silyl)oxy]-10-methyl-*N*-phenyl-2-[(phenylseleno)methyl]-2,9-hexadecadienamide (0.60 g, 0.936 mmol) by the procedure described for the preparation of the amide **7a**: ^{1}H NMR δ 8.72, 8.95 (s, 1 H), 7.00–7.68 (m, 5 H), 6.08, 6.10 (s, 1 H), 5.65 (s, 1 H), 5.11 (t, J = 6.5 Hz, 1 H), 4.35–4.76 (m, 1 H), 3.95 (br s, 1 H), 3.58–3.73 (m, 1 H), 1.79–2.11 (m, 9 H), 1.05–1.61 (m, 17 H), 0.63–1.03 (m, 12 H), 0.08 (s, 6 H); IR (thin film) 3300, 1670, 1600, 1450, 850 cm^{-1}; exact mass calcd for $C_{30}H_{51}NO_3Si$ (M^+) 501.3637, found 501.3617.

(3*R,4*S**)-(*E*)-4-[(*tert*-Butyldimethylsilyl)oxy]-3-(mesyloxy)-10-methyl-2-methylene-*N*-phenyl-9-hexadecenamide (27)**: ^{1}H NMR δ 7.85 (s, 1 H), 7.13–7.54 (m, 5 H), 6.08 (s, 1 H), 5.94 (s, 1 H), 5.48 (d, J = 3.7 Hz, 1 H), 5.09 (t, J = 7.1 Hz, 1 H), 4.11–4.15 (m, 1 H), 3.07 (s, 3 H), 1.91–1.97 (m, 4 H), 1.19–1.55 (m, 17 H), 0.86–0.95 (m, 12 H), 0.12–0.16 (s, 6 H); IR (thin film) 3330, 1670, 1600, 1440, 1360, 1180, 850 cm^{-1}; exact mass calcd for $C_{23}H_{35}NO_5SSi$ ($M^+ - C_4H_9$) 522.2710, found 522.2735.

(3*R,4*R**)-(*E*)-[(*tert*-Butyldimethylsilyl)oxy]-3-(mesyloxy)-10-methyl-2-methylene-*N*-phenyl-9-hexadecenamide (28)**: ^{1}H NMR δ 7.85 (s, 1 H), 7.14–7.54 (m, 5 H), 6.02 (s, 1 H), 5.79 (s, 1 H), 5.33 (d, J = 5.8 Hz, 1 H), 5.08 (t, J = 7.2 Hz, 1 H), 4.09–4.13 (m, 1 H), 3.03 (s, 3 H), 1.92–1.98 (m, 4 H), 1.19–1.59 (m, 17 H), 0.85–0.90 (m, 12 H), 0.74 (s, 6 H); IR (thin film) 3320, 1660, 1590, 1440, 1360, 1180, 850 cm^{-1}; exact mass calcd for $C_{31}H_{53}NO_5SSi$ (M^+) 579.3412, found 579.3387.

(3*R,4*R**)-(*E*)-3,4-Epoxy-10-methyl-2-methylene-*N*-phenyl-9-hexadecenamide (29) (Conocandin *N*-phenylamide)**. From **27** (0.20 g, 0.345 mmol), **29** was obtained in 63% yield (0.08 g, 0.216 mmol): ^{1}H NMR δ 8.73 (s, 1 H), 7.10–7.58 (m, 5 H), 6.27 (s, 1 H), 5.77 (s, 1 H), 5.10 (t, J = 7.0 Hz, 1 H), 3.56 (d, J = 2.4 Hz, 1 H), 3.09–3.13 (m, 1 H), 1.94–2.04 (m, 4 h), 1.21–1.70 (m, 17 H), 0.86–0.92 (m, 3 H); IR (thin film) 3270, 1660, 1660, 1440 cm^{-1}; exact mass calcd for $C_{24}H_{35}NO_2$ (M^+) 369.2668, found 369.2653.

(3*R,4*S**)-(*E*)-3,4-Epoxy-10-methyl-2-methylene-*N*-phenyl-9-hexadecenamide (30)**. From **28** (0.24 g, 0.414 mmol), the amide **30** was obtained in 36% yield (0.06 g, 0.102 mmol): mp 37–40 °C; ^{1}H NMR δ 8.38 (s, 1 H), 7.11–7.59 (m, 5 H), 6.31 (s, 1 H), 5.68 (s, 1 H), 5.06 (t, J = 7.0 Hz, 1 H), 3.90 (d, J = 4.3 Hz, 1 H), 3.25–3.26 (m, 1 H), 1.91–2.04 (m, 4 H), 1.21–1.59 (m, 17 H), 0.88–0.92 (m, 3 H); exact mass calcd for $C_{24}H_{35}NO_2$ (M^+) 369.2668, found 369.2686.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. K.T. thanks the Kurata Foundation for financial support.

Reversible and in Situ Formation of Organic Arsenates and Vanadates as Organic Phosphate Mimics in Enzymatic Reactions: Mechanistic Investigation of Aldol Reactions and Synthetic Applications

Dale G. Drueckhammer, J. Robert Durrwachter, Richard L. Pederson, Debbie C. Crans,† Lacy Daniels,‡ and Chi-Huey Wong*

Department of Chemistry, Texas A&M University, College Station, Texas 77843, Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, and Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

Received June 15, 1988

A synthetic strategy is developed that uses organic phosphate utilizing enzymes as catalysts and a mixture of an organic alcohol and inorganic arsenate or vanadate to replace the organic phosphate substrate. In this process, inorganic arsenate or vanadate reacts with the alcohol reversibly in situ to form a mixture of esters, one of which is accepted by the enzyme as a substrate. Examples of the utility of this approach are demonstrated in enzymatic aldol condensations catalyzed by fructose-1,6-diphosphate aldolase, fuculose-1-phosphate aldolase, and rhamnulose-1-phosphate aldolase with a mixture of dihydroxyacetone and inorganic arsenate as substrate. Several uncommon sugars and deoxy sugars are prepared on 5–17-mmol scales. Mechanistic studies on an aldol reaction indicate that the redox reaction between dihydroxyacetone and inorganic vanadate prohibits the use of such a mixture to replace dihydroxyacetone phosphate in enzymatic aldol condensations.

Introduction

Organic phosphate utilizing enzymes hold potential for the preparation of many multifunctional or complex organic compound, especially sugars.[1] Enzymatic synthesis of organic phosphates that require ATP and ATP regeneration has been successfully developed.[2] When an analogue of the naturally occurring organic phosphate is desired, the enzymatic preparation, however, is limited by the substrate specificity of the enzyme used. In some instances, phosphorylated substrates or products are unstable and difficult to manipulate in solution, and the phosphate moiety of the product may have to be removed. This overall reaction sequence is shown in eq 1.

$$\mathrm{ROH} \xrightarrow[\text{kinase}]{\text{ATP}} \mathrm{ROPO_3^{2-}} \xrightarrow{\text{enzyme}} \mathrm{R'OPO_3^{2-}} \xrightarrow[\text{H}^+ \text{ or phosphatase}]{\mathrm{H_2O}} \mathrm{R'OH} \quad (1)$$

Several organic phosphate utilizing enzymes are known to catalyze transformations of their nonphosphorylated substrates in the presence of inorganic arsenate; the reactions are detected spectroscopically under assay conditions.[3] These enzymes include glucose-6-phosphate dehydrogenase, glucose phosphate isomerase, α-glycerophosphate dehydrogenase, phosphofructokinase, and 6-phosphogluconate dehydrogenase. More recently it has been shown that vanadate also stimulates the oxidation of glucose by glucose-6-phosphate dehydrogenase.[4] These reactions may involve the reversible, nonenzymatic formation of organic arsenate or vanadate esters, which are analogous to organic phosphates and accepted by the en-

† Colorado State University.
‡ University of Iowa.

(1) Whitesides, G. M.; Wong, C.-H. *Angew Chem., Int. Ed Engl.* **1985**, *25*, 617.
(2) Crans, D. C.; Kazlauskas, R. J.; Hirschbein, B. L.; Wong, C.-H.; Abril, O.; Whitesides, G. M. *Methods Enzymol.* **1987**, *136*, 263 and references cited therein.
(3) (a) Lagunas, R.; Sols, A. *FEBS Lett.* **1968**, *1*, 32. (b) Jaffe, K.; Aptiz-Castro, R. *FEBS Lett.* **1977**, *80*, 115. (c) Lagunas, R. *Arch. Biochem. Biophys.* **1980**, *205*, 67.
(4) Nour-Eldeen, A. F.; Craig, M. M.; Gresser, M. J. *J. Biol. Chem.* **1985**, *260*, 6836.

0022-3263/89/1954-0070$01.50/0

Table I. Activities of Enzymes with Their Unphosphorylated Substrate Analogues in the Presence of Arsenate or Vanadate

enzyme	reaction	V_{max}, units/mg enzyme	K_m, mM
glycerophosphate dehydrogenase	DHAP + NADH → G-3-P + NAD	116[a]	0.43
	DHA + As + NADH → glycerol + NAD	110[a]	52.5[b]
	DHA + V + NADH → glycerol + NAD	14[a]	79.0[b]
FDP aldolase	DHAP + D-Glyd-3-P → FDP	25	2.0
	DHA + As + D-Glyd-3-P → Fruc-6-P	3.3	11[b]
phosphogluco-isomerase	Fru-6-P → Glc-6-P	500	0.17
	Fruc + As → Glc	4	100[b]
	Fruc + V → Glc	2	200[b]
glucose-6-phosphate dehydrogenase	Glc-6-P + NAD → gluconate-6-P + NADH	54	0.24
	Glc-6-As + NAD → gluconate-6-As + NADH	33	0.5[c]
6-phosphogluconic dehydrogenase	gluconate-6-P + NAD → Rib-5-P + NADH	5	0.02
	gluconate-6-As + NAD → Rib-5-As + NADH	5	3.0[c]
phosphoribo-isomerase	Rib-5-P → Ribu-5-P	95	2.8
	Rib + As → Ribu	+	
	Rib + V → Ribu	+	
Ribu-5-P 3-epimerase	Ribu-5-P → Xylu-5-P	60	0.5
	Ribu + As → Xylu	+	
	Ribu + V → Xylu	+	
Fucu-1-P aldolase + Fuc isomerase	DHAP + L-lactaldehyde → Fucu-1-P	4	1.2[d]
	DHA + As + L-lactaldehyde → Fucu + Fuc	0.4	
Rhamnu-1-P aldolase + Rham isomerase	DHAP + L-lactaldehyde → Rhamnu-1-P	17	3.0[d]
	DHA + As + L-lactaldehyde → Rhamnu + Rham	0.9	
nucleoside phosphorylase	inosine + P_i → Rib-1-P + hypoxanthine	18	0.38[e]
	inosine + As → Rib + hypoxanthine	1.2	
	inosine + V → Rib + hypoxanthine	1.8	

+ Indicates some activity (<0.1 units/mg enzyme). For details see the Experimental Section. [a] Apparent V_{max} with 0.3 mM NADH and 0.1 M sodium arsenate or 0.5 mM sodium vanadate. [b] K_m is expressed in terms of concentration of DHA or Fruc in the presence of 0.1 M arsenate or 0.5 mM vanadate. [c] K_m for the arsenate ester. [d] K_m for DHAP. [e] K_m for inosine. Abbreviations: DHA, dihydroxyacetone; G-3-P, glycerol-3-phosphate; Glyd, glyceraldehyde; Fruc, D-fructose; Glc, D-glucose; Rib, D-ribose; Ribu, D-ribulose; Xylu, D-xylulose; Fuco, L-fuculose; Fuc, L-fucose; Rhamnu, L-rhamnulose; Rham, L-rhamnose.

zyme. After enzymatic transformation, the product ester hydrolyzes spontaneously to give the free alcohol product. This overall reaction, shown in eq 2,

$$ROH \xrightarrow{HOAsO_3^{2-}} ROAsO_3^{2-} \xrightarrow{\text{enzyme}} R'OAsO_3^{2-} \xrightarrow{H_2O} R'OH \quad (2)$$

is effectively the same as the synthetic sequence in eq 1 except that kinase, phosphatase, and ATP regeneration are eliminated. We envisioned that the reaction sequence of eq 2 could be used for synthetic purposes. This approach was employed in a preliminary study where dihydroxyacetone in the presence of a catalytic amount of arsenate was found to be an effective substrate for fructose-1,6-diphosphate (FDP) aldolase, which accepts dihydroxyacetone phosphate as a natural substrate.[5] This new synthetic scheme eliminates the need for preparing dihydroxyacetone phosphate (DHAP) and for removing the phosphate group of the product to give the unphosphorylated sugar, simplifying the synthetic procedure. The overall reaction scheme is depicted in Figure 1. Since many enzymes that catalyze transformations of organophosphates (e.g. sugar phosphates) are synthetically useful and preparation of the substrates is often complicated, this approach may have potential for preparative synthesis of some natural and unnatural biologically active substances that are now difficult to prepare. To extend the scope of this approach to enzyme-catalyzed organic synthesis, we report here a study of the activity of several synthetically useful organic phosphate utilizing enzymes with their unphosphorylated substrate analogues in the presence of arsenate or vanadate, and the practical-scale syntheses of sugars by using FDP aldolase, fuculose-1-phosphate (Fucu-1-P) aldolase, and rhamnulose-1-phosphate (Rhamnu-1-P) aldolase.

(5) Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812.

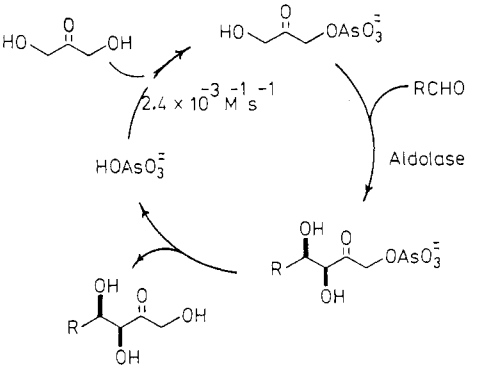


Figure 1. FDP aldolase catalyzed aldol condensation in the presence of DHA/inorganic arsenate.

Results and Discussion

Enzyme Activities. The enzymes studied and their activities with their unphosphorylated substrate analogues in the presence of arsenate and vanadate are shown in Table I. Assays were done in 0.1 M arsenate buffer, pH 8, or in 0.02 M Tris buffer, pH 8, containing 0.5 mM vanadate. Arsenate concentrations as high as 1.0 M may be used, but in synthetic reactions the isolation of product could be a problem so it was desirable to evaluate activity at lower concentration with arsenate being recycled during the reaction. Vanadate polymerizes at concentrations above 0.5 mM, so higher concentrations of the active monomeric vanadate cannot be obtained. For determination of V_{max} and K_m values the rates were measured at varying concentrations of the unphosphorylated substrate analogue in 0.1 M sodium arsenate or 0.5 mM sodium vanadate with fixed concentrations of enzyme, and the kinetic data were determined from the double-reciprocal plot. Conditions were used in which the rate of the enzyme-catalyzed reaction was much slower than the rate of arsenate or vanadate ester formation.

With the first three enzymes in Table I, K_m is expressed in terms of concentration of dihydroxyacetone or fructose in the presence of 0.1 M arsenate or 0.5 mM vanadate. The K_m values for the fourth and fifth enzymes are taken from the literature[3c] and expressed in terms of concentration of the arsenate esters calculated using the equilibrium constant for arsenate ester formation. With the last five enzymes kinetic constants were not determined because activities were too low. The V_{max} values indicated for these five enzymes are the observed rates under the assay conditions. While some of the K_m values are quite high, the V_{max} values are generally good, in some cases almost as high as with the phosphorylated substrate. Thus one can usually achieve good rates if high concentrations of substrate are used. The fact that some of the rates are somewhat lower is not alarming since most of the unnatural substrates transformed enzymatically for synthetic purposes exhibit rates significantly lower than those of the natural substrates.

The enzymes exhibiting the most apparent synthetic usefulness in this process are the three aldolases. The synthesis of sugars using FDP aldolase is well documented[5-7] while Fucu-1-P aldolase and Rhamnu-1-P aldolase can be used to make deoxy sugars. The deoxy ketoses obtained in the latter two enzyme reactions can be isomerized in situ to the corresponding deoxy aldoses catalyzed by fucose isomerase and rhamnose isomerase, respectively (see below). Such multienzyme systems hold promise for one-pot syntheses of uncommon sugar derivatives. These two transformations could not be carried out in one pot by the conventional approach, as a dephosphorylation step would be required between the two steps. The enzyme nucleoside phosphorylase could conceivably be useful in the synthesis of nucleoside analogues of medical importance if the reaction were run in the opposite direction.[8]

Mechanistic Studies of Dihydroxyacetone Arsenate and Vanadate Ester Formation. Due to the interest in the aldolases and the fact that all three aldolases use DHAP as the natural substrate, further experiments were initiated to study the formation of dihydroxyacetone arsenate and vanadate esters. Previous studies using glucose-6-phosphate dehydrogenase have shown that the rate constants for glucose-6-arsenate and glucose-6-vanadate formation at pH 7.0 are 6.3×10^{-6} and 2.4 M^{-1} s^{-1}, respectively, compared to 9×10^{-11} M^{-1} s^{-1} estimated for glucose-6-phosphate formation.[4] Via similar procedures the rates of dihydroxyacetone ester formation were measured with glycerophosphate dehydrogenase. This enzyme-catalyzed reaction is convenient to study since the arsenate and vanadate esters are good substrates for the enzyme, and the rate can be determined directly by measuring the disappearance of NADH spectrophotometrically. The initial rates of glycerophosphate dehydrogenase catalyzed reduction of dihydroxyacetone (DHA) were determined at constant concentrations of

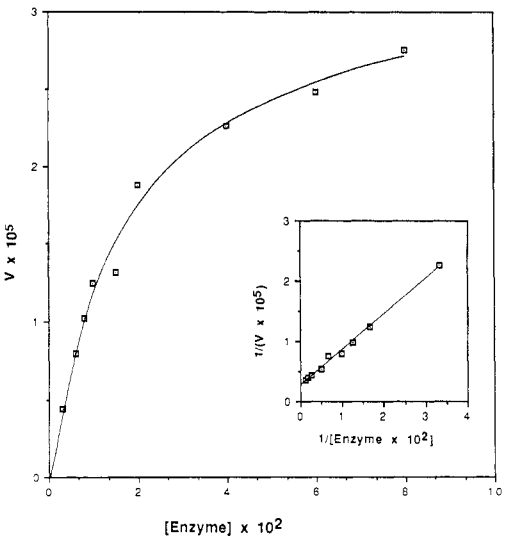


Figure 2. Rate (M/min) of glycerophosphate dehydrogenase catalyzed reduction of dihydroxyacetone in the presence of 5 mM arsenate vs enzyme concentration (mg/mL). Inserted is the reciprocal plot of the above data.

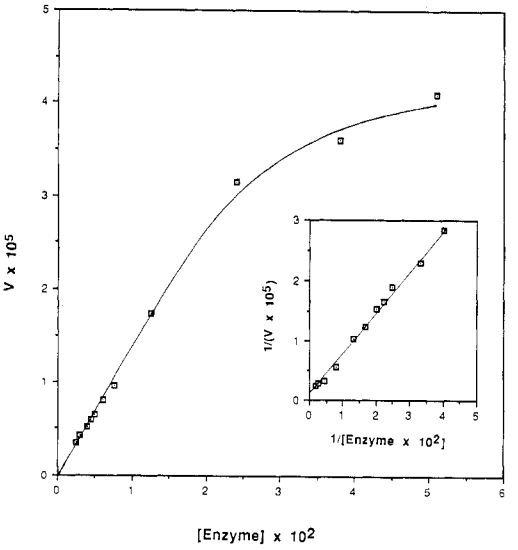


Figure 3. Rate (M/min) of glycerophosphate dehydrogenase catalyzed reduction of dihydroxyacetone in the presence of 0.1 mM vanadate vs enzyme concentration (mg/mL). Inserted is the reciprocal plot.

DHA and arsenate with increasing concentrations of enzyme. The arsenate was added last to initiate the reaction. A plot of the rate versus amount of enzyme (Figure 2) shows a leveling off of the rate at high enzyme concentration. This is consistent with the mechanism of eq 2 with the curve in the rate profile being due to a change in the rate-determining step from enzymatic reduction of dihydroxyacetone arsenate to formation of the dihydroxyacetone arsenate ester. When arsenate and DHA are allowed to react before enzyme is added, a much greater initial rate is observed, consistent with nonenzymatic formation of dihydroxyacetone arsenate, which is rapidly reduced when enzyme is added. A plot of the reciprocal of the rate versus the reciprocal of the enzyme concentration shows a nonzero intercept (Figure 2 insert). This intercept corresponds to the rate at infinite enzyme concentration, which is equal to the rate of formation of the dihydroxyacetone arsenate ester (2.4×10^{-3} M^{-1} s^{-1}). The same enzyme assays using 0.5 mM vanadate in Tris buffer showed a similar although much less pronounced leveling

(6) (a) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199. (b) Wong, C.-H.; Mazenol, F. P.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3493. (c) Durrwachter, J. R.; Sweers, H. M.; Nozaki, K.; Wong, C.-H. *Tetrahedron Lett.* **1986**, *27*, 1261. (d) Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175.

(7) Bednarski, M. D.; Waldmann, H. J.; Whitesides, G. M. *Tetrahedron Lett.* **1986**, *27*, 5807.

(8) Sheit, K. H. *Nucleotide Analogs*; Wiley-Interscience: New York, 1980. Krenitsky, T. A.; Rideout, J. L.; Chao, E. Y.; Kozalka, G. W.; Churney, F.; Crouch, R. C.; Cohn, N. K.; Wolberg, G.; Vinegar, R. *J. Med. Chem.* **1986**, *29*, 138. Utagawa, T.; Morisawa, H.; Yamanaka, S.; Yamazaki, A.; Hirose, Y. *Agric. Biol. Chem.* **1986**, *50*, 121. Kim, C. H.; Marquez, V. E.; Moo, D. T.; Haines, D. R.; McCormack, J. J. *J. Med. Chem.* **1986**, *29*, 1374.

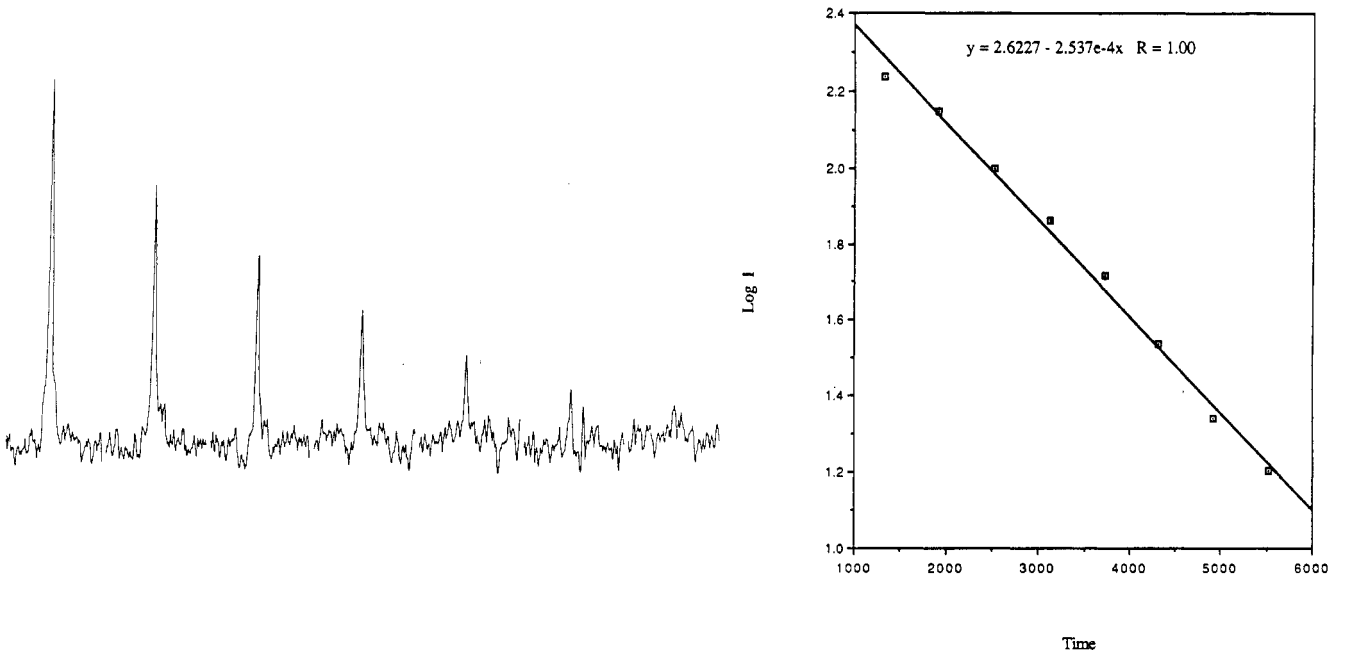


Figure 4. Left, ^{51}V NMR spectra of the disappearance of 0.5 mM vanadate at −550 ppm in the presence of 1 M DHA at pH 7. The sample was deoxygenated with Ar. The time period for each peak is 10 min. Right, plot of data for the disappearance of vanadate (time is in seconds). *I*, intensity of the peak.

off of the rate at high enzyme concentration (Figure 3). This is consistent with a much greater rate of formation of the dihydroxyacetone vanadate ester than the corresponding arsenate ester so that a much higher concentration of enzyme is required to make the vanadate ester formation become partially rate determining. Again the double-reciprocal plot (Figure 3 insert) shows a nonzero intercept corresponding to the rate of formation of the dihydroxyacetone vanadate ester (0.43 M^{-1} s^{-1}).

The interaction of DHA with arsenate was further explored with ^{1}H, ^{13}C, and ^{75}As NMR spectroscopies. However, the concentration of ester is too low to be detected. ^{75}As NMR spectroscopy is notoriously uninformative.[9] The large quadrupole moment and low spin gives rise to very broad line widths. To observe As, the environment of the nuclei must by symmetric, i.e. the ester must be completely deprotonated. Indeed, the signals can only be observed at a pH of >12. Even then, line widths of 1000 Hz are typical. It was interesting to note that a solution of DHA in arsenate buffer colors faster than in phosphate or borate buffers at the same pH and concentration. This is indicative of some interaction unique to arsenate and DHA. A redox reaction is unlikely. A solution of DHA in arsenate, allowed to react for 3 days at room temperature, has the same integrated ^{75}As signal as does the corresponding solution without DHA. The solution with DHA had become very colored by the third day. These results led us to conclude that a DHA–arsenate ester is formed.

^{51}V NMR studies were carried out in an attempt to observe formation of the dihydroxyacetone vanadate ester. It has been reported that in an aqueous solution of ethylene glycol at a concentration of about 1 M, a signal for the ethylene glycol vanadate monoester and several signals for the diester in addition to the larger signal for inoranic vanadate can be observed.[10] However, it was found that in a 1 M aqueous solution of DHA, no ^{51}V NMR signal was observed at all. At lower concentrations of DHA

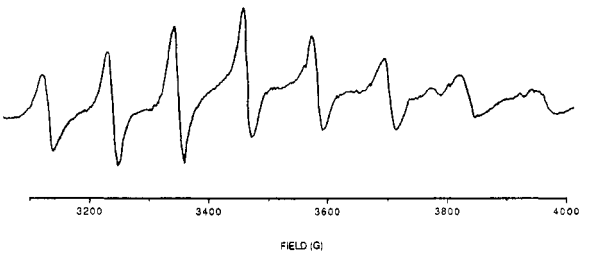


Figure 5. ESR spectrum of V(IV) formed in the reaction of V(V) (0.5 mM) and dihydroxyacetone (0.1 M) in deoxygenated HEPES (0.05 M, 10 mL, pH 7) at room temperature. The spectrum was recorded after addition of 1 mL of 12 N H_2SO_4. Field set, 3300 G; scan angle, 2000 G; time constant, 1 s; modulation amplitude, 1 × 10 G; receiver gain, 8 × 10^3; scan time, 8 min; microwave power and frequency, 5 mW at 9.39 GHz.

(0.1 M), the signal for inorganic vanadate (−550 ppm) and two other signals at −539 and −512 ppm (the ratio of these three peaks is about 4:3:3) could be observed, and their intensities decreased with time. A half-life of about 20 min for the vanadate signal at −550 ppm was observed in 0.1 M DHA at pH 7 (Figure 4). This loss of signal was attributed to reduction of vanadium (V) to the NMR-inactive vanadium (IV). It was reported that the reduction of vanadium (V) by dihydroxybenzene occurred after complexation.[11] The bimolecular rate constant for reduction of vanadate by dihydroxyacetone at pH 7 is about 5.8 × 10^{-3} M^{-1} s^{-1}, 75 times slower than the rate for ester formation determined enzymatically. The reduction of V(V) can also be observed in UV, since the extinction coefficient for V(IV) is smaller than that for V(V). The formation of vanadium (IV) was confirmed by EPR. Upon mixing of dihydroxyacetone and vanadate (V) followed by addition of sulfuric acid, an EPR spectrum containing eight lines was obtained (Figure 5), characteristic of vanadium (IV).[12] Addition of acid was required as, even with authentic

(9) Balimann, G.; Pregosin, P. S. *J. Magn. Reson.* **1977**, *26*, 283. Collins, M. J.; Rao, U. R. K.; Schrobilgen, G. J. *J. Magn. Reson.* **1985**, *61*, 137.
(10) Gresser, M. J.; Tracvey, A. S. *J. Am. Chem. Soc.* **1986**, *108*, 1935.
(11) Ferguson, J. H.; Kustin, K. *Inorg. Chem.* **1979**, *18*, 3349.
(12) Chasteen, N. D.; DeKoch, R. J.; Rogers, B. L.; Hanna, M. W. *J. Am. Chem. Soc.* **1973**, *95*, 1301. Vanadates interact with various buffers including triethanolamine and Tris (Crans, D. C.; Shin, P. K. *Inorg. Chem.*, in press).

vanadyl (IV) sulfate, no signal could be observed at neutral pH presumably because V(IV) formed an EPR-silent complex with Tris buffer.[12]

It was also found that when 0.1 M DHA and vanadate were incubated together and then glycerophosphate dehydrogenase and NADH were added, the enzyme activity decreased slowly as the preincubation time increased. After incubation for 1 h the activity was only about 60% of the activity with freshly mixed vanadate and DHA. Incubation times greater than 1 h did not further decrease the activity. This decrease in activity was attributed to reduction of vanadium (V) to vanadium (IV). Indeed when the enzyme was assayed with DHA and vanadium (IV), the activity observed was about 60% of that with vanadium (V). Thus vanadium (IV) is also an effective, although less effective, inducer of glycerol phosphate dehydrogenase activity. Again, a plot of the reciprocal of the rate against the reciprocal of enzyme concentration showed a nonzero intercept consistent with a mechanism involving dihydroxyacetone vanadium (IV) ester formation followed by enzymatic reduction and nonenzymatic hydrolysis of the ester. The rate constant for the vanadium (IV) ester formation was found to be 0.021 M^{-1} s^{-1}, intermediate between the rates of arsenate and vanadium (V) ester formation. These results indicate that if synthetic reactions are attempted with vanadium (V) and DHA the vanadate will quickly be reduced to vanadium (IV). While vanadium (IV) is 60% as effective as vanadium (V) with glycerophosphate dehydrogenase, it is a poor phosphate mimic and should be much less effective with some other enzymes such as aldolase. Furthermore, V(IV) reacts with molecular oxygen to form superoxide, which reacts with DHA via a radical process.[13] Our initial studies with FDP aldolase indicated that no observable condensation product was formed in 1 day with DHA and vanadate substrate while with arsenate product was formed. However, in an assay measuring the fructose-6-phosphate cleavage by aldolase in the presence of vanadate measured directly by coupling with triosephosphate isomerase and glycerophosphate dehydrogenase, activity was observed. This assay was done in about 40 min in the absence of initial DHA so that little reduction of vanadate occurred. When the same assay was performed with vanadium (IV), no activity was observed after subtraction of the background containing no aldolase. Thus while vanadium (V) is an effective inducer of aldolase activity with fructose-6-phosphate, it is not useful in the condensation direction.

The reaction between DHA and V(V) observed in the ^{51}V NMR study is indeed a redox reaction. The rate of the reaction increased with an increase in pH from 6.5 to 10. At pH 6, the reaction slows considerably, and there is no loss of intensity of the V(V) signal over a period of 90 min. Other compounds were tested for their susceptibility to oxidation by V(V). In the presence of 1 M MeOH, V(V) was stable for over 24 h. Fructose consumed most of the V(V) overnight at a rate faster than glucose. Acetol reduced V(V) with a half-life of 12 h, or approximately 3% of the rate of DHA.

To study the effect of O_2 on the reaction of V(V) and DHA, a sample of DHA was allowed to react until the V(V) signal disappeared. Oxygen was then bubbled through the sample. The V(V) signal had reappeared, was stable for hours, and then began to disappear as before. When a sample of DHA was thoroughly degassed with argon, the rate of disappearance of the vanadate was the same as in

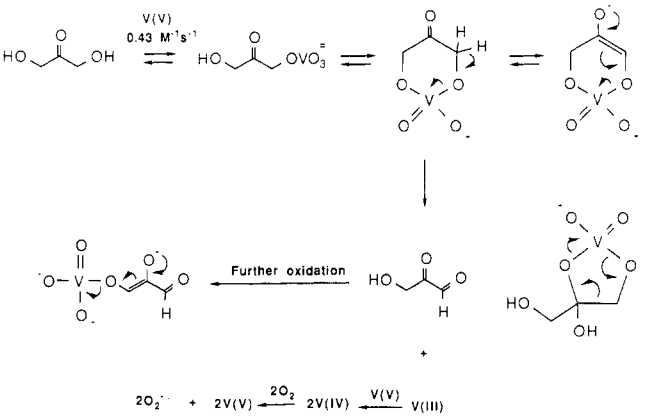


Figure 6. Proposed mechanism for the redox reaction between V(V) and dihydroxyacetone.

a normal sample. The difference was that in a normal oxygenated sample, the signal for vanadate is stable for the first 1000–1500 s, and then the signal starts to fall off. In the deoxygenated sample, the disappearance began immediately. This indicated the vanadate was being turned over in the presence of oxygen. When the oxygen ran out, the V(IV) that was being formed could not be reoxidized to V(V). This oxidoreduction was also observed in UV analyses.

Another experiment was undertaken to exploit this turnover mechanism. A sample of DHA was treated with V(V) and placed under an oxygen atmosphere. An identical sample of DHA except without V(V) was oxygenated as well. A ^{13}C spectrum of each sample was taken every few days. The oxygenated V(V) sample darkened very quickly, while the oxygenated sample in the absence of V(V) only slowly yellowed. The spectra showed only DHA. Gradually the signals grew very weak for the V(V) sample. After a few weeks it was lyophilized. No DHA was detected by ^1H or ^{13}C NMR spectroscopy, and no other peaks were observed in the carbon spectrum between 0 and 120 ppm. Obviously DHA was completely decomposed to carbonyl or other volatile species that were not detected by NMR. A concentrated solution of acrylamide was then used for trapping possible radical intermediates. Formation of a gel would indicate the presence of a radical. This was used in the studies involving higher concentrations of V(V) in strong acid.[14] We found that DHA in the presence of air did gel on acrylamide solution. A thoroughly degassed solution of DHA, acrylamide, and V(V), however, did not gel even after 12 h. When ascorbate is included in the reaction mixture containing 0.1 M DHA and 0.5 mM V(V) or V(IV), the rate of reactions between DHA and V measured enzymatically drops to ~35% for V(V) and 5% for V(IV). Superoxide dismutase does little to affect the rate of reaction in a normal deoxygenated sample.

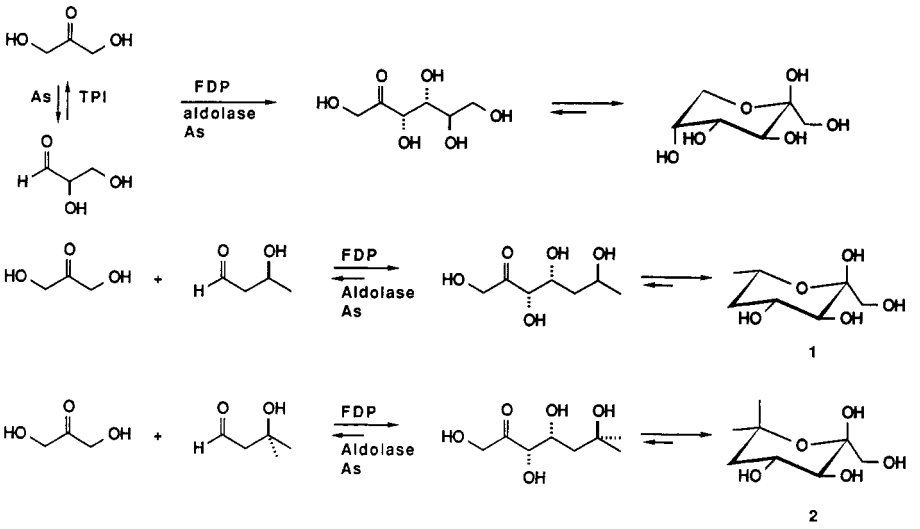
It was known that both Cr(VI) and V(V) complex with diols and oxidize the ligand via an one-electron (radical) or a two-electron process, depending on the structure of the diol.[15] The radical reaction is favorable for substrates containing tertiary OH group. Since DHA has no tertiary OH group and since no radical intermediate was detected under anaerobic conditions, the oxidation of DHA by V(V) may be a two-electron process in which V(III) is generated. Oxidation of V(III) by V(V) should give V(IV), which is EPR active and will react with molecular oxygen to give superoxide radical and V(V). This is also consistent with

(13) Mashino, T.; Fridovich, I. *Arch. Biochem. Biophys.* **1987**, *254*, 547. Liochev, S.; Fridovich, I. *Ibid.* **1987**, *255*, 274; *Biochim. Biophys. Acta.* **1987**, *924*, 319.

(14) Boyd, D. W.; Kustin, K. *Adv. Inorg. Biochem.* **1984**, *6*, 311.

(15) Wilberg, K. B. *Oxidation in Organic Chemistry (Part A)*; Academic Press: New York, 1965; pp 204–6.

Scheme I. FDP Aldolase Catalyzed Aldol Condensations[a]

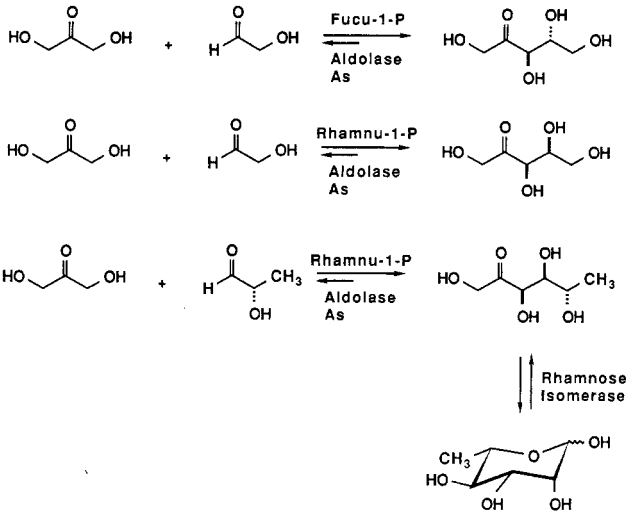


[a] As, inorganic arsenate; TPI, triosephosphate isomerase.

the pH effect. Based on this limited study the overall mechanism is proposed and shown in Figure 6. Both hydrated and nonhydrated DHA may be involved in the reactions. The reaction with nonhydrated DHA to form the 6-membered intermediate, however, would account for the faster reaction compared with the reaction with other substrates. The ^{13}C NMR spectrum of DHA indeed indicated that 66% of DHA was present in its nonhydrated form. In any case, identification of the products from DHA and further kinetic investigations using perdeuteriated DHA as substrate will be necessary in order to determine the details of the mechanism.

Synthesis. It is obvious that the vanadate ester of DHA is not useful for aldol reactions due to its instability. It may find use in other enzyme reactions. The arsenate ester, however, is synthetically useful. As examples of the synthetic utility of this methodology, a mixture of DHA and arsenate was used as a mimic of DHAP in enzyme-catalyzed aldol condensations that require DHAP as a natural substrate. Three reactions were carried out with FDP-aldolase on 5-mmol scales. As shown in Scheme I, dihydroxyacetone in the presence of FDP aldolase and triosephosphate isomerase (TPI) was converted to D-fructose quantitatively as determined by HPLC. This indicates that TPI catalyzes the isomerization of DHA in the presence of arsenate to D-glyceraldehyde, which was then converted to D-fructose through the aldol condensation. In the synthesis of the seven-carbon sugar 1, (*S*)-3-hydroxybutyraldehyde[16] was used as a substrate and the aldol product was purified by chromatography on Dowex 50 (Ba^{2+}) with 50% aqueous ethanol as the mobile phase (80% yield). The sugar was then characterized by ^{13}C NMR analysis including normal and DEPT experiments. The normal spectrum showed only seven major lines, similar to the spectrum of 5-deoxy-D-fructose, which shows only six lines.[5] The DEPT spectrum indicates one methyl carbon (C-7), two methylenes (C-1,5), and three methines (C-3,4,6) as expected with one unprotonated carbon (C-2) not being observed. This sugar seems to exist almost entirely in a pyranose form. When 3-methyl-3-hydroxybutanal was used as the acceptor, 5-deoxy-6,6-dimethyl-D-*threo*-hexulose **2** was produced in 31% yield. We have also found that Fucu-1-P aldolase catalyzes the synthesis

Scheme II. Fuculose-1-phosphate (Fucu-1-P) Aldolase and Rhamnulose-1-phosphate (Rhamnu-1-P) Aldolase Catalyzed Reactions



of D-ribulose from DHA and glycoaldehyde in the presence of arsenate (Scheme II). Similarly, L-xylulose was formed when Rhamnu-1-P aldolase was used as a catalyst. Both reactions were carried out on 14-mmol scales. Our limited study so far indicates that these two aldolases may, like FDP aldolase,[5-7] accept a variety of aldehydes, in addition to the natural acceptor L-lactaldehyde, as substrates. Determination of the substrate specificity is in progress. When L-lactaldehyde was used as acceptor, the product L-rhamnulose was further isomerized to L-rhamnose because the cells contain rhamnose isomerase.

In conclusion, arsenate and vanadate esters are mimics of phosphate esters in many phosphate-depending enzymatic reactions. Unlike phosphate esters, the arsenate and vanadate esters are formed spontaneously and reversibly in aqueous solution. This enables one to carry out enzymatic transformations with a mixture of inorganic arsenate (or vanadate) and the unphosphorylated organic alcohol to replace the corresponding organic phosphate, which is normally the substrate. This approach eliminates the needs for the preparation of organic phosphate substrate and the removal of the phosphate moiety from the product. Of several enzymatic reactions studied, the most synthetically useful are the aldolase-catalyzed reactions with

(16) Wong, C.-H.; Drueckhammer, D. G.; Sweers, H. M. *J. Am. Chem. Soc.* **1985**, *107*, 4028.

DHAP being replaced by DHA and arsenate.

Experimental Section

All enzymes and biochemicals were from Sigma. *E. coli* $0111B_4$[17] and *E. coli* K40[18] were grown on 2-L scales to prepare the cells as sources of Fucu-1-P aldolase and Rhamnu-1-P aldolase, respectively. About 5 g each was obtained in each cycle. NMR data were obtained on Varian XL-200 or XL-400 spectrometers. ESR spectra were recorded on a Varian E-6S spectrometer. HPLC analyses were done with a Gilson chromatography system including a refractive index detector.

Enzyme Assays with Arsenate and Vanadate. Glycerophosphate dehydrogenase and glucose-6-phosphate dehydrogenase activities were determined by measurement of NADH consumption and production, respectively. Ribulose-5-phosphate epimerase was assayed by formation of the trimethylsilyl sugars of the product mixtures, which were analyzed by gas chromatography.[19] The other enzymes could not be assayed directly but were assayed by setting up small reactors and analyzing for product formation, usually after 1 day.

Phosphoglucose Isomerase. The following arsenate, vanadate, and control solutions were used in all enzyme assays unless otherwise mentioned. To 1-mL solutions of sodium arsenate (0.1 M, pH 8.0) or sodium vanadate (0.5 mM) in Tris buffer (0.01 M, pH 8.0) and a control containing only Tris buffer were added fructose (0.1 mmol) and phosphoglucose isomerase (32 units). After 2 days at room temperature a 0.2-mL aliquot of each was added to separate cuvettes containing 0.8 mL of triethanolamine buffer (0.1 M, pH 7.5) and NAD (1.75 mM). Glucose dehydrogenase (5 units) was added, and the change in absorbance at 340 nm was measured to determine the concentration of glucose.

Phosphoribose Isomerase. To 1 mL of the arsenate or vanadate solutions were added ribose (15 mg) and phosphoribose isomerase (1.8 mg, 170 units). After 1 day at room temperature, 50 μL of each solution was added to separate cuvettes, each containing triethanolamine buffer (1 mL, 0.1 M, pH 7.5) and NADH (0.30 mM). Sorbitol dehydrogenase (2 units) was added, and the decrease in absorbance at 340 nm was measured for determination of D-ribulose produced. The As sample contained 0.1 mM D-ribulose, and the V(V) sample contained 0.2 mM D-ribulose.

Ribulose Phosphate Epimerase. To 0.1-mL solutions of arsenate or vanadate were added ribulose (2.5 mg) and ribulose 5-phosphate epimerase (3 units). After 3 days at room temperature the solutions were lypholized. To each of the resulting residues were added pyridine (0.2 mL), hexamethyldisilazane (0.04 mL, 0.22 mmol), and trimethylchlorosilane (0.02 mL, 0.16 mmol). The mixtures were shaken for 30 s and allowed to stand at room temperature for 10 min. The solutions were then centrifuged to remove the white precipitate, and the solutions were analyzed by GC at 125 °C for 1 min followed by an increase in temperature of 1 °C per minute. The mixture containing arsenate showed 6.1% xyulose (peaks at t_R 7.07 and 11.65 min) and 93.9% ribulose (peaks at t_R 8.50, 9.99, and 11.37 min). Both the reactions containing vanadate and the control showed only ribulose.

Fructose Diphosphate Aldolase. As described above, to 1-mL solutions of As, V(V), or V(IV) were added fructose-6-phosphate (15 mg), NADH (0.23 μmol), glycereophosphate dehydrogenase (6.2 units), TPI (60 units) and FDP aldolase (0.10 mg, 1.5 units). The decrease in absorbance at 340 nm corresponds to the amount of DHAP generated from FDP. To assay in the condensation direction, to the 1-mL arsenate and vanadate solutions were added D-glyceraldehyde-3-phosphate (1.0 μmol), DHA (50 μmol), NAD (1.5 μmol), phosphoglucose isomerase (20 units), and glucose-6-phosphate dehydrogenose (20 units). FDP aldolase (2 units) was added, and the change in absorbance at 340 nm was measured vs time.

Fuculose-1-phosphate and Rhamnulose-1-phosphate Aldolases. To 0.2-mL solutions of As or V(V) containing DHA (24 mg) and D,L-lactaldehyde (20 mg) were added the *E. coli* whole cells containing Fucu-1-P aldolase or Rhamnu-1-P aldolase. The reactions were monitored by HPLC using a Waters carbohydrate analysis column with 85% CH_3CN as the mobile phase and a flow rate of 1 mL per minute. After 36 h at room temperature, the amounts of 6-deoxyhexose (t_R = 9.5 min) was determined by HPLC.

Nucleoside Phosphorylase. To 0.1-mL solutions of the arsenate or vanadate were added inosine (2.5 mg) and nucleoside phosphorylase (1.8 units). The reactions were analyzed by reverse-phase HPLC with the following gradient as mobile phase: solution A = 0.1 M NH_4OAc, pH 4.9, and solution B = 30% methanol in A with 10% B initially sloped to 30% B in 15 min; inosine (t_R = 14.7 min); hypoxanthine (t_R = 7.56 min).

Assay of Dihydroxyacetone Arsenate, Vanadate, and Vanadyl Ester Formation Using Glycerophosphate Dehydrogenase. To a Tris buffer (0.9 mL, 0.02 M, pH 8.0) were added NADH solution (19 μL, 12 mM), DHA (50 μL, 1 M solution), and glycerophosphate dehydrogenase in amounts from 1.7 to 77 μg. Sodium arsenate (10 μL, 0.5 M) or sodium vanadate (10 μL, 10 mM) was added to initiate the reaction, and the decrease in absorbance at 340 nm was monitored versus time. With vanadyl sulfate, a decrease in absorbance was observed for about 2 min after addition even in the absence of enzyme, which made the rate of enzymatic reduction difficult to measure. To avoid this problem, deoxygenated solutions were used and vanadyl sulfate was added to the buffer initially, and about 10 min later NADH and enzyme were added followed by addition of DHA.

^{51}V NMR Kinetic Studies. Solutions of DHA and other substrates were prepared in the following manner: HEPES (604.6 mg, 2.54 mmol) and DHA (2.85 mL, 2M, 5.7 mmol) were dissolved in water (45 mL). The pH was adjusted with 2 N NaOH. Sufficient water was added to bring the volume to 50.0 mL. For analysis, to an aliquot of the buffered DHA solution (4.40 mL) in a 10-mm NMR tube was added D_2O (0.5 mL). The solution was mixed, and an aliquot (100 μL) of Na_3VO_4 (25.0 mM, 2.5 μmol) was added. The sample was mixed, placed in the probe, and quickly locked and shimmed. Acquisitions were taken immediately. The time between addition and start of acquisitions is included in the kinetic analysis. There is a 500-s delay (total 600 s between samples since 1000 transients at 0.1 s each = 100 s) between spectra in a typical run. Slow-reacting samples, such as acetol, had longer delays as needed. Acetol had a 4000-s delay (total 4200 s between samples since 2000 transients at 0.1 s each = 200 s).

FDP Aldolase Catalyzed Aldol Condensation: Preparation of D-Fructose, 5,7-Dideoxy-L-*xylo*-heptulose (1), and 5-Deoxy-6,6-dimethyl-D-*threo*-hexulose (2). For preparation of **1**, (*S*)-3-hydroxybutyraldehyde dimethyl acetal[16] (6 mmol) was hydrolyzed in 6 mL of water and 0.2 mL of concentrated HCl. The pH was then adjusted to 7.5 with $NaHCO_3$ followed by addition of sodium arsenate (6 mL, 1.0 M, pH 7.6). DHA (0.54 g, 6 mmol) and aldolase (600 units) were added, and the solution was stirred slowly at room temperature. The reaction was monitored by HPLC with a Waters carbohydrate column with 85% of CH_3CN as the mobile phase with a flow rate of 1.2 mL/min. After 24 h, HPLC showed only a trace of the aldehyde (t_R = 2.3 min) and DHA (t_R = 3.9 min) with one major peak (t_R = 5.9 min). Methanol was added to precipitate arsenate, and the suspension was filtered. The filtrate was applied to a Dowex 50 (Ba^{2+}) column (100–200 mesh, 3 × 75 cm) and eluted with 50% ethanol. Fractions (8 mL) were collected and analyzed by HPLC. The fractions with retention volume 400–800 mL were collected and lyophilized to give 0.76 g of a product: $[\alpha]^{25}_D$ −20.6° (*c* 1.73, H_2O); ^1H NMR (200 MHz, D_2O) δ 1.09 (d, *J* = 6.2 Hz, 3 H, CH_3), 1.27 (m, *J* = 1.20 Hz, *J* = 12.8 Hz, *J* = 11.6 Hz, 1 H, C5-H_{ax}), 3.33 (d, *J* = 9.6 Hz, 1 H, C3-H), 3.39, 3.58 (C1-H8 C1-H′), 3.83 (m, *J* = 5.0 Hz, *J* = 12.0 Hz, 1 H, C6-H); ^{13}C NMR (50 MHz, D_2O) δ 20.29 (C7), 40.20 (C5), 64.10, 65.35, 68.28, 72.19 (C1,3,4,6), 98.03 (C2). Anal. Calcd for $C_7H_{14}O_5$: C, 47.2; H, 7.90. Found: C, 47.51; H, 7.86. All data are consistent with those reported.[6d]

To prepare **2** the conditions were essentially the same. The aldehyde was generated from the precursor acetal, and the reaction was monitored by TLC (silica gel, water-saturated ethyl acetate–methanol, 9:1 v/v, R_f of aldehyde = 0.9, DHA = 0.36, product = 0.45). After reaction, the mixture was diluted with ethanol (24 mL) and cooled to 4 °C overnight. The mixture was then filtered, and the filtrate was evaporated to give a solid, which was chro-

(17) Ghalambor, M. A.; Heath, E. C. *J. Biol. Chem.* **1962**, *237*, 2427.
(18) Chiu, T.-H.; Feingold, D. S. *Biochemistry* **1969**, *8*, 98.
(19) Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497.

matographed on silica gel (water-saturated ethyl acetate) to yield the product, 0.36 g (31% yield): $[\alpha]^{25}_D$ -21.8° (c 0.40, H_2O); ^1H NMR (200 MHz, D_2O) δ 1.03, 1.20 (s, 6 H, CH_3), 1.37 (dd, J = 13 Hz, J = 11.8 Hz, 1 H, C5-H_{ax}), 1.83 (dd, J = 13 Hz, J = 4.7 Hz, 1 H, C5-H_{eq}), 3.15, 3.42 (d, J = 11.8 Hz, 2 H, CH_2OD), 3.27 (d, J = 9.8 Hz, 1 H, C3), 3.91 (ddd, J = 9.8 Hz, J = 11.8 Hz, J = 4.7 Hz, 1 H, C4); ^{13}C NMR (50 MHz, D_2O) δ 27.14, 31.11 (CH_3), 43.66 (C5), 64.21, 65.82, 72.06, 75.08 (C1,3,4,6), 98.92 (C2). Anal. Calcd for $C_8H_{16}O_5$: C, 50.04; H, 8.40. Found: C, 50.02; H, 8.40. All data are consistent with those reported.

To prepare D-fructose, the condition was essentially the same except that no aldehyde was used. Triosephosphate isomerase (500 units) was added to the mixture in addition to the aldolase. The product was identified with HPLC and compared with that of authentic D-fructose.

Fuculose-1-phosphate Aldolase Catalyzed Reaction: Preparation of D-Ribulose. To a Tris buffer solution (85 mL, 10 mM, pH 7.5, containing 6 mM KCl, 6 mM $Co(NO_3)_2$) were added sodium arsenate (200 mmol), glycoaldehyde (1.7 g, 14.2 mmol), and dihydroxyacetone (3.2 g, 17.8 mmol). The solution was adjusted to pH 7.5 followed by adding 100 mg of the enzyme-containing *E. coli* cells and 1 mg of lysozyme (51 000 μm) to break the cells. The mixture was stirred for 24 h. TLC showed no glycoaldehyde was present, R_f 0.75. The mixture was lyophilized and triturated with 4 × 75 mL of methanol and filtered, and the filtrate was evaporated. The product was purified on Dowex 50 (Ba^{2+}) column with water as a mobile phase to give 1.70 g of product, which was identical with authentic D-ribulose (from Aldrich) by HPLC (t_R = 4.9 min) and NMR analyses.

Rhamnulose-1-phosphate Aldolase Catalyzed Reactions. The *E. coli* cells containing this enzyme were used to prepared L-xylulose from DHA and glycoaldehyde under the same conditions as that for the preparation of D-ribulose. The product obtained (1.60 g, 76% yield based on glycoaldehyde) was identical with authentic L-xylulose (from Aldrich) by HPLC (t_R = 5.0 min) and NMR analyses. When L-lactaldehyde (4.75 mmol, prepared by acid hydrolysis of the dimethyl acetal precursor)[16] was used as acceptor and dihydroxyacetone (5.56 mmol) as donor, a mixture of L-rhamnulose and L-rhamnose (0.61 g, 71% yield) was obtained based on ^1H NMR, ^{13}C NMR, and HPLC analyses (t_R rhamnose = 5.2 min; t_R rhamnulose = 4.8 min; the ratio of aldose to ketose is 0.6:0.4).

Acknowledgment. This research was supported by the NSF (Grant CHE 8318217) and the Robert A. Welch Foundation (Grant A-1004).

A Synthetic Approach to Rocaglamide via Reductive Cyclization of δ-Keto Nitriles

George A. Kraus* and James O. Sy

Department of Chemistry, Iowa State University, Ames, Iowa 50011

Received June 16, 1988

The anticancer agent rocaglamide contains a novel bicyclo[3.3.0]octanol structure. The approach to this molecule involved the preparation of a hydroxy ketone intermediate via a samarium-mediated cyclization. This ketone was then converted into an excellent Michael acceptor via novel chemistry. Subsequent steps led to the preparation of an isomer of rocaglamide. An X-ray determination supported our view that cuprate addition occurred with unusual concave selectivity.

Rocaglamide (1) is a novel natural product which was isolated from *Aglaia elliptifolia* Merr. in 1982.[1] Its structure was elucidated by McPhail. Rocaglamide exhibits significant activity against P388 lymphocytic leukemia in CDF_1 mice and inhibitory activity in vitro against cells derived from human epidermoid carcinoma of the nasopharynx.[2] Its challenging structure and potent activity combine to make rocaglamide an attractive synthetic objective. Two approaches have recently been reported. An approach by Trost and co-workers uses a clever trimethylenemethane–palladium(0) cyclization to form a cyclopentene precursor.[3] The approach by Taylor and Davey utilizes an intramolecular dithiane cyclization to form the cyclopentane ring.[4]

Our approach features a samarium-mediated cylization of a keto nitrile. The retrosynthetic analsyis is illustrated in Scheme I. Our expectation was that nucleophiles would add to the convex face of the bicyclo[3.3.0]octane subunits in **2** and **3**. The starting point in our synthesis is the known ketone **5**. It can be prepared in one step by a Hoesch reaction on phloroglucinol.[5] Methylation of **5** provided ketone **6**. Trimethylation to afford a 3,4,6-trimethoxybenzofuran can occur if the conditions are not carefully monitored. Michael addition of ketone **6** with acrylonitrile to form **7** could be achieved by using a catalytic amount of Triton B in *tert*-butyl alcohol. Rigorous exclusion of oxygen was necessary to prevent the formation of byproduct **8**. This byproduct had also been observed by Taylor and Davey (Scheme II).

Initially, the cyclization of **7** to **4** was attempted by using the Zn/Me_3SiCl protocol developed by Corey.[6] These conditions had already been used for the cyclization of a simple keto nitrile. The only product isolated was alcohol **9**. The same result was obtained with the Mg/Me_3SiCl conditions used by Hutchinson.[7] However, when conditions similar to those developed by Molander for the cyclization of halo ketones were used, a good yield of hydroxy ketone **4** was obtained.[8] Alcohol **9** was also obtained. The

(1) McPhail, A. T.; King, M. L.; Chiang, C. C.; Ling, H. C.; Fujita, E.; Ochiai, M. *Chem. Commun.* **1982**, 1150.
(2) King, M. L.; Ling, C. H.; Wang, C. B.; Leu, S. C. *Med. Sci.* **1975**, *1*, 11.
(3) Trost, B. M.; Saulnier, M. G. Presented at the 192nd National Meeting of the American Chemical Society, Anaheim, CA, Sept 7–12, 1986; Abstract 144.
(4) Taylor, R. J. K.; Davey, A. E. *Chem. Commun.* **1987**, 25.
(5) Katamna, C. *Bull. Soc. Chim. Fr.* **1970**, 2309.
(6) Corey, E. J.; Pyne, S. G. *Tetrahedron Lett.* **1983**, *24*, 2821.
(7) Hutchinson, C. R.; Ikeda, T.; Yue, S. *J. Org. Chem.* **1985**, *50*, 5193.
(8) Molander, G. A.; Etter, J. B.; Zinke, P. W. *J. Am. Chem. Soc.* **1987**, *109*, 453. Molander, G. A.; Etter, J. B. *J. Org. Chem.* **1986**, *51*, 1778.

0022-3263/89/1954-0077$01.50/0