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Platinum Derivatives of Pyridoindole

Clemens Wett

M.Sc. Thesis

in

The Department

of

Chemistry

Presented in Partial Fulfillment of the Requirements

for the degree of Master of Science at

Concordia University

Montréal, Québec, Canada

December 1983

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ISBN 0-315-30604-1

Abstract

Platinum Derivatives of Pyridoindole

Clemens Wett

The use of chemotherapy in cancer treatment requires the further development of its selectivity. Pt-derivatives are proposed which have a structural similarity to the known alkaloid harmine. This thesis describes some attempts to synthesize these compounds.

A reaction of potassium tetrachloroplatinate with pyridoindole resulted in a complex reaction system and the analysis of compounds lead to the tentative identification of three complexes. The thin layer chromatography indicated the presence of four complexes in the initial reaction mixture. During the work up with organic solvents, one complex disappeared and two other compounds were formed, suggesting various ligand exchange reactions. This reactivity was monitored using IR-spectra: the positions of the Pt-Cl and Pt-O bands in the region $200-450\text{cm}^{-1}$ allow a fairly conclusive determination of structure and stability of complexes. It was found that $[\text{Pt}(\text{pyin})_2\text{Cl}(\text{OH}_2)]^+$ is a relatively stable product. Other techniques like Mass-spectra, NMR-spectra, UV-spectra and elemental analysis support the interpretation of the IR-spectra. The solubility of the stable complex in water and acetic acid (5%) or alcohol makes its use as possible anti-tumor agent likely only for oral administration in case of malignant tumors in the stomach and intestines.

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Acknowledgements

I wish to express my thanks to the World University Service, which organised the international student exchange and financially supported this research work.

Thanks also due to Dr. C. Kazakof at the University of Ottawa and Dr. O. Mamer at the McGill University for taking the mass spectra.

Finally I would like to thank Dr. P.H. Bird who directed the research. His guidance and cooperation were deeply appreciated.

Abbreviations:

A3'p5'A	Adenine-Adenine dinucleotide, 3p5 sugar bonding
A3'p5'C	Adenine-Cytosine dinucleotide
A-T	Adenine-Thymine
C-G	Cytosine-Guanosine base pair
GMP	Guanosine monophosphate
(1)N	locations of nitrogen atoms
(6)NH ₂	location of an amino
bipy	bipyridine
DMSO	dimethylsulphoxide
dach	diaminocyclohexane
Et	ethyl
en	enamine
gly	glycine
glyc	glycerol
Me	methyl
mal	malonate
ox	oxalate
prop	propanol
pn	propanolamine
TEAM	triethanolamine
amu	atomic mass unit
calc.	calculated
diff.	difference
mix.	mixture

Part A

Platinum Containing and Alkaloid Based

Drugs in Chemotherapy

Introduction

The discovery of the cell growth inhibition properties of metallic platinum, in the form of an electrode¹, lead to intensive investigations of this phenomenon in the seventies. Today, as a result a number of effective anti-tumor drugs are available. The present use of chemotherapy in combination with radiation therapy has lead to a decrease in the death rate from cancer.

Admittedly, the compounds used are still primitive and an improvement in their properties is an urgent concern. Above all, the toxic side effect on organs and muscle coordination are a disturbing feature of the medical treatment. Because of the uncontrolled mutagenic properties of the cytostatica, all cells are affected instead of a selective anti-tumor activity.

The present work aims towards combining further development in Phytochemistry with observations in pharmaceutical experiments about effects of Pt complexes on different sorts of tumor and cell cultures, and to open new directions for an improvement of today's medicines.

To outline the considerations involved in the choice of the ligand, results in relevant areas have been collected in part A of the thesis.

A 1 Biochemistry of Platinum compounds.

A 1.1. Bonding of Pt to DNA

The anti-tumor activity of platinum complexes is a result of its ability to form stable bonds with nitrogen and oxygen². The possibilities for interaction with compounds in a cell are very large: DNA, DNA bases, nucleosides, nucleotides, and the RNA's are the ones which are the most important for cell growth. Other ligands might include amino acids, proteins, enzymes, while a reaction with cytochromes could change the Pt oxidation state. In essence every molecule with nitrogen, oxygen and possibly sulphur could be considered as a potential reagent.

One is forced to recognise, that a precise definition of the anti-tumor mechanism in vivo is not available. The behaviour of a compound is different in humans, in animals, and in cell cultures: the medium must be considered in the evaluation. An important aspect is the difference of the G-C relation to A-T in humans: 60-40%, and mice with a higher A-T content. This has its special relevance in experiments with cell growth inhibitors, when they are tested in rodents.

The first discovery of cell growth inhibition by platinum took place in 1924¹, when an Escheria coli cell culture had been brought into contact with a platinum electrode. In 1965, this observation was connected with the search for anti-tumor agents^{3,4}. During the last decade, there have been many studies on this subject in all domains, from nuclear

chemistry through biology to medicine, and recently, psychological aspects of chemotherapy are under investigation⁵,

The reactions with nucleotides, dinucleotides and DNA show that platinum has special ability to react with the nitrogen of the DNA bases⁶. The ratio of Pt-complexation to DNA, RNA and protein has been investigated in experiments with $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ and the pure cellular constituents both in a solution, and simulated biological conditions in a test tube⁷. The results are:

Table

cellular constituent	Mol. weight	Number of <u>cis</u> -Pt/base mol	Number of <u>trans</u> -Pt/base mol
DNA	109	22/1	125/1
m-RNA	4.106	1/8	2.5/1
r-RNA	1.5-1.106	1/30	1/2
t-RNA		1/1500	1/70
protein		1/1500	

Complexation of trans- $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ with DNA is highest followed by cis- $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ with DNA.

Surprisingly, the trans compound has no cytostatic effect, despite the high complexation ratio with DNA. Different membrane interactions of cis and trans isomers were proposed as an explanation².

Experiments in cell cultures of Ehrlich ascites tumor, human amnion and lymphocytes showed that RNA and protein

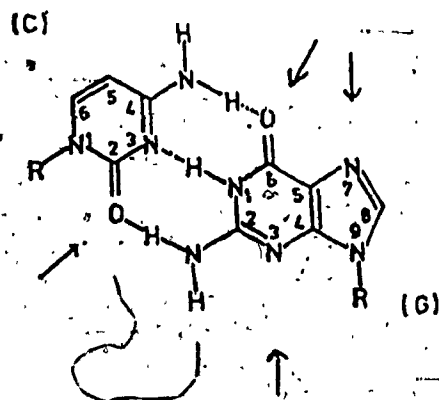
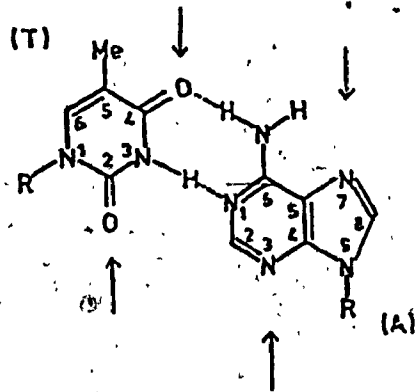
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synthesis were barely affected by treatment with low doses of anti-tumor platinum compounds, whereas DNA synthesis was significantly inhibited¹⁸.

A remarkable finding was that Pt(II)-derivatives enhance the level of DNA polymerase in amnion AV₃ cells in spite of the fact that the same complexes inhibited the enzyme activity in vitro. On the other hand only millimolar concentrations of Pt(en)Br₂ were required to inhibit leucine aminopeptidase in vitro.

It should be noted that over 2000 enzymes have been discovered and their interaction with Pt-drugs is completely unknown. Despite the confusion, one can suggest in general, that inhibition of DNA synthesis, and consequently, the anti-tumor activity results from a direct reaction of Pt-complexes with DNA. For the possible interactions of platinum with DNA, every nitrogen and oxygen atom has to be considered; the possible sites are shown in the following figure (figure 1).

Figure 1



DNA Base Pairs

Bonding to a nitrogen or oxygen of one of the bases would inhibit DNA replication by interference with hydrogen bonds. Bonding to the oxygen of the sugar or phosphate molecule would inhibit the polymerisation.

The most frequently invoked explanation is the crosslinking of DNA by Pt(II). The two replaceable Cl-atoms are 3-4 Å apart, and for crosslinking, the binding sites need to be 3-4 Å apart⁹.

A related experiment was performed with dinucleotides in vitro⁹. Dinucleotides take up one of two possible conformations: a stacked form, with both bases parallel, and vertically above one another with an interplanar separation of 3-4 Å, or an unstacked form with dinucleotides stretched out and the bases separated by a large distance.

The stacked form is favored at low temperature and the circular dichroism spectrum (CD) is highly biphasic, while the unstacked form has a negligible contribution to the CD. If the platinum is able to link together two bases of the same dinucleotide and if the resulting complex is stable over a temperature range between 5-45°C, the temperature dependence of the equilibrium stacked \rightleftharpoons unstacked will be reduced or eliminated. The results of these experiments show that "Cisplatin" ($\text{PtCl}_2(\text{NH}_3)_2$) and cis-Pt(enim)₂Cl₂ form a link between A3'p5'A, or A3'p5'C. No such species has been unambiguously identified in the reaction with A'2p5'A and A2'p5'C. Trans-dichlorodiaminoplatinum reacts with all

four dinucleotides with no temperature independent biphasic signal, which means no crosslink is established. However by measuring the temperature dependence over an extended range, beyond biological conditions, evidence is obtained for the presence of a temperature independent species of a trans- $\text{Pt}(\text{NH}_3)_2(\text{A})_2$. Experiments at different acidities have demonstrated the pH dependence of platinum reactions⁹.

The interaction of platinum(II) with uridine or thymine does not appear to produce Pt-base complexes. The only effect is a conformational change of the dinucleotides from stacked to unstacked. However, a source of error in this experiment could be a crosslink between adjacent dinucleotides.

The conclusions from these experiments are that:

- a) cis Pt(II) bonds to the aminogroup of adenine at N(1) and N(7) of the ring. The crosslink is established by $[\text{PtL}_2(1)\text{N}(6)\text{NH}_2]$ or by $[\text{PtL}_2(7)\text{N}(6)\text{NH}_2]$ complexation of the two adenines.
- b) trans complexes are bound to ring nitrogen (1)N, or (7)N.
- c) further positions of Pt-N bonds are: $(6)\text{NH}_2(5')\text{-Pt-(1)N-(3')}$, $(6)\text{NH}_2\text{-Pt-(6)NH}_2$, or $(7)\text{N}(5')\text{-Pt-(6)NH}_2(3')$ in A-A crosslinks.
- d) in A-C possible crosslinks are: $(3)\text{N}(5')\text{-Pt-(4)NH}_2(3')$, and $(4)\text{NH}_2(5')\text{-Pt-(1)N}(3')$.

In the article⁹, the authors propose as the reason for the anti-tumor activity of the cis-Pt, in contrast to the

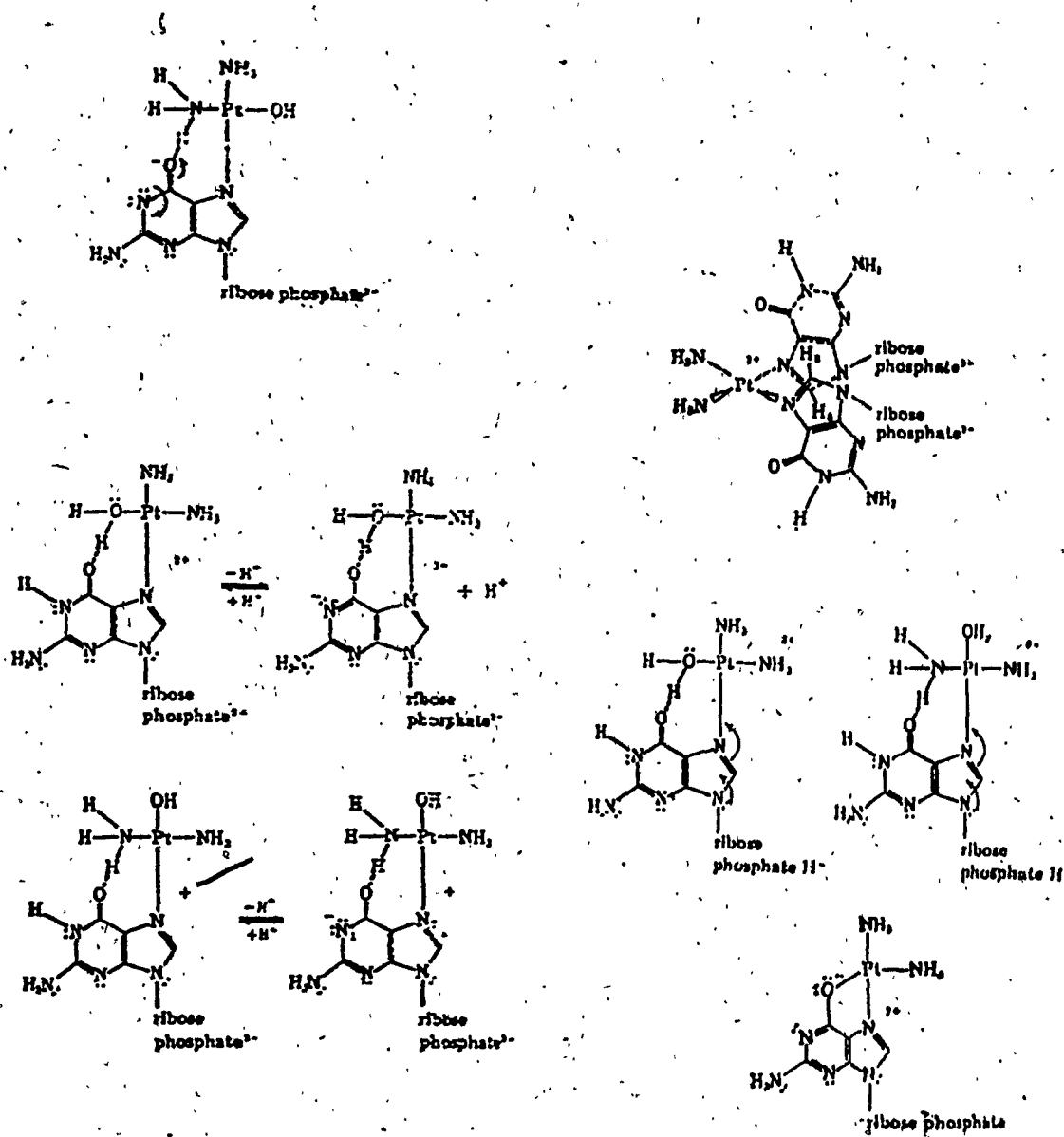
inactivity of the trans-isomer, its peculiar ability to crosslink 3'Ap-dinucleotides.

Other possibilities for the platinum complexes to intervene during the DNA replication, before or after the cell division, have been shown in various experiments and their evaluations^{10,11}.

Thus, the rate of platinum-DNA reaction increases with increasing G+C content, which seems to contradict the statement that the Pt-A3p crosslink is the only reason for the anti-tumor effects of platinum compounds.

The number of different complexes, which are possible between Pt(II) and guanosine-monophosphate (GMP) illustrates the difficulty of this aspect in platinum chemotherapy (figure 2).

Figure 2



Pt-GMP Complexes

Furthermore, bonding between the metal and oxygen of phosphate or ribose during DNA synthesis would prevent the polymerisation. Besides interstrand or inter DNA crosslinks, also shortening, breakage or conformational and configurational changes in the DNA may be the reason for anti-tumor activity^{9,12}. In addition, disturbance of the DNA base sequence via RNA modification, interaction with DNA polymerase and other enzymes of importance for the replication of DNA gives interruptions in the normal cell growth.

The superhelical conformation of DNA can be changed with Pt(II) as detected by viscometric analysis and quantitative densitometric analysis on gel electrophoresis of Pt(II)-complexes. The same techniques were used to prove DNA shortening by Pt(II) whereas Pt(IV) induces a single strand break or double strand break and local microloops⁹.

The difference of cis and trans isomers in their physiological behaviour has been explained by their different ability to build up chelate complexes, see figure 2: Chelates are only possible with cis dichloroplatinum compounds.

The existence of trans Pt(II) and Pt(IV) anti-tumor agents with organic amines as ligands instead of ammonia cannot be explained with the chelate theory². The lability of a Pt-Cl bond as necessity for a DNA interaction is disproved by the activity of dicarboxyl chelates, which are inert against ligand exchange reactions^{2,13}. In copper chelates, the antitumor effect was found to be based on the inhibition

of an enzyme responsible for protein synthesis, without involvement of DNA¹⁴.

A 1.2. The anti-tumor activity of particular platinum compounds:

The procedure for testing activity is usually to implant tumor cells in mice and administer the complexes intraperitoneally beginning the following day². Other procedures have been used with different tumor targets and a modified evaluation system, but most work on this area adopts the following method: To reduce the subjectivity, four different doses of each complex are given to four groups of several mice. In addition there are two control groups, one without treatment, one with "Cisplatin" treatment. The potency of the anti-tumor effect is evaluated by comparing the weights of treated(T) and untreated(C) tumors, the result is expressed as percentage of $T/C \times 100$. The values of the T/C's are classified as significant when less than 45%, the effects are considered marginal when the value is around 45-55%.

A 1.2.1. Classification of platinum complexes:

- a) cis-diaminodichloroplatinum compounds with NR_3 , NR_2 and the corresponding trans complexes
- b) compounds with replaced chloride
- c) chelate complexes of platinum
- d) compounds with heterocycles as ligands
- e) compounds with aminoacids as ligands
- f) compounds with DNA bases bound to platinum

- g) dimer pyrophosphate complexes
- h) complexes with non nitrogen donor atoms
- i) ionic complexes
- a) cis-diaminodichloroplatinum compounds with NR_3 , NR_2 and the corresponding trans complexes;

The geometric arrangements of the chloride ligands characterise this group of compounds. They are highly active and apparently the two adjacent reactive chloro ligands are of importance. Where a corresponding trans isomer has been tested, it has been found to be inactive in comparison to an active cis isomer. A comparison of cis- and trans- PtL_2Cl_2 complexes also shows that the active cis isomer has appreciably lower toxic levels. A notable feature of the comparative chemistry of these compounds is that trans isomers are considerably more reactive than their cis analogs. For example trans-dichlorodiaminoplatinate aquates four times faster than the cis analogue and undergoes ammoniation approximately 30 times faster. This means that trans isomers are likely to react faster and with a wider variety of body constituents, and will be less specific than their corresponding cis compounds. Preliminary distribution and excretion studies involving $^{195\text{m}}\text{Pt}$ enriched dichlorodiaminoplatinate isomers indicated the cis isomer is initially excreted faster than the trans, but the whole body retentions of both, after five days were comparable at approximately 20%. The relative levels of these isomers in the blood indicated a higher initial concentration for trans (three times) coupled

with a higher retention after five days. The compounds that were tested in this study were cis/transPtL₂Cl₂ with L: ammonia, methylamine, ethylamine, dimethylamine, diethylamine, ethanolamine, isopropylamine, phenylamine, and deuterated ammonia.

The amino groups determine the activity only in a secondary manner, the main influence is the dichloro moiety. The replacement of H by D as in the ND₃ complex leads virtually to no change in the activity. The rate of H-D exchange for this system is expected to be relatively slow at 37°C. On substituting H with alkyl groups in ammonia, the response (T/C) becomes less favorable, with the primary amine complexes exhibiting better activity than the corresponding secondary amines. Moreover, the relative dose level required to obtain the optimal effect is in the order: NH₃ · NH₂R · NHR₂. A remarkably higher dose is required for ethanolamine, the most soluble neutral amine analog obtained so far, compared to ethylamine. This reflects, presumably, the greater efficiency, with which the animal system eliminates highly water soluble drugs. Amines, in which the alkyl moiety exceeds two carbon atoms, give rise to highly insoluble compounds (like isopropylamine, diethylamine and n-butylamine) which necessitate a slurry injection. Alternatively a 10% DMSO-saline mixture can be used as a solvent (the so called "salt in" effect).

b) Compounds with replaced chloride:

If the chloride is replaced by bromide or iodide one by one, the tests indicate that the activity is not enhanced. Although cis- $[\text{Pt}(\text{NH}_3)_2(\text{Cl})(\text{Br})]$ is perhaps more active than the dibromo species, it showed a higher and more variable toxicity, i.e., it caused death over a wider dose range. This could be related to the reconversion in vivo of the mixed complex to the dichloro species.

Substitution of halogens by nitrates, nitrites, azides, cyanides, sulphurcyanides and water decreased the activity and enhanced the toxicity considerably.

c) Chelate complexes of platinum:

Remarkable anti-tumor activity is observed for complexes of the type $[\text{Pt}(\text{NH}_3)_2\text{X}]$ where X is a chelated dicarboxylate anion such oxalate, malonate and its methylene H substituted derivatives (with ethyl, cyclobutane etc.).

A special property of these compounds is "their inertness to ligand exchange"², and it is extremely difficult to rationalise the anti-tumor activity in terms of the leaving ability of the chelating ligand. These can only be replaced by strongly bonding ligands (soft bases like sulphur), and the rate of this replacement is expected to be relatively slow, even if the entering group is again a chelate. The large difference in reactivity between these compounds and "Cisplatin" suggests that a different mechanism might be op-

erating. Biological activation, especially enzymatic removal of the chelated groups seems to be an interesting possibility, especially as several cellular enzymes are capable of binding, for example, the free malonate ion. Nonspecific oxidative attack *in vivo* may also be possible, and/or a change of square-planar into square-pyramidal or octahedral configuration.

Malonic acid is a strong skin irritant, and most of the malonate complexes when intraperitoneally injected gave rise to severe peritonitis. A localised irritation was also observed when diaminomalonato platinum was injected subcutaneously. This problem should be readily solved by intravenous administration.

On the other hand, platinum-malonate complexes with diamino or enamine have been tested against 8 day Sarcoma 180 tumors, and have been found to be quite effective in causing long term regression^{15,16}. The complex $[\text{Pt}(1,2\text{-diamminocyclohexane})\text{mal}]$ has a high anti-tumor activity, and higher values of therapeutic indices than "Cisplatin", besides an excellent synergism when administered in combination with cyclophosphamide.

Another class of chelate complexes retains an intact cis-dichloro structure: $[\text{PtACl}_2]$ with A=amines. Whereas the nature of the halogen groups largely determines the reactivity of the amine complex, the nature of the amine group will modify this reactivity, in at least a secondary manner, by

virtue of the differences in steric, electronic, and basicity effects. At present, relatively few kinetic data are available for these systems. Consequently, studies are in progress to evaluate how extensively amine ligands influence aquation and direct reaction kinetics. Preliminary results indicate that, in general, the rates of aquation vary only by a factor of five for the entire series of amine complexes.

When A=enamine in $[\text{PtACl}_2]$, the response (T/C) is good but not quite as favorable as with two amino groups. The more soluble en analogs, A=N-Me-enamine, N,N-Me₂-enamine, show a somewhat better response than the less soluble analogs including 1,2 and 1,3 propanolamine compounds, which require slurry injection. As was noted for the monodentate alkylamines, the more extensive the substitution of alkyl groups on en, the higher the dosage required to obtain the optimal effect.

Some of the bidentate complexes are as active as cis-platin or even more so, for example $\text{Pt}(\text{ox})\text{Cl}_2$. Diaminecyclohexane derivatives (dach) are especially active. Their activity against leukemia L 1210 or P388 is in the order $\text{PtCl}_2(\text{trans-1-dach}) > \text{PtCl}_2(\text{trans-d-dach}) > \text{PtCl}_2(\text{cis-dach})$. Substitution of the dichloro leaving group with oxalate led to an extremely high anti-tumor activity. The most promising platinum complex is $\text{Pt}(\text{ox})(\text{cis-dach})$ because of its high therapeutic index and its good solubility in water. The cyclohexane ring in the cis analogue projects in the

direction of the z axis (chelate ring in the x-y-plane).

The difference to the trans isomer may affect the fitting of these complexes into the DNA. The steric difference allows the separation of the two geometrical isomers.

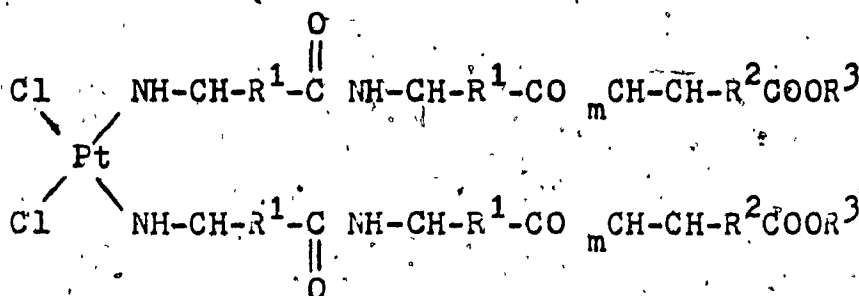
The substitution of the chloride in these complexes has the same effect as discussed in a).

d) Compounds with heterocycles as ligands:

Only a few heterocyclic platinum derivatives were investigated for anti-tumor effects. The following complex ligands show an activity: morpholine, piperazine, pyridine, and nicotinic acid. A remarkable discovery is the fact that cis-dichloro-dipyridineplatinatate has an effect against Ehrlich ascites tumor but no effect against Sarcoma 180, illustrating the very important possibility of selectivity of anti-tumor agents.

e) Compounds with amino acids as ligands:

An example for this type is the activity of¹⁷,



having $\text{R}^1, \text{R}^2 = \text{H}, \text{C}_n\text{H}_{2n+1}$, $\text{R}^3 = \text{C}_n\text{H}_{2n+1}$, alkylgroups and $n=1-4$, $m=1-3$

These compounds show an inhibition of DNA synthesis in

Walker 256 carcinoma at 4.5×10^{-7} mol/l. They are models of metalloproteins and, very specific enzyme-like affects could arise.

cis-PtCl₂(gly)₂ shows a T/C of 79%².

f) Compounds with DNA bases bound to platinum:

One example of PtCl₂(adenosine), which has a higher toxic level than the required dose level for the anti-tumor effect².

complex	dose range	toxic level(LD ₅₀)
"Cisplatin"	8(0.5-20)mg/kg	9mg/kg
<u>cis</u> -Pt(NH ₃) ₂ ox	15(5-20)mg/kg	16-20mg/kg
<u>cis</u> -PtCl ₂ (adenosine)	160(20-220)mg/kg	220mg/kg

A difference from other ligands is that the coordinated DNA base itself can bind to the DNA. Thus the platinum does not necessarily undergo a ligand exchange if a base which is already bound to Pt is polymerised into the growing DNA strand.

The DNA replication can be inhibited by several mechanism in this particular class of compounds.

g) Dimer pyrophosphate complexes:

They are believed to contain a bridging pyrophosphate ligand. These complexes have not been extensively investigated as yet, but slight activity has been observed for the diamine. The potential for in vivo formation of monophos-

phate complexes might have some important biological implications, since a polymerisation with the ribosephosphate of the DNA is possible.

h) Complexes with non-nitrogen donor atoms:

Examples are PPh_3 , DMSO, and Et_2S . The sulphur and phosphorus donor atoms are strong trans labilizing neutral groups and will be more reactive than comparable amine based systems. They show practically no anti-tumor activity. The same observation could be made with DMSO as ligand, and for the parent tetrachloroplatinate.

The postulate that the relative lability of these complex systems is a dominant factor in determining whether an anti-tumor effect will be present, is reinforced.

i) Ionic complexes:

Although the rate of Pt(II)-substitution reactions are largely independent of charge, charge type appears to play a role in the anti-tumor properties. So far only neutral species have shown appreciable activity with the exception of $[\text{Pt}(\text{NH}_3)\text{Cl}_3]^-$, marginal effects were observed in potassium tetrabromoplatinate and potassium dimalonateplatinum.

$[\text{Pt}(\text{dien})\text{Cl}]\text{Cl}$ is an example of a cationic structure with low effectiveness. The explanation for this would appear to be biophysical in nature and is probably related to trans port phenomena and to the greater efficiency with which charged molecules, which are generally water soluble, are

eliminated from the body.

It is not surprising that tetramine complexes such as $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ are inactive and non toxic, as it is known that the four Pt-N bonds are strong and inert to nucleophilic attack.

A 1.2.2. General rules for platinum complexes:

- 1) The cis configuration appears to be a necessary parameter, but trans complexes also react with DNA. Interaction with other cell constituents makes the anti-tumor activity impossible in the case of the most trans complexes.
- 2) Bidentate structures, such as $[\text{Pt}(\text{dach})\text{mal}]$, are assumed to have an alternative mode of DNA inhibition, and a different cell metabolism. Leaving groups like Cl or water are not present in the structures and chelate ligands are practically inert to ligand exchange.
- 3) Amino ligands determine the chemical properties of the complexes.
- 4) The platinum metal, its electronic and geometric constitution, especially in Pt(II), is the main reason for a cytostatic capacity.

A 2 Selectivity of Pt-pyridine complexes:

Results of experiments with cancer cells or cell material:

- a) The effect of cis $\text{Pt}(\text{py})_2\text{Cl}_2$ in a dose range of 5-200mg/kg on Sarcoma 180 implanted in mice shows a treated tumor to control tumor ratio of 91% and is considered as inactivity².

b) *Esherichia coli* ~~bacteria~~ were sensitive to the action of cis-dichlorodipyridineplatinate(II) and virtually total inhibition of growth, without killing the culture, was achieved at a concentration of 8×10^{-5} M/L in the medium. Photomicrographs showed that cell growth in media containing 5×10^{-5} M/L were extremely elongated¹⁸.

c) Using a dose range of 25mg/kg and 1 day treatment, an increase of mean survival times of 147% was observed in rats bearing the Ehrlich ascites tumor¹⁹.

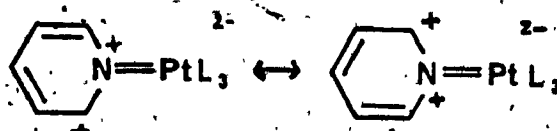
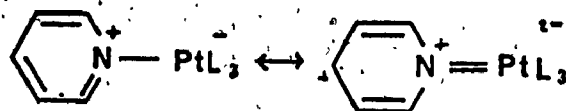
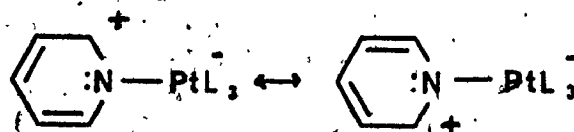
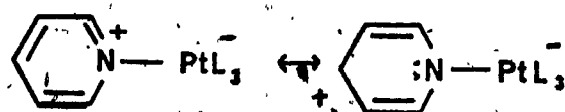
d) Blastogenesis of human lymphocytes was sensitive to the action of the pyridine complex. The concentration that conferred 50% inhibition was almost an order of magnitude lower than that found when the tumor cells were used in a protein free medium. In the paper the author concluded a moderate selectivity against the DNA synthesis in vivo, where "Cis-platin" has no distinct activity¹⁹.

The DNA of different cells consists of four DNA bases, only with a different sequence and length, and Pt-complexes cannot distinguish between different sequences of DNA bases.

It is known that the immune system, the pH-value, enzymes, proteins and membranes distinguish different kind of cells. The interaction of Pt with cell constituents before DNA complexation must be the reason for a selective effect.

Chemically the pyridine complexes are different in their ability to have mesomeric structures (see figure 3). Before

Figure 3



Pt-py Mesomeric Structures

a reaction takes place with DNA, metabolism could change the structure of the platinum complex.

There are some interesting inferences possible from the information about several platinum structures:

- a) If Pt(II) is oxidised to its equivalent Pt(IV) it loses its anti-tumor activity, thus, perhaps planarity is required for activity.
- b) If the cis product is isomerised to a trans form, chelation with DNA is probably excluded, and inactivity results.

The metabolism of the drug is different in various cells, and the nitrogen ligand modifies the properties of the metal complex. If this modification (by mesomerism of py for example) makes the compound sensitive to cell conditions, selectivity may be the result. Pyridines are easy to oxidise and support electron transfer. The electronic structure of pyridine increases the number of options for a reaction and makes the complex more sensitive to cell differences.

A 3 Toxicity of platinum complexes and their ligands²

A 3.1. Toxicity of aquo forms:

The compounds of the form $[\text{PtA}_2(\text{aq})_2]^{2+}$ with A = amines and a dose range of 3mg/kg to 40mg/kg are highly toxic. An apparent side effect is a neuromuscular problem, thus on close observation, some animals went into convulsions as early as five minutes after injection. They convulsed periodically when forced to move, but lay dormant between spasms, which is similar to strychnine poisoning. Perhaps, the use of antidotes could be included in chemotherapy.

The order of toxicity for the amine ligands is:



The lowering of toxicity may be partially due to steric hindrance effects, this presumably reflects the reactivity of the various aquo species.

A 3.2. Toxicity of chelates:

The effect of $[\text{Pt}(\text{en})\text{ox}]$ goes into the opposite direction: in vitro studies suggested curariform activity, which is supported by the synergistic effect of atropine.

Other chelate compounds show similar effects, but to a much lower extent. This tends to support the suggestion, made on the basis of chemical evidence, that chelates act via a different sequence of reactions in comparison to diaquo complexes, although the physical symptoms are identical. It

is likely that the final site of activity is the same.

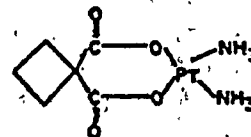
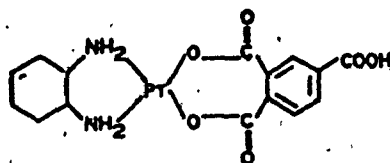
A 3.3. Nephrotoxic potential of platinum anti-tumor agents:

The nephrotoxicity of "Cisplatin" is the dose limiting factor in its clinical use. Therefore the search for decreased nephrotoxic derivatives is a main aspect in the development of new anti-cancer drugs. The indices of nephrotoxicity included blood levels of urea nitrogen and serum creatinine, kidney weight and microscopic alterations. Recently, in order to compare the ligand effects, the following compounds were tested and compared (figure 4)²⁰,

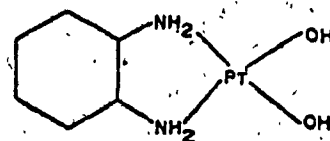
Figure 4



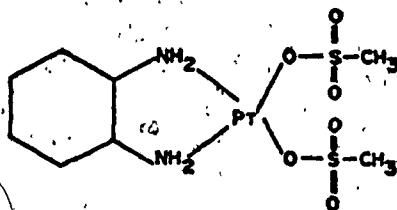
cis - Diamminedichloroplatinum

Diammine [1,1-Cyclobutanedicarboxylato
(2-)-o,o]-(SP 4-2) Platinum4-Carboxyphthalato 1,2-Diamino-
Cyclohexane Platinum

II

(1,2-Cyclohexanediamine-N,N')
Dihydroxy Platinum

III

(1,2-Cyclohexanediamine-N,N') Bis
(Methanesulfonato-O-) Platinum

IV

Structures for different Nephrotoxic Studies

The results obtained indicated that:

"Cisplatin" elevated the biochemical indices of renal function significantly. The blood levels of urea nitrogen and creatinine peaked at day 6 of administration.

The results are:

Amount and compound	urea nitrogen level
8mg/kg "Cisplatin"	176mg/100ml;
44mg/kg of I	17.4mg/100ml;
46mg/kg of II	17.4 mg/100ml;
25mg/kg of III	17.2 mg/100ml;
10.9 mg/kg of IV	39 mg/100ml.

The values of serum creatinine are parallel:

Amount and compound	serum creatinine level
8mg/kg "Cis-platin"	2.83mg/100ml;
61mg/kg of I	0.5mg/100ml;
62mg/kg of II	0.48mg/100ml;
23mg/kg of III	0.48mg/100ml;
10.2mg/kg of IV	0.7mg/100ml.

The order of toxicity in terms of ligands is: (II) diaminedicarbosylate · (I) dicarboxylate · (III) dihydroxydiaminechelate · (IV) diaminechelatebis(sulphonato) dichloro.

The kidney weight increased with the same order of toxicity as the biochemical indices. Changes in histopathology, which are characterised by hydropic degeneration, necrosis, tubular atrophy, regenerative processes with enlar-

ged tubular cells and mitotic figures, had been observed for all complexes except I, the diaminedicarboxylate structure. In a mild degree renal lesions are manifested for complex II with a lack of regenerative processes.

The order of severity in biological changes is: Bis(sulphonato) · dichloro · dihydroxy · chelates.

Summary

There are three toxic side effects observed with platinum treatment: neuromuscular activity, nephrotoxicity and inhibition of cell growth without selectivity.

The neuromuscular activity can be reduced by avoiding aquo and oxalato as ligands, and by increased steric hindrance in the aminogroup. Use of antidotes would be helpful.

The nephrotoxicity tests have suggested the desirability of replacing the chloro ligands. In clinical chemotherapy diuretics are used to diminish the kidney damage.

There is much more to say about platinum drugs, but in this context only the more relevant information with a direct correlation to the practical work can be given. For a deeper treatment of the topic, references 20 and 21 may be read.

The number of possible anti-tumor agents is quite large: The following are the main classes:

- a) alkylating agents, like sulphur mustards, chloroethylamines and epoxides with severe side effects;
- b) hormones, like glucocorticoides and androgens;
- c) antimetabolites, based on synthetic derivatives of amino acids, DNA bases etc.;
- d) peptide antibiotics, for example adriamycin;
- e) antibiotic drugs like, bleomycin;
- f) alkaloids.

In the context of platinum-pyridoindole complexes only the information about alkaloids of relevance like harmine and derivatives are of immediate importance. Further literature is given in reference 22 especially, but see also references 23-26.

The selectivity of the platinum derivatives is most distinct in pyridine derivatives.

A 4 HarimineIntroduction

The compound harmine has been isolated from *peganum harmala*, which was used for centuries against a variety of diseases. Its mutagenic property made it interesting for more research, and development of derivatives with more specific modes of action is under way.

Consideration of structure elements and functional groups in correlation with possible pharmaceutical effects is the classical way to develop new chemotherapeutica. Additional information is offered (among other methods) by the use of minimum energy conformation calculations of molecules. These methods permit some reduction in the ratio of new derivatives to be tested to clinically useful drugs. (About 10 000:1). This section of the thesis describes the structural features of harmine and suggests similarities with other naturally occurring derivatives, and with certain complexes which might be prepared by actually incorporating platinum as well into a harmine-like drug. A generalisation was proposed by Manfred Hesse²⁷ referring to structural contents of plants: There are more than 500 000 different kinds of plants scientifically described and distinguished today. They contain different kinds of plant products, including the nitrogen-heterocyclic compounds. This class of compounds can be subdivided into 18 structure types. One is indole with about 1100 known derivatives, another important

group are quinolines with 900 known derivatives. The indoles are classed in 8 biogenetically related families, of which one is the family of pyridoindoles. Among these, there are hundreds of different structures²⁷.

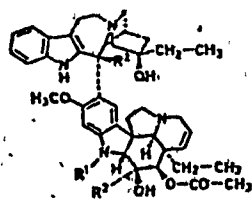
A 4.1. Structure

Structure elements play a crucial role in pharmacodynamics. The chemistry of two reagents is related by the similarity in their structure. Differentiation between the skeleton and functional groups allows modification with specific results. If the effects of a molecule can be optimised by modifying the functional groups (in terms of therapeutical improvement and reduction of toxic side effects), the changes in chemistry and physiological action are small and mostly predictable. The modification of the skeleton has two possible results: The removal of unimportant structure elements by ring opening or closure, omission of ineffective groups and replacement of toxic subunits, or the destruction of the medical value of the drug. The biochemical value of the new derivative is dependent on the effectiveness of the structural modifications. An important aspect is the structural similarity of a framework in relation to body constituents. Because every drug undergoes metabolism, it is easy to understand that an increase of this particular similarity may decrease the toxic side effects. The skeleton of harmine (IX) is a condensed pyridoindole and 12 derivatives are interesting to consider. Their similarity to well known body constituents of medicaments separates

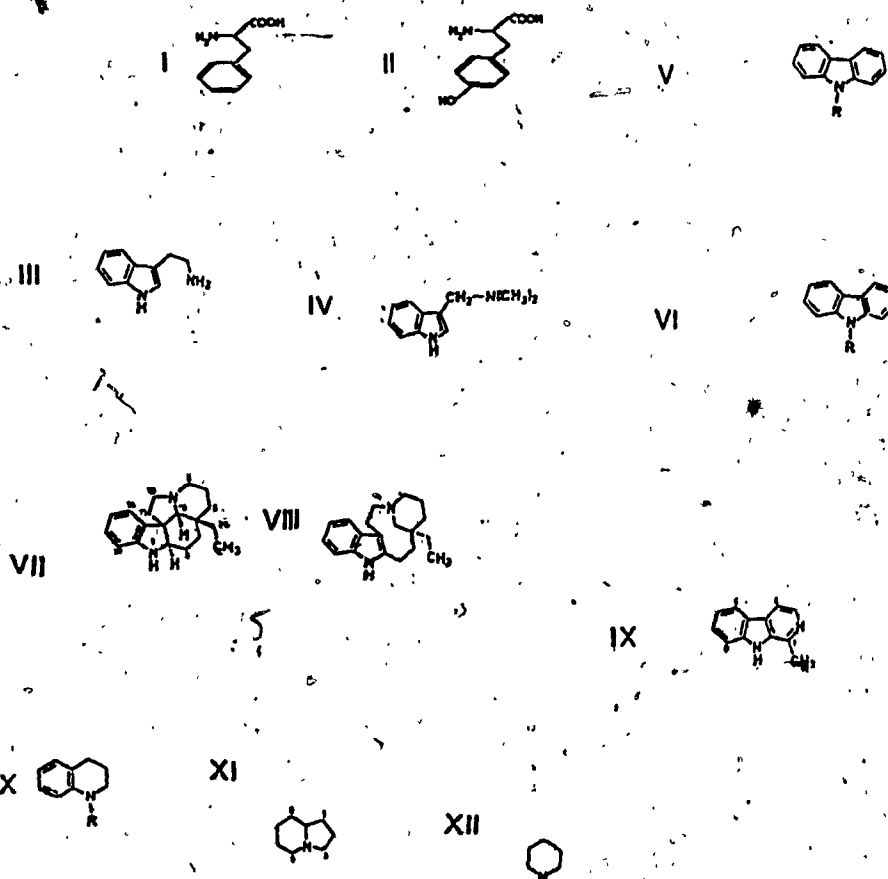
them into three categories which are shown in figure 5²⁷:

Structures I, II, III, IV are essential biochemicals in the functioning of living organisms, the structures V, VI, VII, VIII, and IX are slight modifications of these. Compounds which are similar to molecules X, XI, XII could cause problems according to prior pharmaceutical experience.

Figure 5

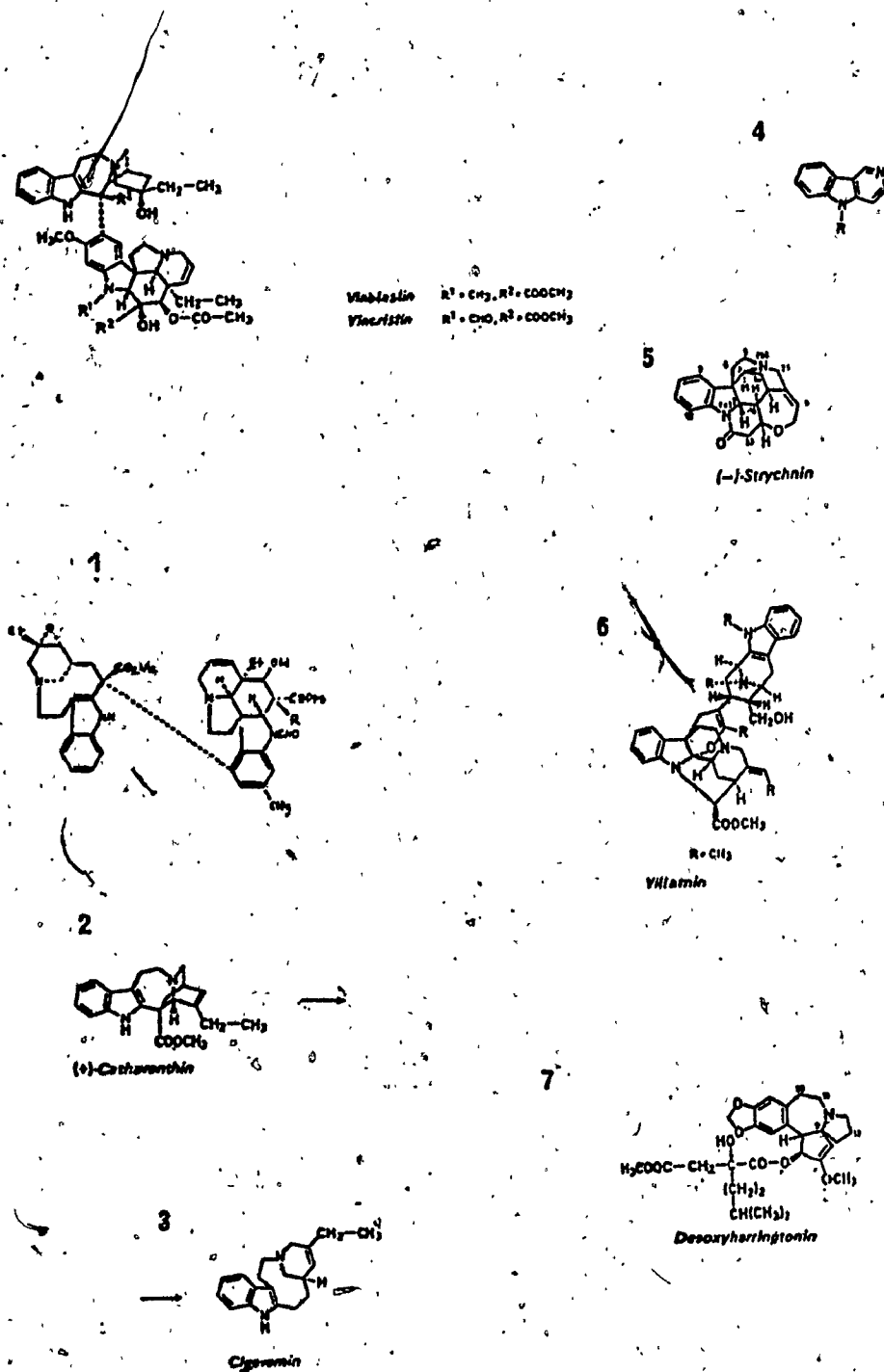


Vinblastine $R^1 = \text{CH}_3, R^2 = \text{COOCH}_3$
 Vincristine $R^1 = \text{CHO}, R^2 = \text{COOCH}_3$



Structures similar to Pyridoindole

Figure 6



Structures distantly related to Pyridoindole

A 4.3. Derivatives

Some alkaloids with anti-tumor activity and a certain structure similarity to pyridoindoles are listed in the following pages. Their different and similar effects should illustrate the not always obvious relation to the compounds in figure 5 and 6.

i) Vincristine and N-formyl leuosin (figure 6, 1) are examples of selectivity in anti-tumor drugs produced by a change in the skeleton and dimerisation. The difference to harmine lies in an extension of the pyridine ring with insertion of an izidine structure element. The drugs were administered to patients for treatment of leukemia and multiple myeloma²⁸.

ii) 4-Methyl-9H-pyrido[3,4-b]indole-2-amine, a methyl amino derivative of carboline (figure 6, 4) is a very strong anti-tumor agent. The author claims that the dosages needed for cytostatic effects are the lowest by comparison to other "chemotherapeutica"²⁹. Its activity is proposed to be related to interaction with RNA.

iii) Methyl-9H-pyrido[3,4-b]indole (figure 5, IX) shows mutagenic effects and should be investigated for cytostatic use³⁰.

iv) Normelionine, which was found in *Strychnos melinoniana*, is an example of a quaternary pyridoindole: the difference from 9H-pyrido[4,3-b]indole is a methyl group on the pyri-

dine nitrogen. It was found to have cytotoxic properties, and the mode of activity is to form intercalation bonds to the DNA. Very close structures like alstonine, serpentine, sempervirine and 9-hydroxy-2-methylellipticinum seem to be attractive candidates as potential antitumor agents and the latter is already undergoing clinical trials¹³.

v) Desoxyharringtonine (figure 6, 7) is a Cephalotaxus alkaloid with antileucemia activity, and it has the indolizidine structure element (XI), significantly also found in vincristine, although it is classed among the Erythrina group, interferes with enzymes giving cytostatic effects^{22,27}.

Conclusions

From the structure derivatives of harmine, compounds structurally related to VI-IX and XI show an anti-tumor activity. The structure element XI has been classed with the toxic group of agents.

A cis-Pt(9H-pyrido[3,4-b]indole)₂ structure would have the structure elements of I- XI, while X-XII are absent and could be expected to have selective cytostatic and less toxic properties.

Structure similarity of alkaloids and Pt-pyridoindole:

Mono alkaloids can dimerise like catharantine (figure 6, 2) with its derivative cleavamine (figure 6, 3) to vincristine, which is biogenetically and structurally related to villamine (figure 6, 6). Villamine is a bis-pyridine structure. The dimerisation is an essential skeletal change with significant pharmaceutical consequences. The compounds 1-6 have tryptamine as their biological origin. For the synthesis of platinum complexes, functional groups with oxygen must be omitted in the first steps of synthesis because they are likely to be an active ligand site for the complexation with the metal and would make any identification of structures virtually impossible. The structure of pyridoindole can be found in harmine like anti-tumor agents and might be recognised more or less in the monomers of the alkaloids of the dogbane and strychnos family.

As quaternary bases like normelionine provide a cytotoxic effect, complexation to a metal, instead of the methyl group on the py-N would not interfere with the ability to intercalate with the DNA. Comparison of reactions of a copper phenanthroline complex with DNA, which have the same intercalation (with DNA), suggests a mechanism which involves oxygen radicals, bonded and activated by the transition metal, which are short lived but can degrade the cell nucleus because of the geometrical vicinity to the DNA. The same mechanism is possible with a platinum-pyridoindole complex, whereby the norharman intercalates to the DNA and the redox-

potential of Pt(II)/Pt(IV) center makes the action of oxygen radicals with the DNA possible, which would otherwise be transformed by Super-oxidismutase or catalase³².

The synthesis of Pt-complexes with 9H-pyrido[3,4-b]indole (pyin) as ligand seems to be promising in terms of reduced toxic side effects in the treatment of cancer. The bulky structure of three condensed rings provides the steric hindrance of the amine group which is helpful in reducing neuromuscular activity. The selectivity of these derivatives is expected because of the relation to cis-dipyridine-dichloroplatinate. The difference in the pyridine complex lies in the similarity to body constituents and drugs with practically no toxicity. Newest results indicate that the amine ligand is labile in vitro³³, thus a further source of poisoning can be eliminated by pyridoindole. The solubility of the complexes should be in the window of polarity: too high a solubility in water removes them from the body before a DNA interaction, too low a solubility reduces the possibility of reaching the cell centers at all. If the solubility of a compound is determined in neutral solution at 20°C, the solubility in blood might be quite different because of the higher temperature of 37°C, different buffer capacity, and additional compounds in solution with possible "salt in/out" effects. The calculation of Hansch π values are not of concern in the beginning of a synthesis work, because the determination of the platinum-nitrogen complexes requires omission of functional groups, which might be nece-

ssary for solubility effects and/or biochemical functions. A comparative pharmacology of the harmala alkaloids found, that omission of the methoxy group abolishes the tremorigenic potency along with the cardiovascular actions. "These differential activities are not readily explained in terms of partition coefficients or pka values"³⁴. Clearly another reason for the large differences in the behaviour of harmine derivatives must exist, a likely possibility being the manner with which the functional groups like methoxy, phenoxy or hydroxy interact with the protein binding sites³⁸.

Part B

Synthesis of Platinum complexes

with 9H-pyrido[3,4-b]indole

Introduction

This part of the thesis describes the attempts to synthesize species containing the $\text{Pt}(\text{pyridoiindole})_2$ moiety with a view to eventual testing as an anti-tumor agent.

B 1 Difficulties in platinum heterocyclic chemistry:

Examples of previous work: The following cases illustrate some of the pitfalls in the synthesis of characterisable Pt complexes:

1 Normal synthesis of cis- PtCl_2L_2 with $\text{L} = \text{pyridine}, \text{NH}_3, \text{NEt}_2, \text{NMe}_2, \text{en}, \text{etc.}$ occurs by mixing equimolar amounts of tetrachloroplatinate(II) with the amine ligand in water at room temperature and pH 7. The reaction product is formed within 15 min. to 3 hrs. in high yield. Isolation is usually possible by evaporation of water and recrystallisation in organic solvents. In the case (water insoluble amines), addition of HCl provides a water soluble salt, but the reaction time is increased by a factor of 10. The reaction of tetrachloroplatinate with amines normally leads to cis-diamine-dichloro complexes. In the case of more complicated heterocyclic bases, difficulties can arise in isolating products.

2 In the case of a reaction from $[\text{Pt}(\text{NH}_3)_3\text{Cl}_3]\text{Cl}$ to trans dichlorodiaminoplatinate, two products are formed: the trans product was identified by IR, but the second complex is not cis equivalent and had an unknown composition³⁵.

3 For the isolation of bipy complexes, careful fractional

recrystallisation and column chromatography was found to be necessary to obtain clean elemental analysis values, even though only one compound was detectable by thin layer chromatography³⁷.

4 cis-[Pt(NH₃)₂Cl(MeCyt)]Cl could be synthesised in a 30% yield mixed together with cis-[Pt(NH₃)₂(MeCyt)₂]Cl₂ in a 7-10% yield. Separation of the compounds was carried out by repeated recrystallisation. At a later stage of the crystallisation process, mixtures of 1:1 and 1:2 complexes and unreacted starting material were obtained³³.

5 Formation of trans-[PtCl₂(NH₃)(Mecyt)] x 1.5H₂O was observed when a sample of the corresponding cis product has been recrystallised from water. The yield of the cis compound varied between 2 and 5%. During the synthesis of hydroxy complexes like [Pt(NH₃)₂(Mecyt)(OH)], three compounds crystallise out of solution, one could not be identified³³.

6 The reaction of 1,2-diaminobenzamide with tetrachloroplatinate(II) gave the cis-diaminodichloro complex identified by IR spectroscopy. In the elemental analysis the values for Cl vary over 4%, C over 2%, N over 2% and H over 1.5% and no values for Pt are available³⁹.

7 With nicotinamide, a similar result is reported with a Pt/Cl ratio of always more than 1:2⁴⁰.

B 2 General summary of synthesis work

The synthesis of the expected cis-dichlorodipyridoindole complex required a reaction time of at least 12 hours. In the crude reaction product there were 4 complexes besides starting materials. The work up with inert solvents like benzene or halogenated hydrocarbons was not successful in separating the complexes and free pyin. Use of elevated temperatures gave brown products, oxygen containing solvents gave ligand exchange reactions.

The reaction of tetrachloroplatinate with pyridoindole was carried out in water and water/chloroform or dichloromethane solvent mixtures. Water had to be present because the starting platinum complex is ionic, while an organic solvent was helpful because of low solubility of pyridoindole in water. Oxygen containing solvents had to be avoided. The conditions of the reactions were varied in temperature, acidity, concentrations, atmosphere, addition of anions and time.

The separation of products was developed over a long period of time until the most successful method was established. The efficiency of these operations was monitored by thin layer chromatography. Typically, the organic layer was extracted with different solvents in a defined sequence, evaporation of solvents on a watchglass gave rings of different fractions, distinguishable by colours and the appearance of the solids ("recrystallisation"). For column chromato-

graphy, the solvent system used in the thin layer chromatography proved to be suitable, but with replacement of acetic acid by chloroform.

Identification:

Out of a variety of products three compounds Ia, IIa, IIb were isolated and tentatively identified by IR-spectroscopy. Further experiments were directed towards the synthesis of compounds with additional ligands and counterions, they are described at the end.

The mass spectra, using both conventional and fast atom bombardment (FAB) sources were obtained for several samples. Comparison of the behaviour of the samples under thermal degradation in the spectrometer was revealing. NMR and UV were also recorded for selected materials, but were of limited usefulness. The results of elemental analysis for certain compounds were only partly useful in the attempt to identify the products.

Assignment of compounds Ia, IIa, IIb

Ia: based on reaction time, TLC, IR-, UV-spectra, elemental analysis, and following products, the most probable Pt containing species in the compound is: $[\text{PtCl}_3(\text{pyin})]^-$

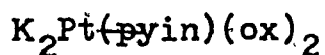
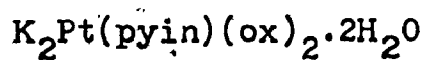
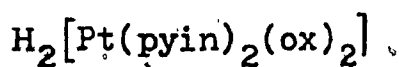
IIa: based on reaction time, TLC, IR-, mass-, NMR-, UV-spectra, elemental analysis and derived products, the compound probably contains the Pt complex ion: $[\text{PtCl}(\text{pyin})_2(\text{OH}_2)]^+$

IIb: based on reaction time, TLC, IR spectra and derived

products, the substance contains: $\text{PtCl}_2(\text{pyin})_2$ and weakly associated pyridoindole.

Additional compounds:

Several complexes with oxalate ligands were tentatively identified, predominantly from their elemental analysis and IR-spectra. They were:



B 3 Practical work

General reaction techniques

This section describes the experimental work performed to prepare and isolate identifiable complexes of Pt with 9H-pyrido [4,3-b]indole.

All reactions were carried out in a nitrogen atmosphere and with deoxygenated solvents, except as noted.

The reactants were weighted out into a 100ml round bottom flask with a two-way inlet connection. The flask was then connected to a high vacuum pump to remove the air, which was subsequently replaced by nitrogen. The deoxygenated solvents were added to the flask while flushing the containers with nitrogen. To remove any remaining oxygen, the reaction mixture was connected again to high vacuum, and then held under a slow stream of nitrogen for the entire reaction period with the N₂ outlet sealed with an oil bubbler. The reaction mixtures were stirred with a magnetic stirrer.

The chemicals used in all of the reactions were obtained from Aldrich. The solvents which were obtained from standard university stocks were distilled under nitrogen.

B 4 Preparation of platinum-pyridoindol complexes

Experiment

3.5g (8.397mmol) potassium tetrachloroplatinate and 0.5 (2.97mmol) 9H-pyrido[3,4-b]indole were stirred in a two phase system of 50ml H_2O and 50ml CH_2Cl_2 . The platinum compound dissolved in water to give a red colour, but, being an ionic salt, it was insoluble in the dichloromethane layer. The colourless pyin was slightly soluble in both solvents. After 15 minutes a grey precipitate was visible, and within 12 hours a yellow colour dominated. The removal of the red water solution gave a dichloromethane suspension which separated into a precipitate and a clear yellow solution. Evaporation of the organic solvent resulted in a yellow powder. Product: 231mg of mixture of the grey and yellow solid materials.

A number of variations of the reaction conditions were tried:

- a) solvent: Absence of an organic solvent gave poorer yields. Oxygenated solvents like methanol and ethanol lead to decomposition.

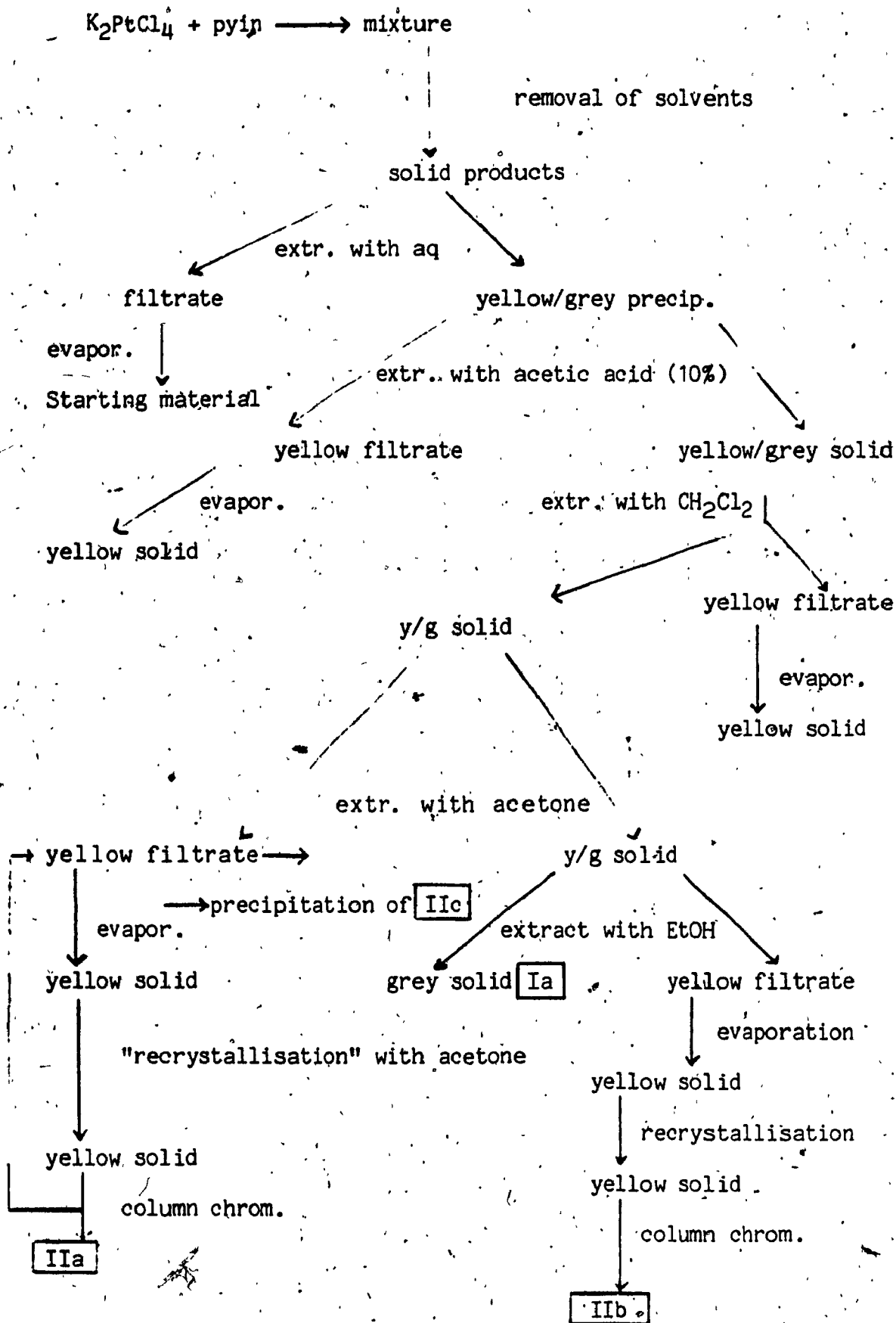
- b) temperature: Heating to 50°C resulted in decomposition. Cooling to 0°C gave poor yields.
- c) acid: Addition of HCl produced a Pt-mirror.
- d) differing concentrations: No changes were observed.
- e) atmosphere: The presence of oxygen caused the formation of additional black and brown components.
- f) time: 15min gave the grey compound only; in the range of 12h-2days, yellow and gray products were observed, and after 5 days partial decomposition occurred giving brown materials.

A reaction of the initial grey material (after isolation) (50 mg) with pyridoindole (20mg) in dichloromethane gave yellow products after 12h-2days.

B.5 Work up methods

After every reaction, the water layer was separated from the organic layer with a separatory funnel. Solids were separated by filtration. The organic solvents were removed in vacuo and the products dried, washed with water several times to completely remove potassium tetrachloroplatinate and potassium chloride, and again dried on the vacuum line. During attempts to isolate pure compounds a rather complicated separation scheme was developed.

The following flow chart summarises the more successful procedures followed (Table):



B 5.1. Elemental analysis

Elemental analyses for all but one of the samples were performed by Galbraith laboratories Inc., Knoxville, Tennessee USA. The elemental analysis for the sample IIId were performed in the "Science Industrial Research Unit (SIRU) in the Department of Chemistry of the Concordia University.

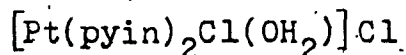
Hydrolysis of IIa (50mg) in the presence of oxalate ions gave a product without Cl and the fingerprint region of pyin. The elemental analysis suggested the following formula: $[\text{Pt}(\text{pyin})_2(\text{OH})_2] \cdot 2\text{H}_2\text{O}$

Found: Pt 32.53% C 43.36% H 2.67% N 9.17%

calc.: 32.41% 43.81% 3.59% 9.32%

diff.: 0.12% 0.45% 0.92% 0.14%

The recrystallisation of IIa in water containing excess malonic acid provided a product with the following composition:



Found: Pt 30.27% C 44.10% H 2.48% N 8.45%

calc.: 31.39% 42.58% 2.90% 9.03%

diff.: 1.12% 1.52% 0.42% 0.58%

A reaction of IIa (50mg) with acetone over a period of 5 days gave the product IIId.

Results of the elemental analysis for Ia, IIc, IIId cannot be assigned to a defined complex:

Results:

	C	H	N	Cl	Pt	R(%)
Ia	38.98	2.75	7.29	14.86	31.30	4.19
IIc	31.01	2.84	7.72	8.27	35.6	14.56
IIId				0.09	22.9	78.01

Elemental ratios:

elements	Ia	IIc	IIId
Pt/Cl	PtCl _{2.6}	PtCl _{1.26}	no Cl
Pt/N	PtN _{3.1}	PtN ₃	
Pt/C	PtC ₂₀	PtC ₁₇	
Pt/H	PtH ₁₆	PtH ₁₆	
Pt/pyin	Pt(pyin) _{1.5}	Pt(pyin) _{1.5}	Pt(pyin) ₃
Pt/C-pyin	PtC _{0.78}	PtC _{0.75}	
Pt/H-pyin	PtH ₂	PtH ₄	
Pt/R	111.8 amu	135.9 amu	

R= oxygen or potassium

B 6 Optical appearances:

The nature of the products was investigated by the use of a microscope. The colour, grain size of solids, and the form of the solids was clearly dependent on the time of exposure and nature of solvent. It was unclear if a modification by solvents or a reaction with them is reflected here, probably both events took place.

Eighteen different fractions could be separated by column chromatography or "recrystallisation" on a watch glass.

Relatively stable forms are:

- a grey powder (Ia),
- a yellow crystalline structure (IIa),
- a bright yellow powder (IIb)
- and the very pale yellow (IIc).

Extraction of the reaction mixture with ether provided an oil or a powder dependent on evaporation time, suggesting that continual modifications were an important source of variations in the nature of the products.

The platinum(II) complexes were precipitated as fine powders in all the conditions which are usually used to produce single crystals. The methods that did not succeed included: slow evaporation of the solvent in a "crystallisation bridge", evaporation by partly exposure to air in the fridge, fume-hood, etc., or slow condensation of a non-solvent into the solution.

B 7 Characterisation Attempts

B 7.1. Thin layer chromatography:

The technique of thin layer chromatography was found to be invaluable to monitor reaction conditions and work up methods.

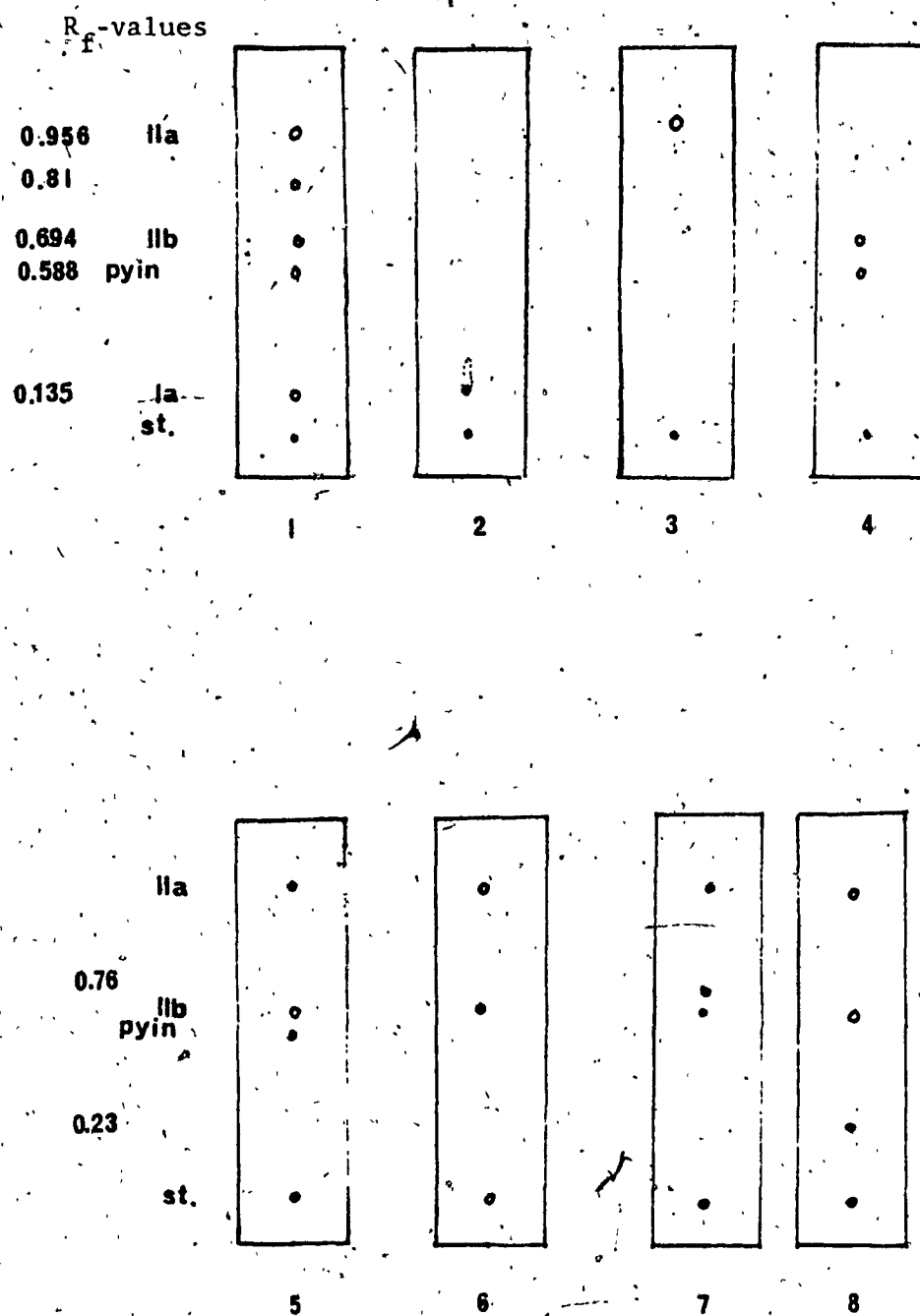
The use of the TLC technique was started relatively late, when it was discovered that instead of $\text{Pt}(\text{pyin})_2\text{Cl}_2$ as principal product, a complex reaction system was leading to multiple products, and further complications were occurring by

ligand exchange reactions between the primary products and the solvents used to isolate them.

The TLC plates consisted of Eastman 13181 silicagel with a fluorescence indicator(6060) and the spots were viewed with UV-light. The best solvent mixture was found to be a mixture of acetic acid, propanol, butanol and ether in a 1:2:1:1 ratio which was suitable for separation of more than five compounds in one chromatogram. The aromaticity of pyridoindole gave an additional fluorescence effect in the UV-light and the pure organic compound was easily distinguished from Pt-complexes. PyinH^+ also shows fluorescence, but it lacks the bright blue phosphorescence, after the UV-light is switched off, which was observed for pyridoindole.

A selection of the most informative chromatograms is depicted diagrammatically in figure 7.

Figure 7



TLC-Sheets obtained during Isolation of Compounds

B 7.1.1. Description of TLC-sheets:

- 1: the crude reaction product, applied as a CH_2Cl_2 suspension on the plate.
- 2: grey residue after washing with miscellaneous solvents
- 3: IIa purified by extractions, "recrystallisation" and column chromatography
- 4: IIb separated from other Pt-complexes
- 5, 6, 7: successive steps in the isolation of IIa
- 8: decomposition of IIa after 3 weeks in acetone

Grey material Ia:

This compound does not seem to be stable to work up with repeated solvent washings. The chromatogram 2 shows a significant pyridoindole tail suggesting that Ia has weakly bound pyridoindole which it slowly loses. The literature contains at least one example of Pt-complexes hydrogen bonded to further "outer sphere" ligands⁴¹.

Yellow product IIa:

This reproducibly produces a single spot on the TLC suggesting a single relatively stable product.

Behaviour of product IIb:

This material appears to be contaminated with excess pyridoindole. Attempts to purify IIb lead to conversion.

Behaviour of primary products with oxygen containing solvents:

During the isolation using 0-solvents a compound with the R_f value 0.81 disappears, but the treatment with these solvents in extraction and recrystallisation process provides two further complexes with the R_f values 0.76 and 0.23.

B 7.1.2. Suitable solvents for Pt-complexes as deducted from several TLC sheets:

solv.	good	partly	insoluble	decomposition
HCl/conc		IIa, b	Ia, IIc	
H ₂ O		IIa	IIb, c, Ia	
10% acetic acid	pyin	IIa	IIb, c, Ia	
MeOH	IIb	IIa	Ia, IIc	
EtOH	IIb	IIa	Ia, IIc	
Propanol		IIa, IIb	Ia, IIc	Pt(IV)
Butanol	IIc	IIa, IIb	Ia	
EtOOCMe		IIa, IIb	Ia, IIc	
acetone	IIa	IIb	Ia, IIc	
PhCH ₂ OMe		IIa, IIb	Ia, IIc	
Ether		IIa, IIb	Ia, IIc	
CH ₂ Cl ₂ /	pyin	IIa, IIb	Ia, IIc	
CHCl ₃				
THF			Ia, IIc	IIa, IIb
phH			Ia, IIa, b, c	
DMSO		IIa, IIb	Ia, IIc	
aqua regia				<u>Ia, IIa, b, c</u>
TEAM	IIc	IIa, b, Ia		
glycerol/crown ether	Pt(IV)			

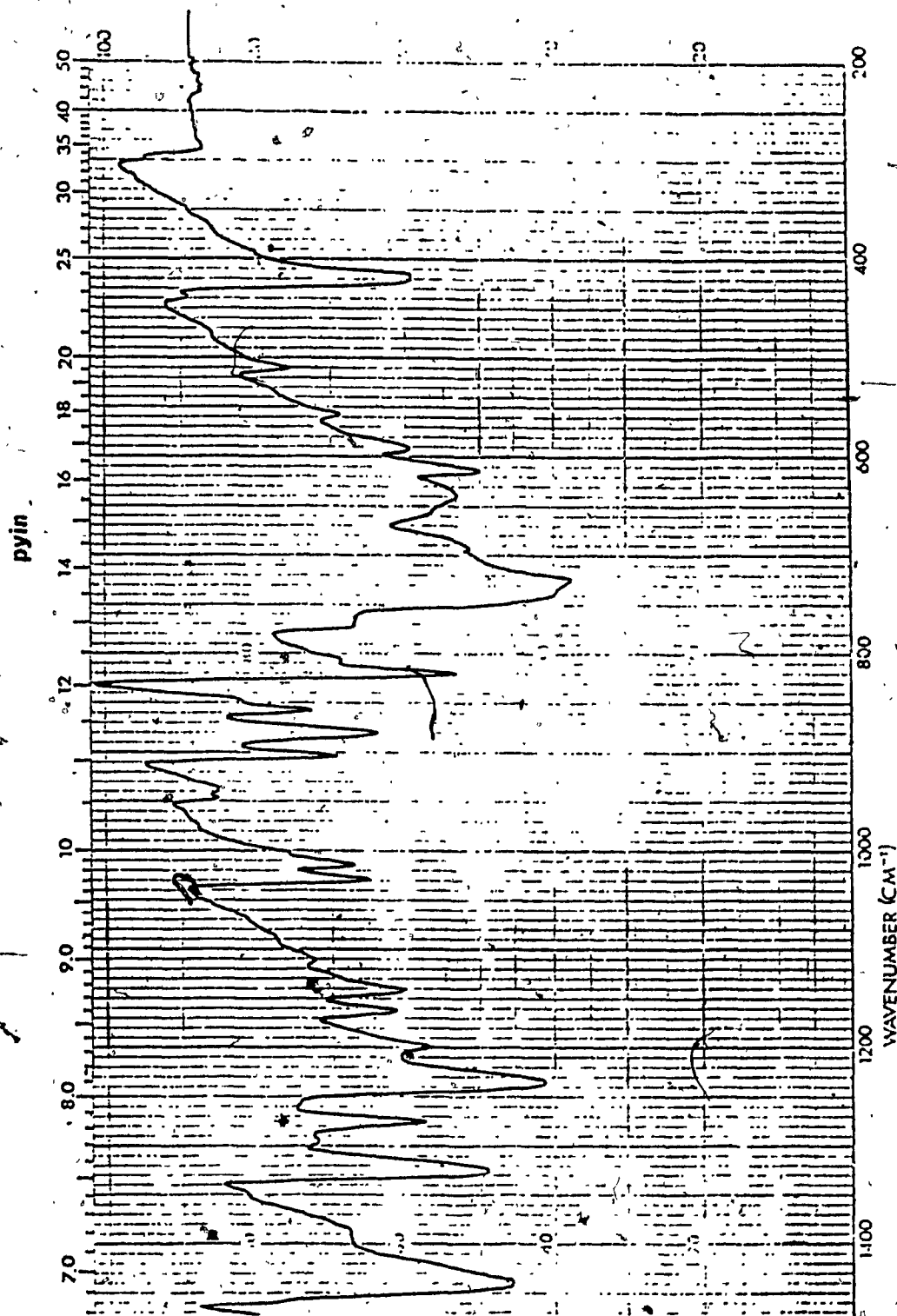
B 7.2.1 IR-spectroscopy:

The small quantities of products suggested KBr pellets as the method of choice for sample preparation. The potassium bromide was dried by storing it in an oven at a 110°C . The pellets were prepared using a pressure of 500 atm.

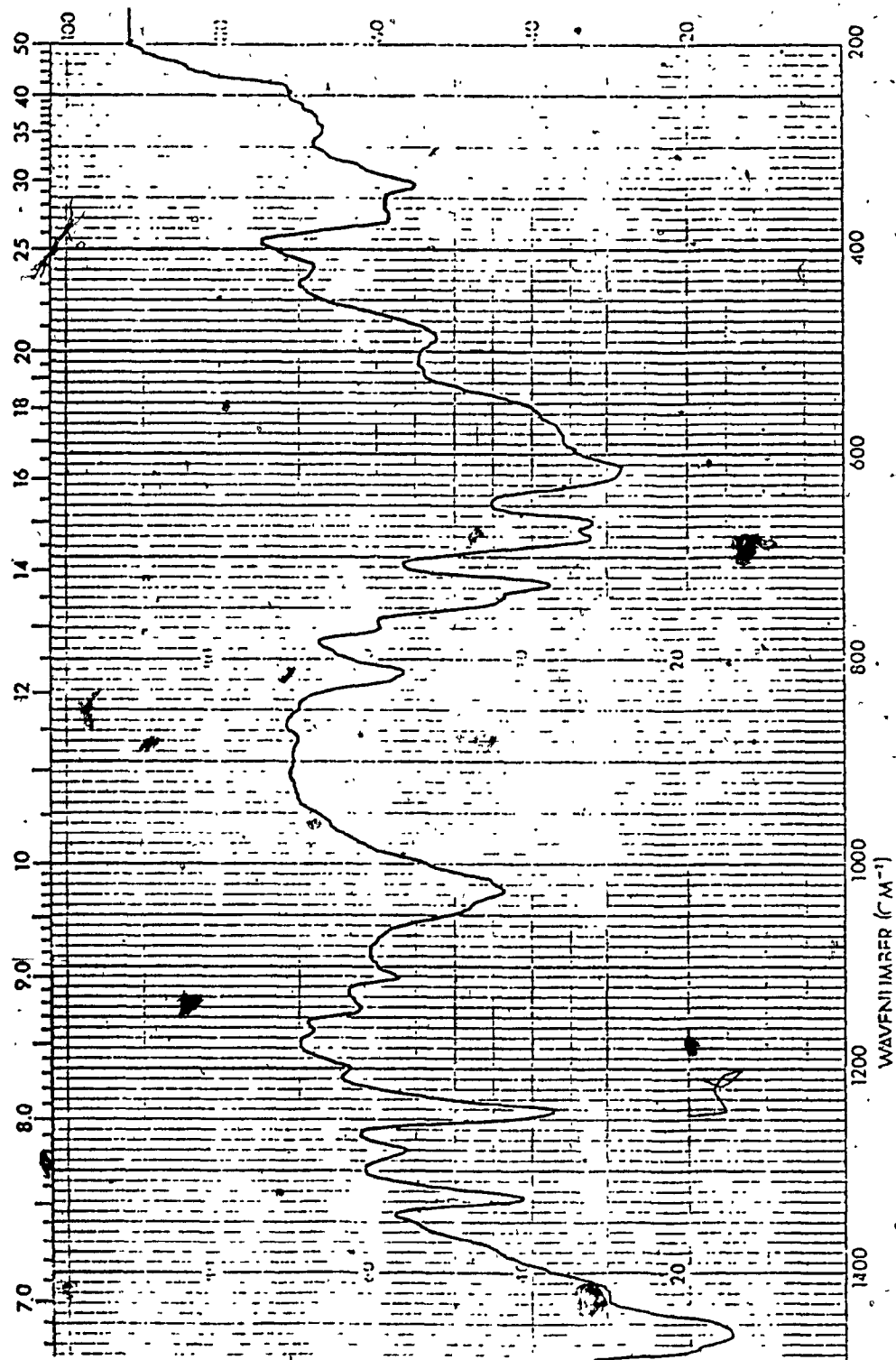
Infrared spectra were recorded on a Perkin-Elmer double beam grating infrared spectrometer (model 457).

The following figures 8-11 show the IR-spectra of pydidoin-dole and its platinum complexes Ia, IIa and IIb in the region $1500\text{-}200\text{cm}^{-1}$. Figures 23 and 13 show the region from $200\text{-}600\text{cm}^{-1}$ for convenience in making comparisons. The interpretation is more successful by considering other analytical information too, and will follow at the end of the chapter.

Figure 8



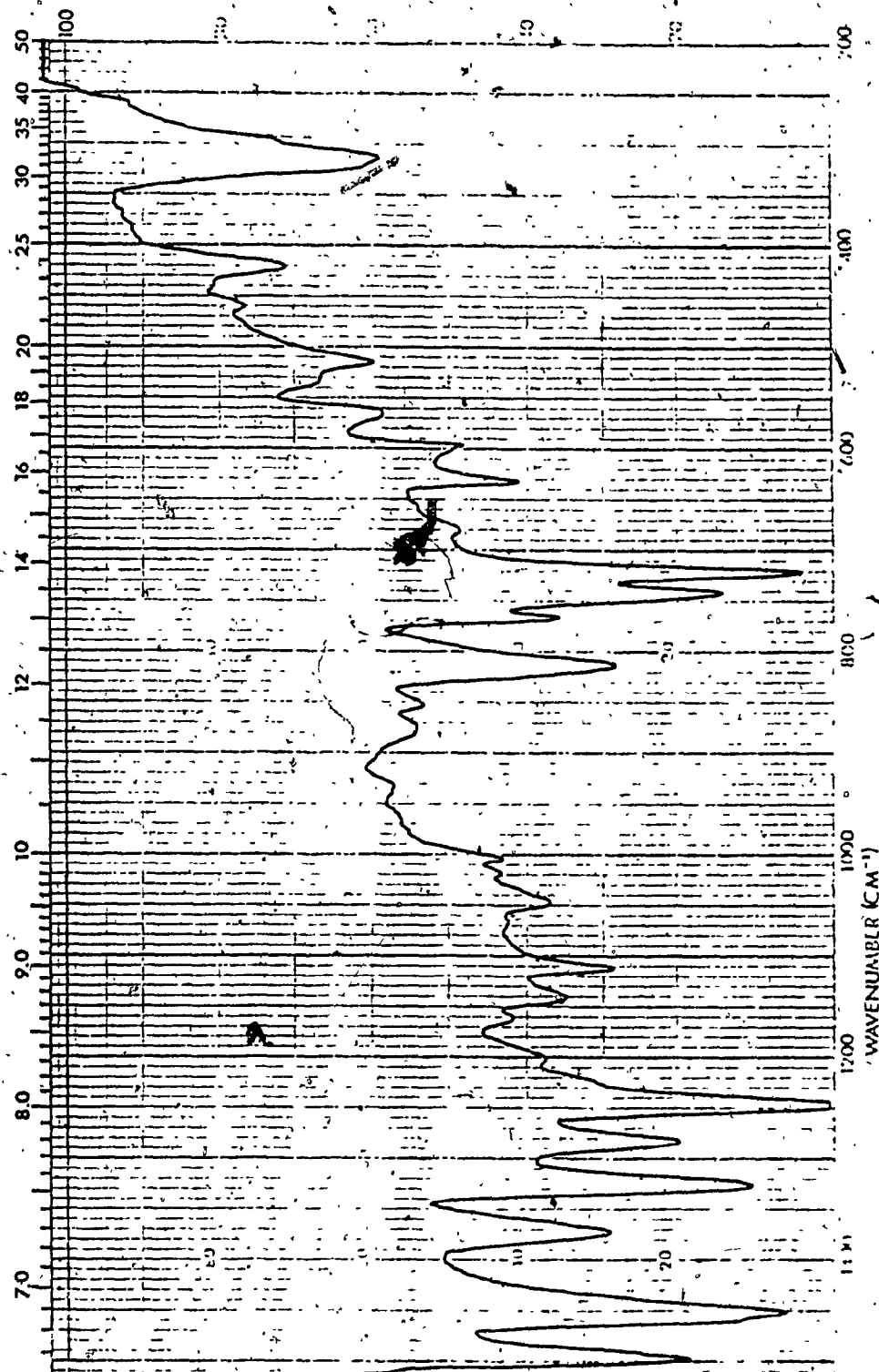
IR-Spectra of Starting Material Pyridindole

Figure 9
IIb

IR-Spectra of Product IIb

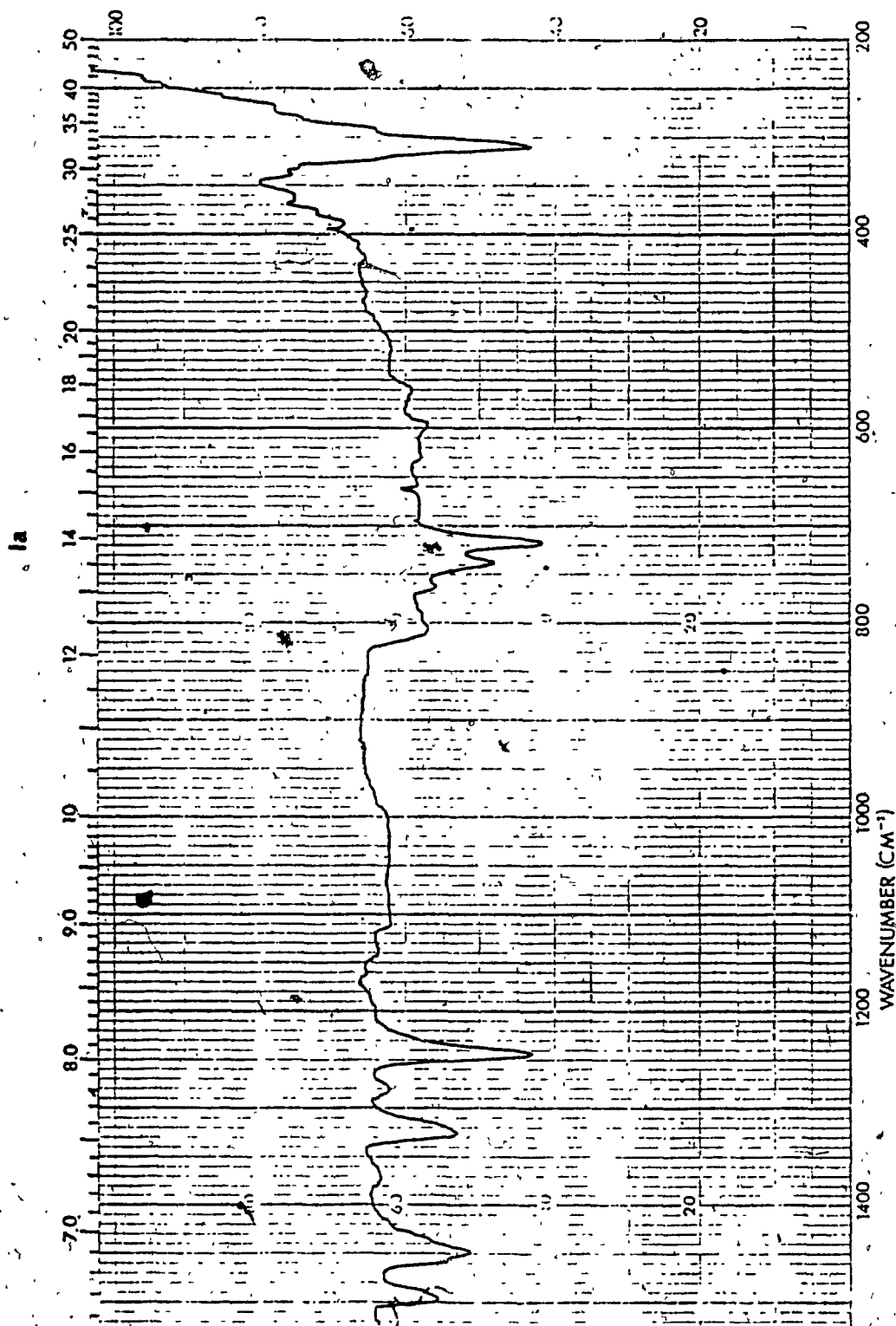
Figure 10

IIa



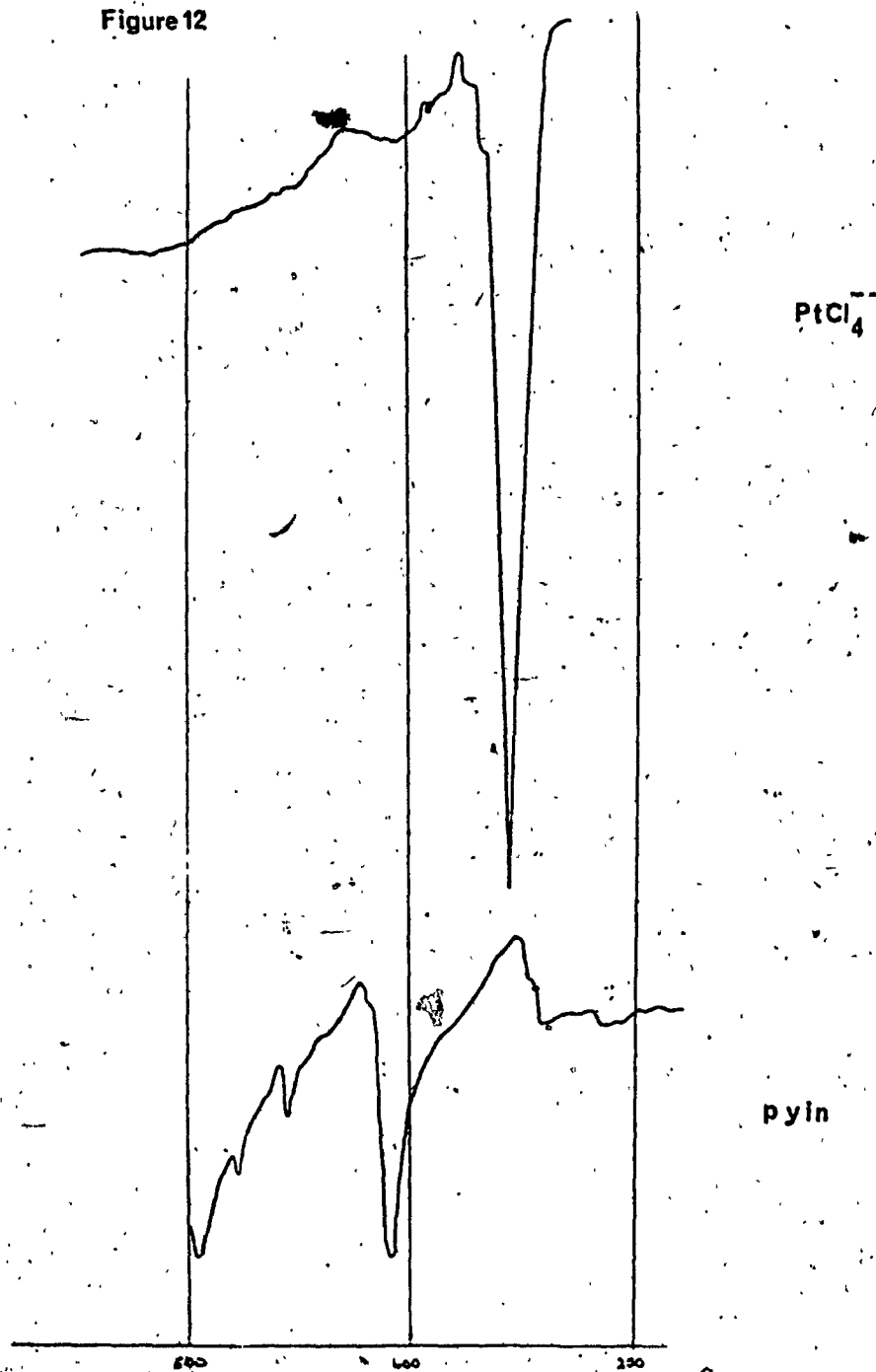
IR-Spectra of Product IIa

Figure 11



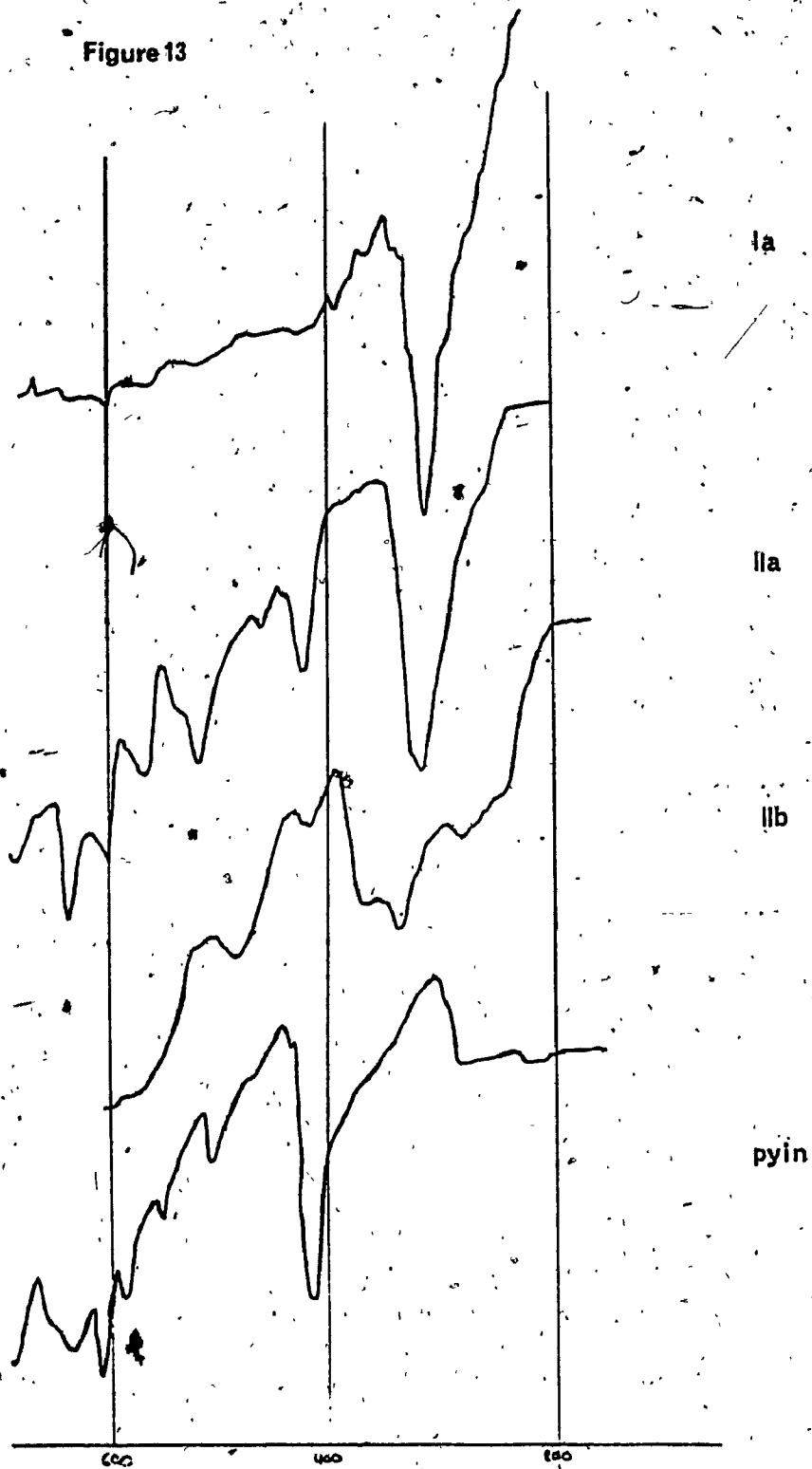
IR-Spectra of Product Ia

Figure 12



IR-Spectra of Starting Material

Figure 13



IR-Spectra of Products and Starting Material

B 7.2.2. Ligand exchange reactions of platinum complexes:

As a consequence of observations during the synthesis and work up procedures, the reactivity of compounds with oxygen containing ligands has been tested.

The Pt-Cl and Pt-O bands in IR spectra are the main diagnostic for structural changes. The Pt-O band lies in between two strong characteristic pyin bands and is absent in structures where solvation by oxygen containing solvents or hydrolysis has been excluded.

Experiments seeking to detect ligand exchange reactions were carried out with 20mg of the starting material (Ia, IIa, IIb, IIc) and 75ml of the solvents. In the case of KOH a 10% water solution was used. GMP was added in excess to purified IIa in a 1:1 acetone/water mixture. Isolation of compounds proceeded by evaporation of solvents until the first precipitate could be collected.

The course of the reactions was followed by the use of IR-spectra: see figures 14-18.

Figure 14

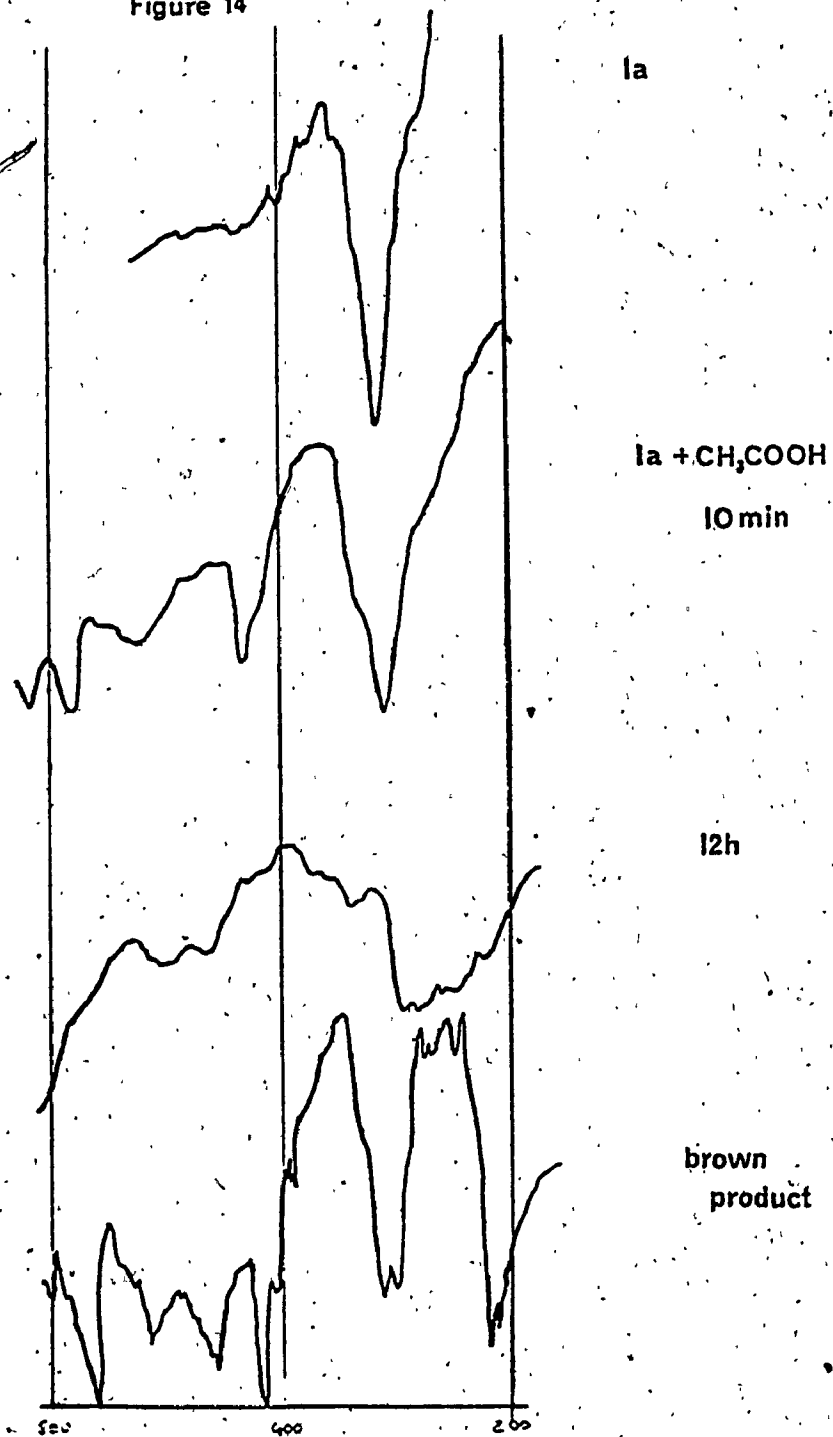
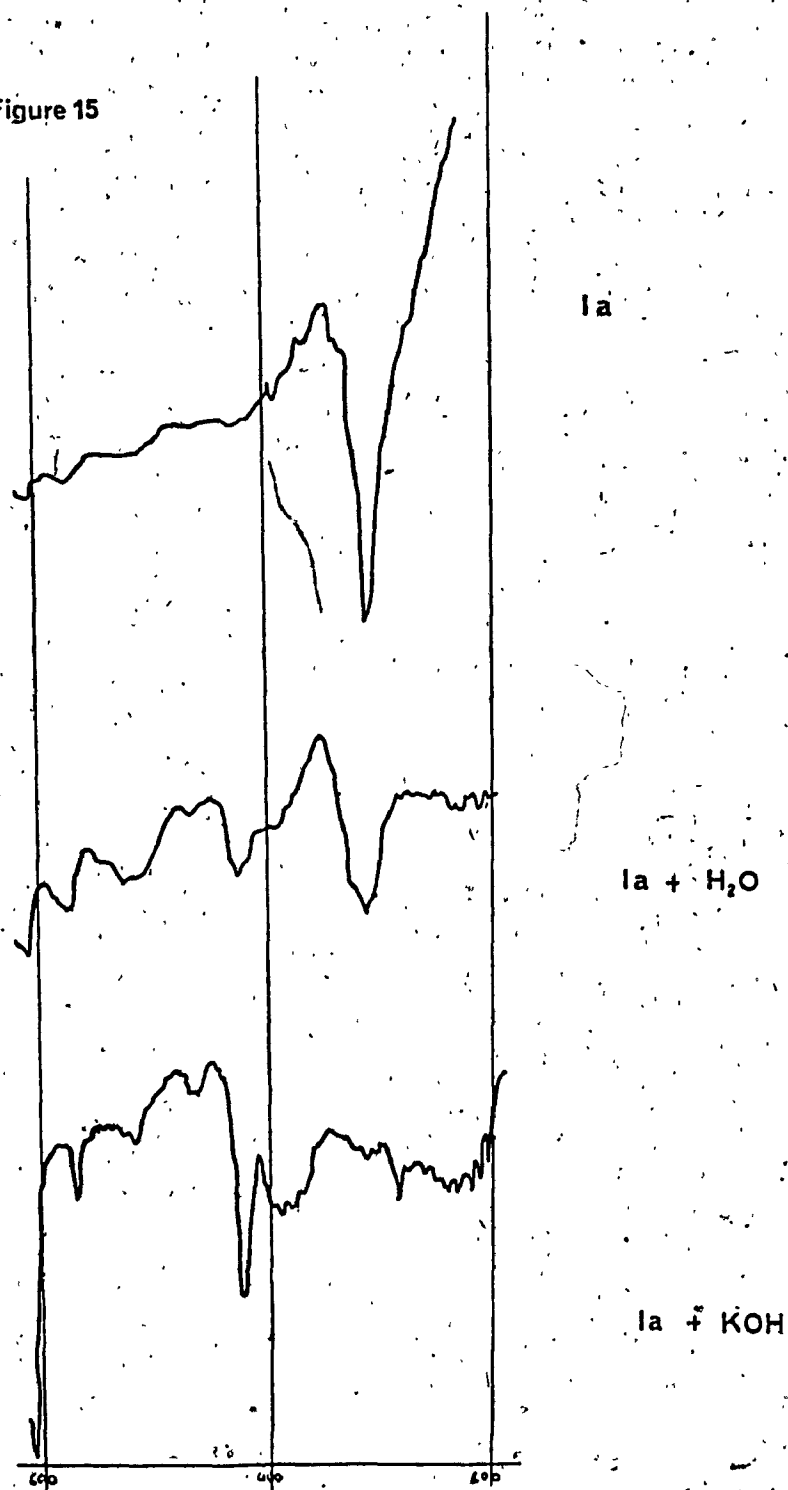
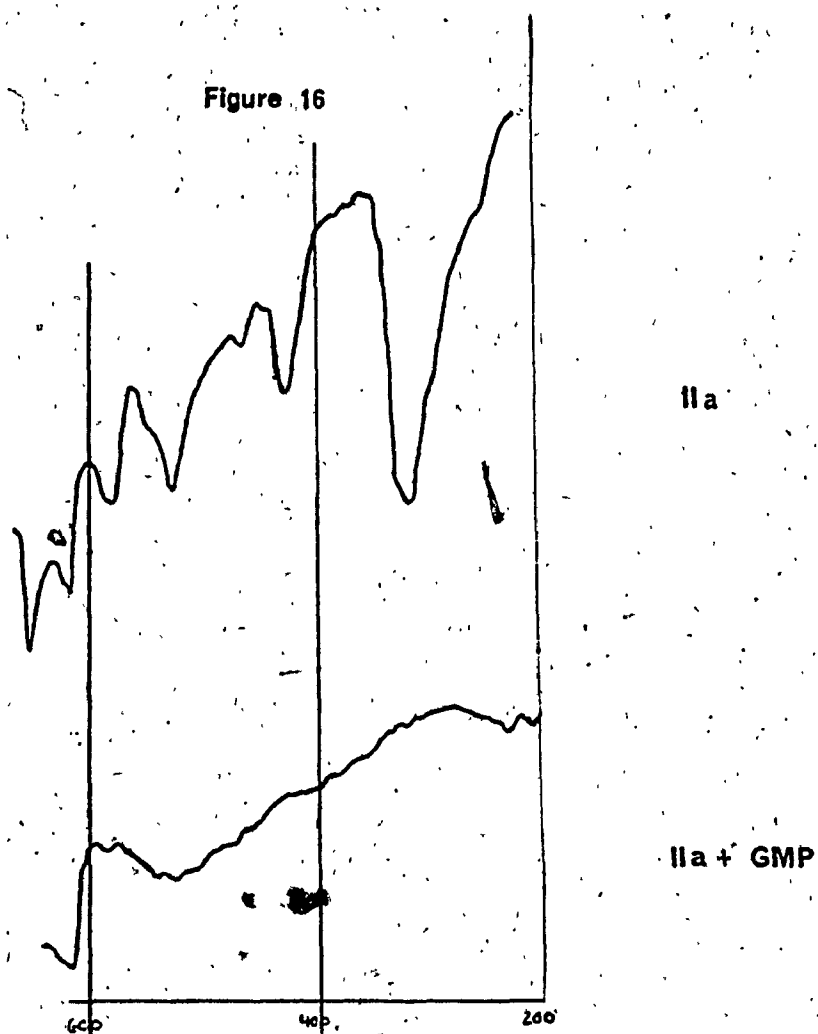
IR-Spectra of Ligand Exchange with CH₃COOH

Figure 15

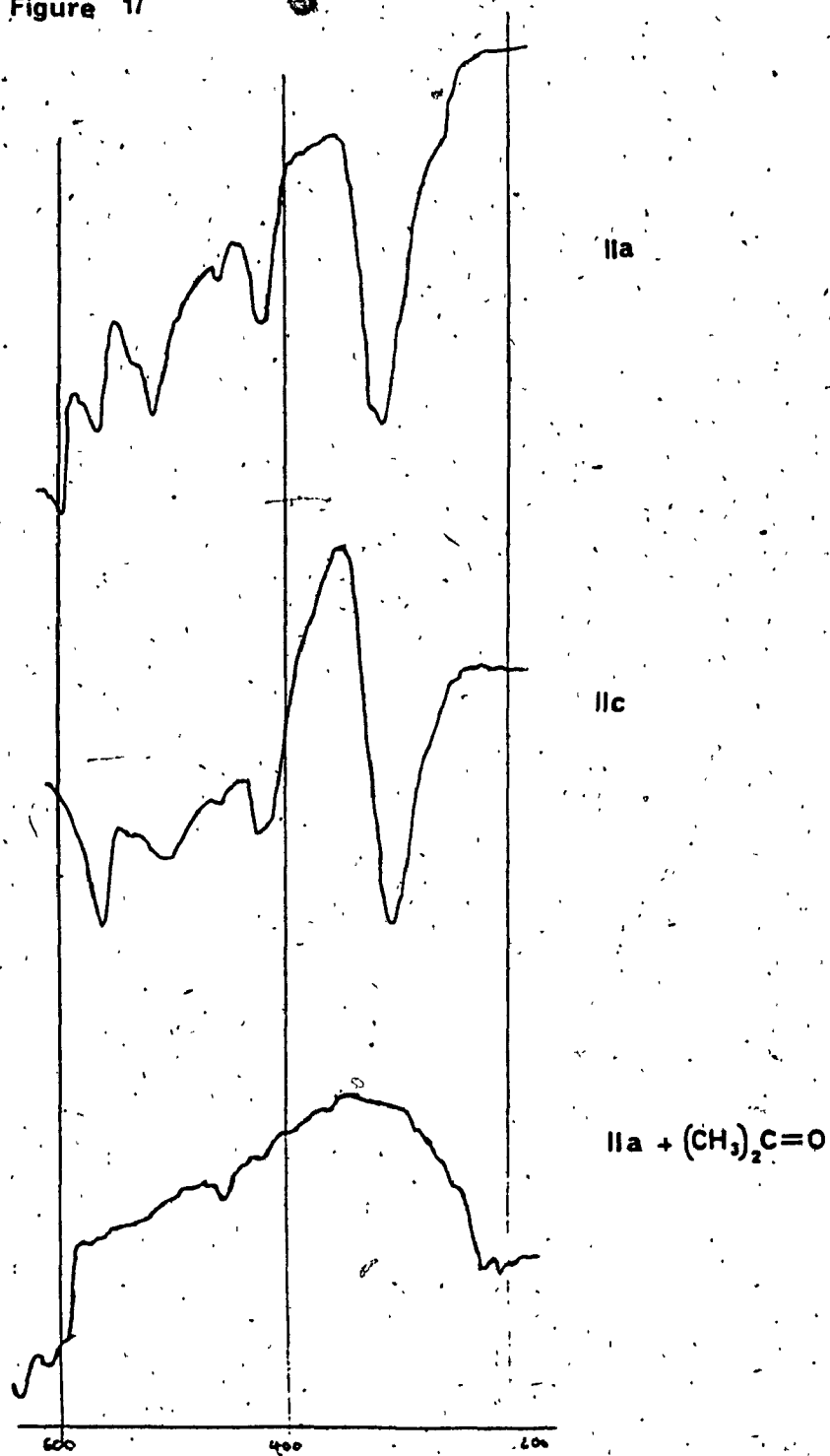


IR-Spectra of Ligand Exchange with H₂O



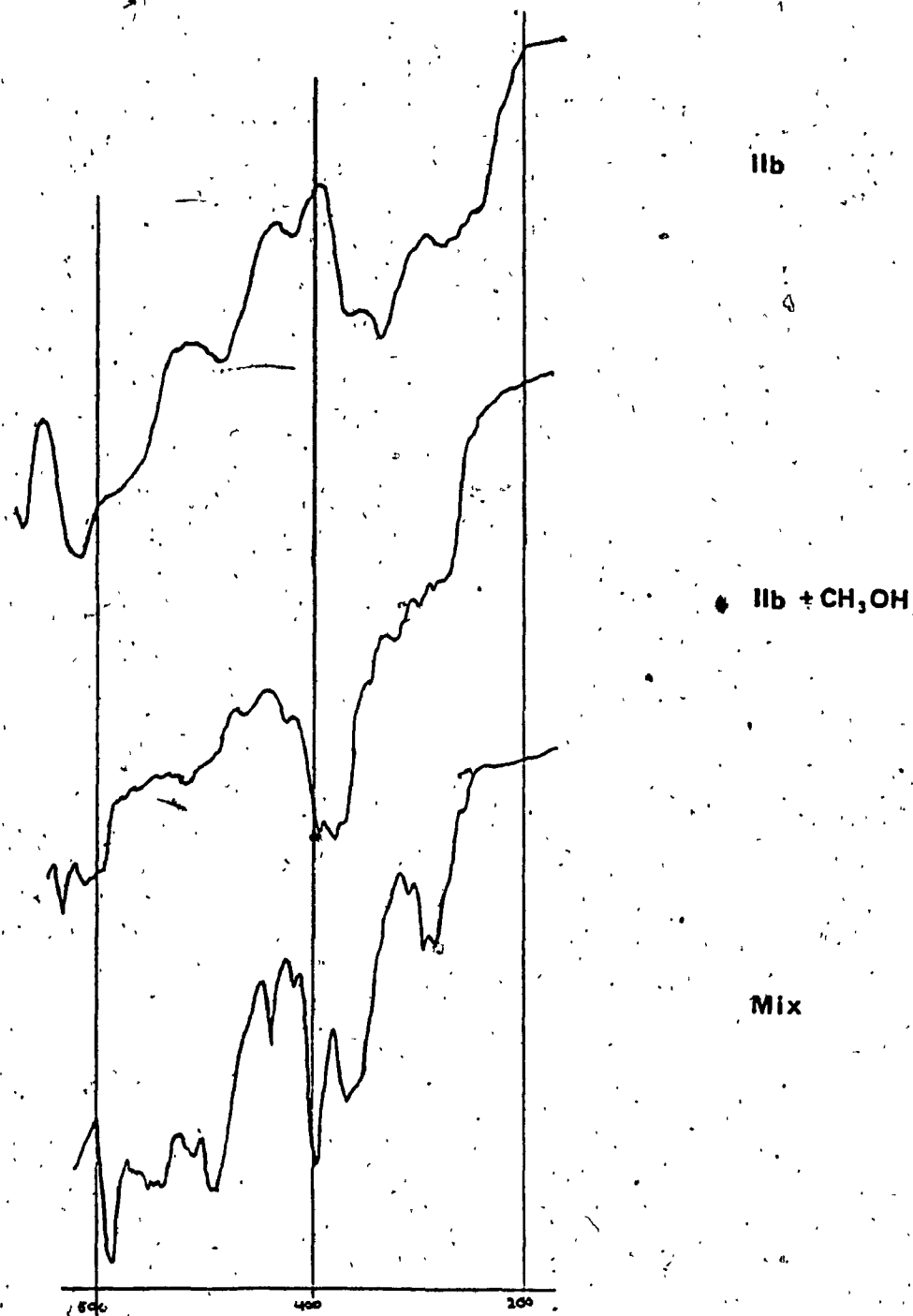
IR-Spectra of Reaction with GMP

Figure 17



IR-Spectra of Ligand Reaction with Acetone

Figure 18



IR-Spectra of Ligand Reaction with Alcohol

Discussion of results:

In the following section only bands between 500cm^{-1} and 200cm^{-1} are discussed:

Reactions of Ia with acetic acid:

Figure 14 shows the changes in the spectrum of Ia. A Pt-O band at 451cm^{-1} appeared after 10min. The Pt-Cl bands were broadened and shifted. The strong Pt-O vibration and several small Pt-Cl bands show the presence of a number of reaction products deriving from a reaction of Ia with acetic acid after 12 hours.

Reaction of Ia with water and hydroxide ion:

Figure 15 shows the result of these reactions. The chloride band weakened from the region near 320cm^{-1} , while a weak Pt-O frequency appeared near 450cm^{-1} . The reaction with KOH appeared to completely remove the chloride.

Reaction of IIa with GMP: Figure 16 shows the complete substitution of the chloride after 20min.

Reaction of IIa with acetone: Figure 17 shows solvation product IIc with no significant changes in the IR spectra, except a small change in the pyridoindole fingerprint region. Total removal of all Cl in the complex IIa after a 3 weeks reaction with acetone lead to the complete disappearance of the Pt-Cl bands.

Reaction of IIb with methanol: Figure 18 shows, that the alcohol soluble complex IIb undergoes a change of its cis-

PtCl₂ band at 338-370 to 400cm⁻¹ and a Pt-O band at 460 cm⁻¹ becomes visible suggesting solvation with methanol. It is possible to distinguish relatively pure compounds from a mixture.

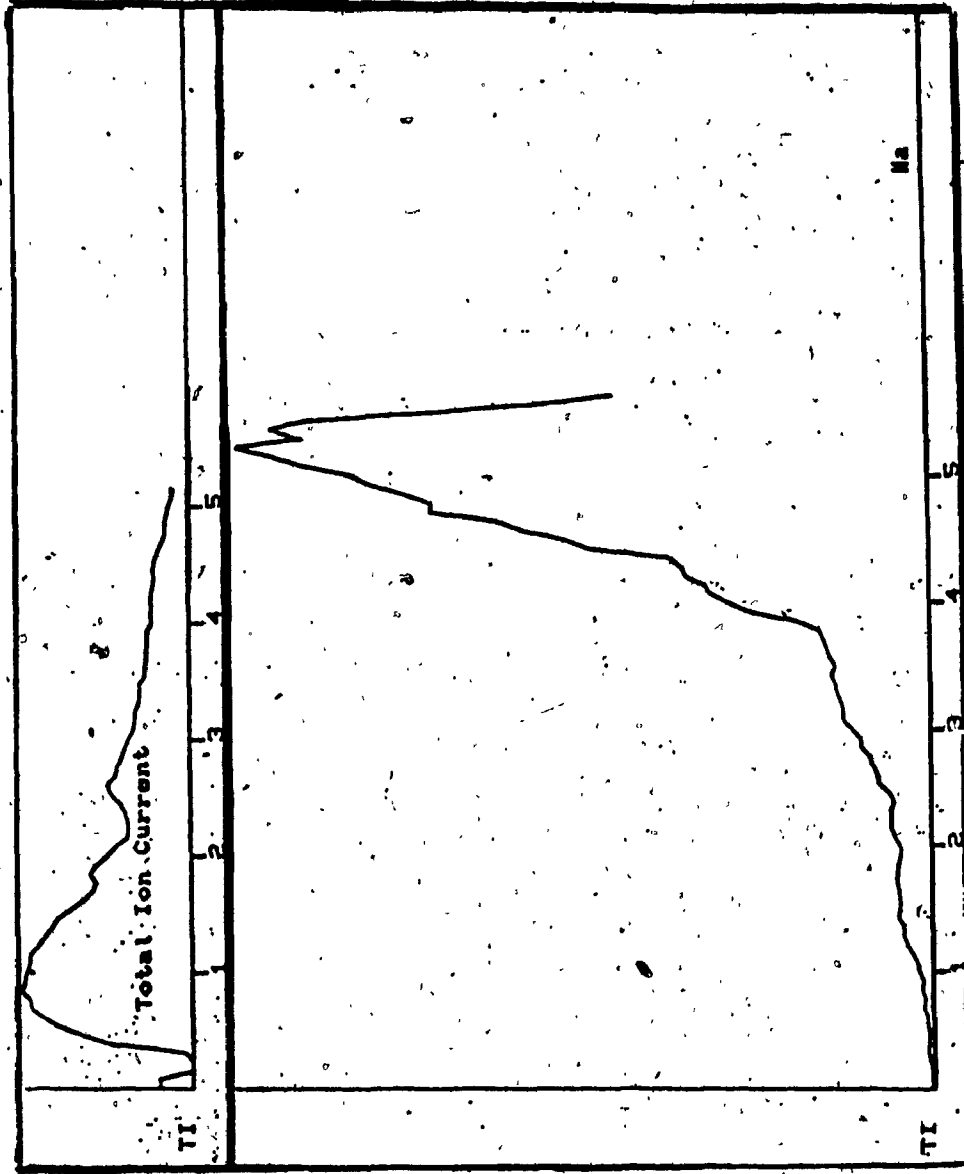
B 7.3. Mass spectroscopy

B 7.3.1 Conventional mass spectra:

The mass spectra obtained by Dr. O. Mamer using a conventional ionisation source show no Pt containing fragments. Nevertheless the results are of some use in postulating a path for the thermal degradation of the complexes. This thermal degradation begins at 37°C in solution, (the solutions became brown) and at 130°C for the solid IIa.

The figure 19 shows the ion current as a function of source temperature/time for IIa and the crude mixture respectively. The mixture releases pyridoindole over a large temperature range from 50 up to 150°C in vacuo with two significant peaks. The compound IIa appears to decompose fairly sharply at 200°C, indicating the possible presence of a single major component.

Figure 19



Temperature dependant Time Response of Mass-spectra

B 7.3.2 FAB Mass spectroscopy

Introduction

Since its introduction in 1981, fast atom bombardment mass ⁴⁵ spectrometry (FAB) has become widely used soft ionisation technique for the investigation of large and/or thermally labile compounds. The sample is bombarded with a neutral particle beam and sample ions are produced as a result of the interaction of the beam of the sample. The usefulness of the FAB technique had been demonstrated for the ionisation of compounds like peptides, antibiotics etc., which had previously been intractable by other forms of mass spectrometry, or had produced molecular species of very low abundance, or fragments only, and from which the molecular weight had to be deduced.

Conventional source mass-spectroscopy failed to produce Pt containing fragments, thus it was felt that the Pt-pyridindole complexes were obvious candidates for the FAB technique. The spectra were run using the spectrometer at the University of Ottawa by Dr. Kazakof.

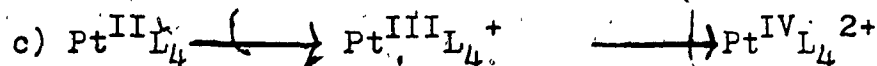
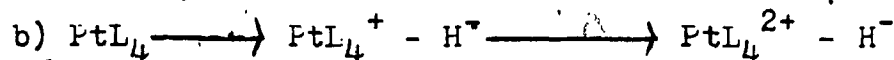
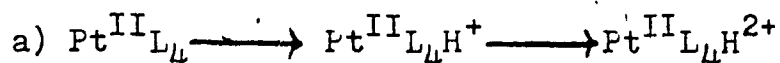
The precise assignment of peaks in the FAB mass-spectra is made somewhat more difficult by several factors: Firstly Pt has 4 fairly common isotopes and Cl has 2 (table),

Table

Isotope distribution:

Isotope	content (%)	atomic mass
Pt-194	32	193.9628
Pt-195	33.8	194.9648
Pt-196	25.3	195.9650
Pt-198	7.21	197.9675
Cl-35	75.53	34.96885
Cl-37	24.47	36.968

Secondly, a particular group of peaks may represent more than one fragment type. Specifically, Cl has mass numbers 35 and 37 while a combination of two of O, OH, and OH₂ groups can give mass numbers from 32 to 36. For this reason no distinction between Cl and OH is made in the following pages. Ions formed in the FAB source are also subject to additional protonation or oxidation:



A) Compound IIa and IIb (figure 20)

The table below shows the composition assignment of the acetone soluble compound IIa based on main isotopes of Pt 195 and Cl 35, and a mass of pyridoinole of 168. In the following table are: $\text{aq} = \text{H}_2\text{O}$, $\text{P} = \text{Cl}_2 = \text{Cl}(\text{OH})_2 = (\text{OH})_4$ and $\text{N} = \text{Cl} = (\text{OH})_2$

m/z(obsd)	m/z(calcd)	diff.amu	%base	composition
866.5	867	-0.5	2.1	Pt(pyin) ₄
735.1	734	+1.1	9.6	Pt(pyin) ₃ N
	735	+0.1		Pt(pyin) ₃ (aq) ₂
621.2	619	-2.2	6.5	Pt(pyin) ₂ P(aq)
	620	-1.2		Pt(pyin) ₂ N(aq) ₃
602.0	601	-1.0	4.7	Pt(pyin) ₂ P
	602	+/-0		Pt(pyin) ₂ N(aq) ₂
567.0	566	-1.0	11.6	Pt(pyin) ₂ H
	567	+/-0		Pt(pyin) ₂ (aq) ₂
545.0	547	+2.0	3.4	Pt(pyin) ₂ O
530.9	531	+0.1	31.6	Pt(pyin) ₂
433.1	433	+0.1	10.9	Pt(pyin)P
	434	-0.9		Pt(pyin)N(aq) ₂
411.1	414	-2.9	5.7	Pt(pyin)N(O)
	413	-1.9		Pt(pyin)(aq) ₂ O
399.1	398	+1.1	45.2	Pt(pyin)H
	399	+0.1		Pt(pyin)(aq) ₂
362.9	363	-0.1	12.5	Pt(pyin)
357.1	362	-4.9	100	Pt(pyin)-H
303	300	-3.0	35.6	PtMP

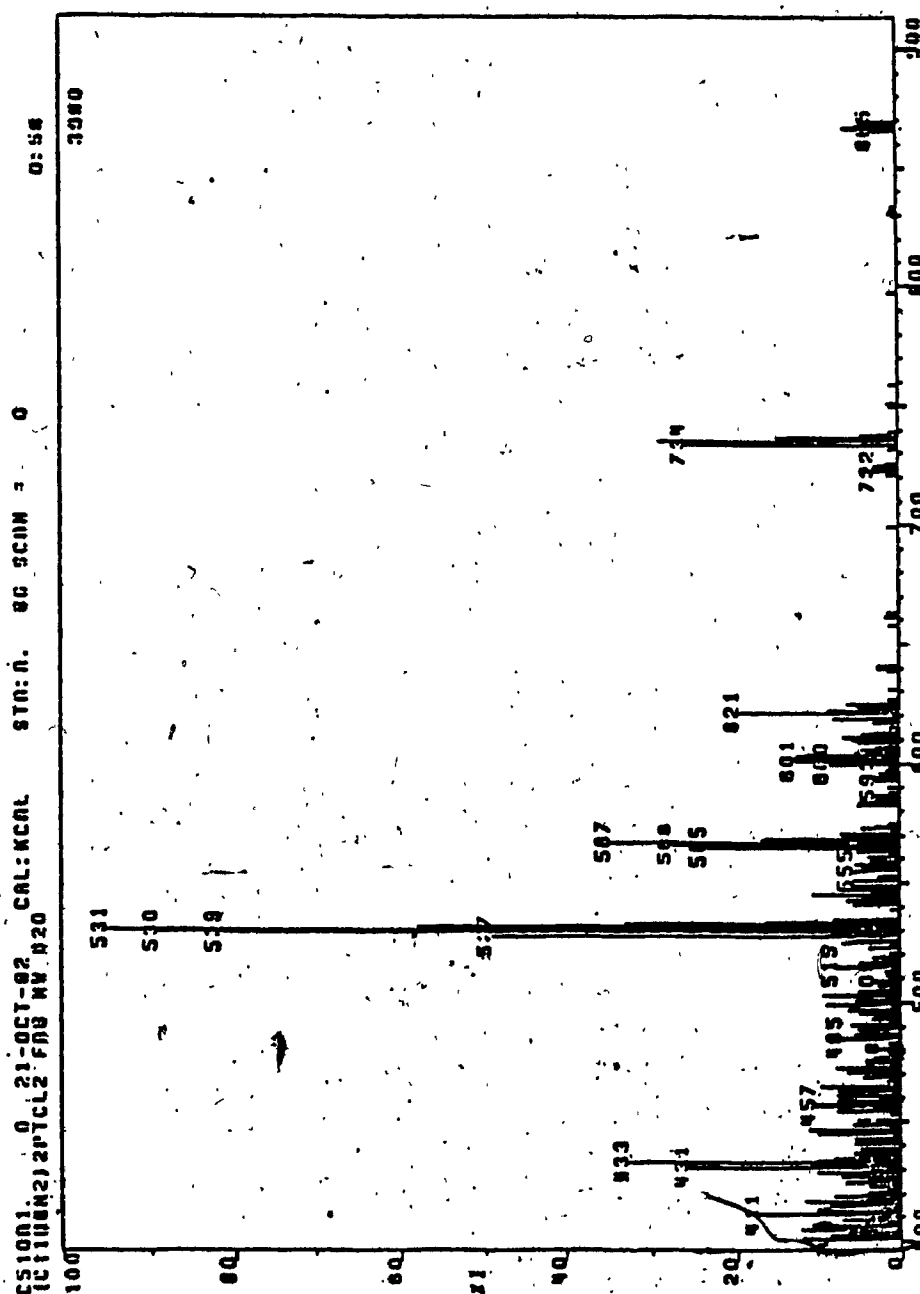


Figure 20

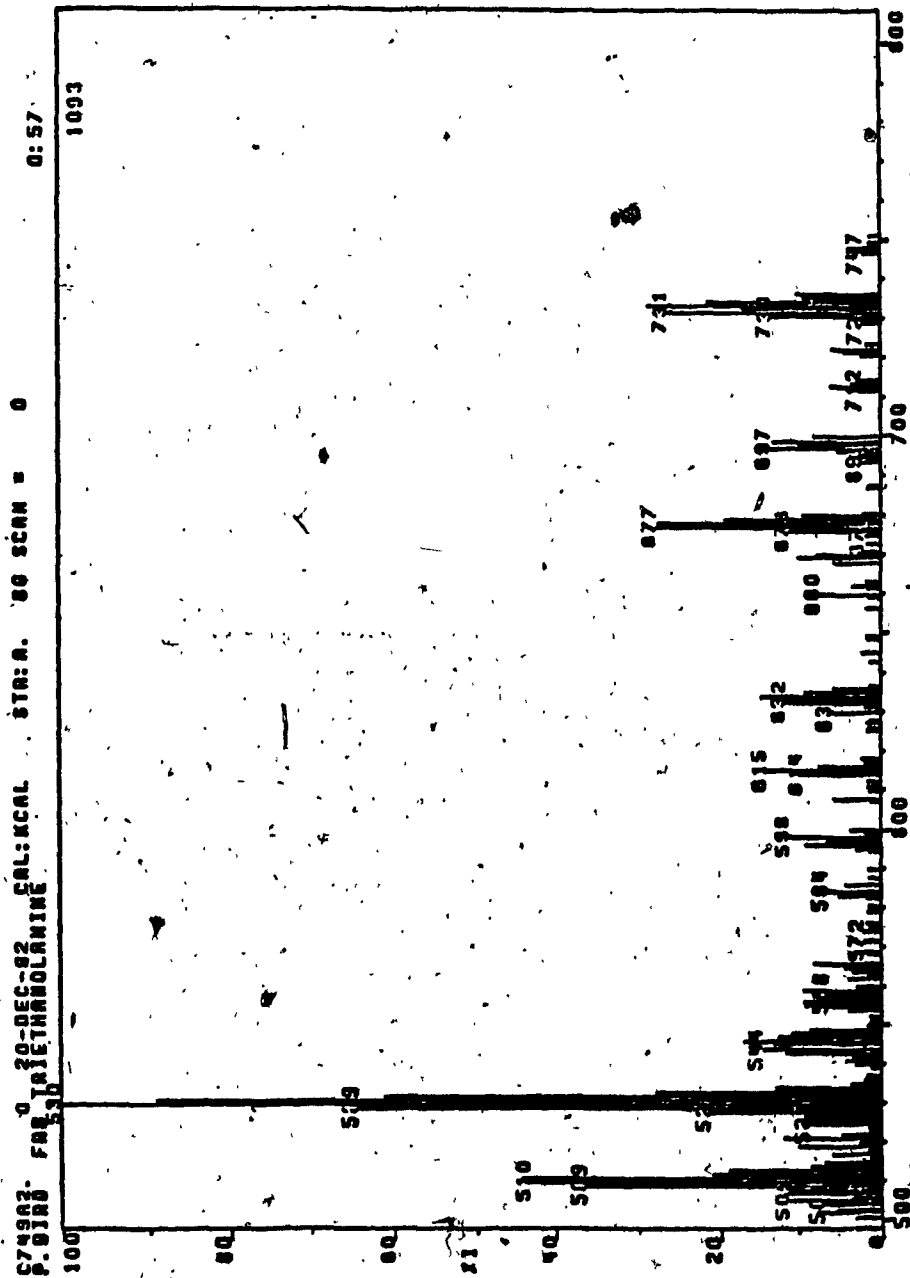
Fab Mass-spectrum of Products IIa, IIb

8) Compound IIc (figure 21, 22)

The table below shows the composition assignment of the acetone insoluble compound IIc based on main isotopes Pt 195 and Cl 35 and a mass of pyridoindole of 168.

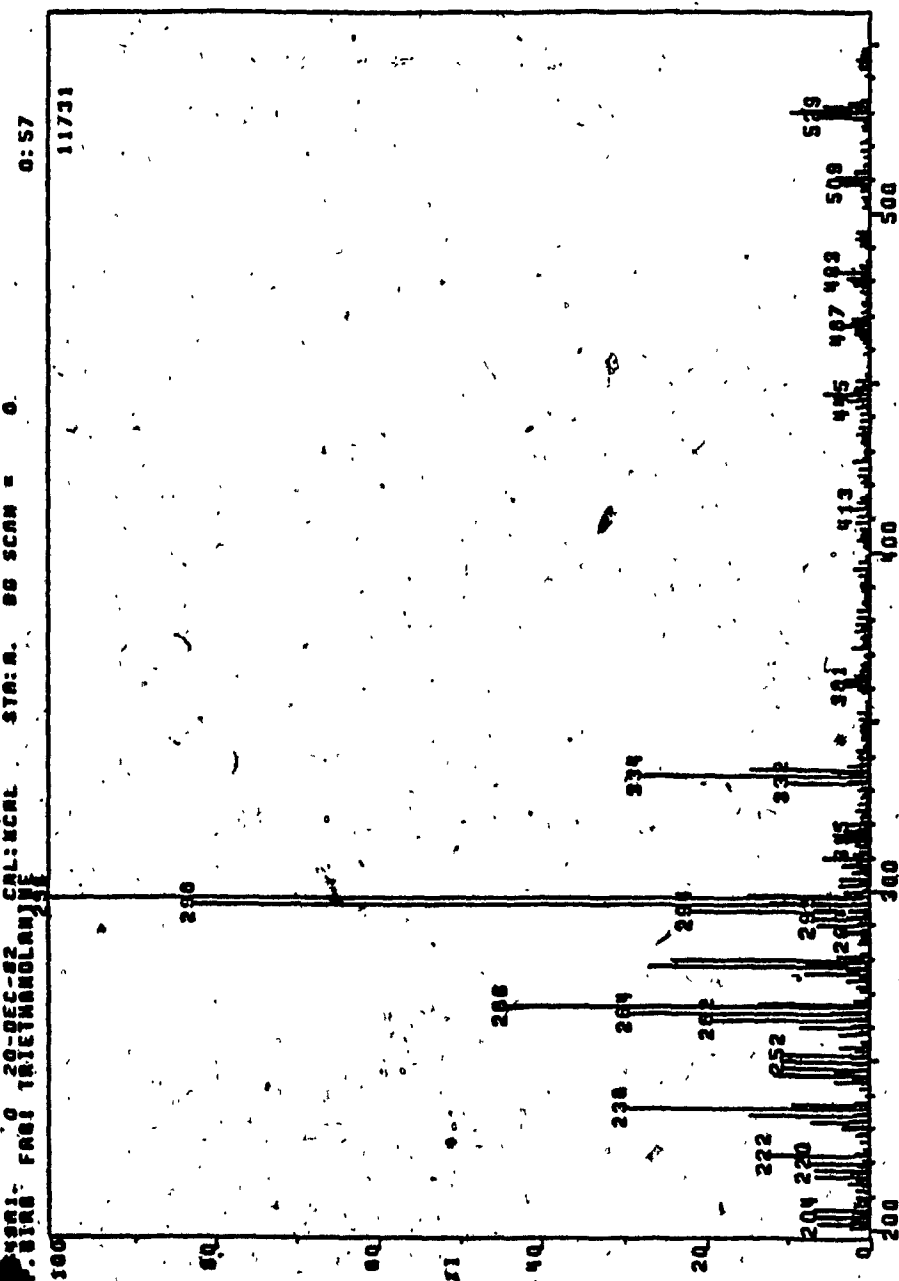
In the following table are: R = prop-2H = acac-2H = acet;
 L = CH₂ = nitrogen; TEAM = triethanolamine; M = Cl(OH) = (OH)₃; N = (OH)₂ = Cl ; P = 2Cl = Cl(OH)₂ = 4(OH)

m/z(obsd)	m/z(calcd)	diff.amu	%base	composition
747.7	750	-2.3	3.48	Pt(pyin) ₃ M
732.9	734	-1.1	28.08	Pt(pyin) ₃ N ^b
712.6	713	-0.4	6.04	Pt(pyin) ₃ L
696.8	699	-2.2	13.54	Pt(pyin) ₃
676.7	680	-3.3	27.63	Pt(pyin) ₂ TEAM
668.8	668	+0.8	10.06	Pt(pyin) ₂ (TEAM-6)
659.5	659	+0.5	7.32	Pt(pyin) ₂ RC1 ₂
633.8	635	-1.2	14.73	Pt(pyin) ₂ 3(O) ₂ L
614.8	617	-2.2	14.09	Pt(pyin) ₂ RL ₂
597.9	601	-3.3	11.16	Pt(pyin) ₂ RC
583.9	584	-0.1	6.40	Pt(pyin) ₂ M
565.8	566	-0.2	8.14	Pt(pyin) ₂ N
555.8	557	-1.2	9.24	Pt(pyin) ₂ LC
545.8	547	-1.2	16.93	Pt(pyin) ₂ O
529.8	531	-1.2	100	Pt(pyin) ₂



Fab Mass-spectrum of Product IIc

Figure 21



Conclusions:

Given the results from the elemental analysis, it seems probable that there are many species, especially $\text{Pt}(\text{pyin})_3$ and $\text{Pt}(\text{pyin})_4$ being formed in the source. Nevertheless it is clear that Pt-pyridoindole complexes are present together with chloride. In both complexes the largest peak cluster seem to be associated with $\text{Pt}(\text{pyin})_2$ and it is probable that this moiety at least was present in the original compounds.

B 7.4 NMR-spectra:

In addition to proton and C^{13} nmr, compounds of platinum present the possibility of Pt^{195} nmr studies. In actual fact it is not possible to obtain useable Pt^{195} spectra because the compound were not sufficiently soluble. This same solubility problem plagued attempts to obtain better proton nmr spectra than those which are presented here:

Results:

Figure 23 shows the H^1 spectrum of the crude reaction products which were soluble in CD_2Cl_2 . The only recognisable features were: peaks between 1.0 and 2.0 ppm which might be solvent impurities and a signal at 5.95 ppm which could be hydroxide bound to Pt. The Pt^{195} (33.8%) should give rise to a superimposed doublet, but this would be probably lost in the background.

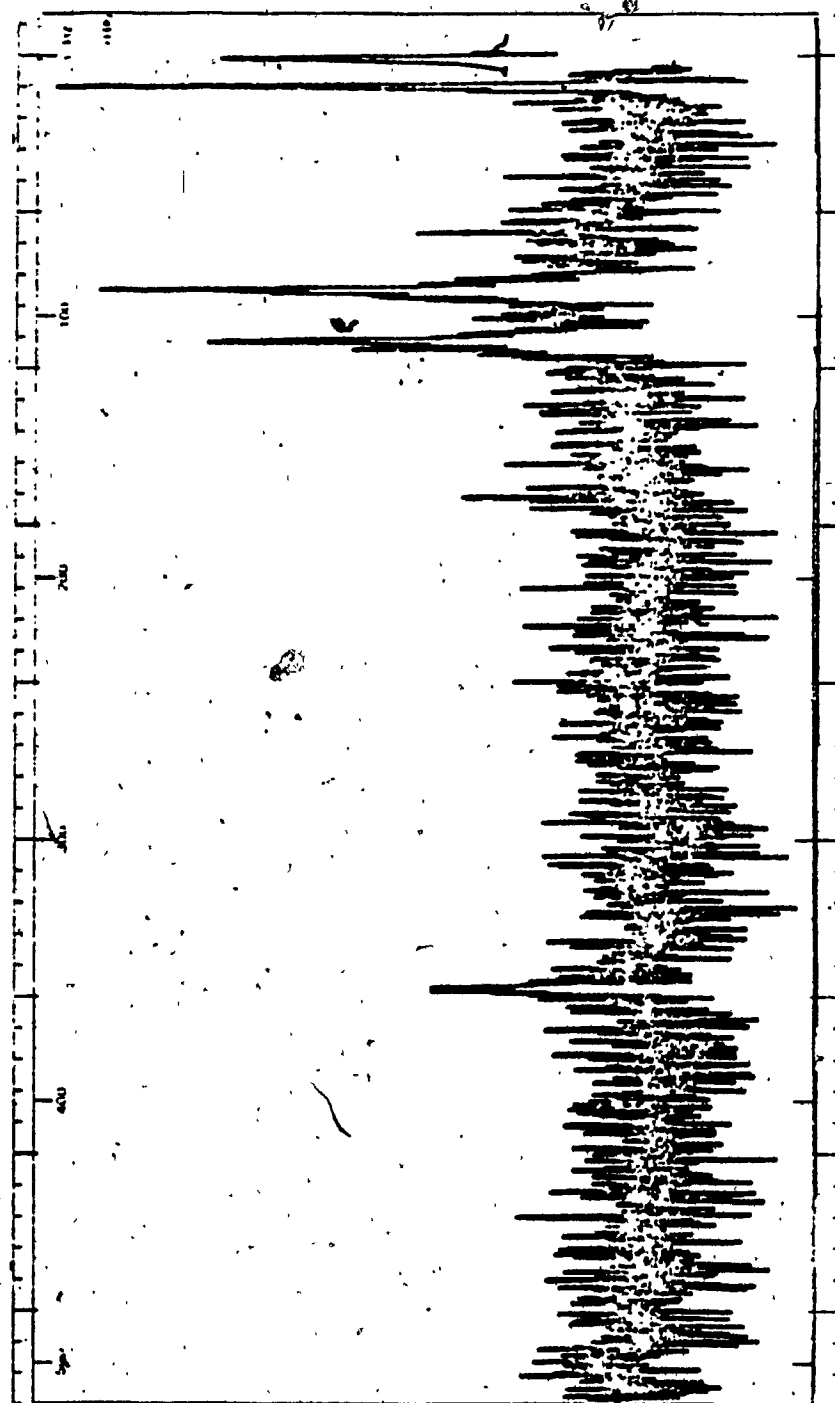
Figure 24 is the H^1 spectrum of IIA in d^6 -acetone which contained water (2.53ppm) and acetone (1.66ppm). This solution was heated to $35^\circ C$, which produced a change of colour from yellow to brown (IIIA):

Figure 25 shows the H^1 -spectrum of this solution with a additional peak at 5.68ppm which could be OH, a shifted peak at 1.40ppm, a reduced, shifted and broadened peak at 2.75ppm and an additional broad peak at 1.82 ppm.

Figure 26 is the H^1 spectrum of the solvation product of IIA with acetic acid in d^6 -acetone with a peak at 5.71ppm. This

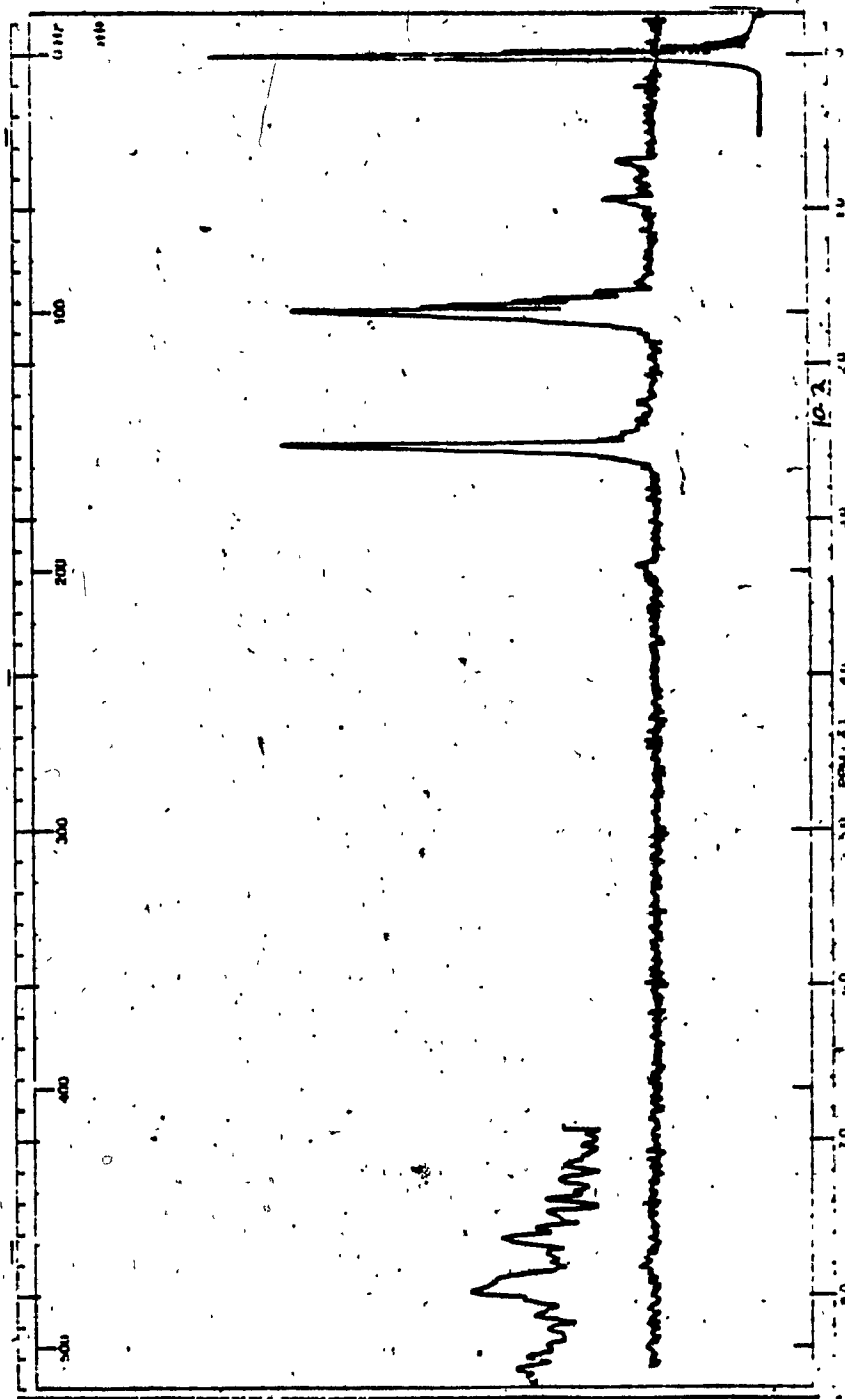
peak again is attributable to OH bound to Pt, though this assignment is far from certain.

Figure 23



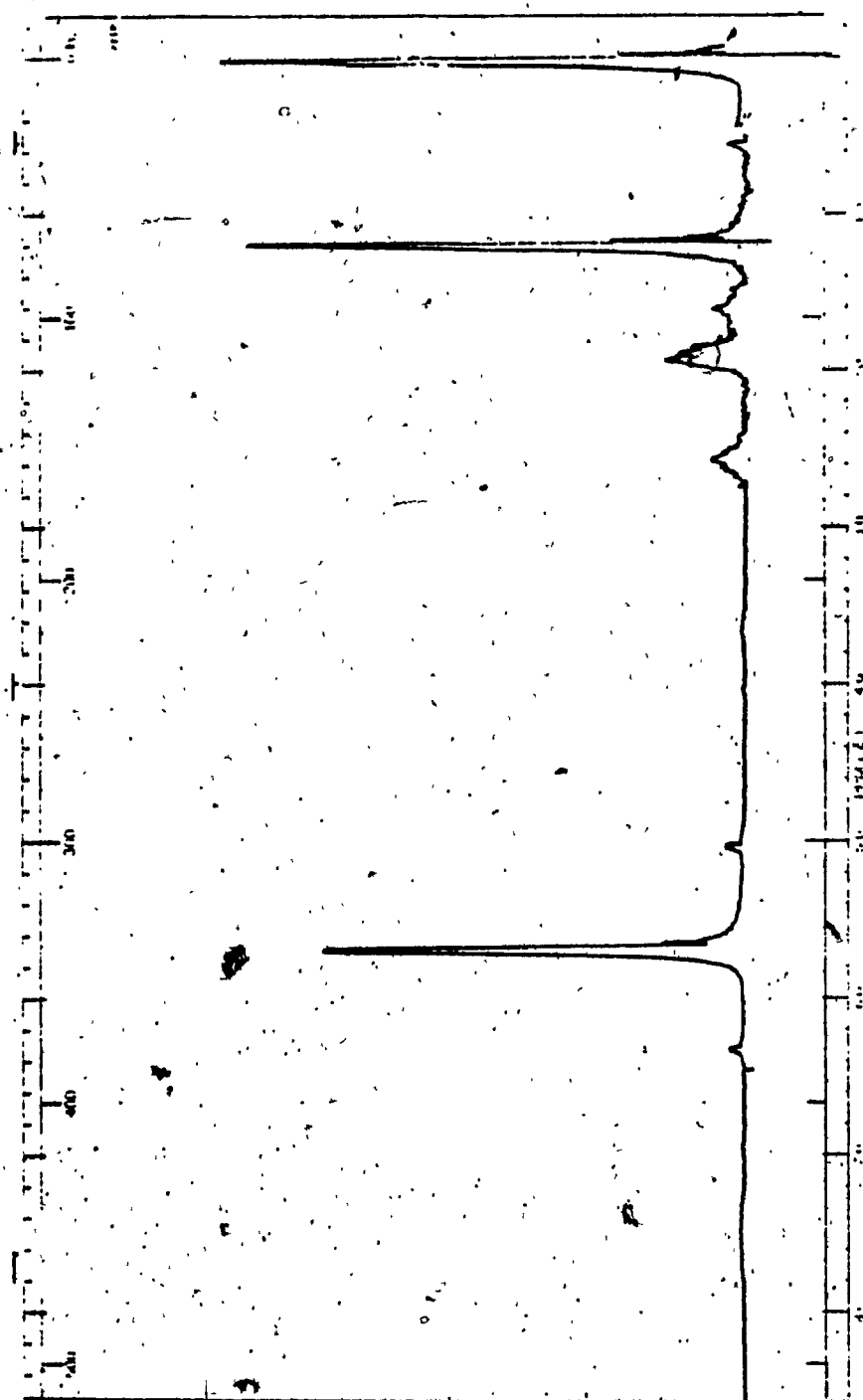
Proton NMR-spectrum of Mixture

Figure 24



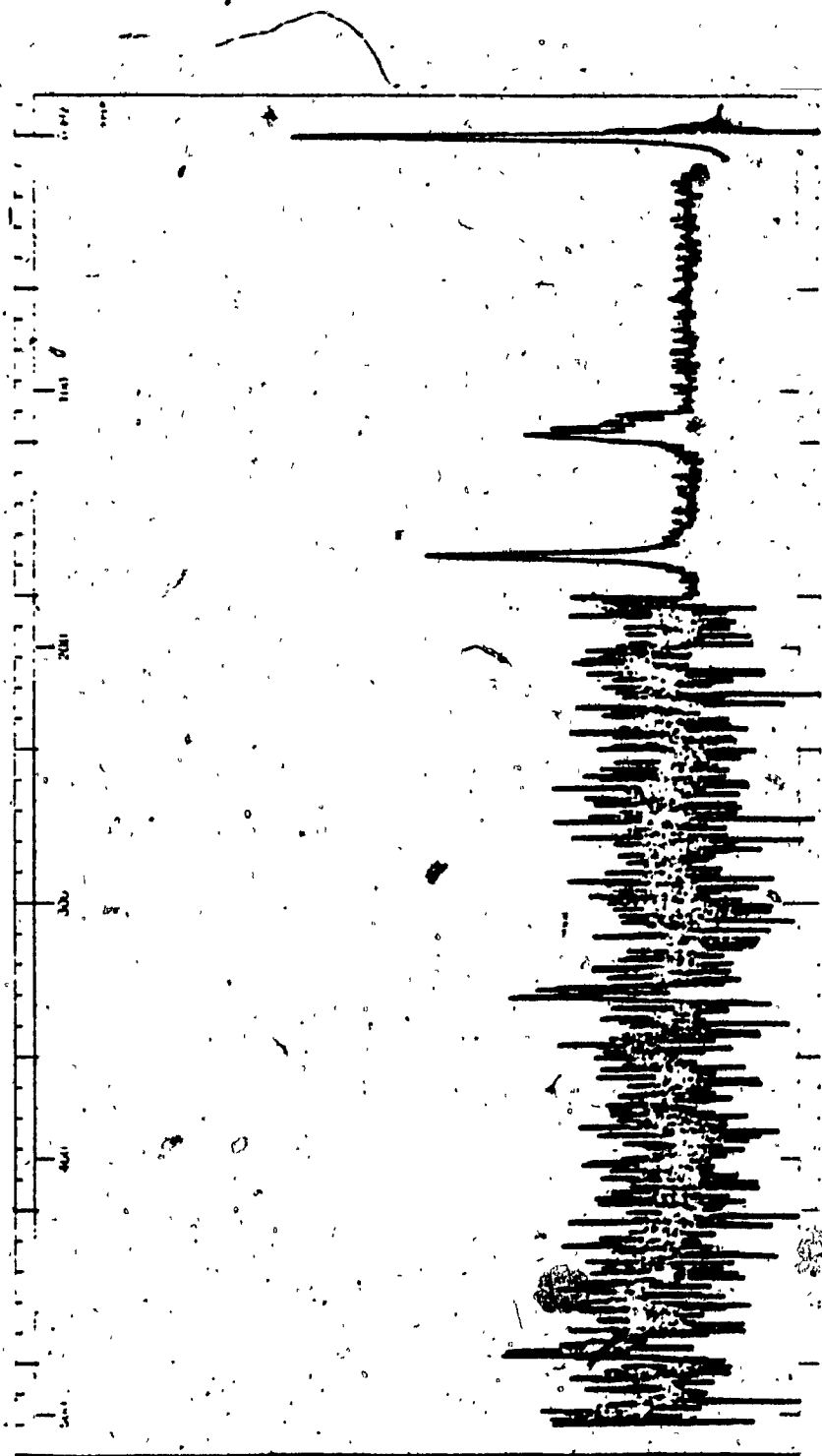
Proton NMR-spectrum of Product Fla

Figure 25



Proton NMR-spectrum of Product III

Figure 28

Proton NMR spectrum of CH_3COOH Product

B 7.5. UV-spectroscopy:

This technique was not likely to provide much information beyond an indication that reaction had occurred.

A reaction of pyin with tertachloroplatinate and the hydrolysis of the reaction products is shown in the first three spectra (figure 27-28):

The first shows a solution of pyin in acetone/water (1:1).

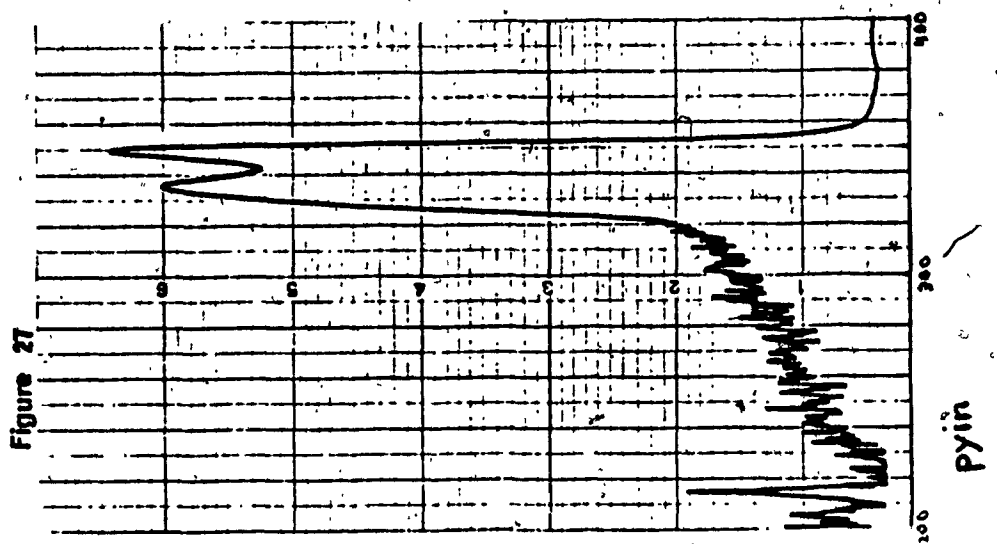
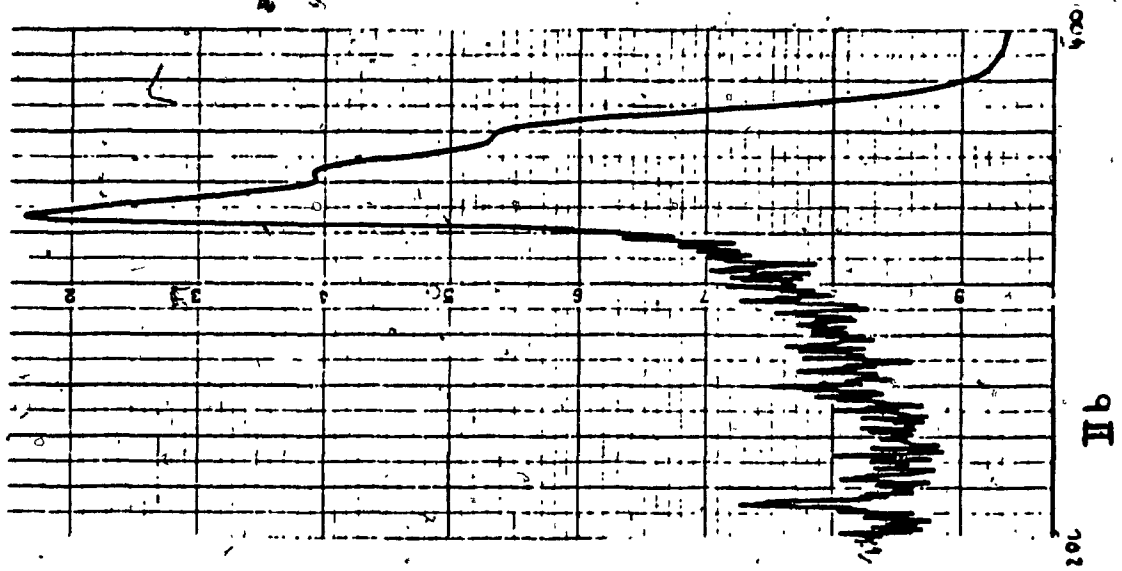
The second is an acetone/water (1:1) extract of a reaction mixture after 12h. The reaction mixture was then stirred

a further 2 days. Its acetone/water extract is recorded in the third spectrum.

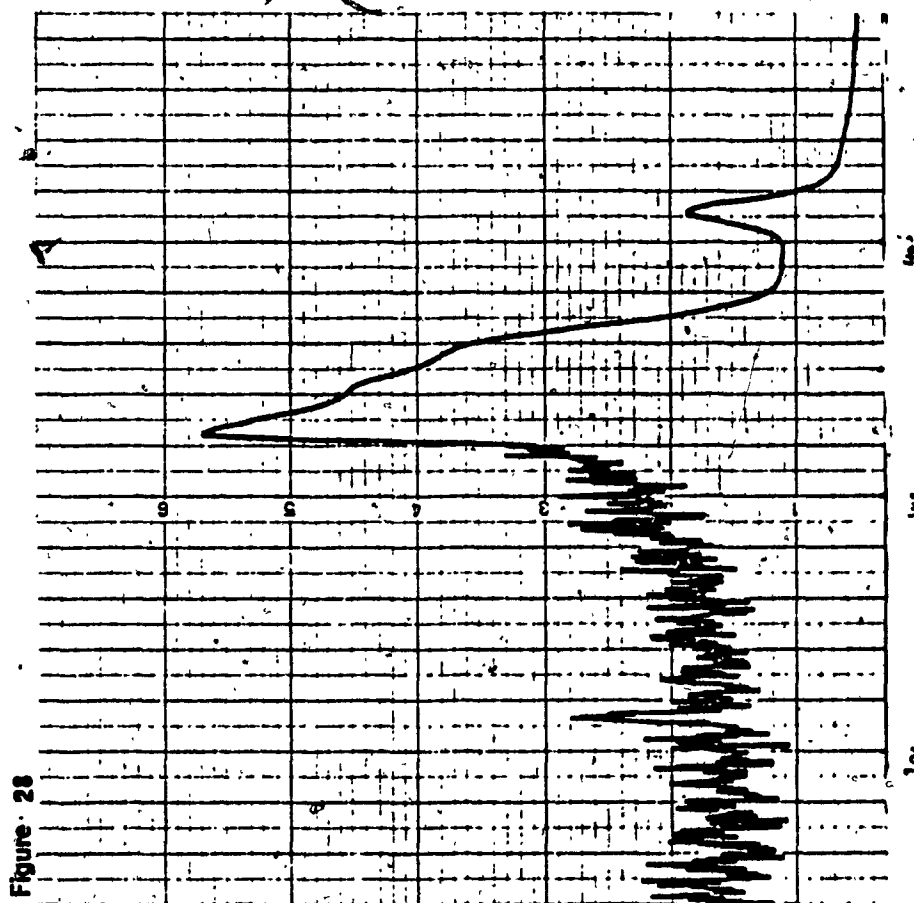
Results:

	Max in nm:
pyin	215, 351, 336
PtCl_4^{2-} + pyin.	208, 316
hydrolysis or solvation	214, 324, 420

The pyin feature is changed after a reaction with PtCl_4^{2-} , the hydrolysis or solvation resulted in broadening of the pyin peak with appearance of an additional peak at 420 nm.



UV-spectra of Ligand Exchange with Pyridindole



UV-spectrum of Product 11a

Figure 29 shows the results of reacting IIa with GMP:

IIa was mixed with GMP in excess in an acetone/water (1:1) mixture and a spectrum was recorded immediately. The second spectrum was recorded after 20 min.

Results

GMP	216, 267
GMP + IIa, t= 0 min	216, 260
GMP + IIa t= 25 min.	218, 264

The very strong peak at 216 is reduced considerably, suggesting a reaction with IIa, which is confirmed also in an IR-spectra see (figure 16).

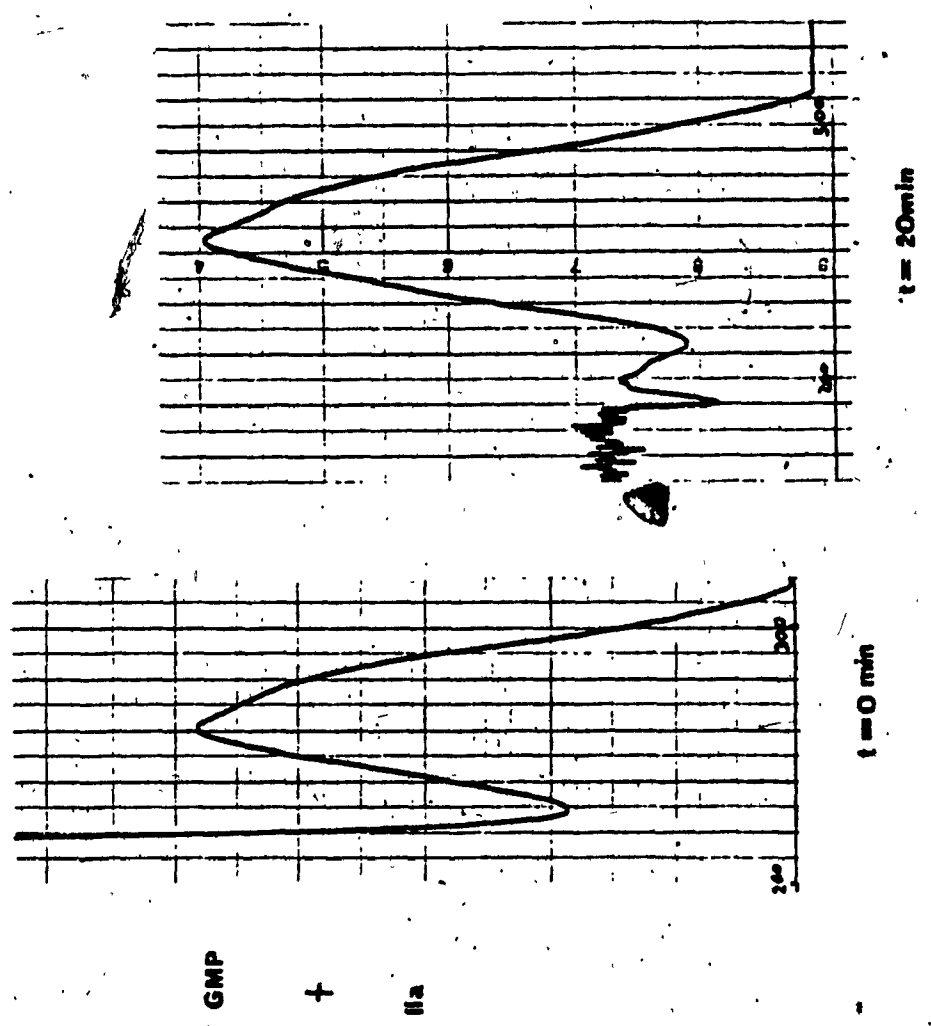


Figure 29

UV-spectra of Ligand Exchange with GMP

B 7.6 Pt(IV) - Chemistry

During the search for single crystals, suitable for a crystal structure analysis, Pt(IV) compounds also were included in the synthesis attempts.

The substance (IIc) was dissolved in fresh prepared aqua regia by adding HCl and HNO₃ to the flask (3:1 ratio). The solution turns brown and hot with NO₂ gas evolution. After 12 hours a yellow solution can be filtered from a precipitate. Slow evaporation of the filtrate by exposure to air in the fumehood for 3 weeks resulted in triclinic single crystals. Unfortunately, the diffraction of X-rays was too weak to allow a crystal structure analysis.

The mass spectra, prepared by fast atom bombardment of a glycerol/crown ether solution, gave the highest mass clusters at m/z 621 suggesting Pt(C₁₁H₈N₂)Cl₃(NO₃)(glyc) as a possible parent ion. The most plausible precursor of this ion is: Pt(C₁₁H₈N₂)Cl₃(NO₃)L, where L could be Cl, NO₃ etc. The most likely ligand to be cleaved by the potential ligand glycerol is the Cl, suggesting [Pt(C₁₁H₈N₂)Cl₄(NO₃)]⁻ as the original ion.

The following table shows the assignment of the mass spectrum (figure 30) of the single crystal based on main isotopes of Pt 195 and Cl 35 and a mass of organic ligand (org.) of 168.

Abbreviations used: nitr = NO₃; glyc = glycerol; aq =

water; L = N = CH₂.

m/z(obsd)	mz/(calcd)	diff.amu	%base	composition
621.1	622	-0.9	7.25	Pt(org.)Cl ₃ (nitr)(glyc)
587.0	587	+/-0	3.00	" -Cl
557.2	557	+0.2	5.36	" -Cl, -L, -O
544.1	543	+1.1	5.09	" -Cl, -2L, -O
527.0	527	+/-0	34.44	" -Cl, -2L, -2O
502.9	502	+0.9	4.52	Pt(org.)Cl ₃ (OH) ₂
485.0	485	+/-0	4.85	" -OH
471.1	469	+2.1	5.57	" -2OH
463.0	461	+2.0	3.58	Pt(org.)(nitr)(aq) ₂
447.0	449	-2.0	4.09	Pt(org.)Cl ₂ (aq)
427.0	425	-2.0	4.61	Pt(org.)(nitr)
409.0	411	-2.0	4.23	" -O
395.0	393	-2.0	6.85	" -2O

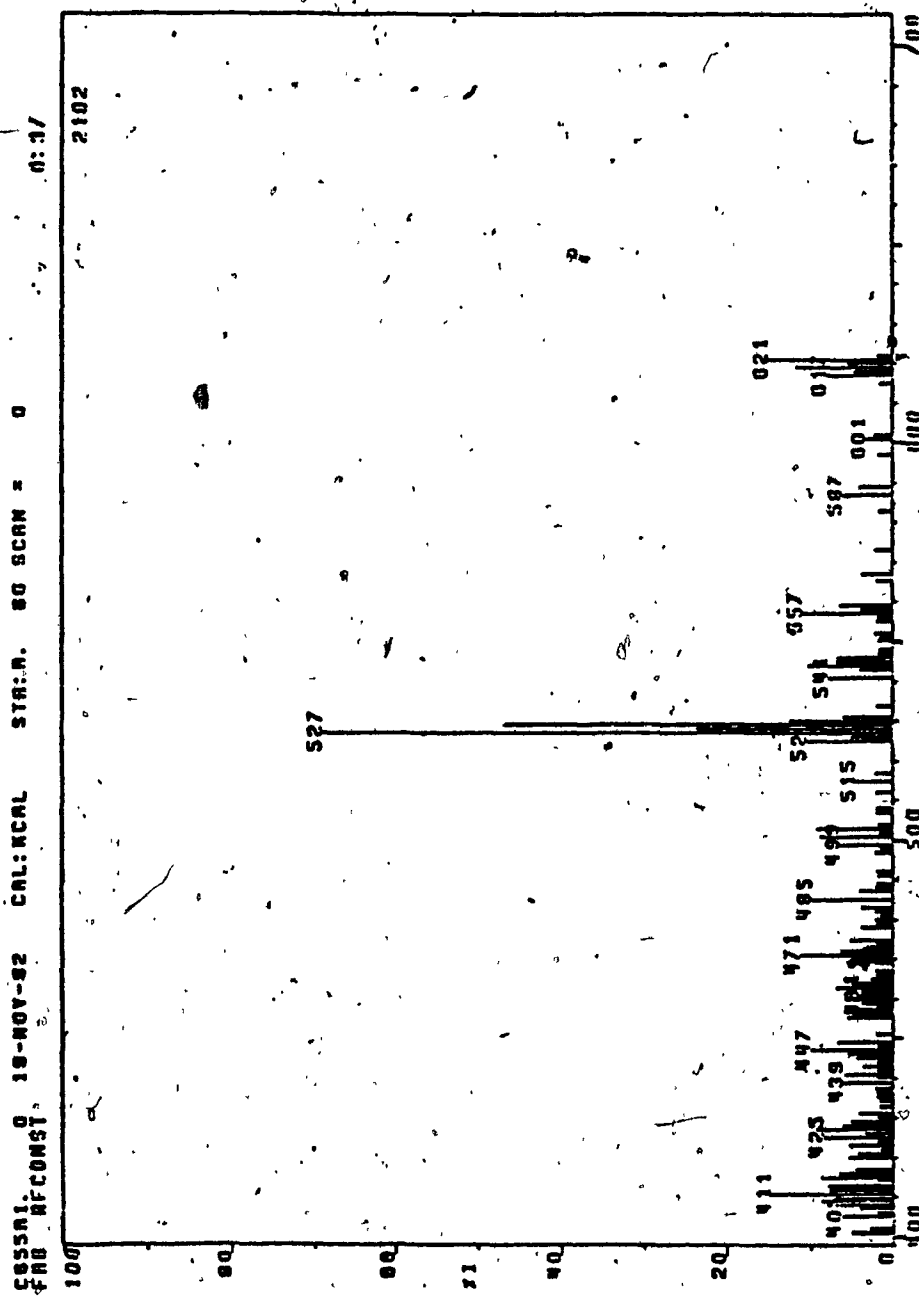


Figure 30

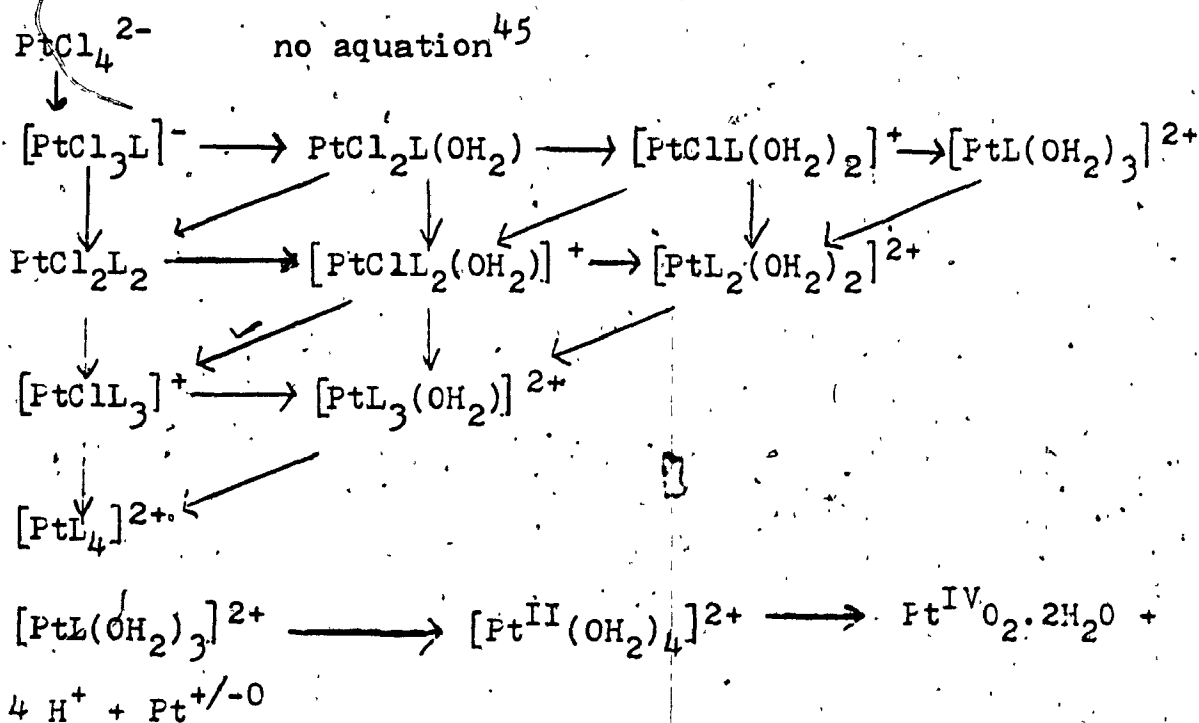
Mass-spectrum of Pt(IV)-Product

B 8 Interpretation of results:

The course of the reaction of PtCl_4^{2-} with pyridoindole and the exact nature of the products is rather uncertain. Nevertheless, the data that have been obtained are amenable to some degree of speculative analysis:

B 8.1. Reaction system

The following scheme shows a rather complete set of reaction possibilities. It is assumed that the chloride ion is the leaving group of preference



B 8.2 Complete list of possible products

The following table shows the type of monomeric complexes which might in principle be obtained if other solvents are also included as potential ligands:

a) Pt(pyin)	Cl ₃	Cl ₂ S	ClS ₂	S ₃
b) Pt(pyin) ₂	Cl ₂	ClS	S ₂	
c) Pt(pyin) ₃	Cl	S		
d) Pt(pyin) ₄				

S: OH₃⁺, OH₂, OH⁻, MeOH, EtOH, Prop, But, glycerol, TEAM, acetone, CH₃COOH, CH₃COO⁻, ox, mal, GMP, NO₃⁻, NO₂⁻

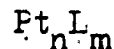
In addition it is necessary to include in the consideration of reactions or schemes of products the following:

Positive counterions: K⁺, H₃O⁺, H⁺, pyinH⁺

Negative counterions: R⁻ = OH⁻, CH₃COO⁻, NO₃⁻, ox, mal

Platinum IV: Pt^{IV}L₆ with L = S, Cl, pyin

Di, oligo, and polymeric complexes of the formula



B 8.3. Elimination of possibilities:

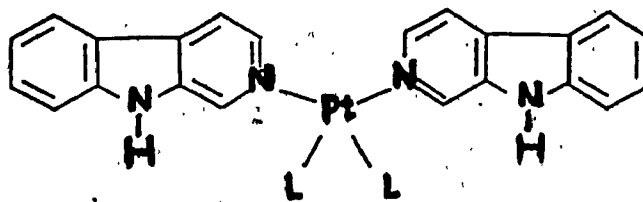
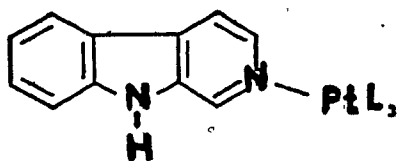
All the analytical techniques prove the presence of Pt-pyin complexes. There are five different types possible: Pt(II) (pyin)₁₋₄ and Pt(IV). The platinum IV complexes are unlikely as products because reaction conditions in the basic preparation experiment do not support oxidation. (Oxidation was observed in reactions with aqua regia and O₂). Therefore

oxidation steps are excluded from the basic reaction scheme. The mass spectra give no evidence for dimeric or oligomeric species. In all cases where elemental analyses were obtained, approximately 4 ligands per platinum are present. In addition, the steric bulk of pyridoindole groups would seem to discourage condensation of oligomers. The basic reaction scheme therefore also excludes such species.

The existence of a $\text{Pt}(\text{pyin})_4$ fragment can be detected in the mass spectra of the acetone soluble fraction IIa as the highest peak, the existence of a $\text{Pt}(\text{pyin})_3$ can be detected in the mass spectra of the acetone insoluble fraction where it is the highest peak. However, the peaks are very small and and the elemental analysis excludes significant quantities of such species, they may be impurities or formed in the source. Therefore substitution beyond PtL_2 is considered minimal in the basic reaction scheme.

The remaining possibilities are now $\text{Pt}(\text{pyin})$ and $\text{Pt}(\text{pyin})_2$ derivatives: see figure 31.

Figure 31



Pt(pyin) Structure Possibilities

According to thin layer chromatography, 4 complexes are the result from the reactions between tetrachloroplatinate, pyin and water, in the initial product mixture, and three different materials can be isolated after work up: Ia, IIa, IIb.

B 8.4. Characterisation of products: Summary

The most useful starting point for the characterisation of those reaction products that have been isolated is the IR spectra:

a) Typical known features:

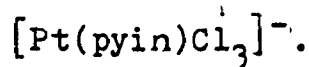
i) PtCl_4^{2-} : This has a strong narrow band at 319cm^{-1} attributed to the Pt-Cl stretching modes (see figure 12).

ii) cis- PtCl_2L_2 with L= amines: The Pt-Cl stretching modes occur at 345cm^{-1} and 327cm^{-1} (44).

iii) trans- PtCl_2L_2 : The Pt-Cl stretching modes are assigned at 340cm^{-1} (36).

iv) Pt-O: The Pt-O stretching mode is assigned at 450cm^{-1} (42).

b) Compound Ia. By comparison with typical known species the single strong narrow band at 310cm^{-1} is probably a Pt-Cl stretching frequency. Its resemblance to the band found in PtCl_4^{2-} , combined with the fact that Ia does contain pyin (elemental analysis, IR fingerprint region of pyin), and does not show a band for Pt-O, leads to the conclusion that most reasonable Pt coordination would be:



Further evidence:

Nitrogen containing compounds easily replace chloride in platinum(II) compounds successively and a $\text{Pt}(\text{pyin})\text{Cl}_3$ would be the first product of the reaction. Substance Ia is indeed the initial product.

The elemental analysis of the complex had a Pt:Cl ratio of 2.6, but poor analysis results are not uncommon in Pt-complex chemistry. Its conversion to dichloro and chloro/oxygen derivatives was observed during ligand exchange reactions with pyridindole and water by IR, UV and during TLC runs.

Compound IIb: By comparison with typical known species, the broad doublet at 370 and 338cm^{-1} probably corresponds to the cis-Pt-Cl₂ stretching modes. Its resemblance to the bands found in cis-PtCl₂(NH₃)₂⁴⁶ leads to the conclusion that, since the compound contains pyin (mass-spectra, IR-spectra) and had no Pt-O bond (IR evidence), the most reasonable Pt-coordination of IIb seems to be:

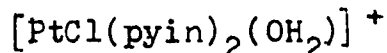


Further evidence:

A mass spectrum of the acetone soluble fraction which contained IIb, had peaks which can only be assigned to the formula above. The compound hydrolyses to complex IIa.

The compound IIa: It has a single strong band at 312cm^{-1} which is probably a Pt-Cl stretching frequency. By comparison with similar species the single weak band at 460cm^{-1} is probably a Pt-O stretching frequency⁴². On the basis of

the pyin fingerprint region, with the increased intensity in IIb by comparison to the intensity in Ia, the $\text{Pt}(\text{pyin})_2$ features in the mass spectra and the presence of chloride, the most reasonable Pt-coordination of IIa seems to be:



Further evidence:

The formation time of IIa exceeded 2 days, hydrolysis is also suggested by UV, and NMR. The mass spectrum shows fragments which can only be assigned to an $[\text{PtCl}(\text{pyin})_2(\text{OH}_2)]$.

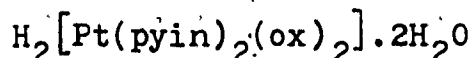
A $[\text{PtCl}_2(\text{pyin})(\text{OH}_2)]$ hydrogen bonded to a second pyridoindole would give the same results. A graph of the total ion current against temperature, of the reaction mixture compared to that for isolated IIa excludes this possibility.

The elemental analysis of IIa, recrystallised from hot water/malonic acid (during an attempted ligand substitution reaction) gave a final proof for the assigned composition.

B 9 Experiments with additional ligands and counterions:

Experiment I

500 mg K_2PtCl_4 was reacted with oxalate and pyridoiindole in a 1:2:2 molar ratio over a period of three days in 50 ml KOH /water (10%). Additional stirring for 12h at pH 2 produced several compounds: a black residue, metallic platinum, 3 water soluble and 2 water insoluble yellow compounds. Extraction of the water insoluble precipitate with acetone provided a residue, which was analysed by IR (pyin, no Pt-Cl) and thin layer chromatography (one spot). An elemental analysis suggests:



Found: Pt 24.87% C 42.35% H 3.36% N 8.24%

Calc.: 26.03% 41.65% 2.93% 7.47%

diff.: 1.16% 0.69% 0.42% 0.76%

Experiment II

The reaction of potassium tetrachloroplatinate with potassium oxalate provided potassium dioxalatoplatinate ⁴⁷. 300mg $K_2Pt(ox)_2$ were reacted with 112mg pyin in a 1:1 molar ratio at 100°C for 8h in 50ml water/butanol (1:1 ratio). After removal of solvents a bright, yellow product was obtained and purified with water, acetone and methanol. The IR spectra gave only the fingerprint region of pyin. An elemental analysis suggests a $K_2Pt(pyin)(ox)_2$

Found: Pt 31.25% C 29.28% H 1.56% N 4.58%

Calc.: 31.50% 29.07% 1.29% 4.52%

diff.: 0.25% 0.21% 0.27% 0.06%

Experiment III:

The reaction of experiment II with a molar ratio of 1:2 for pyin and a reaction period of 7 days resulted in a pale yellow solid with the pyin fingerprint region in the IR spectra.

An elemental analysis suggests a $K_2Pt(pyin)(ox)_2 \cdot 2H_2O$

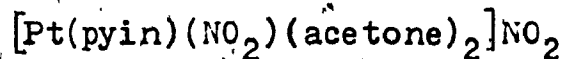
Found: Pt 29.96% C 28.26% H 1.72% N 4.14%

Calc.: 29.81% 27.51% 1.53% 4.28%

diff.: 0.15% 0.75% 0.19% 0.14%

Experiment IV:

500 mg of potassium tetrachloroplatinate were converted into its dichlorodinitrito equivalent with KNO_2 . Two hundred of the green complex was reacted with excess pyin: 500 mg (in H_2O for 12h at $100^\circ C$). Besides unreacted starting material a green insoluble solid appeared, which could be separated from the water solution. In the cold mother liquor a precipitation of organic ligand after 24h indicated lability of pyin. The green insoluble solid was washed by prolonged treatment with acetone. The IR spectra shows pyin and no Pt-Cl. An elemental analysis suggests a:



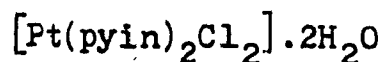
Found: Pt 35.03% C 34.09% H 3.50% N 10.79%

Calc.: 34.15% 35.72% 2.33% 9.87%

diff.: 0.87% 1.63% 1.27% 0.98%

Experiment V

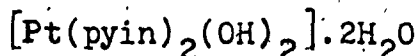
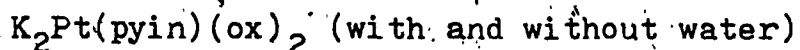
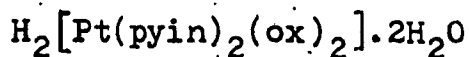
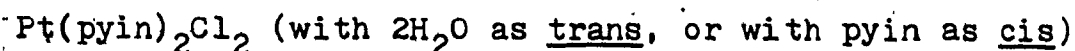
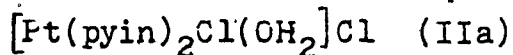
The reaction of potassium tetrachloroplatinate with silver nitrate produced a water insoluble pink precipitate of $\text{Ag}_2[\text{PtCl}_4]$ ⁴⁸, which was easy to purify. 2.8 g $\text{Ag}_2[\text{PtCl}_4]$ was reacted with .854 g pyin in 50 ml chloroform. A colour change from pink to yellow appeared after twelve hours. The separation of AgCl from the product requires the use of water. The IR spectra shows a single Pt-Cl bond and the pyin fingerprint region. An elemental analysis suggests a:



Found:	Pt 30.30%	C 40.44%	H 2.65%	N 8.43%
Calc.:	30.50%	41.30%	3.10%	8.77%
diff.:	0.20%	0.85%	0.45%	0.34%

Summary

The following complexes were identified fairly well during the synthesis work:



Only IIa, administered orally in aqueous acetic acid or alcohol, is likely to be an anti-tumor drug. The partial solubility in water would prevent the complex or its "degradation" products from entering the blood stream, and a further source of toxicity would be prohibited. The other complexes are too instable or too insoluble.

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