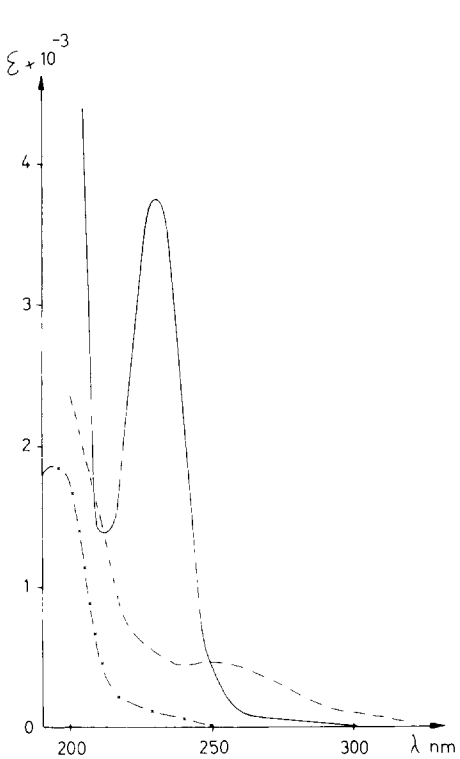
Treatment of *3* with 10% acetic acid at 60°C led to removal of the isopropylidene protecting group and gave 3-S-acetyl-3-thio-*sn*-glycerol (*8*) in 94% yield. Acetylation of *8* with acetyl chloride in benzene-pyridine gave 1,2-di-0-acetyl-3-S-acetyl-3-thio-*sn*-glycerol (*9*) in 43% yield. From *8*, 1,2-di-0-stearoyl-3-S-acetyl-3-thio-*sn*-glycerol (*10*) was also obtained through reaction with stearoyl chloride in an ether/hexane/pyridine mixture. Acylation of *4* with oleyl chloride gave 1,2-0-isopropylidene-3-S-oleyl-3-thio-*sn*-glycerol (*11*), which upon treatment with boric acid in 2-methoxyethanol gave 3-S-oleyl-3-thio-*sn*-glycerol (*12*) in 68% yield. Reaction of *12* with oleyl chloride in the usual way gave 1,2-di-0-oleyl-3-S-oleyl-3-thio-*sn*-glycerol (*13*). Racemic *13* was prepared by reacting commercial freshly distilled racemic l-thioglycerol with oleyl chloride.

III. Chiroptical properties

It has previously been reported that thiol acetates have an absorption maximum at about 235 nm ($\epsilon \sim 4000$) [8]. In connection with investigations of the ORD and CD spectra of some steroidal thiol aceates, Djerassi et al. [9] found an additional weak absorption near 270 nm, which could not be observed in the ultraviolet spectrum. The absorption at 235 nm has been assigned to a $\pi-\pi^*$-transition, while the band at 270 nm, because of its low intensity and its solvent dependence, has been assigned to the $n-\pi^*$-transition of the S–C=0 group [9]. Solvent effects and structural effects on

the latter transition in carboxylate esters have been discussed. The ultraviolet spectra of the thiol acetates *3* and *8–13* are all very similar and show an absorption maximum in hexane solution with ϵ approx. 3700 (cf. fig. 1). The free thiols *4* and *5* show increasing absorption with shorter wave length and a maximum is possibly observed for *5* at 197.5 nm (ϵ = 1700). As is the case with most non-cyclic disulphides, both *6* and *7* show a weak maximum (ϵ = 430) at 250 nm (fig. 1).

The CD spectra for the three triacyl derivatives *9*, *10* and *13* are in principle very similar. They show a positive CD curve with maximum at 262 nm ([θ] = 1500) and two more or less resolved negative maxima at 230 and 250 nm, [θ] approx. 3000–4000. These CD bands can be assigned to the n–π*- and π–π*-transitions, respectively. The CD effects of the 0-acyl groups, which in common triglycerides occur at 235 nm [1], are probably hidden, as the θ-value is around 50. This is drastically demonstrated in fig. 2, which shows the CD curve of 1,2-distearoyl-3-acetyl-*sn*-glycerol, *10* and *13*. Only small solvent effects were observed for the triacyl derivatives of



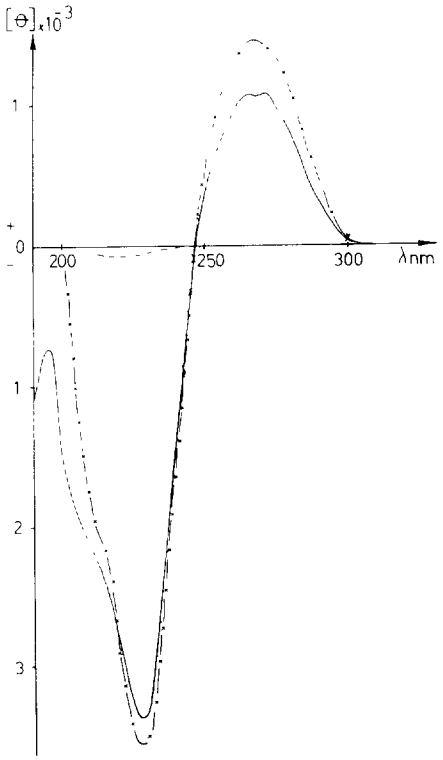


Fig. 1. Ultraviolet spectrum of 1,2-di-0-oleoyl-3-S-oleoyl-3-thio-*sn*-glycerol (hexane) (———), bis-1,2-0-diisopropylidene 3-thio-*sn*-glycerol (ethanol) (– – – –) and 3-thio-*sn*-glycerol (ethanol) (+ – + –).

Fig. 2. CD spectrum in hexane of 1,2-distearoyl-3-acetyl-*sn*-glycerol (– – – –), 1,2-di-0-stearoyl-3-S-acetyl-3-thio-*sn*-glycerol (———) and 1,2-di-0-oleoyl-3-S-oleoyl-3-thio-*sn*-glycerol (+ – + –).

l-thioglycerol, on going from hexane to hexafluoroacetone. It might perhaps be dangerous to draw any conclusions about the usefulness of these CD curves for the determination of absolute configuration as the available material is limited. In the triacyl derivatives a positive effect at 260 nm and a negative effect at 230–250 nm indicates that the thioacyl group in the 3-position is not influenced by chain length.

In the 3-thioacetyl derivatives *8* and *12*, the CD maximum at 260 nm is weak and can be positive or negative depending upon solvent indicating that this is a conformation-sensitive band. The CD maximum at 230 nm is still negative. For comparison, the CD curve of 3-acetyl-*sn*-glycerol is shown (fig. 3). In the 1,2-isopropylidene derivatives *3* and *11*, on the other hand, the band at 270 nm is negative and a positive Cotton effect is observed at shorter wave length (fig. 4).

The ORD spectra of the triacyl derivatives are rather complex and an interpretation is more difficult, as is evident from a comparison of the ORD spectra of *9* and *10* with 1,2-diacetyl-3-propionyl-*sn*-glycerol and 1,2-distearoyl-3-acetyl-*sn*-glycerol (fig. 5). In the ORD curve of *8* the weak Cotton effect at 270 nm cannot be detected which is by the way also true for the Cotton effect at 235 nm in 3-acetyl-*sn*-glycerol illustrating the greater usefulness of CD over ORD for detection of Cotton effects.

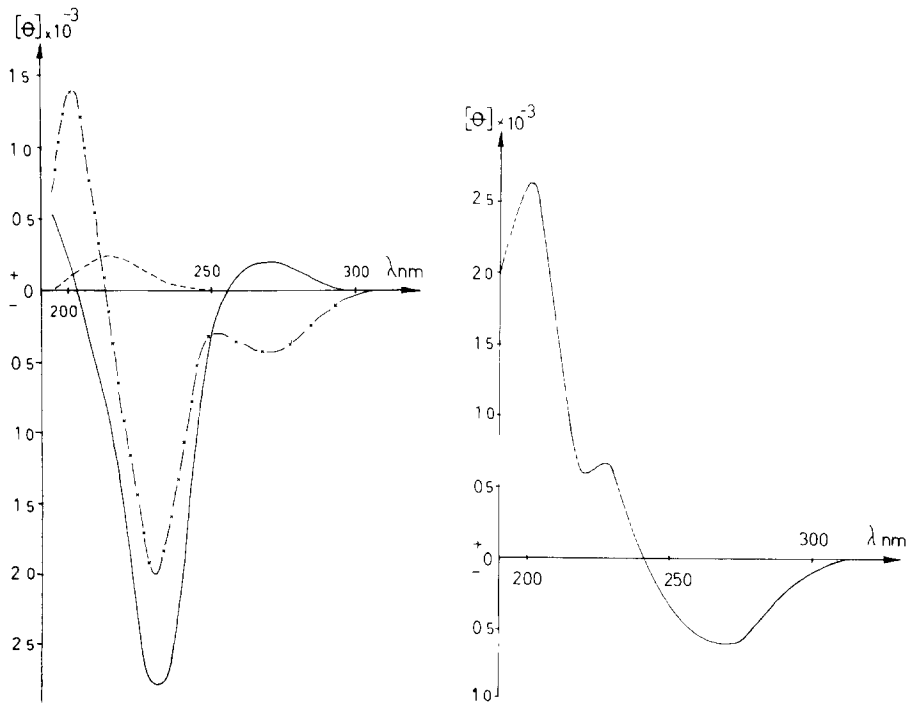


Fig. 3. CD spectrum of 3-acetyl-*sn*-glycerol (ethanol) (– – – –), 3-S-acetyl-3-thio-*sn*-glycerol (ethanol) (———) and 3-S-acetyl-3-thio-*sn*-glycerol (hexane/ether) (+ – + –).

Fig. 4. CD spectrum in hexane of 1,2-di-O-isopropylidene-3-S-acetyl-3-thio-*sn*-glycerol.

Very few thiols containing no other chromophores have been investigated (for review, see ref. 11). (2S)-2-Butanethiol shows a weak positive Cotton effect at 233 nm, which changes sign in its S-ethyl derivative [12]. For (2R)-2-mercaptopropano-1-ol a small negative CD band at 235 nm (methanol) and a positive band at 198 nm and in hexane at 226 nm and 196 nm, respectively, were observed [13]. A very weak positive band was also observed at 257 nm, but was not discussed. For *4*, we can observe an indication of a positive CD effect at 240 nm as a shoulder on a stronger positive effect at 220 nm, which is more pronounced in ethanol than in hexane/ether. 3-Thio-*sn*-glycerol showed in ethanol a negative CD effect at 255 nm (θ = 120), and a positive shoulder at 215 nm, due to a even stronger positive effect at lower wavelength. In hexafluoroacetone the CD spectrum is quite different. A broad, very weak negative maximum centred about 250 nm, and a negative maximum at 210 nm going towards a strong positive maximum at 190 nm are observed (fig. 6). The negative CD

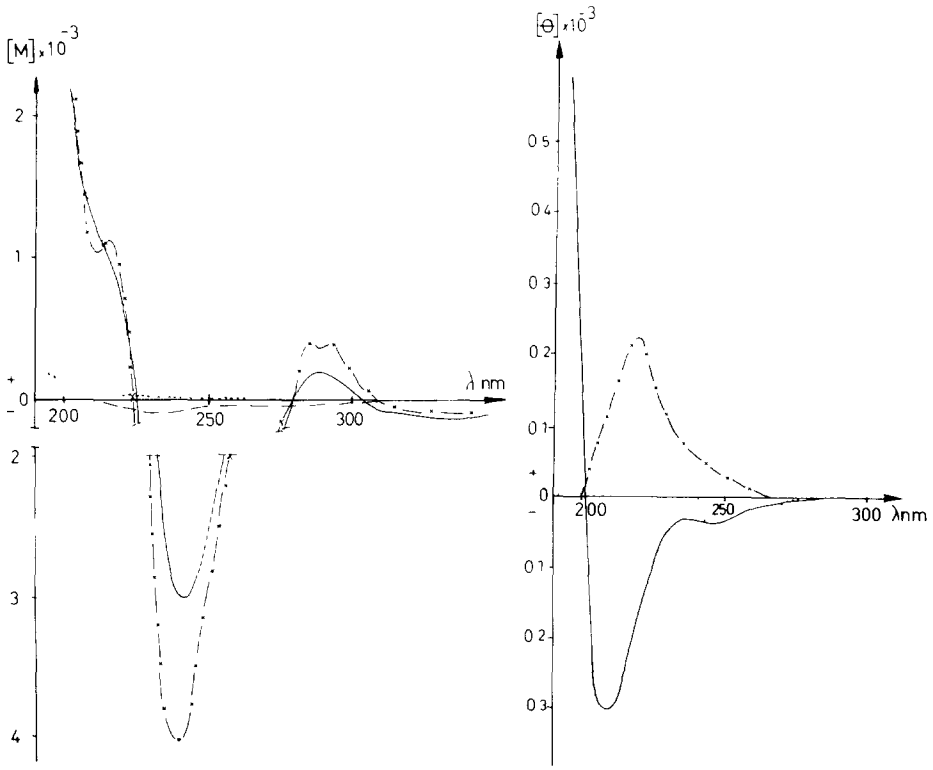


Fig. 5. ORD spectrum (hexane) of 1,2-distearoyl-3-acetyl-*sn*-glycerol (– – – –), 1,2-di-0-stearoyl-3-S-acetyl-3-thio-*sn*-glycerol (———), 1,2-0-diacetyl-3-S-acetyl-3-thio-*sn*-glycerol (+ – + –) and 1,2-diacetyl-3-propionyl-*sn*-glycerol (. . . .).

Fig. 6. CD spectrum of 3-thio-*sn*-glycerol (hexafluoroacetone) (———), 3-thio-*sn*-glycerol (ethanol) (. . . .) and 1,2-0-isopropylidene-3-thio-*sn*-glycerol (ethanol) (+ – + –).

band of *5* at 255 nm is also clearly observed as an anomalous effect in the ORD curves, while for *4* only a plain positive ORD curve is obtained (fig. 7).

It is clear that due to intermolecular hydrogen bonds and similar bonds to the solvent these thiols can have different conformations, which affect the sign of the different CD bands, and the chiroptical curves seem therefore to be of little use for stereochemical determinations. The thiol band at 238 nm is usually assigned to a $n-\pi^*$ transition [11]. Possible assignments for the three lowest-lying singlets in dialkyl sulphides at 240, 220 and 200 nm have been made by Rosenfield and Moscowitz [14]. While *4* showed CD effects at 240 and 220 nm, the band for *5* is shifted towards 255 nm, which probably could be due to hydrogen-bonding. It cannot be due to presence of the disulphide as impurity, as the CD curve of this compound is quite different, cf. below.

The inherent chiral disulphide chromophore has attracted much attention, and a great number of chiroptical studies of such compounds has been carried out (for

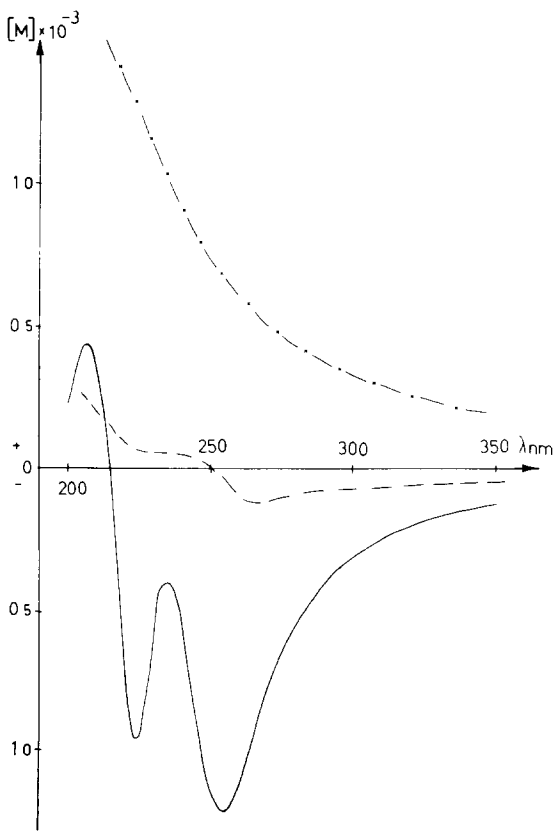


Fig. 7. ORD spectrum of 1,2-O-isopropylidene-3-thio-*sn*-glycerol (ethanol) (+ – + –), 3-thio-*sn*-glycerol (ethanol) (– – – –) and 3-thio-*sn*-glycerol (hexafluoroacetone) (———).

review, see ref. 15). The main absorption, which in acyclic disulphides with a dihedral CSSC angle of about 90° occurs at about 250 nm, is very sensitive to this angle, as found by investigations of cyclic disulphides [16,17]. The sign of this long-wave length CD band has been correlated with the disulphide chirality [18–20]. A quadrant rule has been formulated for the inherent optical activity of organic disulphides [23].

As an example of an optically active disulphide with no other chromophoric group over 200 nm, (2R, 2'R) dithiodipropan-1-ol, can be mentioned, which showed positive CD maxima at 245 nm and 195 nm [13]. In this compound the disulphide group is directly bound to the asymmetric carbon. More related to our compounds *6* and *7* are a recently studied series of S-alkylthio-L-cysteins [22], in which the disulphide group (as in our compounds) is one carbon removed from the asymmetric carbon. These compounds showed a negative maximum between 250–260 nm ($\theta \sim 1000$) assigned to the S-S-chromophore and a positive CD maximum at 220 nm due to the carboxyl chromophore. For *6*, positive CD maxima are observed at 265 nm and 190 nm indicating a predominance of the conformer with P-chirality of the disulphide group (fig. 8).

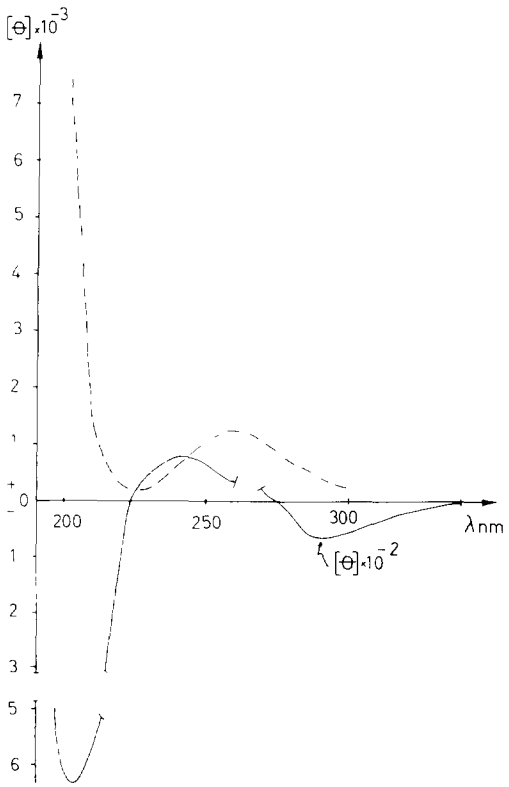


Fig. 8. CD spectrum in ethanol of bis-1,2-0-isopropylidene-3-thio-*sn*-glycerol (– – – –) and bis-3-thio-*sn*-glycerol (———).

For 7 an interesting but very weak negative maximum at 290 nm might indicate that due to hydrogen-bonding a conformer with a C-S-S-C dihedral angle deviating about 30° [23] from 90° is present. A positive maximum at 242 nm (θ 600) and a negative maximum at 205 nm ($\theta \sim 6000$) was also observed for 7.

IV. Experimental

Melting points were determined with a Leitz melting-point microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 infrared spectrophotometer. The ^{1}H NMR spectra were recorded with a Varian A60 or JEOL MH-100 spectrometer. Mass spectra were recorded on an LKB A 9000 spectrometer. GLC analyses were carried out on a Varian aerograph 1400 gas chromatograph equipped with a flame ionization detector. TLC was performed on silica gel plates (Merck, Fertigplatten, Kiselgel 60 F_{254}) using ether/hexane (1 : 4) as eluent. The spots were made visible with 2',7'-dichlorofluorescein (0.1%). Ultraviolet spectra were recorded with a Cary 15 spectrophotometer. Rotations at the D-line were measured with a Perkin-Elmer model 141 spectropolarimeter. ORD measurements were carried out with a Cary 60 spectropolarimeter and CD curves were registered with a JASCO 40 spectropolarimeter at 27°C.

Commercial fatty acids of highest possible purity (99–99.5% GLC) were used. Stearoyl chloride was prepared through reaction with excess thionyl chloride, and oleyl chloride was obtained according to ref. 24. Elemental analyses were carried out by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mulheim, Ruhr.

(a) 1,2-0-Isopropylidene-3-(p-toluenesulphonyl)-sn-glycerol (2) was prepared according to the method of Sowden and Fischer [5] and was purified by chromatography on silica gel using benzene as eluent, yield 84% of the title compound showing the expected GLC, infrared and NMR data.

(b) *1,2-0-Isopropylidene-3-S-acetyl-3-thio-sn-glycerol (3).* A solution of 48.0 g (0.168 mol) of 1,2-0-isopropylidene-3-(p-toluenesulphonyl)-*sn*-glycerol and 24.0 g (0.21 mol) of potassium thioacetate in 300 ml of acetone was refluxed for 18 h. After cooling the mixture was filtered and the solvent evaporated. The viscous residue was treated with ether and shaken for some minutes and the precipitate (potassium thioacetate) filtered off. The ether was evaporated and the residue distilled in vacuo, yielding 20.0 g (63%) of the title compound, b.p. 68–70°C/0.8 mm Hg, $n_D^{20} = 1.4744$, $\alpha_D^{25} = 8.68°$ (neat), $[\alpha]_D^{25} = +13.1$ (0.830 in ethanol), $[\alpha]_D^{25} = -12.15°$ (1.83, $CHCl_3$).
Anal. Calcd. for $C_8H_{14}O_3S$: C 50.51; H 7.42; S 16.85. Mol. wt. 190.3.
Found: C 50.50; H 7.40; S 16.80. Mol. wt. 190.
Ultraviolet (hexane): $\lambda_{max} = 230$ nm, $\epsilon = 3670$.
ORD (hexane): $[M]_{290} = +41.5$ T, $[M]_{235} = +1800$ P, $[M]_{210} = 2700$ P.
CD (hexane): $[\theta]_{310} = 0$, $[\theta]_{265} = -630$, $[\theta]_{241} = 0$, $[\theta]_{225} = +640$, $[\theta]_{200} = +2600$.

NMR ($CDCl_3$): δ 3.5–4.4 (3, m, OCH_2-CH-O), 3.10 (2, d, J = 5.5 Hz, CH_2-S), 2.40 (3, s, $COCH_3$), 1.43 (3, s, CH_3), 1.35 (3, s, CH_3).
Infrared: 1690 cm^{-1} (SCO).
Literature value [7] : n_D^{18} = 1.4750, b.p. 118°C/20 mm Hg, $[\alpha]_D^{19}$ = − 7.4 (14, $CHCl_3$). IR: 1690 cm^{-1} (SCO).

(c) *1,2-0-Isopropylidene-3-thio-sn-glycerol (4).* To a solution of 20% potassium hydroxide in methanol, which had been deoxygenated by a stream of nitrogen 10.73 g (0.0565 mol) of 1,2-0-isopropylidene-3-S-acetyl-*sn*-glycerol was added under nitrogen and the mixture stirred for 20 h. The solution was made weakly alkaline through the addition of acetic acid, and then evaporated. The residue was diluted with water and extracted five times with 50 ml portions of chloroform. The combined organic phases were dried over sodium sulphate and evaporated. Distillation in vacuo gave 6.15 g (72%) of the title compound, b.p. 49–50°C/3.5 mm Hg, n_D^{20} = 1.4645, $[\alpha]_D^{25}$ = +33.5 (1.00 in C_2H_5OH), $[\alpha]_D^{25}$ = +31.8 (2.19 in $CHCl_3$). Literature value [7] : b.p. 82°C/25 mm Hg, n_D^{20} = 1.4632, $[\alpha]_D^{23}$ = +31.4 (5.5 in $CHCl_3$).
Anal. Calcd. for $C_6H_{12}O_2S$: C 48.62; H 8.16; S 21.63. Mol. wt. 148.2.
Found: C 48.65; H 8.20; Cl 21.65. Mol. wt. 148.
NMR ($CDCl_3$): δ 3.7–4.4 (3, m, OCH_2-CH-0), 2.5–2.8 (2, m, CH_2-S), 1.60 (1, s, SH), 1.40 (3, s, CH_3), 1.33 (3, s, CH_3).
Infrared: 2525 cm^{-1} (SH).
ORD (ethanol): plain curve $[M]_{350}$ = +200, $[M]_{300}$ = +340, $[M]_{250}$ = +800, $[M]_{210}$ = +1650.
CD (ethanol): $[\theta]_{220}$ = 220, $[\theta]_{205}$ = 0.

(d) *3-Thio-sn-glycerol (5).* A mixture of 3.14 g (0.0212 mol) of 1,2-0-isopropylidene-3-thio-*sn*-glycerol and 100 ml of 10% deoxygenated acetic acid was stirred under nitrogen for 5 h at 60°C, until a clear solution was obtained. The solution was evaporated and washed until a viscous oil was obtained, which was taken up in chloroform and washed with bicarbonate solution and water. The ether solution was dried over sodium sulphate evaporated and then the residue was distilled azeotropically with benzene. The benzene was evaporated and the residue distilled in vacuo, yielding 1.95 g (85%) of the title compound, b.p. 92–94°C/1.2 mm Hg, n_D^{20} = 1.5245, $[\alpha]_D^{25}$ = −18.68° (0.139 in ethanol). Literature value [7] : b.p. 102°C/0.9 mmHg, n_D^{22} = 1.5230, $[\alpha]_D^{25}$ = −8° (11 in ethanol). NMR ($(CD_3)_2CO$): δ 4.3–3.5 (3, m, O-CH_2-CH-O), 2.65 (2, s, CH_2-S), 1.6 (1, s, SH).
Anal. Calcd. for $C_3H_8O_2S$: C 33.31; H 7.46; S 29.64. Mol. wt. 108.2.
Found: C 33.35; H 7.44; S 29.57.
Ultraviolet (ethanol): λ_{max} = 197.5 nm, ϵ = 1870.
ORD (ethanol): $[M]_{265}$ = −120 T, $[M]_{252}$ = 0.
CD (ethanol): $[\theta]_{295}$ = 0, $[\theta]_{255}$ = −120, $[\theta]_{241}$ = 0.

(e) *Bis-1,2-0-isopropylidene-3-thio-sn-glycerol (6).* To a dispersion of 2.7 g (0.018

mol) of 1,2-0-isopropylidene-3-thio-*sn*-glycerol in 150 ml of saturated sodium bicarbonate solution, iodine was added in small portions until no more iodine was consumed. Excess was reduced with sodium thiosulphate. The reaction mixture was extracted with chloroform. The combined chloroform fractions were dried over sodium sulphate, the solvent evaporated and the residue chromatographed on silica gel (7 g) using hexane/ether (8 : 2) as eluent, yielding 0.79 g of the title compound as a colourless liquid, $[\alpha]_D^{25} = +40.1$ (0.18 in ethanol), NMR ($CDCl_3$): δ 4.4–3.9 (4, m, CH_2-O), 3.8–3.5 (2, m, HC-O), 3.1–2.6 (4, dd, CH_2-S), 1.4–1.3 (6H, $CH_3)_2$).
Ultraviolet (ethanol): shoulder $\lambda = 250$ nm, $\epsilon = 460$.
ORD (hexane) plain curve $[M]_{350} = +1650$, $[M]_{300} = +3200$, $[M]_{250} = +4000$, $[M]_{200} = +22600$, $[M]_{195} = +23300$.
CD: $[\theta]_{340} = 0$, $[\theta]_{260} = +2000$, $[\theta]_{230} = +360$, $[\theta] = +37000$.
Anal. Calcd. for $C_{12}H_{22}O_4S_2$: C 48.95; H 7.53; S 21.80. Mol. wt. 294.4.
Found: C 49.00; H 7.50; S 21.68.

(f) *Bis-3-thio-sn-glycerol (7).* A solution of 0.63 g (0.021 mol) of bis-1,2-0-isopropylidene-3-thio-*sn*-glycerol in 25 ml of 50% acetic acid was heated for 6 h at 75°C. The solvent was evaporated off, water added and the evaporation repeated. This procedure was repeated several times. 0.56 g of a product, m.p. 105–107°C, was obtained. Infrared and mass spectra indicated the presence of some acylated products. The crude product was therefore dissolved in 30 ml of ethanol, 0.36 g of sodium hydroxide was added, and the solution stirred at 60°C for 1 h. The solvent was evaporated to dryness, dissolved in warm absolute ethanol, filtered and evaporated yielding 0.36 g (79%) of the title compound, as colourless crystals, m.p. 105–105.5°C, $[\alpha]_D^{25} = -73.1°$ (0.14 in ethanol), NMR ($(CD_3)_2SO$): δ 4.5 (4, s,-OH), 3.8–3.5 (2, m, CH-O), 3.5–3.2 (4, d, CH_2-O), 3.0–2.5 (4, m, CH_2-S).
Ultraviolet (ethanol): shoulder $\lambda = 250$ nm, $\epsilon = 420$.
ORD (ethanol): $[M]_{215} = -6700$, $[M]_{198} = 0$.
CD: $[\theta]_{345} = 0$, $[\theta]_{290} = -63$, $[\theta]_{275} = 0$, $[\theta]_{240} = +750$, $[\theta]_{227} = 0$, $[\theta]_{205} = 6300$, $[\theta]_{200} = 0$.

(g) *3-S-Acetyl-3-thio-sn-glycerol (8).* A mixture of 9.48 g (0.050 mol) of 1,2-isopropylidene-3-S-acetyl-3-thio-*sn*-glycerol and 45 ml of 10% acetic acid was vigorously stirred for 3 h at 60°C until a clear solution was obtained. The mixture was evaporated at 40°C until a viscous residue was obtained, which was dried in a drying pistol at 10^{-3} nm Hg at room temperature for 5 h, yielding 7.0 g (94%) of title compound, m.p. 24–25°C.
$[\alpha]_D^{25} = -53.66$ (4.23 in pyridine)
Infrared: 1690 cm^{-1} (S–C=O).
NMR ($CDCl_3$): δ 3.5–4.0 (3, m, OCH_2-CH-O), 3.05 (2, d, CH_2-S), 2.4 (3, s, $COCH_3$).
Ultraviolet (hexane): $\lambda_{max} = 235$ nm, $\epsilon = 440$.
ORD (ethanol): $[M]_{245} = -1100$ T, $[M]_{227} = 0$, $[M]_{215} = +670$ P, $[M]_{195} = 0$.
CD (ethanol): $[\theta]_{300} = 0$, $[\theta]_{270} = +185$, $[\theta]_{255} = 0$, $[\theta]_{230} = -2800$, $[\theta]_{203} = 0$.

Anal. Calcd. for $C_5H_{10}O_3S$: C 39.98; H 6.71; S 21.35. Mol. wt. 150.2.
Found: C 40.00; H 6.74; S 21.40.

(h) *1,2-Di-O-acetyl-3-S-acetyl-3-thio-sn-glycerol (9).* To an ice-cooled solution of 5.5 g (0.0366 mol) of 3-S-acetyl-3-thio-*sn*-glycerol and 3.6 ml of anhydrous pyridine in 150 ml of anhydrous benzene, 7.2 g (0.092 mol) of acetyl chloride was added dropwise with stirring. The reaction mixture was stirred at room temperature for 72 h and then washed with water, 5 M hydrochloric acid, sodium bicarbonate solution and finally water. The organic phase was dried over sodium sulphate and evaporated, and the residue dried in a drying pistol for 5 h at 70°C and 10^{-3} mm Hg, yielding 3.7 g (43%) of the title compound, m.p. 25–26°C, $[\alpha]_D^{25} = -14.25°$ (1.704 in hexane).
NMR ($CDCl_3$): δ 5.15 (1, m, CH), 4.2 (2, dd, $-CH_2$-O), 3.18 (2, dd, $-CH_2$-S), 2.35 (3, s, $SCOCH_3$), 2.05 (3, s, $OCOCH_3$).
Infrared: 1690 cm^{-1} (SCO), 1740 cm^{-1} (OCO).
Ultraviolet (hexane): $\lambda_{max} = 230$ nm, $\epsilon = 3662$.
ORD (hexane): $[M]_{310} = 0$, $[M]_{285} = +420$ P, $[M]_{279} = 0$, $[M]_{240} = -4100$ T, $[M]_{222} = 0$, $[M]_{215} = +1100$ P, $[M]_{210} = +1000$ T.
CD (hexane): $[\theta]_{305} = 0$, $[\theta]_{270} = +1400$, $[\theta]_{247} = 0$, $[\theta]_{225} = -4300$ T.
Anal. Calcd. for $C_9H_{14}O_5S$: C 46.14; H 6.02; S 13.69. Mol. wt. 234.3.
Found: C 46.20; H 6.00; S 13.71.

(i). Determination of optical purity of 1,2-O-isopropylidene-3-S-acetyl-3-thio-sn-glycerol.
This compound was prepared from 1,2-O-isopropylidene-3-thio-*sn*-glycerol, and the appropriate amounts of pyridine, benzene and acetyl chloride, following the method described above to yield the title compound with the same spectral properties as the sample described above, $[\alpha]_D^{25} = +12.8$ (0.838 in ethanol).

To 50 mg of 1,2-O-isopropylidene-3-S-acetyl-3-thio-*sn*-glycerol and 50 mg of the racemic compound 0.6 ml of deuterochloroform and 80 mg of Tris-(3-heptafluoro)-propyl-(hydroxymethylene)-d-(camphorato)-europium was added. This amount gave the largest separation. For the optically active compound one peak at δ 3.69 was observed, while the racemic form gave two peaks at δ 3.68 and δ 3.70 for the $SCOCH_3$ resonance. Without shift reagent both the racemic and optically active form showed one peak at δ 2.35. Computer simulation (Lorentzian curves) of the peak from the optically active form indicated the optical purity to be higher than 99%.

(j) 1,2-Di-O-stearoyl-3-S-acetyl-3-thio-sn-glycerol (10). To an ice-cooled solution of 2.0 g (0.013 mol) of 3-S-acetyl-3-thio-*sn*-glycerol and 3 ml of pyridine in 50 ml of ether/hexane (1 . 1), 10.0 g (0.033 mol) of steraoyl chloride in 50 ml of ether/hexane (1 : 1) was added with stirring, the mixture stirred at room temperature for 72 h, and worked up as described above. The crude product was chromatographed on neutral alumina using benzene as eluent, and then recrystallized from acetone to yield 6.0 g (67%) of the title compound, m.p. 48-49°C, $[\alpha]_D^{25} = -3.21°$ (0.468 in hexane).
Infrared: 1690 cm^{-1} (SCO), 1730 cm^{-1} (OCO).

NMR ($CDCl_3$): δ 5.1 (1, m, CH), 4.15 (2, dd, CH_2O), 3.15 (2, dd, CH_2S), 2.3 (3, s, $SCOCH_3$), 1.75 (4, m, $OCOCH_2$), 0.85 (3, m, CH_3).
Ultraviolet (hexane): λ_{max} = 235 nm, ϵ = 3500.
ORD (hexane): $[M]_{335}$ = −120 T, $[M]_{304}$ = 0, $[M]_{290}$ = +190 P, $[M]_{280}$ = 0, $[M]_{245}$ = −3000 T, $[M]_{225}$ = 0.
CD (hexane): $[\theta]_{310}$ = 0, $[\theta]_{265}$ = +1400, $[\theta]_{247}$ = 0, $[\theta]_{230}$ = −3600.
Anal. Calcd. for $C_{41}H_{78}O_5S$: C 72.09; H 11.51; S 4.69. Mol. wt. 683.1.
Found: C 72.13; H 11.55; S 4.73. Mol. wt. 683.

(k) 1,2-0-Isopropylidene-3-S-oleyl-3-thio-sn-glycerol (11). To an ice-cooled solution of 3.0 g (0.021 mol) of 1,2-0-isopropylidene-3-thio-*sn*-glycerol and 4 ml of pyridine in 100 ml of ether/hexane (1 : 1), 7.7 g (0.0256 mol) of oleyl chloride in 100 ml of hexane was added dropwise under nitrogen. The reaction mixture was then stirred for 20 h at room temperature and worked up as described above. The crude product was chromatographed first on neutral alumina using benzene as eluent and then on silica gel using ether/hexane (1 : 4) as eluent, and the product was dried in a drying pistol at 10^{-3} mm Hg, yielding 5.1 g (61%) of the title compound, n_D^{20} = 1.4768, $[\alpha]_D^{25}$ = +8.79 (1.422 in ethanol).
Infrared: 1690 cm^{-1} (SCO).
NMR ($CDCl_3$): δ 5.35 (2, t, CH=CH), 4.3–3.34 (3, m, OCH_2-CH-O), 3.10 (2, d, CH_2S), 2.75–2.35 (2, t, $SCOCH_2$), 2.2–1.8 (4, m, CH_2-C=C-CH_2), 1.46 (3, s, -CH_3), 1.35 (3, s, -CH_3), 1.3 (24, s, $(CH_2)_{12}$), 0.85 (3, m, -CH_3).
ORD (hexane): $[M]_{330}$ = +1200 inflection, $[M]_{295}$ = +93 T, $[M]_{240}$ = +1700 P, $[M]_{230}$ = +1600 T, $[M]_{210}$ = +2500 P.
CD (hexane): $[\theta]_{320}$ = 0, $[\theta]_{270}$ = −620, $[\theta]_{242}$ = 0, $[\theta]_{220}$ = +720 inflection, $[\theta]_{200}$ = +2600, $[\theta]_{195}$ = +2000.
Anal. Calcd. for $C_{24}H_{44}O_3S$: C 69.58; H 10.75; S 7.77. Mol. wt. 412.7.
Found: C 69.88; H 10.77; S 7.80. Mol. wt. 412.

(l) 3-S-Oleoyl-3-thio-sn-glycerol (12). To a solution of 5.0 g (0.012 mol) of 1,2-0-isopropylidene-3-S-oleoyl-3-thio-*sn*-glycerol in 45 ml of 2-methoxyethanol, 15 g of boric acid was added and the mixture heated at 95°C for 7 h with vigorous stirring. After cooling, 55 ml of water and 85 ml of chloroform were added and the mixture shaken for some minutes. The precipitated boric acid was filtered off. The chloroform phase was separated, washed with water and dried over sodium sulphate. The solvent was evaporated and the residue dried in a drying pistol for 3 h at 40°C and 10^{-3} mm Hg, yielding 3.0 g (68%) of the title compound, m.p. 38–39°C, $[\alpha]_D^{25}$ = −15.72 (0.668 in ethanol).
Infrared: 1690 cm^{-1} (SCO).
NMR ($CDCl_3$): δ 5.35 (2, 5, CH=CH), 3.65 (2, m, CHO), 3.1 (2, d, CH_2-S), 2.75–2.40 (2, t, $SCOCH_2$), 2.25–1.80 (4, m, -CH_2-C=C-CH_2), 1.36 (24, s, $(CH_2)_{12}$), 0.85 (3, m, -CH_3).
Ultraviolet (hexane): λ_{max} = 230 nm, ϵ = 3560.

ORD (ethanol): $[M]_{245} = -1000$ T, $[M]_{220} = +770$ P, $[M]_{200} = 0$.
CD (ethanol): $[\theta]_{320} = 0$, $[\theta]_{300} = +6$, $[\theta]_{295} = 0$, $[\theta]_{270} = +92$, $[\theta]_{259} = 0$, $[\theta]_{230} = -2800$, $[\theta]_{200} = 0$.
Anal. Calcd. for $C_{21}H_{40}O_3S$: C 67.69; H 10.82; S 8.61. Mol. wt. 372.6.
Found: C 67.73; H 10.78; S 8.64.

(m) 1,2-Di-0-oleoyl-3-S-oleoyl-3-thio-sn-glycerol (13). To an ice-cooled solution of 2.0 g (0.0054 mol) of 3-S-oleoyl-*sn*-glycerol and 3 ml of pyridine in 100 ml of ether/hexane (1 : 1), 4.0 g (0.0033 mol) of oleyl chloride in 50 ml of ether/hexane 1 : 1 was added dropwise with stirring and was then stirred at room temperature for 72 h and worked up as described above. The crude product was chromatographed on neutral alumina using benzene as solvent and then dried in a drying pistol for 4 h at 70°C in vacuo, yielding 2.0 g (41%) of the title compound, $n_D^{20} = 1.4778$, $[\alpha]_D^{25} = -6°$ (1.481 in hexane).
Infrared: 1690 cm^{-1} (SCO), 1740 cm^{-1} (OCO).
NMR ($CDCl_3$): δ 5.35 (6, t, CH=CH), 5.10 (1, m, CH), 4.15 (2, m, CH_2O), 3.15 (2, dd, CH_2S), 2.5–2.0 (6, m, $COCH_2$), 1.35 (s, $(CH_2)_n$), 0.85 (3, m, CH_3).
Ultraviolet (hexane): $\lambda_{max} = 230$ nm, $\epsilon = 3740$.
ORD (hexane): $[M]_{312} = 0$, $[M]_{295} = +340$ P, $[M]_{280} = 0$, $[M]_{242} = -3600$ T, $[M]_{224} = 0$.
CD (hexane): $[\theta]_{310} = 0$, $[\theta]_{265} = +1400$, $[\theta]_{246} = 0$, $[\theta]_{225} = -3300$, $[\theta]_{195} = -740$ P.
Anal. Calcd. for $C_{57}H_{104}O_5S$: C 75.94; H 11.62; S 3.56. Mol. wt. 901.5.
Found: C 75.89; H 11.56; S 3.59. Mol. wt. 901.

(n) 1,2-Dioleoyl-3-S-oleoyl-3-thio-rac-glycerol. To an ice-cooled solution of freshly distilled rac. 3-thioglycerol and 5 ml of pyridine in 100 ml of ether/hexane (1 : 1) 18 g (0.060 mol) of oleyl chloride dissolved in 100 ml of hexane was added dropwise with stirring. After stirring for 90 h at room temperature the mixture was worked up as described above for the active form yielding 8.78 g (53%) of the title compound, $n_D^{20} = 1.4778$, with the same infrared and NMR spectrum as the optically active form.

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