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Carboxy Uracil and Some of Its Derivatives as Complexant toward Platinum. Platinum Blues

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Platinum complexes of 6-carboxy uracil (orotic acid), 3-methyl orotic acid and 5-nitro-orotic acid and particularly a 'platinum-blue' have been isolated and studied; the different complexation sites of the ligand are discussed, the orotic acid nucleus exhibiting multiplicity and interconversion of binding sites.

Introduction

The first 'anomalous blue complex', called 'Platinblau' by Hofmann and Bugge was reported as early as 1908 [1]. It was obtained by reacting *cis*-dichloro acetonitrile platinum(II) with a silver salt. Its empirical composition $Pt(acetamide)_2 \cdot 2H_2O$ has been assigned to a variety of structures involving either divalent or tetravalent platinum oxidation states [2]. As with all other platinum blues, the nature of this complex remains questionable, mainly because of the difficulty of crystallizing these compounds. So far, only one crystalline blue product has been obtained by using α-pyridone as ligand and the hydrolysis products of *cis*-$(NH_3)_2PtCl_2$ (*cis*-DDP) as platinum source [3–6]. The crystallographically determined structure of this compound consists of tetrameric species where the platinum atoms are bridged by α-pyridonate ligands.

The discovery, a few years ago, that pyrimidine complexes—the so-called 'platinum pyrimidine blues'—exhibit a high index of antitumor activity with a low associated nephrotoxicity has provided added impetus to the study of these singular species [7, 8]. These blue complexes arise from the reaction between the hydrolysis products of *cis*-DDP and uracil, uridine, thymine or other related pyrimidines and therefore belong to Davidson's class I [9]. Despite this interest, most of the literature dealing with blue platinum pyrimidine complexes has been concerned with the origin of the blue color or with the platinum oxidation state, but very few results have been reported concerning the complexation sites. In some recent papers, Lippert investigated the various ways of uracil binding in many uracil platinum complexes, including uracil blues [10, 11]. The main result of this work is to underline the versatility of uracil as a ligand towards platinum. A large variety of complexes are formed with uracil under its neutral, monoanionic or dianionic form bonded to the metal through N(1) or N(3).

Orotic acids (normal, iso- and some of their substituted derivatives) which may be viewed as substituted uracils have not been considered as possible ligands. However, orotic acid (6-carboxy uracil) occupies a singular position among the free pyrimidines, being the only effective precursor for pyrimidine bases of nucleic acids in living organisms [12, 13]. Moreover, it is known to display bacteriostatic and cytostatic properties.

In the course of our own work concerning the blue compounds obtained by reacting potassium tetrachloroplatinate and amide ligands, we obtained not only classical complexes but also blue complexes by using orotic acid and some of its derivatives as ligands.

The present paper reports on the study of those complexes and aims to assess the complexation sites of the ligands.

Experimental

Preparation of the Complexes

The starting materials, *cis*-$(NH_3)_2PtCl_2$, K_2PtCl_4, orotic acid, and 5-nitro-orotic acid potassium salt, were prepared as described below.

3-Methylorotic Acid (a)

This synthesis implies the preparation of a hydantoinic ester (obtained through urea condensation with an oxalacetate) and an intramolecular transposition by enlargement of the heterocycle. The preparation was conducted as described by Clerc-Bory *et al.* [14]. M.p. = 311 °C (lit. M.p. = 306–311), λ_{max} = 280 nm, pH = 7.

[Pt(3-Me-orotic acid)$_2$]K$_2$·6H$_2$O (b)

2×10^{-3} mol of 3-methyl orotic acid in the minimum amount of water at pH 7.2 (the pH was

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adjusted with KOH) were allowed to react with K_2PtCl_4 (10^{-3} *M*) overnight. The resulting yellow precipitate was washed with water, ethanol, and dried *in vacuo*. If the reaction proceeds for several days or several weeks, a light green colour appears but analysis and u.v. spectrum remain unchanged. *Anal.* Calcd. for $Pt(C_6N_2O_4H_4)_2K_2 \cdot 6H_2O$: C, 20.08; H, 2.78; N, 7.81; Pt, 27.19; K, 10.87. Found: C, 20.26; H, 2.79; N, 7.88; Pt, 27.04; K, 10.63.

[Pt(5-nitro-orotic acid)$_2$]$K_2 \cdot 4H_2O$ (c)

The potassium salt of 5-nitro-orotic acid (2×10^{-3} mol) dissolved in 20 ml of warm water was treated with K_2PtCl_4 (1×10^{-3} mol). With time, a yellow precipitate appeared, which was washed rapidly with water, ethanol and dried *in vacuo*. *Anal.* Calcd. for $Pt(C_5N_3O_6H)_2K_2 \cdot 4H_2O$: C, 16.15; H, 1.34; N, 11.30; Pt: 26.24; K, 10.50. Found: 16.45; H, 1.22; N, 10.91; Pt, 25.85; K, 10.34.

[Pt(5-nitro-orotic acid)$_4$]$K_2 \cdot 4H_2O$ (d)

4×10^{-3} mol of the potassium salt of 5-nitro-orotic acid were allowed to react with K_2PtCl_4 (1×10^{-3} mol). The yellow precipitate was successively washed with water and ethanol, and dried *in vacuo*. When the reaction proceeds for one or two months, a green colour appears on the supernatant solution. *Anal.* Calcd. for $Pt(C_5N_3O_6H_2)_4K_2 \cdot 4H_2O$. C, 20.96; H, 1.39; N, 14.67; Pt, 17.03; K, 6.81. Found: C, 20.83; H, 1.11; N, 14.76; Pt, 16.98; K, 7.0.

$(NH_3)_2$Pt(5-nitro-orotic acid)$\cdot 5H_2O$ (e)

A 0.1 *M* solution of *cis*-diammine platinum(II) hydrolysis products was prepared by mixing 0.3 g of *cis*-$(NH_3)_2PtCl_2$ and 0.34 g of $AgNO_3$ in 10 ml of water. The solution was stirred overnight in the dark at room temperature and centrifuged to remove any AgCl precipitate. One equivalent of 5-nitro-orotic acid potassium salt dissolved in 40 ml water was then added, the pH being monitored to 6.5 throughout the course of the reaction. A light yellow precipitate appeared immediately. After two hours, the yellow product was isolated, washed with water, ethanol and dried *in vacuo*. *Anal.* Calcd. for $(NH_3)_2Pt(C_5HN_3O_6) \cdot 5H_2O$: C, 11.58; H, 3.28; N, 13.51; Pt, 37.64. Found: C, 11.42; H, 3.10; N, 14.24; Pt, 35.91.

[Pt(orotic acid)$_2$]K_2 (f)

K_2PtCl_4 (10^{-3} mol) dissolved in H_2O (20 cm^3) was added to two equivalents of orotic acid dissolved in a minimum amount of H_2O and adjusted to pH = 8 with 0.4 *M* KOH. After addition, the reaction mixture was adjusted to pH = 8 and stirred in the dark at 37–40 °C. After a few hours, the mixture displayed a green colour and a yellow-green solid precipitated. During the course of the reaction, the pH fell from 8 to 2.4. The yellow-green species was isolated by filtration and dried *in vacuo*. *Anal.* Calcd. for $Pt(C_5H_2N_2O_4)_2K_2$: C, 20.64; H, 0.69; N, 9.63; Pt, 33.56; K. 13.44. Found: C, 20.0; H, 0.93; N, 9.2; Pt, 34.1; K, 12.9. This yellow species has been observed to dissolve slowly in water to give a green solution which soon turns blue while the pH decreases. With time, a blue powder may be collected by precipitation with an ethanol/ether mixture (80/20 in volume). However, the analytical results for this blue compound are poor and better analytical data are obtained using the preparative scheme described below.

Platinum Orotato-blue (g)

This compound was obtained by equimolecular reaction of K_2PtCl_4 and orotic acid in the minimum amount of water. In this case, the yellow precipitate did not appear and only a blue solution was obtained. The mixture was stirred in the dark for one day, after which a blue powder was precipitated with ethanol/ether (80/20 in volume). It was then redissolved in water and reprecipitated. This procedure was repeated several times and the sample dried *in vacuo*. One typical analysis of the blue compound is reported here, *i.e.*: C, 14.06; H, 1.04; N, 6.8; Pt, 47.5; K, 7.0. These data are not related to calculated data since the experimental results vary slightly from batch to batch. However, it should be emphasized that in any case only one ligand is implied by the platinum atom and that no chlorine can be detected.

Measurements

Spectroscopic measurements were carried out at 25 °C on a Cary 14 recording spectrophotometer. Cells of 10 mm path length were employed. U.v. light absorption studies were carried out using solutions of orotic acid derivatives which were diluted to 10^{-4} *M*. Each solution was adjusted to the desired pH by means of a Copenhagen Automatic Radiometer titration chain using 0.1 *N* aqueous KOH solutions. E.s.r. spectra were obtained using a Bruker ER 200 K with a conventional X-band (9.6 GHz). The microwave frequency was calibrated with diphenylpicrylhydrazyl. All e.s.r. spectra were recorded on frozen aqueous solutions directly on the mother solution and on the powdered sample over a wide range of temperatures.

An oxidative titration was conducted for the platinum blue, the solution of such a complex being discoloured by a number of oxidizing agents. This provides a method for estimating the formal oxidation number of platinum in these compounds. Oxidative titration was carried out using ceric sulphate solution (5.023×10^{-3} *N* in 0.70 H_2SO_4) which was standardized by the normal procedure and monitored by spectrophotometry.

Results and Discussion

Due to the presence of many potential binding sites, the coordination behaviour of orotic acid is controversial. In addition to the two nitrogen atoms of the pyrimidine ring, N(1) and N(3), and to the oxygen atoms of the carbonyl group, the carboxyl group is also a potential binding site which may be involved in a chelating process together with the N(1) site. Moreover, the complexity of the system involving the pyrimidine ring results from the effects of pH changes and group substitutions on the ratio of the tautomeric forms of the dianion HL^{2-} (Fig. 1).

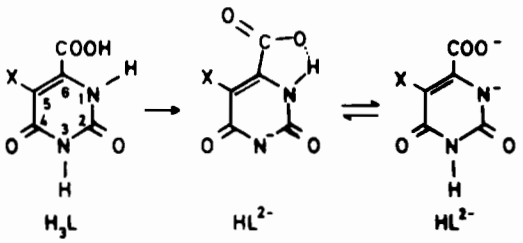

Fig. 1. Tautomeric forms of orotic acid and orotic acid dianions HL^{2-}.

Most of the studies already devoted to orotic acid complexes have been performed on aqueous solutions prepared by mixing appropriate quantities of ligand and metal salt [15–17]. However, the X-ray structure of some complexes has been described [18–20]. These data support the conclusions previously deduced from solution studies: complexation specifically stabilizes the N(3)H tautomer and coordination to the metal occurs via one oxygen of the carboxylate group and the N(1) nitrogen as chelating sites (Fig. 2) [18–20].

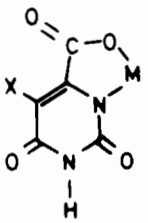

Fig. 2. Classical metal-complexation of orotic acid.

This general scheme may not be extended to all the metals and in two recent papers [21, 22] we have shown that coordination may also occur at the N(3) site in the case of copper and palladium complexes. Moreover, a copper(II) complex of 5-nitro-orotic acid involving jointly the N(1) and N(3) coordination sites of the same ligand has been recently isolated and structurally characterized [21].

From the data already published relating to the two N-methylated orotic acids [23] and N-methylated uracils [24, 25] and to their related anionic forms, one may notice that the spectra of the N(1) substituted anions are blue shifted with respect to the N(3) substituted ones.

For orotic acid itself, there is a very small shift to longer wavelengths when moving from the acidic (λ_{max} = 285 nm) to the pH 12 region (λ_{max} = 290 nm). Keeping in mind the results obtained by Dubois *et al.* [26] as well as those of Tucci and Li [15], one may assume that the absorbance at higher wavelengths is only due to the N(3)H tautomer. In this instance, the orotic acid dianion would be predominantly in a N(1)H form which would be stabilized by hydrogen bonding between N(1) and the carboxylate group.

3-Methyl-orotic acid obviously behaves differently from orotic acid itself. In this case, deprotonation of the pyrimidine nucleus occurs only at the N(1) site and, as expected, a bathochromic shift is observed at pH = 12 (from 280 to 305 nm).

5-Nitro-orotic acid displays a strong bathochromic effect (from 285 to 340 nm) near and above pKa_2 which suggests that the N(3)H structure is favoured. It is likely that this important shift cannot be ascribed only to ionic strength effects; a conjugative stabilization by the nitro group may also be evoked. Similarly, the nitro group exhibits a strong influence on proton dissociation from the carbonyl and pyrimidine ring nitrogen since the pKa values are 2.10 and 9.5 for orotic acid and 1.5 and 4.9 for 5-nitro-orotic acid (Table I).

3-Methyl-orotic Platinum Complex

Due to methylation at N(3), complexation may occur at N(1). As expected, only one complex is obtained, $[PtL_2]K_2$ (b). The λ_{max} at 360 nm (Fig. 3) is

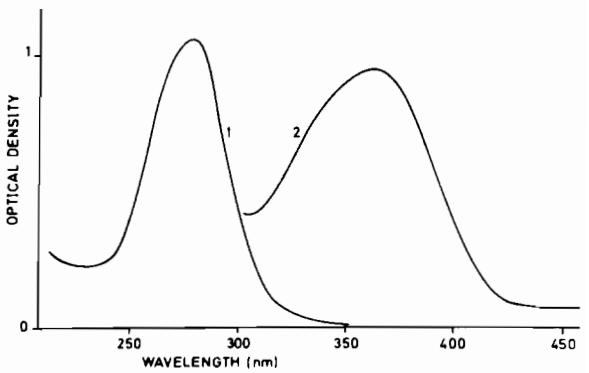

Fig. 3. Ultraviolet absorption spectra at 20 °C of: (1) 3-methyl-orotic acid, in water at pH = 7, (2) $[Pt(3\text{-methyl-orotic acid})_2]K_2 \cdot 6H_2O$.

consistent with complexation occurring at N(1) and at the carboxylate group. It should be emphasized that the observed absorbance (360 nm) is strongly

TABLE I. Ultraviolet Absorption Data for Some Orotic Acid Derivatives Related to Their Deprotonation or Complexation Site.

Compound	Neutral media λ_{max}	Basic media λ_{max}	Deprotonation or complexation site
N(1), N(3)-dimethyl-orotic	275	275	–
N(1)-methyl-orotic	274	280	N(3)
N(3)-methyl-orotic (LH_2)	280	305	N(1)
$[(PtL_2)]K_2 \cdot 6H_2O$ (b)	360		N(1)
5-nitro-orotic (LH_3)	340	340	N(1)
$(NH_3)_2Pt(LH) \cdot 5H_2O$ (e)	340		N(1)
$[Pt(LH)_2]K_2 \cdot 4H_2O$ (c)	375		N(1)
$[Pt(LH_2)_4]K_2 \cdot 4H_2O$ (d)	298		N(3)
orotic (LH_3)	285	290	N(3)
$[Pt(LH)_2]K_2$	315		N(1)
orotato blue	278		N(3)

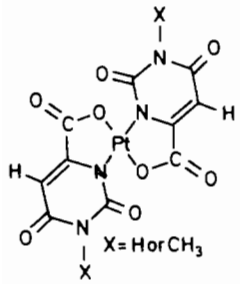

Fig. 4. Tentative molecular structure of a $[Pt(LH^{2-})_2]K_2$ complex.

blue shifted even with respect to the deprotonated ligand itself (λ_{max} = 305 nm). This may be due to an extended electron delocalization on the two coordinated ligands through the platinum itself (Fig. 4). As reported *infra*, this seems to be a general trend for this type of complex involving two ligands.

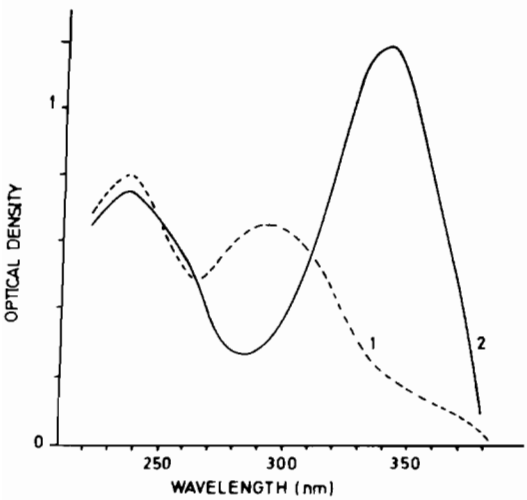

Fig. 5.Ultraviolet absorption spectra at 20 °C of: (1) 5-nitro-orotic acid at pH = 2.5, (2) 5-nitro-orotic acid at pH > 7.

5-Nitro-orotic Acid Complexes

Three complexes have been isolated in the case of 5-nitro-orotic acid. In the cases of the $[Pt(LH)_2]K_2$ (c) and $(NH_3)_2Pt(LH)$ (e) complexes, the stoichiometry and the values observed for λ_{max} (Figs. 5 and 6)

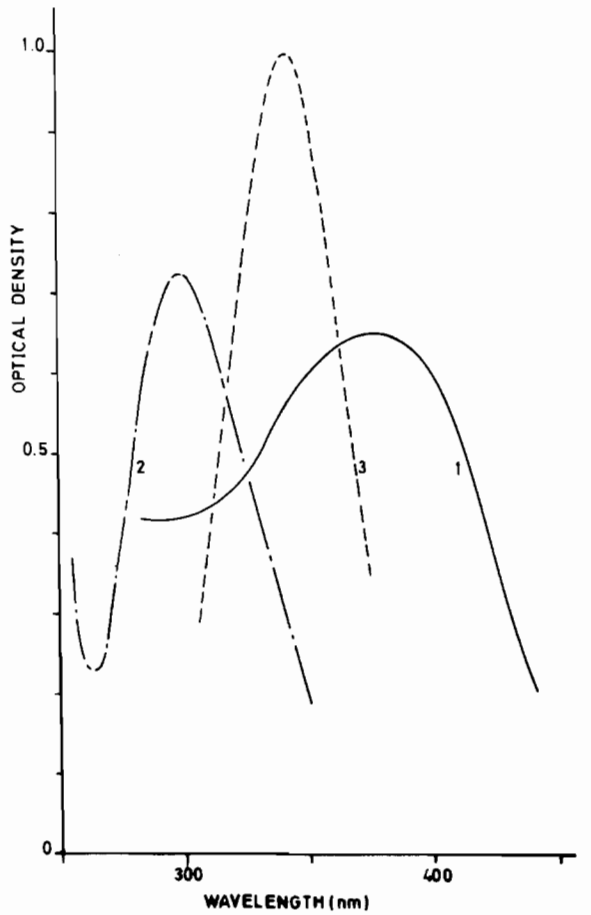

Fig. 6. Ultraviolet absorption spectra at 20 °C of: (1) $[Pt(5\text{-nitro-orotic acid})_2]K_2 \cdot 4H_2O$, (2) $(NH_3)_2Pt(5\text{-nitro-orotic acid}) \cdot 5H_2O$, (3) $[Pt(5\text{-nitro-orotic acid})_4]K_2 \cdot 4H_2O$.

favour coordination of the ligand through N(1) and the carboxylate group. As already mentioned, the complex involving two ligands, *i.e.* (c), displays a larger bathochromic shift than the 1/1 species (e). The $[Pt(LH_4)]K_2$ complex displays a different behaviour since the maximum wavelength is *ca.* 50 nm smaller than the value related to the free ligand. Such a trend has already been observed in the case of the copper(II) complex. In the latter case, the hypsochromic shift has been related unambiguously to N(3) complexation, the X-ray structural data providing the ultimate evidence of this assumption. This conclusion may be extended to the platinum complex (Fig. 7).

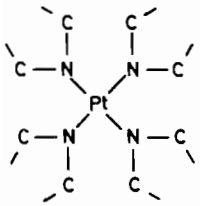

Fig. 7. Tentative molecular structure of a $[Pt(H_2L^-)_4]K_2$ complex.

Orotic Acid Complexes

A platinum complex of orotic acid has already been described [20]. This complex, obtained by reacting the ligand with the hydrolysis product of *cis*-diamminodichloroplatinum(II) is yellow, but blue products are also present in the reaction mixture. The yellow product submitted to X-ray diffraction analysis offers structural characteristics quite similar to those observed for other orotic acid complexes, *i.e.* coordination through N(1) and one oxygen of the carboxylate group.

In our work, using K_2PtCl_4 as platinum source, two types of complexes are obtained. The yellow complex displays a $[Pt(LH)_2]K_2$ (f) formula and is quite similar to the products obtained from 3-methyl-orotic and 5-nitro-orotic acid as ligands. The maximum wavelength is blue shifted with respect to the free ligand supporting a 'classical' coordination by the N(1) deprotonated site of the pyrimidine ring and one carboxylic oxygen. Nevertheless, in the blue complex, the maximum wavelength (278 nm) strongly suggests that N(3) is actually involved in the coordination mode (Fig. 8); moreover, all the blue platinum complexes known involve as complexation sites an amidic oxygen besides a deprotonated nitrogen. Therefore, either O(2) or O(4) may also be considered as coordination sites.* Considering that,

*Tentative determination of carbonyl complexation sites by means of ^{13}C or ^{195}Pt n.m.r. spectroscopy failed due to the poor solubility of the platinum blue.

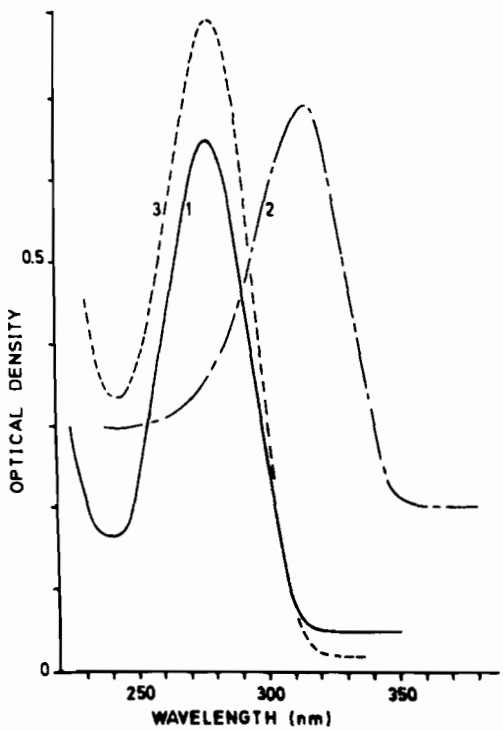

Fig. 8. Ultraviolet absorption spectra at 20 °C of: (1) orotic acid at pH = 7 in water, (2) $[Pt(orotic\ acid)_2]K_2$, (3) 'orotato blue'.

with time, the yellow species (f) slowly dissolves in water to give a blue solution with a subsequent red shift of the λ_{max}, a tentative mechanism for the formation of the blue complex is proposed: (i) the carboxylate group and N(1) act as anchor to chelate the platinum atom and (ii) promote complexation with an amide group. The weak co-ordinating capabilities of amide and the importance of the role played by a primary ligating site have recently been underlined by Sigel and Martin [27]. Considering the interest taken in the blue platinum complexes and especially in complexes involving a pyrimidine nucleus, we have specified some data regarding this product.

Spectral Properties of the Orotato Blue

In addition to the absorption characterizing the pyrimidine nucleus, the visible spectrum of the orotato blue displays a broad transition centered at 625 nm (ϵ = 580 cm^{-1} M^{-1} where M stands for a monomeric formula). The visible spectrum in the solid state is essentially identical to that observed in solution showing that the electronic nature of the compound is not strongly altered by dissolution. The blue solution is discoloured by a number of oxidizing or reducing agents. This suggests that the colour is related either to the simultaneous presence of Pt(II) and Pt(IV) ions or to a strong electronic delocalization along a number of platinum atoms.

Oxidative Titration Data

An oxidative titration by ceric ions discloses a formal oxidation state higher than two and varying from batch to batch between 2.20 and 2.42. These data may be related to those obtained for other platinum blues. The average oxidation state is 2.25 in the case of α-pyridone blue [5] while it varies from 2.9 to 3.2 [28], in the phthalimide-blue and is as high as 3.72 in 1-methylthymine blue [29]. It may be underlined that oxidative titration conducted on the yellow orotato complexes always affords an oxidation state of platinum very close to 2 for these compounds.

E.s.r. Spectroscopy

All known blue compounds exhibit paramagnetism which can be detected by means of e.s.r. spectroscopy. E.s.r. signals are observed when the complex is studied in the solid state and in aqueous frozen solutions. Boths sets of measurements yield practically identical g values: $g_{//} = 1.97$ and $g_{\perp} = 2.47$. The large g shift suggesting considerable spin–orbit coupling of an odd-electron is typical behaviour of paramagnetic heavier transition metal ions. The g values are very similar to those obtained for *cis*-diammine platinum α-pyridone blue [6], Pt(IV) doped Magnus' green salt [30], 'irradiated' Pt(II) complexes [31, 32] and all known platinum blues. The measured g values are interpreted in terms of $5d_{z^2}$ hole states, with admixture of the degenerate dxy, dxz states due to spin orbit coupling.

Thus it appears that orotato blue exhibits the main characteristics of the platinum blues. In this instance, the uv data previously reported are particularly interesting since they support the occurrence of only one coordination mode involving the N(3) site while it has been suggested [5] that 'platinum uracil blue' might contain a mixture of species with different coordination modes.

Recently, Lippert [10] emphasized the versatility of unsubstituted uracil as a ligand of platinum and underlined the multiplicity and interconversion of binding sites. It is likely that the carboxylic group strongly reduces the versatility of the ligand, the N(1) site becomes favoured due to the possibility for chelation. However, N(3) complexation is not completely prohibited. It is worthy of note that for orotic acid complexes, there is an interconversion of the complexation sites on going from the yellow to the blue complex. Consequently, the complexation site N(3) should not be neglected when biological processes are studied. This site may be favoured by the nature of the metal, the nature of the ligand, or even by the stoichiometry of the complex. More subtle factors leading to a stabilization of the blue species may be operative in the case of platinum orotato blues.

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