ferred with some benzene to a Carius tube; 6.68 g. of *m,m'*-ditolyl disulfide was added and the sealed tube was heated for 15 hours at 220°. The contents of the tube was extracted exhaustively with boiling benzene, concentrated at atmospheric pressure using a short Widmer column, and the residue was distilled. It yielded 2.88 g. (58%) of a yellowish liquid, b.p. 273–278° (690 mm.); reported[24] for *m,m'*-bitolyl, b.p. 286–287° (713 mm.), which was oxidized with alkaline 2% potassium permanganate to biphenyl-3,3'-dicarboxylic acid, m.p. 357–360° (reported[25] 357–358°).

Reaction of Raney Nickel Degassed at 200° with 2,2'-Dinaphthyl Sulfide and Diphenyl Sulfide at 220°.—Raney nickel (53 g.) was transferred with benzene to a Carius tube and the solvent was evaporated on the water-pump, while the nickel was held in place by means of a magnet. The dry nickel was degassed by heating for 2 hours at 200° and 2 mm. and 5.05 g. of 2,2'-dinaphthyl sulfide and 3.46 g. of diphenyl sulfide were added under a stream of nitrogen. After heating for 15 hours at 220° the tube was connected to a gas buret and opened. No gas formation was observed. It next was connected to two traps, one at 0° and the other at −40°, and then heated at 95° for some hours. Only traces of a white crystalline substance appeared in the first trap; in the second 0.18 g. of a colorless substance was collected, was identified as benzene by nitration to 1,3-dinitrobenzene, m.p. 89–90° (reported[26] 89–89.5°). The contents of the Carius tube was then extracted with twelve 100-ml. portions of boiling xylene. The extract was concentrated and the residue fractionated. By repeated recrystallizations of the fractions 1.53 g. of biphenyl (m.p. 67–68°), 1.62 g. of 2-phenylnaphthalene (m.p. 97–99°) and 2.56 g. of 2,2'-binaphthyl (m.p. 185–187°) were isolated. The melting points of these substances were not changed by admixture of authentic material. A sulfur free white crystalline product, m.p. 100–120°, 0.27 g., which obviously was a mixture of several substances, and 0.63 g. of a sulfur-containing resin could not be purified or identified.

Reaction of 2,2'-Dinaphthyl Disulfide with Nickel Powder Prepared after Sabatier.—Nickel powder obtained by reducing 20 g. of nickel oxide[27] at 320° for 4 hours in a hydrogen stream was suspended in benzene and transferred to a Carius tube. It then was dried and degassed at 200° and 4.34 g. of 2,2'-dinaphthyl disulfide added as described above. The tube was heated at 220° for 15 hours. By extraction with xylene 3.13 g. (90%) of 2,2'-binaphthyl was obtained which melted at 184.5–185° (from alcohol) and was identified by transformation into the picrate (m.p. 184°).

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The Absolute Configuration of Dihydroxyacetone Phosphate Tritiated by Aldolase Reaction[1]

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The configuration of enantiomorphic tritiated dihydroxyacetone phosphate molecules is established by relation to the stereospecificity of reaction of glycolic acid oxidase and lactic dehydrogenase with glycolic acid. It is concluded that the enzyme–substrate compound formed between aldolase and dihydroxyacetone phosphate is attacked by either a proton or an aldehyde approaching from the same direction.

Isotopic and kinetic evidence has been presented indicating that in the aldol condensation of dihydroxyacetone phosphate (DHAP) with aldehyde, catalyzed by the enzyme aldolase, there occurs a preliminary reaction between DHAP and enzyme to form an enzyme–DHAP compound.[2–5] This intermediate can either react with an aldehyde to form the free condensation product and enzyme or split to free enzyme and DHAP which, when the reaction is performed in tritiated water, acquires one atom of tritium joined to the carbinol-carbon. No radioactivity is found in the condensation product even upon prolonged incubation of the complete system in tritiated water. It has been noted[5] that all products formed by condensation of DHAP with aldehydes and all compounds subject to aldol cleavage by this enzyme have the L-configuration at the third carbon of the phosphoketose.

In the present work, it is of interest to determine whether the tritium atom of the DHAP is placed in a sterically equivalent position to carbon four of the condensation product. This will be called the L-configuration and its enantiomorph, the D-configuration. The absolute configuration has relevance to the mechanism of the aldolase reaction when considered in the following light. Although the chemical structure of the activated DHAP cannot be given in detail, the labilization of one particular hydrogen atom suggests that of a sterically restrained anion, bound firmly to the protein, with an electronic distribution between that of the enol- and keto-forms. Depending on the mechanism of attack of the protein upon the DHAP, the proton may have been either ejected or withdrawn from the α-carbon. In either case it must be in rapid equilibrium with hydrogen ions of the medium (there is no isotope effect)[5] so that the reversal of this process results in labelled DHAP. In the cleavage of the condensation product the resulting aldehyde may likewise be ejected or withdrawn from the α-carbon. If the latter, the subsequent dissociation of the aldehyde does not require the simultaneous dissociation of the enzyme–DHAP compound as shown by the independent turnover of the two "triose parts" of fructose 1,6-diphosphate when incubated with aldolase.[4] Regardless of the mechanism, if both aldehyde and proton follow the same reaction line in their approach to or departure from the intermediate, the absolute configuration of the tritiated DHAP would be L. If, however, they approach along opposite paths,

(1) This work was supported by grant A-912 of the U. S. Public Health Service.

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the D-configuration would result, being opposite that found in the condensation product.

To establish the proper configurational assignment for the asymmetric carbon of tritiated DHAP the following sequence of reactions was carried out: removal of the phosphate, periodate oxidation of the resulting dihydroxyacetone to formaldehyde and glycolic acid and finally the oxidation of the glycolate to glyoxylic acid with glycolic acid oxidase. It will be shown that this enzyme removes only one particular hydrogen atom of the glycolate. The fact that L-lactic acid but not its enantiomorph[6] is also oxidized by glycolic acid oxidase suggests that the hydrogen removed in glycolate is sterically related to the α-hydrogen of L-lactate. To strengthen this assumption, it will be shown that muscle lactic dehydrogenase, which reacts with L-lactic acid but not the D-isomer,[7] labilizes the same hydrogen of the glycolate as does the oxidase. From these arguments it follows that if glyoxylate contains the radioactivity, the tritiated DHAP must be of the L-configuration.

Since it is known that tritiation of DHAP by reaction with triose phosphate isomerase gives rise to the enantiomorph of the aldolase labeled compound[3,8] the present results establish the configuration of this product.

Experimental

Dihydroxyacetone phosphate was labeled with tritium by incubation with aldolase ($DTAP_a$) or with triose phosphate isomerase ($DTAP_t$) and isolated by methods previously reported.[5] The specific activity was determined from those counts which were not held back by Dowex-1, Cl^- before and after treatment with the enzyme used for the labeling. Each compound was virtually free from contamination with its radioactive enantiomorph as determined by measuring the extent of detritiation with the other enzyme.

The DTAP sample was dephosphorylated enzymically with prostate phosphatase kindly supplied by Dr. S. V. Rieder. The reaction was followed by disappearance of substrate as measured with α-glycerophosphate dehydrogenase. The mixture was passed through Dowex-1 Acetate to remove unreacted substrate. The dihydroxyacetone was treated with excess periodic acid at *p*H 4–5 for 15 minutes at 10°. Glycolic acid was isolated by elution from a Dowex-1 Acetate column with 4 *N* acetic acid,[9] the elution being followed by colorimetric analysis.[10] The glycolate, freed of acetic acid by evaporation at 50°, was oxidized with a glycolic acid oxidase preparation from tobacco leaves kindly provided by Dr. I. Zelitch. The oxidation was carried out at *p*H 8.3 with added riboflavin phosphate (0.8 m*M*) and catalase to destroy the H_2O_2 formed in the oxidation. The reaction was followed by disappearance of glycolate until virtual completion. Glyoxylate was isolated by ion exchange under the same conditions used for the glycolate. The separation from glycolate is complete. Glyoxylate was determined by the method of Friedemann and Haugen.[11]

Tritiated glycolic acid was prepared by reaction of lactic dehydrogenase (Worthington) with glyoxylic acid and tritiated reduced diphosphopyridine nucleotide. This latter was prepared by the hydrosulfite reduction of diphosphopyridine nucleotide in tritiated water and isolated by barium–ethanol precipitation.[12]

All counting was done by liquid scintillation as previously detailed.[5]

Results

In Table I are given the specific activities of glycolate and glyoxylate obtained from the two enantiomorphic forms of DTAP. It will be noted that the glycolate and glyoxylate prepared from the aldolase labeled material have the same specific activity and about one-half that of the starting compound. On the other hand the glyoxylate from the isomerase labeled DHAP is without activity. These results are consistent with the assumed stereospecificity of the glycolic acid oxidase if DTAP made by aldolase exchange has the L-configuration. This then supports the view that the aldolase–DHAP compound is attacked by aldehyde or proton approaching from the same direction.

TABLE I

LABELING OF GLYCOLIC AND GLYOXYLIC ACIDS PRODUCED FROM TRITIATED DHAP ENANTIOMORPHS

DTAP Enantiomorph	Specific Activity, c.p.m./μmole		
	DHAP	Glycolate	Glyoxylate
$DTAP_a$	*24,600*	*10,500*	*10,900*
$DTAP_t$	*24,200*	*10,800*	*188*

Tritiated glycolic acid prepared from glyoxylate, lactic dehydrogenase and tritiated reduced diphosphopyridine nucleotide had a specific activity of 7050 c.p.m. per micromole. The glyoxylic acid derived from this by action of glycolic acid oxidase contained 115 c.p.m. per micromole. This result supports the assumption made with respect to the stereospecificity of the two enzymes reacting with glycolate.

In a recent paper, Bloom and Topper[13] have argued that the configuration of a DHAP carbanion postulated to be formed by aldolase is the same as that of C_3 of D-fructose. This is undeniable if one considers an inversion of configuration upon condensation to be unlikely. Likewise, if the formation of the postulated carbanion from DHAP occurs with retention of configuration, it would have the configuration predicted by Bloom and Topper. However, the evidence presented herein does not eliminate the possibility that the anion formed is a bound planar enol, the strictly asymmetric nature of its formation being governed by the enzyme. Thus one need not limit the discussion of intermediates to optically active carbanions. It is to be hoped that evidence on the nature of the intermediates involved in the aldolase and isomerase reactions will be forthcoming.

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