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Rare Iminol Tautomer of 1-Methylthymine through Metal Coordination at N(3)

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*Bis(1-methylthyminato-N3)*cis*-diammineplatinum-(II),* cis*-$Pt(NH_3)_2(C_6H_7N_2O_2)_2$, is readily protonated to give a compound of composition* cis*-$[Pt(NH_3)_2(C_6H_7N_2O_2)(C_6H_8N_2O_2)]^+X^-$ ($X = Cl^-, NO_3^-, ClO_4^-$). The pK value for the protonation is 2.05 ± 0.05. The compound contains an anionic 1-methylthymine ligand and a neutral 1-methylthymine ligand in an unusual iminol tautomer structure, both being coordinated to Pt(II) through N(3). The neutral thymine ligand is only weakly bound to Pt(II) and readily displaced. Spectroscopic data (^{1}H-NMR, UV, IR) are presented. A model for a possible nucleobase mispairing mechanism catalyzed through metal coordination at N(3) of thymidine, is proposed.*

Introduction

Bis(1-methylthyminato-N3)*cis*-diammineplatinum-(II) is a model compound for a hypothetical interaction of the antitumor agent *cis*-$Pt(NH_3)_2Cl_2$ with two thymine bases of DNA. We recently reported on the interesting complex forming properties of this compound which readily binds additional *cis*-Pt(II) [1] or Ag(I) [2] through O(4) of the thymine ligands. It has now been found, that protonation of one of the two thymine ligands of the 2:1 compound is achieved equally well, leading to a product of composition [(1-methylthyminato-N3)(1-methylthymine-N3)*cis*-diammineplatinum(II)]$^+X^-$ ($X = Cl^-, NO_3^-, ClO_4^-$). This compound contains a neutral 1-methylthymine ligand in the unusual iminol tautomer structure and an anionic 1-methylthyminato ligand, both coordinated to Pt(II) through N(3).

The preparation and spectroscopic data are presented. Possible biological implications of this finding are briefly discussed.*

Abbreviations used: TH = 1-methylthymine (usual dioxo-tautomer); TH = 1-methylthymine (unusual oxo, hydroxo-tautomer); T = 1-methylthymine anion; *cis*-a_2Pt = *cis*-$(NH_3)_2Pt$; DMSO = dimethylsulfoxide; DMF = dimethylformamide.

Experimental

Preparation of cis-$[Pt(NH_3)_2T(TH)]^+X^-$

Bis(1-methylthyminato-N3)*cis*-diammineplatinum-(II) hydrate, *cis*-$Pt(NH_3)_2(C_6H_7N_2O_2)_2aq$, was prepared as previously described [2]. The compound was then passed over a Sephadex G10 column to remove any residues of unreacted 1-methylthymine. [*cis*-$Pt(NH_3)_2(C_6H_7N_2O_2)(C_6H_8N_2O_2)$]X (X = Cl^-, NO_3^-, ClO_4^-) was prepared by dissolving *cis*-$Pt(NH_3)_2(C_6H_7N_2O_2)_2$ in a minimum amount of water (1 mmol/20 ml H_2O) and titration with 1 equivalent of 0.2 *N* acid or, with better yield, with 1.5–2 equivalents. The nitrate and the perchlorate readily precipitated as shiny, white microneedles. The chloride was obtained by concentrating the solution under vacuum without warming the solution. All three salts were washed with a small amount of ice cold diluted acid, water, and then extensively with acetone. Yields 45% (NO_3^-), 75% (Cl^-), 80% (ClO_4^-). Elemental analysis in all cases was satisfactory for Pt, C, H, N. *Anal.* chloride. Found: C, 24.21; H, 4.50; N, 13.82; Pt, 31.7. Calcd. for $[Pt(NH_3)_2(C_6H_7N_2O_2)(C_6H_8N_2O_2)]Cl\cdot 3H_2O$: C, 24.10; H, 4.56; N, 14.06; Pt, 32.62%. Nitrate. Found: C, 23.91; H, 4.21; N, 16.10; Pt, 31.9. Calcd. for dihydrate: C, 23.76; H, 4.16; N, 16.17; Pt, 32.16%. Perchlorate. Found: C, 22.71; H, 3.76; N, 13.19; Pt, 30.9. Calcd. for monohydrate: C, 23.02; H, 3.71; N, 13.43; Pt, 31.17%.

Decomposition of cis-$[Pt(NH_3)_2T(TH)]X$

If *cis*-$[Pt(NH_3)_2T(TH)]X$ was suspended in excess acid HX, it slowly (1–3 weeks, 22 °C) redissolved with formation of a yellow (X = Cl^-) and blue solution (X = NO_3^-, ClO_4^-), respectively. From the HCl solution, *cis*-$Pt(NH_3)_2Cl_2$ and 1-methylthymine were obtained upon slow evaporation, from the HNO_3 and $HClO_4$ solutions only one product (1-methylthymine) has been identified.

Thermal decomposition of *cis*-$[Pt(NH_3)_2T(TH)]X$ (X = Cl^-, NO_3^-) without additional acid gave the following results: X = Cl^-: 1 mmol of *cis*-$[Pt(NH_3)_2T(TH)]Cl\cdot 3H_2O$ was dissolved in 20 ml H_2O. pH = 1.7. Heating to 90 °C for 12 min gave a yellow-tan solution of pH = 2.5. Upon slow evaporation (4d,

22 °C, final pH = 2 8) to a small volume, 400 mg of a mixture of TH and *cis*-$[Pt(NH_3)_2TCl]\cdot 1H_2O$ were filtered, washed with H_2O and treated with DMF to remove TH Recrystallization of the remaining product from hot water gave 200 mg of colorless to slightly yellow crystal plates of *cis*-$[Pt(NH_3)_2TCl]\cdot 1H_2O$ IR(Nujol) νPt–Cl at 315 cm^{-1} *Anal* Found C, 17 53, H, 3 67, N, 13 25, Pt, 45 7 Calcd C, 17 08, H, 3 59, N, 13 29, Pt, 46 25% No attempts were made to optimize the yield

X = NO_3^- Analogous treatment of *cis*-$[Pt(NH_3)_2T(TH)]NO_3\cdot 2H_2O$ yielded a mixture of TH *1*, yellow crystals *2* (50% yield), white microneedles *3* (10% yield), *cis*-$Pt(NH_3)_2T_2$aq *4*, yellow-brown dicroic cubes *5*, and a dark green, amorphous compound *6* Separation of the individual compounds was achieved by repeated fractional crystallization due to different solubilities of the components in water *2* and *6* are well soluble, *3*, *4* and *5* are moderately soluble, *1* is only weakly soluble *Anal* yellow crystals *2* Found C, 16 38, H, 3 38, N, 15 72, O, 20 25, Pt, 44 5 Calcd for $[Pt(NH_3)_2T]_2(NO_3)_2\cdot 1H_2O$ C, 16 44, H, 3 00, N, 15 98, O, 20 08, Pt, 44 51% IR(Nujol, KBr) 1655vs, 1570w, 1505vs cm^{-1}

Recrystallization of *3* from water (60 °C, evaporation at 22 °C) led to partial formation of compound *2*

Apparatus and Experimental Conditions

^{1}H-NMR spectra were recorded on a Jeol JNM-FX 60 Fourier-transform spectrometer Depending upon the concentrations, usually 100–2000 transients were accumulated into 8 K data points of memory Temperatures were determined by means of methanol and ethyleneglycole capillaries Chemical shifts are given on the δ scale and referenced to internal TMS (tetramethylsilane) in DMSO-d_6 and DMF-d_7 Chemical shifts in D_2O were measured by means of an internal $[N(CH_3)_4]BF_4$ reference and calculated to TSP (sodium 3-(trimethylsilyl)propanesulfonate) The shift of $[N(CH_3)_4]^+$ relative to TSP was taken as 3 1869 ppm pD-measurements were performed with a glass electrode and 0 4 units were added to the obtained pH meter reading to give the pD [3]

Solvents were purchased from Roth (DMSO-d_6, DMF-d_7, D_2O) and Merck (DMSO-d_6) DMSO and DMF were dried over 4 Å molecular sieves Compounds were dissolved and the water of crystallization removed by means of molecular sieves (DMSO, DMF) The molecular sieves were removed prior to recording the spectra Samples were handled in an atmosphere of dry nitrogen IR spectra were recorded on a Perkin Elmer 580 grating spectrometer as Nujol mulls (CsI windows) and KBr discs between 4000 and 200(300) cm^{-1}

UV spectra were recorded on a Cary 17D spectrophotometer pH titrations were performed by means of a glass electrode and a Radiometer pH meter 26 under N_2 to exclude CO_2 uptake

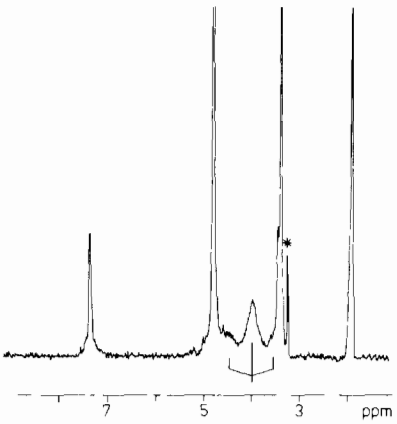


Fig 1 ^{1}H-NMR spectrum of *cis*-$[a_2PtT(TH)]Cl$ aq in D_2O immediately after dissolving the compound c = 0 04 *M* (Pt), pD = 2 34, * = $[N(CH_3)_4]^+$ reference

Results

^{1}H-NMR Spectra

D_2O In Fig 1 the ^{1}H-NMR spectrum of the chloride salt, *cis*-$[a_2PtT(TH)]Cl$, in D_2O immediately after dissolving is given The spectrum consists of single peaks for C(6)H at 7 35 ppm, C(5)CH_3 at 1 83 ppm, N(1)CH_3 at 3 32 ppm as well as a signal of the unexchanged NH_3 protons at 3 93 ppm and an averaged signal due to solvent protons, exchanged NH_3 protons and protons of water of crystallization at 4 76 ppm The spectrum slowly changes with time and rapidly if warmed The appearance of two new sets of signals has been observed This is particularly evident with the C(6)H and the C(5)CH_3 signals In Fig 2 changes of the C(6)H signal are shown, in Fig 3 changes of the C(5)CH_3 signal Signals B and C can unambiguously be assigned to neutral 1-methylthymine TH and to *cis*-$[a_2PtTCl]$, respectively, from comparison with the ^{1}H-NMR spectra of the individual compounds and based on IR spectroscopy and elemental analysis (*cf* Experimental) The C(6)H signals of both B and C consist of quartets due to coupling with the protons of the CH_3 group at the C(5) position The C(5)CH_3 signals of the corresponding compounds are split into doublets as a consequence of coupling with the C(6) proton Coupling constants are identical within experimental error for B and C, 1 22 Hz each

The spectral changes are accompanied by an increase in pD For example, the sample in Fig 2a (3a) had a pD = 2 34, but a pD = 4 30 after 10 minutes at 90 °C (Fig 2d, 3c) The position of the C(6)H signal A is pD dependent and is shifted to higher field with increasing pD until it remains constant at a pD $\geqslant$ 4 It is then identical with that of *cis*-a_2PtT_2 (A*) as can be seen from comparison with the spectrum of the isolated compound

Fig 2 Spectral changes of the C(6)H signal of *cis*-[a_2PtT-(TH)]Cl in D_2O c = 0 04 *M* (Pt) a) Spectrum after dissolving the compound, b) spectrum 18 h after dissolving the compound (sample kept at 22 °C), c) spectrum after 5 min heating to 90 °C, d) spectrum after 10 min heating to 90 °C

The spectroscopic changes, which are in agreement with experimental findings on a preparative scale (*cf* Experimental), are interpreted as follows

The signal set A represents the average signals of the 1-methylthymine ligands of the two platinum species in equilibrium (1)

$$cis\text{-}[a_2PtT(TH)]^+ \rightleftharpoons cis\text{-}[a_2PtT_2] + H^+ \qquad (1)$$

Sets B and C represent the corresponding thymine signals of the decomposition products of *cis*-[a_2PtT(TH)]Cl

$$cis\text{-}[a_2PtT(TH)]Cl \longrightarrow \underset{\|}{cis\text{-}[a_2PtTCl]} + \underset{\|}{TH} \qquad (2)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad C \qquad\quad B$$

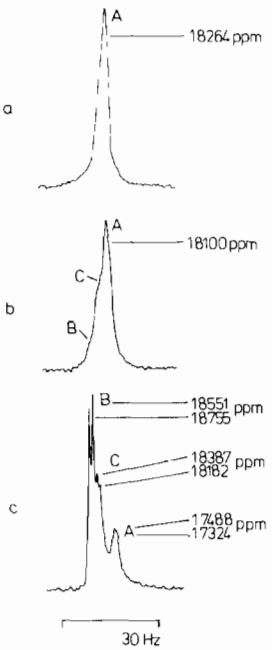


Fig 3 Spectral changes of the C(5)CH_3 signal of *cis*-[a_2PtT(TH)]Cl in D_2O, c = 0 04 *M* (Pt) a) Spectrum after dissolving the compound, b) spectrum 18 h after dissolving the compound (sample kept at 22 °C), c) spectrum after 5 min heating to 90 °C

B and C are formed in equal quantities However, due to the low solubility of *cis*-[a_2PtTCl] in water, this compound is partially precipitating This results in a somewhat smaller signal intensity of C compared to B

As decomposition proceeds, the strongly acidic proton of the platinated TH ligand is more tightly bound in the weakly acidic free TH, thus leading to a rise in pD

From the pD measured immediately after dissolving the compound, that is, before decomposition starts, and the pK of equilibrium (1) (*vide infra*), one obtains approximately a 1 1 ratio for *cis*-[a_2PtT(TH)]$^+$ and *cis*-[a_2PtT$_2$] From this result, and the shift of the signal of pure *cis*-[a_2PtT$_2$] (A*), one can estimate a shift of approximately 7 6 ppm for the C(6)H signal of the *cis*-[a_2PtT(TH)]$^+$ species Of course, this signal again is averaged over the individual T and TH signals of the *cis*-[a_2PtT(TH)]$^+$ compound

DMSO In Fig 4 the ^{1}H-NMR spectrum of the perchlorate salt in DMSO-d_6 is shown If water of crystallization is present and/or the solvent not completely dry (Fig 4a–4c), the acidic proton of *cis*-[a_2PtT(TH)]$^+$ and the protons of water exchange, thus leading to an average signal around 4 5–7 ppm As expected, with increasing H_2O content, the signal is shifted to higher field, but to lower field with decreasing H_2O content With all water removed (Fig 4d), the signal of the acidic proton is broadened beyond resolution and not observed at 30 °C (The

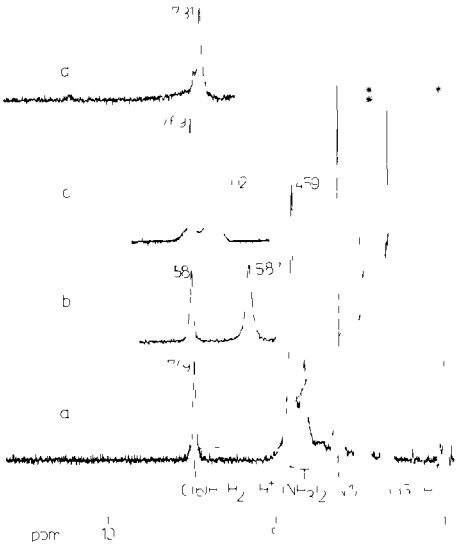


Fig 4 ^{1}H-NMR spectrum of *cis*-[a_2PtT(TH)]ClO_4 with differing water content a) 3H_2O per Pt, b) 1 3H_2O per Pt, c) 0 6H_2O per Pt, d) free of H_2O (partially decomposed, *cf* text) * = TMS, ** = DMSO

very weak signal observed at 11 16 ppm is due to 1-methylthymine and will be dealt with subsequently) From the intensities of the average signals, their shifts and the shift of H_2O protons in DMSO (≃3 35 ppm), a shift of 11 7 ± 0 3 for the acidic proton has been calculated from spectra 4a–4c This value appears reasonable, since it lies within the range of absorption of acidic hydroxo protons of heterocycles

The C(6)H signal exhibits a downfield shift with decreasing H_2O content (Fig 4a–4c) However, there is a substantial upfield shift of this signal with all water being removed (Fig 4d) With time, further spectroscopic changes are observed which qualitatively are similar to those observed in D_2O, *i e* the appearance of two new sets of signals As with the D_2O spectra, the changes are most easily recognized in the low field region of the spectrum Changes of the C(5)CH_3 and the N(1)CH_3 signals are less pronounced although clearly detectable Changes of the ammine proton signals are observed as well, but because of overlapping, splitting due to ^{195}Pt coupling and their broadness, a differentiation at an early stage of the decomposition reaction is not easy With the decomposition of *cis*-[a_2PtT(TH)]$^+$ proceeding, one observes an upfield shift of the original triplet and appearance of a new triplet centered around 4 4 ppm ($^2J_{^{195}Pt-^1H}$ = 56 Hz) In Fig 5 the low field range is given at various times (H_2O-free samples) In the first spectrum (Fig 5a, corresponding to Fig 4d), a broad singlet A is observed at 7 31 ppm together with weak signals B and B′ at 7 50 and 11 16 ppm The original signal A gradually shifts to higher field while decreasing in intensity Simultaneously signals B and B′ increase in intensity, and a new signal C appears

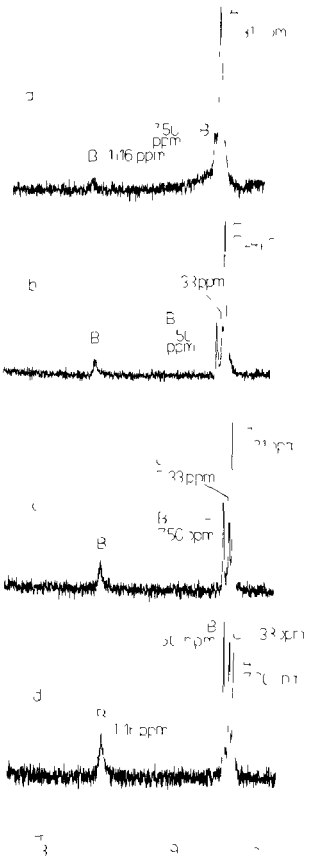


Fig 5 Spectral changes of the C(6)H signal of *cis*-[a_2PtT-(TH)]ClO_4 in DMSO a) 24 h after addition of molecular sieves to remove H_2O, b) H_2O free sample after 1 d at 22 °C; c) H_2O free sample after 3 d at 22 °C, d) H_2O free sample after 5 min at 80 °C Indicated shifts refer to center of signals

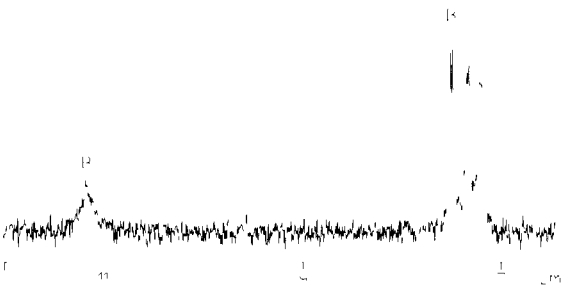


Fig 6 Spectrum of Fig 5d in an extended scale

between signals A and B (Fig 5b) After 3 days at 22 °C signal A has shifted sufficiently so that signal C is completely observable Brief warming (5 minutes, 80 °C) of the solution leads to a further increase of signals B, B′ and C at the expense of A In Fig. 6 the spectrum of Fig 5d is given on an extended scale. The quartet structures of signals B and C, although not ideally resolved, can be recognized

The spectroscopic changes are interpreted in a way analogous to that described above for the behaviour in D_2O signal A represents the average C(6)H signal of the two Pt species in equilibrium (1), B and B' the signals of 1-methylthymine (C(6)H and N(3)H, respectively), signal C is due to species *cis*-$[a_2PtT(DMSO)]^+$ Neutral 1-methylthymine and the 1,1 platinum complex are formed in equal quantities as a result of the decomposition of *cis*-$[a_2PtT(TH)]^+$ according to

$$cis\text{-}[a_2PtT(TH)]ClO_4 \xrightarrow{DMSO} cis\text{-}[a_2PtT(DMSO)]ClO_4 + TH \qquad (2a)$$

With the C(6)H signal of the thymine ligands in *cis*-$[a_2PtT_2]$ absorbing at higher field* compared with the average signal A, the observed downfield shift of A with decreasing H_2O content (Fig 4a–4c) is interpreted in terms of a shift of equilibrium (1) to the left Thus dissociation of *cis*-$[a_2PtT(TH)]^+$ in DMSO is reduced with decreasing H_2O content With this in mind, the observed upfield shift of the C(6)H signal when going from a DMSO solution containing a small amount of water (Fig 4c approximately 0 06 *M* H_2O) to a DMSO solution containing no water at all (Fig 4d) seems to be unlogical However, both the very low solubility of *cis*-$[a_2PtT_2]$ in dry DMSO**, which leads to a partial precipitation of this compound from the solution given in Fig 4d, and the beginning of the decomposition of *cis*-$[a_2PtT(TH)]^+$ according to (2a), lead to a reduction of the concentration of the protonated platinum species As a consequence, the observed average signal of equilibrium (1) is shifted upfield

Prolonged heating (>10 minutes, 90–100 °C) leads to further spectral changes (not shown) For example, a reduction of the signal intensities of C and of the NH_3 resonances is observed Release of ammonia from the *cis*-$[a_2PtT(DMSO)]^+$ species according to

$$cis\text{-}[a_2PtT(DMSO)]^+ \xrightarrow{DMSO} trans\text{-}[aPtT(DMSO)_2]^+ + a$$

would be consistent with this observation

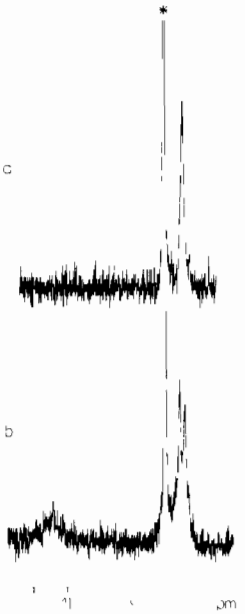


Fig 7 Low field portion of the ^{1}H-NMR spectrum of *cis*-$[a_2PtT(TH)]ClO_4$ in DMF-d_7 after removal of H_2O a) at 30 °C, b) at −20 °C * = DMF solvent

DMF In Fig 7 the low field portion of the ^{1}H-NMR spectrum of *cis*-$[a_2PtT(TH)]ClO_4$ after drying over molecular sieves is shown Fig 7a gives the spectrum at 30 °C It shows a single peak for the resonances of the C(6) protons of the thymine ligands at 7 47 ppm and the proton of the DMF solvent at 8 03 ppm No signal for the acidic proton of the TH ligand is observed, most likely because of fast inter- or intramolecular exchange between the two thymine ligands in *cis*-$[a_2PtT(TH)]^+$ When the sample is cooled to −20 °C, a splitting of the C(6)H signal into two signals of equal intensities (7 61 and 7 45 ppm) is observed, and a new signal appears at 11 4 ppm Simultaneously a splitting of the $N(1)CH_3$ and the $C(5)CH_3$ peaks is observed, although not nearly as clear as that of the C(6)H resonance

The split signals are assigned to the C(6)H resonances of the TH ligand (7 61 ppm) and the T ligand (7 45 ppm) in *cis*-$[a_2PtT(TH)]^+$, the signal at 11 4 ppm to the OH resonance of the TH ligand Thus cooling leads to a freezing of the proton exchange between the two thymine ligands in *cis*-$[a_2PtT(TH)]^+$ An alternative explanation – freezing of the equilibrium (1) – can be ruled out The solubility of *cis*-$[a_2PtT_2]$ in DMF is so extremely low that one would never get a signal nearly as strong as that at 7 45 ppm As with D_2O and DMSO-d_6 as solvents, formation of free 1-methylthymine is observed, accompanied by a shift of the original C(6)H signal to higher field This shift occurs more slowly in DMF and is smaller in magnitude in this solvent compared to D_2O and DMSO, which indicates that

*The signals of *cis*-$[a_2PtT_2]$ in DMSO-d_6 show a peculiar broadening with half-widths of 10–15 Hz for C(6)H, $C(5)CH_3$ and $N(1)CH_3$ which is not observed in D_2O Also, no proton coupling is observed Moreover, the position of C(6)H at 7 04 ppm is shifted upfield in DMSO compared to D_2O (7 1441 ppm, center of quartet)

**Cis*-$[a_2PtT_2aq]$ gives, when treated with DMSO (slurry), a white, very DMSO-insoluble product of composition $a_2PtT_2x2H_2OxDMSO$ The compound has been identified by elemental analysis and IR spectroscopy (νSO = 1030 cm^{-1}) The compound is also partially precipitating from a DMSO solution of *cis*-$[a_2PtT(TH)]ClO_4aq$ upon addition of 4 Å molecular sieves

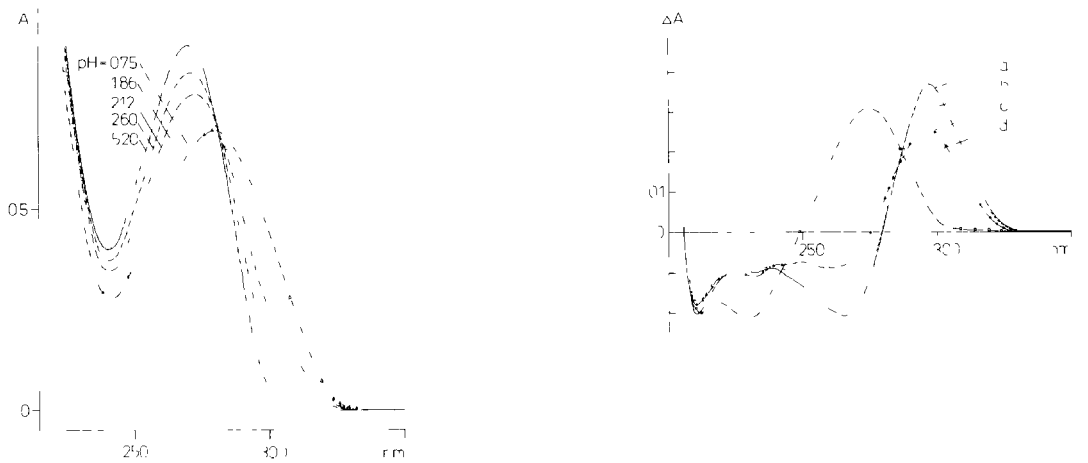


Fig 8 UV spectrum of *cis*-a_2PtT_2 + HCl at various pH values and UV difference spectra of *cis*-a_2PtT_2/HCl at pH = 0 6 *versus* pH = 5 20 after various times a) Immediately after addition of HCl, b) after 3 h at 22 °C, c) after 26 h at 22 °C, d) after 10 min at 90 °C Concentration 2 16 mg *cis*-a_2PtT_2 $2H_2O$/100 ml solution

cis-$[a_2PtT(TH)]^+$ is slightly more stable in DMF and also, that this compound is less dissociated in dry DMF than in the other two solvents

UV Spectra

Protonation of the 1-methylthyminato ligand in *cis*-a_2PtT_2 has been studied spectrophotometrically as well In Fig 8 the UV spectra of an aqueous solution of *cis*-a_2PtT_2 in the pH range 5 20–0 75 is shown One can see that the spectrum is sensitive to the extent of protonation of T Observation of an isosbestic point is consistent with only one reaction occurring and two species absorbing in the pH range studied, namely

$$cis\text{-}a_2PtT_2 + H^+ \rightleftharpoons cis\text{-}[a_2PtT(TH)]^+$$

UV difference spectra show the protonated thymine ligand to absorb at 297 nm compared to 269 nm of the anionic thyminato ligand in *cis*-a_2PtT_2 A gradual decrease of the 297 nm band at 22 °C is observed and a complete disappearance of this band after keeping the sample for 10 minutes at 90 °C This behaviour corresponds to the decomposition reaction

$$cis\text{-}[a_2PtT(TH)]Cl \longrightarrow cis\text{-}[a_2PtTCl] + TH$$

and, because of excess HCl, also to

$$cis\text{-}[a_2PtTCl] + HCl \longrightarrow cis\text{-}a_2PtCl_2 + TH$$

The pK value for the protonation of one thymine ligand of *cis*-a_2PtT_2 could not be obtained from UV spectra because complete protonation of a T ligand could not be achieved up to pH = 0 and because of possible protonation of the second T ligand at negative pH values

Protonation of *cis*-a_2PtTCl (λ_{max} = 272 nm) leading to *cis*-$[a_2Pt(TH)Cl]^+$ (λ_{max} = 297 nm) was observed as well In contrast to *cis*-a_2PtT_2, protonation of the T ligand in this compound is more difficult to achieve At pH = 1, *e g* , a positive peak at 297 nm in the difference spectrum was obtained which was only about 15–20% of the intensity of the corresponding peak in the 2 1 complex

pK_a Value of cis-*$[a_2PtT(TH)]^+$*

The *cis*-$[a_2PtT(TH)]^+$ cation represents an amholytic compound which can interact with water in two ways

$$[a_2PtT(TH)]^+ + H_2O \rightleftharpoons a_2PtT_2 + H_3O^+$$

and

$$[a_2PtT(TH)]^+ + H_2O \rightleftharpoons [a_2Pt(TH)_2]^{2+} + OH^-$$

As can be concluded from the acidic pH of an aqueous solution of *cis*-$[a_2PtT(TH)]^+$, only the first reaction is relevant in H_2O [4]

From a titration curve, obtained by neutralizing *cis*-$[a_2PtT(TH)]^+$ with NaOH, the degree of the actual degree of protonation g of *cis*-$[a_2PtT(TH)]^+$ has been calculated through an equation derived by Schwarzenbach [5] For g = 0 5 a pH = pK_a = 2 05 ± 0 05 was obtained in 0 1 *N* $NaNO_3$ solution and 1 80 ± 0 05 in pure water Thus the acidity of *cis*-$[a_2PtT(TH)]^+$ in aqueous solution can be compared with that of HSO_4^- or H_3PO_4 (pK_{a1})

IR Spectra

In Fig 9 the solid state infrared spectra of the neutral bis(1-methylthyminato)complex, *cis*-a_2PtT_2-aq, its monoprotonated form, *cis*-$[a_2PtT(TH)]Claq$, and of the head-to-head dimer, *cis*-$[a_2PtT_2Pta_2]$-$(NO_3)_2$ [1], in the double bond stretching region are shown One finds that the strong band at 1570 cm^{-1} and the shoulder at 1540 cm^{-1} in the spectrum of *cis*-a_2PtT_2 are shifted to lower energy upon protona-

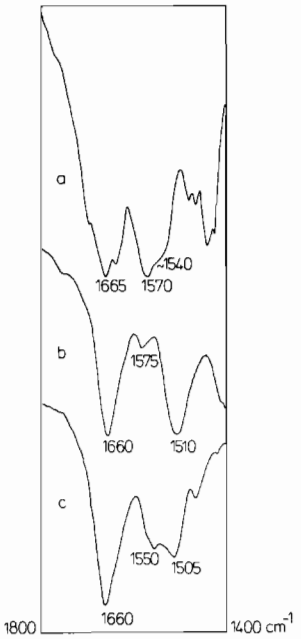


Fig 9 IR spectra (KBr) between 1800 and 1400 cm^{-1} of a) *cis*-a_2PtT_2, b) *cis*-$[a_2PtT_2Pta_2](NO_3)_2$ (head-to-head dimer), c) *cis*-$[a_2PtT(TH)]Claq$

tion of one ligand (1550 and 1505 cm^{-1}) Thus protonation leads to a similar change in the double bond stretching region as covalent Pt binding to an exocyclic keto group [2] This is what one expects if protonation occurs at an exocyclic CO group, because this leads to a reduction of the double bond character as a consequence of contributions of resonance structures of the type, $\geq C = \dot{O}H$ and $\geqslant C{-}OH$ Any protonation at a ring atom – which is unlikely since N(3) is platinated and N(1) is methylated – would cause a shift of the double bond stretching modes to higher energy

In the OH stretching region, no band due to a free OH group is observed because of overlapping with H_2O absorptions In the perchlorate compound, for example, intense bands are observed at 3600, 3500 and 3400 cm^{-1}, which are well separated from the NH_3 stretching modes at 3300 and 3220 cm^{-1} (Nujol) In the spectra of the chloride and nitrate compounds, only broad, intense bands centered around 3440 cm^{-1} are observed They are assigned to OH and HOH stretching modes

Discussion

Our earlier findings on the nucleophilic properties of N(3) platinated 1-methylthymine with respect to other metal cations [1, 2] is herewith confirmed for reaction with the proton As in the case of the former, the reason for this reactivity must primarily be seen in the insufficient ability of the Pt atom at N(3) to localize the double bonds in the heterocycle as compared to the proton at N(3) in the neutral ligand As a consequence, the exocyclic oxygen atoms – or at least one of them – are becoming sites for additional attack of an electrophile. This behaviour is reflected by the IR spectroscopic changes in the double bond stretching region as well as by the large difference in pK values for protonation of free 1-methylthymine and the N(3) platinated 1-methylthyminato ligand Deprotonation of 1-methylthymine occurs with a pK value of 10 3 in aqueous solution [6] The pK value for the protonation of 1-methylthymine has not been determined However, from the results obtained for the closely related 1-methyluracil, for which a pK = –3 40 ± 0 12 has been obtained [7], it is reasonable to assume a very similar value for 1-methylthymine As to the site of protonation of 1-methyluracil, O(4) has been suggested as the most likely atom from comparison of the UV spectra of related, ethoxy substituted compounds [7] In the solid state, protonation at O(4) has been determined for 1-methyluracil unambiguously by X-ray diffraction [8] The protonation of unsubstituted uracil leads to a mixture of O(2) and O(4) protonated tautomers with the latter being the predominant species [9] The pK for the formation of the uracil monocation is –3 38 ± 0 15 [7]

The pK for the protonation of a 1-methylthymine ligand in the bis(1-methylthyminato) complex has been determined as 2 05 ± 0 05 in 0 1 *N* $NaNO_3$ solution This means, that the ability of N(3) platinated to act as a base and accept a proton, has increased by approximately 5 orders of magnitude

In the related compound, *cis*-$[a_2PtTCl]$ with a N(3) coordinated 1-methylthyminato ligand, protonation of this ligand according to

$$cis\text{-}[a_2PtTCl] + H^+ \rightleftharpoons cis\text{-}[a_2Pt(TH)Cl]^+$$

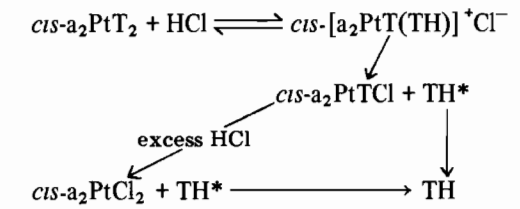
appears harder to be achieved than in the 2 1 complex (*cf* UV spectra) This difference possibly reflects the additional stabilization of a single protonated ligand in the bis-complex through favourable intramolecular hydrogen bonding On the basis of molecular models, three kinds of intramolecular hydrogen bonds should theoretically be possible between two O(4) atoms, between two O(2) atoms, and between one O(4) and one O(2) atom With the present data it is not possible to decide which of these options is the most likely to occur, and if it is indeed taking place Because of time-averaging and the changing ^1H-NMR spectra, it is not possible to find out if there is inter- or/and intramolecular hydrogen bonding However, from X-ray results on the above mentioned platinum dimer [1] and the heteronuclear platinum-silver compound [2], a similar binding principle for the proton seems possible Although O(4) coordination in these two compounds as well as in a related

one [10] has been taken for certain, and therefore O(4) protonation appears likely as well, one can not exclude the possibility of O(2) protonation and/or an equilibrium involving O(2) and O(4) protonated species

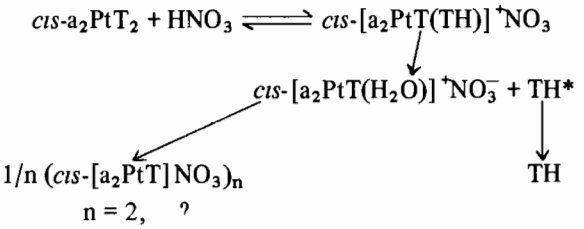
It is interesting to compare the protonation reactions of N(3) platinated thymines with those of N(1) platinated ones Although we have not studied 3-methylthymine complexes of Pt(II) as yet, we recently isolated complexes of unsubstituted thymine [11, 12] and unsubstituted uracil [12] with Pt-coordination at N(1) and determined their structures by single crystal X-ray diffraction In one of these compounds [12], pentahydrodioxonium chloro(uracilato-N1)-(ethylenediamine)platinum(II) chloride, a N(1) platinated uracil compound contains a $H_5O_2^+$ unit connected with the O(4) position of the uracil ring through an extremely short hydrogen bond of 2 47 (2) Å This compound has been isolated from a concentrated aqueous HCl solution The corresponding chloro(thyminato-N1) complex, which has been obtained from a somewhat more diluted HCl solution, does not contain the $H_5O_2^+Cl^-$ unit Preliminary UV studies on the protonation of enPt(uracilato-N1)Cl in aqueous solution do not reveal any spectral changes up to a pH $\simeq$ 0–0 5 The weak new band observed in the UV difference spectrum in this pH range absorbs at 309 nm compared to 288 nm of the starting compound It is indicative of a partial protonation of the N(1) platinated uracil at this pH It also indicates that protonation of N(1) platinated 2,4-dioxopyrimidines occurs less easily than that of N(3) platinated ones Thus a platinum atom at the N(1) position has a greater 'similarity' to a proton at this position than a Pt atom and a proton do have at the N(3) position

This difference in the protonation reactions of N(3) and N(1) platinated 2,4-dioxopyrimidines are closely related to differences in their stability upon acid treatment From ^{1}H-NMR spectra and UV measurements it is evident, that the Pt–N(3)1-methylthymine bond is becoming very weak when the thymine ligand is protonated at an exocyclic oxygen This leads to formation of neutral thymine In contrast, N(1) platinated uracil and thymine complexes are very stable even in concentrated HCl We have previously assumed, that there are definite differences in stability between N(1) and N(3) platinated 2,4-dioxopyrimidines [11, 12] The data presented here verify this assumption Moreover, this finding has now been confirmed using Laser Raman spectroscopy [13]

With regard to the products formed upon acid decomposition of the bis(1-methylthyminato) compound, different 1 1 complexes are obtained, depending upon the acid used With HCl, which contains a well coordinating anion, (1-methylthyminato-N3)chloro-*cis*-diammineplatinum(II) monohydrate is obtained



With HNO_3, an acid containing a poorly coordinating anion, several compounds are obtained (*cf* Experimental) They do not have NO_3^- coordinated to Pt as is evident from the vibrational spectra From IR spectra and elemental analysis it seems possible that one of these compounds (yellow crystals) is the head-to-head dimer bis(μ-1-methylthyminato-N3, O4)bis-(*cis*-diammineplatinum(II)) dinitrate monohydrate, which has recently been described, although prepared in a different way* Formation of higher oligomers can not be excluded Treatment of *cis*-a_2PtT_2 with HNO_3 thus leads to the following reaction sequence



The neutral thymine molecule TH* initially expelled from the Pt complex certainly does not exist in the usual dioxo-tautomer form [14] but rather in the 4-hydroxo,2-oxo or/and the 2-hydroxo,4-oxo form, depending upon the site of protonation (Fig 10) The lifetime of this(these) rare tautomer(s) certainly depends upon parameters such as solvent, temperature and concentration In water, for example, transversion into the usual dioxo tautomer should be extremely fast, whereas in aprotic media the rare tautomer(s) might be detectable The fact that the reported ^{1}H-NMR spectra in dry DMSO and dry DMF

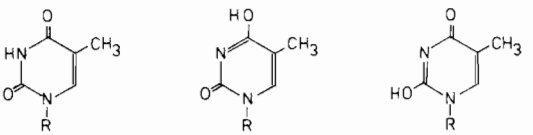


Fig 10 Usual 2,4-dioxo tautomer of 1-methylthymine (a), and rare tautomeric forms 4-hydroxo,2-oxo (b) and 2-hydroxo,4-oxo (c)

Cf ref 10 No space group determination has been possible thus far due to insufficient crystal quality

only indicate the existence of the normal dioxo tautomer of 1-methylthymine, is due to the simultaneous existence of the equilibrium (1) besides the decomposition reaction (2), leading to the formation of neutral thymine The presence of protons from equilibrium (1) in the solution leads to a fast tautomeric interconversion of the rare tautomer to the normal one

Possible Biological Implications

The outlined reaction sequence – metal coordination at N(3) of thymine with replacement of a proton, protonation at O(4) or O(2), removal of the metal and formation of a rare thymine tautomer – can be considered a possible model for a metal-assisted tautomerization mechanism leading to nucleobase mispairing and consequently to mutation It is pointed out, that this model by no means is a unique one to explain the well established mutagenicity of a variety of metals in general [15] and that of *cis*-a_2PtCl_2 in particular [16] A number of other pathways leading to base substitution mutations are feasible and will not be considered [17] It is evident, that a 4-hydroxo,2-oxo tautomer of thymine could mispair with guanine or the normal dioxo tautomer of thymine, and a 2-hydroxo,4-oxo tautomer with normal thymine or cytosine (Fig 11) Additional 'wrong' base pairs are feasible, *e g* between the enol, imino form of guanine and the 2-hydroxo,4-oxo tautomer of thymine, but the chances for two unusual tautomers to pair is so low that it can be neglected. The biological significance of pyrimidine–pyrimidine base pairing as indicated in Fig 11b, c appears questionable because of its unfavourably,

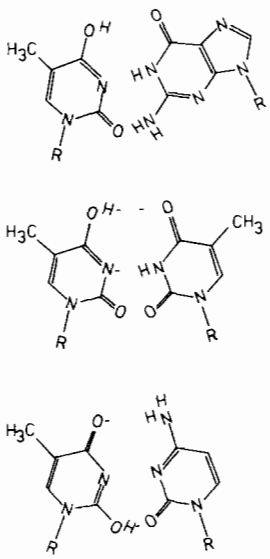


Fig 11 'Wrong' base pairs between guanine and thymine (enol) (a), thymine (dioxo) and thymine (enol) (b), and cytosine and thymine (enol) (c)

short glycosyl bond separation distance, even though interpyrimidine base pairing has been included in theoretical calculations on the stability of RNA molecules [18] Thus guanine–thymine(enol) mispairing as indicated in Fig 11a is the most plausible kind of base mispairing caused by a 'wrong' thymine tautomer The possible importance of this base pair for mutations has been recognized before [19]

Any nucleobase mispairing mechanism presupposes that tautomerization to the 'usual' tautomer does not occur once the 'wrong' base has been formed It is assumed that the exclusion of water and the fixation of the 'wrong' base in the synthetic apparatus enable this [17a]

Provided the proposed model of a metal-assisted tautomerization of thymine is of biological relevance, one should expect those metals to be most active in producing mutations *via* this route, that bind to nitrogen donor atoms preferentially but not too strongly These two requirements should ease both protonation of an exocyclic oxygen and removal of the metal by a proton, In contrast, preferential binding to the exocyclic oxygens of thymine should stabilize the 'normal' lactam tautomer, whereas strong binding to the ring atom (N(3)) should make protonation of an exocyclic oxygen more difficult and consequently also the removal of the metal

The binding of metal ions to the N(3) position of thymine or uracil in nucleic acids under physiological conditions has not been studied in great detail so far Only for Cu(II) [20] and Ag(I) [21] studies are available These studies indicate that metal binding at N(3) occurs with replacement of a proton Despite the relatively high pK_a of 9 8 for poly(U), for example, Ag(I) binding to N(3) at pH = 6 is substantial [21] As to *cis*-a_2PtCl_2, no reaction with poly-(dT) at pH 7 has been detected [22] However, reaction between the diaquo species *cis*-$a_2Pt(H_2O)_2^{2+}$ or aquo-hydroxo species *cis*-$[a_2Pt(OH)]_n^{n+}$ (n = 2, 3) [23] with poly(U) as well as other substituted and unsubstituted pyrimidine-2,4 diones readily occurs [24] Provided a *cis*-a_2PtT_2 complex with two thymidine residues could be formed *in vivo*, and provided the pK_b for accepting a proton were around 12 as in the 2 1-methylthymine complex, chances for protonation of a thymine ligand at a physiological pH 7 3 are extremely small* However, with metal complexes having a smaller pK_b value (*e g* 10–11), protonation of the thymine ligand could occur at a higher frequency. Findings on the striking alteration of base

*Using the formula $x_B = 1/1 + 10^{pK_a - pH}$ with x_B = molar base ratio and pK_a = 2, pH = 7 3 one obtains x_B = 0 999995 and a molar acid ratio $x_A = 1 - x_B = 5 \cdot 10^{-6}$ This means, that in a medium of pH = 7 3 only $0\,5 \cdot 5 \cdot 10^{-6} = 2\,5 \cdot 10^{-6}$ of all platinated thymine ligands in a 2 1 complex are protonated and potentially able to give thymine in its rare tautomeric form

composition of bacteria DNA under the influence of copper [25] – the AT content is very much reduced at the expense of the GC content – would be consistent with the proposed model of a metal-assisted thymine tautomerization

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