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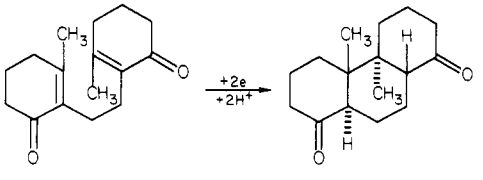
The one-electron electrochemical reduction of α,β-unsaturated carbonyl system has been much studied[4] and found to be a useful method of forming carbon to carbon bonds. We have used this process[5] for the synthesis of the *trans*-anti-*trans*-perhydrophenanthrene ring system in the reduction of the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

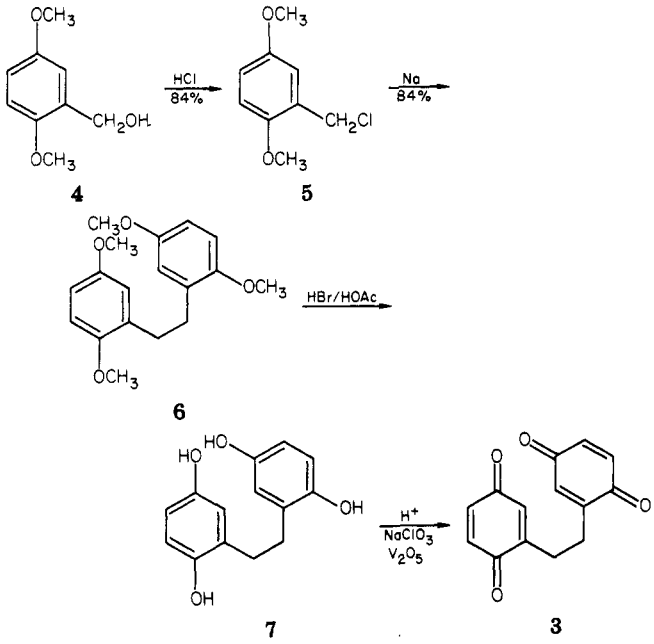


1 **2**

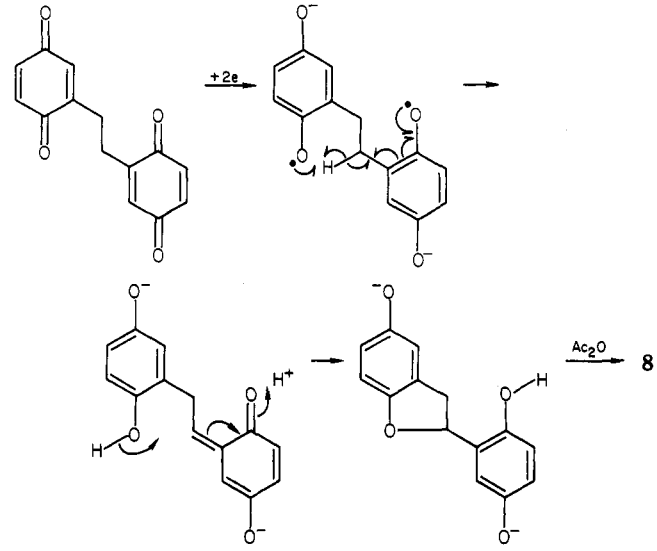
(1) This paper is dedicated to Dr. Charles T. Lester in honor of his retirement.
(2) Work done in partial fulfillment of the Ph.D. requirements, Emory University, 1979.
(3) Senior Scientist, Nicolet Corp., Fremont, CA.
(4) Simmer, J. P.; Richards, J. A.; Turner, J. C.; Evans, D. H. *Anal. Chem.* **1971**, *43*, 1000 and references cited therein. Weinburg N. L. "Technique of Electro-Organic Synthesis"; Rift, M. R., Ed., Wiley: New York, 1974; Part II, Chapter VIII.
(5) Mandell, L., Daley, R. F., Day, R. A., Jr. *J. Org. Chem.* **1976**, *41*, 4087.

0022-3263/83/1948-3132$01.50/0

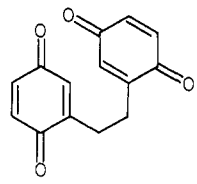
Scheme I



Scheme II



work, we decided to investigate the electrochemical reduction of [2-(1,4-benzoquinonyl)ethyl]-1,4-benzoquinone (3) to see if phenanthrene derivatives might be produced



3

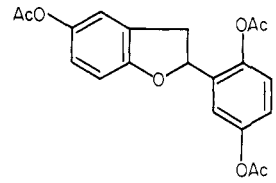
via intramolecular coupling. This report presents the synthesis of 3 by a modification of the route of Wegner et al.[6] and the elucidation of the structure of the major electrochemical reduction product isolated.

The synthesis of the bis quinone 3 is given in Scheme I. Thus, treatment of the benzyl alcohol 4 with hydrochloric acid afforded the chloride 5. Wurtz coupling with sodium in ether produced 6 which was cleaved with HBr/HOAc to *crude* bis hydroquinone 7. The crude hydroquinone was oxidized to the bis quinone by using a procedure developed by Underwood and Walsh[8] which yielded 3 (67%) from the tetraether 6.

The polarography of the bis quinone 3 was studied under a variety of conditions, with the best results being obtained on using 0.1 M tetra-*n*-butylammonium tetrafluoroborate as the electrolyte in either methylene chloride or acetonitrile as the solvent. The half-wave reduction potentials in methylene chloride were found to be -0.450 and -0.830 V, with each polarographic wave corresponding to two-electron additions.

The controlled potential reduction of 3 was performed at a potential of -0.600 V in methylene chloride with 0.1 M n-Bu_4NBF_4 as the electrolyte. Difficulty was experienced in trying to isolate the direct product of this reduction due to solubility problems. To circumvent this, we acetylated the reduction product by the addition of acetic anhydride after the completion of the electrolysis, and the acetylated reduction products were chromatographed on silica gel.

One major product was isolated pure in modest yield (14%). Elemental analysis ($C_{20}H_{18}O_7$) and spectra were compatible with structure 8. Thus, the presence of three



8

acetates was confirmed by NMR (proton and ^{13}C). Six aromatic protons and the ABX pattern of the CH_2–CH(OAc) grouping were also indicated in the proton NMR. The mass spectrum exhibited peaks at (M – CH_2CO), (M – $2CH_2CO$) and (M – $3CH_2CO$), indicating each of the acetates was an aryl acetate[7].

It is possible to picture a logical mechanism by which the structure 8 may be derived (Scheme II). Final confirmation of this structure as the reduction product was provided by X-ray crystal structure determination.

Experimental Section

Proton magnetic resonance spectra were obtained on a Varian Model EM-360 spectrometer with $CDCl_3$ as the solvent and Me_4Si as the internal reference. Carbon-13 magnetic resonance spectra were run on a Varian CFT-20 spectrometer. Mass spectra were recorded on a Finnigan 4000 GC/MS data system mass spectrometer and infrared spectra on a Perkin-Elmer Model 257 spectrometer in spectrograde $CHCl_3$. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Polarography and controlled potential reductions were done by using a Princeton Applied Research Model 170 electrochemistry system.

2,5-Dimethoxybenzyl Chloride (5). To 15 mL (0.18 mol) of concentrated HCl in a 125-mL separatory funnel was added 10 g 0.06 mol) of 2,5-dimethoxybenzyl alcohol (4, Aldrich Chemical Co.). The mixture was vigorously shaken (venting frequently) for 15–20 min. Ether (50 mL) was then added to dissolve the white solid formed during the reaction. The ether layer was separated, washed with saturated bicarbonate solution, dried over Na_2SO_4, and concentrated on a rotary evaporator. The crystalline residue was recrystallized from ether/hexane to afford 5: 9.35 g (84.5%); mp 69–71 °C, NMR δ 3.70 (s, 3 H), 3.80 (s, 3 H), 4.58 (s, 2H), 6.85 (m, 3 H); IR 3000 (m), 2940 (s), 2900 (m), 2830 (s), 1590 (w), 1490

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(s), 1460 (s), 1280 (s), 1220 (s), 1040 (s), mass spectrum, m/e 186 (M^+). Anal. Calcd for $C_9H_{11}ClO_2$: C, 57.95; H, 5.96; Cl, 18.93. Found: C, 57.95; H, 5.96; Cl, 18.93.

Tetraether 6. To a suspension of 1.06 g (0.046 mol) of sodium wire in 125 mL of anhydrous ether in a three-necked, 500-mL, round-bottomed flask equipped with a reflux condenser, mechancial stirrer, and a 125-mL pressure-equalizing addition funnel was added, under a nitrogen atmosphere, a solution of 5.0 g (0.027 mol) of 2,5-dimethoxybenzyl chloride in 100 mL of anhydrous ether. During the addition the solution was rapidly stirred and after the addition the reaction mixture was refluxed 48 h under a nitrogen atmosphere.

The suspension was then cooled to room temperature and methanol slowly added until further addition ceased to cause refluxing. This mixture was refluxed 2 h to assure decomposition and cooled, and water was added until the suspension became a colorless two-layered solution. The ether layer was separated and combined with additional ether extracts of the aqueous layer. the ethereal solution was washed twice with water, dried over sodium sulfate, and concentrated on a rotary evaporator. The solid residue was recrystallized from ether/hexane to afford 3.42 g (84%) of a white crystalline solid: mp 61–69 °C, NMR δ 2.85 (s, 4 H), 3.70 (s, 6 H), 3.75 (s, 6 H), 6.70 (s, 6 H); mass spectrum, m/e 302 (M^+). Anal. Calcd for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.44; H, 7.35.

Bis Hydroquinone 7. A mixture of 100 mL of 48% hydrobromic acid, 100 mL of glacial acetic acid, and 2 g (0.0066 mol) of tetraether **6** was refluxed overnight under a nitrogen atmosphere. The solution was cooled, 250 mL water added, and the mixture extracted with several portions of ether. The combined ether layers were dried over sodium sulfate and concentrated on a rotary evaporator to provide 1.57 g (96.3%) of crude bis hydroquinone **7**. This crude material was used directly in the next reaction: NMR δ 2.80 (s, 4 H), 6.65 (m, 6 H).

[2-(1,4-Benzoquinonyl)ethyl]-1,4-benzoquinone (3). A mixture of 1.57 g of crude bis hydroquinone **7**, 150 mL of 2% H_2SO_4, 15 g (0.14 mol) of sodium chlorate, and 0.1 g of vanadium pentaoxide was warmed to 50 °C, with stirring, until the bluish black suspension of starting material changed to golden yellow. After cooling, the reaction mixture was extracted thoroughly with methylene chloride. The extract was dried over sodium sulfate, filtered through a silica gel column, and concentrated on a rotary evaporator. The golden solid thus obtained was recrystallized from methylene chloride/hexane to yield **3**: 1.07 g (67% from tetraether **6**); decomposition point 185 °C; NMR δ 2.70 (s, 4 H), 6.60 (s, 2 H), 6.80 (m, 4 H); mass spectrum, m/e 242 (M^+); IR 2980 (w), 1660 (s), 1600 (w), 1175 (s). Anal. Calcd for $C_{14}H_{10}O_4$: C, 69.41; H, 4.14. Found C, 69.30; H, 4.19.

Polarography of 3. the polarographic measurements were made with a Princeton Applied Research Model 170 electrochemistry system, using a dropping mercury electrode (DME) and a saturated calomel electrode (SCE) in a 10-mL H-type polarographic cell. The methylene chloride solution, 0.001 M in bis quinone **3** and 0.1 M in tetra-*n*-butylammonium tetrafluoroborate, was purged with nitrogen for 15 min before the run. A blank solution, consisting of 0.1 M *n*-Bu_4NBF_4 in CH_2Cl_2, was run to ensure that the waves observed were actually due to the bisquinone **3**. The half-wave potentials for this compound were found to be -0.450 and -0.830 V.

Controlled Potential Electrolysis of 3. The Princeton Applied Research Model 170 was also used for the controlled potential electrolysis. The electrolysis cell was a conventional three-electrode system: a mercury (instrument grade) pool working electrode (cathode), a saturated calomel reference electrode, and a Ag/AgCl auxiliary electrode (anode), which was separated from the solution by a fritted-glass disk. The reduction was done under a nitrogen atmosphere. The mercury pool was stirred rapidly throughout the electrolysis with a magnetic stirrer. Tetra-*n*-butylammonium tetrafluoroborate was used as the supporting electrolyte. Prior to the electrolysis the system was purged with nitrogen for approximately 20 min before the electrolysis was begun. The cell contained 1.0 g (4.132×10^{-3} mol) of bis quinone **3** dissolved in 250 mL of anhydrous methylene chloride which was 0.1 M in electrolyte. The electrolysis was started at -0.600 V vs. SCE and was complete in 4 h as indicated by a return to background levels of current. After electrolysis was complete, 2.7 g (2.648×10^{-2} mol) of acetic anhydride was added to the electrolysis cell with stirring and with no current passing through the cell. After being stirred overnight, the contents of the cell were poured into a separatory funnel. The mercury was taken off, and the methylene chloride solution was filtered and concentrated on a rotary evaporator. The residue was taken up in a minimum amount of methylene chloride, diluted with ether, and washed with water. The ether layer on being allowed to stand precipitated tetra-*n*-butylammonium tetrafluoroborate. The precipitated salt was filtered off, and the ethereal solution was dried over sodium sulfate and concentrated on a rotary evaporator. The crude material (0.6430 g) was chromatographed on Silicar CC-7 by using 25% ether/hexane. The first component (82.3 mg) was not characterized. The second component (62.6 mg) was not characterized. The third component (204.0 mg, 14%) was characterized. Spectral data are given below. The fourth component was a mixture and was identified as partially acetylated "third component" and constituted 208.3 mg.

Component three: NMR δ 2.16 (s, 3 H), 2.20 (s, 6 H), 3.30 (dd, 2 H), 5.68 (t, 1 H), 6.68, 6.92, 7.05 (m, 6 H), IR 1760 (s), 1480 (s), 1370 (s), 1170 (s), 1180 (s), 1230 (s), 1050 (m), mass spectrum, m/e 370 (M^+), 328, 281, 244, 100 (base peak). Anal. Calcd for $C_{20}H_{18}O_7$: C, 64.85; H, 4.91; O, 30.24. Found : C, 64.79; H, 4.97; O, 30.24.

Crystal Structure Determination. Single crystals of compound **8** ($C_{20}H_{18}O_7$) grown from an ether/hexane solution were used for the measurement of the X-ray diffraction data. Unit cell parameters were determined on a Syntex $P2_1$, four-circle diffractometer equipped with graphite monochromator (Bragg 2θ angle = 12.2°) by using MoKα radiation. Least-squares refinement of 15 reflections with Bragg angles ranging from 5.34° to 12.84° resulted in a = 11.094 (4) Å, b = 7.499 (4) Å, c = 23.141 (7) Å, and β = 98.70 (3°). The unit cell volume is 1903 (1) Å. The systematic absences were consistent with the space group $P2_1/a$. A total of 1911 integrated independent reflections with $4° < 2\theta < 50°$ were measured by using the 2θ–θ scan technique and found to have $I > 3\sigma(I)$. Lorentz and polarization corrections were made in the usual way, and the structure was solved by using the direct-methods procedure implemented in the SHELXTL program system (Nicolet XRD, Fremont, CA).

The positional and anisotropic thermal parameters were refined for all nonhydrogen atoms by using full-matrix least-squares analysis. The hydrogen atoms were placed in their calculated positions. The weights used were $1/\sigma^2(F) + 0.001\,F^2$, and the final reliability index R (defined as $\sum|F_o - F_c|/\sum F_o$) was 0.0741 and R_w (defined as $\sum w^{1/2}|F_o - F_c|\sum F_o w$) was 0.0735.

Registry No. **3**, 20452-50-0; **4**, 33524-31-1; **5**, 3840-27-5; **6**, 20306-76-7; **7**, 10365-14-7; **8**, 86689-90-9; 8 trihydroxy derivative, 86689-91-0.

Supplementary Material Available: A structure (Figure 1) and tables of atomic coordinates for structure **8** (3 pages). Ordering information is given on any current masthead page.

1,3 Acyl Migration to an Epoxide. Reversible Rearrangement of 5,6β-Epoxyepicholesteryl Acetate

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The work of Henbest in the 1950's established the directing influence of the hydroxyl function in epoxidation of allylic and homoallylic alcohols.[1] Thus epicholesterol (**1b**) reacts with peracids to give exclusively the α-epoxide

(1) Henbest, H. B.; Wilson, R. A. L. *J. Chem. Soc.* **1957**, 1958.

0022-3263/83/1948-3134$01.50/0