acterization of cytochrome oxidation-reduction behavior. In particular, it will be of interest to examine the influence of heme substituents through use of heme-substitution techniques. These studies are now in progress.

Acknowledgment. We thank Professors R. A. Scott and H. B. Gray for useful discussions and R.A.S. for listings of several computer programs. We thank Dr. Marcia Mauk and Steve Pelech for helpful suggestions. This research was supported by an operating grant from the Medical Research Council of Canada. The stopped-flow spectrophotometer and associated computer system were obtained through a major equipment grant from the Medical Research Council.

Registry No. Cytochrome b_5, 9035-39-6; $Fe(EDTA)^{2-}$, 15651-72-6.

Supplementary Material Available: Listing of observed first-order rate constants (3 pages). Ordering information is given on any current masthead page.

Communications to the Editor

Stereochemistry and Mechanism of an Acid-Catalyzed 1,2-Phospho Group Migration: Evidence for Pseudorotation in the Reaction of a Phosphoric Monoester

Stephen L. Buchwald,† Diana H. Pliura,‡ and Jeremy R. Knowles*

Department of Chemistry, Harvard University Cambridge, Massachusetts 02138

Received October 13, 1981

In the past few years, there has been much work on the determination of the stereochemical consequence at phosphorus of enzyme-catalyzed reactions that involve esters of phosphoric acid.[1] There have been almost no reports, in contrast, on the stereochemical course of *non-enzymic* reactions of phosphoric monoesters. Such stereochemical results are of particular interest since phosphoric monoesters may in principle undergo nucleophilic substitution at phosphorus by three mechanisms,[2] each of which has a different predicted stereochemical outcome. First, the reaction may follow the unimolecular "dissociative" pathway via a monomeric metaphosphate intermediate. If this species is free and symmetrically solvated, we expect the product will have suffered *racemization* at phosphorus. Secondly, if the "in-line associative" mechanism is followed, the reaction will go through a pentacoordinate transition state, and by analogy with the S_N2 reaction at carbon, *inversion* of the configuration at phosphorus will result. Thirdly, in the "adjacent associative" process, adjacent attack of the entering nucleophile forms a pentacoordinate intermediate that must undergo pseudorotation to allow expulsion of the leaving group from an apical position.[3] The stereochemical course of such a reaction is predicted to be *retention*. We report here experimental results on a reaction where the entering nucleophile is constrained to attack phosphorus "adjacent" to the leaving group and show that the stereochemical results are qualitatively and quantitatively in accord with the above prediction for a pathway that involves pseudorotation of an intermediate that is pentacoordinate at phosphorus.

The reaction we have chosen is the 1,2-phospho group migration that occurs when 2-[(*R*)-^{16}O,^{17}O,^{18}O]phosphopropanediol (**2**) is heated in acid. Many years ago, Bailly[4] described the analogous rearrangement of 2-phosphoglycerol to 1-phosphoglycerol and observed that hydrolysis of the phosphoric ester was slow compared to the isomerization reaction. Subsequently, Fordham and Wang[5] demonstrated that two pathways exist for this isomerization, and in a series of elegant kinetic experiments these workers evaluated the rate constants for the minimal kinetic scheme shown in Scheme I. Most important, while the isomerization route via the cyclic diester resulted in the incorporation of solvent ^{18}O into the product, the direct isomerization caused no such incorporation. This is consistent with the latter path involving a pseudorotating pentacoordinate intermediate and is a necessary (though insufficient) condition for this mechanism.

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

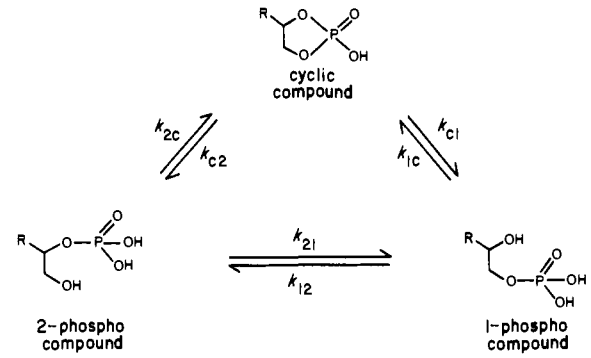


Table I. Kinetic Parameters for the Isomerization of **2** and **1**[a,d]

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

[a] 85 °C, 0.5 N $HClO_4$. [b] See Scheme I. [c] [1]/[2]. [d] The estimated precision of the individual rate constants is ±7%; of the rate ratio, ±5%; and of the equilibrium constant, ±4%.

We first determined conditions under which the rearrangement of **2** to **1** would proceed at a reasonable rate with minimal label incorporation from $H_2{}^{18}O$. At 85 °C in 0.5 M $HClO_4$, the contribution of the upper pathway of Scheme I is minimized, and the rate of loss of phosphoric ester by hydrolysis is negligible. Since, however, the overall equilibrium constant for the **2** to **1** isomerization is not far from unity (K_{eq} = [**2**]/[**1**] = 0.55), the

† National Science Foundation predoctoral Fellow.
‡ Fellow of the National Sciences and Engineering Council of Canada.

(1) Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877-919. Knowles, J. R. *Fed. Proc., Fed. Am. Soc. Exp Biol.* in press. Frey, P. A. *Tetrahedron*, in press.
(2) Kirby, A. J.; Warren, S. G. "The Organic Chemistry of Phosphorus"; Elsevier: Amsterdam, 1967.
(3) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70-78.
(4) Bailly, M. C. *Bull. Soc. Chim. Fr.* **1942**, *9*, 314-339.
(5) Fordham, W. D.; Wang, J. H. *J. Am. Chem. Soc.* **1967**, *89*. 4197-4203. The rate of ^{18}O incorporation at chemical equilibrium was analyzed by an iterative procedure. 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, produced the best fit between the predicted and observed values of f_p. Equation 7 of Fordham and Wang's paper only holds precisely when the fractions of each isomer that is isotopically labeled are equal. In the present case, use of the Fordham and Wang equation produces rate constants that differ by less than 8% from those reported in Table I.

0002-7863/82/1504-0845$01.25/0

Table II. Isotopic Content and Stereochemistry of 2 and 1

	starting 2[a]	reisolated 2[b]	product 1[c]
	Isotopic Content[d] 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[e]		
2:6[e]	1.76	1.73	1/1.11
7:6[e]	1.86	1.71	1/1.25
	% R of Phosphoryl Group[f]		
obsd	97 ± 2.5	97 ± 0.5	72 ± 9
predicted[g]	97 ± 2.5	97 ± 0.2	62 ± 4

[a] 2-[(R)-^{16}O,^{17}O,^{18}O]phospho-(S)-propane-1,2-diol bis(cyclohexylammonium) salt was synthesized by our general route.[8] This material (700 mg) was dissolved in 0.5 N $HClO_4$ and heated in a thick-walled Teflon-capped tube in a constant temperature oil bath at 85 °C for 17 min. The reaction was quenched into an ice-cold solution of Tris buffer. [b] Isolated from the reaction mixture by ion exchange chromatography on AG1-X8 (from BioRad). [c] Purified by high-pressure liquid chromatography on Partisil 10 SAX (Whatman) of the enriched material from b, in 250 mM acetate buffer, pH 4. [d] From repeated scans of the mass spectrum of samples after derivation with bis(trimethylsilyl)trifluoroacetamide. The $M^+ - 15$ peak envelope was used. [e] The stereochemical analysis was done by ^{31}P NMR spectroscopy according to our published procedure.[9] The peak numbers relate to the eight-line spectrum, counting from downfield, up. Peak areas were determined by planimeter from expanded spectra. [f] Calculated from the mean value of the ratios of stereochemically informative peaks of the NMR spectrum [i.e. (2:3 + 7:6)/2]. The error limits quoted only express the difference in apparent %R from the syn (2:3) and anti (7:6) cyclic triesters.[9] [g] Calculated using the rate constants quoted in Table I and the known extent of reaction, by iteration.

first-formed product can readily reform starting material, and we must therefore evaluate the rate constants for Scheme I in order to be able to predict the stereochemical and isotopic composition of the isolated product 1. The necessary rate constants were determined by modification of the methods of Fordham and Wang[5] to provide k_{12}, k_{21}, k_{2c}, k_{1c}, and k_{c1}/k_{c2} (see Table I). These data allow the prediction of the *isotopic content* of 1 and remaining 2, when [^{16}O,^{17}O,^{18}O]-2 is allowed to proceed toward equilibrium in $H_2{}^{16}O$. To predict the *stereochemistry* of the product 1 (and of the remaining 2), we make the reasonable assumption[6] 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[7] of the resulting cyclic diester will have lost ^{17}O or ^{18}O, and when the 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 (Scheme II). In contrast, those molecules of [^{16}O,^{17}O,^{18}O]-2 that are converted to 1 by the direct route (Scheme II, 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 (see Scheme III). In summary, we expect that one-third of the

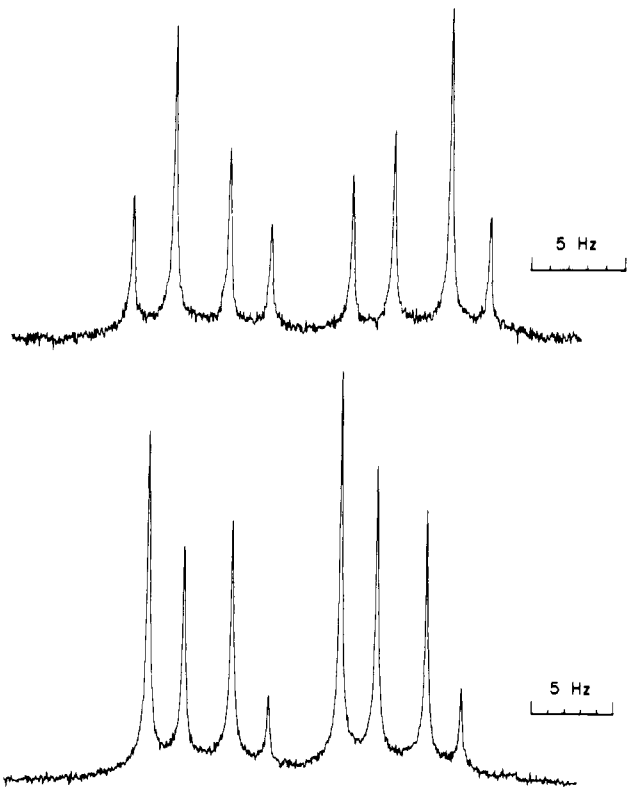
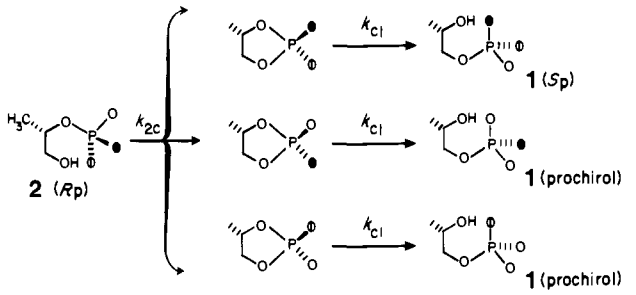
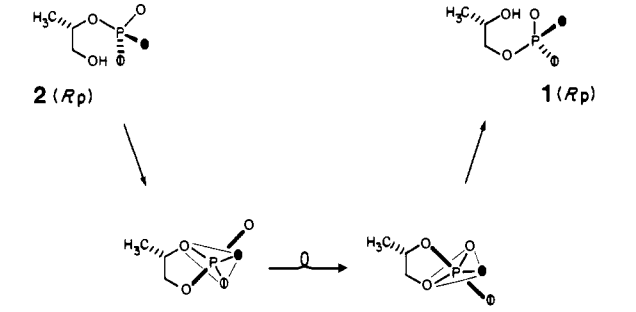


Figure 1. ^{31}P NMR spectra of the products (after purification, "in-line" ring closure, and methylation[9]) from the partial equilibrium of 2-[(R)-^{16}O,^{17}O,^{18}O]phospho-(S)-propane-1,2-diol (2). Upper spectrum, reisolated 2; lower spectrum, product 1. The NMR spectra were performed as described earlier[9] on a Bruker WM-300 WB instrument, at 121.5 MHz.

Scheme II. Predicted Configurational Inversion (of One-Third of the Molecules) and Label Loss (from Two-Thirds of the Molecules) for the Isomerization of 2 to 1 via the Cyclic Diester in $H_2{}^{16}O$



Scheme III. Predicted Retention of Configuration at Phosphorus for the Isomerization of 2 to 1 via a Pseudorotation Path



product 1 molecules formed via the upper route will have suffered inversion, and all of the product molecules deriving from the lower route will have been formed with retention.

Since we know the rate constants for Scheme I from the kinetic analysis, the stereochemistry at phosphorus in 1 and in reisolated

(6) On the basis of literature precedent for monoesters and diesters, see, e.g.: Usher, D. A.; Richardson, D. I.; Eckstein, F. *Nature (London)* **1970**, *228*, 663–665.

(7) Oxygen kinetic isotope effects will be negligible.

(8) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2558–2560. Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Bockhoff, F. M.; McLafferty, F. W.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 4323–4332.

2 can be predicted 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 some 23% to equilibrium. The product **1** and remaining **2** were isolated and purified by ion exchange chromatography followed (for **1**) by HPLC (see Table II). Stereochemical analysis by the ^{31}P NMR method we reported earlier[9] showed that the product **1** is predominantly formed with retention (see Figure 1[10]) and that the extent of racemization is, within experimental error, what is predicted from the rate constants determined independently.

The agreement between predicted and observed values (Table II) indicates that (i) the minimal kinetic scheme (Scheme I) is sufficient to describe the reaction course for the acid-catalyzed equilibration of **2** and **1** and (ii) the direct isomerization path proceeds with *quantitative retention of configuration at phosphorus, as predicted by the pseudorotation mechanism.* The possibility of a mechanism involving either free or caged metaphosphate species is unlikely. These results also provide the first evidence for the validity of the rules for pseudorotation[3] in a simple phosphoric monoester.

Acknowledgment. We are grateful to Dr. Joel Belasco and David Hansen for help with the computer program used to predict the isotopic content and stereochemical outcome. This work was supported by the National Institutes of Health. The Bruker NMR instrument used in this work was purchased with the help of a grant from the National Science Foundation.

Registry No. 1, 66943-89-3; **2**, 80262-60-8.

(9) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1980**, *102*, 6601–6602.

(10) These spectra also demonstrate the power of our analytical method[9] even for a reaction where there is considerable washout of isotopic label.

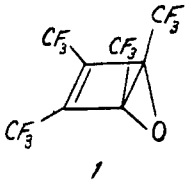
A Cyclobutadiene Oxide (Dewar Furan)

David Wirth and David M. Lemal*

Department of Chemistry, Dartmouth College
Hanover, New Hampshire 03755

Received November 3, 1981

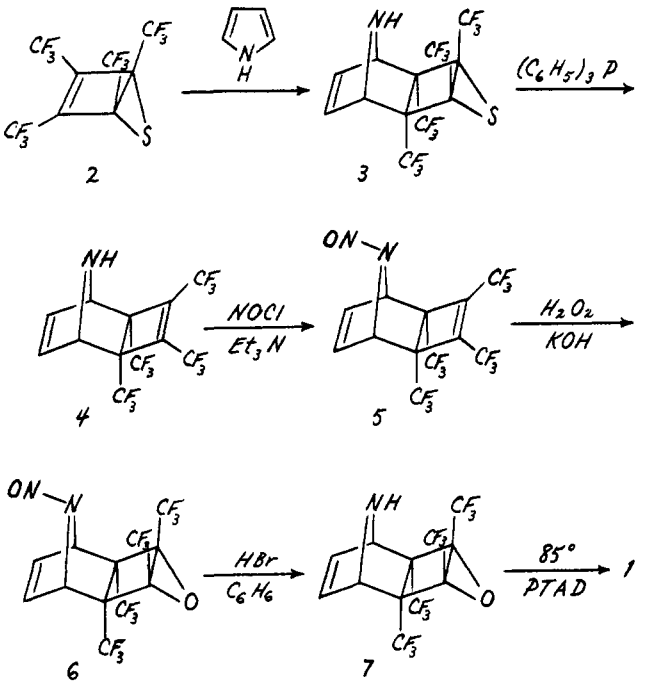
We wish to describe the synthesis and some unusual chemistry of perfluorotetramethylcyclobutadiene oxide [perfluorotetramethyl(Dewar furan), **1**], the first representative of its ring system.



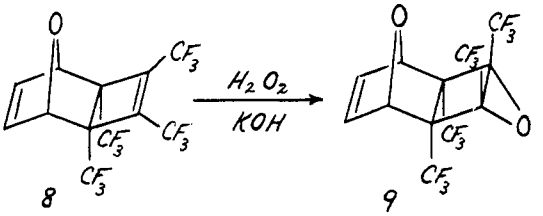
1

Although Dewar thiophene **2**, the sulfur analogue of **1**, is prepared by photocyclization of the thiophene,[1,2] this direct approach failed with the furan.[3,4] Hence **2** was chosen as the starting material from which to fashion **1**, but the disarmingly simple-looking **2** → **1** transformation required the circuitous pathway outlined in Scheme I. Since attempts to epoxidize **2** directly led to destruction of the ring system, its double bond was protected by formation of the known pyrrole adduct **3**.[5] Desulfurization

Scheme I



with triphenylphosphine yielded diene **4**,[6] mp 43–46 °C (70% from **2**). Again attempted epoxidation of the perfluoroalkyl-substituted double bond with alkaline hydrogen peroxide[7] at room temperature resulted in thoroughgoing degradation. The problem was traced to the bridging nitrogen through the observation that the oxygen counterpart **8**[2,8] was successfully oxidized to **9** under these conditions. Accordingly, **4** was protected by nitrosation, giving **5**, mp 108–109 °C (92%). Oxidation of **5** with hydrogen peroxide in methanolic potassium hydroxide yielded oxirane **6**, mp 93–94 °C (60%). Hydrogen bromide in benzene transformed **6** into aminooxirane **7**, mp 53–84 °C[9] (80%). When **7** was heated at 85 °C and 15 torr in 1,2,4-trichlorobenzene containing a fivefold molar excess of 4-phenyl-1,2,4-triazoline-3,5-dione (to trap pyrrole), cyclobutadiene oxide **1** distilled into a cold trap (~55%). This volatile, colorless liquid displayed IR λ_{max} (vapor) 1695 cm^{-1} ($\nu_{C=C}$); ^{19}F NMR[10] ($CDCl_3$) δ 64.35 and 66.80; mass spectrum, *m/e* 321 ($M^+ - F$), 290 ($M^+ - CF_2$), 271 ($M^+ - CF_3$), 243 ($M^+ - COCF_3$), 69 (base, CF_3). The instantaneous reaction of **1** with pyrrole and furan to yield **7** and **9**, respectively, confirmed its structure.



8 *9*

Cyclobutadiene sulfide **2** undergoes a degenerate rearrangement which becomes detectable on the NMR time scale above 100 °C,[11]

(1) Wiebe, H. A.; Braslavsky, S.; Heicklen, J. *Can. J. Chem.* **1972**, *50*, 2721–2724.

(2) Kobayashi, Y.; Kumadaki, I.; Ohsawa, A.; Sekine, Y.; Mochizuki, H. *Chem. Pharm. Bull. Jpn.* **1975**, *23*, 2773–2778. Kobayashi, Y.; Kumadaki, I. *Acc. Chem. Res.* **1981**, *14*, 76–82.

(3) Boriack, C. J.; Laganis, E. D.; Lemal, D. M. *Tetrahedron Lett.* **1978**, 1015–1018.

(4) Kobayashi, Y.; Hanzawa, Y.; Nakanishi, Y.; Kashiwagi, T. *Tetrahedron Lett.* **1978**, 1019–1022.

(5) Kobayashi, Y.; Kumadaki, I.; Ohsawa, A.; Sekine, Y.; Ando, A. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2355–2357.

(6) Satisfactory elemental analyses were obtained for all new compounds except **1**, whose C, H analysis fell within 0.6% of the calculated values.

(7) Tarrant, P.; Allison, C. G.; Barthold, E. C.; Stump, Jr. *Fluorine Chem. Rev.* **1971**, *5*, 102.

(8) Ross, J. A.; Seiders, R. P.; Lemal, D. M. *J. Am. Chem. Soc.* **1976**, *98*, 4325–4327.

(9) Despite the broad melting range, the compound was pure as judged by ^{19}F NMR spectroscopy and gave the following analysis. Anal. Calcd: C, 35.38; H, 1.23; N, 3.44; F, 56.02. Found: C, 35.30; H, 1.30; N, 3.68; F, 56.22.

(10) Chemical shifts are reported relative to internal CCl_3F.

(11) Bushweller, C. H.; Ross, J. A.; Lemal, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 629–631.

0002-7863/82/1504-0847$01.25/0