trans-Ternary Complexes of Pd(II) with Nucleosides and Dipeptides. Models for *trans*-DDP–DNA–Protein Crosslinks

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Abstract

The reactions of complexes of the type *trans*-[Pd(dipeptide)$_2$Cl$_2$] with the nucleosides guo and ino in aqueous solutions, produce the ternary complexes *trans*-[Pd(dipeptide)$_2$(nucl)$_2$]Cl$_2$, which were isolated and characterized as solid adducts, with elemental analysis, conductivity measurements, IR and ^{1}H NMR spectra. Many isomers, corresponding to the head to head, head to tail and tail to tail orientations of the nucleosides, with two major ones called 'closed' and 'opened' forms, are observed with ^{1}H NMR in D_2O solutions, for the complexes. The ratio of the two major isomers being 1:1.5 in D_2O solutions of the 'opened' to 'closed' form, favors the 'opened' form in DMSO-d_6 solutions changing from 1:6 to 1:10 for the guo derivatives, while this form is the only one observed in the ino derivatives. Possible ligand–ligand interactions are detected in the ^{1}H NMR spectra of the compounds. The *anti* conformation of the sugar moiety of guo is found to increase in the *trans*-ternary complexes of the present system and with the presence of the dipeptide, indicating that the model DNA–Pt–protein crosslink may be responsible for the toxicity of platinum drugs.

Introduction

The antitumor drug *cis*-DDP is known to exhibit its activity through interaction with DNA during its replication [1]. It seems therefore highly unlikely to be due to a DNA–Pt–protein crosslink, although such a model had been proposed as possible earlier [2–4]. On the other hand, the action of *cis*-DDP is followed by various toxic side effects, which are believed to be due to the interaction of the drug with other biological molecules and especially to the formation of DNA–Pt–protein crosslinks [5]. For example the *trans*-DDP, not possessing antitumor properties and being more toxic than the *cis* analog, forms such DNA–Pt–protein crosslinks in a much larger proportion [5].

It is also worthwhile noting, that biologically important metal ions may promote selective interactions of nucleic acids with amino acids, through the formation of ternary complexes, even far from the site of the metal attachment [6–11].

With the aim to better investigate simple *cis*- or *trans*-DNA–Pt–protein models and the interactions between the ligands mediated by the metals, we have chosen simple nucleoside(-tide)–M–amino acid(-peptide) systems, with M = Pd(II) or Pt(II). In this respect, we reported the results of the reactions of *cis*-Pt(ino)$_2$Cl$_2$ and *cis*-Pd(guo)$_2$Cl$_2$ with various amino acids of increasing aliphatic chain [12–14], as well as the reactions of the complex (GHL)PdCl, where GHL is glycyl–histidyl–lysine, transporter of metal ions through the membranes in the body, with the nucleotides 5′-IMP and 5′-GMP [15].

Similar studies have also been reported earlier by various investigators. For example, Sigel *et al.* [6, 16, 17] investigated the ternary complexes formed between bivalent metal ions, amino acids with increasing aliphatic side chain and nucleotides. They found hydrophobic ligand–ligand interactions, increasing with the increase of the side chain length of the amino acids. Studies with similar results have also been carried out by Martin *et al.* [18–21], Kozlowski *et al.* [22, 23] and Yamauchi *et al.* [24, 25], in ternary systems of metals with peptides and nucleosides or aromatic diamines.

In the present paper, we report on similar models of *trans* structure, of the general formulae *trans*-[Pd(nucl)$_2$(dipeptide)$_2$]Cl$_2$, where nucl is ino or guo (inosine, guanosine) and dipeptide is gly–gly, gly–L-

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TABLE 1. Elemental analyses and molar conductances of the compounds

No.		Pd (%)	Cl (%)	C (%)	N (%)	H (%)	Λ_M
1a	calc.	10.55	7.04	33.33	19.44	4.16	
	found	10.40	6.70	33.01	19.10	4.39	260.00
1b	calc.	10.87	7.26	34.35	17.18	4.09	
	found	11.10	7.20	34.10	16.80	4.36	285.00
2a	calc.	10.27	6.90	34.74	18.91	4.43	
	found	10.30	6.30	34.55	18.40	4.69	241.35
2b	calc.	10.58	7.06	35.78	16.69	4.37	
	found	10.60	6.50	35.45	16.15	4.58	270.00
3a	calc.	9.74	6.50	37.35	17.94	4.94	
	found	9.80	6.40	36.95	17.44	4.92	238.00
3b	calc.	10.02	6.68	38.41	15.81	4.89	
	found	10.00	6.60	37.95	15.42	4.95	255.00
4a	calc.	9.50	6.34	38.56	17.49	5.17	
	found	9.60	6.30	37.96	17.25	5.22	234.00
4b	calc.	9.76	6.50	39.62	15.41	5.13	
	found	10.00	6.45	39.55	15.30	5.29	244.00

1a: *trans*-[(guo)$_2$Pd(gly–gly)$_2$]Cl$_2$, 1b: *trans*-[(ino)$_2$Pd(gly–gly)$_2$]Cl$_2$, 2a: *trans*-[(guo)$_2$Pd(gly–L-ala)$_2$]Cl$_2$, 2b: *trans*-[(ino)$_2$-Pd(gly–L-ala)$_2$]Cl$_2$, 3a: *trans*-[(guo)$_2$Pd(gly–L-val)$_2$]Cl$_2$, 3b: *trans*-[(ino)$_2$Pd(gly–L-val)$_2$]Cl$_2$, 4a: *trans*-[(guo)$_2$Pd(gly–L-leu)$_2$]-Cl$_2$, 4b: *trans*-[(ino)$_2$Pd(gly–L-leu)$_2$]Cl$_2$.

ala, gly–L-val, gly–L-leu. We also attempt to compare the results with the reported *cis* analogs [12–14].

Results and Discussion

trans-Ternary complexes of Pd(II) with nucleosides and dipeptides were synthesized from the reactions of the corresponding complexes *trans*-[Pd(dipeptide)$_2$Cl$_2$], prepared according to literature methods [26], with the nucleosides ino or guo, in aqueous solutions.

$$\textit{trans}\text{-[Pd(dipeptide)}_2\text{Cl}_2 + 2\text{nucl} \xrightarrow[10\,\text{min}]{50\,^\circ\text{C}} \textit{trans}\text{-[Pd(nucl)}_2\text{(dipeptide)}_2\text{]Cl}_2 \quad (1)$$

The solid adducts isolated with the above reactions correspond to their assigned empirical formulae, as their elemental analyses show (Table 1).

The molar conductance values of the complexes in aqueous solutions, correspond to 1:2 electrolytes, increasing with time, due to the partial ionization of the carboxylate groups of the C-terminal amino acids of the dipeptides [27] (see Table 1).

IR Spectra

The IR spectra of the compounds show strong and broad bands in the region of 3000–3600 cm^{-1} containing their various νNH and νOH bands [28–30]. The broad bands at 2900–2960 cm^{-1} for all the complexes are assigned to the aliphatic νCH motions of both the dipeptides and the nucleosides [31].

The νC=O bands of the free carboxylate group of the dipeptides gly–gly, gly–L-ala, gly–L-val and gly–L-leu are shown at 1675–1700 cm^{-1} [32–35], lower than in the complex *trans*-[Pd(gly-gly)$_2$Cl$_2$] [26]. In the ternary complexes of both series with ino and guo, the νC=O bands of the protonated carboxylates of the dipeptides, are shown near 1700 cm^{-1} as shoulders of the nearby νC=O frequencies of the carbonyl group at the 6th position of both nucleosides, shown at slightly lower frequencies, as strong bands. These results indicate the non-involvement in bonding with Pd(II) of both the O_6 atoms of the purines and the carboxylates of the dipeptides, which also are protonated.

The amide I band of the zwitterionic form of the dipeptides is shown near 1650 cm^{-1} [32, 35]. It is shown at 1630 cm^{-1} for the complex *trans*-[Pd(gly-gly)$_2$Cl$_2$] [26], 1650 cm^{-1} for *trans*-[Pt(val-glyO-Me)$_2$Cl$_2$] [36] and at 1640 cm^{-1} in the present series of the ternary complexes with ino. In the corresponding complexes with guo, this band cannot be distinguished in most of the cases, being covered by characteristic bands of the nucleoside.

The amide II band on the other hand, shown near 1550 cm^{-1} for the zwitterionic form of the dipeptides [32, 37], is also observed near 1540–1550 cm^{-1} in various Pt(II) and Pd(II) complexes of monocoordinated (through $-NH_2$) dipeptides [26, 29, 36]. The position of this band does not change significant-

ly in the *trans*-ternary complexes with ino and dipeptides (near 1530 cm^{-1}), while it cannot be distinguished in most of the cases in the series with guo, being covered by other strong bands of the nucleoside.

The δNH_2 band occurring also in this region, is observed at about 1550 cm^{-1} for the zwitterionic forms of the dipeptides [33, 37] and 1560 cm^{-1} in the complex **1b**, at 1555 cm^{-1} in **2b**, at 1560 cm^{-1} in **3b** and at 1561 cm^{-1} in **4b**.

In conclusion, the positions of all the amides I, II and δNH_2 bands, indicate the retention of the monodentate (through NH_2) coordination of the dipeptides with Pd(II) [26, 29].

The skeletal vibrations of free ino at 788 cm^{-1} and of free guo at 775 cm^{-1}, are observed at slightly higher frequencies in all the complexes, indicating the Pd(II) coordination of the bases [38].

Free guo presents a band at 824 cm^{-1}, indicating predominance of the $C_{2'}$-*endo–anti* conformation [39–42] (^3E = 38%). In the complexes *cis*-$[Pt(NH_3)_2(guo)_2]^{2+}$ and *cis*-$[Pt(en)(guo)_2]^{2+}$ the band at 824 cm^{-1} diminishes in intensity or disappears and a new band appears at 798–800 cm^{-1}, indicating increase of the $C_{3'}$-*endo–anti* conformation, upon Pt(II) coordination at N_7 [39–42]. In the present case of the ternary complexes with guo, the increase in intensity of the band near 800 cm^{-1}, indicates also the increase in the $C_{3'}$-*endo–anti* conformation of the sugar of guo.

The same is true in the series with ino, where the 822 cm^{-1} of the free nucleoside, with a 46% ^3E conformation of the sugar [13] is replaced by a new band near 827–830 cm^{-1}. This is also true for the complex *cis*-$Pt(ino)_2Cl_2$ [13] shown with ^1H NMR spectroscopy.

^1H NMR Spectra

All the complexes of the general formulae *trans*-$[Pd(nucl)_2(dipeptide)_2]Cl_2$ show the presence of many isomers (two major ones) in their ^1H NMR spectra, in D_2O solutions. This is evidenced from the number of the aromatic protons of the purines and it should be due to the relative orientation of the nucleosides (head to head, head to tail, tail to tail) [12, 43–48] or towards the dipeptides ('closed' or 'opened' forms [6, 16, 17]) (Fig. 1), stabilized with strong hydrogen bonds. In DMSO-d_6, where the hydrogen bonds are weakened, the number of isomers is decreased and there is observed only one in the series *trans*-$[Pd(ino)_2(dipeptide)_2]Cl_2$ and two in the series *trans*-$[Pd(guo)_2(dipeptide)_2]Cl_2$. The relative ratio of the two major isomers in D_2O, being about 1:1.5, ('opened' to 'closed') becomes 1:6 or 10 in DMSO-d_6 solutions, favoring the 'opened' form.

Given as an example, the ^1H NMR spectra of the complexes **3a** and **3b** are shown in Fig. 2 and their chemical shifts in Table 2.

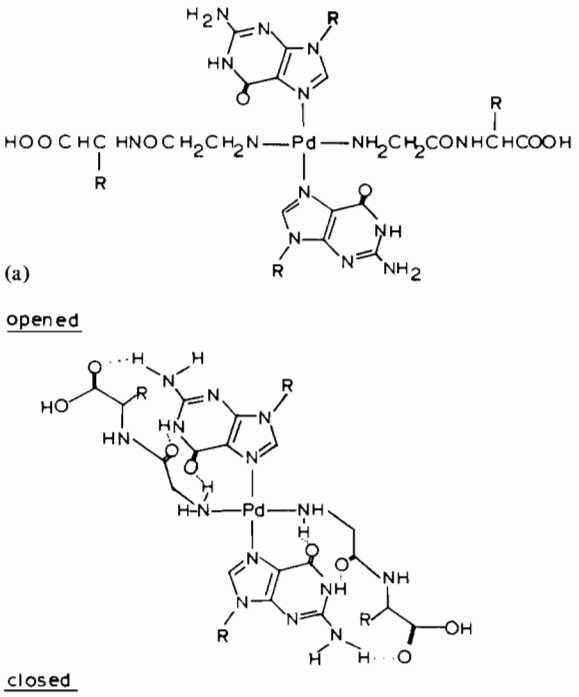


Fig. 1 (a) The 'opened' and (b) the 'closed' forms of the *trans*-$[Pd(guo)_2(dipeptide)_2]Cl_2$ complexes.

The complex **3a** shows three resonances for the H_8 proton of guo in D_2O solutions at 8.2694, 8.1181 and 8.4011 ppm. The first two are the major rotamers in a 1.58:1 ratio. In DMSO-d_6 the ratio changes to 1:7.5 with only two resonances for H_8 at 8.3604 and 8.0559 ppm. They are assigned to the 'closed' and 'opened' forms of the complex respectively [6, 16, 17, 25].

Four isomers are observed, on the other hand, in D_2O solutions for the complex **3b** with the resonances of H_8 at 8.8476, 8.7184, 8.6108 and 8.5370 ppm and of H_2 at 8.1901, 8.1198, 8.0652 and 8.0372 ppm. These isomers are not found in DMSO-d_6 solutions, showing only resonances at 8.4635 ppm for H_8 and 8.1982 ppm for H_2. The values of the chemical shifts of the H_8 for both ino and guo in the two series of complexes, show that the nucleosides bind through their N_7 atoms [49–52].

In the case of the ino complexes, the rapid rotation around the Pd–N_7 bonds of the two nucleosides only allows the observation of the 'opened' form in DMSO-d_6 solutions. None of the many possible rotamers can be stabilized with hydrogen bondings, contrary to the guo complexes, which seem to stabilize the 'closed' form (Fig. 1) in small amounts, due to its $-NH_2$ group at the C_2 position.

The peptide protons of the complex **3a** in its 'opened' form in D_2O solutions are seen upfield

compared to the free peptide [53, 54] in decreasing amounts with distance from the bonding side (see Table 2).

This is a result of the hydrophobic interactions between the protons of the aliphatic side chain of the peptides and the aromatic rings of guo, decreasing with an increase in their distance from the bonding site. The opposite effect was observed in the *cis*-[Pt(guo)$_2$(amac)]Cl complexes [14]. This result is better seen in the plot of Fig. 3 for the complex **3a**, as an example. The 'closed' form shows even larger shifts, though without linear dependence.

In DMSO-d$_6$ solutions, all the peptides protons are shifted downfield as compared to the free peptide for the 'opened' form, due to the predominance of the bonding effect and the decrease of the stacking effects in this solvent. The only one resonance at 3.7954 ppm, observed for the CαH proton of val, (with irradiation of the adjacent CβH proton) for the 'closed' form on the other hand, is shifted upfield.

Downfield shifts are also observed for all the peptide protons of the ternary complex **3b**, in DMSO-d$_6$ solutions, both compared to the free peptide [55] or to the starting complex *trans*-[Pd(gly-L-val)$_2$Cl$_2$] [26].

The proportion of the 'closed' form of the complexes in D_2O solutions increases with the increase of the aliphatic side chain of the dipeptides in the *trans*-[Pd(guo)$_2$(dipeptide)$_2$]Cl$_2$ complexes. The opposite is true for the 'opened' form (see Fig. 4).

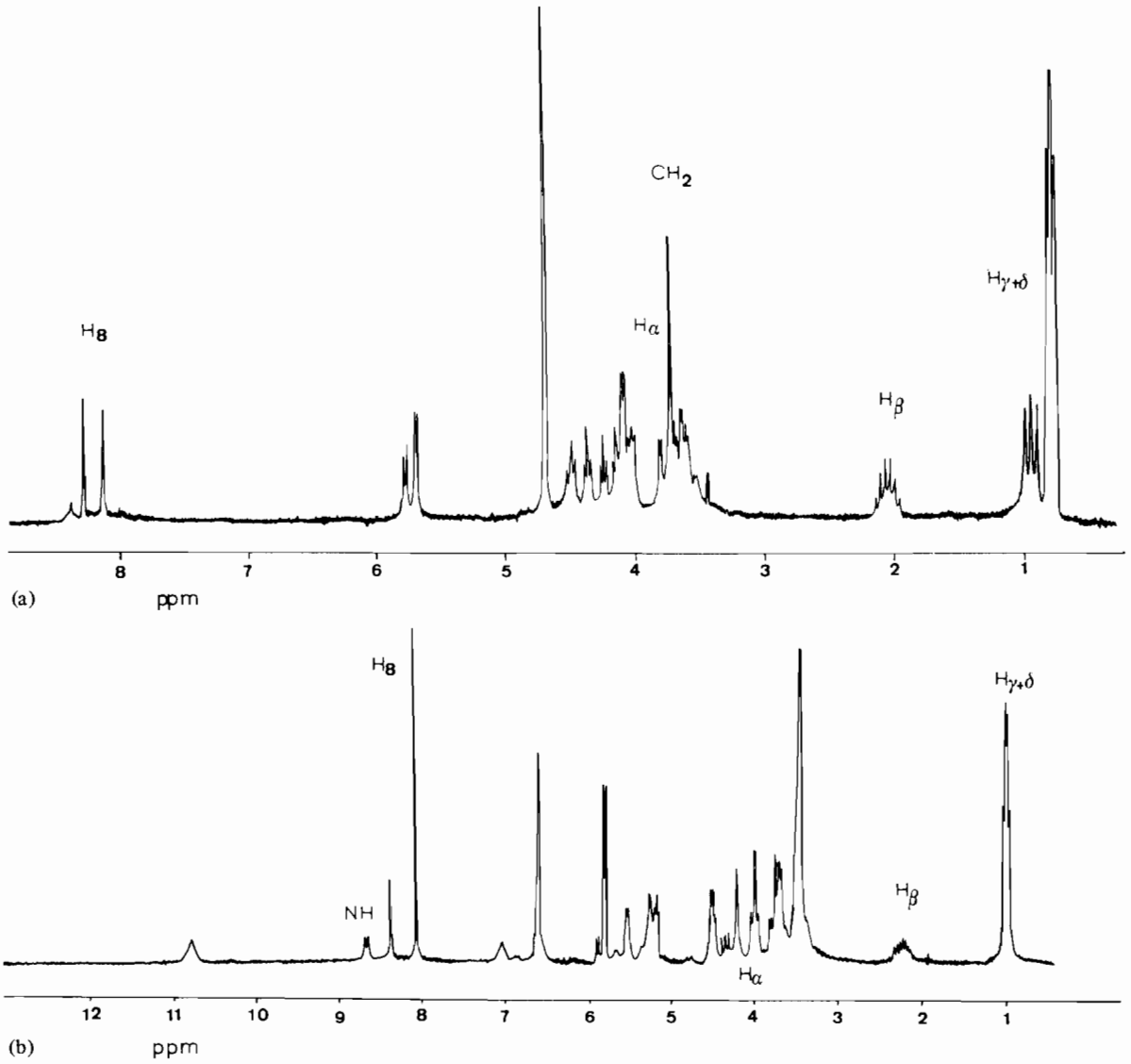
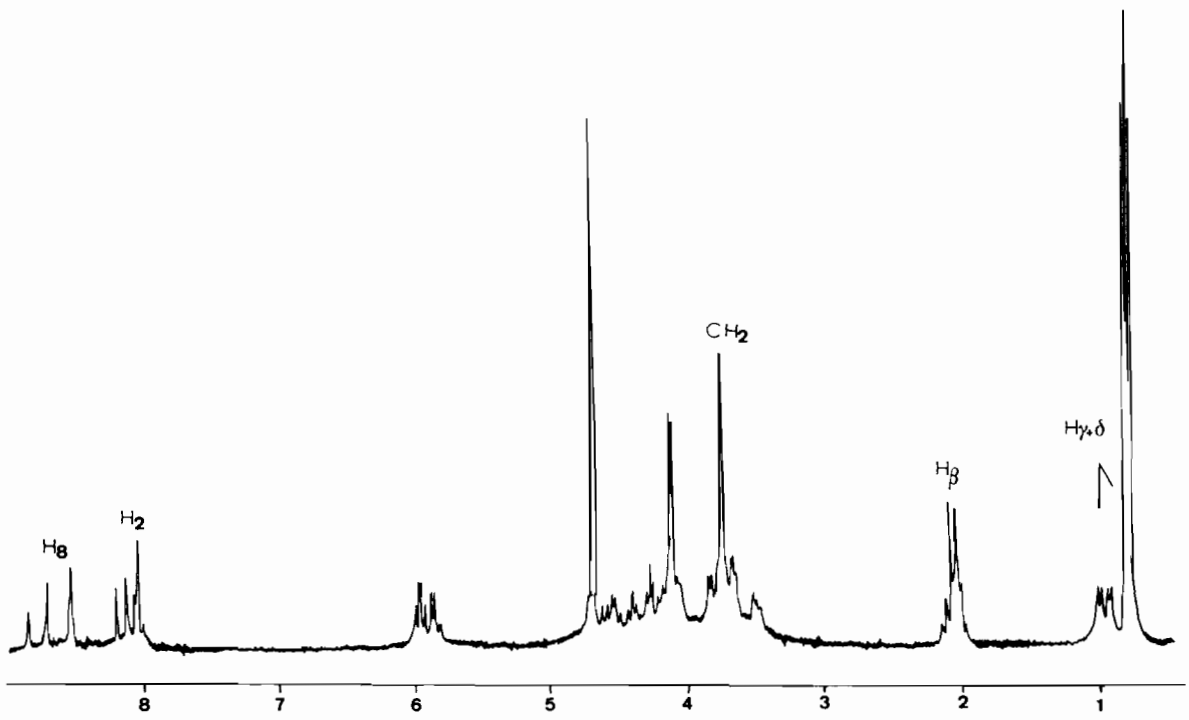


Fig. 2. *(continued)*



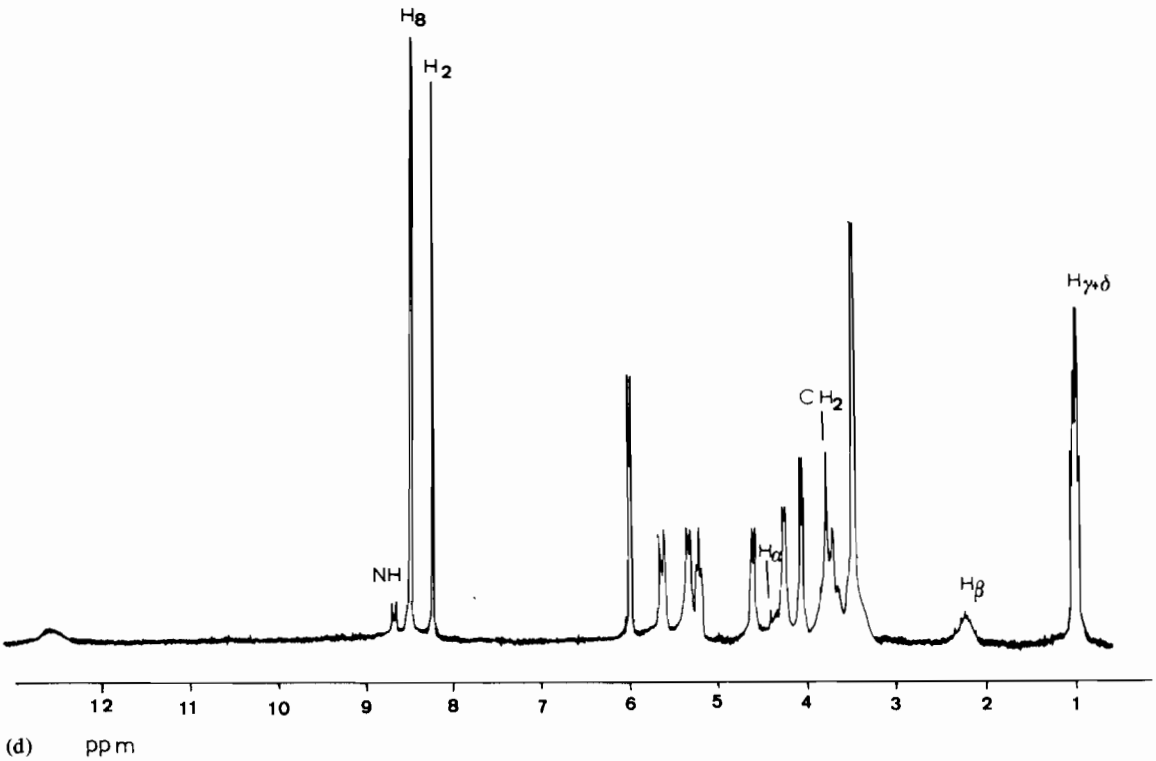


Fig. 2. ^{1}H NMR spectrum of the complex *trans*-[Pd(guo)$_2$(gly–val)$_2$]Cl$_2$ in D_2O (a) and in DMSO-d_6 (b). ^{1}H NMR spectrum of the complex *trans*-[Pd(ino)$_2$(gly–val)$_2$[Cl$_2$ in D_2O (c) and in DMSO-d_6 (d).

TABLE 2. ^{1}H NMR chemical shifts of the compounds 3a, 3b in D_2O and DMSO-d_6 solutions

Compound	Solution	Nucleoside protons						Peptides protons					
		H_8	H_2	H_1	H_2	H_3	H_4	αCH_2	αCH	βCH	γCH	δCH	NH
3a	D_2O	8.4011											
		(i) 8.2694		5.6747	4.3602	4.1640		3.4635	3.7951d	2.0385m		0.7803	
								(+0.3967)	(+0.3849)	(+0.0913)		(+0.1747)	
		(ii) 8.1181		5.7596	4.4857	4.2333		3.7281	4.0949d			0.9460	
								(+0.1321)	(+0.0851)			(+0.0095)	
3b	D_2O	8.8476	8.1901									0.7879	
		8.7184	8.1198					3.7362		2.0488		0.9680	
		8.6108	8.0652										
		8.5370	8.0372										
3a	DMSO-d_6	(i) 8.3604							3.7954m				
		(−0.5604)							(+0.4646)				
		(ii) 8.0559		5.8063	4.5053	4.1940	3.9795	3.7529	4.3431m	2.1934	1.0273	0.9942	8.6524d
		(−0.2559)							(−0.0831)	(−0.2234)	(−0.0473)	(−0.0742)	(−0.7724)
3b	DMSO-d_6	(ii) 8.4635	8.1982	5.9839	4.5982	4.2424	4.0522	3.7541	4.3484m	2.1400m	1.0293	0.9969	8.6518
		(−0.1335)	(−0.0982)						(−0.0889)	(−0.1700)	(−0.0493)	(−0.0769)	(−0.7719)
gly-L-val[a]	D_2O							3.8602	4.1800	2.1300	0.9700	0.9400	
gly-L-val[b]	DMSO-d_6								4.2600	1.9700	0.9800	0.9200	7.8800
trans-[Pd(gly-val)$_2$Cl$_2$][c]	DMSO-d_6							3.6200s	4.2200m	2.0600m	0.9100d	0.8300d	8.2100d
									(+0.0400)	(−0.0900)	(+0.0700)	(+0.0900)	(−0.3300)

The numbers in parentheses represent upfield shifts from the chemical shifts of the free dipeptide (positive shifts) and downfield shifts (negative values), (i): 'closed' form, (ii): 'opened' form, m: multiplet, d: doublet, s: singlet. [a]From ref. 53. [b]From ref. 55. [c]From ref. 26.

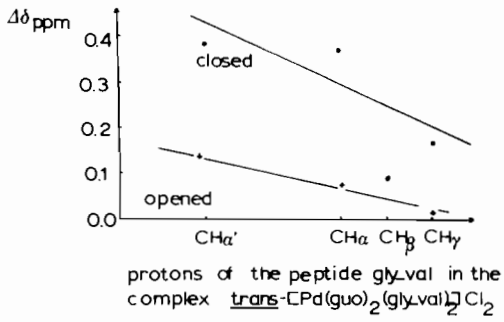


Fig. 3. Difference in chemical shifts Δδ (ppm) of the protons of the zwitterionic form of gly–val and the 'closed' and 'opened' forms of the complex *trans*-$Pd(guo)_2(gly–val)_2]$-Cl_2. The positive Δδ (ppm) values, represent upfield shifts of the protons of the complexes, as compared to the zwitterionic forms of the peptides. The spectra were taken in D_2O solutions.

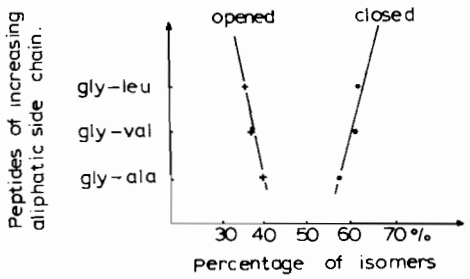


Fig. 4. Variation of the percentage of the 'opened' and 'closed' forms of the complexes *trans*-$[Pd(guo)_2(dipeptide)_2]Cl_2$ with the dipeptide in D_2O solutions.

TABLE 3. K_{ST} for the complexes in D_2O and DMSO-d_6

Compound	'Opened'	'Closed'	K_{ST}
2a[a]	58.3	41.7	1.40
3a[a]	61.4	38.6	1.58
4a[a]	62.3	37.7	1.65
1a[b]	14.5	85.5	0.17
2a[b]	14.3	85.7	0.17
3a[b]	11.8	88.3	0.13
4a[b]	9.1	90.9	0.10

[a]In D_2O. [b]In DMSO-d_6.

This is expressed with the K_{ST} of the equilibrium

'closed' $\overset{K_{ST}}{\rightleftharpoons}$ 'opened'

where the K_{ST} increases with the increase of the aliphatic side chain of the peptides in the complexes (see Table 3).

Yamauchi *et al.* [24] in similar ternary complexes of Pd(II) of the type Pd(L)(DA), with DA = bipy or bphen and L = tyr–glu, tyr–gly, trp–glu and trp–gly, found that the 'closed' form of the complexes was favored according to the following sequence of the side chain of the dipeptide: indole > phenole > benzene > phenyl ester. Sigel *et al.* [6] found also similar results.

The opposite effect is observed in DMSO-d_6 solutions for the predominant 'opened' form, where the K_{ST} of eqn. (2) for the 'closed' form, decreases in the following sequence of the dipeptides: gly–gly < gly–ala < gly–val < gly–leu.

It is finally observed that the H_8 proton of guo in the 'closed' forms shifts more upfield as the aliphatic side chain of the dipeptides increases in DMSO-d_6 solutions. The chemical shift of the 'opened' form on the other hand, remains almost constant. In D_2O solutions, no dependence of the H_8 chemical shift upon the side chain of the peptide is observed.

The percentage of the possible h + g, t conformers [20, 21, 24, 56] calculated from the ^1H NMR spectra, gave the following results.

Compound	Form	g + h (%)	t (%)	Solvent
3a	'opened'	68.0	32.0	D_2O
3a	'closed'	95.0	5.0	D_2O
3a	'opened'	72.0	28.0	DMSO-d_6
3b	'closed'	71.5	28.5	DMSO-d_6

These results are as expected, since in the 'closed' form isomer, the two methyl groups of the dipeptide gly–val should interact more strongly with the aromatic rings of guo, than in the 'opened' form.

The percentages of each of the two possible envelope conformations ^3E and ^2E of the sugar moiety (Fig. 5) of the nucleosides were calculated [57, 58] from the ^1H NMR spectra in D_2O and DMSO-d_6 solutions. The results are given in Table 4.

It is known that platination at the N_7 position of guo increases the percentage of the ^3E conformation (38%) by about 10% [39, 41, 42]. The same is true with ino [13]. In ternary complexes of amino acids with nucleosides of Pt(II) and Pd(II), the ^3E conformation does not change significantly [13, 14] when varying the amino acid, except for a slight decrease with an increase of the aliphatic side chain, reflecting the stronger ligand–ligand interactions in these complexes. Similar results are also found in the present systems, in D_2O solutions, while in DMSO-d_6 solutions where the 'opened' form predominates, the percentage of the ^3E conformer is found much lower and does not vary with the dipeptide.

The conformation around the $C_{4'}$–$C_{5'}$ bond is described with the gg, gt and tg conformers [59–62] (Fig. 5). The percentage of each was calculated on the basis of the estimation of the coupling con-

TABLE 4. Percentage of the ^3E, gg and g/t conformation of the complexes in D_2O and DMSO-d_6

Compound	^3E (%)		$K_{eq} = {}^3E/{}^2E$	gg (%)		gt (%)	
	'opened'	'closed'		'opened'	'closed'	'opened'	'closed'
2a[a]	49.9	51.5	1.00–1.10	63.3	75.0	37.3	25.0
3a[a]	49.7	52.7	0.99–1.10		68.8		31.2
4a[a]	48.8	52.1	0.95–1.10		69.0		31.0
$[Pd(guo)_4]Cl_2$	52						
1a[b]	38.9		0.64				
1b[b]	41.1		0.70	49.0		51.0	
2a[b]	39.9		0.66				
2b[b]	39.7		0.66	49.0		51.0	
3a[b]	38.8		0.63				
3b[b]	39.9		0.66	49.0		51.0	
4a[b]	39.5		0.65				
4b[b]	42.1		0.73	50.0		50.0	
guo[b]	40.9			50.1		49.9	

[a]In D_2O. [b]In DMSO-d_6.

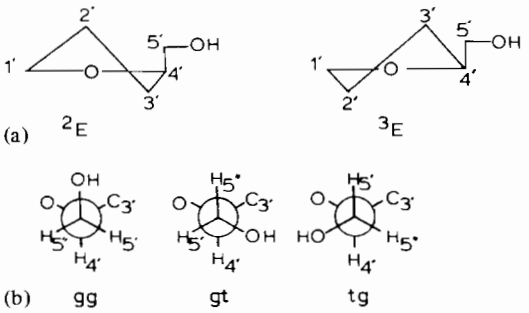


Fig. 5. (a) ^3E and ^2E sugar conformations. (b) Conformations around the C_4'–C_5' bond of the sugar moiety of the nucleosides.

stants $^3J_{HH}$ and application of the Karplus equation [59–62].

The results are also included in Table 4 and show that the gg conformer is favored (~69%) in the case of the ternary complexes of guo with the various peptides; this also happens in free guo (~69%), in D_2O solutions.

Both ternary complexes of guo and ino in DMSO-d_6 solutions, on the other hand, have almost the same ratio (1:1) of the gg and g/t conformers, appreciably lower than in D_2O solutions and not varying with the variation of the peptides.

It should be mentioned that the percentage of the gg conformer of guo is retained at 69%, when the ligand coordinates with the antitumor drug *cis*-DDP at N_7 [39, 63], while it decreases upon reaction of the ligand with 2-acetyl-amino-fluoride, a strong carcinogenic agent.

The *syn–anti* conformation of the sugar moiety of the nucleosides was also examined in the ternary complexes of ino and guo, based on their ^1H NMR spectra. The percentage of each conformer was reported [63] to be related to the chemical shift of the $H_{2'}$ proton of the sugar. Thus, for the complex *cis*-$Pt^7(G_P{}^{5'})_2{}^{2+}$ with the most upfield chemical shift of 4.608 ppm for the $H_{2'}$ proton, a 100% *anti* conformation was supposed to exist in solution [63]. In t-Bu^6guo, with a 5.073 ppm chemical shift, a 100% *syn* conformation was supposed [63]. The relative percentage of the *syn* and *anti* conformations [64] was then calculated from the relation

$$\delta_{obs} = P_{syn}\delta_{syn} + P_{anti}\delta_{anti} \quad (3)$$

In the case of the ternary complexes of the present study however, the $H_{2'}$ proton is found to be more upfield for both the 'opened' and the 'closed forms (about 4.5000 and 4.3700 ppm respectively), than with the $H_{2'}$ sugar proton with a supposed 100% *anti* conformation [63]. We were therefore not able to calculate the percentages of the *syn* and *anti* conformations in our case and we notice that such results [63] should be interpreted with caution. We can only say that the percentage of the *anti* conformation should be larger than in the *syn* and even larger in the 'closed' than in the 'opened' form, without being able to calculate them accurately.

The presence of the peptides in the ternary complexes increases the percentage of the *anti* conformation of guo, compared with the complex $[Pd(guo)_4]Cl_2$ [50].

Conclusions

The *trans*-ternary complexes of nucleosides with dipeptides of increasing aliphatic side chain show:

(i) two main isomers in D_2O solutions, called the 'opened' and 'closed' forms, with weak and strong ligand–ligand interactions, respectively. In DMSO-d_6 solutions the 'opened' form is favored. (ii) The hydrophobic interactions between the protons of the aliphatic side chain of the peptides and the aromatic rings of guo in D_2O solutions, decrease with increasing their distance from the bonding site, contrary to what is observed for the *cis*-[Pd(guo)$_2$(am–ac)]Cl complexes [14]. (iii) The 'closed' form increases with the aliphatic side chain of the peptides. (iv) The ^3E, ^2E and gg–gt conformation of the sugar part of the nucleosides do not seem to depend upon the presence or the nature of the peptide in the *trans*-ternary complexes. (v) The percentage however, of the *anti* conformation seems to increase more in the *trans*-than the *cis*-ternary complexes [14]. A further increase is observed by the presence of the peptide in the *trans* complexes of the present systems and especially of their 'closed' form. This may indicate that the toxicity of *cis*-DDP may be due in a large extent, to the formation of DNA–Pt–protein crosslinks, which cause the increase of the *anti* conformation of DNA; this is more pronounced in the *trans* compounds forming these crosslinks to a larger extent [5]. This is in agreement with the *trans*-DDP being more toxic than the *cis*-analog [5].

Experimental

Materials

The dipeptides, glycine–glycine, glycine–L-alanine, glycine–L-valine and glycine–L-leucine were purchased from Sigma Chemical Company and used without further purification. Palladium chloride was purchased from Degusa A. G. (F.R.G.).

Methods

(i) The elemental analyses of Pd and Cl were performed in our laboratory, while those of C, H and N were performed in the Laboratoire de Chimie de Coordination, Toulouse, France.

(ii) The conductivity measurements were performed in an E365 B Conductoscope, Metrohm Ltd., Herisau, Switzerland.

(iii) The IR spectra were recorded on a Perkin-Elmer model 580 spectrophotometer.

(iv) The ^1H NMR spectra were obtained on a Brucker WM-250 spectrometer equipped with an Aspect 3000 computer. The ^1H NMR chemical shifts were measured in parts per million with DSS or TMS as internal reference.

Preparation of the Compounds

Complexes of the type *trans*-Pd(dipeptide)$_2$Cl$_2$ for the dipeptides gly–gly, gly–L-ala, gly–L-val and gly–L-leu were synthesized according to literature methods [26].

Preparation of trans-[(nucl)$_2$Pd(dipeptide)$_2$]Cl$_2$

One mmol of the complex *trans*-Pd(dipeptide)$_2$-Cl$_2$ is mixed in the solid state with 2 mmol of the nucleosides (ino or guo) and a small volume of water added to the mixture. The mixture is stirred for 10 min at 50 °C whereupon a complete dissolution is achieved. The solvent is then evaporated under vacuum (~2 ml) and the complex precipitated with acetone. It is then filtered and washed with a mixture of acetone and ether. It is then dried first in a dessicator at room temperature and then at 110 °C under vacuum. Yield 85%.

Preparation of the Deuterated Derivatives

One hundred mg of the corresponding complex are dissolved in a few ml of D_2O and stirred for 5 min at room temperature. The deuterated complex is then reprecipitated with acetone and ether, filtered and dried in vacuum.

Supplementary Material

A Table with tentative assignments of several of the various IR bands of the compounds and Tables with ^1H NMR chemical shifts of the various complexes in D_2O and DMSO-d_6 solutions are available from the authors on request.

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