Synthesis and Properties of some New Platinum Blues Derived from the Reaction of *Cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with Pyrimidines and Adenine, and their Inhibition of Two Mitochondrial Enzymes (L-Malate Dehydrogenase and Fumarase) and DNA Synthesis in *E. Coli*

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Several new platinum blue and green materials, with reproducible analyses and properties, have been synthesized from the reaction of cis-*$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with the pyrimidines uracil, thymine and cytosine. New blues have also been obtained from the reaction of $[Pt_2(aden\text{-}H)_2(aden)_2][PtI_4]$ (aden-H = monodeprotonated adenine) with dimethylformamide, dimethylsulphoxide, tetrahydrofuran, acetone and pyridine. Some physical properties of these compounds are described, as well as their ability to inhibit two mitochondrial enzymes (L-malate dehydrogenase and fumarase) and DNA synthesis in* E. Coli.

Introduction

Rosenberg and co-workers [1–4] discovered that a range of 'platinum blues' (blue and green platinum–pyrimidine complexes) formed by the reaction of *cis*-diaquodiammineplatinum(II) with 2,4-dioxopyrimidines are antitumor, antiviral and antibacterial agents with a low level of renal toxicity and a high degree of solubility in water. Very little is known about these interesting materials. Although some EXAFS results on a platinum uridine blue [5] and a powder X-ray diffraction study on platinum acetamide blue [6] are available, the only real insight into their nature comes from the single crystal X-ray study of the platinum-α-pyridone blue [7]. Some *in vitro* studies have shown interesting properties [8, 11], namely: the species are cationic since they travel towards the cathode during electrophoresis and may be precipitated from solution by the addition of bulky anions. Sucrose gradient, gel chromatography, electrophoresis and mass spectral data suggest polymeric products with a continuous distribution of molecular weights from several thousand Daltons to less than a thousand. The oxidation state of the platinum may be non-integral; the 1-methylthymine blue was found to have platinum in the +3.72 oxidation state. Some blues contain paramagnetic components, detected by broadening of ^1H NMR signals and the appearance of an ESR signal, the intensity pattern of which suggests hyperfine interaction of two or more platinum nuclei.

In this study we wish to report the preparation and characterization of some new platinum blue and green complex compounds derived from the reaction of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with the pyrimidines uracil, thymine and cytosine and from the reaction of K_2PtI_4 with adenine. The inhibition of two mitochondrial enzymes, L-malate dehydrogenase and fumarase and DNA synthesis in *E. Coli* by the pyrimidine blues is also reported.

Results and Discussion

The synthesis of the platinum blues is reported in the Experimental Section. While it may be possible that these materials are mixtures, we found that the synthesis was highly reproducible, yielding materials which gave reproducible analyses and physical properties. Table I contains analytical data for the pyrimidine blues and the estimated Pt: pyrimidine ratio based on the analytical data. All blues synthesized in this study were passed through three different columns packed with silica gel, florisil or sephadex, but no separation was achiev-

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TABLE I. Analytical Data and Colors of the Platinum Pyrimidine Blues.

Blue[a]	Color	%Pt	%C	%H	%N	Formulation[b]	Pt/Pyrimidine[c] ratio
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (1:1) [EtOH soluble]	Blue	32.5	19.8	2.6	17.6	$PtC_{9.9}H_{15.6}N_{7.5}O_{10.3}$	1/2
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (1:1) [EtOH insoluble]	Dark Blue	41.2	15.4	2.4	15.9	$PtC_6H_{11.4}N_{5.4}O_{7.4}$	2/3
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (1:2)	Pale Blue	34.5	16.7	2.7	19.2	$PtC_{7.9}H_{15.3}N_{7.7}O_{9.5}$	1/2
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (1:3)	Green	29.6	24.0	2.3	19.9	$PtC_{13.2}H_{15.2}N_{9.4}O_{10}$	1/3.1
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Thymine (1:1)	Blue	37.2	11.6	2.3	16.4	$PtC_5H_{12}N_{6.1}O_{10.6}$	1/1
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Thymine (1:2)	Blue	38.6	19.6	2.8	14.8	$Pt_2C_{17}H_{28.2}N_{10.7}O_{15.3}$	2/3.2
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Thymine (1:3)	Pale Blue	41.7	19.1	2.9	14.2	$Pt_2C_{14.8}H_{27}N_{9.5}O_{13.3}$	2/3
cis-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Cytosine (1:1)	Blue	36.0	11.0	2.8	20.1	$PtC_5H_{15.2}N_{7.8}O_{10.2}$	1/1.25
cis-$[Pt(NH_3)_2(OH_2)_2](ClO_4)_2$/Uracil[d] (2:1)	Dark Blue	37.0	9.1	2.1	13.3	$PtC_4H_{11}N_5O_{10}Cl_{1.2}$	1/1

[a]Ratios between parentheses represent the molar ratios between the aquo sepcies and the ligand used during synthesis. [b]%O was determined by difference. [c]Pt/pyrimidine ratio suggested by the formulation. [d]%Cl = 8.1.

TABLE II. Infrared Spectra of the Platinum Pyrimidine Blues.[a]

Blue[b]	ν(O–H)	ν(N–H)	ν(C=O)	Counterion	
				$(NO_3)^-$	$(ClO_4)^-$
Pt/Uracil 1:1) (EtOH soluble)	3450m,br	3150mS 3225m	1650mS	1785m, 830Sh, 720m	
Pt/Uracil (1:2)	3450m,br	3140mS 3220mS	1650mS	1785m, 830Sh, 720m	
Pt/Uracil (1:3)	3425m,br	3150mS,br 3250m	1650mS	1785m, 830Sh, 720m	
Pt/Uracil (1:1)	3500m,br	3280S,br 3200S,br	1635mS		1085S,br
Pt/Uracil (2:1)	3550mS,br	3300S,br 3200mS	1620mS		1085S,br
Pt/Thymine (1:1)	3450m,br	3260m,br 3150m	1645mS	1785m 830Sh, 720m	
Pt/Thymine (1:2)	3420m,br	3220m 3150m	1640m,br	1785m, 830Sh, 720m	
Pt/Thymine (1:3)	3420m,br	3250Wm 3150Wm	1640m	1785m, 830Sh, 720m	
Pt/Cytosine (1:1)	3450m,br	3300mS 3220mS	1645m,br	1785m, 830Sh, 720m	

[a]S: strong; m: medium; W: weak; Sh: sharp; br: broad. [b]Ratios between parentheses represent the molar ratio between the diaquo species and the ligand used during synthesis.

TABLE III. Electronic Spectra of the Platinum Pyrimidine Blues.

Blue[a]	Aqueous Solutions E_{max} kK	Absorbance	Concentration (g/l)	Solid Reflectance
Pt/Uracil (1:1)	15.0 18.3 35.7	0.41 0.38	0.7	18.9, 34.4
Pt/Uracil (1:2)	15.1 18.3 35.7	0.31 0.27	0.7	17.8, 35.0
Pt/Uracil (1:3)	15.2 18.6 35.7	0.18 0.09	0.7	17.8, 34.5
Pt/Thymine (1:1)	13.8 15.8 sh 34.4	0.33 0.26	0.87	17.2, 33.9
Pt/Thymine (1:2)	13.6 15.6 sh 34.5	0.21 0.17	0.7	18.7, 33.3
Pt/Thymine (1:3)	13.7 15.7 sh 34.5	0.13 0.09	0.5	15.9, 33.9
Pt/Cytosine (1:1)	15.9 sh 18.3 33.9	0.42 0.35	0.2	17.8, 35.0
Pt/Uracil (1:1)[b]	15.3 18.8 sh 35.0	0.44 0.37	0.2	19.2, 35.7
Pt/Uracil (2:1)[b]	15.0 18.3 sh 35.0	0.43 0.38	0.2	18.3, 35.0
Pt/Uracil (2:1)[b]	15.0 18.3 sh 35.1	0.75 0.70	0.2	18.5, 34.5

[a]Ratios in parenthesis represent the molar ratios between *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$ and the ligand used during synthesis. [b]Molar ratios between *cis*-$[Pt(NH_3)_2(OH_2)_2](ClO_4)_2$ and uracil.

ed during elution, as the blue solutions passed through the columns in one fraction having identical elemental composition and electronic spectra to those of the crude blue products. The thymine and uracil blue species seem to be cationic, since the blue color of the aqueous solution appears to require an anion such as nitrate, sulfate or perchlorate. If the pH of a solution containing *cis*-$[Pt(NH_3)_2(OH_2)_2](SO_4)$ and thymine (or uracil) was adjusted to ~7 by the addition of $Ba(OH)_2$, barium sulfate precipitates and the filtrate stays pale yellow/green and does not turn to blue unless excess of an anion is added. On addition of excess sodium nitrate, sulphate or perchlorate to the filtrate, a blue color develops and often the blue precipitates.

It is suggested that the blue color is associated with the cation as the anion could be substituted by other anions by reprecipitating the blue from a solution containing excess amounts of the counterion without loss of color. The uracil blue (nitrate salt) was reprecipitated with $NaClO_4$, with Na_2SO_4, and in each case the precipitate seemed to lose its nitrate anion as the infrared absorptions at 1780, 830, 720 cm^{-1} disappear. Addition of excess NaOH does not give the hydroxy complex but the blue solution decolorizes.

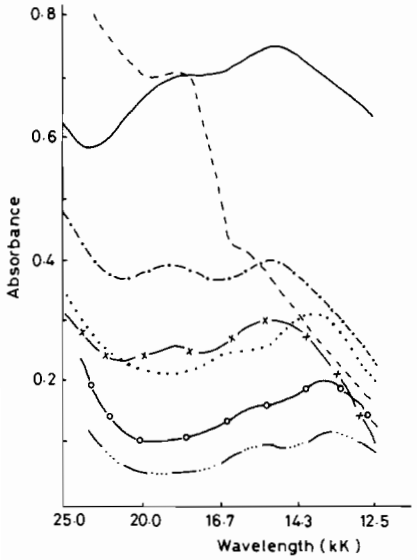


Fig. 1. Electronic Spectra of the Platinum Pyrimidine Blues in Aqueous Solutions. Pt/Uracil (2:1)[b] ——— (0.2 g/l); Pt/Uracil (1:1) –●–●–●– (0.7 g/l); Pt/Uracil (1:2) –X–X–X– (0.7 g/l); Pt/Thymine (1:1) ·····(0.87 g/l); Pt/Thymine (1:2) –○–○–○– (0.7 g/l); Pt/Thymine (1:3) ——·——·— (0.5 g/l); Pt/Cytosine (1:1) – – – – (0.2 g/l).

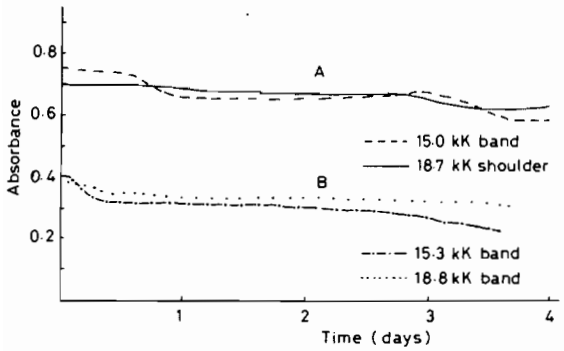


Fig. 2. Time Dependence of the Electronic Spectra of the Uracil Blues in Aqueous Solutions. A = $[Pt(NH_3)_2(OH_2)_2](ClO_4)_2$/Uracil (2:1) [0.2 g/l]; B = $[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (1:1) [0.2 g/l].

The infrared spectra are listed in Table II and bands assignable to ν(OH), ν(NH) and ν(C=O) are clearly observed, as are bands assignable to nitrate and perchlorate.

The instability of the blue complexes in aqueous solutions has been studied at various time periods and temperatures by visible spectroscopy (Figs. 1–5 and Table III). The spectra were found to be sample and concentration dependent. A shift of bands in the visible region was observed at different concentrations. The intensity of these bands decreased with time indicating some kind of dissociative process occurred in solution, and high temperatures seemed

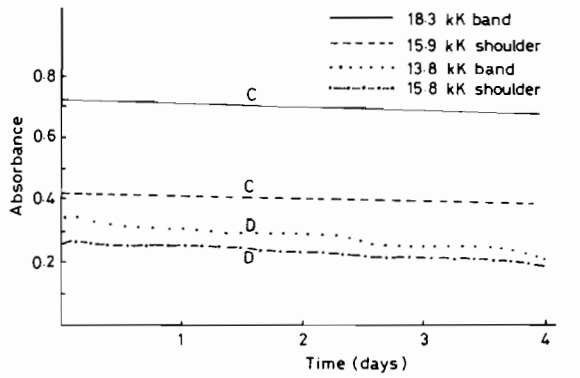


Fig. 3. Time Dependence of the Electronic Spectra of Thymine and Cytosine Blues in Aqueous Solution. C = *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Cytosine (1:1) [0.2 g/l]; D = *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Thymine (1:1) [0.87 g/l].

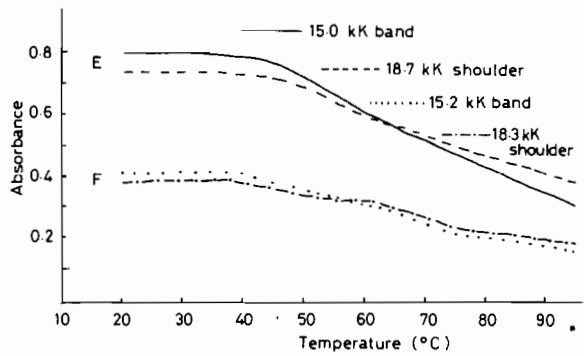


Fig. 4. Temperature Dependence of the Electronic Spectra of the Uracil Blues in Aqueous Solutions. E = *cis*-$[Pt(NH_3)_2(OH_2)_2](ClO_4)_2$/Uracil (2:1) (0.2 g/l). F = *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (2:1) (0.2 g/l).

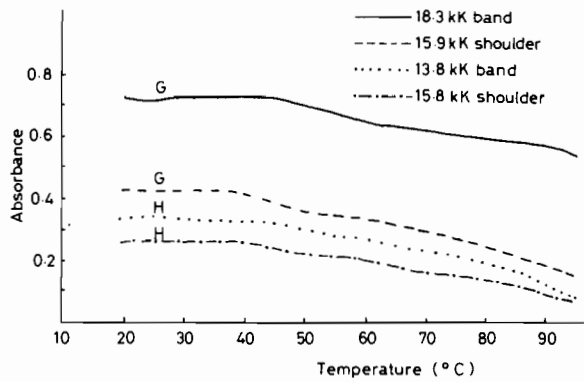


Fig. 5. Temperature Dependence of the Electronic Spectra of the Cytosine and Thymine Blues in Aqueous Solution. G = *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Cytosine (1:1) [0.2 g/l]; H = *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Thymine (1:1) [0.87 g/l].

to accelerate this process. The spectra are characterized by bands at frequencies above 33 kK, presumably due to charge transfer transitions (M ← L), and

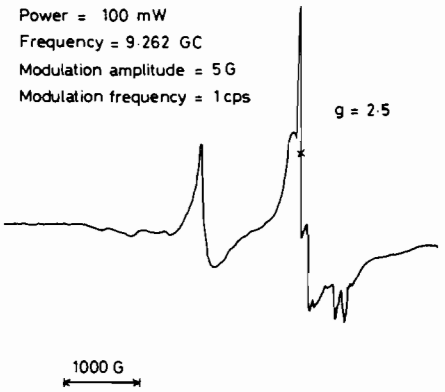


Fig. 6. Powder ESR Spectrum of *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Uracil (1:1).

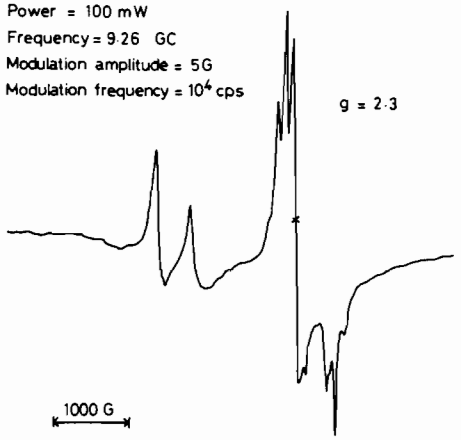


Fig. 7. Powder ESR Spectrum of *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Thymine (1:2).

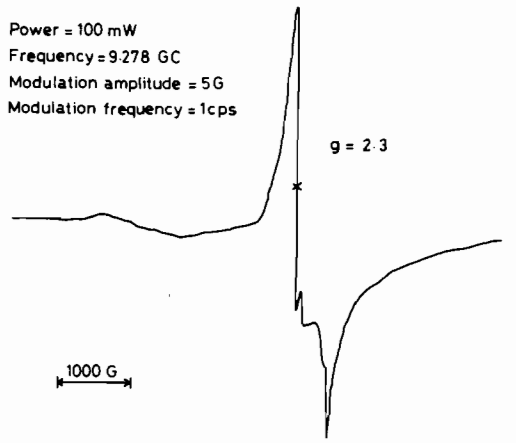


Fig. 8. Powder ESR Spectrum of *cis*-$Pt(NH_3)_2(OH_2)_2](ClO_4)_2$/Uracil (2:1).

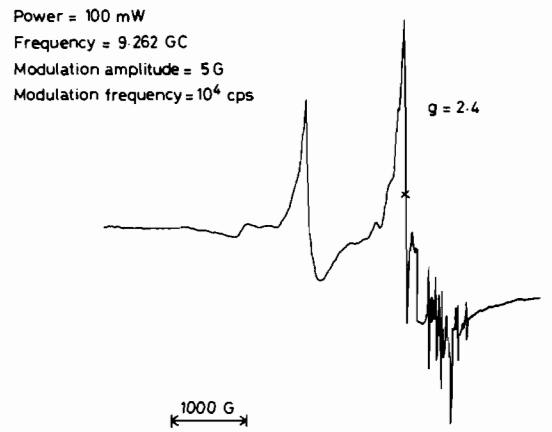


Fig. 9. Powder ESR Spectrum of *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$/Cytosine (1:1).

broad bands in the visible which can be divided roughly into two regions: 14–16 kK and 16–19 kK (usually as a shoulder on the previous bands). These bands may be due to d–d formally forbidden transition bonds but gain intensity by vibrational perturbations and by borrowing from the intense allowed bands. The solid reflectance spectra are characterized by broader bands at 16–20 kK, shoulders observed in this region in solution spectra do not show in the solid reflectance spectra. The formation of the blue color in aqueous solution was also monitored by ^1H NMR spectroscopy. The formation is accompanied by line broadening, decrease in intensity and very often the disappearance of the spectrum especially at relatively high temperatures (65–85 °C). The ^1H NMR spectra of the uracil blues showed two doublets due to the H_6 and H_5 of the free uracil at 6.87 ppm and 5.15 ppm, respectively. The signals decreased in intensity as the blue color developed till the spectrum disappeared after 72 h. The signal at 5.15 ppm shifts to 5.67 ppm after 24 h indicating a deshielded environment around the H_5 which may suggest the co-ordination of the exocyclic oxygen, O_4, or it may be due to the intermolecular hydrogen bonding involving O_4 and H_5.

The spectrum of free thymine exhibited two singlets at 0.85 ppm and 6.37 ppm assignable to the methyl protons and the H_6, respectively. In the ^1H NMR spectrum of the thymine blue (2:1), it seemed that the methyl protons coupled with ^{195}Pt forming a distorted triplet at 1.05 ppm. Moreover, the H_6 proton couple with ^{195}Pt forming a weak broad multiplet centered at 6.48 ppm. It is clear that the downfield shift of the methyl protons signal was greater than that of the H_6 signal, suggesting that the methyl protons were closer to the platinum atom than the H_6 which lead to the coordination via the exocyclic oxygen (O_4).

ESR spectroscopy has provided an explanation for the poor ^1H NMR spectra of these blue complexes.

TABLE IV. Inhibition of Fumarase by Platinum Pyrimidine Blue Complexes. (Enzyme concentration = 0.5 μM).

Blue	Blue/ Enz.	Activity (ΔA/m)	K_I (mM)	Average K_I
Pt/Thymine	0	0.083		
(1:1)	5000	0.051	4.2	
	3000	0.054	2.6	
	2000	0.067	4.2	
				3.70
Pt/Thymine	0	0.083		
(1:2)	3000	0.023	0.67	
	2000	0.038	0.94	
	1000	0.048	0.80	
				0.80
Pt/Thymine	0	0.083		
(1:3)	2000	0.021	0.49	
	1000	0.024	0.30	
	500	0.053	0.64	
				0.50
Pt/Uracil	0	0.078		
(1:1)	20,000	0.017	0.022	
	10,000	0.025	0.037	
	5000	0.037	0.009	
				0.023
Pt/Uracil	0	0.086		
(1:3)	10,000	0.012	2.1	
	6000	0.021	1.2	
	2000	0.034	1.6	
				1.6
Pt/Cytosine	0	0.083		
(1:1)	300	0.012	0.82	
	200	0.020	0.98	
	100	0.043	0.66	
				0.82

TABLE V. Inhibition of L-Malate Dehydrogenase by Platinum Pyrimidine Blue Complexes. (Enzyme concentration = 1.5 μM; Inhibition time = 48 h.).

Blue	Blue/ Enz.	Activity (ΔA/m)	K_I (mM)	Average K_I
Pt/Thymine	0	0.063		
(1:1)	1600	0.012	1.8	
	830	0.024	1.5	
	415	0.040	2.1	
				1.80
Pt/Thymine	0	0.063		
(1:2)	830	0.010	0.84	
	415	0.030	1.20	
	208	0.046	1.39	
				1.14
Pt/Thymine	0	0.063		
(1:3)	415	0.014	0.65	
	208	0.028	0.85	
	83	0.032	0.49	
				0.67
Pt/Uracil	0	0.063		
(1:1)	3300	0.022	0.049	
	1150	0.040	0.039	
	575	0.054	0.084	
				0.057
Pt/Uracil	0	0.063		
(1:3)	2780	0.016	4.0	
	1670	0.028	6.1	
	830	0.040	11.2	7.1

The first derivative ESR spectra in frozen aqueous solutions were obtained at 12 h time intervals up to 36 h during synthesis, and it was seen that the signal increased in intensity mirroring the increase of paramagnetic species as the reaction proceeds. The position of the signal does not change with time or state (powder or solution) of the blue sample. The regular signal pattern at high field observed in the powder spectrum is due to the ^{195}Pt hyperfine coupling interactions. The g values are very similar to those published by Lippert [8], Figs. 6–9.

Enzyme Inhibition and Inhibition of DNA synthesis in E. Coli

The platinum–pyrimidine blue complexes were found to inhibit two mitochondrial enzymes, L-malate dehydrogenase and fumarase, and DNA synthesis in *E. Coli.* The interaction of the platinum blue complexes with fumarase, Table IV, was allowed to proceed over a period of 48 h after which the measured change in activity was found to be fairly constant. The inhibition constant is defined in the equation below:

$$K_I = \frac{[E][I]}{[EI]}$$

[E] is proportional to the activity of the uninhibited enzyme, [I] is the concentration of unbound inhibitor, and [EI] is proportional to the difference between the total enzyme and the uninhibited enzyme concentrations. The general trend of a decrease in K_I with increasing pyrimidine content per molecule was observed for both thymine and uracil. There is roughly a 2 to 5 fold increase in the binding per unit increase in base within each nucleotide series. The uracil and thymine complexes seem to inhibit fumarase approximately equally for similar platinum–pyrimidine ratios. However, the cystosine complex inhibits the enzyme roughly 100 fold better than either the uracil or the thymine blues. Comparison of these complexes with *cis*-dichlorodiammine-platinum(II) [*cis*-DDP], which has a K_I of 0.32 mM under similar experimental conditions [12], suggests that cytosine blue is a far better inhibitor than *cis*-DPP, while the thymine blue (1:3) is about as effective as *cis*-DDP.

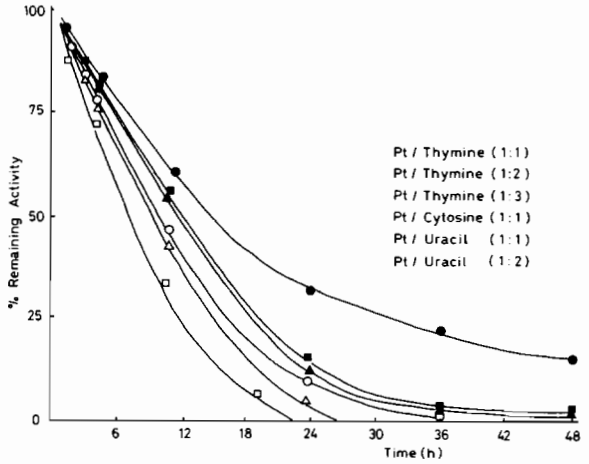


Fig. 10. Inhibition of L-Malate Dehydrogenase by Platinum Blue Complexes as a Function of Inhibition Time.

Table V lists the inhibition constants of the interaction between the blue complexes and L-malate dehydrogenase. The values are similar to those of the fumarase studies with the slight exception that the uracil complexes were only 1/4 as effective in inhibiting the dehydrogenase. The calculated K_I values are approximately the same as those found in the fumarase studies. The results from the kinetic studies made using L-malate dehydrogenase are presented in Fig. 10 and Table VI. From the calculated rate constants, it is observed that the cytosine complex is by far the most reactive while the thymine complexes inhibit better than the analogous uracil complexes. Comparison of the inhibition of these complexes to *cis*-DDP, which has a k value of 3.6 M^{-1} h^{-1} [13] shows that the thymine and cytosine blues are better inhibitors and the uracil complexes are approximately the same.

Table VII lists the % inhibition of DNA synthesis in *E. Coli* by the platinum blues. The data indicates that both the thymine and uracil blues are poor inhibitors of DNA synthesis while the cytosine complex inhibits reasonably well. Comparison of the cytosine complex with *cis*-DDP, which inhibits DNA by 79% at a 100 μM *cis*-DDP concentration [14], is quite favorable. Indications are that as the ratio of pyrimidine to platinum increases then DNA synthesis is more likely to be inhibited as observed in the uracil data.

Since the actual structure of the platinum blues in solution have yet to be determined it is difficult to propose a rational for these results. However, whatever effect the solution structure has on the enzyme, it appears that the higher base composition plays a significant role.

TABLE VI. Measurement of the Rate Constants for the Inhibition of L-Malate Dehydrogenase by the Platinum Pyrimidine Blue Complexes.

Blue	k (M^{-1} h^{-1})
Pt/Thymine (1:1)	6.3
Pt/Thymine (1:2)	16.7
Pt/Thymine (1:3)	40
Pt/Uracil (1:1)	2.1
Pt/Uracil (1:3)	4.6
Pt/Cytosine (1:1)	225

TABLE VII. Inhibition of DNA Synthesis in *E. Coli* by Some Platinum Pyrimidine Blue Complexes.

Blue	Concentration (μM)	% Inhibition
Pt/Thymine (1:2)	25	0
	50	0
	100	5
Pt/Cytosine (1:1)	25	0
	50	11
	100	50
Pt/Uracil (1:1)	250	0
	500	0
	1000	0
Pt/Uracil (1:3)	250	0
	500	2
	1000	10

Platinum–Adenine Blues

The reaction between adenine and $K_2[PtI_4]$ (*in situ*) has been studied by McAuliffe *et al.* [15] who discovered a facile route to a number of platinum blues. The reaction of $K_2[PtI_4]$ with adenine in a 1:2 molar ratio produces a brown complex which is believed to be dimeric containing two types of adenine binding *via* N-3 and N-9 (Table VIII). The coordination of the primary amine group was ruled out since almost identical infrared absorptions in the complex and ligand were observed. The strong absorption at 1640 cm^{-1} was assigned to ν(C=N) and shifted from 1670 cm^{-1} in free adenine. The electronic spectrum of the brown complex shows evidence of metal–adenine binding (Table IX). Free adenine has an absorption at 38.2 kK (π–π^* transition) which shifts to 36.6 kK indicating the expected spectral change on binding of the purine ring to a metal [16]. The band at 19.3 kK suggests

TABLE VIII. Analytical Data[a] and Colors of the Platinum Adenine Complexes.

Complex	Color	%Pt	%C	%H	%N	%I	%S
$[Pt_2(Aden\text{-}H)_2(Aden)_2][PtI_4]$	Dark Brown	36.2(35.9)	14.4(14.7)	1.2(1.1)	16.8(17.2)	29.3(31.1)	–
$[Pt(Aden\text{-}H)_2(Py)_2I_2]$	Dark Blue	35.7(36.5)	21.8(22.4)	1.7(1.7)	14.9(15.7)	22.6(23.7)	–
DMSO Fraction (a)/Silica Gel $Pt_2(Aden\text{-}H)(Aden)(DMSO)_3I_3$	Dark Blue	31.3(30.6)	16.2(15.1)	2.8(2.1)	10.4(10.9)	29.4(29.9)	7.8(7.5)
DMF Fraction IV/Silica Gel $Pt_2(Aden\text{-}H)(Aden)(DMF)_2I_3$	Blue	31.6(32.9)	15.9(16.2)	2.6(1.8)	13.6(14.2)	30.7(32.1)	–
DMF Fraction/Florisil $Pt_2(Aden)(Aden\text{-}H)(DMF)_3I_3$	Blue	32.4(31.0)	17.4(18.1)	2.3(2.4)	15.1(14.4)	31.2(30.2)	–
DMSO Fraction/(c) Florisil $Pt_2(Aden)(Aden\text{-}H)(DMSO)_2I_2$	Blue	31.0(32.6)	14.1(14.0)	2.1(1.7)	10.8(11.7)	30.9(31.8)	4.6(5.3)
$Pt(Aden)_2(THF)_2I_3$	Gray-Blue	34.1(33.0)	17.3(18.2)	2.3(2.1)	12.3(11.8)	31.6(32.1)	–

[a]Found (Calc.).

TABLE IX. Electronic Spectra of the Platinum Adenine Complexes.

Complex	Solvent	E_{max}, kK	Solid Reflectance E_{max}, kK
$[Pt_2(Aden\text{-}H)_2(Aden)_2][PtI_4]$	DMF	36.6, 34.6sh, 28.3sh, 23.5, 19.3	22.2, 18.8
$Pt_2(Aden\text{-}H)_2(Py)_2I_2]$	Pyridine	32.2, 25.3sh, 13.5	24.3
DMSO Fraction (a)/Silica Gel[a] $Pt_2(Aden\text{-}H)(Aden)(DMSO)_3I_3$	DMSO	32.5, 27.5sh, 16.9, 13.7	22.2
DMF Fraction IV/Silica Gel[a] $Pt_2(Aden\text{-}H)(Aden)(DMF)_2I_3$	DMF	33.8, 27.5, 16.9, 13.7	22.2, 17.2
DMF Fraction/Florisil[a] $Pt_2(Aden)(Aden\text{-}H)(DMF)_3I_3$	DMF	34.7, 27.8sh, 17.1sh, 13.0	23.5
DMSO Fraction (c)/Florisil[a] $Pt_2(Aden)(Aden\text{-}H)(DMSO)_2I_2$	DMSO	30.5, 25.3, 17.1sh, 12.9sh	22.3

[a]Actual fractions recovered from the chromatography column; these fractions were warmed at 60 °C until the color changed to blue.

the presence of $[PtI_4]^{2-}$ [17]. This evidence, plus the fact that the deprotonation of the N-9 is most likely, suggested the structure shown in Fig. 11.

Column chromatography has made it possible to separate various blue platinum–adenine complexes, Fig. 12, derived from the brown complex of differing formulation.

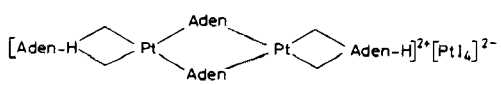
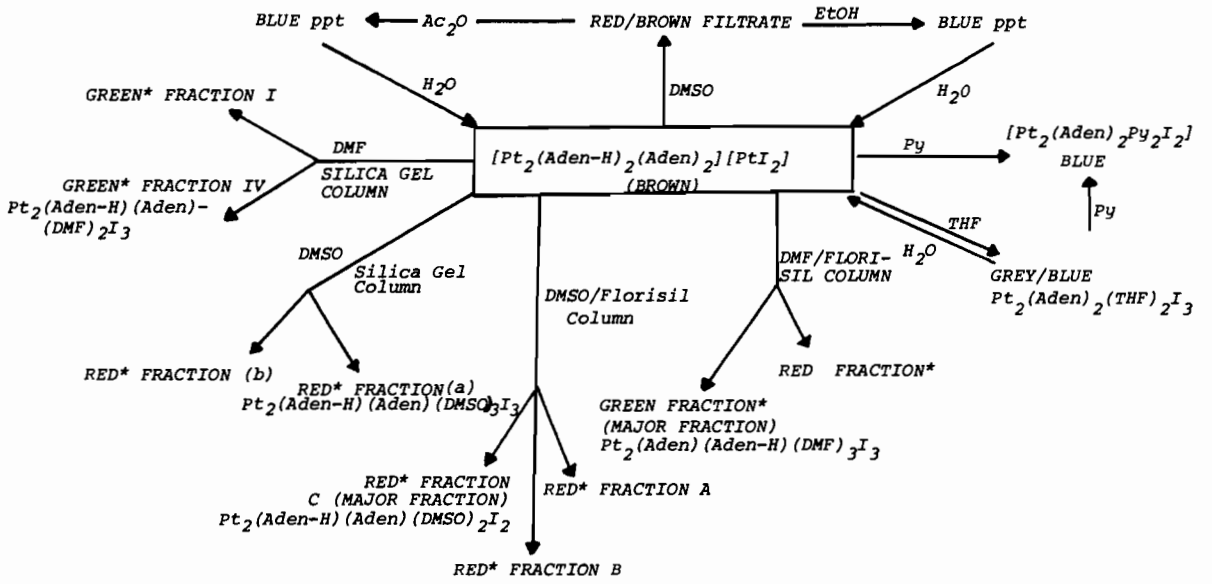


Fig. 11. Proposed Structure of $[Pt_2(Aden)_2(Aden\text{-}H)_2]$-$[PtI_4]$.

The infrared spectra of the blue complexes are similar and indicate a free primary amine group. The ν(C=N) which occurs at 1645–1630 cm^{-1} is hidden by ν(C=O) of the DMF solvate. All blues isolated by the evaporation to dryness of the fractions eluted from the chromatography columns were found to trap solvent molecules in the crystal lattice. The DMSO blues were found to have two types of DMSO molecules; the first coordinated via the oxygen atom since ν(S=O) shifts to 1007 cm^{-1} (1020 cm^{-1}) in free DMSO, and the second was trapped in the lattice. The low solubility of the blue complexes hindered conductivity studies and made structural assignment difficult.



**These fractions turn to blue during concentration.*

Fig. 12. Various Blue Complexes Derived from $[Pt_2(Aden\text{-}H)_2(Aden)_2[PtI_4]$.

Experimental

Synthesis of the Platinum–Pyrimidine Blues

The blue complexes were synthesized using a modification of the procedure of Rosenberg [3].

Uracil Blue (1:1)

Uracil (1 mmol) in water (100 ml) was added to an aqueous solution containing *cis*-$[Pt(NH_3)_2(OH_2)_2]$-SO_4 (1 mmol) and $Ba(OH)_2$ (0.3 *M*) was added to bring the pH to 8. After stirring for 2 h and filtering, the supernatant liquid was concentrated to 100 ml. The mixture was then incubated at 37–40 °C for 2 days in the dark. The pale green solution was then gently concentrated to ~30 ml and excess sodium nitrate (9 g) was then added yielding a dark blue ethanol-insoluble powder which was filtered, washed with boiling ethanol, dry diethylether and dried *in vacuo* at room temperature. On cooling the filtrate to 0 °C an ethanol soluble blue powder separated, was washed with dry diethyl ether and dried *in vacuo* at room temperature.

Uracil Blue {cis-*[Pt(NH$_3$)$_2$(OH$_2$)$_2$](ClO$_2$)$_2$/uracil (1:1)*}

Uracil (1 mmol) in water (100 ml) was added to an aqueous solution containing *cis*-$[Pt(NH_3)_2(OH_2)_2](ClO_4)_2$ (1 mmol in 100 ml) and a solution of NaOH (0.1 *M*) was added to bring the pH to 7. The solution was stirred for 0.5 h, filtered and the filtrate was then concentrated to 100 ml at 40 °C *in vacuo* and incubated at 37–40 °C for 63 h yielding a blue/green solution which was filtered then saturated with sodium perchlorate (110 g) stirred for 0.5 h, concentrated to 50 ml at 40 °C *in vacuo* then cooled in an ice bath. The solution turned to dark blue and a blue solid started to separate, and was filtered off. The filtrate was passed through Sephadex 25 and then more blue product was precipitated by the addition of excess ethanol. The blue product was filtered, washed with ethanol, dry diethyl ether and then dried *in vacuo* at room temperature.

The uracil blue (2:1) {*cis*-$[Pt(NH_3)_2(OH_2)_2]$-$(ClO_4)_2$/uracil (2:1)} was also prepared using the procedure described above.

Uracil Blue (1:3)

Uracil (3 mmol) was suspended in water (100 ml) and dissolved by the addition of sufficient NaOH to adjust to pH 9. To this was added an aqueous solution containing *cis*-$[Pt(NH_3)_2(OH_2)_2](NO_3)_2$ (1 mmol in 100 ml) and the final pH was adjusted to 7–8. The solution was incubated at 37–40 °C for 3 days, cooled to 0 °C and the resulting green solid separated by filtration. Further product was isolated by gentle concentration of the filtrate. The resulting uracil green complex was washed with boiling ethanol, dry diethyl ether and dried *in vacuo* at room temperature. Uracil (1:2); Thymine (1:1), (1:2), (1:3); Cytosine (1:1) were similarly prepared, but washed with cold ethanol, diethyl ether and dried *in vacuo* at room temperature.

Procedures Used in Enzyme Inhibition Studies

E. Coli (ATCC 25922) was used in the experiments and the cells were cultured as described by Kohl *et al.* [14]. Cell concentrations were determined either by counting suspensions of the

bacteria (stained with methylene blue) in a standard Neubauer hemocytometer or turbidimetrically by comparison with McFarland's standard barium sulphate suspension [14].

L-Malate dehydrogenase was assayed using a substrate solution containing 0.1 *M* glycine, 10 mmol L-malate and 0.2 mmol nicotinamide adenine dinucleotide (NAD^+) at pH 9.5 in a 1.0 cm cuvette [18]. Between 20–50 μl of enzyme or enzyme platinum complex solution was added, and the absorbance of fumarate was measured at 240 nm on a Gilford Spectrometer. Protein concentrations were determined by the Lowery method [19].

Enzyme inhibition studies were carried out as follows. Various concentrations of platinum blue complexes were prepared in 0.2 *M* phosphate buffer at pH 7.0. An aliquot of these solutions was mixed with an aliquot of enzyme (between 0.5 and 1.0 μ*M*) and the final volume was brought to 0.5 ml with the phosphate buffer. The concentration of the platinum complex, and therefore the molar ratio of complex to enzyme, was based upon the monomeric molecular weight of the platinum complex suggested by the microanalysis of the blue complex. After mixing, the solutions were assayed for enzymatic activity over a period of several days. Kinetic and equilibrium constants were calculated from this data. All studies were performed at 25 °C in a water bath.

Synthesis of Platinum–Adenine Complexes: $[Pt_2(Aden\text{-}H)_2(Aden)_2][PtI_4]$

$K_2[PtCl_4]$ (2 mmol) was dissolved in water (30 ml), filtered, and then KI (12 mmol) was added and the solution stirred for 5 min in the dark. To this, adenine (4 mmol) was added and the mixture was refluxed at 70–75 °C for 2 h. The dark brown product was filtered, washed with water, ethanol (20 ml) and then dried *in vacuo* at room temperature.

DMF, DMSO, THF, Acetone and Pyridine Blue Complexes

These blues were formed by suspending the brown complex, $[Pt_2(Aden\text{-}H)_2(Aden)_2][PtI_4]$, in the appropriate solvent, and the change in color occurred during the increase in temperature from 40–80 °C.

The blue complex is isolated by filtration and washed with dry diethyl ether and dried *in vacuo* at 60 °C. It is to be noted that these blues (except the pyridine blue) can revert to the brown complex by suspending the blue complex in water at 60 °C for 15–20 mins. The brown complex separated and was isolated by filtration.

Column Chromatography

Column Specification: Length: = 130 cm; Diameter: 2.5 cm; Packings; Florisil 60–100 U.S. Mesh (BDH), Kieselgel 60 (silica gel 60) (Merck). The packings were Soxhlet extracted with water for 48 h, washed with ethanol, dry diethyl ether and dried at 140 °C and stored over phosphorous pentoxide.

Physical Measurements

Infrared spectra of the materials in Nujol and hexachlorobutadiene (HCB) mulls were recorded on a Perkin-Elmer 397 spectrometer. *Electronic Spectra* in solution were obtained using a Beckman Acta M4 spectrophotometer. Diffuse reflectance spectra were obtained by using the standard attachment with barium sulphate as the blank reflector.

Conductivities were measured in redistilled deionized water at room temperature using a portable conductivity measuring set, Model MC1, Mar V.

The ESR Spectra were obtained on a Varian E9 ESR spectrometer using the dual cavity mode and were run in the x-band (~9.3 GHz) in the solid state (ground powder) or in water at 77 K.

The ^1H NMR Spectra were performed in D_2O with a Bruker Spectrospin (80 MHz) spectrometer using TMS as internal reference.

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