Synthesis, Stereochemistry and Inhibition of Tumor Cell Transplantability by the Iron and Copper Complexes of 2-Aminomethyl- and 2-(2′-Aminoethyl)pyridine

T. TUNDE BAMGBOYE
Department of Chemistry, University of Nottingham, Nottingham NG7 2RD, U.K.
(Received October 9, 1987)

Abstract

Complex formation between transition metal chlorides and the ligands 2-aminomethylpyridine (AMP) and 2-(2′-aminoethyl)pyridine (AEP) has been investigated. The complexes were characterized on the basis of elemental analysis, magnetic measurements and spectral studies. The cytotoxicity of the iron and copper complexes of AMP and AEP against Ehrlich ascites tumor cells has been measured. Brief incubation of cells and drugs was followed by implantation into the host mice; subsequent development of tumor cells was a measure of cytotoxicity.

Introduction

There are few reports on the coordination complexes of 2-aminomethylpyridine (compound X and abbreviated as AMP) and 2-(2′-aminoethyl)pyridine (compound Y and abbreviated as AEP) with transition metal halides. Available evidence [1] comes from the square-planar complexes of Pt(II) and Pd(II) halides with AMP and AEP.

In view of our recent observation of antitumor activity among the hexa-coordinate complexes of Fe(II) and Cu(II) halides with AMP and AEP, it was of interest to synthesize several new complexes within this class and investigate their activity. We present in this paper the reactions of a range of transition metal halides with AMP and AEP and the cytotoxicity of their copper and iron complexes against Ehrlich ascites.

Experimental

The chelating ligands X and Y were used without further purification.

TABLE I. Analytical Data[a], Magnetic Moments and Mössbauer Data[b] of the Complexes

Complexes	C (%)	H (%)	N (%)	Cl (%)	M (%)	μ_{eff} (BM)	δ	Δ_{Eq}
$Fe(AMP)_2(NCMe)_2Cl_2$	45.17 (45.19)	5.17 (5.18)	19.76 (19.77)	16.70 (16.71)	13.18 (13.16)	5.38	1.2	3.98
$Fe(AEP)_2(NCMe)_2Cl_2$	47.68 (47.69)	5.73 (5.74)	18.54 (18.55)	15.67 (16.68)	12.36 (12.34)	5.40	1.3	3.97
$Co(AMP)_2(NCMe)_2Cl_2$	44.85 (44.87)	5.14 (5.16)	19.62 (19.63)	16.58 (16.60)	13.78 (13.79)	4.90		
$Co(AEP)_2(NCMe)_2Cl_2$	47.36 (47.38)	5.70 (5.70)	18.42 (18.43)	15.57 (15.57)	12.91 (12.92)	4.92		
$Ni(AMP)_2(NCMe)_2Cl_2$	44.85 (44.89)	5.14 (5.14)	19.62 (19.64)	16.58 (16.60)	13.72 (13.72)	3.12		
$Ni(AEP)_2(NCMe)_2Cl_2$	47.36 (47.39)	5.70 (5.71)	18.42 (18.43)	15.57 (15.55)	12.87 (12.88)	3.15		
$Cu(AMP)_2(NCMe)_2Cl_2$	44.38 (44.38)	5.08 (5.09)	19.41 (19.42)	16.41 (16.41)	14.70 (14.70)	2.00		
$Cu(AEP)_2(NCMe)_2Cl_2$	46.91 (46.89)	5.65 (5.64)	18.24 (18.24)	15.42 (15.41)	13.78 (13.81)	2.01		

[a]Calculated(Found). [b]Values at 80 K in mm s^{-1}.

0020-1693/88/$3.50

TABLE II. Cytotoxicity of Iron and Copper Complexes of AMP and AEP

Compound	Dose[a] (mg/ml)	Solubility[b] (mg/ml)	T/C[c]	Tumor[d] incidence	Day[e]	Long-term[f] survivors (days)
$Fe(AMP)(NCMe)_2Cl_2$	1.10	3.5	0.55	2/3 (3, 19)	21	
	1.05		0.10	0/5	23	
	1.05		0.52	5/5	18	3/5 (42)
	1.00		0.76	3/5	15	
	0.105		0.85	4/4 (2, 18)	20	
$Fe(AEP)(NCMe)_2Cl_2$	1.00	3.5	0.60	3/3 (2, 23)	22	
	1.00		0.50	3/4 (1, 15)	23	
	1.05		0.15	0/5	23	5/5 (45)
	1.10		0.18	0/5	23	5/5 (65)
	0.105		0.85	4/5	22	
$Cu(AMP)(NCMe)_2Cl_2$	0.70	0.1	0.29	0/5	22	5/5 (75)
	0.75		0.25	0/5	21	5/5 (75)
	0.80		0.22	1/5	22	
	0.050		0.13	1/5	22	
	0.75		0.24	0/5	20	
$Cu(AMP)(NCMe)_2Cl_2$	0.75	0.1	0.23	1/5	21	
	0.55		0.26	0/5	23	
	0.80		0.22	0/5	21	
	0.050		0.20	0/4 (1, 19)	22	
	0.75		0.24	0/4	23	

[a]Drug delivered to a 30-g mouse; 13 mg/kg. [b]Solubility at pH 7.5. [c](Δ wt/wk treated)/(Δ wt/wk control). [d]Determined by analysis of weight changes with groups on day of final observation. Death of tumor-bearing animals occurred as shown in parentheses (number, day). [e]Day of final observation. [f]Animal groups with tumors were sacrificed at the end of the period of observation. Others were kept for extended days and analysed as in footnote b for tumor development.

The syntheses of the complexes are similar, but details for the copper and iron complexes are presented.

Copper Complexes

The ligands react completely with copper ion in solution to form 1:2 complexes. Solid materials were formed by reaction of 1.23 g of ligand dissolved in 30 ml of acetonitrile and 1.8 g of copper chloride in 40 ml of acetonitrile. The mixture was stirred and refluxed for 36 h at 60 °C in a three-neck flask. Ingress of moisture was prevented by a calcium chloride drying tube. The precipitated product was filtered and recrystallized from acetonitrile. Analytical data of the product formed are summerized in Table I.

Iron Complexes

As for the copper complexes, solids for these studies were prepared by allowing ferrous chloride and the ligand to react in hot acetonitrile; they were recrystallized from the same solvent. Analytical data in Table I show that the iron complexes have a 1:2 metal-to-ligand ratio.

Tumor Cell Studies

The *in vitro* incubation of complexes with tumor cells was carried out. Ascites fluid from one mouse, about 10 ml, containing around 10^9 cells, was spun at low speed to separate the cells from the plasma. The cells were then resuspended with a volume of Eagle's MEM equal to the ascites fluid removed.

A given amount of drug (Table II) was dissolved in 2 ml of Eagle's MEM and then mixed with the same volume of the cell suspension. The total volume of drug and cell was then incubated at 37 °C for 1 h. Afterwards, 0.4 ml of this drug–cell mixture was injected into each of five mice per class. The same pattern was followed for each drug and for several concentrations of the same drug. Thus, the 0.4 ml of inoculum contained about 10^7 cells and no less than 10^6 viable cells. In every experiment, a control group was employed where the inoculum was exactly 0.4 ml of suspension as described above but the Eagle's MEM replaced the drug solution during the incubation period.

Observation of increase in weight of tumor-bearing animals was used to assess the progress of tumor development. The degree of inhibition of tumor growth by drugs is expressed as a ratio of the average weight gain per week of animals with treated cells

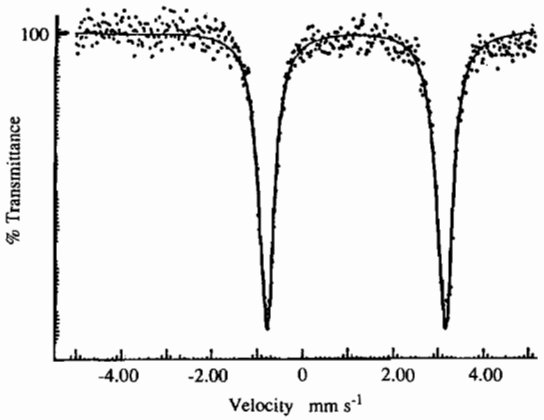

Fig. 1. Mössbauer Spectrum of $Fe(AEP)_2(NCMe)_2Cl_2$.

to that of control animals with untreated cells. Other control animals not injected with cells grew very little over the course of the experiment. The observation period was roughly 25 days, after which time the tumor incidence was recognized on the basis of the change in weight and the general physiognomy of the animals. In cases where there was no incidence of tumor development, the animals were kept for extended periods to see if they were completely free of the tumor.

Physical Measurements

For the magnetic moments, the Gouy method was employed with $Hg[Co(NCS)_4]$ used as the standard. Diamagnetic corrections were also applied using Pascal's constants [2].

Fe, Co, Ni and Cu contents of the complexes were estimated using a Perkin-Elmer 603 atomic absorption spectrophotometer. Carbon, hydrogen, nitrogen and chlorine were determined using a Perkin-Elmer Model 240G elemental analyser.

Electronic absorption spectra of the complexes and the ligand were recorded on a Perkin-Elmer Model 402 spectrophotometer in dimethyl formamide (DMF).

The procedures employed for the collection of ^{119}Sn Mössbauer spectra have been reported previously [3, 4]. The spectra of the iron complexes $Fe(AMP)_2(NCMe)_2Cl_2$ and $Fe(AEP)_2(NCMe)_2Cl_2$ were recorded at room temperature accumulating a minimum of 10^6 counts per channel, and were subsequently fitted to Lorentzian lineshapes by the usual least-squares methods. The results are summarized in Table I and a typical Mössbauer spectrum is presented in Fig. 1.

Infrared spectra in the range 4000–180 cm^{-1} of the ligands and complexes were recorded on a Perkin-Elmer Model 938G spectrophotometer as CsI pellets.

Results and Discussion

Magnetic Properties

$Fe(AMP)_2(NCMe)_2Cl_2$ and $Fe(AEP)_2(NCMe)_2Cl_2$

Magnetic susceptibility measurements yielded an effective magnetic moment of 5.38 BM for $Fe(AMP)_2(NCMe)_2Cl_2$ and 5.40 BM for $Fe(AEP)_2(NCMe)_2Cl_2$, which also support the high-spin ($S = 2$) configuration for the two Fe(II) complexes. The complexes are orbitally triply degenerate and consequently an orbital contribution to magnetic moment is expected, hence $\mu_{eff} > \mu_{s.o}$.

$Co(AMP)_2(NCMe)_2Cl_2$ and $Co(AEP)_2(NCMe)_2Cl_2$

Under an octahedral ligand field, Co(II) is a d^7 system with a T ground state, hence an orbital contribution to the magnetic moment is expected. The μ_{eff} for the complexes are respectively 4.80 and 4.82 BM. These values are greater than the $\mu_{s.o}$ value of 3.87 BM for three unpaired electrons as a result of orbital contribution to the magnetic moment.

$Ni(AMP)_2(NCMe)_2Cl_2$ and $Ni(AEP)_2(NCMe)_2Cl_2$

For these complexes, a Ni(II) d^8 configuration has effective magnetic moment of 3.12 and 3.15 BM respectively. These values are larger than the $\mu_{s.o}$ value of 2.83 BM for two unpaired electrons. Under an O_h environment, Ni(II) is orbitally singly degenerate (ground state A) and as such no orbital contribution to the magnetic moment is expected. But $\mu_{eff} > \mu_{s.o}$ as a result of mixing of the ground state with the excited state via spin–orbit coupling.

$Cu(AMP)_2(NCMe)_2Cl_2$ and $Cu(AEP)_2(NCMe)_2Cl_2$

For the two copper complexes, the effective magnetic moments are respectively 2.00 and 2.01 BM. These values are in excess of the μ_{spin} only value of 1.73 BM. Cu(II) is a d^9 system with a doubly degenerate E ground term and consequently no orbital contribution is expected. The E ground term of the octahedrally coordinate ion is expected to yield a moment of $\mu_{eff} = \mu_{s.o}\ (1 - 2\lambda/10\,Dq)$, in excess of 1.73 BM, because of mixing of the excited T term with the ground term. The high value of λ (-850 cm^{-1}) makes this effect very significant.

Electronic Spectra

In DMF, $Fe(AMP)_2(NCMe)_2Cl_2$ and $Fe(AEP)_2(NCMe)_2Cl_2$ each exhibits a double hump absorption. For the complex $Fe(AMP)_2(NCMe)_2Cl_2$, the double hump is centred at 1280 and 910 nm. This absorption has been assigned to the only spin-allowed transition $^5T_{2g} \rightarrow {}^5E_g$ in O_h high-spin Fe(II) complexes [5, 6]. The two maxima are 380 nm apart due to asymmetry in the crystal field and inherent susceptibility of high-spin Fe(II) towards Jahn–Teller

distortion. In the case of $Fe(AEP)_2(NCMe)_2Cl_2$, the bands appear at 1430 and 390 nm. The blue shift of the absorption maxima confirm that AEP is a stronger ligand than AMP.

Each of the Co(II) complexes should normally exhibit three spin-allowed bands, but only two are observed. One of the spin-allowed transitions, actually $^4T_{1g} \rightarrow {}^4A_{2g}$, is in the strong field limit, that is, a two-electron jump ($t_{2geg}^{5\,2} \rightarrow t_{2geg}^{3\,4}$) and hence the corresponding band is too weak to be observed. The other intense bands centred at 880 and 450 nm respectively correspond to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$. These transitions are in perfect agreement with distortions from regular octahedral symmetry [7].

The spectra of the Ni(II) complexes are similar and the spectrum of $Ni(AMP)_2(NCMe)_2Cl_2$ has the following principal bands, *viz.* (nm) $^3A_{2g} \rightarrow {}^3T_{2g}$ 810, $^3A_{2g} \rightarrow {}^3T_{1g}$ 520 and $^3A_{2g} \rightarrow {}^3T_{1g}$ 340. These transitions conform to octahedral coordination around Ni(II).

For the copper complexes, a single band characteristic of the transition $^2E_g \rightarrow {}^2T_{2g}$, in agreement with perfect O_h symmetry, was not observed. However, the observed bands are very broad and clearly contain several components indicative of distorted octahedral as a result of operation of the Jahn–Teller effect. Consequently, the transitions are by no means easy to assess unambiguously.

Mössbauer Spectra (Fig. 1)

The Mössbauer spectra of the two Fe(II) complexes are similar. The spectrum of the polycrystalline $Fe(AMP)_2(NCMe)_2Cl_2$ consists of a quadrupole doublet with isomer shift ($\delta = 1.2$ mm s^{-1}) and quadrupole splitting ($\Delta Eq = 3.98$ mm s^{-1}). These values are characteristic of high-spin Fe(II) in a crystal field of low symmetry [8]. According to Bancroft and Sham [9], the quadrupole splitting ΔEq of 3.98 mm s^{-1} is consistent with a distorted octahedral geometry.

Vibrational Spectra

We discuss the selective infrared bands of the ligand and the complexes. The highest frequency bands of the free ligand come at 3345 and 3250 cm^{-1}. These bands have been ascribed respectively to unsymmetric and symmetric ν(N–H) stretching vibrations [10]. The band centred at 1585 cm^{-1} is attributable to δ(N–H) deformation of the NH_2 group [11]. The bands at 620 and 430 cm^{-1} in the free ligand are assigned to the in-plane and out-of-plane pyridine ring deformation, respectively [12].

In the complexes, considerable changes occur in the NH_2 group stretching vibrations as expected on complex formation. For example, the unsymmetric and symmetric ν(N–H) vibrations have moved to higher wave numbers, with the implication that the nitrogen of the NH_2 group is involved in bonding [13]. Also, the δ(N–H) deformation of the NH_2 group has shifted for the same reason. The in-plane and out-of-plane pyridine modes have shifted to higher frequencies, suggesting that the ring nitrogen is involved in bonding [10]. Hence the emergence of new bands in the spectra of the complexes between 515 and 485 cm^{-1} are due to ν(M–N) vibrations [7].

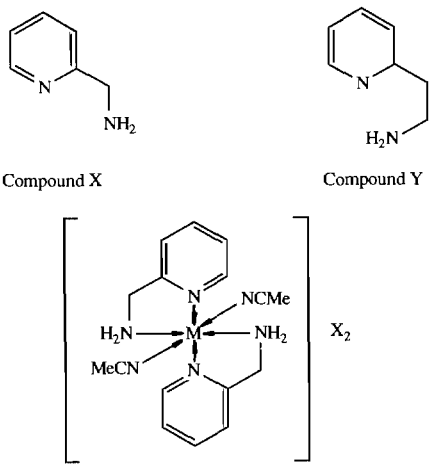

Fig. 2. AEP and AMP structure.

In addition to these stretching vibrations in the complexes, two prominent bands, which are absent in the uncomplexed ligands, come at 2299 and 2276 cm^{-1} respectively. These bands have been ascribed to ν(C≡N) of the coordinated acetonitrile molecule [14, 15]. Thus, we conclude that the ligands AMP and AEP are bidentate and the structure shown in Fig. 2 is therefore proposed.

Cytotoxicity

As a preliminary screening for compounds which are toxic to tumor cells after short exposures, a combination of *in vitro* and incubation of test material and tumor cells followed by implantation of cells into the host organism has been employed. This method provides information on drug-induced tumor cell toxicity which is relatively uncomplicated by attendant host responses to the compounds.

The present study affords examination of the iron and copper complexes of 2-aminomethyl- and 2-(2′-aminoethyl)pyridine. Table II is a summary of several experiments. The average change in weight of treated *versus* controls provided a quantitative basis of comparison of cytotoxic effects upon tumor and animal host. Experiments were reproduced several times and qualitatively similar results were obtained. Only in the case of the iron complexes was there a marked difference of results among the runs. At 1 mg/ml *in vitro* incubation, the relative effectiveness of the iron complexes is not clear-cut. At this

concentration of drug, about 13 mg/kg is delivered to an average 30 g mouse, and neither the AMP nor the AEP iron complex examined at 0.1 mg/ml had activity.

The two copper complexes of AMP and AEP are more active on a molar basis than their analogous iron complexes. In fact, since the solubility of these copper complexes in Eagle's medium is only about 0.1 mg/ml, it may be that their activity extends to this lower total concentration of complex. The marked difference in activity between the ligands, their iron complex and their copper complex, further suggests that three different cytotoxic species are observable.

Of potential significance is the apparent rapidity with which these drugs inactivate tumor cells upon direct interaction. This would be a desirable property for agents used in conjunction with surgery to prevent metastasis from the site of tumor excision.

Hexa-coordinate complexes of diorganotin halides and pseudohalides with certain bidentate N-donor ligands have been the subject of intense investigation as antitumor agents with the implication of their metal-binding capability in proposed mechanisms of action. Thus, the present study opens up possibilities of the design of active compounds which contain iron or copper.

Acknowledgements

The author thanks Professor J. J. Turner and Dr. D. B. Sowerby of the Department of Chemistry, University of Nottingham, U.K. for the provision of research facilities.

References

1 L. K. Rastogi, D. C. Pachauri, K. C. Sharma and M. P. Teotia, *Ind. J. Chem., 15A*, 44 (1977).
2 L. N. Mulay, 'Magnetic Susceptibility', Wiley–Interscience, New York, 1963, p. 1779.
3 P. G. Harrison, N. W. Sharpe, C. Pelizzi, G. Pelizzi and P. Tarasconi, *J. Chem. Soc., Dalton Trans.*, 921 (1983).
4 G. Long and B. W. Dale, *Nucl. Instrum. Methods, 116*, 567 (1974).
5 S. G. Rosenfield, S. A. Swedberg, S. K. Arora and P. K. Mascharack, *Inorg. Chem., 25*, 2109 (1985).
6 A. B. P. Lever, 'Inorganic Electronic Spectroscopy', 2nd edn., Elsevier, Amsterdam, 1984, p. 458.
7 T. T. Bamgboye and O. A. Bamgboye, *Inorg. Chim. Acta, 133*, 247 (1987).
8 N. N. Greenwood and T. C. Gibb, 'Mössbauer Spectroscopy', Chapman and Hall, London, 1971, Chap. VI.
9 G. M. Bancroft and T. K. Sham, *Inorg. Chim. Acta, 14*, 2281 (1975).
10 T. T. Bamgboye and O. A. Bamgboye, *Inorg. Chim. Acta, 105*, 223 (1985).
11 J. R. Dyer, 'Absorption Spectroscopy of Organic Compounds', Prentice-Hall, N.J., 1965, p. 22.
12 K. Nakamoto, 'Infrared and Raman Spectra of Inorganic and Coordination Compounds', 3rd edn., Wiley–Interscience, New York, 1977.
13 T. T. Bamgboye, *Inorg. Chim. Acta*, (1987), in press.
14 R. A. Walton, *Q. Rev. Chem. Soc., 19*, 126 (1965).
15 M. J. Begley, M. F. A. Dove, R. C. Hibbert, N. Logan, M. Nann and D. B. Sowerby, *J. Chem. Soc., Dalton Trans.*, 2433 (1985).