Mechanisms of Carbonyl Participation in Phosphate Ester Hydrolysis and Their Relationship to Mechanisms for the Carboxylation of Biotin

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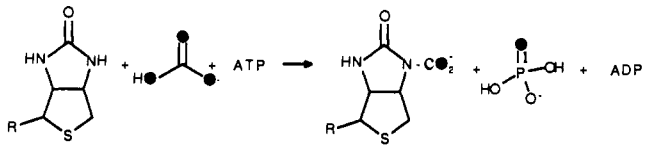
Abstract: The enzymic reaction of biotin with bicarbonate and ATP produces *N*-carboxybiotin, ADP, and phosphate. The intermediates in the reaction are unknown. It has been proposed that the intramolecular reaction of the carbonyl hydrate of methylacetoin diethyl phosphate (**1**) may serve as a model for the latter part of such a mechanism. In basic solution, **1** reacts rapidly to give two sets of products that can be accounted for by a mechanism involving addition of a hydroxyl group of the carbonyl hydrate to the adjacent phosphate. The rapid reaction of the nonenolizable ester with base rules out earlier mechanistic proposals of the reaction involving an enolization pathway. The course of incorporation of water containing $H_2{}^{18}O$ into phosphate ester products was determined by ^{31}P NMR analysis. This reveals that the reversion of the carbonyl hydrate to the ketone occurs at a rate which is competitive with reaction at the phosphate ester since the isotopic enrichment of the phosphate group is less than the content of the solvent (76%). In weakly basic solutions a single set of products is obtained, but in more highly alkaline solutions a second set of products develops. The results are consistent with a competition between a mechanism in which the carbonyl hydrate adduct expels phosphate and one in which ethoxide is expelled to give a cyclic phosphate. Reactions of acetoin diethyl phosphate (**2**) and acetol diethyl phosphate (**3**) show a larger degree of solvent incorporation and a smaller variation in product distribution, consistent with expectations based on steric and inductive effects. It is concluded that the reaction mechanism parallels that which was proposed for the carboxylation of biotin.

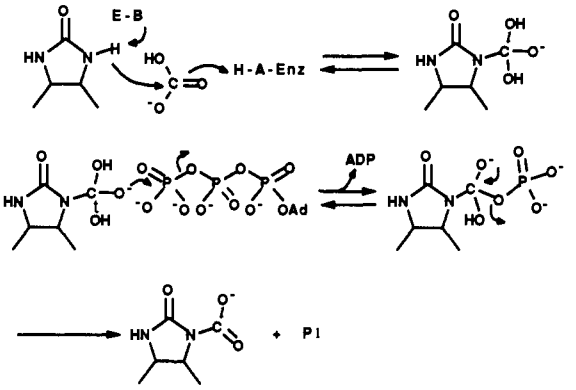
N-Carboxybiotin is an intermediate in the enzymic transfer of an equivalent of carbon dioxide from bicarbonate to an acceptor.[1-3] The formation of *N*-carboxybiotin is accompanied by the conversion of ATP to ADP and inorganic phosphate. The mechanism is not known in detail, but key experiments have limited the possibilities to be considered. Labeling studies have shown that an oxygen atom from bicarbonate is transferred to the inorganic phosphate produced from ATP.[4] Enzymes that promote biotin-dependent carboxylations do not catalyze partial exchange reactions. Thus, a mechanism in which ATP, biotin, and bicarbonate combine in a concerted process is in accord with these properties, but the transition state for such a process is chemically unreasonable[1-3,6] (Diagram I).

Diagram I. Biotin + Bicarbonate + ATP with Label



Alternatively, it can be proposed that intermediates form but are not accessible for exchange with external species. A widely considered mechanism involves an initial reaction between ATP and bicarbonate to form carboxyphosphate followed by transfer of the carboxyl group to biotin.[1,2] This is consistent with available data from stereochemical studies,[6] extrapolations from related chemical reactions,[7] and enzymic reactions with alternative substrates.[8-10] However, other pairwise combinations of the three reactants could also lead to formation of carboxybiotin by what may well be reasonable mechanisms that are also consistent with the data.[5,11-13] Thus, since a mechanism can be considered as valid if it is proven by indisputable criteria or if all other possibilities are ruled out, it is necessary to test the validity of any proposal. We have undertaken tests of the chemical reasonableness of one of the pairwise combination mechanisms which has not been considered in detail, the direct reaction of bicarbonate and biotin followed by reaction of the intermediate with ATP and decomposition of the second intermediate to produce carboxybiotin (Diagram II).

Diagram II. Biotin + Bicarbonate and Then ATP



The first step in the mechanism already has a valid precedent. Blagoeva and co-workers[14] have shown that the conjugate base of a urea will add to an adjacent carboxylate, a reaction which parallels the addition of the conjugate base of biotin to bicarbonate. Studies of proton-exchange reactions show that the conjugate base

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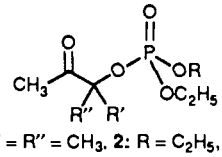
of biotin can be expected to be a kinetically feasible intermediate in an enzymic reaction.[15,16] Therefore, the initial pairwise reaction of biotin and bicarbonate to form a covalent adduct is chemically reasonable. The formation of carboxybiotin by this mechanism requires the adduct to react with ATP, either as a separate step or in concert with attack of biotin on bicarbonate.[5] Would an intermediate generated from the addition of biotin to bicarbonate react with a phosphate derivative to form a carboxylated product by subsequent elimination of phosphate?

While the adduct of bicarbonate and a urea is an unstable species that would only form in small steady-state amounts, carbonyl hydrates form much more readily and should show similar reactivity properties. Ramirez reported that acetoin dimethyl phosphate hydrolyzes in mild basic solution 10^6 times faster than does trimethyl phosphate. One mechanism that Ramirez proposed to account for the rapid hydrolysis rate involved attack of the hydrate form of the carbonyl group on the adjacent phosphate esters.[17,18] Cox and Ramsay suggested that a mechanism in which the reactant is first converted to the enol tautomer is consistent with the observed incorporation of solvent oxygen into the phosphate product.[17,19] However, Witzel et al. showed that β-carbonyl phosphates that could not enolize at the α-position also hydrolyze rapidly in dilute basic solution.[20] They proposed that the reaction may involve C–O cleavage and proceed by epoxide formation, on the basis of an analogous reaction with a Grignard reagent.[20] Frank and Usher showed that the carbonyl hydrate mechanism can effectively explain the differing reaction patterns of related phosphates and phosphonates.[21]

To clarify the details of the mechanism and consider its relevance to that for the carboxylation of biotin, we have now determined the mechanism of the alkaline hydrolysis of methylacetoin diethyl phosphate and related compounds using a combination of kinetic and isotope labeling studies (**1–3**). Our results



1: R = C_2H_5, R′ = R″ = CH_3. **2**: R = C_2H_5, R′ = CH_3, R″ = H, **3**: R = C_2H_5, R′ = R″ = H, **4**: R = H, R′ = R″ = CH_3.

indicate that the mechanism is relevant to that proposed for the carboxylation of biotin and that this mechanistic pathway for carboxylation of biotin is chemically reasonable.

Experimental Section

Instrumentation. ^{13}C and ^1H NMR spectra were obtained with a Varian Gemini-200 spectrometer. Chemical shifts for proton NMR spectra of chloroform-*d* solutions are relative to internal tetramethylsilane. ^{13}C spectra are broad band proton decoupled, and chemical shifts are relative to the central peak of chloroform-*d* at δ 77. For deuterium oxide solutions, ^1H chemical shifts are relative to the residual HDO peak at δ 4.68 and ^{13}C chemical shifts are relative to dioxane at δ 67.4 (internal standard). ^{31}P spectra were recorded on a Varian XL-200 spectrometer with broad band proton decoupling. Chemical shifts are relative to external 85% phosphoric acid.

Materials. All chemicals used in syntheses were purchased from Aldrich Chemical Co. and distilled before use. Solvents were purchased from Caledon Laboratories Ltd. and BDH Chemicals. Deuterium oxide (99.97%) was a gift from Ontario Hydro, Toronto, Canada. Concentrated sodium deuteroxide solutions and ^{18}O-enriched water (97% ^{18}O) were obtained from Aldrich. Dilute solutions of sodium deuteroxide were titrated with standardized hydrochloric acid.

Syntheses. (a) Methylacetoin Diethyl Phosphite. Diethyl phosphorochloridite (73.5 mmol, 11.50 g, prepared by the method of Cook and co-workers[22]) in dry ether (50 mL) was added dropwise to a solution of 3-hydroxy-3-methylbutanone (80 mmol, 8.16 g) and pyridine (80.0 mmol, 6.32 g) in dry ether (300 mL) at 0 °C under dry nitrogen for 1 h. After addition, the mixture was brought to room temperature and stirred for 1 h. Pyridine hydrochloride was removed by filtration and the solvent removed in vacuo. Distillation of the residue resulted in pure product in 85% yield (62.2 mmol, 13.8 g, 40–42 °C, 0.15 Torr): ^1H NMR (chloroform-*d*) δ 3.97–4.11 (m, 4 H), 2.41 (s, 3 H), 1.64 (s, 6 H), 1.37–1.44 (t, 6 H): ^{31}P NMR (chloroform-*d*) δ 142.

(b) Methylacetoin Diethyl Phosphate (1). Methylacetoin diethyl phosphite (9.0 mmol, 2.0 g) was dissolved in 40 mL of dichloromethane at 0 °C. Ozone generated in a stream of dry oxygen was passed through the solution until a downstream solution of potassium iodide darkened (approximately 30 min). The reaction solution was concentrated under vacuum and distilled twice [bp 70–71 °C, 0.10 Torr (lit:[20] bp 109.5–110 °C, 0.30 Torr)]: yield 93% (8.2 mmol, 2.0 g); ^1H NMR (chloroform-*d*) δ 4.09–4.24 (m, 4 H), 2.33 (s, 3 H), 1.62 (s, 6 H), 1.34–1.41 (t, 6 H); ^{31}P NMR (chloroform-*d*): δ −4.4.

(c) Acetoin Diethyl Phosphate (2). This was prepared by a procedure similar to that developed by Ramirez[23] for the dimethyl ester. Thus, 20 g (81.0 mmol) of the adduct of triethyl phosphite and biacetyl was reacted with a solution of anhydrous hydrogen chloride in ether for 1 h. Removal of the ether left a pale yellow liquid which was twice distilled to give pure product (bp 67–69 °C, 0.12 Torr): yield 98% (79 mmol, 17.7 g); ^1H NMR (chloroform-*d*) δ 4.70–4.86 (m, 1 H), 4.10–4.29 (m, 4 H), 2.3 (s, 3 H), 1.49–1.54 (d, 3 H), 1.35–1.45 (t, 6 H); ^{31}P NMR (chloroform-*d*) δ −1.16.

(d) Acetol Diethyl Phosphate (3). This was prepared by the procedure as described for **1** except acetol was used in place of methylacetoin: bp 77–78 °C, 0.85–0.10 Torr; overall yield 63%; ^1H NMR (chloroform-*d*) δ 4.55–4.60 (d, 2 H), 4.13–4.27 (m, 4 H), 2.23 (s, 3 H), 1.34–1.42 (t, 6 H); ^{13}C NMR (chloroform-*d*) δ 202.5 (d, J_{P-C} = 5.2 Hz), 70.4 (d, J_{P-C} = 5.8 Hz), 63.9 (d, J_{P-C} = 5.9 Hz) 25.5 (s), 15.5 (d, J_{P-C} = 6.7 Hz); ^{31}P NMR (chloroform-*d*) δ 1.8.

(e) Methylacetoin Ethyl Phosphate (4). This material was prepared as a standard for comparison in analysis of the products of the reaction of **1** with aqueous base. The reaction of neutral phosphate esters with sodium iodide in acetone leads to C–O cleavage of a single alkyl group and does not involve reaction at phosphorus.[24,25] Methylacetoin diethyl phosphate (**1**) (4.8 mmol, 1.0 g) was added to a solution of sodium iodide (9.6 mmol, 0.72 g) in 50 mL of butanone. The mixture was refluxed for 48 h and then kept in an ice bath for 4 h. The resulting precipitate was collected by filtration. The product was recrystallized from acetone/ethyl acetate (7:3) (−78 °C to room temperature): yield 81% (3.8 mmol, 0.9 g, white powder); ^1H NMR (D_2O) δ 3.74–3.93 (m, 2 H), 2.21 (s, 3 H), 1.37 (s, 6 H), 1.09–1.18 (t, 3 H); ^{13}C NMR (D_2O) δ 216.3 (d, J_{P-C} = 4.3 Hz), 84.0 (d, J_{P-C} = 6.6 Hz), 62.5 (d, J_{P-C} = 5.7 Hz), 25.0 (d, J_{P-C} = 4.5 Hz), 16.4 (d, J_{P-C} = 6.4 Hz); ^{31}P NMR (D_2O) δ −4.4.

Kinetic Measurements. Rates of hydrolysis of compounds **1–3** were determined by using a pH stat (Radiometer pH meter 27, TTT80 titrator, autoburet 13, GK-202B combination electrode) with data collected into a Commodore 8032 microcomputer. Reactions were conducted in a jacketed flask under argon with temperature maintained with ±0.1 °C with a Neslab RTE-80 circulating water bath. A solution of potassium chloride (7 mL, 0.10 M) was stirred and equilibrated in the reaction vessel with the apparatus set to maintain the pH through the addition of a 0.1 M potassium hydroxide solution from the autoburet. The reactant (0.7–1.0 μL) was added with a calibrated syringe. All runs were performed in triplicate. The integrated first-order rate law was fit to the data by using the Swain kinetic overrelaxation algorithm[26] in BASIC. This gave correlation coefficients of at least 0.99999 over four or more half-lives with standard errors of less than 2%. The concentration of hydroxide was derived from pH measurements, the dissociation constant of water,[27] and the activity coefficient of hydroxide ion[28] at 25 °C: $[OH^-] = (K_w/\gamma)(1/a_h)$.

Product Distribution. The product distributions for the hydrolysis of **1–3** in basic solutions were determined by using ^1H NMR spectroscopy. For base concentrations ranging from 0.1 to 4 M NaOD the procedure was as follows: 10 μL of substrate was added to an NMR tube containing 0.74 mL of the appropriate NaOD solution at room temperature.

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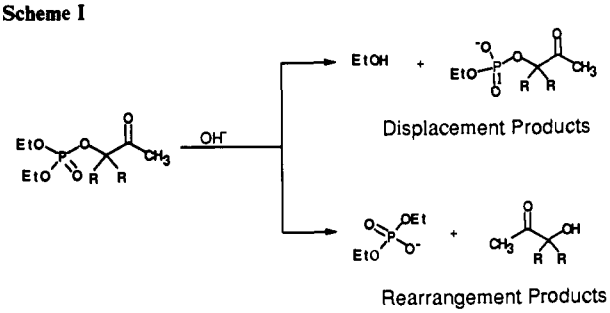
Scheme I



The tube was shaken, and a spectrum was recorded after approximately 10 min. For reactions performed in solution of pD 10.0–10.5 the procedure was as follows: 5 mL of D_2O was added to a flask under argon and then brought to pD 10–10.5 by the addition of 0.1 M NaOD; 0.050 mL of substrate was then added, and the pD was maintained between 10 and 10.5 by the addition of 1 M NaOD solution with a Hamilton syringe.

The hydrolysis of **1** in basic solution yields a total of four products in two sets of two. The first set is ethanol and **4** (the "displacement products"; see Results). The second set is methylacetoin and diethyl phosphate (called the "rearrangement products" since they arise from internal migration of the phosphate ester; see Results). The relative amounts of the sets of products depend upon the reaction conditions. The amount of the displacement products was determined by measuring the ratio of the integrated signal due to the methyl protons of **4** (singlet, δ 1.25) to the combined integrated signals due to the *gem*-dimethyl protons of methylacetoin (singlet, δ 1.10) and the signal of the methyl protons of **4**. Since ethanol and **4** are formed in equal amounts, the fraction of displacement product was also determined by measuring the ratio of the integrated signal of the methylene protons of ethanol (quartet, δ 3.20–3.33) to the combined integrated signals due to the methyl protons of methylacetoin and the methylene protons of ethanol. Both methods gave the same results. The percentage of displacement product was also determined by using proton-decoupled ^{31}P NMR by measuring the ratio of the intensity of the signal due to **4** (δ −3.10) to the combined signal intensities due to diethyl phosphate (δ 0.73) and **4**. The relative amounts of displacement products determined by ^{31}P NMR agreed with those obtained by ^1H NMR.

The hydrolysis of **2** in basic solutions also yields four products in two sets: ethanol and acetoin ethyl phosphate (displacement products) and acetoin and diethyl phosphate (rearrangement products). The relative amount of each set depends upon the concentration of base. The precise fraction of displacement products was difficult to determine since the acetoin is produced in very small amounts and it undergoes polymerization and proton-exchange reactions in base. However, an estimate of the amount of displacement products was made by using ^1H NMR spectroscopy measuring the ratio of the integrated signal of the methylene protons (quartet) of ethanol to the combined integrated signals of the methylene protons of ethanol and half the methylene protons (multiplet, δ 3.48–3.63) of the ethyl groups of diethyl phosphate.

3 hydrolyzes in basic solution to yield only rearranged products (acetol and diethyl phosphate) except in very concentrated base, where very small amounts of displaced products (ethanol and acetol ethyl phosphate) are formed. As was the case with **2**, the amount of displaced products was difficult to determine since the acetol is produced in very small amounts and was subject to polymerization and proton-exchange reactions. However, an estimate of the amount of displaced products was made by using the procedure described above for **2**.

Isotopic Labeling. ^{31}P NMR Analysis. Ten microliters of substrate was added to a 5-mm NMR tube containing 0.5 mL of 1 M NaOD (33% or 47% $H_2{}^{18}O$). The tube was capped and shaken, and after approximately 10 min, 0.25 mL of D_2O was added. An NMR spectrum was then recorded. Under these conditions, **1** produces diethyl phosphate and methylacetoin (83%) plus **4** and ethanol (17%). Although **2** under these conditions produces rearrangement products (acetoin and diethyl phosphate, 95%) as well as displacement products (acetoin ethyl phosphate and ethanol, 5%), only the rearrangement products are produced in sufficient amounts to allow accurate assessment of the amount of incorporation of label into the phosphate component using the effect of ^{18}O on the chemical shift in the phosphorus spectrum.[29,30] Under these conditions **3** produces only rearranged products. The experiments were repeated three times for each compound.

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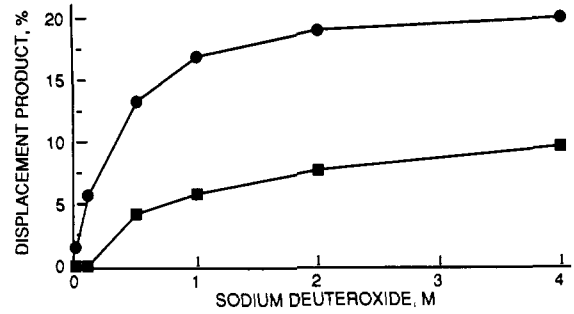


Figure 1. Effect of base concentration on product distribution [percent displacement product set with respect to total of both product sets in the hydrolysis of **1** (●) and **2** (■) (**3** gives only rearrangement products except in 4 M deuteroxide, where approximately 2% of the total is the displacement set)]. The 0 M deuteroxide point is from the distribution at pD 10. Reactions are at 25 °C.

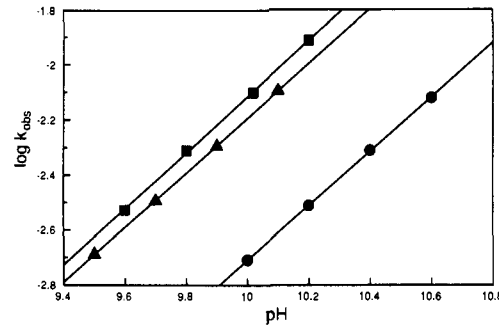


Figure 2. Dependence on solution pH of observed first-order rate constants for hydrolysis of **1** (●), **2** (▲), and **3** (■).

Table I. Second-Order Rate Constants for the Hydroxide-Catalyzed Hydrolysis of β-Carbonyl Phosphates at 25 °C

compd	k_2, M^{-1} s^{-1}
1	14
2	48
3	58
triethyl phosphate[a]	8.5×10^{-6}

[a] From data in: Asknes, G.; Bergesen, K. *Acta Chem. Scand.* **1966**, *30*, 2508.

Results

Product Distribution. The reaction of **1** in alkaline solution produces two sets of products (Scheme I). The first set consists of materials that would result from the hypothetical displacement of one of the ethoxy groups of the phosphate ester by hydroxide: methylacetoin ethyl phosphate (**4**) and ethanol. We call this set displacement products. The identity of **4** was confirmed by cleavage of one of the ester groups of **1** by reaction with sodium iodide, a process that cleaves the C–O bond. The second set consists of the products that result from migration of the diethyl phosphate moiety to the hydrated adjacent carbonyl group followed by decomposition: diethyl phosphate and methylacetoin (rearrangement products). The relative amounts of the two sets of products vary with base concentration (Figure 1).

In dilute basic solutions, only the rearrangement products are observed. In more concentrated basic solution, the fraction of displacement products increases to a maximum of about 20% of the total products in 4 M deuteroxide (Figure 1). The product distribution roughly follows a titration curve with an apparent K_a approximately at 0.5 M deuteroxide. The reaction of **2** and **3** follows a similar pattern of product distribution, but the maximum amount of displacement product from **2** is about 10% and for **3** about 2%.

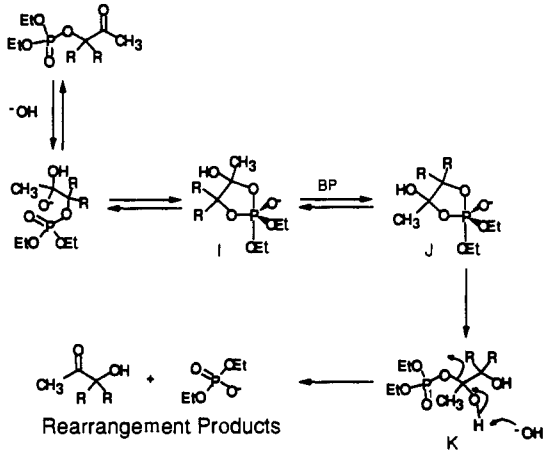
pH Dependence on Rate of Reaction. The rates of hydrolysis of the phosphoacetoin esters in solutions between pH 9 and 11 are presented in Table I and Figure 2. The rates increase linearly with hydroxide concentration (slope = 1.0). The rates are on the

Table II. Extent of Incorporation of Solvent Oxygen into the Phosphate Products for the Basic Hydrolysis of β-Carbonyl Phosphotriesters in 1 M $Na^{18}OD$, $D_2{}^{18}O$

reactant	phosphate ester product	% incorpn of solvent[a]
1	diethyl phosphate	76
1	**4**	76
2	diethyl phosphate	100
3	diethyl phosphate	100

[a] Comparison of total fraction of oxygen isotope (^{18}O) in phosphate product and isotope fraction in $D_2O/D_2{}^{18}O$.

Scheme II



order of 10^6 times greater than that of triethyl phosphate.

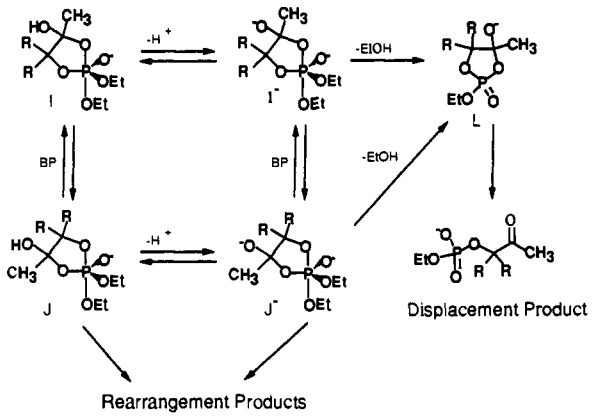
Isotope Labeling. Reactions were conducted in water containing $H_2{}^{18}O$. The reactants initially contain no ^{18}O. The phosphate products were analyzed for ^{18}O content by using ^{31}P NMR.[29,30] For methylacetoin diethyl phosphate (**1**), 76% of solvent oxygen is incorporated into both the rearranged and displaced phosphate products (Table II); 100% of solvent oxygen is incorporated into the phosphate products of **2** and **3**.

Discussion

A mechanism involving the formation of a carbonyl hydrate and its reaction with the internal phosphate can account for both the rapid hydrolysis of the substituted β-carbonyl phosphate esters and the product distribution. A mechanism in which the reactive species may be the enediol phosphate resulting from enolization of the carbonyl function[19] is ruled out on the basis of the rapid reaction of **1**, which cannot produce an enol ester.[20] Mechanisms involving C–O cleavage via epoxide formation[20] may be ruled out since solvent oxygen is incorporated into the phosphate products.

Reaction Pathway in Dilute Basic Solution. In dilute basic solution the rearrangement products constitute 100% of the products for all of the reacting phosphate triesters (**1–3**). The mechanism we propose for the reaction in dilute basic solution is shown in Scheme II. This mechanism is consistent with work by Frank and Usher in which contrasting pathways of reaction of acetoin dimethyl phosphate and acetoin methyl methylphosphonate can be rationalized in terms of electronic requirements of phosphorane intermediates.[21] For the reaction of acetoin dimethyl phosphate they proposed a mechanism in which the conjugate base of the carbonyl hydrate reacts to form a cyclic phosphorane which then undergoes pseudorotation. Thus, in the mechanism in Scheme II, the conjugate base of the carbonyl hydrate attacks the phosphate to form a phosphorane intermediate I. Pseudorotation of I yields phosphorane J. In either phosphorane intermediate (I or J) the ring alkoxide is a better leaving group than ethoxide due to the lower pK_a resulting from the additional oxygen substituent.[31] Therefore, if the initial intermediate I can pseudorotate more rapidly than ethoxide is expelled to form intermediate J, it will expel the ring ligand to form the rearranged phosphate K, which rapidly breaks down via elimination of diethyl phosphate to give exclusively rearranged products.[32]

Scheme III



Reaction Pathway in Concentrated Basic Solution. In concentrated base, a portion of the product is the result of a reaction in which ethoxide is displaced (displacement products, Table I).[33] Since the overall reaction is first order in base concentration, any change in product distribution caused by increased hydroxide concentration requires an additional mechanism to become competitive. Thus, the increase in production of displaced products with increasing hydroxide concentration indicates the involvement of a second-order dependence on hydroxide. Because the amount produced reaches a plateau (Figure 1), it is probable that a group is being titrated or that a kinetic competition is being established which branches to a fixed ratio. Intermediates I and J are monoanions. Removal of a proton from the ring hydroxyl group of intermediates I and J yields the dianionic phosphoranes I^- and J^- as shown in Scheme III. In intermediates I^- and J^- the ring alkoxide is a poorer leaving group than in the monoanionic intermediates I and J since a dianion would be expelled. Therefore, expulsion of ethoxide becomes competitive with ring opening. The cyclic phosphate L resulting from expulsion of ethoxide breaks down via an elimination process to form the phosphate product.[34]

The increase in the amount of displacement products with increasing base concentration depends upon the number of methyl groups at the α-position. For **1** the fraction of displacement products increases to a maximum of about 20% of the total products in 4 M sodium deuteroxide (Table I; Figure 1). However, for **2** the maximum amount of displacement products is only 8% and for compound **3** only about 2%. For **1**, expulsion of ethoxide is competitive with ring opening in intermediates I^- and J^-. However, for **3** the lack of any α-methyl groups results in the formation of intermediates I^- and J^- in which the ring alkoxide is still a better leaving group than ethoxide.[35a,b] Therefore, only rearranged products result from **3**, even in strong base.

Incorporation of Solvent Oxygen into Phosphate Products. Cox and Farmer reported that dimethyl phosphate ion produced in the basic hydrolysis of acetoin dimethyl phosphate contains one oxygen atom derived from solvent.[19] On the basis of these results,

(31) Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795.

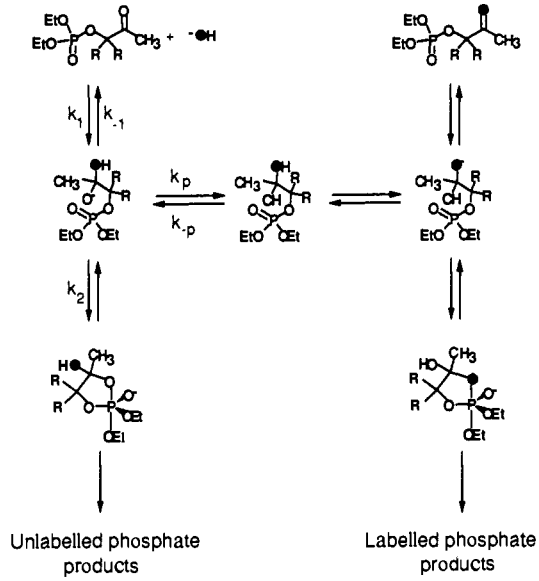
(32) It has been suggested[17] that phosphoranes of type J (Scheme II) break down to form rearranged products by rupture of the hemiacetal C–O bond after protonation of the equatorial oxyanion of I and pseudorotation. However, such a mechanism is inconsistent with the results in ref 21.

(33) **4**, the displaced phosphate product resulting from the hydrolysis of **1**, reacts with base to produce ethanol at a rate of about $^1/_{100\,000}$ that of **1**.

(34) It is unlikely that cyclic phosphate L breaks down by attack of hydroxide at phosphorus since this would result in full incorporation of solvent oxygen into the displaced phosphate products. For **1**, only 76% of solvent oxygen is incorporated into *both* the rearranged and displaced phosphate products.

(35) α-Methyl substitution should increase the basicity of the leaving group, thus decreasing its nucleofugacity. The effect of the basicity of an alkyl leaving group on the reactivity of phosphorus esters has been well documented: (a) Cook, R. D.; Diebert, C. E.; Schwarz, W.; Turley, P. C.; Haake, P. *J. Am. Chem. Soc.* **1973**, *95*, 8088. (b) Cook, R. D.; Farah, F.; Ghawi, L.; Itani, A.; Rahil, J. *Can. J. Chem.* **1986**, *64*, 1630.

Scheme IV

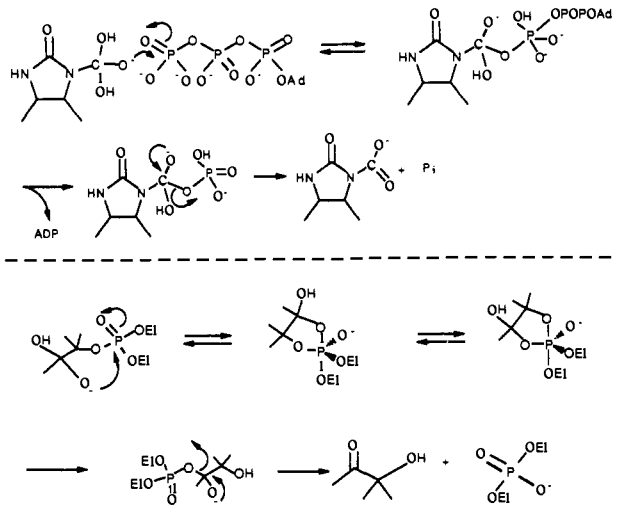


Cox and Ramsay suggested that acetoin dimethyl phosphate reacts via direct attack of hyroxide on the phosphorus of a more reactive enediol phosphate resulting from enolization of the carbonyl function.[19] Our results also indicate that the solvent oxygen is incorporated into the phosphate products derived from compounds **1**–**3** (Table II). However, although **1** cannot be converted to the enol ester since it is methylated, it reacts at a rate comparable to that of **2** and **3** and solvent oxygen is incorporated into its phosphate products.

It is known that isotopic oxygen exchange occurs with carbonyl compounds via a hydration–dehydration mechanism and that such processes are base catalyzed.[36,37] The addition of labeled hydroxide to the carbonyl group of the reactant generates the conjugate base of the carbonyl hydrate (Scheme IV) with a single label. If proton migration is rapid, the maximum extent of transfer of the label into the product is half that of the solvent since there are only two hydroxyls and one is derived from solvent. For the label to be fully incorporated into the hydrate, the unlabeled hydroxide must leave and be replaced by labeled hydroxide. Again, if proton transfers are rapid, there is a 50% chance that the unlabeled species will leave. In the next cycle of addition of labeled hydroxide, the resulting statistical distribution of label will be 75% of the two oxygens of the hydrate. Likewise, a third turnover will lead to 87% incorporation, and a fourth will give 94%, etc. The next step in the mechanism is addition of the oxyanion of the carbonyl hydrate. Since proton transfers are normally relatively fast processes compared to nucleophilic additions, it can be expected that either of the oxygens of the carbonyl hydrate will add to the phosphorus. If the exchange is much faster than addition, then full isotopic incorporation into the phosphate products will be observed. If exchange is slower, then full incorporation of the solvent oxygen into the phosphate product will not occur. We see that for **1** the isotopic content of both the rearranged and displaced phosphate products is 76% that of the solvent, indicating that the rate of cyclization is competitive with exchange.

The presence of α-methyl substituents decreases the rate at which solvent oxygen is exchanged into the carbonyl group of aliphatic ketones.[36] Moreover, the presence of geminal methyl substituents should promote the cyclization process (k_2, Scheme IV) as is expected on the basis of similar effects in reactions of phosphodiesters[38] and in lactonization.[39] Thus, for **1** (which has two α-methyl groups) full incorporation of solvent oxygen does not occur due to the slower rate of exchange of label into the carbonyl group and the faster rate at which the carbonyl hydrate cyclizes to form the phosphorane intermediate J.

Scheme V



We can estimate the rate constant for expulsion of hydroxide from the conjugate base of the carbonyl hydrate of **1** (k_{-1}, Scheme IV). The rate constants for the acid- and base-catalyzed addition of water to acetone are 33 and 110 M^{-1} s^{-1}, respectively.[36b] The rate constant for the acid-catalyzed addition of water to pinacolone is 6 M^{-1} s^{-1}.[36b] Thus, two α-methyl groups reduce the rate of acid-catalyzed addition by a factor of 5.5. If the effect of α-methyl substitution on the rate of the base-catalyzed addition is similar, then the rate constant for addition of hydroxide to **1** should be about 20 M^{-1} s^{-1}, assuming **1** is comparable to pinacolone. The effect of alkyl substitution on the equilibrium for hydration can be incorporated from data for formation of the neutral hydrate of pinacolone by using the linear free energy relationship developed by Greenzaid and co-workers for the hydration of aliphatic ketones:[36a]

$$\log K_h = 1.7(\sigma^*_{R1} + \sigma^*_{R2}) - 2.81 \quad (1)$$

For pinacolone, $\sigma^*_{R1} = 0$ (R1 = Me), $\sigma^*_{R2} = -0.3$ (R2 = *t*-Bu),[40] and K_h is therefore approximately 5×10^{-4}. The pK_a of the hydrate of pinacolone can be estimated by using the linear free energy relationship developed by Hine and Koser for estimating the pK_a of the hydrates of aliphatic aldehydes and ketones:[41]

$$pK_a = 14.19 - 1.32(\sigma^*_{R1} + \sigma^*_{R2}) \quad (2)$$

By use of the above σ^* values, the pK_a of the hydrate of pinacolone is approximately 14.6. Assuming that the equilibrium constant for formation of the neutral hydrate of **1** as well as its pK_a will be similar to those of pinacolone, then the rate constant for the elimination of hydroxide (k_{-1}, Scheme IV) will be about 10^5 s^{-1}.[42] If cyclization of **1** (k_2, Scheme IV) is competitive with expulsion of hydroxide, then the rate constant for cyclization of **1** is about 10^5 s^{-1}. This is well beyond the minimum rate required for the process to be competent in the related enzymic reaction, assuming the availability of acid–base catalysis mechanisms.[15,16]

Relation to Biotin Mechanism. Our results show that the proposed parallel of the mechanism of formation and decomposition of the phosphorylated carbonyl hydrate and a mechanism for carboxylation of biotin is chemically reasonable at every step. As noted in the introduction, the addition of the conjugate base

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(41) Hine, J.; Koser, G. F. *J. Org. Chem.* **1971**, *36*, 1348.
(42) In calculating k_{-1} we have assumed that k_{-p} in Scheme IV is on the order of that for a diffusion-controlled process ($k_{-p} = 10^{10}$ s^{-1}).

of biotin to bicarbonate is related to a cyclization reaction reported by Blagoeva and co-workers.[14] Our work establishes that the tetrahedral adduct of bicarbonate and biotin should react readily with an adjacent phosphate. Such a process, when considered in both forward and reverse directions, is reasonable in comparison with other mechanisms. The reverse reaction involves the attack of phosphate on carboxybiotin, which is analogous to the transfer of the carboxyl group to acceptors in the normal carboxylation process. In the forward direction, the phosphate ester thus generated will decompose by expulsion of the phosphate from the carbonyl hydrate to generate the carbonyl. The parallel reactions are shown in Scheme V.

How Is Biotin Really Carboxylated? The mechanism of carboxylation of biotin remains to be discovered, and experiments to rule out or confirm any mechanism need to be developed. Jencks has proposed that organic reactions "choose" their mechanism simply by finding the lowest energy stepwise pathway involving stable intermediates.[43] On the other hand, the choice of mechanism of an enzyme-catalyzed reaction depends on the structure of the enzyme and the pathway through which the enzyme evolved.[44,45] Evolutionary pressures can direct the development of a pathway without necessarily leading to the overall most rapid pathway if other considerations related to efficiency within the environment take precedence. Consistent with such expectations, biochemical studies are usually aimed at discovering a reaction rather than deducing how a reaction occurs. In this context, what advantages would cause this mechanism to be favored? First, the efficient utilization of ATP is normally an important evolutionary consideration. If ATP is cleaved, it should always lead to formation of the product; otherwise, metabolic processes that led to the generation of ATP will be wasted and the organism will be obliged to provide a new source of energy to permit recoupling of ADP and phosphate. In the present mechanism, as in others, the cleavage of ATP is in a step that produces another intermediate whose reaction is heavily favored toward formation of the product, carboxybiotin. By comparison, mechanisms in which ATP first reacts with bicarbonate to produce carboxyphosphate generate a species that will produce carbon dioxide rapidly.[7] This must be trapped since the lifetime of carboxyphosphate is inherently short.[7] Having the reaction with bicarbonate occur first requires no commitment of ATP until after reaction with bicarbonate has occurred. Thus, the system can be efficient in providing a direct route to utilize the most abundant species.

Conclusions. Our results show that the reaction patterns of dialkyl phosphate esters **1-3** are consistent with mechanisms that involve the reaction of the carbonyl hydrate and the internal phosphate ester. The resulting intermediate reacts at phosphorus and then decomposes via C-O cleavage to form the ketone and expel the dialkyl phosphate ester. The latter process serves as a model for proposed reaction of the adduct of biotin and bicarbonate, which is completed by reaction with ATP to form carboxybiotin. It remains to test the mechanism in an enzymic system where key transition states can serve as targets of probes that provide the necessary differentiation.

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Tetraarylethanediols: Surprisingly Low Energy Requirements for Electron Transfer in Solution and in the Gas Phase

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Abstract: A number of methyl-substituted tetraarylethanediols **I** have been found to undergo facile electron transfer (et) to tris(1,10-phenanthroline)iron(III) complexes ($Fe^{III}L_3$). The products of this reaction are the corresponding benzophenones when an appropriate base is added to the reaction solution. The electron-transfer rate constants (k_{et}) for the reaction of **1** and $Fe^{III}L_3$ have been measured as a function of temperature and are higher than anticipated, based on the energetic predictions derived from model arenes. The oxidation potential, derived from the measured $\Delta G_{et}^{\ddagger}$, is in good agreement with the solution-phase ΔG_{el}°, which can be calculated from the gas-phase ionization potential. Control experiments demonstrate that the reaction proceeds through a normal outer-sphere electron-transfer reaction. The surprisingly low oxidation potentials can only be explained by through-space phenyl-phenyl interactions.

Introduction

There has been much recent activity directed toward understanding the reactivity of radical cations. The utility of radical cations in organic chemistry has been demonstrated in their use in photochemically[1-4] and thermally[5] induced electron-transfer bond-cleavage reactions, thermally activated cycloaddition reactions,[6] and a variety of other electrocyclic reactions.[7-10] Our general interest in bond-cleavage reactions has stimulated our

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