and to Prof. M. Hirama of Tohoku University for discussion. This work was supported by a grant-in-aid for overseas research from the Ministry of Education, Science and Culture, Japan; by the French Ministry of Research and Superior Education; by the Pasteur Institute of Paris; and by the French Polynesia Government.

Supplementary Material Available: Table of ^1H NMR chemical shifts and coupling constants of **1** and **2**, ^1H NMR spectrum and COSY map of **1**, and ^1H and ^{13}C NMR spectra, COSY and DQF-COSY maps, and NOE difference spectra of **2** (14 pages). Ordering information is given on any current masthead page.

Proton Inventory of a Bifunctional Ribonuclease Model

Eric Anslyn[1] and Ronald Breslow*

Department of Chemistry, Columbia University
New York, New York 10027

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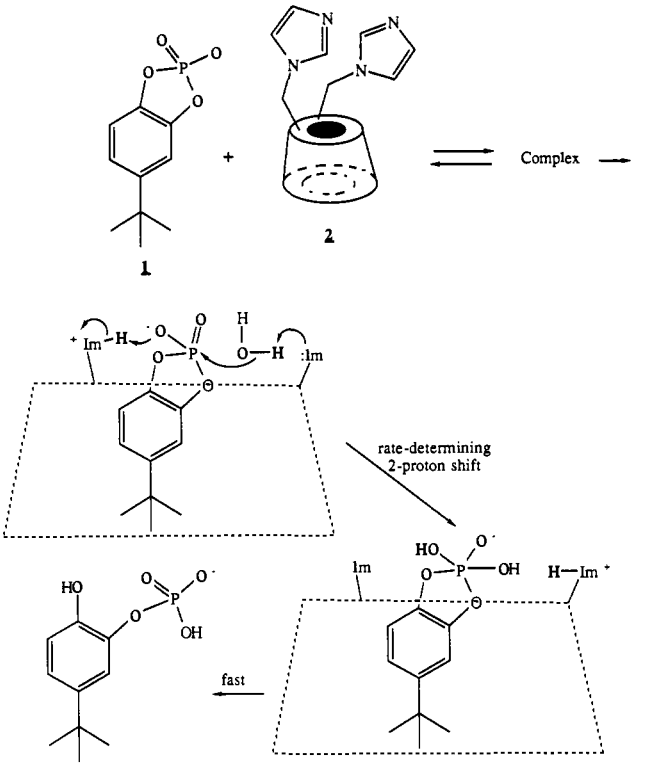
We have described the hydrolysis of 4-*tert*-butylcatechol cyclic phosphate (**1**) catalyzed by β-cyclodextrin 6,6′-bis(imidazoles).[2-4] In our earliest work[2,3] we used mixtures of the A,C, and A,D isomers,[3] but recently we have reported[4] a study of the pure isomers and also of the A,B isomer **2**. Our pH/rate studies[2-4] showed that k_{cat} reached an optimum when the catalyst was partially protonated, so that both a basic imidazole (Im) and an acidic imidazolium (ImH^+) group were present. We have also described a detailed kinetic study of the cleavage of RNA polymers and dimers by imidazole buffers.[5-7] Again a pH optimum showed that both Im and ImH^+ were involved, but in a bifunctional *sequential* mechanism in which the ImH^+ first protonated the substrate phosphate ion and the Im then promoted attack of a hydroxyl group, forming a phosphorane intermediate. This then cleaved in a subsequent fast step. However, we pointed out[6] that what is sequential with separate buffer species could well become simultaneous if the catalytic groups are attached, as in **2** or in the enzyme ribonuclease A for which **2** is a model.

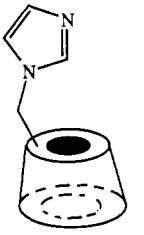
Our finding that the A,B isomer **2** is better[4,8] than the A,C or A,D analogues supports this; in a truly sequential mechanism, the intermediate could rotate in the cyclodextrin cavity and the relative placement of the two catalytic groups should be irrelevant. However, one could imagine other factors that might favor one catalyst over the other, such as simple hydrogen bonding to phosphate without an actual proton transfer by ImH^+ in the transition state. In our mechanistic proposals,[4,6] there should be a true protonation by ImH^+ as the Im delivers H_2O to the phosphate, forming a phosphorane intermediate (Scheme I).

To check this, we have used the well-known proton inventory method,[9] studying the rate in various mixtures of H_2O and D_2O.

Scheme I



This is commonly used to determine whether two protons are actually undergoing bonding changes, whereupon the isotope effect will appear to be second order. We find that indeed the catalyzed hydrolysis of **1** by **2** shows a two-proton inventory, supporting the mechanism of Scheme I. We have also confirmed the results and the method by examining the hydrolysis of **1** by β-cyclodextrin 6-imidazolide (**3**). We had seen,[2] and now confirm, that this acts



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as a simple base catalyst, Im delivering the H_2O to bound substrate. In this case the proton inventory method indicates that only one proton moves in the transition state, as expected. The proton inventory results with bifunctional **2** are similar to those observed[10] when the enzyme ribonuclease A catalyzes a similar reaction.

We have described[4] the pH/rate profile for the cleavage at 25.0 °C of **1** complexed by **2**, a bell-shaped curve with a broad maximum at pH 5.75; in D_2O,[11] a bell-shaped curve was seen with its broad maximum at a pH meter reading of 6.1 (pH 6.5 corrected for the meter isotope effect), and the rate decreased by a factor of 4.0. Thus the proton inventory study was conducted buffered[11] to a pH meter reading of 6.00 (H_2O) in 0.01 increments to 6.10 (D_2O) with nine intermediate mixtures. The substrate was at 1 mM and the catalyst at 5 mM, well in excess of the K_d of 0.18 mM[4] for the complex of **1** with **2**. The results are plotted in Figure 1. The curve shows distinct downward curvature. The points

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(8) We find $\Delta H^* = 6.9 \pm 1.2$ kcal/mol for k_{cat}, while that for cleavage of **1** by the A,C isomer of **2** is 9.2 ± 1.6, and for the A,D isomer, 9.6 ± 1.7 kcal/mol. $\Delta S^* = -49.1 \pm 6.5$ eu for **2**, and −43.9 ± 5.8 and −43.3 ± 5.7 eu for the A,C and A,D isomers, respectively. For catalyzed cleavage of **1** by *N*-methylimidazole, $\Delta H^* = 11.7 \pm 2.1$ kcal/mol and $\Delta S^* = -37.7 \pm 4.9$ eu.
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(11) For experiments in D_2O, all catalysts and buffers were lyophilized from D_2O prior to use. In mixed solvents, account was taken of the density differences of D_2O and H_2O and of the protons contributed by buffers and catalysts.

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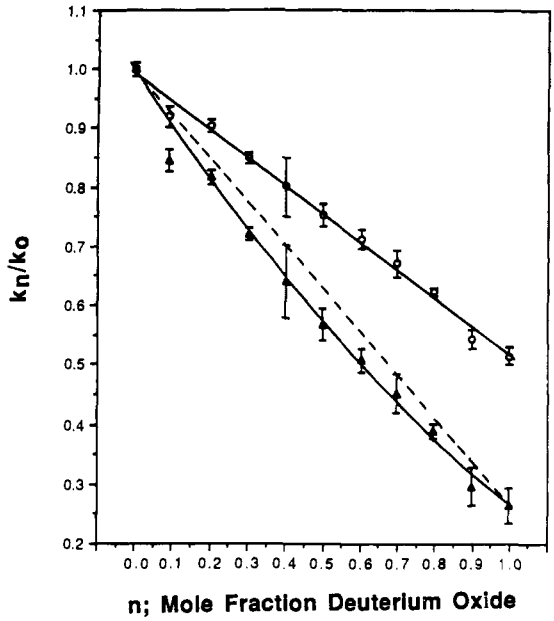

Figure 1. The pseudo-first-order rate constant for the hydrolysis of substrate **1** at kinetic saturation with bifunctional catalyst **2** in water as a function of the mole fraction of D_2O; the temperature was 25.0 °C, and the pH was adjusted to the kinetic maximum (see text). The vertical axis is the rate constant in a given medium relative to that in H_2O. The lower curve is the theoretical curve for a two-proton inventory, one with an isotope effect k_H/k_D of 2.12 and the other with k_H/k_D = 1.90, along with the average (2–3 runs) experimental points (▲), with error bars $\pm 2\sigma$. The dashed line is the theoretical curve for a one-proton inventory connecting the H_2O and D_2O points. The upper curve is a straight-line fit of the experimental points (O) in a plot of $(k_n/k_0)^{1/2}$, which should be linear for a two-proton inventory.

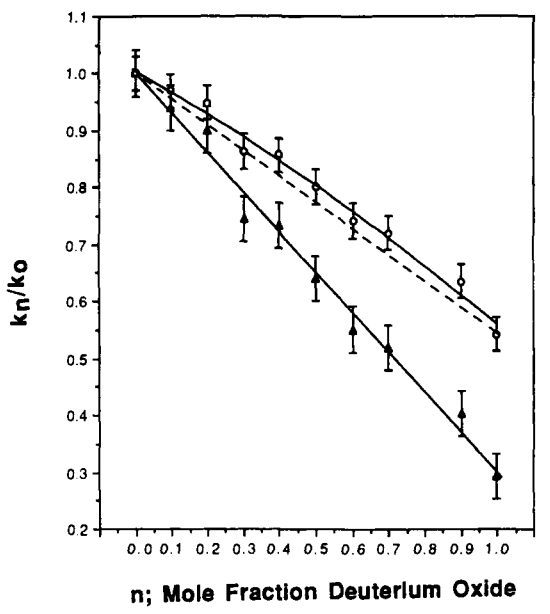

Figure 2. The pseudo-first-order rate constant for the hydrolysis of substrate **1** at kinetic saturation with monofunctional catalyst **3** in water as a function of the mole fraction of D_2O; the temperature was 25.0 °C, and the pH was adjusted to the kinetic plateau maximum (see text). The lower curve is the theoretical straight line for a one-proton inventory along with the experimental points (▲). The upper curve is a least-squares fit of the experimental points (O) in a plot of $(k_n/k_0)^{1/2}$, which should show upward curvature for a one-proton inventory. The dashed line is a straight line connecting the H_2O and D_2O points; it does not fit the points, in contrast to the $(k_n/k_0)^{1/2}$ curve of Figure 1.

can fit a theoretical curve for a two-proton isotope effect with k_H/k_D of 2.12 and 1.90, respectively; 2.12 is the measured k_{HOH}/k_{DOD} for cleavage of **1** by **2** at high pH, where the ImH^+ catalytic group is neutralized.

The curvature is as expected, but it is small, so we checked these conclusions. First of all, a square-root plot is straight (Figure 1), as it should be[9] for a two-proton effect. Secondly, we examined the proton inventory method with catalyst **3**, performed as above but with 10 points ranging between pH readings 6.65 (H_2O) and 6.80 (D_2O) on the high pH plateau for **3**. The data in Figure 2 show clearly that this reaction has a one-proton inventory, with a linear k_{cat} plot and upward curvature in the square-root plot. The results thus support our conclusion that the mechanism of hydrolysis of **1** by the enzyme model **2** is indeed that of Scheme I, with simultaneous proton transfers by both catalytic groups.[12]

The hydrolysis of cytidine 2',3'-cyclic phosphate by ribonuclease A also shows a two-proton inventory, with points that can fit a curve with a k_H/k_D of 1.75 for each proton.[10] The similarity with our findings is striking; as we have described elsewhere,[4] k_{cat}/K_m for the reaction of **1** with **2** is only 230-fold smaller than that for this enzyme reaction. Thus the enzyme may well use a mechanism[6] directly analogous to that of Scheme I.

Acknowledgment. Support of this work by the NIH and the ONR and a NSF Postdoctoral Fellowship to E.A. are gratefully acknowledged.

(12) As the scheme suggests, T. Liu in our laboratory has found that the cleavage of **1** by **2** is ca. 95% selective for the formation of the 2-phosphate, with ca. 5% of the 1-phosphate formed.

Highly Diastereoselective Coupling Reaction of Cyclopentenol Derivatives by Palladium Catalyst

Sigeru Torii,* Hiroshi Okumoto, Fumihiko Akahoshi, and Takayuki Kotani

Department of Applied Chemistry, Faculty of Engineering Okayama University, Tsushima Naka, Okayama 700, Japan

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Since the pioneering works by Mizoroki[1] and Heck,[2] the palladium-catalyzed alkenylation of olefins has been utilized in a wide range of organic syntheses.[3] Although particular attention has been paid to the reaction,[4]only a few investigations concerning the diastereo- and/or enantioselective coupling have been reported, to our knowledge.[5]

In the course of our study to develop a new methodology directed toward the synthesis of prostaglandin (PG) and its analogues, we have been interested in the use of palladium as a catalyst, because the alkenylation of allyl alcohol producing γ,δ-unsaturated carbonyl compounds is regarded as an equivalent of conjugate addition to enone.

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