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LETTER

^{13}C and ^{195}Pt NMR of anticancer platinum pyrimidine greens

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We have synthesized platinum uridine blue and green complexes and found that the green species exhibited strong antitumor activity against L1210 cells both *in vivo* and *in vitro*, while the blues did not [1–3]. We also reported the selective synthesis of platinum uridine greens (Pt-greens) by hydrogen peroxide oxidation [2, 4, 5] and photooxidation reactions [6]. Furthermore, we described their HPLC behavior [7] and anticancer activity against human tumor cells [8]. Since their single crystals are difficult to prepare, we have studied these complexes by several spectroscopic techniques. We reported the structure analysis of Pt-green in solid powder [9] and in solution [10] with the electron spin resonance (ESR) technique. The ESR spectrum of Pt-green is almost the same both in solid powder and in solution, and shows a typical powder pattern of uniaxial symmetry with $g_{\parallel} = 1.99$ and $g_{\perp} = 2.41$. The spectrum derives essentially from the $5d_{z^2}$-like hole state of Pt(III)($5d^7$). The hyperfine structure of the spectrum suggests that the unpaired spin is not localized on one platinum center but has a wide orbital extending over four platinum atoms with zigzag chain structure. Though the molecular structure of Pt-green is thought to resemble that of α-pyridone blue, only information about the Pt atoms could be obtained from these results. As a next step, it was necessary to investigate how the uridine molecules are coordinated with the Pt atoms. Thus, we expanded research of the structure analysis of Pt-greens in solution using the nuclear magnetic resonance (NMR) technique. Though Pt-green is paramagnetic, clear ^{13}C and ^{195}Pt NMR spectra were oberved. In this paper, we show the ^{13}C and ^{195}Pt NMR spectroscopic results which reflect the isomeric structures of the Pt-greens.

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Experimental

Synthesis of platinum uridine greens

Pt uridine green was synthesized from the reaction of *cis*-diiododiammine platinum(II) and uridine (Fig. 1) with hydrogen peroxide under nitrogen at 75 °C by a one-pot method as described previously [5].

NMR measurement

^{13}C and ^{195}Pt NMR spectra were measured on a JEOL GSX-400 spectrometer and ^{13}C NMR spectra were also measured on Bruker ACP200 and JEOL FX90A spectrometers. The NMR measurement of the Pt-green complex was made at 100.4, 50.3 and 22.5 MHz for the ^{13}C nucleus and at 85.8 MHz for the ^{195}Pt nucleus, in 1/15 M Na_2HPO_4–KH_2PO_4 buffer solution at pH = 6.5. The concentration of Pt-green was about 80 mg/ml. The chemical shift data for ^{13}C NMR were recorded relative to TSP-[$(CH_3)_3Si(CD_2)_2COONa$], and a solution of K_2PtCl_6 in D_2O was used as a reference for the ^{195}Pt chemical shift. All the chemical shifts are expressed in ppm and the sample temperature was regulated at 5 or 40 °C using a variable temperature controller.

Results and discussion

The ^{13}C NMR spectrum at 100.4 MHz of the present compound at 5 °C (pH 6.5) is shown in Fig. 2(a). A set of three signals for each corresponding carbon except C-4′ was observed. They consisted of one large signal and two small close signals of nearly equal intensity. Chemical shifts of each signal are

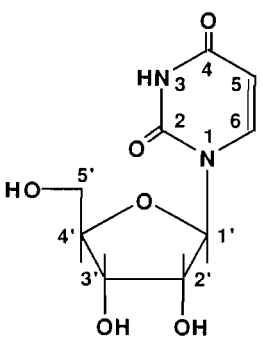

Fig. 1. Molecular structure of uridine.

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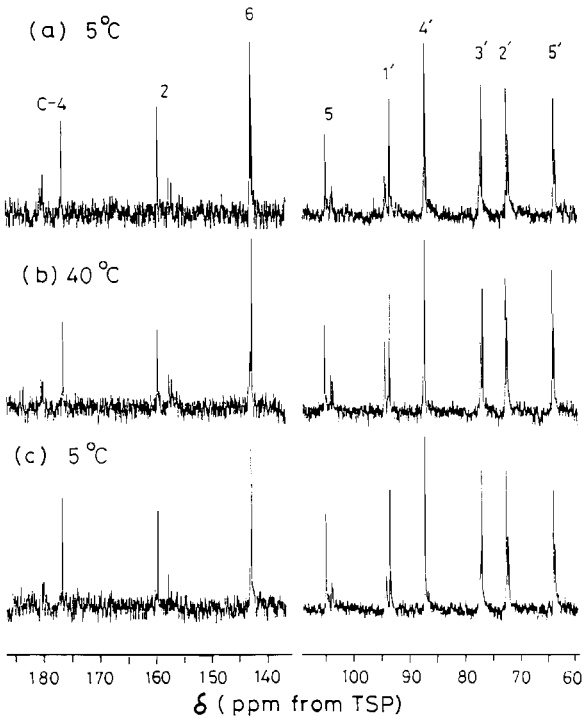

Fig. 2. ^{13}C NMR spectra of Pt-green at pH 6.5(100.4 MHz) showing thermochromism. Measurement conditions: (a) 5 °C, $t = 2$–4 h; (b) 40 °C, $t = 5$–7 h; (c) 5 °C, $t = 8$–9 h; where t means the elapsed time since dissolution of the Pt-green in the buffer solution.

listed in Table 1 together with those of the corresponding signals of uridine, and the differences between them are also shown. The chemical shifts of the Pt-greens are relatively close to those of uridine in most carbons except for the C-4 and C-2 signals, which are remarkably shifted to a lower field (about 9 and 5 ppm, respectively). This means that the C-4 and/or C-2 carbonyl carbons of uridine are coordinated with platinum. In the present complexes, coupling with ^{195}Pt ($I = 1/2$, natural abundance = 33.7%) was not observed, which will be discussed later.

Figure 2(a)–(c) shows thermochromism in the ^{13}C NMR spectra of the Pt-green. The intensity ratio of the two small signals to the large one increases with rising temperature (from (a) to (b)) and, inversely, decreases with lowering temperature (from (b) to (c)). The ratio in (c) does not completely recover to the value in (a), which means that thermal equilibrium is not recognized in the NMR measurement.

Figure 3(a)–(c) shows thermochromism in ^{195}Pt NMR spectra. Three signals were observed: two small signals (−1590, −1760 ppm) and one large one (−2010 ppm). The intensities of the smaller two are comparable and the intensity ratio relative

TABLE 1. ^{13}C NMR of uridine and Pt-green complex (ppm from TSP)

	δ(uridine)	δ(Pt-green)[a]	Δδ[a]
C-4	168.9	180.5	11.6
		180.1	11.2
		176.6*	7.7*
C-2	154.4	160.1*	5.7*
		158.0	3.6
		157.6	3.2
C-6	144.8	143.7	−1.1
		143.5	−1.3
		143.3*	−1.5*
C-5	105.1	104.7*	−0.4*
		103.6	−1.5
		103.3	−1.8
C-1'	92.3	94.0	1.7
		93.7	1.4
		93.1*	0.8*
C-4'	87.1	86.9*	−0.2*
C-3'	76.5	77.0	0.5
		76.9	0.4
		76.7*	0.2*
C-2'	72.3	72.3*	0.0*
		72.0	−0.3
		71.9	−0.4
C-5'	63.6	63.7*	0.1*
		63.4	−0.2
		63.3	−0.3

[a]Asterisk indicates the largest signal among the three.

to the large one increases with rising temperature (from (a) to (b)), and decreases with lowering temperature (from (b) to (c)), as was observed in the ^{13}C NMR spectra.

A set of three signals for each corresponding carbon of uridine was observed in both ^{13}C and ^{195}Pt NMR spectra suggesting that three isomeric structures exist in the Pt-green. The possible structures of the Pt-green are schematically shown in Fig. 4, i.e. two head-to-head (H–H) structures and one head-to-tail (H–T) structure. The ESR study indicates that the Pt-green in solution forms an oligomer equal to or greater than four(tetramer). The three signals observed in ^{13}C NMR consisted of one large signal and two small close signals of nearly equal intensity. It is natural to consider that one H–T and two H–H structures give rise to the former and the latter signals, respectively.

The downfield shift (about 9 ppm in ^{13}C NMR) of the C-4 carbonyl carbon relative to the C-2 indicates that the C-4 carbonyl carbon is coordinated with platinum. This suggests that there are two possible ways for the uridine molecule to be coordinated with platinum: C-4 and N-3, C-4 and C-2. Although we

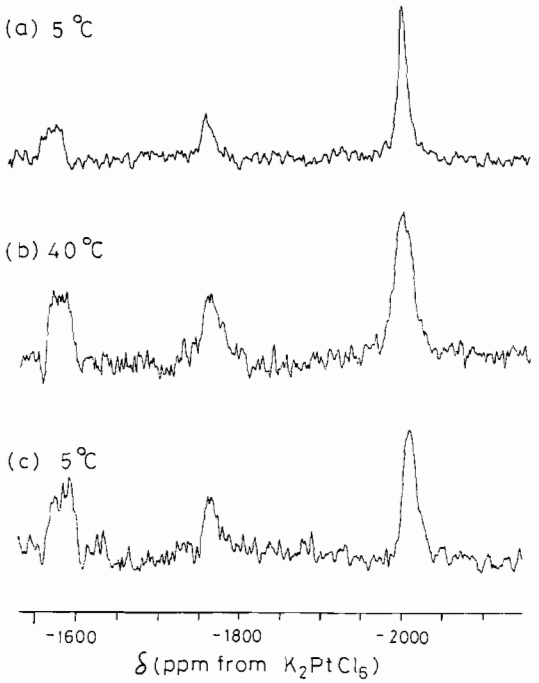

Fig. 3. ^{195}Pt NMR spectra of Pt-green at pH 6.5 showing thermochromism. Measurement conditions: (a) 5 °C, $t = 1.5$–16.5 h; (b) 40 °C; $t = 17.5$–30.5 h; (c) 5 °C; $t = 31$–40.5 h; where t means the elapsed time since dissolution of the Pt-green in the buffer solution.

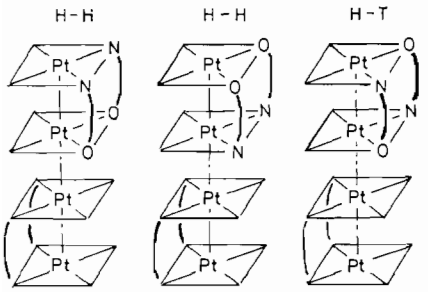

Fig. 4. Expected isomeric structures of Pt-green; two H–H (head to head) structures and one H–T (head to tail) structure are shown.

cannot say which of these we are seeing, it must be a single type. If both existed, the spectrum would be more complicated than that observed.

In the present complexes, the coupling with ^{195}Pt was observed neither in ^{195}Pt NMR nor in ^{13}C NMR. Assuming that a coupling constant is in proportion to the square of the electron densities, the expected coupling constant of the complexes will differ from each other and those for the carbons coordinated with the Pt atom will be larger than those for other carbons. The ^{13}C NMR signals, however, broaden to 20–40 Hz with no splitting. There are several possible reasons for this: such as CSA (chemical shift anisotropy) relaxation, paramagnetic relaxation or dynamic equilibrium.

Regarding the CSA relaxation, Lallemand *et al.* [11] reported that the dominant CSA relaxation mechanism could account for the disappearance of the ^{195}Pt–^{13}C coupling constant at high magnetic field. For instance, in α-pyrrolidonate-bridged binuclear platinum complexes [12], clear triplet signals due to ^{13}C–^{195}Pt coupling were obesrved at 22.5 MHz and they broadened and became less clearly resolved at 50.3 MHz. With this in mind, we also measured the ^{13}C NMR of the present complexes at lower frequencies, at 50.3 (ACP200) and 22.5 (FX90A) MHz. However, no splitting was observed in either case. If the uridine molecule was as strongly coordinated with the Pt atoms as in the α-pyrroridonate-bridged binuclear platinum complexes, splitting should have been observed in our measurements. Therefore, some other mechanisms are also thought to affect the relaxation process.

As reported [9, 10], the present complex has a paramagnetism from the $5d_{z^2}$-hole state of Pt(III) and the unpaired spin does not localize on one Pt center but has a wide orbital extending over four Pt atoms. Therefore, each Pt atom has a paramagnetism and this gives rise to a paramagnetic broadening on ^{13}C NMR of the coordinated uridine molecules.

In the model schematically shown in Fig. 4, two H–H and one H–T structure, in the manner of the uridine molecule coordinated with Pt atoms, are supposed to exchange with each other. Such exchange will affect the relaxation mechanism (dynamic equilibrium).

Thus, there are mechanisms affecting the line broadening of the NMR signal, such as the CSA mechanism, paramagnetic relaxation and dynamic equilibrium. It is, however, difficult to estimate the contribution of each to the line broadening in our measurements.

The intensity ratio of the three isomeric structures of the Pt-green varies not only with varying temperature, but with changing concentration, pH, etc. Detailed experiments under various conditions are necessary as a next step.

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