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An Improved Synthesis of Diammonium Acetyl Phosphate[1]

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We have described a scheme for using ATP-requiring enzymes as catalysts in large-scale organic synthesis, based on the regeneration of ATP from ADP by phosphorylation with acetyl phosphate (AcP) (eq 1 and 2).[3] We have prepared the

$$\text{AMP} + \text{ATP} \rightleftharpoons 2\text{ADP} \quad (1)$$

$$\text{ADP} + \text{AcP} \rightleftharpoons \text{ATP} + \text{Ac} \quad (2)$$

acetyl phosphate required in this scheme by a synthesis based on acylation of anhydrous phosphoric acid with ketene, followed by reaction with anhydrous ammonia.[4] This procedure had the advantage, compared with previous syntheses, that the acetyl phosphate precipitated from a methanol/ethyl acetate solution at the conclusion of the synthesis as its diammonium salt. This material was easily filtered (unlike the phosphate slimes obtained on precipitation of many acetyl phosphate salts from water) and dried. The procedure had two disadvantages. First, it required a ketene generator. Ketene generation using an apparatus of the type normally found in research laboratories is a relatively slow process, and this equipment is, in any event, not universally available. Second, reaction of the initially produced mixture of acylated phosphoric acids with ammonia was carried out by passing ammonia gas over the rapidly stirred reaction mixture. In practice, close attention to detail was required to achieve good yields with this procedure, presumably because the reaction was both rapid and heterogeneous and because acetyl phosphate itself reacts with excess ammonia. Here we describe a modified procedure for the preparation of diammonium acetyl phosphate which differs in two respects from that described earlier: first, acetic anhydride is used as acylating agent rather than ketene; second, reaction of the mixture of acetylphosphoric acids is accomplished by adding this mixture to a saturated *solution* of ammonia in methanol (eq 3). This procedure is much more easily carried out than that based on ketene and is more reproducible when applied to large preparations. It differs from previous preparations employing acetic anhydride as acylating agent in using anhydrous phosphoric acid rather than triethylammonium phosphate as the starting material and in the convenience of the workup procedure.[5]

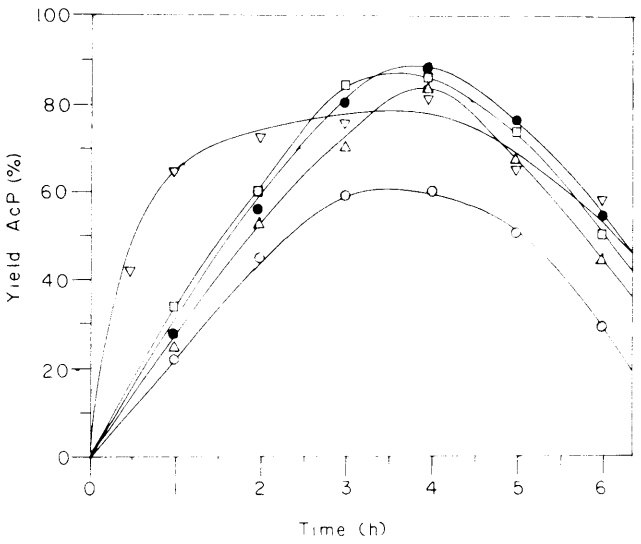

Figure 1. Yields of diammonium acetyl phosphate obtained by acylation of phosphoric acid with acetic anhydride. Reactions were carried out at 0 °C in ethyl acetate, and aliquots of the reaction mixtures were removed and added to methanol saturated with anhydrous ammonia at −10 °C. The molar ratios of acetic anhydride/phosphoric acid used were (○) 1.0, (△) 1.5, (●) 1.8, (□) 2.0, and (▽) 3.0.

$$1.8\text{CH}_3\text{COCCH}_3 + \text{H}_3\text{PO}_4 \xrightarrow[3.7\text{ h, }0\text{ °C}]{\text{EtOAc}} \xrightarrow[-30\text{ to }-10\text{ °C}]{\text{NH}_3/\text{CH}_3\text{OH}} \underset{86\%}{\text{CH}_3\text{COP}(\text{O}^-\text{NH}_4^+)_2} \quad (3)$$

Several experimental variables are important for the success of this new procedure. First, the temperature of the acylation reaction is critical. The best yields were obtained by carrying out the acylation at 0 °C and the reaction with methanolic ammonia at −30 to −10 °C. When the acylation was conducted at −15 °C, yields of acetyl phosphate were low (5%, instead of the 86% observed under optimal conditions); at temperatures higher than 0 °C, the yields also dropped, although less sharply. Second, both the ratio of acetic anhydride/phosphoric acid and the duration of the acylation reaction were important. Figure 1 summarizes data obtained in several variations of these parameters. These plots indicate the fraction of the solid isolated at the end of the reaction, which was diammonium acetyl phosphate, and correspond approximately to the yield of acetyl phosphate; the remainder appeared to be predominantly ammonium phosphates. The best reaction conditions (0 °C, $Ac_2O/H_3PO_4 = 1.8$, $t = 3.7$ h) gave a yield of diammonium acetyl phosphate of 86% based on H_3PO_4; the purity of this material was also 86%.

This acetyl phosphate has been used successfully in our laboratory for enzymatic reactions without further purification. Its storage stability appears to be indistinguishable from that of material obtained using the earlier preparation.[4] In general, ammonium ion is innocuous as a component in enzymatic reactions, although instances are known in which ammonium ion acts as an enzymatic inhibitor.[6] If the ammonium ion should prove undesirable in a particular reaction, it can be exchanged for sodium by ion exchange.[4]

This procedure is the most convenient one available for the preparation of acetyl phosphate, particularly on laboratory scale. For larger scale preparations, however, ketene might still be the preferred acylating reagent for economic reasons. We have examined the applicability of the ammonia treatment described here to the mixture of acylated phosphoric acids prepared from ketene as described previously[4] and found that reaction of this mixture with a solution of ammonia in methanol rather than with ammonia vapor gives yields of acetyl phosphate indistinguishable from that obtained in this work. Since this workup procedure is both more convenient and more reproducible than that described previously,[4] it should be used even when ketene is used as acylating agent.

Experimental Section

General. All chemicals were reagent grade and were not further purified. Enzymes used in the assay of **1**—acetate kinase (EC 2.7.2.1) and a commercial mixture of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and hexokinase (EC 2.7.1.1)—were obtained from Sigma Chemical Co. Anhydrous ammonia was obtained from Matheson, and was used directly from the tank without purification. Phosphoric acid (100%) was made by the slow addition of 191.5 g of phosphorus pentoxide to 500 g of stirred 85% phosphoric acid at −10 °C (ice/acetone bath). Water used in enzymatic assays was distilled twice, the second time using a Corning Model AG-1b distillation apparatus. Ultraviolet absorbance was measured using a Gilford Model 220 spectophotometer. A Radiometer Model PHM 62 pH meter was used to determine pH values.

Enzymatic Assay for AcP. The enzymatic assay used to determine the yield and purity of acetyl phosphate is that previously described.[4]

Diammonium Acetyl Phosphate. Ethyl acetate (2000 mL) and 100% phosphoric acid (294 g, 3.00 mol) were cooled in a 3-L flask to 0 °C in an ice bath. Acetic anhydride (551 g, 5.4 mol) was first cooled in an ice bath to 0 °C and then slowly added to the ethyl acetate/phosphoric acid mixture. The resulting solution was stirred at 0 °C for 3.7 h. A 5-L three-neck flask was fitted with a thermometer, a gas inlet tube, and an overhead stirrer. The stirrer shaft entered the flask through a fitting equipped with a side arm which served as a gas outlet. Methanol (2250 mL) was added, and anhydrous ammonia was allowed to enter with constant stirring. The flask was cooled to −30 °C in a dry ice/acetone bath, and the methanol was allowed to become saturated with ammonia (~30 min). The addition of ammonia was stopped, and the gas inlet tube was replaced with a 3-L addition funnel. The ethyl acetate/acetic anhydride/phosphoric acid mixture was placed in the addition funnel and slowly added to the vigorously stirred methanol solution. This addition took approximately 20 min, during which time the methanol solution rose in temperature to −10 °C. The fine solid which filled the flask was collected by suction filtration on a Büchner funnel. It was washed with 600 mL of methanol and then 600 mL of anhydrous ether. Final drying to constant weight under vacuum gave 524 g of solid. Enzymatic assay showed that the solid contained 86% diammonium acetyl phosphate (2.6 mol) by weight, corresponding to an 86% yield based on phosphoric acid. This material was stored at 0 °C and protected from atmospheric moisture.[4]

Registry No.—**1**, 55660-58-7; phosphoric acid, 7664-38-2; acetic anhydride, 108-24-7; ammonia, 7664-41-7.

References and Notes

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(2) Ida Green Fellow, 1977–1978.
(3) Baughn, R. L.; Adalsteinsson, O.; Whitesides, G. M. *J. Am. Chem. Soc.* **1978**, *100*, 304–6. Shih, Y.-S.; Whitesides, G. M. *J. Org. Chem.* **1977**, *42*, 4165–6. Pollak, A.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1977**, *99*, 2366–7.
(4) Whitesides, G. M.; Siegel, M.; Garrett, P. *J. Org. Chem.* **1975**, *40*, 2516–9.
(5) Porter, R. W.; Modebe, M. O.; and Stark, G. R. *J. Biol. Chem.* **1969**, *244*, 1846–59.
(6) Hooke, J. P.; Laidler, K. J. *J. Am. Chem. Soc.* **1950**, *72*, 2487–9. Sayre, F. W.; Roberts, E. J. *J. Biol. Chem.* **1958**, *233*, 1128–34. Maeba, P.; Sanwal, B. D. *Biochemistry* **1966**, *5*, 525–35. Also see, Williams, R. J. P. *Q. Rev., Chem. Soc.* **1970**, *24*, 331–5; Mildman, A. S. In "The Enzymes"; Boxer, P. D., Ed.; Academic Press: New York; Vol. ii, Chapter 9.