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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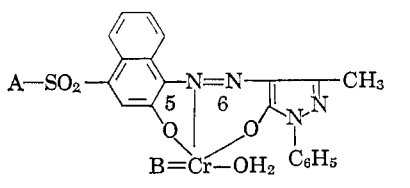
The Stereochemistry of Dye–Metal Complexes

Sir:

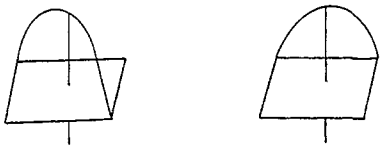
We wish to report the preparation of the metal complex dye I which was separated by alumina chromatog-



I, A =  ; B = $CH_3C{=}CH{-}C{-}CH_3$ (O⁻ and O)

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

raphy into three stereoisomers. A good deal of discussion in the literature, summarized by Schetty[1] and Pfitzner,[2] is concerned with the stereochemistry of metal complex dyes. Schetty's[3] work indicates that *o,o'*-dihydroxyazo dyes with chromium and cobalt form 2:1 octahedral complexes in which the dyes occupy three *mer* positions on the metal (III) rather than the *fac* positions (IV).



III IV

The 1:1 chrome dye II, Inochrome Pink N (Francolor), CI 1876, was converted to the sulfonyl chloride with phosgene in methylene chloride and dimethylformamide. The crude sulfonyl chloride was allowed to react with a large excess of piperidine and drowned in water. The product, a mixture of the piperidide and the starting dye, was dissolved in hot dimethylformamide and 2,5-pentanedione. The products of this reaction were obtained by precipitating in water, filtering, and drying. Chromatography was on Woelm alumina, activity grade I, packed in acetone and washed with benzene. The crude dye was applied and eluted with methylene chloride, giving one fraction; elution with acetone gave a second fraction; and finally elution with 10% methanol in acetone gave a third fraction. No other material could be eluted with pure methanol. The three fractions had identical visible, ultraviolet, and infrared spectra; their X-ray powder diagrams, however, were significantly different from each other. *Anal.* (of one of the chromatographic fractions) for $C_{30}H_{32}CrN_5O_7S$: Calcd: C, 54.7; H, 4.9; Cr, 7.9; N, 10.6; S, 4.9. Found: C, 53.7; H, 5.1; Cr, 8.0; N, 10.9; S, 4.8. The electronic spectrum showed λ_{max} (log ϵ) 256 (4.50), 348 (4.11), 524 (4.33), and 556 mμ (4.34). Dye I is a crystalline substance stable to alkali but less stable to acid.

If the dye formed a complex of structure III, the introduction of the bidentate ligand 2,5-pentanedione could lead to only one isomer (*dl* pair). On the other hand, structure IV leads to three isomers (*dl* pairs). The isolation of three isomers forces us to conclude that the dye I must exist in the *fac* structure IV. It is likely that Inochrome Pink N also exists in the *fac* structure because its visible spectrum is very similar to that of I.[4]

Schetty has shown that 2:1 complexes of *o*-hydroxy-*o'*-carboxyazo dyes may exist in either the *fac* ("sandwich") or *mer* ("Drew–Pfitzner") form depending on the structures of the dyes or the manner in which they were prepared.[4,5] Bearing this in mind, and noting that our 1:1 complex of an *o,o'*-dihydroxyazo dye exists in the *fac* form rather than the *mer* form predicted by Schetty's generalization, we believe that complexes of *o,o'*-dihydroxyazo dyes are capable of existence in either the *fac* or *mer* form.

(1) G. Schetty, *J. Soc. Dyers Colourists*, **71**, 705 (1955).
(2) H. Pfitzner, *Angew. Chem.*, **62**, 242 (1950).
(3) G. Schetty, *Helv. Chim. Acta*, **45**, 1095 (1962).
(4) G. Schetty, *ibid.*, **47**, 921 (1964).
(5) G. Schetty, *ibid.*, **46**, 1132 (1963).

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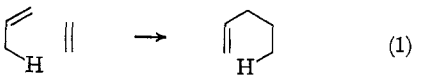
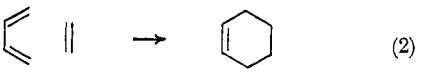
A Preference for Endoid Addition in the Ene Synthesis[1]

Sir:

The ene synthesis or "indirect substitutive addition" [2] (eq 1) is structurally related to the Diels–Alder reaction (eq 2) in that formation of the adduct is accompanied by obligatory movement of a double bond in the ene or diene.[3] Although the question of *endo vs. exo* stereo-



(1)



(2)

chemistry in the Diels–Alder reaction has been studied extensively,[5] the present paper reports the first solutions of the corresponding problem for the ene synthesis.

A benzene solution of *cis*-2-butene and maleic anhydride heated at 225° for 8 hr gives a 20–30% yield of the diastereomeric 3-(1-butenyl)succinic anhydrides in the ratio 15–20% Ia and 80–85% Ib. The observed ratio is essentially the kinetically controlled one, since the product mixture at lower conversion (1 hr at 225°, 6% total yield) also has the composition 15% Ia:85% Ib. With *trans*-2-butene, although the selectivity is lower (57% Ia:43% Ib) the stereochemical direction of the reaction is qualitatively preserved, since the major diastereomer (Ia) in the *trans* series is the minor one in the *cis*. The two 2-butenes do not interconvert significantly under the reaction conditions.

The 1-butene–maleic anhydride adduct 4-(2-butenyl)-succinic anhydride (III)) is present in no more than trace quantities in the 2-butene–maleic anhydride product mixture. Similarly, the 3-butenylsuccinic anhydrides (Ia and Ib) are at most minor components of the 1-butene–maleic anhydride adduct. The virtual absence of cross-products excludes a radical-chain mechanism with initiation by allylic hydrogen abstraction for ene syntheses with 1- or 2-butene.

Fractional crystallization from acetonitrile of a mixture of diastereomeric 2-butylsuccinic acids, obtained by hydrolysis and hydrogenation of the Stobbe condensation product from 2-butanone and succinic ester

(1) This work was supported in part by grants from the Petroleum Research Fund. Grateful acknowledgment is made to the donors of this fund and also to the National Science Foundation for additional support.
(2) K. Alder and H. von Brachel, *Ann.*, **651**, 141 (1962), and references therein cited.
(3) Olefins sometimes react with azodicarboxylic ester to give direct rather than indirect allylic substitution, a result that is consistent with a free-radical chain mechanism. It now seems likely[4] that these reactions should be considered in a separate category from true ene syntheses.
(4) W. A. Thaler and B. Franzus, *J. Org. Chem.*, **29**, 2226 (1964).
(5) For references and discussion, see J. C. Martin and R. K. Hill, *Chem. Rev.*, **61**, 537 (1961); J. A. Berson, Z. Hamlet, and W. A. Mueller, *J. Am. Chem. Soc.*, **84**, 297 (1962).