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THE METABOLISM OF 2-DEOXY-2-FLUOROGLYCERALDEHYDE

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Abstract—2-Deoxy-2-fluoroglyceraldehyde is known to be lethal to rodents probably by being metabolized to fluorocitrate a potent inhibitor of aconitate hydratase. Liver aldehyde dehydrogenase and to a lesser extent xanthine oxidase oxidize 2-deoxy-2-fluoroglyceraldehyde to the corresponding acid. (−)2-Deoxy-2-fluoroglycerate (probably L) causes more citrate to accumulate in guinea-pig kidney particles than does its stereoisomer or 2-deoxy-2-fluoroglyceraldehyde. This is consistent with it being intermediate between 2-deoxy-2-fluoroglyceraldehyde and fluorocitrate and with the hypothesis that the conversion to the intermediate fluoroacetate may be catalysed by L-serine hydroxymethyltransferase. 2-Deoxy-2-fluoroglyceraldehyde unlike other α-ketofluorine compounds does not react with thiol groups at pH 8 and contrary to an earlier report is not phosphorylated by glycerol kinase.

IN EARLIER work on the biochemistry of 2-deoxy-2-fluoroglyceraldehyde O'Brien and Peters[1] showed that 2-deoxy-2-fluoroglyceraldehyde was toxic to the mouse and rat producing convulsions and accumulation of citrate, particularly in the heart and kidney. In view of the similarity between these observations and the *in vivo* effects of fluoroacetate they concluded that 2-deoxy-2-fluoroglyceraldehyde is toxic by being metabolized to fluorocitrate, a powerful inhibitor of the enzyme aconitate hydratase.[2]

In vitro work with guinea-pig kidney particle preparations respiring fumarate indicated that 2-deoxy-2-fluoroglyceraldehyde had a further inhibitory action. 2-Deoxy-2-fluoroglyceraldehyde was observed to inhibit the oxygen uptake of such preparations, the effect increasing with concentration. However, citrate accumulation was substantial only at 330 μM and was less at higher or lower concentrations. The inhibition of respiration increased linearly with time being negligible at zero time. High concentrations of 2-deoxy-2-fluoroglyceraldehyde greatly diminished the amount of citrate caused to accumulate by fluorocitrate. They concluded from this that 2-deoxy-2-fluoroglyceraldehyde also inhibited somewhere between fumarate and citrate. The present work is an extension of the work of O'Brien and Peters[1] and a metabolic route is proposed whereby 2-deoxy-2-fluoroglyceraldehyde is converted to fluoroacetate. A preliminary communication of some of the findings described in this paper has been made.[3]

EXPERIMENTAL

Chemicals

Chemicals were of analytical quality wherever possible. Glass distilled water was used throughout. 2-Deoxy-2-fluoroglyceraldehyde, and quinine 2-deoxy-2-fluoroglycerates

were the gift of Dr. P. W. Kent. The 2-deoxy-2-fluoroglycerates were converted to the sodium salts by adding a 10% excess of sodium hydroxide, extracting the quinine with several washes of ether and neutralizing the solutions with HCl. Diethyl monofluoromalonate was the gift of Dr. A. Chari-Bitron. The free acids from both preparations were not separable on mixed chromatography and each gave a single peak when chromatographed on silicic acid by the method of Bulen *et al.*[4] (kindly carried out by Dr. P. F. V. Ward). NAD was purchased from Boehringer and Soehne GmbH. ATP was purchased from Pabst Laboratories.

Enzyme preparations

Crystalline horse liver alcohol dehydrogenase, crystalline rabbit muscle triose phosphate dehydrogenase, and crystalline horse liver catalase were purchased from Boehringer and Soehne.

Crystals of triose phosphate dehydrogenase were centrifuged and dissolved in tris-hydroxymethylaminomethane-HCl buffer (0·3 M pH 8·3) containing disodium ethylenediaminetetra-acetate (5 mM) and L-cysteine-HCl (4 mM) and stood at 0 °C for 30 min. Aliquots (1 ml E_{280} 1·67) were diluted with the same buffer to 2·5 ml. Sodium arsenate (0·17, M 0·1 ml) and NAD (2·5 mg in 0·25 ml neutralized) were added and the reaction started by addition of substrate (0·1 ml, 0·1 M). Change in absorbancy at 340 mμ was followed using a Beckmann DK2 recording spectrophotometer. Rates of reduction of NAD observed: DL-glyceraldehyde 29·2 mμmoles/min, acetaldehyde and (±) 2-deoxy-2-fluoroglyceraldehyde 0·51 mμmoles min.

Alcohol dehydrogenase was freed from alcohol by centrifuging the suspension, dissolving the crystals in 0·1 M phosphate buffer (KH_2PO_4 adjusted to pH 7·4 with KOH) and passing the solution (10 mg in 2 ml) down a column (1 × 8 cm) of Sephadex G-50, in the same buffer, used according to the maker's instructions (Pharmacia, Uppsala, Sweden). The protein content of the final solution was determined by measuring the extinction at 280 mμ and calculated using the $E^{1\%}_{1\,cm}$ determined by Dalziel.[5]

Pure milk xanthine oxidase was the generous gift of Professor F. Bergel and Dr. R. C. Bray and Dr. D. A. Gilbert. Aldehyde oxidase was prepared from fresh pig liver by the method of Mahler *et al.*[9] as far as the alkaline ammonium sulphate fractionation. The activity of xanthine and aldehyde oxidases were measured manometrically in air at 25 °C. For the xanthine oxidase experiment, Warburg flasks contained sodium pyrophosphate buffer 0·04 M pH 8·2, substrate 10 mM, xanthine oxidase (E_{450} 0·2), catalase 200 μg. Total volume 1 ml. Rate of oxidation of acetaldehyde 2·7 μmoles/hr, of 2-deoxy-2-fluoroglyceraldehyde 0·54 μmoles/hr. The reaction mixture for the aldehyde oxidase experiment contained aldehyde oxidase (E_{450} 0·3), substrate 100 mM, potassium phosphate buffer 0·04 M pH 7·2, final volume 1 ml. Rate of oxidation of acetaldehyde 6·4 μmoles/hr, of DL-glyceraldehyde 0·18 μmoles/hr.

Aldehyde dehydrogenase was prepared from beef liver acetone-dried powder by the method of Racker[6] as modified by Stoppani and Milstein.[7] Serine hydroxymethyltransferase was prepared from rabbit liver acetone-dried powder by the method of Blakley[8] as far as the ammonium sulphate fractionation step. Aldehyde dehydrogenase reaction mixture contained enzyme (0·1 ml of a solution E_{280} 1·35) substrate 10 μmoles in 0·1 ml NAD 1 mg in 0·1 ml (neutralized with KOH), glycine/NaOH buffer pH 9·3 0·2 M. Reaction was started by addition of substrate and followed by

measuring change in extinction at 340 mμ on a Beckmann DK2 recording spectrophotometer. Rates of reduction of NAD. No substrate 4 mμmoles/min, DL-glyceraldehyde 63 mμmoles/min, (±)-2-deoxy-2-fluoroglyceraldehyde 29·6 mμmoles/min and acetaldehyde 16 mμmoles/min. Glycerolkinase was prepared from fresh pig liver by the method of Bublitz and Kennedy.[10]

Citrate estimation

This was carried out by the method of McArdle[11] with the exception that the tubes were cooled before the addition of the bromate–bromide–vanadate mixture, and that the steps involving the removal of the acid layer, and washing and drying the petroleum ether layer were omitted.

Guinea-pig kidney particle preparations. These were prepared as described by Peters and Wakelin[12] with the exception that a Potter–Elvehjem homogenizer was used instead of a mortar and pestle. The particles prepared from two kidneys were suspended in ice-cold KCl (1%) to about 15 ml. Potassium phosphate buffer (pH 7·2 0·5 M 5 ml) was added to the suspension together with $MgCl_2$ (59 μmoles) ATP (12 μmoles neutralized with KOH) and sodium fumarate (328 μmoles). The volume was made up to 26 ml with KCl (1%) and 2 ml of the iced mixture pipetted into 50 ml. Erlenmeyer flasks containing appropriate amounts of inhibitors dissolved in 1 ml KCl (1%). The flasks were incubated with shaking at 38 °C for 60 min and then 1 ml trichloroacetic acid (25%) was added. After standing for about 15 min the flask contents were filtered and washed with trichloroacetic acid (8%). The filtrate was made up to 25 ml with trichloroacetic acid (8%) and citrate was determined upon duplicate 5-ml samples.

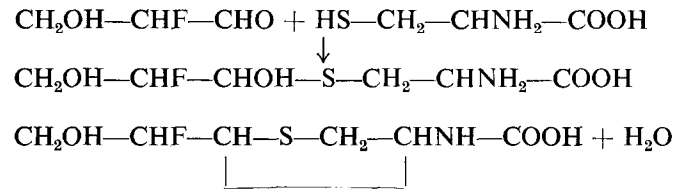
RESULTS AND DISCUSSION

Reaction with cysteine

3-Fluoropyruvate reacts with the thiol group of cysteine[13] with the liberation of inorganic fluoride.[14] The similarity in the structure of 3-fluoropyruvate and 2-deoxy-2-fluoroglyceraldehyde, both having a carbonyl group adjacent to the carbon–fluorine group, suggested that the carbon–fluorine bond in 2-deoxy-2-fluoroglyceraldehyde might be reactive enough to undergo the same sort of reaction.

Sodium 3-fluoropyruvate or 2-deoxy-2-fluoroglyceraldehyde (100 μmoles in 0·1 ml) were added to a solution (4·9 ml) containing L-cysteine HCl or 2-mercaptoethanol (150 μmoles) both at pH 8·0 (KOH). The reactions were followed by recording the addition of 1 N KOH needed to maintain pH 8·0 on a pH-stat (Radiometer, Copenhagen). The reaction vessel (10 ml capacity) was held in a water bath at 25 °C and magnetically stirred. Sodium 3-fluoropyruvate reacted with both L-cysteine and 2-mercaptoethanol, reaching completion within 45 min. The amount of alkali added to maintain pH 8·0 and the amount of inorganic fluoride released (determined on two samples (25 μl) by the method of Belcher *et al.*[15] were both equal to the amount of 3-fluoropyruvate added. 1-Fluoroacetophenone was found to react in a similar manner. However, 2-deoxy-2-fluoroglyceraldehyde reacted with cysteine requiring the addition of alkali to maintain pH 8·0 but did not react in this way with 2-mercaptoethanol. Moreover there was no release of inorganic fluoride. The fluorine on 2-deoxy-2-fluoroglyceraldehyde is, therefore, not reactive towards —SH groups. The

reaction with cysteine is probably the formation of a substituted thiazoldine-4-carboxylix acid by reaction of the aldehyde group with cysteine.[16] Glyceraldehyde gives the same reaction.



Ratner and Clarke[7] showed that the formation of thiazolidine-4-carboxylic acid from cysteine and formaldehyde results in a change of pK_2 of cysteine from 8·18 to 6·21. This alteration in basicity would account for the need for the addition of alkali to maintain pH 8·0.

The report of O'Brien and Peters[1] that 2-deoxy-2-fluoroglyceraldehyde could be phosphorylated by glycerolkinase has been examined in the light of these findings. The criterion of activity used by them was the measurement of the liberation of CO_2 from bicarbonate buffer in Warburg flasks, a method commonly used for following kinase activity. However, a ten-fold molar excess of cysteine over 2-deoxy-2-fluoro-glyceraldehyde was included in the reaction mixture. The results shown in Table 1

TABLE 1. NON-ENZYMIC REACTION BETWEEN CYSTEINE AND 2-DEOXY-2-FLUOROGLYCERALDEHYDE; CO_2 RETENTION BY CYSTEINE AND ATP

Side arm (15 μequiv. in 0·2 ml)	Main compartment	CO_2 released (μmoles)
2-Deoxy-2-fluoroglyceraldehyde	L-Cysteine 20 μmoles in 1 ml	14·6
2-Deoxy-2-fluoroglyceraldehyde	DL-Serine 20 μmoles in 1 ml	0·1
H_2SO_4	L-Cysteine 20 μmoles in 1 ml	14·6
2-Deoxy-2-fluoroglyceraldehyde	L-Cysteine 58 μmoles, ATP 10 μmoles } in 1 ml	8·1
H_2SO_4	L-Cysteine 58 μmoles, ATP 10 μmoles } in 1 ml	8·7

Warburg manometers. Gas phase 95% N_2/5% CO_2 temp. 38°C. Incubation 2 hr. Flasks 1, 2 and 3 contained in addition 1 ml 0·3% sodium bicarbonate. Flasks 4 and 5 contained 1 ml 2·018% sodium bicarbonate. Solutions of amino acids and ATP were neutralized to pH 7·4 before use.

indicate that the non-enzymic reaction is sufficient to account for the reaction observed and that the buffering capacity of the system used by O'Brien and Peters[1] is sufficient to account for their observation that the amount of CO_2 released was equivalent to about 50% of the 2-deoxy-2-fluoroglyceraldehyde added. Accordingly the claim that 2-deoxy-2-fluoroglyceraldehyde could be phosphorylated by glycerolkinase ten times faster than glycerol is phosphorylated and that one optical isomer only is phosphorylated must be discarded. The ability of glycerolkinase to phosphorylate 2-deoxy-2-fluoroglyceraldehyde was tested by the more direct procedure of measuring the disappearance of acid-labile phosphate. The reaction mixture (4 ml) contained enzyme, tris-HCl buffer pH 7·4 (25 μmoles), $MgCl_2$ (1 μmole), ATP neutralized to

pH 7·4 (6 μmoles) KF (25 μmoles) and substrate (100 μmoles). Incubation was carried out at 38 °C for 60 min and samples (0·25 ml) were deproteinized by addition of perchloric acid (20%, 1 ml). After removal of the precipitate by centrifugation the mixtures were heated in a boiling water bath for 10 min. After cooling 0·5 ml was analysed for inorganic phosphate by the method of Fiske and Subbarow.[18] Samples analysed without the hydrolysis step showed that there was no inorganic phosphate present at the end of the incubation. With glycerol as substrate there was 63 per cent of the original acid-labile phosphate at the end of the incubation corresponding to 75 per cent conversion of ATP to ADP whereas with 2-deoxy-2-fluoroglyceraldehyde as substrate acid-labile phosphate at the end of the incubation was within 0·5 per cent of the original level, showing that 2-deoxy-2-fluoroglyceraldehyde is not a substrate for glycerolkinase.

Metabolic reactions

Three possible enzymic reactions which 2-deoxy-2-fluoroglyceraldehyde may undergo as a first step in the pathway of reactions leading ultimately to the lethal synthesis of fluorocitrate, were considered in turn. Phosphorylation by glycerolkinase has been shown not to occur. The results given in Table 2 show that 2-deoxy-2-fluoroglyceraldehyde is reversibly reduced to 2-deoxy-2-fluoroglycerol by crystalline liver alcohol

TABLE 2. ACTION OF LIVER ALCOHOL DEHYDROGENASE UPON 2-DEOXY-2-FLUOROGLYCERALDEHYDE AND 2-DEOXY-2-FLUOROGLYCEROL

Substrate (3 mM)	Rate of oxidation of reduced NAD (mμmoles per min)
Reduction	
Acetaldehyde	880
DL-Glyceraldehyde	28
2-Deoxy-2-fluoroglyceraldehyde	90
(30 mM)	Rate of reduction of NAD (mμmoles per min)
Oxidation	
Glycerol	5·9
2-Deoxy-2-fluoroglycerol	16·7

Reactions were started by addition of substrate and followed by change in extinction at 328 mμ. Total volume 3·3 ml containing 10·6 μg enzyme and either:
NAD 1 mg in 0·1 M glycine/NaOH buffer pH 8·5 or
$NADH_2$ 0·25 mg 0·01 M potassium phosphate buffer pH 7·3.

dehydrogenase. 2-Deoxy-2-fluoroglycerol is unlikely to be an intermediate in the metabolism of 2-deoxy-2-fluoroglyceraldehyde to fluorocitrate since it was observed by O'Brien and Peters[1] to be less toxic. The reverse may hold; 2-deoxy-2-fluoroglycerol may be metabolized to fluorocitrate by way of 2-deoxy-2-fluoroglyceraldehyde. Four enzymes capable of oxidizing aldehydes were examined for their ability to oxidize 2-deoxy-2-fluoroglyceraldehyde. The results given in Table 3 show that liver aldehyde oxidase did not oxidize the compound at all, triosephosphate dehydrogenase oxidized it at 1·7 per cent the rate at which it oxidizes glyceraldehyde. As glyceraldehyde is

oxidized at about 0·1 per cent the rate at which 3-phospho-D-glyceraldehyde is oxidized[19] this a negligible rate. Xanthine oxidase will oxidize 2-deoxy-2-fluoroglyceraldehyde under the conditions at which acetaldehyde is oxidized and could therefore contribute appreciably to the *in vivo* conversion to 2-deoxy-2-fluoroglyceric acid. Aldehyde dehydrogenase oxidizes 2-deoxy-2-fluoroglyceraldehyde readily. Since the K_m for the oxidation of aldehydes by this enzyme is infinitely low[6] this

TABLE 3. ACTION OF ALDEHYDE OXIDIZING ENZYMES UPON 2-DEOXY-2-FLUOROGLYCERALDEHYDE

Enzyme	Relative rates of oxidation CH_3—CHO	CH_2OH—CHOH—CHO	CH_2OH—CHF—CHO
Liver aldehyde oxidase	100	2·7	0
Triose phosphate dehydrogenase	100	5700	100
Xanthine oxidase	100	0	20
Liver aldehyde dehydrogenase	100	495	215

The experimental conditions used for each enzyme are described in the experimental section.

enzyme is likely to be responsible for *in vivo* conversion to 2-deoxy-2-fluoroglyceric acid especially as the concentrations that would occur in an animal after injection would be low. (−)2-Deoxy-2-fluoroglycerate causes more citrate to accumulate in kidney particles (Fig. 1) than O'Brien and Peters[1] reported for 2-deoxy-2-fluoroglyceraldehyde under similar conditions. Moreover, the amount of citrate caused to accumulate by the acid increases with amount of inhibitor added in contrast to the effect of the aldehyde which caused substantial amounts of citrate to accumulate at 330 μM and lesser amounts at higher concentrations of inhibitor.[1] Thus 2-deoxy-2-fluoroglyceric acid could be intermediate between 2-deoxy-2-fluoroglyceraldehyde and fluorocitrate. Injection of (−)2-deoxy-2-fluoroglycerate (120 mg/kg i.p.) produces a condition which is typical of fluoroacetate poisoning[2] and elevated tissue citrate levels (heart 871 μg, kidney 1034 μg/g, liver 811 μg/g fresh weight). Because of scarcity of material this experiment was not repeated. Decarboxylation of 2-deoxy-2-fluoroglycerate to 2-fluoroethanol cannot be the initial step in the conversion of 2-deoxy-2-fluoroglycerate to fluoroacetate since, unlike 2-deoxy-2-fluoroglycerate, 2-fluoroethanol is not toxic to guinea-pig kidney particles.[20] Monofluoromalonate is toxic to rodents causing convulsions and raised tissue levels of citrate. It also causes citrate to accumulate in guinea-pig kidney particle preparations.[21] A direct comparison of monofluoromalonate with (−)2-deoxy-2-fluoroglycerate showed that monofluoromalonate was more toxic to guinea-pig particles (Table 4). This greater toxicity would be consistent with monofluoromalonate being an intermediate between 2-deoxy-2-fluoroglycerate and fluoroacetate. However, the inability of such preparations to oxidize 2-fluoroethanol makes it unlikely that they could oxidize 2-deoxy-2-fluoroglycerate.

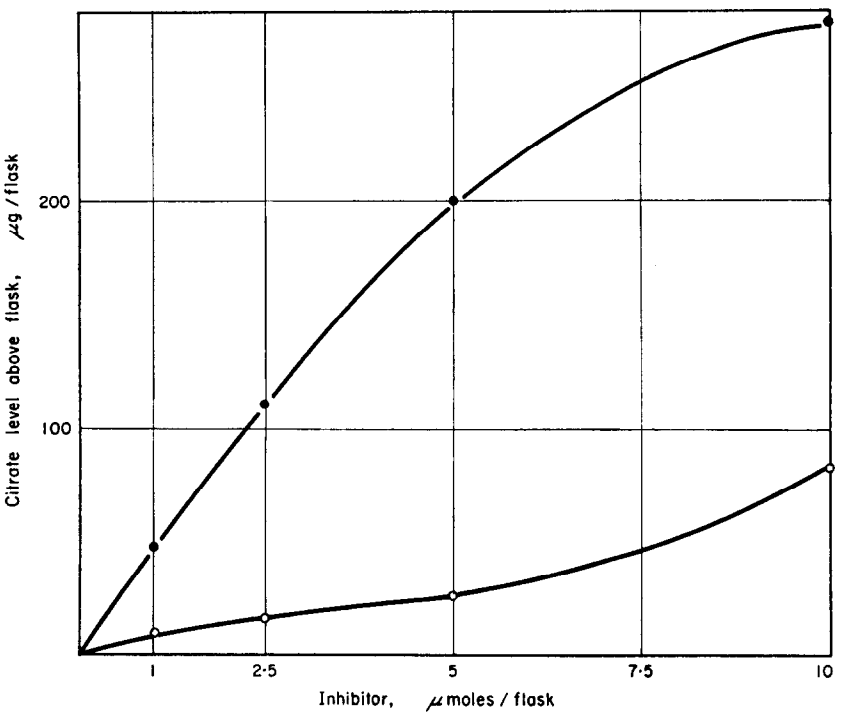


FIG. 1. Optical specificity of 2-deoxy-2-fluoroglycerate on guinea-pig kidney particle preparations. ● (−)2-Deoxy-2-fluoroglycerate, ○ (+)2-deoxy-2-fluoroglycerate. Each point represents at least six determinations.

TABLE 4. EFFECT OF MONOFLUOROMALONATE AND (−)2-DEOXY-2-FLUOROGLYCERATE UPON CITRATE ACCUMULATION BY KIDNEY PARTICLES

Inhibitor	Citrate (μg/flask)
None	58
Fluoroacetate 0·5 μmoles	477
Monofluoromalonate 2·5 μmoles	653
Monofluoromalonate 10 μmoles	718
(−)2-Deoxy-2-fluoroglycerate 2·5 μmoles	96
(−)2-Deoxy-2-fluoroglycerate 10 μmoles	346

Experimental procedure as described in Experimental Section. Figures are average of two experiments both performed in duplicate.

2-Deoxy-2-fluoroglycerate can be regarded as a structural analogue of serine, —F substituted for —NH_2. Serine hydroxymethyl transferase converts serine to glycine; if it acted upon 2-deoxy-2-fluoroglycerate the product would be fluoroacetate. That it does so is suggested by the fact that (−)2-deoxy-2-fluoroglycerate is much more toxic to kidney particle preparations than its stereoisomer. Comparison with the closely related glycerates suggest that it is probably L in configuration.[22] Serine hydroxymethyl

transferase is known to act more strongly on L-serine than D-serine.[23] On incubation with partially purified serine hydroxymethyltransferase (±)-2-deoxy-2-fluoroglycerate formed formaldehyde to the same extent as did D-serine, as determined by the method of O'Dea and Gibbons[24] (Table 5). It seems likely, therefore, that 2-deoxy-2-fluoroglycerate may be converted directly to fluoroacetate by action of this enzyme.

TABLE 5. ACTION OF SERINE HYDROXYMETHYLTRANSFERASE UPON 2-DEOXY-2-FLUOROGLYCERALDEHYDE

Substrate (12·5 mM)	Formaldehyde released (μg) (above corresponding no enzyme control)
None	0·35
L-Serine	3·25
D-Serine	0·78
(±)2-Deoxy-2-fluoroglyceraldehyde	0·75
DL-Glyceraldehyde	0·46

Reaction mixture contains substrate (5 μmoles).
Enzyme preparation (25 mg) Trishydroxymethylaminomethane-HCl 5 mM tetrahydrofolic acid 1 mM pH 7·4. Total volume 0·4 ml.
Incubated 2 hr at 38 °C. Trichloroacetic acid (25% 0·2 ml) added to end reaction. Two samples (0·2 ml) taken after centrifuging for formaldehyde assay.
Results are average of two experiments both done in duplicate.

Thus 2-deoxy-2-fluoroglyceraldehyde can be metabolized to bring about the lethal synthesis of fluorocitrate which inhibits aconitase thereby causing death by action in turn of four enzymes, aldehyde dehydrogenase, serine hydroxymethyltransferase, acetate activating enzyme and condensing enzyme.

The second inhibitory action of 2-deoxy-2-fluoroglyceraldehyde reported by O'Brien and Peters[1] remains unexplained. It is clear, from lack of reactivity towards thiol groups, that it is not acting as an —SH inhibitor as does 3-fluoropyruvate.[14, 25]

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