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# Metal Complexes of Purine-*N*-Oxides. III. Proton Magnetic Resonance Investigation of Cu(II) Complexes of Adenine-$N^1$-Oxides

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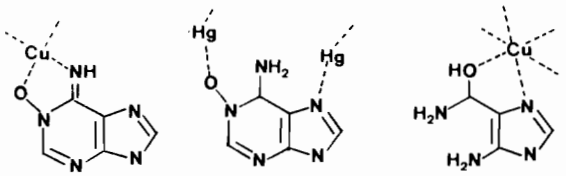
## Abstract

Adenine-$N^1$-oxide (AdNO) is shown to undergo a two-step hydrolysis at pH < 0.2. The reaction mechanism has been elucidated and the intermediate product has been characterized by NMR. Cu(II) coordination has been determined qualitatively based on spin–lattice ($T_1$) measurements at 90 and 400 MHz. At low pH the parent compound binds exclusively at the imidazole part, preferably at nitrogen N(9). In the hydrolyzed intermediate where the N(1)–C(2) bond is broken, the oxide oxygen is the major binding site. In the final hydrolysis product where C(2) is removed, only one non-exchangeable proton (H(8)) is left, prohibiting relative distance calculations.

## Introduction

Modified purine bases have long attracted much attention due to their potential chemotherapeutic value [1]. However, some of these derivatives, e.g. purine-*N*-oxides, are chemical oncogens with potencies comparable to those of the most active oncogenic polyaromatic hydrocarbons [1–2]. From previous studies we have found drastic differences in metal ion affinity between naturally occuring purines and certain derivatives. For example, hypoxanthine (6-oxy-purine) is a monodentate (N7) ligand while the thio-analog is shown to form chelates [3, 4]; an 8-azapurine has been found to bind at N(3) [5], a coordination site not found in the naturally occurring analog. Recent crystal structure determinations of metal–purine-$N^1$-oxides have revealed coordination patterns which are distinctly different from those of the parent compounds. A copper(II) complex obtained in basic solution (**I**) has been shown to be a 2:1 chelate where the metal binds at the $N^1$-oxygen and the deprotonated N(6)-amino group [6]. A Hg(II) complex crystallized from DMSO (**II**) is a dimer where AdNO acts as a bidentate bridging ligand, coordinating through N(7) and O(1) [7].




**I** **II** **III**

At acidic pH, AdNO is degraded in the presence of Cu(II), the C(2)-carbon being removed from the pyrimidine ring. The complex crystallizing from the reaction mixture is shown to be a O(1)–N(7) chelate (**III**) [8]. The crystallographic data imply metal ion catalysis of the degradation reaction, while some of the earlier metalation studies of purine-*N*-oxides in solution do not consider this possibility. Our $^1$H NMR results presented in this paper confirm the instability of the AdNO ligand at acidic pH. This fact complicates the interpretation of the metal binding studies monitored by $^1$H NMR relaxation measurements. These systems have been previously investigated by UV, IR and potentiometric methods [9–11].

## Experimental

Adenine-$N^1$-oxide (AdNO) was used as obtained from Sigma Chemical Co. Salts of Cu(II) were purchased from Fluka. Stock solutions of 0.1 M Cu$(NO_3)_2$ made up in $D_2O$ were adjusted to the appropriate pH by DCl and NaOD. The pH-values are not corrected for $D_2O$ solvent effects. The titration experiments were performed by incremental addition from micro pipettes.

Proton NMR spectra were recorded on 90 and 400 MHz Bruker instruments operating in the Fourier transform mode. The free induction decay over a 1500 Hz bandwidth contained 8000 data

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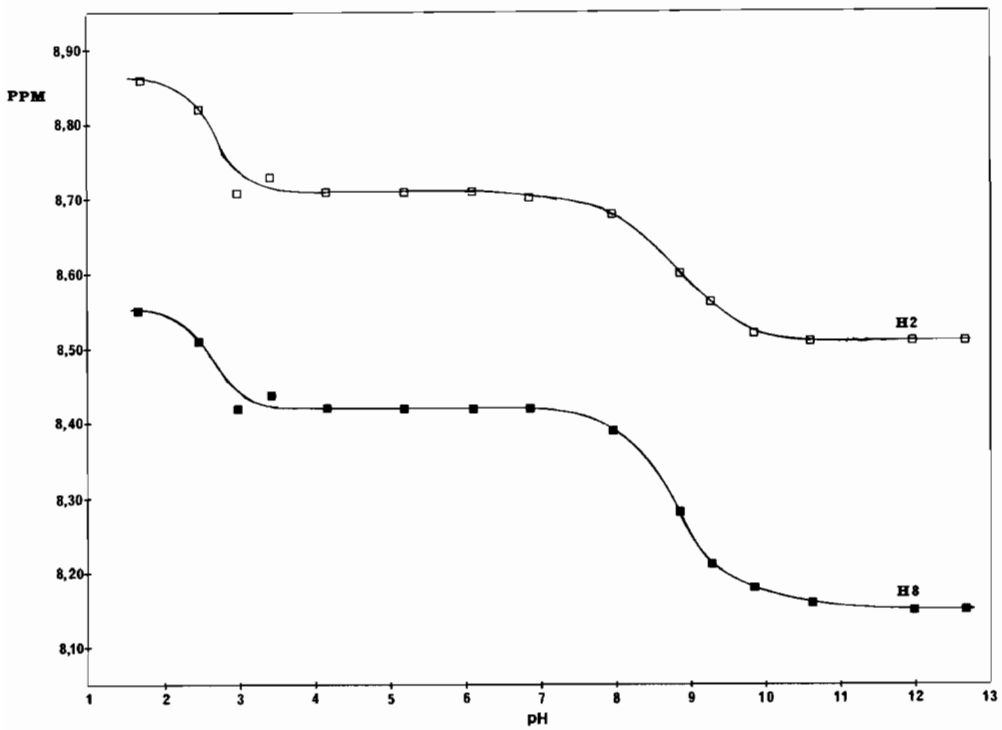



Fig. 1. Measured chemical shift ($\delta$) *vs.* pH profiles for AdNO in $D_2O$ at 27 °C (90 MHz).

points. To improve signal-to-noise, an exponential multiplication with added line broadening of 0.2 Hz was used. Prior to Fourier transform, 8000 zero filling was added. The HDO signal was used as an internal chemical shift reference. Spin–lattice relaxation times ($T_1$) were determined using a $180°-\tau-90°$ pulse sequence. Usually 12 $\tau$-values were used and the peak intensities were fitted to a single exponential decay curve by a three-parameter least-squares program.

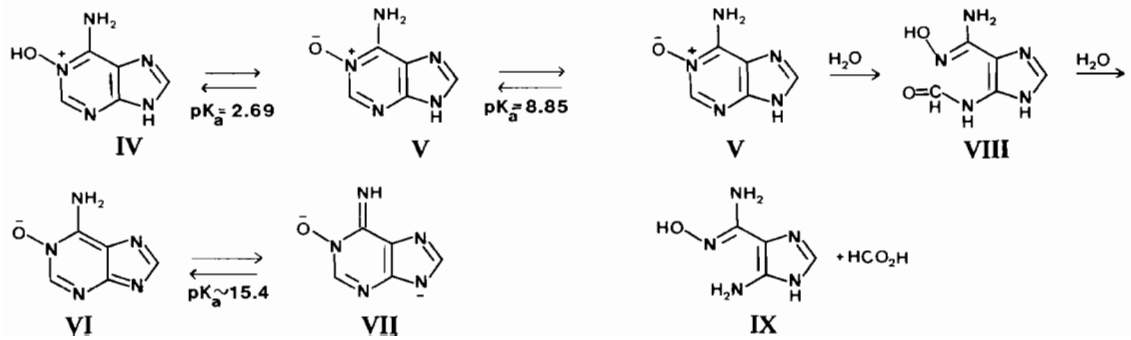
## Results and Discussion

*Stability of AdNO*

The pH-titration curves *vs.* shift of the two carbon bound protons in AdNO are shown in Fig. 1. The two distinct inflection points correspond to $pK_a$-values of 2.73 and 8.83, respectively. Perrin has reported these $pK_a$-values to 2.69 and 8.85 based on potentiometric measurements [11]. At $pK_a$ = 2.73 the $N^1$-oxygen is deprotonated. The influence on the pyrimidine ring is manifested in a relatively large shift of the H(2) resonance. At $pK_a$ = 8.85 the imidazole ring is deprotonated producing a significant shift of the H(8) proton.

Below pH = 0.3 a new pair of H(2), H(8) resonances shows up in the proton spectrum representing a new species (Fig. 2a). The conversion rate is relatively slow, and the reaction can be followed by comparing the integral intensities of the original and the new pair of resonances (Fig. 4). The kinetics at two different temperatures were monitored and gave a pseudo first order rate constant of 0.029 $M^{-1}$ and an activation energy of 123 kJ/M.

The reaction probably involves opening of the pyrimidine ring by an acid-catalysed breaking of the N(1)–



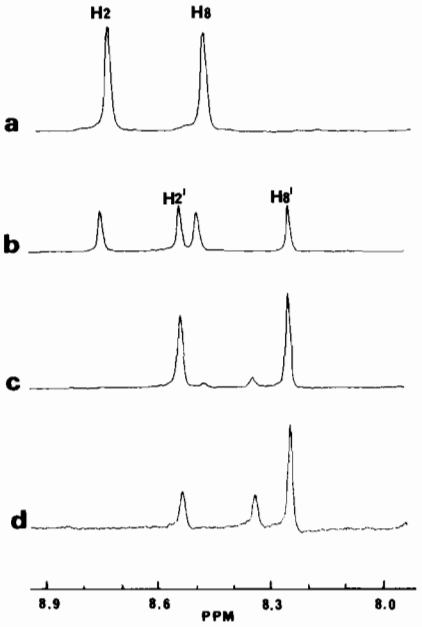



Fig. 2. $^1H$ NMR spectra of 0.05 M AdNO in $D_2O$, pH = 0.2, 90 MHz; (a) freshly prepared sample, (b), (c), (d) after ½, 1 and 3 h reflux, respectively.

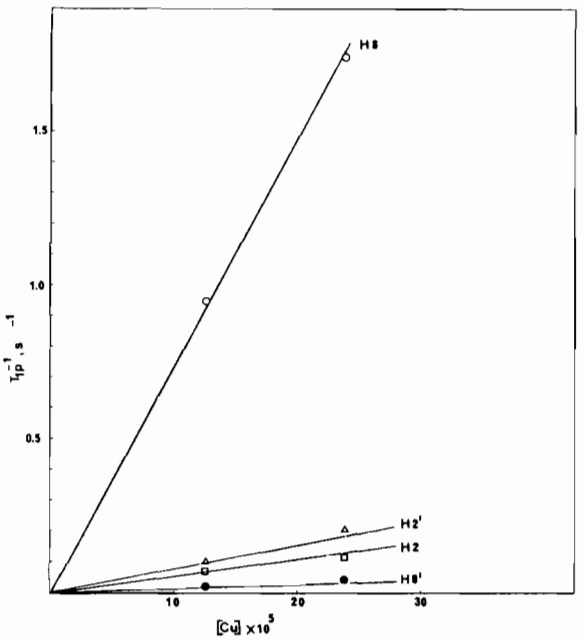



Fig. 3. Paramagnetic induced spin–lattice relaxation *vs.* metal concentration.

C(2) bond. The position of the H(2)′ resonance at 8.54 ppm is in the range for aldehydic protons.

The reaction is taken one step further by prolonged reflux at pH = 0.20 and 100 °C. The H(2)′ signal is seen to disappear (Fig. 2b,c) while a new peak emerges at 8.2 ppm. The hydrolytic splitting of the C(2)–N(3) bond produces formic acid. The C–H

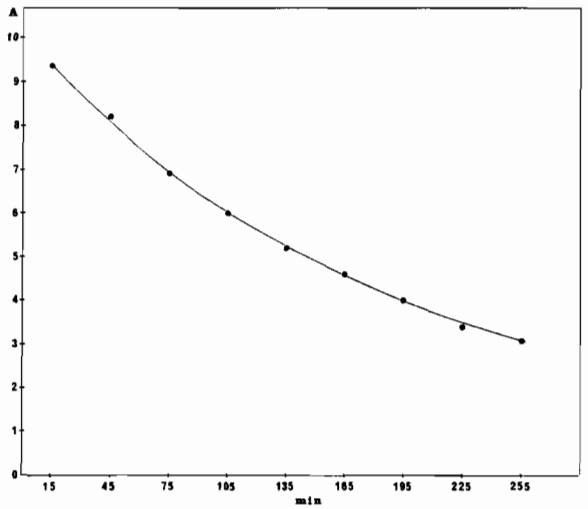



Fig. 4. Plot of the decrease in integral intensities of proton H(2) *vs.* time at pH = 0.2 and 61.7 °C. Each point corresponds to 30 min of data collection.

resonance in formic acid measured in $D_2O$ is 8.22 ppm. The overall degradation reaction of AdNO is reported to proceed to completion by refluxing for 10 min at 100 °C in 3 N HCl [9]. The final product **(IX)** has been shown to be the ligating species in the copper complex **(III)** obtained at pH ~ 0.3 [8]. Apparently the metal ions promote the hydrolytic step in the degradation reaction.

Spin–lattice relaxation times for AdNO protons show some unexpected variations between the two species (Table I). In the initial form at pH = 0.2 and

TABLE I. Proton Spin–Lattice Relaxation Times (s) in Presence of $Cu(NO_3)_2$; pH = 0.20, Temperature = 27 °C, $C_{AdNO}$ = 0.05 M

| $Cu^{++}$ | O | $1.25 \times 10^{-4}$ M | $2.5 \times 10^{-4}$ M |
|---|---|---|---|
| H(2) | 12.15 | 6.59 | 5.10 |
| H(8) | 5.62 | 0.89 | 0.52 |
| H(2)′ | 11.16 | 5.33 | 3.51 |
| H(8)′ | 17.89 | 13.32 | 10.43 |

400 MHz, $T_1$ values for H(2) and H(8) were determined to be 12.14 and 5.62 s, respectively. The corresponding values for the new species are 11.16 and 17.89 s. As can be seen, the $T_1$ values for H(2) and H(2)′ differ by less than 10% while the $T_1$ of H(8)′ has increased by a factor of about three compared to H(8). If the proposed reaction scheme is correct, one would expect a large change in relaxation of the pyrimidine proton H(2) while the imidazole proton H(8) should be less affected. Apparently the relaxation mechanism is more dependent on change in the anisotropic rotational correlation time than in intrinsic electronic changes. In order to

explain the anomaly, one may invoke change in the predominant rotation axis between the two species. If the molecular tumbling is shifted from, say, the C(8)–H(8) axis in **V** to the C(4)–C(5) axis in **VII**, an increase in $T_1$ for H(8) will be observed.

*Metal Binding*

Cu(II) markedly affected the relaxation times ($T_1$ and $T_2$) of bound ligand protons. The origin of this relaxation enhancement is either dipolar coupling between the unpaired electron on Cu(II) and a nucleus or through-bonds scalar interaction. For spin–lattice relaxation ($T_1$) the latter effect is almost always negligible [12]. Thus, in principle it is possible to obtain geometric information from $T_1$ measurements using the Solomon–Bloembergen equation for the inverse spin–lattice relaxation time for nuclei of ligands bound to paramagnetic ions [13, 14].

$$T_{1P}^{-1} = (6/15)pq\gamma_I^2\beta^2S(S+1)\tau_c r^{-6} \quad (1)$$

$T_{1P}$ is the induced paramagnetic relaxation enhancement, $p$ the ratio of molar concentration of paramagnetic metal ion to ligand, $q$ the average number of ligands bound in an identical way, $\tau_c$ the correlation time modulating the dipolar interaction and $r$ the distance between the paramagnetic ion and the measured nucleus.

Since $\tau_c$ is difficult to determine accurately, only relative bond distances between a paramagnetic ion and other ligand nuclei may be calculated.

$$(T_{1P}^{-1})_A/(T_{1P}^{-1})_B = (r_B/r_A)^6 \quad (2)$$

This relation will only give gross qualitative geometric information. Espersen and Martin [12] have pointed out the problem posed by distributed unpaired spin densities on the ligand. If these effects are neglected, an unrealistic degree of precision is anticipated, as has been demonstrated in studies of metal–nucleic acid base complexation [15].

In Fig. 3, a plot of $T_{1P}^{-1}$ *vs.* metal concentration is shown. The large enhancement in relaxation rate for H(8) compared to H(2) is a clear indication that N(7) and/or N(9) is the major coordination site at this pH. Using eqn. (2) a geometric factor of 0.65 is calculated, compared to $r_{H(8)}/r_{H(2)}$ ratios of 0.45 and 0.59 (from model) for N(7) and N(9) coordination, respectively. The corresponding value for $N^1$-oxide binding is about 1.8. Clearly the imidazole ring rather than the pyrimidine ring is involved in binding.

At low pH guanine shows Cu(II) binding at N(9) with N(7) being protonated [16]. In the AdNO complex the $T_1$-ratios also strongly indicate that N(9) is the major binding site. The difference between 0.65 and 0.59 is accounted for by invoking a small amount of N(3)···N(9) dinuclear species.

The metal coordination of the N(1)–C(2) hydrolyzed intermediate is shown by relaxation measurements to be quite different from that of the parent compound. The induced paramagnetic relaxation (Fig. 2) of H(2)′ and H(8)′ is comparable to that of H(2) and appreciably less than for H(8). Accordingly, the two metal–proton distances in the new species have to be of the same order of magnitude, and significantly larger than the $Cu_{N(9)}\cdots H(8)$ distance. Chemically, the most probable bonding pattern that fulfils this geometric requirement is a monodentate O(1) coordination. The $Cu_{O(1)}\cdots H(8)'$ distance is estimated to 6.7 Å from the Hg-AdNO structure where Hg binds O(1). The $Cu_{O(1)}\cdots H(2)'$ distance is more difficult to estimate since after opening of the pyrimidine ring at N(1)–C(2) a consecutive out-of-plane rotation of H(2)′ is expected.

From the $T_1$-data, the distance ratio $r_{H(2)'}/r_{H(8)'} = 0.76$. This value corresponds to $Cu_{O(1)}\cdots H(2)' = 5.1$ Å with $Cu_{O(1)}\cdots H(8)' = 6.7$ Å. From a molecular model, an estimated rotation of approximately 60° around N(3)–C(4) gives the appropriate metal–proton distance. Of course, other conformational changes in the pyrimidine fragments may be envisaged.

The above analysis concerning metal binding sites in AdNO is, as already emphasized, of a purely qualitative nature. In favourable cases one may be able to obtain accuracy in relative distance calculations to ±0.2 Å from $T_1$-data using the existing Solomon–Bloembergen formalism. Normally only gross binding patterns can be deduced with a certain degree of confidence. At low pH, AdNO in its parent state binds Cu(II) in the imidazole part and most strongly at N(9). The intermediate species is pyrimidine-bound, with the most likely binding site being the N(1) oxygen. In the fully hydrolyzed compound where C(2) is removed, relative distance information cannot be obtained from $^1H$ $T_1$- data.

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**References**

1 G. B. Brown, K. Suguira and R. M. Croswell, *Cancer Res., 25,* 986 (1965).
2 K. Suguira and G. B. Brown, *Cancer Res., 27,* 925 (1967).
3 E. Sletten, *Acta Crystallogr., Sect. B, 30,* 1961 (1974).

4 E. Sletten and A. Apeland, *Acta Crystallogr., Sect. B, 31,* 2019 (1975).
5 B. J. Graves and D. J. Hodgson, *Inorg. Chem., 20,* 2223 (1981).
6 E. Sletten, T. Marthinsen and J. Sletten, *Inorg. Chim. Acta, 93,* 37 (1984).
7 M. Damodara Poojary and H. Manohar, *Inorg. Chim. Acta, 93,* 153 (1984).
8 E. Sletten, T. Marthinsen and J. Sletten, *Inorg. Chim. Acta, 106,* 1 (1985).
9 M. A. Stevens and G. B. Brown, *J. Am. Chem. Soc., 80,* 2759 (1958).
10 C. M. Mikulski, F. J. Iaconianni, L. L. Pytlewski, A. N. Speca and N. M. Karayannis, *Inorg. Chim. Acta, 46,* L47 (1980).
11 D. D. Perrin, *J. Am. Chem. Soc., 82,* 5642 (1960).
12 W. G. Espersen and R. B. Martin, *J. Am. Chem. Soc., 98,* 40 (1976).
13 I. Solomon, *Phys. Rev., 99,* 559 (1955).
14 N. Bloembergen, *J. Chem. Phys., 27,* 572, 595 (1957).
15 G. V. Fazakerley, G. E. Jackson, M. A. Phillips and J. C. Van Niekerk, *Inorg. Chim. Acta, 35,* 151 (1979).
16 J. A. Carrabine and M. Sundaralingam, *J. Am. Chem. Soc., 92,* 369 (1970).