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Synthesis of L-1-Deoxyfluoroglycerol and Its 3-Phosphate Ester. Effects of the L and D Enantiomers in BDF_1 Mice†

Thomas P. Fondy,*‡ Richard W. Pero, Katherine L. Karker, Gurdev S. Ghangas, and Frederick H. Batzold

Department of Biology, Syracuse University, Syracuse, New York 13210. *Received December 6, 1973*

L-1-Deoxyfluoroglycerol and L-1-deoxyfluoroglycerol 3-phosphate were synthesized from D-isopropylideneglycerol through D-1-benzyl-3-tosylglycerol and tosyl displacement by KF from D-3-tosyl-1,2-isopropylideneglycerol. The L enantiomers were more toxic than the D by a factor of 2 in BDF_1 mice. At one-half their LD_{50} doses these fluoro organics produced a strong hypothermic effect amounting to 12° within a few hours. Ethanol or pyrazole inhibited the toxicity and hypothermia due to the fluorotriitols but pyrazole did not affect the toxicity or hypothermia due to fluoroacetate, indicating that alcohol dehydrogenase functions in the conversion of the fluorotriitols to fluoroacetate. Toxicities of the fluoro organics were greatly enhanced when hypothermia was prevented by maintaining the ambient temperature at 33°. In the presence of pyrazole the toxicity at 33° was reduced by a factor of 10. The application of hypothermia in the study of the *in vivo* metabolism of fluoro organics and a possible metabolic pathway for conversion of fluorotriitols to fluoroacetate is suggested. These results permit the design of chemotherapeutic protocols for the testing of the 1-deoxyfluoroglycerols and their 3-phosphates in tumor model systems employing BDF_1 mice.

The low activity or total absence of cytoplasmic NAD-linked glycerol-3-phosphate dehydrogenase in rapidly dividing cancer cells[1-4] suggests that important differences may exist between cancer cells and noncancer cells in the pathways by which they interrelate carbohydrate with lipid metabolism. The role of the acyldihydroxy acetone phosphate pathway in phospholipid synthesis in Ehrlich ascites cells[5] and the relationship between that pathway and ether lipid synthesis, in tumor cells and in dividing normal cells in culture, further underscore the potential importance of these differences.[6,7]

We have set forth one possible rationale for attempting to exploit the absence of NAD-linked glycerol-3-phosphate dehydrogenase in cancer cells in the design of chemotherapeutic agents.[1,8] This rationale suggests that 1-fluoro analogs of glycerol 3-phosphate, dihydroxyacetone 3-phosphate, or transportable precursors able to generate them intracellularly might be of particular interest. In following this rationale we have carried out the synthesis of DL-1-deoxyfluoroglycerol 3-phosphate and have established that this fluoro analog is a substrate for NAD-linked glycerol-3-phosphate dehydrogenase.[1] The stereospecific synthesis of D-1-deoxyfluoroglycerol 3-phosphate was subsequently achieved by methods designed to be able to produce, with a few additional steps, the L enantiomorph.[8] The D compound was shown to be inactive as a substrate for NAD-linked glycerol-3-phosphate dehydrogenase and to inhibit the oxidation of L-glycerol 3-phosphate.

This paper details the stereospecific synthesis of L-1-deoxyfluoroglycerol 3-phosphate and of L-1-deoxyfluoroglycerol by modification of the route we employed in the preparation of the D series. Alternative routes to the L series have also been developed by Lloyd and Harrison.[9,10] We have examined the properties *in vitro* of L-1-deoxyfluoroglycerol 3-phosphate with respect to NAD-linked glycerol-3-phosphate dehydrogenase. Since the primary object of this work is to evaluate the chemotherapeutic effect of these fluorotriitols and related fluoro organics in neoplasia, we have examined *in vivo* biochemical and pharmacological effects of the 1-deoxyfluoroglycerols and their phosphates in BDF_1 mice. This study is an essential first step in the evaluation of the effects of these and other fluoro organics on normal and neoplastic cells *in vivo* and in culture. We have chosen to evaluate these compounds in BDF_1 mice since a number of the most thoroughly characterized and well-understood tumor model systems for cancer chemotherapy are based upon the use of this hybrid in testing.

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Experimental Section

Materials. Rabbit muscle NAD-linked glycerol-3-phosphate dehydrogenase was obtained from Sigma Chemical Co. (St. Louis). Dibenzyl phosphite, *N*-chlorosuccinimide, and more common reagents were obtained from Aldrich Chemical Co. (Milwaukee). D-Mannitol was a product of Sigma Chemical Co. (St. Louis). Potassium fluoride (anhydrous) was a product of ROC-RIC Chemical Co. (Sun Valley, Calif.). DL-1-Deoxyfluoroglycerol 3-phosphate as the dicyclohexylammonium salt was prepared from epifluorohydrin and dibenzylphosphoric acid as previously described.[1,8] DL-1-Deoxyfluoroglycerol and D-1-deoxyfluoroglycerol were synthesized as detailed by Ghangas and Fondy.[8] D-1-Deoxyfluoroglycerol 3-phosphate dicyclohexylammonium salt was prepared by slight modification of our previously published procedures.[8] These modifications and the properties of the D-1-deoxyfluoroglycerol 3-phosphate employed in this work are detailed under Syntheses. Mice used were female BDF_1 hybrids (C57BL/6 female × DBA/2 male), 6–10 weeks of age, 18–22 g.

Methods. 1. **Enzyme Assays.** The commercial rabbit muscle glycerol-3-phosphate dehydrogenase obtained as a suspension in 70% ammonium sulfate was collected by centrifugation, dissolved in a buffer of 50 m*M* triethanolamine acetate pH 7.5, 1 m*M* EDTA, and 1 m*M* 2-mercaptoethanol, and dialyzed against the same buffer. Assays for oxidation of 1-fluoro analogs of glycerol 3-phosphate were performed in a 3-ml reaction system within a buffer of 10 m*M* sodium pyrophosphate pH 9.0, 1 m*M* EDTA, 1 m*M* 2-mercaptoethanol, 0.3 m*M* NAD, with 0.1 *M* hydrazine (to react with the product dihydroxyacetone phosphate analog and prevent the reaction from reaching rapid early equilibrium). The enzyme concentration in the reaction system was 10^{-6} *M*. Assay for L-glycerol 3-phosphate oxidation was performed in the identical system except that the enzyme concentration was 10^{-9} *M*.

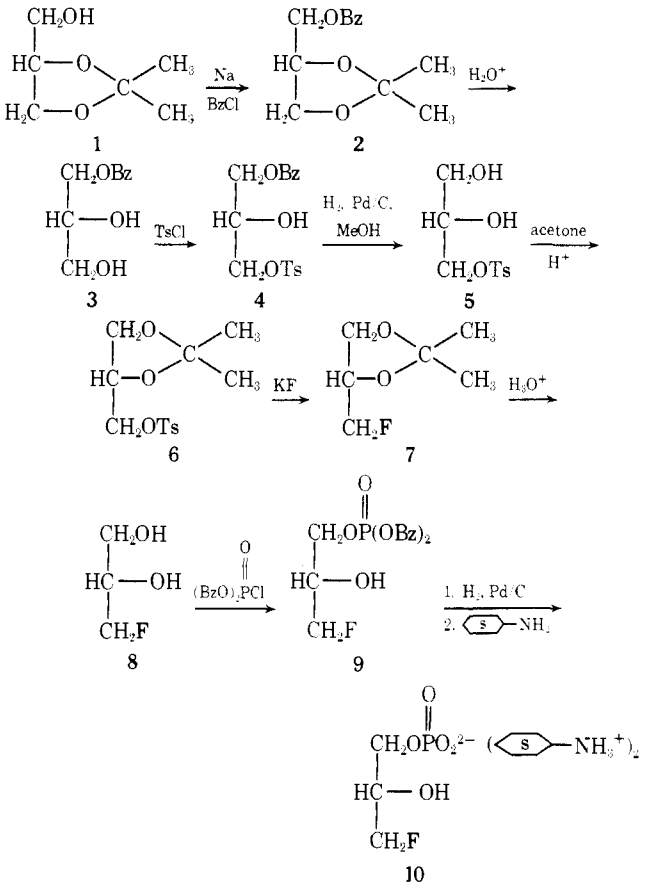
2. **Temperature Monitoring.** Animal temperatures were determined with a Yellow Springs Instrument Co. Tele-thermometer with thermocouple probe no. 423 inserted rectally to approximately 1 cm.

3. **Toxicity Determinations.** Doses were administered intraperitoneally in 0.2 ml of physiological saline and LD_{50}'s estimated by the method of Weil[11] or of Horn.[12]

Syntheses. 1. **L-1-Deoxyfluoroglycerol and L-1-Deoxyfluoroglycerol 3-Phosphate from D-2,3-Isopropylideneglycerol.** D-2,3-Isopropylideneglycerol (1) was prepared from D-mannitol by the procedures devised by Baer,[13] Bird and Chadha,[14] and Lecocq and Ballou[15] as detailed in our previous publication.[8] The synthetic route leading from D-2,3-isopropylideneglycerol to L-1-deoxyfluoroglycerol 3-phosphate *via* L-1-deoxyfluoroglycerol is shown in Scheme I. The compounds are designated as derivatives of the starting material D-isopropylideneglycerol, so that L-1-deoxyfluoroglycerol 3-phosphate (10) is shown as D-3-deoxyfluoroglycerol 1-phosphate.

D-1-Benzyl-2,3-isopropylideneglycerol (2). The title compound was prepared by the method of Howe and Malkin[16] from 38 g (0.29 mol) of compound 1 and 5.75 g (0.25 g-atom) of sodium

Scheme I



to give a clear oil (43.0 g, 0.19 mol, 77%): bp 80–83° (0.05 mm) [lit.[16] bp 93–96° (0.10 mm)]. The product had $[\alpha]^{25}D$ +18.0° (neat). Previously observed $[\alpha]^{25}D$ for the L isomer[17] was −18.3°.

D-1-Benzylglycerol (3). Compound 2 (35.3 g, 0.16 mol) treated as previously described[16] yielded the title compound as a clear oil (27.0 g, 0.15 mol, 93%): bp 130–131° (0.025 mm) [lit.[16] bp 140–145° (0.1 mm)]; $[\alpha]^{25}D$ +5.8° (neat) (lit.[18] +5.3°).

D-1-Benzyl-3-tosylglycerol (4). Compound 3 (36.4 g, 0.20 mol) dissolved in 100 ml of anhydrous pyridine at 4° and treated with 42.0 g (0.22 mol) of *p*-toluenesulfonyl chloride in 100 ml of anhydrous pyridine[19] as described by Belleau and Puranen[20] produced an oil which solidified on prolonged standing to give a white solid (62.3 g, 0.18 mol, 92%): mp 44–48° (lit.[20] mp 48°); $[\alpha]^{25}D$ (in MeOH) +4.8° [lit.[20] $[\alpha]D$ (MeOH) +4.6°].

D-3-Tosylglycerol (5). Compound 4 (22.0 g, 0.065 mol) in 200 ml of MeOH was hydrogenated at 40 psi (3 g of 10% palladium on charcoal) for 15 hr until slightly more than the theoretical hydrogen uptake to produce 15.7 g (0.064 mol, 98%) of an oil.

D-3-Tosyl-1,2-isopropylideneglycerol (6). To compound 5 (15.7 g, 0.064 mol) dissolved in 250 ml of anhydrous Me_2CO was added 3 drops of concentrated H_2SO_4. The mixture was stirred for 3 hr at ambient temperature. $KHCO_3$ (1 g) was added and vigorous stirring continued for 20 min. After filtration, the solvent was removed under reduced pressure leaving 15.2 g (0.054 mol, 84%) of a residual oil. The nmr spectrum confirmed that the product was indeed tosylisopropylideneglycerol by comparison with the spectrum previously published (Ghangas and Fondy, ref 8, Figure 3B); however, the infrared spectrum showed the presence of hydroxyl absorption at 3580 cm^{-1}. When compound 6 containing hydroxyl contaminants was used for displacement of the tosyl group with KF, the yield of fluorinated product was adversely affected. Without additional purification the crude 6 could not be crystallized in the manner detailed in earlier work[8] for the DL compound prepared by the direct tosylation of DL-isopropylideneglycerol. The impure D-3-tosyl-1,2-isopropylideneglycerol (6) (3.0 g) chromatographed on a column of 10 g of neutral alumina (Woelm) and eluted with 70 ml of anhydrous Et_2O yielded 2.3 g of viscous oil free of hydroxyl absorption in the infrared spectrum: $[\alpha]^{25}D$ +7.3° (neat). Previously observed[8] $[\alpha]^{25}D$ for the D-1-tosyl-2,3-isopropylideneglycerol was −7.1°.

D-3-Deoxyfluoro-1,2-isopropylideneglycerol (7). 6 (12.5 g, 0.043 mol) was allowed to react with 17.4 g (0.30 mol) of anhydrous KF in 60 ml of anhydrous ethylene glycol by stirring in an oil bath at 120°. The temperature was raised to 180° and the product removed by distillation to give 2.5 g of a clear liquid (0.019 mol, 45%): bp 124° (760 mm) (lit.[8] for D-1-fluorodeoxy-2,3-isopropylideneglycerol, *i.e.*, L-3-fluorodeoxy-1,2-isopropylideneglycerol, bp 120–123°); $[\alpha]^{25}D$ −10.6° (neat). Previously observed[8] $[\alpha]^{25}D$ for the D-1 isomer was +11.1°. The nmr spectrum of D-3-fluorodeoxy-1,2-isopropylideneglycerol corresponds exactly to that published by us[8] for the racemic mixture.

D-3-Deoxyfluoroglycerol (8). Compound 7 (2.5 g, 0.019 mol) treated as described[8] gave a clear oil weighing 1.2 g. Distillation of the crude product, 56° (0.25 mm) [lit.[9] 53° (0.2 mm)], gave 825 mg (9 mmol, 47%). An ORD spectrum at ambient temperature on a 2.5% solution (v:v) in 95% EtOH showed $[\alpha]_{400}$ +44°, $[\alpha]_{350}$ +76°, $[\alpha]_{300}$ +120°, $[\alpha]_{250}$ +236°. Corresponding values for the D-1-deoxyfluoroglycerol (L-3-deoxyfluoroglycerol)[8] were $[\alpha]_{400}$ −52°, $[\alpha]_{350}$ −81°, $[\alpha]_{300}$ −138°, $[\alpha]_{250}$ −276°. The nmr spectrum is identical with that previously shown for DL-1-deoxyfluoroglycerol.[8]

D-3-Deoxyfluoroglycerol 1-Phosphate Dibenzyl Ester (9) and Dicyclohexylammonium Salt (10). The dibenzyl phosphate ester of D-3-deoxyfluoroglycerol was prepared by reaction of 1.88 g (0.02 mol) of 8 with dibenzyl phosphorochloridate in 100 ml of dry THF and 5 ml of pyridine (distilled from BaO and stored over molecular sieves) and the product isolated as previously described.[1] The dibenzyl phosphorochloridate was prepared from 6.7 g (0.025 mol) of dibenzyl phosphite by the method of Smith[21] and the identity and purity were confirmed by nmr spectrum. The purity of the dibenzyl phosphite is crucial to the success of this reaction. The dibenzyl phosphite should be a freshly prepared crystalline material, with nmr spectrum indicating the absence of benzyl alcohol or other breakdown products.

Hydrogenation, formation of the dicyclohexylammonium salt, and recrystallization from dioxane[1] gave 1.4 g melting at 170–173°. In order to obtain an ultrapure product for pharmacological studies an additional recrystallization was carried out by suspending the product in 20 ml of boiling $CHCl_3$ and adding 95% EtOH dropwise until solution was effected (absolute EtOH will not dissolve the salt). After filtration through a heated sintered glass funnel, the solution was cooled to −15° for at least 24 hr, yielding a pure white crystalline material [450 mg, mp 173–175° (lit.[10] 171–173°)]: ORD (0.5 H_2O) $[\alpha]^{25}$ values $[\alpha]_{400}$ +10°, $[\alpha]_{350}$ +15°, $[\alpha]_{300}$ +20°, $[\alpha]_{250}$ +32°, $[\alpha]_{200}$ +130° [lit.[10] $[\alpha]^{25}$ values $[\alpha]_{400}$ +13.2°, $[\alpha]_{350}$ +16.5°, $[\alpha]_{300}$ +26°, $[\alpha]_{250}$ +50°, $[\alpha]_{200}$ +170°]. Values observed for D-1-deoxyfluoroglycerol 3-phosphate similarly prepared appear below. *Anal.* ($C_{15}H_{34}FN_2O_5P$) H, F, N, P; C: calcd, 48.39; found, 46.73.

2. D-1-Deoxyfluoroglycerol and D-1-Deoxyfluoroglycerol 3-Phosphate from D-2,3-Isopropylideneglycerol. The D enantiomorphs were prepared as previously described.[8] After crystallization from dioxane the D-1-deoxyfluoroglycerol 3-phosphate dicyclohexylammonium salt was recrystallized from $CHCl_3$–95% EtOH as described above for the L enantiomorph (mp 173–175°): ORD (0.05 H_2O) $[\alpha]^{25}$ values $[\alpha]_{400}$ −8.0°, $[\alpha]_{350}$ −12°, $[\alpha]_{300}$ −19°, $[\alpha]_{250}$ −32°. *Anal.* ($C_{15}H_{34}FN_2O_5$) H, F, N, P; C: calcd, 48.39; found, 46.73.

Results

Toxicities in Mice. Toxicities expressed as LD_{50}'s in BDF_1 mice for racemic and optically active deoxyfluoroglycerol and 1-deoxyfluoroglycerol 3-phosphate are shown in Table I. The L enantiomers were significantly more toxic than the D and in the case of L-1-deoxyfluoroglycerol exhibited almost the same toxicity on a mole basis as fluoroacetic acid. On a per mole basis the phosphate esters are slightly less toxic than the corresponding 1-deoxyfluoroglycerols. The dicyclohexylammonium ion had no significant effect on these values since the disodium salt of DL-1-deoxyfluoroglycerol 3-phosphate exhibited on a per mole basis the same LD_{50} as the dicyclohexylammonium salt. Moreover, DL-1-chlorodeoxyglycerol 3-phosphate dicyclohexylammonium salt had an LD_{50} of almost 800 mg/kg (2.1 mmol/kg).

There was no important difference in toxicity by subcutaneous administration of these fluoro organics, although the subcutaneous route did appear to be slightly less toxic.

Table I. Toxicity in Female BDF_1 Mice of 1-Deoxyfluoroglycerols and of Their 3-Phosphate Esters[a]

Deoxyfluoroglycerol	LD_{50} mg/kg	LD_{50} mmol/kg
L-1-	14 (10–19)	0.15
DL-1-	20 (14–24)	0.21
D-1-	26 (20–36)	0.28
Deoxyfluoroglycerol 3-phosphate dicyclohexylammonium		
L-1-	162 (127–205)	0.44
DL-1-[b]	110 (69–177)	0.30
DL-1-[c]	126 (93–171)	0.34
D-1- + L-1-[d]	110 (60–202)	0.30
D-1-	270 (200–369)	0.73
Fluoroacetic acid[e]	8	0.1
DL-1-Chlorodeoxyglycerol 3-phosphate dicyclohexylammonium	794 (584–1080)	2.1

[a]Doses were intraperitoneal in 0.2 ml of saline using 20-g mice at 6–10 weeks of age, in groups of five or six per dose level. LD_{50} and 95% confidence range (in parentheses) calculated according to the method of Weil[11] or of Horn.[12] [b]Prepared from epifluorohydrin and dibenzylphosphoric acid.[1] [c]Prepared from DL-1-deoxyfluoroglycerol and dibenzyl phosphorochloridate.[1,8] [d]0.1-ml injections of identical concentrations of each of the pure enantiomers. LD_{50} dose represents sum of both enantiomers. [e]In sterile water.

Noninbred mice of the HAICR and Carworth populations were more resistant to the effects of these fluoro compounds and showed LD_{50}'s for DL- and L-1-deoxyfluoroglycerols and their phosphates approximately twofold higher than that obtained in BDF_1 mice.

Inhibition of Metabolism of 1-Deoxyfluoroglycerols and the 3-Phosphate Esters. It seemed likely that the 1-deoxyfluoroglycerols and their 3-phosphate esters are rendered toxic by conversion to fluoroacetate in a metabolic pathway involving alcohol dehydrogenase (see Discussion). Therefore, we employed ethanol to inhibit metabolism of the fluorotriitols. Using 3 g/kg of ethanol administered intraperitoneally as a 25% solution in saline simultaneously with the fluoro organics, we observed that toxic reactions were delayed by about 8 hr and that the toxicities of the fluorotriitols (expressed as increases in LD_{50}'s) were reduced by twofold. Ethanol was also effective as a rescue agent when administered up to 10–12 hr after injection of the LD_{50} dose of the fluorotriitols. Ethanol itself at a dose of 3 g/kg could be administered daily or twice daily for up to 5 days apparently without producing any irreversible toxic effects. With simultaneous administration of ethanol, an LD_{50} dose of any of the fluorotriitols could be administered daily for 5 days without incurring any deaths in the test groups.

Ethanol is known to protect directly against fluoroacetate poisoning.[22] In order to distinguish between such direct protective effects exerted by ethanol, and effects of ethanol due to inhibition of the alcohol dehydrogenase catalyzed metabolism of the fluorotriitols, we employed pyrazole, a specific inhibitor of alcohol dehydrogenase.[23] At a dose level of 90 mg/kg, administered intraperitoneally simultaneously with twice the LD_{50} dose of DL-1-deoxyfluoroglycerol 3-phosphate dicyclohexylammonium salt, pyrazole prevented any drug deaths. In addition, it completely eliminated the onset of extreme lethargy characteristic of toxicity induced by the fluorotriitols. Pyrazole itself was nontoxic upon repeated daily doses at 90 mg/kg.

Figure 1 shows the protective effects of either ethanol or pyrazole upon administration of L-1-deoxyfluoroglycerol. It is apparent that repeated daily doses, each approximately

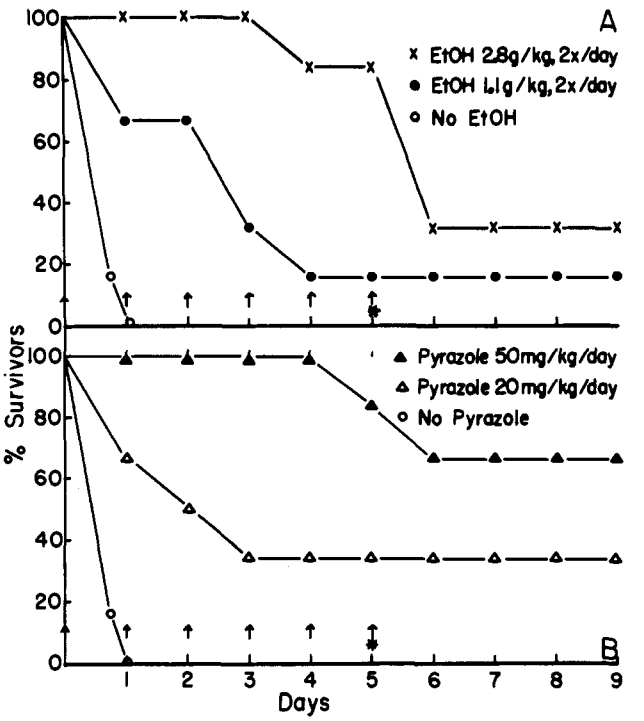


Figure 1. Protection against toxicity in BDF_1 mice of L-1-deoxyfluoroglycerol by ethanol or pyrazole. L-1-Deoxyfluoroglycerol was given ip at 50 mg/kg once daily, day 0–4, to groups of six mice. Arrow denotes injection of 1-deoxyfluoroglycerol and protecting agent. Group receiving ethanol injections twice daily received them at 12-hr intervals. Asterisk denotes injection by protecting agent only.

four times the LD_{50}, could be administered when ethanol or pyrazole was given simultaneously. Pyrazole at the minimum effective protective dose of 50 mg/kg appeared to exert a more complete protective effect than ethanol at 3 g/kg. Similar effects of pyrazole and ethanol have been obtained with racemic and D enantiomers of 1-deoxyfluoroglycerol and with the 3-phosphate esters. Pyrazole did not protect against toxicity by fluoroacetic acid itself.

DL-Glycerol 3-phosphate as the disodium salt (hexahydrate) administered intraperitoneally at 1 g/kg also protected against toxicity by DL-1-deoxyfluoroglycerol 3-phosphate but less well than either ethanol or pyrazole. Glycerol had no protective effect against toxicity by the fluorotriitols. The combination of sodium acetate and ethanol each at 2.5 g/kg employed by Tourtellotte and Coon[24] as an antagonist against fluoroacetate poisoning also exerted a strongly protective effect against toxicity by the deoxyfluorotriitols (see next section).

Induction of Hypothermia by Fluorotriitols and Fluoroacetate. The fact that fluoroacetate causes temperature drop in animals has been observed by others[25] but has not been studied in detail. Stoner[26] employed fluoroacetate in the study of hypothermic effects in rat liver and observed a drop of about 5° within 2 hr after administration of a dose of 5 mg/kg which is in excess of the LD_{50} in rats. During the course of our study of the deoxyfluorotriitols, we noted an enormous temperature drop in BDF_1 mice amounting to almost 12° within 10 hr when a dose of DL-1-deoxyfluoroglycerol 3-phosphate was administered at slightly below the LD_{50} (Figure 2, line 1). Subnormal temperature persisted for more than 2 days. In keeping with its ability drastically to reduce the toxicity of 1-deoxyfluoroglycerols and their phosphates, pyrazole administered simultaneously with a threefold higher dose of DL-1-deoxyfluoroglycerol 3-phosphate substantially altered the temperature effects (Figure 2, line 2). The maximum

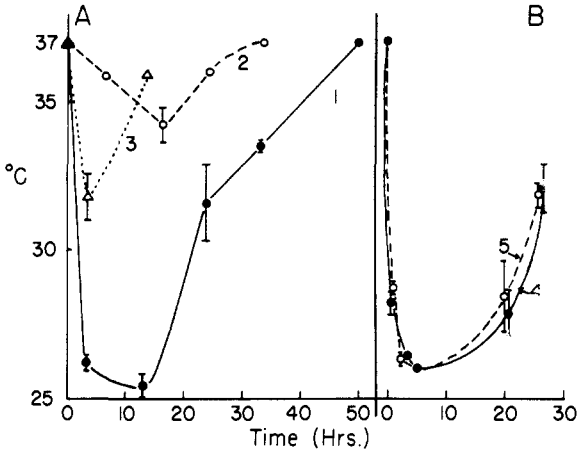


Figure 2. Effect of DL-1-deoxyfluoroglycerol 3-phosphate and of fluoroacetic acid on body temperature in mice. (A) DL-1-Deoxyfluoroglycerol 3-phosphate dicyclohexylammonium salt: line 1, 100 mg/kg into BDF_1 mice; line 2, 300 mg/kg + 75 mg/kg of pyrazole simultaneously into BDF_1 mice; line 3, 100 mg/kg into HAICR mice. (B) Fluoroacetic acid in BDF_1 mice: line 4, 10 mg/kg; line 5, 10 mg/kg + 75 mg/kg of pyrazole simultaneously. S.E.M. indicated.

temperature drop was reduced to about 3°, the minimum point was delayed to about 15 hr, and subnormal temperature ended within 30 hr. HAICR mice treated with DL-1-deoxyfluoroglycerol 3-phosphate in the absence of pyrazole were much less profoundly affected than BDF_1 mice (Figure 2, line 3). Fluoroacetic acid administered at approximately the LD_{50} produced the very rapid and drastic temperature drop shown in Figure 2, line 4, and pyrazole had no effect on the toxicity or on the pattern of induced hypothermia (Figure 2, line 5).

Figure 3A shows the temperature effects upon repeated administration of L-1-deoxyfluoroglycerol 3-phosphate in the presence and absence of pyrazole. Effects with L-1-deoxyfluoroglycerol are shown in Figure 3B. Corresponding results with D-1-deoxyfluoroglycerol 3-phosphate and D-1-deoxyfluoroglycerol are presented in Figure 4A and 4B, respectively. When any of the compounds was administered in a multiple-dose schedule in the absence of pyrazole, the hypothermic effects were greatest with the first dose and diminished with each subsequent dose. This damping effect was fully reproducible and may be related to the observation that survival from an initial dose of fluoroacetic acid can promote tolerance to subsequent doses depending on scheduling.[27] Pyrazole was able to moderate the temperature-drop effects with any of the optically active deoxyfluorotriitols, especially in the first few doses. The D enantiomers in the absence of pyrazole could be administered at approximately twice the dose level tolerated with the L enantiomers as previously noted in the toxicity studies. Roughly comparable temperature effects were obtained for the D and L compounds when the D enantiomers are administered at twice the dose employed for the L series.

Racemic and optically active 1-deoxyfluoroglycerols and their 3-phosphates could be administered in repeated doses several fold above their respective LD_{50}'s when ethanol at 3 g/kg was administered either simultaneously or within 6–10 hr after administration of the fluoro compound. When administered simultaneously, ethanol prevented the initiation of temperature drop for about 4–6 hr, presumably the time required to metabolize the high dose of ethanol. Thereupon, a temperature-drop pattern slightly less drastic than that observed in the total absence of ethanol was observed. When ethanol was administered 6–10 hr after administration of 1-deoxyfluoroglycerol 3-phosphate, rapid recovery from the depths of hypothermia was observed.

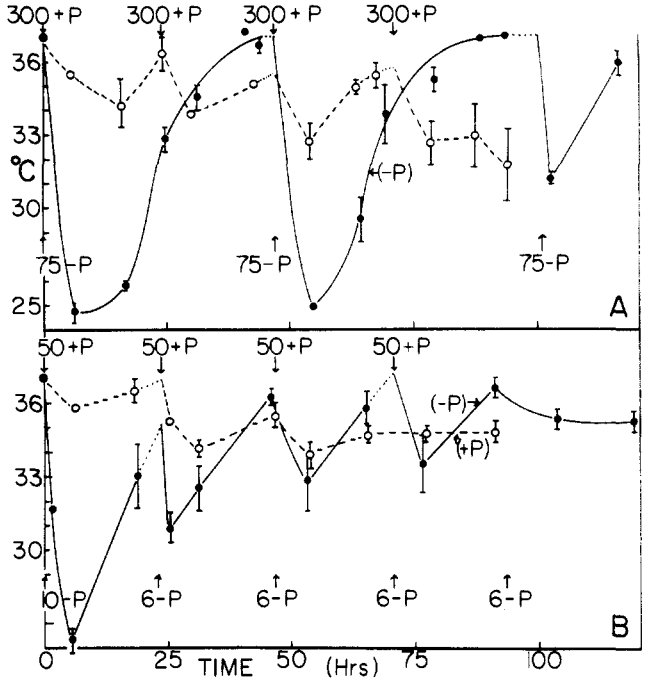


Figure 3. Effect of (A) L-1-deoxyfluoroglycerol 3-phosphate dicyclohexylammonium salt and of (B) L-1-deoxyfluoroglycerol on body temperature in BDF_1 mice in the presence (+P) and absence (−P) of pyrazole: (------) with pyrazole at 75 mg/kg simultaneously; (———) without pyrazole. Arrows indicate times of dosage administration, with dose given in milligrams per kilogram.

The combination of sodium acetate and ethanol each at 2.5 g/kg has been found to be one of the most effective means of preventing fluoroacetate toxicity.[24] Using this combination we were able to administer three doses of DL-1-deoxyfluoroglycerol 3-phosphate at 400 mg/kg spaced at 48-hr intervals, with only 10% deaths, and a hypothermic effect that did not exceed 3° at its greatest depth.

It has been observed that elevated ambient temperature enhances the toxicity of fluoroacetate.[28] We also found that at an ambient temperature of 32° which prevented the drastic temperature-drop observed at 24°, doses of the fluorotriitols that were normally sublethal at 24° ambient were rapidly fatal. The enhanced toxicity at elevated ambient temperature could be curtailed by administering the fluorotriitols in small doses spaced at 6-hr intervals. Alternatively, at elevated ambient temperature, the simultaneous administration of pyrazole permitted use of doses of fluorotriitols that would otherwise have been rapidly fatal. For example, DL-1-deoxyfluoroglycerol 3-phosphate had an LD_{50} of 75 mg/kg (confidence limit, 59–95) at 33° ambient temperature. Simultaneous administration of 75 mg/ml of pyrazole raised the LD_{50} dose to 681 mg/kg. A dose of 300 mg/kg of DL-1-deoxyfluoroglycerol 3-phosphate administered in conjunction with 75 mg/kg of pyrazole to mice kept at an ambient temperature of 33° produced only a 2° depression in temperature at its maximum which occurred at 6 hr after injection. Normal temperature was restored within 18 hr.

L-1-Deoxyfluoroglycerol 3-Phosphate as Substrate *in Vitro* for NAD-Linked Glycerol-3-phosphate Dehydrogenase. The L enantiomorph as the dicyclohexylammonium salt was a substrate for rabbit muscle cytoplasmic glycerol-3-phosphate dehydrogenase with an apparent K_m of 3.8 m*M* at pH 7.5 under the assay conditions detailed under Methods. Under identical conditions the apparent K_m for the racemic mixture was found to be 8 m*M*.[1]

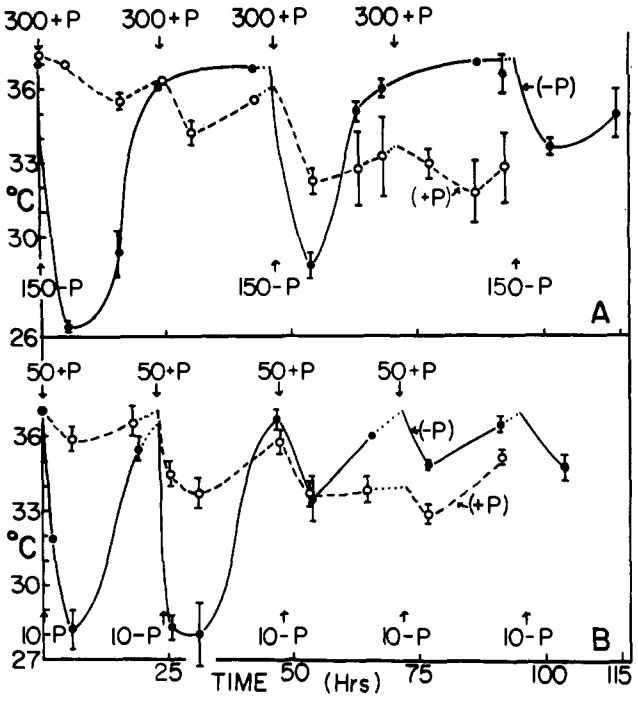


Figure 4. Effect of (A) D-1-deoxyfluoroglycerol 3-phosphate dicyclohexylammonium salt and of (B) D-1-deoxyfluoroglycerol on body temperature in BDF_1 mice in the presence and absence of pyrazole. Symbols as designated in Figure 3.

Discussion

Testing of Deoxyfluorotriitols in Tumor Model Systems. Dose schedules for the use of 1-deoxyfluoroglycerols and their phosphates in tumor model systems employing BDF_1 mice can be designed directly from the studies set forth here. It is apparent that ethanol is effective, and pyrazole is even more effective in moderating the toxicities of the deoxyfluorotriitols. The lower toxicity of D-1-deoxyfluoroglycerol and its 3-phosphate in comparison with the L series is also of importance in planning chemotherapeutic testing of these compounds. The very substantial hypothermic effects observed with the fluoro compounds in BDF_1 mice must be considered in the evaluation of chemotherapeutic results obtained by *in vivo* tests. Fluoroacetate itself has been observed to be carcinostatic against mammary adenocarcinoma 755 grown in C57BL mice;[29] however, the effect of hypothermia on growth rate of the tumor would have to be established in order to obtain an unequivocal evaluation of the observed anticancer effect. Fluoroorganics capable of conversion to fluoroacetate should be evaluated in *in vivo* chemotherapeutic model systems in which the effect of hypothermia on growth rate of cancer cells has been put on a firm quantitative basis. This is particularly true of rapidly growing cancers in which drug-induced hypothermia may persist for a significant proportion of the postinjection survival time. Alternatively, such compounds could be tested by drug exposure of the tumor cells *in vitro* where the temperature can be controlled, followed by subsequent introduction of the treated cells into the host. Ideally both the hypothermia corrected *in vivo* and the combined *in vitro–in vivo* systems should be used.

The effects of hypothermia induced *in vivo* by administration of the deoxyfluorotriitols can be minimized by use of tumor model systems that are tested in random-bred populations such as the HAICR mice in which the temperature-drop effects are much less drastic than in BDF_1 mice.

We have carried out a detailed evaluation of the effect of hypothermia on survival parameters in ascites mouse leukemia L1210.[30]

Proposed Pathway for Metabolism of 1-Deoxyfluorotriitols. DL-1-Deoxyfluoroglycerol has been shown to be converted to fluoroacetate in mice.[31] It is apparent from our work that both optical isomers are so converted, although the D enantiomer may be converted more slowly than the L, or may be metabolized in part by an alternative route, since the D enantiomer is only half as toxic as the L. The 3-phosphate esters behave similarly to the fluorodeoxyglycerols except that the racemic mixture appears to be slightly more toxic than either of the optically pure enantiomorphs. Whether the phosphorylated deoxyfluorotriitols are transported as such or are subject to prior extracellular dephosphorylation is not at present known. The deoxyfluoroglycerols and their 3-phosphates are converted to fluoroacetate by an intermediate reaction that is inhibited by pyrazole. We have observed that pyrazole does not inhibit NAD-linked glycerol-3-phosphate dehydrogenase from rabbit muscle nor does it interfere with the apparent conversion of 1-fluoro-3-hydroxyacetone phosphate to fluoroacetate as evidenced by unimpaired temperature drop and by toxicity.[32] Thus, even though we have shown that L-1-deoxyfluoroglycerol 3-phosphate is a substrate for NAD-linked glycerol-3-phosphate dehydrogenase with an apparent K_m of 3.8 m*M*, oxidation of L-1-deoxyfluoroglycerol 3-phosphate to 1-fluoro-3-hydroxyacetone phosphate catalyzed by cytoplasmic glycerol-3-phosphate dehydrogenase is not likely to be a significant metabolic process *in vivo* in BDF_1 mice. DL-1-Deoxyfluoroglycerol 3-phosphate is a substrate for isolated intact mitochondria, presumably by way of a flavin-linked glycerol-3-phosphate dehydrogenase, but the apparent K_m of 80 m*M* (Holohan and Fondy, unpublished results) suggests that this conversion may be of minor physiological importance.

Metabolism of L- and D-1-deoxyfluoroglycerol 3-phosphate is likely to proceed by phosphatase-catalyzed conversion to the corresponding 1-deoxyfluoroglycerols, followed by oxidation to fluoroacetate perhaps by the route suggested by O'Brien and Peters.[31] This route involves at least one oxidation step presumably catalyzed by alcohol dehydrogenase. Pyrazole protection against toxicity and hypothermia induced by deoxyfluorotriitols is most likely due to inhibition of the proposed oxidation by alcohol dehydrogenase.

Application of Optically Active 1-Deoxyhaloglycerol 3-Phosphates in the Study of Lipid Metabolism. The 1-fluoro analogs of D- and L-glycerol 3-phosphates and the corresponding chloro and bromo compounds may prove to be useful tools in the study of phosphatidic acid biosynthesis. It is possible that these compounds, which do not appear to be very potent inhibitors of NAD-linked glycerol-3-phosphate dehydrogenase[1] might be able to exert selective inhibitory effects at other points in phospholipid metabolism. The optically active 1-halodeoxy analogs might also be of use in the study of the specificity of glycerolphosphate acyl-CoA-transferases in mitochondria and microsomes, because the problem of acyl migration in 2-acylglycerol phosphate[33] would not be present in 2-acyl-1-deoxyfluoroglycerol phosphate. Thus, if 2-acylglycerol phosphates are significant intermediates in the biosynthesis of mitochondrial or microsomal phospholipids, they might accumulate as the 1-deoxyfluoro analog if L-1-deoxyfluoroglycerol 3-phosphate acts as an acyl CoA acceptor.

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Amine Functions of Reduced Basicity. Hypoglycemic and Natriuretic α-Alkoxybenzylamidoximes, Amidines, and Cycloamidines

Denis M. Bailey,* C. George DeGrazia, David Wood, James Siggins,

Chemistry Division

Homer R. Harding, Gordon O. Potts,

Endocrinology Division

and Thomas W. Skulan

Pharmacology Division, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.

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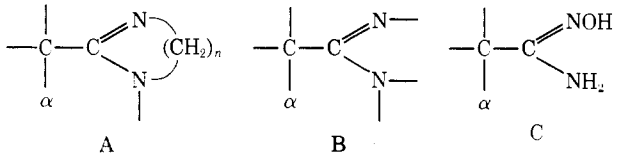
The introduction of α-alkoxybenzyl groups into carboxamidoximes, carboxamidines, 2-imidazolines, 1,4,5,6-tetrahydropyrimidines, and 4,5,6,7-tetrahydro-1*H*-1,3-diazepines predictively lowered the basicity of these nitrogen functions relative to the benzyl-substituted analogs. A general synthesis gave 2-alkoxy-2-arylacetonitriles which served as versatile intermediates for each of the series. Several of the compounds displayed potent natriuretic and/or hypoglycemic activity. One of these, 2-(α-ethoxybenzyl)-1,4,5,6-tetrahydropyrimidine (**32**), proved to be an inhibitor of hepatic drug metabolizing enzymes with a potency equal to or greater than SKF 525-A.

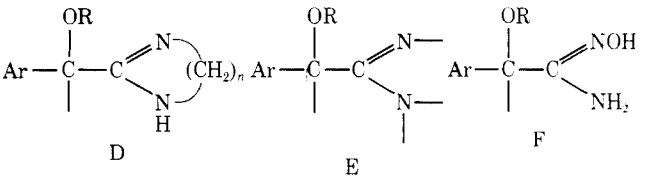
As part of a broad program, we were interested in devising means by which the tissue distribution of certain compounds bearing basic functional groups could be altered by reducing their ionization in solution, *i.e.*, by making them less basic.

In order to preserve the integrity of the functional group being examined and to permit the flexibility for SAR studies, we considered juxtaposing a net "acidifying" function. The basic groups selected for examination were 2-imidazoline and ring homologs, *e.g.*, A (n = 2, 3, 4), amidines B, and amidoximes C. The "acidifying" function selected for this study was the α-alkoxy group.†



† The effect of the alkoxy moiety on amine basicity can be illustrated by a comparison of the pK_a values of ethylamine (10.81) and 2-methoxyethylamine (9.45).[1] For a discussion of the inductive and field effects of the alkoxy group and other functions see ref 2.

Finally, since such diverse biological activity has been attributed to 2-benzyl-2-imidazolines and 2-benzyl-1,4,5,6-tetrahydropyrimidines, an aryl group was added to provide the fundamental structures (D-F) in each series.



Chemistry. Attractive intermediates, useful for all three series, were the 2-alkoxy-2-arylacetonitriles K. Synthesis of the ethyl and methyl ethers of mandelonitrile was first accomplished by Hess and Dorner[3] who dehydrated the corresponding ethers of mandelamide with $SOCl_2$. This procedure was later improved by the use of P_2O_5 as a dehydrating agent.[4,5] Among the procedures considered by the earlier workers was the conversion of benzaldehyde to α-alkoxybenzyl chlorides with HCl and ROH,[3] followed by treatment of the chloro ether with KCN. Although this procedure in their hands was appar-