this coupling on the orientation of the nonbonded electrons on phosphorus with respect to the ring planes. The insignificant changes observed for all $^{13}C-^{31}P$ couplings in going from **6** to **7** (*i.e.*, upon introduction of a para chlorine substituent) are in support of this explanation as are the results for some other non-ortho-substituted triarylphosphines. Thus, evidence has been obtained for a steric interaction caused by the ortho substituents and resulting in different conformational preferences of the aryl rings in some of these phosphines (*e.g.*, in **1**, **2**, and **3**).

The same interpretation was recently invoked to explain the observation of a marked dependence of the ring $^{1}H-^{31}P$ coupling constants on ortho substitution in trithienylphosphines[11] (and also noted for **6** and **7**). CNDO/2 calculations performed for different geometries (rotational isomers) of the primary phosphines, 3-thienylphosphine, 2-thienylphosphine, and phenylphosphine, clearly indicate a considerable angular dependence of the ring $J(^{1}H-^{31}P)$ couplings. Furthermore, taking the experimental data[11] into account these calculations seem to indicate a preferred conformation for the ortho-substituted arylphosphines in which the ring planes are twisted so as to align the substituent toward the phosphorus lone pair of electrons. In view of the recently reported stereospecificity of $^{2}J_{CCP}$ in some cyclic phosphines[8] this predicted conformation is in accordance with the changes observed in this work for $^{2}J_{CCP}$.

In conclusion, the stereospecificity observed for $^{2}J_{CCP}$ appears to be especially useful in conformational analysis of phosphines and proves to be an alternative to the use of $^{1}H-^{31}P$ couplings which are often tedious to obtain.

Acknowledgment. CNDO/2 calculations were kindly performed by Dr. P. Rahn.

(11) H. J. Jakobsen and M. Begtrup, *J. Mol. Spectrosc.*, 40, 276 (1971).

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Hydratase Activity of a Hydrolase. Adenosine Deaminase

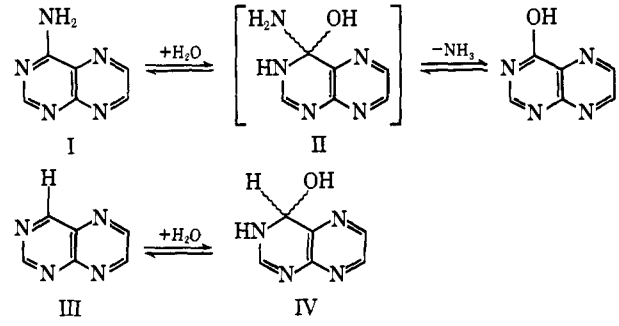
Sir:

In hydrolytic enzyme reactions, one may inquire whether the substrate water enters the reaction (in a chemical sense) before, during, or after the departure of the leaving group from the substrate. For adenosine deaminase, the possibility of direct water attack[1] gained support when it was found that 1,6-dihydro-6-hydroxymethylpurine ribonucleoside, an analog of the hypothetical intermediate formed by addition of water to the substrate adenosine, was an unusually potent inhibitor.[2] We wish to report a novel reaction of this enzyme which strengthens the likelihood that water attacks the substrate directly, resulting in an addition–elimination mechanism without the intervention of a purinyl–enzyme intermediate.

(1) R. Wolfenden, *Biochemistry*, 7, 2409 (1968).
(2) B. Evans and R. Wolfenden, *J. Amer. Chem. Soc.*, 92, 4751 (1970).

Noting that 4-aminopteridine (I) is a substrate for adenosine deaminase,[3] we chose to study the interaction of this enzyme with pteridine, a known inhibitor.[3] In neutral aqueous solution, pteridine (III) exists in stable equilibrium (in a molar ratio of 3.5:1)[4,5] with its 3,4-monohydrate IV (Scheme I). The rate of approach to

Scheme I



equilibrium has been studied in detail by Inoue and Perrin.[6]

In the present experiments, anhydrous pteridine (10^{-4} *M*), freshly dissolved in a buffer (0.1 *M* potassium phosphate, pH 7.5, at 25°), was found to be converted to the equilibrium mixture by a first-order process with a half-time of 22 min; this hydration reaction was followed spectrophotometrically at 318 nm, and gave initial and final spectra identical with those reported.[4] In the presence of calf duodenal adenosine deaminase (33 μg/ml of the enzyme supplied by Boehringer Mannheim Corp.) under conditions which were otherwise identical, conversion of anhydrous pteridine to the equilibrium mixture was found to proceed very much more rapidly with approximately 50% conversion in 30 sec. The enzymatic reaction occurred in two phases, the first considerably more rapid than the second and representing approximately half-conversion to the final equilibrium mixture. To permit study of the reverse reaction, pteridine was first converted to the 3,4-monohydrate by momentary exposure to dilute acid[4] (1–2 sec in HCl, pH 2), then quenched by dilution in buffer (0.1 *M* potassium phosphate, pH 7.5 at 25°). The hydrate reverted to the usual equilibrium mixture with a half-time of approximately 22 min. In the presence of enzyme, this reaction also was found to be markedly more rapid, again following a biphasic course. Both the enzymatic hydration and dehydration reactions were effectively inhibited by competitive inhibitors of adenosine deaminase, indicating that they proceed at the active site of this enzyme.

In the absence of spectrophotometric evidence for any reaction intermediates, it seemed possible that the biphasic nature of these enzymatic reactions might be attributed to a stereochemical preference of the enzyme for one or the other enantiomer of pteridine hydrate (IV). This was substantiated by polarimetric observation (Figure 1). The hydration reaction showed a wave of *levorotation* reaching a maximum on a time scale comparable with that of the spectrophotometric burst, decaying more slowly to zero rotation. The dehydration

(3) R. Wolfenden, J. Kaufman, and J. B. Macon, *Biochemistry*, 8, 2412 (1969).
(4) D. D. Perrin, *J. Chem. Soc.*, 645 (1962).
(5) A. Albert, T. J. Batterham, and J. J. McCormack, *J. Chem. Soc. B*, 1105 (1966).
(6) Y. Inoue and D. D. Perrin, *J. Chem. Soc.*, 2648 (1963).

reaction showed a temporary surge of *dextrorotation*, followed by a slower decay to zero rotation at final equilibrium. These results suggest that in enzymatic hydration, the (−) enantiomer of pteridine hydrate is more rapidly synthesized, giving rise to a spectrophotometric burst and a corresponding levorotation; slower synthesis of the (+) enantiomer results in decay of the *net* optical rotation until the final equilibrium mixture, equimolar in the two enantiomeric hydrates, is reached. In dehydration, the (−) enantiomer is rapidly consumed, leaving in its wake the positive optical rotation of the slower reacting (+) enantiomer; equilibration of the latter leads eventually to the optically inactive equilibrium mixture. The enzymatic hydration of pteridine, as well as the dehydration of pteridine hydrate, thus appears to be stereospecific. However, it should be noted that careful analysis shows that the relatively slow reactions of the (+) enantiomer are also subject to enzyme catalysis; these reactions appear to be approximately one order of magnitude slower than the reactions involving the (−) enantiomer.

The initial rates of enzymatic hydration and dehydration were examined spectrophotometrically as a function of substrate concentration; these rates referred mainly to the reaction of the favored enantiomer. Double reciprocal plots of the observed rates (corrected for a very minor contribution from the nonenzymatic reaction amounting to no more than 5% of the observed rates) were linear, yielding the apparent kinetic parameters listed in Table I. For comparison, the observed

Table I. Enzymatic Reactions in the Pteridine Ring, in 0.1 *M* Potassium Phosphate Buffer, pH 7.5, at 25°

Substrate	4-Amino-pteridine	Anhydrous pteridine	Pteridine hydrate (racemic)
Reaction	Deamination[a]	Hydration	Dehydration
$\Delta\epsilon$ for complete reaction	−3900	+7400	−7400
Wavelength, nm	350	318	318
V_{max}, μmol/(min mg of enzyme)	1.26	3.56	5.00
K_m, m*M*	0.105	0.163	0.071

[a] Reference 3.

kinetic parameters for enzymatic deamination of 4-aminopteridine[3] are shown. It is of interest that the limiting rate constants for hydration and dehydration are somewhat larger than those for hydrolysis of 4-aminopteridine, the analogous substrate.

Although these experiments were carried out with the enzyme from calf duodenum, we have obtained similar results with the fungal enzyme from *Aspergillus oryzae*, which differs considerably from the mammalian enzyme in molecular weight and amino acid composition.[7]

The fact that these enzymes are efficient hydratases suggests that hydration is a partial reaction in the normal *hydrolytic* sequence of reactions (Scheme I). Hydration of pteridine proceeds slightly more rapidly than the overall hydrolysis of I; however, in the case of pteridine, reaction can proceed no further than the hydrate. It is of interest that the presumed reaction intermediate II, like pteridine hydrate (IV), contains a

(7) R. Wolfenden, Y. Tomozawa, and B. Bamman, *Biochemistry*, 7, 3965 (1968).

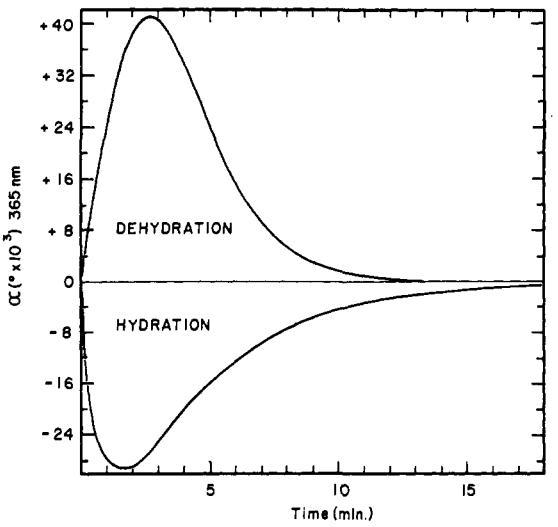


Figure 1. Polarimetric time course for hydration of pteridine (10^{-2} *M*) (lower curve), and dehydration of pteridine 3,4-monohydrate (10^{-2} *M*) (upper curve) in the presence of calf duodenal adenosine deaminase (150 μg/ml) in 0.1 *M* potassium phosphate buffer, pH 7.5 at 25°. Reactions were followed with a Cary Model 60 spectropolarimeter at 365 nm.

chiral center not present in the substrate or products of hydrolysis. The stereoselectivity of the hydration reaction suggests that water attack from one side of the heterocyclic ring is preferred, and this is also consistent with steric effects previously observed[2] for inhibitory analogs of the presumed intermediate in adenosine hydrolysis.

These findings suggest that water attack on substrates for hydrolysis by adenosine deaminase is direct, in contrast with many hydrolytic enzymes which act through double displacement mechanisms. An optically active hydrate of pteridine has been generated for the first time, in a reaction which illustrates the versatility of this enzyme and offers promise as a starting point for the further elucidation of its catalytic function.

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Temperature and Viscosity Effects on the Decay Characteristics of *s-trans*-1,3-Diene Triplets

Sir:

1,3-Diene triplets at room temperature in solution undergo facile rotation about the 1,2 and 3,4 bonds while maintaining geometric integrity with respect to rotation about the 2,3 bond.[1-3] The benzophenone-sensitized interconversion of 2,4-hexadienes can be explained by assuming that twisting at both ends of the diene system gives a common triplet having 1,4 birad-

(1) H. L, Hyndman, B. M. Monroe, and G. S. Hammond, *J. Amer. Chem. Soc.*, **91**, 2852 (1969).
(2) J. Saltiel, L. Metts, and M. Wrighton, *ibid.*, **91**, 5684 (1969).
(3) J. Saltiel, L. Metts, A. Sykes, and M. Wrighton, *ibid.*, **93**, 5302 (1971), and references cited therein.