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C O N T E N T S

Page

1. Nuclear Magnetic Resonance Study of Chicken Ovo-Transferrin

   -- by Gu, Zhi-hong (China) 1

2. Synthesis of Platinum GREEN and BLUE Complexes with 1-Methyluracil and Biological Activity

   -- by Gaber Sayed Mohammed (Egypt) 27

3. Vapour and Liquid Phase Hydrogenation Studies in Catalytic Palladium Membrane Reactors

   -- by Amod Madhukar Sathe (India) 57

4. Immobilization of Activated Sludges and Its Phosphate Treatment Characteristics

   -- by Suk Soon Choi (Korea) 143

5. Chemical Characterization of a Titania Modified CoMo/Al203 Catalyst

   -- by Juan Florencio Gonzalez Mateos (Mexico) 183

6. Effect of Physical Factors on Both the Phospholipid and Fatty Acid Composition of the Marine Bacterium Deleya Marina

   -- by Maria Estela Riveros Meaurio (Paraguay) 199

7. Environmental Measurement Technology and Evaluation

   -- by Victor Eugenio Barreto Medina (Paraguay) 221

8. Photocatalytic Degradation of Aromatic Sulfonic Acids in TiO_2 Aqueous Suspensions

   -- by Benchang Sangchakr (Thailand) 251

Nuclear Magnetic Resonance Study of Chicken Ovo-Transferrin

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Abstract

Transferrins are a family of homologous proteins of about 80 kDa that combine trivalent iron and transport it through the blood either to the reticulocytes or to the liver. Transferrin is a single polypeptide chain with two homologous domains. Each domain has a specific iron binding site. Although the overall structure and function of the two sites (amino (N-) and carboxy(C-) sites) are similar, they demonstrate different behaviors.

For investigating the difference and the cooperativity between these two sites, NMR of ^{51}V bound to the iron binding sites in chicken ovotransferrin was studied and two signals corresponding to the two iron binding sites were found, which are expected to become a high sensitive tool to monitor binding of vanadium to ovotransferrin.

Introduction

General description about iron metabolism by transferrin

Transferrin is a major serum glycoprotein including serum transferrin, ovotransferrin(conalbumin) and lactotransferrin, which transports iron to all tissue cells from the liver, the main site of its storage in the body, and from the intestine, the site of iron absorption. The iron-free form, apotransferrin, binds two Fe(III) ions very tightly forming ferrotransferrin. All growing cells contain surface transferrin receptors that avidly bind ferrotransferrin at neutral pH (Fig.1), after which receptor-bound ferrotransferrin is subject to endocytosis. As shown in Fig.2 (transferrin cycle), after endocytosis, iron is released from the transferrin-receptor complex in the acidic CURL compartment. The transferrin protein remain s bound to its receptor, and they cycle to the cell surface together. When the receptor-transferrin complex encounters the neutral pH of the exterior medium, the iron-free transferrin is released. The secreted apotransferrin is carried in the bloodstream to the liver or intestine, where it is reloaded with iron.

Review of the previous work and purpose of the present study

Ovotransferrin consists of a single polypeptide chain with a molecular mass of 77700 daltons which has the capacity to bind two atoms of iron. The amino- (N-)terminal and and carboxyl- (C-)terminal halves of the chain form independent compact domains(Fig.3), each containing one specific iron-binding site (Fig.4). Although the overall structure and function of the two sites are similar, they demonstrate different behaviours in the kinetics and pH dependence of iron binding(3) and thermal stabilities(4) etc. The functional difference or cooperativity between the two sites may play an important role in iron metabolism.

Moreover recent report by Mason et al. (1), (2) have demonstrated that the interaction between the two half molecules is functionally significant for the recognition by the transferrin receptor of chick-embryo red blood cells.

For investigating the difference and the cooperativity of the two binding sites in transferrin, a lot of experimental works have been done since now(5), however , there are not so many clear works which demonstrated their difference. It is well known that transition metal ions, Fe, Cu, Mn etc. combine preferably to the N-site. By Yamamura et al. , binding of lanthanide ions to ovotransferrin has been systematically investigated.(6) They found that the transferrin-lanthanide complexes at the two sites differ markedly in the rate of the metal release by EDTA. Making use of the properties of transferrin-lanthanide complexes, Uemura et al. studied the electron spin resonance of Gd(III) ion bound to the two iron binding sites in chicken ovotransferrin and found the difference of the ligand field of the two sites(7,8,9,10). Sasaki et al. carried experiments using dynamic loght scattering technique on various lanthanide ions and Cu ion and suggested that the contraction of the transferrin molecule originate from a predominant metal-binding to the C-site compared to the N-site(11).

Vanadium is widely recognized as a biologically important element(12,13) and an attractive method to characterize metal binding sites of metalloproteins is NMR spectroscopy of the bound metal ion which can serve as a highly selective probe, giving information about site symmetries, the nature of the coordinated ligands, exchange kinetics, and the motional properties of the protein. Unlike most other

biologically important isotopes, the NMR receptivity of the ^{51}V nucleus is exceptionally high due to a large magnetic moment, high natural abundance(99.76%) and rapid quadrapolar relaxation in solution. The ^{51}V NMR chemical shifts are very sensitive to changes in the nature of the ligands(14,15,16,17), thereby providing an excellent diagnostic tool for detailed investigation of vanadium(V) coordination environment. In the present report, the ^{51}V NMR study of a V(V)-chicken ovotransferrin complex will be reported.

General principle of nuclear magnetic resonance

The atomic nuclei of many elements have magnetic moment because they are charged and because they behave as if they were spinning. We can investigate this magnetic property by studying the way in which these nuclei interact with an externally applied magnetic field, B_0. Typical magnetic nuclei are those of proton(^{1}H), carbon(the ^{13}C isotope only), nitrogen(^{14}N and ^{15}N), oxygen(^{17}O only), fluorine(^{19}F), and phosphorous(^{31}P).Those nuclei that have magnetic properties give rise to n,m,r. signals. The abundant isotopes of carbon and oxygen, ^{12}C and ^{16}O, have zero spin and therefore do not produce n,m,r. signals. As described above, the science of NMR is dominated by studies of proton and carbon-13 NMR, followed in interest and importance by the NMR properties of elements with high abundance and sensitibity, such as fluorine-19 and phosphorous-31. Nitrogen NMR and oxygen NMR studies owe part of their extensiveness to the central chemical and biochemical importance of these elements. Recently, however, transition metal element NMR and lanthanide element NMR have

attracted an much attention not only in chemists but also in biologists. Because when these elements can be substituted into sites of biologically interest, such as metal binding sites of metal binding proteins, these can be used as probes to investigate the surrounding circumstance of these sites. In the present paper, 51vanadium NMR application to transferrin will be reported. The principle, however, is the same as that of proton NMR. Brief description of the principle of proton NMR will be shown.

The nuclei of hydrogen (proton) behave like a bar magnet in an applied magnetic field, and in the manner of compass needles tend to align themselves along the same direction as the field. According to the quantum mechanics, unlike bar magnets and compass needles, however, magnetic nuclei of proton have quantum restriction which result in two different orientations being allowed-either aligned with the field or opposed to the field. These two orientations clearly have different energies, the aligned position being of lower energy than the opposed. The energy difference (ΔE) between the two nuclear configulations corresponds to a particular, precise electromagnetic frequenzy since, by Bohr relationship, $\Delta E=h\nu$ (Fig.5). If a sample containing proton nuclei is placed in a magnetic field, and the sample then irradiated with the corresponding radio frequency ν, some of the lower energy nuclei absorb radiartion and move up to the higher energy state, that is, they undergo a transitionm from being aligned with the field to become opposed to the field, at the same time, some of the higher energy nuclei are stimulated to emit energy, and they change their opposed orientation and become aligned with the field. These transition will only arise when the magnetic energy gap

between the nuclear energy levels is matched exactly with the incoming radiofrequency, that is, when they are "in resonance" and $\Delta E=h\nu$ with the frequency of radiation absorbed during resonance. The frequency characteristic of n,m,r lie in the radiofrequency region of the electromagnetic spectrum. They are low, typically in the range 1-500 MHz, and are therefore associated with transitions between energy levels that are relatively closely spaced. These levels correspond to different magnetic states of atomic nuclei. The separation of resonance frequencies from an arbitrarily chosen reference frequency is termed the chemical shift, and is expressed in terms of the dimensionless units of parts per million (ppm). The mode of detecting is Fourier transform n,m,r. In Fourier transform n,m,r. the radiofrequency field is applied in short powerful pulses, typically of duration about 20us, the bandwidth is sufficiently large to excite all of the resonances. Its main advantage over the continuous-wave method is that it provides a considerable improvement in sensitivity because the resonances are all detected simultaneously rather than one by one as in continuous-wave n,m,r.

The responses are automatically added in a computer until the required signal-to-noise radio is obtained, and then Fourier transformation of the data is performed using this computer to produce the final spectrum.

Experimental

Sample preparation

Chicken ovo-transferrin was purchased from Sigma Chemical Company as an apo-transferrin. Apoovotransferrin was dissolved in 100mM

HEPES buffer(20% D_2O + 80%H_2O) containing 10mM $NaHCO_3$ (pH7.3). 10mM NH_4VO_3 solution was prepared by dissolving NH_4VO_3 in the same buffer. Vanadium-transferrin was prepared with the required ratio of the metal ion to apoovotransferrin solutions. The pH of the vanadium-transferrin solutions was readjusted to 7.3 and incubated for 12 hours at 37 ^{0}C before measurement.

Determination of the Protein Concentration

The protein concentration of the transferrins was determined by the method of BCA Protein Assay(18). Reagents for this method were purchased from Pierce Chemical Company. The principle of the BCA Protein Assay is described as following; Bicinchoninic acid (BCA) shown in Fig.(6), in the form of its water soluble sodium salt, is sensitive, stable and highly specific reagent for the cuprous ion (Cu^{2+}). Macromolecular structure and four peptides(cysteine, cyctine, tryptphan and tyrosine) have been reported to be responsible for color formation in protein samples when assayes with BCA(19). The reaction scheme is shown in Fig.(7). It shows how the BCA Protein Assay combines the well known biuret reaction (protein reducing Cu^{2+} in alkaline medium to produce Cu^{1+}) with the unique features of BCA. The purple reaction product, formed by the interaction of two molecules BCA with one cuprous ion(Cu^{1+}), is water soluble and exhibits a strong absorbance at 562nm. This allows the spectrophotomeric quantitation of protein in aqueous solition.

Before attempting the assay, a set of protein standards of known concentration by the BSA standard solution (bovine serum albumin) was

prepared, in the same dilutent as the transferrin solution. 0.1 ml of each standard and transferrin sample were pipetted into the appropriately labelled test tube. After 2.0 ml of working reagent was added to each tube and mixed well, all tubes were incubated at 37 ℃ for 30 min. After incubation, all tubes were cooled to room temperature and the absorbance at 562 nm of each was measured v.s. water reference. A standard curve was prepared by plotting the net absorbance at 562 nm vs. protein concentration. Using this standard curve, protein concentration of the transferrin solutuion was determined.

SDS-PAGE electrophoresis of transferrin

The transferrin solution was precipitated with 10% trichloroacetic acid after incubation with 0.02 % deoxycholic acid at room temperature. A pellet obtained by centrifugation for 10 min at 15800g was washed by cold acetone containing 1 % hydrochloride. After centrifugation for 10 min at 15800 g, the pellet was dissolved in the SDS solution(22mM NaPi, pH=7.0, 5.5 % SDS, 220μl glycerol/ml, 0.11% dithiothreitol, 0.037%bromphenol blue) and was incubated for 5 min at 90 ℃. Electrophoresis was carried out using Pharmacia Multiphor II system on a ready made acrylamide gel (ExcelGel SDS, Gradient 8-18 for EPH, from Pharmacia Co. Ltd.). After electrophoresis, the gel was stained with coomasie blue solution. The LMW Calibration kit with a molecular weight range of 14400-94000 was used for calibration.

NMR measurement

^{51}V NMR experiment was performed on about 0.4 ml of sample in a tube of outer diameter 5 mm. ^{51}V NMR spectra were measured on a JEOL α -500 NMR spectrometer at 131.4 MHz. The chemical shift data for ^{51}V were recorded relative to $VOCL_3$. All the chemical shifts are expressed in ppm and the sample temperature was 24.8 ^0C.

Results and Discussion

Purity, molecular weight and concentration of the used samples

The result of SDS-PAGE electrophoresis of transferrin is shown in Fig. 8. Used samples were pure enough for ^{51}V NMR measurements. The molecular weight of chicken ovotransferrin was determined to be 77700 Dalton by extraporation from those of standard sample.

By BCA protein assay, the transferrin concentration of the used samples are determined as shown in Table.1.

Table.1

sample	[transferrin]	$[NH_4VO_3]$	$[NH_4VO_3]$ / [transferrin]
A	0.67 mM	0.63mM	0.94
B	0.73 mM	1.3 mM	1.8
C	0.69 mM	2.0 mM	2.9

^{51}V NMR result

Fugures 9,10 and 11 are ^{51}V NMR spectra of the sample A, B and C, respectively. It is said that vanadium ion binds preferably to N-site in chicken ovotransferrin but no clear evidence has been reported.

The spectrum of the sample A has only one signal at - 530 ppm. If vanadium ion dominantly binds to N-site or C-site, the observed single line is expected to come from N-site or C-site because [V] / [transferrin] = 0.9 < 1. The spectrum of the sample B has almost the same signal with stronger intensity as that of sample A. Since the ratio [V] / [transferrin] = 1.8 > 1 (almost saturated) vanadium ions should bind to both binding sites.

The spectrum of the sample C has two closed signals with line width of 280 Hz (-529.78 ppm and -530.15 ppm) and one signal (-555.23 ppm). Since the ratio [V] / [transferrin] = 2.9 > 2, about one third of the vanadium ions should be free. Comparing Fig.12 with Figs 10 and 11, -555.23 ppm signal can easily be asigned to a signal of free vanadium ions. Two closed signals (-529.78 ppm and -530.15 ppm) should correspond to the signals from ^{51}V bound to two iron binding sites. Unfortunately which signal of the two corresponds to that of N-site or C-site can not be determined at this stage, however, it will be possible by combining other metal ions whose binding properties are well investigated. For example, aluminum dominantly binds to N-site in chicken ovotransferrin and it is very easy to prepare ovotransferrin sample with the N-site occupied by aluminum and with the C-site occupied by vanadium.

Conclusion

For investigating the difference and the cooperativity between two iron binding sites in transferrin, NMR of ^{51}V substitutionally bound to the specific iron binding sites in chicken ovotransferrin was studied. From the NMR spectra we found two signals corresponding to the two iron binding sites, which are expected to become a high sensitive tool to monitor binding of vanadium to ovotransferrin.

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References

1) A. Brown-Mason, S.A.Brown, N.D.Butcher, R.C.Woodworth, Biochem. J. 235, 103 (1987).

2) A.B.Mason, S.A. Brown, W.R.Church, J. Biol. Chem., 262, 9011 (1987).

3)N. Kojima, G.W.Bates, J. Biol. Chem., 254, 8847 (1979).

4) J.W.Donovan, K.D.Ross, J. Biol. Chem., 250, 6022(1975).

5) T.Uemura, Kagaku to Kogyo, 42(4), 111 (1989) , in Japanese.

6) T.Yamamura, K.Ichimura, T.Tsuda, A.Hayashi, T.Taniguchi, R.Toi, H.Kihara, K.Satake, Nippon Kagaku Kaishi 452 (1988).

7) T.Uemura, T.Yamamura, presented at the 10th International Biophysics Congress, Vancouver, Canada, August (1990).

8) T.Uemura, T.Yamamura, presented at the International Conference, Rare Earths '92, Kyoto, June (1992).

9) T.Uemura, T.Yamamura, J. Alloys and Compounds, 193, 216 (1993).

10) T.Uemura, T. Yamamura, Nihon Kagaku Kaishi, 5, 113 (1993), in Japanese.

11) N.Sasaki, H.Honda, H.Yajima, H.Momomi, H.Ichimura, K.Satake, R.Endo, Nippon Kagaku Kaishi, 185 (1990).

12) N.D.Chastem, Struct. Binding, 53, 107 (1983).

13) D.W.Boyd, K.Kustin, Adv. Inorg. Biochem., 6,311 (1984).

14)M.J.Gresser, A.S. Tracey, J. Am. Chem. Soc., 107,4215 (1985).

15) M.A.Habayek, O.E.Hileman, Jr, Can. J. Chem., 58, 2255 (1980).

16) E. Heath, O.W. Howarth, J. Chem. Soc., Dalton Trans., 1105 (1981).

17) D.Rehder, Bull. Magn. Reson., 4, 33 (1982).

18) P.K.Smith, R.I.Krohn, G.T.Hermanson, A.K.Mallia, F.H.Gartner, M.D.

Provenzano, E.K.Fujimoto, N.M.Goeke, B.J.Olson, D.C.Klenk, Anal. Biochem., 150, 76 (1985).

19) K.Wiechelman, R.Braun, J.Fizpatrick, Anal. Biochem., 175, 231 (1988).

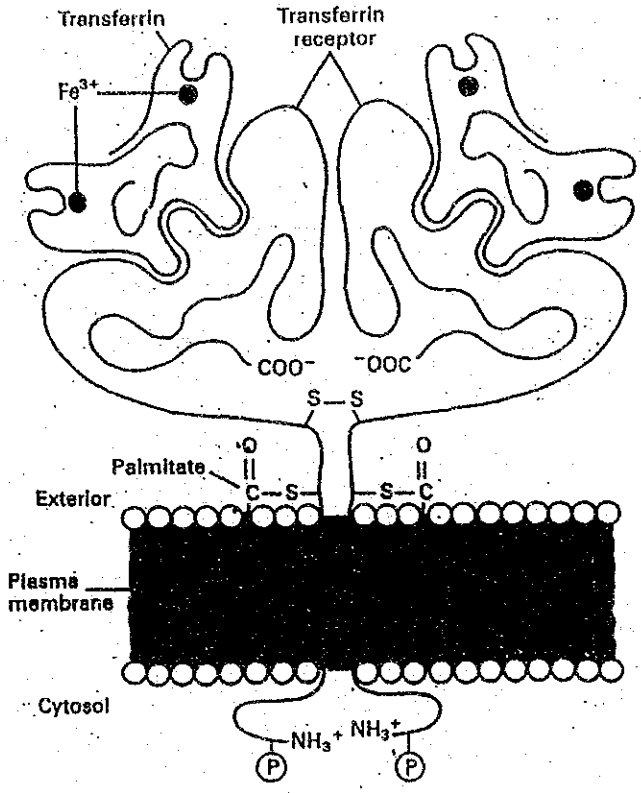

Fig.1 Structure of the transferrin receptor as deduced from the sequence from the cloned cDNA.

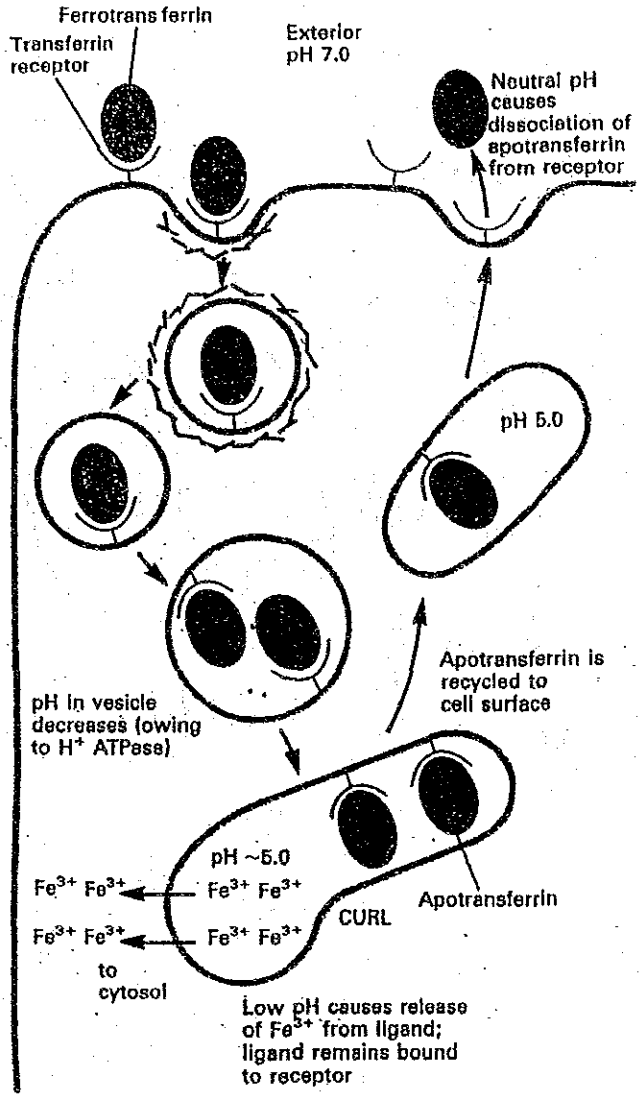

Fig.2 The transferrin cycle.

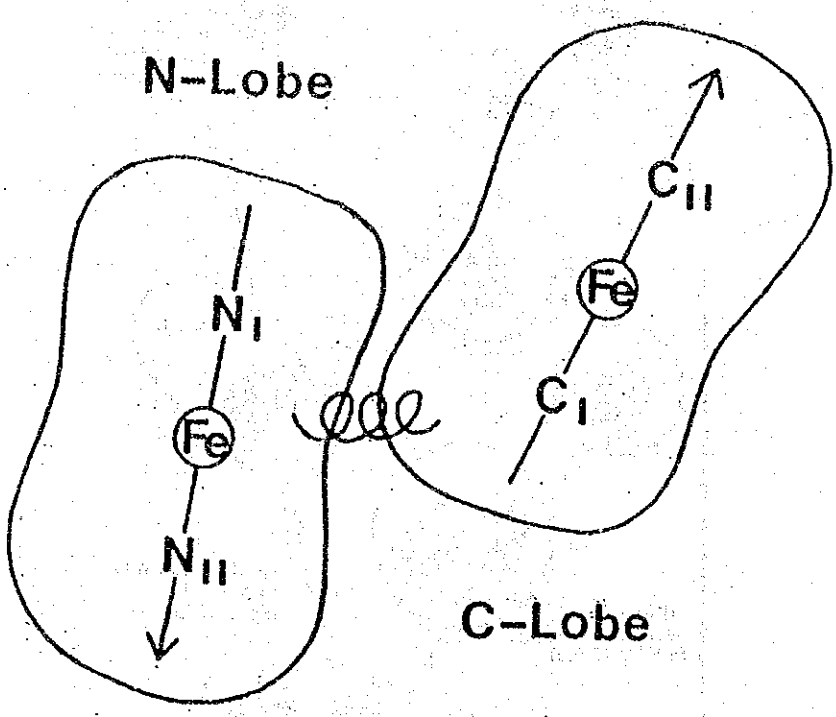

Fig.3 Schematic representation of two domains in transferrin.

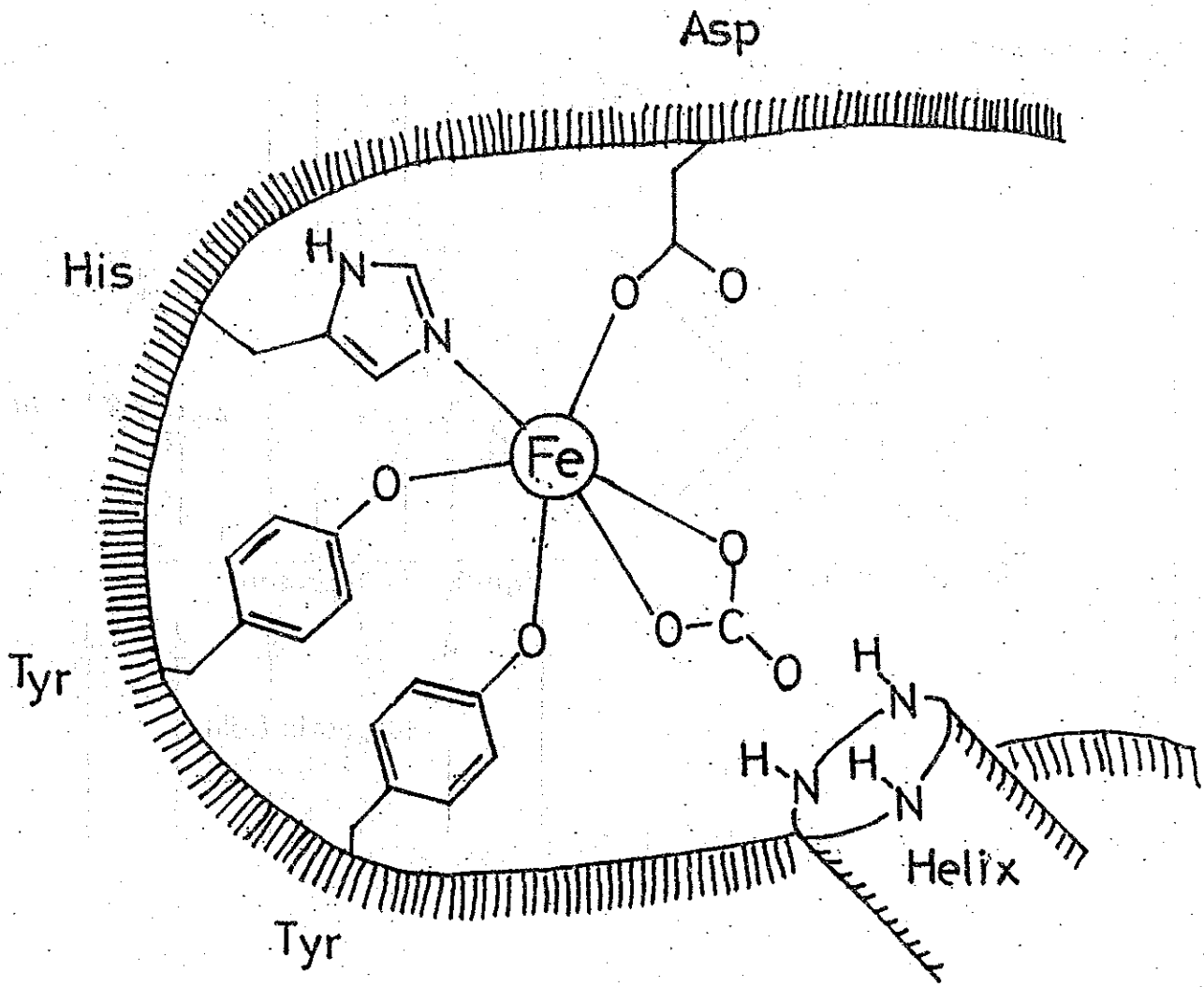

Fig.4 Schematic representation of the iron binding site in the N-domain of human lactotransferrin.

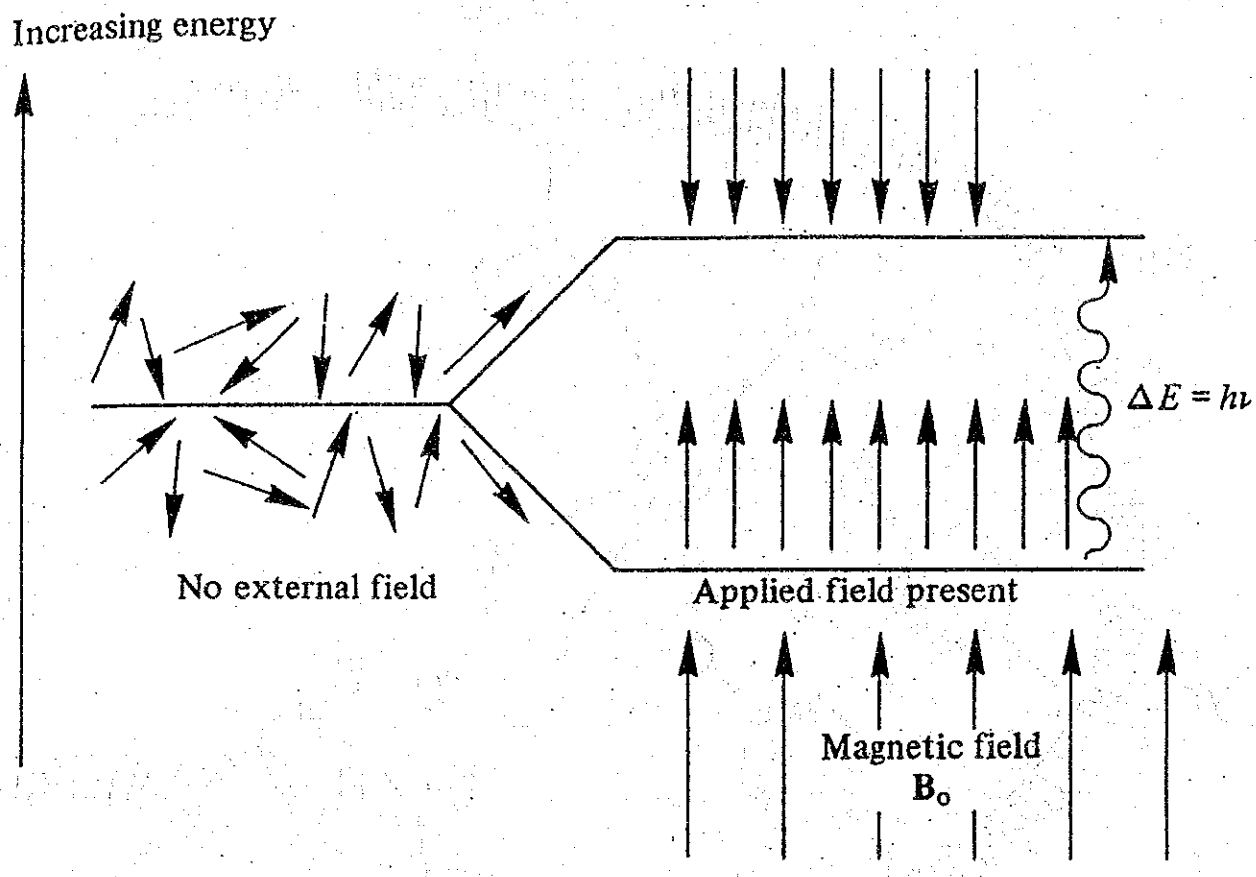

Fig.5 In the absence of B_0, the magnetic nuclei all have the same energy. When B_0 is applied, the aligned and opposed orientations correspond to different energies, the energy difference,ΔE, having the dimension of hν.

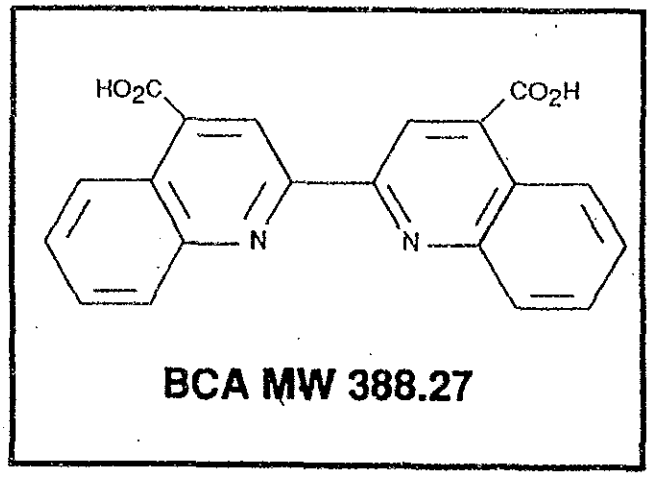

Fig.6 Molecular structure of BCA.

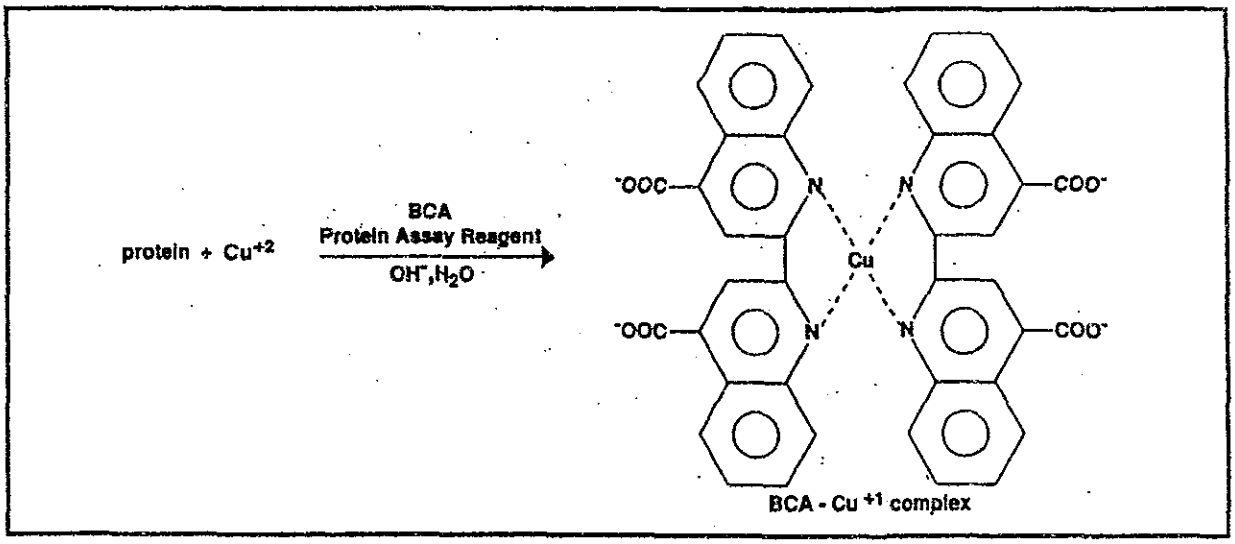

Fig.7 The reaction scheme of the BCA protein assay.

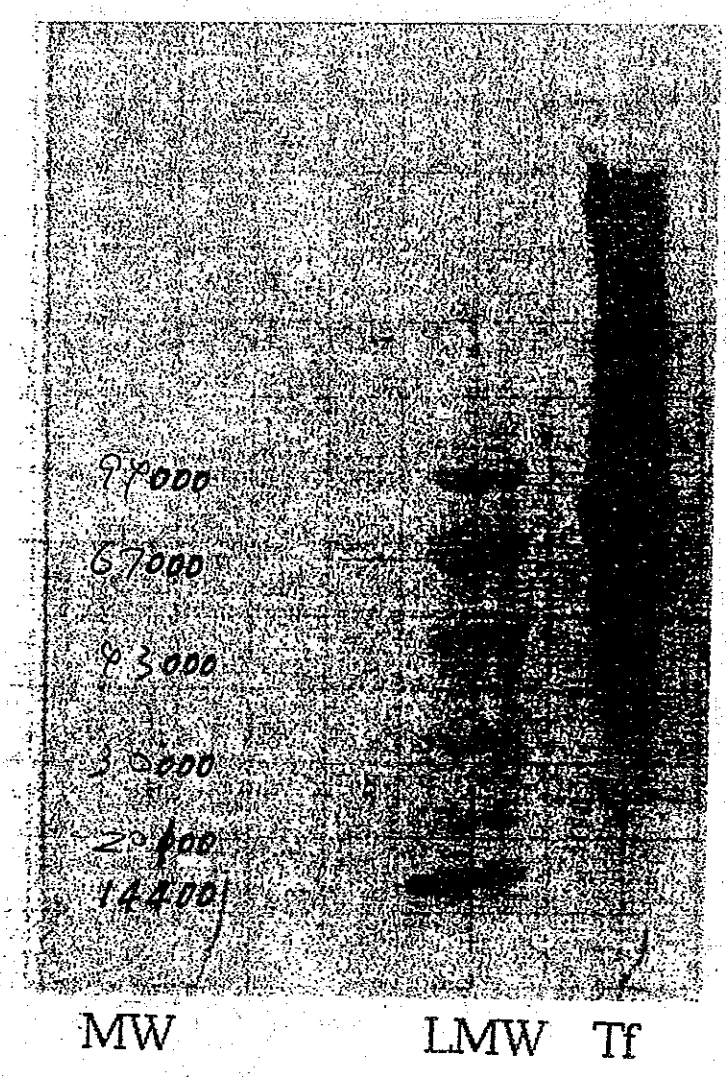

Fig.8 SDS-PAGE of chicken ovotransferrin.

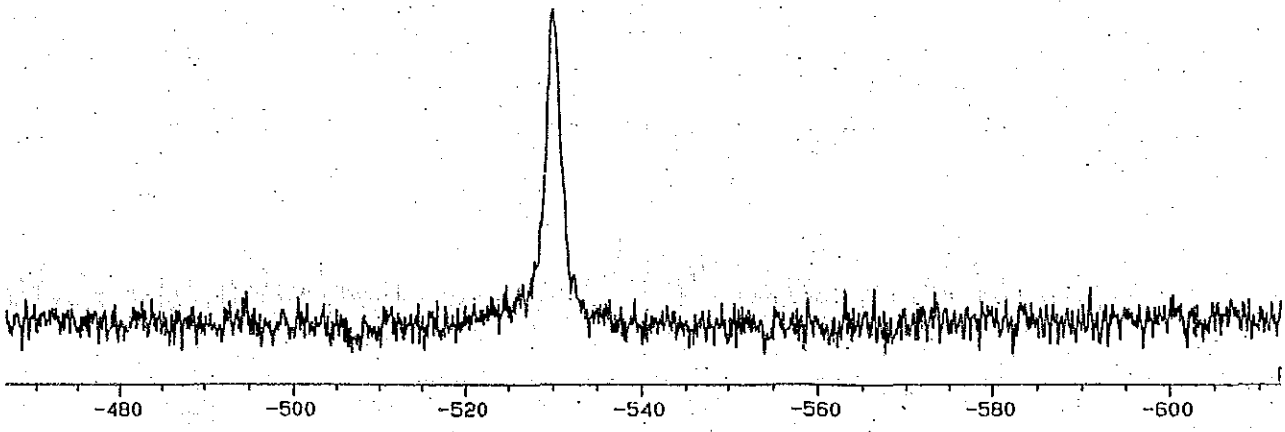

Fig.9 ^{51}V NMR spectrum of sample A.

$[NH_4VO_3]/[\text{transferrin}] = 0.94$

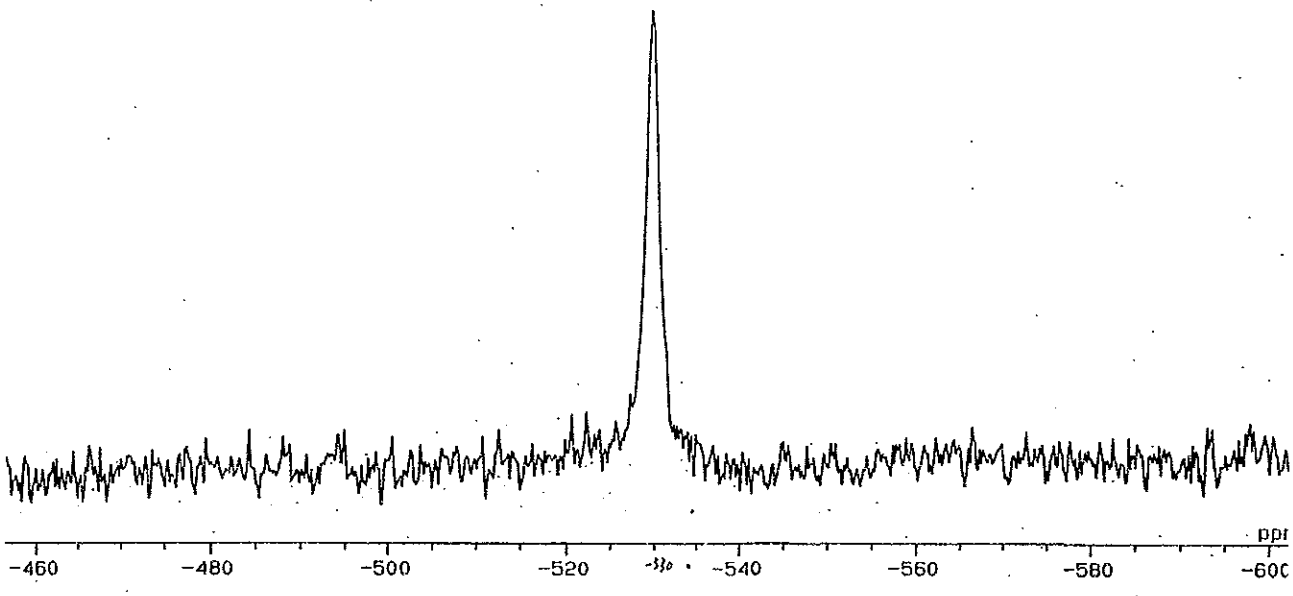

Fig.10 ^{51}V NMR spectrum of sample B.

$[NH_4VO_3]$ / [transferrin] = 1.8

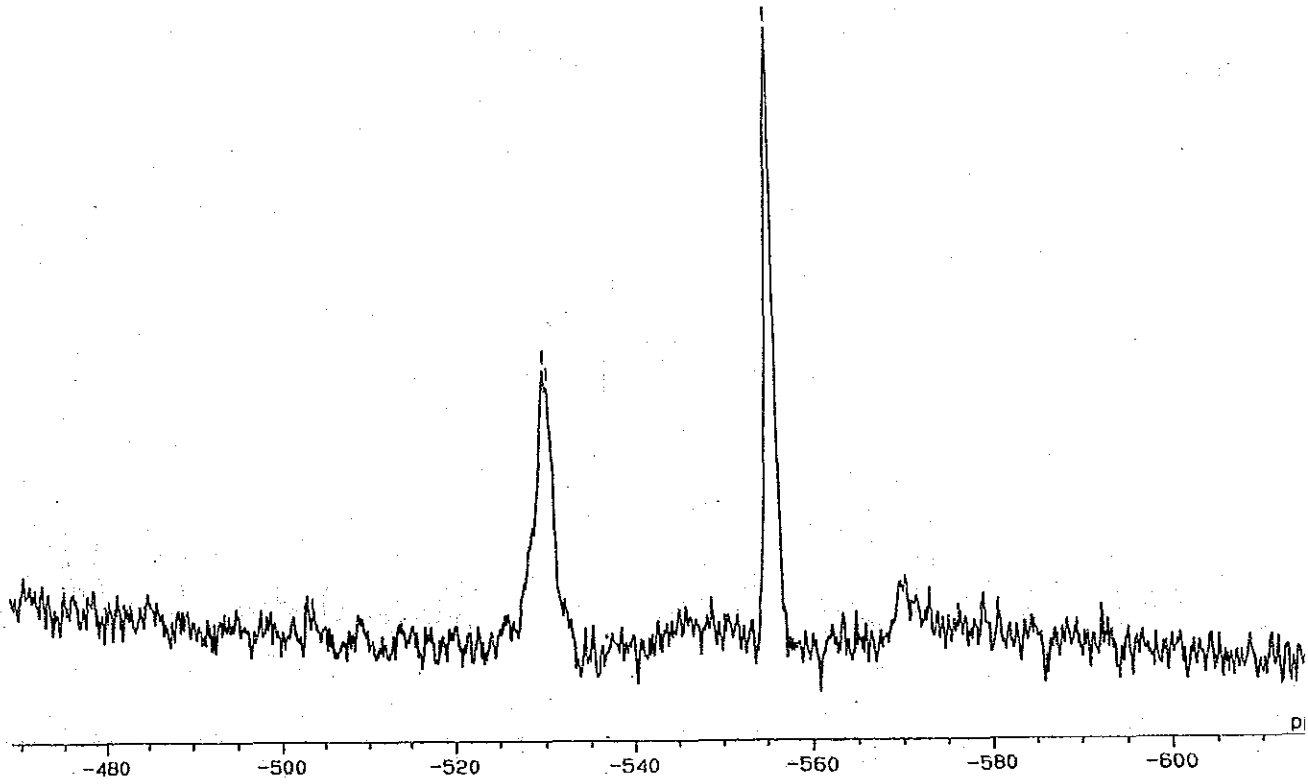

Fig.11 ^{51}V NMR spectrum of sample C.

$[NH_4VO_3]$ / [transferrin] = 2.9

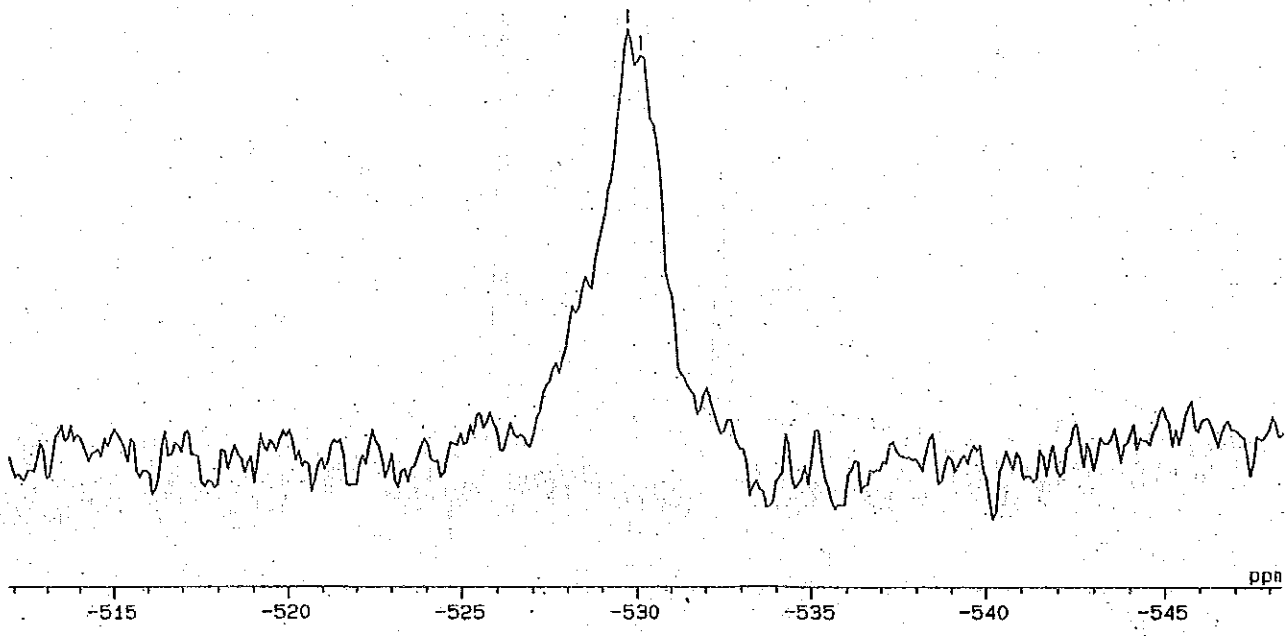

Fig.12 ^{51}V NMR spectrum of sample C with different scale of chemical shift from that of Fig.11.

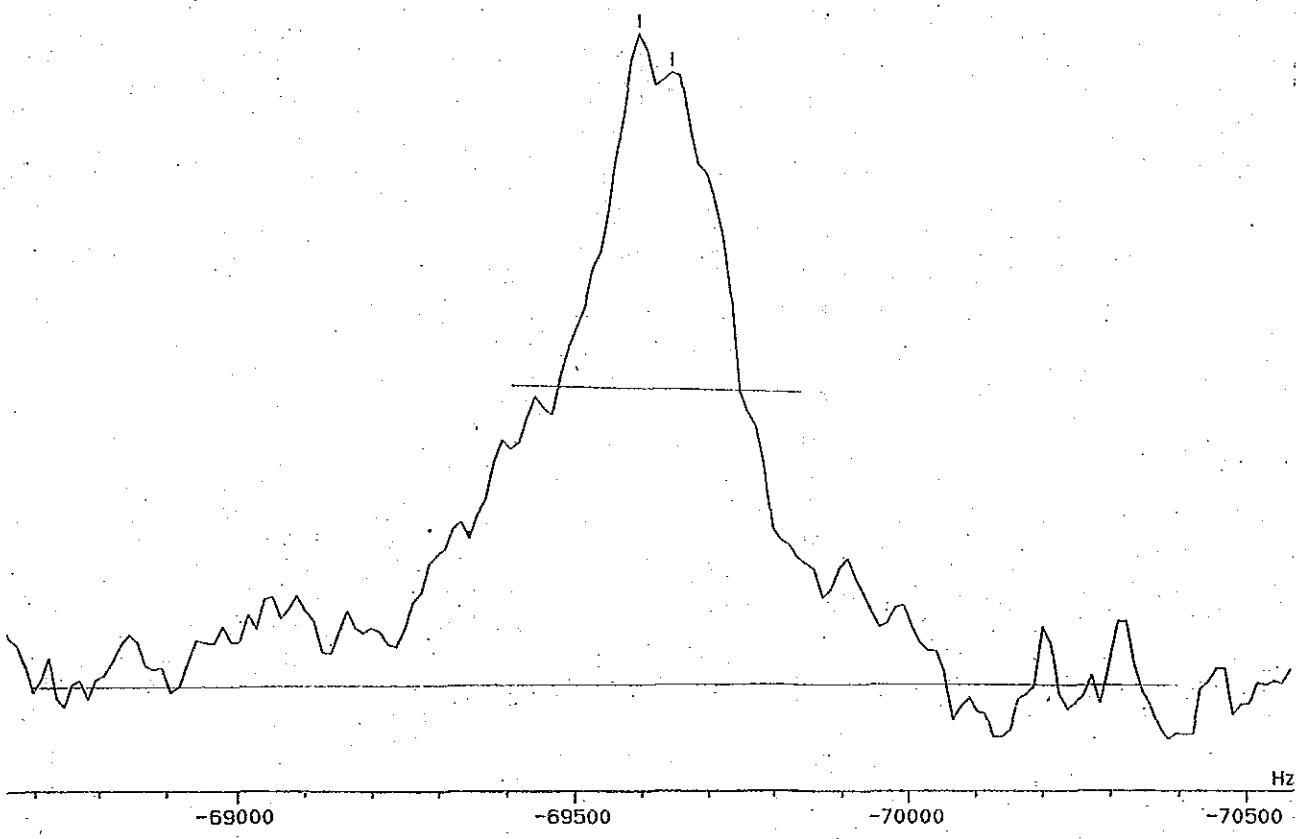

Fig 13.

Synthesis of Platinum GREEN and BLUE Complexes with 1- Methyluracil and Biological Activity

BY

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Japan International Cooperation Agency
JICA

JAPAN
1993

1－メチルウラシルを配位子とする白金グリーン、ブルー錯体の合成と生理活性

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要旨

1－メチルウラシル（1-MUH）を配位子とした白金モノマー錯体、ダイマー錯体、白金グリーン錯体を合成した。グリーン錯体は緑色針状結晶であった。その結晶のX線解析結果から、$[(NH_3)_8Pt_4(1\text{-}MU)_4](NO_3)_5 \cdot 5H_2O$の構造を有する4核錯体であり、従来ブルー錯体として知られていたものと同一物であることが判明した。白金グリーンと同じ反応液からブルー錯体を新たに得た。この錯体をゲル濾過などの方法で精製したが、X線構造解析に必要な単結晶は得られなかった。錯体中の炭素：白金：NO_3の比率はグリーン錯体とブルー錯体とで同一であったが、ブルーの方がわずかに分子量が大きい事を示す結果が得られた。

グリーン錯体は279, 480, 740 nmに、ブルー錯体は268, 680 nmに吸収極大を有した。モノマー、ダイマー錯体は紫外部にのみ吸収を有し、それぞれ268, 279 nmであった。

ダイマー錯体とグリーン錯体（テトラマー）の間には平衡関係があり、pHが低いとグリーンが、中性付近ではダイマーが安定な事が示唆された。

ここで得られた4種類の白金錯体の抗腫瘍活性を数種類の組織培養細胞をつかって測定したところ、全ての細胞に対して、ダイマーとグリーン（テトラマー）は高活性を示したが、ブルーは低活性であり、さらにモノマーは活性がないという結果が得られた。

活性と構造との関係は目下検討中であるが、高活性な錯体と低活性な錯体とでは紫外領域の吸収位置が若干異なる（279, 268 nm）こと、配位子のH_6のケミカルシフトが異なる（7.42, 7.6 ppm）結果が得られた。

Abstract:

Here we reported two-step and one-pot synthesis of the tetranuclear platinum Green complex with 1-Methyluracil in the formula $[(NH_3)_8Pt_4(1\text{-}MU)_4](NO_3)_5.5H_2O$, which was reported before in the literature as Blue. From the same reaction mixture, after obtaining the green complex, we could obtain blue complex and purified by gel chromatography, but no single crystal was obtained for X-Ray study. The ratio of C : Pt : nitrate was obtained and also the molecular weight was determinated by gel chromatography calibrated with Poly Ethylene Glycol. It was found that the Blue complex has the same ratio of C : Pt : nitrate as in the case of Green and also molecular weight little more than that of green one, which means that the blue has oligomeric structure of the tetranuclear green.

The bioassay study showed that the complexes of dinuclear and tetranuclear green gave higher antitumor activity, but the mononuclear and blue complexes have much lower activity compared with them, against several types of cancer cells.

The visible spectra showed λ_{max} of green at 740 and 480 nm and of blue at 680 nm . The spectra in U.V. region of monomer and blue (less active) showed a similar λ max at 268 nm, and also the dinuclear and green (more active) gave the maxima at 279 nm. In the nmr, the chemical shift of H_6, in 1-MU, is the same for the less active complexes at 7.60 PPM, and the same for the more active complexes at 7.42 PPM.

The colorless solution of the dinuclear complex turned green color of the tetranuclear complex upon acidification, and the room temperature was the optimum practical temperature for this conversion.

Introduction

The mononuclear platinum complex CDDP, (cis-platin, cis-DDP)(1), Figure 1, exhibits strong antitumor activity particularly against ovarian, gastric and prostate cancers[1], but also it possesses inherent serious problems of nephrotoxicity and other side effects[2].

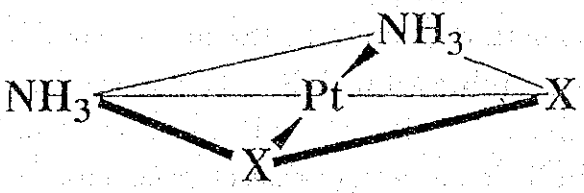
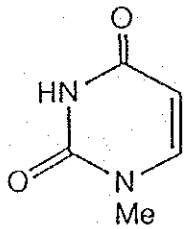


Figure 1 CDDP x=I cis-diiododiammine platinum (II)
x=Cl cis-dichlorodiammine platinum (II)

The platinum pyrimidine complexes were reported as anticancer since they have high antitumor activity with reducing the undesirable effects[3]. It was reported in the literature that the tetranuclear platinum complex with 1-Methyluracil (1-MUH) (2), Figure 2, is blue in color with the characteristic visible spectra maxima at 740, 520, and 480 nm[4,5]. The trinuclear mixed Pt_2Pd complexes containing bridging 1-methyluracilato and 1-methylminato ligands (L) with different amine ligands (A) (NH_3, ethylenediamine), and different counter ions (NO_3^-, CLO_4^-) have been synthesized and characterized as blue complexes with the general formula cis-$[(A)_2Pt(L)_2Pd(L)_2Pt(A)_2]^{3+}$ [6,7] .



1-Methyluracil

Figure 2 (1MeUH) (2)

Recent work from our laboratory showed for the first time an efficient and reproducible preparation of platinum pyrimidine blues through air oxidation under heating[8], and successful purification by gel-filtration. Oxidation reactions with a controlled amount of H_2O_2 as oxidizing agent under nitrogen yielded, green chelates[9], and the bioassay results showed that the green complexes are active as anticancer compounds and that the blue complexes are less active compared with the green complexes[10].

In this work our aim is the synthesis of the platinum green complex with 1-Methyluracil, since we expect that it has higher antitumor activity, and so with determination its structure and study of its bioassay with the comparison with other platinum complexes of 1-Methyluracil.

Experimental

Analytical instruments:

1- HPLC with O.D.S column using JASCO 870-UV detector
HPLC with gel column using RI detector R403
HPLC with Shoden ICI-613 column using JASCO 880-PU ion detector

2- IR spectra recorded on JASCO FT/IR-7300, KBr pellet.

3- ^{1}H-NMR spectra recorded on BRUKER spectrophotometer in D_2O with [3-(Trimethylsilyl)propan] sulphonic acid Na salt as an internal standard.

4-UV and visible spectra recorded on a HITACHI U-3200 spectrophotometer

5- Atomic Absorption, HITACHI 170-70 Zeeman-Effect, Spectrophotometer.

Materials:

- 1-Methyluracil(1-MUH) was prepared as reported[11,12] using uracil and MeI
- cis platin, cis-diiododiammine platinum (II) from Nippon Engelhard
- All other materials and reagents are analytical grade.

-The mononuclear platinum complex with 1-Methyluracil was prepared as reported previously[13] by the reaction of the aquated complex cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, 1 mmol, (3) prepared in situ from cis-$Pt(NH_3)_2I_2$ with $AgNO_3$[14], with 1-MUH, 3 mmol, and NaOH, 2 mmol, in water at pH 8 and 60°C for 88h. The product has the formula $[Pt(NH_3)_2(1\text{-}MU)_2]_2 .4H_2O$ (4). Also, the dinuclear derivative $[Pt(NH_3)_2(1\text{-}MU)]_2.(NO_3)_2.H_2O$ (5) was prepared as repoted previously[15,16].

Synthesis of the platinum Green complex with 1-Methyluracil:

This reaction was carried out in two-step and one-pot reactions and the product were typically the same.

Two-step reaction;

Step 1

A solution of cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ (3) (1 mmol-0.389 g) in 30 ml water was prepared by the reaction of cis-$Pt(NH_3)_2I_2$ (1) (1 mmol-0.483 g) with $AgNO_3$ (2 mmol-0.340 g) at 60°C for 1h, filtered off the silver iodide and a colorless solution of the aquated complex (3) was obtained.

Step 2

To the solution of the aquated complex, from step 1, 1 mmol-0.551 g of the mononuclear platinum complex (4) was added, and the reaction mixture was stirred at 60°C, pH 3.2 for 24 h, blue-green color of the reaction mixture was obtained. In the room temperature, acidified to pH 1 using 7N HNO_3 with addition of 1 g $NaNO_3$, allowed to standing at 5°C, for 2h, filtered, green-yellow crystals of the dinuclear platinum complex were obtained, gave bright yellow crystals of the dinuclear complex (H-H) $[Pt_2(NH_3)_2(1\text{-}MU)_2](NO_3)_2.H_2O$ (5) which reported previously[15,16].

To the filtrate, 20 drops of a 7M HNO_3 solution and 3g of $NaNO_3$ were added and allowed to standing at 5°C over night, filtered, dark green needles were obtained , with blue filtrate, dried over P_2O_5 at 40°C. The yield was 20% of the tetranuclear platinum green complex with 1-Methyluracil $[Pt(NH_3)_2(1\text{-}MU)]_4(NO_3)_5.5H_2O$ (6)

One-pot reaction

Synthesis of the tetranuclear platinum complex green by one-pot reaction gave the same product with nearly the same yield of that produced in two-step reaction. This reaction was carried out by adding the mononuclear platinum complex (4)(mmol-0.551 g) to CDDP (1) (1 mmol-0.483 g) and $AgNO_3$ (2 mmol-0.340 g) in 30ml water , stirred at 60°C for 24h, filtered off AgI and blue-green solution was obtained, treated similarly to that in case of two-step reaction.

The dark green needles gave absorption maxima at 740 and 480 nm in the visible region and at 279 nm in UV region in a 0.01M HNO_3 solution.

- IR spectra; 1634, 1586, 1522, and 1485 cm^{-1}.
- ^1H-NMR (D_2O); δ=3.36 (s,12H,CH3), 5.87(d, 4H, H5) and 7.41(d, 4H, H6).
- Elemental analysis;

The sample which submitted to the elemental analysis was found that it has $8H_2O$ molecules, so the calculated values for the formula with $8H_2O$;

calc. % C 12.83 , H 3.23, N 15.72, Pt 41.70.

found % C 12.50, H 2.96, N 15.73. Pt 41.14 (determinated from ash).

- X-Ray data; a dark green needle was examined and the data of the X-ray diffraction studies are shown in Tables 1, 2, and 3.
- Ion chromatography indicated that ; nitrate % = 17.26 which is equivalent to $-(NO_3)_5$ of the formula (6).

Table 1 The crystal parameters for Green (6) obtained from the X-ray diffraction studies

a= 10.120Å,	α= 92.24,	V= 1409.55 Å^3
b=13.087Å,	β= 101.15,	
c=19.529Å,	γ= 107.34	

Table 2 Selected interatomic distances (Å) and angles (deg.) for Green (6)

ATOM 1	ATOM 2	SYM	DIST
PT1	-PT2	(1)	2.8167
PT1	-N1	(1)	2.1118
PT2	-PT3	(1)	2.8636
PT2	-N3	(1)	2.0963
PT3	-PT4	(1)	2.7997
PT3	-O33	(1)	1.7492
PT4	-N7	(1)	2.0927
PT4	-N41	(1)	2.0339
NO6	-O6C	(1)	1.1537
N11	-PT1	(1)	1.9962
C12	-N11	(1)	1.6628
PT1	-N21	(1)	2.1081
PT2	-PT1	(1)	2.8167
PT2	-O23	(1)	2.0214
PT3	-PT2	(1)	2.8636
PT3	-N6	(1)	1.6510
PT4	-PT3	(1)	2.7997
PT4	-N31	(1)	2.0330
NO6	-O6B	(1)	1.2275
NO5	-O5C	(1)	1.3900
N11	-C18	(1)	1.2027
C12	-C14	(1)	1.4389
PT1	-N11	(1)	1.9962
PT1	-N2	(1)	2.0764
PT2	-O13	(1)	1.9262
PT2	-N4	(1)	1.8916
PT3	-N5	(1)	1.9022
PT3	-O43	(1)	1.9409
PT4	-N8	(1)	2.1136
NO6	-O6A	(1)	0.9455
NO5	-O5B	(1)	1.1058
N11	-C12	(1)	1.6628
C12	-O13	(1)	1.1767

ATOM 2(SM)	-ATOM 1	-ATOM 3(SM)	ANGLE	SIG(ANG)
PT2 (1)	-PT1	-N11 (1)	88.67	1.50
PT2 (1)	-PT1	-N1 (1)	99.35	1.48
N11 (1)	-PT1	-N21 (1)	90.34	2.07
N11 (1)	-PT1	-N2 (1)	176.87	2.16
N21 (1)	-PT1	-N2 (1)	89.53	2.19
PT1 (1)	-PT2	-PT3 (1)	165.08	0.16
PT1 (1)	-PT2	-O23 (1)	83.30	1.28
PT1 (1)	-PT2	-N4 (1)	102.03	1.33
PT3 (1)	-PT2	-O23 (1)	86.97	1.34
PT3 (1)	-PT2	-N4 (1)	88.90	1.40
O13 (1)	-PT2	-N3 (1)	86.35	2.11
O23 (1)	-PT2	-N3 (1)	176.68	2.56
N3 (1)	-PT2	-N4 (1)	93.66	2.27
PT2 (1)	-PT3	-N5 (1)	93.38	1.99
PT2 (1)	-PT3	-O33 (1)	92.02	1.46
PT4 (1)	-PT3	-N5 (1)	97.70	1.94
PT4 (1)	-PT3	-O33 (1)	77.28	1.38
N5 (1)	-PT3	-N6 (1)	87.95	2.79
N5 (1)	-PT3	-O43 (1)	177.48	2.23
N6 (1)	-PT3	-O43 (1)	90.70	2.43
PT3 (1)	-PT4	-N7 (1)	99.39	1.47
PT3 (1)	-PT4	-N31 (1)	86.69	1.87
N7 (1)	-PT4	-N8 (1)	91.90	2.48
N7 (1)	-PT4	-N41 (1)	175.79	1.81
N8 (1)	-PT4	-N41 (1)	90.17	2.35
O6A (1)	-NO6	-O6B (1)	121.29	14.44
O6B (1)	-NO6	-O6C (1)	66.55	9.41
PT1 (1)	-N11	-C12 (1)	113.03	2.85
C12 (1)	-N11	-C18 (1)	118.22	5.38
N11 (1)	-C12	-C14 (1)	107.95	4.55
PT2 (1)	-O13	-C12 (1)	135.45	4.40
C14 (1)	-C15	-N15 (1)	118.44	5.97
C15 (1)	-N15	-C18 (1)	117.64	5.10
N11 (1)	-C18	-N15 (1)	129.15	7.41
N15 (1)	-C18	-O19 (1)	116.10	5.77

Table 3. Positional parameters for Green (6)

N	ATOM	X	Y	Z	N	ATOM	X	Y	Z
1	PT1	0.9855	0.6001	0.7768	40	C38	0.7134	-0.1279	0.5855
2	PT2	0.9364	0.3762	0.7603	41	O39	0.7224	-0.2116	0.6071
3	PT3	0.9650	0.1654	0.7483	42	N41	0.7555	-0.0702	0.7715
4	PT4	0.9162	-0.0558	0.7204	43	C42	0.7152	0.0184	0.7984
5	N06	1.0000	0.5000	1.0000	44	O43	0.7900	0.1216	0.7818
6	N05	0.5088	0.5191	0.5355	45	C44	0.5915	-0.0054	0.8240
7	N11	1.1377	0.6100	0.8612	46	C45	0.5246	-0.0995	0.8318
8	C12	1.1630	0.4923	0.8760	47	N46	0.5654	-0.1880	0.8126
9	O13	1.0985	0.4144	0.8375	48	C47	0.4866	-0.3062	0.8215
10	C14	1.2792	0.5104	0.9354	49	C48	0.6913	-0.1651	0.7832
11	C15	1.3417	0.5975	0.9781	50	O49	0.7309	-0.2400	0.7664
12	N15	1.3061	0.6940	0.9613	51	N01	1.4644	0.1382	0.6998
13	C17	1.3750	0.7922	1.0085	52	O1A	1.5118	0.2067	0.7525
14	C18	1.2035	0.6865	0.9032	53	O1B	1.5377	0.1173	0.6637
15	O19	1.1647	0.7673	0.8943	54	O1C	1.3369	0.0863	0.6963
16	N21	1.1278	0.6001	0.7110	55	N02	1.4520	0.3865	0.8341
17	C22	1.1305	0.5017	0.6751	56	O2A	1.3980	0.3251	0.8704
18	O23	1.0617	0.4141	0.6900	57	O2B	1.3861	0.4080	0.7772
19	C24	1.2335	0.5232	0.6314	58	O2C	1.5887	0.4337	0.8503
20	C25	1.3044	0.6183	0.6211	59	N03	0.7569	-0.1551	0.9720
21	N26	1.2889	0.7074	0.6527	60	O3A	0.8518	-0.1607	0.9291
22	C27	1.3694	0.8204	0.6364	61	O3B	0.7227	-0.2565	0.9885
23	C28	1.1960	0.6982	0.6971	62	O3C	0.7685	-0.0699	1.0019
24	O29	1.1804	0.7776	0.7275	63	N04	0.9595	-0.1815	0.4938
25	N1	0.8489	0.6058	0.8451	64	O4A	1.0924	0.2755	0.5584
26	N2	0.8310	0.5988	0.6894	65	O4B	1.0700	0.1004	0.5463
27	N3	0.8135	0.3311	0.8355	66	O4C	1.0094	-0.2242	0.5503
28	N4	0.7782	0.3308	0.6839	67	O5A	0.5458	0.5225	0.4446
29	N5	1.1384	0.2143	0.7175	68	O5B	0.6093	0.5027	0.5563
30	N6	1.0581	0.1992	0.8297	69	O5C	0.6073	0.5090	0.4971
31	N7	1.0720	-0.0511	0.6630	70	O6A	0.9629	0.5486	0.9742
32	N8	1.0493	-0.0723	0.8142	71	O6B	0.9728	0.4061	0.9762
33	N31	0.7873	-0.0470	0.6285	72	O6C	0.9102	0.4746	0.9503
34	C32	0.7930	0.0670	0.6094	73	OW1	0.5691	0.4548	0.6782
35	O33	0.8665	0.1378	0.6619	74	OW2	0.8321	0.3468	0.5426
36	C34	0.7237	0.0649	0.5417	75	OW3	0.3090	0.0828	0.8690
37	C35	0.6528	-0.0246	0.4955	76	OW4	0.1187	0.4253	0.4491
38	N36	0.6506	-0.1255	0.5213	77	OW5	0.9005	0.1282	0.9503
39	C37	0.5756	-0.2231	0.4719					

The platinum Blue complex with 1-Methylureacil

The blue filtrate was concentrated to about 5 ml under vacuum at 60°C and large amount (200 ml) of acetone was added to the concentrated solution, allowed to standing for 2h, a blue material was collected by filtration contains most of the contents of the reaction mixture, which was purified by gel-chromatography (see purification of the blue material) over Toypearl HW-40S, followed by concentration and reprecipitation from water-acetone, filtered, dried over P_2O_5 at 40°C with yield about 10% of the blue complex with the ratio of Pt: 1MU: NO_3 is 4: 4: 5 (7), the molecular weight was determinated by gel-chromatography and it was found to be near to that of the green with a little more .

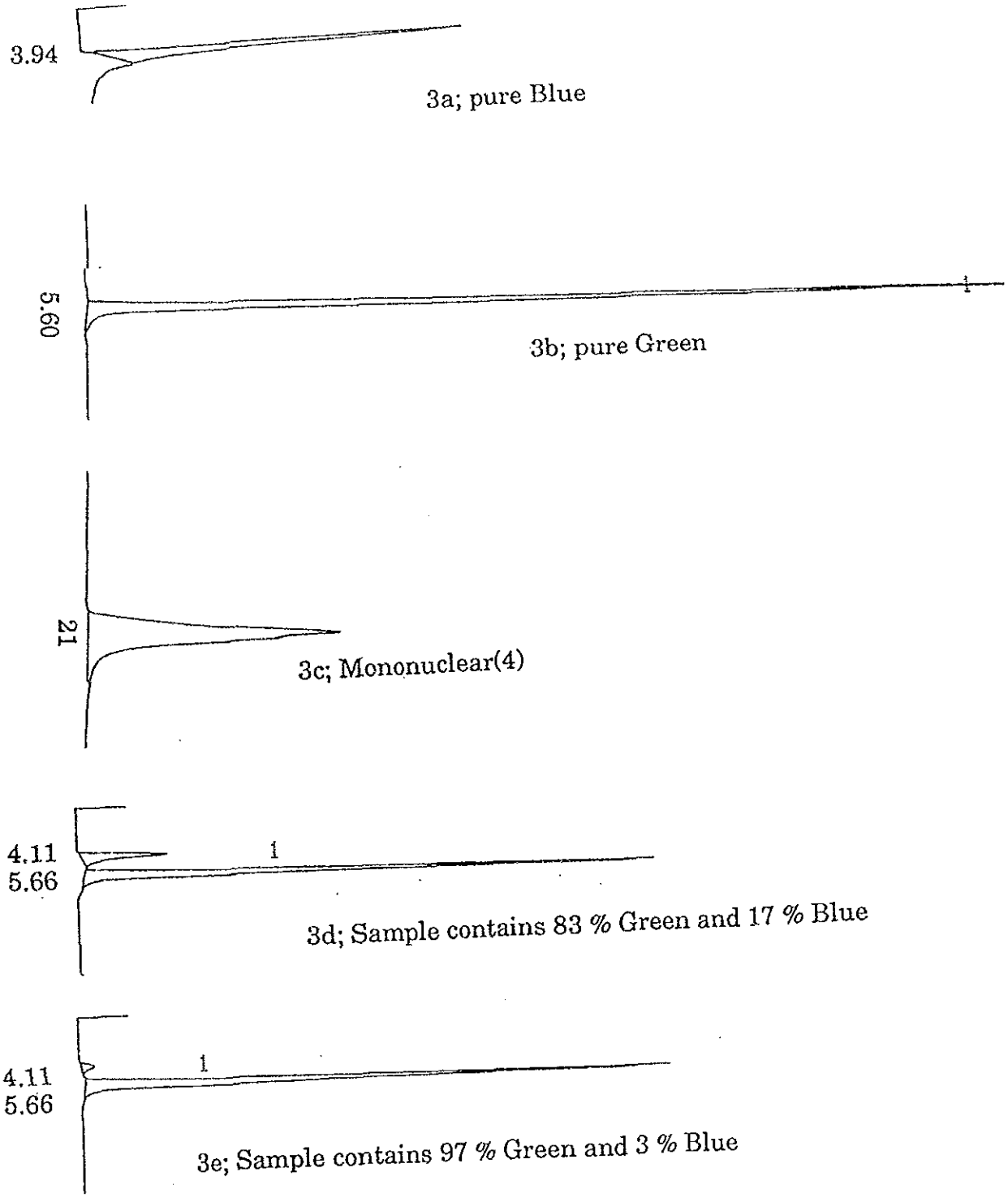
Purification of the blue material:

Addition of acetone to the blue materials gave large amount of the contents and this amount was submitted to gel-column chromatography (a 20mm x500mm column packed with Toyoperal HW-40S) using a 1 mM HNO_3 solution as an eluant. The purity of the collected blue fraction was evaluated by using HPLC with O.D.S column (octadecylsilane) which gave only one peak on the characteristic retention time, Figure (3a).

It was suitable to use O.D.S column chromatography for the evaluation of purity of blue and green complexes, since it was able to distinguish between them even some contaminates of each other or contaminates of the mononuclear (4) and this shown by some examples in figures (3b-e).

Figures 3

The O.D.S chromatography of pure blue, green , mononuclear, and some samples contain both green and blue with deferent ratio as indication for its efficiency.



The purified blue fraction gave absorption maxima at 680 nm in the visible region and at 270 nm in the UV region in 0.01M HNO_3

- IR spectra; 1635, 1526, 1486 cm^{-1}.
- ^1H-NMR(D_2O); δ=3.36 (s,12H,CH3), 5.78 (d, 4H, H5) and 7.60 (d, 4H, H6).

- The ratio of platinum : 1-MU : NO_3;

The ratio of Pt : 1-MU : NO_3 is 4 : 4 : 5 .

C % was determinated by " Total organic carbon analyzer" calibrated with phthalic acid, and from which the amount of 1MU was calculated.

Pt % was determinated by the atomic absorption spectrophotometer; at 265 nm with the calibration using standared platinum solutions in different concentrations.

The amount of nitrate was determined by the gel-chromatography with the green as a reference.

Determination of the molecular weight by gel-chromatography:

Gel- column chromatography was used for determination of the molecular weight of the blue complex [Toyosoda G 3000SW, 7.5 mm x 60cm, using solution of 1 mM HNO_3+ 0.1 M KCl as an eluant] which was calibrated with standared P.E.G. in different molecular weights, from the relation between log MW and time, the molecular weight of both green and blue was obtained from the calibration graph, Figure 4. MW of blue was found to be $1940^{+}150$, and of green $1750^{+}150$, which means that the molecular size of both is nearly the same with a little large in case of blue.

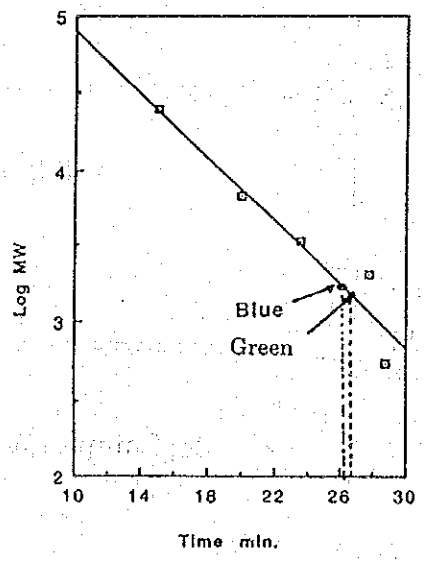


Figure 4. Gel chromatography graph
Calibration of Toyosoda G 3000SW (7.5 mmx60 cm) with P.E.G. (Poly Ethylene Glycol) in different MW using 1 mM HNO_3+0.1 M KCl as an eluant with a 0.8 ml/min flow rate.

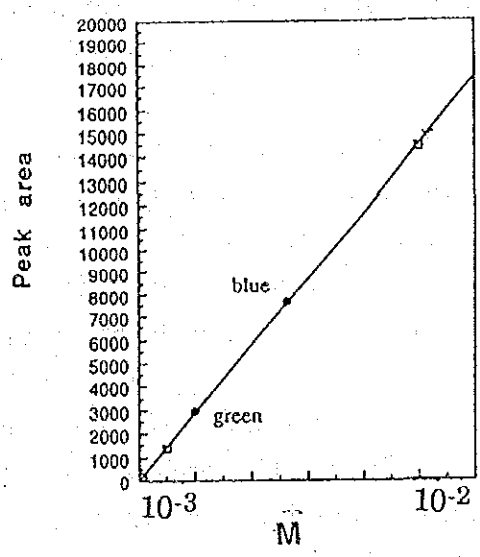
The Ion chromatography:

Ion chromatography using a Shodex ICI-613 column and KNO_3 as a standard solution, with different concentrations, 10^{-2}, 10^{-3}, 10^{-4} M, showed successive quantitative technique for the nitrate anion in both green and blue. From the calibration graph, Figure (5), using KNO_3; the amount of the nitrate could be determined by plotting its peak area against the corresponding concentration as a linear relationship.

Figure 5: Ion chromatography for nitrate determination; calibrated with KNO_3. using a mixture of 0.1 mM phathalic acid, 0.35 mM tetrabutyl ammonium hydroxide and 0.5 % tetrahydrofuran as an eluant in a 1.5 ml/min flow rate.

For the green (6); the sample gave peak area equivalent to nitrate = 17.26%, which means that green(6) contains 5 nitrate anions.

For the blue (7); the sample gave peak area equivalent to nitrate = 15.85%, which means that blue (7) contains 5 nitrate anions, since it has MW around 1940.



The perchlorate and sulphate salts of the green and blue were obtained by using $HClO_4$ or H_2SO_4 and $NaClO_4$ or Na_2SO_4 insted of HNO_3 and $NaNO_3$ during the prepration, but in case of perchlorate the green complex was obtained with contamination of some blue, and so it was submitted to gel-chromatography for purification, and the products gave the same absorption spectra of that of the nitrate salts, and in case of sulphate; the reaction mixture was precipitated by addition of acetone and then submitted to gel-chromatography for purification. Also, the dinuclear of the perchlorate salt was obtained by the same manner in case of nitrate salt.

Biological activity :

The growth inhibitory activity of the mononuclear (4), the dinuclear (5), the green (6), and the blue (7) were tested with different types of cancer cells:

- L1210 cells; mouse white blood cell (Leukemia),
- S 180 cells; mouse Sarcoma,
-KB cell, U 937, HL 60, Daudi cells, Colo 320 cells, and LX 830 cells; Human cancer cells.
-NIH3T3 cells; which is not true cancer cell, established from mouse.

in vitro: Each tumor cell line [$1x10^{-5}$/ml; MEM (for S180) or RPMI (for others) medium containing, penicillin or streptomycin, 20 mM HEPES (N-2-hydroxyethylpiperazine-N`-2-ethanesulfonic acid), and fetal bovine serum] was cultured in the presence or absence of the compounds for 48h in case of S180 and 72h for the others at 37°C in 5% CO_2.

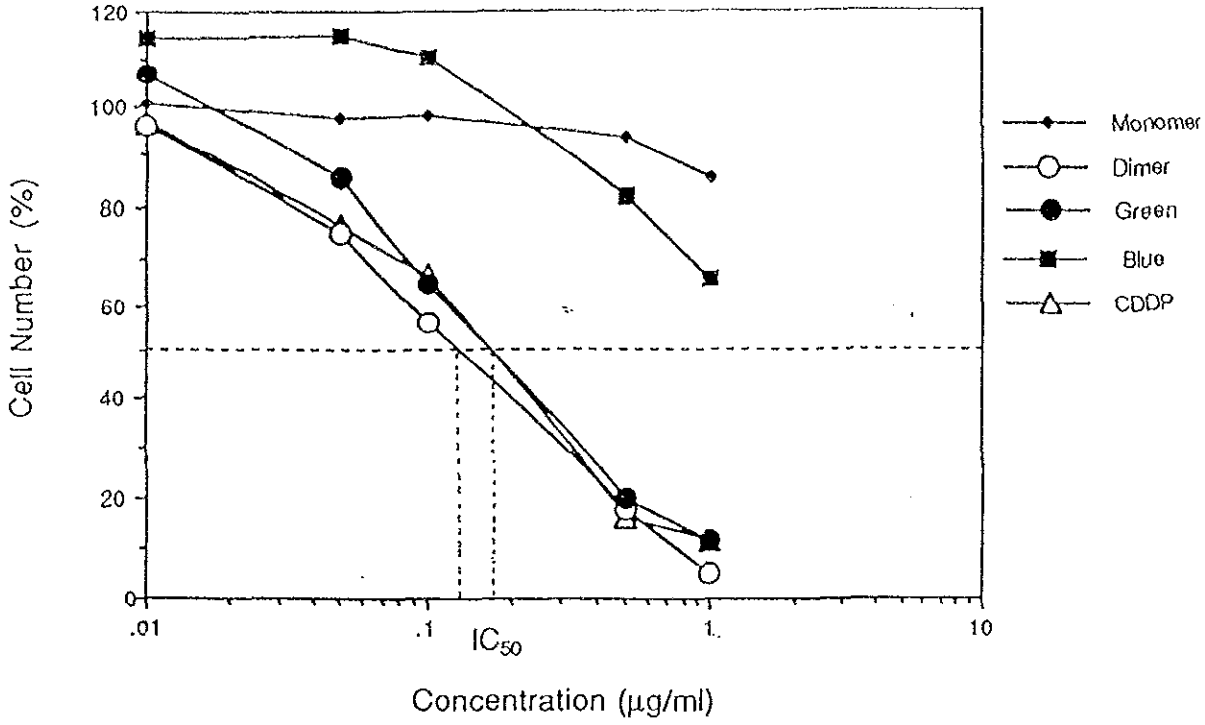
In case of KB cells and L1210 cells; % of cell growth inhibition was calculated by the following formula: % of cell growth inhibition= (1-T/C)x 100, where C is the mean absorbance at 540 nm of the control group and T is that of the treated group. The 50% inhibition (IC_{50}) was determined graphically from the dose-response curve with at least 3 drug concentration points.

In case of other cells; the number and size distribution of cells were assayed with a Coulter Channelyzer, the cells between 6.4 and 16 μm in diameter were counted. Activity was expressed as IC_{50} values defined as the concentration at 50% inhibition of cell growth and the percent inhibition was determined as follows:

% inhibition = [1-(cell numbers of sample well / cell number of control well)]x100

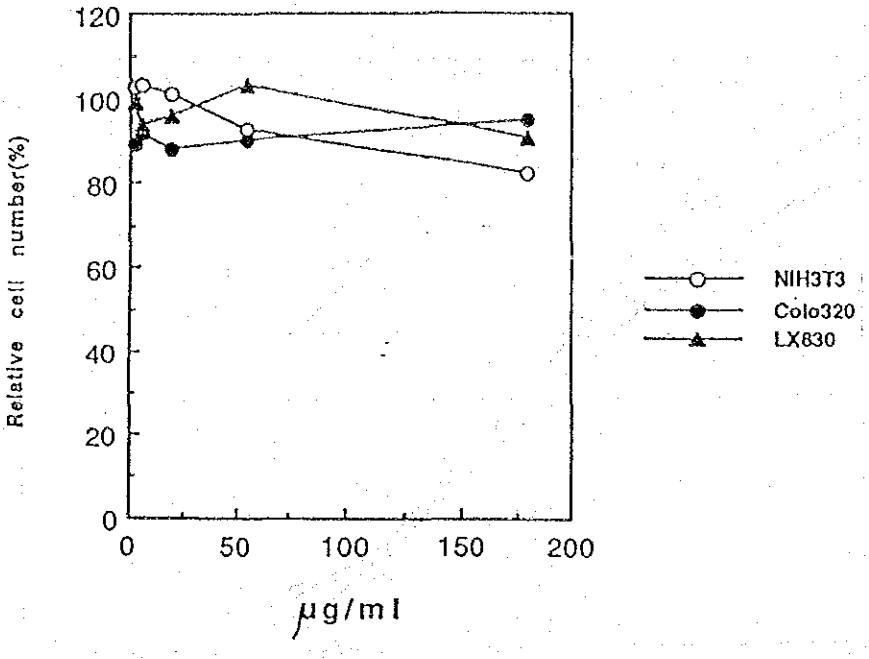
The results of cytotoxic activity of platinum complexes with some cancer cells as a relation between the concentration, μg/ml, and the relative cell number % are shown in Figures 6-10 as the method for IC_{50} determinaion.

Figure 6 Cytotoxic activity of platinum complexes against S-180 cells: monomer and blue have lower activity compared with green, dimer, and CDDP.



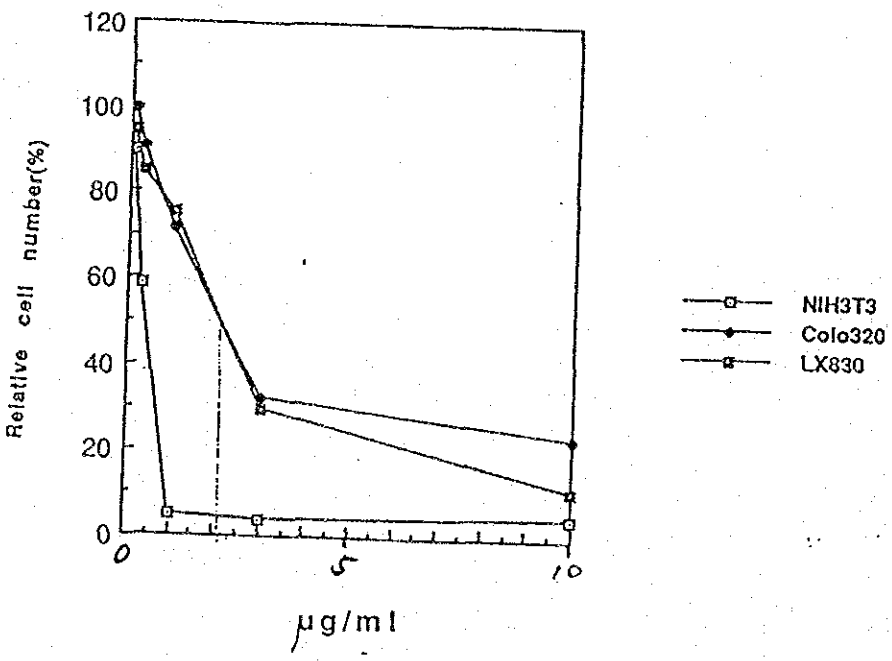
Figures 7

Cytotoxic activity of Monomer against different types of cancer cells.



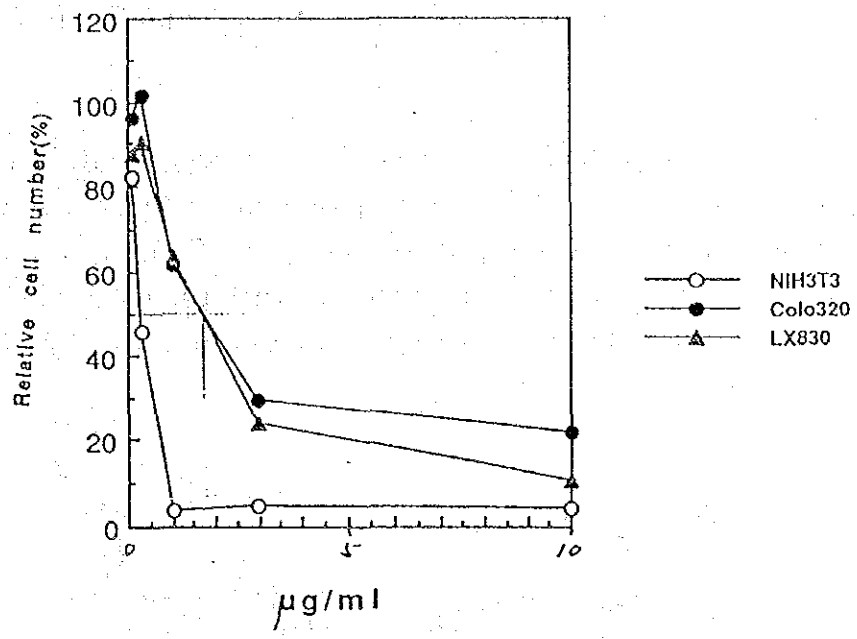
Figures 8

Cytotoxic activity of Dimer against different types of cancer cells.



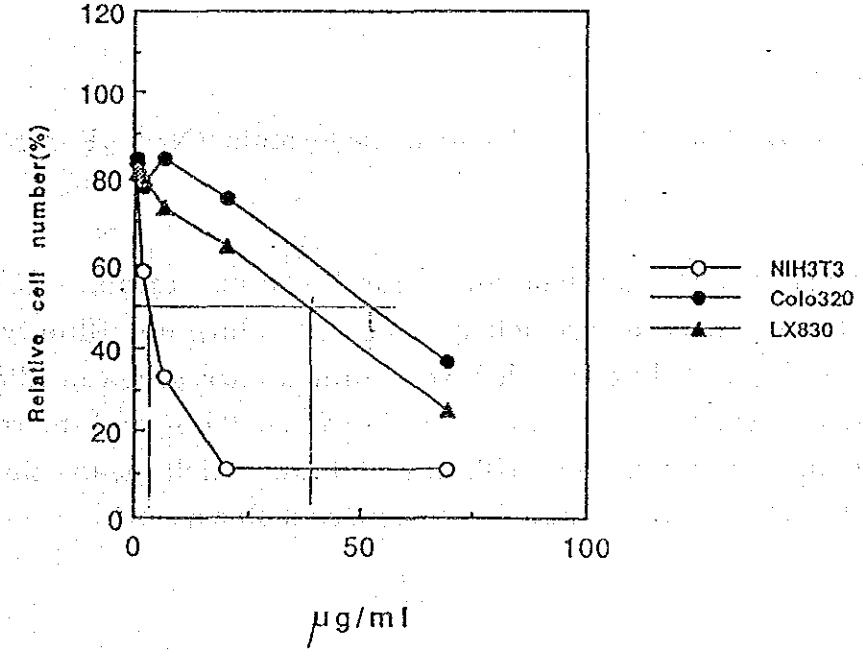
Figures 9

Cytotoxic activity of Green against different types of cancer cells.



Figures 10

Cytotoxic activity of Blue against different types of cancer cells.



Results and discussion:

The tetranuclear platinum **Green** complex was prepared by the reaction of the aquated complex (3) with the mononuclear complex (4) at 60°C, pH 3 for 24h, under air, and from the same reaction mixture the platinum **Blue** complex was obtained.

The structure of the green complex (6) was identified by IR, ^{1}H-NMR, elemental analysis, and the X-ray diffraction, also using the ion chromatography for the determination of the nitrate amount, and O.D.S column chromatography to indicate about the purity of the structure with the formula $[Pt(NH_3)_2(1\text{-}MU)]_4(NO_3)_5.5H_2O$ (Figure 11) with average oxidation number 2.25 for each platinum atom.

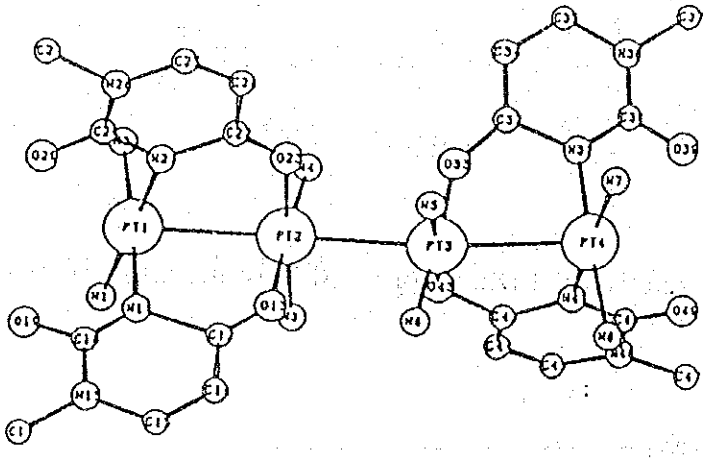


Figure 11

The crystal structure of Green (6) with the formula $[(NH_3)_2Pt(1\text{-}MU)]_4^{5+}$

Surprisingly, the data obtained for the green complex (6) are perfectly consistent with those of previously reported "Platinum Blue with 1-MU" by Lippard and Lippert, but actualey we obtained dark green needles which have characteristic visible spectra maxima at 740 and 480 nm, and the reported blue in literature has maxima at 740, 520, and 480 nm, which means that there is one additional absorption maximum for the reported blue at 520 nm (Figure 12).

The structure of the Blue complex(7) was estimated by IR, ^{1}H-NMR, Pt%, C%, and ion chromatography for the nitrate amount after purification by gel-chromatography, and the purity of the compound was checked by using O.D.S column chromatographay. Its molecular size estimated near to that of the green, but no strong evidance for the crystal structure. So from all data which we had , we guess that the molecular structure of both green and blue is the same with the differance in the crystal structure. The blue compound gave characteristic visible spectra maximum at 680 nm. Figure 12 shows the visible spectra of green (6), blue (7), and the reported blue.

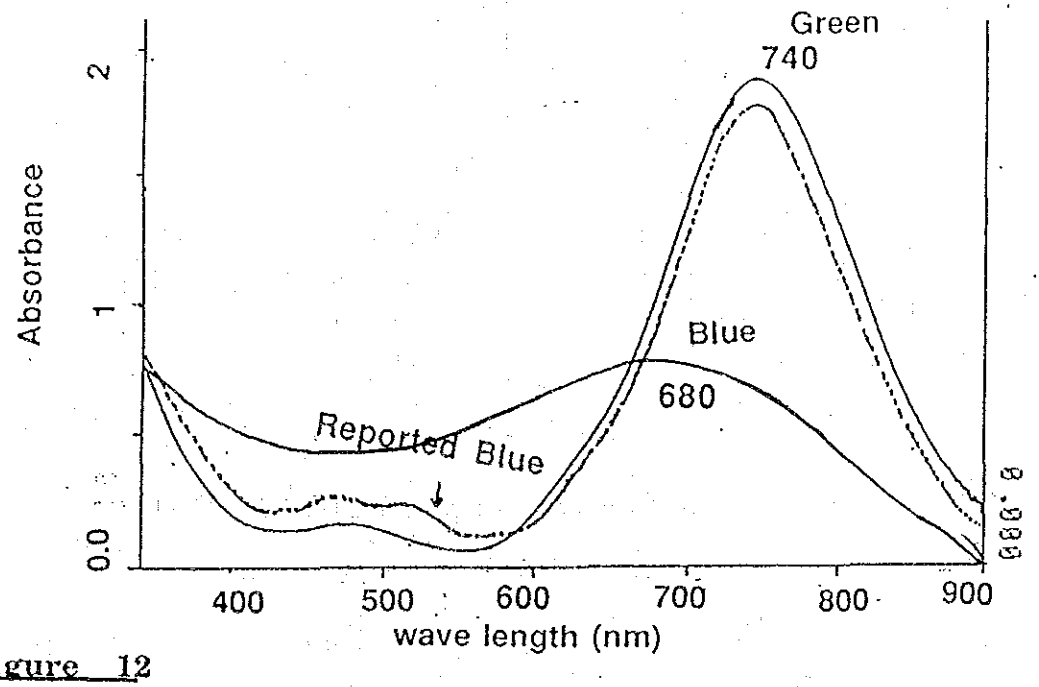


Figure 12

Visible spectra of platinum green and blue complexes with 1-Methyluracil, 1 mg / 0.5ml 0.01M HNO_3

Comparing the UV spectra of the green (6) with the Blue (7), there was a maximum at 279 nm for the green (6) but at 270 nm for the blue (Figure 13). Also ^1H NMR study showed that H_6 of 1-MU in blue complex has a chemical shift at 7.60 but in green at 7.42 PPM, with a little difference in case of H_5, Figure 14.

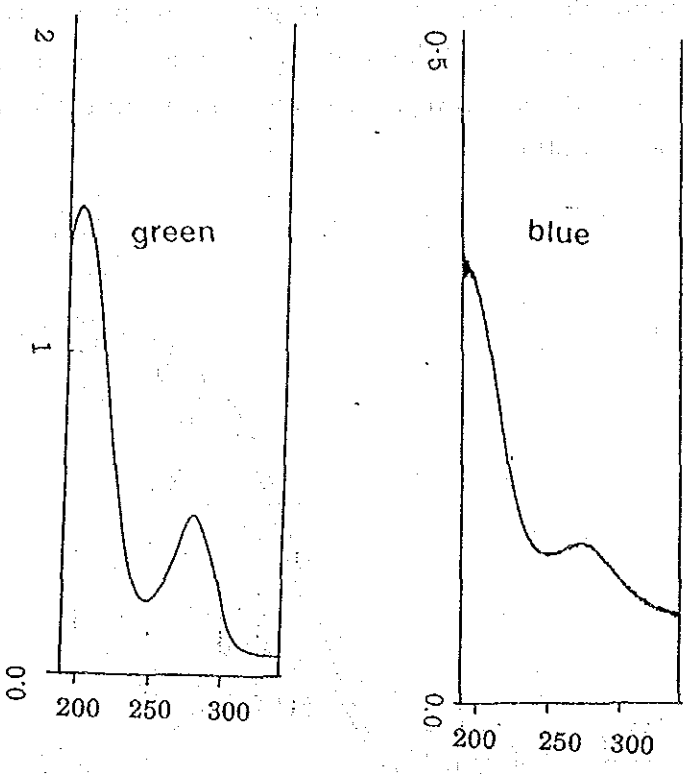


Figure 13

U.V. spectra of Grren (6) and Blue (7) ; 1mg/ 0.5ml 0.01 M HNO_3

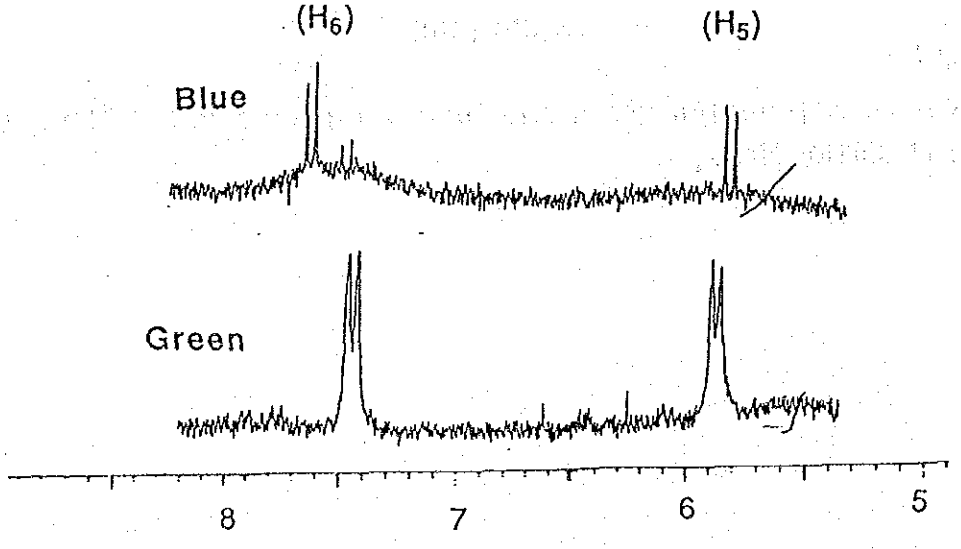


Figure 14

H_5 and H_6 chemical shifts in green (6) and blue (7) complexes

The stability of the green (6) and the blue (7) in 0.01M HNO_3 with the time was studied by measuring the decrease in the absorbance at λ_{max}. The blue complex (7) was more stable, since the rate of decreasing in the absorbance at λ_{max} was slower than that of the green (6), Figure 15.

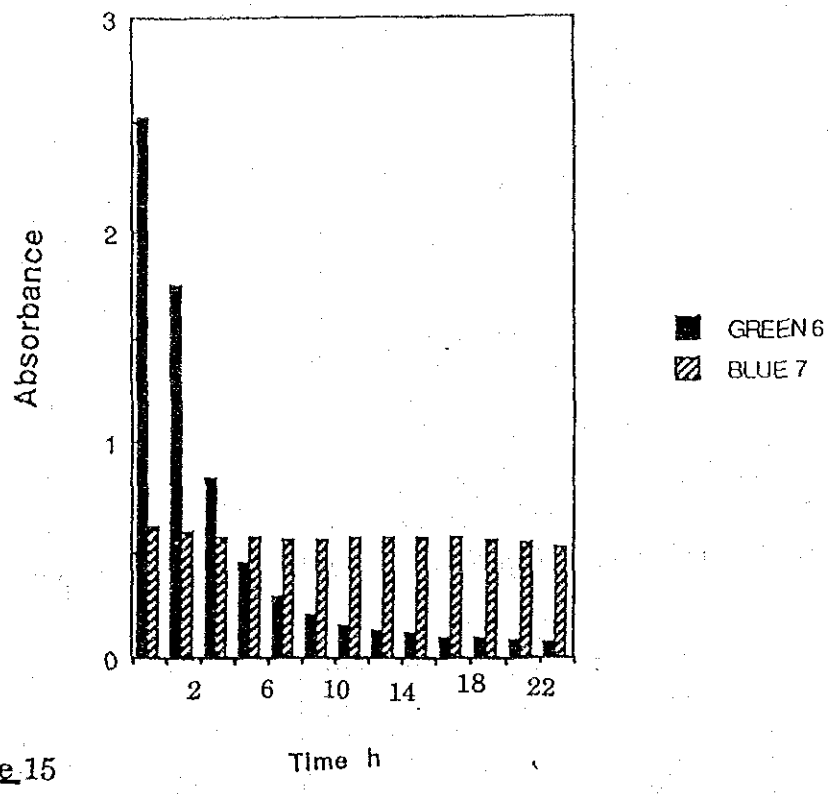
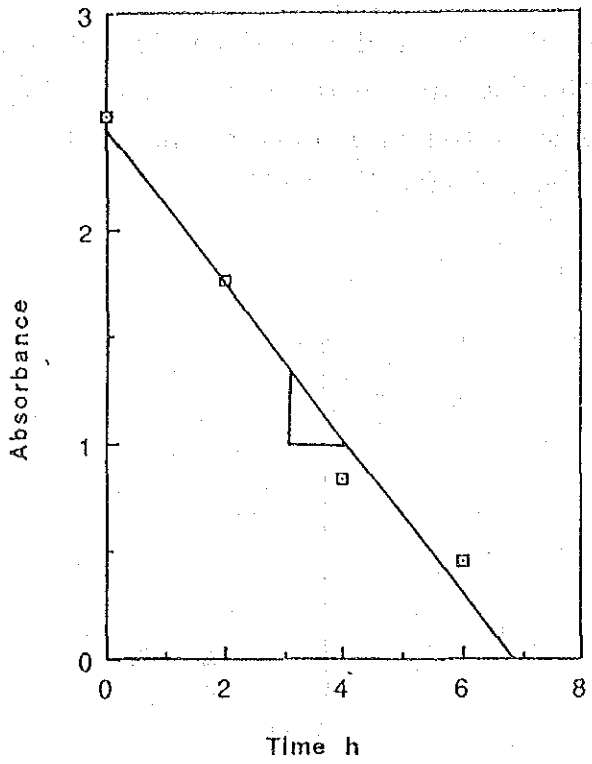


Figure 15

The absorbance at λ_{max} of Green (6), 740 nm, and Blue (7) , 680 nm, in 0.01M HNO_3 solution for 24h.

From this study, we could calculate the decompostion rate of green in 0.01M HNO3, during the first 6 h after preparing the solution, the rate was faster, 0.164mg/h, but after 16 h the rate was slower, 0.0021mg/h, and this calculation was obtained from plotting the absorbance with the time for each interval, Figures 16 and 17, with using the linear relation between absorbance and concentration .



Figures 16

Absorbance change of Green (6) at 740 nm in 0.01M HNO_3 for the first 6 h

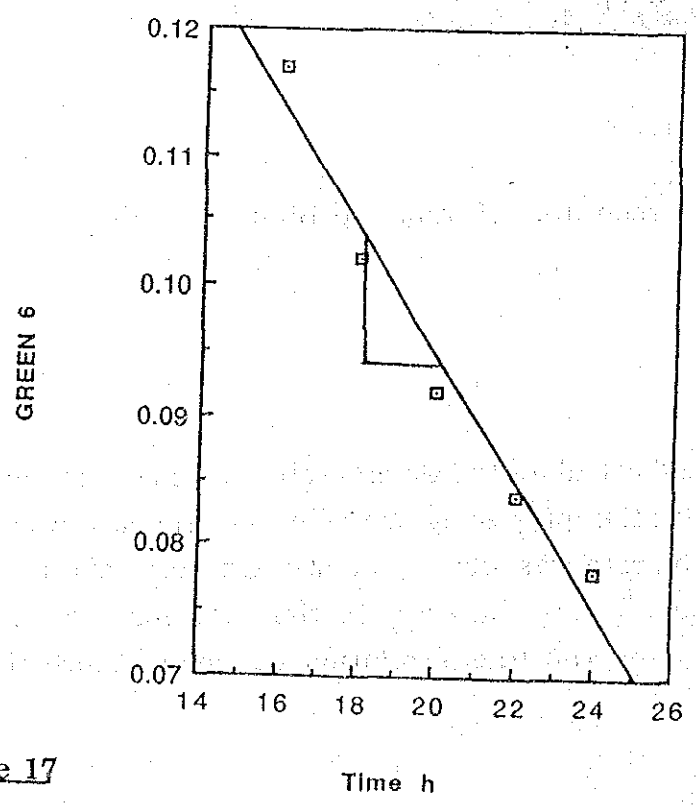


Figure 17

Absorbance change of Green (6) at 740 nm in 0.01M HNO_3 for the first after 16 h

Interestingly, a colorless aqueous solution of the dinuclear platinum complex (5) turned a green solution instantly upon acidification, in 0.01M HNO_3, whose maxima λ max were at 278 and 730 nm, and gave the retention time of the green (6) by the O.D.S column chromatography. This conversion from the dinuclear (5) to the green (6) was studied by measuring the increasing of the absorbance of λ_{max} , 730nm, with the time in different temperatures. It was found that the room temperature was the optimum temperature for this conversion, and also it is time dependent, since it increased with the time, Figures18, 19.

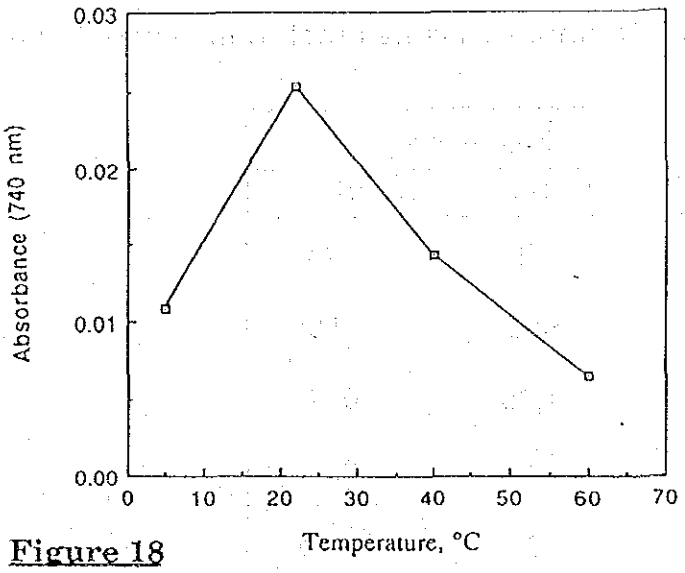


Figure 18

The intensity of the visible absorption maxima for 0.01M HNO_3 solutionof the dinuclear platinum complex with 1-methyluracil with temperatures after 1 h

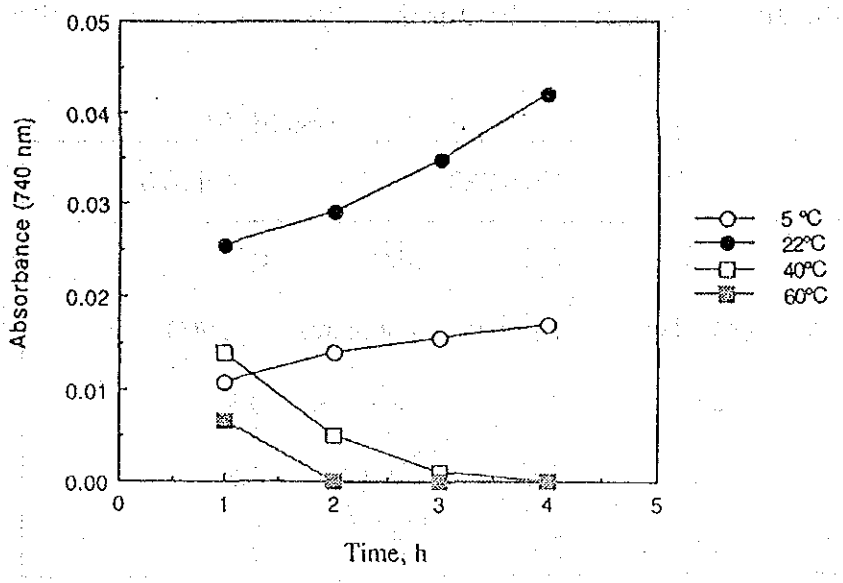


Figure 19

Absorbance at 740 nm in 0.01M HNO_3 solution of the dinuclear platinum complex with 1-methyluracil with the time in different temperatures

The biological activity

The IC_{50} (concentration at 50% inhibition of cell growth) values for these compounds are summarized in Table 4 and 5. From all the data; it was found that the green complex (6) and the dinuclear (5) are active aganist all the types of the cancer cells but the blue (7) and the mononuclear complexes (4) are much less active.

Table 4 Antitumor activity of Platinum 1-Methyluracil complexes in vitro

Sample	IC_{50} μg/ml		
	S-180	L1210	KBcell
CDDP	0.02	1.85	0.82
Mononuclear(4)	>200	>50	>50
Dinuclear (5)	0.16	1.65	0.48
Green (6)	0.07	2.05	0.5
Blue (7)	3.2	33	16.5

Table 5: IC_{50} of the platinum complexes with another types of cancer cells

	IC_{50} μg/ml					
Complex	U937	HL60	Daudi	Colo 320	LX 830	NIH 3T3
CDDP	1.8	1.4	0.9	2.5	3.0	0.7
Monomer	inactive	inactive	inactive	inactive	inactive	>200
Dimer	1.0	0.8	0.8	2.1	2.1	0.5
Green	0.9	0.6	0.6	1.8	1.8	0.3
Blue	33	19	42	53	39	4

By comparing the activity of these complexes with the chemical shift of H_6, it was found that the dinuclear (5) and the green (6) gave chemical shift for H_6 at 7.42, but that of the mononuclear (4) and the blue (7) at 7.6 PMM, and so we can say that the active compounds have the same ^{1}H-NMR chemical shift and the less active compounds have another same one, this is shown in Table 6, as well as the λ_{max} in the UV region, which also indicated that the dinuclear (5) and green (6), active compounds, gave nearly the same value, but the mononuclear (4) and blue (7), less active compounds, have λ_{max} at lower wave length near to that of the ligand itself.

Table 6 λmax in U.V. and H_6 chemical shift for platinum complexes

Compound	H-NMR δ H6	UV nm λ max
1-MUH	7.60	265
Mononuclear	7.60	266
Dinuclear	7.43	278
Green	7.42	279
Blue	7.60	270

Green and dimer, active , λmax=279-278 and δ H6 = 7.42-7.43

Blue and monomer, less active, λ max is in lower wave length near to λmax 1-MUH and so do for chemical shifts of δ H_6.

For the different counter anions of the green, in case of S 180 cells, it was found that the perchlorate is more active (lower IC_{50}), than nitrate or sulphate salts. Also, IC_{50} was higher in case of the dinuclear than that in case of green in both perchlorate and nitrate salts as shown in Table 7.

Table 7 IC_{50} values with different anions

Anion	IC_{50} µg/ml	
	Green	Dinuclear
NO_3^-	0.07	0.16
CLO_4^-	0.02	0.12
SO_4^{2-}	0.2	ND

IC_{50} for CLO_4 is lower than that for NO_3 in both green and dimer

Conclusions

1- The tetranuclear platinum complex with 1-Methyluracil in the formula $[(NH_3)_8Pt_4(1\text{-}MU)_4](NO_3)_5.5H_2O$ is **GREEN** with the characteristic visible spectra at 740 and 480 nm (λ_{max}), and U.V. spectra at 279 nm, which has been reported in literature as blue.

2- The tetranuclear platinum **BLUE** complex with 1-Methyluracil has the same Pt: 1MU: NO_3 ratio (and amounts) as in the green one with the characteristic visible spectra at 680 nm, and U.V. spectra at 270 nm (λ_{max}).

3- The solution of tetranuclear green, in 0.01M HNO_3, is less stable compared with that of the blue with the time.

4- The tetranuclear green and dinuclear complexes have higher antitumor activity, but the tetranuclear blue and mononuclear complexes have much lower activity aganist all types of cancer cells tested here.

5- It was found that the active compounds have the same λ_{max} in U.V. and ^1H-NMR chemical shift, and the less active compounds also have another same values, which means that the ligands orientation in the structure of the complex has a big rule in the activity of the complex.

6- Acidification of the colorless solution of the dinuclear complex turned a green solution of the tetranuclear complex with its characteristic visible absorption at 740 nm. We studied this conversion, and it was found that the room temperature was the practically optimum temperature for this conversion which was time dependent.

References :

1) Rosenberg, B., VanCamp, L., Krigans T. *Nature*, 205, 689, 1960.

2) Hollis, L.S., Stern, E.W., Amundsen, A.R., Miller, A.V., Doran, S.L., *J. Am. Chem. Soc.*, 109, 39, 1987.

3) Loehrer, P. J., Einhorn, L.H. *Ann .Intern. Med.*, 100, 704, 1984.

4) Pradip K. Mascharak, Lan D. Williams, Lippard, S., J., *J.Am.Chem.Soc.* 106, 6428, 1984.

5) Thomas V. Ohalloran, Pradip K. Mascharak, Lan D.Willams, Michael M. Roberts. *Inorg.Chem.*, 19987, 26, 1261.

6) Micklitz, W., Muller, G., Riede, J., and Lippert. B.,*J. Chem. Soc., Chem Commun.*, 1987, 76-78.

7) Micklitz, W., Muller, G., Huber, B., Riede, J., Rashwan, F., Heinze, J., Lippert. B., *J. Am. Chem. Soc.*, 110, 7084, 1988.

8) Okuno, Y., Tonosaki, K., Inoue, T., Yonemitsu, O., Sasaki. T., *Chemistry Letters,* 1947-1950, 1986.

9) Shimura, T., Tomohiro, T., Laitalainen, T., Moriyama, T., Venura, T., Okuno, Y., *Chem. Pharm. Bull.,* 36, 448, 1988.

10) Shimura,T., Tomohiro, T., Maruno, K., Fujimoto, Y., Okuno, Y., *Chem. Pharm. Bull.*, 35, 5028, 1987.

11) Sakai, T., T., Alfonsol. Pogolotti, Jr., and Daniel V. Santi. *J.Heterocyclic Chem.*, 5, 849, 1968.

12) Micklitz, K., Lippert, B., *J. Heterocyclic Chem.*, 26, 1499, 1989.

13) Lippard, B., Neugebauer, D., *Inorg. Chem.*, 21, 451, 1982.

14) Dhara, S., C., *Indian J. Chem.*, 8, 193, 1970.

15) Lippert, B., Neugebauer, D., Schubert, U., *Inorg. Chem. Acta*, 46, L11-L14, 1980.

16) Lippert,B., Neugebauer, D., Raudaschl, G., *Inorg. Chem. Acta*, 73, 161, 1983.

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