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SELECTIVE SYNTHESIS OF NOVEL ANTICANCER PLATINUM PYRIMIDINE GREENS
BY HYDROGEN PEROXIDE OXIDATION

Takehiko Shimura, Takenori Tomohiro, Tarja Laitalainen,¹⁾ Hiroshi Moriyama,
Toshimasa Uemura and Yohmei Okuno*

National Chemical Laboratory for Industry (NCLI), Tsukuba 305, Japan

Platinum greens can be synthesized effectively and selectively from aquated cis-diiododiammineplatinum(II) and pyrimidine derivatives with hydrogen peroxide under argon. By adjusting reaction temperature, time and the amount of oxidizing agent, various sizes of the molecule have been obtained. This method can be used with the different substrates and counter anions of the complexes.

KEYWORDS ——— platinum pyrimidine green; selective synthesis; hydrogen peroxide oxidation; selective green formation; antitumor activity

The mononuclear platinum compounds such as cis-DDP, carboplatin, and complexes of vitamin C and diaminocyclohexane, are a family of compounds with current interest because of their remarkable antitumor activities.²⁾ But they have serious problems of nephrotoxicity and emesis. To reduce the undesirable side effects, a series of related oligomers have been synthesized and studied.³⁾ Recently, we have reported efficient preparations of platinum pyrimidine blues, and found that "green" species obtained as a minor product after gel filtration were responsible for the anticancer activity against leukemia L1210 cells rather than the corresponding blue ones.⁴⁻⁶⁾ To examine these further in detail, development of productive methods for synthesizing the green complexes was highly required. We now present here the first selective synthesis of platinum pyrimidine greens and newly observed structural information about the greens. The molecular size of uridine blue complex is obviously larger than that of the green, as gel chromatographic behavior shows. Moreover the extent of average Pt-oxidation states in the blues is higher than that in green species. Therefore, a strictly controlled amount of the oxidizing agent seemed to be crucial for the selective synthesis of platinum greens. After some exploratory efforts, very efficient and selective methods were discovered for preparing Pt-greens. Typical procedures are as follows.

An aqueous solution containing uridine (73.3 mg, 0.3 mmol), aqua-complex (3 ml, 0.3 mmol, SO₄²⁻ as a counter anion), and hydrogen peroxide (336 μ l, 1%) was heated under argon at 40 °C for 3.7 h with magnetic stirring. The aqua-complex was derived from cis-diiododiammineplatinum(II)⁷⁾ (144.9 mg, 0.3 mmol) and Ag₂SO₄ (93.5 mg, 0.3 mmol). The mixture was allowed to cool to room temperature, submitted to gel filtration (Toyopearl HW-40), and then reprecipitated from

water-acetone. After drying at 60 °C over P₂O₅ in vacuo, the yield was 63.9 mg (40.2%; see run 4 in Table I).

As long as exposure to air is carefully excluded during these purification processes, very uniform results can be obtained, and no Pt-blue formation was observed at all. This is in sharp contrast with simple air oxidation which produces the blue materials exclusively.⁴⁾ Aqueous solutions of Pt-uridine green give a characteristic absorption maximum at 730 nm in a visible region. The green complexes showed a C=O stretching band at 1645 (cf. 1690 in uridine) and a Pt-N band at 870, 815 and 585 cm⁻¹ in infrared spectra (KBr pellet), and gave [θ] (λ max nm) values⁸⁾ 37,800 (272) and -37,800 (219) in circular dichroism (in water). ¹³C-NMR measurements gave slightly complicated spectra (in D₂O, DSS as a reference): two signals for each corresponding carbon except C-3' and C-4'. The chemical shifts of Pt-greens were very close to those of uridine in most carbons. However, those corresponded to the C-2 and C-4 carbons were largely shifted to a lower field (6.0 & 4.3, and 11.2 & 8.1 ppm from the original positions, 154.2 for C-2 and 168.6 ppm for C-4 in uridine, respectively). The above results strongly suggest that the C-4 and/or C-2 of uridine are coordinated with platinum (II) and/or (III). Electron spin resonance (ESR) of Pt(III) in platinum uridine green is observed. The spectra showed a typical powder pattern of uniaxial symmetry, and g_{||} and g_⊥ were determined to be 1.991 and 2.413, respectively. A hyperfine structure of the spectrum has suggested that the unpaired spin was not localized on one platinum center but that it had a wide orbital extended over several platinum atoms. Full details of the matter are given in a separate paper.⁹⁾ Representative results for preparing Pt-greens under characteristic reaction conditions are listed in Table I.

Microanalysis provides very important information on the components especially for the present situation: the numbers of uridine (and nitrogen in uridine) and SO₄ in the molecule are easily determined by analyzing carbon and sulfur, respectively.

Consequently, the numbers of NH₃ can be unequivocally obtained. The results clearly showed that a part of the NH₃ groups primarily coordinated with Pt could have been substituted by different ligands, which should involve an oxygen element.

Recently, a rather

Table I. Synthesis of Platinum Uridine Greens

Run	Temp °C	Reagent ^{a)}	Concn ^{b)} M	Time	Yield ^{c)} %
[1]	2	A	0.1	3 wk	49.5
[2]	"	"	"	4 wk	29.5
[3]	25	"	"	48 h	12.6
[4]	40	"	"	3.7 h	40
[5]	50	"	"	2 h	36.6
[6]	75	"	0.01	3 h	17.9
[7]	"	"	0.1	45 min	41.9
[8]	"	"	"	"	32
[9]	"	"	"	90 min	35.8
[10](Blue)	60	B	0.1	41 h	40

a) A: 1% H₂O₂ used, B: Air oxidation. b) Substrate concentration. c) Computed as tetranuclear complex with overall charge 5. See ref. 4 for more details.

facile substitution of NH_3 ligands in a diplatinum(III) complex has been reported¹⁰⁾ as well as in a platinum green component with 1-methyluracil.¹¹⁾ The data of microanalysis for run 4, assuming an OH ligand as the oxygen-involving one,⁴⁾ are as follows: Calcd for $[\text{Pt}_9(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_4(\text{NH}_3)_{16}(\text{OH})_3(\text{H}_2\text{O})_9]-(\text{SO}_4)_7/2\text{H}_2\text{O}$: C, 11.02; H, 3.01; N, 8.57; S, 5.72; Pt, 44.76; Found: C, 11.39; H, 2.91; N, 8.61; S, 5.77; Pt, 44.57. The elemental analysis results for other complexes are given in the reference part.¹²⁾ It was proved that by changing reaction conditions (see Table I) such as temperature, time, amount of oxidizing agent etc., Pt-greens with various sizes of molecule could be synthesized. Typical examples are summarized in Table II together with some selected data.

Reactions at lower temperatures required longer reaction time for green formation, and were entirely repeatable.

For example, when the oxidation was performed at 2°C for 3 weeks, the identical green products which showed the equivalent elemental compositions (Pt:Ud:NH₃ = 10:6:20 for [1]) were always obtained from several independent runs. Longer reaction resulted a slightly

larger molecule ([1] & [2] in Table II).

Reactions at 25°C for 48 h gave Pt-green with a component quite

similar to that in run 1, but the average platinum oxidation state was slightly lower (see [3]). In the same manner, green species with various compositions can now be prepared by adjusting reaction conditions (cf. [4] - [7]). At 75°C (0.1 M concentration), reactions are very fast, but it is difficult to control the products accurately (see [7] & [8]). More drastic conditions tend to afford larger molecules (see [9]).

The present method can be used with different counter anions of the diaquated compound like SO_4^{2-} , NO_3^- and ClO_4^- , and also with various substrates such as uridine, uracil, thymidine, thymine, 2'-deoxyuridine, uridine 5'-monophosphate, 5-fluorouracil, and others. These consequences will be described later in a full paper. Interesting results on the biological activities of the platinum greens have been observed. These have been discussed in an earlier publication.¹⁴⁾ Thus we have found that anticancer platinum pyrimidine greens can be synthesized efficiently and selectively by hydrogen peroxide oxidation, and that various sizes of the molecule can be obtained by adjusting the reaction

Table II. Selected Data for Platinum Uridine Greens

Run ^{a)}	UV, nm $\lambda_{\text{max}}(\log \epsilon)^b)$	mp °C	Calcd No. of Pt : Ud : NH ₃ ^{c)}	Av Pt oxdn ^{c)}
[1]	268 (4.85)	>300	10 : 6 : 20	2.40
[2]	265 (4.86)	"	11 : 9 : 20	2.36
[3]	270 (4.81)	"	10 : 8 : 19	2.30
[4]	266 (4.55)	"	9 : 4 : 16	2.33
[5]	268 (4.66)	"	10 : 5 : 18	2.30
[6]	265 (4.61)	"	8 : 5 : 15	2.38
[7]	267 (5.00)	"	13 : 12 : 23	2.38
[8]	267 (5.06)	"	14 : 14 : 23	2.36
[9]	265 (5.14)	"	16 : 16 : 28	2.38
[10](Blue)	272 (5.37)	"	28 : 28 : 29	2.82

a) The figures coincide with those in Table I. b) Computed from the estimated molecular weights obtained through microanalytical results. See text for more details. c) From elemental analysis.

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REFERENCES AND NOTES

- 1) Present address; Department of Chemistry, University of Helsinki, Vuorikatu 20, 00100 Helsinki, Finland.
- 2) cf. B. Rosenberg, L. VanCamp, J.E. Trosko and V.H. Mansour, *Nature*, **222**, 385 (1969); B.C. Behrens, T.C. Hamilton, H. Matsuda, K.R. Grotzinger, J. Whang-Peng, K.G. Louie, T. Knutsen, W.M. McKoy, R.C. Young and R.F. Ozols, *Cancer Res.*, **47**, 414 (1987); L.S. Hollis, E.W. Stern, A.R. Amundsen, A.V. Miller and S.L. Doran, *J. Am. Chem. Soc.* **109**, 3596 (1987); Y. Kidani, *Yakugaku Zasshi*, **105**, 909 (1985).
- 3) J.P. Davidson, P.J. Faber, R.G. Fisher, Jr., S. Mansy, H.J. Peresie, B. Rosenberg and L. VanCamp, *Cancer Chemother. Rep. Part 1*, **59**, 287 (1975).
- 4) Y. Okuno, K. Tonosaki, T. Inoue, O. Yonemitsu and T. Sasaki, *Chem. Lett.*, **1986**, 1947.
- 5) Y. Okuno, T. Inoue, O. Yonemitsu, T. Tomohiro and T. Laitalainen, *Chem. Pharm. Bull.*, **35**, 3074 (1987).
- 6) T. Tomohiro, T. Laitalainen, T. Shimura and Y. Okuno, "The Role of Oxygen in Chemistry and Biochemistry," Elsevier, Amsterdam, 1988, in press.
- 7) S.C. Dhara, *Indian J. Chem.*, **8**, 193 (1970).
- 8) Molecular weight is provisionally estimated as a tetranuclear complex with an overall charge of 5. See ref. 4 for further details.
- 9) T. Uemura, T. Tomohiro, K. Hayamizu and Y. Okuno, *Chem. Phys. Lett.*, **142**, 423 (1987).
- 10) B. Lippert, H. Schöllhorn and U. Thewalt, *Inorg. Chem.*, **25**, 407 (1986).
- 11) W. Micklitz, G. Müller, J. Riede and B. Lippert, *J. Chem. Soc. Chem. Commun.*, **1987**, 76.
- 12) For run [1]: Calcd for $[\text{Pt}_{10}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_6(\text{NH}_3)_{20}(\text{OH})_2(\text{H}_2\text{O})_6](\text{SO}_4)_8/7\text{H}_2\text{O}$: C, 13.55; H, 3.24; N, 9.36; S, 5.36; Pt, 40.75; Found: C, 13.84; H, 3.21; N, 9.29; S, 5.35; Pt, 40.72. For [2]: Calcd for $[\text{Pt}_{11}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_9(\text{NH}_3)_{20}(\text{OH})_3(\text{H}_2\text{O})_3](\text{SO}_4)_7/8\text{H}_2\text{O}$: C, 17.38; H, 3.31; N, 9.51; S, 4.01; Pt, 38.34; Found: C, 17.21; H, 3.19; N, 9.50; S, 3.94; Pt, 38.47. For [3]: Calcd for $[\text{Pt}_{10}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_8(\text{NH}_3)_{19}(\text{OH})(\text{H}_2\text{O})_4](\text{SO}_4)_7/10\text{H}_2\text{O}$: C, 16.75; H, 3.40; N, 9.50; S, 4.35; Pt, 37.80; Found: C, 16.96; H, 3.19; N, 9.37; S, 4.32; Pt, 38.22. For [5]: Calcd for $[\text{Pt}_{10}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_5(\text{NH}_3)_{18}(\text{OH})_2(\text{H}_2\text{O})_{10}](\text{SO}_4)_8/2\text{H}_2\text{O}$: C, 12.03; H, 3.03; N, 8.73; S, 5.71; Pt, 43.43; Found: C, 11.98; H, 2.95; N, 8.79; S, 5.71; Pt, 43.34. For [6]: Calcd for $[\text{Pt}_8(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_5(\text{NH}_3)_{15}(\text{OH})_2(\text{H}_2\text{O})_5](\text{SO}_4)_6/3\text{H}_2\text{O}$: C, 14.27; H, 3.14; N, 9.25; S, 5.08; Pt, 41.22; Found: C, 13.94; H, 3.10; N, 9.24; S, 4.92; Pt, 41.42. For [7]: Calcd for $[\text{Pt}_{13}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_{12}(\text{NH}_3)_{23}(\text{OH})(\text{H}_2\text{O})_4](\text{SO}_4)_9/18\text{H}_2\text{O}$: C, 18.21; H, 3.48; N, 9.24; S, 4.05; Pt, 35.60; Found: C, 17.76; H, 3.14; N, 9.23; S, 4.03; Pt, 35.59. For [8]: Calcd for $[\text{Pt}_{14}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_{14}(\text{NH}_3)_{23}(\text{OH})_3(\text{H}_2\text{O})_2](\text{SO}_4)_8/18\text{H}_2\text{O}$: C, 19.63; H, 3.48; N, 9.27; S, 3.33; Pt, 35.44; Found: C, 19.24; H, 3.22; N, 9.16; S, 3.30; Pt, 35.72. For [9]: Calcd for $[\text{Pt}_{16}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_{16}(\text{NH}_3)_{28}(\text{OH})_2(\text{H}_2\text{O})_2](\text{SO}_4)_{10}/25\text{H}_2\text{O}$: C, 19.28; H, 3.55; N, 9.37; S, 3.57; Pt, 34.80; Found: C, 19.42; H, 3.20; N, 9.16; S, 3.62; Pt, 34.31. For [10]: Calcd for $[\text{Pt}_{28}(\text{C}_9\text{H}_{11}\text{N}_2\text{O}_6)_{28}(\text{NH}_3)_{29}(\text{OH})_{27}](\text{SO}_4)_{12}/9\text{H}_2\text{O}$: C, 20.82; H, 3.03; N, 8.19; S, 2.65; Pt, 37.58; Found: C, 20.55; H, 3.02; N, 8.20; S, 2.64; Pt, 37.97.
- 13) Calculated from unburned ash.
- 14) T. Shimura, T. Tomohiro, K. Maruno, Y. Fujimoto and Y. Okuno, *Chem. Pharm. Bull.*, **35**, 5028 (1987).

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