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TITLE STUDIES OF 3-HYDROXYPYRID-4-ONES AND RELATED LIGANDS

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STUDIES OF 3-HYDROXYPYRID-4-ONES AND RELATED LIGANDS

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by

Azita Dodd

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DECLARATION

While registered as a candidate for this degree the author has not been a registered candidate for any other award.

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Azita Dodd
Studies of 3-hydroxypyrid-4-ones and related ligands

ABSTRACT

It has been established that the previous synthetic route to 3-benzyloxy-1,2-dimethylpyrid-4-one and 1,2-dimethyl-3-hydroxypyrid-4-one, under the reported conditions, does not lead to their free bases but to their protonated forms. The structures of these pyridones in both free base and protonated forms have been clarified by spectroscopic techniques and a hydroxypyridinium structure is proposed for the salts. The direct synthesis of 1,2-dimethlyl-3-hydroxypyrid-4-one, has also been achieved by modifying a reported route.

Two new, and general synthetic routes have been investigated in a preliminary way. One route involving the reaction of 1-bromobutane-2,3-dione with N-methylbenzamide, has led to the recovery of the starting amide and decomposition of the bromo compound. The second route involves the use of pyridine as the starting material. In this study, 1-ethoxycarbonylpiperidine-3,4-diol was synthesised using a previously reported procedure. The oxidation of this diol with Jones' reagent gave a mixture of products, one of which was identified as the desired product, 1-ethoxycarbonylpiperidine-3,4-dione using G.C.-mass spectrometry techniques.

Metal(II) and Co(III) complexes of 1,2-dimethyl-3-hydroxypyrid-4-one (dmpH) have been prepared and characterised by spectroscopic and magnetic studies. The structure of the zinc(II) complex has been established by X-ray crystallography. The complexing behaviour of 1,2-dimethyl-3-hydroxypyrid-4-one with iron(III) has been investigated and has been shown to be dependent on pH and molar ratio of the ligand-to-iron. Reactions with $FeCl_3$ in 1:1 and 2:1 molar ratios at low pH's gave products of tentative formulations $[Fe_2Cl_2(dmp)(dmpH)_2]^{3+}$ $3Cl^-$ and $[Fe_2Cl_2(dmp)(dmpH)_4]^{3+}$ $3Cl^-$, respectively. Reaction in 3:1 ratio at low pH gave $[Fe_2Cl_2(dmp)(dmpH)_4]^{3+}$ $3Cl^-$, whereas at high pH the tris-chelate, $Fe(dmp)_3.4H_2O$ resulted. The formulations are based on elemental analyses, magnetic and conductance measurements, and Moessbauer and i.r. spectroscopic studies.

Genotoxicological evaluation of 1,2-dimethyl-3-hydroxypyrid-4-one and its HCl salt has been carried out using a recently developed bioassay, which involves monitoring the chemically induced mutation frequencies in continuous cultures of the fission yeast, *Schizosaccharomyces pombe*. Both, the free base and the salt have demonstrated antimutagenic activity.

ABBREVIATIONS

aq. = aqueous

b.p. = boiling point

cap^R = chloramphenicol resistant

C.I. = chemical ionization

cyl^R = cycloheximide resistant

decomp. = decomposition

dist. = distance

dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one

E.I. = electron impact

EMS = ethyl methanesulphonate

G.C. = gas chromatography

h.p.l.c. = high performance liquid chromatography

i.r. = infra-red

m.p. = melting point

m.s. = mass spectrometry

n.m.r = nuclear magnetic resonance

pmpH = 1-phenyl-2-methyl-3-hydroxypyrid-4-one

ref. = reference

tcpH = 1-(4-tolyl)-6-carbethoxy-3-hydroxypyrid-4-one

temp. = temperature

t.l.c = thin layer chromatography

tmpH = 1-(4-tolyl)-2-methyl-3-hydroxypyrid-4-one

vis. = visible spectrum

CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 The role of metal ions in biological systems

Within living systems, metals play a vital role in the mechanisms of physiological regulation and hence biological functions. Metals are present in biological systems in two loosely categorised groups:[1,2]

i) Trace metals which occur in very minute quantities but nevertheless are essential for normal physiological functions. For example, many enzymes which act as catalysts in critical biochemical reactions, are highly complex protein structures containing trace metals. Carboxypeptidase, for instance, is a zinc complex with a molecular weight of 34,475 containing only one zinc atom.[1] This enzyme is secreted from the pancreas and acts as a catalyst in the hydrolysis of peptide linkages.

ii) Bulk metals, namely sodium, potassium, magnesium and calcium, which make up 99% of the total metal ion content of living systems are involved in various biological functions.[1] Sodium and potassium exist as their hydrated ions, and are important in certain physiological controls.

For instance, the ionic imbalance of potassium ions within the nerve cell and of sodium ions outside the cell induces the conductance of electrical impulses along the nerve fibre. Calcium and magnesium, on the other hand, are more strongly bound and are involved in the activation of certain enzymes, in maintaining the structure of an organism, and in trigger mechanisms.

The concentration of the metal ions occurring in biological systems is controlled within fine limits. Nevertheless, there are cases in which this balance is disturbed which could lead to disorders. The deficiency of essential metal ions could cause the disturbance of the biological processes associated with these ions. Iron deficiency anaemia is a classic example which is related to a lack of haemoglobin as a result of insufficient iron for the red blood cells. Such conditions can be treated with ferrous sulphate mixed with ascorbic acid which helps the absorption of the iron. Sometimes complexes are used to release iron slowly in order to avoid the toxic effects of iron accumulation.[1,2]

On the other hand, an abnormally high concentration of a vital metal can be toxic and cause disruption of functions associated with it. In other cases, the accumulation of heavy metals such as lead or mercury, which have no apparent biological activity, could also become poisonous.

The accumulation of these heavy metal ions often results from environmental pollution caused by industrial problems. The concentration level beyond which a metal ion can be toxic depends on its physiological location. For instance, a doubling of the normal extracellular concentration of potassium ions can lead to heart disorders, while the intracellular concentration of this ion is still well below the normal.[2]

Wilson's disease is an example of deposition and localisation of an excess of a metal ion. In such conditions the body's total copper content, which is normally about 100-150 mg (with the highest concentration in the brain), increases up to a hundred times. The accumulation of copper in the liver, brain and kidneys leads to symptoms such as lack of coordination and progressive mental deterioration. Wilson's disease can be treated by a copper-restricted diet and chelation therapy.

Chelation therapy involves the use of a chelating agent, which by forming a complex with the excess metal, and through excretion, reduces the concentration of that metal to its normal limits. A chelating drug has to fulfil several criteria which are as follows:

i) It should be selective towards the toxic metal.

ii) The chelator and its complex should be non-toxic.

iii) It must not be easily metabolised.

iv) It must be capable of penetrating to metal storage sites.

v) It should be able to form a stable metal complex which must be readily excreted.

Iron-overload is another example of deposition of an excess metal which is of special interest to this work. While the presence of iron is vital for biological processes such as oxygen transport and electron transfer, its accumulation in certain tissues could lead to serious complications. Iron is present in a number of proteins which are involved in a variety of biological functions. Possibily one of the most well-known iron containing proteins is haemoglobin which gives the red colour to the blood and is responsible for transport of oxygen around the blood system. There is no regulatory mechanism for excretion of iron; thus its concentration within the body is controlled primarily by absorption from the gut, and not by excretion.[2] The absorbed iron is transported to the plasma by transferrin. Transferrin is an iron-binding protein which has the function of distributing iron between absorption, storage, utilisation, and haemoglobin degradation sites. The excess iron is stored by ferritin and haemosiderin. Ferritin is a water soluble protein, capable of binding up to 4500 iron atoms, although it does not have to be fully saturated with iron. It is widely distributed in various organs of mammals but mainly

concentrated in liver, spleen and bone marrow. Haemosiderin is an insoluble molecule with variable composition which is not well understood in comparison to ferritin.[2,3]

The lack of a regulatory mechanism for the excretion of iron in special cases could lead to iron-overload disorders. Iron-overloading may arise from either increased absorption of dietary iron or more importantly through regular blood transfusions. One of the most common cases of iron-overloading is in patients receiving regular blood transfusions as a treatment for β-thalassaemia.

1.2 Iron-overload and Thalassaemia

Thalassaemias are hereditary anaemias, caused by a mutation which affects the haemoglobin synthesis.[4] Haemoglobin consists of four polypeptide chains, each of which is combined with an iron-containing substance known as haem. A normal adult haemoglobin has two identical α-chains and two identical β-chains. The production of these chains is controlled by their respective genes and in a normal condition they are produced equally in the bone marrow.[4,5] However, in the case of thalassaemia, an inadequate amount of one or more of the globin chains is synthesised. This imbalance in production of the globin chains leads to the reduction of haemoglobin synthesis and

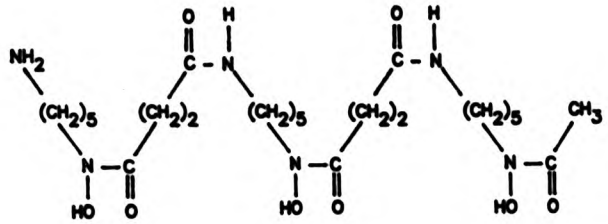
more significantly to the precipitation of those chains which are produced in excess.[4] There are many different types of thalassaemia which can be classified according to which globin chain is affected or to their clinical phenotypes. In a majority of cases, both clinical and molecular classifications are combined. The severest type of this disease is a form of α-thalassaemia in which all the α chains are inactive. This leads to the development of a severe abnormality in the foetus during pregnancy which often results in stillbirth or death shortly after birth. The more common forms are β-thalassaemias, which are mainly two types, β-thalassaemia trait (or minor) and β-thalassaemia major. The former is the heterozygous form which normally results in a mild anaemia indicated by apparent iron deficiency symptoms. Thalassaemia major, however, is the homozygous type in which two inactive β genes have been inherited, and clinically this is the most important type.[3] People with thalassaemia trait are carriers and therefore the disease could be passed on from parents to children. If only one of the parents is a carrier, there is no chance that their children will have thalassaemia major, but they have a 50% chance of being carriers. However, if both parents are carriers then there is a one in four chance that their child will have β-thalassaemia major.[4]

The β-thalassaemias are widely spread throughout the world, but are mainly concentrated in the Mediterranean countries, parts of the Middle East, and Southeast Asia. A recent survey has shown that 7% of the Greek mainland population are carriers of β-thalassaemia; therefore it is expected that about 200 new cases of homozygous β-thalassaemia will be born in Greece each year.[7] Approximately, 15-20% of the population of Cyprus and Sardinia are carriers, and one in a hundred babies have β-thalassaemia major. This disease has become clinically important in Britain since 1957 due to the immigration of 56,000 Cypriots, 10% of the total population of Cyprus. Currently, there are about 300-500 cases of β-thalassaemia major in Britain.[6,7]

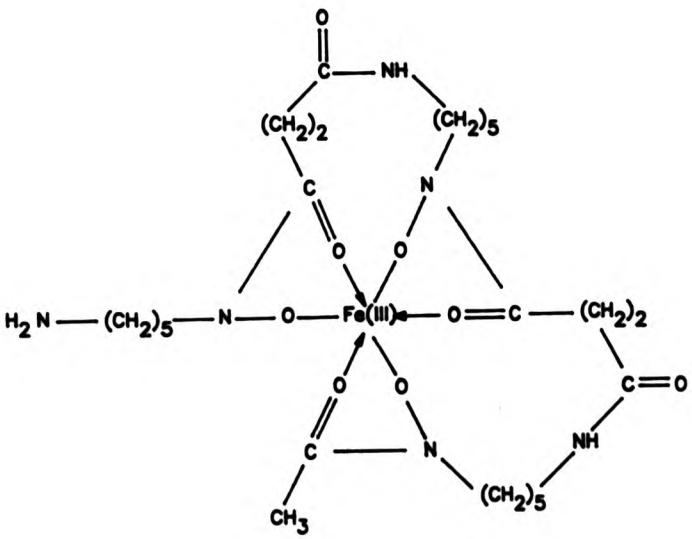
People with β-thalassaemia major suffer from severe anaemia from the early stages of their lives. The majority of homozygous patients depend on regular (every 4-6 weeks) blood transfusions in order to sustain life. This treatment helps the patients to live a relatively normal life into their twenties, but by then they may start developing complications due to accumulation of excess iron. The donated blood cells contain a considerable amount of iron; for example there is about 200 mg of iron in a pint of blood whereas the normal requirement of iron for an adult is only 4 mg per day. The accumulation of this excess iron in a number of tissues such as the liver

leads to diabetes, liver disease, cardiac failures and in some cases inadequate pituitary development. The effects of iron-overload can be reversed by chelation therapy. The only chelating agent being widely used, clinically for iron-overload, is desferrioxamine (1). This compound is a naturally occurring hydroxamate siderophore prepared from ferrioxamine (2). Desferrioxamine, like all other siderophores, has a great affinity for iron and is highly selective towards this metal. It forms a highly stable complex (2) which allows the excretion of iron from the body *via* both urine and faeces.[4]

Desferrioxamine can be given intramuscularly or by slow intravenous or subcutaneous infusion. However, intramuscular infusion cannot achieve the required iron balance in chronically transfused patients. By contrast, when this drug is given intravenously, a significantly greater amount of iron is excreted in the urine. Despite these facts, continuous subcutaneous infusion is preferred because, unlike the intravenous method, it does not have to be performed at a hospital. The drug can be given *via* a portable light-weight pump slowly over 10 to 12 hours.[4,5]



(1)- Desferrioxamine



(2)- Ferrioxamine

1.3 Alternative Chelating agents

Although desferrioxamine provides an effective method of treating iron-overload disorders, there are several disadvantages associated with this drug which are listed below:

i) The drug itself and the pump for its administration make this therapy highly expensive.

ii) Desferrioxamine is not orally active.[4]

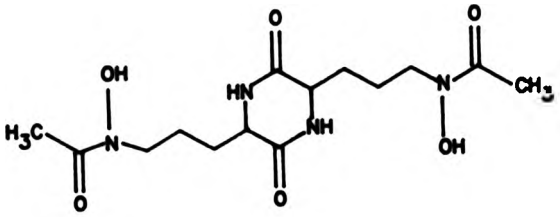
iii) The method of infusion is uncomfortable, as patients are required to carry the pump for several hours everyday.

iv) Several toxic side effects such as reversible retinal abnormalities have been reported which are associated with high doses of this drug.[9,10]

As a result of these drawbacks extensive research has been carried out in order to develop effective and orally active iron chelators. A large number of iron-chelators have been screened for their activity *in vivo*. These include both the naturally occurring siderophores and synthetic chelators. These compounds can be generally classified into four groups:[9, 11-18]

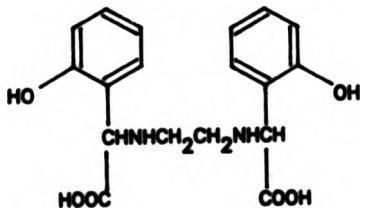
i) Compounds with hydoxamate groups- Rhodotorulic acid (3) is an example of compounds of this group; it is a naturally occurring hydroxamic acid. Although this compound is not orally active it is twice as effective as desferrioxamine when administered intraperitoneally to

rate. Unfortunately, its administration to humans caused persistent swelling and pain at the site of injection.[13]



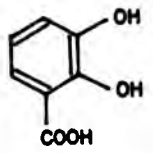
(3)-Rhodotorulic acid

ii) Compounds with phenolic groups- Ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid) (4) is a hexadentate water soluble chelator. This compound has been shown to be orally active and more effective than desferrioxamine. The disodium and dihydrochloride salts of this compound are more soluble but not as potent. Unfortunately, in humans this compound did not induce significant excretion of iron.[14]



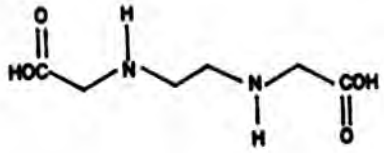
(4)- Ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid)

iii) Compounds with catecholic groups- 2,3-dihydroxy benzoic acid (5) removes iron through urine in rats when it is given orally.[11] However, this compound has been shown to be inactive and toxic when administered intraperitoneally to mice.[8]

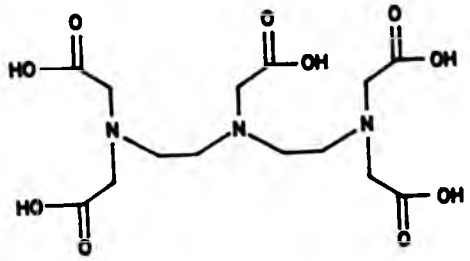


(5)- 2,3-dihydroxy benzoic acid

iv) Compounds of other types- Ethylenediamine acetic acid (6) and diethylenetriaminepenta-acetic acid (7) have been shown to increase iron excretion, but both have shown a high affinity for divalent metals such as calcium and magnesium.[8]

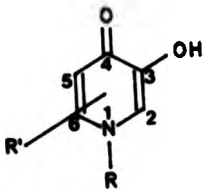


(6)- Ethylenediamine acetic acid



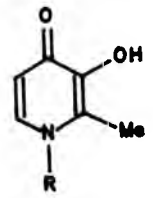
(7)- Diethylenetriaminepenta-acetic acid

Recently, considerable attention has been given to hydroxypyridones (8) which are the main concern of this thesis. The chelating ability of these compounds and its involvement in various biological systems have been discussed by a number of workers (see Chapter 3). However, their potential application as iron chelators for the treatment of iron-overload was first identified by Hider *et al* in 1982.[18]



(8)- 3-hydroxypyrid-4-ones

Hydroxypyridones are bidentate ligands capable of forming a five-membered chelate ring with a metal ion. Their relatively strong acidities (pK_a's = 3-9) enable them to chelate with ferric iron more effectively than catechol or hydroxamates in neutral and acidic solutions.[17] Some of the most promising derivatives of hydroxypyridones are the 1-alkyl-3-hydroxy-2-methylpyrid-4-ones (9). These compounds are orally active iron chelators, capable of forming stable water soluble iron complexes at physiological pHs.[16,18,19] They have been shown to remove iron from transferrin and ferritin *in vitro* and from iron-loaded animals *in vivo*.[20]



(9)- 1-alkyl-3-hydroxy-2-methylpyrid-4-one

However, before the study for this thesis had commenced, very limited information was available on these compounds in relation to their:

i) synthesis;

ii) structures of free base and protonated forms;

iii) mode of chelation, and the nature, structure, and properties of their chelates;

iv) selectivity towards iron;

v) toxic effects.

Concurrent with this work, several reports dealing with some of the above questions have been published.[21-31] Nevertheless, there is still only limited information on the properties and the structure of these chelators and their complexes. One of the compounds of this group, 1,2-dimethyl-3-hydroxypyrid-4-one (9, R = Me), has been given the most attention. Although there are disputes with regard to the toxic effects of this compound,[32-33] clinical trials with iron-overload patients are being carried out.[34]

The aim of this thesis is the synthesis and the study of the chemistry, structure, complexing behaviour, and toxic effects of 3-hydroxypyrid-4-ones (8). This thesis describes work which has led to:

i) the clarification and improvement of the established synthetic route to 1,2-dimethyl-3-hydroxypyrid-4-one;[20]

ii) the elucidation of the structures of the free base and the hydrochloride salts of 1,2-dimethyl-3-hydroxypyrid-4-one and 3-benzyloxy-1,2-dimethylpyrid-4-one;[20]

iii) a possible new, general route for preparation of 3-hydroxypyrid-4-ones;

iv) the isolation and characterisation of Co(II), Ni(II), Cu(II), Zn(II), Ca(II), Co(III), and Fe(III) complexes of 1,2-dimethyl-3-hydroxypyrid-4-one;[27,30]

v) the establishment of the structure of the Zn(II) complex of 1,2-dimethyl-3-hydroxypyrid-4-one by X-ray crystallography;[29]

vi) the illustration of antimutagenic effects of 1,2-dimethyl-3-hydroxypyrid-4-one and its hydrochloride salt in a chronic toxicity test in *Schizosaccharomyces pombe*.

Separate chapters of this thesis deal with the above topics.

CHAPTER TWO

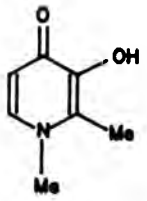
SYNTHESIS AND PROPERTIES OF 3-HYDROXYPYRID-4-ONES

2.1 Introduction

Syntheses of many types of 3-hydroxypyrid-4-ones have been described in the literature[36,37] with the first being reported in 1879.[38] This group of compounds has been the subject of study in a wide variety of research fields, ranging from agriculture[39-41] to medicine.[16,42-44] In these studies, several activities such as depilatory,[45-47] fungicidal[39] and inhibition of certain enzymes[40,48,49] have been reported for 3-hydroxypyrid-4-ones, which have been suggested to be related to their chelating ability. As stated earlier in chapter one, 1-alkyl-2-methyl-3-hydroxypyrid-4-ones (9) are effective iron chelators having potential application in the treatment of iron-overload. However, at present, there is insufficient knowledge available on their properties to warrant their use as a replacement for desferrioxamine, the currently used iron-chelator. Furthermore, although several methods of synthesis have been reported for these compounds, the routes are limited in their versatility. Hence further

study of their properties and synthetic pathways is of considerable importance.

The studies in this thesis have been mainly concentrated on 1,2-dimethyl-3-hydroxypyrid-4-one (10). In this chapter, an investigation of the existing synthetic routes to this compound is presented and discussed. These studies have led to elucidation of the structures of the free base and protonated forms of 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its O-benzyloxy analogue. In addition, an account is given of attempts to develop a new general synthetic pathway to 3-hydroxypyrid-4-ones.



(10)

2.2 Investigation of the published synthetic routes to 1,2-dimethyl-3-hydroxypyrid-4-one (10)

Several methods of preparation for 1,2-dimethyl-3-hydroxypyrid-4-one (10) have been described,[18,19,21,22,50,51] the earliest being 1970, by Yasue *et al.*[50] All involve the

use of 3-hydroxy-2-methyl-4-pyrone (11), otherwise known as maltol, or its derivatives as the starting material. Table 1 outlines these methods and the data reported for the pyridone (10). It should be noted that routes (iv) and (v) were published after the present studies had commenced.

Table 1- Previously reported methods of preparation of 1,2-dimethyl-3-hydroxypyrid-4-one

Route no.	Starting material	Route type	Data reported	Yield
i	3-β-D-glucopyranosyloxy -2-methyl-4-pyrone[30]	indirect	m.p., e.a.	-
ii	Maltol[31]	direct	n.m.r. i.r.	55%
iii	Maltol[19,16]	indirect m.p. m/z of parent ion	n.m.r. i.r.	60%
iv	Maltol[21]	direct	n.m.r.	50%
v	Maltol[22]	indirect i.r.*	n.m.r., m.p	51%

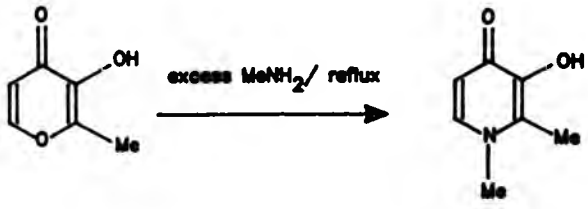
e.a. = elemental analysis;
* X-ray crystallographic data also reported.

The synthetic route (i) uses maltol glucoside, a naturally occurring material as the starting pyrone.[30] On heating this compound with methylamine the pyridone derivative, 3-β-D-glucopyranosyloxy-1,2-dimethylpyrid-4-one was isolated. This in turn was treated with hydrochloric acid and neutralised with sodium hydrogen carbonate to give

1,2-dimethyl-3-hydroxypyrid-4-one (10). The product was purified by recrystallisation from water. This report does not give the yield of the final product and therefore the efficiency of the method cannot be assessed.

The most attractive method for synthesis of 1,2-dimethyl-3-hydroxypyrid-4-one (10) is probably route (ii) which involves the direct conversion of a pyrone ring to the required pyridone. In this method 3-hydroxy-2-methyl-4-pyrone (11) was heated with methylamine in the presence of acetic acid to give the corresponding pyridone (10) in 55% yield. However, the purification of the product involved several steps: extraction, column chromatography, and sublimation. In the present study, the modification of this route has led to a simple and useful method for preparation of 1,2-dimethyl-3-hydroxypyrid-4-one (10). Using h.p.l.c., the progress of the direct reaction of maltol (11) with methylamine was monitored. The h.p.l.c. results have shown that using the reported procedure, complete conversion of the starting material (11) is not achievable even when long refluxing times are used. However, when the ratio of methylamine to maltol (11) is increased a complete reaction of the maltol occurs. The desired product, 1,2-dimethyl-3-hydroxypyrid-4-one (10) (m.p. 263-265 °C; lit.[80] 266-268 °C), is conveniently obtained on removal of the solvent and recrystallisation

from water. A similar modified route (route iv) was later reported by Kontoghiorghes and co-workers.[21]



(11) (10)

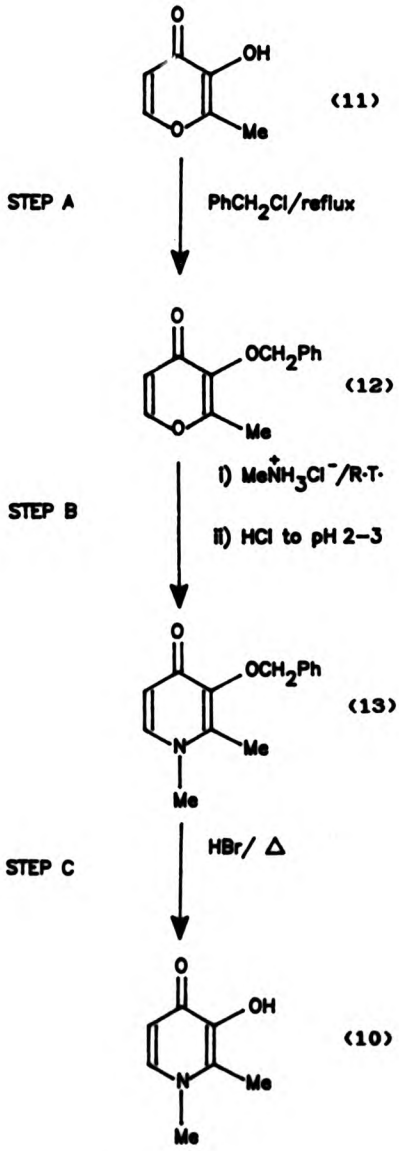
The synthetic route (iii) (Scheme 1) reported by Kontoghiorghes and co-workers,[16,19] describes the indirect production of this pyridone (10) from maltol (11) *via* 3-benzyloxy-2-methyl-4-pyrone (12) and 3-benzyloxy-1,2-dimethylpyrid-4-one (13). Finally, the removal of the benzyl group on treatment with hydrobromic acid yielded the desired pyridone (10) (Scheme 1). These reports[16,19] do not give any account of the characterisation of the intermediate pyridone (13), and the final product was only characterised by n.m.r. spectroscopy. The present study has shown that the method (iii), under the reported conditions, does not lead to the free bases of the pyridones (10) and (13), but to their protonated forms. These results are discussed below.

Concurrent with our work, another method of synthesis (route v) for 1,2-dimethyl-3-hydroxypyrid-4-one (10) was reported,[22] which also involves the use of the benzyloxy derivatives (12) and (13). However, this route used different reaction conditions from those stated by Kontoghiorghes and co-workers[16,19] and the results are in accord with those obtained during our work.

2.2.1 Reassessment of Synthetic Route (iii) to 1,2-dimethyl-3-hydroxypyrid-4-one

One of the early objectives of this work was to prepare 1,2-dimethyl-3-hydroxypyrid-4-one (10) for further studies, using the synthetic route (Scheme 1) described by Kontoghiorghes and co-workers.[16,19] However, attempts to repeat this route led to some complications requiring a detailed investigation.

3-Benzyloxy-2-methyl-4-pyrone (12), was synthesised without problem using Harris's method[45] and was purified by distillation (53% after purification, lit. 92% crude yield). The product was identified by ^1H n.m.r. spectroscopy, exhibiting characteristic doublets at 6.20 and 7.52 ppm (J = 7.1 Hz) due to hydrogens on the pyrone ring, and singlets at 5.05 and 7.35 ppm typical of a benzyl group. However, the conversion of this pyrone (12) to 1,2-dimethyl-3-hydroxypyrid-4-one (10) through


O
OH
O
Me
(11)
STEP A
PhCH2Cl/reflux
O
OCH2Ph
O
Me
(12)
STEP B
i) MeNH3Cl−/R·T·
ii) HCl to pH 2–3
O
OCH2Ph
N
Me
Me
(13)
STEP C
HBr/ Δ
O
OH
N
Me
Me
(10)

Scheme (1)

3-benzyloxy-1,2-dimethylpyrid-4-one (13) produced a highly water soluble white solid. Although the n.m.r. spectrum of this solid was in accordance with that of the expected pyridone, the elemental analysis and melting point were not. As a result, it was decided to investigate steps B and C (Scheme 1) more closely and to attempt to purify and characterise the intermediate pyridone (13).

2.2.1.1 Investigation of Step B

In the literature[15,19] it was reported that, on stirring the 3-benzyloxy-2-methyl-4-pyrone (12) with excess methylammonium hydrochloride in the presence of sodium hydroxide, followed by the acidification of the reaction mixture with HCl (pH 2.5-3.0), the crude solid, 3-benzyloxy-1,2-dimethylpyrid-4-one (13) was produced. This conclusion was based on the isolation of the product; no analytical data or melting point were provided. When this procedure was repeated , during the present study, a white solid was isolated which was recrystallised from a mixture of ethanol and water. The n.m.r. spectrum of this solid was in agreement with the structure of 3-benzyloxy-1,2-dimethylpyrid-4-one (13), but the elemental analysis (Entry (iv),Table 2) was not. The absence of organic impurities was indicated by the n.m.r. spectrum. Similarly, using qualitative analysis for sodium, it was shown that the compound was not contaminated with sodium

chloride (the expected inorganic impurity). A positive test for chlorine together with a high water solubility suggested that the product (m.p. 176-178 °C) could be the hydrochloride salt of 3-benzyloxy-1,2-dimethylpyrid-4-one (13) rather than the free base. This is expected for pyridones, since they are readily protonated due to a lone pair of electrons present on the nitrogen (the structure of protonated 4-pyridones is discussed in section 2.3). The quantitative elemental analysis for carbon, hydrogen, nitrogen and chlorine (Table 2) confirmed that the product was the hydrochloride salt of pyridone (13)(compare entries (ii) and (iv), Table 2).

In order to prepare the neutral form, i.e. compound (13), step B was repeated without the acidification stage. As a result a white solid was isolated, which on drying over calcium chloride under vacuum, converted into a viscous yellow oil. This oil showed highly hygroscopic behaviour. On its exposure to atmospheric moisture, or on addition of a few drops of water it changed into the solid form again. The thermal gravimetric analysis of this solid indicated a loss of 18.4% suggesting that it is a trihydrated compound. The elemental analysis (Entry (v), Table 2) and n.m.r spectroscopic data (see section 2.3.2) were consistent with the expected 3-benzyloxy-1,2-dimethylpyrid-4-one trihydrate, m.p. 39-40 °C. While this work was in progress, the synthesis of 3-benzyloxy-1,2-

dimethylpyrid-4-one (13) was reported[22] from the direct reaction of the corresponding pyrone (12) with methylamine. The procedure does not involve the use of sodium hydroxide or acidification. Although no analytical data were provided[22] for this compound, it was described as an oil; Kontoghiorghes,[19] on the other hand, has described this compound as solid. The results obtained in the current study clarify these contradictory statements.

Table 2- Elemental analyses of the hydrated and hydrochloride salts of pyridone (13)

	Entry no.	Compound	C%	H%	N%	Cl%
	(i)	pyridone (13)	73.4	6.5	6.1	0.0
Expected for:	(ii)	HCl salt of pyridone (13)	63.3	6.0	5.3	13.4
	(iii)	trihydrate salt of pyridone (13)	59.4	7.4	4.9	0.0
	(iv)	presence of HCl (pH 2-3)	63.5	6.6	5.4	13.5
Observed for compound isolated in the:	(v)	absence of HCl	59.9	7.3	5.1	0.0

2.2.1.2 Investigation of Step C

When the hydrochloride salt of 3-benzyloxy-1,2-dimethylpyrid-4-one (13) was treated with hydrogen bromide, under the reported conditions,[16,19] highly water

soluble white crystals, m.p. 189-191 °C, (lit.[18] 230 °C) were isolated. In the previous reports[18,19] this compound was formulated as 1,2-dimethyl-3-hydroxypyrid-4-one (10) on the basis of n.m.r., i.r., and mass spectra (only parent ion reported). However, in the present study, it was found that this compound contained a considerable amount of bromine (see Entry (iv), Table 3 for elemental analysis). It was, therefore, suspected that the crystals isolated were in the form of the hydrobromide salt of the pyridone (10) for the same reasons as described for its precursor. The elemental analysis agreed very closely with the formulation of the hydrobromide salt (Entries (iii) and (IV) Table 3).

Table 3- Elemental analyses of pyridone (10) and its protonated forms

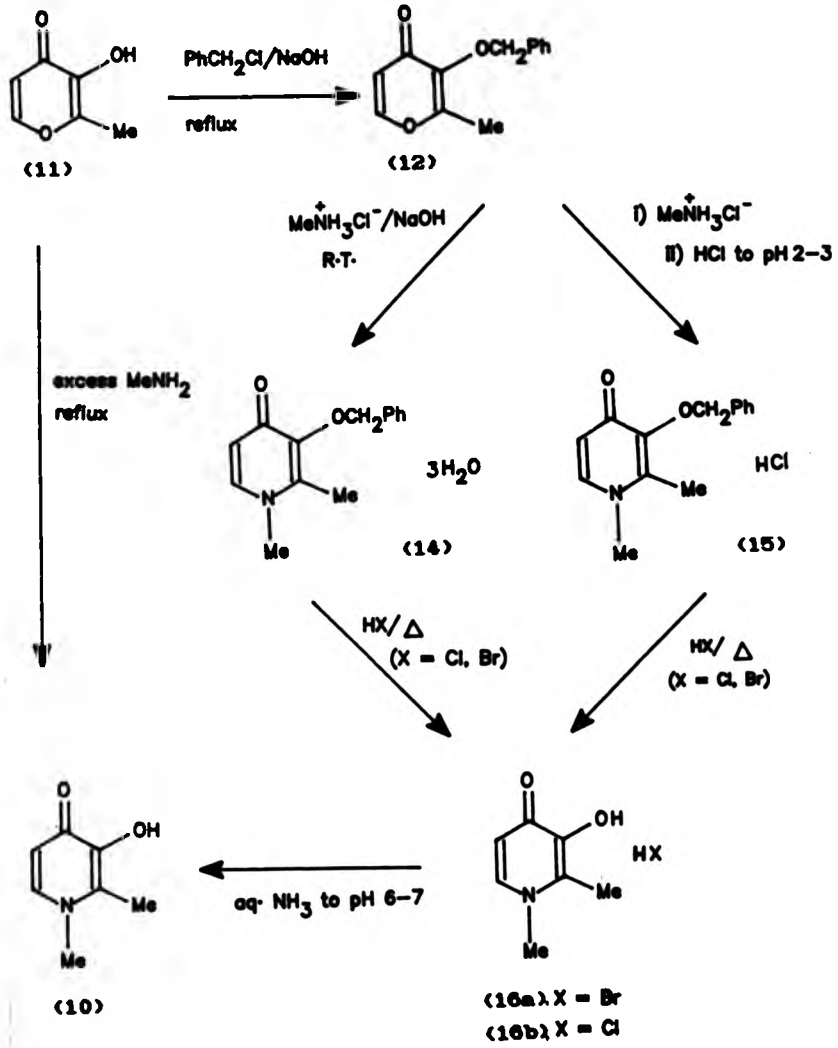
	Entry no.	Compound	C%	H%	N%	Br%	Cl%
	(i)	pyridone (10)	60.4	6.5	10.1	0.0	0.0
Expected for:	(ii)	HCl salt of pyridone (10)	47.9	5.7	7.9	0.0	20.2
	(iii)	HBr salt of pyridone (10)	38.2	4.5	6.4	36.4	0.0
Observed for compound isolated from reaction of HCl salt of pyridone (13) with:	(iv)	HBr	38.6	4.5	6.5	36.2	0.0
	(v)	HCl	47.8	5.9	7.8	0.0	20.4
	(vi)	a) HCl b) aq. NH$_3$	60.1	6.7	10.2	0.0	0.0

The hydrochloride salt of the pyridone (10) was also prepared by carrying out the deprotection step C in aqueous hydrochloric acid. The elemental analysis for carbon, hydrogen, chloride and nitrogen were in close agreement with the expected hydrochloride salt of 1,2-dimethyl-3-hydroxypyrid-4-one, m.p. 174-176 °C (Entries (ii) and (v), Table 3).

In this study, the free base form (10) was successfully isolated by neutralising the 1,2-dimethyl-3-hydroxypyrid-4-one hydrochloride with ammonia solution or with sodium hydrogen carbonate to pH 6-7 (m.p. 265-266 °C, lit.[80] 266-268 °C).

In the light of the foregoing results a new scheme can be proposed as shown in Scheme 2. This scheme gives the reaction conditions required for the formation of free bases (10) and (14) and their protonated forms (16a, 16b, and 15). It is concluded that the best method for preparation of pyridone (10) is by direct reaction of maltol (11) with methylamine.

A comparison of the n.m.r. spectra of the free base pyridones (10 and 14) with their hydrochloride salts has shown considerable differences which have allowed for some structural inferences (see section 2.3.2). Furthermore, the comparison of the X-ray crystallographic


PhCH2Cl/NaOH
reflux
(11)
(12)
MeNH3Cl⁻/NaOH
R·T·
I) MeNH3Cl⁻
II) HCl to pH 2–3
excess MeNH2
reflux
3H2O
HCl
(14)
(15)
HX/Δ
(X = Cl, Br)
HX/Δ
(X = Cl, Br)
aq· NH3 to pH 6–7
(10)
(16a) X = Br
(16b) X = Cl

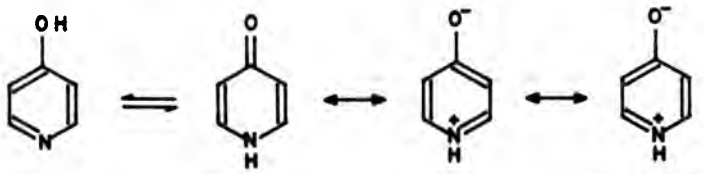
Scheme (2)

data of the free base (10)[22] with its hydrobromide salt (16a)[62] has confirmed the structural deductions derived from n.m.r. studies. These results and their significance in the potential application of these compounds as iron-removing agents are discussed below (section 2.3).

It should also be noted that during this study the hydrochlorides of 1-ethyl-2-methyl-3-hydroxypyrid-4-one and 3-benzyloxy-1-ethyl-2-methypyrid-4-one were prepared (see Chapter 5) using the above established route, for mass spectrometry studies which are discussed in section 2.3.4. No attempts were made to isolate their free bases.

2.3 Properties and structures of 3-hydroxypyrid-4-ones

In general, pyridones are expected to have pronounced aromatic character. Figure 1 represents the resonance and equilibrium forms of 4-pyridinol (17).

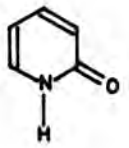


(17) (17a) (17b) (17c)

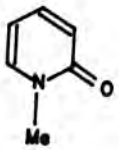
Figure 1- Resonance and equilibrium structures of 4-pyridinols

Like phenols, the oxygen atom of pyridinols shows nucleophilic properties. Thus they are expected to have typical phenolic behaviour. This expectation is realised for 3-pyridinol, as it gives a deep purple colour with ferric chloride, undergoes nuclear electrophilic substitution, and participates in Mannich condensation.[33] 2-Pyridinols and 4-pyridinols have similar chemical characteristics and they also show certain phenolic properties, but not as strongly as 3-pyridinols. Unlike 3-pyridinols, 2- and 4-pyridinols exist in tautomeric forms (e.g. 17 and 17a, Figure 1). Ultraviolet spectroscopic studies have revealed that the predominant tautomer depends on pH. Thus it has been shown that the ultraviolet spectra of 2-pyridinol (18) and its N-methyl (19) and O-ethyl derivatives (20) in hydrochloric acid are almost identical.[83] Since the O-ethyl derivative (in the free base or protonated form) can only have the "pyridinol" structure, the close resemblance of the spectra suggests that the hydrochlorides of these three compounds have similar structures (21, 22, 23).

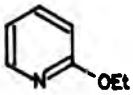
N.m.r. spectroscopic studies of 1-methylpyrid-4-one (24) have shown that this compound in aqueous solution exists predominantly in the pyridone form (24), whereas the spectrum in an acidic medium (10 M H_2SO_4) suggested the presence of the 4-hydroxypyridinium ion (24a) as the dominant species.[84]



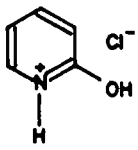
(18)



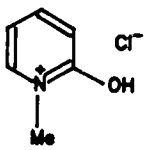
(19)



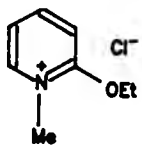
(20)



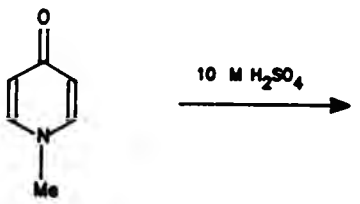
(21)



(22)



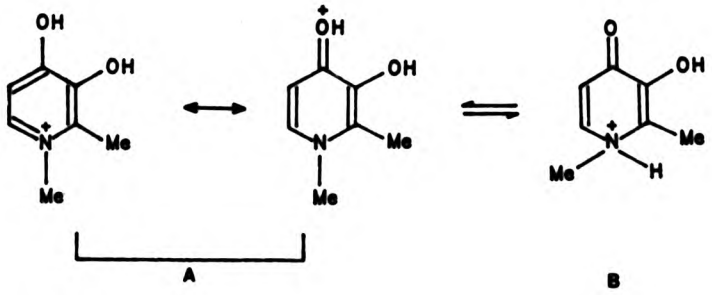
(23)



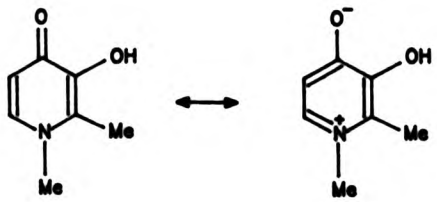
(24)

(24a)

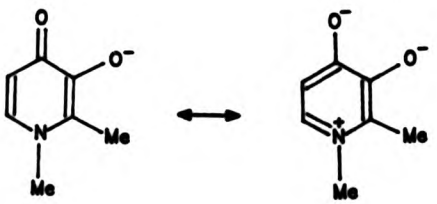
Acidic pH



Neutral pH



Basic pH



Scheme (3)

Some possible structures and their relationships for 1,2-dimethyl-3-hydroxypyrid-4-one (10) at different pHs are depicted in Scheme 3. As apparent, in acidic medium this compound (10) can exist in either tautomeric forms A or B.

In our studies, the structures of the free bases (10) and (14), and their protonated forms have been clarified by spectroscopic techniques, as discussed in the following sections.

2.3.1 Infra-red spectra

The i.r spectra of the free base forms bear some resemblance to those of the protonated forms. This similarity is more significant in the region 2000-600 cm^{-1}, which suggests that the protonation does not produce a drastic change in the structure of the molecule. Table 4 summarises some of the most prominant bands of spectra of the five compounds (10, 14, 15, 16a, and 16b). The spectra of the salts (15, 16a, and 16b) exhibit several strong bands between 2500-3000 cm^{-1} which are absent from the spectra of the corresponding analogous free bases (10 and 14). These peaks are characteristic for strong intramolecular hydrogen bonding.[88] The spectra of the hydrobromide (16a) and hydrochloride (16b) salts of

pyridone (10) are almost identical, suggesting that both compounds have a similar structure.

Table 4- Significant absorption bands (cm^{-1}) of i.r. spectra of pyridones (10) and (14) and their protonated forms (KBr disc)

Compound	vOH	vCH	vO=C and/or vC=C
HCl salt (15)	3336	2829 w, 2867 w	1629, 1552, 1517, 1455, 2924, 3100 1396
free base (14)	2400-2600 b	2675 w, 3034 w	1631, 1522, 1501, 1450 w, 1418 w
free base (10)	3150 b	2947 sh, 2858 sh, 3012	1631, 1569, 1531 w 1514, 1462 ,1420 w
HCl salt (16b)	3470, 3382 3300-2500 b	3300-2500 b	1637, 1537, 1505 1425 w
HBr salt (16a)	3472, 3385 3300-2500 b	3300-2500 b	1641, 1626 sh, 1537, 1506, 1457, 1428 w

b = broad, sh = shoulder, v = stretching vibrations, w = weak.

In the region 1700-1400 cm^{-1}, all the compounds exhibit several bands which can be assigned to vC=O and/or vC=C or possibly vC=N$^+$ in the case of the salts. It should be noted that in the case of 3-benzyloxy-1,2-dimethylpyrid-4-one trihydrate (14) and its hydrochloride (15) the peaks due to the benzene ring would also appear in the same region. Separate assignments of the C=O and C=C bands were attempted by comparing the previously reported[66-64] data for the 4-pyridones and related compounds. However, it was discovered that there are inconsistencies in the literature in relation to the assignments of the C=O and

C=C bands. The spectra of these previously reported 4-pyridones, in the solid state, exhibit bands at approximately 1635 and 1535 cm^{-1}. Some authors have assigned the band at 1635 cm^{-1} to C=O stretching vibrations and 1535 cm^{-1} to C=C stretching[86,87,88] which in some cases have led to structural conclusions for the protonated form where the protonation occurs on the nitrogen.[63] In contrast, others have made a reverse assignment for these two bands,[88,89] and suggested oxygen protonation for the pyridone salts.[88,43] Other groups, however, have suggested that there is a strong mixing between C=O and C=C stretchings, which makes the separate assignments of these bands impossible.[80-82] Similarly, in the present study, it is believed that the mixing of the two vibrations (C=C and C=O stretchings) is likely to occur; hence the separate assignments have not been attempted. It is concluded that i.r. spectra are not helpful in determining the detailed structures of the free bases and salts of pyridones (10) and (14).

2.3.2 N.m.r. spectra

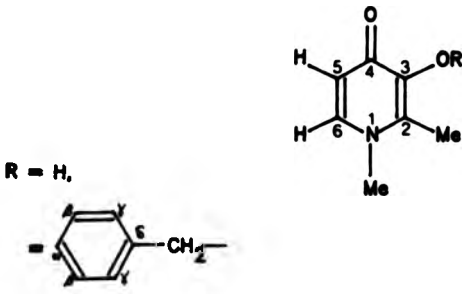
The ^{1}H and ^{13}C n.m.r. spectra (see Appendix, spectra 1-9) of the hydrochloride salts (15 and 16b) and the free bases (10 and 14) were obtained in D_2O under similar conditions. In this section a comparative study of these spectra is presented. The assignments have been made by analogy to

n.m.r. data reported for 4-pyridones and pyridinium salts.[65]

2.3.2.1 ^{1}H n.m.r. spectra

The ^{1}H n.m.r. spectra of the free bases (10) and (14) and their hydrochloride salts are listed in Table 5.

Table 5- ^{1}H n.m.r. chemical shifts of the pyridones (10) and (14) and their hydrochloride salts



	δ (ppm)				
Compound R =	14 CH_2Ph	15 CH_2Ph	10 H	16b H	Assignment
	2.06 (s)	2.38 (s)	2.39 (s)	2.56 (s)	2-Me
	3.58 (s)	3.91 (s)	3.76 (s)	4.05 (s)	N-Me
	4.97 (s)	5.08 (s)			CH_2 of benzyl group
	6.51 (d)	7.12 (d)	6.48 (d)	7.03 (d)	H on C5
	7.39 (s)	7.44 (s)			benzene ring
	7.61 (d)	8.12 (d)	7.61 (d)	8.03 (d)	H on C6

The spectra were obtained in D_2O (TSP = 0 ppm) at 250 MHz;
s = singlet, d = doublet

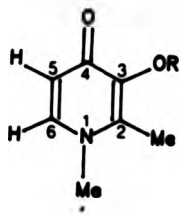
The ^1H signals for the hydrochloride salts show a general downfield shift when compared to those of the corresponding free bases. This shift is more significant for the ring protons indicating delocalisation of the electrons and consequently deshielding of these protons. This in turn may reflect a significant contribution of the pyridinol form(e.g. A, Scheme 3) to the structure of the salts in solution. This downfield feature is also evident in the ^1H n.m.r. spectrum of N-methyl-4-pyridone (24) in acidic medium.[84]

2.3.2.2 ^{13}C n.m.r spectra

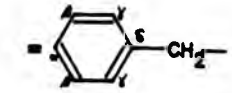
Table 6 shows the proposed assignments of the ^{13}C spectra of the free bases (10) and (14) and their protonated forms. The spectra of the salts (16a) and (16b) are almost identical.

The downfield shifts of C-2 and C-6, and the upfield shift of C-5, in the protonated forms compared to the free bases indicate the presence of a positive charge on the nitrogen atom. The peak due to C-3 cannot be detected in the spectra of the hydrochlorides (15 and 16b), but it is present at 145.71 ppm for the hydrobromide compound (16a) (see Chapter 5) which indicates an upfield shift compared to its free base analogue (10). In the case of the salts this peak may also have shifted upfield and possibily it

Table 6- ^{13}C n.m.r. chemical shifts and suggested assignments of the pyridones (10) and (15) and their protonated forms (D_2O solution).



R = H,



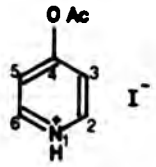
	δ (ppm)					
Compound R =	14 CH_2Ph	15 CH_2Ph	10 H	16a H	16b H	Suggested Assignment
	15.48	16.12	14.65	15.51	15.42	2-Me
	44.94	47.02	45.02	47.23	47.08	N-Me
	76.97	78.34	–	–	–	O-CH_2
	118.58	115.59	114.90	113.48	113.53	C5
	131.54	131.71	–	–	–	Ph ring
	131.67	132.07	–	–	–	Cα,Cβ,Cγ
	132.37	132.35	–	–	–	
	144.24	145.57	138.42	142.02	141.99	C6
	148.94	153.61	141.79	145.16	145.23	C2
	147.60	–a	147.29	145.71	–a	C3
	139.04	138.22				Cδ
	175.83	166.95	171.45	161.25	161.68	C4

The spectra were obtained in D_2O (TSP = 0 ppm) at 250 MHz
a This peak could not be observed, probably it is masked by peaks in the region 139-145 ppm.

is masked by peaks in the region 139-145 ppm. The chemical shift of the quaternary C-4 shows the largest upfield shifts (*ca* 10 ppm) in the salts indicating the loss of carbonyl character and delocalisation of the π-electrons.

This suggests that the hydroxypyridinium structure (e.g. A, Scheme 3) is the dominant species for the protonated salts in D_2O solutions. These data compare closely with those of the N-methyl-4-acetoxypyridinium ion (25, Table 7) (although in a different solvent).

Table 7- Selected chemical shifts[a,b] for the ^{13}C n.m.r. spectrum of 4-acetoxypyridinium iodide (in $CDCl_3$)



(25)

δ(ppm)	Reported assignments[a,b]
151.48	C2 and C6
117.01	C3 and C5
159.77	C4

The spectrum was obtained in $CDCl_3$ (TMS = 0 ppm) at 200 MHz.

In conclusion, the comparison of the n.m.r. spectra of the D_2O solutions of free bases (10 and 14) and their salts (16b and 15) suggests a hydroxypyridinium structure for the salts.

2.3.3 Ultraviolet spectra

1,2-Dimethyl-3-hydroxypyrid-4-one (10) is amphoteric, being capable of both accepting and donating a proton. This amphoteric behaviour can be demonstrated by a general relationship involving two equilibrium reactions (Equation 1). The equilbrium constants (pK_a) of these reactions have been published for a variety of 4-pyridones.[36] In the case of 1,2-dimethyl-3-hydroxypyrid-4-one (10), the reported[19] pK_a1 and pK_a2 values are 3.3 and 9.7, respectively.

$$HAH^{+} \rightleftharpoons HA \rightleftharpoons A^{-}$$

acidic pH Neutral pH Basic pH

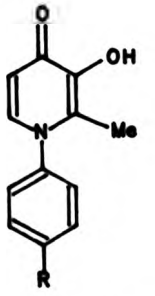
Equation 1

The amphoteric behaviour of pyridone (10) and its derivatives is of great importance with regard to their potential application in the treatment of iron-overload, particularly in considering which species is:

i) present in the biological systems, when the test chelator is administered;

ii) involved in the chelation.

In this study, the amphoteric behaviour of 1,2-dimethyl-3-hydroxypyrid-4-one (10) has been demonstrated by ultraviolet spectroscopic techniques. Spectra of the solutions of pyridone (10) in water, sodium hydroxide, and hydrochloric acid at the same concentration were recorded (Figure 2). The spectrum in neutral aqueous solution (concentration = 1.63×10^{-4} M, pH = 7) shows two absorption maxima at 278.3 and 216.6 nm. The spectrum in sodium hydroxide solution (pH > 12) exhibits two strong absorption peaks at 310 and 230 nm indicating a shift to higher wavelengths in comparison with the neutral solution. On addition of more base no further change was observed, indicating the full dissociation of the pyridone into its anionic form. These results are in accord with those previously published for 4-pyridones of type (26).[66,67]



R = H, CH_3, Cl, NO_2

(26)

As was shown earlier, 1,2-dimethyl-3-hydroxypyrid-4-one (10) acts as a weak base by accepting a proton. At low pH's the pyridone is protonated and exists in a cationic form. The spectrum of this form in aqueous hydrochloric acid (pH = 2) shows a weak peak at 243.3 nm, and two intense peaks at 275.0 and 210.0 nm (Figure 2). The addition of more acid does not change the spectrum, indicating that the protonation of the pyridone is completed. The spectrum of the hydrochloride salt in aqueous solution (pH = 5) was also obtained. The spectrum shows peaks at 278.0 and 216.1 nm similar to those of the free base, except that for the hydrochloride salt (16b), the peak at 278.0 nm appears with a small shoulder at about 245.0 nm. When acid is added to this solution of the hydrochloride salt (16b) the shoulder resolves as a peak at 243.8 nm. This suggests that at pH's around 5 the pyridone exists as an equilibrium between both neutral and cationic forms and at lower pH's (pH < 2) it exists in the cationic form.

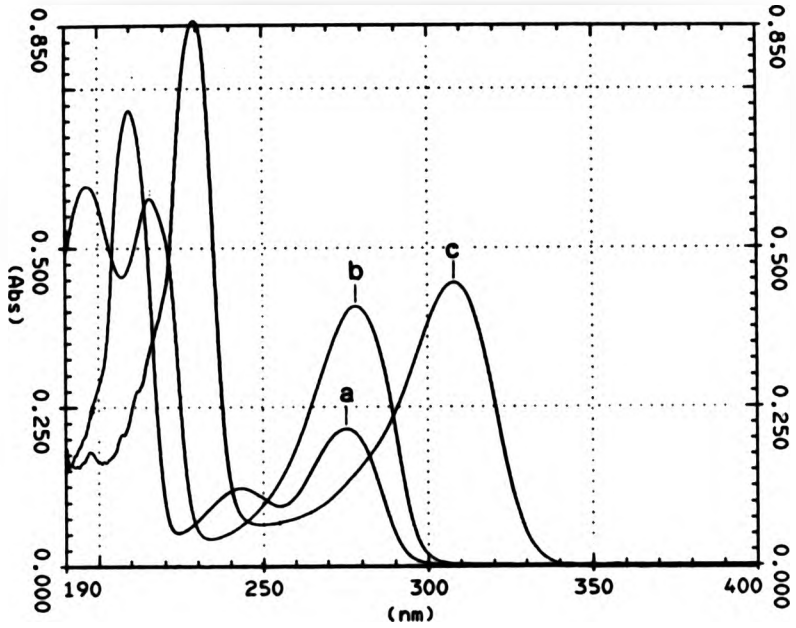


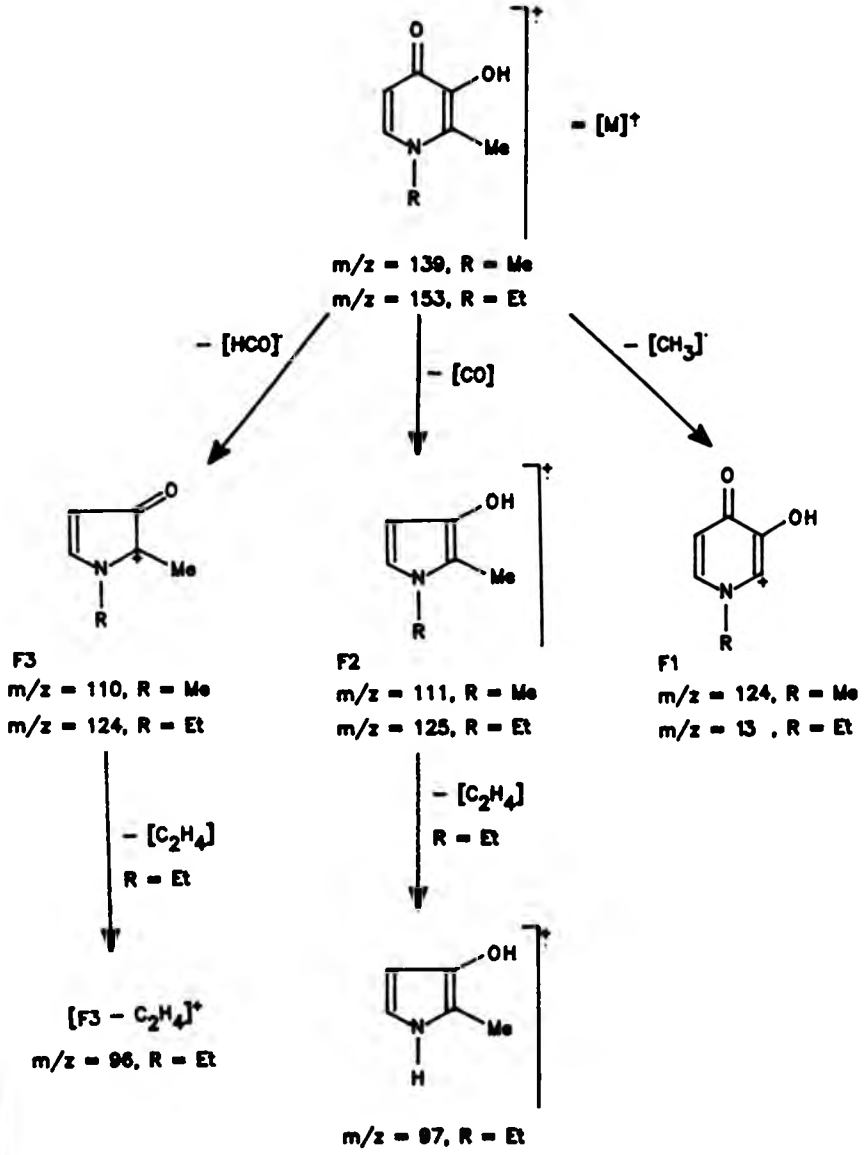
Figure 2- Ultra-violet spectra of the pyridone (10) in:
(a) aq. HCl (1 M);
(b) water;
(c) aq. NaOH (1 M);

2.3.4 Mass spectra

The assignments of the electron impact mass spectra of 1,2-dimethyl-3-hydroxypyrid-4-one (10) and 1-ethyl-2-methyl-3-hydroxypyrid-4-one (9, R = Et) are listed in Table 8. Both compounds show intense parent ion peaks indicating their stable character. In each case the parent ion forms fragments by loss of Me, CO, and HCO. In the case of the 1-ethyl derivative the losses of CO and HCO are followed by the loss of C_2H_4 which is not observed for the 1-methyl derivative, suggesting that C_2H_4 is lost from the ethyl substituent. A fragmentation pathway is proposed for these two pyridones as shown in Scheme 4.

Table 8- Mass spectra of 1-methyl and 1-ethyl,-2-methyl-3-hydroxypyrid-4-ones

m/z (relative abundance)		
1-Me	1-Et	Assignment
139 (68.3)	153(96.2)	$[M]^+$
124 (5.3)	138(1.9)	$[M - Me]^+$ = F1
111 (20.3)	125(100.0)	$[M - CO]^+$ = F2
110 (100.0)	124(17.5)	$[M - HCO]^+$ = F3
-	97(15.1)	$[F2 - C_2H_4]^+$
-	96(32.7)	$[F3 - C_2H_4]^+$


O
OH
Me
N
R
= [M]+
m/z = 139, R = Me
m/z = 153, R = Et
- [HCO]
- [CO]
- [CH3]
F3
m/z = 110, R = Me
m/z = 124, R = Et
F2
m/z = 111, R = Me
m/z = 125, R = Et
F1
m/z = 124, R = Me
m/z = 13 , R = Et
- [C2H4]
R = Et
- [C2H4]
R = Et
[F3 - C2H4]+
m/z = 96, R = Et
H
m/z = 97, R = Et

Scheme (4)

2.3.5 Comparison of the X-ray crystallographic structures of 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its hydrobromide salt (16a)

The above n.m.r. studies have suggested that 1,2-dimethyl-3-hydroxypyrid-4-one (10) has a different structure from its protonated derivative in D_2O solutions. It is important to distinguish between these two forms, particularly with regard to their potential application as orally active iron chelators. Although at physiological pH (pH ≈ 7.4) the pyridone (10) exists largely in the free base form, the possible differences in its properties (e.g. solubility, toxicity) from its hydrochloride salt should not be ignored. Water solubility is one of the properties required for an orally active iron chelator. The hydrochloride salt, as might be expected, is highly soluble in water whereas the free base is only moderately soluble. Furthermore, recent studies have reported[34] that this pyridone (10) has several toxic effects in mammals. The demethylation of the N-methyl substituent and therefore the production of catechol-like metabolites has been proposed as a possible explanation for the toxic effects of this compound. In addition, it has been suggested that other N-substituted (e.g. 9; R = Et, i-Pr) derivatives are more suitable, since the cleavage of larger substituents is less likely to occur. However, this report[34] does not give any explanation of the toxic mode

of action of catecholic compounds. It should be borne in mind that we propose a catechol-like structure for the N-substituted 3-hydroxypyrid-4-ones in acidic medium. Furthermore a number of natural products such as dopamine and noradrenalin are catechols.

N.m.r. studies give insight into structures in solution; by contrast X-ray crystallographic studies provide detail of structures in the solid state. In this section, a comparison of the X-ray crystallographic data of the free base (10)[22] with those of its hydrobromide salt (16a) have revealed that the salt has a hydroxypyridinium (catechol-like) structure in the crystalline state. (The crystallographic data for the hydrobromide salt was kindly provided by Prof. Hider through personal communication.[52]) Table 9 lists the bond length values for 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its hydrobromide salt (16a). In comparing bond lengths, if the difference in two bond lengths is greater than 3x the standard deviation (σ, see Table 9), then the difference is regarded significant.

In the case of the free base,[22] the double bond, O(1)-C(4) (Figure 3), is significantly shorter than the single bond O(2)-C(3), suggesting a ketonic structure for this compound. Similarly, the distances between C(2)-C(3) and C(5)-C(6) are significantly shorter than those between C(4)-C(5) and C(3)-C(4). Nevertheless, all C-C bond

lengths are intermediate between those expected for single and double C-C bonds (*ca* 1.34, and 1.54 Å, respectively.),[ee] suggesting a partial delocalisation of the double bonds.

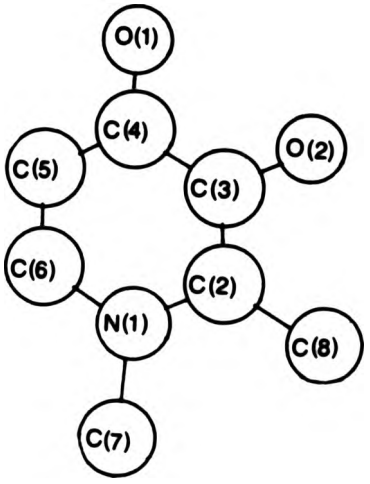


Figure 3- Atom numbering of 1,2-dimethyl-3-hydroxypyrid-4-one (10)

Table 9- Bond lengths (Å) of 1,2-dimethyl-3-hydroxypyrid-4-one[22] and its hydrobromide salt[52]

Bond	length for the free base Å (σ) (10)	length for the HBr salt Å (σ) (16a)
O(1)-C(4)	1.272(3)	1.328(5)
O(2)-C(3)	1.360(3)	1.343(5)
N(1)-C(2)	1.369(4)	1.362(6)
N(1)-C(6)	1.352(4)	1.347(6)
C(2)-C(3)	1.376(4)	1.384(6)
C(3)-C(4)	1.430(4)	1.385(6)
C(4)-C(5)	1.407(4)	1.390(6)
C(5)-C(6)	1.364(4)	1.359(7)
C(2)-C(8)	1.489(4)	1.475(7)
N(1)-C(7)	1.483(4)	1.479(7)
O(1)-H(1)	--	1.021(7)
O(2)-H(2)	--	0.990(7)

σ = standard deviation

In contrast, for the salt,[52] all the C-C bond distances are similar and the differences are within the experimental errors, suggesting a complete delocalisation. The C-O distances are also similar to each other and in between those expected for C-O single and double bonds (*ca* 1.43 and 1.23 Å, respectively)[66]. This strongly supports a hydroxypyridinium structure (A, Scheme 3) for the protonated pyridone, in which there is a complete delocalisation of the C=C and C=O bonds. In addition, the two expected hydroxy hydrogens have been located which establishes the structure of this compound as 1,2-dimethyl-3,4-dihydroxypyridinium bromide. Figure 4 illustrates a packing diagram of this compound (16a).

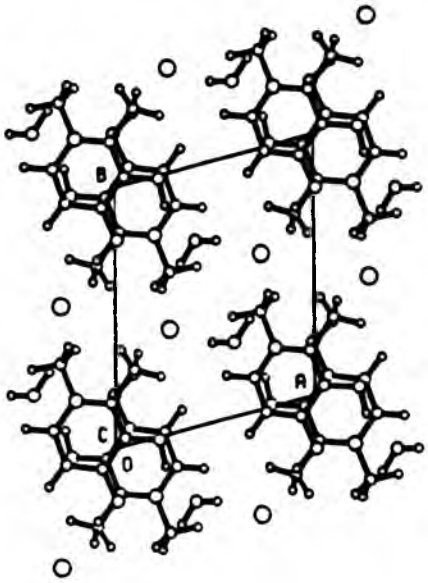


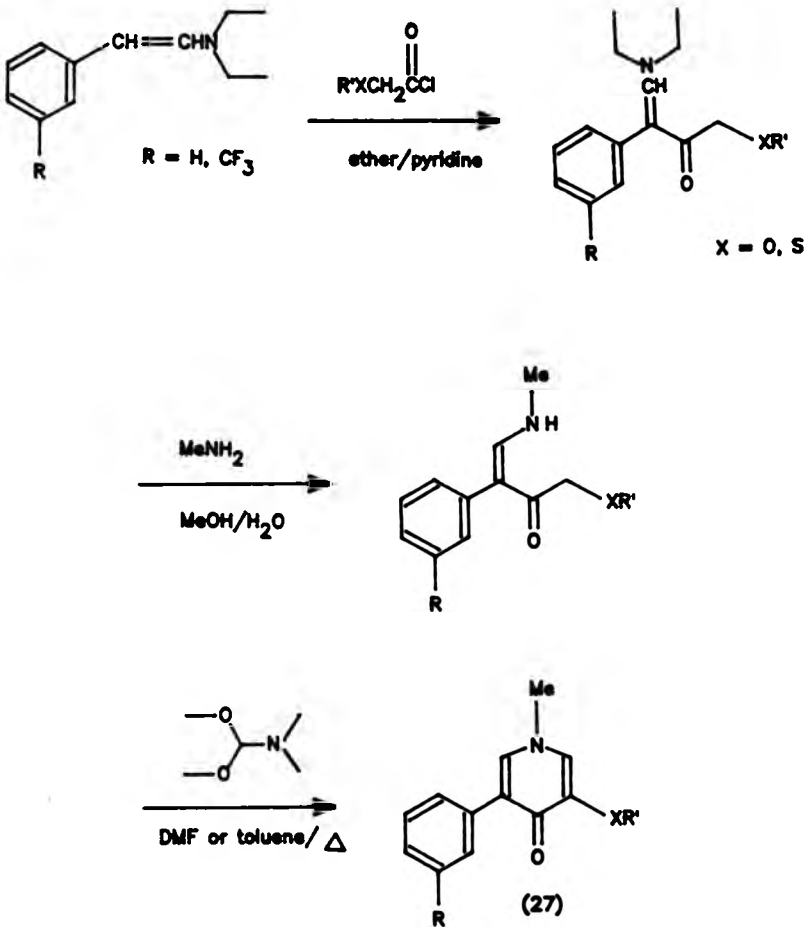
Figure 4- A packing diagram of the HBr salt of 1,2-dimethyl-3-hydroxypyrid-4-one (10)[82]

In summary, it is concluded that hydroxypyridones of type (10) are capable of accepting a proton to form a salt, which differs in properties and structure from the free base form. The free base, for example compound (10), exists as a partially delocalised keto-diene structure, whereas the salt, for example compound (16a), exists both in solution and in the solid state as a hydroxy pyridinium structure. These differences could be of pharmaceutical and clinical importance.

2.4 Attempted new synthetic approaches to 3-hydroxypyrid-4-ones

There are many literature methods for the synthesis of pyridones in general,[36,37] but only a few publications have been concerned with 3-hydroxypyrid-4-ones. All the existing synthetic routes to these compounds are limited in their versatility, since they involve the use of the corresponding pyrone (e.g. maltol) as the starting material. There is only one synthetic pathway to pyridones of type (27) described in the literature which involves the use of an acyclic ("non-pyrone") starting material (Scheme 5).[49] The cleavage of the O-alkyl bond could readily lead to the respective hydroxypyridone. However, the versatility of this route has not been exploited. We therefore concluded that there is a need for a new, efficient, and versatile method of synthesis for these hyroxypyridones. Consequently, attempts are described herein to develop a new general synthetic pathway to 3-hydroxypyrid-4-ones from readily available starting materials.

Several synthetic approaches have been examined at the Polytechnic of North London. A summary of the routes attempted by other students is presented in sections 2.4.1-2.4.3. Section 2.4.4 describes the synthetic approaches which were examined as part of this work.


CH=CHN
R
R = H, CF3
R'XCH2COCl
ether/pyridine
N
CH
XR'
O
R
X = O, S
MeNH2
MeOH/H2O
Me
NH
DMF or toluene/ Δ
(27)

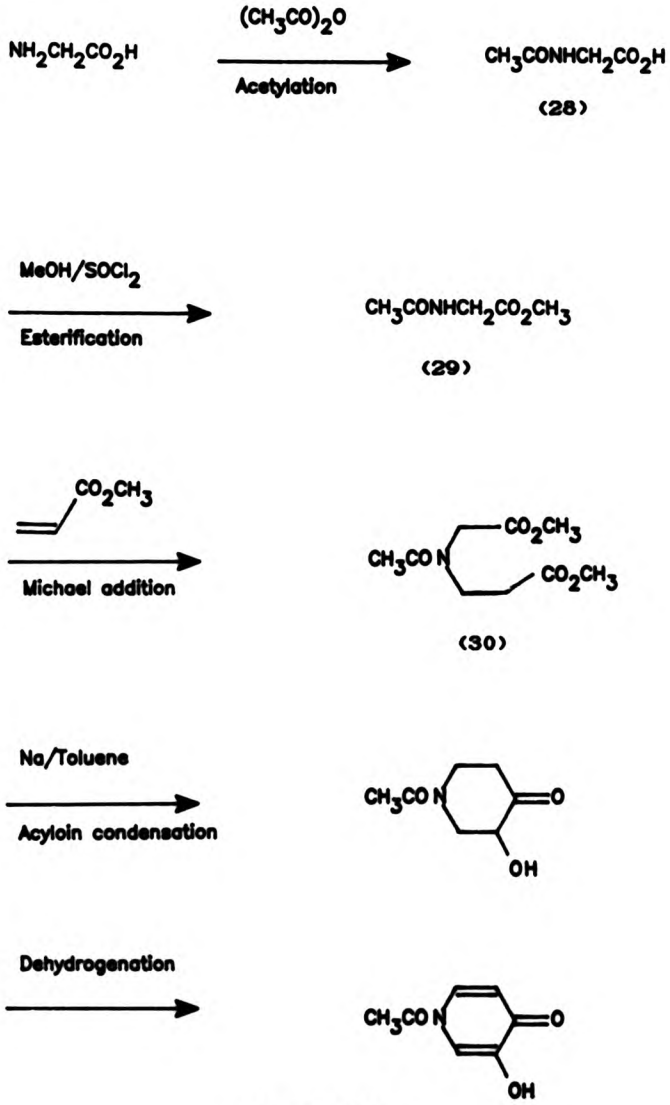
Scheme (5)

2.4.1 Routes involving acyloin condensation

The acyloin condensation involves "dimerisation" of a carboxylic ester. There are numerous examples of synthetic pathways involving acyloin condensation and its use as a method of cyclisation has been shown for a variety of compounds.[70,71] Application of this type of reaction in cyclisation of nitrogen substituted aminodiesters has also been described in the literature.[72-74] However, there are no examples for the synthesis of even-membered nitrogen containing rings using the acyloin condensation.

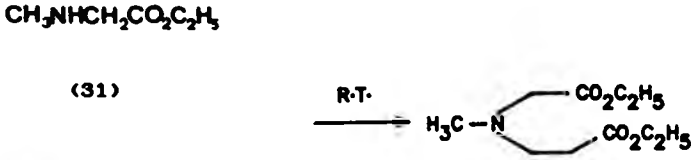
The proposed synthetic route illustrated by Scheme 6 has been examined.[75] However, the conversion of N-acetylglycine methyl ester (29) to the corresponding diester (30) by Micheal addition, using methyl acrylate in the presence of sodium hydride, was unsuccessful. It has been reported that under the reaction conditions used, the N-acetylglycine methyl ester (29) was hydrolysed. The same reaction in the presence of sodium methoxide also afforded the hydrolysed ester together with a crude oil which could be the desired product (30).

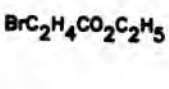
An alternative route, also involving acyloin condensation has been attempted.[75] In this route the amino diester (33) has been prepared from the reaction of the corresponding ethyl N-methylaminoacetate (31) and ethyl


NH2CH2CO2H
(CH3CO)2O
Acetylation
CH3CONHCH2CO2H
(28)
MeOH/SOCl2
Esterification
CH3CONHCH2CO2CH3
(29)
CO2CH3
Michael addition
CH3CO N
CO2CH3
CO2CH3
(30)
Na/Toluene
Acyloin condensation
CH3CO N
O
OH
Dehydrogenation
CH3CO N
O
OH

Scheme (6)

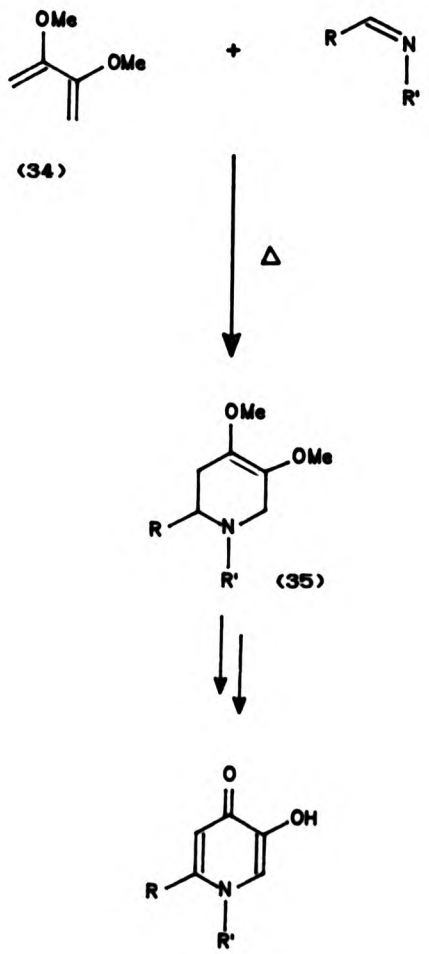
3-bromoproprionate (32). The acyloin condensation of the resulting diester (33) has not yet been clarified.





2.4.2 Synthetic approach using a Diels Alder cyclisation

It has been shown previously that 2,3-dioxygenated butadiene undergoes Diels Alder cycloaddition with imines or nitriles.[77] Scheme 7 presents the approach using this reaction as a key step for the synthesis of 3-hydroxypyrid-4-ones. The starting materials, 2,3-dimethoxybutadiene (34)[78] and imines such as EtCH=N CH_3 and CCl_3CH=NCH_2Ph, are readily obtainable. The reactions of these imines with 2,3-dimethoxybutadiene have been attempted; in all cases the reactions afforded a

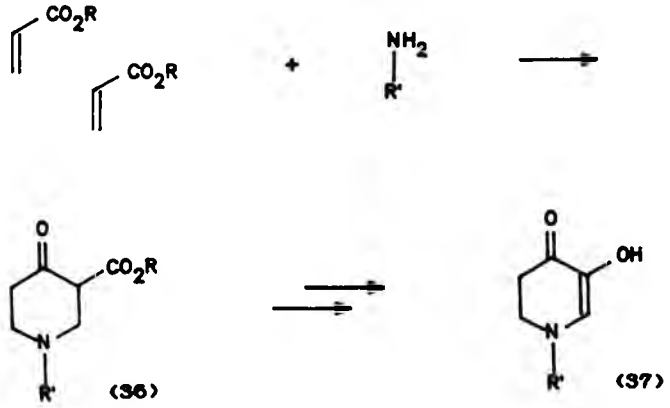


Scheme (7)

mixture of products none of which were identified as the expected tetrahydropyridine (35).[79a] It is known that the symmetrical dienes such as compound (34) are not reactive in Diels Alder reactions[78,80] which may justify the above results.

2.4.3 Synthetic approach using a Dieckmann Cyclisation

Scheme 8 illustrates an attempted synthetic route using Dieckmann cyclisation.[79] 4-Piperidones of type (36) are readily obtained from the condensation of primary amines with acrylate esters.[81,82] Investigations are in progress in order to determine whether the ester (37) can be converted to a hydroxypyridone as indicated in Scheme 8.



Scheme (8)

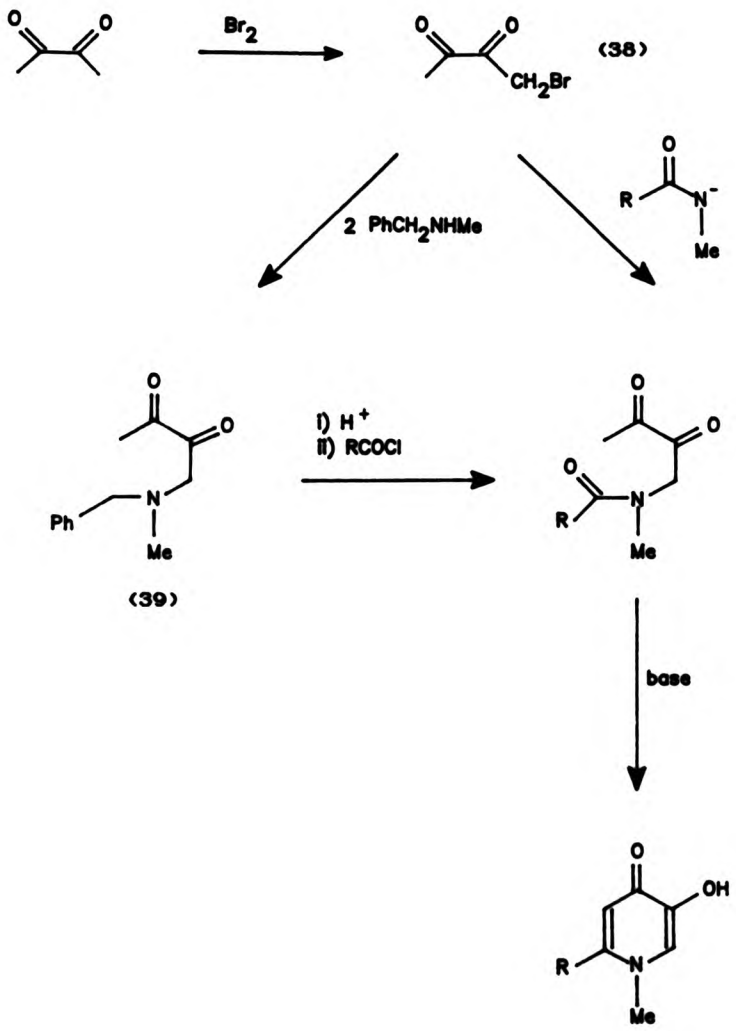
2.4.4 Attempted Synthetic approaches undertaken during the present study

During this work two synthetic routes illustrated by Schemes 9 and 10 were examined. The results of these investigations are presented and discussed in the following sections.

2.4.4.1 Route from 1-bromobutane-2,3-dione

The proposed synthetic method (Scheme 9) involves the use of 1-bromobutane-2,3-dione (38) in nucleophilic substitution reaction with an amide. This bromo compound (38) is readily obtained from the reaction of butane-2,3-dione (diacetyl) with bromine.[83] However, there is only limited information available in the literature about the use of this compound (38). In most cases, investigations have been concerned with the formation of five-membered heterocycles.[84]

For this study, the reactions of 1-bromobutane-2,3-dione (38) with N-methylbenzamide and N-methylbenzylamine were examined. The bromo diketone (38), b.p.$_{1.2}$ 20-22 °C (lit.[83] b.p.$_{4.5}$ 54 °C) and the amide, m.p. 75-80 °C (lit.[85] 78-80 °C) were prepared using previously described procedures. Both compounds were purified for these



Scheme (9)

reactions and their purity was assessed by n.m.r. spectroscopy.

The reaction of 1-bromobutane-2,3-dione (38) with N-methylbenzamide was carried out in two stages:
i) Use of a base to form the anion of the amide;
ii) addition of 1-bromobutane-2,3-dione.

Initially the reaction was attempted using sodium ethoxide as the base. The addition of the bromo diketone to a solution of the amide in dried absolute ethanol/NaOEt yielded a dark brown solution instantly. After 24 hours stirring at room temperature, thin layer chromatography (t.l.c.) indicated the presence of the starting materials plus a material retained on the base line. The removal of the solvent from the reaction mixture yielded a brown viscous residue from which white crystals of the starting amide were extracted. The recovery of the unreacted amide (68%) suggested that the formation of the anion using sodium ethoxide was unsuccessful. The presence of water, either in the reaction mixture or as water of recrystallisation, could prevent the formation of the sodium salt of the amide. Nevertheless, precautions were taken to exclude water from the apparatus and the reaction was carried out under anhydrous conditions. The formation of the anion using sodium hydride in dry diethyl ether was also unsuccessful. The production of the salt in the

presence of sodium hydride is indicated by evolution of hydrogen (Equation 2). However, in the case of the reaction of N-methylbenzamide with sodium hydride no hydrogen was evolved.

Equation 2

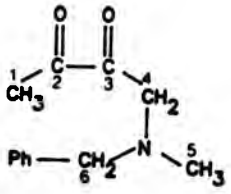
The preparation of the sodium salt of N-methylbenzamide was attempted by refluxing the amide in toluene, in the presence of finely divided sodium. Addition of 1-bromobutane-2,3-dione (38) to the suspension of this salt in toluene yielded an orange solution which became progressively darker. After two days stirring a dark brown solid, identified as impure sodium bromide, separated. The formation of sodium bromide (116%, crude yield) indicated that a reaction had taken place. However, the t.l.c. analysis of the reaction mixture in various solvents showed the presence of the starting amide and traces of the bromo compound (38) only. The evaporation of the solvent from the reaction mixture gave a viscous tarry residue from which no product was separated. No further attempts were made towards the investigation of this reaction. It is believed that under the conditions used

most of the bromo-diketone decomposed. This compound is unstable at room temperature and decomposes slowly within a few days, even when it is stored under nitrogen.

In order to examine the nucleophilic displacement of the bromine of the 1-bromobutane-2,3-dione (38), its reaction with N-methylbenzylamine was next studied. This reaction at room temperature afforded a white precipitate, identified as the hydrobromide salt of N-methylbenzylamine on the basis of n.m.r., i.r. and bromine analyses. The remaining reaction mixture was concentrated to give a viscous orange oil. The proton n.m.r. spectrum (Table 10) of this oil indicated the possible presence of the desired product (39) plus a substantial amount of impurities. The peak at 5.4 ppm could be due to NH of the unreacted amine or its salt.

Parallel to this study, the same reaction was also carried out by an undergraduate student.[66] In this case, the purification of the oil was attempted by extraction with ether from its solution in aqueous HCl. H.p.l.c. analysis of the ether extracts showed the presence of unreacted starting materials as well as several other components. The proton n.m.r. spectrum of this extract appears to be almost identical to that listed in Table 10, suggesting that the purification failed. No further work was undertaken on the route from 1-bromobutane-2,3-dione (38).

Table 10- ^1H n.m.r. spectral data of the product isolated from the reaction of compound (38) with N-methylbenzylamine

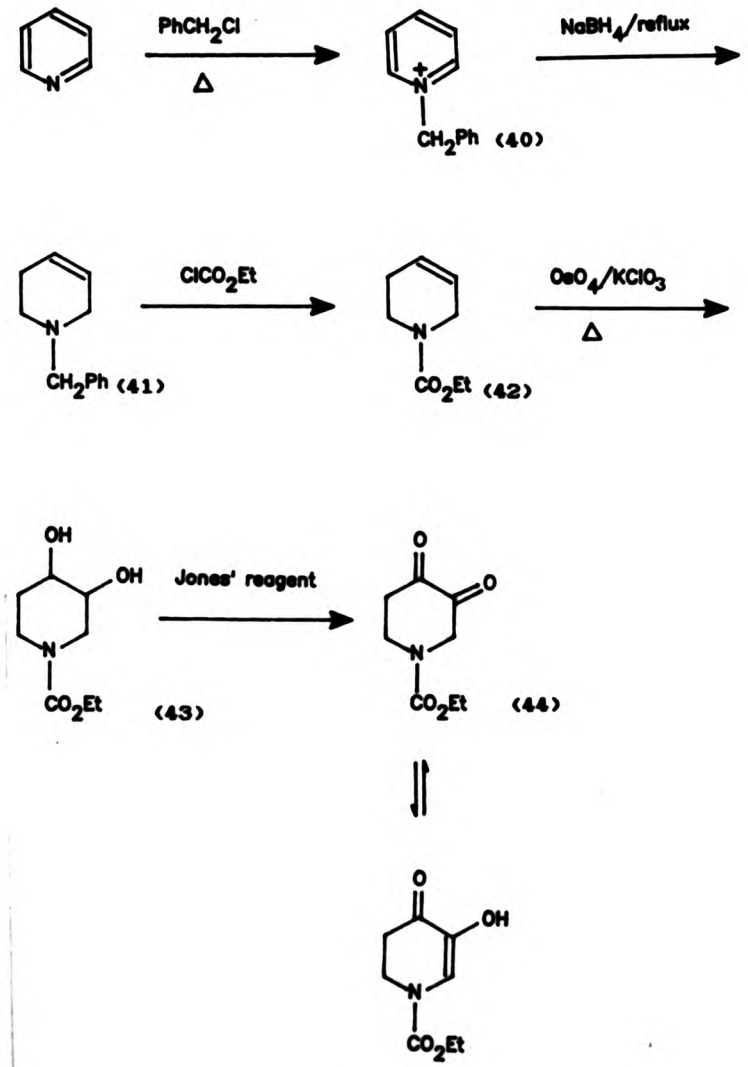


δ (ppm)	integral (mm)	Tentative assignments
2.1 (s)	23	CH_3 group- C1
2.3 (s)	20	CH_3 group- C5
3.1 (s)	14	CH_2 group- C4
3.8 (s)	15	CH_2 group- C6
4.5 (s)	8	impurities
5.4 (b)	12	typical of NH or OH, disappeared on D_2O shake
7.23 (s)	69	Ph group, the product and possibily of the unreacted amine or its salt

The spectrum was obtaind in $CDCl_3$ (TMS = 0 ppm) at 60 MHz;
s = singlet, b = broad

2.4.4.2 Synthetic approach from pyridine and its derivatives

This route involves the use of pyridine as a readily available starting material. The reduction of pyridinium salts to tetrahydropyridines is a well documented process and widely used in the manufacture of various


PhCH2Cl
Δ
NaBH4/reflux
CH2Ph (40)
ClCO2Et
CH2Ph (41)
CO2Et (42)
OsO4/KClO3
Δ
OH
OH
Jones' reagent
CO2Et (43)
O
O
CO2Et (44)
O
OH
CO2Et

Scheme (10)

analgesics.[87] The oxygenation of tetrahydropyridines to diols of type (43) (Scheme 10) has also been described in the literature.[88,89] However, there is only limited information available on the application and reactions of these compounds.

During this study, the pyridinium salt (40) was prepared from the reaction[88] of pyridine with benzyl chloride. The reduction of this salt with sodium borohydride afforded N-benzyl tetrahydropyridine (41) in a good yield (46%, lit. 48%), b.p.$_{10}$ 129-31 °C (lit.[88] b.p.$_{17}$ 127-128 °C). The N-ethoxycarbonyl derivative (42) of this compound (41) was prepared from its reaction with ethyl chloroformate[88] and purified by fractional distillation. The gas chromatographic (G.C.) analysis of the product showed the presence of benzyl chloride (the second product). However, when the distillation was carried out on a larger scale, a purer product (b.p.$_{10}$ 104-6 °C, lit.[88] b.p.$_{10}$ 84-94 °C) and a better yield (82%, lit. 75%) was obtained than those previously reported. The purity of the product was assessed by G.C. and n.m.r. spectroscopy (see Chapter 5 for the experimental results). The product (42) showed some instability at room temperature as it turned yellow overnight. Therefore it is suggested that the product should be stored under nitrogen at 0 °C, or preferably used immediately. The bis-hydroxylation of N-ethoxycarbonyl tetrahydropyridine (42) was achieved with

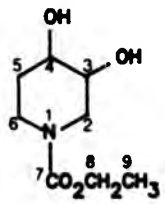
osmium tetroxide in the presence of potassium chlorate. Distillation gave the pure product (43) in a very poor yield (10.8%, lit. 57%). Therefore the purification method was modified. Column chromatography was found to be a more convenient method of purification and a better yield (40%) of the desired product (43) was obtained. A total of 5.5 g of the compound (43) was synthesised for this study. The purity (95%) of the product (43) was assessed with gas chromatography. The ^{1}H and ^{13}C n.m.r. spectra of this compound (43) in $CDCl_3$ were recorded. Although the ^{1}H n.m.r. spectrum of this product was in accord with the reported data,[88] overlapping of the peaks makes the assignment and therefore the identification of the product difficult. The ^{13}C n.m.r.spectrum (Table 11), on the other hand, is considerably simpler and provides a better method for its identification. The assignments have been supported by the off-resonance decoupling technique.

The oxidation of the 1-ethoxycarbonylpiperidine-3,4-diol (43) was attempted using Jones' Reagent (chromium trioxide solution in H_2SO_4). Jones' Reagent is commonly used for the oxidation of alcohols to ketones. This reagent provides mild conditions which are applicable to a variety of compounds.[90]

In this study, the reaction of the diol compound (43) with Jones' Reagent in acetone yielded a green precipitate.

Extraction of the aqueous solution of the reaction mixture and the precipitate with diethyl ether gave a mixture of products. G.C. analysis (Table 12) indicated the presence of three major components, one of which was the starting diol compound (retention time 8.6 min.). The ultraviolet spectrum (Figure 5) of this mixture in methanol exhibited a prominent absorption band at 286 nm. Since the diol compound is expected to have a very weak or no absorption

Table 11- Suggested assignments of the peaks of the broad band decoupled ^{13}C n.m.r. spectrum of compound (43)



Resonance peak (ppm)	Assignments
14.62	C9
29.55	C5
39.89	C6
45.96	C2
61.64	C8
68.04 & 68.53	C3 and C4
156.29	C7

The spectrum was obtained in $CDCl_3$ (TMS = 0 ppm) at 80 MHz.

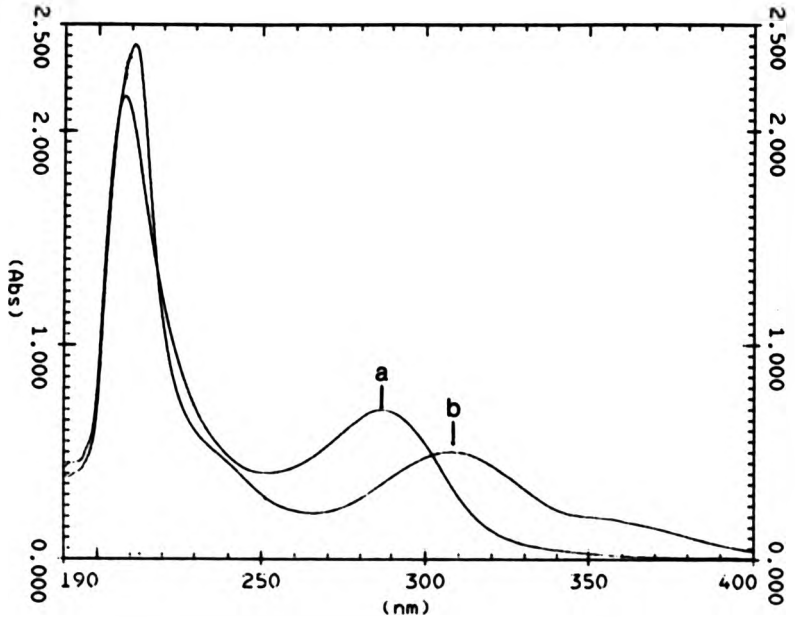
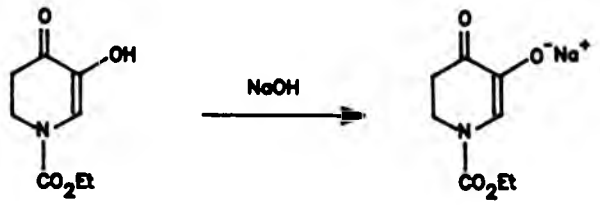


Figure 5- Ultra-violet spectra of the product mixture isolated from the oxidation of 1-ethoxycarbonylpiperidine-3,4-diol (43) in: (a) methanol; (b) methanol + aq. NaOH (pH = 12)

in the ultraviolet region, the observed absorption peaks could be due to the desired product (44). Addition of sodium hydroxide resulted in a shift to higher wavelength (*ca* 310 nm). Such behaviour is expected for the compound (44) as a result of the formation of the sodium salt (45).



(44) (45)

On the basis of the u.v. absorption, the purification of the product mixture by extraction of a diethyl ether solution with aqueous sodium hydroxide was attempted. The G.C. analysis of the organic phase showed the presence of all the three components at the same ratio as that prior to the extraction. The neutralisation of the aqueous layer, follwed by ether extraction yielded a brown oil which also contained the three components.

The oxidation reaction was repeated, and the separation of the product(s) was attempted using column chromatography. As indicated by G.C. analysis, the fractions eluted with a mixture of toluene and ethyl acetate (1:1) contained the major products (components a and c, Table 12) plus a small amount of impurities.

Table 12- Gas chromatographic analysis of the product mixture isolated from the oxidation reaction of the diol (43)

Components	Retention time (min)	Area under the peaks (mm^2)	% of the component
a	6.5	322.0	63.8
b	8.6	52.5	10.4
c	10.2	117.0	23.2
d	5.7	9.0	1.8
e	4.1	12.5	2.5

In order to obtain some information on the nature of these products, the above mixture was analysed by G.C. mass spectrometry (G.C.-MS). The mass spectra were obtained at the SERC mass spectrometry centre, by the chemical ionization (CI) method using ammonia as the reactant gas. Thus the presence of peaks due to $[MH]^+$ and $[M + NH_4]^+$ ions in the spectra is expected [9] (where, M = molecular ion).

Figure (6) represents the gas chromatograph of this mixture. It should be noted that the conditions used for G.C.-MS at the SERC mass spectrometry centre are different from those used for G.C. analysis of the reaction mixture at the Polytechnic of North London. The CI spectrum (Figure 7) of the major component suggests the presence of two compounds of molecular weights 187 and 185, indicated by the prominent peaks at m/z 188 and 186 corresponding to

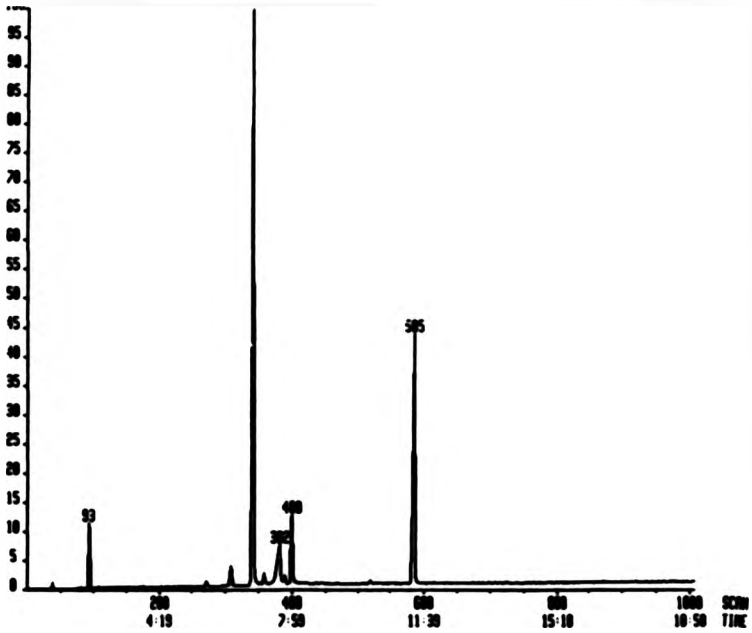
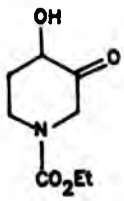


Figure 6- G.C. chromatograph of the product mixture isolated from the oxidation of 1-ethoxycarbonylpiperidine-3,4-diol (43)

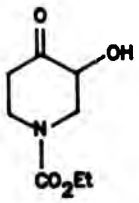
Figure 7- C.I. mass spectrum of the product mixture isolated from the oxidation of 1-ethoxycarbonylpiperidine-3,4-diol (43)

$[MH]^+$ ions of each compound, respectively. This conclusion is drawn from the fact that the peak at m/z 186 is unlikely to be due to fragmentation of the ion at m/z 188, since the loss of two mass numbers is highly unusual. In addition, the spectrum exhibits peaks at m/z 205 and 203 which are assignable to $[M + NH_4]^+$ ions for each compound. The total ion current and that of individual ions of m/z 186 and 188 maximised at the same scan number, thus suggesting that both compounds have the same retention time under the conditions used for G.C.

The molecular mass of 185 corresponds to the expected diene (44, Scheme 10) and the m/z 187 is consistent with compound (46) or (47). The ions due to the latter (i.e. m/z 187) are more abundant in the spectrum of the mixture. However, the relative quantity of the products (43) and either (46) or (47) cannot be determined from the above results.



(46)



(47)

The CI mass spectrum of the second major component observed in the gas chromatograph (Figure 6) gave peaks at m/z 216 and 233 which can be assigned to $[M + H]^+$ and $[M + NH_4]^+$, where M is 215. However, no structural conclusions can be deduced from the CI spectrum.

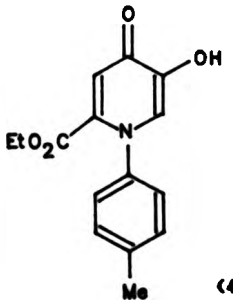
On the basis of the above results, it can be concluded that the preparation of 1-ethoxycarbonylpiperidine-3,4-dione (44) from the oxidation of piperidine diol (43) can be achieved. However, further investigations are required in order to improve the purity and the yield of this product. This proposed route would be versatile and relatively inexpensive given the wide availabilty of substituted pyridines.

CHAPTER THREE

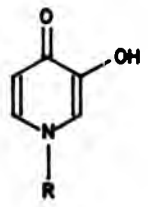
COMPLEXATION BEHAVIOUR OF 3-HYDROXYPYRID-4-ONES

3.1 Introduction

The study of the complexation behaviour of 3-hydoxypyrid-4-ones towards a wide range of metals has been given considerable attention. Previous studies are summarised in Table 13. In the majority of cases the studies have been concerned with measurements of stability constants, partition coefficients, and spectroscopic properties rather than with the isolation and characterisation of the complexes. These studies have been concerned primarily with N-substituted-2-methyl-3-hydroxypyrid-4-ones (9), N-(4-tolyl)-6-carbethoxy-3-hydroxypyrid-4-one (48), and 3-hydroxypyrid-4-ones of type (49).

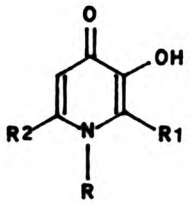


(48)



(49)

Table 13 - Metal complexes of 3-hydroxypyrid-4-ones



a- Complexes studied in solution only

Ligand			Metal	Proposed composition	Reported data	Ref
R	R1	R2		Metal:ligand		
H	H	H	Al(III)		s.c., vis.	92
$CH_2CHNH_2CO_2H$	H	H	"	1:3, 1:2, 1:3	s.c.	"
H	H	H	Ca(II)		s.c.	92, 47
Me	H	H	"		s.c.	47
$CH_2CHNH_2CO_2H$	H	H	"		s.c.	92, 47

(continued)

	Ligand		Metal	Proposed composition	Reported data	Ref
R	R1	R2		Metal:ligand		
H	H	$CH_2CH(NH_2)CO_2H$	Ca(II)		s.c.	47
H	H	H	Cd(II)		s.c.	46
$CH_2CH(NH_2)CO_2H$	H	H	"		s.c.	"
$C_2H_4CO_2H$	H	H	"		s.c.	"
H	H	H	Co(II)		s.c.	92
$CH_2CH(NH_2)CO_2H$	H	H	"		s.c.	"
Me	H	H	Cu(II)		s.c., vis.	46
$CH_2CH(NH_2)CO_2H$	H	H	"	1:1 (monomer)	s.c.	92
$CH_2CH(NH_2)CO_2H$	H	H	"	2:2 (dimer)	s.c., vis.	46, 47
$C_2H_4CO_2H$	H	H	"		s.c.	46
H	H	$CH_2CH(NH_2)CO_2H$	"		s.c.	47
H	H	H	Fe(III)		s.c., vis.	92
$CH_2CH(NH_2)CO_2H$	H	H	"	3:1, 2:1 1:1	s.c., vis	"
Me	Me	H	"	3:1, 1:1	s.c., vis.	19
Ph	Me	H	"	3:1, 2:1, 1:1	vis., p.c.	93
4-tolyl	Me	H	"	2:1, 3:1	vis., p.c.	94
4-tolyl	H	CO_2Et	"	3:1, 2:1, 1:1	vis.	95
Ph	Me	H	Hf(IV)		p.c.	96
H	H	H	Mg(II)		s.c.	92, 47

(continued)

Ligand			Metal	Proposed composition	Reported data	Ref
R	R1	R2		Metal:ligand		
$CH_2CHNH_2CO_2H$	H	H	Mg(II)		s.c.	92, 47
H	H	$CH_2CHNH_2CO_2H$	"		s.c.	47
Ph	Me	H	Pa(V)		p.c.	97
4-tolyl	H	CO_2Et	"		p.c.	98
H	H	H	Pb(II)		s.c.	92
$CH_2CHNH_2CO_2H$	H	H	"		s.c.	92, 46
Me	H	H	"		s.c.	46
Ph	Me	H	Pu(IV)	4:1, 3:1, 2:1	p.c.	99
Ph	Me	H	Ti(IV)	2:1	vis., p.c.	100, 101
4-tolyl	Me	H	Th(IV)	3:1	p.c.	102
Ph	Me	H	"		vis., p.c.	97
Ph	Me	H	V(V)	1:1	vis.	103
4-tolyl	Me	H	"	1:1	vis.	103
Ph	Me	H	V(VI)	2:1	vis.	104
H	H	H	Zn(II)		s.c.	92, 47
Me	H	H	"		s.c.	46, 47
$CH_2CHNH_2CO_2H$	H	H	"		s.c.	92, 46,
H	H	$CH_2CHNH_2CO_2H$	"		s.c.	47
$C_2H_4CO_2H$	H	H	"		s.c.	46

(continued)

b- The isolated metal complexes of 3-hydroxypyrid-4-one

Ligand			Metal	Proposed formulae	Reported data	Ref
R	R1	R2				
H	Me	H	Al(III)	AlL_3	e.a., w.s., i.r, n.m.r., m.s.(FAB), m.p.	23
Me	Me	H	"	$AlL_3.12H_2O$	e.a., w.s., i.r. n.m.r, m.p., m.s.(FAB), X.S.	23, 24
Hex	Me	H	"	"	e.a., w.s., i.r., n.m.r., m.s.(FAB), m.p.	23
H	Me	H	B(III)	$BL(Ph)_2$	e.a., i.r., n.m.r. X.S.	26
Me	Me	H	"	"	e.a., i.r. n.m.r., X.s.	26
Ph	Me	H	Cu(II)	CuL_2	e.a., i.r., p.c.	105
4-tolyl	Me	H	"	CuL_2	e.a., i.r., p.c.	105
Ph	Me	H	Fe(III)	FeL_3	e.a, i.r., vis.	93
4-tolyl	Me	H	"	FeL_2Cl_2	e.a, i.r., vis.	95
4-tolyl	H	CO_2Et	"	FeL_3	e.a, i.r., vis.	94
H	Me	H	Ga(III)	GaL_3	e.a., i.r., n.m.r.,m.s.(FAB)	23
Me	Me	H	"	$GaL_3.12H_2O$	e.a., i.r. n.m.r., m.s.(FAB), X.s.	23
Hex	Me	H	"	"	e.a, i.r., n.m.r., m.s.(FAB)	23

(continued)

Ligand			Metal	Proposed formulae	Reported data	Ref
R	R1	R2				
4-tolyl	H	CO_2Et	Ga(III)	GaL_3	e.a, i.r. p.c.	106
H	Me	H	In(III)	$InL_3.\frac{1}{2}H_2O$	e.a, i.r., n.m.r., m.s.(FAB)	25
Me	Me	H	"	$InL_3.12H_2O$	e.a, i.r., n.m.r., m.s.(FAB), X.s., w.s.	25
4-tolyl	Me	H	Th(IV)	$ThCl_3L(LH)$	e.a., vis., p.c.	102
4-tolyl	H	CO_2Et	"	$ThClL_3(LH)$	e.a., vis., p.c.	98
Ph	Me	H	U(VI)	$UO_2L_2(LH)$	e.a., vis., p.c.	107
Ph	Me	H	V(V)	$VO_2L(LH)_2$	e.a., i.r., vis.	108
				$VO_2Cl(LH)_3$	e.a., i.r., vis.	
4-tolyl	Me	H	"	$VO_2L(LH)_2$	e.a., i.r., vis.	109
				$VO_2Cl(LH)_3$	e.a., i.r., vis.	
				$VO_2(ClO_4)(LH)_3$	e.a., i.r., vis.	
4-tolyl	H	CO_2Et	"	$VO_2L(LH)_2$	e.a., i.r., vis.	110
			"	$VO_2Cl(LH)_3$		
Ph	Me	H	V(VI)	VO_2L_2	e.a., vis., i.r. p.c.	111
Me	Me	H	Zn(II)	ZnL_2	i.r., n.m.r., m.p.	112

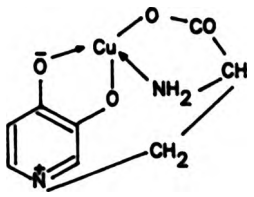
Abbreviations: LH = neutral ligand, L = anionic ligand, e.a. = elemental analysis, i.r. = infra-red spectrum, m.p. = melting point, m.s.(FAB) = fast atom bombardment mass spectrum, n.m.r. = nuclear magnetic resonance spectrum, vis. = visible spectrum, p.c. = partition coefficient, s.c. = stability constant, w.s. = water solubility, X.s. = X-ray structural analysis.

The chelation ability of mimosine (49, R = $CH_2CH(NH_2)CO_2H$), a naturally occurring pyridone with an amino acid substituent, with various metals has been implicated in the inhibition of metal-containing enzymes[40,48,49] and of DNA synthesis of wool follicles,[45-47] and in several other biological systems.[39,113,114] As a result of this, considerable attention has been paid to the chelation of this ligand and its analogues, and to the stability of their complexes with various divalent and trivalent metals.[46,47,92] However, none of the complexes have been isolated. Potentiometric measurements of stability constants of Fe(III), Al(III), Cu(II), Pb(II), Zn(II), Ni(II), Co(II), Ca(II), and Mg(II) complexes derived from mimosine have indicated that this ligand forms the most stable chelate with Fe(III).[92] The order of stabilities of the chelates have been found to be as following:

Fe(III) >Al(III) >Cu(II) >Pb(II) >Zn(II), Ni(II) >Co(II) >Ca(II), Mg(II).

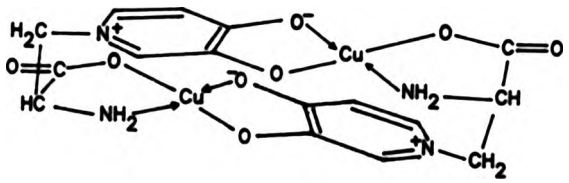
Comparative study of stability constants of complexes derived from mimosine and 3,4-dihydroxypyridine (49, R = H) has shown that mimosine chelates primarily through the hydroxypyridone moeity rather than through the amino carboxylate groups.[92,46,47] In the case of the reaction of Cu(II) salt with mimosine and its derivatives the chelation involves both amino carboxylate and

hydroxypyridone moieties. The monomeric structure (50) has been proposed for the resulting complex.[92]



(50)

However, these suggestions have been disputed in other studies concerned with the products of the reactions of mimosine (49, R = $CH_2CH(NH_2)CO_2H$) and its analogues (49; R = H, CH_3, $(CH_2)_2CO_2H$) with various metals. Thus it was proposed that the mimosine derivative (49; R = $(CH_2)_2CO_2H$) forms a monomeric complex by chelating through the hydroxypyridone moiety.[46] With mimosine the formation of a dimeric complex (51), which requires the implication of both chelating groups, was suggested to be dominant.



(51)

There has also been much interest in the complexation behaviour of hydoxypyridones of types (9, R = Ph, and 4-tolyl) and (48) with various metals (Table 13). These ligands have been reported as effective extractants for a variety of metals[93] and suitable for the direct spectrophotometric determination of metals such as V(V),[102,108] Fe(III),[93-98] Ti(IV),[100,101] U(VI),[107] and W(VI).[111] Spectrophotometry techniques (Job's method) have been used to demonstrate product dependence on pH and to determine the composition of the complex species in solution. It has been shown that the nature of the product is dependent on the pH of the reaction medium, and products containing anionic and/or neutral pyridonato ligands can be formed. Several complexes derived from these ligands have been isolated and characterised. For example, from the reaction of N-phenyl-2-methyl-3-hydroxypyrid-4-one (pmpH = 9; R = Ph) with ammonium vanadate, complexes of types, $VO_2(pmp)(pmpH)$, $VO_2Cl(pmpH)_2$ and $VO_2ClO_4(pmpH)_2$ have been isolated.[108] Similar vanadium complexes have been obtained from the related ligand N-(4-tolyl)-2-methyl-3-hydroxypyrid-4-one (9, R = 4-tolyl)[108] and complexes of several metals derived from N-(4-tolyl)-6-carbethoxy-3-hydroxypyrid-4-one (48) have been isolated.[110] In all cases the complexes have been obtained by shaking a chloroform solution of the ligand with an aqueous solution of the metal salt and the product has been isolated from the organic phase (Table 13b,

references 23, 93-95, 102, 98, 106-111). The complexes have been characterised on the basis of elemental analysis and their electronic spectra. Their infra-red spectra have been recorded, but only in a few cases have the spectra been discussed.[105,107,111]

Recently, the interest in complexes of hydroxypyridones has increased considerably because of their potential application as chelating agents for the treatment of iron overload disorders.[16,19-21] Therefore, the study of their coordination chemistry has become increasingly more important, requiring a more detailed investigation. Consequently, the complexing behaviour of 1,2-dimethyl-3 hydroxypyrid-4-one (10 = dmpH) towards Co(II), Ni(II), Cu(II), Zn(II), Ca(II), and Fe(III) has been systematically examined, as part of the work undertaken for this thesis. While this work was in progress further interest in these ligands has arisen because of their potential value as chelators for the treatment of aluminium overload, as *in vivo* directors of ^{67}Ga(III), and as *in vivo* transport agents for Al(III).[25] As a consequence of this interest, B(III),[26] Al(III),[23,24] Ga(III),[24] and In(III)[25] complexes of several 3-hydroxypyrid-4-ones (9, R = H, Me, Hex) have been isolated and characterised and in the case of the complexes derived from 1,2-dimethyl-3-hydroxypyrid-4-one (10), their X-ray crystallographic structures have been determined.[24-26]

3.2 Synthesis and characterisation of Co(II), Co(III) Ni(II), Cu(II), Zn(II), and Ca(II) complexes derived from 1,2-dimethyl-3-hydroxypyrid-4-one (10)

With the exception of the zinc complex,[112] there is no literature precedent for the isolation of divalent metal complexes derived from 1,2-dimethyl-3-hydroxypyrid-4-one (10). In this section the studies concerned with the reaction of this ligand (10) with Co(II), Ni(II), Cu(II), Zn(II) and Ca(II), and the isolation and characterisation of the resultant complexes are presented. Section 3.3 deals with the X-ray crystallographic study of the zinc(II) complex derived from this ligand (10).

The reaction of 1,2-dimethyl-3-hydroxypyrid-4-one (dmpH = 10) with calcium nitrate in aqueous methanol in the presence of ammonium hydroxide afforded the complex $Ca(dmp)_2$, which was formulated on the basis of elemental analysis. Similarly, reactions of this pyridone with nickel(II), copper(II), and zinc(II) acetates yielded the complexes; $Ni(dmp)_2.4½H_2O$, $Cu(dmp)_2$, and $Zn(dmp)_2.3½H_2O$, respectively (see Chapter 5, Table 43 for elemental analyses). In all cases the complex was obtained by filtration after concentration of the reaction mixture. The anhydrous complex, $Ni(dmp)_2$ was isolated by heating a suspension of the hydrated complex in toluene under reflux. The anhydrous complex showed hygroscopic character

and readily reverted to the hydrated form on exposure to the atmosphere.

The reaction of pyridone (10) with cobalt(II) acetate in the presence of ammonium hydroxide after one hour gave a red solution from which the pink complex, $Co(dmp)_2.3H_2O$ was isolated. However, when the same reaction was allowed to proceed for 24 hours a green solution resulted from which a green crystalline solid, identified as $Co(dmp)_3.12H_2O$, was isolated. The formulation of this solid is based on the crystal data (see Chapter 5, section 5.4.19 for the crystal data), which suggested that this compound is isomorphous with $Fe(dmp)_3.12H_2O$.[27] The formation of this cobalt(III) complex was accelerated by heating the reaction mixture and/or carrying out the reaction in the presence of charcoal. Formation of cobalt(III) complexes from the reaction of cobalt(II) salts with various ligands is commonly observed and can be explained in terms of atmospheric oxidation.[3] For instance, the reaction of a Co(II) salt (CoX_2, X = Cl, Br, NO_3) and the corresponding ammonium salt in the presence of ammonia, activated charcoal and air yields the corresponding cobalt(III) hexamine complex (Equation 3).

$$4CoCl_2 + 4NH_4Cl + 20NH_3(aq.) + O_2 \rightarrow 4[Co(NH_4)_6]Cl_3 + 2H_2O$$

Equation 3

It should be noted that for the elemental analysis, spectroscopic, and magnetic studies the crystals of $Co(dmp)_2.12H_2O$ were dried under vacuum (80 °C, 0.1 mmHg) to give the anhydrous $Co(dmp)_2$.

The hydrated complex, $Co(dmp)_2.3H_2O$ readily afforded the anhydrous species by either heating it in toluene under reflux or by macro-scale pyrolysis at 80 °C/0.1 mmHg. In contrast to $Ni(dmp)_2$, the anhydrous cobalt complex was found to be non-hygroscopic.

All the complexes obtained during the present study were characterised by elemental analysis and where appropriate by using spectroscopic and magnetochemical techniques, and by thermal gravimetric analysis. A summary of some properties of these complexes together with the thermal gravimetric analyses of the hydrated compounds are given in Table 14.

The mass spectra of the complexes, $Ni(dmp)_2$, $Co(dmp)_2$, $Cu(dmp)_2$, and $Zn(dmp)_2$ support the proposed formulations. In all cases the spectra show peaks due to the molecular ion, $[M(dmp)_2]^+$ and fragments arising by loss of $[dmpH]^+$, $[dmp]^+$, and $[dmp - H]^+$. The spectra are discussed more fully in section 3.2.1.

Table 14- Thermal gravimetric analysis and some properties of M(II) and M(III) (M = Co(II), Ni(II), Cu(II), Zn(II), Ca(II), Co(III), & Fe(III)) complexes derived from the pyridone (10)

Complex	Colour	T.G.A. wt. loss (mg)		T.G.A. Temp. (°C) of		Solubility in		
		Calc.	Found	H_2O loss	Decomp.	H_2O	MeOH	CH_2Cl_2
$Co(dmp)_2.3H_2O$	pink	14.1	13.9	80-100	220	ss	ss	i
$Co(dmp)_2$	purple	00.0	00.0	--	310	ss	ss	i
$Ni(dmp)_2.4\%H_2O$	green	27.6	28.0	110-180	250	ss	ss	i
$Ni(dmp)_2$	yellow	00.0	00.0	--	250	ss	ss	i
$Cu(dmp)_2$	green	00.0	00.0	--	300	s	s	ss
$Zn(dmp)_2.3\%H_2O$	white	15.4	15.6	100-120	320	ss	ss	i
$Ca(dmp)_2$	white	00.0	00.0	--	300	i	i	i
$Co(dmp)_3$	green					hs	hs	ss
*$Fe(dmp)_3.4H_2O$	red					hs	hs	s
*$FeCl_3(dmp)(dmpH)_4$	violet					hs	hs	ss
*$FeCl_3(dmp)(dmpH)_2$	violet/red					hs	hs	ss

ss = slightly soluble, i = insoluble, s = soluble, hs = highly soluble
*The Fe(III) complexes of the pyridone (10) are discussed in section 3.4.

Thermal gravimetric analysis on all the hydrated complexes showed that water was lost quantitatively between 105-180 °C to give the anhydrous species which decomposed at approximately 250 °C (Table 14). In the case of $Ni(dmp)_2.4H_2O$, the loss occurred in two stages, with the first loss occurring around 100-120 °C and the second at 125-170 °C. The former corresponds to the loss of 2.5 water molecules and the latter to two water molecules. These observations suggest that the water molecules which were liberated at a higher temperature are co-ordinated to the nickel atom, which in turn implies an octahedral environment for the nickel atom.

All the complexes gave well resolved infra-red spectra. The spectra of the free ligand and complexes in the region of 1450-1650 cm^{-1} showed several prominent bands. These are listed in Table 15 together with suggested assignments. The i.r. spectra of the hydrated complexes also exhibited a strong, broad band at approximately 3300-3400 cm^{-1} due to the water of crystallisation. The bands in the region of 1650-1450 cm^{-1} are characteristic for 4-pyridones and as described earlier (Chapter 2, section 2.3.1) are assignable to the vC=O and vC=C and their mixed vibrations.

Table 15- Assignment of i.r. spectra of M(II), (M = Co, Ni, Cu, Zn, and Ca) and Co(III) complexes derived from the pyridone (10)

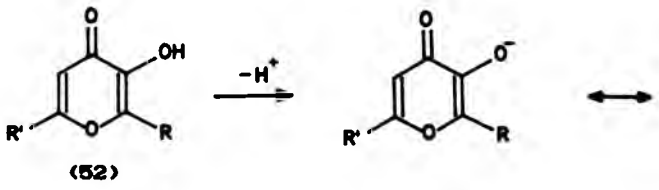
		Wave no. (cm^{-1})
No.	Compound	ν(C=O) and ν(C=C) of ring
1	dmpH	1631, 1570, 1531w, 1514, 1462
2	$Co(dmp)_2.3H_2O$	1600, 1550, 1500, 1457
3	$Co(dmp)_2$	1599, 1546, 1498, 1455
4	$Ni(dmp)_2.4½H_2O$	1598, 1548, 1503, 1454
5	$Ni(dmp)_2$	1597, 1548, 1503, 1454
6	$Cu(dmp)_2$	1600, 1550, 1505, 1456
7	$Zn(dmp)_2.3½H_2O$	1600, 1550, 1505, 1457
8	$Ca(dmp)_2$	1600, 1550, 1500,
9	$Co(dmp)_3$	1595, 1540, 1500, 1455

w = weak

Comparison of the spectra of the complexes with that of the ligand revealed that bands at 1631, 1570, 1514, and 1462 cm^{-1} in the free ligand have shifted by 20-30 cm^{-1} to lower frequencies on complexation. Such bathochromic shifts have also been observed in the spectra of the complexes derived from 3-hydroxy-4-pyrones (52)[116-117] and M(III) (M = B, Al, Ga, and In)[28-26] complexes of 1,2-dimethyl-3-hydroxypyrid-4-one (10).

Generally, for ligands which chelate through a O=C-C-OH group a shift of νC=O to lower frequencies is observed upon chelation as a result of the involvement of the C=O group in the bonding with the metal. However, in the case of 3-hydroxypyrid-4-ones the situation is more complicated

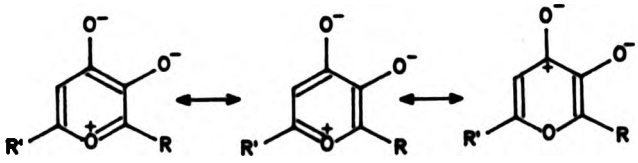
since the vC=O vibrations cannot be distinguished from vC=C. Nevertheless, the involvement of C=O in chelation has been established by X-ray crystallographic studies of M(III) (M = B, Al, Ga, In, and Fe)[25-27] and Zn(II) complexes of 1,2-dimethyl-3-hydroxypyrid-4-one (10). In the case of 3-hydroxy-4-pyrones (52), the decrease in the frequencies of bands due to vC=C and vC=O on complexation has been explained by assuming contribution from resonance structures (52a) and (52b) (Figure 8).[116,117]



(52)

R = H, R' = CH_2OH; Kojic acid

R = Me, R' = H; Maltol



(52a) (52b)

Figure 8- Resonance structures of 3-hydroxy-2-methyl-4-pyronato ions

Analogous resonance structures of chelated 1,2-dimethyl-3-hydroxypyrid-4-one (structures a and b, Figure 9) could similarly explain the observed shift of the νC=O and νC=C bands on complexation. This conclusion is supported by the X-ray crystallographic study of $Zn(dmp)_2.3½H_2O$ (see section 3.3) and several other complexes derived from pyridone (10).[23-27]

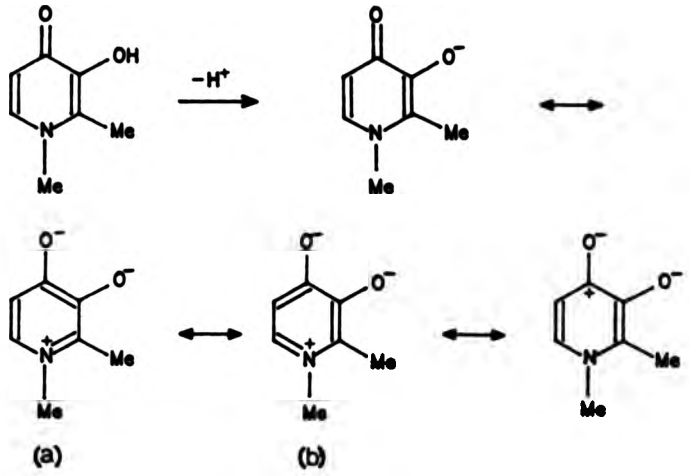


Figure 9- Resonance structures of 1,2-dimethyl-3-hydroxypyrid-4-onato ion

For the complexes involving the transition metals, Co(II), Co(III), Ni(II), and Cu(II) some structural information has been obtained from the study of their magnetic properties and electronic spectra (Table 16). The results have been compared with those reported for complexes of the related ligands of type (52).[115-116]

Table 16- Spectral and magnetic results of Co(II), Ni(II), Cu(II) and Co(III) complexes of pyridone (10)

Complex	λ_{max} (nm) Solution spectra (solvent)	λ_{max} (nm) Solid spectra	μ_{eff} (B.M.)
$Ni(dmp)_2.4H_2O$	684.6, 393.5 (methanol)	ca700 406.0, 495.0(w)	3.13
$Ni(dmp)_2$	-- --	530.0(sh) 456.0(sh)	3.16
$Co(dmp)_2.3H_2O$	542.5, 472.0 (methanol)	542.4, 505.0	4.60
$Co(dmp)_2$	544.0, 471.0 (methanol)	560.0, 602.1	4.50
$Cu(dmp)_2$	677.0 (water)	672.0	1.82
$Co(dmp)_3$	640.0 (methanol)	641.0	dia

sh = shoulder, w = weak

The observed room temperature magnetic moments for both $Ni(dmp)_2.4H_2O$ (3.13 B.M.) and $Ni(dmp)_2$ (3.16 B.M.) are within the expected range for six co-ordinate Ni(II).[8] Similar magnetic properties have been observed for both hydrated and anhydrous Ni(II) complexes of 3-hydroxy-2-methyl-4-pyrone (11) which have also been suggested to be octahedral.[116,117] For the hydrated complex,

Ni(dmp)$_2$.4½H$_2$O, this geometry is also indicated by thermal gravimetric analysis (see Table 14) and its electronic spectrum. The solution spectrum in methanol exhibits bands at 684.6 and 393.5 nm which could be assigned to the d-d transitions, $^3T_{1g}(F) \leftarrow {}^3A_{2g}$ and $^3T_{1g}(P) \leftarrow {}^3A_{2g}$, respectively. The band at 684.6 nm is split probably as the result of mixing the $^3T_{1g}(F)$ and ^1E_g states through spin-orbit coupling.[3] The solid state spectrum of Ni(dmp)$_2$.4½H$_2$O is very similar to that of the solution spectrum, except that a shift of the absorption bands to slightly lower energies is observed. No structural information could be obtained for the anhydrous complex from electronic spectroscopic studies. The insolubility of this complex in suitable solvents prevented measurement of the spectrum in solution. In the solid state this compound gave an ill defined spectrum probably due to its highly hygroscopic nature. Nevertheless, as suggested earlier on the basis of the magnetic moment, Ni(dmp)$_2$ has an octahedral structure which indicates association, and this is consistent with the insolubility of this compound.

The room temperature magnetic moments of Co(dmp)$_2$ and Co(dmp)$_2$.3H$_2$O are 4.7 and 4.5 B.M., respectively. These are within the expected range for tetrahedral complexes (i.e. 4.2-4.8 B.M.),[117] but only slightly below the range expected for octahedral complexes (i.e 4.7-5.2 B.M.)[3]. Thus no definite structural conclusions could be obtained

from the magnetic moment. In solution the electronic spectra of both compounds exhibit a strong absorption at approximately 543 nm which can be assigned to the transition $^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$ and is typical of octahedral Co(II) complexes. The spectra also show several poorly resolved bands that could not be assigned. The reflectance spectrum of the hydrated form is similar to that in solution suggesting a similar geometry for cobalt in solution and solid state. In the reflectance spectrum of the anhydrous complex, however, the principal absorptions at 542.5 and 472.0 nm appear at 560.0 and 595.0(sh) nm. This shift to lower energy compared with the absorptions in solution suggests a tetrahedral structure in the solid state. In general, tetrahedral Co(II) complexes are known to absorb in the region of 600-750 nm, and octahedral complexes in the region of 550-600 nm.[3]

The Co(III) complex of pyridone (10) was found to be diamagnetic, as generally expected for octahedral Co(III) complexes, which is in accordance with the crystal data of the hyrated form ($Co(dmp)_3.12H_2O$) of this complex. The reflectance and solution spectra show a single band with a maximum at about 640.0 nm which is assignable to the transition $^1T_{1g} \leftarrow {}^1A_{1g}$.[3]

The magnetic moment of the copper complex (1.84 B.M.) is indicative of Cu(II) and the absence of Cu-Cu

interactions.[3] The electronic spectrum of $Cu(dmp)_2$ in methanol and in the solid state exhibits a single band of maximum absorption at approximately 677 cm^{-1}. These results do not allow any definite structural inferences to be made.

3.2.1 Mass spectra of metal(II) complexes

Table 17 presents some of the metal-containing ions observed in the spectra of metal(II) complexes of pyridone (10).

Table 17- Some ions in the mass spectra of M(II) (M = Co, Ni, Cu, and Zn) complexes of pyridone (10)

Ion	M in $M(dmp)_2$ (m/z, rel. abundance)			
	Co	Ni	Cu	Zn
$[M(dmp)_2]^+$	335, 47.8	334, 40.0	339, 31.0	340, 69.9(3.8)
$[M(dmp)_2 - H]^+$	-	333, 0.7	-	-
$[M(dmp)_2 - CH_3]^+$	320, 0.9	-	-	325, 3.4
$[M(dmp)_2 - CO]^+$	307, 0.2	-	-	312, 2.0
$[M(dmp)_2 - HCO]^+$	306, 2.8	-	-	311, 4.4
$[M(dmp)_2 - CH_3 - CO]^+$	292, 0.9	-	-	297, 2.0
$[M(dmp) + H]^+$	198, 13.2	197, 37.5	202, 32.9	203, 2.9
$[M(dmp)]^+$	197, 29.9	196, 10.2	201, 18.3	202, 10.4
$[M(dmp) - H]^+$	-	195, 8.5	200, 13.4	201, 1.3
$[M(dmp) - CH_3]^+$	182, 0.5	-	-	-
$[M(dmp) - HCO]^+$	168, 1.0	167, 1.9	172, 1.8	-
$[MC_2OH]^+$	100, 2.8	99, 2.4	104, 2.5	-

Figure in the bracket indicates the abundance of doubly charged species

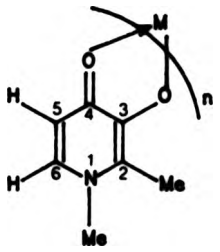
Ion-abundances are expressed as percentage of the ion-current due to the metal-containing ions and are corrected for the isotopic abundances. The spectra of all complexes show intense peaks due to the molecular ions which fragment mainly by loss of an intact ligand radical to give the fairly abundant $[M(dmp)]^+$ ion. Other prominent ions correspond to $[M(dmp) + H]^+$ and $[M(dmp)_2 - dmp]^+$. Additionally, the zinc complex showed reactions involving loss of CH_3, CO, and HCO fragments. The peak with m/z 170, in the spectrum of the zinc complex is due to the doubly charged molecular ion. Although the doubly charged ion is fairly abundant in the case of the zinc complex, it is absent in the spectra of the other complexes.

3.2.2 ^1H n.m.r. spectra of 1,2-dimethyl-3-hydroxypyrid -4-one (10) and its Zn(II) and Co(III) complexes

The ^1H n.m.r. chemical shifts of $Zn(dmp)_2.3½H_2O$ and $Co(dmp)_3$ and the free ligand (dmpH) in dimethyl sulfoxide solutions are given in Table 18. The spectra are consistent with the formulations. The characteristic H5-H6 doublets (J = 6-7 Hz) are observed which are in accord with those reported for M(III) (M = Al, Ga, and In)[23-25] complexes of this ligand and Zn(II) complexes derived from 3-hydroxy-4-pyrones (52).[119] The peak at 4.11 ppm in the

spectrum of the free ligand is assigned to the OH group which, as expected, is absent in the spectra of the complexes, confirming the loss of the H of the OH group upon chelation. Generally, all the resonance peaks (except that due to H6) of the ligand are shifted downfield in the complexes. Similar downfield shift has also been reported for 3-hydroxy-2-methyl-4-pyrone (11)[117] and could be indicative of the delocalisation of C=C bonds of the ligand ring in the complexes.

Table 18 - ^{1}H n.m.r. Resonance peaks (ppm) of pyridone (10) and its hydrated Zn(II) and Co(III) complexes



n = 2, M = Zn
n = 3, M = Co

dmpH	$Zn(dmp)_2.3H_2O$	$Co(dmp)_3$	Assignments
2.26	2.34	2.37	2-Me (s)
3.62	3.70	3.70	N-Me (s)
4.11	----	----	OH
6.03	6.21	6.31	H5 (d)
(J = 7.3 Hz)	(J = 6.8 Hz)	(J = 6.4 Hz)	
7.54	7.35	7.35	H6 (d)
(J = 7.2 Hz)	(J = 6.8 Hz)	(J = 6.2 Hz)	

s = singlet; d = doublet;
The spectra were obtained in DMSO (TMS = 0 ppm) at 200 MHz

3.3 X-ray structural study of hydrated Bis(1,2-dimethyl-3-hydroxypyrid-4-onato)Zn(II) $Zn(C_7H_8NO_2)_2.3½H_2O$

The formation of the zinc(II) complex of 1,2-dimethly-3-hydroxypyrid-4-one (10) together with its infra-red and nuclear magnetic resonance spectra have been reported earlier.[111] Although no analytical data was reported this compound was formulated as $Zn(dmp)_2$. During the present work the Zn(II) complex of pyridone (10) was isolated and formulated as $Zn(dmp)_2.3½H_2O$ on the basis of elemental (Chapter 5, Table 43) and thermal gravimetric analyses (Table 14). This formulation has subsequently been confirmed by single crystal X-ray studies.[29] As mentioned earlier, the X-ray crystallographic structure of several M(III) (M = B, Al, Fe, Ga, In) complexes derived from this ligand (10) and its derivatives have been reported.[23-27] However, there is no X-ray data published for Zn(II) complexes of this pyridone (10) or related ligands.

Crystal preparation: The crystals of bis(1,2-dimethyl-3-hydroxypyrid-4-onato)zinc(II) $3½H_2O$ were obtained by recrystallisation of the crude complex from hot water. A crystal of dimensions 0.32 x 0.28 x 0.15 mm was selected for the crystallographic study. The structure was determined using the single crystal X-ray diffraction technique (in collaboration with an undergraduate

student)[120] and the data was collected on a Philips PW1100 four-circle diffractometer in the θ range 3-25°, with a scan width of 0.80° using graphite monochromated Mo-K_α radiation. A total of 2016 unique reflections with $I > 3\sigma(I)$ (I = intensity of the reflections) were collected.

Structure solution and refinement: Crystal data for $Zn(C_7H_6NO_2)_2.3\frac{1}{2}H_2O$ are as following : Relative molecular mass = 402.55, monoclinic, space group $P2_1/c$, a = 16.252, b = 15.392, c = 7.132, α = 90.0°, β = 92.63°, γ = 90.0°, Volume (U) = 1782 $Å^3$, μ(Mo-K_α) = 0.67 cm^{-1}, Z = 4, F(000) = 844, density (D_c) = 1.508 gcm^{-3}.

The structure was solved initially using a Patterson synthesis from which the coordinates of the zinc atom were located. Subsequently, difference-Fourier maps enabled the localisation of all the remaining atoms, including the hydrogen atoms (except H atoms of the water molecules). During the final stage of structure refinement, anisotropic thermal parameters were assigned to the Zn and all the oxygen atoms of both the coordinated ligand rings and of the water molecules. Final cycles of least squares refinement on all the non-hydrogen atom parameters converged at R and R_w values of 0.0564 and 0.0582, respectively ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = \Sigma||F_o| -$

$F_c|w^{1/2}/\Sigma|F_o|w^{1/2}$ with the weight of $w = 1/\sigma^2(F_o)$ assigned to individual reflections).

The atomic coordinates, inter and intra-molecular distances, temperature factors, and the intra bond angles are listed in Tables I-VI in the Appendix.

A stereoview of the molecule of hydrated bis(1,2-dimethyl-3-hydroxypyrid-4-onato)Zn(II) is diplayed in Figure 10. Figure 11 illustrates the atom numbering of the structure of this compound. The overall structure may be described as distorted square pyramidal with the central zinc atom coordinated to the oxygen atoms O(11), O(12) (Ring 1), O(21), and O(22) (Ring 2) which form the base of the pyramid, and the water molecule (O(1w)) occupying the apical site. The ligand rings and the substituent atoms are planar to within 0.09 Å in relation to the central zinc atom. The distortion from regular square pyramidal is evident from the basal bond angles; with O(21)-Zn-O(22) (95.2(2)°) being larger than O(11)-Zn-O(12) (91.4(2)°).

The complex molecule was found to contain 3.5 water molecules, one of which was coordinated to the Zn(II) atom. The others were incorporated within the lattice by hydrogen bonding (Table 19). The hydrogen bonds are mainly observed between the oxygens of the water molecules and

the hydrogens of the ligand rings and of the methyl group of N-CH_3.

Table 19 - Selected hydrogen bonding distances in $Zn(C_7H_6NO_2)_2.3\frac{1}{2}H_2O$

Hydrogen bond	Length (Å)
O(2w)...H(28a)	2.724
O(2w)...H(26)	2.255
O(3w)...H(17b)	2.901
O(3w)...H(16)	2.595
O(4w)...H(27a)	2.776
O(4w)...H(28b)	2.900

O(nW), n = the numbering for the lattice water;

H(mi), m = the ring no, i = the no. of atom to which the H is bonded

Table 20 - Bond length (Å) for $Zn(C_7H_6NO_2)_2.3\frac{1}{2}H_2O$

Ring 1		Ring 2	
Bond	length(σ)	Bond	length(σ)
Zn-O(11)	2.062(5)	Zn-O(21)	2.051(5)
Zn-O(12)	2.011(5)	Zn-O(22)	2.042(5)
Zn-O(1w)	2.041(6)		
O(11)-C(14)	1.300(8)	O(21)-C(24)	1.289(8)
O(12)-C(13)	1.319(9)	O(22)-C(23)	1.340(8)
C(13)-C(14)	1.428(10)	C(23)-C(24)	1.414(10)
C(14)-C(15)	1.391(10)	C(24)-C(25)	1.420(11)
C(15)-C(16)	1.383(11)	C(25)-C(26)	1.372(11)
N(11)-C(16)	1.326(10)	N(21)-C(26)	1.314(10)
N(11)-C(12)	1.379(10)	N(21)-C(22)	1.381(10)
C(12)-C(13)	1.361(10)	C(22)-C(23)	1.381(10)
C(17)-N(11)	1.512(11)	C(22)-C(28)	1.496(11)
C(12)-C(18)	1.489(11)	N(21)-C(27)	1.495(10)

σ = standard deviation

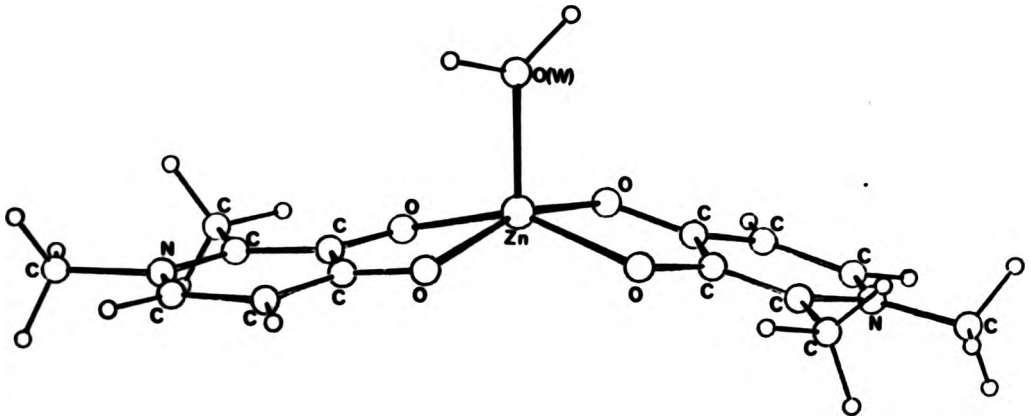


Figure 10- A stereoview of the molecule of hydrated bis(1,2-dimethyl-3-hydroxypyrid-4-onato)Zn(II)

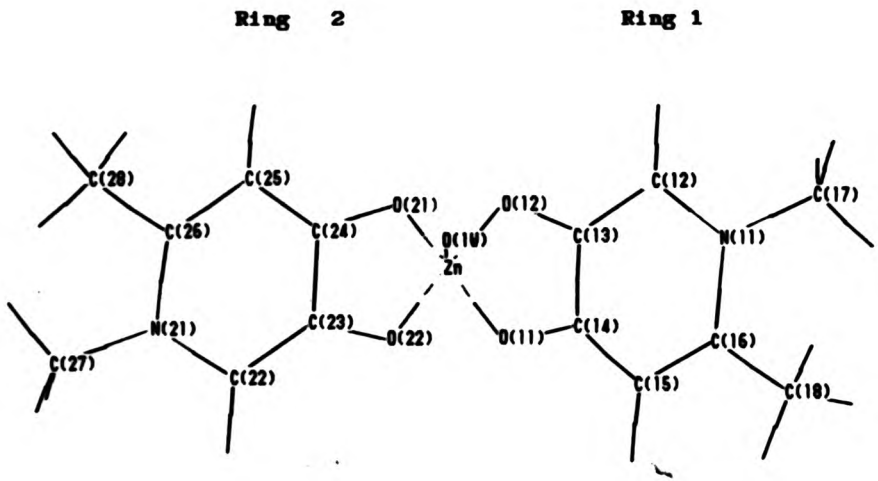


Figure 11- Atom numbering of hydrated
bis(1,2-dimethyl-3-hydroxypyrid-4-onato)Zn(II)

Comparison of equivalent bond lengths in the two ligand rings reveals that they are the same within the limits of the experimental errors (i.e. the difference in any two equivalent bond lengths is less than 3σ).

A notable trend is observed in Zn-O bond lengths (Table 20), with the Zn-O(hydroxy) being markedly longer than the Zn-O(ketonic) of each coordinated ring; i.e. Zn-O(11), 2.062(5) > Zn-O(12), 2.011(5) (Ring 1) and Zn-O(21), 2.051(5) > Zn-O(22), 2.042(5). Such variations in the Zn-O bond lengths indicate that some of the ketonic character of the ligand has been retained upon coordination to the zinc atom. This is also evident from C-O distances, where the average of the C-O(hydroxy) (1.329 Å) of the two rings is significantly longer than that of the C-O(ketonic) (1.290 Å). Nevertheless, all the C-O bonds of the chelated ligands are intermediate between those expected for C-O single and double bonds (C-O, 1.43 Å, and C=O, 1.23 Å),[88] suggesting delocalisation of the π electrons. Previous crystallographic studies for the free ligand have shown a partial delocalisation of the C=O and C=C bonds.[22] Comparison between the bond lengths of the coordinated and the free ligand (10) [22] (see page 50) indicate that this delocalisation is more significant for the former. The difference between C-O(hydroxy) and C-O(ketonic) is decreased from 0.088 Å in the free ligand to 0.039 Å in the coordinated ligand (mean values of C-O(hydroxy) and

C-O(ketonic) are used for the coordinated ligands). Furthermore, there is a significant averaging of the C-C and C=C bonds within the coordinated ligand rings compared to the free ligand.[23-27] Similar bond length patterns have also been observed for the M(III) (M = B, Al, Ga, and Fe) complexes of this ligand.[23-27]

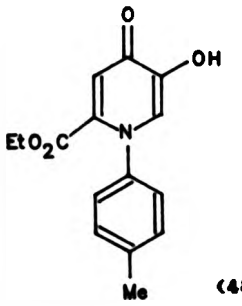
In Chapter two (section 2.3.5) it was concluded that the hydrobromide salt of the pyridone (10) has a hydroxypyridinium structure, where a complete delocalisation occurs. Comparison of the bond lengths between this salt (16a) and the coordinated ligand in $Zn(dmp)_2.3½H_2O$ indicates that the latter has some ketonic character.

The range of the corresponding C-C bond lengths of the two ring systems (1.361(10)-1.495(11) Å) and C-N distances are similar to each other and to those reported for M(III) complexes derived from this ligand.[23-27]

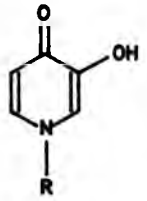
The above crystallographic results and spectroscopic studies suggest that the chelated ligand (10) is stabilised by the predominating canonical forms a and b (Figure 9, page 92).

3.4 Synthesis and characterisation of iron(III) complexes derived from 1,2-dimethyl-3-hydroxypyrid-4-one (10)

As noted in the previous section there is currently a considerable interest in the complexing behaviour of hydroxypyridones towards iron. This interest arises primarily because of the potential application of these ligands in the treatment of iron-overload.



(48)



(49)

Previous spectroscopic studies have indicated that several hydroxypyridones (9, R = Me,[19] Ph,[93] 4-tolyl,[94] 48,[95] and 49, R = H, $CH_2CH(NH_2)CO_2H$[92]) form iron(III) complexes of different compositions, depending on the pH and the ligand-to-iron ratio. In all cases the composition of the complexes has been determined using the Job's method. These studies have shown that 1,2-dimethyl-3-hydroxypyid-4-one (dmpH = 10) forms an iron complex with a 3:1 ratio of ligand-to-iron at physiological pH (*ca.* pH 7.4) and a 1:1 dmpH-iron(III) species at approximately pH 2.[19] Similarly, 1-phenyl-2-methyl-3-hydroxypyrid-4-one (pmpH =

9, R = Ph) yields three iron complexes depending on the reaction conditions. These complexes have been formulated as $[Fe(pmp)]^{2+}$, $[Fe(pmp)_2]^{+}$ and $Fe(pmp)_3$ amongst which only $Fe(pmp)_3$ has been isolated.[93] In the case of the reaction of 1-(4-tolyl)-2-methyl-3-hydroxypyrid-4-one (tmpH = 9, R = 4-tolyl) with iron(III), the formation of the complexes $[Fe(tmp)]^{2+}$ and $[Fe(tmp)_2]^{+}$ has been reported.[94] A complex formulated as $Fe(tmp)_2Cl$ has been isolated.[94] It has also been shown that 1-(4-tolyl)-6-carbethoxy-3-hydroxypyrid-4-one (tcpH = 48) gives a complex with a ligand-to-iron ratio of 3:1 at pH 2.0-3.5. This complex has been isolated and formulated as $Fe(tcp)_3$. In all cases the isolated complexes, i.e. $Fe(pmp)_3$, $Fe(tmp)_2Cl_2$, and $Fe(tcp)_3$, have been characterised on the basis of elemental analysis only. Mimosine (49, R = $CH_2CH(NH_2)CO_2H$) has also been shown to form three different iron(III) complexes, depending on the pH of the reaction mixture.[92] It has been reported that at low pH values, a complex containing Fe(III) and the ligand in a 1:1 ratio was formed, the composition of which was changed into 2:1 and 3:1 of ligand-to-iron ratios at higher pH values. However, none of these complexes has been isolated.

In view of the limited data reported on the iron-pyridone complexes noted above, and also because of their significance, a systematic study of their synthesis and

characterisation has been carried out as part of this work.

Initially, the formation of the iron(III) complexes of the pyridone (10) was investigated qualitatively using spectroscopic methods. The visible spectra of a series of aqueous solutions containing a mixture of ligand and iron(III) chloride of 1:1, 2:1, and 3:1 molar ratios, at variable pH values were recorded. In highly acidic (pH < 1) solutions, the spectra of all three solutions were identical and showed a prominent absorption at 576 nm. Addition of sodium hydroxide to each of the solutions led to colour changes from blue to purple and finally orange. Table 21 summarises the results together with those previously reported[93] for the 1-phenyl-2-methyl-3-hydroxypyrid-4-one/iron(III) chloride system. The similarity of colours and of the changes of absorption characteristics with pH indicate that both ligands behave in a similar manner. In the case of the 1-phenyl derivative (pmpH), the composition of the complexes has been determined, by Job's method, for solutions containing equal molar concentrations of Fe(III) and the ligand at pH 1.0, 2.5,and 4.0.[93] The results indicated that at pH 1.0 only one complex, formulated as $[Fe(pmp)]^{2+}$, with maximum absorption at 570 nm was formed. At pH = 2.5 and 4.0, the existence of three complexes, $[Fe(pmp)]^{2+}$, $[Fe(pmp)_2]^{+}$, and $Fe(pmp)_3$, has been suggested. On the basis of these

Table 21- Variation λ_{max} of Fe(III) complexes of the pyridones (9 = pmpH, R = Ph) and (10 = dmpH) with pH and molar ratio of the ligand to Fe(III)

pH	λ_{max} (nm) reported[20] for pmpH-Fe(III)				Product formulae	pH	λ_{max} (nm) observed for dmpH-Fe(III)		
	Fe(III):pmpH						Fe(III):dmpH		
	1:1	2:1	3:1	10:1			1:1	2:1	3:1
0.6	570	570	570	570	a	0.7	576	576	576
1.0	570	570	570	570	"	0.9	575	576	576
1.5	565	560	560	560	"	1.8	560	558	557
2.0	560	540	515	510	a,b,c	--	--	--	--
2.5	555	510	505	500	"	--	--	--	--
3.0	555	505	505	480	"	3.0	546	516	500
3.5	530	505	490	470	"	--	--	--	--
4.0	510	490	470	470	c	--	--	--	--
5.0	480	475	470	470	"	5.3	467	467	458
--	--	--	--	--		7.6	447	457	457
--	--	--	--	--		10.0	457	457	457

a = $[Fe(pmp)]^{2+}$, b = $[Fe(pmp)_2]^{+}$, and c = $Fe(pmp)_3$

results and those listed in Table 21, the following conclusions have been reported:[89]

i) At pH values between 0.6 and 1.5, only one complex is formed independent of the molar ratio of the reactants.
ii) At pH > 1.5 a mixture of the three complexes is present and their concentrations are dependent on both pH and molar ratio of the reactants.
iii) At high pH values and ligand:iron(III) molar ratios, the complex $Fe(pmp)_3$, with maximum absorption at 470 nm, is the dominant species.

Because of the similar behaviour of this ligand (9, R = Ph) and of the pyridone (10) towards iron(III) chloride similar formulations are suggested for the complexes derived from these two ligands (9, R = Ph; and 10). On this basis the complex with absorption at 457 nm is formulated as $Fe(dmp)_3$. This complex is formed at pH values above 5.3 where the molar ratio of ligand to iron is 3:1. The formation of this complex is also indicated in the solutions containing a 2:1 concentration ratio of iron to the pyridone (10) at pH > 7.6. As will be shown later (page 113), the solution spectrum of the isolated complex $Fe(dmp)_3.4H_2O$ also absorbs at 457 nm supporting the above statement.

In order to isolate and characterise the complexes arising from pyridone (10) and iron(III), the reaction between this ligand and iron(III) chloride in aqueous methanol at different pHs and molar ratios of the reactants were investigated. The conditions used and the composition of the resulting complexes are given in Table 22. In all cases the product composition has been determined by elemental analysis (see Chapter 5, Table 43).

Table 22 - A summary of reactions of the pyridone (10) with iron(III) chloride in aqueous methanol

molar ratio of dmpH : Fe	pH of reaction mixture	product composition Fe : ligand : Cl
1 : 1	low (< 1.0)	2 : 3 : 5
2 : 1	low (≈ 1.5)	2 : 4 : 5
3 : 1	low (≈ 1.5)	2 : 4 : 5
3 : 1	high (≈ 12)	1 : 3

The reaction of pyridone (10) with iron(III) chloride in aqueous methanol, at a 3:1 molar ratio of ligand to iron(III) in the presence of sodium hydroxide (pH ≈ 12) gave a dark red solution. From this solution a red solid, identified as $Fe(dmp)_3.4H_2O$, was extracted using dichloromethane. The infra-red spectrum of this complex is similar to those of metal(II) complexes derived from this ligand, indicating involvement of the carbonyl group in the bonding to the metal on the basis of the arguments put

forward in section 3.2. The room temperature magnetic moment of this complex (5.5 B.M.) suggested a high spin Fe(III) complex with the metal ion in an octahedral environment. The electronic spectrum of this complex in methanol and in the solid state gave a strong absorption band at approximately 457 nm, suggesting a similar structure for the complex in both solution and solid state. However, no structural deductions can be made from the electronic spectrum of this complex. The observed absorption band at 457 nm is likely to be due to charge transfer transitions, since in iron(III) complexes the Fe^{3+} ion is a d^5 species and would be expected to have very weak d-d spin-forbidden transitions. In practice, the bands due to these transitions are too weak to be observed and are often obscured by strong charge transfer bands. Nevertheless, a tris chelate structure, with Fe(III) in an octahedral environment, is indicated for this complex from elemental analysis, magnetic measurements and i.r spectroscopy. This structure has been confirmed by X-ray crystallography.[27]

The reactions of the pyridone (10) with iron(III) chloride in 3:1, 2:1 and 1:1 molar ratios of the ligand to iron(III) were examined in aqueous methanol, in the absence of sodium hydroxide.

The reactions involving 2:1 and 3:1 ligand:metal ratios gave purple solutions of pH *ca* 1.5. In both cases the addition of diethyl ether to the solution precipitated a purple solid with a metal : chlorine : ligand ratio of 2:5:5. This solid showed high solubility in polar solvents such as water and methanol but was insoluble in non-polar solvents such as diethyl ether and dichloromethane. Each of the reactions was repeated three times and it was established that the results are reproducible. Charge requirements suggest that this product must involve both neutral and anionic ligand moieties and its composition could be described as $Fe_2Cl_5(dmp)(dmpH)_4$. However, it must be stressed that other possibilities also exist.

The reaction in 1:1 molar ratio, on the other hand, gave a blue/violet solution of pH less than 1. Precipitation with diethyl ether yielded a water soluble red solid with a metal : chlorine : ligand ratio of 2:5:3. As in the case of the reactions involving 2:1 and 3:1 ligand:metal ratios it was shown that this solid can be prepared reproducibly. On the basis of elemental analysis and charge requirements the composition $Fe_2Cl_5(dmp)(dmpH)_2$ is tentatively proposed for this product.

In order to determine the structural characteristics of these products, several chemical and physicochemical techniques such as infra-red and Mossbauer spectroscopy,

magnetic and conductance measurements, and ion-exchange behaviour were investigated. However, the results do not allow definite structural conclusions.

Figures 12 and 13 display the i.r. spectra of the products with compositions $Fe_2Cl_6(dmp)(dmpH)_2$ and $Fe_2Cl_6(dmp)(dmpH)_4$, respectively. In the region 1650-400 cm^{-1}, both spectra are generally similar to those of metal(II) complexes derived from this ligand, indicating the presence of the anionic ligand (dmp^-). In addition, the spectra show a band at *ca* 1635 cm^{-1}. Such a band is also observed in the spectrum of the free ligand and its protonated form, thus suggesting the presence of either free ligand or cationic ligand ($dmpH_2^+$) or both in these products. In the region 4000-2500 cm^{-1} the spectra of the products are different from each other and from the spectrum of the free ligand. A close resemblance of spectra of the hydrochloride salt of the ligand (10) and of $Fe_2Cl_6(dmp)(dmpH)_4$ (Figure 14) indicates the presence of protonated ligand(s) in the latter. The spectrum of $Fe_2Cl_6dmp(dmpH)_2$, on the other hand, exhibits a broad band at *ca* 3500 cm^{-1} which is indicative of the presence of water.

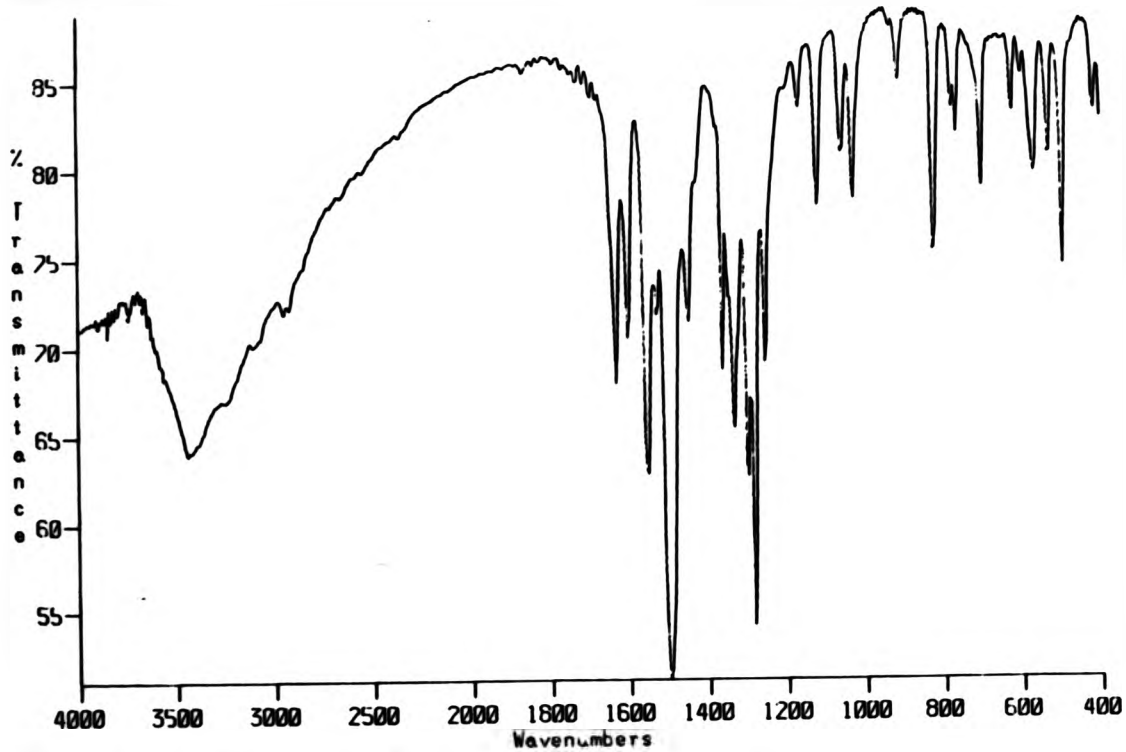


Figure 12- Infra-red spectrum of $Fe_2Cl_6(dmp)(dmpH)_2$ (dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one) (KBr disc)

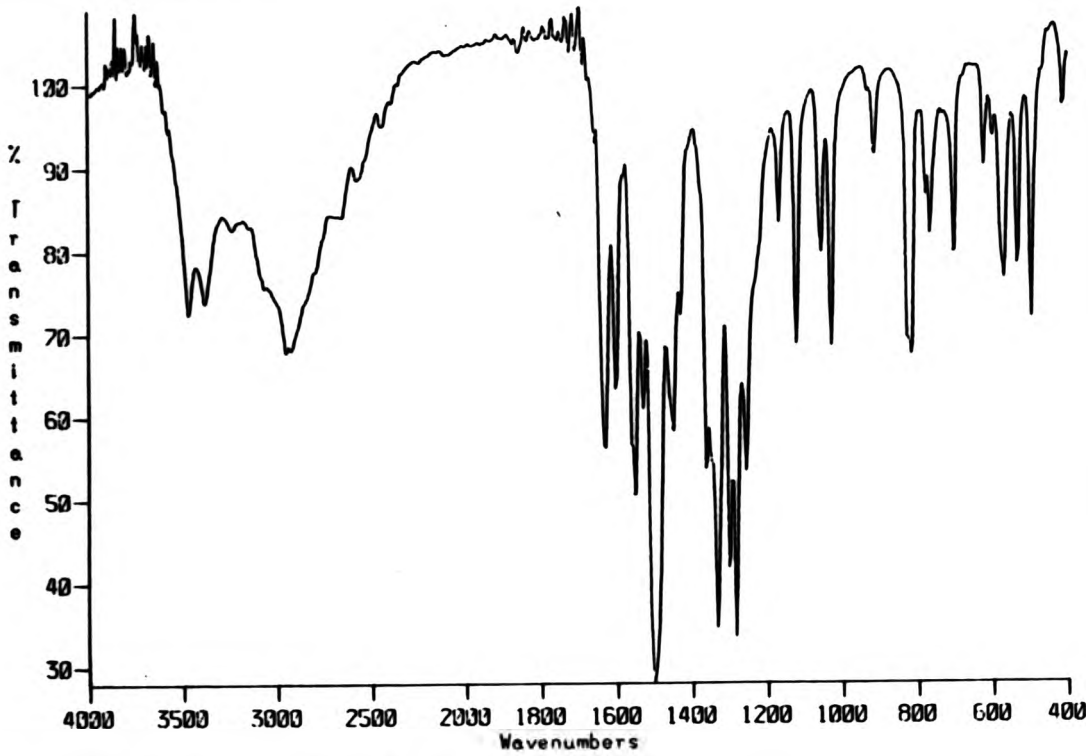


Figure 13- Infra-red spectrum of $Fe_2Cl_4(dmp)(dmpH)_4$ (dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one) (KBr disc)

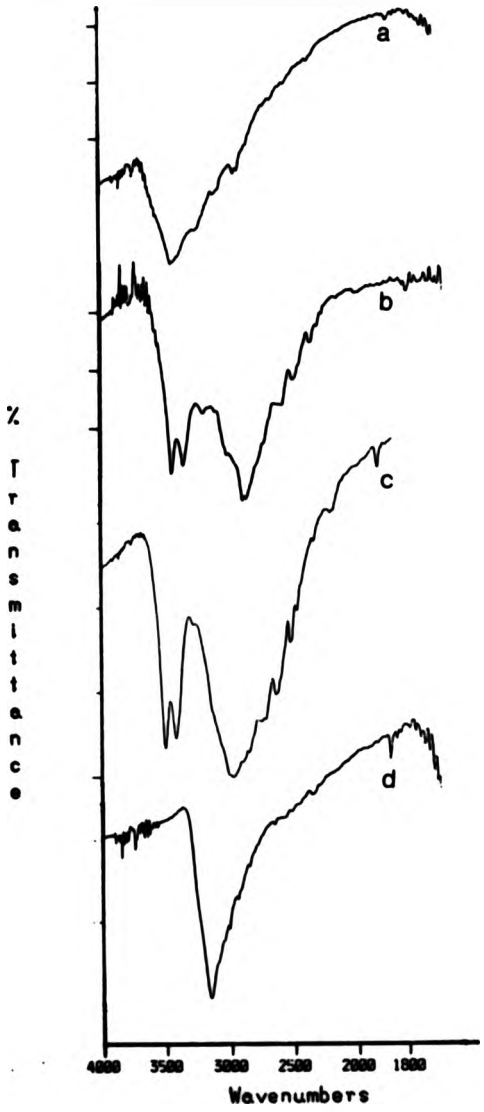


Figure 14- Infra-red spectra of: (a) $Fe_2Cl_6(dmp)(dmpH)_2$;
(b) $Fe_2Cl_6(dmp)(dmpH)_4$;
(c) dmpH.HCl (16b); (d) dmpH (10)
(dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one)

Room temperature magnetic measurements of the products $Fe_2Cl_6(dmp)(dmpH)_4$ and $Fe_2Cl_6(dmp)(dmpH)_2$ indicate moments of 5.4. and 5.8 B.M. per iron atom, respectively, suggesting the presence of iron(III) in a magnetically dilute environment.

The Moessbauer spectra of both products which are presented in Table 23 in each case suggest the presence of one iron species in an asymmetrical environment. In both cases the isomer shifts and quadrupole splitting parameters are in the range expected for six coordinate high spin Fe(III),[121] which is consistent with magnetic measurements.

Table 23 - Moessbauer spectra of iron complexes of the pyridone (10)

Product	Temp. (°C)	δ (mm s^{-1})	Δ (mm s^{-1})
$Fe_2Cl_6(dmp)(dmpH)_2$	20	0.517 ± 0.030	0.857
	-196	0.606 ± 0.007	0.870
$Fe_2Cl_6(dmp)(dmpH)_4$	20	0.401 ± 0.009	0.899
	-196	0.436 ± 0.006	0.982

δ = Isomer Shift; Δ = Quadrupole Splitting

The spectrum of $Fe_2Cl_6(dmp)(dmpH)_2$ shows two very dissimilar peaks which sharpen on lowering the temperature from 20 °C (Figure 15) to -196 °C (Figure 16). This is characteristic of high spin Fe(III) species for which

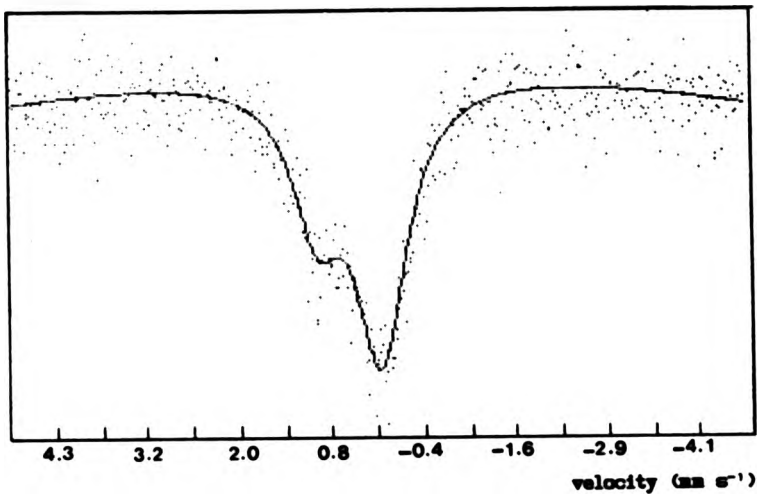


Figure 15- Moessbauer spectrum of
$Fe_2Cl_5(dmp)(dmpH)_2$ at 20 °C
(dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one)

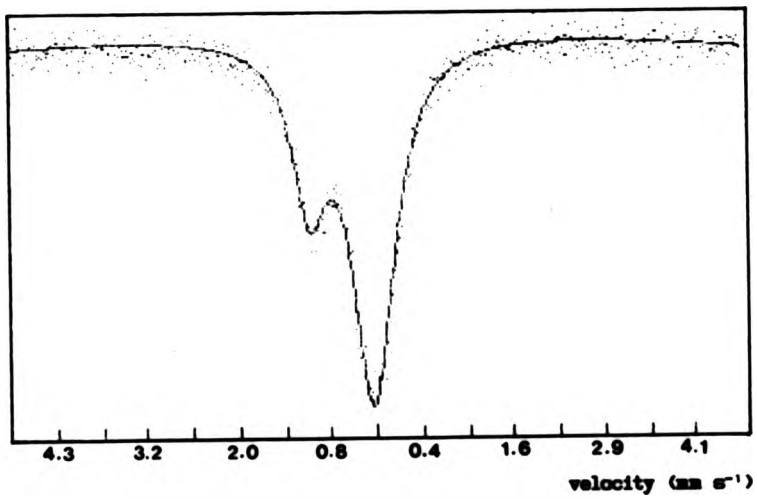


Figure 16- Moessbauer spectrum of
$Fe_2Cl_5(dmp)(dmpH)_2$ at -196 °C
(dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one)

spin-lattice relaxation of the ground state is very slow and spin-spin relaxation is the dominant mechanism. Such an effect has been mainly observed for five coordinate species such as $Fe(acac)_2Cl$; (acacH = acetylacetone).[120] However, the effect also occurs in six coordinate species such as $FeCl_3.6H_2O$ which exists as $[FeCl_2(H_2O)_4]Cl.2H_2O$.[120]

The Mossbauer spectrum of $Fe_2Cl_6(dmp)(dmpH)_4$, at room temperature, exhibits an unsymmetrical doublet (Figure 17) with quadrupole splitting and isomer shift values compatible with high spin Fe(III) in an octahedral environment; the doublet became perfectly symmetrical at -196 °C (Figure 18). The presence of only a doublet suggests that there is only one iron site.

The above results do not allow definite structural inferences. The high water solubility of these products suggests ionic structures which were supported by conductance measurements. The values of conductance of both $Fe_2Cl_6(dmp)(dmpH)_4$ and $Fe_2Cl_6(dmp)(dmpH)_2$ in methanol (at concentration of *ca* 10^{-3} M) at 21 °C were found to be 309 and 313 $\mu S\ cm^2\ mol^{-1}$, respectively. These values are within the range reported for 1:3 electrolytes in methanol.[122] In order to obtain more information on the nature of the ions present, the complex $Fe_2Cl_6(dmp)(dmpH)_4$ was selected for further investigations.

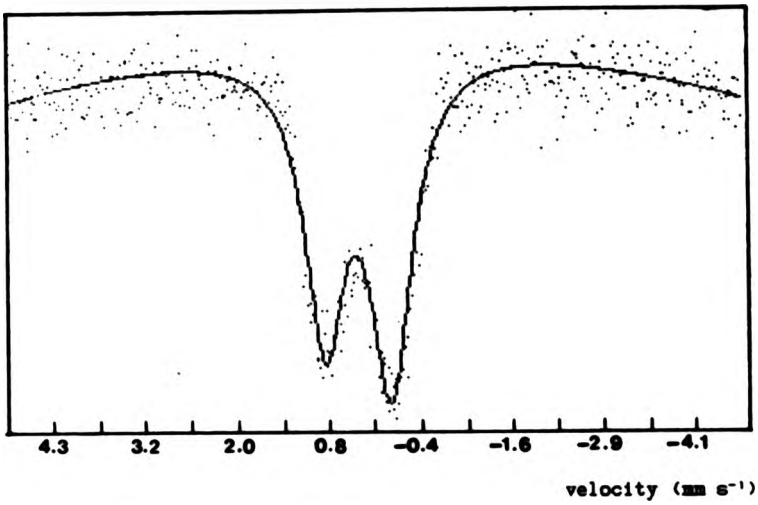


Figure 17- Moessbauer spectrum of
$Fe_2Cl_6(dmp)(dmpH)_4$ at 20 °C
(dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one)

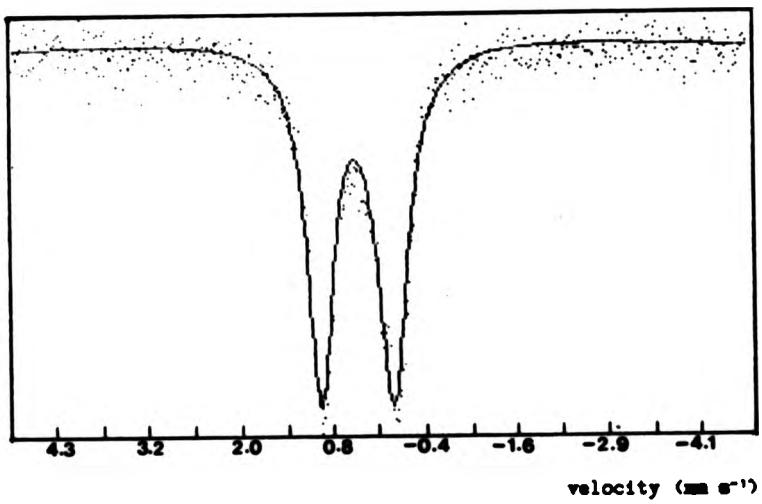


Figure 18- Moessbauer spectrum of
$Fe_2Cl_6(dmp)(dmpH)_4$ at -196 °C
(dmpH = 1,2-dimethyl-3-hydroxypyrid-4-one)

A methanolic solution of this product was passed through a cationic ion exchange column with $-SO_3^-H^+$ as the active group. Elution with methanol and water gave a colourless solution which did not contain the ligand or iron ions, but contained chloride ions. This suggests the presence of a cation species incorporating iron, the ligand, and possibly chlorine. Attempts to remove this cation by addition of dilute hydrochloric or nitric acid to the column failed. Addition of more concentrated acid led to decomposition of this ion as indicated by the colourless eluate.

On the basis of the above results the products $Fe_2Cl_6(dmp)(dmpH)_4$ and $Fe_2Cl_6(dmp)(dmpH)_2$ can be tentatively formulated as $[Fe_2(dmp)(dmpH)_4Cl_2]^{3+}$ $3Cl^-$ and $[Fe_2dmp(dmpH)_2Cl_2]^{3+}$ $3Cl^-$.

In conclusion, it has been shown that 1,2-dimethyl-3-hydroxypyrid-4-one (10) forms different complexes with iron(III). At high pH values a complex containing an iron(III):ligand ratio of 1:3 is formed which can be isolated. At low pH values, on the other hand, the formation of complexes with 1:2 and 1:1 ratios of iron(III):ligand is evident in solution, but the complexes could not be isolated under the conditions used in this study. However, a product of tentative formula $[Fe_2(dmp)(dmpH)_2Cl_2)]^{3+}$ $3Cl^-$ was isolated from acidic

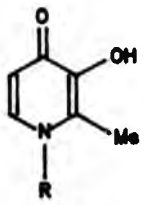
solutions containing a 1:1 ratio of iron(III) and the ligand. Similarly, a product of possible formula $[Fe_2(dmp)(dmpH)_4Cl_2]^{3+}$ $3Cl^-$ was obtained from acidic solutions of iron(III) and the ligand at 1:2 or 1:3 ratios.

CHAPTER FOUR

GENOTOXICOLOGICAL STUDIES OF 1,2-DIMETHYL-3-HYDROXYPYRID-4-ONE

4.1 Introduction

For any compound to be used as a medicine, one of the important aspects that should be considered is its toxic effects. With regard to 3-hydroxypyrid-4-ones of type (9), there are numerous reports with encouraging results on their iron removal abilities, both *in vivo* and *in vitro*,[19,20,123-129] but little is known about their toxic effects. There are many outstanding questions about both the long and short term toxicity of these compounds which necessitate more research prior to their clinical use in man.



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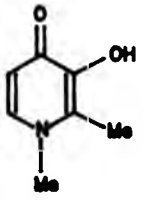


(10)

Previous toxicological studies have been mainly concerned with relatively short term toxic effects of these chelators. Generally, both *in vitro* and *in vivo* studies have shown that these chelators are highly selective towards iron and therefore, side effects arising from the removal of other essential metals are unlikely to occur.[19,123,130]

In evaluating the potential toxicity of a chemical, one of the general procedures is the assessment of acute toxicity (i.e. high dose of the chemical for a short period of time) for the determination of the lethal dose. This usually is obtained by measuring the LD_{50} of the test chemical. LD_{50} is defined as the single dose which kills 50% of the treated animals, normally mammals, over a selected period of time.[131] This dose has not yet been determined for any of the hydroxypyridones of type (9). However, in a study primarily concerned with iron excretion in response to different doses of these chelators (9, R = Me, and Et), some preliminary toxicological data have been reported.[131] In this report it has been noted that no toxic effects were observed following the continuous daily administration of a single dose (10 mg) of these compounds (9, R = Me, and Et) in iron overloaded mice, over 24 days and of double dose for a further 7 days.[132] In the case of 1,2-dimethyl-3-hydroxypyrid-4-one (10), the effect of a single dose of

1 g/kg has also been studied on four normal mice, two of which died, but no adverse effects have been reported for the other two.[132] On the basis of these preliminary studies an LD_{50} value of 1 g/kg for a single dose has been estimated for this compound (10).[132]

Cytotoxicological and DNA synthesis inhibitory studies of 1,2-dimethyl-3-hydroxypyrid-4-one (10) have shown that this compound has no toxic effects in these tests.[133] In another study related to iron chelation in rats, a pronounced salivation was observed in the animals treated with this compound either parenterally or orally.[130]

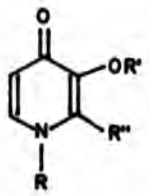
Nevertheless, this chelator (10) has been selected for clinical trial in man, and for nearly the past three years has been given experimentally to iron-overloaded patients.[31] So far there appears to be only one case of a serious toxic reaction in a patient but its relationship to this drug is not yet established.[34] Concurrent with these clinical trials, several other toxic effects associated with this compound, have been reported in animals[32,33]. These include interaction with barbiturates, retinal toxicity in rats (similar to those caused by desferrioxamine), severe sweating, muscular spasms and hyperactivity in other animals. However, there are some disputes between different workers with regard to these side effects. In addition, it has been shown that this

compound (10) at doses of 200 mg/kg causes a significant reduction in haemoglobin and white-cell counts in non-overloaded mice.[33] Similar effects have been observed in rats treated with high doses (200 mg/kg) of compound (10) but not with low doses (60 mg/kg).[33] Other derivatives of these hydroxypyridones (9, R = Et, $(CH_2)_2OCH_3$), however, have been suggested to be more suitable for further studies than the N-methyl derivative (10), since they have an acceptable balance between iron removal ability and toxicity.[32]

Nevertheless, while the extent of the toxicity of pyridone (10) in animals has not yet been agreed, its clinical trial is still being continued.[34] Furthermore, in none of these previous studies, have the <u>long term</u> effects of this chelator and its potentially useful derivatives been examined. In the treatment of iron overload disorders, iron chelating agents, such as hydroxypyridones, can be expected to be administered to patients regularly over their entire lifetime. Hence, it is essential to be aware of their long term (i.e. chronic) toxicity. The long term effects are generally thought to arise from chronic exposure, i.e. low doses over a long time period. Genotoxicity is an important example of such long-term effects which is the main concern of this chapter. The term genotoxic is given to a chemical which is capable of damaging DNA in a chemical sense.[100].

In the past thirty years because of the increased public concern about the presence of new environmental chemicals and their relation with cancer, the identification of potential carcinogens has become more important.[134] During the last decade a high degree of correlation between carcinogenicity and mutagenicity has been established. Many chemicals which are known to be carcinogenic in mammals have also been shown to induce mutation in microorganisms.[134]

In a general literature survey on hydroxypyridones, it was discovered that both 2- and 4-pyridones of types (53) and (54), respectively have shown potential antitumour activities.[43,44,135] These results may be alarming since the majority of antitumour agents, depending on their mode of action, are also potential carcinogens and therefore possible mutagens.[134]



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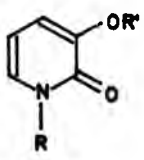

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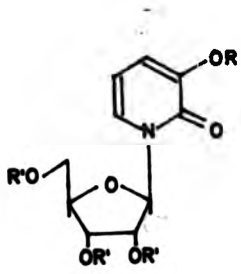
(R, R', and R'' = H, alkyl, or acyl)

The mode of action of these pyridones as antitumour agents has not yet been established. The activity of several derivatives of both 2- and 4-pyridones was examined *in vivo* and it was shown that "at least two oxygen-containing functional groups are required for their activity".[43] In a later report it was suggested that pyridones could undergo anabolic reactions to give analogs of naturally occurring nucleosides, which may be responsible for their antitumour activity.[44] However, this possibility has been ruled out since ribo- (e.g. 55) and arabinofuranosyl (e.g. 56) nucleosides of the pyridones (53) and (54) showed no activity *in vivo*.[44] Several other N-substituted derivatives of the 2-pyridone series (53, R' = $COCH_3$, R = CH_3, and R = R' = $CONHCH_3$) have also been evaluated for their antitumour activity,[43,135] but the results do not indicate a direct correlation between N-substitution and antitumour activity.



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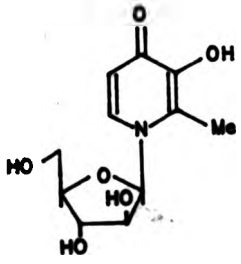

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During the present study the potential mutagenicity of 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its hydrochloride salt (16b) was evaluated using a recently developed bioassay.[136] This assay monitors the increase in chemically induced mutations in continuous cultures of the fission yeast *Schizosacchromyces pombe* and has several advantages over the conventional bacterial tests. This new assay benefits from utilising a test organism with a cell structure much more closely related to human cells than are those of bacteria. In addition, by employing continuous culture, it allows the detection of mutagenic effects caused by chronic levels of the test chemicals, whereas the bacterial tests such as the Ames test, can only measure the effects of acute exposure.

4.2 The concept of continuous cultures

A continuous culture system consists of four basic parts (Figure 21); a growth chamber, a nutrient supply system, a drainage system, and an agitation mechanism.[137] In this system fresh medium enters the growth chamber at a constant rate. The volume of the cell culture is kept constant using an overflow mechanism which removes the excess volume of culture from the growth chamber at the same rate at which the fresh medium is added. After a period of adjustment, the cell population reaches a steady state at which the number of cells and the rate of cell

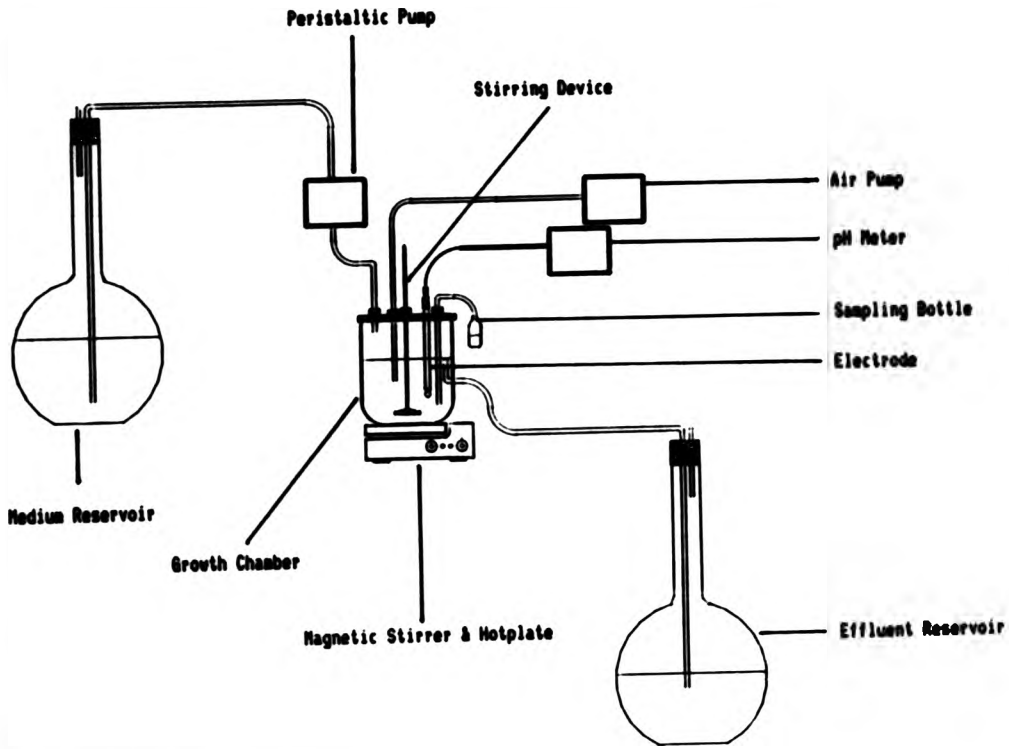


Figure 10- Chemostat apparatus

division remains constant with time. There are two types of continuous culture systems, the turbidostat and the chemostat. In a turbidostat the growth is controlled by the internal cellular reactions and it approaches the maximum possible under particular cultural conditions. In a chemostat the growth rate is limited by the concentration of a critical growth factor. All other growth factors must be supplied in excess in a defined medium. Consequently, under these conditions, the growth rate is controlled externally; that is by the rate of the medium supply. Hence, in a chemostat the growth rate is independent of cell concentration and relatively independent of temperature and pH. Therefore, a chemostat provides greater flexibility for mutagenic studies than a turbidostat.[136]

In a chemostat, at steady state the specific growth rate (μ) is equal to the dilution rate (D):

$$\mu = D \qquad \text{Equation 4}$$

The dilution rate (D) is calculated from the following relationship:

$$D = F/V \qquad \text{Equation 5}$$

F = flow rate of fresh medium - cm^3h^{-1}

V = working volume of the growth chamber - cm^3

In order to determine the working volume, the standard conditions of the chemostat such as the gaseous systems and agitation mechanisms should be in operation.

At steady state, the cell concentration of the suspension which leaves the growth chamber is the same as that remaining in the chamber. Therefore, one volume (V) of the culture is washed out in the time taken for the cell number to double. This time is referred to as the culture generation time (τ) and is calculated as following:

$$\tau = V/F = 1/D = 1/\mu \qquad \text{Equation 6}$$

It is important to distinguish between the culture generation time and the cell generation time. In any culture there are always some non-viable cells and also some of the cells leave the growth chamber before they can divide. Therefore, the cell number would increase if cells were washed into a medium in which they could continue to grow at the same rate. Consequently, the total cell number would increase. Thus the cell generation time is less than the culture generation time (t_d) and is estimated as:

$$t_d = (ln\ 2)/\mu = (ln\ 2)/D \qquad \text{Equation 7}$$

In all continuous culture experiments in this work, standard conditions of cell generation time 6.9 hours were maintained and glucose (0.5%) was used as the growth limiting factor.

4.3 Mutagenic studies in continuous cultures

Continuous culture systems are suitable for mutagenic studies because they can provide a constant source of cells in their exponential growth phase, and also permit continuous exposure of cells to the low concentrations of test compounds. Furthermore, under the conditions provided by these systems, the selection factors can be easily determined and eliminated.[137]

For mutagenic studies in a continuous culture system, a forward mutation system has to fulfil three major criteria:[138]

i) Mutants should be suitable for effective screening. This implies that in practice mutants should be distinguishable from non-mutants either by eye or a simple test.

ii) Both mutants and non-mutants should have the same growth rate, that is the mutants should be under no positive or negative selection. Under these conditions mutants will accumulate linearly with time, and therefore,

the slope of the graph, number of mutants against time, represents the mutation rate.

iii) Both spontaneous and induced rates of mutation must be high enough to allow the isolation of mutants from cell concentrations used in the chemostat.

In these studies, the mutagenic effects of the test compounds were determined by measuring the rate of accumulation of mutants resistant to the antibiotics, cycloheximide and chloramphenicol. Forward mutation to these antibiotics fulfil the above three criteria.[28]

4.4 Results and Data analysis

4.4.1 Preliminary studies

In evaluating the potential mutagenecity of a chemical in a continuous culture system, cells are exposed to the test chemical over a long period. Therefore, it is important to establish that the chemical under test does not reduce the cell viability. In the present study, preliminary batch culture experiments were carried out in order to determine the maximum non-lethal concentrations of 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its hydrochloride salt (16b).

As optical density (OD) is directly proportional to cell growth; a decrease in OD indicates a decrease in cell concentration. The maximum non-lethal concentration of the

chelators (10 and 16b) was found by measuring the optical density of *S. pombe* cultures in Edinburgh minimal medium number 2 (EMM2) containing different concentrations of the test compounds (Table 24). This concentration for both pyridone (10) and its hydrocloride salt (16b) was found to be approximately 100 ppm. This is indicated by the reduction of the optical density of the cell suspensions containing more than 100 ppm of the test compounds, when compared with the control cultures.

The optical density measurements, however, only represent the total cell count; that is the number of both viable and non-viable cells. In order to confirm the above results a series of batch cultures containing the free base (10 = dmpH) were prepared and the number of viable cells for both the control and test cultures were determined (Table 25). The viable count of cultures grown in media supplemented with more than 100 ppm of the test compound decreased, which is consistant with the results obtained from OD measurements.

In addition, these preliminary studies indicated that the ligand in both free base and hydrochloride forms did not completely inhibit the growth of *S. pombe* even at concentrations as high as 5000 ppm.

Table 24 - Optical density measurements of batch cultures of *S. pombe* grown in EMM2 containing different concentrations of pyridone (10 = dmpH) and its HCl salt (16b = dmpH.HCl)

conc. of test compound (ppm)	O.D of dmpH cultures	O.D of dmpH.HCl cultures
0	0.34	0.34
1	0.30	0.32
10	0.32	0.31
100	0.29	0.31
250	0.23	0.19
500	0.22	0.20
750	0.21	0.19
1000	0.20	0.19
2000	0.15	0.13
2500	0.13	0.12
3000	0.11	0.10
3500	0.11	0.09
4000	0.10	0.09
5000	0.10	0.07

Table 25 - Viable count of batch cultures of *S. pombe* grown in EMM2 containing different concentrations of pyridone (10 = dmpH)

conc of dmpH (ppm)	no. of viable cells X 10^7
0	1.32
10	1.92
25	2.11
50	1.55
100	1.57
125	1.20
200	0.93
1000	0.68

4.2.2 Continuous culture experiments

4.2.2.1 Determination of mitochondrial and nuclear mutations

It has been suggested that some carcinogens have greater mutagenic effect on mitochondrial DNA than on nuclear DNA.[139] Therefore, during this work, both mitochondrial and nuclear mutations were measured by monitoring the mutation rates to resistance to the antibiotics chloramphenicol and cycloheximide. Chloramphenicol, by binding to the mitochondrial ribosomes prevents protein synthesis.[140] Thus mitochondrial mutations may be monitored by measuring the accumulation of chloramphenicol resistant mutants. On the other hand, cycloheximide inhibits cytoplasmic protein synthesis by blocking polypeptide chain elongation, thus immobilizing the polysomes.[141] Therefore, nuclear mutation can be followed by measuring the increase in the yield of the cycloheximide resistant mutant. In order to confirm the nature of the mutants isolated from the chemostat experiments during our studies, a genetical analysis was carried out. Crosses were made between antibiotic resistant mutants and a histidine requiring mutant. In all the crosses the number of histidine requiring progeny (*his*$^-$) and histidine independent progeny (*his*$^+$) were equal, indicating that the crosses had been accomplished

Table 26- Results from crosses between *cyl*^{R} *his2*$^{+}$ *h*$^{-}$ and *cyl*^{S} *his2*$^{-}$ *h*$^{+}$

Experiment number	No. of *his*$^{-}$ colonies	No. of *cyl*^{R} colonies	No. of *his*$^{-}$ & *his*$^{+}$ colonies	No. of *cyl*^{R} & *cyl*^{S} colonies
1	32	38	100	100
2	68	67	100	100
3	25	25	50	50
4	20	16	37	37
5	52	50	90	90
6	29	32	53	60
7	25	24	55	55
Total	251	252	485	492

Total number of *cyl*^{S} colonies = 492 - 252 = 240

The ratio of no. of *cyl*^{R} to *cyl*^{S} = 252 : 240 = 1 : 1

Table 27-Results from crosses between *cap*^{R} *his2*$^{+}$ *h*$^{-}$ and *cap*^{S} *his2*$^{-}$ *h*$^{+}$

Experiment# number	No. of *his*$^{-}$ colonies	No. of *cap*^{R} colonies	No. of *his*$^{-}$ & *his*$^{+}$ colonies	No. of *cap*^{R} & *cap*^{S} colonies
1	52	0	100	100
2	52	0	100	100
3	62	0	100	100
4	65	0	120	120
5	65	0	120	120
6	62	0	94	102
7	28	0	55	60
Total	386	0	690	702

The *cyl*^{R} and *cap*^{R} mutants were isolated from different chemostat experiments.

successfully. Both cycloheximide resistant (*cyl*^{R}) and cycloheximide sensitive (*cyl*^{S}) cells were dissociated at approximately equal proportions with *cyl*^{R} cells forming nearly half the total cell population in each case (Table 26), which indicated that cycloheximide resistance was due to nuclear mutation. The absence of chloramphenicol resistants (*cap*^{R}) after the crosses (Table 27) suggested that chloramphenicol resistance was the result of mitochondrial mutation.

4.2.2.2 Mutagenic evaluation of 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its hydrochloride salt (16b)

In the chemostat experiments, the mutation frequencies have been expressed as the number of mutants per million viable cells. In each individual continuous culture experiment this ratio was plotted against the time at which a particular sample was collected. The best straight line was fitted by calculating the least square linear regression line. The slope of this line represents the rate of mutation to a particular antibiotic resistance.

A negative control was carried out by measuring the rate of spontaneous mutation to cycloheximide and chloramphenicol resistance in continuous cultures of *S. pombe* grown in unsupplemented EMM2 (Tables 28 and 29).

Table 28- Accumulation of spontaneous cycloheximide resistant (*cyl*^{R}) mutants

Sample no.	No. of mutants/ 10^{6} viable cells (Y)	Time (hr.) (X)
1	0.51	64.7
2	0.79	72.8
3	0.45	88.9
4	0.40	96.5
5	0.81	112.3
6	0.72	120.1
7	0.62	137.0
8	0.51	144.3
9	1.16	160.2
10	1.19	168.5
11	1.95	187.0
12	3.04	232.5

Regression equation : $Y = -0.73278 + 0.01322 \times X$
Correlation Coefficient = 0.8489, Degree of freedom = 11

Table 29- Accumulation of spontaneous chloramphenicol resistant (*cap*^{R}) mutants

Sample no.	No. of mutants/ 10^{6} viable cells (Y)	Time (hr.) (X)
1	0.00	64.7
2	0.00	72.7
3	0.04	88.9
4	0.11	96.5
5	0.15	112.2
6	0.35	137.0
7	0.83	144.3
8	0.78	160.2
9	0.22	168.5
10	0.56	187.0
11	1.78	232.5

Regression equation : $Y = -0.73469 + 0.80968 \times 10^{-2} \times X$
Correlation Coefficient = 0.8534, Degree of freedom = 10

The induced mutation frequencies were determined by measuring the rate at which antibiotic resistant mutants accumulated in *S.pombe* cultures grown in EMM2 supplemented with appropriate (maximum non-lethal) concentrations of the test chemicals. The mutagenic potential of the test compounds was quantified by comparing the rate of accumulation of mutants in the presence, and the absence of the test chemical. If the induced mutation rate was double or more than the respective spontaneous mutation frequency, then that compound was regarded as being mutagenic in *S. pombe*.

In order to confirm the sensitivity of the system a positive control was carried out by measuring mutation induced by a known mutagen, ethyl methanesulphonate (EMS). This compound has been used as a positive control in many other mutational studies.[142,143] It has also been shown, previously, to induce mutations in this assay.[136] EMS is an alkylating agent which induces mutation by DNA base pair substitution. The addition of ethyl groups from EMS to guanine causes that base to behave as an analogue of adenine and thus produces pairing errors.[144]

Ethyl methanesulphonate at its highest non-lethal concentration (120 $\mu g\ cm^{-3}$)[136] induced mutations to both cycloheximide (Figure 20, Table 30) and chloramphenicol

resistances (Figure 21, Table 31), increasing yields by several times those of the respective spontaneous mutation rates. The rates for both spontaneous and induced mutations were different from previously published data.[134] With the exception of spontaneous mutation to chloramphenicol resistance, the level of mutation was lower for reported data than those obtained during this work. This variation could be due to several reasons:

i) The continuous culture apparatus used for these experiments was different from that previously used. While in earlier studies some parts of the apparatus were made of stainless steel, all the parts of the chemostat apparatus used in our experiments were made in glass since the test chemicals react with metal.

ii) In this study the samples were collected directly from the growth chamber, whereas in previous work the samples were obtained from the overflow system.

iii) The continuous culture experiments for this work were carried out over a longer time than corresponding previous work. Most of the data for these experiments were collected after 48 hours (i.e. after steady state was reached) whereas the reported data were obtained after 7-10 hours.

Table 30 - Accumulation of *cyl*^{r} mutants induced by EMS

Sample no.	No. of mutants/ 10^{8} viable cells (Y)	Time (hr.) (X)
1	0.90	64.8
2	0.88	71.8
3	1.41	89.0
4	2.56	96.4
5	3.82	113.0
6	2.03	136.8
7	4.21	143.8
8	7.21	161.3
9	5.81	165.0

Regression equation : Y = -2.83823 + 0.05219 x X
Correlation Coefficient = 0.8799, Degree of freedom = 8

Table 31 - Accumulation of *cap*^{r} mutants induced by EMS

Sample no	No. of mutants/ 10^{8} viable cells (Y)	Time (hr.) (X)
1	6.2	64.8
2	6.5	71.8
3	16.0	89.0
4	22.9	96.4
5	15.9	113.0
6	26.8	119.8
7	27.8	136.8
8	43.0	143.8
9	27.4	165.0

Regression equation : Y = -10.5575 + 0.28740 x X
Correlation Coefficient = 0.7180, Degree of freedom = 8

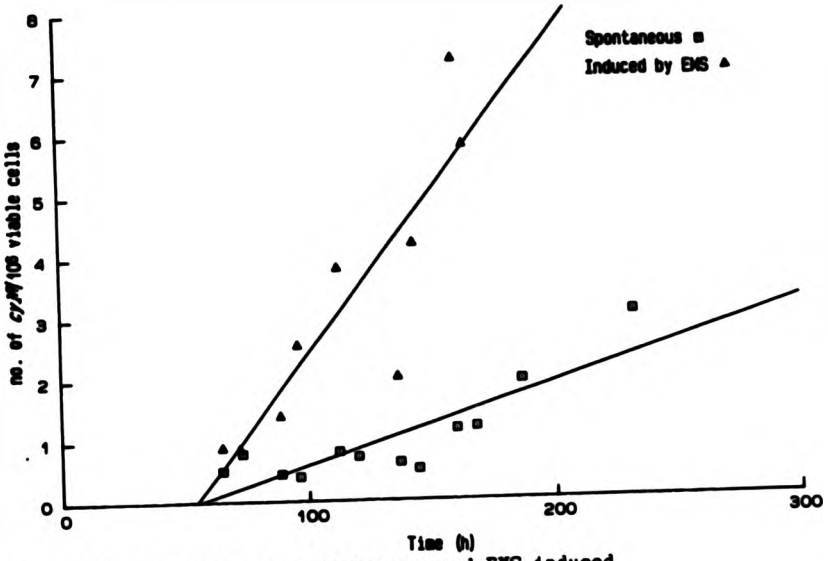


Figure 20- Comparison of spontaneous and EMS induced mutations to *cyl*$^{+}$

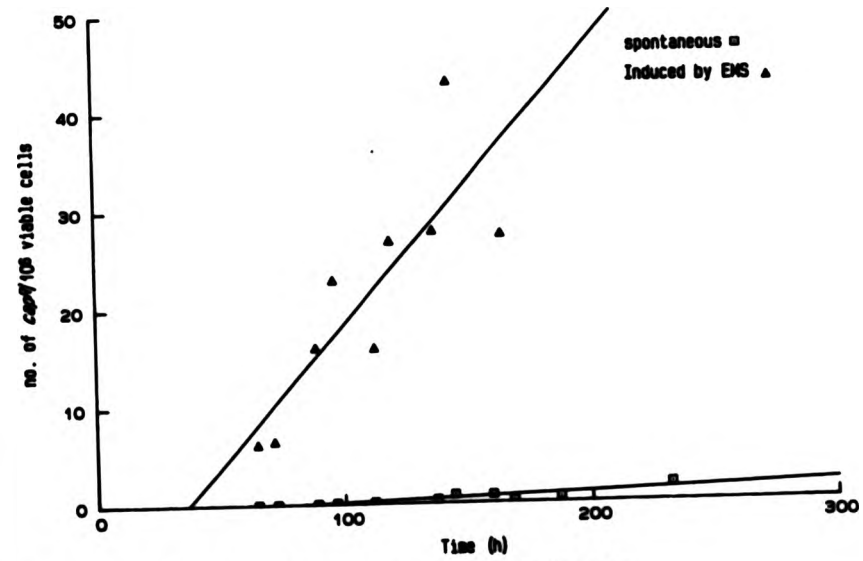


Figure 21- Comparison of spontaneous and EMS induced mutations to *cap*$^{+}$

The mutagenic effects of each test compound (10 and 16b) were tested at their maximum non-lethal concentrations (100 ppm). Figures 22 (Table 32) and 23 (Table 33) illustrate the mutation rates to cycloheximide resistance in the presence of pyridone (10) and its hydrochloride salt (16b), respectively. In both cases the mutation rates of cells were reduced to half or less, those of the corresponding spontaneous mutation rate, indicating that the above chelators have antimutagenic effects on *S. pombe*.

Table 32 - Accumulation of *cyl*^{R} in the presence of pyridone (10)

Sample no.	No. of mutants/ 10^{8} viable cells (Y)	Time (hr.) (X)
1	0.03	44.4
2	0.02	89.8
3	0.04	97.2
4	0.09	113.5
5	0.34	120.5
6	0.45	137.5
7	0.35	144.6
8	0.67	161.5
9	0.25	185.1
10	0.58	193.6

Regression equation : $Y = -0.22954 + 3.97269 \times 10^{-3} \times X$
Correlation Coefficient = 0.7654, Degree of freedom = 9

Table 33 - Accumulation of *cyl*^{R} in the presence of the hydrochloride salt (16b)

Sample no.	No. of mutants/ 10^{8} viable cells (Y)	Time (hr.) (X)
1	0.28	66.2
2	0.28	70.2
3	0.38	90.3
4	0.53	167.7
5	0.42	186.0
6	0.71	190.2
7	0.70	209.2
8	0.53	215.2
9	0.97	233.4
10	0.86	240.4
11	0.86	257.2
12	0.97	304.7

Regression equation : $Y = 0.07728 + 3.12645 \times 10^{-3} \times X$
Correlation Coefficient = 0.8872, Degree of freedom = 11

The chloramphenicol resistant mutation rates were also reduced by the free base (10) (Figure 24, Table 34) but increased by the hydrochloride salt (Figure 25, Table 35) in relation to the appropriate spontaneous mutation rate. However, the differences in both cases were not significant, suggesting that the mutation to chloramphenicol resistance is not significantly affected by these compounds.

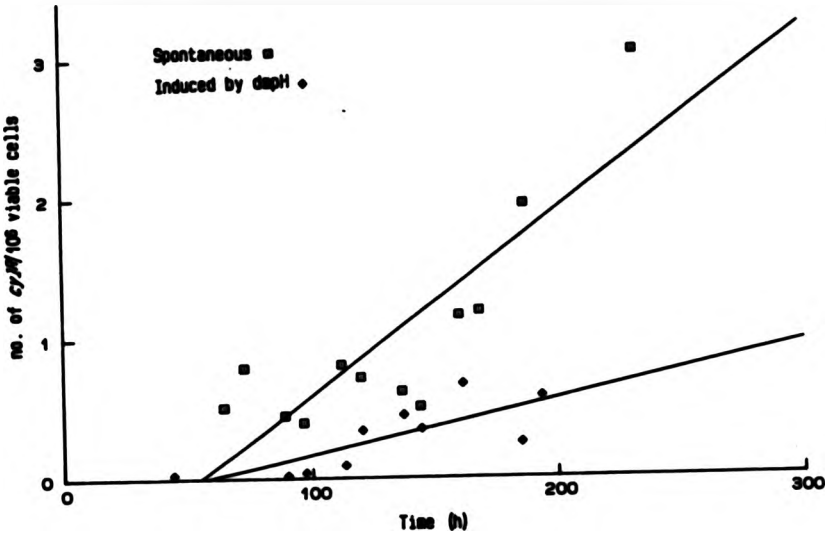


Figure 22- Comparison of spontaneous and dmpH induced mutations to *cyl*$^{+}$

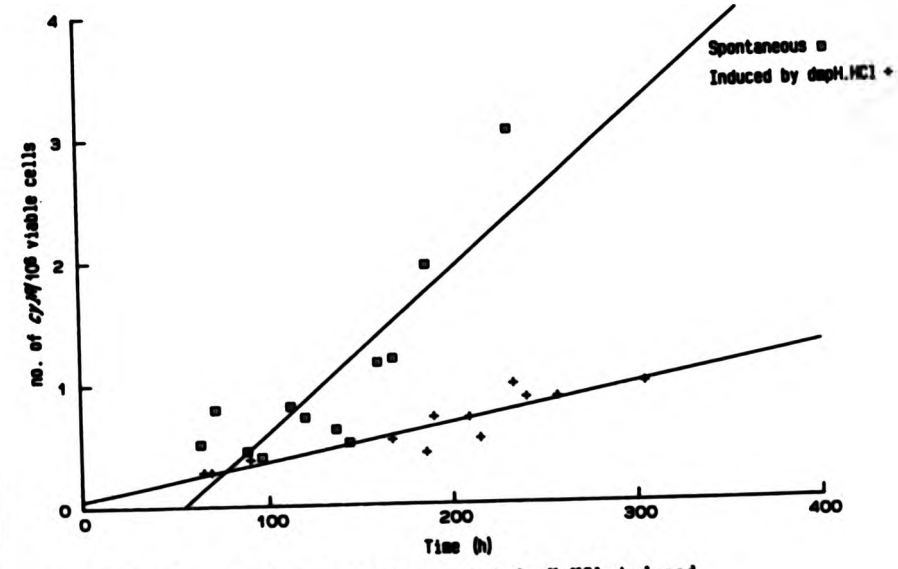


Figure 23- Comparison of spontaneous and dmpH.HCl induced mutations to *cyl*$^{+}$

Table 34- Accumulation of *cap*^{R} in the presence of pyridone (10)

Sample no.	No. of mutants/ 10^6 viable cells (Y)	Time (hr.) (X)
1	0.00	25.8
2	0.13	44.7
3	0.79	89.7
4	0.63	97.2
5	0.38	113.5
6	0.57	120.5
7	0.67	137.5
8	0.74	144.6
9	1.04	161.5
10	1.66	169.3
11	0.63	185.1
12	1.33	193.6

Regression equation : $Y = -0.14583 + 6.95854 \times 10^{-3} \times X$
Correlation Coefficient = 0.7879, Degree of freedom = 11

Table 35- Accumulation of *cap*^{R} in the presence of the hydrochloride salt of pyridone (10)

Sample no.	No. of mutants/ 10^6 viable cells (Y)	Time (hr.) (X)
1	0.37	66.2
2	0.45	70.2
3	1.90	90.2
4	2.90	141.3
5	1.10	186.0
6	0.83	190.2
7	1.77	209.2
8	3.17	215.2
9	4.09	233.4
10	3.30	240.4
11	4.78	257.2

Regression equation : $Y = -0.49414 + 0.01894 \times X$
Correlation Coefficient = 0.7944, Degree of freedom = 10

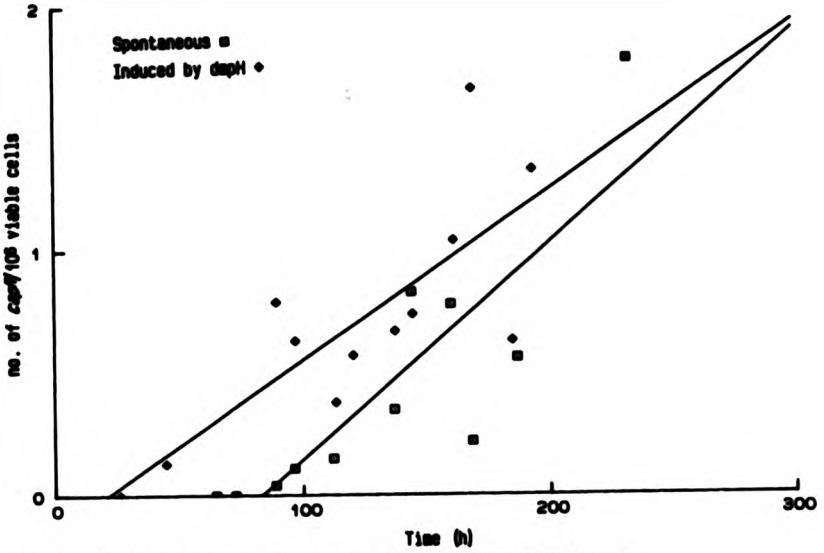


Figure 24- Comparison of spontaneous and dmpH induced mutations to *cap*$^{-}$

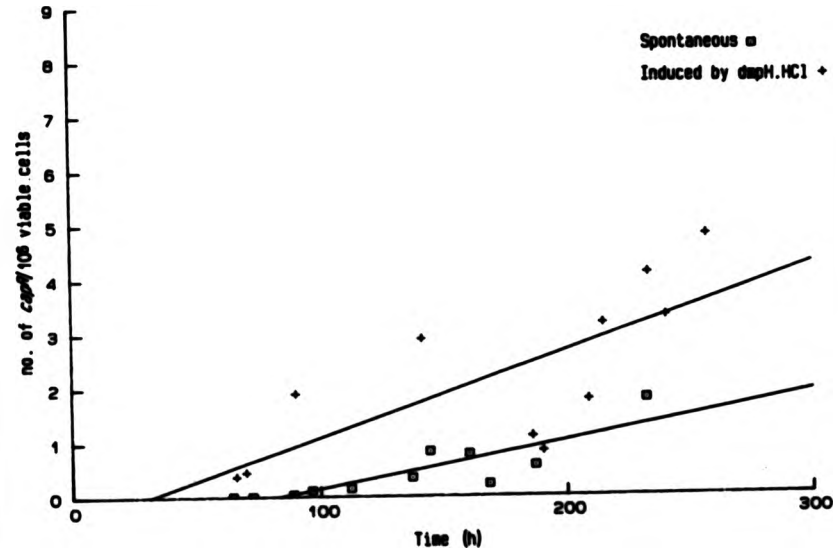


Figure 25- Comparison of spontaneous and dmpH.HCl induced mutations to *cap*$^{-}$

In order to investigate the antimutagenic property of the free base, the rate of mutation induced by a mixture of EMS and this compound, both present at their maximum non-lethal concentrations, was determined. The results showed that this pyridone had striking antimutagenic effects. The frequencies of accumulation of both cycloheximide and chloramphenicol resistant mutants were reduced significantly when compared with those induced by EMS alone. These results are summarized in Table 36, Figure 26; and Table 37, Figure 27, respectively. The rates of mutation to both cycloheximide and chloramphenicol resistances were higher than those of the corresponding spontaneous mutation rate (Figures 26 and 27). However, in both cases these differences were insignificant. Therefore, it can be concluded that the EMS induced mutation rates for both antibiotics were reduced to their spontaneous level in the presence of the free base (10). These findings are encouraging and show the need for further investigations in order to confirm them and to establish the mechanism involved in the antimutagenicity of this pyridone (10).

Generally, the term antimutagen has been given to agents that reduce the apparent frequency of spontaneous or induced mutations.[145] Antimutagens have been categorised into two general groups : desmutagens and bioantimutagens.[145] The desmutagens are defined as

Table 36 - Accumulation of *cyl*^{R} in the presence of the pyridone (10) + EMS

Sample no.	No. of mutants/ 10^{8} viable cells (Y)	Time (hr.) (X)
1	0.43	47.4
2	0.77	54.3
3	0.88	71.0
4	0.97	79.3
5	1.12	95.8
6	0.98	103.6
7	0.85	119.8
8	1.01	127.8
9	2.12	143.6
10	2.36	152.0
11	1.86	166.8

Regression equation : Y = -0,13674 + 0,01279 x X
Correlation Coefficient = 0,8341, Degree of freedom = 10

Table 37 - Accumulation of *cap*^{R} in the presence of the pyridone (10) + EMS

Sample no.	No. of mutants/ 10^{8} viable cells (Y)	Time (hr.) (X)
1	1.51	47.4
2	1.65	54.3
3	1.63	79.3
4	1.51	95.8
5	1.57	103.6
6	1.50	119.8
7	1.65	127.8
8	2.13	143.6
9	2.80	152.0
10	3.22	166.8

Regression equation : Y = 0,68345 + 0,01131 x X
Correlation Coefficient = 0,7475, Degree of freedom = 9

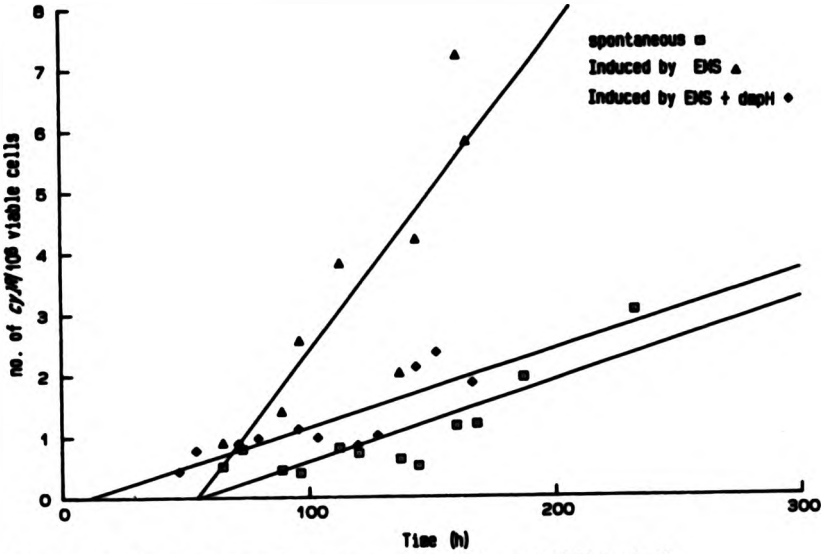


Figure 26- Comparison of spontaneous, EMS, and EMS + dmpH induced mutations to *cyl*$^{+}$

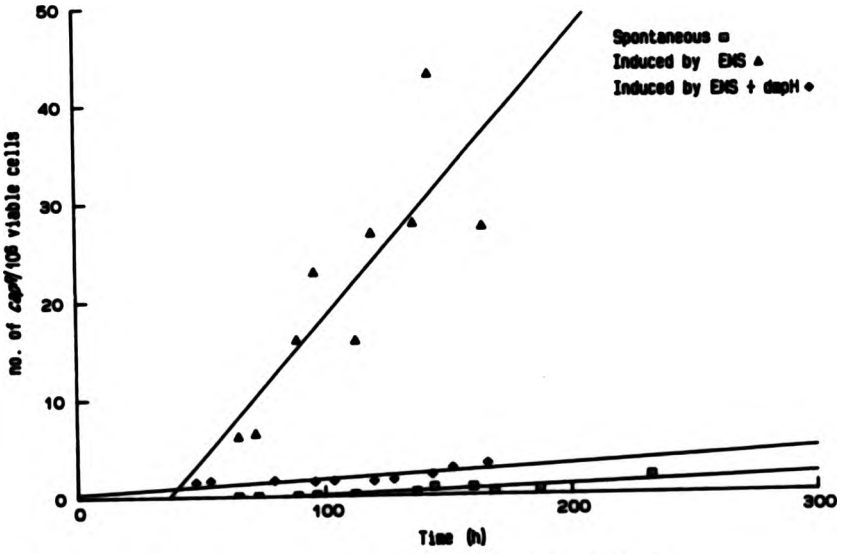
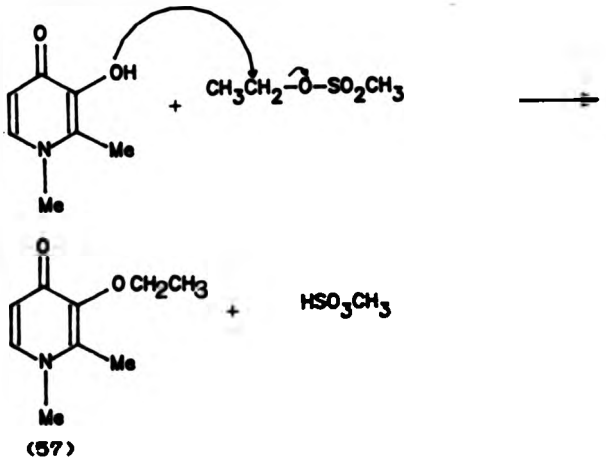


Figure 27- Comparison of spontaneous, EMS, and EMS + dmpH induced mutations to *cap*$^{+}$

compounds that reduce the frequency of induced mutation, by chemical or biochemical modifications of mutagens outside cells, whereas, bioantimutagens are agents which interfere with the mutagenic processes. In other words, the term bioantimutagenic is given to those factors that are biologically active.

The reduction of mutation rates induced by EMS in the presence of this compound (10) could suggest that it behaves as a desmutagen. This in turn implies a direct interaction between the ligand and EMS, which leads to inactivation of EMS. However, prior to the above experiment, the possibility of chemical reaction of this pyridone with EMS was considered. Figure 28 illustrates a possible reaction which could inactivate EMS.



(57)

Figure 28- A possible reaction of 1,2-dimethyl-3-hydroxypyrid-4-one with ethyl methanesulphonate

The reaction of EMS with the free base (10) in water at room temperature was investigated. The reaction was carried out at concentrations both present in continuous cultures and ten times higher, and their progress was monitored by h.p.l.c.; the results indicated that no reaction had taken place. Under the conditions used for h.p.l.c. analysis only the pyridone (10), but no EMS, could be detected. Under these conditions it is also likely that the expected product (57) would be detected. It should be noted that these analyses were run in parallel to the measurement of a standard solution containing the same concentration of pyridone (10) as that in the reaction mixture. Therefore, any diminution in the concentration of this pyridone (10) in the reaction mixture, could have been detectecd by comparing the area under the peaks of the standard and reaction solutions. This concentration remained the same over the reaction period of two weeks. These observations suggest that EMS is not inactivated by a simple chemical reaction with this pyridone. However, the possibility that the EMS is inactivated by the metabolites of pyridone (10), before its interaction with DNA should not be excluded.

The reduction of spontaneous mutation rates by both free base (10) and its HCl salt (16b) could be due to chelating properties of these ligands. It has been shown previously that metals have mutagenic effects on *S. pombe*[196] and

hence so-called "Spontaneous mutation" could be partly due to the mutagenic effects of metals. Therefore, both compounds (10 and 16b), might by simply chelating with these metals, reduce their concentrations and thereby decrease observed spontaneous mutation. This mechanism also suggests that these compounds behave like desmutagens.

However, it is believed that the pyridone (10) (and possibly its hydrochloride salt) more likely employs the same mechanism in reducing both the spontaneous and EMS induced mutations. On this basis, it is improbable that these ligands behave as desmutagens. While more specific experiments have to be carried out, the combination of these observations lead to the conclusion that the pyridone (10) acts as a bioantimutagen. The possible mechanisms for antimutagenic activities of chemicals have been previously discussed[146]. In this previous report bio-antimutagens, on the basis of their mode of action, are categorised as following:

1) agents which increase the fidelity of DNA replication;
2) agents that promote repair of DNA damage; and
3) chemicals which inhibit the error-prone repair system.

Assuming that pyridone (10) has bioantimutagenic effects, it may employ one of the above mechanisms or a completely different route may be involved.

Finally, in order to determine the amount of pyridone (10) metabolised by the *S. pombe* cells a series of batch cultures were prepared containing known concentrations of the ligand. The cultures were incubated for 48 hours and the concentrations of the ligand were determined using h.p.l.c. Initially, the chromatogram was standardised using solutions of the ligand in EMM2, and the area under the peak due to the ligand was measured (Table 38). The least square line for the plot of area against concetration was calculated (Figure 29), and using this graph the concentrations of this compound in the batch culture samples were determined (Table 39).

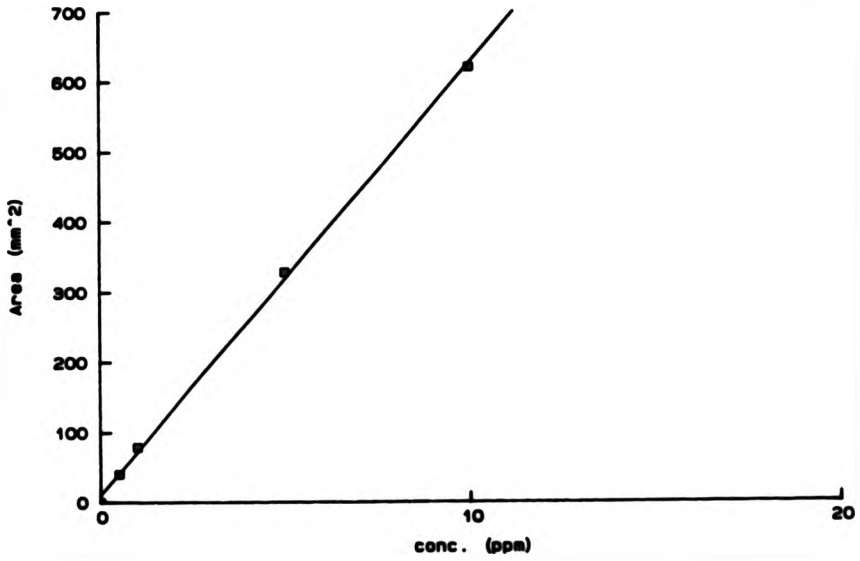


Figure 29- Standardisation of h.p.l.c. chromatogram with solutions of pyridone (10) in EMM2

Table 38 - Standardisation of the chromatogram with solutions of the pyridone (10) in EMM2 only

Sample no.	Concentration (ppm)	Area of the ligand's peak*	Average of Area (mm^2)
1	10.0	606, 627, 627	620.0
2	5.0	328.5, 327.3	327.9
3	1.0	77.6, 80.0	78.8
4	0.5	40, 40	40.0
5	0.0	0.0, 0.0	0.0

* Average of 2 readings

Table 39 - The concentration of the pyridone (10) in batch cultures of *Sch. pombe*

Sample no.	Initial conc. (ppm)	Area of the ligand's peak* (mm^2)	Calc. conc. (ppm)
1	0.00	0.0	0.00
2	1.00	78.3	1.11
3	5.00	328.1	5.17
4	10.00	618.5	9.88

* Average of 2 readings of 3 replicates

The results indicated that there is no significant change in the concentration of the pyridone (10), possibly due to the fact that the method of detection was not sensitive enough to detect very small changes. Alternatively, this pyridone could be dynamically entering and leaving the yeast cells so that the changes in concentrations cannot be detected, under the above experimental conditions.

In summary, these studies indicated that both 1,2-dimethyl-3-hydroxypyrid-4-one (10) and its hydrochloride salt (16b) act as antimutagens in *S. pombe*. Although it is too early to state any firm conclusion, the results suggest that these compounds act as bicantimutagens. These observations not only encourage the application of pyridones of type (9) as iron-chelating agents but also lead to a new avenue of investigation, as potential antimutagens.

CHAPTER FIVE

EXPERIMENTAL

5.1 Materials

All the chemicals were obtained from commercial manufacturers and they were used without further purification. In the case of chemical reactions the chemicals were of G.P.R. or 'Analar' grades. For microbiological experiments, on the other hand, all the chemicals were of 'Analar' grade.

5.2 Analytical techniques

Carbon, hydrogen, and nitrogen analyses were carried out by the microanalytical services at the Polytechnic of North London.

Metal content analyses were obtained by atomic absorption spectrophotometry using a Pye Unicam SP9 spectrophotometer. The sample solutions were prepared by the wet oxidation method. This was achieved by heating an accurate quantity (ca 0.1 g) of the material in a mixture

of concentrated sulphuric and nitric acid, plus a few drops of 100 volume hydrogen peroxide. Then the samples were diluted to appropriate volume.

Chlorine and bromine analyses were carried out using a gravimetric method.[147] In the case of metal complexes, the chlorine contents were determined titrimetrically after combustion of the sample using the oxygen flask method.[147]

5.3 Physical techniques

5.3.1 Infra-red spectroscopy

The infra-red spectra of all solid materials were recorded as KBr discs over the region 400-4000 cm^{-1} using a BIORAD digilab (FTS-40) spectrophotometer.

5.3.2 Electronic spectroscopy

Ultraviolet and visible spectra of the solution samples were recorded on a Schimadzu uv-2100 spectrophotometer, and the reflectance spectra were obtained on a Varian (DMS 90) uv-visible spectrophotometer.

5.3.3 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectra were obtained using a Perkin-Elmer RB12 60 MHz spectrometer, a Bruker WP80 MHz

Fourier transform, or a Bruker AM 250 MHz. spectrometer. Tetramethylsilane was used as internal standard (δ = 0 ppm). In the case of solutions in deuterated water, the sodium salt of 3-(trimethylsilyl)-1-propanesulphonic acid was used as the standard reference (δ = 0 ppm).

5.3.4 Mass spectrometry

Mass spectra of organic compounds were recorded using an AEI/MSS MS9 double focusing spectrometer, at the Polytechnic of North London. The spectra of metal complexes were obtained on a VG Analytical 7070 EQ mass spectrometer at the Royal Armament Research and Development Establishment (London). The G.C.-mass spectra were obtained at the SERC mass spectrometry centre (Swansea).

5.3.5 Mossbauer spectroscopy

The mossbauer spectra were recorded at the Thames Polytechnic (London) using a Cryophysics Mossbauer spectrometer with a CO_2/Xe detector. ^{57}Co/(Rhodium) was used as the source. The spectra were recorded at 20 °C and -196 °C.

5.3.6 Chromatographic techniques

5.3.6.1 High performance liquid chromatography

High performance liquid chromatography (h.p.l.c.) was carried out using a Varian 2000 instrument.

The conditions used for separation of maltol (11) and 1,2-dimethyl-3-hydroxypyrid-4-one (10) were as follows:- Column RP-Select B, 25 cm x 4 mm; eluent: methanol: aqueous KH_2PO_4 buffer (50 mM, pH 3.6) of ratio 35:65; flow rate: 0.5 cm^3 min^{-1}; detector: u.v. (280 nm); chart speed: 5 mm min^{-1}; volume injected: 10 µl; temperature: ambient; pressure: 100 atm.

5.3.6.2 Gas chromatography

Gas chromatography (G.C.) was carried out on a Varian 3700 gas chromatograph.

The conditions used for experiments (5.4.16), (5.4.17), and (5.4.18) were as follows:
Column 5i. OV-1 (glass), O.D. 6 mm, I.D. 4 mm ; Flame ionisation detector; column temp. 175 °C; injector temp. 250 °C; detector temp. 300 °C; pressure 12 psi; chart speed 5 mm min^{-1}; carrier gas nitrogen; flow rate 10 mm min^{-1}.

5.3.6.3 Column and thin layer chromatography

In all experiments using column chromatography, Merck silica gel 60 of 70-230 mesh was used as the stationary phase. Merck Kieselgel 60 F_{254} pre-coated (0.2 mm thickness) aluminium sheets were employed for thin layer chromagraphy (t.l.c.).

5.3.7 Thermal gravimetric analysis (t.g.a)

A Stanton thermobalance (HT-SM) was used for thermal gravimetric analysis at a heating rate of 100 °C per hour.

5.3.8 Magnetic moment measurements

Room temperature magnetic moments were measured using a Johnson Matthey magnetic susceptibility balance. The instrument was calibrated with a solution of magnesium(II) chloride. Diamagnetic corrections were made using Pascal's constants.[148]

5.3.9 Conductance measurements

Conductance measurements were made at 21 °C in methanol as the solvent using a PTI-18 digital conductivity meter.

5.4 Chemical reactions

The expression 'concentrated under vacuum (90 °C, 15 mmHg)' refers to use of a rotary evaporater.

5.4.1 Preparation of 3-benzyloxy-2-methyl-4-pyrone (12)[48].-

3-Benzyloxy-2-methyl-4-pyrone was prepared from the reaction of 3-hydroxy-2-methyl-4-pyrone (44.4 g, 0.35 mol) with benzyl chloride (52.1 g, 0.41 mol) using a previously described method.[48]

The product (53.2 g, 70%) was identified using physical techniques (b.p. 153-155 °C at 2.8 mmHg). N.m.r.: ^{1}H(CDCl$_3$, 20 °C), δ 1.9 (3 H, s, 2-Me), 5.05 (2 H, s, benzylic CH$_2$), 6.2 (1 H, d, J 7.0 Hz, 5-H), 7.35 (5 H, s, aromatic H), and 7.52 ppm (1 H, d, J 7.1 Hz, 6-H).

5.4.2 Preparation of the monohydrochloride salt of 3-benzyloxy-1,2-dimethylpyrid-4-one (15).-

A solution of 3-benzyloxy-2-methyl-4-pyrone (5.0 g, 23 mmol) in IMS (100 cm^3) was added to an aqueous solution (100 cm^3) of sodium hydroxide (2.4 g, 60 mmol), followed by addition of methylammonium hydrochloride (1.7 g, 25 mmol). The reaction mixture was stirred at room

temperature for 7 days and then acidified to pH 2.5-3.0 with concentrated hydrochloric acid. The solvent was removed under vacuum (90 °C, 15 mmHg) to give a yellow solid, which was washed with dichloromethane to give a white solid. The dichloromethane extract afforded the unreacted starting material, 3-benzyloxy-2-methyl-4-pyrone, which was identified by n.m.r spectroscopy. The solid product insoluble in CH_2Cl_2, was recrystallised from water to give white crystals of the hydrochloride salt of 3-benzyloxy-1,2-dimethylpyrid-4-one (15) (3.75 g, 75%) (m.p. 178-180 °C) (Found, C, 63.5; H, 6.55; Cl, 13.45; N, 5.4 Calc. for $C_{14}H_{16}ClNO_2$: C, 63.3; H, 6.0; Cl, 13.4; N, 5.3%) (see Chapter 2, sections 2.3.1 and 2.3.2 for i.r. and n.m.r spectral details).

5.4.3 Preparation of 3-benzyloxy-1,2-dimethylpyrid-4-one trihydrate (14).-

The procedure described in 5.4.2. was repeated, except that in this case the reaction mixture was not acidified. After 7 days stirring, the reaction mixture was concentrated to 1/5 of its original volume under vacuum (90 °C, 15 mmHg) to give a white solid. The formation of solid was assisted by addition of a few crystals of the product. The resultant solid was collected and washed with water. Recrystallisation from water afforded the hydrated form of the free base of 3-benzyloxy-1,2-dimethylpyrid-4-

one trihydrate (15)(4.0 g, 80%) (m.p. 39-40 °C)(Found: C, 59.9; H, 7.25; N, 5.1. Calc. for $C_{14}H_{21}NO_8$: C, 59.4; H, 7.4; N, 4.9%) (see Chapter 2, sections 2.3.1 and 2.3.2 for i.r. and n.m.r. spectral details).

5.4.4 Preparation of the hydrobromide salt of 1,2-dimethyl-3-hydroxypyrid-4-one (16a)[16,19].-

Hydrobromic acid (50 cm^3, sp.gr. 1.46-1.49 g cm^{-3}) was added to a solution of 3-benzyloxy-1,2-dimethylypyrid-4-one monohydrochloride (1.5 g, 6 mmol) and the mixture was heated on a steam bath for one hour. The reaction mixture was concentrated to 1/10 of its original volume under reduced pressure (90 °C, 15 mmHg). After bubbling nitrogen into the resultant solution for a few minutes a white solid appeared which was washed with ethyl acetate (30 cm^3) and dried in the desiccator over anhydrous calcium chloride to yield 1,2-dimethyl-3-hydroxypyrid-4-one monohydrobromide (16a) (1.0 g, 82%) (m.p. 189-191 °C)(Found: C, 38.55 ; H, 4.5; Br, 36.2; N, 6.5. Calc. for $C_7H_{10}BrNO_2$: C, 38.2; H, 4.5; Br, 36.4; N, 6.4%) (see Chapter 2, sections 2.3.1 and 2.3.2 for spectral details).

5.4.5 Preparation of the hydrochloride salt of 1,2-dimethyl-3-hydroxypyrid-4-one (16b).-

Water (10 cm^3) and concentrated hydrochloric acid (15 cm^3) were added to the hydrochloride salt of 3-benzyloxy-1,2-dimethylpyrid-4-one (10 g, 37 mmol) and the mixture was heated on a steam bath for 1½ h. After cooling, dichloromethane (15 cm^3) was added to remove the by-product, benzyl chloride. The aqueous layer was separated and concentrated under vacuum to give a white solid. The resultant solid was recrystallised from water yielding colourless crystals of the hydrochloride salt of 1,2-dimethyl-3-hydroxypyrid-4-one (16b) (6 g, 60%) (m.p. 174-176 °C) (Found: C, 47.75; H, 5.9; Cl, 20.4; N, 7.8. Calc. for $C_7H_{10}ClNO_2$: C, 47.9; H, 5.7; Cl, 20.2; N, 7.9%) (see Chapter 2, sections 2.3.1 to 2.3.3 for spectral details).

5.4.6 Preparation of 1,2-dimethyl-3- hydroxypyrid-4-one (10) from its hydrochloride salt .-

The hydrochloride salt of 1,2-dimethyl-3-hydroxypyrid-4-one (5.0 g, 29 mmol) was dissolved in the minimum amount of cold water and neutralised with concentrated ammonium hydroxide to pH 6.5-7.0. On reducing the volume a white solid crystallised out, and was collected on filtration.

The resultant solid was recrystallised from water to give white crystals of 1,2-dimethyl-3-hydroxypyrid-4-one (10) (5 g, 90%) (m.p. 265-266 °C; lit.[80] 266-268 °C)(Found: C, 60.1; H, 6.7; N, 10.2. Calc. for: $C_7H_9NO_2$: C, 60.4; H; 6.5; N, 10.1%) (see Chapter 2, sections 2.3.1 to 2.3.4 for n.m.r. spectral details).

5.4.7 Direct synthesis of 1,2-dimethyl-3-hydroxypyrid-4-one (10) from Maltol.-

3-Hydroxy-2-methyl-4-pyrone (Maltol) (5.0 g, 38 mmol) was dissolved in a mixture of water (100 cm^3) and glacial acetic acid (1.2 g, 20 mmol), followed by addition of a 25% aqueous solution of methylamine (40 cm^3, 312 mmol). The reaction mixture was heated under reflux. The progress of the reaction was monitored by h.p.l.c.(see section 5.3.6.1 for conditions). After 10 h reflux, h.p.l.c. showed that all of the maltol (retention time 11.5 min.) had reacted. The reaction mixture was then evaporated under reduced pressure to give a brown solid which was recrystallised from water, with decolourisation by charcoal, to yield a white crystalline solid. The resultant solid was further purified by recrystallisation from water to give 1,2-dimethyl-3-hydroxypyrid-4-one (10) (2.5 g, 37%). The product was identified by h.p.l.c. (retention time 7.6 min.), n.m.r., i.r. spectroscopy and

m.p (in comparison with compound (10) obtained in section 5.4.6).

5.4.8 Preparation of the hydrochloride salt of 3-benzyloxy-1-ethyl-2-methylpyrid-4-one .-

A solution of 3-benzyloxy-2-methyl-4-pyrone (10.0 g, 23 mmol) in IMS (200 cm^3) was added at room temperature to an aqueous solution (100 cm^3) of sodium hydroxide (24.0 g, 60 mmol), followed by addition of ethylammonium hydrochloride (20.4 g, 25 mmol). The reaction mixture was stirred at room temperature for 7 days and then acidified to pH 2.5-3.0 with concentrated hydrochloric acid. The solvent was removed under vacuum. The resultant solid was washed with diethyl ether and recrystallised from a mixture of ethanol and dichloromethane (9:1) to give white crystals of the hydrochloride salt of 3-benzyloxy-1-ethyl-2-methylpyrid-4-one (6.1 g, 49%) (m.p. 171-172 °C)(Found: C, 64.8; H, 6.7; Cl, 12.3 ; N, 5.1. Calc. for $C_{15}H_{16}ClNO_2$: C, 64.4; H, 6.4; Cl, 12.7; N, 5.0%). N.m.r.: ^1H(D_2O, 20 °C) δ 1.42(3 H, t, *J* 7.3 Hz, Me of Et group), 2.40(3 H, s, 2-Me); 4.29(2 H, q, *J* 7.3 Hz, CH_2 of Et group), 5.06(2 H, s, CH_2 of benzyl group), 7.26(1 H, d, *J* 7.0 Hz, 5-H), 7.42(5 H, Ph group), 8.14 ppm(1 H, d, *J* 7.0 Hz, 6-H).

5.4.9 Preparation of the hydrochloride salt of 1-ethyl-2-methyl-3-hydroxypyrid-4-one (9, R = Et).-

The procedure described in section 5.4.6. was repeated for the hydrochloride salt of 3-benzyloxy-1-ethyl-2-methylpyrid-4-one. In this case the reaction mixture was heated for 20 minutes. A white crystalline solid was separated which was recrystallised from 50% aqueous ethanol to give the hydrochloride salt of 1-ethyl-2-methyl-3-hydroxypyrid-4-one (9, R = Et)(20 % yield) (m.p 192-3 °C); (Found: C, 50.9; H, 6.3; Cl, 18.5; N, 7.5. Calc. for $C_8H_{12}ClNO_2$: C, 50.6; H, 6.3; Cl, 18.7; N, 7.4%). N.m.r.: ^1H(D_2O, 20 °C) δ 1.55(3 H, t, J 7.3 Hz, Me of Et group), 2.64(3 H,s, 2-Me); 4.44(2 H, q, J 7.3 Hz, CH_2 of Et group), 7.14(1 H, d, J 7.0 Hz, 5-H), 8.14 ppm(1 H, d, J 7.0 Hz, 6-H); n.m.r.: ^{13}C(D_2O, 20 °C), δ 14.79(Me of Et group), 17.85(2-Me), 54.60(CH_2 of Et group), 114.20(C-5), 140.75(C-2), 143.09(C-6), 145.83(C-3), 163.0 ppm(C-4).

5.4.10 Preparation of 1-bromobutane-2,3-dione (38)[88].-

1-Bromobutane-2,3-dione was prepared from the reaction of bromine (19.0 g, 119 mmol) with diacetyl (15.0 g, 174 mmol) using a previously described procedure.[89] The product (9.9 g, 50%) was purified by fractional vacuum distillation (b.p.$_{12}$ 20-22 °C). N.m.r.^1H ($CDCl_3$, 25 °C), δ 2.35(3 H, s, Me), 4.21 ppm(2 H, s, CH_2).

5.4.11 Preparation of N-methylbenzamide.[ref]-

N-Methylbenzamide was prepared from the reaction of methylamine (25-30% aq., 50 cm^3) with benzoyl chloride (8.4 g, 60 mmol).[ref] The white solid of N-methylbenzamide (4.5 g, 55%) (m.p. 75-80 °C, lit[ref] 76-78 °C) was recrystallised from a mixture of IMS and water (1:2 volume ratio). N.m.r. $^1H(D_2O$, 20 °C), δ 2.9(3 H, s, Me group), 7.5-7.8 ppm(5 H, m, Ph group).

5.4.12 Reaction of 1-bromobutane-2,3-dione (38) with N-methylbenzamide in the presence of sodium ethoxide.-

Freshly cut sodium (0.5 g, 20 mmol) was added to dry absolute ethanol (150 cm^3) and stirred until all the sodium was converted to sodium ethoxide. N-Methylbenzamide (2.4 g, 18 mmol) was then added and the mixture was stirred at room temperature for 10 mins. After addition of 1-bromobutane-2,3-dione (3.0 g, 18 mmol) a brown solution resulted instantly. The reaction mixture was stirred for 4 h at 20 °C. and its progress was examined by t.l.c. (solvent system : methanol:toluene:ethylacetate at 3:4:3 ratio). The t.l.c. analysis showed the presence of three components of Rf values 0.89, 0.70, and 0.15 (streaking). The first two components were shown to be 1-bromobutane-2,3-dione and N-methylbenzamide, respectively. The solvent

was removed under reduced pressure to give a brown residue. On leaving this residue overnight at room temperature crystals of N-methylbenzamide (1.6 g, 68% recovery) separated; the crystals were washed with diethyl ether and characterised on the basis of n.m.r. spectroscopy.

5.4.13 Reaction of 1-bromobutane-2,3-dione (38) with N-methylbenzamide in the presence of sodium metal.-

A mixture of sodium (0.2 g, 10 mmol) in dry toluene was stirred vigorously under reflux until all the sodium had melted; on cooling very small particles (1-2 mm) of sodium were produced. The experiment was carried out under a dry inert atmosphere. A suspension of N-methylbenzamide (1.4 g, 10 mmol) in dry toluene (50 cm^3) was added and the mixture was heated under reflux until all the sodium disappeared. After cooling, a solution of 1-bromobutane-2,3-dione (1.6 g, 10 mmol) in dry toluene (20 cm^3) was added slowly with stirring. After 48 h stirring at room temperature, a fine brown precipitate appeared which was recrystallised from methanol to give impure sodium bromide (1.1 g, 116%) (identified by i.r. spectrum and bromine analyses). The filtrate was dried to give a brown residue which was shown by t.l.c. to contain the starting materials only.

5.4.14 Reaction of 1-bromobutane-2,3-dione (38) with N-methylbenzylamine.-

A solution of 1-bromo-2,3-butanedione (1.6 g, 10 mmol) and N-methylbenzylamine (2.4 g, 24 mmol) in diethyl ether (50 cm^3) was stirred at room temperature for 2 h. The reaction mixture was filtered to yield a white solid of the hydrobromide salt of N-methylbenzylamine (1.6 g, 40%), which was identified by n.m.r. spectroscopy and bromine test. N.m.r. ^1H(CD_3OD, 25 °C) δ 2.58(3 H, s, Me), 4.05 ppm(2 H, s, CH_2), 7.36(5 H, s, Ph). The filtrate was dried to give a viscous orange liquid (2.4 g)(see Chapter 2, page 62 for n.m.r. spectral details).

5.4.15 Preparation of 1-benzyl-1,2,5,6-tetrahydropyridine (41)[ee].-

The reaction of benzyl chloride (80 g, 0.6 mol) with pyridine (50 g, 0.6 mol) under the reported[ee] conditions gave the red gelatinous solid of 1-benzylpyridinium hydrochloride. The reduction of this salt with sodium borohydride (50 g, 1.3 mol) using the previously described procedure[ee] yielded 1-benzyl-1,2,5,6-tetrahydropyridine (41). The product (39 g, 36%) was purified by distillation (b.p.$_{17.8}$ 129-131 °C; lit bp$_{17}$ 127-128 °C).

5.4.16 Preparation of 1-ethoxycarbonyl-1,2,5,6-tetrahydropyridine (42)[88].-

1-Ethoxycarbonyl-1,2,5,6-tetrahydropyridine (42) was prepared from the reaction of ethyl chloroformate (32.5 g, 0.300 mol) with 1-benzyl-1,2,5,6-tetrahydropyridine (41) (33.5 g, 0.2 mol) using reported procedures.[88] The pure product (24.5 g, 82%) was obtained by fractional vacuum distillation (b.p.$_{17.3}$ 104-106 °C; lit. b.p.$_{16}$ 80-94 °C).

5.4.17 Preparation of 1-ethoxycarbonylpiperidine-3,4-diol (43)[88].-

1-Ethoxycarbonylpiperidine-3,4-diol (43) was prepared by hydroxylation of 1-ethoxycarbonyl-1,2,5,6-tetrahydropyridine (42) (5.0 g, 26.5 mmol) with OsO_4 (12 mg) (care should be taken in handling OsO_4) using reported procedures.[88] The pure product (43) (0.54 g, 10.8%) was obtained by distillation (b.p.$_{0.7}$ 150-151 °C; lit. b.p.$_{0.7}$ 115-125°C (bath temperature). N.m.r. ^1H($CDCl_3$, 20 °C), δ 1.25(3 H, t, J 7.1 Hz, Me of ethoxy group), 1.75-1.8(2 H, m, 5-CH_2), 3.29-3.56(2 H, broad, 2 OH), 3.56-3.73(4 H, m, 2-CH_2 and 6-CH_2), 3.73-3.98(2 H, m, 3-H and 4-H), 4.12 ppm(2 H, q, J 7.1 Hz, CH_2 of ethoxy group), (see Chapter 2, page 68 for ^{13}C n.m.r. spectrum).

The same reaction was repeated but the purification was carried out by column chromatography. The crude product was placed on a 25 cm silica chromatography column and 27 fractions (*ca* 25 cm^3) were eluted as shown in Table 40. The elutants were analysed by G.C., using the product obtained from the previous reaction as the standard. The product (43) (2.3 g, 46%) was eluted with the toluene-ethyl acetate (1:1) fractions.

Table 40 - Column chromatographic purification of 1-ethoxycarbonylpiperidine-3,4-diol (43)

Fraction	eluting solvent
1-3	pet ether:toluene (8:2)
4-7	pet ether:toluene (1:1)
8-12	pet ether:toluene (1:9)
13-17	toluene:ethylacetate (8:2)
18-23	toluene:ethylacetate (1:1)
24-27	ethylacetate

5.4.18 Reaction of 1-ethoxycarbonylpiperidine-3,4-diol with Jones' reagent.-

Jones' reagent[149] (10 cm^3) was added slowly, from a micro burette, to a solution of 1-ethoxycarbonylpiperidine-3,4-

diol (43) (2.0 g, 5.3 mmol) in acetone (75 cm^3), with stirring at room temperature. Then the solvent was removed under reduced pressure to give a green solid which was dissolved in water (35 cm^3) and extracted with diethyl ether. The ether layer (layer A) was dried over $MgSO_4$ and analysed by G.C.

The dried ether layer was extracted with sodium hydroxide (10% w/v, 2x50 cm^3) which gave a yellow aqueous layer and a colourless ethereal layer (layer B). The aqueous layer was neutralised with aqueous HCl (1 M) and extracted back into diethyl ether (layer C). All ethereal layers (A, B, and C) were analysed by G.C. and showed to contain the same mixture of products.

The same reaction was repeated at half the scale, and after the first extraction with ether, the product mixture was subjected to silica column chromatography (20 cm x 2 cm). The eluted fractions (30-40 cm^3) were concentrated and analysed by G.C.(Table 41). The elution with toluene-ethyl acetate (1:1) contained the major product plus a small amount of impurity. These fractions (30-33) (0.1 g) were analysed further by G.C.-mass spectrometry which are discussed in Chapter 2, page 71.

Table 41 - Column chromatography of the product mixture from experiment 5.4.19

Fraction	eluting solvent (ratio)	no. of components by g.l.c.
1-6	pet ether:toluene (8:2)	none
7-12	pet ether:toluene (1:1)	none
13-15	pet ether:toluene (1:9)	none
16-19	toluene	two
20-27	toluene:ethylacetate (8:2)	two
28-29	toluene:ethylacetate (1:1)	three
30-33	toluene:ethylacetate (1:1)	three
34-40	toluene:ethylacetate (8:2)	two
41-42	ethylacetate	two
43-46	ethylacetate:methanol (8:2)	four

5.4.19 Preparation of M(II) complexes (M = Co, Ni, Cu, Zn, Ca) of 1,2-dimethyl-3-hydroxypyrid-4-one (10).-

A suspension of 1,2-dimethyl-3-hydroxypyrid-4-one (5.56 g, 40 mmol) in 1:2 methanol:water (200 cm^3) was added to a stirred solution of the metal(II) acetate (metal = Ni, Co, Cu, or Zn) (20 mmol) in water (100 cm^3). While stirring, the pH of the reaction mixture was adjusted to approximately 10 using dilute (0.1 M) ammonium hydroxide solution. After stirring for one hour the volume of the

reaction mixture was reduced to 1/2 to give a precipitate. The mixture was filtered to give hydrated bis(1,2-dimethyl-3-hydroxypyrid-4-onato)metal(II). (Metal = Co, Ni, Zn) or anhydrous bis(1,2-dimethyl-3-hydroxypyrid-4-onato)copper(II). Tables 42 and 43 present a summary of the above reactions together with the obtained results.

In the case of the reaction with cobalt(II) acetate tetrahydrate, the filtrate changed colour from pinkish red to brown and then green overnight. On leaving this solution for several days at room temperature green crystals of tris(1,2-dimethyl-3-hydroxypyrid-4-onato)Co(III) dodecahydrate ($Co(dmp)_3.12H_2O$) were isolated. The product was formulated on the basis of crystal data and was shown to be isomorphous with tris(1,2-dimethyl-3-hydroxypyrid-4-onato)Fe(III) dodecahydrate[27].

The crystal data for $Co(dmp)_3.12H_2O$ were collected on a Philips PW1100 four-circle diffractometer using graphite monochromated Mo-K_α radiation. These data are as following:
$a = 16.653$ Å, $b = 16.623$ Å, $c = 6.875$ Å, $\alpha = \beta = 90°$, and $\gamma = 120°$.

On drying the crushed crystals of $Co(dmp)_3.12H_2O$ under reduced pressure (80 °C, 0.1 mmHg) the anhydrous $Co(dmp)_3$

Table 42- The appearance and yield of the metal(II) (Metal = Co, Ni, Cu Zn, Ca) and Co(III) complexes derived from pyridone (10 = dmpH)

Product no.	Section	Metal containing reagent used	product formulation	colour	yield (%)
1	5.4.19	$Ni(CH_3COO)_2.4H_2O$	$Ni(dmp)_2.4½H_2O$	pale green	93.7
2	"	$Co(CH_3COO)_2.4H_2O$	$Co(dmp)_2.3H_2O$	pale pink	51.3
3	"	$Cu(CH_3COO)_2.H_2O$	$Cu(dmp)_2$	green	75.0
4	"	$Zn(CH_3COO)_2.7H_2O$	$Zn(dmp)_2.3½H_2O$	white	87.0
5	"	$Ca(NO_3)_2.4H_2O$	$Ca(dmp)_2$	white	83.4
6	"	$Co(CH_3COO)_2.4H_2O$	$Co(dmp)_3$	green	78.0
7	5.4.20	$Ni(dmp)_2.4H_2O$	$Ni(dmp)_2$	yellow	100.0
8	"	$Co(dmp)_2.3H_2O$	$Co(dmp)_2$	purple	100.0

Table 43- The elemental analyses of the metal(II) (Metal = Co, Ni, Cu, Zn, Ca) and Co(III) complexes derived from pyridone (10 = dmpH)

Product no.	Found				Expected			
	%C	%H	%N	%M	%C	%H	%N	%M
1	40.3	6.2	6.6	14.3	40.4	6.0	6.7	14.1
2	43.4	5.6	7.1	15.6	43.2	5.7	7.2	15.2
3	49.5	4.7	8.2	18.4	49.5	4.7	8.2	18.7
4	41.0	5.6	6.5	16.6	41.6	5.7	6.9	16.1
5	52.9	5.1	8.8	12.5	53.1	5.1	8.9	12.7
6	53.4	5.1	8.9	12.3	53.8	5.2	8.8	12.2
7	50.1	4.9	8.3	17.3	50.2	4.8	8.4	17.5
8	50.2	4.8	8.5	17.5	50.2	4.8	8.4	17.6

was isolated. The elemental analysis (Table 43) was only carried on the anhydrous compound.

The anhydrous bis(1,2-dimethyl-3-hydroxypyrid-4-onato)calcium(II) was prepared similarly using calcium nitrate as the metal salt (see Table 43 for the analysis).

5.4.20 Preparation of anhydrous M(II) (M= Co or Ni) complexes of 1,2-dimethyl-3-hydroxypyrid-4-one (10).-

A suspension of the hydrated bis(1,2-dimethyl-3-hydroxypyrid-4-onato)metal(II) (M = Co or Ni) in dry toluene (200 cm^3) was heated under reflux for three hours, in an apparatus equipped with a water separator. Filtration afforded anhydrous bis(1,2-dimethyl-3-hydroxypyrid-4-onato)metal(II) (see Table 43 for analyses).

5.4.21 Alternative method of preparation of tris(1,2-dimethyl-3-hydroxypyrid-4- onato)Co(III).-

Dilute ammonium hydroxide was added to a mixture of 1,2-dimethyl-3-hydroxypyrid-4-one (4.17 g, 30 mmol), cobalt(II) acetate tetrahydrate (2.49 g, 10 mmol) and charcoal (0.5 g) in 1:2 methanol:water till the pH was approximately 10. After stirring for 2 hours, the

filtration gave a green filtrate. The resultant solution was concentrated to give a green solid which was dried under reduced pressure (80 °C, 0.1 mmHg) to give tris(1,2-dimethyl-3-hydroxypyrid-4-onato)Co(III) (6.8 g, 43%).

5.4.22 Reaction of 1,2-dimethyl-3-hydroxypyrid-4-one (10) with $FeCl_3$ at high pH.-

A suspension of 1,2-dimethyl-3-hydroxypyrid-4-one (1.4 g, 10 mmol) in methanol (20 cm^3) was added to a methanolic solution of ferric chloride (0.53 g, 3 mmol). While stirring at room temperature, an aqueous solution (25% w/v) of sodium hydroxide (2 cm^3) was slowly added to the reaction mixture. The mixture was then heated under reflux for 1 hour. The solvent was removed under reduced pressure to yield a red solid. The product was extracted into dichloromethane (50 cm^3). On removal of the solvent <u>tris(1,2-dimethyl-3-hydroxypyrid-4-onato)iron(III) tetrahydrate</u> was isolated as a dark red solid (1.0 g, 97%) (Found: C, 46.6; H, 6.5; Fe, 10.7; N, 7.6; Calc. for $C_{21}H_{32}FeN_3O_{10}$: C, 46.5; H, 6.5; Fe, 10.3; N, 7.7%). Thermal gravimetric analysis indicated a loss of 12.5% by weight at 80-100 °C.

5.4.23 Reaction of 1,2-dimethyl-3-hydroxypyrid-4-one (10) with $FeCl_3$ at low pH (3:1 and 2:1 molar ratio).-

A suspension of 1,2-dimethyl-3-hydroxypyrid-4-one (1.4 g, 10 mmol) in water (20 cm^3) was added to a methanolic solution of ferric chloride (0.53 g, 3 mmol) and the mixture was refluxed for ½ h. The reaction mixture was concentrated to a third of the volume. The solution was then added dropwise to diethyl ether (300 cm^3) with stirring at room temperature. Filtration afforded a dark purple solid of $Fe_2Cl_5(dmp)(dmpH)_4$ (2.9 g, 66%). (Found: C 42.6; H 4.1; Cl 18.5; Fe, 11.6; N, 7.0; Calc. for: $C_{28}H_{44}Cl_5Fe_2N_5O_{10}$: C, 42.8; H, 4.5; Cl, 18.1; Fe, 11.4; N, 7.1%).

The reaction was repeated at 2:1 molar ratio of ligand to ferric chloride and the same product was isolated.

The product $Fe_2Cl_5(dmp)(dmpH)_4$ (0.3 g) was dissolved in methanol and was applied to an ionic exchange column (packed with Dowex 50W-X8(H); particle size 0.39-1.00 mm; 16-40 mesh). Elution with methanol (300 cm^3) followed by water (300 cm^3) yielded colourless solutions which were qualitatively tested for chlorine, the free ligand (dmpH) and iron. The product gave a positive test for chlorine but negative for both iron and the ligand.

5.4.24 Reaction of 1,2-dimethyl-3-hydroxypyrid-4-one (10) with $FeCl_3$ at low pH (1:1 molar ratio)

The procedure described in section 5.4.23 was repeated except that in this case the molar ratio of the ligand to ferric chloride was 1:1. The product formulated as $Fe_2Cl_5(dmp)(dmpH)_2$ was isolated as a purple/blue solid (72%). (Found: C, 35.5; H, 3.5; Cl 24.6; Fe, 15.7; N, 6.1; Calc. for $C_{21}H_{25}Cl_5Fe_2N_2O_6$: C, 35.7; H, 3.5; Cl, 25.1; Fe, 15.9; N, 5.9%).

5.4.25 Reaction of 1,2-dimethyl-3-hydroxypyrid-4-one (10) with ethyl methanesulphonate.-

Ethyl methanesulphonate (0.1 g, 0.8 mmol) was added to a solution of 1,2-dimethyl-3-hydroxypyrid-4-one (10) (0.1 g, 0.7 mmol) in water (1000 cm^3). The mixture was then stirred at room temperature for one week. The progress of the reaction was monitored by h.p.l.c (using conditions described in section 5.3.6.1) which showed that no reaction had taken place.

5.5 Genectoxicological Experiments

5.5.1 Strains

The *Schizosacchromyces pombe* wild type strain 972h$^-$ was used for preliminary and continuous culture experiments. The histidine auxotroph *his* 2h$^-$ was used for genetical analyses.

5.5.2 Media and Chemicals

Yeast extract liquid (YEL) was used as complete medium. This was prepared by dissolving a mixture of glucose (5.0 g) and yeast extract (3 g) powder (Oxoid Ltd.) in distilled water (1000 cm^3). This medium was solidified when required by the addition of 2% agar to give yeast extract agar (YEA). For isolation of antibiotic resistant mutants YEA was supplemented with the appropriate antibiotic. For isolation of chloramphenicol resistant mutants the glucose was replaced by glycerol as the carbon source.[136]

Malt extract agar (MEA, supplied by Oxoid Ltd.) was used as sporulation medium in genetical analyses.[136]

Edinburgh minimal medium number 2 (EMM2)[149] was used as minimal medium and when required was solidified by the addition of 2% agar.

All dilutions were made in Giese's Salts Vitamins Buffer (GSVB).[160] In the case of both EMM2 and GSVB the salts, vitamins and trace elements (only for EMM2) were made separately as concentrated solutions (eg. 100x) and stored at 5 °C until required.

5.5.3 Sterilization

Sterilization of the media and the apparatus were carried out at 121 °C, 115 lbs for 15 min. For the volumes of media which were greater than 1000 cm^3 the sterilization time was increased by approximately 5 minutes per 1000 cm^3. The antibiotics and EMS were filter sterilized (using millipore filters; pore size 0.45 μm) and added to sterilised media.

In the case of continuous culture experiments, the individual components of the apparatus (i.e. culture vessel with all the essential tubings attached to it, medium reservoir and effluent reservoir) were sterilized separately.

5.5.4 Preliminary experiments

Several batch cultures of *S. pombe* was prepared by inoculating YEL (10 cm^3) with a single colony and incubating for 48 h at 32 °C. Then a series of solutions

of (10 cm^3) containing 0, 1, 10, 100, 250, 500, 750, 1000, 2000, 2500, 3000, 3500, 4000, and 5000 ppm of 1,2-dimethyl-3-hydroxypyrid-4-one were prepared and then sterilized. Each solution was inoculated with a 48 h batch culture (0.1 cm^3) of *S. pombe*. Similarly, a series of solutions of the hydrochloride salt of 1,2-dimethyl-3-hydroxypyrid-4-one was prepared. After 48 h incubation at 32 °C the extent of the growth was determined by optical density measurements.

In the case of the free base (dmpH) a series of batch cultures containing 0, 10, 25, 50, 100, 125, 200, and 1000 ppm of the test chemical (i.e. dmpH) were prepared as described above. After 48 h incubation at 32 °C the cells were harvested by centrifugation (Centaur 2) at 3000 rev./min for 15 min, and then washed twice with GSVB (10 cm^3). The cells were then resuspended in GSVB (10 cm^3) and this concentration was taken as 10^0 from which the suspensions of dilutions 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were prepared. Aliquots (0.1 cm^3) of dilutions 10^{-4} and 10^{-5} were plated on YEA and incubated for 48 h at 32°C after which the number of colonies were counted (it is assumed colonies = viable cells).

5.5.5 H.p.l.c. experiments

Series of EMM2 solutions containing 0.0, 0.5, 1.0, 5.0, and 10.0 ppm of the ligand (10) were prepared and then filter sterilized. Similarly, Series of the hydrochloride salt solutions in EMM2 were prepared. All the solutions were inoculated with a 48 h batch culture (0.1 cm^3) of *S. pombe* and incubated for 48 h. at 32°C. After this period the cultures were filtered and analysed by h.p.l.c.

5.5.6 Continuous culture experiments

5.5.6.1 Apparatus

a) Culture vessel: A modified LH Fermentor (501/C series) was used for all the chemostat experiments in this study. All parts of the apparatus which were in contact with the medium were made in glass.

b) Nutrient supply: The nutrients were supplied at a constant rate from the medium reservoir (containing 4-5 x 10^3 cm^3 of the fresh medium) using a peristaltic pump (Watso-Marlow Ltd., type MHRE200). The rate of the medium supply (flow rate) was measured and adjusted so that the required cell generation time (see Chapter 4 for details) was obtained.

c) Overflow system: The medium was removed from the culture vessel through a weir tubing of a fixed height so

that the volume (600 cm^3) of the medium in the culture vessel remained constant.

d) Effluent reservoir: The effluent reservoir (a 10^3 cm^3 glass container) was connected to the culture vessel via the weir tubing.

e) Air flow system: The culture vessel, nutrient and effluent reservoir were equipped with air-inlet and air-outlet glass tubings, which were plugged with non-absorbent cotton wool to prevent aerial contamination. The filtered air was supplied into the culture vessel by a hyflo air-pump (Gallenhemp ltd.)

f) Connections: All parts of the chemostat were connected by silicon tubing, as these are heat resistant and remain intact during autoclaving.

5.5.6.2 Temperature and pH conditions

The experiments were carried out at room temperature (25-27 °C). The pH of the growth medium was measured twice daily, using an autoclaveable electrode (Russel ACWL/200) which showed to be fairly constant throughout the experiment (5.0-5.2). The electrode was immersed in the medium prior to sterilization.

5.5.6.3 Operation and Sampling

The growth chamber was inoculated with a 48 h batch culture of *S. pombe* and incubated at room temperature overnight. Then all the components of the chemostat were connected and the air flow, nutrient supply, and stirring mechanism were started under the required conditions (i.e flow rate, temperature, etc.). After allowing 48 hours for the steady state to be reached, samples (*ca* 20 cm^3) were collected twice daily directly from the culture vessel. The samples were stored (maximum 48 h) at 5 °C.

5.5.6.4 Isolation of mutants

The mutants were isolated using reported procedures.[136]

5.7 Genetic analysis

The genetic analysis of crosses between antibiotic resistant mutants (cyl^R and cap^R) and (*his* $2h^-$ were carried out according to procedures described previously.[136]

REFERENCES

1- Bell, C.F.,
"Metal Chelation Principles and Applications"
Clarendon Press, Oxford, 1977.

2- Hughes, M.N.,
"The Inorganic Chemistry of Biological Processes",
2nd.Ed., John Wiley and Sons, New York, 1981.

3- Cotton, F.A. and Wilkinson, G.,
"Advanced Inorganic Chemistry", 4th Ed., John Wiley
and Sons, New York, 1980.

4- Clegg, J.B. and Weatherall, D.J.,
"Blood and its disorders", Blackwell Scientific
Publications, 2nd. Ed., Oxford, 1982.

5- Modell, B., *Br. Med. Bull.*, 32, 270, 1976.

6- Weatherall, D.J.,
"The New Genetics and Clinical Practice", 2nd. Ed.
Oxford Medical Publications,Oxford, 1985.

7- World Health Organisation, Report on the community
Control of Hereditary Anaemias. Memorandum from a WHO
Meeting, *Bull. World Health Org.*, 63, 1983.

8- Cerami, A., Grady, R.W., Patterson, C.M., and
Bhargava, K.K., *Annals. New York Academy of Sciences*,
344; 425; 1980.

9- Arden, G.B., Davies, S.C., Hungerford, J.L., Hueghns, E.R., Marcus, R.E., and Miller, M.H., *Lancet*, II, 181, 1983.

10- Dehon, C., Doppee, J.P., Ducobu, J., Dupont, P., Hainaut, J., and Rubinstein, M., *Lancet*, I, 817, 1985.

11- Grady, R.W., Graziano, J.H., Akere, H.A., and Cerami, A., *J. Pharmacol. Exp. Ther.*, 196, 478, 1976.

12- Grady, R.W., Graziano, J.M., White, G.P, Jacobs, A., and Cerami, A., *J. Pharmacol. Exp. Ther.*, 205, 757, 1978.

13- Hoy, T., Humphrys, J., Jacobs, A., Williams, A., and Ponka, P., *Br. J. Haematol.*, 43, 443, 1979.

14- Pitt, C.G., Gupta, G., Estes, W.E., Rosenkrantz, H., Matteville, J.J., Crumbliss, A.L., Palmer, R.A., Nordquest, K.W., Sprinkle Hardy, K.A., Whitcomb, D.R., Byers, B.R., Arceneaux, J.E.L., Gains, C.G., and Sciortino, C.V., *J. Pharmacol. Exp. Ther.*, 208, 12, 1979.

15- Grady, R.W., Peterson, C.M., Jones, R.L., Graziano, J.H., Bhargava, K.K., Berdoukas, V.A., Kokkini, G., Loukopoube, D., and Cerami, A., *J. Pharmacol. Exp. Ther.*, 209, 342, 1979.

16- Hider, R.C., Kontoghiorghes, G.J., and Silver, J., U.K. Patent no. GB2118176, 1982.

17- Scarrow, R.C., Riley, P.E., Abu-Dari, Whik, D.L., and Raymond, K., *Inorg. Chem.*, 24, 954, 1985.

18- Kontoghiorghes, G.J., *Lancet*, I, 517, 1985.

19- Kontoghiorghes, G.J., "The design of orally active iron chelators for the treatment of thalassaemia", PhD thesis, Essex University, 1982, British Library Micro Film D 66194/86.

20- Kontoghiorghes, G.J., *Br. J. Haematol.*, 62, 607, 1986.

21- Kontoghiorghes, G.J. and Sheppard, L., *Inorg. Chim. Acta*, 136, L11, 1987.

22- Nelson, O.W., Karpishin, T.B., Rettig, S.J., and Orvig, C., *Can. J. Chem.*, 66, 123, 1988.

23- Nelson, O.W., Karpishin, T.B., Rettig, S.J., and Orvig, C., *Inorg. Chem.*, 27, 1045, 1988.

24- Nelson, O.W., Rettig, S.J., and Orvig, C., *J. Am. Chem. Soc.*, 109, 4121, 1987.

25- Nelson, O.W., Matsuba, C.A., Rettig, S.J., and Orvig, C., *Inorg. Chem.*, 27, 3935, 1988.

26- Nelson, O.W., Rettig, S.J., Orvig, C., and Trotter, J., *Can. J. Chem.*, 66, 132, 1988.

27- Charalambous, J., Dodd, A., McPartlin, M., Matondo, S., Pathirana, N.D., and Powell, H., *Polyhedron*, 7, 2235, 1988.

28- Charalambous, J., Dodd, A., McAthey, P., and Turner, S., *Thalassaemia Conference*, London, 1987.

29- Charalambous, J., Dodd, A., Gwatidzo, N., and McPartlin, M., *Thalassaemia Conference*, London, 1987.

30- Charalambous, J., Dodd, A., and Pathirana, D.N., *Inorganic biochemistry discussion group*, London, 1988.

31- Kontoghiorghes, G.J., Aldouri, M.A., Hoffbrand, A.V., Barr, M.J., Wonke, B., Kourouclaris, T. and Sheppard, L., *Br. Med. J.*, 295, 1509, 1987.

32- Hughs, E.R., Porter, J.B., and Hider, R.C., *Haemoglobin*, 12, 593, 1988.

33- Porter, J.B., Hoyes, K.D., Abeysinghe, R., and Hughs, E.R., *Lancet*, II, 156, 1989.

34- Hoffbrand, A.V., Bartlett, A.N., Veys, P.A., O'Connor, N.T.J., and Kontoghiorghes, G.J., *Lancet*, II, 457, 1989.

35- Kontoghiorghes, G.J., Nasseri-Sina, P., Goddard, J.G., Barr, M.J., Nortey, P., Sheppard, L.N., *Lancet*, II, 457, 1989.

36- Klingeberg, E.(Editor), "Pyridine and its Derivatives", Part III, Interscience Publishers, New York, 509, 1962.

37- Katritzky, A.R.(Editor), "Handbook of heterocyclic chemistry", Pergamon, New York, 1985.

38- Ost, H., *J. prakt. Chem.*, 19, 177, 1879. CA A., 307, 1879.

39- Fredrick, J.F., U.S. Patent 2,918,402, 1959. CA 54:9217d.

40- Smith, I.K. and Fowden, L., *J. Exper. Bot.*, 17, 750, 1966.

41- Nickell, L.G. and Gordon, P.N., *Agr. Biol. Chem.*, 27, 65, 1963. CA 58:10666f.

42- Christie, G.S., Lee, C.P., and Hererty, M.P., *Endocrinology (Baltimore)*, 105, 342, 1979. CA 91:186580w.

43- Hwang, D.R. and Driscoll, J.S., *J. Pharm. Sci.*, 68, 816, 1979.

44- Mao, D.T., Driscoll, J.S., and Marquez, V.E., *J. Med. Chem.*, 27, 160, 1984.

45- Harris, R.L.N., *Aust. J. Chem.*, 29, 1329, 1976.

46- Stunzi, H., Perrin, D.D., Teitei, T. and Harris, R.L.N., *Aust. J. Chem.*, 32, 21, 1979.

47- Stunzi, H., Perrin, D.D., Teitei, T. and Harris, R.L.N., *Aust. J. Chem.*, 33, 2207, 1980.

48- Hashinguchi, H. and Takahashi, H., *Mol. Pharmacol.*, 13, 362, 1976.

49- Fredrick, J.F., *Physiol. Plantarum*, 11, 493, 1958. CA 54:21352d.

50- Yasue, M., Kawamura, N., and Sakakibura, J., *Yakugaku Zasshi*, 90, 1222, 1970.

51- Severin, T. and Loidl, A., *Z. Lebensm.-Untersu. Forsch.*, 161, 119, 1976.

52- Hider, R.C., Personal Communication, 1987.

53- Specker, and Gaurosch *Ber.*; 75; 1338; 1942.

54- Katritzky, A.R., Jones, R.A., and Lagowski, A., *Chem. and Ind.*, 870, 1960.

55- Williams, D.H. and Fleming, I. "Spectroscopic methods in Organic Chemistry", 3rd. Ed., McGraw-Hill Book Company (U.K.) Ltd., U.K., 1980.

56- Leonard, N.J. and Locke, D.M., *J. Am. Chem. Soc.*, 77, 1852, 1955.

57- Mason, S.F., *J. Chem. Soc.*, 4874, 1957.

58- Katritzky, A.R. and Jones, R.A., *J. Chem. Soc.*, 2947, 1960.

59- Bellamy, L.J. and Rogasch, P.E., *Spectrochim. Acta*, 16, 30, 1960.

60- Albert, A. and Spinner, E., *J. Chem. Soc.*, 122, 1960.

61- Spinner, E., *J. Chem. Soc.*, 3119, 1962.

62- Batta, B.D. and Spinner, E., *Aust. J. Chem.*, 22, 2581, 1969.

63- Spinner, E., *J. Chem. Soc.*, 1226, 1960.

64- Cook, D., *Can. J. Chem.*, 41, 2575, 1963.

65- Giudice, M.R.; Settimj, G.; and Delfini, M.; *Tetrahedron*, 40, 4067, 1984.

66- Tamhina, B., Jakopcic, K, Zorko, F., and Herak, M.J., *J. Inorg. Nucl. Chem.*, 36, 1855, 1974.

67- Tamhina, B., Jakopcic, K, Zorko, F., and Herak, M.J., *J. Inorg. Nucl. Chem.*, 39, 1201, 1977.

68- Tables of Interatomic Distances and Configuration in Molecules and Ions, p. S.17, Chemistry Society, 1958.

69- Abdulla, R.T., Fuhr, K.H., and Williams, J.C., *J. Org. Chem.*, 44, 1349, 1979.

70- Finely, K.T., *Chem. Rev.*, 64, 573, 1964.

71- McElvain, S.M., *Org. Reactions*, 4, 262, 1948.

72- Leonard, N.J. and Oki, M., *J. Am. Chem. Soc.*, 77, 6237, 1955.

73- Leonard, N.J. and Oki, M., *J. Am. Chem. Soc.*, 77, 6241, 1955.

74- Leonard, N.J. and Oki, M., *J. Am. Chem. Soc.*, 77, 6245, 1955.

75- Matthews, C.N.,
"A potential new general method for the synthesis of 3-hydroxypyrid-4-ones", BSc. Project, Polytechnic of North London, 1988.

76- Sohal, A.S.,
"The synthesis of 1-methyl-3-hydroxypyrid-4-one", BSc. Project, Polytechnic of North London, 1989.

77- Shishido, J.K., Light, L., and Davis, L., *Tetrahedron Letters*, 22, 4607, 1981.

78- McDonald, E., Sukkamran, A., and Wylie, R.D., *J. Chem. Soc.*, Perkin Trans, I, 1893, 1979.

79- (a) Pathirana, N.D., Unpublished work, Polytechnic of North London, 1988.
(b) Tulu, D.,
"The synthesis of six membered heterocyclic compounds", BSc. Project, Polytechnic of North London, 1989.

80- Scharf, H.D., Plum, H., Fleischhauer, J., and Schleker, W., *Chem. Ber.*, 112, 862, 1979.

81- Elpern, B., Vetterau, W., Carabateas, P., and Grumbach, L., *J. Am. Chem. Soc.*, 80, 4916, 1958.

82- Bloomfield, J.J., *Org. Chem.*, 15, 1, 1967.

83- Dow Chemical Co., U.S. Patent, 2 821 555, 1958. CA 52:P9198i.

84- For example: (a) Higashigawa, T., Jpn. Kokai Tokkyo Koho, JP 60 92,257, 1985. CA 103:P195838e.

(b) Lin, Y.I., Seifert, C.M., Kang, S.M., Dueza, J.P., and Lang, S.A., *J. Heterocycl. Chem.*, 16, 1377, 1979. CA 92:181064m.

(c) Pittet, A.O., Hruza,D.E., Ger.Offen. 2,166,575, 1977. CA 81:P120609z.

85- (a) Furniss, B.S., "Vogel's Textbook of Practical Organic Chemistry", 4th. Ed., Longman, London, 1978.

(b) Aldrich Catalogue, 1988.

86- Sampat, M.D., "A potential New Synthesis of 3-Hydroxypyrid-4-ones", BSc. Project, Polytechnic of North London, 1987.

87- Ager, J.H. and May, E.L., *J. Org. Chem.*, 27, 245, 1962.

88- Takemura, S., Miki, Y., Ueno, M., Yoshimura, K., Kuroda, M., and Suzuki, A., *Chem. Pharm. Bull.*, 29, 3026, 1981.

89- Petrow, V. and Stephenson, O., *J. Pharm. Pharmacol.*, 14, 306, 1962.

90- Allinger, N.L., Cava, M.P., De Johgh, D.C., Johnson, C.R., Lebel, N.A., and Stevens, C.L., "Organic Chemistry", 2nd. Ed., Worth Publishers Inc., New York, 1976.

91- Harrison, A.G., "Chemical Ionization Mass Spectrometry", CRC Press Inc., Florida, 1983.

92- Tasai, W.C., and Ling, K.H., *J. Chin. Biochem. Soc.*, 2, 70, 1973.

93- Herak, M.J., Tamhina, B., *Croat. Chem. Acta*, 45, 603, 1973.

94- Herak, M.J., Janko, M., and Tamhina, B., *Mikrochim. Acta*, 783, 1973.

95- Herak, M.J. and Tamhina, B, *Croat. Chem. Acta*, 46, 237, 1974.

96- Vojikovic, V. and Tamhina, B, *Solvent Extr. Ion Exch.*, 4, 27, 1986.

97- Herak, M.J., Prpic, I., and Tamhina, B., *CIN special*, 21, 462, 1979.

98- Herak, M.J. and Janko, M., *J. Inorg. Nucl. Chem.*, 34, 2627, 1972.

99- Herak, M.J. and Kolarik, Z., *Solvent Extr. Ion Exch.*, 3, 427, 1985.

100- Tamhina, B, Vojkovic, V., and Herak, M.J., *Croat. Chem. Acta*, 49, 533, 1977.

101- Vojkovic, V., Tamhina, B, and Herak, M.J., *Z. Anal. Chem.*, 285, 266, 1977.

102- Tamhina, B, Gojmerac, A., and Herak, M.J., *J. Inorg. Nucl. Chem.*, 40, 335, 1978.

103- Tamhina, B, Vojkovic, V., and Herak, M.J., *Microchem. J.*, 25, 8, 1980.

104- Tamhina, B and Herak, M.J., *Microchem. J.*, 22, 144, 1977.

105- Gojmerac, A., Tamhina, B, and Herak, M.J., *J. Inorg. Nucl. Chem.*, 41, 1475, 1979.

106- Herak, M.J., Tamhina, B, and Jakopcic, K., *J. Inorg. Nucl. Chem.*, 35, 1665, 1973.

107- Tamhina, B and Herak, M.J., *J. Inorg. Nucl. Chem.*, 38, 1505, 1976.

108- Vojkovic, V., Tamhina, B, and Herak, M.J., *Z. Anal. Chem.*, 276, 377, 1975.

109- Tamhina, B, Vojkovic, V., and Herak, M.J., *Croat. Chem. Acta*, 48, 183, 1976.

110- Tamhina, B and Herak, M.J., *Mikrochim. Acta*, 2, 45, 1975.

111- Tamhina, B and Herak, M.J., *J. Inorg. Nucl. Chem.*, 39, 391, 1977.

112- Hider, R.C., Kontoghiorghes, G.J., Silver, J., and Stockham, M.A., U.K. Patent GB 2 148 896 A, 1985.

113- Matsumotu, H., Smith, E.G., and Sherman, G.D., *Arch. Biochem. Biophys.*, 33, 201, 1951.

114- Tsai, W.C. and Ling, K.H., *J. Formosan. Med. Assoc.*, 71, 739, 1972.

115- Murakami, Y. and Mera, K., *Bull. Chem. Soc. Jap.*, 39, 396, 1966.

116- Morita, H., Shimomura, S., and Kawaguchi, S., *Bull. Chem. Soc. Jap.*, 49, 2461, 1976.

117- Gerard, C., *Bull. Soc. Chim. Fr.*, 11-12, I-451, 1979.

118- Allan, J.R., Baillie, G.M., Bowley, H.J., and Gerrard, D.L., *Eur. Polym. J.*, 24, 1149, 1988.

119- Finnegan, M.M., Lutz, T.G., Nelson, W.O., Smith, A., and Orvig, C., *Inorg. Chem.*, 26, 2171, 1987.

120- Gwatidzo, N.,
"X-ray Structural Analysis of bis(1,2-dimethyl-3-hydroxypyrid-4-onato)zinc(II) complex", BSc. Project, Polytechnic of North London, 1986.

121- Greenwood, N.N. and Gibb, T.C.,
"Mossbauer Spectroscopy", Chatman and Hall Ltd., London, 1971.

122- Geary, W.J., *Coord. Chem. Rev.*, 7, 81, 1971.

123- Kontoghiorghes, G.J., *Acta Haemat.*, 78, 212, 1987.

124- Hider, R.C., Kontoghiorghes, G.J., and Silver, J. Patent GB 2 146 989, 1985.

125- Collingham, B., Hider, R.C., Kontoghiorghes, G.J., and Stockham, H.A.Eur. Patent EP 159 194, 1985.

126- Kontoghiorghes, G.J. and Hoffbrand, A.V., *Br. J. Haematol.*, 62, 607, 1986.

127- Kontoghiorghes, G.J. *Molec. Pharmac.*, 30, 670, 1986.

128- Kontoghiorghes, G.J., *Biochem. Biophys. Acta*, 882, 267, 1986

129- Kontoghiorghes, G.J. and Evan, R.W., *FEBS Lett.*, 189, 141, 1985.

130- Kontoghioghes, G.J., Sheppard, L., Hoffbrand, A.V., Charalambous, J., Tikerpoe, J. and Pippard, M.J., *J. Clin. Pathol.*, 40, 404, 1987.

131- Kontoghioghes, G.J. *Scand. J. Haematol.*, 37, 63, 1986.

132- Kontoghiorghes, G.J., Piga, A., Hoffbrand, A.V., *FEBS Lett.*, 204, 208, 1986.

133- Hedle, J.A., "Mutagenecity", Academic Press, New York, 3, 1982.

134- Moutschen, J., "Introduction to Genetic Toxicology", John Wiley and Sons, Chichester, New York, 1985.

135- Hwang, D.R., Proctor, G.R., and Driscoll, J.S. *J. Pharm. Sci.*, 69, 1074, 1980.

136- McAthey, P. and Patel, B. in: "Industrial Microbiological Testing", Society for Applied Bacteriology Technical series 23, (J.W Hopton and E.C. Hill, Eds.), Blackwell Sc. Publications Ltd., Oxford, 133, 1987.

137- Kubitschek, H.E. "Introduction to research with continuous cultures", Prentice-Hall Inc., New York, 1970.

138- Eglieson, V., Evans, I.H. and Wilkie, D., *Molec. Gen. Genet.*, 174, 39, 1979.

139- McAthey, P. and Kilbey, B.J., *Biol. Zbl.*, 95, 415, 1976.

140- Linnane, A.W., Lamb, A.J., Christodolou, C., and Lukins, H.B., *Proc. Natl. Acad. Sci.* (USA), 59, 1288, 1968.

141- Gale, E.F., Condliffe, E., Reynolds, P.E., Richmond, M.H. and Waring, M.J.
"The Molecular Basis of Antibiotic Action", John Wiley and Sons, London, 1981.

142- Westmann, E.R., *Mut. Res.*, 28, 323, 1975.

143- Mohn, G.R., *Mut. Res.*, 87, 191, 1981.

144- Sega, G.A., *Mut. Res.*, 134, 113, 1984.

145- Kada, T. in:
"Environmental Mutagens and Carcinogens", (Sugimura T. *et. al., Eds.*), Alan R. Liss, Inc. and University of Tokyo Press, New York, 1982.

146- Kada. T., Inoue, T., Ohta, T. and Shirasu, Y. in:
"Antimutagenesis and Anticarcinogenesis Mechanisms" , (Shankel, D.M. *et. al., Eds.*), 39, 181, Plenum, New York, 1986.

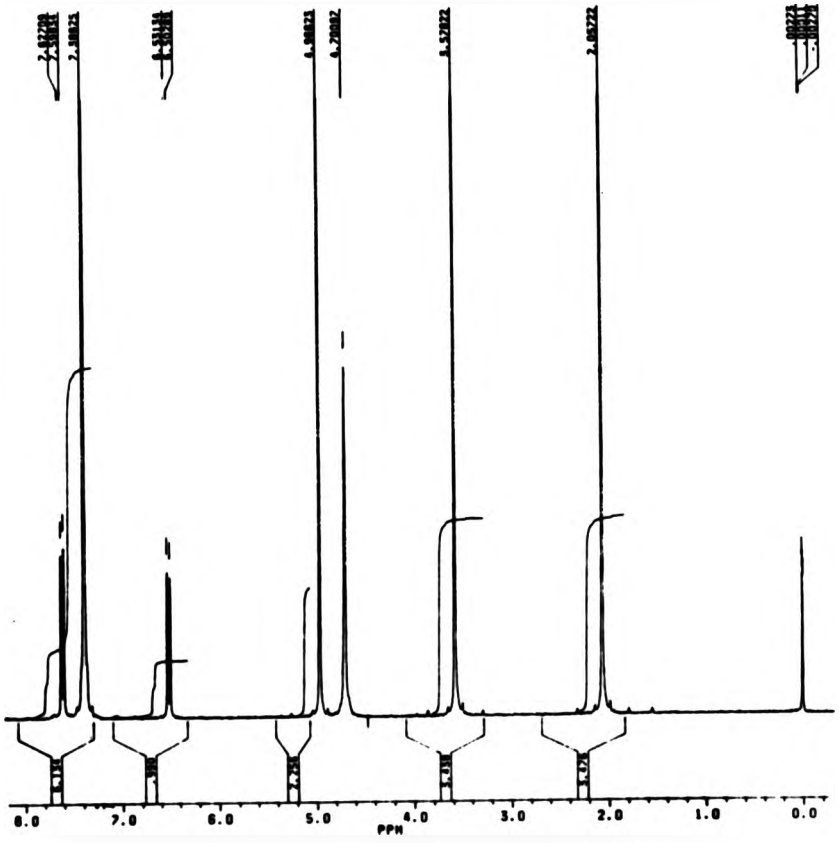
147- Vogel, A.,
"Text Book of Quantitative Inorganic Analysis", 4th Ed., Longman, London, 1978.

148- Barnshaw, A.
" Introduction to Magnetochemistry", Academic Press, London, 1968.

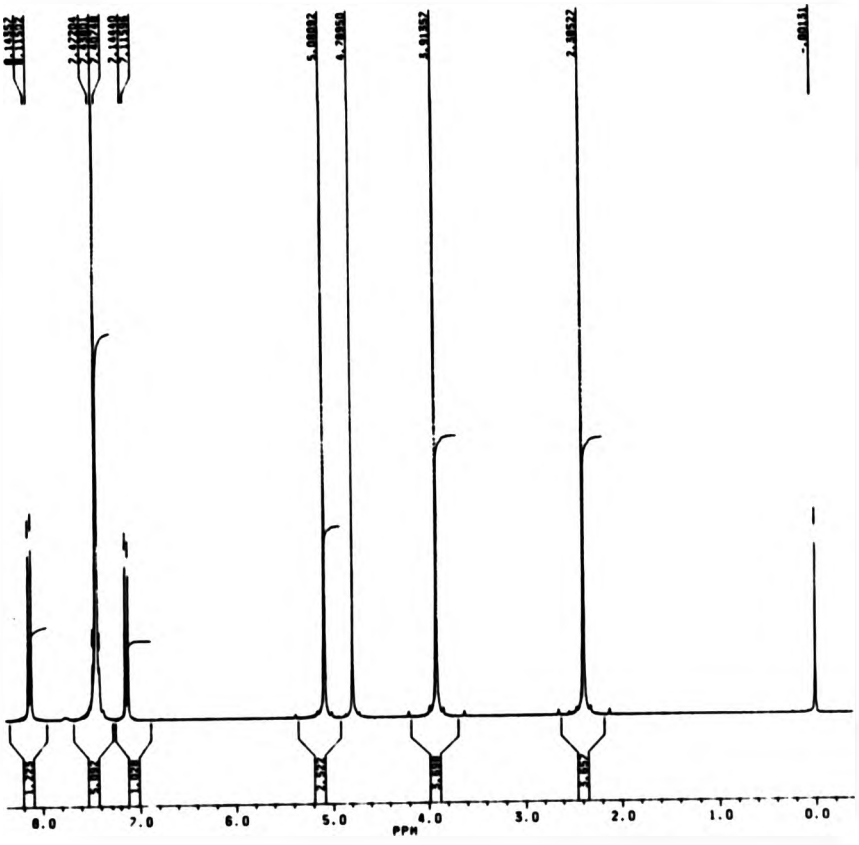
149- Bowers, A., Halsall, T.G., Jones, E.R., and Lemin, A.J., *J. Chem. Soc.*, 2548, 1953.

150- Mitchison, J.M., in:
"Methods in cell physiology", (Prescott, D.M.), 4, Academic Press, New York, 1970.

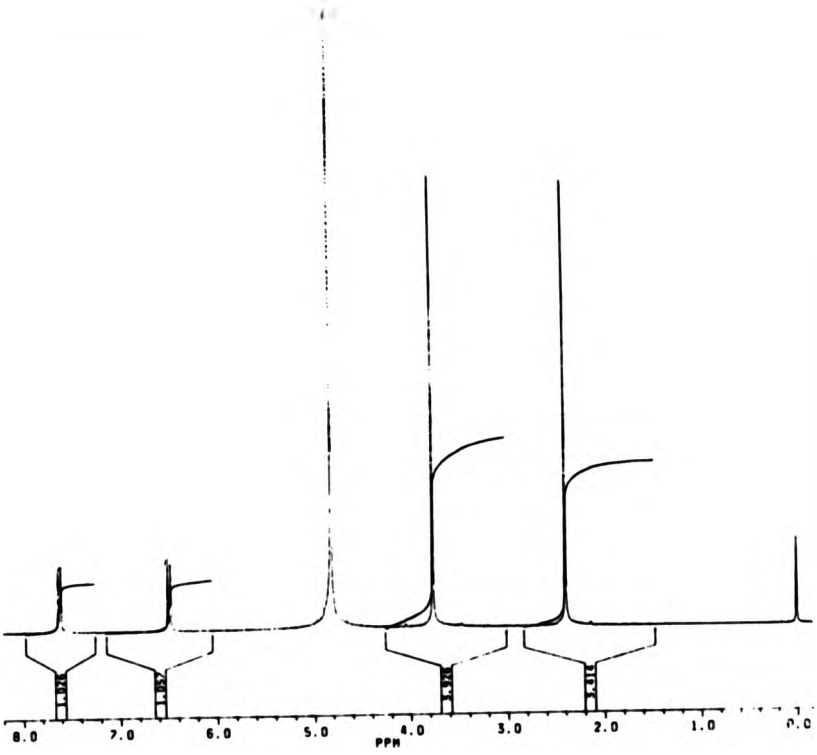
APPENDIX



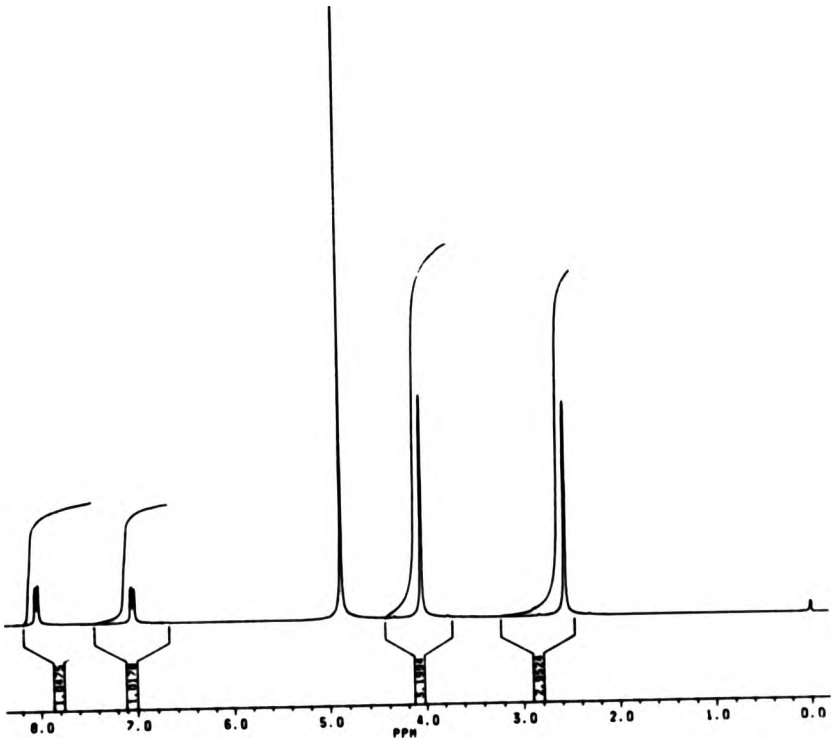
Spectrum 1- ^1H n.m.r. spectrum of 3-benzyloxy-1,2-dimethylpyrid-4-one trihydrate (14)



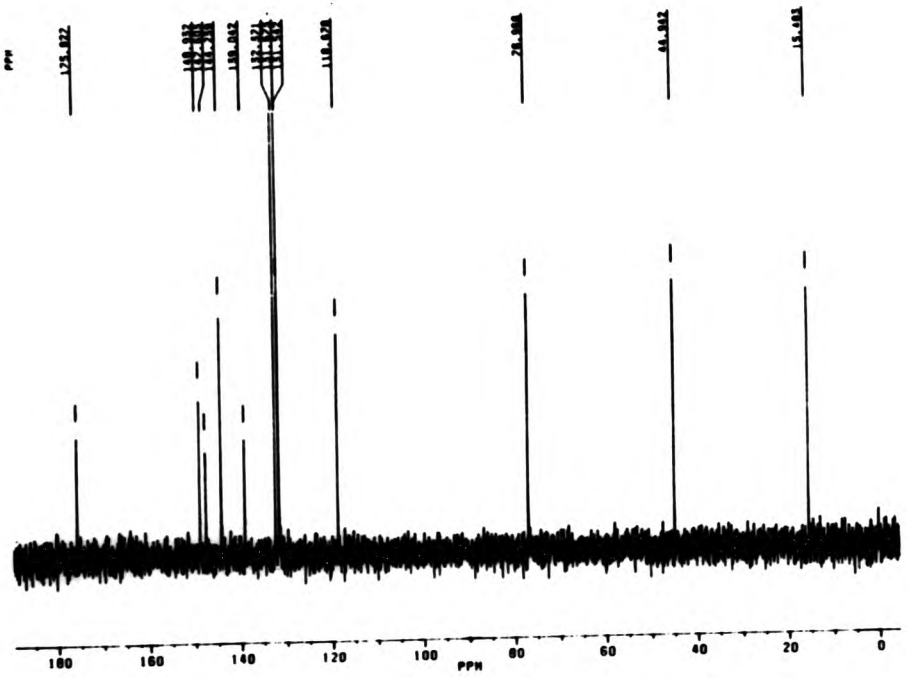
Sepctrum 2- ^1H n.m.r. spectrum of 3-benzyloxy-1,2-dimethylpyrid-4-one hydrochloride (15)



Spectrum 3- ^1H n.m.r. spectrum of 1,2-dimethyl-3-hydroxypyrid-4-one (10)



Spectrum 4- ^1H n.m.r. spectrum of 1,2-dimethyl-3-hydroxypyrid-4-one hydrochloride (16b)



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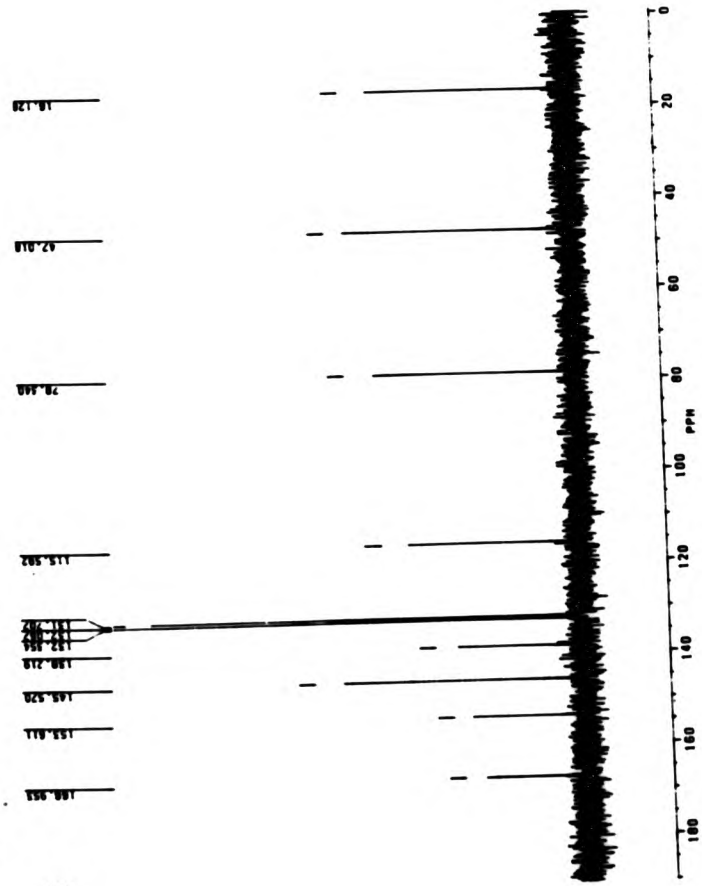

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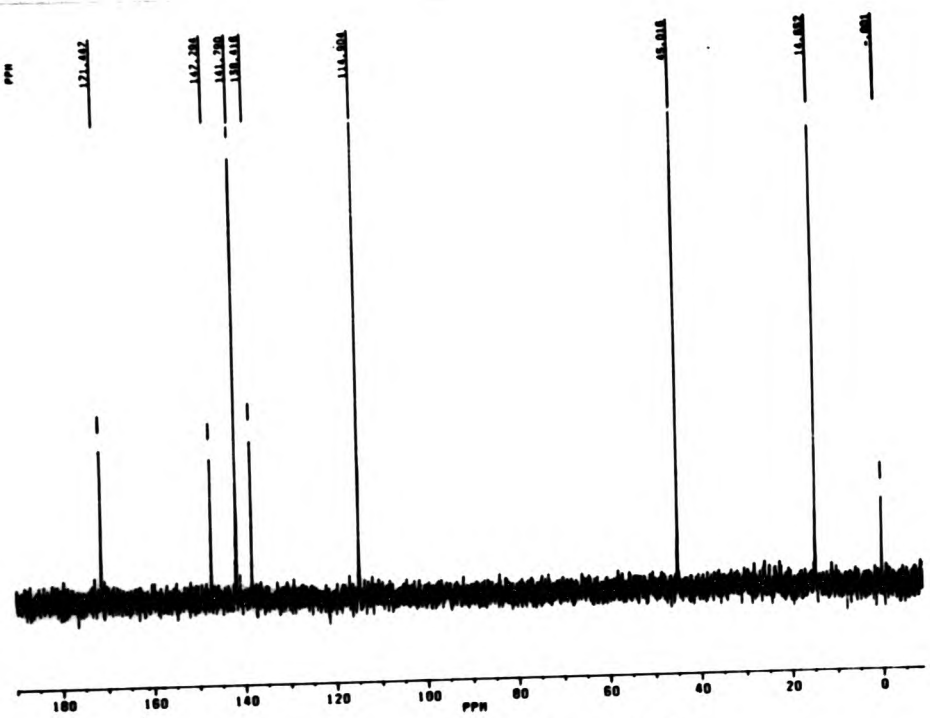
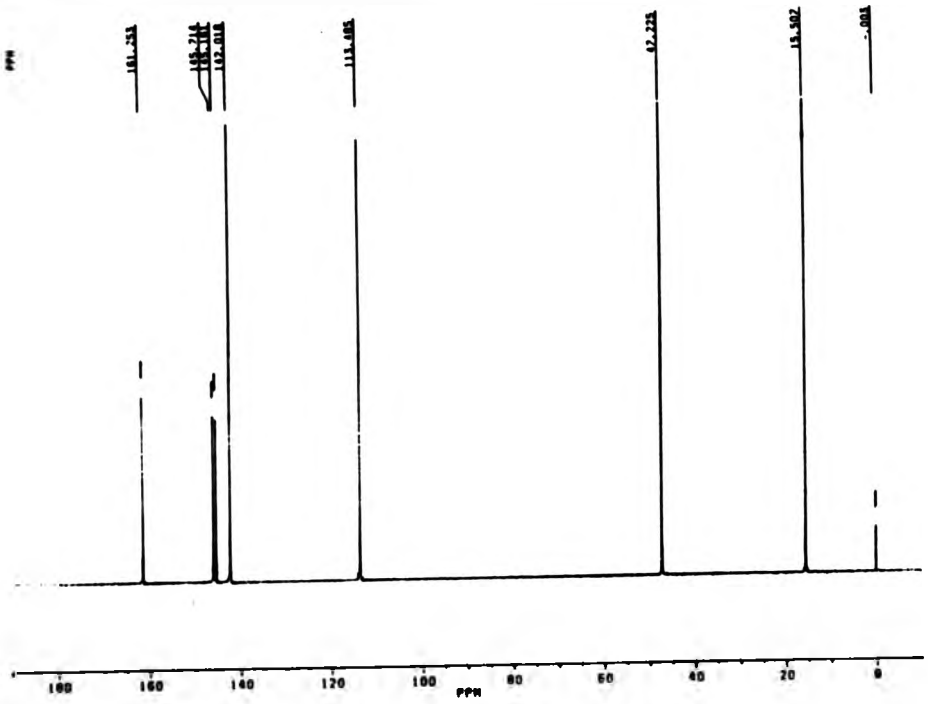
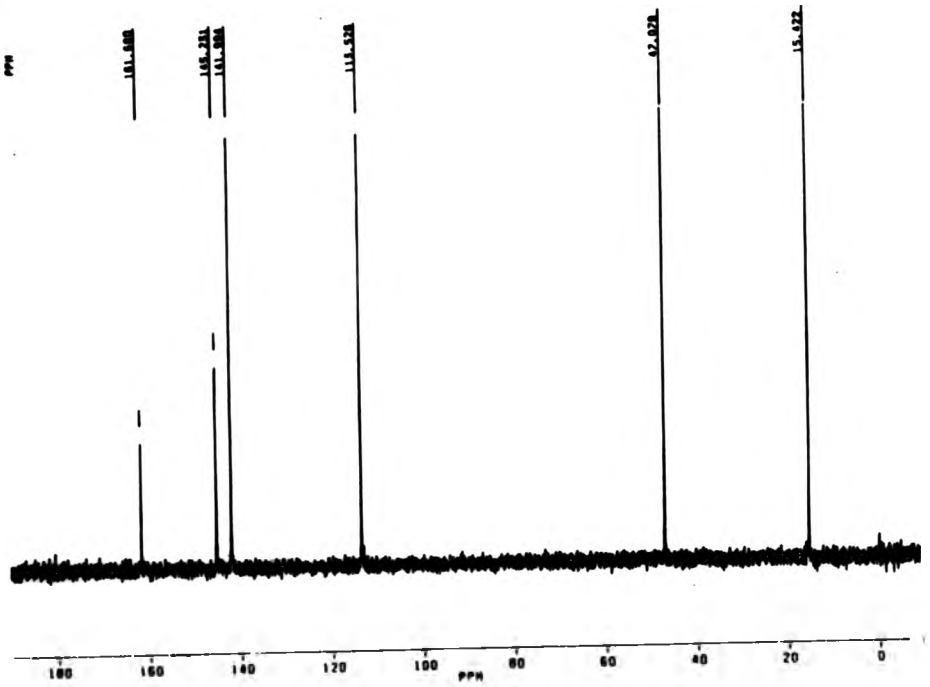


Spectrum 7- ^{13}C n.m.r. spectrum of 1,2-dimethyl-3-hydroxypyrid-4-one (10)



Spectrum 8- ^{13}C n.m.r. spectrum of 1,2-dimethyl-3-hydroxypyrid-4-one hydrobromide (16a)



Spectrum 9- ^{13}C n.m.r. spectrum of 1,2-dimethyl-3-hydroxypyrid-4-one hydrochloride (16b)

X-ray Crystallographic Data for

$Zn(C_7H_8NO_2)_2.3\frac{1}{2}H_2O$

Table I- Fractional atomic coordinates and thermal parameters ($Å^2$) for $Zn(C_7H_8NO_2)_2.3.5H_2O$

Atom	x	y	z	U_{iso} or U_{eq}
Zn	0.23005(5)	0.10282(6)	0.16794(14)	0.01311(5)
O(11)	0.1614(3)	0.1938(3)	0.3041(8)	0.040(3)
O(12)	0.1295(3)	0.0304(3)	0.2092(8)	0.034(3)
O(21)	0.2998(3)	-0.0061(3)	0.1277(9)	0.042(3)
O(22)	0.3415(3)	0.1490(3)	0.2671(8)	0.031(3)
O(1w)	0.2167(3)	0.1509(3)	-0.0985(8)	0.041(3)
O(2w)	0.3381(3)	0.1806(4)	-0.3413(8)	0.041(3)
O(3w)	0.1903(5)	-0.1385(4)	0.144(13)	0.084(6)
O(4w)	0.2889(7)	-0.2469(9)	0.3969(21)	0.063(8)
C(13)	0.0681(4)	0.0781(5)	0.2665(11)	0.029(2)
C(14)	0.0866(4)	0.1657(5)	0.3195(11)	0.029(2)
C(15)	0.0232(5)	0.2171(6)	0.3828(12)	0.039(2)
C(16)	-0.0551(5)	0.1822(6)	0.3867(13)	0.044(2)
N(11)	-0.0710(4)	0.1007(4)	0.3365(9)	0.039(2)
C(12)	-0.0098(4)	0.0466(5)	0.2772(11)	0.031(2)
C(17)	-0.1584(6)	0.0680(7)	0.3470(16)	0.059(3)
C(18)	-0.0305(5)	-0.0446(6)	0.2237(13)	0.044(2)
C(23)	0.4003(4)	0.0889(5)	0.2499(11)	0.030(2)
C(24)	0.3758(4)	0.0063(5)	0.1805(11)	0.032(2)
C(25)	0.4372(5)	-0.0591(6)	0.1735(12)	0.042(2)
C(26)	0.5169(5)	-0.0391(6)	0.2290(12)	0.041(2)
N(21)	0.5387(4)	0.0394(4)	0.2842(9)	0.037(2)
C(22)	0.4821(4)	0.1058(5)	0.2970(11)	0.032(2)
C(27)	0.6283(5)	0.0564(6)	0.3249(13)	0.047(2)
C(28)	0.5107(5)	0.1929(5)	0.3657(13)	0.044(2)

Table II- Fractional atomic coordinates for the hydrogen atoms for $Zn(C_7H_8NO_2)_2.3.5H_2O$

Atom	x	y	z
H(15)	0.0348	0.2831	0.4281
H(16)	-0.1049	0.2225	0.4321
H(17a)	-0.1666	0.0126	0.4541
H(17b)	-0.1772	0.0448	0.2087
H(17c)	-0.1962	0.1227	0.3628
H(18a)	0.0244	-0.0772	0.1611
H(18b)	-0.0762	-0.0448	0.1088
H(18c)	-0.0546	0.0781	0.3425
H(25)	0.4214	-0.1238	0.1251
H(26)	0.5630	-0.0896	0.2277
H(27a)	0.6369	0.1232	0.3680
H(27b)	0.6621	0.0444	0.2006
H(27c)	0.6506	0.0139	0.4364
H(28a)	0.4591	0.2371	0.3673
H(28b)	0.5559	0.2179	0.2732
H(28c)	0.5381	0.1867	0.5058

Table III- Intermolecular distances (Å) for

$Zn(C_7H_6NO_2)_2.3.5H_2O$

	dist.	S	a	b	c
H(17a)...Zn	3.43	-1	0.0	0.0	1.0
H(17b)...Zn	3.59	-1	0.0	0.0	0.0
H(18b)...Zn	3.24	-1	0.0	0.0	0.0
O(2w)....Zn	3.77	2	0.0	0.0	-1.0
H(17a)...O(12)	2.54	-1	0.0	0.0	1.0
H(18b)...O(12)	2.40	-1	0.0	0.0	0.0
O(1w)....O(11)	2.64	2	0.0	0.0	-1.0
O(2w)....O(22)	2.84	1	0.0	0.0	-1.0
O(2w)....O(22)	2.73	2	0.0	0.0	-1.0
H(27b)...O(21)	2.52	-1	1.0	0.0	0.0
H(18b)...O(1w)	2.81	-1	0.0	0.0	0.0
H(17a)...C(13)	2.86	-1	0.0	0.0	1.0
H(18b)...C(13)	2.73	-1	0.0	0.0	0.0
H(18c)...C(13)	2.81	-1	0.0	0.0	1.0
H(18c)...C(14)	2.83	-1	0.0	0.0	1.0
H(15)....C(14)	2.98	2	0.0	0.0	0.0
H(18c)...C(15)	2.93	-1	0.0	0.0	1.0
C(18)....H(15)	2.87	-2	0.0	0.0	1.0
H(18c)...C(16)	3.03	-1	0.0	0.0	1.0
O(3w)....H(16)	2.60	-2	0.0	0.0	1.0
H(18c)...N(11)	3.02	-1	0.0	0.0	1.0
C(16)....C(12)	3.49	-1	0.0	0.0	1.0
H(18c)...C(12)	2.90	-1	0.0	0.0	1.0
C(27)....C(17)	3.47	1	1.0	0.0	0.0
H(27b)...C(17)	3.08	1	1.0	0.0	0.0
O(3w)....H(17b)	2.90	-1	0.0	0.0	0.0
C(27)....H(17c)	3.04	1	1.0	0.0	0.0
O(4w)....H(17c)	2.93	-1	0.0	0.0	1.0
H(27c)...C(23)	2.89	-1	1.0	0.0	1.0
H(27b)...C(24)	2.87	-1	1.0	0.0	0.0
H(27c)...C(24)	2.80	-1	1.0	0.0	1.0
C(24)....C(25)	3.36	-1	1.0	0.0	0.0
N(21)....C(25)	3.32	-1	1.0	0.0	0.0
H(27b)...C(25)	3.06	-1	1.0	0.0	0.0
H(26c)...C(25)	3.03	-1	1.0	0.0	1.0
C(26)....H(25)	3.03	-2	1.0	1.0	1.0
O(2w)....C(26)	3.28	-1	1.0	0.0	0.0
O(2w)....H(26)	2.26	-1	1.0	0.0	0.0
O(4w)....H(27a)	2.78	-1	1.0	0.0	1.0
O(2w)....H(26a)	2.72	2	1.0	0.0	-1.0
O(4w)....H(26b)	2.90	-2	1.0	0.0	1.0
O(4w)....O(3w)	3.01	2	0.0	-1.0	0.0

Table IV- Intramolecular distances (Å) for

$Zn(C_7H_6NO_2)_2.3.5H_2O$

Atom 1	Atom 2	dist. (Å)	Atom 1	Atom 2	dist. (Å)
C(13)	Zn	2.78	C(14)	Zn	2.79
C(23)	Zn	2.81	C(24)	Zn	2.79
O(3w)	Zn	3.77	O(11)	O(12)	2.65
O(21)	O(12)	2.91	O(1w)	O(12)	3.25
C(14)	O(12)	2.34	C(16)	O(12)	2.35
C(18)	O(12)	2.85	H(18a)	O(12)	2.38
O(3w)	O(12)	2.83	O(22)	O(11)	3.03
O(1w)	O(11)	3.12	C(13)	O(11)	2.35
C(15)	O(11)	2.37	H(15)	O(11)	2.66
O(21)	O(22)	2.66	O(1w)	O(22)	3.23
C(24)	O(22)	2.36	C(22)	O(22)	2.38
C(28)	O(22)	2.89	H(28a)	O(22)	2.42
O(1w)	O(21)	3.17	C(23)	O(21)	2.33
C(25)	O(21)	2.39	H(25)	O(21)	2.68
O(3w)	O(21)	2.71	O(2w)	O(1w)	2.72
C(15)	C(13)	2.42	C(16)	C(13)	2.73
N(11)	C(13)	2.36	C(18)	C(13)	2.49
H(18a)	C(13)	2.56	H(15)	C(14)	2.15
C(16)	C(14)	2.39	N(11)	C(14)	2.76
C(12)	C(14)	2.42	H(16)	C(15)	2.13
N(11)	C(15)	2.37	C(12)	C(15)	2.78
C(16)	H(15)	2.14	C(12)	C(16)	2.36
C(17)	C(16)	2.44	H(17c)	C(16)	2.47
N(11)	H(16)	2.08	C(17)	H(16)	2.59
H(17a)	N(11)	2.25	H(17b)	N(11)	2.10
H(17c)	N(11)	2.10	C(18)	N(11)	2.48
H(18b)	N(11)	2.77	H(18c)	N(11)	2.77
H(17a)	C(12)	2.94	C(17)	C(12)	2.51
H(17b)	C(12)	2.74	H(18a)	C(12)	2.11
H(18b)	C(12)	2.11	H(18c)	C(12)	2.11
C(18)	H(17a)	2.95	C(18)	C(17)	2.87
H(18b)	C(17)	2.81	H(18c)	C(17)	2.81
C(18)	H(17b)	2.75	O(3w)	H(18a)	2.88
C(25)	C(23)	2.42	C(26)	C(23)	2.74
N(21)	C(23)	2.38	C(28)	C(23)	2.52
H(28a)	C(23)	2.60	H(25)	C(24)	2.18
C(26)	C(24)	2.41	N(21)	C(24)	2.76
C(22)	C(24)	2.43	H(26)	C(25)	2.12
N(21)	C(25)	2.35	C(22)	C(25)	2.77
C(26)	H(25)	2.13	C(22)	C(26)	2.36
C(27)	C(26)	2.41	H(27b)	C(26)	2.70
H(27c)	C(26)	2.70	N(21)	H(26)	2.07
C(27)	H(26)	2.57	H(27a)	N(21)	2.12
H(27b)	N(21)	2.12	H(27c)	N(21)	2.11

(Continued)

Atom 1	Atom 2	dist. (Å)	Atom 1	Atom 2	dist. (Å)
C(28)	N(21)	2.46	H(28b)	N(21)	2.76
H(28c)	N(21)	2.77	C(27)	C(22)	2.49
H(27a)	C(22)	2.56	H(28a)	C(22)	2.12
H(28b)	C(22)	2.11	H(28c)	C(22)	2.12
C(28)	C(27)	2.86	H(28b)	C(27)	2.77
H(28c)	C(27)	2.83	C(28)	H(27a)	2.31
O(4w)	O(3w)	2.89			

Table V- Anisotropic thermal parameters ($Å^2$) for

$Zn(C_7H_6NO_2)_2.3.5H_2O$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Zn	0,027(1)	0,028(1)	0,039(1)	-0,001(1)	0,003(1)	-0,001(1)
O(11)	0,035(3)	0,030(3)	0,054(4)	-0,009(3)	0,011(3)	-0,005(2)
O(12)	0,030(3)	0,027(3)	0,044(3)	-0,005(3)	0,006(3)	-0,003(2)
O(21)	0,034(3)	0,031(3)	0,062(4)	-0,004(3)	0,000(3)	0,003(2)
O(22)	0,026(3)	0,026(3)	0,042(3)	-0,003(3)	0,003(2)	0,005(2)
O(1w)	0,048(3)	0,034(3)	0,041(4)	0,007(3)	0,008(3)	0,005(3)
O(2w)	0,043(3)	0,037(3)	0,042(4)	0,003(3)	0,009(3)	0,010(3)
O(3w)	0,094(5)	0,046(4)	0,112(7)	-0,007(4)	0,023(5)	-0,011(4)
O(4w)	0,064(8)	0,044(7)	0,080(10)	0,013(7)	0,013(8)	0,006(7)

Table VI- Bond angles (degree) for $Zn(C_7H_6NO_2)_2.3.5H_2O$

	Angle
O(11)-Zn-O(12)	81.2(2)
O(22)-Zn-O(11)	95.2(2)
O(21)-Zn-O(11)	159.4(2)
O(1w)-Zn-O(12)	106.5(2)
O(1w)-Zn-O(22)	104.5(2)
C(13)-O(12)-Zn	111.6(4)
C(23)-O(22)-Zn	110.6(4)
C(14)-C(13)-O(12)	117.0(6)
C(12)-C(13)-C(14)	120.5(7)
C(15)-C(14)-O(11)	123.2(7)
C(16)-C(15)-C(14)	118.9(8)
C(12)-N(11)-C(16)	121.4(7)
C(17)-N(11)-C(12)	120.4(7)
C(18)-C(12)-C(13)	121.5(7)
C(24)-C(23)-O(22)	117.6(6)
C(22)-C(23)-C(24)	120.4(7)
C(25)-C(24)-O(21)	123.4(7)
C(26)-C(25)-C(24)	119.1(8)
C(22)-N(21)-C(26)	121.9(7)
C(27)-N(21)-C(22)	120.2(7)
C(28)-C(22)-C(23)	122.0(7)
O(22)-Zn-O(12)	148.9(2)
O(21)-Zn-O(12)	91.4(2)
O(21)-Zn-O(22)	81.1(2)
O(1w)-Zn-O(11)	98.9(2)
O(1w)-Zn-O(21)	101.6(2)
C(14)-O(11)-Zn	110.0(5)
C(24)-O(21)-Zn	111.5(5)
C(12)-C(13)-O(12)	122.5(7)
C(13)-C(14)-O(11)	118.6(7)
C(15)-C(14)-C(13)	118.2(7)
N(11)-C(16)-C(15)	121.9(8)
C(17)-N(11)-C(16)	118.2(7)
N(11)-C(12)-C(13)	119.1(7)
C(18)-C(12)-N(11)	119.4(7)
C(22)-C(23)-O(22)	122.0(7)
C(23)-C(24)-O(21)	119.0(7)
C(25)-C(24)-C(23)	117.6(7)
N(21)-C(26)-C(25)	122.1(8)
C(27)-N(21)-C(26)	117.8(7)
N(21)-C(22)-C(23)	118.8(7)
C(28)-C(22)-N(21)	119.2(6)

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