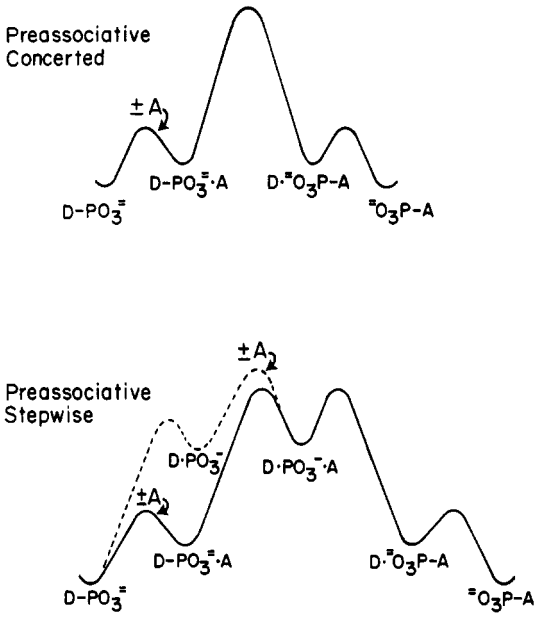

Figure 3. Free energy profiles for preassociative processes. In a preassociative concerted reaction (e.g., an S_N2 process: upper profile) the acceptor A diffuses to the phosphorylated donor D and phospho group transfer occurs via a single associative transition state. A reaction is preassociative stepwise (lower profile, full line) rather than dissociative (lower profile, dashed line) if the intermediate PO_3^- in the complex $D{\cdot}PO_3^-{\cdot}A$ is so unstable that it collapses back to $D{-}PO_3^{2-}$ faster than the acceptor A can diffuse away. [Charges on D and A are omitted for clarity.]

of substituted pyridines and have concluded that the absence of a "break" in the Brønsted plots is most consistent with a concerted reaction, the single, relatively symmetrical transition state involving weak bonding to both the entering and leaving groups. There is no evidence for a change in rate-limiting step as the pK_a of the acceptor nucleophile becomes less than that of the leaving group, as would be expected for a preassociative stepwise path. In so far as these conclusions can be applied more generally, it is likely that the transfer of phospho groups from phospho monoamidates in protic media is best viewed as a preassociative concerted mechanism where the single transition state is a loose one in which neither acceptor nor donor nucleophile is closely associated with phosphorus.

The above conclusion is consistent with the stereochemical results reported in this paper and with the mechanistic evidence cited in the introduction for reactions in protic solvents (items 1-7). For such processes, there are no data that demand the existence of a free metaphosphate intermediate, and it now seems clear that no such postulate is required. In the case of aprotic media, however, the evidence that metaphosphate can be a liberated intermediate is more compelling (see items 8-10 of the introduction). Thus the transfer of phospho groups in the three-phase reactions of Rebek and his group,[10] the phosphorylation of *tert*-butyl alcohol during solvolytic reactions of aryl phosphoric esters studied by Ramirez and collaborators,[11] and the products from the fragmentation reactions of β-halophosphonates investigated by Westheimer and co-workers[12,13] are all strongly suggestive of free (or possibly, in the case of dioxane and acetonitrile, specifically solvated) monomeric metaphosphate ion. Stereochemical investigations on these systems will, it is hoped, provide evidence for the explicit intermediacy of metaphosphate in reactions in aprotic media and set some limits on the freedom of metaphosphate in such solutions.

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Stereochemical Evidence for Pseudorotation in the Reaction of a Phosphoric Monoester

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Abstract: The phospho group of 2-phosphopropane-1,2-diol migrates to the 1-position on heating in aqueous acid. This migration occurs by two routes. The direct route is believed to proceed via a pentacoordinate intermediate that must, by Westheimer's rules, pseudorotate at least once to yield product. In the phospho diester route, the cyclic 1,2-phospho diester is formed as an intermediate. Four experiments have been performed to determine the rate constants for each route. These experiments involve the measurements of (a) the overall equilibrium constant, (b) the partition ratio (to the 1- or 2-phospho monoesters) for the hydrolysis of the cyclic diester intermediate, (c) the overall rate of isomerization, and (d) the rate of solvent ^{18}O incorporation into an equilibrium mixture of 1- and 2-phospho compounds. By using chiral 2-[(*R*)-^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol as substrate and determining the configuration both of the 1-phospho product isomer and of the remaining 2-phospho substrate, the direct route has been shown to proceed with *retention* of configuration at phosphorus, in accord with the predicted behavior for reaction via a pseudorotating pentacoordinate intermediate.

In the mid 1960's, Westheimer[1] laid the foundation for our understanding of many previously enigmatic reactions of phosphate esters, phosphonates, phosphinates, and phosphoramidates. He recognized the importance of and requirements for pseudorotatory processes in these reactions and proposed a set of predictive rules governing the stereochemical disposition of entering groups, departing groups, and other ligands to phosphorus, in the formation and breakdown of pentacovalent phosphorus intermediates.[1] During the past 15 years, much evidence has accumulated in support of Westheimer's proposals for the reactions of phosphoric esters. Many of these data necessarily derive from work on

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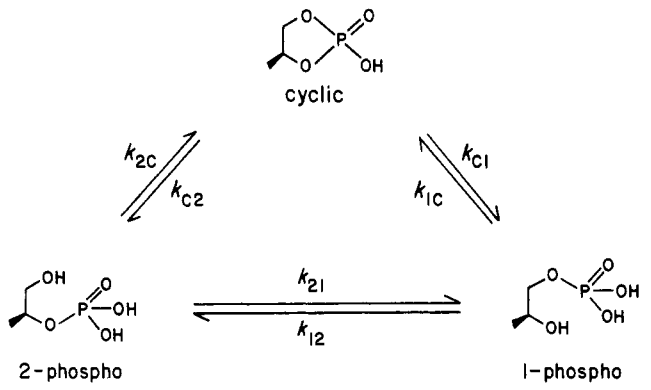
Scheme I. Pathways for the Isomerization of 2-Phosphopropane-1,2-diol and 1-Phosphopropane-1,2-diol



phosphoric triesters and diesters, and the predictions have, economically, been extrapolated to include phosphoric *mono*esters, despite the fact that no direct experimental tests were available for this, perhaps the most important, class.

Phosphoric monoesters may in principle undergo nucleophilic reactions at phosphorus in three ways,[2] each of which has a different predicted stereochemical outcome. First, the dissociative pathway would produce monomeric metaphosphate as an intermediate, and if this species were a "liberated" intermediate[3] and became symmetrically solvated, a product *racemic* at phosphorus would result. Second, if the displacement were "in-line associative" and proceeded by way of a trigonal-bipyramidal transition state in which the entering and departing groups were apical, the stereochemical course of the reaction would be *inversion*. Third, in the "adjacent associative" process, the entering nucleophile would attack phosphorus to form a pentacoordinate intermediate which, since ligands may only enter or leave the intermediate apically,[1] would necessarily suffer at least one pseudorotation to allow expulsion of the leaving group. The stereochemical outcome of such a reaction is in general inversion or retention, depending upon the number of pseudorotations that the intermediate suffers before the leaving group departs. In the case studied here, however, in which two phosphorus coordination sites are linked in a 5-membered ring, only *retention* of the configuration at phosphorus is predicted. We report in this paper an example of the third class of nucleophilic displacement at phosphorus, where the entering nucleophile is constrained to attack phosphorus "adjacent" to the ligand that will become the leaving group, in order to evaluate the stereochemical predictions of the pseudorotatory path in a phosphoric *mono*ester.

The reaction chosen is the 1,2-phospho group migration that occurs when 2-[(*R*)-^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol (**2**) is heated in aqueous acid. Forty years ago, Bailly[4] studied the mechanism of the acid-catalyzed rearrangement of 2-phosphoglycerol to 1-phosphoglycerol and observed that hydrolysis of the phosphoric esters was slow compared to the rate of isomerization.[4] Fordham and Wang subsequently demonstrated in a series of elegant kinetic experiments that two pathways existed for this isomerization.[5] These workers determined the rate constants for the minimal kinetic scheme shown in Scheme I. Importantly, it was demonstrated that while isomerization via the cyclic diester naturally resulted in the incorporation of solvent ^{18}O into the product (upper path, Scheme I), isomerization by the direct route (lower path, Scheme I) caused no incorporation of solvent isotopic label. This latter fact is a necessary (though insufficient) condition for a path involving a pentacoordinate intermediate that must pseudorotate before product is formed. Our decision to study the acid-catalyzed isomerization of 2-phosphopropane-1,2-diol derived from the ready applicability of the methods we developed earlier for the asymmetric synthesis[6] and stereochemical analysis[6,7] of chiral [^{16}O,^{17}O,^{18}O]phospho groups attached to the propanediol skeleton. A preliminary report of some of this work has been published.[8]

Experimental Section

Materials. All chemicals were obtained from Aldrich, Sigma, or Alfa/Ventron unless otherwise noted, and were used as received unless specified otherwise. Isotopically enriched water was purchased from either Bio-Rad Laboratories ($H_2{}^{18}O$) or Mound Research Laboratories ($H_2{}^{17}O$ or $H_2{}^{18}O$) and was used without purification.

Methanol was distilled from $Mg(OMe)_2$ prepared in situ; tetrahydrofuran, acetonitrile, diethyl ether, pyridine, and triethylamine were distilled from CaH_2; dimethylformamide was distilled from CaH_2 under reduced pressure and stored over 4 Å molecular sieves; dioxane was distilled from sodium; methylene chloride, carbon tetrachloride, benzyl bromide, diisopropylamine, and tri-*n*-octylamine were passed through neutral alumina immediately prior to use; trifluoroacetic anhydride was distilled immediately prior to use; cyclohexylamine was distilled from CaH_2 under nitrogen and stored under nitrogen; diazomethane was prepared from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine;[10] sodium hydride was obtained as a 50% oil dispersion and washed with hexane prior to use; pyridinium tosylate was a gift from W. McWhorter; molecular sieves were Linde type 4 Å and were washed exhaustively with methanol, dried at 100 °C, and activated by heating at 250 °C for 24 h under vacuum.

Alkaline phosphatase (from *E. coli* or from calf intestine) was obtained from Sigma as an ammonium sulfate suspension and was either used as received or dialyzed at 4 °C against the buffer in which it was subsequently used. Phosphatases were assayed by observing the change in absorbance at 405 nm when 4-nitrophenyl phosphate was hydrolyzed.[11]

Unless otherwise specified, all mention of phosphate monoesters, including weights of material, refer to the bis(cyclohexylammonium) form of the compound.

Ethyl *O*-Tetrahydropyranyl-(*S*)-lactate. To a slurry of DL-camphor sulfonic acid (5.8 g, 25 mmol) in anhydrous diethyl ether (250 mL) was added ethyl (*S*)-lactate (59 g, 500 mmol) and then dihydropyran (50.5 g, 600 mmol). The resulting solution was cooled in an ice bath and stirred vigorously. After the initial exothermic reaction was complete (approximately 5 min), the solution was stirred overnight at room temperature. Ether (250 mL) was then added and the solution washed successively with portions (100 mL) of aqueous sodium bicarbonate, water, and saturated brine. The ether solution was dried over anhydrous $MgSO_4$ and the solvent then removed under reduced pressure to yield ethyl *O*-tetrahydropyranyl-(*S*)-lactate (101 g, 100%), which was used without further purification. TLC R_f 0.66 (ether–hexane, 7:3 v/v).

2-*O*-Tetrahydropyranyl-(*S*)-propane-1,2-diol. To a suspension of $LiAlH_4$ (19.5 g, 500 mmol) in dry ether (1 L) at 0 °C was added, dropwise, the crude ester from the previous step (101 g, 500 mmol) in dry ether (300 mL) over a period of 165 min. The reaction mixture was refluxed for 2 h and then stirred overnight at room temperature. The solution was then cooled to 0 °C and the reaction was quenched by the successive dropwise addition of water (19.5 mL), 15% aqueous NaOH (19.5 mL), and water (58.5 mL). The resulting white precipitate was isolated by filtration and triturated with ether (500 mL). The combined ethereal solutions were concentrated under reduced pressure to a volume of approximately 500 mL, and this solution was dried over anhydrous $MgSO_4$. The solvent was then removed under reduced pressure to yield crude 2-*O*-tetrahydropyranyl-(*S*)-propane-1,2-diol (81.2 g, 100%). This material was used without further purification.

1-*O*-Benzyl-2-*O*-tetrahydropyranyl-(*S*)-propane-1,2-diol. To a slurry of sodium hydride (7.2 g, 300 mmol) in dry tetrahydrofuran (200 mL) was added crude alcohol (40 g, 250 mmol) in dry DMF (50 mL). The mixture was stirred for 30 min at 0 °C. Benzyl bromide (47 g, 275 mmol) was added dropwise over 15 min at 0 °C. The reaction mixture was then stirred at room temperature for 3.5 h. At this point, exami-

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(11) Bessey, O. A.; Lowry, O. H.; Brock, M. J. *J. Biol. Chem.* **1946**, *164*, 321–329.

nation by TLC (ether–hexane, 7:3 v/v) indicated the absence of starting material. Excess sodium hydride was quenched by the addition of dry methanol (25 mL), and the reaction mixture was partitioned between ether (500 mL) and water (250 mL). The ether layer was washed with brine and dried over anhydrous $MgSO_4$. Removal of solvent yielded crude product (62.5 g, 100%). This material was used without further purification. TLC R_f 0.7 (ether–hexane, 7:3 v/v).

1-*O*-Benzyl-(*S*)-propane-1,2-diol. Crude 2-*O*-tetrahydropyranyl-1-*O*-benzyl-(*S*)-propane-1,2-diol (62.5 g, 250 mmol) was dissolved in absolute ethanol (750 mL), and pyridinium tosylate (2.1 g, 9 mmol) was added. The reaction mixture was stirred for 12 h at room temperature. An additional portion of pyridinium tosylate (3 g, 12.5 mmol) was then added. The solution was refluxed for 3.25 h, after which examination by TLC (ethyl acetate) indicated the absence of starting material. The solution was concentrated to 250 mL and ether (500 mL) was added. The resulting solution was washed successively with 1 N HCl, saturated aqueous $NaHCO_3$, water, and saturated brine. The ethereal solution was then dried over anhydrous $MgSO_4$ and filtered and the solvent was removed under reduced pressure. The yellow oil so obtained was distilled to yield 1-*O*-benzyl-(*S*)-propane-1,2-diol [28.51 g, bp 85 °C (0.45 mmHg)]. This corresponds to a 69% overall yield from ethyl (*S*)-lactate: ^1H NMR ($CDCl_3$) δ 7.25 (s, 5 H), 4.45 (s, 2 H), 3.87 (m, 1 H), 3–3.45 (m, 3 H), 1.08 (d, J = 6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 137.86, 128.09 (2 C), 127.4 (3 C), 75.64, 72.97, 66.12, 18.64. The optical purity of the alcohol was determined by the method of Dale et al.[12] and was found to be >99.5%.

Reaction of the Cyclic Ephedrine Phosphorochloridate with 1-*O*-Benzyl-(*S*)-propane-1,2-diol. To a solution of 1-*O*-benzyl-(*S*)-propane-1,2-diol (3.37 g, 20 mmol) in dry tetrahydrofuran (100 mL, containing a crystal of 1,10-phenanthroline as an indicator) was added, at −78 °C, a solution of *n*-butyllithium (2.4 M in hexane) until a red color (indicative of free alkyllithium) persisted. The mixture was stirred at −78 °C for 30 min and then transferred by cannula under argon pressure, over 35 min, to a solution of ^{17}O-labeled cyclic phosphorochloridate [2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxaphosphilidin-2-[^{17}O]one: prepared from reaction of [^{17}O]-$POCl_3$ with (−)-ephedrine[6]] (4 g, 16 mmol) in dry tetrahydrofuran (250 mL) at −78 °C. This mixture was stirred for 1 h at −78 °C, and then between 0 °C and room temperature for 6 h. The mixture was diluted with ether (500 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and filtered and the solvent removed to yield a yellow oil (5.7 g). The desired syn isomer was isolated by preparative thin-layer chromatography. A total of 14 2000-μm plates were used. Each was developed three times with ether as eluant. A total of 2.15 g (38%) of the cyclic diester amidate was isolated in this manner. TLC R_f 0.41 (ether); ^1H NMR ($CDCl_3$) δ 7.3 (s, 10 H), 5.61 (dd, J = 2.6, 5.5 Hz, 1 H), 4.8 (m, 1 H), 4.6 (s, 2 H), 3.32–3.87 (m, 3 H), 2.65 (d, J = 10 Hz, 3 H), 1.41 (d, J = 6 Hz, 3 H), 0.75 (d, J = 7 Hz, 3 H); ^{31}P NMR (proton decoupled) ($CDCl_3$) δ 19.86 (s), 19.82 (s) (^{18}O shift).

Acid-Catalyzed Ring Opening of the Cyclic Diester Amidate. The diester amidate (2.35 g, 6.2 mmol) was dissolved in dry dioxane (8 mL) and added via cannula to a mixture of trifluoroacetic anhydride (440 μL, 3.1 mmol) and $H_2{}^{18}O$ (2 mL, 98.37 atom % ^{18}O). After 30 min, TLC (ethyl acetate) showed the absence of starting material. Solid NH_4CO_3 (0.5 g) was added, and the reaction mixture was diluted with aqueous ethanol (10 mL, 1:1 v/v) and subjected to hydrogenolysis as described[6] for the 1-phosphopropanediol isomer.

2-[(*R*)-^{16}O,^{17}O,^{18}O]Phospho-(*S*)-propane-1,2-diol. The product from the hydrogenolysis reaction was purified by ion-exchange chromatography on AG1-X8 (100 mL) to yield the bis(cyclohexylammonium) salt (1.35 g, 61%). ^{31}P NMR (proton decoupled) δ 3.5035 (s), 3.4754 (^{18}O shift) (s); undecoupled (d, J = 7.9 Hz); mass spectrum (of the tris(trimethylsilyl) derivative) m/z (M^+ − 15) (1.3%), 358 (3.5%), 359 (16.4%), 360 (44.5%), 361 (34.3%).

Propane-1,2-diol Cyclic Phosphate. To a suspension of 1-phosphopropane-1,2-diol (30 mg, 0.085 mmol) in dry pyridine (2 mL) was added neat diphenylphosphochloridate (approximately 2 equiv) until ^{31}P NMR indicated that no starting material remained. The reaction mixture was diluted with 100 mM aqueous triethylammonium bicarbonate, pH 7 (2 mL), and then washed 10 times with CH_2Cl_2. The aqueous layer was divided into two portions, and each was lyophilized to yield a white solid that was used immediately for the kinetic ring-opening experiment. The ^{31}P NMR showed the absence of either 1- or 2-phosphopropane-1,2-diol. ^{31}P NMR (proton decoupled) (D_2O) δ 17.9 (s).

Methods. ^1H NMR spectra were recorded on a Varian HFT-80 or XL-100 spectrometer or a Bruker WM-300 spectrometer. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (for samples in $CDCl_3$) or ((trimethylsilyl)propane)sulfonic acid (for samples in D_2O). ^{31}P NMR spectra were measured on a Varian XL-100 or a Bruker WM-300 spectrometer. Chemical shifts are reported in ppm relative to external 85% H_3PO_4. Downfield shifts are positive. ^{13}C NMR spectra were recorded on a Bruker WM-300 spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane. Mass spectra were measured on an AEI MS-9 or a Kratos MS-50 spectrometer. Phosphate monoesters were derivatized for mass spectral analysis by heating a sample in a mixture of an excess of pyridine and bis(trimethylsilyl)trifluoroacetamide (1:2, v/v) at 80 °C for 10 min. High-pressure liquid chromatography (HPLC) was performed on a Waters Associates ALC/201/R-401/6000 system equipped with a differential refractometer detector. A Waters Associates μ-Porasil column was used for separations in organic solvents and a Whatman M-9 Partisil-10 SAX column was used for separations with aqueous eluents. Optical rotations were measured on a Perkin-Elmer 451 polarimeter. Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected.

Thin-layer chromatography was performed on Analtech silica gel GHLF plates (250 μm) for analytical separations and Analtech Silica Gel GF (1000 or 2000 μm) for preparative separations. Column chromatography was performed on either E. Merck Silica Gel 60 (63–200 μm) or for "flash chromatography" E. Merck Silica Gel 60 (40–63 μm). Ion-exchange chromatography was performed on AG1-X8 or Dowex 1 (200–400 mesh) for anion exchange and Dowex 50 (200–400 mesh) for cation exchange.

For a column of n mL of packing material (wet volume), a linear gradient of $5n$ mL each of 50 and 400 mM triethylammonium bicarbonate buffer, pH 7, was used. In instances where aromatic phosphates were being separated, the final buffer concentration was 500 mM. Phosphate-containing fractions were located in the eluate by treatment of 50-μL portions of every other fraction with calf intestine alkaline phosphatase (0.5 unit) for 15 min at room temperature and then assayed by the method of Ames.[12] Fractions containing the organic phosphates were pooled and evaporated to dryness. The resulting syrup was dissolved in a small volume of water and loaded onto a Dowex 50 (H^+) column (the amount of packing material used was 10 mL/mequiv charge). The column was then washed with 5 column volumes of water. The pH of the eluate was raised to >10 with cyclohexylamine, and this solution was lyophilized to yield the white crystalline bis(cyclohexylammonium) salt. These salts were recrystallized from acetone–water (20:1, v/v).

Isomerization of 2-[(*R*)-^{16}O,^{17}O,^{18}O]Phospho-(*S*)-propane-1,2-diol. 2-[(*R*)-^{16}O,^{17}O,^{18}O]phospho-*S*-propane-1,2-diol (700 mg, 1.96 mmol) was dissolved in 0.5 N $HClO_4$ (25.65 mL) in a resealable tube equipped with a Teflon-lined screw top. The tube was immersed in a constant temperature oil bath at 85 °C for 17.5 min. The tube was then removed from the oil bath and plunged into a bath of ice water. ^{31}P NMR analysis of a small portion of the reaction mixture indicated that 13.7% of the total amount of phosphopropane-1,2-diols was now the 1-phospho isomer. Further immersion in the oil bath for 10 min was followed by cooling in an ice bath. The tube was then opened, and the reaction was quenched by the addition of ice-cold 1 M tris(hydroxymethyl)aminomethane (38.5 mL, free base form). The pH of the resulting solution was 8.8. The solution was then diluted with water to 500 mL and loaded onto a column (150 mL) of AG1-X8. Ion-exchange chromatography in the usual manner partially separated the 1- and 2-phosphopropane-1,2-diol. The column fractions containing phosphopropanediols were divided into three batches. The first batch (which contained the majority of the 1-phospho isomer) was collected and rechromatographed on AG1-X8. This treatment failed to yield pure 1-phospho isomer, and those fractions in which this isomer predominated were pooled and the material isolated as the bis(cyclohexylammonium) salt, for further purification (see below). The second batch was pooled and stored. The third batch was collected and contained the 2-phospho isomer (130 mg) contaminated by <1% of the 1-isomer. The recovered **2** had the following: ^{31}P NMR (proton decoupled) (D_2O) δ 3.536 (s), 3.50 (s) (isotope shift); undecoupled (d, J = 7.9 Hz) (for each signal); mass spectrum (of the tris(methylsilyl) derivative) m/z (M^+ − 15) 357 (4.2%), 358 (5.6%), 359 (20.5%), 360 (39.2%), 361 (30.4%).

The material from the first batch, containing mainly the 1-phosphopropanediol product, was subjected to high-pressure liquid chromatography on an M-9 Partisil-10 SAX column in 250 mM sodium acetate buffer, pH 4.0. It was necessary to recycle two times per run to achieve adequate separation. By employing this strategy, an 8:1 mixture of **1**:**2** (65.9 mg) was isolated. This material was concentrated and then resubjected to the HPLC purification. After each HPLC separation, acetate was removed by the method of Blättler and Knowles.[13] The resulting aqueous solution was finally rechromatographed on AG1-X8 (150 mL), and a 99:1 mixture of **1**:**2** (34 mg of the bis(cyclohexylammonium)salt) was isolated. The product **1** had the following: ^1H NMR (D_2O) δ 1.19 (d, J = 6.4 Hz) (**2**), 1.15 (d, J = 6.4 Hz) (**1**), relative

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intensity 1:99; ^{31}P (proton decoupled) (D_2O) δ 5.05 (s); mass spectrum (of the tris(trimethylsilyl) derivative) m/z ($M^+ - 15$) 357 (13.8%), 358 (11.1%), 359 (32.4%), 360 (24.7%), 361 (17.8%).

Equilibrium Constant. To a thick-walled glass tube, sealed at one end, was added **2** (20 mg, 0.056 mmol) and 0.5 N $HClO_4$ (1 mL). The tube was sealed and heated for 10 h in a constant temperature oil bath at 85 °C. At the end of this time, the tube was plunged into ice water for 10 s and then into dry ice–acetone for 10 s and was then left for 5 min at 0 °C. The tube was opened and the reaction mixture diluted with D_2O (2 mL). The ^{31}P NMR spectrum of the mixture was recorded with an inverse gated decoupling pulse sequence to eliminate nuclear Overhauser effects. Peak areas were determined by digital integration of the signals for **1** and **2**. A value of 1.81 ± 0.08 was measured for the ratio **1**:**2**.

Kinetic Partitioning. To a sample of the cyclic propane-1,2-diol phosphate prepared as described above was added 0.5 N $HClO_4$ (2 mL, preheated to 85 °C), and the flask was swirled for 1 min ± 10 s in an oil bath at 85 °C. The reaction flask was then quickly chilled and the ratio of products determined by ^{31}P NMR. The ratio of **1**:**2** was 0.59 ± 0.03, as measured from three spectra each from two separate experiments.

Regioisomer Content vs. Time. Compound **2** (21.4 mg, 0.064 mmol) was converted to its disodium salt by passage down a column of Dowex 50 (Na^+). The resulting material, after lyophilization, was dissolved in 0.5 N $HClO_4$ (1.5 mL, prepared from 0.5 mL of 1.5 N $HClO_4$ and 1 mL of D_2O). Portions (0.3 mL) of this solution were placed in each of five 5-mm NMR tubes, and these were sealed. The tubes were then heated in a constant temperature oil bath at 85 °C for the appropriate time, and then quickly cooled. The ratio of **1**:**2** was determined by ^1H NMR (D_2O) at δ 1.20 (2-phosphopropanediol) and δ 1.14 (1-phosphopropanediol). The proportion of **1**:**2** varied as follows: 0 s, 0; 900 s, 0.203; 1800 s, 0.572; 2700 s, 0.798; 3600 s, 1.00; 4500 s, 1.24.

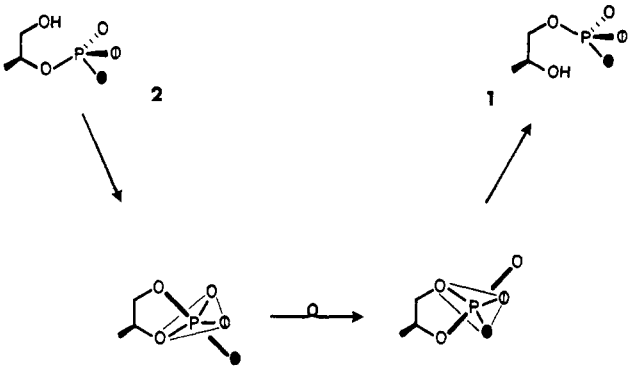
^{18}O Content of the Phospho Groups of 1 and 2 vs. Time. An equilibrium mixture of **1** (19.3 mg) and **2** (10.7 mg) was dissolved in a mixture of $H_2{}^{18}O$ (0.666 mL, 94.9 atom % ^{18}O) and 1.5 N $HClO_4$ (0.333 mL) in a 1-dram glass vial fitted with a Teflon septum. A portion (0.05 mL) of the reaction mixture was removed for the determination of the ^{18}O content (see below). The reaction vial was then placed in a constant temperature oil bath at 85 °C. Portions (0.15 mL) were removed after 10, 20, 30, 45, and 60 min. Each sample was treated as follows. The reaction was quenched by the addition of cold 1 N NaOH (0.1 mL) followed by 60 mM potassium bicarbonate buffer, pH 9.9 (0.3 mL). Dialyzed *E. coli* alkaline phosphatase (1.5 units) was then added, and the mixture was left at room temperature for 45 min. The reaction was stopped by the addition of 1 N HCl (0.2 mL), followed by distilled water (2 mL). The resulting solution was loaded onto a column (1 × 4 cm) of Dowex 1. The column was washed with distilled water (10 mL). The inorganic phosphate was eluted with 40 mM HCl (10 mL). This eluate was concentrated under reduced pressure, ethanol (2 mL) was added, and the solution was taken to dryness under reduced pressure. Silylation then provided the tris(trimethylsilyl) derivative which was analyzed by mass spectroscopy. The ^{18}O content of the peripheral phospho group oxygens varied as follows: 0 s, 0 (% excess over natural abundance); 600 s, 3%; 1200 s, 7.8%, 1800 s, 9.4%; 2700 s, 12%; 3600 s, 17.1%. Complete exchange of all three peripheral oxygens would, under the conditions of the experiment, have led to an ^{18}O content of 63.1%.

^{18}O Content (f_w) of the Solution. The portion (0.05 mL) of the reaction mixture (see previous section) that had been removed before heating was added to freshly distilled trimethylorthobenzoate (0.025 mL) in dry acetonitrile (0.05 mL). After 15 min, dry ether (0.1 mL) was added and this solution was analyzed directly by mass spectrometry. Comparison with unlabeled methyl benzoate (using M^+:136 and $M^+ + 2$:138) indicated that the ^{18}O content of the isomerization solution was 63.1%.

Results and Discussion

The stereochemical course of a phospho group transfer that proceeds via a pentacoordinate intermediate that must pseudorotate is depicted in Scheme II. It is clear from this scheme that if the phospho group transfer proceeds via a pathway involving the pseudorotation of a pentacoordinate intermediate, the product will be formed with retention of configuration at phosphorus. A stereochemical study can therefore distinguish between a pathway involving pseudorotation and other mechanistic possibilities. Alternative mechanistic courses for this reaction include an "in-line" attack of the primary hydroxyl group with expulsion of the secondary hydroxyl group. This process, which would result in inversion of configuration at phosphorus, is unlikely because the attainment of the in-line geometry required at the transition state should be precluded by the size of the five-membered ring. A second mechanistic alternative involves the transfer of a caged metaphosphate moiety. This process could proceed with racemization at phosphorus. The requirement that any metaphosphate species so formed be caged is that if such a reactive species as metaphosphate were free, substantial amounts of inorganic phosphate should be produced as the intermediate reacted with the solvent water. Similarly, the belief that this reaction proceeds by an intramolecular rather than an intermolecular course is predicated on the belief that any intermolecular mechanism would require the formation of inorganic phosphate and/or 1,2-diphosphopropane-1,2-diol, neither of which is observed.

Scheme II. Predicted Retention of the Configuration at Phosphorus for the Isomerization of **2** to **1** via a Pseudorotatory Path



Our initial goal was to establish conditions under which the rearrangement of 2-phosphopropanediol (**2**) to 1-phosphopropanediol (**1**) proceeds at a reasonable rate with minimal label incorporation when the reaction is run in ^{18}O-enriched solvent. It was desirable to find conditions which minimized label incorporation (for a given amount of product formed) because this would mean that the proportion of the product produced via the direct route (lower path, Scheme I) was being maximized. At 85 °C, in 0.5 N $HClO_4$, the contribution of the lower pathway of Scheme I was substantial and, moreover, the rate of loss of the phosphopropanediols by hydrolysis was negligible.

Since the reaction product can be formed by two routes and we are concerned to establish the stereochemical course of only one of them, the proportion of product deriving from each path must be determined independently. Accordingly, we need to know the rate constants for the minimal kinetic scheme, Scheme I, in order to be able to predict the stereochemical and isotopic composition of the phospho groups of the product **1** and the recovered **2**. These kinetic studies are described first.

In their study of the isomerization of 2-phosphoglycerol and 1-phosphoglycerol, Fordham and Wang[5] performed four experiments, the information from which allows an essentially complete kinetic description of the events shown in Scheme I. In determining the analogous rate constants for the isomerization of **1** and **2**, we have followed the experimental approach of Fordham and Wang, though the results from one of the four experiments have been analyzed in a different way. The modification is necessary because of an error in the kinetic derivation presented by Fordham and Wang.[5] The details of the correct analysis are given in the Appendix.

The four experiments that are required are outlined below. Each experiment was done at 85 °C in 0.5 N $HClO_4$.

(1) The overall equilibrium constant, K_{eq}, for the isomerization reaction was determined to be 1.81 by allowing a solution of 2-phosphopropane-1,2-diol to equilibrate for 10 h and then measuring the ratio of the ^{31}P NMR signals for **1** and **2**. A complementary experiment, the equilibration of a solution of 1-phosphopropane-1,2-diol, gave an identical result for K_{eq}.

(2) The ratio k_{c2}/k_{c1} was determined to be 1.7 by determining the ratio of **1**:**2** formed after short times (approximately 1 min)

(14) Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.*, preceding paper in this issue.

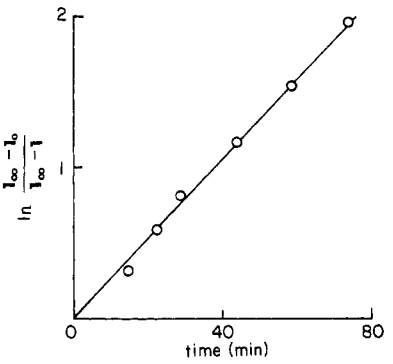


Figure 1. Time course of the isomerization of 2-phosphopropane-1,2-diol (**2**) plotted according to eq A7 of the Appendix.

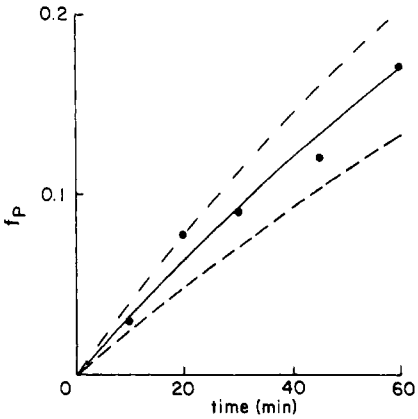


Figure 2. Time course of the incorporation of solvent ^{18}O into an equilibrium mixture of **1** and **2**. The solid line is that calculated by using the best-fit rate constants of Table I. The dashed lines are those calculated for a change in k_{1c} of ±25% (see Appendix).

when a sample of the cyclic phosphopropane-1,2-diol diester was exposed to the reaction conditions.

(3) The time course of the overall equilibration of **2** was determined by sampling a reaction mixture at various times. The samples were quenched and the ratio of **1**:**2** was determined by ^1H NMR. Values of $[\mathbf{1}]_\infty/([\mathbf{1}]_\infty - [\mathbf{1}])$ were plotted against time, according to eq A7 of the Appendix. The slope of this plot (see Figure 1) gives the quantity $[k_{12} + k_{1c}/(1 + k_{c1}/k_{c2})](1 + K_{eq}^{-1})$.

(4) The rate of incorporation of ^{18}O into a mixture of **1** and **2** at chemical equilibrium was determined by sampling a solution of the reaction mixture in $H_2{}^{18}O$ at regular intervals and analyzing the ^{18}O content of the phospho groups. This was done by neutralizing the sample, excising the phospho groups by hydrolysis catalyzed by alkaline phosphatase, and analyzing the inorganic phosphate so formed by mass spectrometry. The atom fraction of ^{18}O in the phospho group, f_p, was compared with the atom fraction of ^{18}O in the solvent, f_w. A plot (see Figure 2) of f_p vs. time was made. The value of k_{1c} was found that, combined with the independently determined parameters K_{eq}, k_{c1}/k_{c2}, and the overall isomerization rate from (3), above, produced the best fit between the predicted and observed values of f_p. In this manner k_{1c}, k_{2c}, k_{12}, and k_{21} could be calculated (see Appendix). The kinetic parameters in Table I describe the time course of the equilibration and provide the data necessary to predict the overall stereochemical consequence of the reaction of 2-[(*R*)-^{16}O,^{17}O,^{18}O]propane-1,2-diol. The rate constants of Table I provide the free energy profile of Figure 3, which shows that under the conditions used, the initial rates of isomerization of the 2-phospho isomer by the direct route and by the route via cyclic phospho compound are in the ratio of 1:1.75. About 36% of the product **1** initially formed from **2** comes by the direct route.

To predict the isotopic composition and configuration of product **1** and of remaining starting material **2** when isotopically labeled **2** (chiral at phosphorus) is used as the substrate, we need not only to know the rates of all processes leading to and from **1** and **2** (as

Table I. Kinetic Parameters for the Isomerization of **2** and **1**[a,b]

k_{21},[c] s^{-1}	1.02×10^{-4}	k_{1c},[c] s^{-1}	1.57×10^{-4}
k_{12},[c] s^{-1}	5.64×10^{-5}	k_{c2}/k_{c1}[d]	1.70
k_{2c},[c] s^{-1}	4.84×10^{-4}	K_{eq}[e,f]	1.81

[a] 85 °C, 0.5 N $HClO_4$. [b] See Scheme I. [c] ±7%. These errors derive from the fit of the best-fit line to the data in Figure 2. See Appendix. [d] ±5%.[g] [e] [**1**]/[**2**]. [f] ±4%.[g] [g] From replicate experiments.

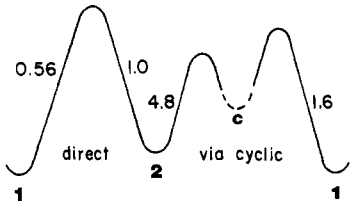


Figure 3. Free energy profile for the isomerization of **1** and **2** by the direct (pseudorotatory) path and by the phospho diester path via the cyclic diester, c. Barrier height differences are exaggerated for clarity. The small numbers are the rate constants for that step in s^{-1}, $\times 10^4$.

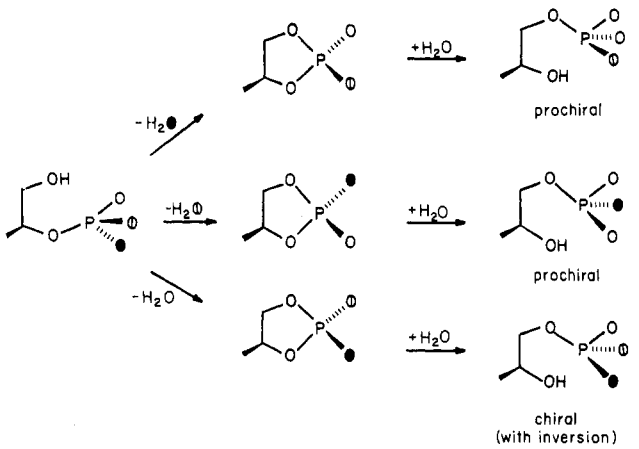


Figure 4. Predicted configurational inversion (of one-third of the molecules) and isotopic label loss (from two-thirds of the molecules) for the isomerization of **2** to **1** by the phospho diester path via the cyclic diester.

described above) but also the predicted stereochemical course of every reaction at phosphorus. On the basis of literature precedent for the reactions of phosphoric monoesters and diesters,[9] we make the reasonable assumption that both the cyclizations (k_{2c} and k_{1c}) and the ring-opening reactions (k_{c2} and k_{c1}) proceed with "in-line" geometry. This means that when **2** cyclizes, two-thirds of the resulting cyclic diester will have lost either ^{17}O or ^{18}O, and when the resulting cyclic compounds hydrolyze in $H_2{}^{16}O$ these two species will yield *pro*chiral phosphoric esters containing two peripheral ^{16}O atoms. The remaining one-third of the cyclic diester (that which lost ^{16}O on cyclization) will regain ^{16}O from the solvent on hydrolysis to give labeled **1** that is chiral at phosphorus and has suffered inversion. The labeled **2** that reforms from this cyclic diester is unchanged in configuration (Figure 4). In contrast, those molecules of [^{16}O,^{17}O,^{18}O]-**2** that are converted to **1** by the *direct* route (Scheme I, lower path) will retain all their isotopic labels, and *if* this route follows the pseudorotation mechanism, the product **1** will be formed with retention of configuration at phosphorus (Scheme II). In summary, we expect that one-third of the product **1** molecules deriving from the route via the cyclic diester will have suffered inversion, and all of the product molecules from the direct route will have been formed with retention of configuration at phosphorus.

On the basis of the rate constants for Scheme I listed in Table I, and of the stereochemical behavior outlined above, we can predict the isotopic composition and the configuration at phosphorus for both the product **1** and the reisolated isomer **2** for any extent of reaction. In Table II are listed the predicted and observed values for the configuration at phosphorus for the reaction of 2-[(*R*)-^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol that had proceeded 23% to chemical equilibrium. The product **1** and remaining

Table II. Isotopic Content and Stereochemistry of **2** and **1**

	starting **2**	reisolated **2**[a]	product **1**[a]
	Isotopic Content Relative Intensities, %		
M^+	1.3	4.2	13.8
$M^+ + 1$	3.5	5.6	11.1
$M^+ + 2$	16.4	20.5	32.4
$M^+ + 3$	44.5	39.2	24.7
$M^+ + 4$	34.3	30.4	17.8
	Ratio of ^{31}P NMR Peaks[b]		
2:6	1.76	1.73	1/1.11
7:6	1.86	1.71	1/1.25
	% R of Phospho Group		
observed	97 ± 2.5	97 ± 0.5	72 ± 9
predicted[c]	97.2 ± 2.5	97.2 ± 0.2	62 ± 4

[a] Extent of reaction, 23%. [b] Numbering from downfield, up (see ref 7 and 8). [c] See text.

2 were isolated from the quenched reaction, and purified by ion exchange chromatography followed (for **1**) by high-pressure liquid chromatography. Stereochemical analysis of each of these samples using the ^{31}P NMR method[7] showed that the product **1** is formed predominantly with *retention of configuration at phosphorus*. The extent of racemization is, within experimental error, that predicted from the rate constants determined independently. If the direct route goes with retention, a product that is 62% *R* is predicted (see Table II); if the direct route had gone with inversion, a product that was 0% *R* would be predicted; and if the direct route had led to racemization, the product would have been 31% *R*. The observed value of 72 ± 9% *R* is clearly consistent only with retention as the stereochemical course of the direct pathway of isomerization. [One may wonder in passing why the intermolecular reactions studied in the previous paper[14] all proceed by either a loose associative transition state or a preassociative path involving caged metaphosphate, yet the intramolecular rearrangement investigated in the present paper apparently goes via a pseudorotating pentacoordinate intermediate. We must evidently conclude that, in systems and under conditions where "in-line" attack is impossible, the pseudorotatory path becomes the most favored route for nucleophilic displacement.]

The agreement between predicted and observed values (Table II) indicates (i) that the minimal kinetic scheme (Scheme I) is sufficient to describe the reaction course for the acid-catalyzed equilibration of **2** and **1** and (ii) that the direct isomerization proceeds with retention of configuration at phosphorus, as predicted by the pseudorotation mechanism. While it is true that stereochemical retention would be predicted both for a putative "adjacent concerted" process in which the entering nucleophile attacks phosphorus from the same face as that from which the leaving group departs and for a hypothetical "adjacent stepwise" reaction where metaphosphate slides from one hydroxyl group to the other without ever leaving the solvent cage, it must be said that both of these possibilities would run counter to all that we know about the stereochemical behavior of phosphorus in phosphoric esters generally. These results, then, constitute the first direct evidence for the pseudorotation mechanism[1] in the reaction of a phosphoric monoester.

Acknowledgment. We are grateful to Dr. Joel Belasco and David Hansen for help with the computer program. This work was supported by the National Institutes of Health and Merck, Sharp and Dohme. The Bruker NMR instrument used for the high-resolution ^{31}P NMR spectra was purchased with the help of a grant from the National Science Foundation.

Appendix

Presented in this Appendix is the derivation of the kinetic equations that describe the processes illustrated in Scheme I and allow the quantitative prediction of the isotopic content and configuration of the phospho groups in **1** and **2**.

Rate of Approach to Equilibrium Starting with 2. Let the concentration of 1-phosphopropane-1,2-diol be [**1**], that of 2-phosphopropane-1,2-diol be [**2**], that of the cyclic diester be [c], and that of the *sum* of **1** and **2** be [a], and let k_{c1}/k_{c2} be θ. Now, the rates of change of [**1**], [**2**], and [c] are

$$d[\mathbf{1}]/dt = -(k_{12} + k_{1c})[\mathbf{1}] + k_{21}[\mathbf{2}] + k_{c1}[c] \quad (A1)$$

$$d[\mathbf{2}]/dt = k_{12}[\mathbf{1}] - (k_{21} + k_{2c})[\mathbf{2}] + k_{c2}[c] \quad (A2)$$

$$d[c]/dt = k_{1c}[\mathbf{1}] + k_{2c}[\mathbf{2}] - (k_{c1} + k_{c2})[c] \quad (A3)$$

and at the steady state

$$d[c]/dt = 0 \quad (A4)$$

From eq A1, A3, and A4, and using the equilibrium conditions

$$K_{eq} = k_{1c}k_{c2}/k_{c1}k_{2c} = k_{12}/k_{21} \quad (A5)$$

we obtain

$$\frac{d[\mathbf{1}]}{dt} = \left(\frac{k_{1c}}{1+\theta} + k_{12}\right)\left(\frac{[a] - [\mathbf{1}]}{K_{eq}} - [\mathbf{1}]\right) \quad (A6)$$

integration of which gives

$$\ln \frac{([\mathbf{1}]_\infty - [\mathbf{1}]_0)}{([\mathbf{1}]_\infty - [\mathbf{1}])} = \left[(1 + K_{eq}^{-1})\left(\frac{k_{1c}}{1+\theta} + k_{12}\right)\right]t \quad (A7)$$

Equation A7 (which is identical with eq 17 of ref 5) allows the evaluation of the term in large square brackets from the linear plot shown in Figure 1.

Rate of Incorporation of ^{18}O from Solvent into 1 and 2 at Equilibrium. Let [$\mathbf{1}^0$], [**1**′], [**1**″], and [**1**‴] be the concentrations of 1-phosphopropanediol containing zero, one, two, or three peripheral ^{18}O labels, respectively. Let [$\mathbf{2}^0$], [**2**′], [**2**″], and [**2**‴] apply similarly to the 2-phospho isomer, and let [c^0], [c′], and [c″] apply similarly to the exocyclic oxygens of the cyclic diester. Further, let

$$A = [\mathbf{1}^0] + [\mathbf{1}'] + [\mathbf{1}''] + [\mathbf{1}''']$$

$$B = [\mathbf{2}^0] + [\mathbf{2}'] + [\mathbf{2}''] + [\mathbf{2}''']$$

and if f_1 is the atom fraction of ^{18}O in the phospho group of **1**, f_2 is the atom fraction of ^{18}O in the phospho group of **2**, and f_p is the atom fraction of ^{18}O in the phospho groups of the equilibrium mixture of **1** and **2**

$$f_1 = ([\mathbf{1}'] + 2[\mathbf{1}''] + 3[\mathbf{1}'''])/3A$$

$$f_2 = ([\mathbf{2}'] + 2[\mathbf{2}''] + 3[\mathbf{2}'''])/3B$$

$$f_p = [[\mathbf{1}'] + [\mathbf{2}'] + 2([\mathbf{1}''] + [\mathbf{2}'']) + 3([\mathbf{1}'''] + [\mathbf{2}'''])]/3(A + B)$$

Then we may write

$$d[\mathbf{1}']/dt = k_{21}[\mathbf{2}'] + k_{c1}[c^0]f_w + k_{c1}[c'](1 - f_w) - (k_{1c} + k_{12})[\mathbf{1}'] \quad (A8)$$

where f_w is the atom fraction of ^{18}O in the solvent. Analogous equations can be written for $d[\mathbf{2}']/dt$, $d[\mathbf{1}'']/dt$, $d[\mathbf{2}'']/dt$, $d[\mathbf{1}''']/dt$, and $d[\mathbf{2}''']/dt$. At the steady state

$$(k_{c2} + k_{c1})[c^0] = k_{1c}([\mathbf{1}] + [\mathbf{1}']/3) + k_{2c}([\mathbf{2}] + [\mathbf{2}']/3) \quad (A9)$$

and similarly for [c′] and [c″].

Now, f_p can be expressed by

$$3f_p(A + B) = [\mathbf{1}'] + [\mathbf{2}'] + 2([\mathbf{1}''] + [\mathbf{2}'']) + 3([\mathbf{1}'''] + [\mathbf{2}'''])$$

and the *rate* of incorporation of label into **1** and **2** becomes

$$3(A + B)\frac{df_p}{dt} = \frac{d[\mathbf{1}']}{dt} + \frac{d[\mathbf{2}']}{dt} + 2\left(\frac{d[\mathbf{1}'']}{dt} + \frac{d[\mathbf{2}'']}{dt}\right) + 3\left(\frac{d[\mathbf{1}''']}{dt} + \frac{d[\mathbf{2}''']}{dt}\right) \quad (A10)$$

Using eq A8 and A9 (and their analogous forms for more heavily labeled species) and substituting for f_1 and f_2, we obtain:

$$3(A + B)\ (df_p/dt) = (k_{1c}A + k_{2c}B)f_w - (k_{1c}Af_1 + k_{2c}Bf_2) \quad (A11)$$

This equation becomes identical with eq 21 of Fordham and Wang[5]

$$3(A + B)\ (df_p/dt) = (k_{1c}A + k_{2c}B)(f_w - f_p) \quad (A12)$$

only if $f_1 \approx f_2 \approx f_p$. These equalities only hold under two circumstances, *either if*

$$k_{c1}/k_{c2} = [A/B]_{eq}$$

which states that the cyclic diester partitions to **1** and **2** in the same ratio as the equilibrium proportions of **1** and **2**, *or if*

$$k_{12},\ k_{21} \gg k_{1c},\ k_{2c}$$

which states that **1** and **2** equilibrate much more rapidly than any wash-in of solvent label occurs via c. Since neither of these limiting conditions holds, the equation of Fordham and Wang (A12) cannot be used. The rate constants were therefore evaluated as follows.

The term in large square brackets in eq A7 is known from the slope of Figure 1. Since we know K_{eq} and θ from independent direct measurement, we can express k_{12} in terms of k_{1c}. Now with use of eq A5, k_{21} and k_{2c} are determined. We therefore vary k_{1c} (and in accord, k_{12}, k_{21}, and k_{2c}) and, using a simple computer program, derive a best fit to the plot of f_p vs. time (Figure 2). The solid line plotted in Figure 2 is the "best-fit" line obtained with this procedure, and the kinetic constants listed in Table I are derived from this fit. For the sensitivity of the experimental data to the value of k_{1c} to be illustrated, Figure 2 contains the predicted lines if k_{1c} is 25% larger than the best-fit value (upper dashed line) or 25% smaller than the best-fit value (lower dashed line).

Aliphatic Hydroxylation by Cytochrome P-450. Evidence for Rapid Hydrolysis of an Intermediate Iron–Nitrene Complex

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Abstract: Studies with synthetic metalloporphyrin-based hydrocarbon oxidation systems have suggested that the cytochrome P-450 class of heme-containing enzymes may be able to transfer a nitrene equivalent to a hydrocarbon substrate in direct analogy to their currently known ability to transfer an oxene equivalent. Exposure of cyclohexane to the nitrenoid reagent (*N*-(*p*-toluenesulfonyl)imino)phenyliodinane (**1**) in the presence of a purified cytochrome P-450 isozyme ($P\text{-}450_{LM2}$) did not result in any amidation of the hydrocarbon. Instead, cyclohexane was efficiently hydroxylated to cyclohexanol. Hydroxylation was also observed with two other hydrocarbons, methylcyclohexane and *p*-xylene. This reaction is a true catalytic activity of the P-450. Anaerobic and isotope-labeling studies demonstrated that the oxygen in cyclohexanol derived from the water of the reaction medium. These results are interpreted by a scheme whereby the nitrenoid reagent generates a transient iron(V)–nitrene complex which is rapidly hydrolyzed in the aqueous environment to the corresponding iron(V)–oxo complex, which then hydroxylates the substrate.

The cytochromes P-450 are a class of heme-containing enzymes able to catalyze the introduction of a single oxygen atom into an unactivated substrate from atmospheric dioxygen. During this process the other oxygen atom from dioxygen is reduced to water at the expense of reduced pyridine nucleotide.[1]

$$RH + O_2 + NAD(P)H + H^+ \rightarrow ROH + H_2O + NAD(P)^+ \quad (1)$$

This monooxygenation reaction is formally an oxene transfer and is suggestive of sequestration of a single reactive oxygen atom at the enzyme's catalytic site as, for instance, a high-valent iron–oxo species ($Fe^V{=}O$). Unfortunately, characterization of the P-450 reactive oxygen intermediate has been hindered by the lack of a stable, readily observable intermediate as is available in the peroxidase series (i.e., compound I).[2] Thus, the only information that has been gleaned about the P-450 intermediate is the result of indirect inferences from studies of special substrates, artifical oxidants, and synthetic models. A number of the model systems have employed iodosylarenes as oxygen donors with striking success.[3] Indeed, the P-450 enzymes themselves may use iodosylbenzene to supplant dioxygen and NADH in aliphatic hydroxylation.[4] Recently, Groves[5] and Breslow[6] have revealed metalloporphyrin-based catalytic systems capable of transferring a nitrene equivalent to aliphatic and olefinic hydrocarbons. We now report the results of similar experiments using an isolated P-450 enzyme and (*N*-(*p*-toluenesulfonyl)imino)phenyliodinane (**1**), one of the nitrene sources used by Breslow.

Experimental Section

Analytical Procedures. Infrared spectra were recorded on a Perkin-Elmer 735 instrument with potassium bromide pellets with use of the 1601-cm^{-1} band of polystyrene for calibration. Proton NMR spectra were determined on a Varian EM-360 instrument at 60 MHz, referenced

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