慶北大農學誌(Agric. Res. Bull. Kyungpook Natl. Univ.) 5 : 168～172 (1987)

Mercaptoglycerol중 cis－alkenyl thioethers의 입체 특이적 합성

김 득 진 · 여 영 근

대구대학교 식품가공학과 · 경북대학교 낙농학과

Stereospecfic Synthesis of Cis－Alkenyl Thioethers of Mercaptolycerol

Kim, Deuk Jin ·Yeo, Young Kun

Dept. of Food Science Taegu Natl. Univ.
*Dept. of Dairy Science Kyungpook Natl. Univ.

Summary

Methods were developed to synthesize optically active mercaptoglycerol from optically active isopropylidene glycerols.

1, 2－Isopropylideneglycerol was tosylated and the tosyl group displaced with thiolacetate. Base hydrolysis and oxidation gave 1, 1'－dithiobis－2, 3－isopropylidene－2, 3－propanediol. This compound could be used as a source of mercaptoglycerol, or reacted with 1－decenyl lithium to form cis－1－S－dec－1'enyl－2, 3－isopropylidene－1－mercapto－2, 3－propanediol. The latter is a stereospecific synthetic route to cis－alkenyl thioethers of protected mercaptoglycerol, and it may be useful for the preparation of a thioplasmalogen substrate for plasmalogenase.

요 약

광학성체인 isopropylidene glycerols 로 부터 mercaptoglycerol을 합성하였다.

1, 2－Isopropylideneglycerol을 tosyl화 하고 다시 tosyl group을 thiolacetate로 치환하였다. 염기성 가수분해와 산화에 의해 1, 1'－dithiobis－2, 3－isopropylidene－2, 3－propanediol이 생성되었다. 이 화합물을 mercaptoglycerol 합성을 위해 사용하므로써 1－decenyl lithium으로 cis－1－S－dec－1'－enyl－2, 3－isopropylidene－1－mercapto－2, 3－propanediol을 생성하였다. 본 생성물은 plasmalogenase용 thioplasmaloger 기질 조제에 사용된다.

INTRODUCTION

A rapid and convenient enzyme assay would be of clinical importance. Plasmalogenase is the enzyme that hydrolyzes the enolether linkage of plasmalogens to produce fatty aldehyde and lysophospholipid (Ansell and Spanner, 1965). It was described the use of sulfur substituted analogs of phospholipids as spectrophotometric substrates for phospholipase A1 and C (Cox et al., 1979). These analogs contain thioesters and thiophosphoester bonds, and when hydrolyzed by the enzyme they release thiols that react with colorimetric

thiol reagents. The assay of this type was reported for acetylcholinesterase, phospholipase, and plasmalogenase (Ellman et al., 1961 ; Aarsman and van den Bosch, 1977; Aarsman et al., 1976).

The organic synthesis of plasmalogens is a difficult problem (Paltauf, 1973). Besides the presence of two centers for isomerism, the asymmetric C-2 carbon of glycerol and the cis-double bond of the enolether linkage, the acid lability of the enolether group severely restricts the types of reactions that can be employed. Hirsch (1976) prepared a cis/trans mixture of the 1-heptenyl thioether of mercaptoglycerol by reaction of the Li/NH_3 reduction product of 1-ethylthioheptene with 1-bromo-2, 3-propanediol. Because of the vast array of reactions in sulfur chemistry, it seemed probable that an alternative route could be devised that would yield only the cis-isomer. This study was carried out to take adventage of the configurational stability of cis-1-alkenyl lithium compounds and their reaction with disulfides to produce cis-1-S-dec-1'-enyl-2, 3-isopropylidene-1-mercapto-2, 3-propanediol.

MATERIALS AND METHODS

The chemicals were purchased from the following companies : n-BuLi and catecholborane (Aldrich Chemical Co., Milwaukee, Wisconsin) ; mercaptoglycerol (Evans Chemetics, Darien, Connecticut) ; thiolacetic acid (Eastman Organic Chemicals, Rochester, New York) ; Li metal (Ventron Co., Beverly, Massachusetts). Tetrahydrofuran and diethyl ether were refluxed for 2 hours over Na chips prior to distillation. Methylene chloride (CH_2Cl_2) was dried over $CaSO_4$, distilled, and collected over molecular sieve.

Infrared spectroscopy was performed on a Beckman Model 4230 IR Spectrophotometer, and GC/MS was performed on Hewlett Packard HP 5985. Analytical GLC was done with a Varian Aerograph Model 920 Gas Chromatograph equipped with a column of 15 % ethylene glycol succinate on Gas Chro P, 80/100 mesh. Gas flow rate was 60 ml/min and all runs were isothermal.

One mol glycerol was dissolved in 2 mol acetone and 700 ml benzene. p-Toluenesulfonic acid (2 g) was added the solution refluxed overnight with a Dean Stark trap. After rotary evaporation of the solution to a viscous oil, 400ml pyridine and 0.95mol p-toluenesulfonyl chloride were added. The mixture was stirred for two days and then added to diethyl ether and the resulting mixture extracted twice with one portion of water, 500ml 5 % $NaHCO_3$, and 500ml saturated NaCl. The ether phase was filtered through Na_2SO_4, dried over $CaSO_4$, filtered and rotary evaporated to give a yellow oil solidified on standing. Thioacetic acid (1.32 mol) was added dropwise to a 40 % solution of KOH in methanol (1.32 mol KOH) cooled in an ice bath. Solvent was evaporated with several additions of benzene to remove water. The residue was taken up in 1.5l ethanol and heated to reflux. The tosylated isopropylidene glycerol (1.0 mol) was added and the solution refluxed for 1 hour. It was then cooled in an ice bath and suction filtered through a Buchner funnel. The filterate was rotary evaporated and taken up in 400ml water. A reddish oil separated out as a lower phase and was drained off. The aqueous phase was washed three times with 200ml diethyl ether, and the combined organic phases were extracted with 150ml saturated NaCl. The ether was evaporated from a one round bottomed flask and 400ml 20 % NaOH added for overnight thioester hydrolysis. The aqueous solution was then extracted with

3

200ml diethyl ether to remove any material insoluble in aqueous base, and the aqueous phase was sampled for sulfhydryl group determination with 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB). It was found to contain 0.54 mol sulfhydryl groups. 0.27 mol of I_2 was added. A dark brown oil separated during the reaction and was collected by draining the lower aqueous phase off through a separatory funnel. Distillation of the oil through a 10 cm vacuum jacketed Vigreaux column gave around 46g of a pale yellow oil (Fig. 2). *The IR spectrum of the yellow oil was* identical to isopropylidene glycerol except for the absence of hydroxyl absorption bands. *The molecular weight* was estimated at 303 on the basis of sulfhydryl groups liberated by Zn^{2+} / HCl reduction. Thus, the pale yellow oil which is 1, 1'-dithiobis-2, 3-isopropylidene-2, 3-propanediol (Fig. 2) was yielded around 30% of the total solution used.

To prepare cis-1-S-dec-1'-enyl-2, 3-isopropylidene-1-mercapto-2, 3-propanediol (Fig. 3) using n-BuLi, cis-1-decenyl bromide (10 mol) was placed in a flame dried, Ar atmosphere, three neck flask equipped with a gas inlet and rubber stopple. Dry THE (20 ml) was transferred in to the flask with a double ended needle and cooled to -70℃. While rapidly stirring, 5.95ml n-BuLi (1.6M) was added dropwise over a 20 minute period. Stirring was continued for 1 hour at -70℃ and the disulfide (Fig. 2) of 10 mmol added dropwise. After stirring for an additional hour, the flask was warmed to room temperature and the contents transferred to a separatory funnel with 100ml hexane. After washing with three 50ml portions of water, the organic phase was dried over Na_2SO_4, and evaporated to give 4g of yellow oil (Fig. 3).

RESULTS AND DISCUSSION

Sulfur substituted analogs of some glycerolipids have been chemically prepared and used to spectrophotometrically assay phospholipases A_1 including plasmalogenase and phospholipase C, and monoglyceride lipase (Fig. 1). The first step in this synthesis was the preparation of the symmetrical disulfide of isopropylidene mercaptoglycerol (Fig. 2). The sulfide is an intermediate in a reaction pathway proposed to provide optically active mercaptoglycerol, which was warranted by successful assays of other phospholipases with thioester substrates (Aarsman et al., 1976). The tosylate of rac-1, 2-isopropylideneglycerol was prepared as described by Sowden and Fisher (1942) and displaced the tosyl group with thiolacetate in refluxing ethanol. Rather than work up the product at this stage, the acetate thioester was hydrolyzed in strong base to give the sodium thiolate salt of rac-1 mercapto-2, 3-isopropylidene-2, 3 propanediol. Unreacted material was removed at this point by ether extraction, and since the condition were already alkaline, the disulfide was formed *in situ* by I_2 oxidation. The product separated as an insoluble oil that was collected and distilled in 31% overall yield. Starting from racemic glycerol, a pair of diastereomers is formed (Fig. 2), and this may account for the broad boiling point. sn-1-Mercapto or sn-3-mercaptoglycerols can be prepared by starting from 2, 3-or 1, 2-isopropylidene-sn-glycerol. After the completion of this work, a full paper describing the synthesis of optically active mercapto glycerol using the same synthetic route was communicated by Gronwitz et al. (1978).

Because of the low percentage of side reactions during the reaction of alkynyl lithium

compounds with disulfides to form alkynyl thioethers, it could be stereospecifically reduced to the cis-alkenyl thioether (Fig. 3) by making another initial compound. When the alkynyl thioether was reacted successively with disiamylborane and acetic acid for the reduction of alkynes to cis-alkenes, several products were formed. Preparative TLC and IR analysis indicated the presence of about 20% of unreacted starting material and about an equal amount of a compound that migrated immediately behind the alkynyl thioether, stained more intensely with I_2 vapor, and contained a double bond. This material was confirmed to be the alkenyl thioether (Fig. 3).

A cleaner reaction yielding the alkenyl thioether as the main product was the reaction of cis-1-decenyl lithium with the disulfide (Fig. 2). Starting with decenyl bromide that was greater than 99% cis, the alkenyl thioether was assayed in the drude reaction mixture as 91% cis. Some of the alkynyl thioether was also formed, presumably by reaction of the decenyl lithium with unreacted decenyl bromide causing elimination of HBr to form the alkyne. Abstraction of the acidic alkynyl proton by a second decenyl lithium molecule yielded the alkynyl lithium intermediate. Thus, a maximum of 3 mol of decenyl lithium is lost in the formation of 1 mol of alkynyl lithium. To make matters worse, the two products are so similar that the separation requires careful and repeated column chromatography. After two columns of silicic acid, the cis-alkenyl thioether (Fig. 3) was isolated in about 25% yield and contained 0.7% alkynyl thioether and 5% trans-isomer. The ability of simple column chromatography to separate these compounds, which are structurally quite similar, is probably due to the position of the unsaturation and its contribution to the overall polarity of the molecule. These compounds possess only two functional groups that allow absorption to silica gel. These are the isopropylidene group and the unsaturation-thioether linkage. The position of the unsaturation is such that it may affect interaction of the chromatography support with the isopropylidene group. Thus, there is even some resolution of the

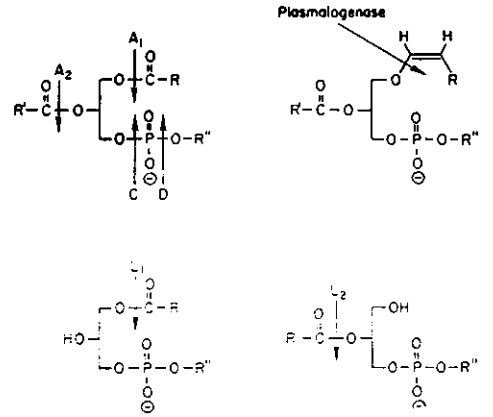

Fig. 1. Sites of action of the various phospholipases.

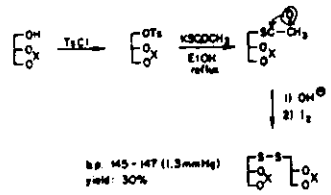

Fig. 2. Synthetic scheme for acetonated mercaptoglycerol disulfide.

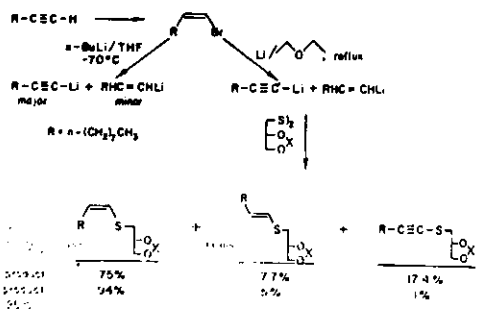

Fig. 3. Synthetic scheme for alkenyl thioethers of mercaptoglycerol.

cis and trans isomers after two silicic acid columns. Separation of alkynyl and alkenyl thioethers may result from the different resonance contribution of the sulfur in alkynyl compared to alkenyl thioethers (Silverstein et al., 1974). Once the isopropylidene group is removed, the polarity of the glycol group is likely to dwarf any contribution from the unsaturated thioether linkage and prevent separation of alkynyl and alkenyl thioether (Fig. 3).

REFERENCES

1. Aarsman, A. J., van Deenen, L. L.M., and van den Bosch, H. 1976. Bioorganic Chem. 5, 241.
2. Aarsman, A. J., and van den Bosch, H. 1977. FEBS Lett. 79, 317.
3. Ansell, G. B., and Spanner, S. 1965. Biochem J. 94, 252.
4. Cox, J. W., Snyder, W. R., and Horrocks, L. A. 1979. Federation Proc. 38, 237.
5. Ellman, G. L., Courtney, D., Andres, Jr., V., and Featherstone, R. N. 1961. Biochem. Pharmacol. 7, 88.
6. Gronowitz, S., Herslof, B., Michelson, P., and Akesson, B. 1978. Chem. Phys. Lipids 22, 307.
7. Hirsch, A. F. 1976. Chem. Phys. Lipids 17, 399.
8. Paltauf, F. 1973. Chem. Phys.Lipids 11, 270.
9. Silverstein, R. M., Bassler, G. C., and Morril, T. C. 1974. Spectrometric Identification of Organic Compounds, 3rd Ed., p. 216, John Wiley and Sons, New York.
10. Sowden, J. C., and Fischer, H. O. L. 1942. J. Am. Chem. Soc. 64, 1291.