The carbanilides with substituents in the 2 and 5 positions were markedly more insoluble than the corresponding 3,4-substituted compounds. The amino-substituted carbanilides require prolonged drying under vacuum since they retain solvent tenaciously.

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The Synthesis of Dihydroxyacetone Phosphate

BY CLINTON E. BALLOU AND HERMANN O. L. FISCHER

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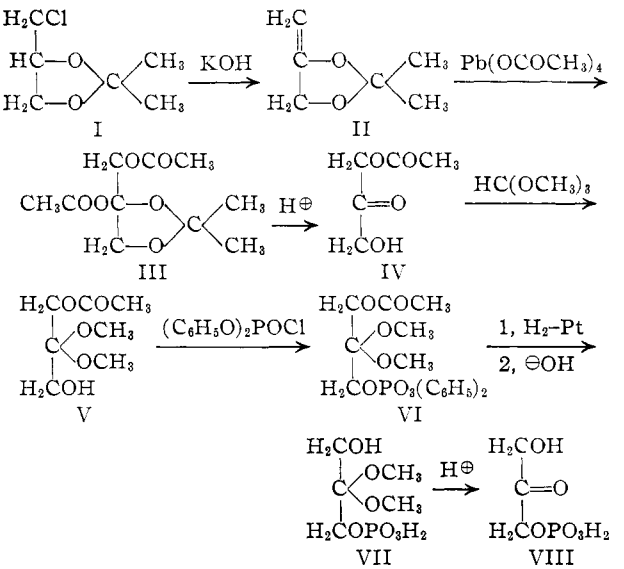
A new method for the synthesis of dihydroxyacetone phosphate, as its stable dimethyl or diethyl ketal, is described. The ketal can be converted in a 95% yield to the free compound by mild acid hydrolysis. This synthesis, which makes this important glycolytic intermediate readily available as a pure substance for the first time, should facilitate future studies on the role of this substrate in carbohydrate metabolism.

Although the Embden–Meyerhof glycolytic scheme is one of the most completely investigated metabolic pathways, until recently at least half of the intermediates were known chemically only as impure, poorly defined substances. We have undertaken the preparation of a number of these intermediates, and have recently described syntheses of D-glyceric acid 2-phosphate,[1] D-glyceric acid[2] 3-phosphate, and D-glyceraldehyde 3-phosphate.[3] The latter, normally an unstable substance, was prepared as the dimethyl acetal, which, although perfectly stable as its cyclohexylamine salt, hydrolyzed readily in dilute acid to give the free aldehyde in high yield. The success of this approach has led us to the new synthesis of dihydroxyacetone phosphate outlined below.



The starting material for this synthesis was prepared, with some modifications, according to published procedures.[4] Isopropylidene glycerol monochlorohydrin (isopropylidene 3-chloro-1,2-propanediol) (I)[5] was dehydrohalogenated, and the resulting isopropylidene 2-propen-1,2-diol (II) was oxidized with lead tetraacetate. Acid hydrolysis of the product III gave acetyl dihydroxyacetone (1-acetoxy-3-hydroxy-2-propanone) (IV)[4] which was then converted to its dimethyl ketal (1-acetoxy-2,2-dimethoxy-3-propanol) (V). Phosphorylation of the ketal V, with diphenyl phosphorochloridate gave the phosphorylated intermediate 1-acetoxy-2,2-dimethoxy-3-diphenylphosphonyloxypropane (VI), from which the phenyl groups were removed by hydrogenolysis and the acetyl group by saponification. The dihydroxyacetone phosphate dimethyl ketal (2,2-dimethoxy-1,3-propanediol phosphate) (VII) was isolated as a crystalline cyclohexylammonium salt. The ketal, as its free acid in aqueous solution, undergoes ready hydrolysis (4 hours at 40°) to give a 95% yield of dihydroxyacetone phosphate (VIII). This synthesis has been used in the preparation of 5–15 gram quantities of the product without modification.

The corresponding diethyl ketal of dihydroxyacetone phosphate (IX) also was prepared, and the rates of hydrolysis of the ketal structures in the two substances are compared in Fig. 1.

The dihydroxyacetone phosphate prepared by this definitive method has been characterized by the acid and alkali lability of the phosphate group; by its reduction in the presence of glycerol phosphate dehydrogenase and reduced diphosphopyridine nucleotide[6]; and by its oxidation in the presence of triose phosphate isomerase, glyceraldehyde phosphate dehydrogenase and diphosphopyridine nucleotide.[7] All tests applied gave results that were qualitatively and quantitatively those expected for dihydroxyacetone phosphate.

Other methods for the preparation of dihydroxyacetone phosphate involve the enzymatic dismutation of D-fructose 1,6-diphosphate,[8] and the indiscriminate phosphorylation of dihydroxyacetone with phosphorous oxychloride.[9] The synthesis

(1) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **76**, 3188 (1954).

(2) C. E. Ballou and H. O. L. Fischer, Abstracts of Papers, 126th Meeting, American Chemical Society, 7D (1954).

(3) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **77**, 3329 (1955).

(4) H. O. L. Fischer, E. Baer and L. Feldman, *Ber.*, **63**, 1732 (1930); H. O. L. Fischer and E. Baer, *ibid.*, **65**, 345 (1932).

(5) E. Fischer and B. Pfähler, *ibid.*, **53**, 1608 (1920).

(6) G. Beisenherz, T. Bücher and K.-H. Garbade, in "Methods in Enzymology," Vol. I, edited by S. P. Colowick and N. O. Kaplan, Academic Press, Inc., New York, N. Y., 1955.

(7) As described by G. Cori, M. Slein and C. Cori, *J. Biol. Chem.*, **173**, 605 (1948), for D-glyceraldehyde 3-phosphate, except that triose phosphate isomerase was also added.

(8) O. Meyerhof and K. Lohmann, *Biochem. Z.*, **271**, 89 (1934).

(9) W. Kiessling, *Ber.*, **67**, 869 (1934).

described here has obvious advantages, and makes this important biochemical intermediate available in large amounts, in a pure, stable form.

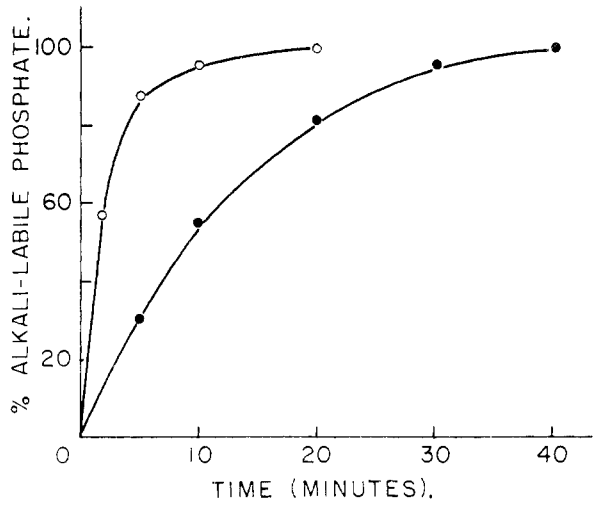


Fig. 1.—Rates of acid hydrolysis (0.1 *N* hydrochloric acid at 40°) of ketal structures of the diethyl ketal (open circles) and dimethyl ketal (solid points), as determined by formation of alkali-labile phosphate.

Experimental

Acetyl Dihydroxyacetone.—This substance was prepared according to published procedures.[4,5] The product was ketalated, as described in the next paragraph, immediately after distillation, otherwise a poor yield was obtained probably due to dimerization on standing.

Acetyl Dihydroxyacetone Dimethyl Ketal (V).—One gram of ammonium chloride was dissolved in 50 ml. of boiling dry methanol. The solution was cooled to room temperature and mixed with 50 ml. of redistilled trimethyl orthoformate.[10] To this mixture was added 20 g. of acetyl dihydroxyacetone, and the reaction was allowed to stand at room temperature for 7 days.

To the slightly yellow solution was added 200 ml. of ether and 75 ml. of 0.2 *N* ammonium hydroxide. The mixture was shaken in a separatory funnel and the ether layer was separated. The water layer was extracted twice more with 100-ml. portions of ether, and the combined ether extracts were dried over sodium sulfate.[11] The extract was then concentrated to a thin sirup at the water pump, and distilled in a high vacuum giving 25–28 g. of the ketal with b.p. 65–73° at 0.1–0.2 mm. This crude product was redistilled, to yield 23–25 g. with b.p. 70–72° at 0.1 mm.

Anal. Calcd. for $C_7H_{14}O_5$ (178): C, 47.1; H, 7.9; OCH_3, 34.8; sapon. equiv., 178. Found: C, 47.0; H, 8.0; OCH_3, 34.8, sapon. equiv., 183.

Phosphorylation of V with Diphenyl Phosphorochloridate.—To 3.0 g. of V in 10 ml. of dry pyridine cooled in ice-water was added 6.0 g. of diphenyl phosphorochloridate, dropwise, over a period of 5 minutes. The reaction was then stoppered and left overnight at 5°.

A few drops of water were added to destroy the excess phosphorylating reagent, and the solution was concentrated at the water pump to remove most of the pyridine. The resulting sirup was dissolved in 75 ml. of benzene and washed successively with 50 ml. each of water, cold 1 *N* hydrochloric acid, cold 1 *M* potassium bicarbonate and water. The benzene layer was dried over sodium sulfate and concentrated to a sirup that weighed 6.7 g. The theoretical yield of the phosphorylated intermediate is 6.9 g.

The phenyl groups were removed from the above crude product by hydrogenation at atmospheric pressure in 250 ml. of absolute ethanol containing 1.0 g. of platinum oxide. The hydrogen uptake was 3050 ml. in 30 minutes; the theoretical uptake being 2930 ml. The catalyst was removed by centrifugation, and to the alcohol solution was added 7.5 g. of barium hydroxide dissolved in about 100 ml. of hot water. The mixture was concentrated (to remove the alcohol) to a volume of about 50 ml. After an additional hour to allow for deacetylation (the solution should be strongly basic) a solution of 7.15 g. of cyclohexylammonium sulfate[12] in 25 ml. of water was added. The mixture was warmed to 80° to facilitate the filtration, then filtered with suction through Filtercel on a hardened filter paper. The filtrate was concentrated *in vacuo* to dryness, and the residue was extracted with 25 ml. of warm absolute ethanol. Filtration removed a small amount of the insoluble salt of inorganic phosphate, and the alcoholic filtrate was concentrated to a dry white crystalline solid. This was stirred up in 50 ml. of acetone, filtered off and washed on the funnel with more acetone. The air-dry material weighed 5.5 g. This cyclohexylamine salt of dihydroxyacetone phosphate dimethyl ketal was dissolved in 15 ml. of water, 25 ml. of acetone was added, and the solution was filtered by suction. Acetone (about 40 ml.) was added to the filtrate to cause turbidity. The compound crystallized as needles, and was collected by filtration after 18 hours at 5°. It was washed on the funnel with acetone, and dried in air. The yield was 4.5 g., and another 0.5 g. was obtained by concentrating the mother liquors (to remove most of the water) and adding acetone. For analysis the product was dried in a vacuum desiccator over calcium chloride for 12 hours. The m.p. was 183–185° dec.

Anal. Calcd. for $C_{17}H_{39}O_7PN_2 \cdot H_2O$ (432): C, 47.2; H, 9.5; N, 6.5; P, 7.2; OCH_3, 14.35; H_2O, 4.16. Found: C, 47.5; H, 9.8; N, 6.5; P, 7.3; OCH_3, 14.20; H_2O, 4.0.

Phosphorylation of acetyl dihydroxyacetone diethyl ketal in an identical manner led to dihydroxyacetone phosphate diethyl ketal, isolated as the crystalline cyclohexylamine salt. The substance melted at 180° dec.

Anal. Calcd. for $C_{19}H_{43}O_7N_2P \cdot H_2O$: C, 49.6; H, 9.9; N, 6.1; P, 6.7; OC_2H_5, 19.5. Found: C, 50.0; H, 9.9; N, 6.2; P, 6.8; OC_2H_5, 19.5.

The diethyl ketal hydrolyzed more readily than the dimethyl ketal to give dihydroxyacetone phosphate. It is more difficult to prepare, because of the lower yield in the preparation of acetyl dihydroxyacetone diethyl ketal (note 10). The diethyl ketal is, however, the preferred derivative for the preparation of phosphoryl hydroxypyruvic acid, in which the greater lability of the diethyl ketal is an asset.[13]

Dihydroxyacetone Phosphate.—A solution of 100 mg. of cyclohexylammonium dihydroxyacetone phosphate dimethyl ketal in 5.0 ml. of water (0.046 *M*) was swirled with 2 ml. of Dowex-50 (H^-) resin for 30 seconds, then filtered. The filtrate was kept at 40° for 4 hours, when hydrolysis of the ketal was complete. The solution of dihydroxyacetone phosphate was 0.042 *M* in alkali labile phosphate (1 *N* sodium hydroxide for 20 minutes). It assayed 0.044 *M* in dihydroxyacetone phosphate with the enzyme glycerol phosphate dehydrogenase, the yield of biologically active product being 95%.

The acid solution may be brought to *p*H 4.5 with potassium bicarbonate solution and stored frozen without decomposition. If the methanol formed in the hydrolysis is undesirable it may be removed by distillation *in vacuo*. Since the dihydroxyacetone phosphate can be prepared so easily and in such good yield from its stable ketal, no attempt has been made to prepare a crystalline salt of the free compound.

Enzymatic Assays.—The assays of dihydroxyacetone phosphate were carried out by the procedures[6,7] referred to, using purified triose phosphate dehydrogenase (rabbit muscle) and crystalline glyceraldehyde phosphate dehydrogenase (rabbit muscle). The rates of oxidation and reduction were comparable to those for dihydroxyacetone phosphate prepared enzymatically from D-fructose 1,6-diphosphate.

Acknowledgment.—This work was supported by

(10) Ketalation with triethyl orthoformate according to the original procedure of Fischer and Baer[4] gave a poor yield in our hands.

(11) Additional water washings of the ether extracts should not be made since the product is quite soluble in water.

(12) Prepared by mixing a solution of 50 g. of cyclohexylamine in 1 liter of absolute ethanol with 50 ml. of 10 *N* sulfuric acid. After the mixture is cooled to 0°, the salt is filtered off, washed on the funnel with absolute ethanol, and dried.

(13) C. E. Ballou and R. Hesse, unpublished.

grants from the Nutrition Foundation, and from the United States Public Health Service (Grant A-884). A gift of glycerol monochlorohydrin was made by Dr. W. F. Ross of Shell Development Company.

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE NAVAL RESEARCH LABORATORY]

The Base-catalyzed Reaction of Dialkyl Phosphonates with Isocyanates[1]

BY ROBERT B. FOX AND DAVID L. VENEZKY

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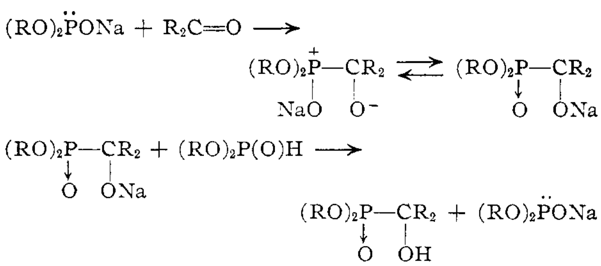
A series of carbamoylphosphonates, $RNHCOPO(OR)_2$, has been prepared by the base-catalyzed reaction of isocyanates with dialkyl phosphonates.

In recent years, the addition of dialkyl phosphonates to unsaturated groupings such as carbonyl, imine or to activated carbon–carbon unsaturation has been extensively investigated.[2] These reactions are generally catalyzed by alkali metals or their alkoxides. Catalysis by other bases has not been reported.[3] It has been assumed that the mechanism of these reactions involves the polarization of the unsaturated group by the metal salt of the phosphonate, followed by addition of the dialkoxyphosphinyl radical. For example, reaction with ketones may take the course[4]



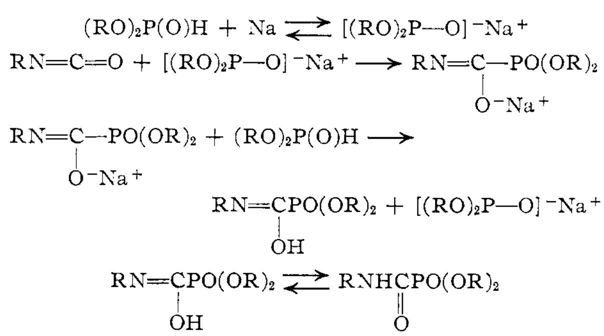
Since this course of reaction seems to be typical of the addition reactions of phosphonates, it would appear that the nucleophilic reactivity of the dialkoxyphosphinyl group, $(RO)_2P(O)^-$, would be better demonstrated through an addition reaction subject to general base catalysis.

The high reactivity of the isocyanate group toward nucleophilic reagents is well known.[5] Pseudo acids, such as aliphatic nitro compounds, have been shown to react with isocyanates in the presence of bases.[6] Indeed, dialkyl phosphonates themselves have been reported recently to react directly with aliphatic isocyanates in the absence of bases at temperatures on the order of 135°.[7] It would therefore seem probable that isocyanates and dialkyl phosphonates would react readily in the presence of bases to form carbamoylphosphonates.[7,8]

$$RNCO + HPO(OR)_2 \xrightarrow{\text{base}} RNHCOPO(OR)_2$$

This has indeed been found to be the case, and the reaction appears to be a general one. Not only the more reactive ethyl isocyanate, but aromatic isocyanates having varying substituents in the *para*-position react rapidly at room temperature with dialkyl phosphonates in the presence of catalytic quantities of strong bases such as sodium, sodium dialkyl phosphonates, triethylamine and cyclohexyldiethylamine. A less vigorous reaction takes place in the presence of weaker bases such as sodium carbonate, sodium cyanide, tributylamine or α-picoline; no catalysis was observed with dialkylanilines. Under our reaction conditions, little if any of the "spontaneous" reaction shown by Reetz, *et al.*,[7] took place.

At least in the case of sodium as catalyst, the mechanism of the reaction is probably similar to that cited for ketones



Since no significant changes were observed in the infrared spectra of mixtures of diethyl phosphonate and triethylamine, and since weak bases such as α-picoline were effective catalysts, an alternative mechanism may be operating. One such mechanism might involve an initial attack by the tertiary amine on the isocyanate, as proposed[9] for the base-

(1) Presented before the Division of Organic Chemistry at the 128th National Meeting of the American Chemical Society, Minneapolis, Minnesota, September, 1955.

(2) This work has been reviewed by A. N. Pudovik, *Uspekhi Khim.*, **23**, 547 (1954).

(3) A. N. Pudovik and L. I. Sidnikhina, *Zhur. Obshchei Khim.*, **24**, 1193 (1954), have indicated that no reaction takes place between O,O-di-*n*-butyl phosphonothioate and diethyl 2-propylidenemalonate in the presence of triethylamine, whereas a normal reaction takes place in the presence of sodium butylate to yield the addition product.

(4) V. S. Abramov, *Doklady Akad. Nauk., S.S.S.R.*, **73**, 487 (1950).

(5) H. Saunders and R. J. Slocumbe, *Chem. Revs.*, **43**, 203 (1948).

(6) R. N. Boyd and R. Leshin, THIS JOURNAL, **75**, 2762 (1953).

(7) T. Reetz, D. H. Chadwick, E. E. Hardy and S. Kaufman, *ibid.*, **77**, 3813 (1955).

(8) Using other synthetic routes, Reetz, *et al.*,[7] have described the series $RR'NCOPO(OC_2H_5)_2$, where R and R' are hydrogen or alkyl groups; B. A. Arbusov and N. I. Rizpolozhenskii, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 847 (1952), have prepared the series $(C_2H_5)_2NCOPO(OR)_2$ and $(C_2H_5)_2NCOPO(OR)C_2H_5$ (*ibid.*, 631 (1954)), where R is an alkyl group.

(9) J. W. Baker and J. Gaunt, *J. Chem. Soc.*, 9 (1949).