

**Figure 2.** Mims ENDOR spectra of [4Fe-4S]<sup>+</sup> cluster of aconitase (A) without substrate and (B) with substrate (citrate). Spectra are plotted as  $\delta\nu = \nu - \nu_D$ . Experimental conditions:  $T = 2$  K;  $\nu_e = 9.14$  (A), 9.09 (B) GHz;  $H_0 = 3525$  (A), 3645 (B) G; microwave pulse width, 16 ns; rf pulse width, 50  $\mu$ s;  $\tau_{12} = 380$  (A), 440 (B) ns; (A) 168, (B) 880 scans.

ENDOR spectra from which hyperfine and quadrupole splittings can be determined directly. Figure 1B is a single-crystal-like<sup>5a,b</sup> <sup>2</sup>H Mims ENDOR spectrum taken<sup>7b</sup> at the high-field ( $g_3$ ) edge of the *Anabaena* Fd EPR absorption envelope, using the same small sample used at the Q-band.<sup>5d</sup> It shows a pair of peaks centered at  $\nu_D$  and separated by 0.60 MHz. This splitting, which is far too large to be associated with the quadrupolar interaction, represents a deuteron hyperfine coupling,  $A^D \approx 0.60$  MHz.<sup>10</sup> The peaks further show a partially resolved quadrupole splitting of  $2P^D \approx 0.1$  MHz. As no exogenous ligands are associated with the cluster, this local deuteron can be assigned to a hydrogen N-H...S bond, presumably one of the two putative strong H bonds seen in the crystal structure of the oxidized protein:<sup>6a</sup> arginine 42 H-bonded to a cysteinyl mercaptide sulfur bound to iron or arginine 258 H-bonded to a bridging cluster S<sup>2-</sup>.

The <sup>2</sup>H splitting varies little in the ENDOR spectra taken at magnetic field values across the EPR envelope (data not shown), which indicates that the coupling is nearly isotropic.<sup>5a,b</sup> This is equivalent to an isotropic proton interaction,  $A^H = (g_H/g_D)A^D = 6.51A^D \approx 3.9$  MHz, whose size indicates that this H bond must have significant covalency. Variations in the quadrupole term indicate that  $K^D \approx 3e^2qQ/2h \lesssim 0.2$  MHz, in agreement with the expectation from model compounds.<sup>10</sup>

The explanation for the difference in resolution of the CW and pulsed deuterium signals is likely as follows. In the former, distant ENDOR signals from noninteracting deuterons<sup>9,5d</sup> overwhelm the local ENDOR of H-bonded deuterons. In pulsed ENDOR, an individual pulse sequence ( $\leq 50$   $\mu$ s) is shorter than the time for the spin diffusion processes that are the basis of the distant CW ENDOR response; thus the distant ENDOR response is "quenched", and local ENDOR signals become visible.

The [4Fe-4S]<sup>+</sup> cluster of aconitase has one iron ion (Fe<sub>a</sub>) that has been shown by <sup>17</sup>O CW ENDOR to be coordinated to a solvent species, H<sub>2</sub>O.<sup>2a,5c</sup> The enzyme shows a rhombic EPR signal, with  $g_{1,2,3} = 2.06, 1.93, \text{ and } 1.86$  in the absence of substrate, and  $g_{1,2,3} = 2.04, 1.85, \text{ and } 1.78$  in the presence of substrate (citrate). Figure 2A is a single-crystal-like <sup>2</sup>H Mims ENDOR spectrum of the enzyme in D<sub>2</sub>O without substrate, taken at the high-field edge

( $g_3 = 1.86$ ) of the EPR absorption envelope. It shows one pair of peaks from D<sub>2</sub>O at  $\delta\nu_{\pm} = \nu_{\pm} - \nu_D \approx \pm 0.25$  MHz, with each showing a further small splitting of  $\sim 0.12$  MHz. We assign the spectrum to a deuteron with hyperfine interactions,  $A^D \approx 0.50$  ( $A^H \approx 3.25$ ) MHz, and quadrupole splitting,  $2P^D \approx 0.12$  MHz. The pulsed ENDOR spectrum taken at  $g_3 = 1.78$  for the enzyme with substrate (nondeuterated citrate) in D<sub>2</sub>O (Figure 2B) shows a more complex pattern, with four deuterium peaks at  $\delta\nu_{\pm} \approx \pm 0.61$  and  $\pm 0.23$  MHz. An assignment to a single deuteron with  $A^D = 0.84$  MHz and quadrupole splitting  $2P^D = 0.38$  MHz can be discounted because the latter is improbably large;<sup>10</sup> instead, the peaks are assigned to two inequivalent nuclei that have  $A^D = 1.22$  and 0.46 ( $A^H = 7.9, 3.0$ ) MHz and no observable quadrupole splitting at this field. Comparison with the <sup>1</sup>H resonances lost upon H/D exchange<sup>5c</sup> confirms the latter assignment. The <sup>2</sup>H Mims ENDOR spectrum of enzyme without substrate has significantly better resolution than the corresponding Q-band CW ENDOR spectrum; that of enzyme with substrate also is improved.<sup>5c</sup> These high-resolution spectra support the earlier suggestion that the H<sub>2</sub>O species coordinated to the [4Fe-4S]<sup>+</sup> cluster of the enzyme with substrate is a water molecule, whereas it is a hydroxyl ion in the enzyme without substrate.<sup>2a,5c</sup>

The data presented here clearly show that <sup>2</sup>H Mims pulsed ENDOR examination of H bonds and H<sub>2</sub>O coordinated to metal clusters provides an important complement to multifrequency CW ENDOR<sup>5</sup> and its pulsed analogue, Davies ENDOR,<sup>4d,7b</sup> which typically do best with larger couplings, and to ESEEM, which is well-suited for measuring distances to and numbers of dipole-coupled exchangeable protons in the active-site vicinity.<sup>3c</sup>

**Acknowledgment.** This work could not have been performed without the expert assistance of Mr. Clark E. Davoust and supportive discussions with Dr. Peter E. Doan. The *Anabaena* 7120 Fd was kindly provided by Professor John L. Markley. This work was supported by the NIH [HL 13531 (B.M.H.), GM 34812 (H.B.)], NSF [DBM-8907559 (B.M.H.)], and USDA [90-37120-5604 (B.M.H.)]. Equipment was purchased with a grant from the NIH (DRR 04936) and received support from the Materials Research Center of Northwestern University (DMR-88 21571).

### Stereochemical Analysis of Totally Stereoselective, Competing [1,2]- and [2,3]-Wittig Rearrangements. Inversion at the Lithium-Bearing Carbon Atom

Erik J. Verner and Theodore Cohen\*

Department of Chemistry, University of Pittsburgh  
Pittsburgh, Pennsylvania 15260

Received July 18, 1991

We present the first stereochemical analysis of [1,2]-<sup>1</sup> and [2,3]-Wittig rearrangements which includes the stereochemical change that occurs at the lithium-bearing carbon atom. Counterintuitively, the [2,3]-rearrangement requires a trans disposition of the two substituents in a 2-lithio-6-vinyltetrahydropyran, and both rearrangements occur with *inversion of configuration at the lithium-bearing carbon atom*.<sup>3</sup> This inversion in the case of the

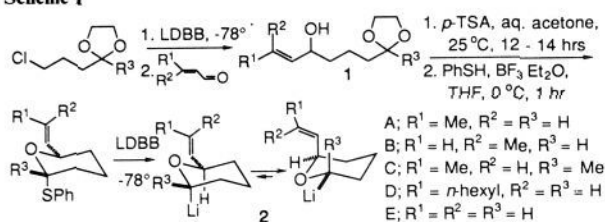
(10) (a) Recall (refs 5a,b) that ENDOR frequencies for <sup>2</sup>H ( $I=1$ ) have the form  $\nu_{\pm}(m) \approx |\pm A^D/2 + \nu_D + P^D(2m-1)|$ ,  $m = 0, 1$ , for a given field orientation. (b) Studies on model compounds (ref 10c) show that the maximum deuterium quadrupole splitting falls in the range  $2|P_{zz}| \approx K^D \approx 0.26\text{--}0.30$  MHz for R<sub>2</sub>ND that is H-bonded,  $2|P_{zz}| \approx 0.20\text{--}0.36$  MHz for OD<sup>-</sup>, and  $2|P_{zz}| \approx 0.30\text{--}0.38$  MHz for D<sub>2</sub>O. (c) Edmonds, D. T. *Phys. Rep.* **1974**, 29(4), 233.

(1) (a) Review: Schöllkopf, U. *Angew. Chem., Int. Ed. Engl.* **1970**, 9, 763. For references to more recent work, see: (b) Schreiber, S. L.; Goulet, M. T. *Tetrahedron Lett.* **1987**, 28, 1043. Adam, S. *Tetrahedron* **1989**, 45, 1409. Azzena, U.; Denurra, T.; Melloni, G.; Piroddi, A. M. *J. Org. Chem.* **1990**, 55, 5532. (c) Schlosser, M.; Strunk, S. *Tetrahedron* **1989**, 45, 2649.

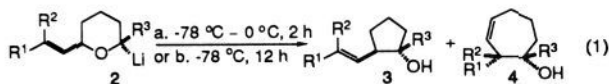
(2) Reviews: Reference 1a. Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1979**, 18, 563. Nakai, T.; Mikami, K. *Chem. Rev.* **1986**, 86, 885.

(3) Carbon nearly always displaces lithium at nonplanar carbon with retention. Fleming, I.; Rowley, M. *Tetrahedron* **1986**, 42, 3181 and citations therein. Chassaing, G.; Lett, R.; Marquet, A. *Tetrahedron Lett.* **1978**, 471 and citations therein.

## Scheme I



**Table I.** Wittig Rearrangements of 2-Lithio-6-(1-propenyl)tetrahydropyrans **2**



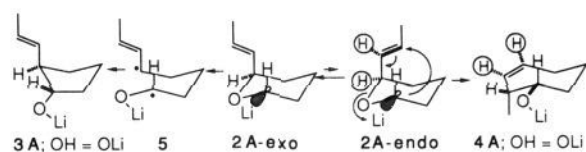
compd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	3, %	4, %	method
<b>2A</b>	CH <sub>3</sub>	H	H	21	45	a or b
<b>2B</b>	H	CH <sub>3</sub>	H	28	3 ( <b>4A</b> )	a
<b>2C</b>	CH <sub>3</sub>	H	CH <sub>3</sub>	79	0	a

[2,3]-rearrangement has recently been anticipated by Wu, Houk, and Marshall<sup>4</sup> on the basis of theoretical calculations; we have arrived at essentially the same transition state on the basis of experimental results.

Earlier work<sup>5</sup> indicated that a wide variety of  $\alpha$ -lithio ethers could be generated by reductive lithiation of  $\alpha$ -(phenylthio) ethers, and we<sup>6</sup> and others<sup>7</sup> have used the resulting anions in Wittig rearrangements. The finding<sup>8</sup> that the proximate product of reductive lithiation of readily produced 2-(phenylthio)tetrahydropyrans possesses an axial carbon–lithium bond has now allowed the determination of the stereochemistry of these rearrangements. The method of preparation of the 2-lithio-6-vinyl-tetrahydropyrans used in the present study is outlined in Scheme I (LDBB is lithium 4,4'-di-*tert*-butylbiphenylide<sup>9</sup>).<sup>10</sup> The expected<sup>8,11</sup> trans arrangement of the Li and vinyl substituents was shown to be virtually complete by <sup>1</sup>H NMR analysis of the products obtained by quenching **2D** with CH<sub>3</sub>OD and **2C** with CH<sub>3</sub>OH, processes that occur with configurational retention.<sup>8,12,13</sup>

Upon being warmed, **2A–C** yield as the only identified compounds [1,2]- and [2,3]-rearrangement products (**3** and **4**, respectively) (eq 1, Table I).<sup>14</sup> In the ring contraction product **3**, one of the two chiral centers of the substrate has been inverted and one retained. Since the literature contains many examples of partial retention of the migrating group in [1,2]-Wittig rearrangements,<sup>1</sup> it is likely that this group retains its configuration and that the lithium-bearing carbon atom becomes inverted, as

## Scheme II



indicated in eq 1. This was shown to be the case by conducting the rearrangement of nonracemic **2E** prepared by the use of nonracemic **1E**, generated by Sharpless resolution.<sup>15,16</sup>

The usual assumption<sup>2</sup> that the [2,3]-rearrangement is concerted seems particularly likely in the case of **2A** since it is the major reaction path despite the undoubtedly more crowded conformation of the precursor (see below). Since the propenyl group must undergo a suprafacial change, an inversion must have occurred at the lithium-bearing carbon atom. These stereochemical results are readily rationalized in Scheme II.

The equatorial C–Li bond is aligned antiperiplanar to the C–O bond that cleaves in both processes, and in fact, the  $\sigma, \sigma^*$  overlap is a substantial contributor to the stabilization of these conformers.<sup>17</sup> The widely accepted Lansbury<sup>18</sup> mechanism nicely explains the stereochemistry of the [1,2]-process. Unlike other cases whose stereochemistry has been studied, the two radical centers of **5**, generated by homolysis of the C–O bond, are held together by a tether, accounting for the fact that recombination, very rapid in any case,<sup>1c,19</sup> occurs before bond rotations.<sup>19</sup> Thus retention at the migrating chiral center and inversion at the terminus occur. The undoubtedly far less stable endo conformer of **2A** is the only one capable of undergoing a concerted [2,3]-Wittig rearrangement, assuming the necessity of an equatorial C–Li bond. Such a process yields a *cis* olefin as can be seen by noting the juxtaposition of the circled hydrogen atoms. This six-electron process is consistent with the rules of orbital symmetry<sup>20</sup> in that it involves a suprafacial process and two inversions, one at the carbon atom bearing the lithium and the other at the oxygen atom ( $\sigma_{2s} + \sigma_{2a} + \sigma_{2a}$ ). The importance of this transfer of Li<sup>+</sup> from C to O during the [1,2]-process has been highlighted by the depression in rate that is observed when Li<sup>+</sup> is replaced by K<sup>+</sup>.<sup>1c</sup>

This picture of the [2,3]-process leads to several testable predictions. The endo conformation should be greatly destabilized by steric congestion if a *cis*-propenyl group were used as in **2B** or if the hydrogen atom on the lithium-bearing carbon atom were replaced by a methyl group as in **2C**. In fact, the concerted [2,3]-process is inhibited in both cases (Table I); only **2B** produces a trace of cycloheptanol, and it is not **4B** but **4A**, presumably formed by a nonconcerted process from the exo conformer. Finally, we have found<sup>21</sup> that an analogue in which the 2-lithio and 6-propenyl substituents are *cis* to each other and *trans* to a 4-*tert*-butyl group undergoes no [2,3]-rearrangement even though it is undoubtedly born in the 2,6-diaxial conformation. This result rules out the possibility that such a conformer, generated by a thermal inversion<sup>8a,11</sup> of the lithium-bearing carbon atom, is an intermediate in the rearrangements of **2A–C**.

The full paper will explore the synthetic aspects of these rearrangements, including the preparation of the hydrazulene ring system by this type of ring expansion.

(4) Wu, Y. D.; Houk, K. N.; Marshall, J. A. *J. Org. Chem.* **1990**, *55*, 1421. Marshall and Lebreton, without explanation, also appear to assume such an inversion in [2,3]-Wittig rearrangements: Marshall, J. A.; Lebreton, J. *J. Am. Chem. Soc.* **1988**, *110*, 2925. Marshall, J. A.; Lebreton, J. *J. Org. Chem.* **1988**, *53*, 4108.

(5) Cohen, T.; Matz, J. R. *J. Am. Chem. Soc.* **1980**, *102*, 6900.

(6) Lin, M. T. Ph.D. Thesis, University of Pittsburgh, 1984.

(7) Broka, C. A.; Shen, T. *J. Am. Chem. Soc.* **1989**, *111*, 2981. Kruse, B.; Brückner, R. *Chem. Ber.* **1989**, *122*, 2023. Kruse, B.; Brückner, R. *Tetrahedron Lett.* **1990**, *31*, 4425.

(8) (a) Cohen, T.; Lin, M.-T. *J. Am. Chem. Soc.* **1984**, *106*, 1130. (b) Lancelin, J.-M.; Morin-Allory, L.; Sinay, P. *J. Chem. Soc., Chem. Commun.* **1984**, 355.

(9) Freeman, P.; Hutchinson, L. *J. Org. Chem.* **1980**, *45*, 1924.

(10) Reductive lithiation of acetals of 3-chloro aldehydes and ketones: Ramón, D. J.; Yus, M. *Tetrahedron Lett.* **1990**, *31*, 3763, 3767.

(11) Rychnovsky, S. D.; Mickus, D. E. *Tetrahedron Lett.* **1989**, *30*, 3011. Rychnovsky, S. C. *J. Org. Chem.* **1989**, *54*, 4982.

(12) Sawyer, J. S.; Kucerovy, A.; Macdonald, T. L.; McGarvey, G. J. *J. Am. Chem. Soc.* **1988**, *110*, 842.

(13) There is a strong tendency for the C–Li bond to become equatorial.<sup>8a,11</sup>

(14) Ring contractions and expansions by [1,2]- and [2,3]-Wittig rearrangements, respectively, are rare and have only involved stabilized anions: Mazaleyrat, J.-P.; Welvert, Z. *Nouv. J. Chim.* **1983**, *7*, 491. Thomas, A. F.; Dubini, R. *Helv. Chim. Acta.* **1974**, *57*, 2084.

(15) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

(16) The use of L-(+)-diisopropyl tartrate provided 55% of unreacted **1** of ~48% ee by (+)-Eu(hfc)<sub>3</sub> resolution. The derived **3** was hydrogenated to *trans*-2-ethyl-1-cyclopentanol of ~50% ee [(+)-Eu(hfc)<sub>3</sub>]. The optical rotation of  $\alpha_{D}^{25} = +10.6$  ( $c = 0.5$ , C<sub>2</sub>H<sub>5</sub>OH) indicated the major isomer to be 1*S*,2*S*; Brown, H. C.; Jadhav, P. K.; Mandal, A. K. *J. Org. Chem.* **1982**, *47*, 5074.

(17) Lehn, J. M.; Wipff, G. *J. Am. Chem. Soc.* **1976**, *98*, 7498.

(18) Lansbury, P. T.; Pattinson, V. A.; Sidler, J. D.; Bieber, J. B. *J. Am. Chem. Soc.* **1966**, *88*, 78 and citations therein.

(19) Dervan, P. B.; Dougherty, D. In *Radicals*; Borden, W. T., Ed.; Wiley: New York, 1982; p 107.

(20) Woodward, R. B.; Hoffmann, R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 781.

(21) Verner, E. J.; Cohen, T., submitted for publication.

**Acknowledgment.** We thank the National Institutes of Health for financial support and Steven Norton and Professors Peter Wipf and Russell Petter for helpful suggestions.

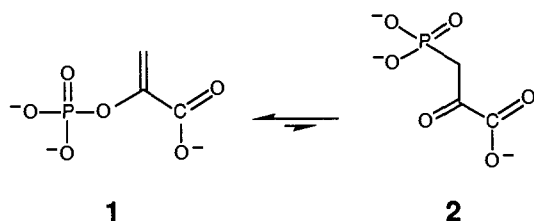
**Supplementary Material Available:** Experimental procedures and spectral data (5 pages). Ordering information is given on any current masthead page.

### Synthesis of the Unusual Metabolite Carboxyphosphoenolpyruvate. Cloning and Expression of Carboxyphosphoenolpyruvate Mutase

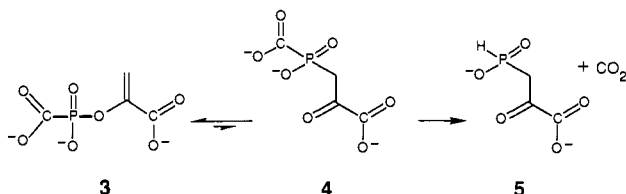
Sally Freeman,<sup>†</sup> Scott J. Pollack, and Jeremy R. Knowles\*

Departments of Chemistry and Biochemistry  
Harvard University, Cambridge, Massachusetts 02138  
Received September 10, 1991

The carbon-phosphorus bond of most naturally occurring phosphonates derives from a 1,3-phospho group transfer reaction between phosphoenolpyruvate (**1**) and phosphonopyruvate (**2**).<sup>1</sup> The equilibrium for this rearrangement, which is catalyzed by phosphoenolpyruvate mutase (EC 6.4.2.9), lies predominantly toward **1**.<sup>1</sup> This enzyme is not, however, responsible for the



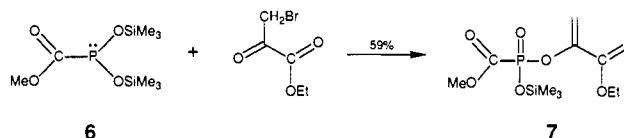
formation of either of the two carbon-phosphorus bonds in bialaphos, a powerful herbicide isolated from *Streptomyces hygroscopicus*. Thus Seto and his collaborators<sup>2,3</sup> have shown that the biosynthetic pathway to bialaphos involves carboxyphosphoenolpyruvate mutase (CPEP mutase), which catalyzes the formation of (hydroxyphosphinyl)pyruvate (**5**) from carboxyphosphoenolpyruvate (**3**, CPEP). By analogy with phosphoenolpyruvate mutase, the first step of the CPEP mutase reaction presumably generates the new carbon-phosphorus bond by carboxyphospho group migration to give **4**. This reaction seems likely



to be energetically unfavorable (as is the conversion of **1** to **2**). In the second step, the intermediate **4** would decarboxylate, thus driving the reaction toward **5**. Mechanistic studies on this interesting enzyme will be possible only with a supply of the substrate **3**, a continuous product assay for **5**, and ready access to the enzyme. We report here the chemical synthesis of the unusual phosphonate **3**, the cloning of the mutase gene from *S. hygroscopicus* and its expression at high levels in *Escherichia coli*, and a convenient assay for the product, **5**.

Despite earlier suggestions that **3** is "extremely unstable",<sup>3</sup> the chemical synthesis of **3** proceeded smoothly, the key step<sup>4</sup> involving

a Perkov reaction between bis(trimethylsilyloxy)(methoxycarbonyl)phosphine<sup>5</sup> (**6**) and ethyl bromopyruvate to give the triester **7**. Although recent studies on the hydrolysis of trialkyl



esters of phosphonofosphate have shown that the carbon-phosphorus bond is readily cleaved by attack at carbonyl,<sup>6</sup> treatment of **7** with aqueous base first removes the reactive SiMe<sub>3</sub> group to give the diester, in which the carbon-phosphorus bond is much less vulnerable. The triester **7** was readily converted to **3** by the careful addition of 3 equiv of aqueous NaOH.<sup>7</sup> The spectroscopic data<sup>7</sup> were consistent with those reported for the natural product.<sup>2</sup> The trisodium salt of **3** is stable in water over several days at room temperature. Carboxyphosphoenolpyruvate (**3**) will be useful for studies of both CPEP mutase and the enzyme that catalyzes CPEP formation from phosphoenolpyruvate and phosphonofosphate.<sup>8</sup>

To develop an assay for CPEP mutase, a sample of **5**<sup>9,10</sup> was prepared by the transamination of (hydroxyphosphinyl)alanine<sup>11</sup> with glyoxylic acid-Cu(OAc)<sub>2</sub>.<sup>12</sup> We had earlier shown that **2** is a relatively poor substrate for malate dehydrogenase (MDH), having a high *K*<sub>m</sub> of 11 mM. In terms of both size and charge, **5** should be a better mimic for oxaloacetate than **2**. As predicted, **5** is a good substrate for MDH, having a *K*<sub>m</sub> of 0.68 mM and a *k*<sub>cat</sub> of 164 s<sup>-1</sup>.<sup>13</sup> Interestingly, the product of this reaction, (hydroxyphosphinyl)lactate, has been found in extracts of *S. hygroscopicus*.<sup>14</sup> Using MDH/NADH, therefore, a continuous coupled enzyme assay can be established for CPEP mutase.

Using the partial gene sequence reported by Hidaka et al.,<sup>2</sup> we have cloned the gene for CPEP mutase from *S. hygroscopicus* into *E. coli*. The open reading frame encodes a protein of 295 amino acids, the calculated molecular weight of which (32 700) agrees with that determined for the purified enzyme (32 000 ± 1000).<sup>2</sup> The gene was expressed in *E. coli* using the T7 pET11 vector, which produced CPEP mutase at 20% of the total cell

(4) Sekine, M.; Futatsugi, T.; Yamada, K.; Hata, T. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2509.

(5) Issleib, K.; Mögelin, W.; Balszuweit, A. *Z. Anorg. Allg. Chem.* **1985**, 530, 16.

(6) Krol, E. S.; Davies, J. M.; Thatcher, G. R. J. *J. Chem. Soc., Chem. Commun.* **1991**, 118. Mitchell, A. G.; Nicholls, D.; Walker, I.; Irwin, W. J.; Freeman, S. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1297.

(7) Addition of methanol to the aqueous solution caused precipitation of trisodium carboxyphosphoenolpyruvate (**3**) in 53% yield: mp 275–280 °C dec; <sup>1</sup>H NMR (250.1 MHz, D<sub>2</sub>O) δ 5.54 (t, *J*<sub>PH</sub> = *J*<sub>gem</sub> = 1.7 Hz, 1H), 5.20 (t, *J*<sub>PH</sub> = *J*<sub>gem</sub> = 1.8 Hz, 1H); <sup>13</sup>C NMR (62.9 MHz, D<sub>2</sub>O) δ 179.6 (d, *J*<sub>PC</sub> = 235.4 Hz, PCOO<sup>-</sup>), 173.4 (d, *J*<sub>PC</sub> = 6.1 Hz, CCOO<sup>-</sup>), 151.8 (d, *J*<sub>PC</sub> = 9.2 Hz, C=CH<sub>2</sub>), 106.0 (d, *J*<sub>PC</sub> = 4.8 Hz, C=CH<sub>2</sub>); <sup>31</sup>P NMR (101.3 MHz, D<sub>2</sub>O) δ -1.77 (s); IR (KBr) 1603 cm<sup>-1</sup>. Calcd for C<sub>4</sub>H<sub>2</sub>O<sub>7</sub>PNa<sub>3</sub>: C, 18.34; H, 0.77; P, 11.82. Found: C, 18.00; H, 1.01; P, 11.48.

(8) Imai, S.; Seto, H.; Sasaki, T.; Tsuruoka, T.; Ogawa, H.; Satoh, A.; Inouye, S.; Niida, T.; Otake, N. *J. Antibiot.* **1984**, 37, 1505.

(9) Meiji Seika Kaisha, Ltd. Japanese Patent 58219192, 1983, Japan; *Chem. Abstr.* **1984**, 100, 210149q.

(10) **5** was purified by ion-exchange chromatography (on AG1-8X, formate form, eluting with triethylammonium bicarbonate buffer, then on Dowex-50, sodium form, eluting with water) to give **5** in 30% yield: <sup>1</sup>H NMR (500 MHz, H<sub>2</sub>O) δ 7.33 (dt, *J*<sub>PH</sub> = 550.3, *J*<sub>HH</sub> = 1.7 Hz, 1H), 3.37 (dd, *J*<sub>PH</sub> = 18.9, *J*<sub>HH</sub> = 1.7 Hz, 2H); <sup>13</sup>C NMR (100.6 MHz, H<sub>2</sub>O) δ 199.9 (br s, CH<sub>2</sub>C(O)), 168.9 (s, COO<sup>-</sup>), 46.7 (d, *J*<sub>PC</sub> = 71.7 Hz, PCH<sub>2</sub>); <sup>31</sup>P NMR (121.5 MHz, H<sub>2</sub>O) 17.7 ppm (dt, *J*<sub>PH</sub> = 550.3, *J*<sub>PH</sub> = 18.9 Hz, <sup>1</sup>H coupled); MS (negative-ion FAB, glycerol matrix) *m/z* 172.9623 (172.9616 calcd for C<sub>3</sub>-H<sub>3</sub>NaO<sub>5</sub>P, M - Na).

(11) Dingwall, J. G.; Ehrenfreund, J.; Hall, R. G. *Tetrahedron* **1989**, 45, 3787.

(12) Sparkes, M. J.; Rogers, K. L.; Dixon, H. B. F. *Eur. J. Biochem.* **1990**, 194, 373.

(13) Malate dehydrogenase (0.56 μg) was added to a solution containing 50 mM MES buffer, pH 6.5 (1.0 mL), NADH (0.1 mg), **5** (0.038–7.7 mM) at 30 °C. The A<sub>340nm</sub> was monitored with time. The *k*<sub>cat</sub> was calculated using 37 000 as the molecular weight for MDH: Wolfenstein, C.; England, S.; Listowsky, I. *J. Biol. Chem.* **1969**, 244, 6415. The *K*<sub>m</sub> of oxaloacetate is 4 μM at pH 6.4: Cassman, M.; England, S. *J. Biol. Chem.* **1966**, 241, 793.

(14) Seto, H.; Imai, S.; Tsuruoka, T.; Ogawa, H.; Satoh, A.; Sasaki, T.; Otake, N. *Biochem. Biophys. Res. Commun.* **1983**, 111, 1008.

\* Permanent address: Pharmaceutical Sciences Institute, Aston University, Birmingham, B4 7ET, UK.

(1) 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.

(2) Hidaka, T.; Seto, H.; Imai, S. *J. Am. Chem. Soc.* **1989**, 111, 8012.

(3) Hidaka, T.; Imai, S.; Hara, O.; Anzai, H.; Murakami, T.; Nagaoka, K.; Seto, H. *J. Bacteriol.* **1990**, 172, 3066.