Stereospecific Synthesis of D-1-Fluorodeoxyglycerol 3-Phosphate and Its Effects on Glycerol 3-Phosphate Dehydrogenase*

Gurdev S. Ghangas and Thomas P. Fondy†

ABSTRACT: D-1-Fluoro-1-deoxyglycerol 3-phosphate was prepared starting from D-mannitol by the intermediate synthesis of D-2,3-isopropylideneglycerol, D-1-tosyl-2,3-isopropylideneglycerol, D-1-fluorodeoxy-2,3-isopropylideneglycerol, D-1-fluorodeoxyglycerol, and D-1-fluorodeoxyglycerol 3-phosphate dibenzyl ester. The corresponding racemic compounds were also prepared starting from glycerol. The identity of DL-1-fluorodeoxyglycerol, DL-1-fluorodeoxyglycerol 3-phosphate dibenzyl ester, and DL-1-fluorodeoxyglycerol 3-phosphate was confirmed by comparison of chemical and physical properties of the compounds synthesized by this route, to the corresponding compounds prepared by ring opening of epifluorohydrin by aqueous acid, dibenzylphosphoric acid, or phosphoric acid, respectively. Nuclear magnetic resonance spectra of intermediates and of the final product are presented and analyzed. Racemic 1-fluorodeoxyglycerol 3-phosphate synthesized from glycerol is a substrate for rabbit muscle NAD-linked glycerol 3-phosphate dehydrogenase, and is indistinguishable in its kinetic behavior from the compound synthesized from epifluorohydrin. D-1-Fluorodeoxyglycerol 3-phosphate possesses no substrate activity with the enzyme, and inhibits the oxidation of L-glycerol 3-phosphate by the enzyme. The stereospecific synthetic route to D-1-fluorodeoxyglycerol 3-phosphate is readily applicable with slight modification to synthesis of the L enantiomorph. Availability of optically pure forms of D- and L-1-fluorodeoxyglycerol 3-phosphate will permit an examination of our suggestion that 1-fluoro analogs of glycerol 3-phosphate or dihydroxyacetone 3-phosphate or precursors that could generate them *in vivo* may be selectively toxic to cells lacking NAD-linked glycerol 3-phosphate dehydrogenase, which is a characteristic of many types of cancer cells.

Studies in many laboratories over the past decade have shown that a wide variety of human cancer types have unusually low activity for cytoplasmic NAD-linked glycerol-3-P dehydrogenase or lack the enzyme entirely. We have listed the papers detailing this general observation in our previous work (Fondy *et al.*, 1970). Subsequently, Hilf *et al.* (1970) in a comprehensive study of the enzymology of infiltrating ductal carcinoma of human breast have shown that NAD-linked glycerol-3-P dehydrogenase is again substantially depressed whereas other dehydrogenases show elevated activity. Since glycerol-3-P dehydrogenase is a key enzyme linking carbohydrate and lipid metabolism, alteration in its activity may be a fundamental feature in neoplastic processes in the development of some types of cancer. In any event, this depressed glycerol-3-P dehydrogenase level in human cancer cells is sufficiently general to constitute a possible point of exploitable biochemical difference between cancer cells and their normal cells of origin. Moreover, successful exploitation of this biochemical difference might introduce a class of cancer chemotherapeutic agents that function as glycolytic or Krebs cycle anti-metabolites and are thus likely to be less specifically immunosuppressive than agents directed against nucleotide and nucleic acid metabolism, protein synthesis, or cell division. We have suggested that 1-halo analogs of glycerol-3-P or of dihydroxyacetone-P or precursors that could generate them *in vivo* may be valuable as agents specifically toxic to cells lacking glycerol-3-P dehydrogenase and have set forth our rationale for this suggestion in our previous paper (Fondy *et al.*, 1970).

If the 1-halo analogs of glycerol-3-P or of dihydroxyacetone-P are to be metabolized differently in tissues lacking NAD-linked glycerol-3-P dehydrogenase as compared to those which have the enzyme, it is necessary that the analogs act as substrates for the enzyme under physiological conditions. We have shown that the 1-fluoro analog of DL-glycerol-3-P (DL-1-fluorodeoxyglycerol-3-P) is a substrate for NAD-linked glycerol-3-P dehydrogenase with an apparent K_m of 8 mM at pH 7.5 (Fondy *et al.*, 1970). It is assumed that only the L isomer in the DL-1-fluorodeoxyglycerol-3-P preparation is actually a substrate for L-glycerol-3-P dehydrogenase and that the pure optical isomers should be used in any in-depth study of the value of these analogs as potential cancer chemotherapeutic agents.

It is the purpose of this paper to detail the stereospecific synthesis of D-1-fluoro-1-deoxyglycerol-3-P by methods which we have designed for ready application, with some modification, for subsequent synthesis of the L isomer. It is our further purpose to examine the chemical and biochemical properties of the D isomer that will be important in the synthesis and comparative study of the L isomer, and in the investigation of both the D and L isomers as parent compounds for cancer chemotherapeutic agents.

Materials

Enzyme. Rabbit muscle NAD-linked glycerol-3-P dehydrogenase was obtained from Sigma Chemical Co. (St. Louis) (lot 70C-9590).

Chemicals. Dibenzyl phosphite, *N*-chlorosuccinimide, and epifluorohydrin were obtained from Aldrich Chemical Co

* From the Department of Biology, Syracuse University, Syracuse, New York 13210. *Received March 26, 1971.* This work was supported by Grant CA-10250 from the National Cancer Institute of the National Institutes of Health, and by Grant GB-20943 from the National Science Foundation.

† To whom to address correspondence.

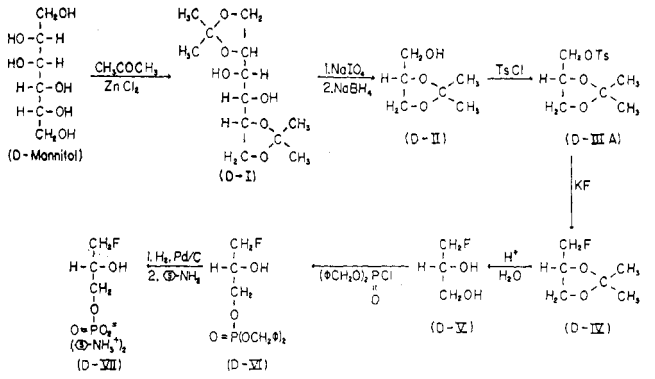

FIGURE 1: Stereospecific synthesis of D-1-fluoro-1-deoxyglycerol-3-P from D-mannitol *via* 2,3-isopropylidene-D-glycerol.

Potassium fluoride (anhydrous) was purchased from ROC–RIC Chemical Co., Sun Valley, Calif. More common reagents were obtained primarily from Aldrich and Eastman Organic Chemicals (Rochester). Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Methods

Enzyme Assays. The commercial rabbit muscle glycerol-3-P dehydrogenase was dialyzed against Tris-acetate buffer at 4° for 2 days and then appropriately diluted with the Tris buffer (50 mM Tris, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 7.5).

Enzyme assays were done at pH 9.0 using 10 mM pyrophosphate, 1 mM EDTA, and 1 mM 2-mercaptoethanol. Concentration of NAD^+ was 0.3 mM for assays with 1-fluorodeoxyglycerol-3-P and 1.5 mM for assays with L-glycerol-3-P as substrate. The assays were performed at room temperature (23°) by monitoring absorbance at 340 mμ, using a Gilford Model 2400 spectrophotometer with 0.1 *A* unit as full scale. When L-glycerol-3-P was employed as substrate, 0.1 M hydrazine was added to the assay system to react with the product dihydroxyacetone-P and prevent the enzymatic reaction from attaining rapid equilibrium.

Optical rotatory dispersion and circular dichroism studies were carried out at 25° on solutions of D-1-fluorodeoxyglycerol (D-V) in 100% ethanol and on solutions of D-1-fluorodeoxyglycerol-3-P (D-VII) in H_2O–EtOH (1:2, v/v) mixture. D-1-Fluorodeoxyglycerol was measured at concentrations of 1.0–5.0% (by volume) in a 1-cm path-length cell. The concentration of dicyclohexylammonium salt of D-1-fluorodeoxyglycerol-3-P (D-VII) was 5% by weight and measurements were also made in a path length of 1 cm. Measurements were made with a Jasco Model ORD–UV-5 spectrometer equipped with Sproul Scientific SS-107 CD accessory. Specific rotations reported as $[\alpha]_D^{25}$ were measured in a Rudolph polarimeter.

Synthesis of DL- *and* D-*1-Fluorodeoxyglycerol-3-P.* The stereospecific synthesis of D-1-fluorodeoxyglycerol-3-P involved replacement by fluoride of the hydroxyl group of D-isopropylideneglycerol (D-II) as the tosyl derivative (D-III) using nucleophilic displacement by KF in ethylene glycol. The synthetic routes are shown in Figure 1. The resulting 1-fluorodeoxy-D-isopropylideneglycerol (D-IV) was deketalized to D-1-fluorodeoxyglycerol (D-V) which was then phosphorylated by dibenzyl phosphorochloridate with subsequent removal of the benzyl ester groups by catalytic hydrogenation to yield the 1-fluoro analog of D-glycerol-3-P (D-VII) which was isolated as the dicyclohexylammonium salt.

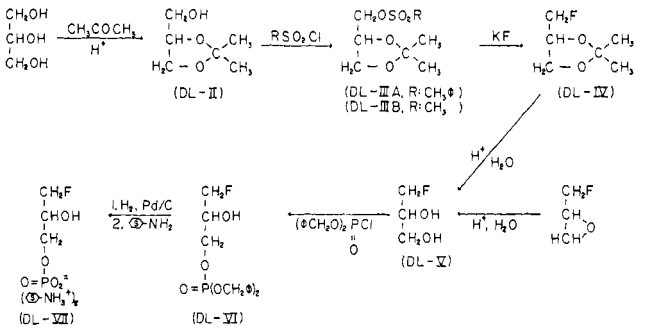

FIGURE 2: Synthetic route for preparation of DL-1-fluoro-1-deoxyglycerol-3-P from glycerol *via* DL-isopropylideneglycerol.

In order to develop the synthetic procedures, the steps were first carried out beginning with DL-isopropylideneglycerol (DL-II) prepared from glycerol and acetone (Figure 2).

SYNTHESIS OF DL-1-FLUORODEOXYGLYCEROL-3-P FROM DL-ISOPROPYLIDENEGLYCEROL. DL-Isopropylideneglycerol (DL-II). To 50 g of glycerol (0.545 mole) was added 200 ml of acetone, 170 ml of benzene, and 1.5 g of *p*-toluenesulfonic acid. The mixture was heated in an oil bath at 75–80° while stirring under reflux for 50 hr. The cooled reaction mixture was neutralized with slightly more than the equivalent amount of Na_2CO_3. The lower layer, mainly unreacted glycerol, was separated and the upper layer was subjected to fractional distillation. The fraction distilling at 45–57° (4 mm) was obtained in 50% yield (36.2 g, 0.274 mole), density 1.04 g/cm³. The refractive index, n_D^{23}, was 1.430 ± 0.002. The nmr spectrum is shown in Figure 3A.

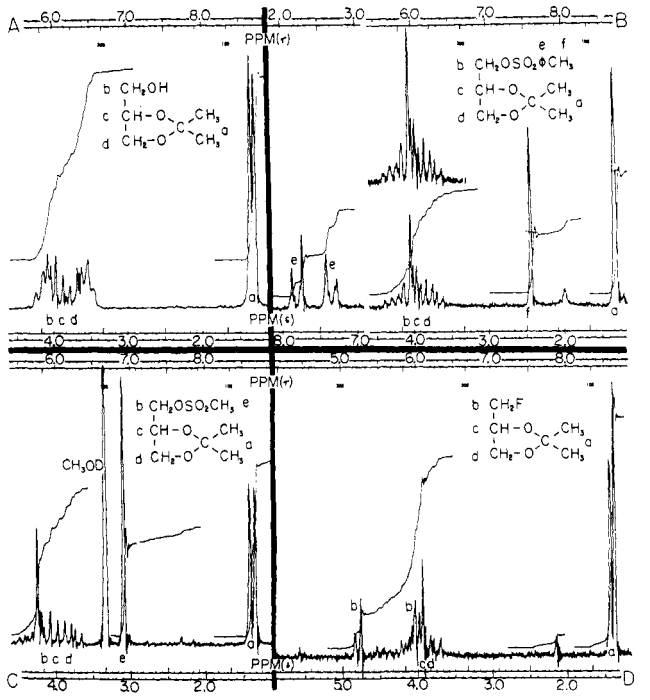

FIGURE 3: 60-MHz nuclear magnetic resonance spectra of 2,3-isopropylidene derivatives of glycerol (spectrum A), 1-*p*-toluenesulfonyl-DL-glycerol (spectrum B), 1-methanesulfonyl-DL-glycerol (spectrum C), and 1-fluoro-1-deoxy-DL-glycerol (spectrum D). Spectrum A run without solvent, spectra B and D in deuteriochloroform, and spectrum C in CH_3OD. Absorptions are assigned by lower case letters in the nmr spectra corresponding to protons labeled in the structures.

In a similar experiment use of petroleum ether (bp 30–60°) in place of benzene (Newman and Renoll, 1945), resulted in a 54% yield and facilitated the distillation of the product.

DL-1-Tosylisopropylideneglycerol (DL-IIIA). DL-Isopropylideneglycerol (DL-II) (13.2 g, 100 mmoles) in 5 ml of dry pyridine was cooled in an ice bath. To this constantly stirred solution was added over a period of 2 hr 20.5 g of *p*-toluenesulfonyl chloride dissolved in 17 ml of dry pyridine. The mixture was kept at 4° for 1 day and then at room temperature for another day. The whole of the reaction mixture (including a white precipitate) was taken up in 250 ml of distilled water at 0°. The heavy oil at the bottom was recovered by decanting off the supernatant. This heavy oil was dissolved in 400 ml of ethyl ether and washed twice with 100-ml portions of cold 5% Na_2CO_3 and the ether was removed by rotary evaporation. The residue which included some crystalline material and weighed 33 g, was dissolved in 150 ml of ethyl ether and to this was added 300 ml of petroleum ether. The temperature was lowered to −70° and the white precipitate filtered on a cold Büchner funnel. The residue, dried under vacuum, weighed 24 g (85%) and had a melting point (uncorrected) of 49–50° (lit. (Freudenberg and Hess, 1926) mp 47°). The nmr spectrum is shown in Figure 3B. *Anal.* Calcd for $C_{13}H_{18}O_5S_1$: C, 54.54; H, 6.35; O, 27.94; S, 11.20. Found: C, 54.75; H, 6.36; O, 28.08; S, 11.02.

DL-1-Methanesulfonylisopropylideneglycerol (DL-IIIB). DL-Isopropylideneglycerol (DL-II) (13.2 g, 100 mmoles) in 5 ml of dry pyridine was cooled in an ice bath. To this constantly stirred solution was added 11.85 ml (17.18 g, 150 mmoles) of methanesulfonyl chloride in 20 ml of dry pyridine over a period of 2–3 hr. The reaction mixture was left at 4° for 2 days with occasional stirring. The reaction mixture was taken up in about 300 ml of cold, distilled water. A small amount of oil at the bottom was separated by decanting. The remaining fraction was extracted three times with 150-ml portions of ethyl ether. This ether extract was added to the oily residue and the combined ether fraction was washed twice with 5% Na_2CO_3. The washed ether layer was dried with anhydrous Na_2SO_4, and concentrated by rotary evaporation. The concentrate was taken up in 400 ml of ether and a small amount of brownish residue, insoluble in ether, was removed. Addition of two volumes of petroleum ether to the ether extract followed by cooling to −78° produced a gummy precipitate which was recovered by decanting off the liquid. This gummy material melted to a brownish yellow liquid at room temperature. The yield was 15.6 g (74%). The nmr spectrum appears in Figure 3C.

DL-1-Fluorodeoxyisopropylideneglycerol (DL-IV). A. From DL-1-Tosylisopropylideneglycerol (DL-IIIA). To a flask containing 240 ml of anhydrous ethylene glycol and 68 g of anhydrous KF was carefully added 57.2 g (200 mmoles) of DL-1-tosylisopropylideneglycerol (DL-IIIA). The flask was fitted with a distillation unit and immersed in a preheated oil bath (120°) on a hot plate equipped with a magnetic stirrer. The contents were stirred under anhydrous conditions and the bath temperature raised to 180 ± 10° and maintained at this temperature for 20–25 min. The fraction distilling at 122–126° was collected in a flask immersed in Dry Ice–acetone. The distillate did not freeze completely even at −60 to −70° but by lowering the temperature below −10° the contaminating ethylene glycol which solidifies could be removed by careful decanting. The yield of the remaining product was 18.44 g (68%). The nmr spectrum is shown in Figure 3D.

B. From DL-1-Methanesulfonylisopropylideneglycerol (DL-IIIB). To 8.4 g (40 mmoles) of DL-IIIB in 60 ml of ethylene glycol was added 11.60 g of anhydrous KF. The flask containing the reactants was fitted for distillation under anhydrous conditions. The flask was immersed in an oil bath with the contents being stirred with a magnetic stirrer. The reaction mixture turned brown at bath temperature (140–150°). The bath temperature was raised to 180–185° and the fraction distilling at 120–123° was collected in a Dry Ice–acetone bath as a partially crystalline whitish fluid. The temperature was raised to −25° and contaminating ethylene glycol (mp −11.5°) was removed by decanting. The yield of DL-1-fluorodeoxyisopropylideneglycerol (DL-IV) thus obtained was 3.4 g (63%). The nmr spectrum was identical with that obtained from the tosyl derivative (DL-IIIA) and shown in Figure 3D.

DL-1-Fluorodeoxyglycerol (DL-V). To 9.38 g (70 mmoles) of DL-IV was added 25 ml of 0.2 N H_2SO_4 in 80% ethanol. The covered solution was left at room temperature for 24 hr and then refluxed for 5 min. The solution was cooled and neutralized with $Ba(OH)_2$. After removing the $BaSO_4$ precipitate by centrifugation, the water was removed with the addition of absolute ethanol followed by rotary evaporation. The residue was subjected to vacuum distillation. The fraction distilling at 78° at 4–5 mm (bath temperature 150°) was collected. The product weighed 4.9 g (yield 75%). The nmr spectrum is shown in Figure 4A. The structure of DL-1-fluorodeoxyglycerol was confirmed by synthesis from epifluorohydrin as follows. Epifluorohydrin (2.7 g) was mixed with 2.7 ml of 1.5 N H_2SO_4 and refluxed for 1 hr (Gryszkiewicz-Trochimowski, 1947; Pattison and Norman, 1957). The refluxed solution was allowed to cool and stand at room temperature overnight. The mixture was refluxed again for a few minutes, cooled, and neutralized with $NaHCO_3$. Water was removed by rotary evaporation after the addition of absolute ethanol. Fluorodeoxyglycerol was recovered by distillation *in vacuo*. The yield was 2.5 g (73%). The nmr spectrum of DL-1-fluorodeoxyglycerol obtained from epifluorohydrin is shown in Figure 4C. The stability of 1-fluorodeoxyglycerol is discussed under synthesis of D-1-fluorodeoxyglycerol and under Results.

DL-1-Fluorodeoxyglycerol-3-P Dibenzyl Ester (DL-VI). DL-1-Fluorodeoxyglycerol (DL-V) was phosphorylated with dibenzyl phosphorochloridate by the same procedure employed for phosphorylation of 1-chlorodeoxyglycerol (Fondy *et al.*, 1970). The dibenzylphosphoryl ester of DL-1-fluorodeoxyglycerol (DL-VI) was isolated in 45% yield. The nmr spectrum is shown in Figure 4D along with the spectrum of DL-1-fluorodeoxyglycerol-3-P dibenzyl ester (Figure 4B) obtained by reaction of epifluorohydrin with dibenzylphosphoric acid following previously published procedures (Fondy *et al.*, 1970).

DL-1-Fluorodeoxyglycerol-3-P Dicyclohexylammonium Salt (DL-VII). The dibenzylphosphoryl ester (3.1 g) prepared from 1-fluorodeoxyglycerol (DL-VI) was debenzylated by catalytic hydrogenation with palladium on charcoal in absolute ethanol. The hydrogenated product was neutralized with freshly distilled cyclohexylamine. This helped to remove contaminating P_i, which precipitates in ethanol as the dicyclohexylammonium salt. Removal of ethanol by rotary evaporation gave 2.3 g of the dicyclohexylammonium salt of DL-1-fluorodeoxyglycerol-3-P (yield 75%), mp (uncor) 159–163°. The nmr spectrum was identical to that obtained for the D compound and shown later.

SYNTHESIS OF D-1-FLUORODEOXYGLYCEROL-3-P (D-VII) FROM D-MANNITOL *via* D-ISOPROPROPYLIDENEGLYCEROL. 1,2,5,6-Di-*O*-isopropylidene-D-mannitol (D-I). The modified method of Baer (Baer, 1952; Bird and Chadha, 1966) was used as fol-

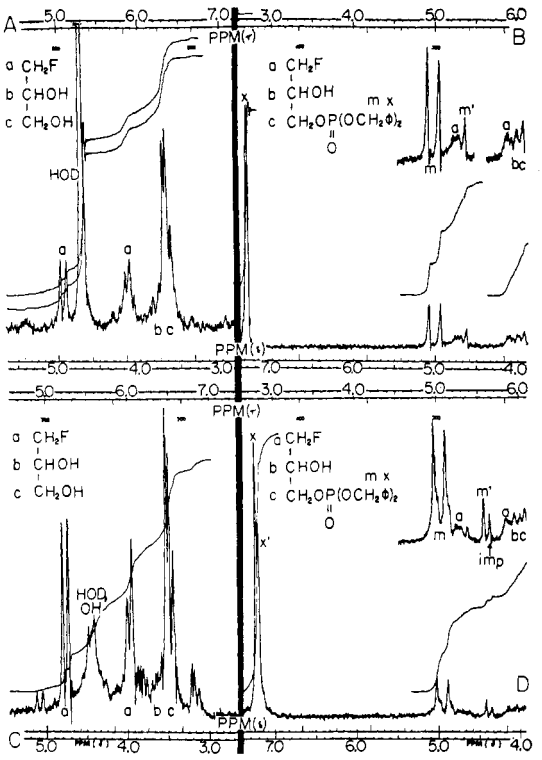

FIGURE 4: 60-MHz nuclear magnetic resonance spectra of DL-1(3)-fluorodeoxyglycerol (spectra A and C) and DL-1-fluorodeoxyglycerol-3-P dibenzyl ester (spectra B and D). Compounds shown in spectra A and D were prepared starting for glycerol as shown in Figure 2. Compounds shown in spectra B and C were prepared independently from epifluorohydrin by ring opening in aqueous acid (spectrum C) or from epifluorohydrin and dibenzylphosphoric acid (spectrum B). Spectra A and C were run in deuterium oxide, but exchangeable protons on compound in spectrum C were not fully exchanged by preliminary washing in deuterium oxide. Spectra B and D were run in deuteriochloroform. Lower case letters identify absorption peaks as detailed in legend to Figure 3. Peak designated "m'" in spectra B and D is benzyl alcohol released by intramolecular deesterification. Peak designated "x'" in spectrum D and shoulders on doublet "m" are due to dibenzylphosphoric acid.

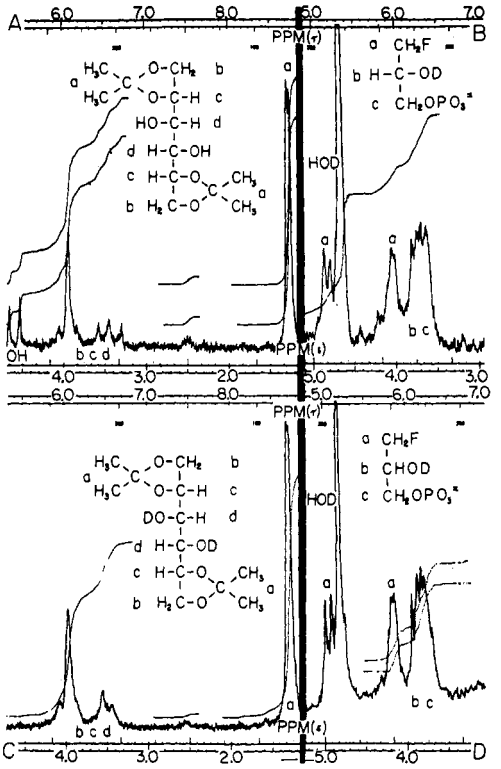

FIGURE 5: 60-MHz nuclear magnetic resonance spectra of 1,2,5,6,-di-*O*-isopropylidene-D-mannitol (spectra A and C), of D-1-fluorodeoxyglycerol-3-P dilithium salt (spectrum B) synthesized from D-mannitol as shown in Figure 1, and of DL-1-fluorodeoxyglycerol-3-P dilithium salt (spectrum D) synthesized from epifluorohydrin and phosphoric acid. Spectra A and C were run in deuterated dimethyl sulfoxide-D_6. Compound in spectrum A was analyzed without prior exchange of hydroxyl protons. Compound in spectrum C was lyophillyzed several times from deuterium oxide. Spectra B and D were run in deuterium oxide.

lows. To 680 ml of recently distilled acetone (previously dried with anhydrous K_2CO_3) was added 135 g of anhydrous $ZnCl_2$ and the mixture was stirred gently to get the $ZnCl_2$ into solution. This solution was left aside for cooling to room temperature. To a separate flask containing 85 g of D-mannitol was added the above $ZnCl_2$–acetone solution by decanting, leaving the residue behind. The contents were shaken under anhydrous conditions at room temperature (23°) for 2 hr. The material was then filtered and the residue (unreacted mannitol) discarded. The filtrate was rapidly added to a vigorously stirring K_2CO_3 solution (170 g of anhydrous K_2CO_3 dissolved in 170 ml of H_2O and cooled to room temperature) covered with 675 ml of ethyl ether. The reaction mixture was stirred vigorously (motor-driven paddle stirrer necessary) for 40 min and then filtered. The $ZnCO_3$ precipitate was washed with 200 ml of 1:1 mixture of acetone–ether and acetone and ether removed from the combined filtrate and washings by rotary evaporation on a water bath at 25°. The remaining liquid was left in the cold room at 0°. A white precipitate started appearing after a few minutes and the contents eventually turned to a solid. After about 2 hr, the solid contents were broken up in cold *n*-hexane and filtered. The residue was washed with hexane and dried under vacuum against P_2O_5. The yield was 62 g (51%). The nmr spectra of the product with unexchanged and with deuterium-exchanged hydroxyl groups are shown in Figure 5A,C, mp (uncor) 113–114°.

D-2,3-Isopropylideneglycerol (D-II). The method described here is essentially that developed by Lecocq and Ballou (1964) with slight modifications. In 500 ml of cold water was dissolved 40 g of sodium metaperiodate. To this was added 34.2 g of 1,2,5,6-di-*O*-isopropylidene-D-mannitol (D-I) and the mixture stirred vigorously in an ice bath. The 1,2,5,6-di-*O*-isopropylidene-D-mannitol (D-I) dissolved within 2 min. The stirring was continued for another 13 min, after which sodium iodate was precipitated out with 1 l. of absolute ethanol and removed by filtration. To the above filtrate immersed in an ice bath, was added 10 g of sodium borohydride dissolved in cold water and the solution was stirred occasionally. After 2 hours the pH of the solution was brought to 7.5 with 12 ml of glacial acetic acid. The small amount of residue which appeared during borohydride reduction was removed by decanting. The ethanol from the clear solution was removed by rotary evaporation. The resulting solution was extracted with 300-ml portions of $CHCl_3$ totalling to about 2 l. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and then concentrated to a syrup weighing 32.7 g with $[\alpha]_D^{25}$ +12.4° (neat). The redistilled product weighed 23.8 g (69%) and had $[\alpha]_D^{25}$ +14.7° (neat). The nmr spectrum was identical with that previously shown for DL-II (Figure 3A).

D-1-Tosylisopropylideneglycerol (D-IIIA). The conditions for tosylation of D-2,3-isopropylideneglycerol are essentially the same as those described for the preparation of the DL derivative (DL-IIIA). The tosyl derivative (D-IIIA) was isolated as a white semisolid in approximately 80% yield by recrystallization (Baer and Fischer, 1948) from ether–petroleum ether at −78°. The solid changes to a viscous liquid at room temperature with $[\alpha]_D^{25}$ −7.1° (neat), −4.5° (50% v/v in dry EtOH), −6.7° (neat), −4.6° (in dry EtOH) (Baer and Fischer, 1948). The nmr spectrum was identical with that obtained for the DL compound (Figure 3B).

D-1-Fluorodeoxyisopropylideneglycerol (D-IV). Conditions for preparation of D-IV are identical with those for the corresponding DL derivative (DL-IV). The material distilling at 120–123° was isolated in 72.4% yield $[\alpha]_D^{25}$ +11.1° (neat), +11.9° in dry ethanol, 50% (v/v); density (at 23°) = 1.03 g/cm³. The nmr spectrum was identical to that obtained for the DL compound (Figure 3D). *Anal.* Calcd for $C_6H_{11}F_1O_2$: C, 53.71; H, 8.28; F, 14.16; O, 23.85. Found: C, 53.73; H, 8.20; F, 14.01; O (not assayed in presence of fluorine).

D-1-Fluorodeoxyglycerol (D-V). The details of the reaction were identical to those used for the synthesis of DL-fluorodeoxyglycerol (DL-V) from the isopropylidene derivative (DL-IV). D-Fluorodeoxyglycerol obtained had $[\alpha]_D^{25}$ −7.6° in dry ethanol (50% v/v). ORD results showed $[\alpha]_{400}$ −52°, $[\alpha]_{350}$ −81°, $[\alpha]_{300}$ −138°, $[\alpha]_{250}$ −276°, and $[\alpha]_{210}$ −690°. The nmr spectrum was identical with that obtained for DL-1-fluorodeoxyglycerol (DL-V) prepared starting either from glycerol or from epifluorohydrin (Figure 4A,C). *Anal.* Calcd for $C_3H_7F_1O_2$: C, 38.29; H, 7.51; F, 20.19; O, 34.01. Found: C, 38.66; H, 7.66; F, 18.0; O (not assayed in presence of fluorine). The sample of D-1-fluorodeoxyglycerol (D-V) sent for elemental analysis had been prepared 5 weeks earlier. 1-Flurodeoxyglycerol was observed to lose fluoride ion slowly on standing as will be detailed under Results.

D-1-Fluorodeoxyglycerol Phosphate Dibenzyl Ester (D-VI) and Dicyclohexylammonium Salt (D-VII). Synthesis of compounds (D-VI) and (D-VII) was achieved using the same procedures as for the corresponding DL derivatives (DL-VI and DL-VII). ORD results for D-1-fluorodeoxyglycerol-3-P dicyclohexylammonium salt showed $[\alpha]_{400}$ −5.2°, $[\alpha]_{350}$ −7.0°, and $[\alpha]_{320}$ −9.0°. The uncorrected meling point was 162–166°. The nmr spectrum of D-VI was identical with that shown in Figure 4B for the DL compound. The nmr spectrum of the dilithium salt of D-1-fluorodeoxyglycerol-3-P (D-VII) is shown in Figure 5B along with the spectrum in Figure 5D of DL-1-fluorodeoxyglycerol-3-P prepared from epifluorohydrin and phosphoric acid (Fondy *et al.*, 1970). *Anal.* Calcd for $C_3H_6F_1Li_2O_5P_1$: C, 19.37; H, 3.26; F, 10.21; Li, 7.46; O, 43.02; P, 16.66. Found: C, 17.83; H, 3.18; F, 7.49; P, 16.63. The dilithium salt of D-1-fluorodeoxyglycerol-3-P that was subjected to elemental analysis was synthesized from a preparation of D-1-fluorodeoxyglycerol (D-V) that was several weeks old. Fluorodeoxyglycerol slowly loses fluoride ion as mentioned previously, which accounts for the below theoretical value of fluorine content in the final product.

Results

Synthesis of D-1-Fluorodeoxyglycerol-3-P. The overall yield in several preparations of 1-fluorodeoxyglycerol-3-P (D- and DL-VII) was 12% beginning from isopropylideneglycerol (D- and DL-II). The yield of D-1-fluorodeoxyglycerol-3-P (D-VII) starting from D-mannitol was 4%. Confirmation of the identity of the intermediates and of the final products was established by nmr and infrared spectra, by melting points, by elemental analyses as detailed under Methods, and by syntheses of three of the compounds by independent routes. The structure of the final product in the DL series was also confirmed by enzymatic analysis as detailed in the following section. Optical activity in the stereospecific synthesis of D-1-fluorodeoxyglycerol-3-P from D-mannitol was preserved as determined by examining intermediates for specific rotation at the sodium D wavelength or by optical rotatory dispersion spectra. Optical purity of the final product was established by enzymatic analysis as set forth below.

Nmr Spectral Comparisons. The nmr spectra of the intermediates in the synthetic routes to both DL- and D-1-fluorodeoxyglycerol-3-P (DL- and D-VII) were consistent with the proposed structures at each point (Figures 3, 4, and 5). The assignment of absorption peaks is designated in the figures with lower case letters, although in some instances it was not possible to resolve peaks and assign absorptions to specific protons with very similar chemical shifts. Absorptions due to protons in the CH_2F group in various compounds is, however, readily identifiable by the characteristic coupling constant of 47 cycles.

Confirmation of the structures in the DL series was established at three points by independent synthesis (1) of DL-1-fluorodeoxyglycerol (DL-V) from epifluorohydrin in aqueous acid, (2) of DL-1-fluorodeoxyglycerol-3-P dibenzyl ester (DL-VI) from epifluorohydrin and dibenzylphosphoric acid, and (3) of the final product DL-1-fluorodeoxyglycerol-3-P (DL-VII) from epifluorohydrin and phosphoric acid. These three compounds synthesized independently were indistinguishable in nmr and infrared spectra and in other physical properties from the corresponding compounds synthesized by the synthetic route shown in Figure 2. (1) The spectrum of DL-1-fluorodeoxyglycerol (DL-V) obtained from DL-isopropylideneglycerol (DL-II) and shown in Figure 4A was identical to the compound DL-V obtained from epifluorohydrin by ring opening in aqueous acid (Figure 4C), except that the extent to which exchangeable protons had been washed out by repeated freeze-drying from deuterium oxide was not the same for both samples. (2) The spectrum of the dibenzyl ester of DL-1-fluorodeoxyglycerol-3-P (DL-VI) prepared from DL-1-fluorodeoxyglycerol (DL-V) and dibenzyl phosphorochloridate (Figure 4D) was identical to the spectrum of the compound (DL-VI) obtained from epifluorohydrin and dibenzylphosphoric acid (Figure 4B) except that the former product was contaminated with dibenzylphosphoric acid. This dibenzylphosphoric acid arises from unreacted dibenzyl phosphorochloridate and produces the peak designated "x'" and the shoulder on the doublet designated "m" in Figure 4D. The contaminant is removed from the final product as dicyclohexylammonium phosphate after catalytic hydrogenation to phosphoric acid. The peak designated "m'" in Figure 4B,D is due to benzyl alcohol which is slowly released by intramolecular deesterification catalyzed by the free hydroxy group on C_2 as discussed earlier (Fondy *et al.*, 1970). (3) D-1-Fluorodeoxyglycerol-3-P (D-VII) obtained as the final product in the stereospecific synthesis produced an nmr spectrum (Figure 5B) that was identical to the spectrum (Figure 5D) of DL-1-fluorodeoxyglycerol-3-P (DL-VII) synthesized from epifluorohydrin and dibenzylphosphoric acid, and presented in our previous paper (Fondy *et al.*, 1970).

Stability of 1-Fluorodeoxyglycerol. The fluoro group content of 1-fluorodeoxyglycerol slowly decreased from a value of 1 g-atom of fluoro group per mole of compound in 1-fluorodeoxyisopropylideneglycerol to values less than unity. The

theoretical fluoro group content of 1-fluorodeoxyglycerol is 20.19%. After 5 weeks at room temperature this value decreased to 18.00%, at 9 weeks the value was 15.86%, and at 12 weeks, 11.86%. Freshly prepared D-1-fluorodeoxyglycerol displayed a plain ORD curve with no Cotton effect in the range 200–700 mμ. Upon standing, a negative Cotton effect appeared at 270 mμ, but did not appear in the same sample immediately after redistillation. The appearance of the Cotton effect was accompanied by changes in the nmr spectrum. Although the exact nature of the chemical alteration remains to be determined, it is likely that neighboring group participation by the free hydroxyl group (Kun and Dummel, 1969) in 1-fluorodeoxyglycerol promotes the loss of HF which in turn reacts with the glass containers and does not appear in the elemental analyses. This possibility is supported by the fact that the fluoro group in 1-fluorodeoxy-2,3-isopropylideneglycerol (D- and DL-IV) is stable indefinitely.

Enzymatic Analyses. Rabbit muscle NAD-linked glycerol-3-P dehydrogenase in the presence of NAD^+ catalyzed the oxidation of DL-1-fluorodeoxyglycerol-3-P (DL-VII) prepared by procedures detailed in this paper from DL-isopropylideneglycerol (DL-II). The rate of oxidation was identical to that obtained for DL-1-fluorodeoxyglycerol-3-P (DL-VII) synthesized from epifluorohydrin and characterized earlier (Fondy *et al.*, 1970). D-1-Fluorodeoxyglycerol-3-P (D-VII) prepared from D-mannitol *via* D-isopropylideneglycerol (D-II) was not a substrate for the enzyme as shown in Figure 6. Detailed conditions for the enzymatic analyses are presented in the legend to Figure 6. D-1-Fluorodeoxyglycerol-3-P (D-VII) at a concentration of 10 mM did not inhibit the oxidation of 10 mM DL-1-fluorodeoxyglycerol-3-P (DL-VII), thus demonstrating that the failure of the D enantiomorph to exhibit substrate properties is not due to the presence of extraneous inhibitor in the preparation of the D compound. Moreover, the complete absence of substrate properties in the preparation of the D compound confirms that the compound is optically pure.

When 1 mM L-glycerol-3-P was used as the substrate for glycerol-3-P dehydrogenase, 0.1 M hydrazine was incorporated into the buffer to react with the product dihydroxyacetone-P and prevent the reaction from reaching rapid equilibrium. Under these conditions, the rate of oxidation of 1 mM L-glycerol-3-P was slightly inhibited by 10 mM D-1-fluorodeoxyglycerol-3-P (D-VII) as shown in Figure 6.

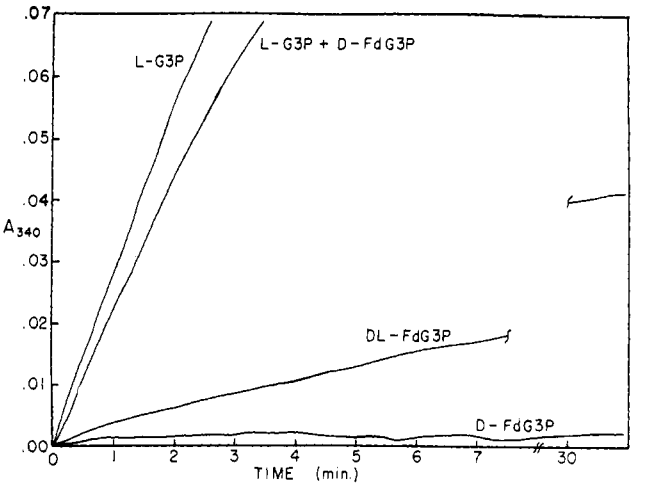

FIGURE 6: D- and DL-1-fluoro analogs of glycerol-3-P as substrate and inhibitor for rabbit muscle NAD-linked glycerol-3-P dehydrogenase. D-FdG3P: D-1-fluoro-1-deoxyglycerol-3-P dicyclohexylammonium salt (D-VII of Figure 1), 10 mM in enzyme reaction system. DL-FdG3P: DL-1-fluoro-1-deoxyglycerol-3-P dicyclohexlammonium salt (DL-VII) prepared from glycerol by the reaction scheme shown in Figure 2, 10 mM in enzyme reaction system. L-G3P: L-glycerol-3-P dicyclohexlammonium salt, 1 mM in enzyme reaction system. Buffer: 50 mM pyrophosphate (pH 9.0), 1 mM EDTA, and 1 mM 2-mercaptoethanol. Enzyme concentration: 5×10^{-7} M in assays not involving L-G3P; 5×10^{-9} M in assays with L-G3P as substrate. Assays with L-G3P as substrate employed 0.1 M hydrazine in buffer. NAD^+ concentration was 0.3 mM except in assays involving 1 mM L-glycerol-3-P in which case NAD^+ concentration was raised to 1.5 mM. The increase in absorbance at 340 nm is shown as a direct tracing of recorder scans form a Gilford Model 2400 spectrophotometer with 0.100 *A* unit full scale.

Discussion

Projected Synthesis of L-1-Fluorodeoxyglycerol-3-P and Pharmacological Studies of 1-Fluoro Analogs of Glycerol-3-P. The successful synthesis of D-1-fluorodeoxyglycerol-3-P (D-VII) detailed in this paper employs a synthetic route which is readily applicable to synthesis of the L compound. The synthesis of the L compound can be achieved by protection of the primary hydroxyl group (on C_1) in D-isopropylideneglycerol (D-II) followed by substitution of a fluoro group at the hydroxyl group on C_3 that is initially blocked by the isopropylidene group. The optical purity of D-1-fluorodeoxyglycerol-3-P (D-VII) obtained in our work indicates that L-1-fluorodeoxyglycerol-3-P (L-VII) can be obtained in optically pure form by the modified route suggested.

We have suggested (Fondy *et al.*, 1970) that 1-fluoro analogs of glycerol-3-P, dihydroxyacetone-3-P, or precursors that could give rise to them *in vivo* might be toxic to cells lacking glycerol-3-P dehydrogenase. Testing of this suggestion requires the availability of optically pure L-1-fluorodeoxyglycerol-3-P (L-VII). Moreover, it is not possible to establish the values of apparent kinetic constants and of maximal rates of oxidation of L-1-fluorodeoxyglycerol-3-P (L-VII) when only the racemic mixture and the optically pure D enantiomorph are available. When 10 mM D-1-fluorodeoxyglycerol-3-P (D-VII) was added to 10 mM DL-1-fluorodeoxyglycerol-3-P (DL-VII) there was no measureable inhibition of the already slow rate of oxidation of the racemic mixture. However, it is possible that inhibition of oxidation of the L enantiomorph by the D compound could be measured at concentrations of the D enantiomorph that are less than the 5 mM concentration present in a 10 mM solution of the DL compound.

Nmr Comparison of Isopropylidene Derivatives. *cis*- and *trans*-methyl groups appear as two distinct singlets in the nmr absorptions due to the isopropylidene group in Figure 3A,C,-D. In contrast, the absorptions due to both of the methyl groups in the isopropylidene group in Figure 3B fuse into what appears as a single peak. This observation suggests that the *p*-toluenesulfonyl moiety in DL-1-tosylisopropylidene glycerol (DL-IIIA) (Figure 3B) interacts with the *cis*-methyl groups of the isopropylidene group and shifts the methyl resonances either downfield or upfield slightly so that it overlaps the resonance peak due to the *trans*-methyl group. Although the exact nature of the interaction of the *p*-toluenesulfonyl moiety with the isopropylidene group is not clear at this time, examination of the nmr spectra of 1,2,5,6-di-*O*-isopropylidene-D-mannitol (D-I) in Figure 5A,C may be helpful. In these spectra the absorption due to the methyl groups in the isopropylidene group appear almost to overlap, suggesting that steric bulk is at least partly responsible for the change in chemical shift of the isopropylidene group in these various derivatives.

Metabolic Studies on D-*1-Fluorodeoxyglycerol.* DL-1-Fluorodeoxyglycerol (DL-V) has been synthesized on a preparative scale by Dummel and Kun (1969) from glycidol (1,2-epoxy-3-hydroxypropane) and KHF_2. Our synthetic route to D-1-fluorodeoxyglycerol-3-P (D-VII) now provides a route to D-1-fluorodeoxyglycerol (D-V). O'Brien and Peters (1958) observed that DL-1-fluorodeoxyglycerol (DL-V) is highly toxic in rats and mice due to a metabolic conversion which takes place in the liver. The availability of the optically pure D enantiomorph of 1-fluorodeoxyglycerol and the prospect of synthesis of the L enantiomorph as suggested above, provides an opportunity to investigate the comparative metabolism and toxicology of optically active forms of 1-fluorodeoxyglycerol.

1-Fluoro Analogs of Glycerol-3-P and Dihydroxyacetone-3-P as Potential Cancer Chemotherapeutic Agents. A key element in our rationale for the use of 1-fluorodeoxyglycerol-3-P and 1-fluorodeoxydihydroxyacetone-3-P as anti-cancer agents is the postulate that these analogs would be substrates for NAD-linked glycerol-3-P dehydrogenase. The substrate properties of the glycerol-3-P analog have been established in our previous paper using a DL mixture of 1-fluorodeoxyglycerol-3-P (Fondy *et al.*, 1970). We have recently shown that 1-fluorodeoxydihydroxyacetone-3-P is also a substrate for the enzyme (Fondy *et al.*, 1971). The metabolism of 1-fluorodeoxyglycerol-3-P or of 1-fluorodeoxydihydroxyacetone-3-P might be quantitatively and qualitatively different in cells which lack NAD-linked glycerol-3-P dehydrogenase compared to cells which have normal levels of the enzyme. Thus, it is conceivable that specific cancer cell types might be found in which the fluoro analogs are toxic at lower concentrations than in cells which possess glycerol-3-P dehydrogenase activity.

Several complicating features of the rationale deserve mention. (1) What is the likely effect of the mitochondrial flavin-linked glycerol-3-P dehydrogenase which probably does not exhibit exploitable differences in activity between normal and cancer cells? We have determined that DL-1-fluorodeoxyglycerol-3-P is a substrate for intact mitochondria, but exhibits an apparent K_m almost tenfold higher than for the NAD-linked enzyme (P. D. Holohan and T. P. Fondy, unpublished observations). Thus concentrations of the fluoro analog that might be acted upon by the NAD-linked enzyme, may be too low to be affected by the mitochondrial glycerol-3-P oxidase. (2) How might the potentially toxic fluoro analogs gain access to cancer cells and their toxic metabolites remain sufficiently localized to produce significant differential toxicity? The problem of permeability remains to be investigated after the synthesis of the analogs has been achieved. However, it is possible that precursors which could generate the fluoro analogs intracellularly and might themselves more readily gain access to the cells would be of most interest as potential chemotherapeutic agents. Likely possibilities in this regard are 3-fluorodeoxyfructose and 3-fluorodeoxypsicose which after *in vivo* phosphorylation could generate 1-fluorodeoxydihydroxyacetone-3-P if the fluorohexoses are subject to cleavage by aldolases. Similarly, phosphoryl esters of 1-fluorodeoxyglycerol-3-P and 1-fluorodeoxydihydroxyacetone-3-P might be useful precursors. Specific cancer cell types which might preferentially transport such precursors suggest themselves as likely candidates for initial investigation. Whether sufficient concentration of locally generated toxic metabolites can be maintained to achieve useful selective toxicity must be examined experimentally. (3) By what route might the fluoro analogs generate toxic metabolites? O'Brien and Peters (1958) have suggested possible routes for the metabolism of DL-1-fluorodeoxyglycerol to toxic metabolites. It is possible that 1-fluorodeoxyglycerol-3-P might itself be dephosphorylated to 1-fluorodeoxyglycerol by the action of phosphatases, and perhaps follow the pathway suggested by O'Brien and Peters. In this case, cancer cell types with elevated alkaline phosphatases might be particularly susceptible to the potential toxicity of 1-fluorodeoxyglycerol-3-P. Alternatively, 1-fluorodeoxydihydroxyacetone-3-P might itself be significantly more toxic than 1-fluorodeoxyglycerol-3-P, since the latter might be converted in part into lipid derivatives which could reduce the level of acute toxicity of the 1-fluorodeoxyglycerol-3-P. This alternative is particularly attractive because the reduction of dihydroxyacetone-3-P is strongly favored by the equilibrium catalyzed by NAD-linked glycerol-3-P dehydrogenase.

Acknowledgments

We are grateful to Mr. Thomas Meteyer and Dr. Richard Matthews for the nmr analyses, and to Dr. Charles Olive for helpful discussions on the enzymatic analyses. Dr. Jean-Pierre Merle and Dr. Anatole Sarko kindly provided us with the use of the ORD instrumentation and instruction in its operation.

References

Baer, E. (1952), *Biochem. Prepn. 2*, 31.

Baer, E., and Fischer, H. O. L. (1948), *J. Amer. Chem. Soc. 70*, 609.

Bird, P. R., and Chadha, J. S. (1966), *Tetrahedron Lett.*, 4541.

Dummel, R. J., and Kun, E. (1969), *J. Med. Chem. 12*, 347.

Fondy, T. P., Ghangas, G. S., Basloe, J. L., and Mahajan, K. P. (1971), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 30*, 1253Abs.

Fondy, T. P., Ghangas, G. S., and Reza, M. J. (1970), *Biochemistry 9*, 3272.

Freudenberg, K., and Hess, H. (1926), *Ann. Chem.* 448.

Gryszkiewicz-Trochimowski, E. (1947), *Recl. Trav. Chim. Pays-Bas 66*, 427.

Hilf, R., Goldenberg, H., Michel, I., Orlando, R. A., and Archer, F. L. (1970), *Cancer Res. 30*, 1874.

Kun, E., and Dummel, R. J. (1969), *Methods Enzymol. 13*, 624.

Lecocq, J., and Ballou, C. E. (1964), *Biochemistry 3*, 976.

Newman, M. S., and Renoll, M. (1945), *J. Amer. Chem. Soc. 67*, 1621.

O'Brien, R. D., and Peters, R. A. (1958), *Biochem. J. 70*, 188.

Pattison, F. L. M., and Norman, J. J. (1957), *J. Amer. Chem. Soc. 79*, 2311.