Traces of acid isomerize **5** ($n = 8$) into an approximate 1:1 equilibrium mixture with **11** ($n = 8$). ^{1}H NMR: 5.44 (d, $J \approx 6$, 1 H); remainder are unresolved. ^{13}C NMR: 139.89 (q); 126.92 (CH); 41.99 (CH_2); 36.94 (CH_2); 36.53 (CH_2); 35.06 (CH_2); 33.45 (CH_2); 31.98 (CH_2); 30.59 (CH); 28.50 (CH); 27.30 (CH_2); 25.90 (CH_2); 25.25 (CH); 25.17 (CH_2); 24.70 (CH_2); 24.13 (CH_2); 22.61 (CH_2). MS: m/z = 232 (M^+, 35), 93 (58), 92 (100), 91 (75), 79 (51). On GLC isomer **11** ($n = 8$) has the shorter retention time.

***exo*-7-Methyl-3-methylenebicyclo[3.3.1]nonane (13).** This alkene has been reported[26] from the partial hydrogenation of the dimethylene analogue but is more easily prepared by methylenation of the ketone with Lombardo's reagent.[27] To a solution of the ketone (113.3 mg, 0.75 mmol) in methylene chloride (2 mL) and THF (0.5 mL) was added the methylenation reagent as a gray slurry. The starting material had disappeared in the GLC after 30 min. Standard workup and flash chromatography with pentane yielded **13** as a colorless oil (74.8 mg, 67%), pure by GLC and NMR.

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(26) Martynova, E. N.; Yurchenko, A. G. *Khim. Tekhnol. (Kiev)* **1973**, *2*, 3-5.

(27) Lombardo, L. *Tetrahedron Lett.* **1982**, *23*, 4293.

Electrochemical Oxidation of Enamines Related to the Key Intermediate on Thiamin Diphosphate Dependent Enzymatic Pathways: Evidence for One-Electron Oxidation via a Thiazolium Cation Radical

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Abstract: Electrochemical experiments were performed in Me_2SO on a number of 2-alkyl and 2-benzylthiazolium ions in the absence and presence of sodium bis(trimethylsilyl)amide ($pK = 26$). Under the latter conditions there is quantitative deprotonation at the C2α position leading to enamines that are structurally similar to the key enzyme-bound enamine intermediate present on all thiamin diphosphate dependent enzymes (Jordan, F.; Kudzin, Z. H.; Rios, C. B. *J. Am. Chem. Soc.* **1987**, *109*, 4415). For enamines derived from 2-alkylthiazolium salts in the presence of the strong base, but not in its absence, there was observed a cyclic voltammogram that is characteristic of irreversible processes. The relative peak potentials indicated that all enamines examined were easier to oxidize than ferrocene. The oxygen substituent at the C2α position facilitated oxidation compared to the hydrogen substituent by ca. 250 mV, and by ca. 120 mV compared to the methyl substituent. A series of para-substituted 2-(1-methoxy-1-phenylmethyl)thiazolium salts, when deprotonated at the C2α position, underwent reversible one-electron oxidation and gave a Hammett $\rho = -7.61$. The remarkably facile oxidation of all enamines was shown to proceed by a single-electron transfer according to controlled potential coulometry. The resulting cation radical undergoes dimerization according to spectroscopic analysis of the predominant product pursuant to electrolysis. Especially, the enamine derived from 2-(1-methoxyethyl)-3,4-dimethylthiazolium ion is a close analogue of the thiamin-bound intermediate. The results suggest that those enzymes responsible for oxidation at the C2α position may proceed by a stepwise redox mechanism via a thiazolium cation radical. These results constitute the first electrochemical determination of the redox properties of this key enamine intermediate and appear to have direct relevance to at least one enzymatic oxidative decarboxylation of pyruvic acid,pyruvate-ferrodoxin oxidoreductase, reported to proceed by a radical mechanism (Docampo, R.; Moreno, S. J.; Mason, R. P. *J. Biol. Chem.* **1987**, *262*, 12417).

There are a number of distinct thiamin diphosphate (TDP) dependent enzymes known to catalyze the oxidative decarboxylation of α-keto acids: (1) the α-keto acid dehydrogenases that utilize lipoamide as the immediate oxidizing agent and yield acetyl-coenzyme A;[1] (2) pyruvate oxidase utilizes flavin adenine dinucleotide as the oxidizing agent and yields acetate;[2] and (3) pyruvate-ferredoxin oxidoreductase[3a] and pyruvate-NADP oxidoreductase,[3b] also yielding acetyl-coenzyme A. All of these enzymes probably follow a common pathway through the decarboxylation step, forming an enamine or 2α-carbanion intermediate, similar in structure to **2**. We reported recently the generation of models for this key enamine intermediate,[4a,b] as well as pK_a's in Me_2SO for the precursor 2-(1-hydroxyethyl)thiazolium salts in which the alcohol function was protected.[5] Those models can also be used to help delineate the oxidative pathways. Several issues need to be resolved. One concerns whether the oxidation of an aldehyde to an acyl equivalent takes place by a series of two single-electron-transfer steps, or by a concerted two-electron transfer. A second issue is whether the oxidation involves a distinct covalent intermediate between the oxidant and the reductant. Yet a third concerns the true substrate for oxidation.

We here report that the enamines derived from a number of 2-alkyl and 2-benzylthiazolium salts, and not the salts themselves, undergo facile electrochemical oxidation in Me_2SO.

Experimental Section

Synthesis of 2-alkyl and 2-benzylthiazolium salts was reported elsewhere.[4a,5,6] Briefly, to synthesize the 2-(1-hydroxybenzyl)thiazolium ions, the thiazole was condensed with the appropriate benzaldehyde, the hydroxy functional group was methylated with NaH/MeI then the ni-

(1) Reed, L. J. *Acc. Chem. Res.* **1974**, *7*, 40.
(2) Hager, L. P. *J. Biol. Chem.* **1957**, *229*, 251.
(3) (a) Kerscher, L.; Nowitzki, S.; Oesterhelt, D. *Eur. J. Biochem.* **1982**, *128*, 223. (b) Inui, H.; Ono, K.; Miyatake, K.; Nakano, Y.; Kitaoka, S. *J. Biol. Chem.* **1987**, *262*, 9130.
(4) (a) Jordan, F.; Kudzin, Z. H.; Rios, C. B. *J. Am. Chem. Soc.* **1987**, *109*, 4415. (b) Jordan, F.; Kudzin, Z. H.; Rios, C. B. *Stud. Org. Chem.* **1987**, *31*, 461.
(5) Bordwell, F. G.; Satish, A. V.; Jordan, F.; Rios, C. B.; Chung, A. C. *J. Am. Chem. Soc.* **1990**, *112*, 792.
(6) Rastetter, W. H.; Adams, J.; Frost, J. W.; Nummey, L. J.; Frommer, J. E.; Roberts, K. B. *J. Am. Chem. Soc.* **1979**, *101*, 2752.

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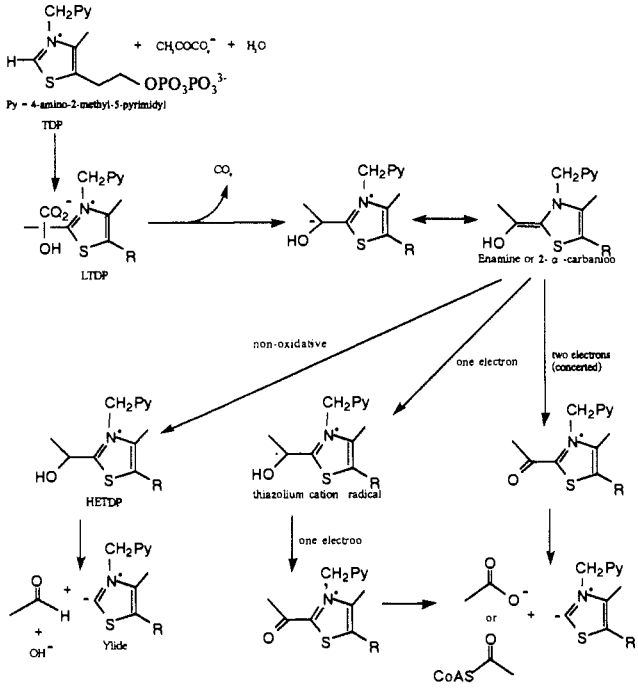
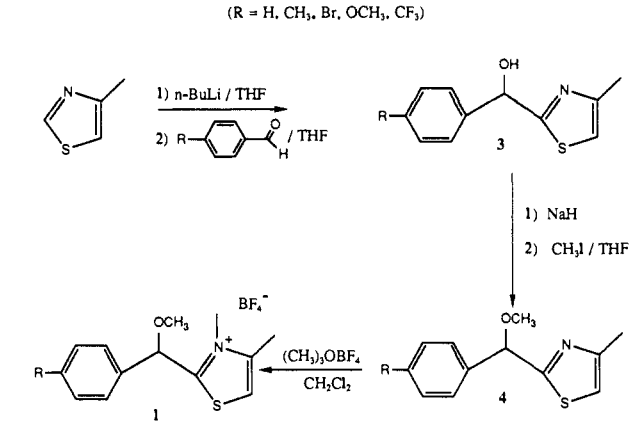
Scheme I. Oxidative vs Nonoxidative Decarboxylation of Pyruvate



Scheme II



trogen was quaternized with Me_3OBF_4 according to Scheme II. The synthesis and analytical data on the new compounds are summarized below. Thiazole precursors were purchased from Pyrazine Specialties, Atlanta, GA.

2-(1-Hydroxy-1-phenylmethyl)-4-methylthiazole (3g). 4-Methylthiazole (6.0 mL, 60.0 mmol) in 5 mL of dry THF was added dropwise to a solution of 60.0 mmol *n*-BuLi in 6.0 mL of THF at −78 °C under Ar. The reaction mixture was stirred for 30 min; then benzaldehyde (6.37 g, 60.0 mmol) in THF was added to the stirred solution in three portions. The mixture was stirred for 2 h and then quenched with 15 mL of H_2O–EtOH (1:1). The reaction mixture was concentrated in vacuo. The crude yellow product was dissolved in anhydrous ether and the precipitate was filtered by using a fine sintered-glass funnel. The solution was concentrated in vacuo to afford 11.2 g (91%) of **3g** as an oil, which was crystallized from ether–hexane (9:1): ^{1}H NMR (400 MHz, $CDCl_3$–TMS) δ 7.44 (m, 2 H, Ar), 7.33 (m, 3 H, Ar), 6.82 (s, 1 H, C5-H), 5.99 (s, 1 H, C2α-H), 3.88 (br, 1 H, OH), 2.37 (s, 3 H, C4-CH_3).

2-(1-Methoxy-1-phenylmethyl)-4-methylthiazole (4g). To a stirred solution of **3g** (2.0 g, 9.79 mmol) in 30 mL of dried THF at 0 °C under Ar was added NaH (0.26 g, 10.77 mmol) in one portion. The mixture was stirred 30 min and then a solution of CH_3I (1.53 g, 10.77 mmol) in 5 mL of THF was added. After 2 h the mixture was quenched with 20 mL of H_2O–EtOH (1:1) and concentrated in vacuo to afford an oil, which was extracted into ethyl ether–H_2O. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to afford 2.0 g (94%) of **4g**. The crude yellow product was chromatographed on 350 g of silica gel and eluted with ether–hexane (1:1): ^{1}H NMR (400 MHz, $CDCl_3$–TMS) δ 7.44 (m, 2 H, Ar), 7.40 (m, 3 H, Ar), 6.82 (s, 1 H, C5-H), 5.51 (s, 1 H, C2α-H), 3.43 (s, 3 H, OCH_3), 2.39 (s, 3 H, C4-CH_3).

2-(1-Methoxy-1-phenylmethyl)-*N*,4-dimethylthiazolium Fluoroborate (1g). To a solution of compound **4g** (0.5 g, 2.3 mmol) in 20 mL of anhydrous CH_2Cl_2 at 0 °C, was added $(CH_3)_3OBF_4$ (0.34 g, 2.3 mmol) in one portion. The suspension was stirred for 20 h and then concentrated in vacuo. The resulting thick slurry was washed several times with anhydrous ether until compound **1g**, 0.6 g (81%), precipitated as a white powder: ^{1}H NMR (200 MHz, Me_2SO–TMS): δ 7.77 (s, 1 H, C5-H), 7.46 (m, 5 H, Ar), 5.92 (s, 1 H, C2α-H), 3.80 (s, 3 H, N-CH_3), 3.45 (s, 3 H, OCH_3), 2.48 (s, 3 H, C4-CH_3). Anal. Calcd for $C_{13}H_{16}NOS\cdot BF_4\cdot H_2O$: C, 46.04; H, 5.34; N, 4.12; S, 9.45. Found: C, 46.21; H, 4.89; N, 3.98; S, 9.39.

2-[1-Hydroxy-1-(*p*-bromophenyl)methyl]-4-methylthiazole (3i). In a similar manner as shown for the synthesis of **3g**, 13 g (76%) of **3i** was obtained as a yellow oil, which was then chromatographed on 350 g of silica gel and eluted with ether–hexane (1:2). The combined fractions were concentrated in vacuo and crystallized from ether–hexane to afford 5.6 g (33%) of product: ^{1}H NMR (200 MHz, $CDCl_3$–TMS) δ 7.52 (d, 2 H, J = 8.4 Hz, Ar), 7.36 (d, 2 H, J = 8.4 Hz, Ar), 6.82 (s, 1 H, C5-H), 5.95 (s, 1 H, C2α-H), 4.39 (br, 1 H, OH), 2.36 (s, 3 H, C4-CH_3).

2-[1-Methoxy-1-(*p*-bromophenyl)methyl]-4-methylthiazole (4i). In a similar manner as shown for the synthesis of **4h**, compound **4i** was obtained as a yellow oil, which was purified by column chromatography (1:1 ether–hexane, 300 g of silica gel) to afford 0.6 g (60%) of product: ^{1}H NMR (200 MHz, $CDCl_3$–TMS) δ 7.38 (d, 2 H, J = 8.5 Hz, Ar), 7.28 (d, 2 H, J = 8.5 Hz, Ar), 6.85 (s, 1 H, C5-H), 5.47 (s, 1 H, C2α-H), 3.42 (s, 3 H, OCH_3), 2.39 (s, 3 H, C4-CH_3).

2-[1-Methoxy-1-(*p*-bromophenyl)methyl]-*N*,4-dimethylthiazolium Fluoroborate (1i). In a similar manner as shown for the synthesis of **1h**, 0.4 g (60%) of **1i** was obtained as a white powder: ^{1}H NMR (200 MHz, $CDCl_3$–TMS) δ 7.63 (d, 2 H, J = 8.5 Hz, Ar), 7.38 (d, 2 H, J = 8.5 Hz, Ar), 7.58 (s, 1 H, C5-H), 5.94 (s, 1 H, C2α-H), 3.88 (s, 3 H, N-CH_3), 3.49 (s, 3 H, OCH_3), 2.52 (s, 3 H, C4-CH_3). Anal. Calcd for $C_{13}H_{15}NOSBr\cdot BF_4$: C, 39.03; H, 3.78; N, 3.50; S, 8.01. Found: C, 38.78; H, 3.66; N, 3.24; S, 7.74.

2-[1-Hydroxy-1-[*p*-(trifluoromethyl)phenyl]methyl]-4-methylthiazole (3h). In a similar manner as shown for the synthesis of **3g**, compound **3h** was obtained as a yellow oil, which was crystallized from ether–hexane to afford 1.85 g (12%) of white crystals: ^{1}H NMR (200 MHz, $CDCl_3$–TMS) δ 7.53 (s, 4 H, Ar), 6.79 (s, 1 H, C5-H), 6.05 (s, 1 H, C2α-H), 5.65 (s, 1 H, OH), 2.35 (s, 3 H, C4-CH_3).

2-[1-Methoxy-1-[*p*-(trifluoromethyl)phenyl]methyl]-4-methylthiazole (4h). In a similar manner as shown for the synthesis of **4g**, 0.9 g (85%) of **4h** was obtained as an oil, which was then chromatographed on 250 g of silica gel and eluted with ether–hexane (1:1). The combined fractions containing the product were concentrated in vacuo to afford 0.55 g (55%) of a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$–TMS) δ 7.60 (s, 4 H, Ar), 6.65 (s, 1 H, C5-H), 5.59 (s, 1 H, C2α-H), 3.47 (s, 3 H, OCH_3), 2.41 (s, 3 H, C4-CH_3).

2-[1-Methoxy-1-[*p*-(trifluoromethyl)phenyl]methyl]-*N*,4-dimethylthiazolium Fluoroborate (1h). In a similar manner as shown for the synthesis of **1g** (with the exception that the suspension was stirred for 72 h), 0.210 g (94%) of **1h** was obtained as a white powder: ^{1}H NMR (200 MHz, $CDCl_3$–TMS) δ 7.80–7.64 (m, 4 H, Ar), 7.58 (s, 1 H, C5-H), 6.00 (s, 1 H, C2α-H), 3.91 (s, 3 H, N-CH_3), 3.51 (s, 3 H, OCH_3), 2.53 (d, 3 H, C4-CH_3). Anal. Calcd for $C_{14}H_{15}NOS\cdot BF_4\cdot 0.3\ H_2O$: C, 42.62; H, 3.98; N, 3.55. Found: C, 42.69; H, 3.58; N, 3.68.

2-[1-Hydroxy-1-(*p*-methylphenyl)methyl]-4-methylthiazole (3k). In a similar manner as shown for the synthesis of **3g**, 7 g (50%) of **3k** was obtained as a dark-yellow oil, which was then crystallized from ethyl ether: ^{1}H (400 MHz, $CDCl_3$–TMS) δ 7.36 (d, 2 H, J = 8.7 Hz, Ar), 6.86 (d, 2 H, J = 8.7 Hz, Ar), 6.73 (s, 1 H, C5-H), 5.89 (s, 1 H, C2α-H), 4.41 (br, 1 H, OH), 3.73 (s, 3 H, Ar-OCH_3), 2.37 (s, 3 H, C4-CH_3).

2-[1-Methoxy-1-(*p*-methoxyphenyl)methyl]-4-methylthiazole (4k). In a similar manner as shown for the synthesis of **4g**, 1 g (95%) of **4k** was obtained as an oil. According to thin-layer chromatography on silica gel (ether–hexane 2:1), this material was sufficiently pure to proceed to the next step without further purification: ^{1}H (200 MHz, $CDCl_3$–TMS) δ 7.36 (d, 2 H, J = 8.7 Hz, Ar), 7.61 (s, 1 H, C5-H), 6.88 (d, 2 H, J = 8.7 Hz, Ar), 5.47 (s, 1 H, C2α-H), 3.77 (s, 3 H, $ArOCH_3$), 3.42 (s, 3 H, OCH_3), 2.39 (s, 3 H, C4-CH_3).

2-[1-Methoxy-1-(*p*-methoxyphenyl)methyl]-*N*,4-dimethylthiazolium Fluoroborate (1k). In a similar manner as shown for the synthesis of **1g**, 0.50 g (71%) of **1k** was obtained as a white powder: ^{1}H (200 MHz, $CDCl_3$–TMS) δ 7.64 (s, 1 H, C5-H), 7.35 (d, 2 H, J = 8.7 Hz, Ar), 6.95 (d, 2 H, J = 8.7 Hz, Ar), 5.84 (s, 1 H, C2α-H), 3.83 (s, 3 H, N-CH_3), 3.81 (s, 3 H, $ArOCH_3$), 3.43 (s, 3 H, OCH_3), 2.49 (s, 3 H, C4-CH_3). Anal. Calcd for $C_{14}H_{18}NO_2S\cdot BF_4\cdot 0.2H_2O$: C, 47.40; H, 5.17; N, 3.95; S, 9.04. Found: C, 47.38; H, 5.16; N, 3.94; S, 9.32.

Table I. Summary of Electrochemical Data[a]

	R_1	R_2	peak potential, mV vs ferrocene[b]	peak potential, mV vs SCE[c]	no. of electrons transferred
2a	H	H	−270	144	
2b	H	CH_3	−336	78	1.12
2c	CH_3	CH_3	−450	−36	1.03
2d	OCH_3	CH_3	−583	−169	0.98
2e	H	Ph	−340	74	0.92
2f	O-pyran	Ph	−447	−33	
2g	OCH_3	Ph	−426	−12	
2h	OCH_3	*p*-CF_3Ph	−320	94	
2i	OCH_3	*p*-BrPh	−392	22	
2j	OCH_3	*p*-CH_3Ph	−447	−33	
2k	OCH_3	*p*-OCH_3Ph	−461	−47	

[a] Counterions is BF_4^-. [b] Counter electrode, Pt wire; reference electrode, $Ag/AgNO_3$ (0.1 M) in CH_3CN; working electrode, Pt disk; scan rate, 100 mV/s; solvent, Me_2SO; supporting electrolyte, $(CH_3)_4N^+BF_4^-$. Ferrocene was used as an internal standard. [c] Potentials obtained by adding 414 mV to column b. [d] Counter electrode, Pt mesh separated from bulk solution; reference electrode, $Ag/AgNO_3$ (0.1 M) in CH_3CN; working electrode, Pt mesh; supporting electrolyte, KNO_3; solvent, Me_2SO.

2-(1-Hydroxy-1-*p*-tolylmethyl)-4-methylthiazole (3j). In a similar manner as shown for the synthesis of **3g**, compound **3j** was obtained as a yellow oil (which could not be crystallized from ether–hexane). The product was chromatographed on 400 g of silica gel and eluted with ether–hexane (1:1). The combined fractions were concentrated in vacuo to afford 5 g (38%) of an oil, which was then crystallized from ether–hexane: ^1H (200 MHz, $CDCl_3$–TMS) δ 7.33 (d, 2 H, J = 8.1 Hz, Ar), 7.15 (d, 2 H, J = 8.1 Hz, Ar), 6.79 (s, 1 H, C5-H), 5.95 (s, 1 H, C2α-H), 4.26 (br, 1 H, OH), 2.36 (s, 3 H, C4-CH_3), 2.34 (s, 3 H, Ar-CH_3).

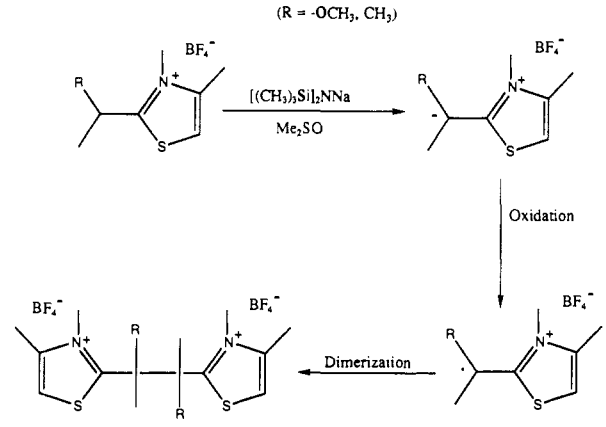
2-(1-Methoxy-1-*p*-tolylmethyl-4-methylthiazole (4j). In a similar manner as shown for the synthesis of **4g**, 0.95 g (45%) of **4j** was obtained as an oil: ^1H NMR (200 MHz, $CDCl_3$–TMS) δ 7.35 (d, 2 H, J = 8.0 Hz, Ar), 7.16 (d, 2 H, J = 8.0 Hz, Ar), 6.83 (s, 1 H, C5-H), 5.55 (s, 1 H, C2α-H), 3.43 (s, 3 H, OCH_3), 2.40 (s, 3 H, C4-CH_3), 2.32 (s, 3 H, Ar-CH_3).

2-(1-Methoxy-1-*p*-tolylmethyl)-*N*,4-dimethylthiazolium Fluoroborate (1j). In a similar manner as shown for the synthesis of **1g** (with the exception that the suspension was stirred for 72 h), 0.50 g (67%) of **1j** was obtained as a yellow oil: ^1H NMR (200 MHz, $CDCl_3$–TMS) δ 7.53 (s, 1 H, C5-H), 7.22–7.16 (m, 4 H, Ar), 5.78 (s, 1 H, C2α-H), 3.74 (s, 3 H, N-CH_3), 3.38 (s, 3 H, OCH_3), 2.42 (s, 3 H, C4-CH_3), 2.31 (s, 3 H, Ar-CH_3). Anal. Calcd for $C_{14}H_{18}NOS{\cdot}BF_4{\cdot}0.5H_2O$: C, 48.86; H, 5.56; N, 4.07; S, 9.32. Found: C, 49.12; H, 5.33; N, 4.22; S, 9.25.

Electrochemistry. Cyclic voltammetry and controlled-potential electrolysis were carried out on a Princeton Applied Research potentiostat–galvanostat, Model 273. The working electrode was a 1.6 mm diameter platinum disk embedded in Kel-F. A platinum mesh was used as the working electrode for the controlled-potential electrolysis experiments. The counter electrode was a platinum wire (for cyclic voltammetry) or a platinum mesh in a tube separated from the bulk solution with a glass frit (for controlled-potential electrolysis). The reference electrode was $Ag/AgNO_3$ (0.1 M) in MeCN separated from solution by a glass frit. The Me_2SO solution containing 0.1 M KNO_3 supporting electrolyte was purged and then blanketed with dry argon during the experiment to prevent traces of moisture from entering the system. Typical concentrations of thiazolium compounds used in the cyclic voltammetry experiments were 0.5–1.0 mM with an equivalent concentration of base added to generate the enamine. For the controlled-potential electrolysis experiments in which the product of electrolysis was isolated, the concentrations of thiazolium compound and base were in the range of 4–10 mM.

After cyclic voltammograms were recorded for each compound, ferrocene was added to the solution, and its recorded cyclic voltammogram provided us with an internal standard. The ferrocene–ferrocenium couple was measured first against the Ag/Ag^+ reference and then against SCE in Me_2SO. The anodic peak potential was found to shift from 0.067 V vs Ag/Ag^+ to 0.481 V vs SCE. In both cases the anodic–cathodic peak separation was 0.064 V. The peak potentials in Table I are reported vs the internal standard ferrocene, and vs SCE, $E_{SCE} = E_{ferrocene} + 0.414$ V. Equilibrium potentials can be obtained directly $[(E_{anodic} - E_{cathodic})/2]$ for compounds (**2f–2k**) whose voltammograms exhibit a cathodic peak. These values are nearly the same as those obtained by subtracting 32 mV from their peak potentials. The peak potentials are reported in Table I to allow a uniform comparison of all compounds studied. Since compounds **2f–2k** exhibit electrochemical reversibility, it may be assumed that **2a–2d** also undergo electrochemically reversible oxidation, and their equilibrium potentials can also be obtained by subtracting 32 mV from their peak potentials.

Scheme III

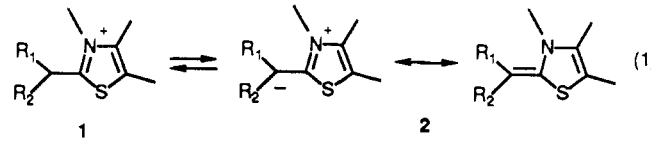


Isolation and Characterization of the Product of Electrolysis of 1d on Addition of $(TMS)_2$ NNa. After the controlled-potential electrolysis CH_2Cl_2 (70 mL) was added. This mixture was centrifuged, and the solution was decanted from the precipitate (mainly KNO_3). The solution was then concentrated in vacuo to an oil, which was then dissolved in 10 mL of $CHCl_3$. The insoluble product was filtered by using a fine sintered glass funnel: ^1H NMR (200 MHz, CD_3CN–TMS) δ 7.86 (s, 1 H, C5-H), 7.71 (s, 1 H, C5-H′), 4.09 (s, 3 H, N-CH_3), 4.03 (s, 3 H, N-CH_3′), 3.41 (s, 3 H, OCH_3), 3.35 (s, 3 H, OCH_3′), 2.54 (s, 3 H, C4-CH_3), 2.53 (s, 3 H, C4-CH_3′), 1.98 (s, 3 H, C2β-H_3), 1.78 (s, 3 H, C2β-H_3′). The spectrum is consistent with the presence of a *d,l*-racemic pair and a meso diastereomer of the symmetrical dimer (Scheme III) since there are two chiral centers produced in the dimer. MS (FAB) for $C_{16}H_{26}N_2O_2S_2{\cdot}BF_4$ m^+/e 429. For the mononitrate m^+/e 404 was apparent (the latter is the result of complexation with the counterion of the supporting electrolyte), as well as m^+/e 342 for the dication.

Isolation and Characterization of the Product of Electrolysis of 1c on Addition of $(TMS)_2$ NNa. The product was isolated in a manner analogous to that described above for **1d**: ^1H NMR (200 MHz, Me_2SO–TMS) δ 8.1 (s, 2 H, C5-H), 3.98 (s, 6 H, NCH_3), 2.52 (s, 6 H, C4-CH_3), 1.74 (s, 12 H, C2β-H_3). Note that in the starting material the last methyl group is a doublet by virtue of the coupling to the C2α proton. The remarkably clean and simple spectrum is consistent with the symmetrical dimer only (Scheme III); in this case there are no chiral centers in the dimer. MS (FAB) showed a time-dependent decrease in the masses corresponding to the mononitrate (m^+/e 372) and monofluoroborate (m^+/e 397) of the symmetrical dimer. It would appear that this dimer (a highly hindered butane) undergoes decomposition during the mass spectral measurements.

Results and Discussion

Generation of Electroactive Species. Cyclic voltammograms were recorded for compounds listed in Table I. Sodium bis(trimethylsilyl)amide $[(TMS)_2NNa]$ was used to generate the enamines **2** quantitatively according to eq 1 and confirmed by UV



(1)

spectroscopy (the λ_{max} shifted to longer wavelength going from **1** to **2**). In all cases examined there was an oxidation peak observed only upon addition of the base. The base itself, as well as its conjugate acid was shown not to give rise to the oxidation peaks. A typical set of experiments is presented in Figure 1. In the absence of base, none of the thiazolium ions **1** underwent oxidation (dashed curve of panel A, Figure 1). The potential was scanned out to the limiting potential of the solvent, which is nearly 1.0 V more positive than the oxidation potentials we observed when the base was added. On addition of $(TMS)_2NNa$ to the solution there was observed an oxidation peak (solid curve of panel A, Figure 1) whose potential was compared to the oxidation potential recorded for ferrocene after addition of a small amount of fer-

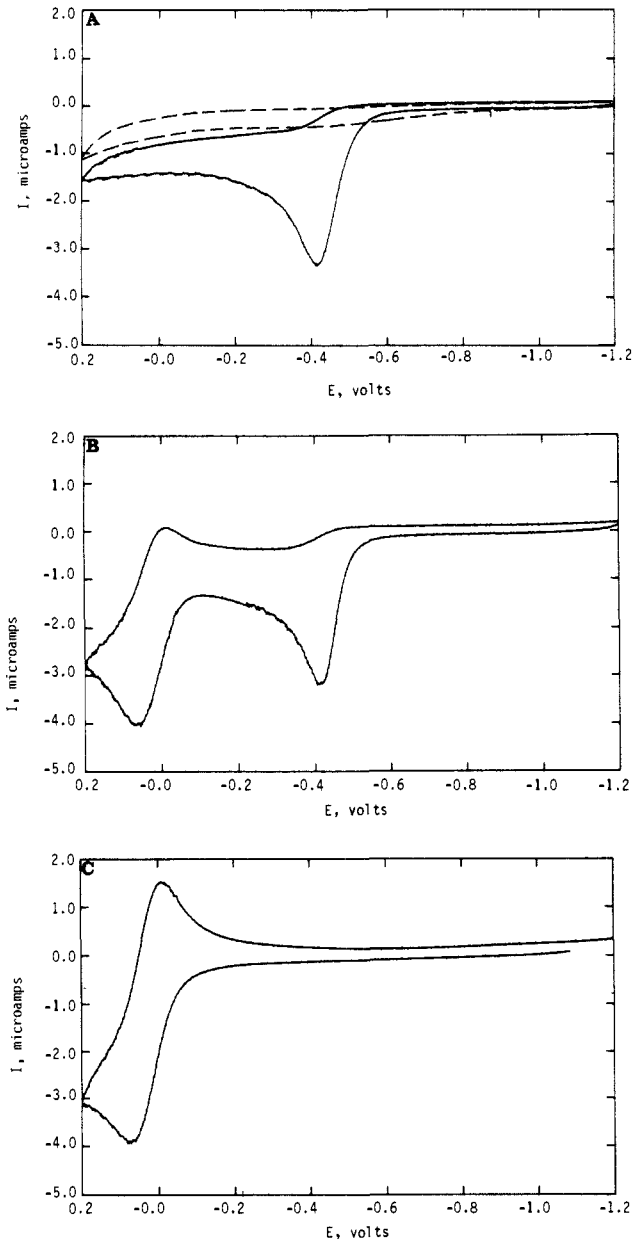


Figure 1. Panel A. Cyclic voltammogram for ion **1c** in Me_2SO (dashed curve) and on the addition of a limiting amount of $(TMS)_2NNa$ (solid curve). The supporting electrolyte was 0.1 M KNO_3, the scan rate was 0.10 V/s. Panel B shows the same solution on subsequent addition of a small amount of ferrocene, while panel C shows the same solution on subsequent addition of excess nitric acid.

rocene to the enamine solution (panel B, Figure 1). Sufficient ferrocene was added to obtain a peak of about the same magnitude as observed for the enamine, thus correcting for uncompensated resistance when comparing peak potentials vs the ferrocene couple. On addition of acetic or nitric acid (sufficient to neutralize the base), the oxidation peak vanished (panel C, Figure 1). One should note, however, that so long as any enamine is present, it will reduce some of the ferrocenium formed upon oxidation of ferrocene, and the cathodic peak response will be diminished. Addition of acid reverses eq 1 to thiazolium ion and the intensity of the ferrocene–ferrocenium cathodic peak is restored to its expected value. These observations clearly demonstrate that it is the enamine and not the precursor thiazolium ion that undergoes oxidation. Similarly, a cyclic voltammogram is presented for **2d**, the closest analogue to thiamin-bound enamines, clearly showing an anodic wave.

Evidence for One-Electron Oxidation. Scan rates were varied from 0.01 to 5 V/s. The dependence of peak potential on scan rate, v, supports an EC (electrochemical oxidation followed by

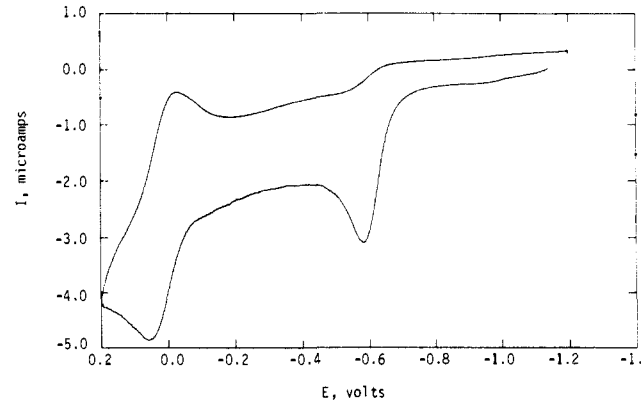
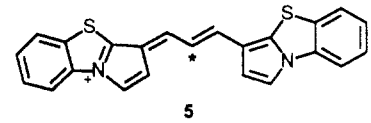


Figure 2. Cyclic voltammogram for **1d** in Me_2SO on the addition of a limiting amount of $(TMS)_2NNa$.

chemical reaction) mechanism.[7] A plot of E_p vs log v gave slopes that ranged from 17 mV for compound **2j** to 37 mV for **2b**. This dependence does not clearly distinguish between first order (20 mV/decade) and second order (30 mV/decade) kinetics of the follow-up reactions under study. However, at higher scan rates uncompensated resistance effects contribute to progressively larger E_p values and as a result larger dE_p/d (log v) slopes will be obtained. Therefore, many of the slopes recorded are on the high side. Most likely, the follow-up chemical reaction is dimerization of the intermediate cation radical (see below).

Controlled-potential electrolysis was performed on compounds **2b–2e**, and in all cases studied, one-electron oxidation was shown to occur at the electrode. For compounds **2c** and **2d** the product(s) were isolated and the predominant product was identified by ^1H NMR and FAB-MS as the symmetrical dimer formed by the dimerization of two radical cations at their C2α atoms (see Scheme III). Similar dimerization subsequent to electrochemical oxidation was reported for the related compound at the starred atom derived from a benzothiazolium enamine shown below.[9a]



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We reasoned that such dimerization may be slowed down by steric crowding at the C2α position. We therefore also studied the electrochemical behavior of a number of compounds bearing a phenyl, or para-substituted phenyl ring at this position, with the assumption that the enamines derived and the subsequent cation radical may by virtue of steric crowding provide insight to the electrochemical process. All enamines with phenyl substituents at the C2α position gave a small cathodic peak, which increased in magnitude relative to the anodic peak with increasing scan rate. The separation between the cathodic and anodic peaks was between 60 and 80 mV, again indicative of a (in these cases reversible) one-electron oxidation, for which the theoretical value would be 59 mV/electron transferred. As a representative for this group of compounds, Figure 3 presents cyclic voltammograms at increasing scan rates for compound **2h**. The current scale is normalized to the peak current at a scan rate 1.60 V/s. The separation of anodic and cathodic peaks is 60–80 mV and the anodic peak potential shifts 25 ± 5 mV for each ^{1}0-fold increase in scan rate (Figure 4). This result is the expected classical behavior for an EC mechanism in which the electron transfer is reversible and the chemical reaction is irreversible. Figure 5 presents the cyclic voltammogram of a solution of compound **2h** in the presence of excess $(TMS)_2NNa$ and some ferrocene at the scan rate of 1.0 V/s. Sufficient ferrocene was added such that

(7) Bard, A.; Faulkner, L. *Electrochemical Methods, Fundamentals, and Applications*; John Wiley and Sons, Inc.: New York, 1980; Chapter 11.
(8) Schoeller, W. W.; Neimann, J.; Rademacher, P. *J. Chem. Soc., Perkin Trans. 2* **1988**, 369.
(9) (a) Lenhard, J. R.; Parton, R. L. *J. Am. Chem. Soc.* **1987**, *109*, 5808. (b) Huenig, S. *Pure Appl. Chem.* **1967**, *15*, 109.

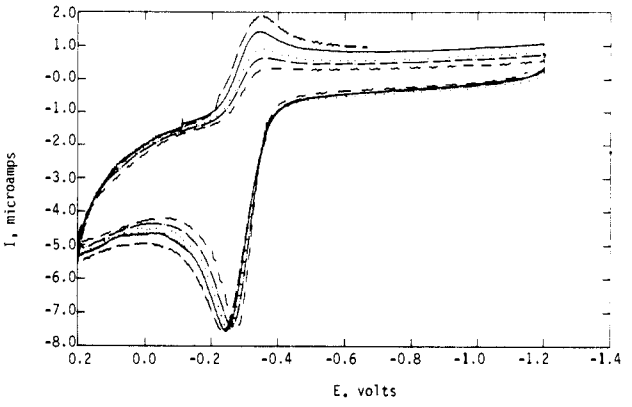

Figure 3. Effect of scan rate on the cyclic voltammograms of **1h** in Me_2SO on the addition of a limiting amount of $(TMS)_2NNa$ in the presence of 0.1 M KNO_3 supporting electrolyte. The normalized magnitudes of the increasing cathodic curves correspond to 100, 400, 1000, 1600, and 4000 mV/s scan rates.

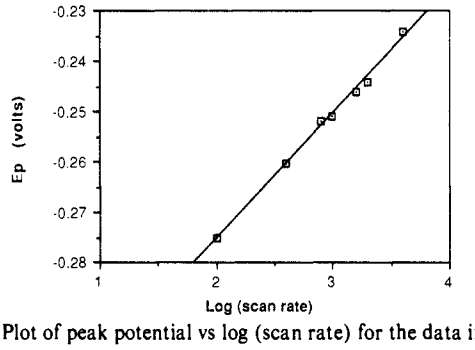

Figure 4. Plot of peak potential vs log (scan rate) for the data in Figure 3.

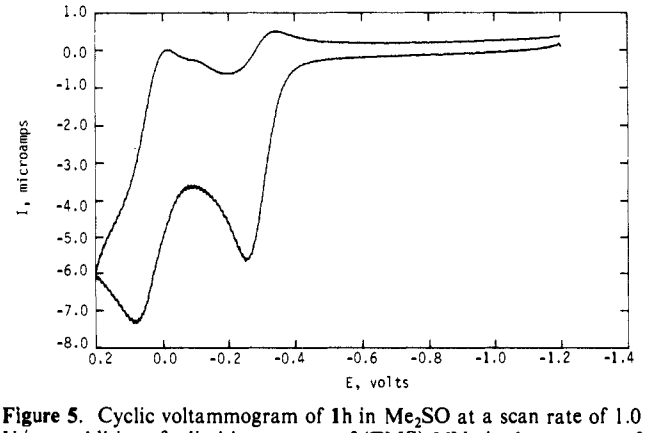

Figure 5. Cyclic voltammogram of **1h** in Me_2SO at a scan rate of 1.0 V/s on addition of a limiting amount of $(TMS)_2NNa$ in the presence of 0.1 M KNO_3 supporting electrolyte and after addition of ferrocene.

the oxidation peaks for ferrocene and the enamine are of the same magnitude. Ferrocene is known to undergo one-electron oxidation in Me_2SO. The separation of the anodic and cathodic peaks for the enamine and ferrocene is the same. This result confirms that the enamine undergoes reversible one-electron oxidation to form a cation radical followed by an irreversible chemical reaction. The difference between the observed separation of cathodic and anodic peaks (80 mV) and the theoretical one (59 mV) is most likely due to a shift in the potential due to uncompensated resistance. Similar findings were recently reported by Schoeller et al. on enamines produced from cyclic ketones and cyclic secondary amines.[8]

Substituent Effects on Peak Potentials and E_o's. To compare the relative ease of oxidation of enamines **2**, peak potentials recorded at 100 mV/s scan rate are presented in Table I (since all compounds with aliphatic substituents gave the same electrochemical response). The peak potentials ranged from −583 mV for **2d** (most easily oxidized) to −270 mV for **2a**. Two trends can be discerned for substitution at C2α, the effect of alkyl substituents

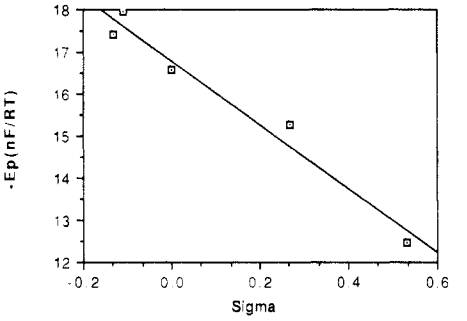

Figure 6. Hammett plot of E_o vs σ for the electrochemical oxidation of **2g–2k**. σ values were taken from Wells, P. R. *Linear Free Energy Relationships*; Academic Press: New York, 1968; p 14.

vs H vs OMe and the effects of para substituents on the phenyl ring. In general, electron donation to C2α should stabilize the cation radical and enhance the ease of oxidation of the enamine. A comparison of peak potentials for **2a–2d** indicates that the relative ease of oxidation increases in the order (the two groups represent the two substituents at C2α in the enamine) H, H < H, Me < Me, Me < Me, OMe. We also have evidence to indicate that a Me group enhances the ease of oxidation compared to the phenyl. Furthermore, variation of the para substituent in a series of 2-(1-methoxy-1-phenylmethyl)thiazolium salts enabled us to construct a Hammett plot giving a ρ value of −7.61 (Figure 6; $r^2 = 0.996$ for the five points), again an indication that electron donation enhances the ease of oxidation. Qualitatively, the ease of oxidation varies inversely with enamine stability. Resonance interactions in the enamines between the phenyl and thiazolium rings and via the C2α atom can be inferred both from pK's of the corresponding thiazolium ions[5] and from the shift to longer wavelength of the λ_{max} in the UV spectrum going from **2d** (290–300 nm) to **2f** (360–370 nm). This resonance stabilization of the enamine is also obvious if one compares our results with those on carbocyanine dye **5** (closely related in electronic structure to **2**), which also undergoes one-electron oxidation followed by dimerization.[9] Apparently the conjugation that is very much a factor in providing enamine stability is no longer present in the cation radical. This cation radical could be pyramidal at C2α, thereby hindering conjugation between the phenyl and thiazolium rings. This would imply that the free energies of the cation radicals are rather similar, and the differences in peak potentials, or E_o's (where such are available), are primarily a result of differences in the free energies of the enamines from which the redox process originates. So far as nonconjugated substituents are concerned, the ability of **2d** to undergo more facile oxidation than **2b** may be related to the radical-stabilizing effect of the OMe substituent compared to H.[10] Chemical oxidation of **2d** by ferrocenium cation radical resulted in a product whose UV spectrum indicated that the thiazolium ring remained intact, i.e., dimerization at C2α may have resulted. Enamines **2** are uniformly more easily oxidized than simple enamines reported by others[8] or related cyanine dyes.[9]

Conclusions and Relevance to Biochemical Pathways. While the mechanisms of electron transfer from the enamine to lipoic acid or flavin are not yet clear, there is evidence for a single-electron transfer from the enamine to an Fe_4S_4 cluster from spin-trapping of an intermediate radical (and subsequent ESR detection) during the reaction of pyruvate-ferrodoxin oxidoreductase,[11] for which our results constitute an appropriate model. According to that report, the second electron during the redox process originates from one-electron oxidation of coenzyme A (CoASH) to CoAS$^{\bullet}$ or a thiyl radical, which then rapidly combines with the thiazolium cation radical yielding a tetrahedral intermediate that collapses to thiazolium and acetyl-CoA. Application of a different mechanistic tool, attempted cleavage of a cyclopropyl substrate that would be characteristic of a free-radical process,

(10) Viehe, H. G.; Janousek, Z.; Merenyi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *18*, 148.
(11) Docampo, R.; Moreno, S. J.; Mason, R. P. *J. Biol. Chem.* **1987**, *262*, 12417.

failed to provide positive evidence in favor of stepwise electron transfer for this or the other oxidative reactions listed in the introduction.[12]

In a recent report by Ciurli et al. models were synthesized for the Fe_4S_4 cluster.[13] Equilibrium redox potentials vs SCE were reported for a variety of ligands in Me_2SO, the solvent and standard employed in our study. Only a few of the clusters reported possess a potential in the range appropriate for spontaneous oxidation of **2d**. Data reported in Table II of that study, in conjunction with our data on **2d**, suggest some limitations for the Fe_4S_4 cluster in pyruvate-ferredoxin oxidoreductase. In particular, the $Fe_4S_4^{3+/2+}$ couple, but not the $Fe_4S_4^{2+/+}$ couple, is capable of oxidizing **2d** and specifically employing only those ligands listed for clusters **1**, **4-6**, and **8**.[13]

These results constitute the first model for one-electron oxidation of the thiamin diphosphate bound enamine intermediate and demonstrate at least the possibility of a thiazolium cation radical intermediate during the redox process performed by the enzymes quoted. The peak potentials, especially on the most relevant **2d**, demonstrate for the first time the ease with which the enamine can be oxidized.

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(12) Livingston, D. L.; Shames, S. L.; Gennis, R. B.; Walsh, C. T. *Bioorg. Chem.* **1987**, *15*, 358.
(13) Ciurli, S.; Carrie, M.; Weigel, J. A.; Carney, M. J.; Stack, T. D. P.; Papaefthymiou, G. C.; Holm, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 2654.

Phosphonate Biosynthesis: The Stereochemical Course of Phosphoenolpyruvate Mutase

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Abstract: Chiral [^{16}O,^{17}O,^{18}O]phosphonopyruvate has been synthesized and used to determine the stereochemical outcome at phosphorus of the reaction catalyzed by phosphoenolpyruvate mutase, the enzyme that catalyzes the interconversion of phosphonopyruvate and phosphoenolpyruvate. The mutase-catalyzed reaction proceeds with overall retention of the configuration at phosphorus. This result restricts the range of mechanistic possibility for this unusual enzymic reaction.

The enzyme phosphoenolpyruvate mutase (EC 6.4.2.9), which catalyzes the formation of many of the carbon-phosphorus bonds found in naturally occurring phosphonates,[1] has recently been isolated and purified.[2] This enzyme catalyzes the interconversion of phosphoenolpyruvate (PEP) and phosphonopyruvate (Figure 1), the equilibrium for which lies predominantly toward PEP. The mechanism of this rearrangement has yet to be unravelled, though enzymological and chemical precedent suggests four mechanistic possibilities.

First, by analogy with phosphomutases such as phosphoglucomutase and the phosphoglycerate mutases, reaction via a phosphoenzyme intermediate is an attractive possibility. By this pathway, an enzyme nucleophile (phosphoglucomutase uses serine,[3] while phosphoglycerate mutase uses histidine[4]) would attack phosphonopyruvate at phosphorus as illustrated in Figure 2A. The oxygen atom of the resulting enolate anion of pyruvate would then attack the phosphoenzyme to yield the product, phosphoenolpyruvate. Such enolate attack on a phosphoenzyme (or on an enzyme-bound phosphoric anhydride such as ATP) is well precedented in the reactions catalyzed by phosphoenolpyruvate synthase,[5] by pyruvate, phosphate dikinase,[6] and by pyruvate kinase.[7]

A second possible pathway is suggested by the presumed mechanisms for the Wadsworth-Emmons and the Wittig reactions.[8] In this case, an oxaphosphetane, pentacoordinate at phosphorus, would be formed from phosphonopyruvate by intramolecular attack of the carbonyl oxygen at phosphorus (Figure 2B). After a necessary pseudorotation (so that the methylene group becomes apical for departure), the oxaphosphetane would collapse to produce PEP. Although this pathway with its strained four-membered ring may seem improbable, it is true that the Wadsworth-Emmons reaction involves a similar 1,3-migration of a phospho group from carbon to oxygen,[9] and an analogous four-membered cyclic intermediate has been proposed. The Wittig reaction also is believed to proceed via an oxaphosphetane intermediate,[10] and in exotic cases, such species have been isolated.[11]

Third, is the formal possibility that the reaction is a concerted 1,3 sigmatropic rearrangement, as shown in Figure 2C. In this case, the constraints of orbital symmetry,[12] which have been validated in the rearrangement of an alkyl group across an allylic system,[13] require that the phosphorus suffer the equivalent of an

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(1) Hori, T.; Horiguchi, M.; Hayashi, A. *Biochemistry of Natural C-P Compounds*; Maruzen: Tokyo, 1984. Hilderbrand, R. L. *The Role of Phosphonates in Living Systems*; CRC Press, Inc.: Boca Raton, FL, 1983.
(2) Bowman, E.; McQueney, M.; Barry, R. J.; Dunaway-Mariano, D. *J. Am. Chem. Soc.* **1988**, *110*, 5575. Seidel, H. M.; Freeman, S.; Seto, H.; Knowles, J. R. *Nature (London)* **1988**, *335*, 457. Hidaka, T.; Mori, M.; Imai, S.; Hara, O.; Nagaoka, K.; Seto, H. *J. Antibiot.* **1989**, *42*, 491.
(3) Milstein, C.; Sanger, F. *Biochem. J.* **1961**, *79*, 456.
(4) Rose, Z. B. *Arch. Biochem. Biophys.* **1970**, *140*, 508. Rose, Z. B. *Arch. Biochem. Biophys.* **1971**, *146*, 359. Rose, Z. B.; Hamasaki, N.; Dube, S. *J. Biol. Chem.* **1975**, *250*, 7939.
(5) Cooper, R. A.; Kornberg, H. L. *Biochim. Biophys. Acta* **1967**, *141*, 211. Cooper, R. A.; Kornberg, H. L. *Biochem. J.* **1967**, *105*, 49c.
(6) Spronk, A. M.; Yoshida, H.; Wood, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4415.
(7) Kuo, D. J.; O'Connell, E. L.; Rose, I. A. *J. Am. Chem. Soc.* **1979**, *101*, 5025.
(8) Wadsworth, W. S., Jr.; Emmons, W. D. *Org. Synth.* **1965**, *45*, 44. Maercker, A. *Org. React.* **1965**, *14*, 270.
(9) Wadsworth, W. S., Jr. *Org. React.* **1977**, *25*, 73.
(10) Vedejs, E.; Snoble, K. A. J. *J. Am. Chem. Soc.* **1973**, *95*, 5778.
(11) Mazhar-Ul-Haque; Caughlan, C. N.; Ramirez, F.; Pilot, J. F.; Smith, C. P. *J. Am. Chem. Soc.* **1971**, *93*, 5229.
(12) Woodward, R. B.; Hoffmann, R. *The Conservation of Orbital Symmetry*; Verlag Chemie: Weinheim, FRG, 1970.

0002-7863/90/1512-8149$02.50/0