4837.
Naiki, M., Marcus, D. M., and Ledeen, R. (1974), *J. Immunol. 113,* 84.
Preud'homme, J. L., and Seligmann, M. (1972), *J. Clin. Invest. 51,* 701.
Reif, A. E., and Allen, J. M. (1966), *Nature (London) 209,* 521.
Saito, T., and Hakomori, S. (1971), *J. Lipid Res. 12,* 257.
Schwarting, G. A., and Marcus, D. M. (1977), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36,* 1241.
Segel, G. B., Lichtman, M. A., Gordon, B. R., MacPherson, J. L., and Nusbacher, J. (1977), *Transfusion (Philadelphia)* (in press).
Siakotos, A. N., and Rouser, G. (1965), *J. Am. Oil Chem. Soc. 42,* 913.
Steel, C. M., Evans, J., Joss, A. W. L., and Arthur, E. (1974), *Nature (London) 252,* 604.
Stein, K. E., Schwarting, G. A., and Marcus, D. M. (1977), submitted for publication.
Stein-Douglas, K. E., Schwarting, G. A., Naiki, M., and Marcus, D. M. (1976), *J. Exp. Med. 143,* 822.
Svennerholm, L. (1957), *Biochim. Biophys. Acta 24,* 604.
Vance, D. E., and Sweeley, C. C. (1967), *J. Lipid Res. 8,* 621.
Yu, R. K., and Ledeen, R. W. (1970), *J. Lipid Res. 11,* 506.

Absolute Configuration of Tritiated *O*-Alkylglycerol Synthesized Enzymatically from [1,3-$^3H_2$, 1,3-$^{14}C_2$]Dihydroxyacetone Phosphate†

Samuel J. Friedberg* and Rene D. Alkek

ABSTRACT: *O*-Alkyldihydroxyacetone phosphate is synthesized enzymatically from hexadecanol and acyldihydroxyacetone phosphate. In this process there is a hydrogen exchange in which the *pro-R* hydrogen of C-1 of the *sn*-glycerol moiety is lost. This hydrogen is replaced by a hydrogen from the medium. In order to obtain additional information on the mechanism of ether bond formation, it would be of interest to know whether or not the hydrogen exchange results in a change of configuration in the product, *O*-alkyldihydroxyacetone phosphate. By using *O*-alkylglycerol prepared both chemically and enzymatically from isomerase-treated [1,3-$^3H_2$, 1,3-$^{14}C_2$]dihydroxyacetone phosphate and an *O*-alkylglycerol cleavage enzyme system, it was shown that the hydrogen exchange occurs with retention of configuration of the substituents of C-1 of the *sn*-glycerol moiety.

The conversion of acyldihydroxyacetone phosphate to *O*-alkyldihydroxyacetone phosphate is an important step in glycerol ether biosynthesis (Hajra, 1970; Wykle et al., 1972). In this enzymatic reaction, the fatty acid moiety of acyldihydroxyacetone phosphate is lost and a fatty alcohol is gained, resulting in the formation of *O*-alkyldihydroxyacetone phosphate. We have previously shown that there is a simultaneous stereospecific loss of hydrogen from carbon one of the dihydroxyacetone phosphate of acyldihydroxyacetone phosphate (Friedberg et al., 1971). The hydrogen which is lost is the same one which is exchanged in the triosephosphate isomerase reaction (Friedberg et al., 1972). The dihydroxyacetone phosphate moiety then gains a hydrogen from the medium (Friedberg and Heifetz, 1975). Thus when acyldihydroxyacetone phosphate, hexadecanol, and Ehrlich ascites tumor cell microsomes are incubated in the presence of tritiated water, the *O*-alkyl lipid formed becomes tritiated. A question which is immediately suggested is whether or not the configuration of the substituents of carbon one of the dihydroxyacetone phosphate of *O*-alkyldihydroxyacetone phosphate retains the same configuration as the substrate dihydroxyacetone phosphate. The answer to this question would be important in determining the mechanism of ether bond formation.

In the present investigation we demonstrate that the hydrogen exchange occurs with retention of configuration at carbon one of *sn*-glycerol of *O*-alkylglycerol. Thus, the *pro-R* hydrogen is initially lost and is replaced by a hydrogen from the medium in the *pro-R* position.

Davis and Hajra (1977) recently presented data which confirm that there is a hydrogen exchange in the conversion of acyldihydroxyacetone phosphate to *O*-alkyldihydroxyacetone phosphate. These workers also indicate that they have preliminary data suggesting that this occurs with retention of configuration.

Materials and Methods

[1,3-$^3H_2$]Dihydroxyacetone phosphate and [1,3-$^{14}C_2$]-dihydroxyacetone phosphate were synthesized as previously described except that triethanolamine was used instead of glycine in the buffer solution (Friedberg et al., 1971).

The preparation of Ehrlich ascites tumor cells and *Tetrahymena pyriformis* microsomes was carried out as previously described (Friedberg et al., 1971; Friedberg and Heifetz, 1975).

Biosynthetic Preparation of O-Alkyl[S-1-$^3H_1$, 3-$^3H_2$, 1,3-$^{14}C_2$]-sn-glycerol. A mixture of [1,3-$^3H_2$]- and [1,3-$^{14}C_2$]-dihydroxyacetone phosphate, 33.12 μCi and 2.24 μCi, respectively, with an absolute ratio of 14.8 (0.098 μmol, total) was incubated at 37 °C with 16 mL (4 mg of protein per mL) of Ehrlich ascites tumor cell microsomes in a final volume of 21.76 mL. The mixture contained: ATP, 7.35 mM; magnesium

† From the Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78283. *Received April 25, 1977.* This work was supported by Research Grant 1 R01 CA15047 from the National Cancer Institute, United States Public Health Service.

chloride, 7.34 mM; coenzyme A, 0.735 mM; and hexadecanol, 0.0735 mM. After 2 h, 80 μmol of NADPH was added and the incubation was continued for another hour. The lipids were extracted by the method of Bligh and Dyer (1959). The chloroform phase was dried and the *O*-alkylglycerol was isolated and purified by thin-layer chromatography on silica gel. The developing solvent was ethyl ether–hexane–acetic acid (20:80:1 v/v/v). The *O*-alkylglycerol obtained had lost 25% of the tritium as expected (Friedberg et al., 1971). The yield was approximately 3%.

Chemical Synthesis of O-Alkyl[S-1-$^3H_1$*, 3-*$^3H_2$*, 1,3-*$^{14}C_2$*]-sn-glycerol.* *O*-Alkylglycerol was prepared chemically starting with a mixture of [1,3-$^3H_2$]glycerol and [1, 3-$^{14}C_2$]-glycerol of known ratio (0.733 μmol total). The labeled substrate was dried under nitrogen and reacted with ATP, 10 μmol; NAD, 4 μmol; magnesium chloride, 2.4 μmol; α-glycerophosphate dehydrogenase (0.1 mg, 17.5 units), glycerokinase (0.02 mg, 3.2 units), triosephosphate isomerase (0.04 mg, 100 units), and 0.1 M potassium phosphate buffer (pH 7.6), in a final volume of 1.3 mL. All enzymes were obtained from the Sigma Chemical Co., St. Louis, Mo. The mixture was incubated for 2 h at 37 °C. The reaction was stopped by immersing the mixture in boiling water for 1 min. In this reaction [1,3-$^3H_2$, 1,3-$^{14}C_2$]glycerol is converted to [1,3-$^3H_2$, 1,3-$^{14}C_2$]dihydroxyacetone phosphate via α-glycerophosphate. In the equilibrium reaction between α-glycerophosphate and dihydroxyacetone phosphate catalyzed by α-glycerophosphate dehydrogenase, triosephosphate isomerase was used to exchange the *pro-R* tritium of carbon 1 of dihydroxyacetone phosphate with hydrogen from the medium. The result is the synthesis of an equilibrium mixture of α-glycerophosphate and dihydroxyacetone phosphate specifically labeled with tritium at the *S* position of carbon 1 of both compounds. This was confirmed by finding a reduction of 25% in the ^3H to ^{14}C ratio of the starting glycerol and also by the final result of these experiments as detailed in the next section. The pH was then adjusted to 5.0 and 20 mg of acid phosphatase (potato, Sigma Chemical Co., St. Louis, Mo.) was added. The incubation mixture was then incubated for 3 h at 37 °C and the reaction was stopped by immersion in boiling water for 1 min. Thin-layer chromatography on cellulose with development in *tert*-butyl alcohol–water–formic acid (60:15:2) revealed 80% conversion of α-glycerophosphate to glycerol. The incubation mixture was then filtered and treated with a mixed bed ion-exchange resin (Bio Rad, Richmond, Calif.) to remove the buffer. NAD and ATP were removed by filtration through charcoal. To this mixture, 762 μmol of unlabeled glycerol was added. The mixture was dried under nitrogen. The yield of labeled glycerol was 74%. The labeled glycerol was then converted to its isopropylidene derivative. This was done by a modification of a method described by Fischer and Pfähler (1920). One milliliter of acetone and 0.04 mL of concentrated hydrochloric acid were added to the dried glycerol. This was shaken until the glycerol dissolved in the acetone. To this were added 0.5 mL of petroleum ether and 0.5 g of anhydrous sodium sulfate. The reaction was carried out at 60 °C for 5 h. The mixture was then neutralized with 0.1 g of fused sodium acetate and 0.2 g of anhydrous sodium carbonate. The mixture was then filtered and the residue was washed twice with a small amount of ethyl ether. The filtrates were combined and dried. The yield was 42% of the glycerol used in the glycerokinase reaction. The product was identified by thin-layer chromatography on silica gel. The developing solvent was hexane, ethyl ether, acetic acid (20:80:1). A single product was obtained which had the mobility of authentic isopropylidene glycerol. The isopropylidene derivative of *O*-alkylglycerol was prepared by the method of Baumann and Mangold (1964). Accordingly, the ether solution of isopropylidene glycerol was dried and KOH (0.2 mg), xylene (4 mL), and sodium sulfate (0.5 mg) were mixed in a 25-mL pear-shaped reaction flask fitted with a dropping funnel and a reflux condenser. The mixture was refluxed for 1 h and hexadecylmethanesulfonate, 0.145 g, in 3 mL of xylene, was added dropwise. Refluxing was continued for an additional 4 h. Hexadecylmethanesulfonate was prepared by the method of Baumann and Mangold (1964) except that all quantities were reduced by a factor of 20. The liquid phase was removed from the reaction flask and the residue was rinsed with a small amount of xylene. The solution was concentrated to 3 mL, and 15 mL of ethyl ether and 15 mL of water were added. The ether phase was removed and the aqueous phase was extracted with 9 mL of ethyl ether. The extracts were combined and dried after an aliquot was taken for thin-layer chromatography on silica gel. The developing solvent was hexane–ethyl ether–acetic acid (90:10:1 v/v/v). Authentic isopropylidene derivative of hexadecenyl glyceryl ether was used as a standard. The free glyceryl ether was generated by reacting the product in 2.75 mL of methanol, concentrated HCl (10:1 v/v). The reaction mixture was heated for 1 h at 80 °C in a sealed tube. After cooling, 5 mL of water was added and the mixture was extracted twice with ethyl ether. The ethyl ether phase was washed once with water, and once with 1% potassium carbonate, and then finally with water. The ethyl ether solution was dried with sodium sulfate. The product was identified by thin-layer chromatography on silica gel. The developing solvent was hexane–ethyl ether–acetic acid (20:80:1 v/v/v) and the product was purified by thin-layer chromatography in the same system. The final yield was 20% of the starting material. With respect to the *O*-alkylglycerol formed in the chemical procedure, it is essential to note that formation of the isopropylidene derivative of glycerol occurs randomly at the 1,2 or 2,3 positions of *sn*-glycerol. Therefore, the effect of the initial isomerase treatment would be the formation of *O*-alkylglycerol molecules half of which would have half of the tritium missing from the ether linked carbon and half of which would have half of the tritium missing from the non-ether-linked primary carbinol carbon.

Enzymatic Cleavage of O-Alkylglycerol and Recovery of the Glycerol Moiety. *O*-Alkylglycerol, approximately 20 000 cpm, dispersed in 0.02 mL of 1% Tween 80, and with a known ^3H to ^{14}C ratio, was incubated with 1 mL of *Tetrahymena* microsomes (4 mg of protein per mL) at 37 °C for 1 h. The incubation mixture contained added glycerol (0.837 mM), NADP (0.837 mM), glucose 6-phosphate (4.18 mM), glucose-6-phosphate dehydrogenase (0.837 unit), and NAD (0.837 mM) (Kapoulas et al., 1969). The reaction mixture was extracted by the method of Bligh and Dyer (1969). The aqueous layer was filtered and dried in vacuo at 50 °C. Ten milliliters of water and 2 mL of mixed bed resin (Bio Rad, Richmond, Calif.) were added. The mixture was shaken and filtered and the solution was dried in vacuo at 50 °C. Two milliliters of water was added. The yield was approximately 10%. In order to obtain sufficient specifically labeled material for further experiments, ten incubations were carried out simultaneously and the extracts were pooled. The labeled water soluble cleavage product migrated as a single peak and was identified as glycerol by thin-layer chromatography on silica gel. The developing solvent was acetone–chloroform–30% ammonium hydroxide (80:10:10 v/v/v)[1]. The use of this system results in wide separation between glycerol and glyceraldehyde.

[1] Amersham/Searle Technical Data Sheet No. 16907.

TABLE I: Stereospecificity of ^3H Activity at C-1 of *sn*-Glycerol from Biosynthetic *O*-Alkylglycerol Synthesized from [1,3-$^3H_2$, 1,3-$^{14}C_2$]Dihydroxyacetone Phosphate.[a]

Experiment	1	2	3	4	5	Mean
1. ^3H:^{14}C of C-1 of *sn*-glycerol of *O*-alkylglycerol	2.3	2.2	2.2	2.3	2.4	2.3
2. Same as No. 1 treated with isomerase	2.2	2.4	2.3	2.0	2.0	2.2
3. Same as No. 1 treated with aldolase	0.27	0.40	0.11	0.20	0.09	0.21

[a] Glycerol obtained from the enzymatic cleavage of biosynthetic *O*-alkyl[1,3-$^3H_2$, 1,3-$^{14}C_2$]glycerol was incubated 2 h at 37 °C with: ATP, 20 μmol; $MgCl_2$, 5 μmol; NAD, 14 μmol; α-glycerophosphate dehydrogenase, 17.5 units; glycerokinase, 48 units; and isomerase, 40 units or aldolase, 1 unit as indicated in a final volume of 0.5 mL. The pH was adjusted to 7.6 with NaOH prior to the addition of the enzymes. After incubation the mixture was passed through a 0.5-g charcoal column and dried with nitrogen. One milliliter of 0.05 N sodium metaperiodate in 25% acetic acid was added and the solution was kept in the dark for 30 min. One milliliter of 0.5 N sodium arsenite was added. The pH was adjusted to 6.0 with NaOH and 15 mL of 0.4% dimedon was added. The solution was kept at 4 °C for 12 h. The precipitate of formaldehydodimedon was extracted four times with petroleum ether, dried in a counting vial, and counted in 10 mL of 2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazolyl)]benzene–toluene.

Other Methods. Periodate oxidation, dimedon precipitation of formaldehyde, thin-layer chromatography, and liquid scintillation counting were carried out as previously described (Friedberg et al., 1971, 1972; Friedberg and Heifetz, 1973, 1975).

Results

Biosynthetic *O*-alkylglycerol which had lost the *pro-R* tritium from carbon one of the glycerol moiety was cleaved enzymatically as described in Materials and Methods. As detailed in Table I, this glycerol was converted to α-glycerophosphate and then to dihydroxyacetone phosphate. The incubation mixtures also contained either triosephosphate isomerase, or fructose-1,6-diphosphate aldolase, or neither. The overall reaction favors the formation of α-glycerophosphate. Carbon one of α-glycerophosphate and dihydroxyacetone phosphate was isolated by treatment with periodate and precipitation of the released formaldehyde with dimedon to form formaldehydodimedon which was then assayed for radioactivity.

In order to interpret these experiments, it must be recalled that glycerol is phosphorylated asymmetrically by glycerokinase (Bublitz and Kennedy, 1954). The dihydroxyacetone phosphate used to synthesize *O*-alkylglycerol in these experiments was obtained enzymatically by the action of glycerokinase and α-glycerophosphate dehydrogenase on [1,3-$^3H_2$]-glycerol and [1,3-$^{14}C_2$]glycerol. The ether bond formed from this substrate becomes linked to the unphosphorylated carbinol carbon of dihydroxyacetone phosphate (carbon one of *sn*-glycerol). When the glycerol of the *O*-alkylglycerol is split by the *Tetrahymena* cleavage enzyme, the glycerol carbon (carbon 3 of *sn*-glycerol) which is phosphorylated a second time in these experiments by glycerokinase is the same one which had been previously phosphorylated in the biosynthesis of the starting dihydroxyacetone phosphate substrate used in the biosynthesis of the *O*-alkylglycerol. This is because the enzyme is specific for C-3 of *sn*-glycerol, which means that all of the hydrogen labilized in the formation of *O*-alkylglycerol is conveniently found on the unphosphorylated carbon of α-glycerophosphate or dihydroxyacetone phosphate generated from the cleavage of the *O*-alkylglycerol.

TABLE II: Stereospecificity of ^3H Activity at C-1 of *sn*-Glycerol from Chemically Synthesized *O*-Alkylglycerol Prepared from Isomerase-Treated [1,3-$^3H_2$, 1,3-$^{14}C_2$]Dihydroxyacetone Phosphate.[a]

Experiment	1	2	3	Mean
1. ^3H:^{14}C of C-1 of *sn*-glycerol of *O*-alkylglycerol	4.44	4.49	4.55	4.49
2. Same as No. 1 treated with isomerase	3.73	3.69	4.50	3.97
3. Same as No. 1 treated with aldolase	0.25	0.04	0.22	0.17

[a] Glycerol obtained from the enzymatic cleavage of *O*-alkyl[1,3-$^3H_2$, 1,3-$^{14}C_2$]glycerol (prepared chemically from isomerase-treated [1,3-$^3H_2$, 1,3-$^{14}C_2$]DHAP) was treated as described in Table I.

The results (Table I) show that, when the sequence described above takes place in the presence of isomerase, there was no loss of tritium from C-1 in relation to ^{14}C. However, when aldolase was substituted for isomerase, almost all of the tritium remaining on C-1 was lost. These results show that there is retention of configuration of C-1 substituents in relation to C-1 of the starting dihydroxyacetone phosphate substrate and that the hydrogen which is lost is replaced in the same position by another hydrogen from the medium.

In order for this conclusion to be valid, it would be necessary to show that no inversion of configuration occurs at C-1 of *sn*-glycerol liberated in the enzymatic cleavage of *O*-alkylglycerol. Accordingly, *O*-alkylglycerol was prepared chemically as described in Materials and Methods. This *O*-alkylglycerol was prepared from [1,3-$^3H_2$, 1,3-$^{14}C_2$]dihydroxyacetone phosphate treated with isomerase to exchange the *pro-R* tritium at C-1 with hydrogen from the medium. The synthetic *O*-alkylglycerol was then cleaved enzymatically by means of the *Tetrahymena* system described above. The cleaved product was examined for the positional specificity of the isomerase and aldolase labilized hydrogens. The results in Table II show that the absolute configurations of the substituents of C-1 of *sn*-glycerol are not altered during the enzymatic cleavage. The ^3H to ^{14}C ratio of the chemically synthesized *O*-alkyl[1,3-$^3H_2$, 1,3-$^{14}C_2$]glycerol was 6.08 after labilization of the *pro-R* tritium of C-1 (isomerase labile tritium). Since the glycerol molecule is labeled with ^{14}C in the 1 and 3 positions, the expected ^3H to ^{14}C ratio at C-1 would be 4.06 and 8.11 at C-3. The results of the experiment are in agreement since the measured ratios at C-1 were 4.44, 4.49, and 4.55 (Table II).

If inversion of configuration had occurred with enzymatic cleavage of the ether bond at both C-1 and C-3, the expected result would be that half of the tritium remaining at C-1 would have been labilized by isomerase and half by aldolase (see Figure 1). This is the case because half of the chemically synthesized *O*-alkylglycerol would have the ether linkage at C-3 of *sn*-glycerol and this would not be detected in the cleavage reaction since the C-3 position retains both tritiums. However, it is not known if the cleavage reaction occurs with –OR at C-3. Nevertheless, with reference to Figure 1, it can be seen that the results would not have been altered if ether bond cleavage does not occur at C-3. If cleavage of the ether bond does not occur at C-3, and inversion at C-1 had occurred during cleavage, all the tritium at C-1 would have been labilized by isomerase rather than aldolase.

Discussion

The absolute configuration of the enantiotopically paired hydrogens of C-1 of dihydroxyacetone phosphate has been

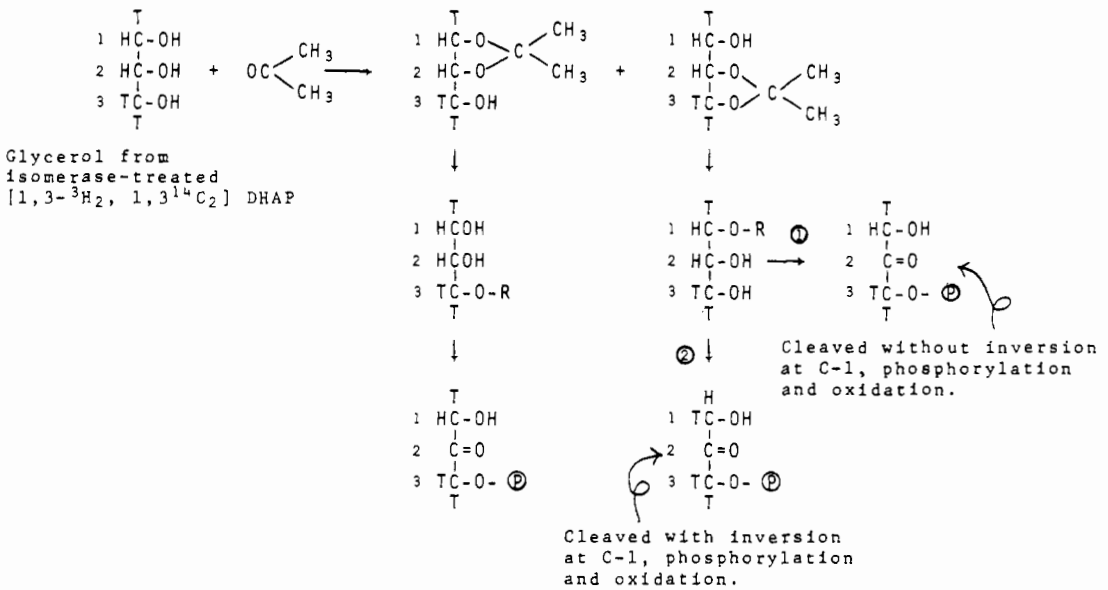

FIGURE 1: Postulated effect of isomerase or aldolase treatment of DHAP generated from *O*-alkylglycerol synthesized chemically from isomerase-treated [1,3-$^{3}H_2$, 1,3-$^{14}C_2$]DHAP. (①) Without inversion at C-1 during cleavage, all ^{3}H of C-1 would be labilized by aldolase. (②) With inversion at C-1 during cleavage half of ^{3}H at C-1 would be labilized by isomerase and half by aldolase.

established. It has been shown by Bloom and Topper (1956), Rose and Rieder (1958), and by Rieder and Rose (1959) that triosephosphate isomerase and fructose-1,6-bisphosphate aldolase labilize different hydrogens from C-1 of dihydroxyacetone phosphate. The absolute configuration of these paired hydrogens was established by experiments based on the known stereospecificity of the glycolic acid oxidase reaction (Rose, 1958) and by Johnson et al. (1965) by neutron and x-ray diffraction. These studies, therefore, assign a *pro-S* configuration to the hydrogen labilized by fructose-1,6-bisphosphate aldolase and a *pro-R* configuration to the hydrogen labilized by triosephosphate isomerase.

The present study utilizes the known stereospecificity of isomerase and aldolase to establish that the hydrogen exchange which takes place during the synthesis of *O*-alkyldihydroxyacetone phosphate from acyldihydroxyacetone phosphate occurs without change of configuration. The hydrogen which is lost is *pro-R*. A hydrogen from the medium is then gained by C-1 of *sn*-glycerol of *O*-alkylglycerol and assumes the *R* configuration. Several enzymatic and chemical reactions were involved in the conversion of *O*-alkylglycerol to the formaldehydodimedon containing the C-1 of the glycerol liberated from *O*-alkylglycerol. It was considered that one of these reactions, the glycerol ether cleavage enzyme, might possibly, by an unknown mechanism, produce an inversion of configuration of the substituent at C-1. This was ruled out by reacting the cleavage enzyme with *O*-alkylglycerol chemically synthesized and specifically labeled with tritium in the *S* position of C-1 of the glycerol moiety of *O*-alkylglycerol.

Acknowledgments

We gratefully acknowledge the assistance of Mrs. Constance Stahl.

References

Baumann, W. J., and Mangold, H. L. (1964), *J. Org. Chem. 29,* 3055.

Bligh, E. G., and Dyer, W. J. (1959), *Can. J. Biochem. Physiol. 37,* 911.

Bloom, B., and Topper, Y. J. (1956), *Science 124,* 1956.

Bublitz, C., and Kennedy, E. P. (1954), *J. Biol. Chem. 211,* 963.

Davis, P. A., and Hajra, A. K. (1977), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36,* 850.

Fisher, E., and Pfähler, E. (1920), *Ber. Dtsch. Chem. Ges. 53,* 1607.

Friedberg, S. J., and Heifetz, A. (1973), *Biochemistry 12,* 1100.

Friedberg, S. J., and Heifetz, A. (1975), *Biochemistry 14,* 570.

Friedberg, S. J., Heifetz, A., and Greene, R. C. (1971), *J. Biol. Chem. 246,* 5822.

Friedberg, S. J., Heifetz, A., and Greene, R. C. (1972) *Biochemistry 11,* 297.

Hajra, A. K. (1970), *Biochem. Biophys. Res. Commun. 39,* 1037.

Johnson, C. K., Gabe, E. J., Taylor, M. R., and Rose, I. A. (1965), *J. Am. Chem. Soc. 87,* 1802.

Kapoulas, V. M., Thompson, G. A., Jr., and Hanahan, D. J. (1969), *Biochim. Biophys. Acta 176,* 250.

Rieder, S. V., and Rose, I. A. (1959), *J. Biol. Chem. 234,* 1959.

Rose, I. A. (1958), *J. Am. Chem. Soc. 80,* 5835.

Rose, I. A., and Rieder, S. V. (1958), *J. Biol. Chem. 231,* 315.

Wykle, R. L., Piantadosi, C., and Snyder, F. (1972), *J. Biol. Chem. 237,* 2944.