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A NOVEL CONVENIENT PREPARATION OF DIHYDROXYACETONE PHOSPHATE AND ITS USE IN ENZYMATIC ALDOL REACTIONS

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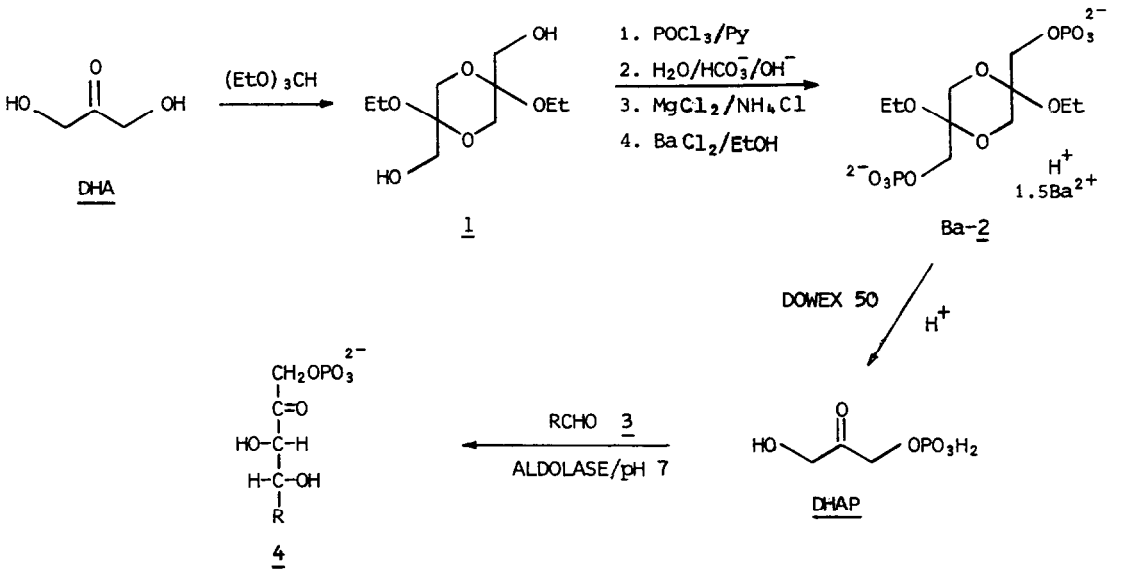
Abstract: A new preparation of the stable barium salt of 2,5-bis(phosphonooxymethyl)-2,5-diethoxy-1,4-dioxane Ba-2 is described, which by treatment with DOWEX 50 H^+ gives dihydroxyacetone phosphate (DHAP) in high yield. DHAP prepared by this method was used for aldolase-catalyzed condensations.

An increasing number of papers dealing with enzymatic aldol reactions demonstrates their synthetic utility[1,2,4]. Rabbit muscle fructose-1,6-diphosphate aldolase (EC 4.1.2.13), which has been used as a catalyst, is rather specific for dihydroxyacetone phosphate (DHAP) reacting as a nucleophile while accepting a wide variety of aldehydes as electrophiles. However, the prohibitively high costs (in 1986, the price of 1 mmol DHAP was 175 ₰) of DHAP have discouraged the use of this highly stereospecific C-C-bond formation method as an efficient preparative tool in organic synthesis.

DHAP can be synthesized by enzymatic phosphorylation of dihydroxyacetone (DHA) with ATP, the latter being regenerated with some loss with acetylphosphate[3]. In comparison, an *in situ* generation of DHAP from fructose-1,6-diphosphate in the presence of triose phosphate isomerase affords in many cases lower yields in the subsequent aldolase-catalyzed sugar synthesis[4].

In attempting chemical routes to DHAP, the polyfunctionality of DHA has until now required complicated multistep syntheses[5]. A typical 8-step synthesis proceeding via the monoacetylated derivative of DHA yields the dimethyl ketal of DHAP which can be isolated as its stable cyclohexylammonium salt[5]. Another way[6] involves first ketalating the DHA-dimer with triethyl orthoformate[7] to 1. Phosphorylation of 1 with $(PhO)_2POCl$ followed by catalytic hydrogenation gives 2, isolated as its cyclohexylammonium salt[6]. Our attempts at reproducing a reported direct phosphorylation of DHA with $POCl_3$[1] resulted in complex reaction mixtures containing only small amounts of DHAP which were not useful for enzymatic aldol reactions.

We now report a successful direct phosphorylation of 1 with $POCl_3$/pyridine to form 2 which is isolated as its very stable barium salt Ba-2 in 85% yield. From Ba-2 DHAP can be generated easily by treatment with acid.



Aldolase-catalyzed reactions of aldehydes 3 with DHAP obtained by the described method give yields of condensation products 4 comparable with those from DHAP obtained enzymatically[3], as demonstrated in the case of the reaction with DL-glyceraldehyde.

Table 1. Aldolase-catalyzed Reactions of Aldehydes 3 with DHAP from Ba-2

3 R	Product 4 Yield/%	3 R	Product 4 Yield/%
$CH_3CH_2CH_2$-	80	CH_3-CH-CH- (O)	94
$(CH_3)_2CH$-	65		
$(EtO)_2P(O)CH_2$-	90	2-Pyridyl-	95
HO_2C-	100	3-Pyridyl-	84
OCH-	94	$C_6H_5CH_2$-	73
$OCHCH_2CH_2CH_2$-	89	C_6H_5CO-	90

The utility of Ba-2 as DHAP precursor in aldolase-catalyzed reactions was investigated with a great number of aldehydes 3. In Table 1 the results of aldol reactions not yet described in the literature are summarized.

The progress of the reaction was monitored by enzymatic determination of residual DHAP. The decrease of DHAP correlates exactly with the increase of product formation, proven by measurement of the generated optical activity in several cases. Using DHAP generated *in situ* from fructose-1,6-diphosphate is not desirable in determining the suitability of various aldehydes as substrates because the starting material is also optically active. This would needlessly complicate our chosen method of simply using polarimetry to follow generation of the newly formed sugars.

Aromatic aldehydes and α,β-unsaturated aldehydes did not react with DHAP under these conditions. However, 1,2,3,6-tetrahydrobenzaldehyde was an active substrate. Aliphatic aldehydes with increasing chain lengths as well as chain-branching gave lower yields of addition products. Pivalaldehyde, for example, was inactive.

Dihydroxyacetone Phosphate. DHA was ketalated according to ref.[7] yielding 1 (50%). A solution of $POCl_3$ (11.84 ml, 127 mmol) in 84 ml abs. pyridine was maintained at -10 to -2°C while 1 (11.81 g, 50 mmol) in 84 ml pyridine was dripped in over 45 min. The solution was stirred at room temperature for 30 min and then poured into 700 ml of ice-cold 0.5 M $NaHCO_3$ (the pH was kept at 7 by addition of 2N NaOH). After stirring for 10 h at room temperature followed by degassing, 60 ml magnesia mixture[8] was added to precipitate inorganic phosphate (P_i), which was removed by centrifugation after 12 h of standing at 4°C. To the supernatant (free of P_i according to ^{31}P-NMR) 60 ml of a solution of 40 g $BaCl_2 \cdot 2\ H_2O$ in 100 ml CO_2-free water was added and the pH was adjusted to 8.2 by the addition of 2N NaOH. After 10 h at 4°C the solution was filtered, the barium salt of 2 was precipitated by the addition of 1,2 l of ethanol to the filtrate and the resultant mixture was kept at 0°C for 3 h. The salt was collected by centrifugation, washed twice with ethanol (80%, then absolute) and ether and dried *in vacuo* to yield 28.13 g (85%) of Ba-2[10]. This salt (9.0 g , 13.80 mmol) was treated with 90 ml of DOWEX 50 WX $8H^+$ in a total volume of 210 ml of water and hydrolyzed at 65°C, pH 1.0 for 4 h[6], to give DHAP in 80% yield. The solution can be frozen and stored for months without noticeable decomposition. It should be neutralized with 2N NaOH just before use, because of the instability of DHAP at this pH.

Aldolase-catalyzed Reactions. An aqueous solution of DHAP (5.55 mmol), DL-glyceraldehyde (590 mg, 6.55 mmol) and aldolase (100 u) was incubated according to ref.[9] until a turnover of 100% had been achieved. The resulting sugar phosphates were hydrolyzed to give 912 mg (91%) of D-fructose and L-sorbose. Although being present in a 4-fold excess, some other aldehydes reacted

quiet slowly and then only at relatively low concentrations (10-50 mM), due to inhibition of aldolase at higher substrate levels.

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10) Ba-2 is actually $Ba_{1,5}H$-2 · 3 H_2O.
^{1}H-NMR (D_2O), 1.19, 1.12 (2t,J=7Hz,6H,2C<u>H</u>$_3$CH$_2$O-,ax/eq), 3.94-3.53 (m,12H, 2CH$_3$C<u>H</u>$_2$, 4CH$_2$), 4.78 (s,6H,<u>H</u>$_2$O);

^{13}C-NMR (D_2O), δ = 17.56, 17.68 (<u>C</u>H$_3$CH$_2$O, ax/eq), 60.85, 60.45 (CH$_3$<u>C</u>H$_2$O, ax/eq), 64.39, 65.60 (ring-<u>C</u>H$_2$-), 66.38, 66.87 (<u>C</u>H$_2$OP, ax/eq), 97.84 (d, $^3J_{CCOP}$=10.5Hz), 100.50 (d, $^3J_{CCOP}$=10.8Hz).

Spectra were recorded at pH 4 after treatment with D_2SO_4 (supernatant).

After acid hydrolysis of Ba-2, all spectra proved to be identical with those obtained from commercial DHAP (with 1 mol ethanol).

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