# High- and Low-Potential Flavin Mimics (Based on the Pyrimidino[5,4-g]pteridine and Imidazo[4,5-g]pteridine System). 1. General Chemistry

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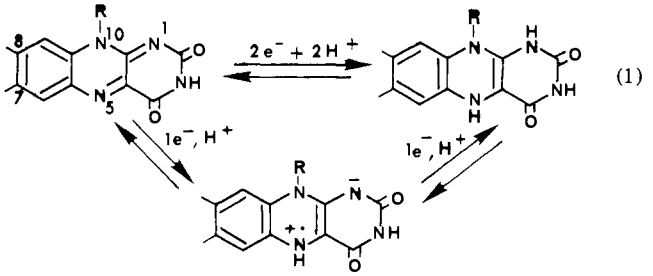
**Abstract:** 3,7,10-Trimethyl-(3*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone ($PPT_{ox}$) dissociates to its anion ($PPT_{ox}^-$) with a p$K_a$ of 1.18. This very low p$K_a$ for the uracil moiety of $PPT_{ox}$ is due to the large charge delocalization in the base $PPT_{ox}^-$. Reduction of $PPT_{ox}$ (2 $e^-$, 2 $H^+$) provides 3,7,10-trimethyl-(1*H*,3*H*,5*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone ($PPTH_2$). Acid dissociation of the two pyrimido rings of $PPTH_2$ occurs simultaneously (p$K_a$s 5.51 and 5.56) to provide the dianion $PPT^{2-}$. At pH 7.0, the two-electron reduction of $PPT_{ox}^- \rightarrow PPT^{2-}$ is associated with an $E^{\circ\prime}$ of −0.346 V (NHE). This reduction potential is 148 mV more negative than the corresponding reduction potential for a flavin. The $PPT_{ox}^-/PPT^{2-}$ couple is offered as a low-potential flavin mimic. Removal of the negative charge of $PPT_{ox}^-$ by introduction of a methyl group at $N^1$ provides $PPTMe_{ox}$. The $E^{\circ\prime}$ for two-electron reduction of $PPTMe_{ox}$ is −0.127 V. The change in potential on comparing $PPT_{ox}^-$ and $PPTMe_{ox}$ is discussed. The kinetics and products formed in the hydrolysis of $PPT_{ox}^-$ and $PPTMe_{ox}$ are described. $PPT_{ox}^-$ is rather stable, hydrolyzing via $HO^-$ attack at the 10a-position to provide 3,8-dimethyl-6-(*N*-methylcarboxamido)-(1*H*,3*H*,8*H*)-pteridine-2,4,7-trione anion ($III^-$). Protonation of $III^- \rightarrow IIIH$ is associated with a p$K_a$ of 2.96. The solvolysis of $PPTMe_{ox}$ under anaerobic conditions also occurs by formation of a 10-hydroxyl adduct ($IMe^-$) which undergoes 10a−1 ring opening to yield $IIMe^-$. The intermediate $IMe^-$ [formed by $H_3O^+$, $H_2O$, and $HO^-$ reactions with $PPTMe_{ox}$, as well as general base and general acid catalyzed addition of $HO^-$ (Scheme II)] has been characterized spectrally and $IIMe^-K^+$ has been isolated. The p$K_a$ for dissociation of $IIMeH \rightarrow IIMe^-$ is 2.79. Under aerobic conditions $IIMe^-$ undergoes oxidative ring contraction and decarboxylation (Scheme IV) to provide 3,6,8,9-tetramethyl-(3*H*,6*H*,8*H*,9*H*)-imidazo[4,5-*g*]pteridine-2,4,7-trione (IVMe). This intermediate undergoes an intramolecular rearrangement first order in IVMe (apparent suprafacial [1,3] sigmatropic N → O methyl migration) to yield VII (Scheme VI, path B). The sequence of events leading from $IIMe^-$ to IVMe is described in relation to Scheme V and eq 21. The anion $IIMe^-$ (when treated with strong base and then acidified) also undergoes a ring contraction in the absence of $O_2$ to yield $IVMeH_2$ which can be oxidized ($O_2$/Pt) to IVMe (eq 19). The p$K_a$ for dissociation of $IVMeH_2 \rightarrow IVMeH^-$ has been determined to be 8.5. Acid-catalyzed hydrolysis of $PPTMe_{ox}$ also yields $IVMeH_2$, presumably via the sequence of reactions shown in Scheme III. The $E^{\circ\prime}$ potential for two-electron reduction of IVMe to $IVMeH_2$ is +0.400 V vs. NHE. The compound IVMe is suggested as a possible high-potential flavin mimic.

Insights into the mechanisms of cofactor requiring enzymes have been obtained by so called "model studies", wherein the details of reactions of cofactor and cofactor analogues with substrates are determined. Of current interest is the "model approach" to the mechanisms of the diverse reactions mediated by flavoenzymes in their oxidized and reduced state.[1] As in the instance of any physical organic approach to mechanisms, the queries to be posed deal with the importance and stability of intermediates and the identity of the rate determining step(s). Because the principal reactions of flavoenzymes are redox in nature, it is of particular importance to determine the mode of transfer of electrons between flavin moiety and substrate species (i.e., one-electron transfer, hydride transfer, or two-electron transfer by way of a covalent intermediate, eq 1). To assist in the pursuit of these investigations,




(1)

it would be most useful to possess flavins (or isoalloxazines in general) or flavin mimic molecules which possess either unusually high or unusually low redox potentials. The reasons for this assessment are 2-fold: (i) the potentials of enzyme-bound flavin cofactor are matched to their catalytic role (presumably by preferential binding of oxidized or reduced forms)[2] so that the potential of the flavoenzyme may be far removed from that of the flavin cofactor in aqueous solution (pH 7.0); (ii) the possession of high- and low-potential flavins, or close mimics, would allow investigation of model (and possible enzymatic) reactions in both the forward and reverse directions. Representative examples of the control of the flavin potential by apoenzyme are found in glucose oxidase,[3] which changes the $E^{\circ\prime}$ (two-electron reduction potential at pH 7) for flavin adenine dinucleotide reduction from −0.219[4] to 0.0 V, and thiamine dehydrogenase, in which case the one-electron transfer potentials ($E^{\circ}_1{}'$ and $E^{\circ}_2{}'$) are +0.08 and +0.03 V, respectively.[5] Useful changes in the potentials of isoalloxazines have been obtained by the introduction of substituents to the fused benzo ring. Notably useful have been the 7- and 8-cyano analogues,[6] which display the ability to oxidize nitroalkane anions.[7] Changes in the fused pteridine ring of the isoalloxazine structure are also accompanied by marked changes in potential and, in some cases, by profound changes in chemistry. Thus, the two electron transfer potentials ($E^{\circ\prime}$) of 1-carba-1-deazariboflavin, 5-carba-5-deazariboflavin, and 1,5-dicarba-1,5-dideazariboflavin analogues are −0.28, −0.31, and −0.37 V, respectively.[8,9] The 1-carba-1-deazaflavin analogues resemble the flavins as cofactors in that the flavin-like qualities of hydrogenation–dehydrogenation, electron transfer, and oxygen activation are exhibited.[8] In model studies, the 1-carba-1-deaza-1,5-dihydro-$N^5$-ethyllumiflavin has been shown to provide a stable

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(2) For a recent and informative review of flavoenzyme reactions, see: Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148.
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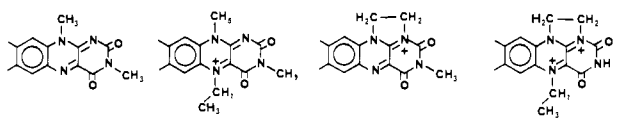
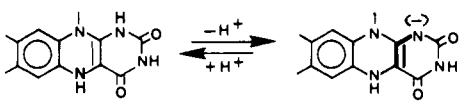
4a-hydroperoxide on reaction with oxygen as does 1,5-dihydro-$N^5$-ethyllumiflavin.[10] The potentials of the biologically relevant 1-carba-1-deazaflavin are not far removed from the potentials of flavins. The 5-carba-5-deazaflavins undergo oxidation and reduction by "hydride transfer" to and from the 5-position.[11-15] Though of considerable biochemical interest,[14-16] the 5-carba-5-deazaflavins are best considered analogues of nicotinamide, as is evident from their structure. The 1,5-dicarba-1,5-dideazaflavins[9] do not possess the reactivity patterns of flavins and are, therefore, not considered flavin analogues.[17] The most pronounced effect on the redox potentials of isoalloxazine is obtained upon $N^1$- and $N^5$-alkylation. The standard potentials for transfer of the first ($E^\circ_1{}'$) and second ($E^\circ_2{}'$) electron to the oxidized flavin analogues are for the series of structures A:[18]



| | | | | |
|---|---|---|---|---|
| $E_{o1}'$ | −0.265 V | +0.335 V | −+0.070 V | +0.545 V |
| $E_{o2}'$ | −0.130 V | +0.065 V | −+0.105 V | +0.280 V |

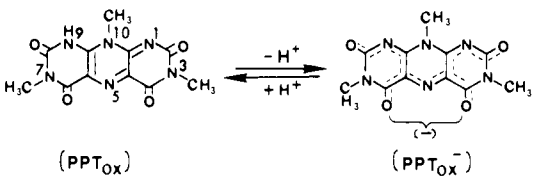
A

Notably absent among the various known flavin analogues are compounds which possess the chemistry of flavins and exhibit reduction potentials much lower than flavins. To preserve flavin chemistry an analogue must contain, in its reduced state, the central 8$\pi$ electron 1,4-dihydropyrazine ring. This is most clearly established through studies of the carbadeaza analogues (loc. cit.). The importance of the pyrazine structure to the chemistry of reduced, radical, and oxidized states of the flavins (i.e., Hoffman orbital splitting, antiaromaticity and homoaromaticity) has been discussed by Bruice and Yano.[19] A feature which contributes to the negative potential for one- and two-electron reduction of flavin at neutrality and at high pH is the enamine anion[20] nature of the two electron reduced isoalloxazine ring. This feature destabilizes the 8$\pi$-dihydropyrazine moiety and thereby increases the free energy of the reduced form.



(2)

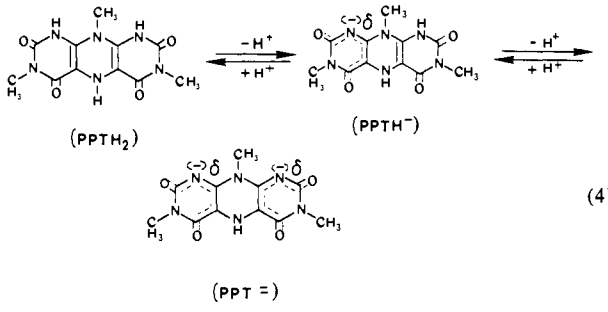
Our approach to the preparation and study of a low-potential flavin analogue is based simply on the idea that if one ionized pyrimidine ring is good (eq 2), two might be better. The chemistry of a pyrimido[5,4-*g*]pteridine system is described herein. In the anion of 3,7,10-trimethyl-(3*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]-pteridine-2,4,6,8-tetrone ($PPT_{ox}^-$) the negative charge may be distributed over 15 atoms (eq 3). In the two-electron reduced



(3)

($PPT_{ox}$) ($PPT_{ox}^-$)

state ($PPTH_2$), the dianion ($PPT^{2-}$) should, a priori, be reached at moderate pH. In the dianion, the two negative charges are localized to their respective pyrimidine rings (eq 4). The reso-

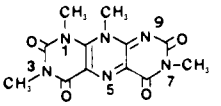


(4)

($PPTH_2$) ($PPTH^-$) ($PPT^=$)

nance stabilization of $PPT_{ox}^-$ and its greater electron density, as compared to those of oxidized flavin ($Fl_{ox}$), would be anticipated to decrease the free energy content of $PPT_{ox}$ relative to that of oxidized flavin ($Fl_{ox}$). On the other hand, the increased electron density of $PPT^{2-}$ relative to that of the anion of reduced flavin ($FlH^-$) should increase the free energy content of $PPT^{2-}$ relative to that of $FlH^-$. These various considerations lead to the conclusion that $PPT^{2-}$ should be a much better reducing agent than is dihydroflavin. Our anticipation that in $PPT^{2-}$ we would realize a flavin-like analogue of low redox potential is borne out by the present and following investigations.

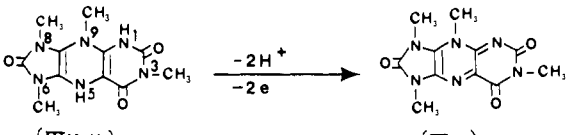
Replacement of the ionizable proton of $PPT_{ox}$ with a methyl group provides $PPTMe_{ox}$ (structure B). An entrance to the



$PPTMe_{ox}$

B

synthesis of a high-potential flavin analogue might be based on ring contraction of $PPTMe_{ox}$ to provide 3,6,8,9-tetramethyl-(1*H*,3*H*,5*H*,6*H*,8*H*,9*H*)-imidazo[4,5-*g*]pteridine-2,4,7-trione ($IVMeH_2$) which could then be oxidized to IVMe. The conceptual basis for the ring contraction stems from the proposal by Taylor, Maki, and McKillop[23] that imidazo[4,5-*g*]pteridines are intermediates in the base-catalyzed hydrolysis of pyrimido[5,4-*g*]-pteridine $N^5$-oxides (vide infra, eq 18). The expectation of a high-potential for two-electron transfer to IVMe to yield $IVMeH_2$ (eq 5) is based on considerations of strain and electronic effects



(5)

($IVMeH_2$) (IVMe)

in IVMe when compared to flavins. Peri strain between $N^8$- and $N^9$-methyls of $IVMeH_2$ is expected to distort the antiaromatic 8$\pi$ pyrazine ring, perhaps resulting in increased homoaromaticity.[19] The oxidized form IVMe, on the other hand, cannot distort to relieve this strain. Thus, stabilization of the reduced form and destabilization of the oxidized form in this manner should increase $E^{o\prime}$ over that of flavins. Furthermore, the electron-withdrawing fused imidazolone ring will serve to stabilize the overly electron-rich 8$\pi$ pyrazine ring of $IVMeH_2$.

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(18) (a) Eberlein, G.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 1449. (b) Hemmerich, P.; Massey, V.; Michel, H.; Schug, C. *Struct. Bonding (Berlin)* **1981**, *48*, 93. (c) See ref 44.
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In this paper, we describe the synthesis, solution chemistry, and electrochemistry of $PPT_{ox}$ + $PPTH_2$, $PPTMe_{ox}$ + $PPTMeH_2$, and IVMe + $IVMeH_2$. In the course of these investigations an apparent N → O suprafacial 1,3-sigmatropic shift for methyl group transfer was discovered. Some details of this reaction are provided. In part 2 (following paper in this issue),[24] there are described studies of the reduction of carbonyl functions, *N*-alkylpyridine cations ($NAD^+$ models), and alkyl disulfide by $PPT^{2-}$ and in part 3[25] there will be described studies of carbon–carbon double bond reductions by $PPT^{2-}$.[26]

## Experimental Section

Elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, TN. IR spectra were taken with KBr pellets or Nujol mull on a Perkin-Elmer 132 spectrophotometer. NMR spectra were taken on a Varian FT20 or a Varian T60 spectrometer. UV and visible spectra were obtained with a Cary 118 spectrometer or a Perkin-Elmer λ 3 spectrophotometer. Measurements of pH were made with a Radiometer Model M26 pH meter equipped with a Radiometer GK 2402C glass–calomel combination electrode. All kinetics were followed at 30 ± 0.2 °C with a Cary 118 or a Perkin-Elmer λ 3 spectrophotometer. Buffers and stock solutions were prepared with doubly distilled water.

**3,7,10-Trimethyl-(1*H*,3*H*,7*H*,9*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetraone ($PPT_{ox}$).** To a Parr bottle 10.0 g (0.049 mol) of 3-methyl-5-nitro-6-(methylamino)uracil[27] 0.5 g of 10% palladium on charcoal and 300 mL of water were added. Reduction to 3-methyl-5-amino-6-(methylamino)uracil under 90 psi $H_2$ was carried out for 1 day. The catalyst was filtered off under $N_2$ and the filtrate evaporated under high vacuum at low temperature. Crystallized product was washed from the catalyst with glacial acetic acid and combined with the residue from the evaporation above. Care was taken to minimize air contact. The total volume of the amine–acetic acid solution was 350 mL.

To this solution 15.0 g (0.096 mol) of *N*-methylalloxan was added, and heating at 140 °C was carried out for 2 h after which time a yellow precipitate formed. The reaction was cooled to room temperature and filtered. The yield after drying was 13.9 g (97%). a 1.0-g sample was recrystallized from 300 mL of water for analysis and kinetic studies to yield 0.74 g of yellow needles: NMR (TFA) δ 3.61 [s, 3 H, N(10) methyl], 3.20 [s, 6 H, N(3) and N(7) methyl]; IR (KBr) 3000, 1700, 1640, 1290, and 1175 $cm^{-1}$; TLC [$CCl_3H$–MeOH (7:3)] on silica gel showed a single fluorescent blue spot with $R_f$ 0.91. Anal. Calcd for $C_{11}H_{10}N_6O_4 \cdot 1.5H_2O$: C, 41.63; H, 4.12; N, 26.49. Found: C, 41.61; H, 4.16; N, 26.53.

**3,7,10-Trimethyl-(1*H*,3*H*,5*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone ($PPTH_2$).** A suspension of 5 g (0.017 mol) of $PPT_{ox}$ and 1 g of Pd/C in 200 mL of $H_2O$ was hydrogenated under 30 psi $H_2$ in a Parr apparatus for 2 days. The reaction vessel was transferred to a $N_2$ glovebox and the crystalline $PPTH_2$ plus catalyst collected by filtration. Separation of product from catalyst was carried out by dissolution of the latter with ~50 mL of $O_2$-free KOH, filtration, and acidification of the filtrate to pH <5 with $O_2$-free HCl. The tan product (which immediately precipitates from solution) was collected by filtration, washed 2× with $O_2$-free water, and dried under $N_2$ [yield 3.57 g (72%)]. The compound $PPTH_2$ is very reactive with $O_2$, being converted to $PPT_{ox}$.

**1,3,7,10-Tetramethyl-(1*H*,3*H*,7*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetraone ($PPTMe_{ox}$).** To 25 mL of glacial acetic acid, dried by azeotroping with benzene and storage over molecular sieves, 1.1 g of 1,3-dimethyl-5-amino-6-(methylamino)uracil[28] (5.97 mmol) and 1.1 g of *N*-methylalloxan (7.04 mmol) was added. Heating at 80 °C was carried out for 30 min. After the mixture was cooled, the bright yellow precipitate was collected and washed with acetic acid and diethyl ether. The yield was 1.6 g (88%). Recrystallization was carried out from water with a 45% loss by decomposition, but the crude product is pure enough for synthetic purposes: NMR (TFA) δ 4.50 [s, 3 H, $N^{10}$-methyl] 4.00 [s, 3 H, $N^1$-methyl] and 3.63 [s, 6 H, $N^3$- and $N^7$-methyls]; IR (Nujol) 1660, 1600, 1525, 1250 $cm^{-1}$; TLC [1-butanol, acetic acid, water (5:2:3)] on silica gel gave an $R_f$ of 0.48. Anal. Calcd for $C_{12}H_{12}N_6O_4 \cdot 0.5H_2O$: C, 46.00; H, 4.19; N, 26.82. Found: C, 45.72; H, 4.19; N, 26.82.

**$PPTMe_{ox}$: Alternate Method.** To 40 mL of dried glacial acetic acid 9.6 g (0.048 mol) of 1,3-dimethyl-5-nitroso-6-(methylamino)uracil[27] and 5.7 g (0.05 mol) of *N*-methylbarbituric acid were added. This was refluxed for 1 h after which the reaction mixture contained a bright yellow precipitate. The mixture was cooled to room temperature and filtered. Solids were washed with $H_2O$, then methanol, and finally diethyl ether [yield = 2.0 g (13.5%)].

**3,8-Dimethyl-6-(*N*-methylcarboxamido)-(1*H*,3*H*,8*H*)-pteridine-2,4,7-trione (III).** To a solution of 500 mg of KOH in 50 mL of degassed distilled water 0.056 g (0.19 mmol) of $PPT_{ox}$ was added and the reaction stirred for 24 h at room temperature under $N_2$. The solution was acidified with concentrated HCl and chilled in the refrigerator overnight. Colorless needles were filtered off and dried under high vacuum. The yield was 0.027 g (55%). Recrystallization was carried out by dissolving in dilute ammonium hydroxide and acidifying to pH 1 with 3 N HCl: NMR (TFA) δ 3.56 [d, 3 H, *N*-methyl of amide], 3.86 [s, 3 H, N(3) methyl], 4.06 [s, 3 H, N(8) methyl]; Ir (KBr) 3500, 3300, 2900, 1750, 1650, 1550, and 1440 $cm^{-1}$; TLC [1-butanol–acetic acid–water (5:2:3)] on silica gel gave an $R_f$ of 0.52. Anal. Calcd for $C_{10}H_{11}N_5O_4 \cdot 1.1H_2O$: C, 42.13; H, 4.66; N, 24.57. Found: C, 42.09; H, 4.73; N, 24.40.

**3,8-Dimethyl-6-(*N*,*N*′-dimethylcarboxyureido)-(1*H*,3*H*,8*H*)-pteridine-2,4,7-trione Potassium Salt ($IIMe^-K^+$).** A 500 mg (1.64 mmol) sample of $PPTMe_{ox}$ and 50 mL of 2 M, pH 6.0, acetate buffer were degassed in the port of a nitrogen glovebox overnight. After transfer into the $N_2$ box, the $PPTMe_{ox}$ was added to the buffer and the mixture stirred for 4 days. At this time, the formerly yellow suspension had been converted to a colorless fibrous mass which was filtered and washed 2× with 50-mL portions of oxygen-free water. After the mixture was dried in vacuo, 0.35 g (59%) of $IIMe^-K^+$ was obtained. Spectral investigation of the filtrate and washings indicated a considerable amount of $IIMe^-K^+$, the isolation of which was not practical under the circumstances. Isolated product was stored at room temperature under $N_2$: NMR ($D_2O$, anaerobic with DDS as a reference) δ 3.70, 3.32, 3.06, 2.81 [4 s, methyls at N(3), N(8), and N and N′ of carboxyureide—no assignments made]; Ir (Nujol) 1725, 1650 $cm^{-1}$; TLC (aerobic, protonated) (30% methanol in chloroform) on silica gel, $R_f$ 0.39. Anal. Calcd for $C_{12}H_{13}KN_6O_5$: C, 39.99; H, 3.63; K, 10.84; N, 23.32. Found: C, 39.84; H, 3.71; K, 10.87; N, 23.17.

**3,6,8,9-Tetramethyl-(1*H*,3*H*,5*H*,6*H*,8*H*,9*H*)-imidazo[4,5-*g*]pteridine-2,4,7-trione ($IVMeH_2$).** To a suspension of 1.6 g (5.25 mmol) of 1-$MePPT_{ox}$ in 20 mL of deoxygenated water was added 0.4 g (7.1 mmol) of KOH pellets. The suspension was rapidly transformed to a slightly red solution. Acidification to pH 1.0 was then carried out with concentrated HCl and the reaction mixture stored in the refrigerator overnight. The colorless crystalline product was filtered and washed with water. Drying yielded 1.01 g (65%) of $IVMeH_2$. Recrystallization from methanol gave colorless needles: NMR ($Me_2SO$-$d_6$) δ 3.35, 3.22, 2.88, and 2.73 (4 s, 3,6,8,9-tetramethyl—no assignments made); IR (KBr) 3250, 1800, 1750, 1700, 1625, 1550, 1480, 1360 $cm^{-1}$; TLC (10% methanol in chloroform) on silica gel gave an $R_f$ value of 0.26. Anal. Calcd for $C_{11}H_{14}N_6O_3 \cdot H_2O$: C, 44.58; H, 5.44; N, 28.36. Found: C, 44.88; H, 5.37; N, 28.76.

**Isolation of 3,8-dimethyl-(1*H*,3*H*,8*H*)-pteridine-2,4,7-trione-6-carboxylic acid** (IVH) was carried out by evaporation of the liquor of $IVMeH_2$ in vacuo. Extraction of the dry residue with 50 mL of hot methanol, filtration, and concentration to ~10 mL yielded a small amount of $IVMeH_2$ which was filtered off. Treatment of the methanol filtrate with concentrated HCl yielded IVH as needles. Recrystallization was carried out by dissolution in ammonium hydroxide and acidification with concentrated HCl: yield 17 mg (1%); NMR (TFA) δ 8.23 (s, 1 H, $N^1$H), 3.90 (s, 3 H, $N^8$-methyl), 3.60 (s, 3 H, $N^3$-methyl); IR (KBr) 2800 (COOH), 1740, 1650, 1550, 1450 $cm^{-1}$; TLC [1-butanol, acetic acid, water (5:2:3)] on silica gel gave an $R_f$ of 0.48. Anal. Calcd for $C_9H_8N_4O_5 \cdot 2H_2O$: C, 37.41; H, 4.15; N, 19.39. Found: C, 37.74; H, 4.29; N, 19.53.

**Large-Scale Preparation of IVH.** To 25 mL of 1.0 N KOH 0.75 g (2.46 mmol) of $PPTMe_{ox}$ was added. The reaction was refluxed for 12 h and then cooled to room temperature and the pH adjusted to ~7 with concentrated HCl. This solution was diluted to 500 mL with water and added to a 100 mL of AG1-x2 200–400-mesh $Cl^-$ resin column (Bio-Rad). After the column was eluded with 1 L of distilled water, the product was removed with 0.1 N HCl. The residue obtained on evaporation of the product fractions was dissolved in ~2 mL of 1 N $NH_4OH$ and acidified to ~pH 1 with concentrated HCl. Light tan needles crystallized out on chilling, yielding 164 mg (23%) on filtration and drying. TLC indicated this was the only product formed in the reaction and along with NMR showed identity with the product isolated from the synthesis of $IVMeH_2$.

**3,6,8,9-Tetramethyl-(3*H*,6*H*,8*H*,9*H*)-imidazo[4,5-*g*]pteridine-2,4,7-trione (IVMe).** To 100 mL of water containing 0.90 g (32 mmol) of $IVMeH_2$ was added 0.1 g of 5% Pt on asbestos. Oxygen was bubbled into the reaction over a period of 2 h. The red crystalline precipitate was

(24) Skibo, E. B.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, following paper in this issue.
(25) *Ibid.*, to be submitted.
(26) A preliminary communication dealing with these topics has appeared. See: Skibo, E. B.; Bruice, T. C. *J. Am. Chem.* **1982**, *104*, 4982.
(27) Pfleiderer, W.; Walter, H. *Justus Liebigs Ann. Chem.* **1964**, *677*, 113.
(28) Pfleiderer, W.; Schündehütte, K.-H. *Justus Liebigs Ann. Chem.* **1958**, *612*, 158.

then removed by filtration. Solids were extracted with DMF to separate IVMe from the catalyst. DMF extracts were evaporated in vacuo until only 5 mL of liquid remained. The addition of 45 mL of water resulted in the formation of fibrous red crystals of IVMe. The yield after filtration and drying was 0.467 g (53%): NMR ($Me_2SO$-$d_6$) $\delta$ 3.31, 3.13, 3.10, and 2.43 (4 s, 3,6,8,9-tetramethyl); IR: (Nujol) 1700, 1675, 1600 $cm^{-1}$. TLC (10% methanol in chloroform) on silica gel gave an $R_f$ value of ~0.5. Anal. Calcd for $C_{11}H_{12}N_6O_3 \cdot 2H_2O$: C, 42.30; H, 5.16; N, 26.91. Found: C, 42.45; H, 5.30; N, 26.71.

**Synthesis of IVMe from $IIMe^-K^+$.** Preparation of $IIMe^-K^+$ was carried out without isolation by the method described above. After complete reaction, the $IIMe^-K^+$ suspension in 2 M, pH 6.0, buffer was removed from the $N_2$ box and the reaction diluted to 100 mL with water. Oxygen was bubbled in for 2 days, resulting in formation of a red crystalline precipitate. Isolation by filtration and drying in vacuo yielded 0.15 mg (35%) of material identified as IVMe by comparison of TLC and NMR spectrum with those obtained for IVMe synthesized from $IVMeH_2$. In addition, considerable amounts of IVH and V were observed by TLC and UV in the liquor.

**3,6,9-Trimethyl-7-methoxy-(3*H*,6*H*,9*H*)-imidazo[4,5-*g*]pteridine-2,4-dione (VII). (a) In Methanol.** IVMe, 158 mg (0.57 mmol), was refluxed in 20 mL of methanol for 3 h after which the reaction mixture assumed a yellow-green fluorescence. Cooling overnight yielded 68 mg (43%) of bright yellow crystals: NMR ($Me_2SO$-$d_6$) $\delta$ 4.18 (s, 3 H, 7-OMe), 3.77, 3.40, and 3.32 (3 s, 9 H, methyls on $N^3$,$N^6$, and $N^9$—no assignments made); IR (KBr) 1625, 1475 $cm^{-1}$; TLC (30% methanol in chloroform) on silica gel gave an $R_f$ of 0.33. Anal. Calcd for $C_{11}H_{12}N_6O_3$: C, 47.82; H, 4.37; N, 30.42. Found: C, 47.74; H, 4.47; N, 30.48.

**(b) In Ethanol.** IVMe, 50 mg (0.18 mmol), was refluxed in 5 mL of ethanol for 12 h after which the reaction mixture assumed a yellow-green fluorescence and contained crystallized solids. The ethanol was evaporated and ~1 mL of DMF added to dissolve the residue. This was diluted to 100 mL with chloroform and added on to ~20 mL of 230–400-mesh silica gel in a 25-mL medium fritted glass funnel equipped with suction. Elution with 20% DMF in chloroform removed VMeH; the fluorescent product, VII, was removed with 100% DMF.

The former fraction containing *3,8-dimethyl-6-(N,N'-dimethylureido)-(1H,3H,8H)-pteridine-2,4,7-trione* (VMeH) was evaporated to 1 mL (DMF) and ~10 mL of diethyl ether added. Crystallization occurred after chilling overnight and yielded, after filtration and drying, 16 mg (30%) of VMeH as a colorless solid: TLC (20% DMF in chloroform) on silica gel gave an $R_f$ of 0.5; NMR ($Me_2SO$-$d_6$) $\delta$ 8.14 (q, 1 H, N-H of ureido group, $J$ = 4.8 Hz), 3.71 (s, 3 H, $N^8$-methyl), 3.23, and 3.20 (2 s, 6 H, ureido $N$-methyl and $N^3$-methyl—no assignments made) 2.63 (d, 3 H, $N'$-methyl, $J$ = 4.8 Hz); IR (KBr) 3200, 1725, 1625, 1525, 1420, 1350, 1250, 1080, 1025 $cm^{-1}$. Anal. Calcd for $C_{11}H_{14}N_6O_4$: C, 44.89; H, 4.79; N, 28.56. Found: C, 44.84; H, 4.86; N, 28.39.

The fluorescent fraction was evaporated to yield a yellow residue which was triturated with diethyl ether, filtered, and dried, yielding ~20 mg (40%) of VII. TLC (20% DMF in chloroform) on silica gel gave an $R_f$ of 0.11, identical with that obtained from VII synthesized in methanol. NMR ($Me_2SO$-$d_6$) displayed chemical shifts identical with those of VII synthesized in methanol and contained no ethyl protons. *In Benzyl Alcohol:* IVMe, 200 mg (0.72 mmol), was heated in benzyl alcohol at 80–90 °C for 12 h after which time the reaction mixture was a fluorescent yellow-green and a crystalline precipitate had formed. Isolation of products was carried out as described for the reaction of IVMe with ethanol, yielding 100 mg (47%) of VMeH and only trace amounts of VII. NMR ($Me_2SO$-$d_6$) and TLC (20% DMF in chloroform) on silica gel indicated that the latter was identical with VII synthesized in methanol and ethanol.

**3,8-Dimethyl-6-(*N'*-methylureido)-(1*H*,3*H*,8*H*)-pteridine-2,4,7-trione (VH).** A solution of 0.15 g (0.54 mmol) of $IVMe_{ox}$ in 100 mL of pH 7.00 (0.33 M phosphate) buffer was incubated at 30 °C for 4 days after which the formerly deep red solution was fluorescent blue. TLC indicated only one product present. The reaction mixture was diluted to 500 mL and placed on a 100 mL of AG 1-x2 200–400-mesh $Cl^-$ resin column (BioRad). After the column was washed with 500 mL of distilled water, elution with 500 mL of 0.01 N HCl and then 500 mL of 0.1 N HCl was carried out. The product was removed during the latter elution. Evaporation of product fractions yielded an oily residue which solidified when triturated with methanol–ether: crude yield 78 mg (43%). Crystallization was carried out by diluting the crude product in ~3 mL of $H_2O$ and adding a drop of concentrated HCl. After the mixture was chilled for 2 days, light yellow needles were obtained: NMR ($Me_2SO$-$d_6$) $\delta$ 3.48 (s, 3 H, $N^8$-methyl), 3.22 (s, 3 H, $N^3$-methyl), 3.07 (s, 3 H, ureido $N^1$-methyl); IR (KBr) 3400, 3000, 1725, 1650, 1600, 1550, 1460, 1020 $cm^{-1}$; TLC [1-butanol–acetic acid–water (5:2:3)] on silica gel gave an $R_f$ of 0.48. Anal. Calcd for $C_{10}H_{12}N_6O_4 \cdot 3.5H_2O$: C, 34.98; H, 5.57. Found: C, 35.15; H, 5.54. The nitrogen analysis was 1.48% less than that calculated: MS amu (%) 281 ($M^+$ + 1, 3.4), 207 (M + 1 - $CH_3NHC(=O)NH$–, 11), 169 (12).

**p$K_a$ determinations** for compounds $PPT_{ox}$, IIIH, IIMe, VII, VMeH, and VH were carried out as follows: A 25-mL aqueous solution, $\mu$ = 1.0 with KCl, of the compound being studied was added to a thermostated 50-mL cup with quartz windows (path length = 3.387 cm) mounted on a Cary 118 spectrophotometer. The cup was equipped with a magnetic stirrer, a glass–calomel electrode, and an argon inlet. Concentrations were chosen so that the absorbances of the ionized and unionized species were between 0 and 1. After the solution was equilibrated at 30 °C, titration was carried out with acid or base. A known amount of titrant was added, and the pH and the absorbance at one or more wavelengths were measured. Absorbance values were then corrected for the volume change. The resultant pH vs. absorbance data were fit to eq 6

$$\text{absorbance} = \frac{A_T a_H \epsilon_{HA} + A_T \epsilon_A K_a}{a_H + K_a} \quad (6)$$

where $A_T$ is the total concentration of the compound being studied, $a_H$ is the proton activity determined with a glass electrode, $\epsilon_A$ and $\epsilon_{HA}$ are the extinction coefficients of the ionized and unionized forms, respectively, and $K_a$ is the equilibrium constant determined by the fit.

The p$K_a$ values for $PPTH_2$ were also calculated by fitting absorbance vs. pH data to eq 6. Because of the air sensitivity of dilute solutions of $PPTH_2$, each point represented a separate experiment carried out under anaerobic conditions. To the bottom port of a Thunberg cuvette there was added 3.5 mL of a buffer at a selected pH containing $2.43 \times 10^{-5}$ M $PPT_{ox}$ and $2.85 \times 10^{-3}$ M EDTA, $\mu$ = 1.0 (KCl). The Thunberg was sealed and polyethylene tubes were threaded into the top and bottom ports through a side arm. Argon, deoxygenated with an Oxiclear filter and humidified, was passed into the Thunberg for 1 h. The tubes were removed and the cuvette was closed. Irradiation of the bottom port for 30 min with a water-cooled 300-W lamp reduced $PPT_{ox}$ to $PPTH_2$. After equilibration to 30 °C, the absorbance at 292 nm and pH (aerobic) was measured. For points in the $H_0$ region, a slightly different methodology was used. The bottom port contained 3.0 mL of an acid solution of desired strength and the top port contained 0.4 mL of $2.13 \times 10^{-4}$ M $PPT_{ox}$ in 1 M KCl and 0.1 mL of 0.1 M EDTA buffered at pH 7.00. The $H_0$ value obtained on mixing both ports was obtained from Paul and Long.[29] The contents of the Thunberg were degassed as described above. The top port was then irradiated for 30 min with a focused, water-cooled beam from a 75-W xenon source mounted in an Oriel housing. The $PPTH_2$ solution was combined with the bottom port and the absorbance at 292 nm measured at 30 °C.

Verification that two protons dissociate from $PPTH_2$ with p$K_a$s of ~5.5 was obtained by volumetric titration. A pH 12 solution containing 0.223 mmol of $PPT^{2-}$ was titrated with 1.0 N HCl to pH 2.0 under the strictest anaerobic conditions. The pH was measured after each addition of titrant. A plot of volume vs. pH indicated a p$K_a$ of ~5.5 and that 0.42 mequiv of HCl was required to protonate $PPT^{2-}$. Therefore, 2.0 protons per molecule of $PPT^{2-}$ were necessary for neutralization.

The p$K_a$ of $IVMeH_2$ was determined volumetrically because significant spectral changes in the ionized and unionized species were lacking. An 0.248 mequiv solution of $IVMeH^-$ was prepared by diluting 69 mg of $IVMeH_2$ and 2.5 mL of 0.1 N KOH to 25 mL with $CO_2$-free $H_2O$. Titration with 0.05 N HCl, $\mu$ = 1.0 with KCl, were carried out at 30 °C (under argon) from pH 10.5 to pH 2.5. A plot of pH vs. volume of 0.05 N HCl indicated a p$K_a$ of ~8.4 and that ~0.91 proton per mol of $IVMeH^-$ was required for neutralization.[30]

Cyclic voltammetric experiments were carried out with a modified Princeton Applied Research Model 174 polarographic analyzer. The voltammograms were recorded on a EAI 1133 variplotter for slow scans. For fast scans, the oscilloscope display was photographed and peak potentials determined with a Hewlett-Packard digitizer.

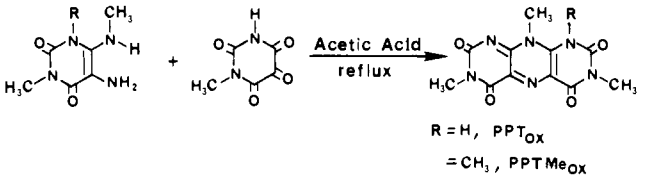
The working electrode material was a carbon paste consisting of 3 g of 325-mesh carbon graphite and 2 mL of paraffin oil.[31] The working electrode assembly was fashioned from a Pasteur pipet by fusing the capillary end around an inserted platinum wire. A well is left at the tip of the pipet, 1 mm long, into which the wire projected 0.5 mm. The paste was packed into this well and the surface was smoothed with glassine paper. The area of the active surface was ~0.8 $mm^2$. After each use the old paste was removed and the tip repacked. The auxilliary electrode was a platinum wire isolated in a compartment containing 1 M NaCl.

(29) Paul, M. A.; Long, F. A. *Chem. Rev.* **1957**, *57*, 15.
(30) Albert, A.; Serjeant, E. P. "Ionization Constants of Acids and Bases"; Methuen & Co., Ltd.: London, 1962; New York.
(31) (a) McCreery, R. L.; Dreiling, R.; Adams, R. N. *Brain Res.* **1974**, *73*, 23. (b) Kissinger, P. T.; Hart, J. B.; Adams, R. N. *Brain Res.* **1973**, *55*, 209.

The buffered solution containing the compound being studied was separated from this compartment by a porous frit. The reference electrode was an Ag/AgCl (1 M NaCl) electrode, 0.222 V vs. NHE.

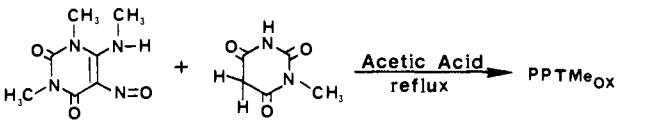
## Results

**Synthesis** of 3,7,10-trimethyl-(3*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone ($PPT_{ox}$) and 1,3,7,10-tetramethyl-(3*H*,7*H*,9*H*,10*H*)-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone ($PPTMe_{ox}$) was carried out by condensation of the appropriate diaminouracil with *N*-methylalloxan (eq 7). The methodology



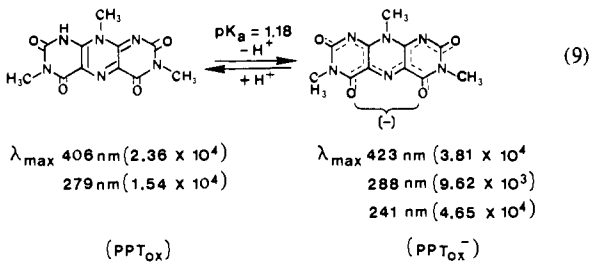

(7)

used is based on literature precedents.[32] Condensation with a [5,4-g] fusion occurs as a consequence of the high reactivity of the 5-amino group of the diaminouracil and the 5-carbonyl of *N*-methylalloxan. Unambiguous proof of [5,4-g] fusion has been obtained by condensation of a 5-nitroso-6-aminopyrimidine with a barbituric acid to yield a pyrimido[5,4-*g*]pteridine.[32] In a parallel experiment, we condensed 1,3-dimethyl-6-(methylamino)-5-nitrosouracil with *N*-methylbarbituric acid in refluxing acetic acid and obtained a compound identical with $PPTMe_{ox}$ (eq 8). The



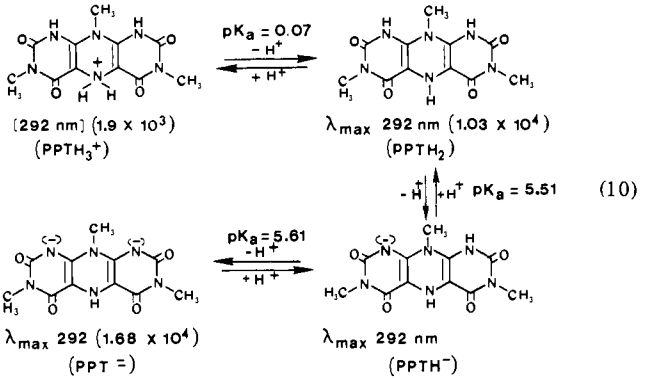

(8)

$pK_a$ of $PPT_{ox}$ was determined spectrophotometrically (423 nm, eq 9). The low $pK_a$ value is consistant with the high degree of




(9)

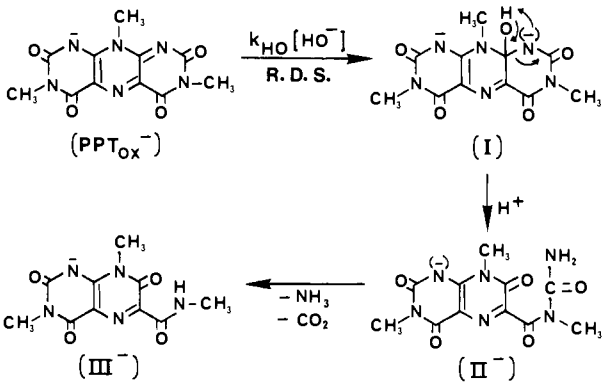
delocalization of the anion. Reduction of $PPT_{ox}$ yields $PPTH_2$ (Experimental Section). A priori, $PPTH_2$ should display one $pK_a$ for dissociation at protonated $N^5$ and another two for ionization at $N^1$ and $N^9$. Spectrophotometric determination of the $pK_a$ values was carried out by following OD changes at 292 nm vs. pH. A $pK_a$ of 0.07 for loss of a proton from protonated $N^5$ was obtained, but only one additional $pK_a$, with a value of 5.80, was found spectrophotometrically (eq 10). The simultaneous dissociation




(10)

(32) Taylor, Jr. E. C.; Cain, C. K.; Loux, H. M. *J. Am. Chem. Soc.* **1953**, *76*, 1874.

Scheme I

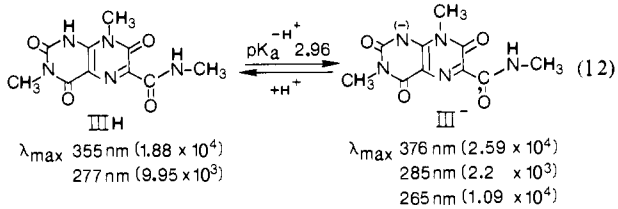



of two protons ($pK_a \sim 5.5$) from $PPTH_2$ was verified by volumetric titration. The inability to detect two $pK_a$ values by spectral titration may result from too little OD change at 292 nm for either first or second ionization of $N^1$ and $N^9$ protons and/or $pK_a$ values which are almost identical. In a following study,[24] the equation required to fit the pH rate profile for the reduction of formaldehyde by $PPTH_2$ gave $pK_{app}$ values of 5.5 and 5.6. Since these are close in value to the spectrophotometric $pK_a$, they may be considered to be the $pK_a$ values for $N^1$- and $N^9$-ionization.

**$PPT_{ox}^-$ Hydrolysis** was studied between pH 11.85 and pH 12.95 by following its disappearance at its $\lambda_{max}$ at 423 nm. These hydrolytic studies were carried out at 30 °C with $\mu = 1.0$ (with KCl). Because $PPT_{ox}$ exists as its anion ($PPT_{ox}^-$) through most of the pH range, it is rather resistant to hydrolysis. The disappearance of $PPT_{ox}^-$ was found to be coincidental to the appearance (376 nm) of the anion of 3,8-dimethyl-6-(*N*-methylcarboxamido)-(1*H*,3*H*,8*H*)-pteridine-2,4,7-trione ($III^-$). Both the disappearance of $PPT_{ox}^-$ and appearance of $III^-$ followed the same first-order rate law. When the log of the pseudo-first-order rate constants ($k_{obsd}$) were plotted vs. pH, a straight line of unity slope was obtained from which there was calculated the second-order rate constant for reaction of $HO^-$ with $PPT_{ox}^-$ (eq 11) where $k_{HO}$

$$k_{obsd} = k_{HO}K_W/a_H \qquad (11)$$

$= 5.78 \times 10^{-4}\ M^{-1}\ s^{-1}$, $pK_W = 13.86$, and $a_H$ is the hydrogen ion activity determined with a glass electrode. The compound IIIH has been isolated and characterized (see Experimental Section) and its $pK_a$ determined by spectrophotometric titration (eq 12).

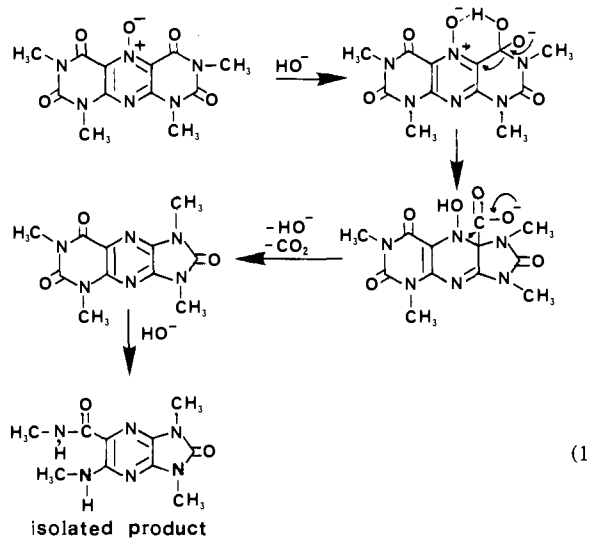



(12)

The formation of $III^-$ from $PPT_{ox}^-$ is most reasonably envisioned as occurring by addition of $HO^-$ to the 10a-position of $PPT_{ox}^-$ followed in turn by ring opening to yield a carboxyureide ($II^-$) which then undergoes hydrolysis (Scheme I).

**Hydrolysis of $PPTMe_{ox}$** ($4.9 \times 10^{-5}$ M) under anaerobic conditions was studied between pH 1.0 and pH 11.0. Replacement of the ionizable proton on $N^1$ of $PPT_{ox}^-$ with a methyl group greatly increases its susceptibility to hydrolysis. A description of the novel chemistry which accompanies this change follows.

The hydrolysis of $PPTMe_{ox}$ under anaerobic conditions is depicted in Scheme II. The $IIMe^-K^+$ salt was isolated from the preparative hydrolysis of $PPTMe_{ox}$ in 2 M, pH 6.00, acetate buffer under anaerobic conditions. Elemental analysis (see Experimental Section) does not readily distinguish between the proposed structure resulting from 10a attack (Scheme II) and those arising from 4, 6, and 9a attack (structures C). However, the $pK_a$ of 2.79 for dissociation of IIMeH to $IIMe^-$, eq 13, is not consistent




(18)

in the absence of oxygen, it is converted by oxygen oxidation to the hydrolytically labile species, IVMe.

The preparation and characterization of IVMe and VH have been carried out (for details see Experimental Section) under conditions which parallel the kinetic experiments. The solvolyisis of 0.5 g of $IIMe^-$ in 50 mL of oxygenated 2 M acetate buffer (pH 6.0) provided a mixture of products from which IVMe readily crystallized (31% yield). From the supernatant, the highly fluorescent IVH (430 nm) could be detected on TLC, $r_f$ 0.36 on silica gel [1-butanol–acetic acid–$H_2O$ (5:2:3)]. Attempts to isolate IVH by silica chromatography provided only VH (as shown by UV–vis spectroscopy).

Kinetic investigations in support of the reaction sequence of Scheme IV follow. The product of solvolysis of $PPTMe_{ox}$ under anaerobic conditions (i.e., $IIMe^-$ $\lambda_{max}$ 351 nm) does not accumulate to any great extent under aerobic conditions. In addition, very little, if any, $IMe^-$ (368 nm), seen under anaerobic conditions, accumulates in the presence of oxygen. Thus, the intermediates in the conversion of $PPTMe_{ox}$ → IVMe are, in essence, at steady-state concentration. The spectra of authentic $IIMe^-$ and IVMe have a common extinction at ~388 nm. Repetitive spectral scanning at pH 6.0 of authentic $IIMe^-$ in air-saturated 1 M acetate buffer showed that $IIMe^-$ → IVMe proceeds with isobestic points at 388 and 308 nm. A shift in isobestic points is noted toward the end of the reaction along with an absorbance decrease at >460 nm (Figure 5). This observation is consistent with the conversion IVMe → IVH. Absorbance vs. time plots at 430 nm for the reaction of $IIMe^-$ in oxygen-saturated, pH 6.00, acetate buffer (total buffer concentration range from 0.1 to 1.0 M) exhibited a zero-order phase followed by a first-order phase. A third phase, first-order decrease at 430 nm to zero absorbance, was observed after several days. This triphasic kinetic behavior supports the reaction scheme $IIMe^-$ → IVMe → IVH → V. Neither of the first two phases exhibited buffer catalysis. The initial zero-order appearance of IVMe is considered in conjunction with Scheme V and eq 21.

Investigation of the hydrolysis of authentic IVMe provides evidence consistent with the sequence IVMe → IVH → V (Scheme IV). The hydrolysis of IVMe ($6.5 \times 10^{-5}$ M) at pH 6.0 (acetate buffers 0.1 to 1.0 M) and at pH 7.0 (phosphate buffer 0.033–0.33 M) when followed spectrally, showed that absorbance of IVMe (445 nm) decreases with the concomitant increase in absorbance at 430 nm (IVH) with an isosbestic point at 460 nm (Figure 6). The reaction does not exhibit buffer catalysis at pH 6.0 and 7.0, but $k_{ly}$ is greater by a factor of 10 at the higher pH ($1 \times 10^{-4}$ $s^{-1}$ vs. $9.7 \times 10^{-4}$ $s^{-1}$), supportive of specific base catalysis. The isolation of IVMe in the preparative solvolysis of $IIMe^-$ (loc. cit.) may have been possible only because its insolubility removed it from the reaction mixture. When formation of IVH reaches its highest concentration, there ensues a slower reaction which does not exhibit buffer catalysis and for which $k_{ly}$ is greater by

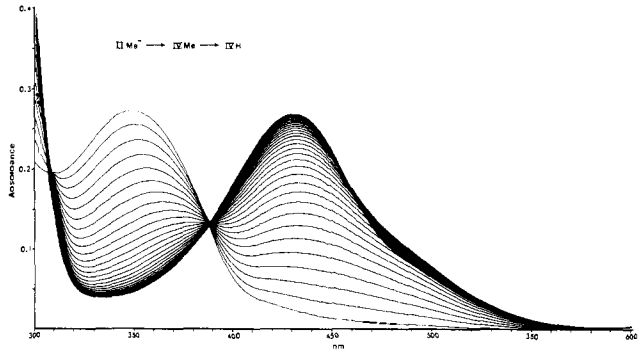



**Figure 5.** Repetitive scan of the hydroxide-catalyzed conversion of $IIMe^-$ ($4.9 \times 10^{-5}$ M) to yield IVH. (aerobic, pH 6.00, 1 M acetate buffer, $\mu$ = 1.0 with KCl, at 30 °C. Scan times are 815 s).

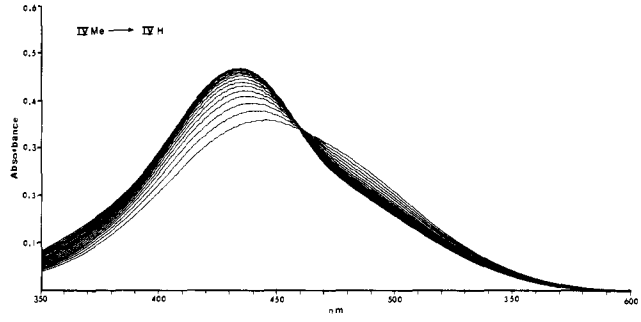
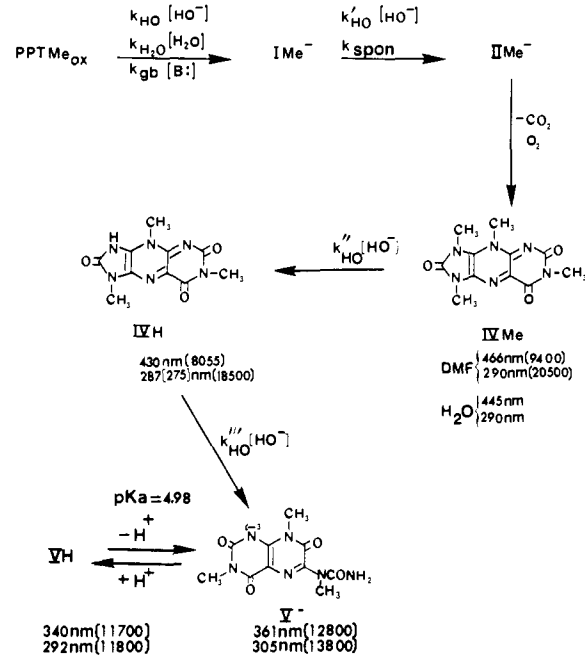



**Figure 6.** Repetitive scan for the hydrolysis of $6.5 \times 10^{-5}$ M IVMe to IVH in pH 6.00, 1 M acetate buffer, $\mu$ = 1.0 with KCl, at 30 °C. Scan times are 815 s.

Scheme IV




1 order of magnitude at pH 7 when compared to pH 6 ($k_{ly}$ = 2.5 $\times 10^{-5}$ $s^{-1}$ vs. $2.0 \times 10^{-6}$ $s^{-1}$). Thus, at neutrality, IVMe → IVH and IVH → VH are specific base catalyzed. To obtain an accurate $\epsilon$ for IVH, the absorbance vs. time data were fit to eq 14 for two consecutive first-order reactions. The values of $k_1$ and $k_2$ at pH 7.0 obtained by this means ($1 \times 10^{-3}$ $s^{-1}$ and $2.8 \times 10^{-5}$ $s^{-1}$) agree, within experimental error, with the values of $k_1$ and $k_2$ when determined separately. The extinction coefficient for IVH was determined as 8055 $cm^{-1}$ $M^{-1}$ on the basis of the known extinction coefficients for IVMe and VH and the computer-generated values of $X$, $Y$, and $Z$ (eq 14). These considerations provide strong support for the sequence of events depicted in Scheme IV.

$$OD_{obsd} = Xe^{-k_1 t} + Ye^{-k_2 t} + Z$$
$$X = \epsilon_A[A_0] - \epsilon_C[A_0] + (\epsilon_B[A_0] - \epsilon_C[A_0])[k_1/(k_2 - k_1)]$$
$$Y = \epsilon_C[A_0] - \epsilon_B[A_0][k_1/(k_2 - k_1)]$$
$$Z = \epsilon_C[A_0] \quad (14)$$

reactions (A → B → C). (In eq 14, $\epsilon_A[A_0]$, $\epsilon_B[A_0]$, and $\epsilon_C[A]$ are the maximum possible absorbances of A, B, and C in the process A → B → C, $[A_0]$ is the initial concentration of A, and $\epsilon$'s are extinction coefficients.) The effect of 10-fold changes in buffer concentration on the rate processes observed between pH 2.0 and pH 9.0 was investigated by employing the following acids and their potassium salts as buffer pairs: chloroacetic acid ($pK_a$ = 2.88), acetic acid ($pK_a$ = 4.76), phosphoric acid ($pK_{a_2}$ = 6.85, $pK_{a_3}$ = 11.44, and carbonic acid ($pK_{a_2}$ = 9.66). Above pH 7.0, the disappearance of $PPTMe_{ox}$ and appearance of $IMe^-$ was found to be buffer independent but $[HO^-]$ dependent. The conversion of $IMe^- \rightarrow IIMe^-$ was not buffer-concentration dependent at any pH. Buffer dilution plots for the rate of disappearance of $PPTMe_{ox}$ in the acetic acid–acetate buffer system are shown in Figure 2.

The pH rate profile for hydrolysis of $PPTMe_{ox}$ to $IMe^-$ by lyate species ($k_{ly}$) between pH 1 and pH 11 is given in Figure 3. Values of $k_{ly}$ for Figure 3 were obtained as intercepts of the buffer dilution plots of Figure 2 between pH 2.0 and pH 9.0 and as the observed rate constants at higher and lower pH values. The solid line of Figure 3 was computer generated from eq 15 where $k_H$ = 1.64

$$k_{ly} = k_H a_H + k_{H_2O} + k_{HO}\text{-}K_W/a_H \quad (15)$$

$\times 10^{-4}$ $M^{-1}$ $s^{-1}$, $k_0 = 2.7 \times 10^{-6}$ $M^{-1}$ $s^{-1}$, and $k_{HO} = 1.5 \times 10^3$ $M^{-1}$ $s^{-1}$. The first-order rate constants for the formation of $IMe^-$ follow the same rate law. The ratio of the rate constants for the $HO^-$-mediated hydrolysis of $PPTMe_{ox}$ and $PPT_{ox}^-$ ($1.5 \times 10^3$ $M^{-1}$ $s^{-1}$ and $5.8 \times 10^{-4}$ $s^{-1}$) of $\sim 10^6$ ($\Delta\Delta G^{\ddagger} \cong 39$ kJ $M^{-1}$) deserves comment. No more than ~8 kJ $M^{-1}$ can be attributed to the formal negative charge of $PPT_{ox}^-$.[34] The remaining ~31 kJ $M^{-1}$ must be attributed to the release of peristrain on formation of the tetrahedral intermediate by 10a attack of $HO^-$ on $PPTMe_{ox}$ and to the resonance stabilization of $PPT_{ox}^-$ due to delocalization of the negative charge.

The pH–rate profile for conversion of $IMe^-$ to $IIMe^-$ between pH 4 and pH 8 is given in Figure 4. The points of Figure 4 were fit by eq 16 where $k_{spon} = 9.55 \times 10^{-5}$ $M^{-1}$ $s^{-1}$ and $k_{HO} = 1.35$

$$k_{ly} = k_{spon} + k_{HO}K_W/a_H \quad (16)$$

$\times 10^4$ $M^{-1}$ $s^{-1}$. The value of $k_{spon}$ pertains to a spontaneous reaction in which $H_2O$ is not involved as a base. This is shown by the lack of general base catalysis in $IMe^- \rightarrow IIMe^-$.

For the buffer-sensitive conversion of $PPTMe_{ox}$ to $IMe^-$, buffer dilution plots obtained with acetate buffer evidence general base catalysis only, while those obtained with chloroacetate buffer exhibited both general base and general acid catalysis. The observed pseudo-first-order rate constant ($k_{obsd}$) may then be expressed as in eq 17, where $k_{gb}$ and $k_{ga}$ are the second-order general

$$k_{obsd} = k_{ly} + k_{gb}\left(\frac{K_a}{K_a + a_H}\right)[B_T] + k_{ga}\left(\frac{a_H}{K_a + a_H}\right)[B_T] \quad (17)$$

base and general acid catalyzed rate constants respectively, $[B_T]$ is the total concentration of buffer species ($[B^-] + [BH]$), and $K_a$ is the acid dissociation constant of the buffer. A plot of $(k_{obsd} - k_{ly})/[[B_T]/(K_a + a_H)]$ vs. $a_H$ for a buffer system at a series of pH values gave as slope $k_{ga}$ and as intercept $k_{gb}K_a$. The calculated values for $k_{gb}$ are $7.11 \times 10^{-5}$ $M^{-1}$ $s^{-1}$ and $5.05 \times 10^{-6}$ $M^{-1}$ $s^{-1}$, for acetate and chloroacetate, respectively. The value of $k_{ga}$ for chloroacetic acid was determined to be $6.97 \times 10^{-6}$ $M^{-1}$ $s^{-1}$. Presumably the role of general base and general acid catalysis

(34) Bruice, T. C.; Holmquist, B. *J. Am. Chem. Soc.* **1967**, *89*, 4028. *Ibid.* **1969**, *91*, 2982, 2985.

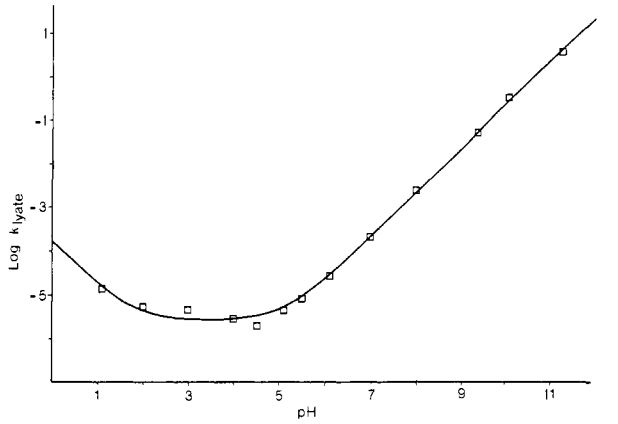



**Figure 3.** pH–rate profile for the hydrolysis of $PPTMe_{ox}$ to $IMe^-$ (anerobic conditions at 30 °C, $\mu$ = 1.0 with KCl).

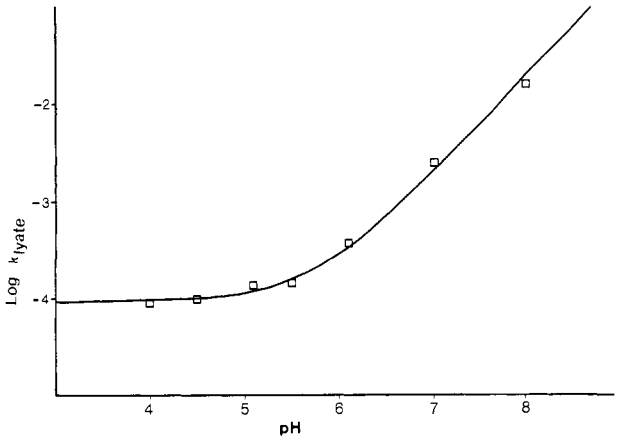
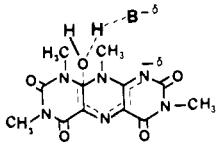
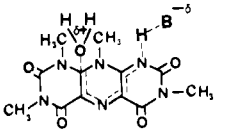



**Figure 4.** pH–rate profile for the hydrolysis of $IMe^-$ to $IIMe^-$ (anerobic conditions at 30 °C, $\mu$ = 1.0 with KCl).

in the conversion of $PPTMe_{ox}$ to IMeH is proton removal from $H_2O$ (by $H_2O$ and general base $B^-$) and proton donation to N-1 (by $H_3O^+$ and general acid BH) as shown in transition-state structures D and E.



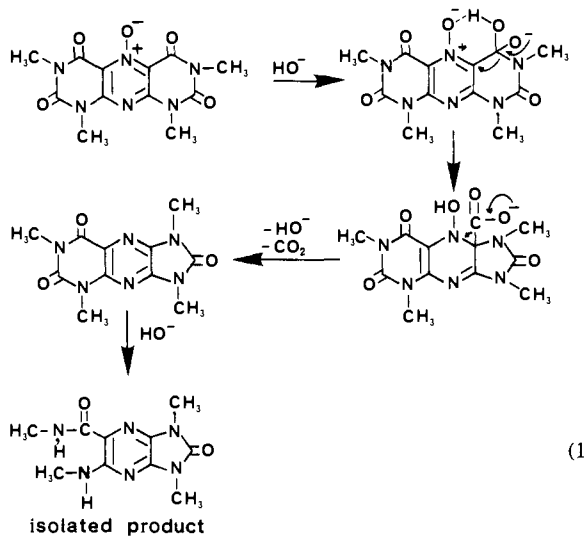
D



E

The specific acid catalyzed ($k_H[H_3O^+]$ of Scheme II) hydrolysis of $PPTMe_{ox}$ to yield $IVMeH_2$ (eq 15) does not involve IIMeH as an intermediate. In an independent experiment, the pseudo-first-order rate constant for $IIMeH \rightarrow IVMeH_2$ was determined (pH ~0) to be $5 \times 10^{-7}$ $s^{-1}$. This value is much smaller than the pseudo-first-order rate constant (pH ~0) for the conversion $PPTMe_{ox} \rightarrow IVMeH_2$ which is $2 \times 10^{-5}$ $s^{-1}$. The mechanism of Scheme III is proposed. An analogous base-catalyzed ring contraction of 1,3,7,9-tetramethylpyrimido[5,4-*g*]pteridine 5-oxide has been recorded by Taylor, Maki, and McKillop (eq 18).[23]

**Hydrolysis of $PPTMe_{ox}$ under aerobic conditions** has been investigated. In acetate buffers the rate constants for hydrolysis of $PPTMe_{ox}$ ($k_{gb}$ and $k_{ly}$) are not influenced by the presence of oxygen. However, the products formed under anaerobic and aerobic conditions differ. Whereas under anaerobic conditions the final product is $IIMe^-$, in the presence of oxygen the final product is $V^-$ (and its conjugate acid VH). Experimental evidence (vide infra) supports the reactions of Scheme IV for the aerobic solvolysis of $PPTMe_{ox}$. The rate-determining step under both anaerobic and aerobic conditions is the formation of $IMe^-$. Inspection of Schemes II and IV shows that whereas $IIMe^-$ is stable




(18)

in the absence of oxygen, it is converted by oxygen oxidation to the hydrolytically labile species, IVMe.

The preparation and characterization of IVMe and VH have been carried out (for details see Experimental Section) under conditions which parallel the kinetic experiments. The solvolysis of 0.5 g of $IIMe^-$ in 50 mL of oxygenated 2 M acetate buffer (pH 6.0) provided a mixture of products from which IVMe readily crystallized (31% yield). From the supernatant, the highly fluorescent IVH (430 nm) could be detected on TLC, $r_f$ 0.36 on silica gel [1-butanol–acetic acid–$H_2O$ (5:2:3)]. Attempts to isolate IVH by silica chromatography provided only VH (as shown by UV–vis spectroscopy).

Kinetic investigations in support of the reaction sequence of Scheme IV follow. The product of solvolysis of $PPTMe_{ox}$ under anaerobic conditions (i.e., $IIMe^-$ $\lambda_{max}$ 351 nm) does not accumulate to any great extent under aerobic conditions. In addition, very little, if any, $IMe^-$ (368 nm), seen under anaerobic conditions, accumulates in the presence of oxygen. Thus, the intermediates in the conversion of $PPTMe_{ox}$ → IVMe are, in essence, at steady-state concentration. The spectra of authentic $IIMe^-$ and IVMe have a common extinction at ~388 nm. Repetitive spectral scanning at pH 6.0 of authentic $IIMe^-$ in air-saturated 1 M acetate buffer showed that $IIMe^-$ → IVMe proceeds with isobestic points at 388 and 308 nm. A shift in isobestic points is noted toward the end of the reaction along with an absorbance decrease at >460 nm (Figure 5). This observation is consistent with the conversion IVMe → IVH. Absorbance vs. time plots at 430 nm for the reaction of $IIMe^-$ in oxygen-saturated, pH 6.00, acetate buffer (total buffer concentration range from 0.1 to 1.0 M) exhibited a zero-order phase followed by a first-order phase. A third phase, first-order decrease at 430 nm to zero absorbance, was observed after several days. This triphasic kinetic behavior supports the reaction scheme $IIMe^-$ → IVMe → IVH → V. Neither of the first two phases exhibited buffer catalysis. The initial zero-order appearance of IVMe is considered in conjunction with Scheme V and eq 21.

Investigation of the hydrolysis of authentic IVMe provides evidence consistent with the sequence IVMe → IVH → V (Scheme IV). The hydrolysis of IVMe ($6.5 \times 10^{-5}$ M) at pH 6.0 (acetate buffers 0.1 to 1.0 M) and at pH 7.0 (phosphate buffer 0.033–0.33 M) when followed spectrally, showed that absorbance of IVMe (445 nm) decreases with the concomitant increase in absorbance at 430 nm (IVH) with an isosbestic point at 460 nm (Figure 6). The reaction does not exhibit buffer catalysis at pH 6.0 and 7.0, but $k_{ly}$ is greater by a factor of 10 at the higher pH ($1 \times 10^{-4}$ $s^{-1}$ vs. $9.7 \times 10^{-4}$ $s^{-1}$), supportive of specific base catalysis. The isolation of IVMe in the preparative solvolysis of $IIMe^-$ (loc. cit.) may have been possible only because its insolubility removed it from the reaction mixture. When formation of IVH reaches its highest concentration, there ensues a slower reaction which does not exhibit buffer catalysis and for which $k_{ly}$ is greater by

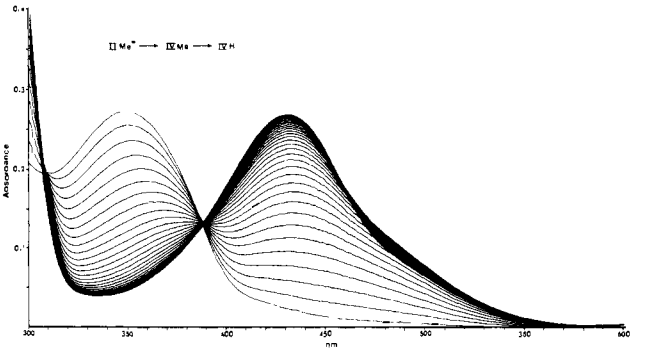



**Figure 5.** Repetitive scan of the hydroxide-catalyzed conversion of $IIMe^-$ ($4.9 \times 10^{-5}$ M) to yield IVH. (aerobic, pH 6.00, 1 M acetate buffer, $\mu$ = 1.0 with KCl, at 30 °C. Scan times are 815 s).

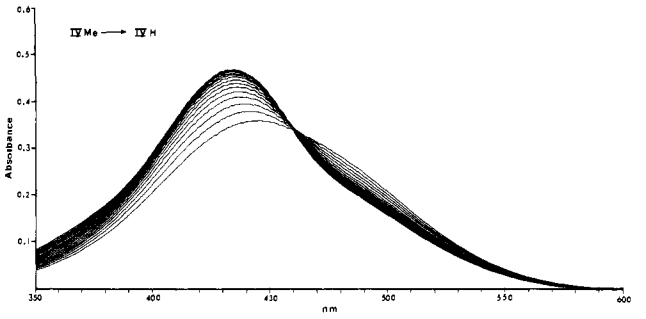
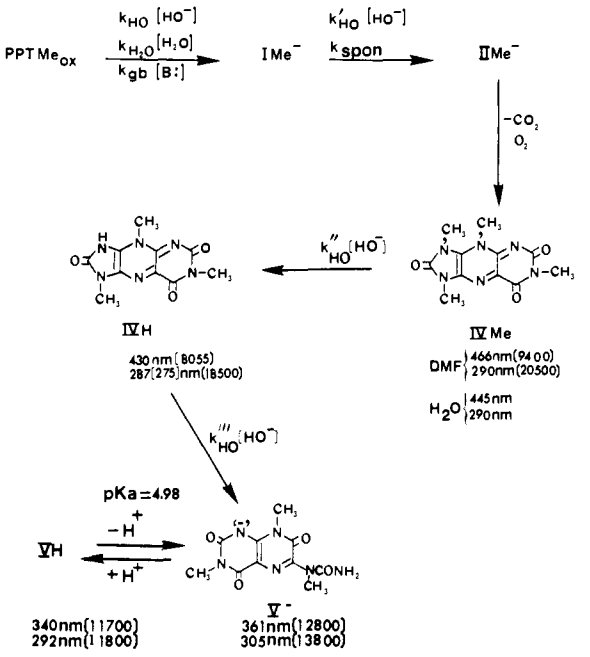


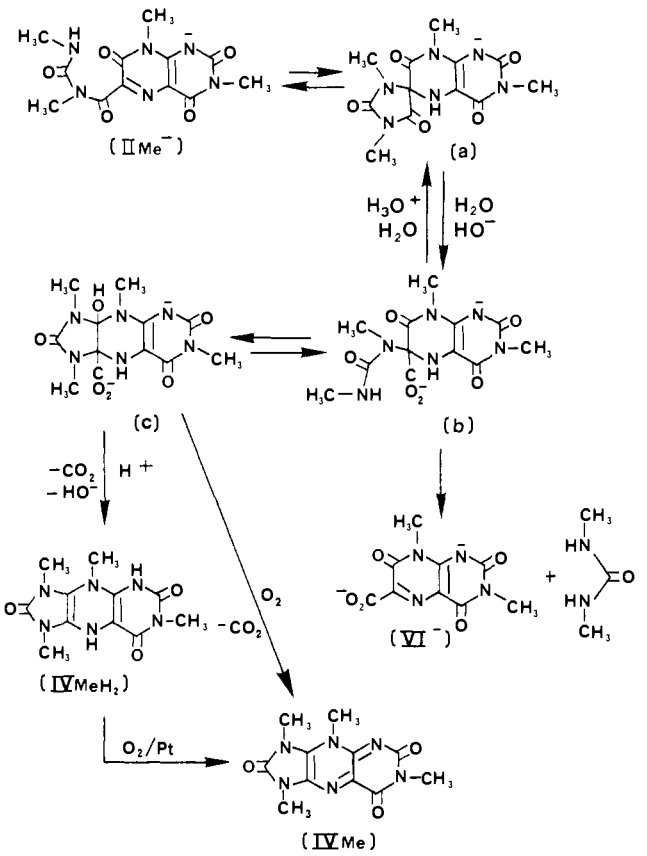

**Figure 6.** Repetitive scan for the hydrolysis of $6.5 \times 10^{-5}$ M IVMe to IVH in pH 6.00, 1 M acetate buffer, $\mu$ = 1.0 with KCl, at 30 °C. Scan times are 815 s.

Scheme IV

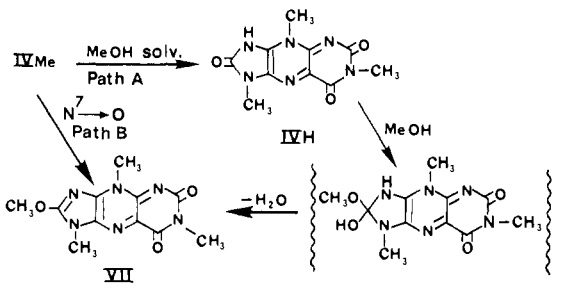



1 order of magnitude at pH 7 when compared to pH 6 ($k_{ly}$ = $2.5 \times 10^{-5}$ $s^{-1}$ vs. $2.0 \times 10^{-6}$ $s^{-1}$). Thus, at neutrality, IVMe → IVH and IVH → VH are specific base catalyzed. To obtain an accurate $\epsilon$ for IVH, the absorbance vs. time data were fit to eq 14 for two consecutive first-order reactions. The values of $k_1$ and $k_2$ at pH 7.0 obtained by this means ($1 \times 10^{-3}$ $s^{-1}$ and $2.8 \times 10^{-5}$ $s^{-1}$) agree, within experimental error, with the values of $k_1$ and $k_2$ when determined separately. The extinction coefficient for IVH was determined as 8055 $cm^{-1}$ $M^{-1}$ on the basis of the known extinction coefficients for IVMe and VH and the computer-generated values of $X$, $Y$, and $Z$ (eq 14). These considerations provide strong support for the sequence of events depicted in Scheme IV.

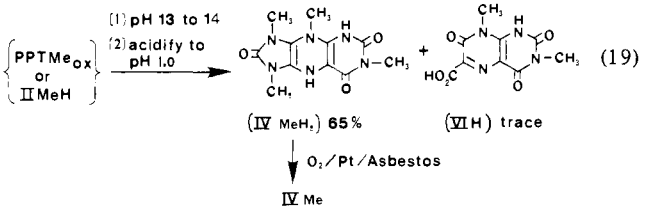
Scheme V



Scheme VI

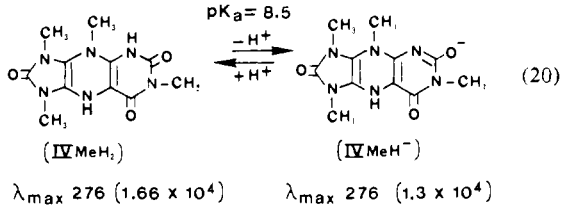


**The remainder of the discussion of hydrolytic reactions deals with the mechanisms of the specific base catalyzed ring contractions** and conversions stemming from IIMe$^-$ (Scheme IV). The formation of IVMe from IIMe involves an oxidative ring contraction with the loss of $CO_2$. However, IIMe$^-$ also undergoes a ring contraction in the absence of oxygen to provide $IVMeH_2$. This is brought about by treating $IIMeH_2$ (or $PPTMe_{ox}$) with strong base followed by treatment with acid (eq 19). The $pK_a$ of IV-



(19)

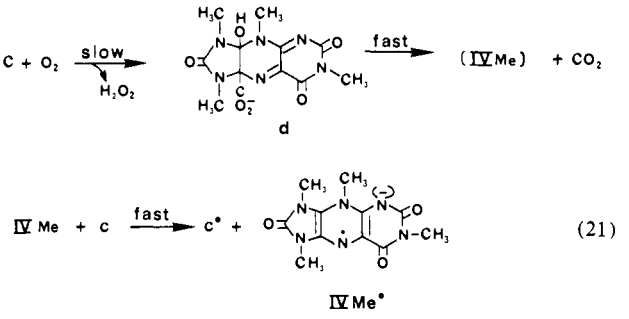
$MeH_2$ was determined by volumetric titration since both conjugate acid and its base exhibit indistinguishable spectra (eq 20).



(20)

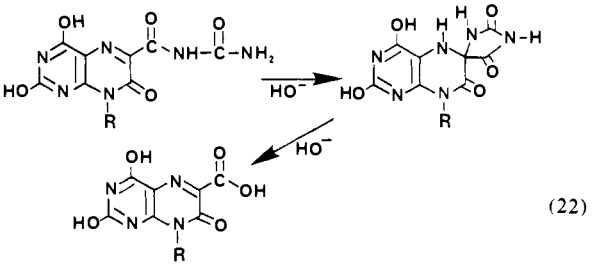
Compound $IVMeH_2$ exhibits resistance to oxidation in the solid form and oxidizes only slowly in neutral solution. Preparative oxidation of $IVMeH_2$ to IVMe was carried out with $O_2$ in the presence of Pt/asbestos (eq 19).

In Scheme V, species IIMe$^-$, a, b, and c are envisioned as being in equilibrium at pH 13 with the free energy contents of a, b, and c exceeding that of IIMe$^-$. If IIMe$^-$ is dissolved in an alkaline solution which is then immediately acidified to pH 1, there is obtained $IVMeH_2$ and a trace of VIH (eq 19). On the other hand, allowing the alkaline solution to stand, or better yet, reflux prior to acidification provides only VIH. While IIMe$^-$ is stable in pH 6.00 buffer under anaerobic conditions, it is converted to $IVMe_{ox}$ (430 nm) via a rate process which is zero order in IIMe$^-$ in the presence of oxygen (Figure 5). We propose that IIMe$^-$ plus a, b, and c are also in equilibrium at pH 6.0 and that IVMe arises by the autocatalytic oxidation of the 1,5-dihydropteridine c (eq 21). Ample precedence exists for the proposed sequence of eq 21.[35]



(21)

Formation of the postulated intermediates a, b, and/or c is supported by the shift in the $\lambda_{max}$ of IIMe$^-$ from 351 nm (pH 6.0) to 274 nm when added to 0.1 N KOH. The large hypsochromic shift is expected given the pyrimidine-like character of these intermediates. Spirohydantoin formation from carboxy ureides, as in IIMe$^-$ → a, have been observed with other pteridine carboxy ureides,[36] as well as quinoxaline carboxy ureides[37,38] under alkaline conditions. A large hypsochromic shift for carboxyureide → spirohydantoin was observed in both these systems. The chemistry of pteridine N-methylated spirohydantoins in strong base has been explored by Taylor and Loux[36] (eq 22). In agreement with our

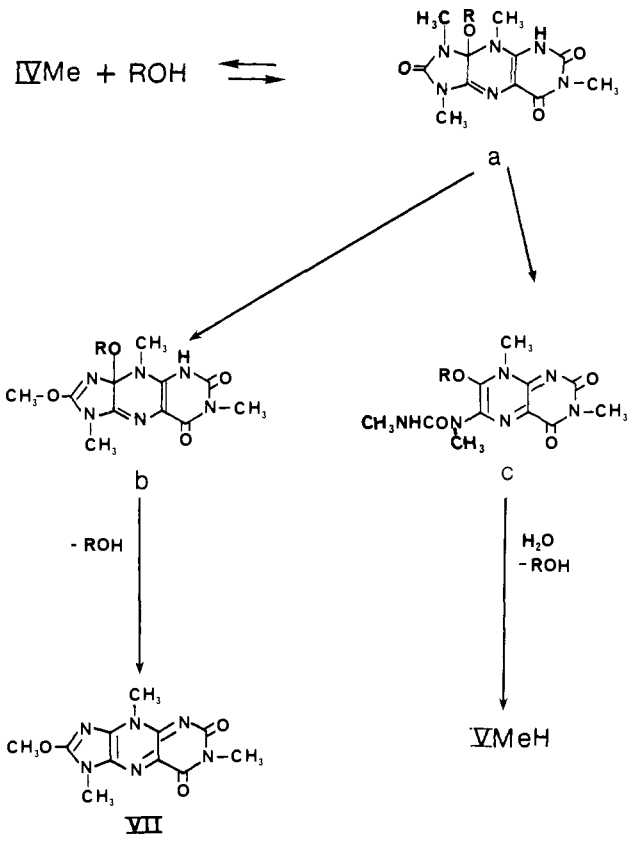


(22)

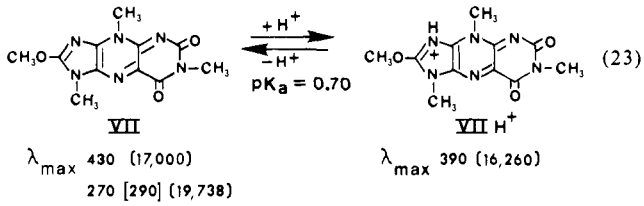
finding, b → VI$^-$, the formation of a pteridine carboxylic acid was observed. The lack of expected IVMeH$^-$ hydrolysis products supports the contention that this product could only arise from acid-catalyzed decarboxylation of c.

(35) Kemal, C.; Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 7272.
(36) Taylor, E. C.; Loux, H. M. *J. Am. Chem. Soc.* **1959**, *81*, 2427.
(37) Clark-Lewis, T. W. *J. Chem. Soc.* **1957**, 422.
(38) Guttman, D. E.; Platek, P. E. *J. Pharm. Sci.* **1967**, *56*, 1423.

Scheme VII

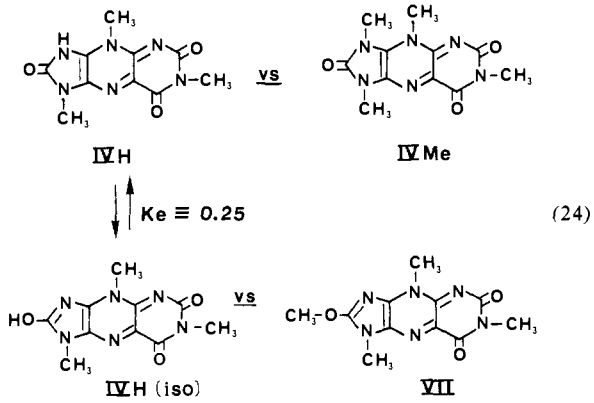



**Methanolysis of IVMe** ($6.5 \times 10^{-5}$ M) at 30 °C results in the formation of a fluorescent product with $\lambda_{max}$ 430 nm. The first-order rate constant for this process is $2.55 \times 10^{-5}$ $s^{-1}$. Preparative solvolysis of IVMe in boiling methanol yielded VII (Scheme VI). Solutions of VII in strong acid exhibit a loss of fluorescence due to protonation (eq 23). The species VII and




(23)

$VIIH^+$ are reasonably stable at acidic pH. The similarity in visible spectra of IVH and VII is not unexpected since VII is the stable isoimidazolone form of IVH (eq 24). Similarly, IVMe is the

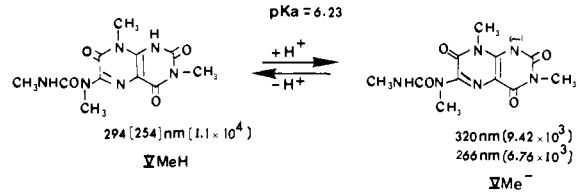



(24)

stable keto form of IVH. The $K_e$ for the keto–enol tautomerization of IVH was calculated to be 0.25 by using $\epsilon_{430}$ values in 0.33 M, pH 7.0, phosphate buffer for IVH (8000), VII (17 000), and IVMe (5700). Similarly, the benzoimidazolones and imidazo[4,5-*b*]-pyrazin-2-ones exist predominately in their keto forms.[39]

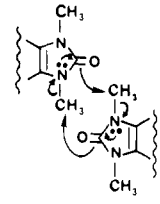
As expected, VII is hydrolyzed to IVH (pH 7.00, 0.33–0.033 M phosphate buffer), and from IVH, there arises VH. From repetitive spectral scans of VII ($6.63 \times 10^{-5}$ M) in aqueous 0.33 M phosphate buffer (pH 7.0), it was seen that the decrease in [VII] observed at 430 and 270 nm was accompanied by an increase in absorbance at 287 nm. The product had the spectrum of IVH. In time, this product hydrolyzed to give a spectrum identical with that of VH. Absorbance vs. time data for the hydrolysis of VII ($6.63 \times 10^{-5}$ M) in pH 7.00 (0.33–0.033 M) phosphate buffer was fit to a biphasic consecutive two first-order equation (eq 14). The pseudo-first-order rate constant for VII → IVH was $6.3 \times 10^{-4}$ $s^{-1}$ and that for IVH → VH was $1.75 \times 10^{-5}$ $s^{-1}$. No dependence of either rate on $[B_T]$ was found. By use of eq 14, the value of $\epsilon_{430}$ for IVH was calculated to be 7670 $cm^{-1}$ $M^{-1}$ in good agreement with the value of 8055 $cm^{-1}$ $M^{-1}$ calculated earlier. The $R_f$ of this intermediate on silica gel [1-butanol–acetic acid–$H_2O$ (5:2:3)] was identical with that of the intermediate observed during the hydrolysis of IVMe. Formation of VII could occur by $N^8$-demethylation of IVMe to give IVH, which upon addition of methanol and elimination of water, results in VII. Alternatively, an $N^8$ to oxygen methyl migration may be considered (Scheme VI).

**Intramolecular methyl migration from N → O** in the conversion of IVMe → VII was shown by the following experiments. The fluorescent product VII ($\lambda_{max}$ 430 nm) is formed from IVMe ($6.5 \times 10^{-5}$ M) in ethanol. The reaction is first-order in IVMe ($3.4 \times 10^{-5}$ $s^{-1}$ at 30 °C). Preparative solvolysis of IVMe in refluxing ethanol (Experimental Section) allowed the isolation of VII along with a new product VMeH (Scheme VII and eq 25). That –OMe



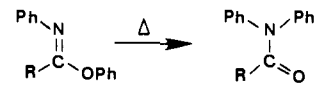

(25)

was not exchanged for –OEt excludes pathway A of Scheme VI. Furthermore, the first-order conversion of IVMe to VII in ethanol and methanol excludes the bimolecular methyl transfer of eq 26:



(26)

To the best of our knowledge, N → O four center methyl migrations involving isomerization of a urea structure to an isourea are unknown. The sterically less stringent O → N four center intramolecular acyl migrations of *O*-acylisoureas to provide *N*-acylureas are of course facile.[40] The O → N *intermolecular* methyl migrations which accompany the heating (200 °C, etc.) of many *O*-methyl heterocycles are common knowledge.[41] The Chapman rearrangement of *N*-arylbenzimidates to *N*-aryldiphenylamines (eq 27) has been shown to be intramolecular[42] as



(27)

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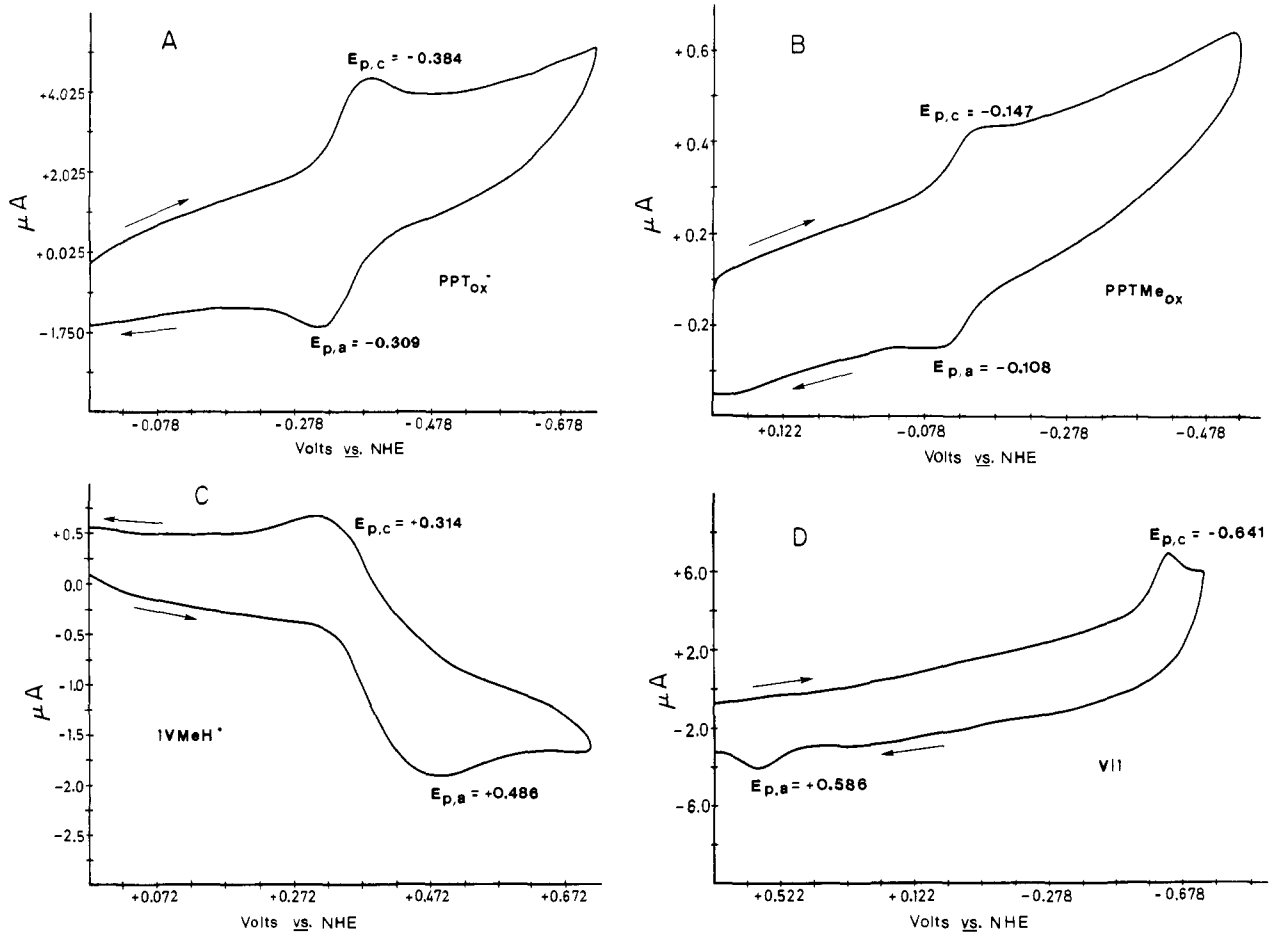
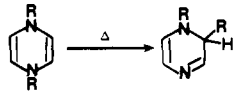


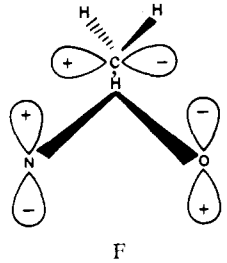

**Figure 7.** Cyclic voltammograms vs. NHE of $5 \times 10^{-4}$ M compound in 0.33 M, pH 7.00, phosphate buffer, $\mu$ = 1.0 with KCl, at room temperature. (A) For $PPT_{ox}^-$, measurement was made with a cathodic scan at a rate of 20 mV/s. (B) For $PPTMe_{ox}$, measurement was made with a cathodic scan at a rate of 200 mV/s. (C) For IVMeH, measurement was made with an anodic scan at a rate of 50 mV/s. (D) For VII, measurement was made with a cathodic scan at a rate of 5000 mV/s.

have thermal [1,3] alkyl shifts in 1,4-dialkyl-1,4-dihydropyrazines (eq 28).[41] The observation that inversion of the migrating R group



(28)

[R = (*S*)-CHDPh] occurs in the latter is consistent with a concerted [1,3] sigmatropic shift with suprafacial enamine utilization.[43] The intramolecular nature of IVMe → VII suggests that this process is in operation. A probable driving force is the relief of peristrain between the $N^8$- and $N^9$-methyl substituents rather than an increase in conjugation. Thus, the imidazolone form of IVH is favored over the iso form (eq 24). A requirement for suprafacial N → O methyl migration is distortion of the planar $N^8$-methyl C-7 oxygen grouping since the methyl would use both lobes of an antisymmetric orbital (structure F). The facility of



F

the rearrangement in methanol and ethanol is not completely understood. Perhaps there are two mechanisms; the first involving a thermal distortion of the amide bond and the second involving addition of alcohol at position 8a of IVMe to form species a of Scheme VII. Nucleophilic addition of this nature could destablize the planar amide by loss of conjugation with the 5a–8a carbon double bond. In the former process, VII is obtained directly on methyl migration, while in the latter species, b is the result of methyl migration which then yields VII on elimination of alcohol (Scheme VII). The rate of this elimination reaction would be required to exceed the addition of ROH to the 7,8-imine double bond of IVMe in order to preclude exchange of the methoxy group of species b. The much slower reactions with bulky alcohols may be accounted for by $N^8$- and $N^9$-methyl group steric inhibition. Evidence that attack at the 8a-position of IVMe by alcohols does occur is found in the formation of VMeH. It is, indeed, difficult to envision how VMeH might arise without invoking 8a-addition (Scheme VII). Of course, the formation of VMeH may involve 8a-substitution and the N → O methyl migration may not. The origin of VMeH is postulated to be from elimination of the $N^8$ in species a giving rise to species c which hydrolyses in the presence of adventitious water during workup (silica gel chromatography). Aqueous hydrolysis of IVMe or VII in pH 7.00, phosphate buffer yields exclusively VH. As stated previously, IVH is proposed to be formed on hydrolysis of either of these compounds. This is based on the finding that the hydrolysis reaction when followed spectrophotometrically (430 nm) or by TLC indicated the presence of a common intermediate. The formation of IVH from IVMe by $HO^-$ addition (Scheme IV) may then involve N → O methyl migration as shown for ROH addition (Scheme VII). When methyl migration is followed by hydrolysis and elimination of $HO^-$, IVH would be obtained (Scheme IV). That VII ($\epsilon_{430}$ = 1 000 cm

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$M^{-1}$) does not build up during hydrolysis of IVMe is apparent from the calculated $OD_{430}$ of the intermediate ($\epsilon_{430} \cong 8000$ cm $M^{-1}$). This was borne out by an analogue simulation (not shown) of a reaction sequence involving the intermediacy of VII in the hydrolysis of IVMe. A possible explanation is the rapid hydrolysis of the imidate (species b in Scheme VII, R = H). The complex chemistry of IVMe described here begs further investigation.

**Cyclic voltammetry of $PPT_{ox}$, $PPTMe_{ox}$, $IVMeH_2$, and VII** in $5 \times 10^{-4}$ M, pH 7.00, 0.33 M phosphate buffer, $\mu = 1.0$ (with KCl), was carried out with a carbon paste working electrode with an Ag/AgCl/1 M NaCl reference electrode (0.222 V vs. NHE). The reversibility of the couples was evaluated by varying scan speeds from 20 to 5000 mV/s and measuring $E_{p,c}$ and $E_{p,a}$ at these speeds. A reversible 2e couple has a maximum $E_{p,c} - E_{p,a}$ difference of 0.056 V, and this difference is independent of scan time. A dependence of $E_{p,c} - E_{p,a}$ on scan time and/or differences in $E_{p,c} - E_{p,a}$ greater than 0.056 V is evidence for a quasi-reversible process. Irreversible processes will not have either an $E_{p,c}$ or $E_{p,a}$ peak for the couple being studied.[44]

With $PPT_{ox}$ a cathodic scan (20 mV/s) revealed a quasi-reversible couple with $E_{p,c} = -0.384$ V vs. NHE and $E_{p,a} = -0.309$ V vs. NHE on the reverse scan (Figure 7A). Scan speeds of 5000, 500, 100, and 20 mV/s for $PPT_{ox}$ displayed $E_{p,c} - E_{p,a}$ values which increased with speed, showing the quasi-reversible properties of this system. The midpoint potential, $E^{\circ\prime}$ ($PPT_{ox}^{-}/PPT^{2-}$) = $(E_{p,c} + E_{p,a})/2 = -0.346$ V vs. NHE, was obtained from the 20 mV/s scan. The potential of −346 mV for two-electron reduction of $PPT_{ox}^{-} \rightarrow PPT^{2-}$ is ~150 mV more negative than that for riboflavin[45] and about equal to the $E^{\circ\prime}$ for $NAD^{+}$.[46] The magnitude of the negative potential for the couple $PPT_{ox}^{-}/PPT^{2-}$ reflects the resonance stability of $PPT_{ox}^{-}$ and the dienamine anion nature of $PPT^{2-}$. In the following study,[24] $PPT^{2-}$ is shown to be a good low-potential mimic for the monoanion of 1,5-dihydroflavin. Studies with $PPTMe_{ox}$ at 5000 and 50 mV/s revealed a significant difference in $E_{p,c} - E_{p,a}$, giving evidence of a quasi-reversible system. A negative scan with $PPTMe_{ox}$ gave an $E_{p,c} = -0.147$ V vs. NHE and $E_{p,a} = -0.108$ V vs. NHE on the reverse scan at 200 mV/s (Figure 7B). An $E^{\circ\prime}$ of −0.127 V vs. NHE was calculated. Replacement of a negative charge of the couple $PPT_{ox}^{-}/PPT^{2-}$ with a methyl group to provide the couple $PPTMe_{ox}/PPTMe^{-}$ destabilizes the oxidized species and reduces the reductive driving force. The result is that the $E^{\circ\prime}$ for $PPTMe/PPTMe^{-}$ is more positive by 219 mV than is the $E^{\circ\prime}$ for the couple $PPT_{ox}^{-}/PPT^{2-}$.

Because of the instability of IVMe in aqueous media, cyclic voltammetry was done with the reduced form, $IVMeH_2$. A positive scan of 50 mV/s gave $E_{p,a}$ of +0.486 V vs. NHE and $E_{p,c}$ of +0.314 on the reverse scan (Figure 7C). Scan speed had little effect on $E_{p,a} - E_{p,c} = 0.172$ V vs. NHE. However, the oxidation–reduction of $IVMeH_2$ is considered quasi-reversible because $E_{p,a} - E_{p,c} > 0.056$ V. The $E^{\circ\prime}$ for $IVMe_{ox}/IVMeH_2$ of +0.400 V vs. NHE was calculated from these data. The compound $IVMe_{ox}$ may prove to be a useful high potential mimic of oxidized flavin.

The only example of irreversible behavior was found with VII. A negative scan at 5000 mV/s gave an $E_{p,c}$ of −0.456 V vs. NHE and $E_{p,a}$ of +0.500 V vs. NHE on the reverse scan (Figure 7D). A rearrangement of $VIIIH_2$ to a compound with high redox potential is suspected.

**Acknowledgment.** This work was supported by grants from the National Institutes of Health and the National Science Foundation.

**Registry No.** $IMe^{-}$, 85282-64-0; $IIMe^{-}$, 85282-65-1; $IIMe^{-}K^{+}$, 85282-66-2; IIMeH, 85282-67-3; IIIH, 85282-63-9; $III^{-}$, 85282-62-8; IVMe, 85282-68-4; $IVMeH_2$, 85282-70-8; IVH, 85282-71-9; $IVMeH^{-}$, 85282-76-4; VMeH, 85282-72-0; VH, 85282-73-1; $VMe^{-}$, 85282-75-3; $V^{-}$, 85282-77-5; VII, 85282-69-5; $VIIIH^{+}$, 85282-74-2; $PPT_{ox}$, 82634-45-0; $PPTH_2$, 82639-48-3; $PPT^{\cdot}_{ox}$, 82639-46-1; $PPT^{2-}$, 82639-47-2; $PPTMe_{ox}$, 82639-53-0; $PPTH^{-}$, 82639-49-4; $PPTH_3^{+}$, 85282-78-6; 3-methyl-5-nitro-6-(methylamino)uracil, 944-48-9; 3-methyl-5-amino-6-(methylamino)uracil, 2278-13-9; 1,3-dimethyl-5-amino-6-(methylamino)uracil, 61541-46-6; *N*-methylalloxan, 2757-83-7; 1,3-dimethyl-5-nitroso-6-(methylamino)uricil, 5770-10-5; *N*-methylbarbituric acid, 2565-47-1.

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