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Efficient Preparation of Anticancer Platinum Pyrimidine Blues. On the Highly Reproducible Reaction Conditions and Purification by Gel Filtration

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The employments of dianion such as SO_4^{2-} and nucleosides as a substrate resulted prevalent formation of platinum blues at 75 °C for 20 h. Gel filtration method was successfully used for purification of the products. It is notable that green species rather than blue complexes gave remarkably high antitumor activity against L1210 cells.

cis-Diamminedichloroplatinum(II), cis-DDP, is a potent antitumor agent, but has strong side effects of nephrotoxicity and emesis. According to an earlier report,[1] "platinum blues" formed in the slow reaction with diaquo derivative of cis-DDP and pyrimidine bases, such as uracil and thymine, at 37 °C and pH 7 under air for weeks show lower toxicity, improved activity and high water solubility as compared with cis-DDP itself. Since then, various attempts pertinent to preparing the materials and clarifying the structure have been made but with limitted success. First successful crystallization and structural analysis of the analog complex were achieved by Lippard et al[2,3] using α-pyridone as a substrate which has a single coordination site; a tetranuclear zigzag chain structure with average Pt oxidation state 2.25 was determined by X-ray diffraction analysis (Fig. 1). Similar structures have been subsequently reported with α-pyrrolidone,[4] 1-methyluracil,[5] 1-methylhydantoin,[6] and so on.

It can therefore be presumed that platinum pyrimidine blue is also a polynuclear mixed-valent complex containing Pt(II) and Pt(III). However, little is known so far about efficient synthetic procedures and structural information due to the amorphous precipitate. Presented here are the results on the efficient synthesis of platinum blue with water soluble nucleosides as a substrate and purification by gel filtration as well as on anticancer activities.

After considerable exploratory work, the pH value of 4.5 was found to be optimum, which is consistent with analog preparation.[7] It is notable that an employment of dianion such as SO_4^{2-} instead of NO_3^- that is thus far extensively used accelerates the reaction remarkably. A typical example is given below. To the hydrolysis products which were prepared from cis-DDP and Ag_2SO_4, uridine (1 mol eqv.) was added, then adjusting pH 4.5 with 0.5 M NaOH (1 M=1 mol dm^{-3}), and

reacted at 60+1°C under air in the dark. Color of the entirely homogeneous mixture turns blue slightly in half an hour, which gives visible absorption maxima at 575 nm. The color intensity increases with reaction time, and finally reaches plateau after 15-20 hr as illustrated in Fig. 2. The amorphous blue powder obtained after evaporation of water under 50 °C in vacuo was then purified by gel filtration (Toyopearl HW-40) eluted with 12 mM H_2SO_4 solution to yield three fractions; blue, dark red, and green. To the each fraction, ethanol or acetone was added and kept at 4 °C overnight. Filtrations through membrane filter (0.45 μm) gave blue (1), dark red (2), and green (3) amorphous solids in 15.3, 7.7, and 10.5% yields,[8] respectively. When the reaction was carried out at 75 °C for 20 h, blue complex was predominantly isolated (31.1%) together with green (4.5%), dark red (4.1%), and newly obtained brown (4, 5.4%) ones.

The purified uridine blue gave a characteristic absorption spectra in visible region as shown in Fig.3 (490sh, 575, 660sh nm), and C=O stretching band at 1640 cm^{-1} (cf. 1690 cm^{-1} in uridine) and Pt-N band at 590 and 610 cm^{-1} in ir spectra.

Elemental analysis showed precise 1:1 ratio of Pt and uridine in the molecule but considerably lower nitrogen, which suggested substitution of NH_3 by different ligand containing oxygen element. The data of microanalysis, when considered to OH^- ligand, indicate that the blue complex involves the constituent of $[Pt_4(C_9H_{11}N_2O_6)_4(NH_3)_4(OH)_4](SO_4)_{1.73}/H_2O$; Calcd C, 20.85; H, 3.01; N, 8.11; S, 2.67; Pt, 37.65; Found C, 20.55; H, 3.02; N, 8.20; S, 2.64; Pt, 37.96.[9] By the same consideration, the following components are given for; dark red compound, Calcd for $[Pt_4(C_9H_{11}N_2O_6)_4(NH_3)_5(OH)_3](SO_4)_2$; C, 20.77; H, 3.00; N, 8.75; S, 3.08; Pt, 37.49; Found C, 20.88; H, 3.06; N, 8.90; S, 3.06; Pt, 37.03. green complex, Calcd for $[Pt_4(C_9H_{11}N_2O_6)_4(NH_3)_7(OH)](SO_4)_{2.34}/3H_2O$; C, 19.95; H, 3.35; N, 9.69; S, 3.46; Pt, 36.01; Found C, 19.96; H, 3.43; N, 9.62; S, 3.46; Pt, 36.46.

Gel chromatographic behavior exhibits the degree of oligomerization of blue, dark red, and green complexes, which decreases in that order. The extent of oxidation is parallel to this observation, and tentatively employing the formulas given, average platinum oxidation states of compounds 1, 2, and 3 are 2.86, 2.75, and 2.42, respectively. Weak absorption around at 730 nm in an early reaction stage (Fig. 2) suggested the blue formation via oligomerization of green complex at least to some extent, which was proven by successful conversion of isolated green chelate to the blue. Attempts to obtain crystals of these derivatives suitable for an X-ray structural study have so far been unsuccessful.

The biological properties of synthesized complexes were then examined on the survival time of mice bearing L1210 (10^4 cells; CDF_1 mice). The sample was injected on days 1 and 5, and ratio of the median survival time of the treated vs control (T/C %) was determined, which is summarized in Table 1. The bioassay results are somewhat beyond expectation, because purified uridine blue showed no antitumor activity against L1210 cells in vivo, while the green one exhibited noteworthy high activity. The activity of a crude sample which contains approximately 30% of green complex was comparable to that of the green itself (Table 1). Furthermore, it should be emphasized that with the green material there observed the examples of complete cure defined as animals alive 60 days after tumor inoculation.

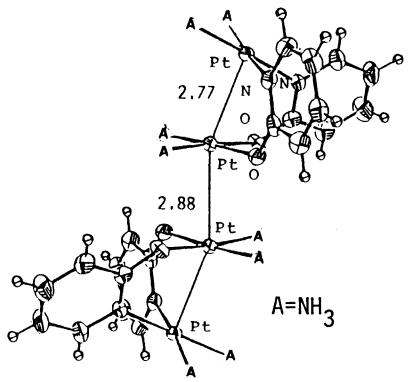

Fig. 1. α-Pyridone blue.

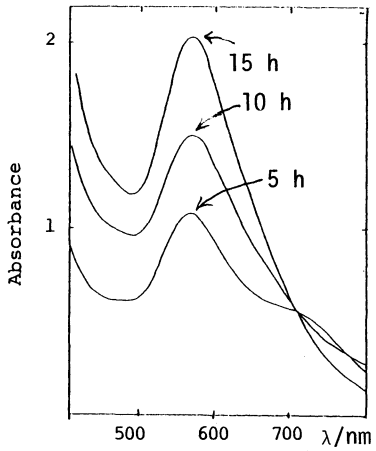

Fig.2. Time courses of absorption spectrum in uridine blue formation at 60 °C.

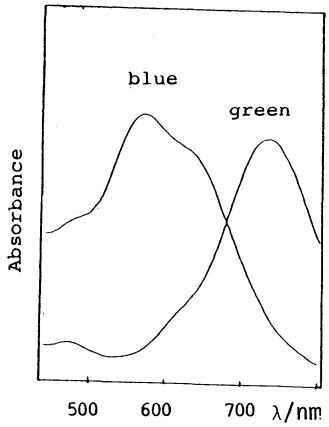

Fig.3. Visible absorption spectrum of uridine blue and green complexes in water.

Table 1. Antitumor Activity of Platinum Uridine Blues and Related Complexes against L1210[a)]

Sample	Dose[b)] (mg/kg)	MST[c)] (day)	T/C[d)] (%)	LTS[e)]
Uridine blue	100	10.5	105	0/6
"	260	12.0	126	"
dark red	100	11.0	110	"
green	75	21.0	221	2/6
crude sample[f)]	200	19.5	205	0/4
Uracil blue	75	10.5	111	0/6

a) CDF_1 mice, weighing 18-20 g, were used, and L1210 cells ($1x10^4$) were transplanted intraperitoneally. b) The sample was intraperitoneally injected on days 1 and 5. c) Median survival time, days. d) Ratio of the MST of the treated vs control. e) Long-term surviving animals defined as animals alive 60 days after tumor inoculation. f) Before gel filtration, contains approx. 30% green complex.

The relationship between the activity and structure, and the mode of reaction in biological activities will be the next targets.

Conclusively, we have developed efficient methods of preparation for platinum pyrimidine blues and related complexes. From our bioassay results we propose that the real antitumor active species against L1210 in vivo are green complexes rather than the blue one. Related work with various kinds of nucleosides has been under progress as well as selective synthesis of green complexes,[10] which is in preparation for publication.

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8) Yield is calculated as a tetranuclear complex as shown in a model compound with overall charge 5.
9) Calculated from unburned ash.
10) A part of the related work has been presented by Y. Okuno, T. Inoue, K. Tonosaki, and O.Yonemitsu at the 106th Annual Meeting of Japan Pharmaceutical Society at Chiba, 1986. See p. 448 of the Abstract.

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