Note

UDC 547.455.3-118.5

Tyunosin Ukita and Kinzo Nagasawa: A Novel Synthesis of DL-Glyceraldehyde 3-Phosphate.

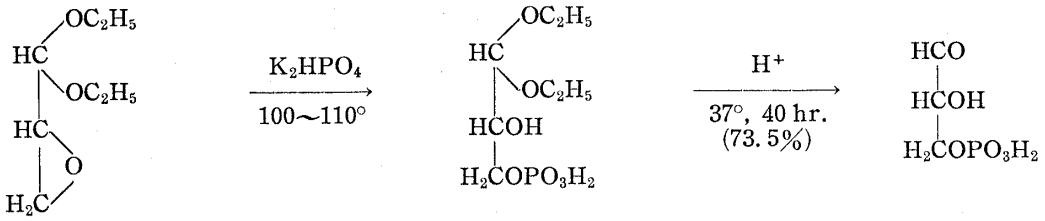
(*Faculty of Pharmaceutical Sciences, University of Tokyo**)

Several procedures have hitherto been reported for the preparation of biologically important glyceraldehyde 3-phosphate. Thus, in 1943, Meyerhof, *et al.*[1] prepared enzymatically the D-form of this phosphate and recently, Ballou and Fischer[2] reported the chemical synthesis of this compound. The most practical method in the preparation of this compound (for the purpose of biological chemistry) is that given by Baer and Fischer for the preparation of DL-form of this phosphate.[3]

However, the Baer's method is inconvenient in some points such as the difficulty of preparing the starting material in this method, DL-glyceraldehyde, or purchased at a high cost, and a rather complicated series of reactions must be followed for subsequent phosphorylation of this aldehyde.

A series of simple reactions which gave DL-glyceraldehyde 3-phosphate more easily and in an excellent yield was developed and described in this paper.

In the phosphorylation of polyhydroxy compounds including several sugars, Lardy,[4] Davis,[5] and the present authors[6] reported that the phosphorolysis of epoxide derivatives of polyhydroxy compounds is one of the most useful methods. By the application of a similar method, glyceraldehyde diethylacetal 3-phosphate (II) was obtained in a quantitative yield from glycidaldehyde diethylacetal[7] (I) and dipotassium hydrogen phosphate.



b.p$_{17}$ 69~71°
(I)

Cyclohexylammonium salt
m.p. 180~181°(decomp.)
(II)

(III)

The aqueous solution of the components was warmed in a pressurized bottle. The percentage of phosphorus esterified to the amount of (I) is given in Fig. 1. Thus, an almost quantitative phosphorylation was observed when 10 times the equivalent of (I) against dipotassium hydrogen phosphate was used. The total amount of the phosphate in the reaction mixture was converted to barium salts, the soluble barium salt of (II) was separated from inorganic phosphate, and converted into stable needle crystals of

* Hongo, Tokyo (浮田忠之進，長沢金蔵).

1) O. Meyerhof, R. Junowicz-Kocholaty: J. Biol. Chem., **149**, 71(1943).
2) C. E. Ballou, H. O. L. Fischer: J. Am. Chem. Soc., **77**, 3329(1955).
3) E. Baer, H. O. L. Fischer: J. Biol. Chem., **150**, 223(1943); E. Baer: Biochemical Preparations, **1**, 50(1949).
4) G. P. Lampson, H. A. Lardy: J. Biol. Chem., **181**, 693, 697(1949).
5) W. Davis, W. C. J. Ross: J. Chem. Soc., **1952**, 4296.
6) T. Ukita, K. Nagasawa, M. Irie: This Bulletin, **5**, 215(1957).
7) D. I. Weisblat, *et al.*: J. Am. Chem. Soc., **75**, 5895(1953).

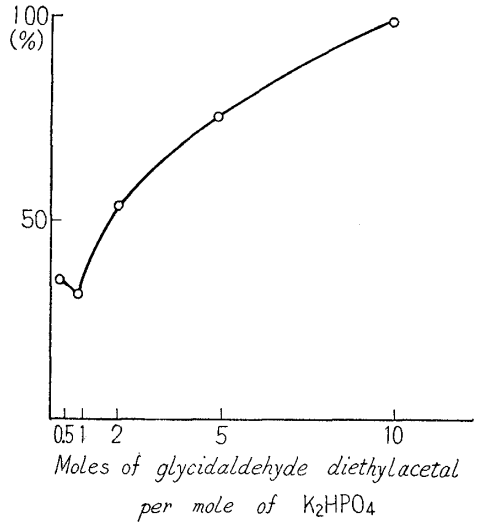


Fig. 1.

The Percentage of Phosphorus Esterified to the Amount of Glycidaldehyde Diethyl Acetal

cyclohexylammonium salt which showed m.p. 180~181°(decomp.). When 0.1*M* aqueous solution of the free glyceraldehyde diethylacetal 3-phosphoric acid (II) was kept at 37° for 40 hours, a quantitative hydrolysis of the diethylacetal occurred (Fig. 2) giving

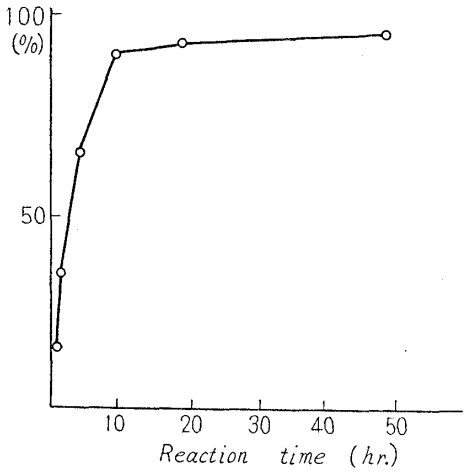


Fig. 2.

Hydrolysis Rate of DL-Glyceraldehyde 3-Phosphate Diethylacetal to DL-Glyceraldehyde 3-Phosphate

DL-glyceraldehyde 3-phosphoric acid which was isolated as calcium salt according to the procedure reported by Baer and Fischer.[3] The calcium salt thus obtained consumed 0.98 mol. equivalent of periodate to furnish glycolaldehyde phosphate as an only phosphorus product detected by paper chromatography. These observations gave the evidence that no migration of the phosphoryl group has occurred during hydrolytic reaction and the sole product was the desired DL-glyceraldehyde 3-phosphate (III).

The synthetic method for DL-glyceraldehyde 3-phosphate described above is prominent in its simple reactions and good yield, especially for the preparation of (III) containing isotopic phosphorus, and in the preservability of the intermediate material (II) which is obtained by one-step reaction from the starting glycidaldehyde diethylacetal (I) and converted quantitatively into (III) by simple treatment with cation exchange resin. Further, the cyclohexylammonium salt of (II) should be especially convenient for preparation of an aqueous solution containing quantitative amount of DL-glyceraldehyde 3-phosphate.

The authors are indebted to Miss S. Ohno for carrying out the microanalyses.

Experimental

DL-Glyceraldehyde 3-Phosphate Diethylacetal (II)—A mixture of 2.92 g.(0.02 mole) of glycidaldehyde diethylacetal (b.p_{17} 69~71°) and 40 cc. of aqueous solution containing 0.01 mole of K_2HPO_4 was heated in a pressurized bottle with shaking at 100~110° for 5 hr. The reaction mixture was extracted with Et_2O (20 cc. × 3) and the aqueous layer was decationized by passing through a column of Amberlite IR-120 (H^+) in a cold room. The acidic effluent was immediately neutralized with satd. $Ba(OH)_2$ to remove the insoluble $Ba_3(PO_4)_2$ by filtration. The filtrate was again decationized with Amberlite IR-120 (H^+) in a cold room and the acidic effluent was adjusted to pH 9 with aq. cyclohexylamine. The mixture was evaporated below 40° *in vacuo* to a syrup and the residue was added with a small volume of Et_2O to separate crystals (1.85 g., 41.8%) which were dissolved in a minimum volume of hot *iso*-PrOH and precipitated by addition of 2 volumes of Et_2O; yield, 1.2 g. (27.1%), m.p. 180~181°(decomp.). *Anal.* Calcd. for $C_{19}H_{43}O_7N_2P \cdot H_2O$: C, 49.56; H, 9.86; N, 6.08; P, 6.74. Found: C, 49.66; H, 10.04; N, 6.19; P, 7.00. Rf_1 0.32, Rf_2 0.78, Rf_3 0.86.

Detection of Hydrolysis Rate of DL-Glyceraldehyde 3-Phosphate Diethylacetal (II) to DL-Glyceraldehyde 3-Phosphate (III) (Fig. 2)—15.3 mg. of (II) was dissolved in 0.7 cc. of water (total P= 1.473 mg./cc.) and to the solution was added 0.2 g. of dried Amberlite IR-120 (H^+) resin. The acid mixture (0.05 *M*) was kept at 37° and 0.05-cc. aliquots were taken at intervals into a measuring flask (25 cc.). To the series of each hydrolysate, 0.5 cc. of *N* NaOH was added and set aside at 37° for 30 min. DL-Glyceraldehyde 3-phosphate produced by acid hydrolysis was quantitatively decomposed to orthophosphate and methyl glyoxal. As the unreacted DL-glyceraldehyde 3-phosphate diethylacetal was inert to alkaline treatment, the reaction mixtures were diluted with water after alkaline hydrolysis exactly to 25 cc. and inorganic phosphorus was determined.[8]

DL-Glyceraldehyde 3-Phosphate (III)—0.92 g.(0.002 mole) of (II) was dissolved in 15 cc. of water and passed through a column of Amberlite IR-120 (H^+) (5 × 2 cm.). The acid effluent and washings were combined (ca. 20 cc., 0.1*M*) and kept at 37° for 40 hr. to complete hydrolysis. The hydrolysate was lyophilized and the residue was redissolved in 2.1 cc. of water. The acid solution was mixed with 0.8 g. of anhyd. $Ca(OAc)_2$ dissolved in 3.6 cc. of water and followed by gradual addition of 4.2 cc. of dehyd. EtOH. After keeping this mixture in a refrigerator over night, crystals that precipitated out were collected and washed with 40% hydr. EtOH (2 cc. × 3). The sample for analysis was dried over anhyd. $CaCl_2$ *in vacuo* for 4 hr.; yield, 0.36 g.(73.5%). *Anal.* Calcd. for $C_3H_5O_6CaP \cdot 2H_2O$: C, 14.72; H, 3.70; P, 12.7. Found: C, 14.75; H, 3.89; P, 13.1. Rf_3 0.24~0.30.

On periodate oxidation, 0.98 mol. equivalent of the reagent was consumed. The phosphate compound found in the oxidation mixture was identified by paper chromatography[9,10] with authentic glycolaldehyde phosphate (Table I).

Table I.

	Rf_2	Rf_3
Periodate oxidation product	0.39	0.42
Glycolaldehyde phosphate	0.38	0.42

Paper Chromatography—Samples were applied on Toyo Roshi No. 53 filter paper and run ascendingly with solvent systems (1) and (2), and by descending technique with solvent system (3). Solvent systems employed: (1) *iso*-PrOH : conc. NH_3 : H_2O (7:1:2), (2) 80% EtOH containing 0.8% AcOH, (3) *tert*-BuOH : H_2O : picric acid (80 cc. : 20 cc. : 4 g.). Spots were detected according to the method of Bandurski and Axelrod for phosphorus[11] and Schiff's reagent for reducing group, and the Rf values of phosphorus compounds found for each of these solvent systems are represented with the abbreviations Rf_1, Rf_2, and Rf_3, respectively.

(Received October 23, 1958)

8) R. J. L. Allen: Biochem. J., **34**, 858(1940).
9) J. Baddiley, *et al.*: *Ibid.*, **64**, 601(1956).
10) H. S. Loring, *et al.*: J. Am. Chem. Soc., **78**, 3724(1956).
11) R. S. Bandurski, B. Axelrod: J. Biol. Chem., **193**, 405(1951).