even more favorable since LaPlaca, Hamilton, and Ibers[9] have determined all of the structural parameters except the position of the hydrogen. They have shown, in agreement with infrared[12] and Raman[13] studies, that $HMn(CO)_5$ has C_{4v} symmetry and that pairs of $HMn(CO)_5$ molecules are oriented such that their C_{4v} symmetry axes intersect with a 135° angle and that the distance between the two manganese atoms is 5.167 A. They assume that the hydrogens lie on the C_{4v} symmetry axes and on the basis of this assumption they obtained the relation

$$r_{Mn-H} = 2.804 - 0.543r_{H-H} \quad (1)$$

where the distances are expressed in angstroms, r_{Mn-H} is the intramolecular Mn–H bond distance, and r_{H-H} is the intermolecular H–H distance.

It follows from the work of Van Vleck[14] that the second moment, ΔH_2^2, of the proton nmr absorption spectrum of a polycrystalline sample of $HMn(CO)_5$ can be written as

$$\Delta H_2^2 = 358.071r^{-6}_{H-H} + 114.064[r^{-6}_{Mn-H,\,inter} + r^{-6}_{Mn-H,\,intra}] \quad (2)$$

The r_{H-H} term of eq 2 arises from the intermolecular H–H interaction. The second and third terms arise from the inter- and intramolecular Mn–H interactions, respectively. The intermolecular Mn–H interaction accounts for less than 0.5% of the contribution to ΔH_2^2 and can be neglected. From eq 1 and 2 one can obtain an equation expressing r_{Mn-H} as a function of ΔH_2^2; the equation has two real roots.

A value of 26.6 ± 0.6 G^2 {10^4 gauss (G) = 1 tesla (T)}[15] was determined for the second moment (this value was corrected for modulation broadening[16]) of a carefully purified[17] polycrystalline sample at −165°. This value for ΔH_2^2 corresponds to Mn–H distances of 1.281 or 1.950 ± 0.02 A. By eq 1, the longer distance gives an intermolecular H–H distance of 1.57 A, which is much less than the normal van der Waals contact of 2.2 A[10] and is therefore extremely unlikely. Furthermore, since (see eq 2) the intramolecular Mn–H interaction accounts for more than 97% of the observed value of ΔH_2^2, our result does not depend upon the validity of the assumption that the hydrogens lie along the C_{4v} symmetry axes.[18]

Further studies of other transition metal carbonyl hydrides are in progress.

(12) H. D. Kaesz and D. K. Huggins, *J. Am. Chem. Soc.*, 86, 2734 (1964).
(13) A. Davison and J. W. Faller, to be published.
(14) J. H. Van Vleck, *Phys. Rev.*, 74, 1168 (1948).
(15) All of the errors reported here are average errors; see, for example, H. Margenau and G. M. Murphy, "The Mathematics of Physics and Chemistry," 1st ed, D. Van Nostrand Co., New York, N. Y., 1943, p 493.
(16) This value has not been corrected for zero point vibration. This correction, however, will not change the value of ΔH_2^2 by more than 2 or 3% and would change the value of the Mn–H bond distance by less that 1%. ΔH_2^2 was obtained by integrating the proton nmr absorption spectrum over a 40-gauss interval centered about the absorption maximum.
(17) No impurities were observed in microwave, infrared, or high-resolution proton nmr spectra. The value for ΔH_2^2 is virtually constant from about −40 to −165°.
(18) Even in the Mn–H bond distance range 1.5–1.6 A, where ΔH_2^2 = 11.95 to 9.91 gauss^2, the Mn–H dipole–dipole contribution to ΔH_2^2 is very large (84 to 69%).

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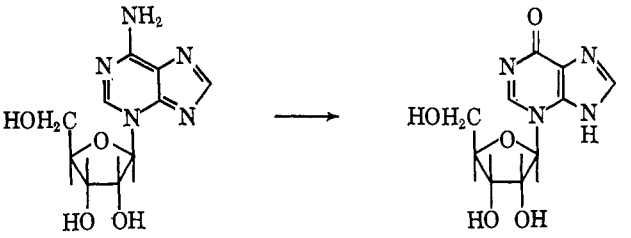
Enzymatic and Chemical Deamination of 3-(β-D-Ribofuranosyl)adenine[1]

Sir:

3-Isoinosine (3-(β-D-ribofuranosyl)hypoxanthine) (II) has been prepared from 3-isoadenosine (3-(β-D-ribofuranosyl)adenine) (I)[2] in our two laboratories by enzymatic and chemical procedures, respectively. The deamination of isoadenosine by an adenosine deaminase from *Aspergillus oryzae*, unexpected in view of the somewhat altered electronic configuration of the purine ring, renders less likely one mechanism for the action of the enzyme on adenosine. The independent chemical synthesis of isoinosine, which presents unusual requirements because of the lability of the N_3–C_1' bond, made possible direct identification of the enzymatic product.



I II

The adenosine deaminase of *Aspergillus oryzae*[3] has been purified approximately 5000-fold by methods which will be described in a subsequent communication.[4] The purified enzyme shows no ultraviolet absorption maxima above 300 mμ. Its activity is unaffected by the presence of 0.001 *M* ethylenediaminetetraacetic acid or by dialysis against neutral phosphate buffer, and it is not detectably inhibited by 0.001 *M* hydrazine or 0.05 *M* cysteine, suggesting that pyridoxal is not a cofactor. A mechanism for deamination of adenosine, not requiring Schiff base formation with the enzyme or cofactors, would involve protonation by the enzyme of the purine ring at N_1 or N_3, shifting adenosine to its 6-imino tautomer and potentially facilitating hydrolysis. It was of particular interest to test the possible activity of the deaminase on 3-isoaden-

(1) The work at Princeton University was supported by Research Grant USPHS-GM-12725 from the National Institute of Health, U. S. Public Health Service. The work at the University of Illinois was supported by Research Grant USPHS-GM-05829-06/07) from the National Institutes of Health, U. S. Public Health Service.
(2) N. J. Leonard and R. A. Laursen, *Biochemistry*, 4, 354 (1965).
(3) H. K. Mitchell and W. D. McElroy, *Arch. Biochem.*, 10, 351 (1946); T. P. Wang and N. O. Kaplan, *J. Biol. Chem.*, 206, 311 (1954).
(4) T. K. Sharpless and R. Wolfenden, in preparation.

osine, in which protonation of N_1 or N_3 would not assist formation of the imino tautomer.[5]

Incubation of the deaminase with 10^{-4} *M* isoadenosine (λ_{max} 277 mμ) in 0.05 *M* Tris–HCl buffer, pH 7.0, resulted in a shift to a final absorption maximum at 265 mμ. When an aliquot was subjected to paper chromatography (isobutyric acid–concentrated ammonia–water, 66:10:33) after approximately 50% reaction, two ultraviolet-absorbing products were found, one identical with isoadenosine (R_f 0.78). The other (R_f 0.57), which reacted with periodic acid and yielded hypoxanthine and ribose after acid hydrolysis, was directly identified with chemically synthesized 3-isoinosine (see below) by ultraviolet absorption spectra in acid, neutral, and alkaline solutions, by paper chromatography in two systems, and by paper electrophoresis at pH 8.5.

The kinetics of deamination of isoadenosine could be conveniently followed at 285 mμ and consistently showed good pseudo-first-order kinetics at substrate concentrations well below saturation. Whereas the rate of deamination of adenosine is virtually constant through the pH range from 5 to 9 in 0.05 *M* potassium acetate and Tris maleate buffers, the rate of deamination of isoadenosine increases with pH, reaching a plateau near pH 7, above which the rate is constant. The pH–rate profile is consistent with the titration of a nonreactive acid with $pK_a' = 5.4$, in close agreement with the measured pK_a' (5.5)[2] of isoadenosine. These results, which indicate that the free base of isoadenosine is the form which reacts with the enzyme, are of special interest since the pK_a of the protonated form of adenosine itself lies well below the range of stability of the enzyme.

The deaminations of both adenosine and isoadenosine appear to be catalyzed by the same enzyme; thus, the relative rates of deamination of the two substrates by the enzyme did not change during enzyme purification or during heat inactivation of the purified enzyme. Isoadenosine weakly inhibited the deamination of adenosine when the two substrates were present in equimolar concentration. At pH 7.1 (Tris–HCl buffer, 0.05 *M*) the Michaelis constant for isoadenosine was found to be approximately 5×10^{-3} *M*, as compared with approximately 2×10^{-4} *M* for adenosine, whereas V_{max} for isoadenosine was approximately 25 times lower than that for adenosine. No reversal of the deamination of adenosine or isoadenosine was detected; thus, no change in spectrum occurred when either inosine or isoinosine was incubated with the enzyme in 1 *M* ammonium acetate buffer, pH 8.1.

The unusual biological activity observed for 3-isoadenosine (I)[2] and the ability of coenzyme analogs derived from 3-isoadenosine to replace the corresponding natural coenzymes in certain enzymatic reactions[6] suggested that chemical deamination of I would be useful in providing 3-isoinosine (3-β-D-ribofuranosylhypoxanthine) (II) in quantity sufficient for biochemical study. The first application realized for synthetic 3-isoinosine was the direct identification of the product of enzymatic deamination of 3-isoadenosine, as described above. For the synthesis, 3-β-(2′,3′,5′-tribenzoyl-D-ribofuranosyl)adenine[2,7] in pyridine–chloroform at 55–60° was treated with 5 molar equiv of nitrosyl chloride in chloroform during 2 hr.[8] The 3-β-(2′,3′,5′-tribenzoyl-D-ribofuranosyl)hypoxanthine obtained after extraction with ethyl acetate, washing the extract with water, and evaporation was debenzoylated with ammonia in dimethylformamide–methanol. The crude 3-isoinosine isolated following concentration *in vacuo* was dissolved in 50% aqueous ethanol brought to pH 10 with ammonia. The solution was absorbed on a Dowex 1-X8 formate column, which was eluted with water (discarded), then with 0.01 *N* formic acid. Ultraviolet monitoring indicated the fractions which were to be combined. Evaporation of these, followed by recrystallization of the residue from 50% aqueous ethanol, yielded II in 43% over-all yield, mp 178° (dec at 218°) with ultraviolet spectra characteristic of a 3-substituted hypoxanthine[9] (in mμ (ε)): $\lambda_{max}^{0.1N HCl}$ 254 (ε 10,950), λ_{min} 231 (5000); $\lambda_{max}^{pH7 (H_2O)}$ 265 (13,200), λ_{min} 232 (4100); $\lambda_{max}^{0.1N NaOH}$ 270 (10,950), λ_{min} 241 (4750). *Anal.* Calcd for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.55; H, 4.74; N, 21.10.[10]

(5) It should be recognized that formation of the 6-amino tautomer could be assisted, in the case of isoadenosine, by enzymatic protonation at N_7 or N_9. This alternative, which cannot be excluded, would require remarkable flexibility of action of the binding and catalytic sites of the deaminase.

(6) N. J. Leonard and R. A. Laursen, *Biochemistry*, 4, 365 (1965).

(7) N. J. Leonard and R. A. Laursen, *J. Am. Chem. Soc.*, **85**, 2026 (1963).

(8) H. J. Thomas and J. A. Montgomery, Abstracts of Papers, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964, p 24M, have reported similar reaction conditions for the conversion of 3-benzyladenine to 3-benzylhypoxanthine.

(9) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 28, 2304 (1963).

(10) It is of interest that 3-isoinosine could not be prepared by a method of deamination found applicable to the synthesis of 3-benzylhypoxanthine, mp 278–281° dec (reported 250°),[9] from 3-benzyladenine, and of 3-(γ,γ-dimethylallyl)hypoxanthine, mp 233–237° dec (ultraviolet spectra, nmr spectra, and analysis satisfactory), from triacanthine,[11] namely, treatment with sodium nitrite in buffered acetate–acetic acid at 80–85°, due to the ease of hydrolysis of the N_3–C_1' bond in I and II.

(11) N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, 84, 2148 (1962).

(12) U. S. Public Health Service Predoctoral Trainee on Grant No. 5T-15H-962.

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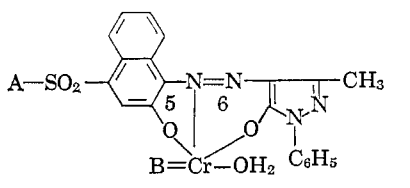
The Stereochemistry of Dye–Metal Complexes

Sir:

We wish to report the preparation of the metal complex dye I which was separated by alumina chromatog-



I, A = N⟨ ⟩; B = $CH_3C(O^-)$=CH–C(=O)–CH_3

II, A = O^-; B = $2H_2O$