vacuum line, assayed, and characterized by vapor-phase infrared spectrophotometry (PE 1330), electron-impact (70 eV) mass spectrometry (Hewlett-Packard GC/MS, 5710A GC, 5980A MS, 5934A computer), and ^1H and ^{19}F nuclear magnetic resonance (JEOL FX90Q, omniprobe) in $CDCl_3$ with 1% $CFCl_3$ as an internal standard. Elemental Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY.

Aerosol Fluorination of Adamantane. Adamantane (Aldrich, 99+%) was used as received. Adamantane (1.39 g, 11.2 mmol) was loaded into the sublimator. The main helium carrier flow (Figure 1) was set at 600 cm^3/m. This flow is directed through the nucleating particle (NaF) furnace (A) and the liquid nitrogen heat exchanger (B) and enters one side of the aerosol generator (C) where it is mixed in the aerosol generator (C) with the hydrocarbon carrier containing the adamantane vapor. The hydrocarbon carrier consists of one upper, primary (150 cm^3/m) and two lower, secondary (20 cm^3/m) helium flows entering into the sublimator (K) at the top (L) and bottom (M, N) of the sublimator body (K), respectively. The reactor modules (E–G) were cooled to −30 °C, −20 °C and ambient temperature (−10 °C) while the copper coil (I) preceding the photochemical stage (J) was heated to 100 °C. Fluorine flows into the reactor modules were 20 cm^3/m, 50 cm^3/m and zero, respectively. The photochemical lamp was ignited and the sublimator was then heated to 150 °C. After 7 h the reaction was stopped. When the reactor was opened, 1.23 g of unreacted adamantane was recovered (0.300 g, 2.2 mmol, reacted); 0.361 g of crude product was collected, dissolved in perfluoropentane, and separated on the Fluorosilicone QF-1 column (70 °C, 15 min; 30 °C/min to 180 °C), producing 0.259 g (74%) of *F*-adamantane, a 28% yield based on theoretical input. It should be noted that significant quantities of unfluorinated adamantane were found inside the reactor. Anal. Calcd for $C_{10}F_{16}$: C, 28.32; F, 71.68; H, 0.00. Found: C, 28.60; F, 71.88; H, 0.0. The fluorine-19 NMR consists of a pentet of intensity 12 at −121.20 ppm and a near symmetrical multiplet of ~13 prominent diminishing maxima of intensity 4 at −223.53 ppm relative to internal $CFCl_3$; a coupling constant of 6 Hz is a best fit.

Acknowledgment. This work was supported in part by the Office of Naval Research whose support is gratefully acknowledged.

Registry No. Adamantane, 281-23-2; *F*-adamantane, 69064-33-1.

Supplementary Material Available: A reproduction of the ^{19}F NMR spectra and a complete characterization (EI MS, IR) (2 pages). Ordering information is given on any current masthead page.

A Convenient Synthesis of Disodium Acetyl Phosphate for Use in in Situ ATP Cofactor Regeneration[1]

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Many enzyme-catalyzed reactions useful in organic synthesis consume ATP.[3] We routinely regenerate ATP in situ by procedures in which acetyl phosphate[4,5] and phosphoenol pyruvate[6,7] are the ultimate phosphorylating agents. A number of syntheses of acetyl phosphate have been reported,[8] of which the best, a synthesis of crystalline diammonium acetyl phosphate,[5] is still less than ideally convenient. This synthesis involves several steps which require careful experimental control and which are accordingly difficult to carry out on large scale. Further, the ammonium ion (used in this preparation to confer crystallinity to the solid product) has two disadvantages. First, it reacts with acetyl phosphate in solution.[9] Second, it forms an insoluble precipitate (magnesium ammonium phosphate) under the reaction conditions. This precipitation both removes from the solution the magnesium ion which is required for activity[10] of the enzymes and occludes particles of immobilized enzyme.

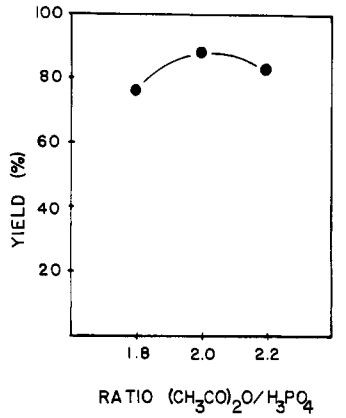

Figure 1. Yield of acetyl phosphate as a function of the molar ratio of acetic anhydride to phosphoric acid (1:1 phosphoric acid/ethyl acetate, room temperature, 25–30-min reaction time).

This manuscript describes a simple procedure for the preparation of aqueous solutions of acetyl phosphate as its sodium or potassium salt; no solid derivative of acetyl phosphate is isolated. This synthesis circumvents many of the disadvantages of previous preparations, and provides a convenient source of acetyl phosphate for use in nucleoside triphosphate cofactor regeneration.

Results

This synthesis of acetyl phosphate involves four steps: first, acylation of phosphoric acid with acetic anhydride in ethyl acetate; second, extraction of acetyl phosphate into water by treatment of the reaction mixture with cold aqueous bicarbonate solution; third, extraction of acetic acid from the resulting aqueous mixture with ethyl acetate; fourth, neutralization of the remaining aqueous solution of acetyl phosphate to pH 7 for storage and use. If one uses sodium hydroxide and sodium bicarbonate as bases, the acetyl phosphate is generated as its sodium salt.

(1) Supported by the National Institutes of Health, Grant GM 30367.
(2) Address correspondence to G.M.W. at Harvard University.
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(9) The relative half-lives for acetyl phosphate as the diammonium and disodium salt in water at room temperature are respectively: pH 7.2, 15 and 21 h; pH 8.4, 8 and 23 h; pH 9.4, 1 and 20 h.
(10) General: Mildvan, A. S. In Paul D. Boyers; "The Enzymes"; Boyers, P. D., Ed.; Academic Press: New York, 1970; Vol. 2, pp 445–536. For acetate kinase: Rose, I. A.; Grunberg-Manago, M.; Vorey, S. R.; Ochoa, S. *J. Biol. Chem.* **1953**, *211*, 737–56.

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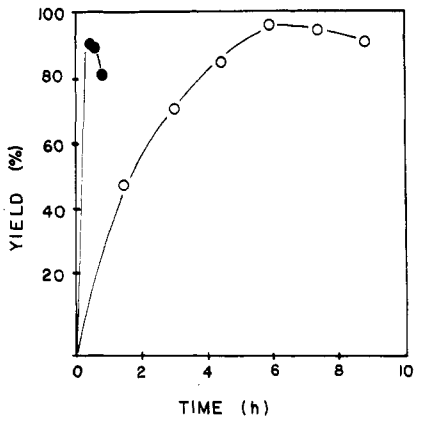

Figure 2. Yield of acetyl phosphate as a function of reaction time. The molar ratio of $(CH_3CO)_2O$ to H_3PO_4 used was 2. Reactions carried out in concentrated solution (moles of H_3PO_4/moles of CH_3CO_2Et = 1) are indicated by ●; those in dilute solution (moles of H_3PO_4/moles of CH_3CO_2Et = 6) are indicated by ○.

Similar procedures using potassium hydroxide and potassium bicarbonate generate potassium acetyl phosphate. We presume that salts of other cations can also be easily obtained, if desired. The sodium salt is particularly useful in applied enzymology, because sodium ion is generally innocuous as a component of enzyme-containing solutions or suspensions. The solution can be used without further purification or manipulation as a source of acetyl phosphate for cofactor regeneration; a representative procedure for the preparation of glucose-6-phosphate is given in the Experimental Section. The storage stability of these solutions of acetyl phosphate is good:[11] at pH 7 and 0 °C, less than 5% of the acetyl phosphate decomposes per month. At room temperature, the half-life of acetyl phosphate in solution (pH 7.2) is 21 h.

Both 85% and 100% phosphoric acid can be used as starting materials for the reaction. The 85% material is generally more convenient but requires larger quantities of acetic anhydride to achieve corresponding yields. Figure 1 summarizes yields of acetyl phosphate obtained as a function of the stoichiometry of the reaction; Figure 2 summarizes yields as a function of the reaction time. The best yield of acetyl phosphate from 85% phosphoric acid is obtained by using 2 mol of acetic anhydride/mol of phosphoric acid. The species actually formed in this acylation reaction have not been rigorously identified, but the ^{31}P NMR spectrum suggests the presence of monoacetyl-, diacetyl-, and pyrophosphoric acids.[12] Diacetyl phosphate may serve directly as a substrate for acetate kinase, or it may hydrolyze under the conditions of the enzymatic reactions to monoacetyl phosphate.[13]

The conditions for formation of acetyl phosphate which consume the least time involve reaction of a concentrated solution of 85% phosphoric acid in ethyl acetate (1:1 molar ratio) with acetic anhydride (2 mol/mol of H_3PO_4) at 24–27 °C; this reaction requires 20–30 min to complete and gives acetyl phosphate in 85–90% yield based on phosphoric acid. Longer reaction times give lower yields of acetyl phosphate and higher yields of pyro- and polyphosphoric acids. Although this procedure is rapid, it requires attention to detail: if the temperature becomes too high, the yield may drop to 70%. An alternative procedure involves the same stoichiometry for the reagents but uses more dilute solutions and longer (~6 h) reaction times. This procedure is very reliable, reproducible, and insensitive to details of experimental procedure. It routinely generates acetyl phosphate in yields >90%. Separate descriptions of each procedure are included in the Experimental Section.

The greater part of the acetic acid produced during either reaction must be removed before the solution of acetyl phosphate can be employed in cofactor regeneration: acetate is a substrate for acetate kinase (K_m = 0.3 M)[14] and reverses the formation of ATP at high concentrations. We separate acetic acid from acetyl phosphate by extraction from an aqueous solution (pH 3) with ethyl acetate.[15]

Conclusion

This synthesis represents a more practical solution to the problem of acetyl phosphate generation for the specific application of cofactor regeneration than that reported previously.[5] It has several advantages. First, it starts with commercial 85% phosphoric acid and does not require conversion of this material to 100% phosphoric acid. Second, it generates the sodium salt of acetyl phosphate directly and avoids the problems which attend the use of the ammonium salt. Third, the synthesis involves no filtrations; the only manipulations required in purification of the acetyl phosphate are liquid–liquid extractions. Fourth, the synthesis involves no experimental steps requiring anhydrous solvents and should be amenable to scaling up to generate larger quantities of acetyl phosphate than that given in the experimental procedure described in the Experimental Section (1.8 mol).

This procedure and a related one involving direct reaction of acetic anhydride and phosphoric acid in water[16] are the most practical procedures now available to prepare

(11) For studies of the hydrolytic stability of acetyl phosphate see: Kurz, J. L.; Gutsche, C. D. *J. Am. Chem. Soc.* **1960**, *82*, 2175–81. Di Sabato, G.; Jencks, W. P. *Ibid.* **1961**, *83*, 4393–400, 4400–5. Oestreich, C. H.; Jones, M. M. *Biochemistry* **1966**, *5*, 2926–31. Koshland, D. E. *J. Am. Chem. Soc.* **1952**, *74*, 2286–92.

(12) In the reaction mixture ^{31}P NMR established the presence of phosphoric acid (−1.4 ppm), acetyl phosphoric acid (7.2 ppm), pyrophosphoric acid (13.0 ppm), and a material assigned the structure diacetyl phosphoric acid (+17.6 ppm) (85% phosphoric acid was the external reference). The pyrophosphate fraction of the reaction mixture increases as reaction temperature increases.

(13) Enzymatic assays indicate that more acetyl phosphate moieties are available in phosphate transfer reactions than are calculated to be present from the intensity of the monoacetyl phosphate peak observed in ^{31}P NMR spectroscopy. The peak assigned to diacetyl phosphate is still present after workup and does not seem to diminish in aqueous solution in the course of 1 h at room temperature (pH ~3). The reactivity of the diacetyl phosphate is in accord with the fact that acyclic phosphodiesters demonstrate slower hydrolysis or displacement reactions than phosphomonoesters and triesters (Cox, J. R.; Ramsay, O. B. *Chem. Rev.* **1964**, *64*, 317–52).

(14) The Michaelis constant for acetate is 0.3 M. (Bergmeyer, H. U. "Methods of Enzymatic Analysis", 2nd ed.; Verlag Chemie: Weinheim, FRG, 1965; Vol. 1, p 425). We observe significant inhibition of the rate of conversion of ADP to ATP by acetyl phosphate when the acetate concentration exceeds 0.6 M.

(15) Ethyl acetate was chosen as solvent for extraction of acetic acid for a number of reasons: it is already part of the acetyl phosphate synthesis; it is nontoxic; residual ethyl acetate dissolved in the acetyl phosphate solution does not seem to inactivate immobilized enzymes. The partition coefficient of acetic acid between water and ethyl acetate at pH ~2.4 and 18 °C is reported to be 0.89 (Eaglesfield, P.; Kelly, B. K.; Short, J. F. *Ind. Chem.* **1953**, *29*, 147, 243–250). This coefficient is in qualitative accord with the extraction efficiency we observe.

The extraction of acetic acid from water by using trioctylphosphine oxide (TOPO)/hydrocarbon mixtures (Kohn, P. M.; *Chem. Eng.* **1978**, *83*, 58–60) is not employed for two reasons. First, it adds another component to the system and seems unnecessary in the light of the efficiency of ethyl acetate as the extractant. Second, although the efficiency of TOPO/hydrocarbon mixtures is very high for dilute acetic acid, it decreases at higher concentrations of acetic acid (0.2 wt % acetic acid, K_d = 3.4; but at 7.5 wt % acetic acid, K_d = 0.5. Richer, N. L.; Michaelis, J. N.; King, C. J. *J. Separ. Proc. Technol.* **1979**, *1*, 36–41). In our acetyl phosphate synthesis the initial acetic acid concentration is 18–20 wt %.

Although TOPO has been reported to extract stronger acids more efficiently than acetic acid (Wardell, J. M.; King, C. J. *J. Chem. Eng. Data* **1978**, *23*, 144–8), it extracts the acetyl phosphoric acid considerably less efficiently than acetic acid at pH ≃3.

(16) Kazlauskas, R. J.; Whitesides, G. M., unpublished.

the acetyl phosphate required in ATP cofactor regeneration for synthetic organic applications. Because the stability in solution of the sodium acetyl phosphate is so much higher than that of ammonium acetyl phosphate,[9,11] one advantage previously ascribed to phosphoenol pyruvate as the ultimate phosphorylating agent in cofactor regeneration (its solution stability) becomes less important. PEP remains much less readily hydrolyzed than acetyl phosphate, but the difference does not justify the greater inconvenience and expense of the PEP synthesis. PEP remains the reagent of choice when a strongly phosphorylating system is required (for example, to drive an unfavorable equilibrium[6]).

Experimental Section

General Methods. Chemicals were reagent grade and were used without further purification. Phosphoric acid (85%) was converted to 100% phosphoric acid when required by treatment with P_2O_5.[17] Enzymes, acetyl phosphate (Li–K salt), ADP (Na salt), and NAD (Na salt) were obtained from Sigma. The PAN employed in enzyme immobilization was synthesized by a procedure described previously.[18] Water was distilled twice, the second time from glass. A Horizon pH controller, Ecology Co., Chicago, was used to control pH in the preparation of glucose-6-phosphate. The enzymatic assay used to determine yield and purity of acetyl phosphate was that described previously,[19] with the substitution of NAD for NADP.

Disodium Acetyl Phosphate (Procedure in Concentrated Solution). Phosphoric acid (85%, 2.0 mol, 135 mL) was mixed with ethyl acetate (2 mol, 196 mL) in a 1-L flask. The flask was immersed in an ice bath. When the temperature of the solution had reached 13–15 °C, acetic anhydride (4 mol, 376 mL) was added. The addition rate was regulated to keep the temperature of the reaction mixture between 24 and 27 °C. All the acetic anhydride was added within 25 min. The solution was left for 5 min at room temperature and then added to a mixture of 1 L of water, 500 g of ice, and 168 g of sodium bicarbonate in a 5-L flask. The suspension was stirred until no more carbon dioxide was evolved (~30 min). The resulting solution (pH ≃3) was extracted twice with 1.8-L portions and once with a 1.0-L portion of ethyl acetate. After neutralization of the aqueous solution of acetyl phosphate with 10 M sodium hydroxide, ~40 mL of ethyl acetate separated as a second phase. The ethyl acetate layer could be separated by using a separatory funnel or removed by decantation if the aqueous solution was frozen for storage. The acetyl phosphate concentration in the final solution (1.7 L) was 1.02 M by enzymatic assay; the yield was 1.73 mol (87%). The acetate concentration was 0.35 M.[20]

These aqueous solutions of disodium acetyl phosphate were stored at 0 °C or frozen at −17 °C. In solution, enzymatic assays indicated a loss of <1% of acetyl phosphate over a 2-week period.

Disodium Acetyl Phosphate (Procedure in Dilute Solution). Phosphoric acid (85%, 2.0 mol, 135 mL) was dissolved in 1.2 L of ethyl acetate in a 2-L flask. The solution was cooled to 0 °C, and precooled (0 °C) acetic anhydride (4.0 mol, 376 mL) was slowly added over 40 min. The mixture was stirred for 6 h at 0 °C and added to a suspension of ca. 1 L of water, 500 g of ice, and 168 g of sodium bicarbonate in a 5-L flask. The resulting mixture was stirred at 0 °C until no more carbon dioxide was evolved. The organic layer was separated and discarded. The resulting solution (pH ≃3.0) was washed with one 1.8-L portion and one 1.0-L portion of ethyl acetate to remove most of the acetic acid. After neutralization of the aqueous solution of acetyl phosphate with 10 M sodium hydroxide, ~40 mL of ethyl acetate separated as a second phase. The ethyl acetate layer was removed as described above. The concentration of acetyl phosphate in the final solution (1.68 L) was 1.10 M by enzymatic assay; the yield was 1.86 mol (93%). The acetate concentration was 0.4 M.[20]

Glucose-6-phosphate.[21] A 1-L aqueous solution of glucose (1 mol), ATP (7 mmol), $MgCl_2$ (30 mmol), and 2-mercaptoethanol (17 mmol) was adjusted to pH 7 and deoxygenated. This solution was added to a suspension of immobilized[21] hexokinase (E.C. 2.7.1.1, 500 U) and acetate kinase (E.C. 2.7.2.1, 700 U) and left at ambient temperatures under argon. Disodium acetyl phosphate (1.1 mol in 1.2 L of solution) was added over 7 days. The reactor was left for 2 days after the end of acetyl phosphate addition, after which enzymatic assay showed 97% conversion of glucose to glucose-6-phosphate and no significant remaining acetyl phosphate. The solution was separated from the enzyme-containing gel by decantation. A solution of barium chloride (0.25 mol in 200 mL of water) was added, and the precipitated barium phosphate was separated by filtration. An additional quantity of barium chloride (1.3 mol in 700 mL of water) was added, and the barium salt of glucose-6-phosphate was allowed to precipitate for 2 days at 4 °C. After filtration and drying, a total of 0.92 mol (92%) of glucose-6-phosphate was obtained (520 g of solid containing 93% barium glucose-6-phosphate as determined by enzymatic assay). The turnover number for ATP during the synthesis was 140, and the activities of enzymes recovered in the gel were as follows: hexokinase, 92%, and acetate kinase, 83%.

Registry No. Disodium acetyl phosphate, 55660-60-1; glucose-6-phosphate, 56-73-5; ATP, 56-65-5.

(17) Conversion of 85% phosphoric acid to 100% phosphoric acid is described in ref 5.
(18) Pollak, A.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1977**, *99*, 2366–7.
(19) Whitesides, G. M.; Siegel, M.; Garrett, P. *J. Org. Chem.* **1975**, *40*, 2516–9.
(20) The acetate content in the acetyl phosphate solution can be reduced by modifying the ethyl acetate extraction procedure. Four extractions with 1.8-L portions of ethyl acetate or two 3.6-L portions of ethyl acetate followed by one 1.8-L portion will in both cases lead to less than 0.1 M acetate in the acetyl phosphate solution.
(21) Pollak, A.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1977**, *99*, 2366–7.

Synthesis and Electrochemical Reduction of [2-(1,4-Benzoquinonyl)ethyl]-1,4-benzoquinone[1]

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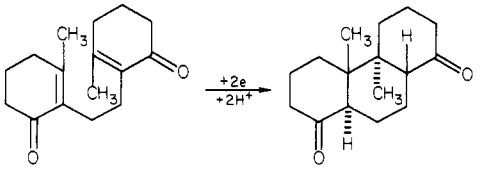
The one-electron electrochemical reduction of α,β-unsaturated carbonyl system has been much studied[4] and found to be a useful method of forming carbon to carbon bonds. We have used this process[5] for the synthesis of the *trans*-anti-*trans*-perhydrophenanthrene ring system in the reduction of the dienedione **1** to **2**. As an extension of this



1 **2**

(1) This paper is dedicated to Dr. Charles T. Lester in honor of his retirement.
(2) Work done in partial fulfillment of the Ph.D. requirements, Emory University, 1979.
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