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Inorganica Chimica Acta 223 (1994) 131–137

Inorganica Chimica Acta

# Analysis of $[(dien)Pd]^{2+}$ binding to uracil and azauracils by proton NMR spectroscopy

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Received by Editor 28 February 1994; received by Publisher 20 April 1994

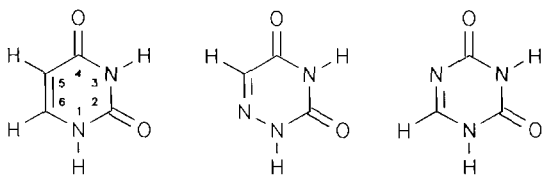
**Abstract**

An analysis of the binding of $[(dien)Pd]^{2+}$ to uracil, 6-azauracil and 5-azauracil has been performed by potentiometric pH titrations and proton NMR spectroscopy. Species of the type MBH predominate in the pH range 2–11 for a 1:1 molar ration of $[(dien)Pd]^{2+}$ and uracil. A doubly metalated complex $M_2B$ is observed at a significant concentration for pH values above 6 and reaches a maximum at pH≅11. A similar macroscopic species distribution is also observed for the 1:1 $[(dien)Pd]^{2+}$/6-azauracil system. N3 is the preferred binding site for both the uracilate and 6-azauracilate anions in complexes of the type MBH; whereas an N1 coordinated species MBH is observed for uracil this is not the case for 6-azauracil. In contrast to 6-azauracil and its anions for which no N6 coordinated species are registered in the $^1H$ NMR titrations, N5 coordinated complexes of the type $M_2BH$ and $MBH_2$ are observed as important species for the $[(dien)Pd]^{2+}$/5-azauracil system. Satisfactory agreement is obtained between the potentiometric and proton NMR spectrometric formation constants $\beta$ for the three equilibrium systems studied.

*Keywords:* Palladium complexes; Uracil complexes; Azauracil complexes; NMR spectroscopy

## 1. Introduction

Replacement of the 5-CH or 6-CH functions of uracil ($UH_2$) by an isoelectronic aza nitrogen leads to marked alterations in both biological activity and chemical properties of the resultant bases [1]. For instance, 6-azauracil ($6AUH_2$) inhibits the growth of solid animal tumours [2] and has been employed, in the form of its nucleoside 6-azauridine, for the treatment of leukemia [3]. Both 6-azauracil [4] and 5-azauracil ($5AUH_2$) [5] have also been shown to exhibit bacteriostatic and fungistatic properties.



$UH_2$ $6AUH_2$ $5AUH_2$

The introduction of a third nitrogen atom into the six-membered pyrimidine ring of uracil causes a significant perturbation of the endocyclic charge distribution. An MNDO calculation on 6-azauracil indicates that N6 in this azapyrimidine carries effectively no residual charge [6]. The absence of hydrogen bonding to N6 in the crystal structures of 6-azauracil, 6-azathymine and 6-azauridine [7] is in accordance with this finding. In contrast, N5 of 5-azauracil displays a charge similar to those of the other endocyclic nitrogen atoms in this modified pyrimidine base [6].

It has been postulated that an enhanced conformational flexibility of 6-azapyrimidines at the glycosidic N–C bond may be responsible for their altered biological activity in comparison to the parent bases [8]. The heterocyclic bases in 6-azauridine [7c] and 6-azacytidine [9] both adopt an unusual 'high-*anti*' conformation relative to the sugar ring ($\chi_{CN} = 76.5$–$99.1°$). This conformational range is energetically unfavourable for the parent pyrimidines, in which the 6-position carries a proton. Alterations in the charge distribution within the six-membered ring could, however, also play an important role. Respective values for the thermodynamic first dissociation constants of uracil, 1-methyluracil and 3-methyluracil are 9.43, 9.72 and 9.85 [10]. The $pK_{a1}$ values for the N-methyl derivatives indicate that the


*SSDI* 0020-1693(94)04008-J

basicity of N1 and N3 in uracil is very similar. Aza substitution of either the 5- or the 6-position leads to a marked reduction in the value of $pK_{a1}$ to 6.73 for $5AUH_2$ [11] and 7.00 for $6AUH_2$ [12]. The second dissociation constants of $5AUH_2$ and $6AUH_2$ have been reported as 12.2 [13] and 12.9 [10], respectively, values which are once again lower than that of 13.2 for uracil [10]. These $pK_{a2}$ values were determined spectrophotometrically at appropriate pH values and given as practical or Brønsted constants [14], i.e. no activity corrections were applied. In contrast to the parent base, however, the basicities of N1 and N3 in the azauracils are no longer similar to one another. Whereas N1 in 6-azauracil is more basic than N3, the reverse state of affairs is observed for 5-azauracil. The relevant $pK_{a1}$ values are 6.99 and 9.52 for 1-methyl- and 3-methyl-6-azauracil [15], 8.15 and 6.58 for 1-methyl- and 3-methyl-5-azauracil [11]. These findings suggest that the monoanions of $5AUH_2$ and $6AUH_2$ will be of importance at biologically relevant pH values, with N1 being preferentially deprotonated in the former and N3 in the latter case.

We have previously reported a systematic study of the interaction of the methylmercury(II) cation $CH_3Hg^+$ with 6-azauracil and 5-azauracil [6]. N3 coordination was established by X-ray structural analysis for [(CH$_3$Hg)6AUH], which may be prepared at pH values between 6 and 8. Using an excess of $CH_3HgOH$, 2:1 complexes with N1,N3 coordination may be isolated for $6AUH_2$ and $5AUH_2$ over a wide pH range (4–12 and 6–12, respectively). A 3:1 complex $[(CH_3Hg)_3$-5AU]$NO_3$, which displays N1,N3,N5 coordination, can be synthesised at pH values between 3 and 4. We were unable to isolate complexes of 6-azauracil or its anions in which N6 is coordinated, a finding which is in accordance with the predicted lack of a residual charge on this aza nitrogen.

To our knowledge further studies on the coordination properties of the azauracils have not been reported. As a result of the multiplicity of potential metal binding sites, potentiometric titrations will, as a rule, yield only macroscopic formation constants. Proton NMR spectroscopy allows the determination of individual formation constants for diamagnetic metal cations if the exchange of such ions between competing binding sites is slow on the NMR time scale. Martin and co-workers [16] have demonstrated that these preconditions are met by the cation $[(dien)Pd(H_2O)]^{2+}$ (dien = diethylenetriamine), which also offers the advantages of being a uniligating ligand. The electronic similarity between Pd(II) and its homologue Pt(II) suggest that studies with $[(dien)Pd(H_2O)]^{2+}$ should allow conclusions to be drawn for Pt(II) complexes, several of which (e.g. *cis*-$PtCl_2(NH_3)_2$) are well known as effective cancerostatica. We now report a potentiometric and proton NMR spectrometric study of the interaction of $[(dien)Pd(H_2O)]^{2+}$ with uracil, 6-azauracil and 5-azauracil in aqueous solution.

## 2. Experimental

Uracil, 6-azauracil and 5-azauracil were purchased from Sigma GmbH and used as received. Stock solutions of $[(dien)Pd(H_2O)]^{2+}$ at appropriate molar strength for potentiometric pH titrations were prepared by addition of 2 equiv. of $AgNO_3$ to a solution of [(dien)PdCl]Cl [17] in $H_2O$ followed by stirring for 12 h in the dark. After filtration of the precipitate, the yellow solution was diluted with $H_2O$ to give the required molar strength and used immediately. Potentiometric titrations were performed with a fully automated microprocessor controlled pH-titration unit (Metrohm 682 with Dosimat 665) in a thermostated vessel at 25 °C under argon with carbonate-free 0.1 M NaOH. A background ionic strength of 0.5 M $KNO_3$ (p.a.) was employed for all titration solutions. A double-junction glass electrode (Metrohm 6.0219.100) containing a saturated $KNO_3$ solution between the membrane of the internal silver chloride reference electrode and an outer membrane was used to prevent diffusion of chloride ions into the titration solution, which could lead to precipitation of [(dien)PdCl]Cl. Calibration of the pH meter was performed with standard buffer solutions (Riedel–de Haën pH 4.008, 6.865, 9.180). The use of such buffer solutions means that the measured pH value ($pH_{meas}$) will depend on the electrode, the background ionic strength, the junction potential and the activity coefficient of $H^+$ [14]. As proposed by Sigel et al. [14b], the conversion factor $A$,

$$A = pH_{meas} - p[H]_{calc/conc}$$

for adjustments between the practical and concentration pH scales, was determined by pointwise evaluation of titration curves of $HNO_3$ with NaOH at a background ionic strength of 0.5 M $KNO_3$. A value for $A$ of $0.053 \pm 0.024$ was provided by the average difference between the pH values measured with the calibrated electrode, $pH_{meas}$, and the concentration value, $p[H]_{calc/conc}$, calculated for points on the $HNO_3$/NaOH titration curve. Formation constants $\beta$ (reported in Table 1) were calculated by use of the program MINIQUAD [18] with potentiometric pH-titration data for 1:1 and 2:1 stoichiometric ratios of $[(dien)Pd(H_2O)]^{2+}$ and the nucleobases uracil and 5-azauracil. These molar ratios were supplemented by a 1:2 titration in the case of 6-azauracil. The reliability indices $R$ (Table 1) are defined as

$$R = \left[ \sum_i (T_i^{obs} - T_i^{calc})^2 / \sum_i (T_i^{obs})^2 \right]^{1/2}$$

in which $T_i$ represents the total concentration of component $i$ (M, B or H). Stock solutions of $[(dien)Pd(D_2O)]^{2+}$ for proton NMR spectrometric titrations were prepared in an analogous manner to $[(dien)Pd(H_2O)]^{2+}$. Titrations were performed with carbonate-free 1 M NaOD for equimolar solutions of $[(dien)Pd(D_2O)]^{2+}$ and the appropriate nucleobase at a background ionic strength of 0.5 M $KNO_3$. The equipment used was identical to that employed for the potentiometric studies. The potential alteration in the total cation and nucleobase concentration caused by addition of NaOD was immediately compensated for by the addition of an identical volume of a $D_2O$ solution of $[(dien)Pd(D_2O)]^{2+}$ and base at double molar strength. After addition of NaOD, the solution was allowed to stand until pH constancy had been achieved (at least 20 min) before samples were taken for $^1H$ NMR measurements on a Bruker AM 400 spectrometer at 25 °C. Evaluation of the proton NMR spectrometric titrations was performed with the program GLKONST [19]. An initial calculation of acidity constants for the deprotonation of species $M_xB_yH_z$ (M = $[(dien)Pd]^{2+}$, B = nucleobase, H = proton), as evidenced by a definite step in the chemical shift $\delta$, is carried out by a least-squares fit of $\delta$ values versus pH. The program subsequently minimises the function

$$\sum_i^I \left[ \left\{ \sum_j^J ([M_s]_{obs})_j - \sum_j^J ([M_s]_{calc})_j \right\}^2 \right]_i$$

for $I$ pH values, where $([M_s]_{obs})_j$ represents the observed concentration and $([M_s]_{calc})_j$ the calculated concentration for each of the $J$ species, whose formation constants $\beta$ are refined by least-squares. Two reliability indices $R_1$ and $R_2$ can be employed for hypothesis testing during the process of establishing species distribution diagrams.

$$R_1 = \left( \sum_i^I \left[ \left\{ \sum_j^J ([M_s]_{obs})_j - \sum_j^J ([M_s]_{calc})_j \right\}^2 \right]_i \Big/ \sum_i^I \left[ \left\{ \sum_j^J ([M_s]_{obs})_j \right\}^2 \right]_i \right)^{1/2}$$

$$R_2 = \left( \sum_i^I \left[ \sum_j^J \left\{ ([M_s]_{obs})_j - ([M_s]_{calc})_j \right\}^2 \right]_i \Big/ \sum_i^I \left[ \sum_j^J \left\{ ([M_s]_{obs})_j \right\}^2 \right]_i \right)^{1/2}$$

It is conventional to define $pH_D = pH_{meas} + 0.40$. Acidity constants $pK_D$ determined in $D_2O$ are approximately related to those in $H_2O$ ($PK_H$) by the equation

$$pK_D = 1.015(pK_H) + 0.45$$

plus a possible term for charge effects [20]. Since the constant terms in the above equations effectively cancel each other for many molecules, the observed $pK_a$ in $D_2O$ resembles that determined in water. In the present work we have, therefore, adopted the convention of Martin and co-workers [21] and report uncorrected $\beta$ values for the H NMR spectrometric titrations. The deprotonation of the coordinated water in the cation $[(dien)Pd(H_2O)]^{2+}$ proceeds via a hydroxo-bridged dinuclear cation $[(dien)Pd(\mu\text{-}OH)Pd(dien)]^{3+}$ for which we determined a formation constant $\log K_1$ of $-5.49$ by potentiometric pH titration. The value of the acidity constant for $[(dien)Pd(H_2O)]^{2+}$ calculated in the course of our work was 7.67. The presence of the species $[(dien)Pd(\mu\text{-}OH)Pd(dien)]^{3+}$ and $[(dien)Pd(OH)]$ was allowed for in the analysis of the potentiometric and proton NMR spectrometric titrations reported in this paper.

## 3. Results and discussion

Macroscopic formation constants $\log \beta$ and acidity constants $pK_a$ determined at 25 °C for $[(dien)Pd]^{2+}$ complexes of uracil, 6-azauracil and 5-azauracil by potentiometric pH titrations are listed in Table 1. The $pK_a$ values may be converted to the activity scale by addition of $0.10 = -\log \gamma(H^+, I = 0.5\ M) - A$. Corrected acidity constants $pK_{a1}$ (9.44, 7.00 and 6.72) obtained for the three bases studied in this work are in very good agreement with the literature values of 9.43, 7.00 and 6.73 [10,12]. Table 2 contains macroscopic and

Table 1
Macroscopic formation constants $\log \beta$ and acidity constants $pK_a$ determined for $[(dien)Pd]^{2+}$ complexes of uracil and azauracils at 25 °C by potentiometric pH titrations (background ionic strength 0.5 M $KNO_3$)[a]

| Base | Uracil | 6-Azauracil | 5-Azauracil |
|---|---|---|---|
| $\log \beta(BH_2)$ | 22.54(1) | 19.80(1) | 6.62(1) |
| $\log \beta(BH)$ | 13.2[b] | 12.9[b] | 12.2[b] |
| $\log \beta(MBH_2)$ | | | 21.7(1) |
| $\log \beta(MBH)$ | 21.07(1) | 19.97(3) | 18.91(7) |
| $\log \beta(MB)$ | 9.93(4) | 9.88(3) | 10.18(7) |
| $\log \beta(M_2BH)$ | | | 22.7(2) |
| $\log \beta(M_2B)$ | 17.56(5) | 17.31(4) | 17.3(1) |
| $\log \beta(M_3B)$ | | | 22.4(1) |
| $pK_a(BH_2/BH)$ | 9.34(1) | 6.90(1) | 6.62(1) |
| $pK_a(MBH_2/MBH)$ | | | 2.8 |
| $pK_a(MBH/MB)$ | 11.14 | 10.09 | 8.73 |
| $pK_a(M_2BH_2/M_2B)$ | | | 5.4 |
| $R$ | 0.010 | 0.014 | 0.007 |

[a]Reported on the practical pH scale; the conversion term $A$ to the concentration scale was determined experimentally for the pH titration unit as $0.053 \pm 0.024$ [14].

[b]Spectrophotometrically determined literature values [10,13].

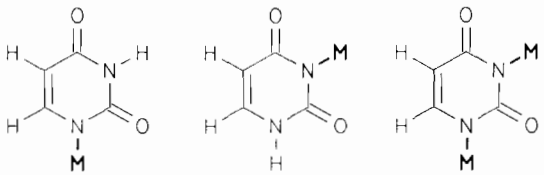
Table 2
Macroscopic and microscopic formation constants log $\beta$ and acidity constants p$K_a$ determined for [(dien)Pd]$^{2+}$ complexes of uracil and azauracils at 25 °C by proton NMR spectroscopy (background ionic strength 0.5 M $KNO_3$)[a]

| Base | Uracil | 6-Azauracil | 5-Azauracil |
|---|---|---|---|
| Macroscopic constants | | | |
| log $\beta$($MBH_2$) | | | 21.65 |
| log $\beta$(MBH) | 21.08 | 20.23 | 18.96 |
| log $\beta$(MB) | 9.79 | 9.45 | 10.85 |
| log $\beta$($M_2BH$) | | | 23.15 |
| log $\beta$($M_2B$) | 17.44(1) | 17.34(1) | 16.98 |
| p$K_a$($MBH_2$/MBH) | | | 2.69 |
| p$K_a$(MBH/MB) | 11.25 | 10.78 | 8.11 |
| p$K_a$($M_2BH_2$/$M_2B$) | | | 6.17 |
| Microscopic constants | | | |
| log $\beta$($M^1BH$) | 20.33(1) | | 18.83(1)[b] |
| log $\beta$($M^3BH$) | 21.00(1) | 20.23(1) | [b] |
| log $\beta$($M^5BH$)[c] | | | 18.36(5) |
| log $\beta$($M^1B$) | 9.40(1) | 9.27(1) | 10.85(10)[b] |
| log $\beta$($M^3B$) | 9.55(8) | 8.96(6) | [b] |
| log $\beta$($M^1M^5BH^3$)[c] | | | 23.04(1) |
| log $\beta$($M^1M^3BH^5$) | | | 22.49(1) |
| log $\beta$($M^1M^3B$) | 17.44(1) | 17.34(1) | 16.98(1) |
| p$K_a$($M^1BH$/$M^1B$) | 10.93 | | |
| p$K_a$($M^3BH$/$M^3B$) | 10.45 | 10.96 | |
| p$K_a$($M^1M^3BH$/$M^1M^3B$) | | | 5.51 |
| $R_1$ | 0.069 | 0.040 | 0.096 |
| $R_2$ | 0.084 | 0.052 | 0.166 |

[a]Reported on the practical pH scale; the conversion term $A$ to the concentration scale was determined experimentally for the pH titration unit as $0.053 \pm 0.024$ [14].
[b]Signals for $M^1BH$/$M^3BH$ and $M^1B$/$M^3B$ could not be resolved.
[c]Tentative assignment of the coordination sites.

microscopic formation constants log $\beta$ determined by proton NMR spectroscopy.

The potentiometric study of the [(dien)Pd]$^{2+}$/uracil system demonstrates that for a 1:1 molar ratio of the components (0.01 M), species of the type MBH predominate in the pH range 2–11. Such complexes may then be deprotonated in strongly alkaline solution to give species MB, which assume an important role at pH values above 11. A doubly metalated complex $M_2B$ is observed at a significant concentration for pH values above 6 and reaches a maximum at pH $\cong$ 11. This latter species was ignored by Lim, who performed a previous potentiometric pH study of the [(dien)Pd]$^{2+}$/uracil system [22]. Four separate H6 doublets were observed in the proton NMR spectra of [(dien)Pd]$^{2+}$/uracil solutions, (1:1 molar ratio, 0.01 M) which were recorded in the pH range 2.8–11.5. These display chemical shifts between 7.3 and 7.6 ppm and were employed in preference to the H5 doublets for the analysis of [(dien)Pd]$^{2+}$ binding to uracil with the program GLKONST. They were assigned the signal numbers 1–4 in the order of increasing shift to higher field and a similar convention was used for the [(dien)Pd]$^{2+}$/6-azauracil and [(dien)Pd]$^{2+}$/5-azauracil systems. Resonance 2 ($\delta$ = 7.52 ppm at pH = 2.8) belongs to uracil itself ($BH_2$) and surprisingly displays no evaluable chemical shift step on deprotonation to the monoanion BH (p$K_{a1}$ = 9.34(1)). Signals 1 and 4 ($\delta$ = 7.56 and 7.33 at pH = 2.8), of which the latter represents the major species over the entire pH range studied, may be assigned solely to species MBH for pH values below 9. Resonance 3 ($\delta$ = 7.39 at pH = 6) belongs to the 2:1 species $M_2B$, which must exhibit N1,N3 coordination. It is only observed for pH values above 6. The species distribution for [(dien)Pd]$^{2+}$ binding to uracil is displayed in Fig. 1 with appropriate microscopic formation constants log $\beta$ listed in Table 2. Inspection of the integral values for signals 1 and 4 at pH values above 10 indicates that these must be average values for species MBH/MB. Inclusion of MB(1) and MB(4) in the refinement model leads to an improvement in $R_1$ from 0.075 to 0.069 and $R_2$ from 0.094 to 0.084. Agreement between the macroscopic formation constants log $\beta$ obtained by potentiometric pH and proton NMR titrations for the major species $M_2B$, MBH and MB is very satisfactory (Tables 1 and 2) and indicates that the latter method will be capable of providing reliable microscopic formation constants for complexes present in solution at relatively high concentrations.




Through an analysis of $^1H$–$^{195}Pt$ coupling constants and a study of N1- and N3-methyluracils Lippert et al. [23] were able to assign H6 resonances at 7.7 and 7.4 ppm to N1- and N3-platinated uracil, respectively. Both complexes displayed H5 signals at 5.7 ppm. Resonances 1 and 4 in the [(dien)Pd]$^{2+}$/uracil system display H6/H5 chemical shifts of 7.56/5.66 and 7.33/5.66 ppm, respectively, in the pH range 2.8–7. On the basis of the results for the analogous platinum complexes, we assign signal 1 to the species $M^1BH$, signal 4 to the species $M^3BH$, which predominates in the pH range 2.8–10.5 (log $\beta$($M^3BH$) − log $\beta$($M^1BH$) = 0.67). Our study indicates, however, that the ratio of the N3- to the N1-metalated species for MB at higher pH values is much smaller (log $\beta$($M^3B$) − log $\beta$($M^1B$) = 0.15). At a 2:1 molar ratio, the doubly metalated complex $M^1M^3B$ is the major species at pH values above 6.

As a result of the lower p$K_{a1}$ value for the first deprotonation of 6-azauracil in comparison to uracil (6.90(1)] versus 9.34(1)), the distribution curve for the

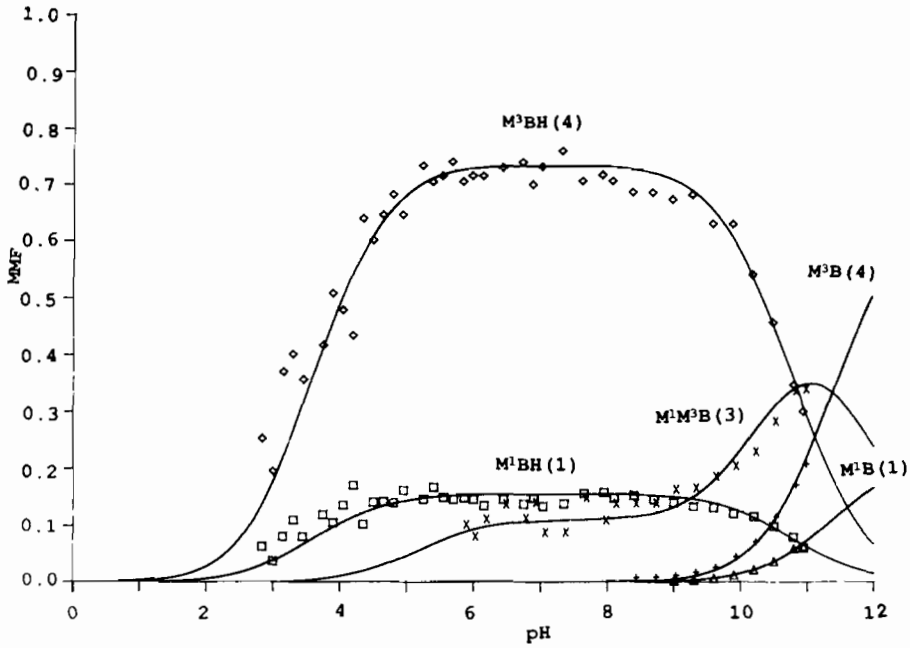



Fig. 1. Species distribution for uracil ($BH_2$) and $[(dien)Pd]^{2+}$ (M) at 0.01 M presented as metal cation mole fraction (MMF) vs. pH. The assigned $^1H$ NMR signals are given in parentheses.

species MBH is shifted approximately 2 units to lower pH values (Fig. 2). For a 1:1 molar ratio, the doubly metalated complex ($M^1M^3B$) is the major species in the $[(dien)Pd]^{2+}$/6-azauracil system at pH values above 9.4. The proton NMR spectroscopic study demonstrates that, in contrast to the $[(dien)Pd]^{2+}$/uracil system, only one MBH species, presumably $M^3BH$, is present at a measurable concentration in $[(dien)Pd]^{2+}$/6-azauracil solutions (Fig. 2). Four separate H5 resonances were observed for species present in this equilibrium system (1:1 molar ratio, 0.005 M) in the pH range 1.23–11.91. Signal 1 belongs to 6-azauracil ($BH_2$) itself ($\delta = 7.51$ at pH = 1.23) and disappears between pH values of 5 and 8, during which a shift of 0.18 ppm to higher field occurs, as a result of deprotonation to the monoanion BH. Signal 4 ($\delta = 7.23$ at pH = 1.23) belongs to a species

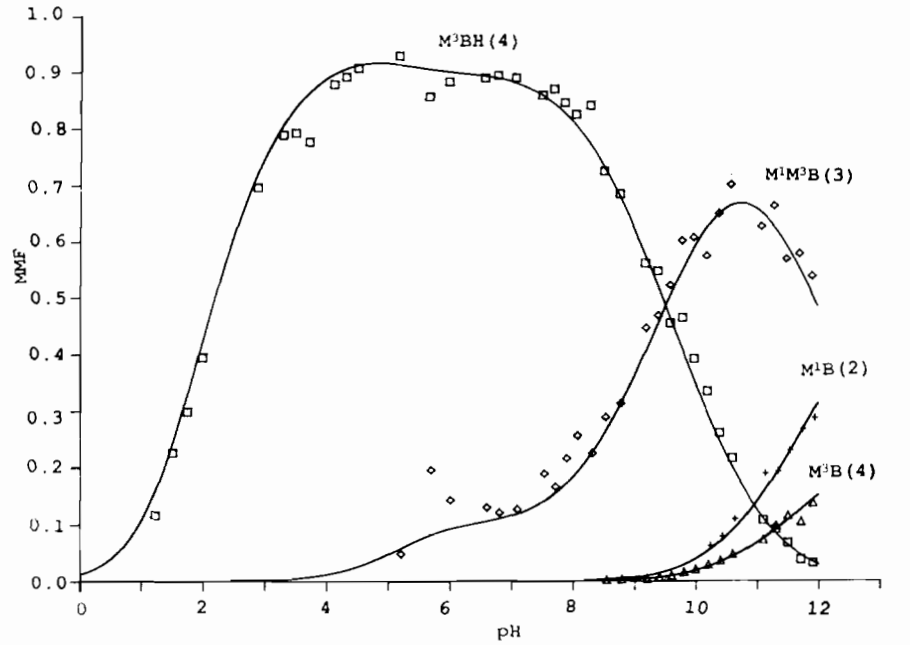



Fig. 2. Species distribution for 6-azauracil ($BH_2$) and $[(dien)Pd]^{2+}$ (M) at 0.01 M presented as metal cation mole fraction (MMF) vs. pH. The assigned $^1H$ NMR signals are given in parentheses.

MBH/MB, which is the major component at pH values below 9.4. At pH values above 10, the deprotonated species MB is present at appreciable concentrations, leading to a marked shift of the H5 resonance to lower field. Although a definitive assignment is not possible on the basis of the $^1$H NMR data, it is reasonable to assign signal 4 to the complexes $M^3BH/M^3B$. As discussed in the Introduction, N3 is the preferred deprotonation site for 6-azauracil [15] and has been established as the binding site in [$(CH_3Hg)$6AUH] [6]. Resonance 2 ($\delta = 7.46$ at pH = 10), which is only observed at pH values above 10, must then be assigned to the complex $M^1B$. As for the analogous uracil complex, the species $M_2B$, for which signal 3 is recorded at pH values above 4.8 ($\delta = 7.28$ at pH = 4.8), may be assumed to display N1,N3 coordination. Inclusion of MB(4) in the refinement model leads to an improvement in $R_1$ from 0.044 to 0.040 and $R_2$ from 0.075 to 0.052. Agreement between the potentiometric and proton NMR spectrometric formation constants log $\beta$ (Tables 1 and 2) may be regarded as satisfactory.

The present study indicates that N6 in 6-azauracil is not competitive as a coordination site for $[(dien)Pd]^{2+}$, confirming, thereby, our previous findings for the methylmercury cation. Although no species $M^1BH$ was observed, N1 is more attractive than N3 at high pH values in $[(dien)Pd]^{2+}$ complexes of the dianion $[6AU]^{2-}$ ($\log \beta(M^1B) - \log \beta(M^3B) = 0.31$).

A total of six separate H6 resonances was observed for species present in the equilibrium system $[(dien)Pd]^{2+}$/5-azauracil, in the pH range 1.06–7.56 (Fig. 3). Signal 4 ($\delta = 8.27$ at pH = 2.2), which may be assigned to $5AUH_2$ itself, shifts to a final value of 8.07 ppm at pH = 7.56, owing to deprotonation of the base at N1 in the pH range 5.5 to 8.0. Resonances 1 and 2 ($\delta = 8.68$ and 8.43 at pH = 1.06) both exhibit very pronounced steps to higher field, the former singlet shifting to 8.07 between pH values of 2.0 and 4.5, the latter to 7.86 between pH values of 4.0 and 6.5. As these alterations (−0.61 and −0.67 ppm) are much greater than that experienced for the free base itself (−0.20 ppm) upon deprotonation of N1, it is reasonable to assume that the associated species formally carry a positive charge at N5 in adjacent position to C6. Unfortunately signal 1 overlaps with signal 5 ($\delta = 8.07$ at pH = 3.7) at pH values above 4.5. Although it was not possible to include their individual contributions in the refinement by GLKONST, it may safely be assumed that both belong to 1:1 species MBH/MB. As signal 5 disappears at pH values below 3.7, it may be assigned to an N3-coordinated complex $M^3BH/M^3B$, with the major signal (1) belonging to species $M^1BH_2/M^1BH$, in which the preferred site N1 is used for metal binding. Signal 2 corresponds to $M^1M^3BH/M^1M^3B$ with the protonation site, as discussed earlier, being at H5. A second $M_2BH$ species produces resonance 3 ($\delta = 8.29$ at pH = 1.06) and predominates over the complex $M^1M^3BH(2)$ at pH values below 6. It does not, however, give rise to a deprotonated complex $M_2B$ and disappears at a pH value of 6.8. N5 must be coordinated in this species and it seems reasonable to assume that the second binding site will be the preferred nitrogen N1. Signal 6 belongs to a third MBH species and is only observed at pH values above 3.05 ($\delta = 8.05$ at pH = 3.05). It exhibits a very similar chemical shift

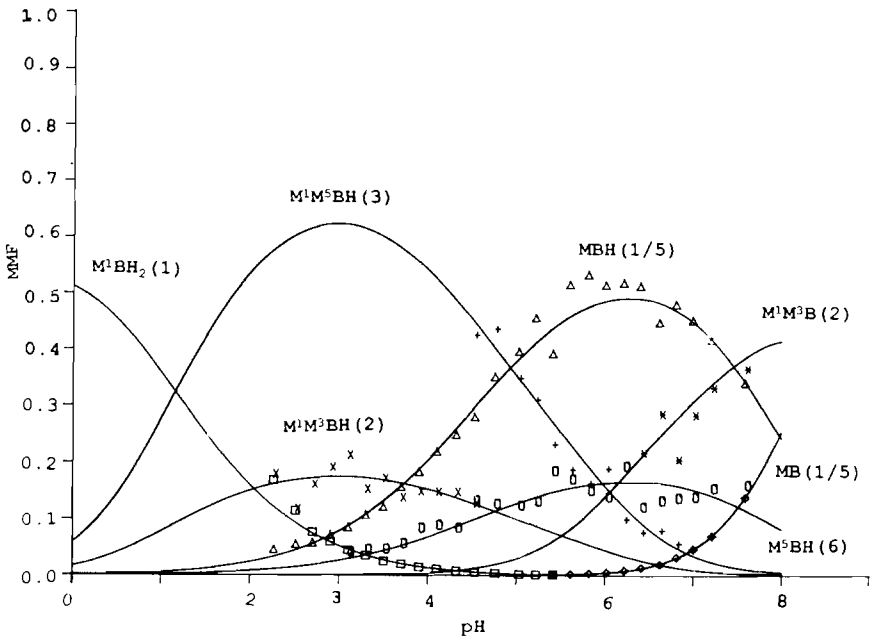
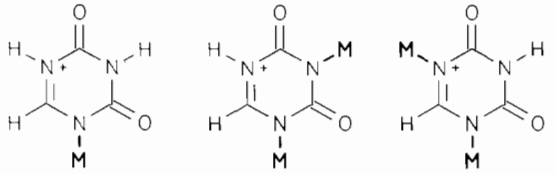



Fig. 3. Species distribution for 5-azauracil ($BH_2$) and $[(dien)Pd]^{2+}$ (M) at 0.01 M presented as metal cation mole fraction (MMF) vs. pH. The assigned $^1$H NMR signals are given in parentheses.

to those of $M^1BH$ and $M^3BH$ ($\delta = 8.07$) and may be assumed to belong to $M^5BH$.

The analysis of the proton NMR data was rendered more difficult by the fact that signals 3 ($M^1M^5BH$) and 4 ($BH_2$) overlap in the pH range 2.3–5.0 and cannot be separated to yield individual integrals. In the model used to obtain the log $\beta$ values reported in Table 2, the formation constant for species $M^1M^5BH(3)$ was, therefore, only refined in the pH range 5.0–6.8. Although the $R_1$ and $R_2$ values of 0.096 and 0.166 indicate



$M^1BH_2(1)$ $M^1M^3BH(2)$ $M^1M^5BH(3)$

that the quality of the proton NMR analysis is relatively moderate, the macroscopic formation constants log $\beta$ obtained by this method for species $MBH_2$ and MBH are not significantly different from those determined by potentiometric pH titrations. The differences in the log $\beta$ values listed in Tables 1 and 2 for species $M_2B$ and MB are not surprising, as the NMR titration data used in their determination is limited to resonances recorded between pH values of 6.45 and 7.56. Evaluation of the proton NMR spectra was not possible for pH values above 7.56 owing to overlapping of the signals 4 (BH), 5 and 6. Inclusion of a species $M_3B$ present at very low concentration in the pH range 2–7 for 1:1 titrations leads to a significant improvement in the $R$ factor for the potentiometric pH titrations performed with 1:1 and 2:1 molar ratios of $[(dien)Pd(H_2O)]^{2+}$ and 5-azauracil.

Inspection of the species distribution diagrams for uracil, 6-azauracil and 5-azauracil (Figs. 1–3) underlines the importance of N5 as both a coordination and protonation site in the latter modified pyrimidine. Complexes of the type $M_2BH$ in which N5 is either coordinated (signal 3) or protonated (signal 2) dominate in the pH range 1–5. At lower pH values, $M^1BH_2$, in which both N3 and N5 are protonated, is the major species. The present work demonstrates that proton NMR spectroscopy can provide reliable log $\beta$ values for complexes present over an adequate pH range at relatively high concentrations. This finding underlines the importance of this method for the determination of microscopic formation constants.

## 4. Supplementary material

Figures displaying the proton chemical shifts versus pH for aqueous solutions of $[(dien)Pd(H_2O)]^{2+}$ and the bases uracil (0.01 M), 6-azauracil (0.005 M) and 5-azauracil (0.01 M) are available from the authors upon request.

## Acknowledgement

This work was supported by the Fonds der Chemischen Industrie, Frankfurt.

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