ASPECTS OF THE CHEMISTRY OF SOME IRON - NITROSYL COMPOUNDS.

A thesis presented for the degree of

DOCTOR of PHILOSOPHY

in the University of St. Andrews

by

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October 1983.

The A 134

To my Mother and Father.

Declaration

I declare that this thesis is my own composition, that it is a record of my own work, and that it has not previously been submitted in application for a higher degree.

Joseph McGinnis

October 1983

Certificate

I hereby certify that Joseph McGinnis has spent twelve terms of research work under my supervision, has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court, 1967, No. 1, and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Dr. C. Glidewell

Research Supervisor

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CHAPTER ONE.

INTRODUCTION.

A very large number of complexes exist in which the nitrosyl group, NO, is bonded to a transition metal. Such compounds are known for most transition metals, and a wide range of other ligands may be present, including CO, CN, halogen, sulphur and cyclopentadiene. The chemistry of metal nitrosyl complexes has been widely reviewed[1-8]. This introductory chapter presents a brief account of some general features of transition metal nitrosyl chemistry, and discusses the background to the work described in succeeding chapters.

1.1 Bonding in metal nitrosyls.

Nitric oxide possesses a single unpaired electron in an antibonding orbital, which leads to considerable versatility in its coordination chemistry. Bonding in metal nitrosyls has previously been considered as arising from one of four different possibilities for coordination of NO:

i) Transfer of the odd electron from NO to the metal, followed by electron pair donation from NO^+. In this case the bonding is similar to that in metal carbonyls, with a σ bond between the metal and the donor atom and π back bonding resulting from overlap of filled metal d_π orbitals with π^* antibonding orbitals on the ligand. However where CO is formally a two-electron donor,

NO, as a result of the prior transfer of the odd electron, is a three-electron donor.

ii) Retention of the odd electron and coordination by donation of two electrons from $NO^{\bullet}$.

iii) Transfer of an electron from the metal to NO, followed by coordination of NO^{-}; in this case NO is formally a one-electron donor.

iv) Bridging of two or three metal atoms by NO. This type of bonding is known among the organometallic nitrosyls, but is uncommon.

Infra-red nitrosyl stretching frequencies have been used to differentiate between the different types of bonding. The stretching frequency of nitric oxide itself is 1878 cm^{-1}, while that of NO^{+} in nitrosonium salts is 2200 - 2300 cm^{-1} [2,9]. These frequencies are lowered on coordination to a transition metal atom as a result of the back donation of electrons into an antibonding orbital on NO. Lewis, Wilkinson and co-workers[9] measured the stretching frequencies in a large number of transition metal nitrosyls, and found them to lie in a wide range from 1980 to 1045 cm^{-1}. Frequencies above 1550 cm^{-1} were given by complexes regarded as containing NO^{+}, while those around 1100 cm^{-1} were given by complexes thought to contain NO^{-}. Classification in this manner was not totally satisfactory, and it was suggested[10] that frequencies in the 1500 - 1700 cm^{-1} range might be better assigned to NO^{-}.

It is now recognised that ν(NO) is not a good diagnostic criterion for the nature of the M-NO bond. X-ray studies have shown that structurally, metal nitrosyls fall into two broad classes - those in which the M-N-O fragment is linear or nearly so, and those in which the M-N-O bond angle is around 120°. The former were regarded as being complexes of NO^+ and the latter of NO^-.[4] A recent n.m.r. study[11] has shown that the ^{15}N chemical shifts of nitrosyl complexes fall into two very distinct groups according to whether the MNO fragment is bent or nearly linear. However a molecular orbital approach to the bonding in metal nitrosyls suggests that this classification is inadequate to account for the observed chemical and physical properties. Enemark and Feltham[5] provide an alternative bonding description using a molecular orbital correlation method. For mononitrosyls this involves treating the MNO fragment as an "inorganic functional group" and examining the effect of perturbing the hypothetical triatomic MNO by coordination of additional ligands to M. In this approach it is not necessarily the case that all linear MNO fragments are due to bonding of NO^+ and all bent fragments due to bonding of NO^-. The geometry depends on the nature of the highest occupied molecular orbital. Effectively, if this is bonding or non-bonding, a linear species results; if it is antibonding, a net gain in energy can be achieved if the system distorts from linearity and so a bent species is formed.

The bending of the MNO fragment has important consequences for the reactivity of metal nitrosyls. Firstly, it arises out of a formal transfer of an electron pair from the metal to the ligand, resulting in coordinative unsaturation of the metal atom, with important implications for catalysis[8]; and secondly the electron pair goes into an orbital largely localised on the nitrogen atom, rendering the NO group susceptible to attack by electrophilic reagents.

The formalism by which the metal is assigned an integral oxidation state and the nitric oxide is regarded as NO^+ or NO^- can still be useful and will be used from time to time in this work, though it is recognised that it does not necessarily represent physical reality.

1.2 Sodium nitroprusside and its medical applications.

Perhaps the best known of nitrosyl complexes is the nitroprusside anion, $[Fe(CN)_5NO]^{2-}$. Sodium nitroprusside was first prepared by Playfair in 1850[12], and its chemistry has been reviewed by Swinehart[13]. The ion is generally regarded as containing Fe^{II} and NO^+, though it has been suggested[14] that it might be better formulated as an Fe^{III} complex. Many of the reactions of the complex, including the well-known colour test for the presence of sulphur in organic compounds, involve nucleophilic attack at the nitrosyl group, so in this sense the

NO^+ formalism is reasonable. Swinehart[13] has compared the relative ease of conversion of NO^+ to nitrite by reaction with hydroxide when the NO^+ is present as the free ion and when it is complexed by $Fe(CN)_5^{3-}$. Before leaving the question of the $Fe^{II}-NO^+$ description it is worth noting that a molecular orbital calculation on nitroprusside[15] gives a charge distribution of $Fe^{+0.32}-NO^{+0.46}$, quite different from the formal assignment of Fe^{2+} or Fe^{3+}.

Interest in the chemistry of nitroprusside in this department arose as a result of reports of its use as a hypotensive agent in anaesthesia[16,17]. Infusion of solutions of sodium nitroprusside into patients results in a rapid lowering of blood pressure. The compound is extremely useful in this respect because the effect is very quick, the blood pressure can be controlled according to the rate of infusion, and when infusion is stopped the blood pressure returns to normal without overshooting. It has been suggested that the physiological effect of nitroprusside may be due to reaction of the NO^+ ligand with amine[18] or thiol[19,20] groups in cell receptor sites in the smooth muscle membrane. Studies of the mechanisms of sodium nitroprusside reactions have been undertaken in the hope of eventually gaining some understanding of the physiological effect, and also for comparison with other nitrosyl compounds which, if they showed similar <u>in vitro</u> behaviour, might be expected to show a similar hypotensive effect <u>in vivo</u>. Such a mechanistic study is described in Chapter Four of this work.

The clinical use of sodium nitroprusside suffers from a serious drawback in that it has been reported[21] that it breaks down in a reaction with haemoglobin, releasing free cyanide , with potentially disastrous consequences for the patient. In view of this, studies of nitrosyl complexes which could act as safe alternatives to nitroprusside, either because they do not contain cyanide or do not release it, are clearly important. In this respect the complexes $Cr(H_2O)_5NO^{2+}$ [22] and $Ru(CN)_5NO^{2-}$ (the ruthenium analogue of nitroprusside)[23], among others, have been investigated recently in this department. The reported breakdown of nitroprusside is a very surprising reaction, in view of its very high thermodynamic stability, and recent work has suggested that the cyanide release is due not to a reaction with haemoglobin, but to a photolytic reaction of nitroprusside resulting from exposure of infusion solutions to light[18,24,25]. This conclusion has led to disagreement[26,27], and in an attempt to settle this point the study described in Chapter Five was undertaken.

1.3 Roussin's salts and iron-sulphur clusters.

The earliest known iron nitrosyls containing sulphur are the Roussin salts, the black $M[Fe_4S_3(NO)_7]$ and the red $M_2[Fe_2S_2(NO)_4]$. These compounds were first prepared in 1858[28], and analysed in 1882[29]. Crystal structure determinations[30,31] of the black salt show that the structure is based on a tetrahedron

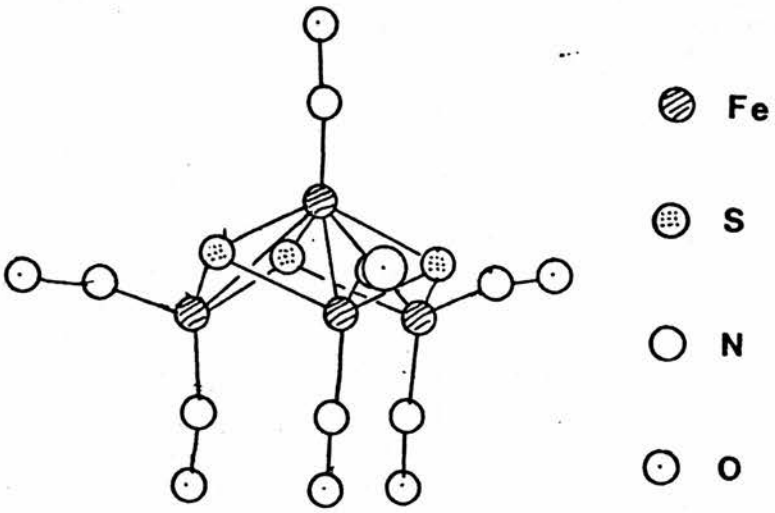

Fig. 1.1 The structure of the anion of Roussin´s black salt. (based on ref. 31)

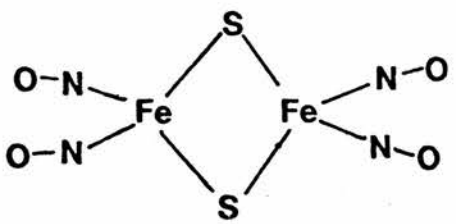

Fig. 1.2 The anion of Roussin´s red salt. (Based on ref. 33)

of iron atoms, three of which carry two nitrosyl groups while the other has only one (Fig. 1.1). The unique Fe-NO group is essentially linear with an angle of 176^{o}, while the others, though deviating slightly from linearity ($\angle$Fe-N-O 165 - 171^{o}), do not fall into the class of bent nitrosyls. The small but significant distortion from linearity was explained[31] on the basis of the bonding ideas of Enemark and Feltham[5]. A determination of the ^{15}N n.m.r. spectrum of Roussin's black salt[32] has shown peaks in the area expected[11] for linear groups.

The crystal structure of the red salt has been determined only recently[33]; it consists of a planar Fe_2S_2 rhombus with the NO groups in a plane perpendicular to that containing the Fe_2S_2 ring (Fig. 1.2). This result was expected by analogy with the ethyl ester of the red salt, $Fe_2(SEt)_2(NO)_4$, the structure of which was determined in 1958[34].

On the basis of ν(NO) in the infra-red spectrum being greater than 1700 cm^{-1},[9,31] and the near linearity of the FeNO fragments, the complexes can be <u>formally</u> regarded as containing NO^{+} (bearing in mind the comments made earlier). If the sulphur is regarded as S^{-II}, then the formal oxidation state of iron in the red salt and its esters is -I, while the black salt formally contains one Fe^{I} and three Fe^{-I}. This assignment of formal oxidation states is used by Chu and Dahl[31] in a qualitative cluster molecular orbital model which describes the bonding in the black salt anion.

There is an interesting connection between Roussin's salts and nitroprusside. Comparison of the compounds would suggest they have little in common beyond the possession of nitrosyl groups bound to iron which may be formally regarded as NO^+. However Roussin considered them to be close analogues. This conclusion was based on similarities in the preparative methods and a surprisingly simple interconversion of the two, since he obviously could not know the structures or the true formulae of the compounds. This point is considered further in Chapter Two.

As well as being metal nitrosyl compounds, the Roussin salts and esters are also of interest as iron-sulphur clusters. This is an area of chemistry which has been attracting considerable interest in recent years[35-37], as a result of the rôle of this type of cluster in biological systems. Iron-sulphur proteins are involved in a wide range of biochemical processes, including electron transport sequences in photosynthesis, reduction of nitrite, hydroxylation of steroids, respiration and nitrogen fixation. The first iron-sulphur protein to be discovered, ferredoxin, is an electron donor in the process of reducing nitrogen to ammonia. The active sites of these protein molecules are iron-sulphur clusters of the types FeS_4, Fe_2S_2 and Fe_4S_4, which can exist in oxidised and reduced forms, enabling them to participate in electron transfer reactions. While the Fe_4S_4 sites have electrons delocalised over the whole cluster, the reduced form of the Fe_2S_2 site in putidaredoxin has been shown to contain distinct Fe^{II} and Fe^{III} atoms, which is an interesting

observation in view of the above discussion regarding formal iron oxidation states.

1.4 Roussin´s red methyl ester and carcinogenesis.

The methyl ester of Roussin´s red salt, possibly derived from naturally occurring iron-sulphur clusters, has recently been implicated in causing cancer. In China, a survey was undertaken of the distribution of the various types of cancer throughout the country. In the case of cancer of the oesophagus, a remarkably high incidence was found in the Linxian region, where one adult in four suffered from this type of cancer. An investigation was begun to find out why this should be , and several possible causes were discovered[38]. The water supply was found to be high in nitrite and nitrate, while the soil was low in molybdenum, and an imporant constituent of the local diet was a particular type of cabbage-like vegetable. This was "pickled" by soaking in water and pressing down with stones, and consumed after some weeks by which time it was essentially rotted and contaminated with fungus. This foodstuff was considered a great local delicacy, but its consumption was shown to be connected to the high incidence of oesophageal cancer.

Chinese chemists investigating this matter have isolated the methyl ester of Roussin´s red salt, $Fe_2(SCH_3)_2(NO)_4$, from the pickled vegetable[39]. This is believed to be the first isolation of such iron-sulphur nitrosyls from natural sources. Extracts of

the pickled vegetable were shown to contain mutagenic compounds[40], and studies on the red salt ester itself indicated that though only weakly mutagenic it may act as a tumour promoter[41] (i.e. it cannot cause a tumour itself but it can assist one to grow once started). It has also been reported[42] that the red salt ester acts as a nitrosating agent, leading to the formation of nitrosamines, which are known carcinogens.

It is conceivable that the vegetable iron-sulphur proteins are broken down during the peculiar pickling process, and that the clusters react with the nitrite present in the water to form the Roussin ester. The physiological effect of this compound may then be due to its acting as a nitrosating agent to produce carcinogens, and also to its action as a tumour promoter.

Studies of the chemistry of the Roussin esters and related nitrosyl compounds in the light of the above are currently being undertaken in this department[43]. This present work has not been directly concerned with the possible nitrosation reactions of these compounds; however in Chapter Three are presented the results of a study which arose out of a comparison of the Roussin esters with analogous carbonyl compounds.

1.5 Historical Note.

Francois Zacharie Roussin is remembered now primarily as the discoverer of the compounds which bear his name. However a short biography[44] written in the 1890's reveals that Roussin lived quite an interesting life. He worked as a pharmacist in the French army during the third quarter of the nineteenth century, having become interested in chemistry at the age of 17 (and caused a few explosions at home as a result of his early experiments!) and having taken a course as an intern in a Paris hospital. He was also a chemical consultant at the Palais de Justice in Paris, and was involved with a number of famous criminal cases, including a bomb plot against Napoleon III. During the rule of the Commune in Paris in 1871, Roussin was arrested and imprisoned for six days before his wife and a colleague, fearing for his life, managed to secure his release. Within a few hours an order for his re-arrest was issued, but in the interim he had managed to escape from Paris by bluffing his way past the guards.

Roussin's chemical researches included work on naphthalene and colouring materials for the paint industry, this latter despite the fact that he was colour blind and had to rely on his wife to tell him the true colour of a sample! In 1878, after 20 years' service in the army, he was awarded the Légion d'Honneur.

Roussin died in rather bizarre circumstances in 1894. The account of his death given in Chasles' biography is worth reproducing:

"One Sunday evening ..... Roussin, in spite of his being so punctual, did not return for dinner at the usual hour. Madame Roussin, full of anxiety, went to his laboratory. A terrible sight awaited her: her husband lying dead in an asphyxiating atmosphere of gas issuing from the gas mantle. A sense of smell, which would have alerted Roussin, had been destroyed by his long researches on naphthalene. .....

"Roussin fell on the field of honour, victim of his profound love of science."

A final curious fact about Roussin is the trouble modern authors have with his initials[45]. As mentioned above, his Christian names were Francois Zacharie, though he seemed to use the second of these preferentially. However he has been cited as Z. Roussin[46], J. Roussin[34,39], and M.L. Roussin[31]. "Z" is correct, while "J" appears to be a simple error, but the paper containing the account of his discovery of the red and black salts is published as by M.L. Roussin. It is thought[45] that the "M" stands for "Monsieur" while the "L" results from a misreading of Roussin's signature on the manuscript, which at that time would probably have been submitted handwritten. A copy of Roussin's signature in Chasles' biography reveals that the

handwritten "Z" could easily be mistaken for "L".

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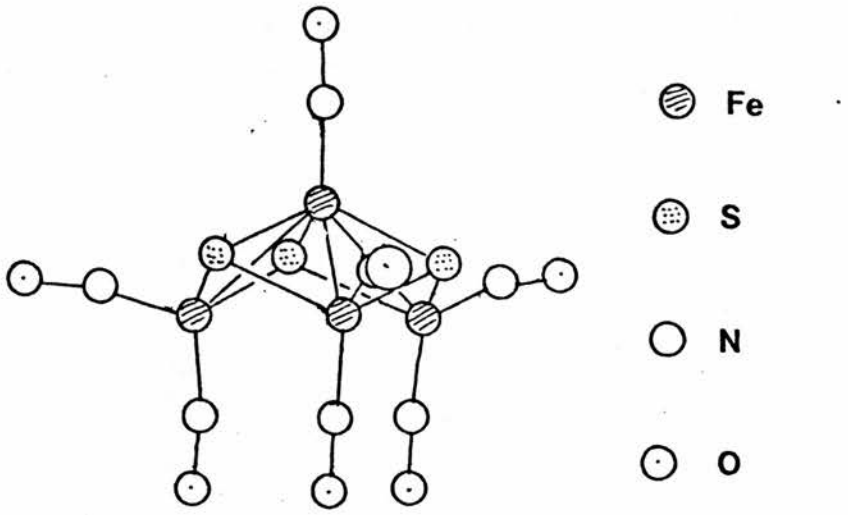

Fig. 2.1 The structure of the anion of Roussin´s black salt. (Based on ref. 4)

CHAPTER TWO.

TRANSFORMATIONS OF ROUSSIN'S BLACK SALT.

2.1 Introduction.

Salts of the black anion $[Fe_4S_3(NO)_7]^-$ were first synthesized by Roussin[1] in 1858, in an attempt to prepare analogues of the recently discovered nitroprusside ion; the correct empirical formula was given 24 years later by Pawel[2]. The crystal structure of the caesium salt has been determined by Johanson and Lipscomb[3], and more recently that of the tetraphenylarsonium salt was determined by Chu and Dahl[4]. The structure of the anion is shown in Fig. 2.1 . It consists of four iron atoms in a tetrahedron, with a triply-bridging sulphur atom above each of three faces of the tetrahedron. The iron atom at the apex of the tetrahedron is bonded to one nitrosyl group, the three sulphur atoms and the three basal iron atoms, whereas each basal iron atom is bonded to two nitrosyl groups, two sulphurs and the apical iron. There are no bonds between the basal iron atoms.

Roussin's salts, both the black $M[Fe_4S_3(NO)_7]$ and the red $M_2[Fe_2S_2(NO)_4]$, have attracted relatively little attention since their discovery. The work which has been done has been largely concerned with structural or biochemical aspects. Early work[5]

was concerned with the constitution of the compounds, and specifically with whether or not the iron was present as Fe^{I}. As a result of a spectroscopic study, Manchot and Linckh[6] concluded that Fe^{I} was present; they also proposed that the structure of the black salt could be represented by the formula $[Fe(NO)_2S]_3.FeNO.K$, which is quite close to the correct structure. A similar representation had been proposed earlier by Bellucci and de Cesaris[5]. Spectroscopic and magnetic susceptibility studies by Cambi and Szego[7,8] led them to formulate the compounds as containing Fe^{II} and Fe^{III}. Cambi[9] also published a paper attempting to explain the constitution of the Roussin salts by valence theory, the conclusions of which were challenged by Hieber and Nast[10].

From the modern viewpoint, the debate about the presence or absence of Fe^{I} is essentially irrelevant as the bonding in the cluster is best considered in molecular orbital terms. Dahl and co-workers have developed a qualitative cluster molecular orbital model for cubane-like Fe_4S_4 systems[11], and have given an analogous description of the bonding in the black salt anion[4]. This involves occupation of 17 out of 20 tetrairon cluster molecular orbitals, the 34 valence electrons coming from one apical d^7 iron atom (formally Fe^{I}) and three basal d^9 iron atoms (formally Fe^{-I}). Under a localised valence bond representation, this is equivalent to there being an electron-pair bond between the apical iron atom and each basal iron atom, as described above.

The infra-red[12,14] and Mössbauer[13,14,15] spectra of the complex have been obtained. Kostiner et al. in their Mössbauer study[14] claimed to be able to differentiate between the two different types of iron atom, but more recent work by Sedney and Reiff[15] indicates that this is not possible.

Biochemical investigations have shown that the black salt displays germicidal and antibacterial properties[16-19]. It has been used in the determination of biological amines[20], and its interaction with the amino groups of enzyme active sites has been studied[21,22]. An early electrochemical investigation by Treadwell and Huber[23] involving potentiometric titrations showed that one mole of the black salt reduced three moles of ferricyanide ion, with the cluster breaking down completely in the process. Preliminary cyclic voltammetry results obtained by Wharton and McCleverty[24] show the existence of both a one-electron oxidation and a one-electron reduction product.

In his original paper[1], Roussin considers the black salt to be an analogue of nitroprusside (pentacyanonitrosylferrate(2-), $Fe(CN)_5NO^{2-}$) and reports that the two compounds can be very easily interconverted. In view of their considerably different structures, this was a surprising result. There is another early report[25] of the conversion of nitroprusside to the black salt, and more recently Wharton and McCleverty[24] obtained the black salt as a result of an attempt to prepare a completely different type of iron-sulphur-nitrosyl from the reaction of nitroprusside

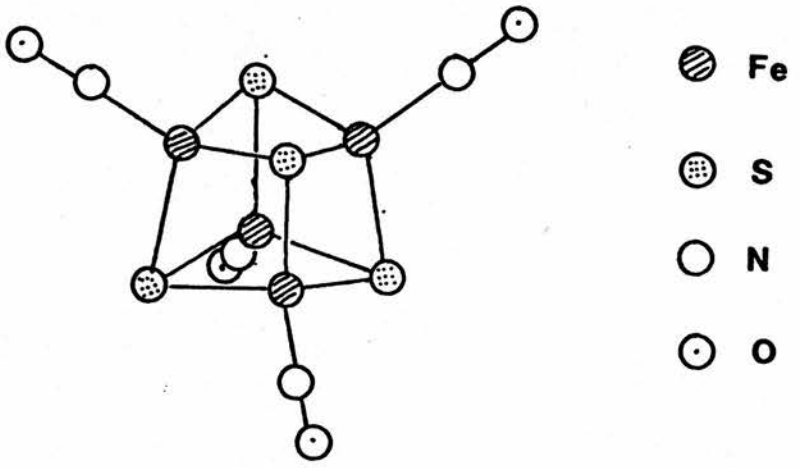

Fig. 2.2 The structure of the cubane-like tetranitrosyltetra-μ_3-thioxotetrairon. (Based on ref. 39)

with dithioacetoin. However it was felt to be worthwhile to re-investigate Roussin's very simple transformations and to put them on a quantitative basis.

Chu and Dahl[4] also unexpectedly obtained the black salt by a sodium amalgam reduction of the cubane-like tetranitrosyltetra-μ_3-thioxotetrairon, $Fe_4S_4(NO)_4$. The structure of this compound (Fig. 2.2) consists of a cube with iron and sulphur atoms at alternate vertices, with a linear nitrosyl group attached to each iron atom. This structure is closely related to that of Roussin's black salt, which can be considered to be derived from it by removal of a sulphur atom and its replacement by three nitrosyl groups, together with an electron to form the monoanion. In view of this structural similarity, and of Chu and Dahl's result, an attempt was made to synthesize the cubane starting from the black salt. The analogous reaction of the black salt with selenium was also investigated, and the selenium analogue of the black salt was prepared.

The original preparation of the cubane[26] involved reaction of elemental sulphur with bis(irontricarbonylnitrosyl)mercury, $Hg[Fe(CO)_3NO]_2$. The reaction of this compound with polysulphide was investigated, and was found to give Roussin's black salt.

Benfey[27] has recently considered the possibility of iron-sulphur clusters as antidotes for mercury poisoning, and Roussin's original paper[1] mentions a reaction of the black salt with mercury oxide; the results of some preliminary experiments

in this line are given here. Finally in this chapter are described the results of attempts to repeat the synthesis of the anion $Fe_3S_2(NO)_5^-$, reported by Dymicky[28] to be formed by a modification of the method of synthesis of Roussin's black salt. This ion would be of interest as a new type of iron-sulphur-nitrosyl compound.

2.2 Experimental.

2.2.1 Materials and instruments.

All chemicals were reagent grade and were used without further purification. Roussin's black salt was prepared by a slight adaptation of the method given by Brauer[29], bis(irontricarbonylnitrosyl)mercury by the method given by King[30], and authentic tetranitrosyltetra-μ_3-thioxotetrairon by the method of Gall, Chu and Dahl[26]. Sodium hydrogen sulphide had been prepared by Mr. V. Chaipanich.

Infra-red spectra were obtained either as Nujol mulls or KBr discs on a Perkin-Elmer 257 spectrometer. Ultra-violet and visible spectra were obtained on a Unicam SP-800 spectrometer using 1 cm cells.

Stock nitroprusside solutions were kept in the dark.

2.2.2 Conversion of nitroprusside into black salt.

$Na_2[Fe(CN)_5NO].2H_2O$ (1.0 g) was dissolved in 150 ml. water. H_2S was bubbled through the solution for about 10 minutes, after which the colour had changed to a deep cloudy blue. The solution was boiled for about 5 minutes. As it came to the boil its colour changed to a muddy green. It was filtered hot, left to cool, and evaporated to dryness.

The residue was extracted with ether. Some unidentified blue material and elemental sulphur remained undissolved. The very dark ether extracts were evaporated to dryness. 0.21 g of crystalline black material was recovered. The i.r. and u.v./visible spectra of this material were identical with those of Roussin's black salt. Assuming the material to be the anhydrous sodium salt, $Na[Fe_4S_3(NO)_7]$, this represents a yield of 78% based on NO.

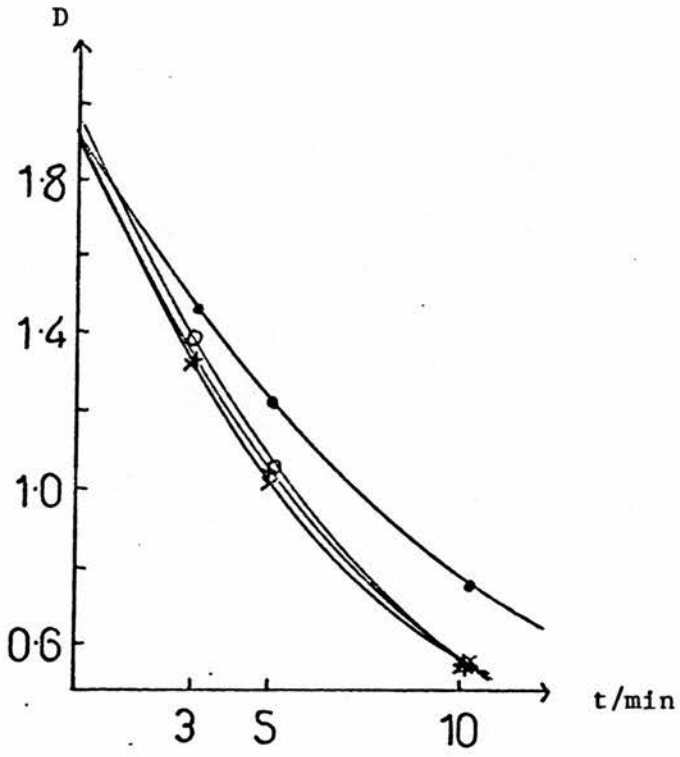

Fig. 2.3 Standard absorbance vs. time curves for nitroprusside + sulphide.

2.2.3 Conversion of black salt into nitroprusside.

2.2.3.1 Standardisation of the analytical method for nitroprusside.

1 ml. of a stock solution of sodium nitroprusside (1.75×10^{-2}M) was pipetted into a 25 ml. volumetric flask. NaSH (0.08 g, 1.43×10^{-3} mol., >80x excess), dissolved in about 5ml. water, was added and a stop-clock started simultaneously with the addition. The solution was made up to the mark with distilled water and wrapped in aluminium foil to protect it from light as much as possible. The optical density of the solution at the absorption maximum was measured 3, 5, and 10 minutes after adding the NaSH. These data were plotted and the resultant curve extrapolated to zero. Four runs of the experiment gave reproducible values on extrapolation (Fig. 2.3) from which a "t=0 extinction coefficient" of 2740 l mol^{-1} cm^{-1} was calculated.

2.2.3.2 Reaction of black salt with cyanide.

0.10 g black salt was dissolved with stirring in ~35 ml water. The solution was transferred to a 50 ml. volumetric flask, 0.5 g KCN was added and the solution was made up to the mark and wrapped in aluminium foil. After about one hour (preliminary semi-quantitative experiments had indicated that the

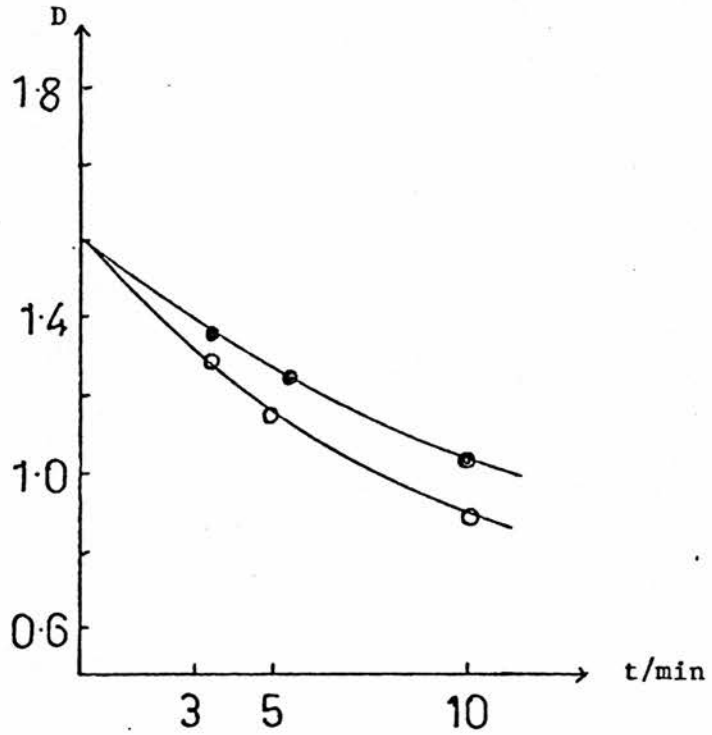

Fig. 2.4 Absorbance vs. time curves for black salt/cyanide reaction mixtures.

nitroprusside concentration was at a maximum at about this time), 1 ml was withdrawn and the analytical method for nitroprusside described above was applied.

The results obtained from two runs are plotted in Fig. 2.4. The curves give the same intercept when extrapolated to zero. From this value and the above extinction coefficient, the total yield of nitroprusside was calculated to be ~100%, $\pm$ the limits of error of the analytical method.

2.2.3.3 Discussion of the method of analysis for nitroprusside.

The method described above of determining the concentration of nitroprusside was not intended to give precise results, merely a reasonable approximation to the percentage conversion. The reaction of nitroprusside with sulphide has been investigated by Rock and Swinehart[31]. They observe an absorption maximum at 540 nm whereas the observed maximum in this work was at 574 nm; Mulvey and Waters[31b] observe a maximum at 572 nm at pH values at which SH^- is present. Rock and Swinehart also report that the initial very intense colour gradually fades, but that the spectrum of the faded solution is "moderately stable" with an extinction coefficient of about 2100 l mol^{-1} cm^{-1}. In this work no stabilisation of the colour was observed. However Rock and Swinehart worked under "subdued light", whereas the solutions used here were exposed to normal laboratory lighting while they were being made up and during transfer to the spectrophotometer

cell. This is one of the main potential sources of error in the method. Another is the extrapolation of a curve based on only three points. The weighing of 0.08 g NaSH was done on a top-pan balance, and so was not very precise, but this is not likely to be such a serious error as what was required was a greater than 80x excess of SH^- ion over nitroprusside - this is necessary to convert the nitroprusside quantitatively to the intensely-coloured species, according to Rock and Swinehart.

Calculation of the yield is based on the assumption that the purple complex is the only absorbing species at 574 nm, whereas the black salt itself has an extinction coefficient at this wavelength of about 2400 1 mol^{-1} cm^{-1}.

In spite of the above drawbacks, the method is reproducible - the intercepts of the four standard curves were within 3% of the mean. It is felt that the method is good enough for its intended purpose, even though the error may be as high as 15-20%.

2.2.4 Preparation of cubane cluster compounds from Roussin's black salt.

(These experiments were carried out by Mr. M.J. Greenhill-Hooper and Mr. Alan J. Kennedy as part of Senior Honours undergraduate projects under the supervision of Dr. C. Glidewell.)

Roussin's black salt (1 g) and elemental sulphur (1 g) were refluxed in 100 ml of toluene for 16 hours under nitrogen. The solvent was evaporated and the product purified by chromatography on a 6 cm silica column, using chloroform as solvent. Evaporation of the solvent yielded 0.6 g of shiny black plates, m.p. 95-97°C and showing a single sharp nitrosyl band in the i.r. at 1790 cm^{-1}, identical with the authentic tetranitrosyl-tetra-μ_3-thioxotetrairon. This represents a yield of about 72%, though there is some doubt about whether the product was obtained completely free from solvent.

Roussin's black salt was reacted with red selenium (obtained by reaction of SeO_2 with sulphite) in the same way. Filtration of the toluene solution through a bed of Hyflo proved sufficient to purify the product. Evaporation of the solvent gave a material which decomposed at about 130°C and which showed the characteristic single sharp NO stretching vibration at 1790 cm^{-1} in the i.r.. This is believed to be the cubane $Fe_4S_3Se(NO)_4$, though an analytically pure sample was not obtained. The yield was 24%.

2.2.5 Preparation and characterisation of the selenium analogue of Roussin's black salt.

A solution of sodium hydrogen selenide was prepared by the method of Klayman and Griffin[32] as follows: a mixture of 7 g grey selenium and 50 ml water was stirred under nitrogen, and a solution of 7 g sodium borohydride in 50 ml water was added dropwise with stirring. Vigorous evolution of H_2 occurred; when this had subsided the mixture was allowed to cool, giving a clear grey solution above a white precipitate of $Na_2B_4O_7.10H_2O$.

$NaNO_2$ (8 g) was then added with stirring; the solution turned dark red. The solution was boiled, and 20 g $FeSO_4.7H_2O$ in 160 ml water was added in one portion, followed by gradual addition of 35 ml 25% ammonia solution. The solution was then boiled for 30 minutes, filtered hot, cooled and filtered a second time; the cold filtrate was extracted with ether, and the extracts dried (Na_2SO_4) and evaporated to yield a black solid. The i.r. and u.v. spectra of this material were similar to those of the authentic sulphur-containing species $[Fe_4S_3(NO)_7]^-$, and the presence of an i.r. absorption at ~1400 cm^{-1} suggested that this was the ammonium salt $NH_4[Fe_4Se_3(NO)_7]$. The yield at this stage was about 17%.

TABLE 2.1 - U.V./Visible Spectra of black salts.

Compound	λ/nm (ε /$M^{-1}cm^{-1}$)		
$NH_4[Fe_4Se_3(NO)_7].H_2O$	570 (2700)	360 (16 000)	279 (27 000)
$NH_4[Fe_4S_3(NO)_7].H_2O$	560 (2400)	350 (15 400)	260 (26 700)

The form of these spectra is a series of shoulders; only the band at lowest wavelength shows a distinct maximum. The selenium compound also shows a discernible inflection at 432 nm (ε 10 000).

TABLE 2.2 - Infra-red Spectra of black salts.

Compound	ν(NO)/cm^{-1}.
$Ph_4As[Fe_4Se_3(NO)_7]$	1790m, 1720s, 1690m.
$Ph_4As[Fe_4S_3(NO)_7]$	1790m, 1730s, 1690m.

The crude ammonium salt was converted to the tetraphenylarsonium salt as follows: equimolar quantities of $NH_4[Fe_4Se_3(NO)_7]$ and $Ph_4AsCl.H_2O$ were dissolved in water and the solutions mixed. The whole was extracted with chloroform and the dried extracts evaporated. The resulting solid was recrystallised from methanol to yield $Ph_4As[Fe_4Se_3(NO)_7]$ as shiny black crystals.

Anal. : Found: C, 27.2%; H, 1.8%; N, 9.2%; Fe, 22.3%; Se, 22.6%.

Calc. for $C_{24}H_{20}N_7O_7AsFe_4Se_3$:

C, 27.4%; H, 1.9%; N, 9.3%; Fe, 21.2%; Se, 22.5%.

The crude ammonium salt was recrystallised from water and its u.v./visible spectrum obtained; the i.r. spectrum of the tetraphenylarsonium salt was also obtained. The data are presented in Tables 2.1 and 2.2, together with those for the sulphur compound for comparison.

2.2.6 Reaction of $Hg[Fe(CO)_3NO]_2$ with polysulphide.

Bis(irontricarbonylnitrosyl)mercury (1.5 g, 2.8 mmol) was dissolved with stirring and warming in 80-90 ml methanol. A solution of 1 g $Na_2S.9H_2O$ and 0.5 g S_8 in 10 ml water (previously dissolved by stirring and heating) was added. The red solution quickly turned black and there was steady gas evolution. The mixture was stirred under nitrogen for about an hour. It was then filtered and the filtrate was evaporated to dryness. The

solid residue was extracted with ether until the extracts were almost colourless (~300 ml). The extracts were dried over sodium sulphate and evaporated to dryness, giving 0.3 g of black powder which on recrystallisation from water was identified as Roussin's black salt by its characteristic i.r. and u.v. spectra. The yield of crude product was about 68% based on NO.

2.2.7 Reaction of Roussin's black salt with mercury compounds.

One of several qualitative observations made by Roussin on reactions of the black salt was that its solutions were decomposed by mercury oxide. In this investigation the initial experiments were carried out with mercuric chloride and nitrate, and mercurous nitrate. The investigation was not pursued beyond the preliminary stages.

A series of qualitative experiments was carried out, involving mixing solutions of Roussin's black salt with solutions of $HgCl_2$, $Hg(NO_3)_2$ and $Hg_2(NO_3)_2$. On a millimole scale it was found that $HgCl_2$ reacted with the black salt with evolution of NO and formation of a sand-coloured precipitate. Excess $HgCl_2$ and standing for some days were necessary to effect complete decolourisation of the black salt solution. Mass spectrometry suggested that the precipitate could contain elemental sulphur and mercurous chloride Hg_2Cl_2.

A solution of Roussin's black salt was divided into 4 equal portions. To the first was added $HgCl_2$ solution, to the second $Hg(NO_3)_2$ solution, to the third $Hg_2(NO_3)_2$ solution and to the fourth water to maintain equal volumes and so provide a reference for comparing colour intensity. All three solutions to which mercury salts had been added decolourised rapidly; the extent of decolourisation was not the same in the three cases, but no attempt had been made to add the same quantities of mercury salts. In the solution to which $HgCl_2$ had been added, a grey-white precipitate formed and there was a smell of NO; in the other solutions the precipitate was dark and there was no noticeable smell. On standing overnight the latter two solutions were completely colourless and the precipitate appeared lighter in colour, while the first solution showed signs of precipitated ferric oxide.

Spot tests on various reaction mixtures consistently showed the absence of nitrite and sulphide or any sulphur oxo-anions, nor were any black or red precipitates of mercury sulphides observed. Tests for Fe^{2+} and Fe^{3+} indicated the presence of Fe^{2+} in mixtures containing the mercury nitrates, but of Fe^{3+} in mixtures containing mercuric chloride, with some indication that the amount of Fe^{3+} present increased on standing overnight.

Attempts to put this investigation on a quantitative basis were relatively unsuccessful, though they did indicate that the decolourisation was fast at concentrations of ~0.005M but very much slower (overnight) at ~0.0005M.

2.2.8 Attempted preparation of salts of $[Fe_3S_2(NO)_5]^-$.

Solutions of NaSH, KSH and NH_4SH were prepared by saturating solutions of Na_2S, KOH and NH_4OH with H_2S. $(NH_4)_2S$ was then prepared by adding NH_4OH to the solution of NH_4SH.

The preparations were then carried out as described by Dymicky[28]. 75 ml of MSH solution (M = K,Na) containing 0.25 mol, MNO_2 (0.25 mol) and 275 ml water were placed in a 1 l. conical flask, and the mixture stirred and heated to boiling. The colour changed to yellow, deepening to orange-brown on reaching the boiling point (in the case of the K^+ salt but not the Na^+). The mixture was cooled, filtered and placed in a 3 l. beaker. A solution of 69.5 g $FeSO_4.7H_2O$ in 500 ml water was then added in portions, with stirring. The pH was kept above 5 by addition of 20% MOH solution. The mixture was then heated to ~85°C for 30 minutes; nitric oxide was given off at this point unless the pH was first adjusted to 7.1. The solution was filtered hot and left to stand overnight at 4°C and alkaline pH. The crystals which formed were filtered off and dried under vacuum over P_2O_5; further crude product was obtained by concentrating the mother

Table 2.3 - Electronic spectra of M^I salts. (ϵ/l $mol^{-1} cm^{-1}$)

M^I	Solvent	λ/nm:	560	350	260
NH_4^+	H_2O		2400	15400	26700
Na^+	H_2O		2380	14400	25500
K^+	H_2O		2460	15800	27400
	EtOH		2660	16400	28200
Ph_4As^+	EtOH		2530	15500	29450[a]

[a] This band displays fine structure presumably due to the Ph_4As^+ ion.

liquor and extracting with ether. For M = K, the total yield was about 2 g; for M = Na, about 7.5 g was obtained.

The ammonium salt was prepared similarly, except that the pH was kept above 9 by addition of concentrated ammonia solution. The products were recrystallised from water and quantitative u.v./visible spectra obtained. A sample of the K^+ salt was converted to the Ph_4As^+ salt as being the most conveniently analysed:

Found, C, 31.54% ; H, 2.19% ; N, 10.59% .

Calc. for $C_{24}H_{20}N_5O_5AsFe_3S_2$:

C, 37.68% ; H, 2.63% ; N, 9.15% .

Calc. for $C_{24}H_{20}N_7O_7AsFe_4S_3$:

C, 31.57% ; H, 2.21% ; N, 10.74% .

These results, together with the identity of the spectra (Table 2.3), show that what has been formed is in fact Roussin´s black salt.

2.3 Results and discussion.

2.3.1 The interconversion of Roussin's black salt and nitroprusside.[33]

This re-investigation of the original work, using procedures essentially the same as those described by Roussin, has confirmed that his results are correct, and has shown that the products are obtained in high yields and not as minor by-products. This point was not clear from Roussin's work as no yields were quoted, and indeed in the reaction of the black salt with cyanide, no mention was made of how nitroprusside was identified as a product; presumably it was isolated as a solid. In this work, the presence of nitroprusside was confirmed qualitatively, prior to the quantitative experiments, by testing the reaction mixture with the carbanion of malononitrile. Formation of a deep red colour indicated the presence of nitroprusside[34]; the black salt itself does not give a colour with malononitrile carbanion.

The black salt/cyanide reaction mixtures turned completely colourless after two days' standing in the dark. The u.v. spectrum at this stage was found to be identical with that of hexacyanoferrate(II), and a quantitative spectrum indicated that all the iron originally in the black salt was now present as hexacyanoferrate(II). The formation of hexacyanoferrate(II) is a consequence of the further reaction of nitroprusside in the presence of excess cyanide at the high pH (~11) of this reaction

mixture. This occurs via the nitro species $Fe(CN)_5NO_2^{4-}$ formed by the reaction of nitroprusside with hydroxide[35,36]:

$$[Fe(CN)_5NO]^{2-} + 2OH^- \text{-----} > [Fe(CN)_5NO_2]^{4-} + H_2O$$

$$[Fe(CN)_5NO_2]^{4-} \xrightarrow{\text{slow}} NO_2^- + [Fe(CN)_5]^{3-}$$

$$[Fe(CN)_5]^{3-} + CN^- \xrightarrow{\text{fast}} [Fe(CN)_6]^{4-}$$

At pH < 10, it was found that cyanide ions showed no reaction with nitroprusside.

In the reaction of Roussin´s black salt with cyanide, therefore, nitroprusside is formed virtually quantitatively, but at the pH of the reaction mixture it slowly goes on to give hexacyanoferrate(II). The sulphur from the black salt reacts with excess cyanide to form thiocyanate, according to Roussin, and thiocyanate was detected qualitatively in the reaction mixture, as was nitrite, which is expected from the reaction of nitroprusside with hydroxide.

In the reaction of nitroprusside with H_2S, the limiting factor in the yield of black salt is the number of nitrosyl groups, as the NO:Fe ratio in nitroprusside is 1:1 but in the black salt is 7:4. The fate of the "excess" iron and cyanide ligands was not investigated; the insoluble blue material formed is probably Prussian Blue or a related substance, while in a solution saturated with H_2S, thiocyanate and iron sulphides are also reasonable possible products.

Wharton and McCleverty[24] have also obtained the black salt from nitroprusside, in yields of 63% or greater, in an attempt to prepare a dithiolene complex by reaction of nitroprusside with dithioacetoin. It seems possible that under their reaction conditions the dithioacetoin is hydrolysed to give H_2S in solution, and that this is the reacting species leading to formation of the black salt.

These results testify to the remarkable stability of Roussin´s black salt. The relatively complex structure forms very readily, and its synthesis from Fe^{II}, NO_2^- and S^{2-} can probably be considered the first example of "spontaneous self-assembly"[37] of an iron-sulphur cluster. The reports of its formation from nitroprusside seemed so surprising because of the known thermodynamic stability of that ion as well as the considerable structural differences.

The one structural feature which nitroprusside and the black salt have in common is the linear Fe-NO group. Whether or not these groups remain intact during the transformations is not known. In the reaction of the black salt with cyanide it seems intuitively likely that four of the seven nitrosyl groups remain bound to Fe throughout, the other three being excess to the formation of nitroprusside. In the reverse reaction, breaking of Fe-NO bonds must occur to some extent, because of the greater NO:Fe ratio in the product, and it may be that all such bonds in the nitroprusside are broken and the black salt then forms by

self-assembly, as in the method of synthesis.

2.3.2 Cubane-like cluster compounds.

Iron-sulphur cluster compounds are currently attracting considerable interest as a result of their rôle in biological systems[37,38]. The active sites of many enzymes contain "cores" of the type FeS_4, Fe_2S_2 or Fe_4S_4, and these play an important part in biological redox reactions. Synthetic analogues which can act as model compounds for the natural iron-sulphur cores have been made, and shown to exist in several stable oxidation states.

A related compound is the nitrosyl $Fe_4S_4(NO)_4$, prepared and studied by Gall, Chu and Dahl[26,39]. This compound contains the cubane-like Fe_4S_4 core, though it is not a direct analogue of a biochemical site. The initial attempts to characterise the monoanion of this species resulted in the unexpected formation of Roussin's black salt[4]. It has now been shown that the reverse transformation is also possible. The reaction of the black salt with elemental sulphur in refluxing toluene is analogous to the original preparation of the cubane from bis(irontricarbonyl-nitrosyl)mercury, and is a more convenient synthetic route, as the yield is greater (70% vs. 20%[39]) and Roussin's black salt is readily available from simple starting materials, whereas the preparation of bis(irontricarbonylnitrosyl)mercury requires overnight reflux and the use of iron pentacarbonyl and mercuric

cyanide[30].

It is of interest to speculate on mechanistic possibilities for this transformation. It is possible to conceive of the black salt framework remaining intact, and a sulphur atom being added to the base of the iron tetrahedron, with loss of three NO groups. It is also possible, and perhaps more likely, that the cluster first breaks down completely and then is re-assembled. When elemental sulphur is added to a solution of Roussin's black salt in DMF, an e.s.r. spectrum corresponding to the presence of Fe(NO) and $Fe(NO)_2$ species is observed[40]; as the black salt itself does not give an e.s.r. spectrum under these conditions, this observation lends support to the idea of initial breakdown of the cluster. This possibility must occur in the reverse transformation, as there are not enough nitrosyl groups available in the cubane for formation of the black salt. Dahl's group have now succeeded in characterising the monoanion of the cubane[39], and have observed that solutions of both the neutral and the reduced species disproportionate on standing in air to give FeS and Roussin's black salt.

Reaction of the black salt with red selenium instead of sulphur also yields a product with a sharp nitrosyl absorption at 1790 cm^{-1} in the i.r., characteristic of the cubane species. Attempts to prepare and isolate the selenium cubane $Fe_4Se_4(NO)_4$ indicated that this compound was rather labile (though it has now been prepared by Dahl's group[41]); the relatively stable product of the black salt/ selenium reaction is believed to be

$Fe_4S_3Se(NO)_4$ or a more complex mixture of species $Fe_4S_{4-x}Se_x(NO)_4$, depending on the reaction mechanism.

The selenium analogue of the black salt, $[Fe_4Se_3(NO)_7]^-$, was synthesized by a method analogous to the synthesis of the sulphur compound, involving reaction of a mixture containing sodium hydrogen selenide and sodium nitrite with $FeSO_4$. The yields obtained were poor, less than 20%; however no attempt was made to develop the synthesis and it may well be possible to improve the yield. The compound was characterised by analysis of its tetraphenylarsonium salt; it was intended to use it in analogous reactions to the above, leading to the formation of cubane species, but in the event this line was not pursued further.

2.3.3 Miscellaneous reactions.

The reaction of bis(irontricarbonylnitrosyl)mercury with polysulphide resulted in the formation of Roussin´s black salt. This result is further evidence of the stability and ease of formation of the black salt. As in the case of nitroprusside, the only common structural feature between reactant and product is the Fe-NO group; the comments made above about the extent to which these groups may remain intact during the transformation are also applicable here.

The conditions of this experiment are not as vigorous as those required to convert the black salt to the cubane. However it is possible that the cubane is in fact the initial product, but that it rapidly decomposes in the aqueous alcohol solution.

The possibility that the iron-sulphur clusters of spinach protein might be antidotes for mercury poisoning has been suggested by Benfey[27], while among various reactions of the black salt recorded by Roussin[1] is that with mercury oxide, which is reported to decompose solutions immediately with formation of nitrogen oxide. A preliminary investigation into the reaction of Roussin's black salt with mercury salts was therefore begun. However the reactions turned out to be rather more complex than anticipated. Any hypothesis based on the somewhat inconclusive qualitative observations recorded must be very tentative; however what seems to be occurring is that the black salt reduces mercury(II) ions initially to mercury(I), which when mercuric chloride is the reagent precipitates as Hg_2Cl_2. In the absence of chloride ions, mercury(I) also reacts with the black salt, perhaps being further reduced to elemental mercury. The first step in this process may be a one-electron transfer from the cluster to the mercury ion, leaving an oxidised form of the cluster still intact. This possibility is consistent with the observation of a one-electron oxidation product in the cyclic voltammetric investigation of the black salt[24]. This hypothetical oxidised species may then react further with mercury and break up. These reactions could prove susceptible to study

by means of a new electrochemical technique[42], which is capable of determining reactant stoichieometries and reaction rates without interference when the reaction leads to formation of a precipitate. Further electrochemical studies on the black salt could also prove of interest, particularly isolation and characterisation of the two species detected by cyclic voltammetry. It is noted that the selenium-containing dianion $Fe_4Se_3(NO)_7^{2-}$ has been prepared[40].

The ion $[Fe_3S_2(NO)_5]^-$ has been reported[28] to be formed by a modification of the method of synthesis of Roussin's salts, using a slightly acidic reaction medium. This anion was of interest as a new iron-sulphur-nitrosyl compound related to Roussin's salts; indeed it could represent a new type of iron-sulphur cluster, as the Fe_3S_2 skeleton is not known to occur naturally, and in the synthetic compound $Fe_3S_2(CO)_9$[43], the structure of the cluster is different from that proposed[28] for $[Fe_3S_2(NO)_5]^-$.

Certain points in the report seem rather unusual. The synthesis of the ammonium salt $NH_4[Fe_3S_2(NO)_5]$ involves alkaline reaction conditions and is very similar to the literature synthesis[29] of Roussin's black salt $NH_4[Fe_4S_3(NO)_7]$. The analytical data presented for the complex purport to differentiate between Fe(II) and Fe(III), which seems an irrelevant concept in a cluster; the Fe(II):Fe(III) ratio is reported as 1.3:1 in one case and 1:1 in another which is remarkable in a 3-iron compound. The esr spectrum reported for the complex at high pH is identical to that[44] of the species

$[Fe(NO)_2]^+$; if $[Fe_3S_2(NO)_5]^-$ were to break down at high pH to give $[Fe(NO)_2]^+$, it should also release sulphide ion and McDonald, Phillips and Mower[44] have shown that $[Fe(NO)_2]^+$ in the presence of sulphide gives a different spectrum, identical to that obtained at high pH from Roussin's black salt.

In the present work[45], the potassium salt was prepared according to the method of Dymicky and converted to the tetraphenylarsonium salt for more convenient elemental analysis. The analytical results demonstrated that what had been formed was in fact Roussin's black salt. The tetraphenylarsonium, potassium, sodium and ammonium salts were shown to contain the same chromophore by the identity of their u.v./visible spectra; these were identical to that of genuine Roussin's black salt.

So far it has not proved possible to confirm the synthesis of $[Fe_3S_2(NO)_5]^-$.

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CHAPTER THREE.

ESTERS OF ROUSSIN'S RED SALT AND RELATED CARBONYL COMPOUNDS.

3.1 Introduction.

Esters of Roussin's red salt, bis(μ-organylthio)bis-(dinitrosyliron), have the general formula $Fe_2(SR)_2(NO)_4$. They were first studied by Hofmann and Wiede[1] in 1895. As in the case of Roussin's black salt, early studies[2-8] were mainly concerned with the structure and constitution of the compounds, and the question of whether or not they contained Fe^I was again the subject of some debate. The crystal structure of the ethyl ester was determined in the late 1950's[9], and force constant calculations based on the NO stretching frequencies in the infra-red spectrum have been carried out[10]. The mass spectra of a number of the red salt esters have also been examined[11]. Analogous compounds containing selenium[10,12,13] and tellurium[13,14] are known.

The chemistry of Roussin's red salt has begun to attract rather more attention recently[13,15,16]. Various esters have been synthesized by different methods, and the "acid" $Fe_2(SH)_2(NO)_4$ has been prepared[16]. Several synthetic methods leading to the formation of these compounds are available[17]; these include

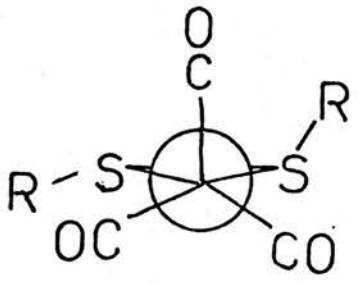

Fig. 3.1 anti- $Fe_2(SR)_2(CO)_6$ (Fischer projection along Fe-Fe bond)

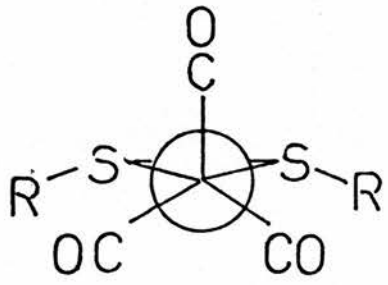

Fig. 3.2 syn- $Fe_2(SR)_2(CO)_6$

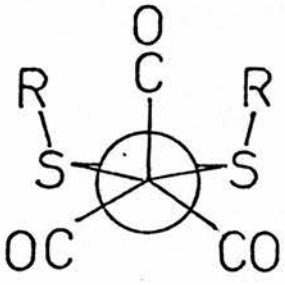

Fig. 3.3

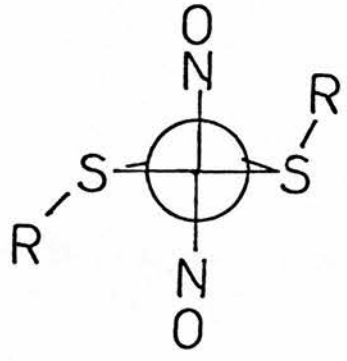

Fig. 3.4 C_{2h} $Fe_2(SR)_2(NO)_4$

reaction of Fe^{II} with nitric oxide and a thiol[18], alkylation of the red salt with alkyl halides[15], and reaction of a thiol with iron dinitrosyl iodide and triethylamine[13]. Of particular interest is the report[18] of the isolation of the methyl ester $Fe_2(SMe)_2(NO)_4$ from a rotted vegetable used as a foodstuff in the Linxian region of China. The compound is reported to be a nitrosating agent, and is implicated in the production of cancer of the oesophagus.

Carbonyl compounds isoelectronic with the red salt esters, $Fe_2(SR)_2(CO)_6$, are known[2-5,19,20]. The analogy between the two classes of compound was noted by Reihlen and co-workers[2-5], and has been quoted as an example of the general analogy between metal carbonyls and nitrosyls[21]. King[22] has shown that the carbonyl compounds exist in two isomeric forms, and the crystal structure of one of these isomers has been determined[23]. It was found to have the alkyl groups in an anti configuration (Fig. 3.1); the other isomer is presumed to have the syn configuration shown in Fig. 3.2 . The alternative syn configuration (Fig. 3.3) is considered unlikely on stereochemical grounds[23]. By contrast, the crystal structure of the nitrosyl[9] showed only one isomer, of C_{2h} symmetry analogous to the anti carbonyl (Fig. 3.4). Evidence from dipole moment measurements suggested the possibility that non-centrosymmetric isomers might exist in solution[17].

In this work, the direct conversion of $Fe_2(SR)_2(CO)_6$ compounds to the corresponding red salt esters was attempted, with a view to seeing if the different isomers of the carbonyl complexes would give different isomers of the nitrosyls. The conversion was found to proceed readily, but it was at first concluded that the C_{2v} nitrosyl isomer (analogous to the syn carbonyl) could not be detected[24]. Other workers then reported the observation of signals in the proton n.m.r. spectra of red salt esters which were attributed to the presence of both isomers[13]. This point was re-investigated and the detection of two isomers by n.m.r. was confirmed. Variable temperature n.m.r. experiments, which were carried out in conjunction with Mr. Andrew Hyde, enabled the approximate energy barriers for isomer interconversion to be calculated.

3.2 Experimental.

3.2.1 Materials and Instruments.

All chemicals were reagent grade and were used without further purification. Literature methods were employed for the syntheses of syn- and anti- $Fe_2(SR)_2(CO)_6$[22] and $Fe_2I_2(NO)_4$[26]. The Roussin esters were prepared either from the carbonyls as described here or from $Fe_2I_2(NO)_4$ as described by Rauchfuss and

Weatherill[13]. Infra-red spectra were obtained as Nujol mulls on a Perkin-Elmer 257 spectrometer. Mass spectra were obtained on an AEI MS 902. N.m.r. spectra were recorded using Varian CFT-20 and Bruker WP-80 instruments.

3.2.2 Reaction of $Fe_2(SMe)_2(CO)_6$ with nitric oxide.

2.4 g of an equilibrium mixture of the isomers of $Fe_2(SMe)_2(CO)_6$ were dissolved in 100 ml methylene chloride. The solution was flushed with nitrogen, then treated with nitric oxide for about 30 minutes. The solvent was evaporated and the residual oil dissolved in 60/80 petrol. This solution was concentrated until a precipitate began to form, then cooled in ice and left to crystallise. The infra-red spectrum of the crystals at this stage showed absorption due to both carbonyl and nitrosyl groups. The product was recrystallised from petrol in the same way as above; the combined mother liquors were concentrated further to give a second crop of crystals. This gave $Fe_2(SMe)_2(NO)_4$ in 28% yield.

Melting point 92-94°C (lit.[18] 93.5°C); I.r.: $\nu(NO)/cm^{-1}$ 1815w, 1780s, 1750s. (lit. 1775, 1750, 1730 [18]; 1778, 1755 [15].) Mass spectrum: M/z 326 (molecular ion); 296, 266, 236, 206 (sequential loss of 4 NO); 191, 176 (sequential loss of 2 Me). Anal.: Found, C, 7.65%; H, 1.84%; N, 15.48% . Calc. for $C_2H_6N_4O_4S_2Fe_2$: C, 7.37%; H, 1.86%; N, 17.19%.

Thin-layer chromatography of the product on silica using 40/60 petrol as eluant showed a single dark brown spot with an R_f value identical to that of anti- $Fe_2(SMe)_2(CO)_6$.

$Fe_2(SMe)_2(CO)_6$ (0.5 g, single isomer) was dissolved in 50 ml methylene chloride and the solution flushed with nitrogen and cooled to ~-80°C in an acetone/CO_2 bath. Nitric oxide was then passed through the solution for 2 or 3 hours. The solvent was removed by freeze drying. The product, a brown powder, was shown by i.r. to be a mixture of the carbonyl and nitrosyl compounds. T.l.c. showed only one spot when the starting material was anti- $Fe_2(SMe)_2(CO)_6$, but two spots when the syn isomer was used, one for the product nitrosyl and one for unreacted starting material.

3.2.3 Reaction of $Fe_2(SEt)_2(CO)_6$ with $NaNO_2$ and NaOH.

$Fe_2(SEt)_2(CO)_6$ (4.0 g, 10 mmol) was dissolved in 50 ml ethanol and the solution stirred under nitrogen. $NaNO_2$ (2.8 g, 40 mmol) in 25 ml water was then added, followed by addition of NaOH (4.6 g) in 25 ml water. The mixture was refluxed under N_2 for 2 hours, cooled to room temp. and acidified with 1:1 (v/v) glacial acetic acid/water. Vigorous gas evolution occurred at this stage. The acidified mixture was filtered through a glass sinter and the residue extracted with methylene chloride. The extracts were concentrated to low volume, cooled in ice and

Key to Table 3.1.

a. Data at 308 K, chemical shift in p.p.m. from TMS; values for centres of multiplets.

b. (^{1}H) at 233 K: 1.23, 1.27, 2.47 p.p.m.

c. No splitting observed even at 223 K.

d. Spectrum in CD_2Cl_2.

e. Not studied.

Table 3.1 - N.m.r. assignments for $Fe_2(SR)_2(NO)_4$[a]

R	Solvent	$\delta(^1H)$/p.p.m.	$\delta(^{13}C)$/p.p.m.	Assignment
Me	$CDCl_3$	2.83±	27.45	CH_3
	$C_6D_5CD_3$	2.16, 2.23	26.95, 27.18	CH_3
Et	$CDCl_3$	1.53, 1.58	19.14	CH_3
		3.07, 3.10	39.45, 40.15	CH_2
	$C_6D_5CD_3$	1.13, 1.16	18.98	CH_3
		2.53, 2.63	39.49, 40.19	CH_2
Pr^n	$CDCl_3$	1.11	13.11	CH_3
		1.96	27.33	$CH_3\underline{CH_2}CH_2$
		3.02, 3.05	47.33, 47.66	$CH_3CH_2\underline{CH_2}$
	$C_6D_5CD_3$	0.84	12.93	CH_3
		1.61	27.50	$CH_3\underline{CH_2}CH_2$
		2.64, 2.68	47.48, 47.91	$CH_3CH_2\underline{CH_2}$
Pr^i	$CDCl_3$	1.54, 1.57	27.64	CH_3
		3.04, 3.07	49.70, 50.63	CH
	$C_6D_5CD_3$	1.26[b]	27.47	CH_3
		2.69[b]	49.94, 50.92	CH
Bu^t	$CDCl_3$	1.45	34.14	CH_3
		-	52.95	$Me_3\underline{C}$
	$C_6D_5CD_3$	1.23[c]	33.96	CH_3
		-	57.05	$Me_3\underline{C}$
CH_2Ph	$CDCl_3$	4.18, 4.22	48.63, 49.37	CH_2
		7.43	128.2, 129.1[d]	Ph
	$C_6D_5CD_3$	3.82, 3.85	e	CH_2
		7.07	e	Ph

Table 3.2 - Variable temperature n.m.r. results for $Fe_2(SR)_2(NO)_4$.

R	Signal	T_c/K	$\Delta\nu$/Hz	$\Delta G^{\ddagger}$/kJ mol^{-1}
Me	CH_3	357(1)	5.8(3)	80.4(4)
Et	$\underline{CH_3}CH_2$	332(1)	2.0(2)	77.5(5)
	$CH_3\underline{CH_2}$	348(1)	6.9(3)	77.8(4)
Pr^n	$CH_3CH_2\underline{CH_2}$	338(1)	3.4(3)	77.4(5)
Pr^i	$(\underline{CH_3})_2CH$	263(1)	2.8(4)	60.1(6)
CH_2Ph	$Ph\underline{CH_2}$	341(1)	2.7(2)	78.8(5)

Data from proton spectra in $C_6D_5CD_3$; estimated errors in brackets.

treated with cold methanol to precipitate $Fe_2(SEt)_2(NO)_4$ in 33% yield. Further product could be obtained by extraction of the aqueous filtrate with methylene chloride.

Melting point 77-80°C (lit.[15] 78.5-80°C). I.r.: ν(NO)/cm^{-1} 1810w, 1780s, 1750s. (lit.[15] 1778, 1750).

3.2.4 N.M.R. Data.

(This section was carried out in conjunction with Mr. Andrew Hyde, who prepared most of the compounds and obtained their spectra.)

Proton and carbon-13 chemical shifts for a series of Roussin esters in both $CDCl_3$ and octadeuterotoluene are recorded in table 3.1. Variable temperature n.m.r. data are presented in table 3.2. Energy barriers to isomer interconversion were calculated by the approximate method given by Abraham and Loftus[27].

Some decomposition of some of the samples occurred on dissolving (in chloroform) or on heating (in toluene). This was considerably reduced by flushing the solution and n.m.r. tube with nitrogen.

The coalescence behaviour was reversible with temperature. It has been shown[26] that the Roussin esters do not dissociate to monomeric $Fe(NO)_2SR$ fragments, nor do they ionise to $Fe(NO)_2^+$ and RS^-; it is therefore almost certain that the rate processes observed here are indeed those for the $C_{2h} \rightleftharpoons C_{2v}$ isomerisation.

3.3 Results and Discussion.

Two different methods were examined for the replacement of the terminal carbonyl ligands in bis(μ-organylthio)bis(tricarbonyliron) complexes $Fe_2(SR)_2(CO)_6$ by nitrosyl groups. Direct reaction of $Fe_2(SMe)_2(CO)_6$ with nitric oxide was found to yield the corresponding red salt ester; the analogous reaction of the trifluoromethyl compound has been reported by Davidson and Sharp[28]. Alkaline sodium nitrite in alcoholic solution can also be used to nitrosate carbonyl compounds, as in the preparations of the ion $Fe(CO)_3NO^-$ and of $Fe(CO)_2(NO)_2$ from iron pentacarbonyl[29]. This method was applied to bis(μ-organylthio)bis(tricarbonyliron) complexes, and again the corresponding nitrosyl was found to be produced. There is at least one statement in the literature to the effect that these carbonyl complexes cannot be converted to the Roussin esters[21a].

As described here, the reactions do not represent very good synthetic methods as the yields are rather low. The reaction with nitric oxide as described by Davidson and Sharp[28] gives a much better yield than was obtained in this work; however further investigation of this reaction in this Department by Mr. A.R. Hyde has shown that by refluxing the reaction mixture, yields of over 90% can be obtained[25].

The initial reason for investigating the direct conversion of the carbonyl compounds to the Roussin esters was to see if the unknown C_{2v} isomers of the nitrosyl complexes could be isolated. In the case of the carbonyls, at least for R = Me, Et, Bz, the two isomers, <u>syn</u> and <u>anti</u>, are readily separable by chromatography and can be obtained as pure solids with quite different physical properties[20,22]. In the reaction of $Fe_2(SMe)_2(CO)_6$ with nitric oxide, the product obtained was the same regardless of whether the starting material was pure <u>syn</u> or pure <u>anti</u> carbonyl or a mixture of both; it was homogeneous by t.l.c., with an R_f value identical to that of the <u>anti</u> carbonyl. The proton n.m.r. spectrum in $CDCl_3$ was interpreted as a single peak at 2.83, and it was therefore concluded that only one isomer of $Fe_2(SMe)_2(NO)_4$ was formed. By analogy with the ethyl compound, whose crystal structure was known[9], this was presumed to be the C_{2h} isomer. It is now recognised that this conclusion was wrong.

Key to Table 3.1.

a. Data at 308 K, chemical shift in p.p.m. from TMS; values for centres of multiplets.

b. (^{1}H) at 233 K: 1.23, 1.27, 2.47 p.p.m.

c. No splitting observed even at 223 K.

d. Spectrum in CD_2Cl_2.

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Et	$CDCl_3$	1.53, 1.58	19.14	CH_3
		3.07, 3.10	39.45, 40.15	CH_2
	$C_6D_5CD_3$	1.13, 1.16	18.98	CH_3
		2.53, 2.63	39.49, 40.19	CH_2
Pr^n	$CDCl_3$	1.11	13.11	CH_3
		1.96	27.33	$CH_3\underline{CH_2}CH_2$
		3.02, 3.05	47.33, 47.66	$CH_3CH_2\underline{CH_2}$
	$C_6D_5CD_3$	0.84	12.93	CH_3
		1.61	27.50	$CH_3\underline{CH_2}CH_2$
		2.64, 2.68	47.48, 47.91	$CH_3CH_2\underline{CH_2}$
Pr^i	$CDCl_3$	1.54, 1.57	27.64	CH_3
		3.04, 3.07	49.70, 50.63	CH
	$C_6D_5CD_3$	1.26[b]	27.47	CH_3
		2.69[b]	49.94, 50.92	CH
Bu^t	$CDCl_3$	1.45	34.14	CH_3
		-	52.95	$\underline{Me_3C}$
	$C_6D_5CD_3$	1.23[c]	33.96	CH_3
		-	57.05	$\underline{Me_3C}$
CH_2Ph	$CDCl_3$	4.18, 4.22	48.63, 49.37	CH_2
		7.43	128.2, 129.1[d]	Ph
	$C_6D_5CD_3$	3.82, 3.85	e	CH_2
		7.07	e	Ph

Rauchfuss and Weatherill have synthesized a number of Roussin esters by the reaction of thiols with iron dinitrosyl iodide and triethylamine[13]. In the proton n.m.r. spectra of their products, they observed doubling of some of the signals, which they interpreted in terms of equally abundant C_{2h} and C_{2v} isomers. As this was in contradiction to our observation, a more detailed study of the n.m.r. spectra of these compounds was undertaken. The data are shown in Table 3.1. Considering firstly the spectra in chloroform, it can be seen that in many cases the signals are doubled, indicating the presence of both isomers.

The above conclusion that only one isomer of the methyl compound was formed arose through expecting too close an analogy with the carbonyl compound. It had been assumed that the nitrosyl isomers would separate by tlc as distinctly as did the carbonyl isomers; when no separation was observed, and when the same product appeared regardless of starting material, it was inferred that only a single isomer was present. This belief was strengthened by the quite sharp (2^{o}) melting point, agreeing with the literature value[18]; the carbonyl isomers have widely different melting points and an equilibrium mixture is low-melting. Again by analogy with the carbonyl, a distinct separation of n.m.r. signals for the two isomers was expected. The proton chemical shift of the <u>syn</u> carbonyl is 2.13 ppm, while that of the corresponding methyl group of the <u>anti</u> isomer is 2.17 ppm. In the nitrosyl compound, however, the separation is so

small that the peaks are virtually superimposed. This would suggest that the difference between the environments of the methyl groups in the two isomers is less in the nitrosyl than in the carbonyl. The Fe_2S_2 ring in the carbonyls is puckered, whereas in the nitrosyls it is a planar rhombus. The sulphur atoms in the nitrosyls are hence further apart than in the carbonyls, and it would seem reasonable that the S-methyl groups would have less effect on each other. (The effect of this stereochemical difference on the chemistry of the anions $Fe_2S_2(CO)_6^{2-}$ and $Fe_2S_2(NO)_4^{2-}$ has been discussed[15].) However in the ethyl compounds, the chemical shift difference between the isomers for the methyl protons is the same for the carbonyl as for the nitrosyl, and for the methylene protons is in fact much larger for the nitrosyl than for the carbonyl.

It is interesting to note that Wang and co-workers describe the n.m.r. spectrum of the methyl ester in an unspecified solvent as "a single peak at δ2.8."[18] Seyferth and Gallagher[15] make no comment on the presence or absence of doubled peaks in the spectra of their products, while Davidson and Sharp[28] report only a single peak in the ^{19}F n.m.r. spectrum of the trifluoromethyl compound.

Some of the signals for the other esters do not appear split. In the isopropyl compound, the methyl groups give one signal at room temperature which splits in two on taking the temperature down to 233 K. In the t-butyl compound, no splitting of the signal is observed even at 223 K; this may be due to rapid

isomer interconversion or it may be that in this case steric hindrance by the bulky t-butyl groups prevents formation of the C_{2v} isomer. In the ^{13}C spectra similar effects are observed; for example the methyl ester shows only a single peak as do the methyl carbons of the ethyl ester, while two peaks are observed for the methylene carbons of the ethyl and benzyl esters. (The two peaks observed for the phenyl carbons of the benzyl ester are not in 1:1 ratio and are due to different carbon atoms in the ring rather than isomers.)

The observation of separate signals for the two isomers led to consideration of the possibility of studying the $C_{2h} \rightleftharpoons C_{2v}$ isomerisation process by variable temperature n.m.r.. Toluene-$\underline{d}_8$ was chosen as solvent because of its high boiling point. It was found that this solvent caused an increase in the separation between the signals for the different isomers of the methyl compound; the two peaks in the spectrum now showed up quite distinctly. This effect is also observed for the methylene protons of the ethyl ester and for the ^{13}C signal of the methyl ester.

A considerable difference in the proton chemical shifts for all the compounds studied occurred on changing the solvent to toluene. All the signals were shifted upfield, by as much as 0.6 ppm in the case of the methyl ester. This may be due to some effect of the paratropic ring current in the toluene molecules, though this would require a very specific orientation of the solvent molecules about a solute molecule. The solvent

Table 3.2 - Variable temperature n.m.r. results for $Fe_2(SR)_2(NO)_4$.

R	Signal	T_c/K	$\Delta\nu$/Hz	$\Delta G^{\ddagger}$/kJ mol^{-1}
Me	CH_3	357(1)	5.8(3)	80.4(4)
Et	CH_3CH_2	332(1)	2.0(2)	77.5(5)
	CH_3CH_2	348(1)	6.9(3)	77.8(4)
Pr^n	$CH_3CH_2CH_2$	338(1)	3.4(3)	77.4(5)
Pr^i	$(CH_3)_2CH$	263(1)	2.8(4)	60.1(6)
CH_2Ph	$PhCH_2$	341(1)	2.7(2)	78.8(5)

Data from proton spectra in $C_6D_5CD_3$; estimated errors in brackets.

dependence of these spectra will be the subject of further study by Dr. C. Glidewell and Mr. A.R. Hyde in this department.

The results of the variable temperature study are shown in Table 3.2. Replacement of one hydrogen in the methyl compound by an alkyl group to give the ethyl or n-propyl derivative causes a small reduction in $\Delta G^{\ddagger}$ of about 2.5 kJ mol^{-1}; in the case of the benzyl ester the difference is even less. When two hydrogen atoms are replaced, as in the isopropyl compound, $\Delta G^{\ddagger}$ falls by a further 17 kJ mol^{-1}, and when all three hydrogens are replaced to give the t-butyl compound, no splitting of the signal is observed even at 223 K. This would suggest, for reasonable values of $\Delta\nu$, an upper limit for $\Delta G^{\ddagger}$ of about 50 kJ mol^{-1}, if both isomers do in fact exist.

These observations are curious in that it would have seemed more likely that the isomerisation process would be more difficult with bulkier substituents. The observation that replacement of H with Ph to give the benzyl derivative has less effect than replacement with Me to give the ethyl compound is also surprising.

The measured activation energies $\Delta G^{\ddagger}$ are the values at the coalescence temperatures. In the case of the ethyl ester, splitting of the signals for both the CH_3 and CH_2 protons was observed, with quite different values of $\Delta\nu$. It was possible to measure distinct coalescence temperatures for each of the two sets of protons, and hence calculate $\Delta G^{\ddagger}$ at two different

temperatures. The values obtained were identical within experimental error, i.e. the effect of a 16 K rise in temperature on $\Delta G^{\ddagger}$ was too small to be detected. The variation of $\Delta G^{\ddagger}$ with temperature depends on $\Delta S^{\ddagger}$ ($\partial\Delta G/\partial T = -\Delta S$). The entropies of the two isomers must be very similar, and although the nature of the transition state is not known it is very unlikely that its entropy will be greatly different. Hence $\Delta S^{\ddagger}$ will be very small, and $\Delta G^{\ddagger}$ would not be expected to vary greatly with temperature.

The values of $\Delta G^{\ddagger}$ measured here can be compared for example with those for sulphur inversion in organosulphur ligands coordinated to chromium and tungsten carbonyls[30], which are considerably lower at around 50 kJ mol^{-1}.

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List of abbreviations used in Chapter Four.

MH = Malononitrile

M^- = Malononitrile carbanion

DMM = Dimethyl malonate

DMM^- = Dimethyl malonate carbanion

EM = Ethylmalononitrile

EM^- = Ethylmalononitrile carbanion

SNP = Sodium nitroprusside

$[\ ]_e$ = Equilibrium concentration

$[\ ]_t$ = Total concentration

k_{obs} = Observed pseudo-first order rate constant

CHAPTER FOUR.

THE KINETICS OF THE REACTIONS OF SODIUM NITROPRUSSIDE WITH THE CARBANIONS OF MALONONITRILE, DIMETHYL MALONATE AND ETHYLMALONONITRILE.

4.1 Introduction.

It has long been known that sodium nitroprusside reacts very rapidly in the presence of a base with organic compounds having "acidic" hydrogen atoms attached to carbon, to produce bright colours, usually red. This is known as the Légal reaction[1]. There is no reaction unless there is base present; this is taken as indicating that the reacting species is the carbanion of the organic compound. The red colour fades after a time to give a yellow solution.

Swinehart and Schmidt[2] have investigated the reactions of the ketones acetone, butanone and pentan-3-one; in the case of acetone, they obtain a rate law for the formation of the red species which is consistent with a mechanism involving rate-determining attack of the carbanion on the nitroprusside ion. The fading of the red species was found to be first order in the concentration of the red species and independent of hydroxide and acetone concentrations. In the cases of the other ketones, the fading reaction was rather more complicated; the formation of the red species in these cases was not

investigated.

The acetone system has also been studied by Loach and Turney[3]. Their experimental conditions were considerably different from those of Swinehart and Schmidt, and they found the rate determining step to be the formation of the carbanion.

As part of a general investigation into the chemistry of sodium nitroprusside, the kinetics of its reactions with the thiol group of cysteine and with certain amines have recently been studied in this department[4]. The carbanion reactions of creatinine[5] and malononitrile[5,6] have also been investigated. In the case of creatinine, the kinetic results indicated that the rate determining step was attack of the carbanion on nitroprusside, as had been observed for acetone. For malononitrile, quite different results were obtained. In the present work the reaction of malononitrile carbanion with nitroprusside was reinvestigated at constant ionic strength, and the mechanism which had been proposed in the earlier work[5] was confirmed. Following on from this, the reactions of dimethyl malonate and ethylmalononitrile were investigated. Although the results obtained in these cases were less conclusive, mechanistic deductions were still possible. A comparison of these results with those obtained for malononitrile is made.

4.2 Experimental.

4.2.1 Materials.

Sodium nitroprusside and potassium chloride were Analar grade; all other materials were reagent grade. Sodium hydroxide solutions were prepared by dilution of BDH concentrated volumetric solutions, other solutions by weighing of reagents and dissolving in distilled water. Sodium nitroprusside solutions were kept wrapped in aluminium foil to prevent undue exposure to light; all parts of the stopped-flow apparatus containing these solutions and exposed to light were similarly protected.

4.2.2 Preparation of Ethylmalononitrile.

Diethyl ethylmalonate $C_2H_5CH(CO_2C_2H_5)_2$ was treated with excess concentrated ammonia and the mixture stirred for 48 hours. The fine white precipitate which formed was filtered off, washed with the mother liquor and a little water, and dried under vacuum over P_2O_5. This gave a 69% yield of ethylmalonic acid diamide, which was used without further purification.

To a 250 ml 3-necked flask equipped with mechanical stirrer, oil bath and condenser was added 22 g of ethylmalonic acid diamide (0.17 mol), 30 g NaCl and 100 ml of 1,2-dichloroethane. The mixture was stirred for 15 minutes, then $POCl_3$ (42 g, 0.27 mol) was added and the mixture refluxed. A solid mass separated after about 35 minutes; this interfered with the stirring and so the reaction was continued without stirring. After 8 hours refluxing was stopped and the solution decanted. The residue was washed with further dichloroethane and the two solutions were combined and evaporated to yield 9 g of orange oil. Further product was extracted from the solid residue by dissolving it in water and extracting the aqueous solution with 1,2-dichloroethane. The extracts were dried over Na_2SO_4 and evaporated to yield a further 7 g of oil. The crude product was distilled twice at the oil pump, rejecting a small forerun each time, to give ethylmalononitrile as a clear colourless liquid, b.p. 92°C/19 mm (lit[7]. 90-91°C/20 mm) in 51% yield based on amide.

(These preparative methods are analogous to those for cyanoacetamide[8] and malononitrile[9] given in "Organic Syntheses".)

4.2.3 Kinetic Experiments.

Rate constants were measured using a Canterbury SF-3A stopped-flow spectrophotometer connected to a Commodore model 4016 microcomputer via a transient recorder. For malononitrile, the computer program calculated first order rate constants using infinity values; for the other carbon acids, a program using the Kezdy-Swinbourne method was used as the red colour faded too quickly to allow for infinity readings.

Pseudo-first order conditions were used with carbanion in excess. Correlation coefficients were always better than 0.99 and in the case of dimethyl malonate usually better than 0.999. In a typical experiment a solution of sodium nitroprusside and the carbon acid was placed in one arm of the instrument and reacted in turn with each of five or six solutions of sodium hydroxide of varying concentration, made up to constant ionic strength with potassium chloride. Five such experiments were carried out with malononitrile, five with dimethyl malonate and three with ethylmalononitrile, varying the concentration of carbon acid between each experiment.

Preliminary experiments had been carried out[5,6] in which the malononitrile and hydroxide were mixed in one reactant solution, while the other contained only nitroprusside. This amounted to having the carbanion pre-formed in solution. For the same concentrations of reagents, there was no difference in the observed rate constants whichever way the reagents were mixed;

this indicates that formation of carbanion is not rate determining under these conditions. Malononitrile/hydroxide solutions were found to be unstable over a period of a few hours, so the alternative arrangement of solutions described above was used.

The red intermediate whose appearance is followed has an absorption maximum around 500 nm. In the case of dimethyl malonate, in order to obtain a suitable trace on the instrument it was necessary to work at 460-475 nm. It was found experimentally, for all three compounds, that changes in operating wavelength even of this magnitude made no significant difference to the observed rate constants.

For dimethyl malonate and ethylmalononitrile, the instrument settings had to be much more sensitive to follow the reaction than was the case for malononitrile, which gives a very much more intense colour. In the case of ethylmalononitrile, the disappearance of the red colour was so fast that calculations could not be made over very many half-lives - as few as 2 in some cases. However it was possible to obtain consistent results, and the observed rate constants are probably accurate enough for the present purposes.

The instrumentation displayed an absorbance vs. time curve as a trace on an oscilloscope, with the data fed automatically to the transient recorder. The computer program allowed for repeated calculations on the same set of data over different

ranges of the curve. For each rate constant determination, several traces were examined until at least two gave consistent values of the rate constant. Each rate constant quoted is an average of several calculations on at least two consistent traces.

4.2.4 Attempted isolation of products.

To an ice-cold solution of malononitrile (0.74 g, 11 mmol) and NaOH (0.9 g, 22 mmol) in 10 ml water was added a solution containing sodium nitroprusside (3.3 g, 11 mmol) in 20 ml water. There was an immediate blood-red colouration. The mixture was swamped with ice-cold methanol and left in an ice-salt bath for 5-10 minutes. A red oil precipitated; the supernatant liquid was decanted off and the oil triturated repeatedly with methanol, eventually yielding a filtrable powder which was dried over P_2O_5. The product remained dry in a tightly capped bottle, but was very deliquescent on exposure to the atmosphere. Satisfactory analytical results were not obtained, though analysis of an earlier isolated sample[5,6] had suggested the formula $Na_4[Fe(CN)_5N(O)C(CN)_2].3H_2O$.

Similar attempts to isolate the red product of the dimethyl malonate reaction were unsuccessful; the red colour changed to brown-yellow immediately on adding the methanol.

TABLE 4.1 Observed rate constants (s^{-1}) for reaction of nitroprusside with malononitrile carbanion. $[Fe(CN)_5NO^{2-}]_{initial}$ = 1.03 - 1.08 mM, I = 0.1 M (KCl); T = 25 ± 0.5°C.

10^2 $[CH_2(CN)_2]_t$/M / $[OH^-]_t$/M	1.005	2.00	3.01	4.10	5.25
0.01	1.2	1.0	1.1	0.75	0.7
0.02	4.4	4.2	3.6	3.1	2.1
0.03	8.6	9.8	9.1	7.2	5.0
0.04	14.8	18.9	17.5	15.7	11.4
0.05	22.8	27.9	29.8	27.4	19.0

4.3 Results.

4.3.1 Malononitrile.

The results obtained for malononitrile are shown in Table 4.1, where the subscript "t" indicates the total added concentration of the particular reagent. It can be seen that, for a given concentration of hydroxide, varying the concentration of malononitrile does not cause a parallel variation in the observed rate constant; however for a given malononitrile concentration, varying the concentration of hydroxide has a dramatic effect on the rate constant. These observations suggest that the rôle of hydroxide ions in this reaction is more than just the formation of the carbanion.

Since the formation of the carbanion is not the rate-determining step, it must be postulated as a fast initial equilibrium, and so further analysis of the data must be based, not on <u>total</u> $[OH^-]$ and $[CH_2(CN)_2]$, but on the <u>equilibrium</u> concentrations of hydroxide and malononitrile carbanion, which will be denoted by the subscript "e". Knowing the values of K_w for water and K_a for malononitrile[10], these quantities can be calculated from the equation

$$[M^-]_e = K_a/K_w\{[MH]_t - [M^-]_e\}\{[OH^-]_t - [M^-]_e\}$$

where MH represents malononitrile and M^- the carbanion. This gives a value for $[M^-]_e$ and subtracting this from $[OH^-]_t$ gives

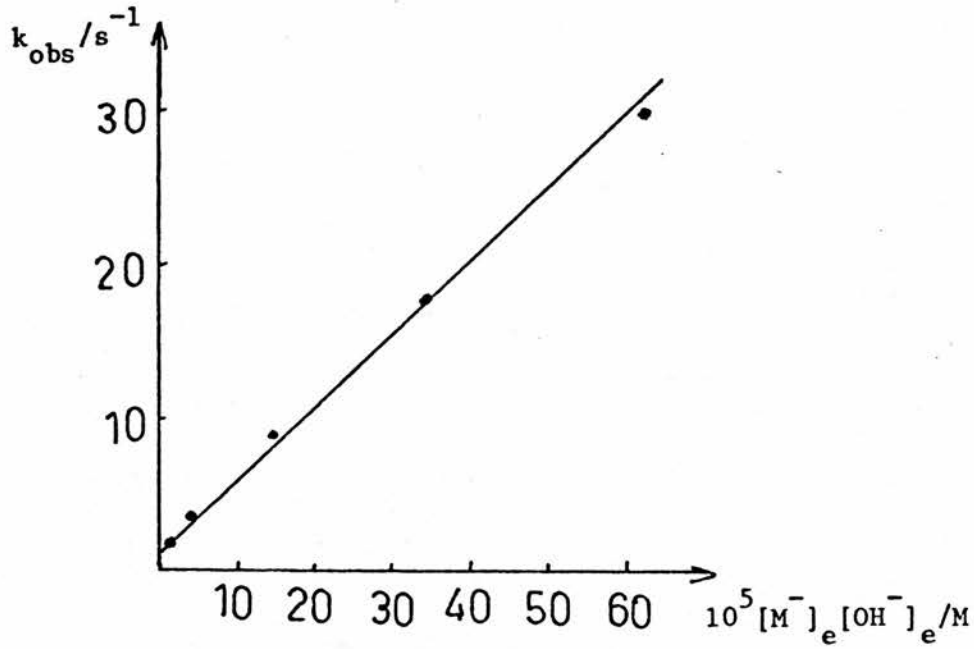

Fig. 4.1 Typical plot of k_{obs} vs. $[M^-]_e[OH^-]_e$ for malononitrile.
$[MH]_t$ = 0.0301 M; $[SNP]_{initial}$ = 1.07 mM.

$$CH_2(CN)_2 + OH^- \underset{}{\overset{K'}{\rightleftharpoons}} CH(CN)_2^- + H_2O \qquad K' = K_a/K_w$$

$$CH(CN)_2^- + [Fe(CN)_5NO]^{2-} \overset{K}{\rightleftharpoons} [(NC)_5FeN(O)CH(CN)_2]^{3-}$$

I

$$[(NC)_5FeN(O)CH(CN)_2]^{3-} + OH^- \xrightarrow[rds]{k} [(NC)_5FeN(O)C(CN)_2]^{4-} + H_2O$$

II

SCHEME 4.1

$[OH^-]_e$.

Analysis of the data in this way showed no linear correlation between k_{obs} and the equilibrium concentration of carbanion, as would have been expected for a mechanism involving rate-determining attack of carbanion on nitroprusside. Instead a linear correlation was found between k_{obs} and the product $[M^-]_e[OH^-]_e$; a typical plot is shown in Fig. 4.1.

A mechanism consistent with these results is shown in Scheme 4.1. The rate determining step is removal of a second proton from the initial adduct species I to give the species II, whose appearance is followed. The rate of the reaction is therefore given by

$$d[II]/dt = k_2[I][OH^-]_e$$
$$= k_2K[(NC)_5FeNO^{2-}][CH(CN)_2^-]_e[OH^-]_e$$

Since pseudo-first order conditions were used ([I] cannot be greater than $[(NC)_5FeNO^{2-}]_{initial}$, and although $[OH^-]_e$ may not always be in <u>excess</u>, it will remain essentially <u>constant</u> because it is an <u>equilibrium</u> concentration with the species involved in the equilibrium in excess) we have

$$k_{obs} = k_2K[M^-]_e[OH^-]_e,$$

i.e. a plot of k_{obs} <u>vs.</u> $[M^-]_e[OH^-]_e$ should be linear, as observed. The slope of the line should give a value for the product of the second-order rate constant k_2 and the equilibrium constant K; it is not possible to separate this value into the two components.

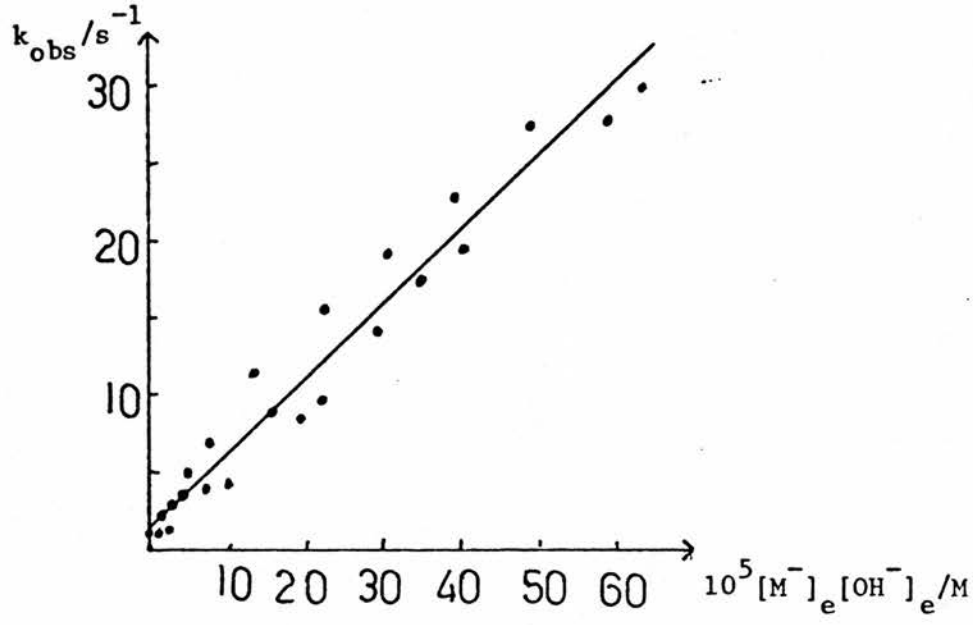

Fig. 4.2 k_{obs} vs. $[M^-]_e[OH^-]_e$ for malononitrile;
data at K′ = 640.

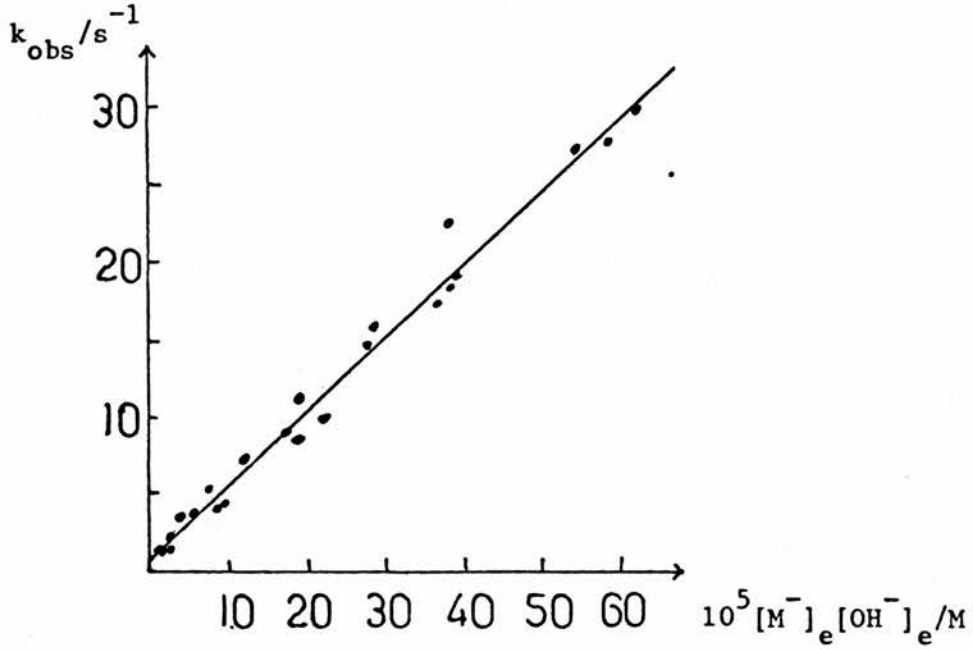

Fig. 4.3 k_{obs} vs. $[M^-]_e[OH^-]_e$ for malononitrile;
data adjusted by computer to K′ = 340.

A slight problem with this interpretation arises when all the data are taken together. In theory all the points should lie on a single straight line. In fact, although a straight line can be drawn through all the points, the scatter is rather large - see Fig. 4.2.

It was decided to see what effect small changes in the value of pK_a for malononitrile would have on this graph. The K_a value used, 6.5×10^{-12} M, was that given by Pearson and Dillon[10], determined by pH measurements of aqueous solutions of malononitrile at unspecified dilution or ionic strength; it may be that under the experimental conditions used here the correct K_a value is rather different. Talvik and co-workers[7] have determined the pK_a of malononitrile by two different methods and found values of 11.35 and 11.39, compared with Pearson and Dillon's value of 11.19; there is an example[11] of a literature pK_a being adjusted by ~0.8 to fit data from kinetic experiments. Thus this procedure does not seem unreasonable.

With the aid of a computer program to solve the quadratic equations and evaluate the products $[M^-]_e[OH^-]_e$ for variable values of the equilibrium constant K', correlation coefficients were calculated for plots of k_{obs} *vs.* $[M^-]_e[OH^-]_e$, for values of K' from 140 to 1040 in steps of 100. The best correlation coefficient was obtained at K' = 340; a plot of the data obtained with this value is shown in Fig. 4.3.

TABLE 4.2. k_{obs}/s^{-1} for reaction of dimethyl malonate carbanion with sodium nitroprusside.

$[SNP]_{initial} = 1.44 \times 10^{-3}$ M; I = 0.25 M (KCl)

T = 25 ± 0.2°C

10^2 $[DMM]_t$/M	2.58	4.93	7.57	9.97	15.0
$[OH^-]_t$/M					
0.03	8.5	9.9	10.6	12.4	13.4
0.05	9.9	12.2	13.4	15.5	16.8
0.07	-	13.8	15.5	18.0	19.6
0.10	12.7	16.0	-	21.2	23.5
0.15	15.1	19.0	21.7	25.6	-
0.20	16.7	21.4	24.7	27.6	30.4

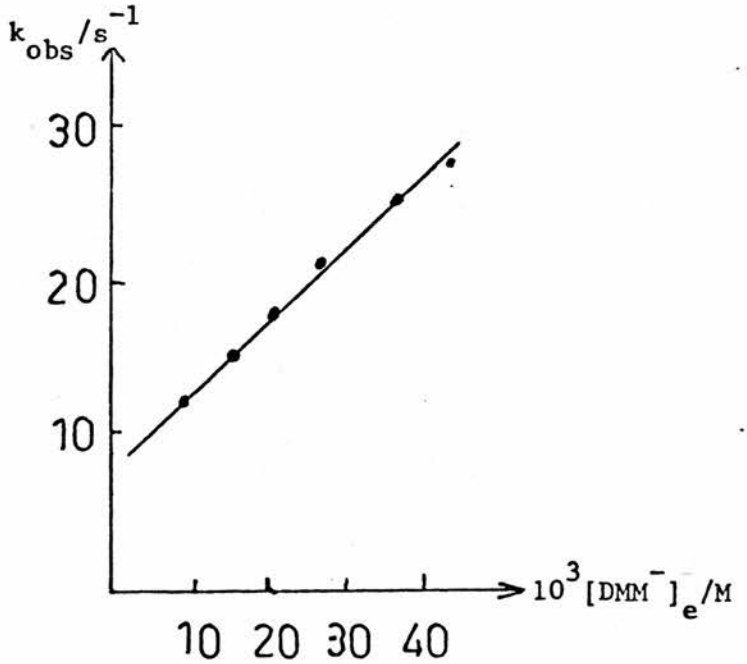

Fig. 4.4 Typical plot of k_{obs} vs. $[carbanion]_e$ for dimethyl malonate; $[DMM]_t$ = 0.10 M; $[SNP]_{initial}$ = 1.44 mM.

The correlation coefficient of this line is 0.993 compared with 0.986 for the previous line with $K' = 640$. Generally 0.99 is the required value for a good straight line. This value of K' represents a pK_a for malononitrile of 11.47.

4.3.2 Dimethyl Malonate.

The results for dimethyl malonate are shown in Table 4.2. In this case, in contrast to the malononitrile results, there is a smooth increase in k_{obs} both across and down the table.

Again, equilibrium values of $[OH^-]$ and $[DMM^-]$ were calculated. The value of K_a used, 5×10^{-14} M, was that for diethyl malonate[12]; it was assumed that the value for dimethyl malonate would not differ significantly.

It was found that plots of k_{obs} vs. $[DMM^-]_e$ were linear with positive intercepts - a typical plot is shown in Fig. 4.4. This type of behaviour is that expected for an equilibrium $A + B \rightleftharpoons C$ under pseudo-first order conditions[13]; the slope of the line is the second-order rate constant for the forward reaction, and the intercept is the first-order rate constant for the reverse reaction.

$$CH_2(CO_2Me)_2 + OH^- \overset{K'}{\rightleftharpoons} CH(CO_2Me)_2^- + H_2O$$

$$CH(CO_2Me)_2^- + [Fe(CN)_5NO]^{2-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [(NC)_5FeN(O)CH(CO_2Me)_2]^{3-}$$

SCHEME 4.2

$$EtCH(CN)_2 + OH^- \overset{K'}{\rightleftharpoons} EtC(CN)_2^- + H_2O$$

$$EtC(CN)_2^- + Fe(CN)_5NO^{2-} \xrightarrow{k} (NC)_5FeN(O)CEt(CN)_2^{3-}$$

SCHEME 4.3.

TABLE 4.3. k_{obs}/s^{-1} for reaction of ethylmalononitrile carbanion with sodium nitroprusside.

$[SNP]_{initial}$ = 2.29 - 2.43 mM; I = 0.25 M (KCl);

T = 25 ± 0.2°C

10^2 $[EtCH(CN)_2]_t$/M	3.30	5.13	10.0
$[OH^-]_t$/M			
0.07	4.8	4.5	3.7
0.10	6.0	5.8	4.7
0.15	7.5	7.4	6.7
0.20	-	8.3	7.9
0.25	8.5	9.0	8.7

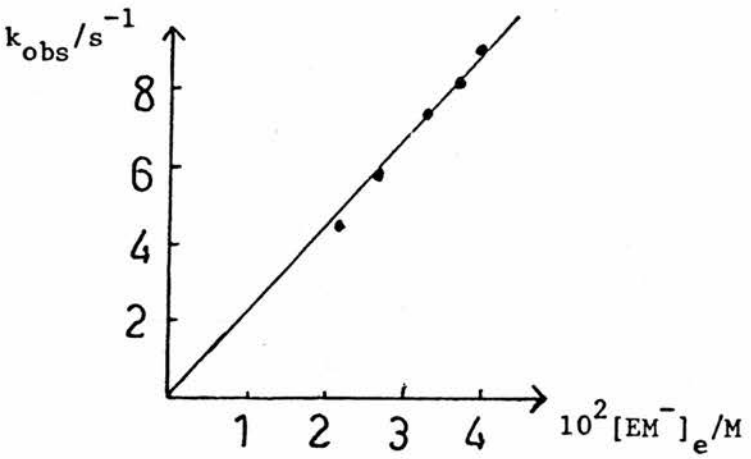

Fig. 4.5 Typical plot of k_{obs} vs. $[carbanion]_e$ for ethylmalononitrile; $[EM]_t$ = 0.051 M; $[SNP]_{initial}$ = 2.3 mM.

The mechanism proposed for this reaction is shown in Scheme 4.2. The rate-determining step is the (equilibrium) attack of the carbanion on nitroprusside.

As in the malononitrile experiments, a problem arose when attempting to reconcile all the data. Each individual experiment gave a good straight line, but the agreement between experiments was not so good. The correlation coefficient when all data were plotted together was only 0.96. Varying K′ from 1 to 10 using the computer program in this case gives best correlation coefficients at K′ = 5, 6, and 7 (value used initially = 5) but these are only 0.967.

4.3.3 Ethylmalononitrile.

The results for ethylmalononitrile are shown in Table 4.3. Equilibrium hydroxide and carbanion concentrations were calculated using a pK_a for ethylmalononitrile of 12.84[7]. It was found that plots of k_{obs} *vs.* equilibrium carbanion concentration were linear through the origin (Fig. 4.5), implying a mechanism involving rate-determining irreversible attack of carbanion on nitroprusside (Scheme 4.3). However from the three experiments, three completely different lines were obtained, which could not be reconciled at all by variations in K′.

TABLE 4.4. Fading reaction - malononitrile.

$[SNP]_{initial} = 3.5 \times 10^{-4}$ M

$[CH_2(CN)_2]_t = 2.55 \times 10^{-2}$ M

$[OH^-]_t$/M	0.75	0.075	0.0075	0.00075
$10^3\ k_{obs}/s^{-1}$	5.49	4.6	6.46	5.31

$$(NC)_5FeN(O)C(CN)_2^{4-} \xrightarrow{k} (NC)_5Fe^{3-} + (NC)_2CNO^-$$

$$(NC)_5Fe^{3-} \xrightarrow[\text{fast}]{H_2O} (NC)_5FeOH_2^{3-}$$

SCHEME 4.4.

TABLE 4.5. Fading reaction - dimethyl malonate.

$[SNP]_{initial} = 5.2 \times 10^{-3}$ M; $[DMM]_t = 4.87 \times 10^{-2}$ M

$[OH^-]_t$/M	0.01	0.1
k_{obs}/s^{-1}	0.14	0.12

4.3.4 Fading reactions.

The disappearance of the red colour was followed in the case of malononitrile using a Pye-Unicam SP8-100 spectrophotometer as this reaction was too slow to be studied by stopped-flow techniques. The reaction was found to be first order in the concentration of the red species and essentially independent of hydroxide concentration over a thousand-fold concentration range (Table 4.4). This is in agreement with earlier findings[5] and is consistent with a mechanism involving unimolecular breakdown of the red compound (Scheme 4.4). It is known[14] that the ultimate products of nitroprusside-carbanion reactions are the aquapentacyanoferrate(II) ion and the oxime of the organic compound.

For the case of dimethyl malonate the fading reaction was within the stopped-flow range. The rate was again found to be first order in the concentration of the red species and was unchanged by a 10-fold increase in $[OH^-]_t$ (Table 4.5); a further 10-fold increase led to non-first order kinetics.

The fading reaction of ethylmalononitrile was not investigated.

4.3.5 Effect of the direct reaction of nitroprusside with hydroxide.

No allowance has been made in these results for the direct reaction of OH^- with nitroprusside. The second order rate constant for this reaction is known[15] to be 0.55 $M^{-1}s^{-1}$; at the highest hydroxide concentration used in these experiments, 0.25 M, this process would therefore have a pseudo-first order rate constant of 0.14 s^{-1}, fully an order of magnitude below anything observed for dimethyl malonate or ethylmalononitrile. Although lower rate constants were observed for malononitrile, the highest total hydroxide concentration used in that case was 0.05 M, corresponding to a maximum pseudo-first order rate constant for the direct reaction of 0.03 s^{-1}. These values are sufficiently low as to be negligible, particularly as the lowest rate constants in the carbanion reactions are observed at low $[OH^-]_t$, where the rate of the direct reaction would be slower still.

4.4 Discussion.

It is thought likely that the above mechanistic interpretations for dimethyl malonate and ethylmalononitrile are broadly correct, though the differences between individual experiments remain unexplained. These may be due to some form of systematic error, possibly because of the very sensitive instrument setting required to follow these reactions, or due in some way to small impurities in the reagents. However the

possibility of there being some mechanistic significance to these observations cannot be ruled out. This is considered rather unlikely in view of the very good linear correlations obtained between k_{obs} and $[carbanion]_e$, and the fact that these were the only linear correlations found. It is also difficult to conceive an alternative mechanism, especially in the case of ethylmalononitrile.

Assuming the mechanisms to be correct, comparison of the results reveals that the kinetic behaviour of both dimethyl malonate and ethylmalononitrile in their carbanion reactions with nitroprusside is qualitatively different from that of malononitrile. The former compounds show linear correlations between the observed pseudo-first order rate constants and the equilibrium concentration of carbanion. Malononitrile does not show this relationship, but does show a linear correlation between the rate constants and the product $[M^-]_e[OH^-]_e$. This is explained by the different mechanism proposed for the malononitrile reaction, i.e. rate-determining removal of the second proton. It was as a result of observing this unexpected mechanism that the investigation of the other two carbanions was undertaken, for comparison.

Ethylmalononitrile has only one ionisable hydrogen atom and so was expected to behave as it did. Dimethyl malonate has two ionisable hydrogens, and it was thought that it might behave similarly to malononitrile. One possible reason why it did not is that the acidity of the second proton could be too low to

allow removal. It is known that the $-CO_2Me$ group is much less effective at delocalising a negative charge than the -CN group, and the acidity of malononitrile (pK_a 11.19) is some 2 orders of magnitude greater than that of dimethyl malonate (pK_a ~13.3). However these are pKs for the first ionisation of the free compounds, whereas the process under consideration here is a second ionisation with the compound complexed by nitroprusside; the effect of this complexation on the acidity of the proton attached to carbon is unknown.

An alternative explanation arises when the behaviour of dimethyl malonate is compared with that of acetone. The kinetic study of the acetone/nitroprusside/hydroxide reaction by Swinehart and Schmidt[2] found the system to obey a rate law of the form

$$\text{Rate} = k[\text{SNP}][OH^-][\text{acetone}]$$

consistent with a mechanism involving rate-determining attack of the acetone carbanion (or enolate anion) on nitroprusside. Since acetone is such an extremely weak acid, pK_a ~20[2], equilibrium concentration of hydroxide in this case is virtually identical with total concentration.

Swinehart and Schmidt assign the red colour to an adduct species $(NC)_5Fe(C_3H_5NO_2)^{3-}$, where the ligand structure is unspecified. However in a more recent review of the nitroprusside ion[14], Swinehart attributes the red colour to a species $(NC)_5FeNO[=CHCOCH_3]^{4-}$; red salts of this anion have been isolated, and it is reported[3,16] that acidification results in

the formation of a blue colour, attributed to the 3- species[14]. Similar observations have been made with other ketones.

If the red colour is due to the 4- species, then the mechanism of the acetone reaction must involve rapid loss of a second proton subsequent to the rate determining step. If this can occur for acetone, which is some 6-7 orders of magnitude weaker an acid, then the above argument based on the relatively low acidity of dimethyl malonate is invalid, and the second proton could be removed from it too. If this does in fact occur, then the overall reaction of dimethyl malonate is the same as that of malononitrile, except that in the former case removal of the second proton is faster than attack of the carbanion on nitroprusside, whereas in the latter case it is attack of the carbanion which is faster.

Removal of the second proton from the malononitrile adduct is likely to be energetically more favourable than in the case of dimethyl malonate. However from the relative values of the pK_as nothing can be deduced about the rate of proton removal. It may be that removal of the second proton from the malononitrile adduct is a slower process than for dimethyl malonate, or it may be that these rates are comparable and it is the rate of attack of carbanion which is very much faster in the case of malononitrile. In comparing rates of proton removal, it must be borne in mind that in order to obtain any results at all for dimethyl malonate, it was necessary to work at considerably higher total hydroxide concentrations than had been used for

malononitrile. Equilibrium hydroxide concentrations were 1-2 orders of magnitude higher. Since the actual rate of removal of the second proton depends on $[OH^-]_e$, this step could well be very much faster for the dimethyl malonate adduct than for the malononitrile adduct under these experimental conditions, even if the rate constant for the malononitrile reaction were the greater of the two.

An approximate idea of the relative rates of attack of the two different carbanions can be gained by considering the fastest rate constant observed in the malononitrile case. This is 29.8 s^{-1} and occurs at a carbanion concentration of ~2.8 x 10^{-2} M. This is a rate constant for removal of a proton from the adduct; the rate of attack of malononitrile carbanion is neccessarily faster than this. At a comparable carbanion concentration ($[DMM]_t$ = 0.0757 M, $[OH^-]_t$ = 0.15 M, $[DMM^-]_e$ = ~2.9 x 10^{-2} M), the rate constant for attack of the dimethyl malonate carbanion is 21.7 s^{-1}, slower than that measured for the proton removal step. Although this treatment is not rigorous it suggests that attack of malononitrile carbanion on nitroprusside is faster than the corresponding attack of dimethyl malonate carbanion.

In the case of ethylmalononitrile, there is no possibility of a second proton removal, so the red adduct must be a 3- species such as $(NC)_5FeN(O)C(Et)(CN)_2^{3-}$. The fact that this species is red rather than blue may be of significance, but would have to be considered carefully in view of the nature of the

$$CH_2(CN)_2 + OH^- \overset{K'}{\rightleftharpoons} CH(CN)_2^- + H_2O \qquad K' = K_a/K_w$$

$$CH(CN)_2^- + [Fe(CN)_5NO]^{2-} \overset{K}{\rightleftharpoons} [(NC)_5FeN(O)CH(CN)_2]^{3-}$$

I

$$[(NC)_5FeN(O)CH(CN)_2]^{3-} + OH^- \xrightarrow[rds]{k} [(NC)_5FeN(O)C(CN)_2]^{4-} + H_2O$$

II

SCHEME 4.1

$$CH_2(CO_2Me)_2 + OH^- \overset{K'}{\rightleftharpoons} CH(CO_2Me)_2^- + H_2O$$

$$CH(CO_2Me)_2^- + [Fe(CN)_5NO]^{2-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [(NC)_5FeN(O)CH(CO_2Me)_2]^{3-}$$

SCHEME 4.2

chromophores. Comparison is being made between a β-dicarbonyl compound, two β-dinitriles and a simple ketone, and although structures such as $[(NC)_5FeN(O)CH(CO_2Me)_2]^{3-}$ and $[(NC)_5FeNO(=CHCOCH_3)]^{4-}$ are drawn freely in this work and in the literature, the actual electronic structure of the adduct complexes is unknown.

The red intermediate obtained from the malononitrile reaction is much more intensely coloured and persists much longer in solution than the other two, to the extent that it is isolable as a solid product. This may be due to the sort of major difference in electronic structure which would be expected between a 3- species and a 4- species, which is the likely explanation in the ethylmalononitrile case; in the dimethyl malonate case the same explanation is possible but it may also be that both are 4- species and the differences are due to the effects of substituting $-CO_2Me$ for -CN. Satisfactory analysis of the isolated red malononitrile compound was not obtained, and the red dimethyl malonate compound could not be isolated.

This study has provided further evidence that the mechanism of the reaction of malononitrile carbanion with sodium nitroprusside is that shown in Scheme 4.1, as proposed earlier[5]; the rate determining step at the hydroxide ion concentrations used is the removal by hydroxide of the second ionisable hydrogen atom in malononitrile from the initial adduct. The reaction of dimethyl malonate carbanion under the experimental conditions used here is as shown in Scheme 4.2. There may be a subsequent

$$EtCH(CN)_2 + OH^- \overset{K'}{\rightleftharpoons} EtC(CN)_2^- + H_2O$$

$$EtC(CN)_2^- + Fe(CN)_5NO^{2-} \xrightarrow{k} (NC)_5FeN(O)CEt(CN)_2^{3-}$$

SCHEME 4.3.

loss of a second proton in this case also, which would be very fast at the high concentrations of hydroxide employed. The reaction mechanism of ethylmalononitrile carbanion cannot be deduced with certainty, but is probably as shown in Scheme 4.3.

Further information on reactions of sodium nitroprusside with carbanions could perhaps be obtained by extending the malononitrile investigation to high [OH^-]; this may have the effect of making the carbanion attack rate determining. The behaviour of e.g. ethyl cyanoacetate $NCCH_2CO_2Et$ in this system may be worthy of study.

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CHAPTER FIVE.

^{13}C N.M.R. STUDY OF NITROPRUSSIDE/BLOOD REACTIONS.

5.1 Introduction.

Sodium nitroprusside is widely used as a hypotensive agent in anaesthesia. Infusion of a solution of nitroprusside into a patient results in a very rapid lowering of the blood pressure, which can then be maintained at the desired level by adjusting the rate of infusion. When infusion is stopped, the blood pressure rapidly returns to normal without overshooting, which can be a drawback with the use of other hypotensive agents. The activity of nitroprusside as a vasodilator has been known since 1929[1]; it was first introduced to British anaesthetic practice in 1968[2].

There are a considerable number of reports that nitroprusside breaks down in a reaction with blood to release free cyanide. For example, Vesey and co-workers[3,4] report increased cyanide concentrations in the blood and expired air of patients undergoing nitroprusside therapy, while Smith and Kruszyna[5] and Spiegel and Kucera[6] state that nitroprusside reacts *in vitro*, with haemoglobin or whole blood respectively, with the release of cyanide. This is clearly a very serious drawback to the medical usage of sodium nitroprusside; indeed a number of deaths have been attributed to this effect. As a consequence a

low maximum dosage has been recommended[7,8].

From a thermodynamic viewpoint a reaction involving the complete breakdown of nitroprusside with the liberation of cyanide is very surprising. The nitroprusside ion is a very stable species - from the known[9] formation constants of $Fe^{II}(CN)_6^{4-}$ and $Fe^{III}(CN)_6^{3-}$ it can be estimated that nitroprusside has $\beta_5 = \sim 10^{30}$. Formation of an equally stable species would be required to make the overall process energetically favourable. Reactions of nitroprusside generally involve the nitrosyl group, with the $Fe(CN)_5$ moiety remaining intact.

Recent investigations in this department have suggested that the apparent cyanide release may be a consequence of a photolytic reaction of nitroprusside rather than a reaction with haemoglobin[10-12]. No release of cyanide from nitroprusside on contact with blood was detected when the mixture was kept dark, and intact nitroprusside was found in the plasma. Release of cyanide was found to occur only when the nitroprusside solution was exposed to light; the presence or absence of blood made no difference.

These results were explained on the basis that nitroprusside contains low spin iron(II), a d^6 species in which the ligands are kinetically inert. Photolysis[13], however, results in the formation of aquapentacyanoferrate(III); the electronic configuration is now d^5 and the ligands are labile.

(As pointed out in Chapter One, the formalism by which nitroprusside is generally regarded as an iron(II) complex has been questioned[14]; however it has been shown[15] to undergo ligand exchange with labelled $^{14}CN^-$ only slowly, though the process is accelerated by light.)

The technique used[4,10] to isolate the cyanide for assay involves acidification of the cyanide-containing solution and removal of the volatile HCN thus formed by passing a stream of nitrogen through the solution. The HCN is then trapped in sodium hydroxide solution. This is a non-equilibrium process, thus although $Fe(CN)_5OH_2^{2-}$ is also a stable species (est. $\beta_5 = 10^{36}$)[9], it will eventually release all its cyanide as the labile ligands are protonated and swept out of solution.

This explanation for the detection of cyanide following mixing of nitroprusside and blood has resulted in a disagreement with the original workers[16]; the experiments described here were therefore undertaken with a view to clarifying further what is clearly a very important aspect of nitroprusside chemistry.

Scott[17] has performed experiments which involved taking n.m.r. spectra of blood samples or of bacteria which contained substrates labelled with carbon-13. This technique seemed particularly suitable for study of the present problem, as it is non-intrusive, i.e. it would enable any reaction to be monitored in situ, without the necessity for further chemical manipulations

such as the lengthy cyanide analysis procedure.

The synthesis of sodium nitroprusside labelled with C-13 was therefore undertaken, and its behaviour in the presence of blood was studied by n.m.r..

5.2 Experimental.

5.2.1 Materials and Instruments.

Sodium cyanide (90 atom % ^{13}C) was purchased from MSD Isotopes Ltd. (Canada); all other chemicals were reagent grade or better. Human blood was obtained from Dr. A.R. Butler, and stored in heparinised tubes until required; experiments were carried out on the same day the blood was withdrawn.

U.v./visible spectra were obtained on a Unicam SP8-100; i.r. spectra were obtained as KBr discs on a Perkin-Elmer 1330. N.m.r. spectra were obtained on a Varian CFT-20 for the preliminary work (carbon resonance at 20 MHz in a field of 1.9 T), and a Bruker WH-360 for the definitive experiments. (This was the S.E.R.C. high field instrument at Edinburgh University; carbon resonance at 90 MHz in a 7.5 T field.)

The preparation of sodium ferrocyanide was found not to give very good yields; in order to make the process economic when using the isotopically labelled material it was necessary to recycle the unrecovered material by conversion into Prussian Blue. The formation of ferrocyanide by decomposition of Prussian Blue with alkali is mentioned by Sharpe[18] as being carried out on a technical scale.

Repeated attempts to prepare nitroprusside from ferrocyanide by either of the literature methods[19,20] were unsuccessful; the method eventually adopted, though similar to that given by Brauer[19], is an adaptation of an industrial process.

All reactions involving cyanide were carried out in a good fume cupboard. All nitroprusside solutions were prepared and handled in such a way that exposure to light was minimal. "Cyanide", "Ferrocyanide" and "Nitroprusside", as used in the rest of this Experimental section, refer to the 90 atom % labelled species unless otherwise stated.

5.2.2 Preparation of sodium ferrocyanide.

Sodium cyanide (4 g, 80.2 mmol) and sodium hydroxide (1.1 g, 28 mmol) were dissolved with stirring in ~15 ml water. Ammonium ferrous sulphate (A.R. grade, 5.1 g, 13 mmol) was added in portions, with continuous stirring, gradual warming and occasional addition of water (<5 ml total). The resultant clear brown solution was boiled for a few minutes, then filtered hot through a wide glass sinter. The filtrate was set aside to crystallise in the dark. If crystallisation did not occur on standing overnight, it could be induced by scratching. The yellow crystals were filtered off, washed with a little water and dried between filter papers. The yield at this stage varied from 36-60%.

The filtrate and any cyanide- or ferrocyanide-containing washings were combined and treated with solutions of ammonium ferrous sulphate followed by ferric chloride, which resulted in the formation of Prussian Blue. The precipitate was filtered off under gravity, washed with water and allowed to stand for 1-2 days to dry out partially before conversion into ferrocyanide.

The Prussian Blue was treated by stirring for about an hour with an aqueous solution containing ~3 g sodium hydroxide. The resultant suspension was filtered twice, once through Hyflo, to remove the very fine precipitate of ferric oxide, then concentrated on the rotary evaporator and left to crystallise. The crystals were collected as above; the filtrate was generally

set aside for the next recycling. Using this procedure over 90% of the cyanide used was recovered as ferrocyanide.

The infra-red spectrum of the product showed CN bands at 2020, 2000 and 1980 cm^{-1} compared with 2060, 2045 and 2020 cm^{-1} in the unlabelled material. The shift is as predicted in the diatomic molecule approximation[21]. The product spectrum also contained bands attributable to the presence of 10% ^{12}C.

5.2.3 Conversion of ferrocyanide into nitroprusside.

(This procedure is adapted from the industrial process, details of which were kindly supplied by Mr. C. Garnsworthy of BDH Chemicals Ltd.)

A mixture of concentrated nitric acid (2.25 ml) and water (1 ml) was stirred and cooled in an ice/water bath. To the stirred solution was added 2.5 g sodium ferrocyanide; a further 1.54 g ferrocyanide was added in portions over the next four hours. (The total amount of ferrocyanide added corresponded to 8.26 mmol.) The mixture was then stirred for an hour, during which it was allowed to come to room temperature. It was then heated to 60° C and sodium carbonate (0.22 g) was added in portions over 15 - 30 minutes, allowing the effervescence to die down somewhat between each addition. The mixture was then heated to 75° C and a further 0.18 g sodium carbonate was added, again in portions and allowing the effervescence to die down. The mixture was then

stirred for approximately one more hour, during which it was allowed to cool to < 65° C. It was then treated with a mixture of 3 ml water and 10 ml methanol and left to stand at 50 - 60° C for 48 hours, protected from light and under a gentle stream of nitrogen.

After 48 hours the mixture was filtered through a bed of Hyflo (1 - 2 cm x 4.25 cm); the reaction flask and the Hyflo bed were washed with methanol until the washings were almost colourless. The deep red filtrate was filtered again through a glass sinter and concentrated on the rotary evaporator at < 80° C until crystals began to form. Just enough water to redissolve the crystals in the hot solution was then added, and the solution set aside in the dark to crystallise.

The small red crystals which formed were filtered off and dried between filter papers. The yield at this stage was typically about 0.9 g . Further product could be obtained from the mother liquor by evaporating to dryness, extracting with a little warm methanol by stirring for 30 - 60 minutes, filtering off the solid residue and crystallising the filtrate as described above. Total yields were around 50 - 60% based on iron, though the product was shown by i.r. to contain sodium nitrate as an impurity at around 2 wt.% .

The nitrate impurity could be removed by careful recrystallisation and washing of the crystals with a little water; however as this resulted in a considerable lowering of yield it was undertaken only for some more heavily contaminated samples, since small amounts of nitrate should not affect the n.m.r. experiments.

The u.v./visible spectrum of the product was qualitatively identical to that of authentic unlabelled sodium nitroprusside; the i.r. spectrum showed the expected differences due to the presence of ^{13}C (see sect. 5.3.1).

Analysis of a purified sample gave the following result:

Found: C, 20.86%*; H, 1.30%; N, 27.21%.

Calc. for $C_5H_4N_6O_3FeNa_2$ (90 atom % ^{13}C):

C, 21.33% ; H, 1.33%; N, 27.79%.

This sample was used in the high field n.m.r. experiments.

(*Since the analyser employs a g.l.c. detector it effectively "counts" CO_2 molecules and cannot allow for the presence of ^{13}C; the percentage of carbon found is corrected for this effect.)

5.2.4 N.m.r. experiments.

5.2.4.1 Experiments on 20 MHz spectrometer.

A series of preliminary experiments was carried out in which nitroprusside was dissolved in phosphate buffer (pH ~7.3 cf. blood pH 7.2) at various concentrations and the quality of the spectra obtained after various acquisition times was examined. It was found that a nitroprusside concentration of about 0.05 M gave a spectrum with a reasonable signal-to-noise ratio after five minutes' acquisition.

A solution of sodium nitroprusside in D_2O of concentration 0.5 M was prepared. 0.2 ml of this solution was added to 2 ml blood in a 10 mm n.m.r. tube. This diluted the nitroprusside to the desired level and provided a 10% concentration of D_2O as a lock for the spectrometer. A series of spectra was obtained by accumulating for 5 minutes at fifteen-minute intervals over a period of about 1.5 hours. The characteristic pattern of the nitroprusside spectrum remained unchanged.

The sample was left overnight at $4^{o}C$; a spectrum accumulated over 2 hours the following day still showed the presence of intact nitroprusside and no evidence for the presence of free cyanide.

The concentration of nitroprusside used in this experiment, however, was too high to be meaningful (see discussion); the result does demonstrate the absence of a catalytic process causing the breakdown of nitroprusside, but such a process is not what has been claimed to occur.

5.2.4.2 Experiments on 90 MHz spectrometer.

A similar experiment was carried out at tenfold greater dilution using the S.E.R.C. 90 MHz spectrometer at Edinburgh University. A solution of nitroprusside in D_2O of concentration 0.054 M was made up. 0.5 ml of this solution was added to 5 ml of phosphate buffer in a 15 mm n.m.r. tube and a spectrum obtained. A further 0.5 ml was then mixed with 5 ml blood, and a series of spectra obtained as before, accumulating for 5 minutes at intervals over a period of 2 hours. The spectrometer probe was thermostatted to $37^{o}C$. These spectra were not of very high quality due to the inhomogeneity and viscosity of the blood, and the quality deteriorated further as the red cells and plasma separated during the 2 hour period; nonetheless it was clear that there was no obvious change in the spectrum of the nitroprusside. After two hours the tube was removed and shaken to re-mix the plasma and red cells; a spectrum was then accumulated for 45 minutes. This spectrum clearly showed the presence of intact nitroprusside.

Spectra of cyanide, in both phosphate buffer and blood at a concentration of 0.028 M, were then obtained; these each showed a single peak at δ119 (buffer) and δ121 (blood). This is similar to the chemical shift of organic nitriles[22], and was taken as indicating the presence of HCN.

The chemical shift range over which the nitroprusside spectra were acquired was not wide enough to cover this region, so if there had been any small decomposition the consequent increase in free HCN would not have been observed. Accordingly a second nitroprusside/blood mixture was examined; the spectrum was accumulated overnight over a chemical shift range which was large enough to include the HCN region. Cumulative spectra were recorded every hour. These spectra show no evidence for the release of cyanide.

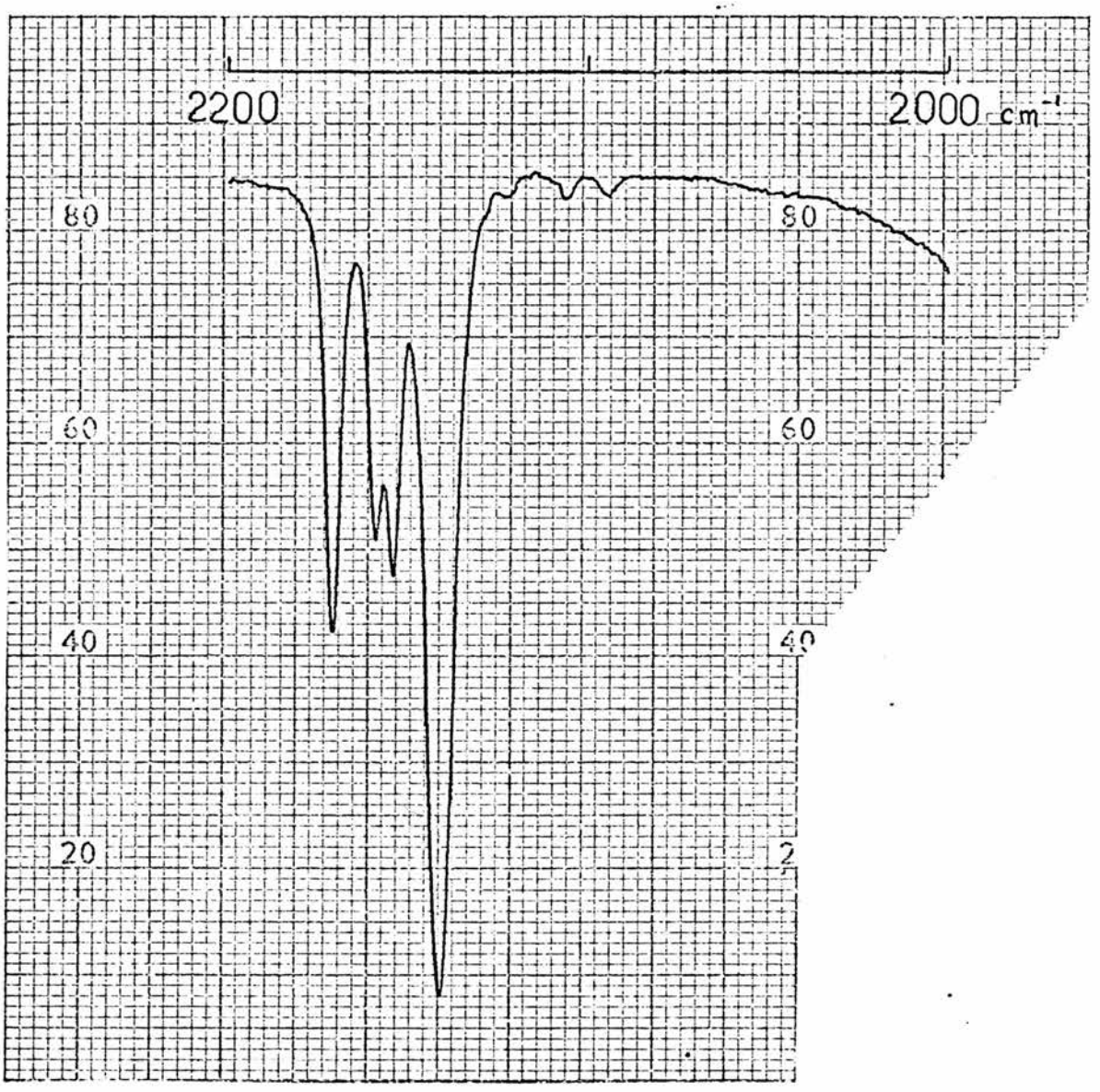

Fig. 5.1 The C≡N stretching region of the i.r. spectrum of sodium nitroprusside.

5.3 Results and Discussion.

5.3.1 The infra-red spectrum of C-13 labelled sodium nitroprusside.

The infra-red spectrum of sodium nitroprusside has been studied by several workers, and bands due to the presence of the carbon-13 isotope at natural abundance have been observed and assigned[23]. The positions of these bands relative to the ^{12}C bands are in good agreement with calculations based on treating the CN group as isolated from the rest of the ion and applying the formula for isotopic shifts in diatomic molecules[21]:

$$\nu^{i}_{osc} / \nu_{osc} = \sqrt{\mu/\mu^{i}}$$

ν_{osc} is the absorption frequency and μ is the reduced mass; the superscript "i" refers to the isotopic species.

Figure 5.1 shows the CN stretching region of the i.r. spectrum of unlabelled sodium nitroprusside. Four major bands are visible, at approximately 2172, 2159, 2154 and 2141 cm^{-1}, agreeing closely with literature values. Weak bands at about 2123, 2106 and 2094 cm^{-1} are also observed; these are the natural abundance ^{13}C bands[23b].

The wavenumbers of the bands observed in this work were not measured with a high degree of precision; as the spectra were primarily used for diagnostic purposes this was not considered necessary.

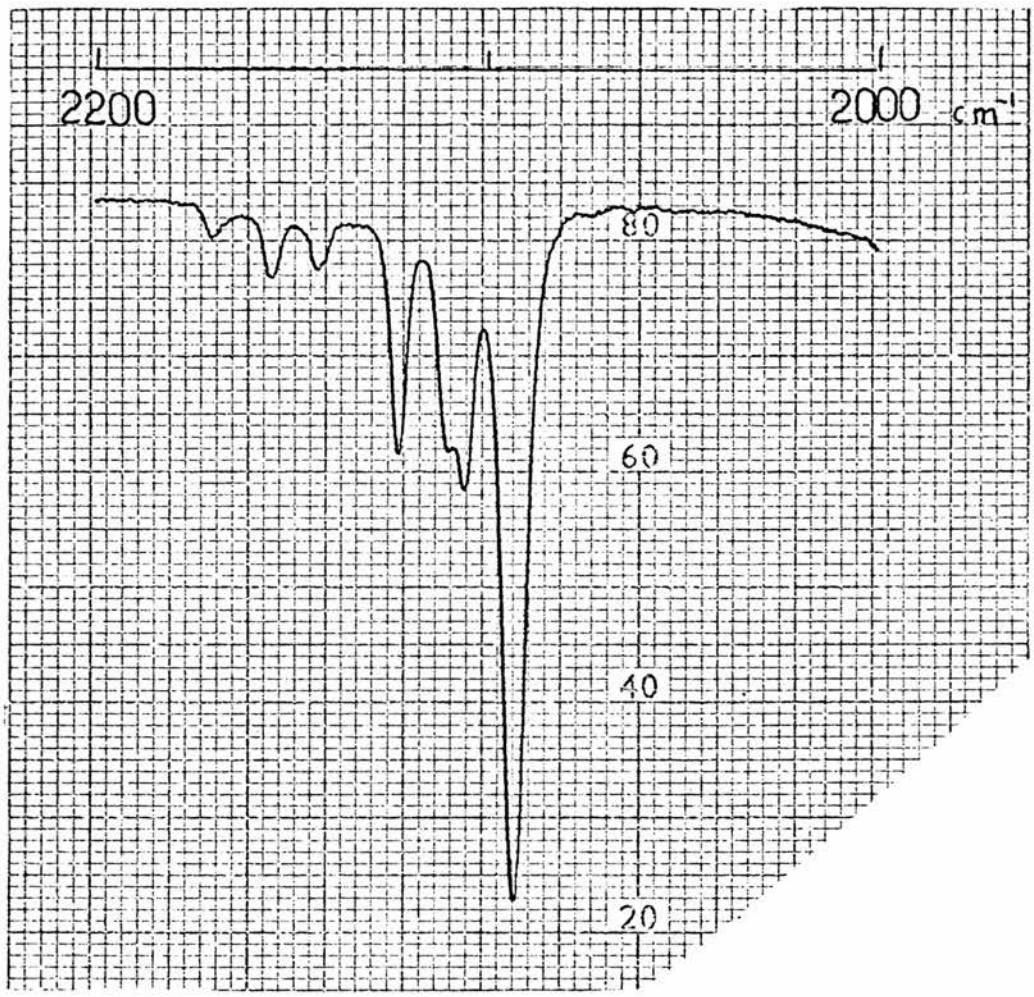

Fig. 5.2 The C≡N stretching region of the i.r. spectrum of 90% C-13 sodium nitroprusside.

In figure 5.2 the spectrum of the 90% labelled material in the same region is shown. The four major bands appear at 2122, 2110, 2105 and 2093 cm^{-1}, with bands at 2171, 2155 and 2143 cm^{-1} due to the 10% ^{12}C. The sizes of the isotopic shifts are not in exact agreement with those calculated by the diatomic molecule approximation; however the spectrum of Fig. 5.2 is clearly consistent with the species $Fe(^{13}CN)_5NO^{2-}$ in which 10% of the carbon atoms are C-12.

A noteworthy feature of this spectrum is the complete absence of absorption at ~2038 cm^{-1}. Cyanide anion absorbs at 2080 cm^{-1} [18]; carbon-13 cyanide is calculated to absorb at 2038 cm^{-1} in the diatomic molecule approximation. The absence of a band in this region indicates that the material as prepared is free from cyanide, as far as can be determined by i.r.. This is not unexpected but is important in view of the nature of the problem under investigation.

Spectra of the labelled product showed a peak at 1385 cm^{-1} not due to nitroprusside. This was assigned to sodium nitrate present as an impurity. Spectra of unlabelled sodium nitroprusside doped with $NaNO_3$ at 1 and 5 weight % were obtained, and comparison of the sizes of the nitrate peaks with those of the strong NO bands enabled the amount of the nitrate impurity in the prepared samples to be estimated at 1-2 wt.%. Samples purified by recrystallisation showed no i.r. absorption due to nitrate.

5.3.2 The C-13 n.m.r. spectrum of labelled sodium nitroprusside.

Natural abundance C-13 n.m.r. spectra of various iron cyano complexes including nitroprusside have been obtained[24,25]. As would be expected, the nitroprusside spectrum showed two singlets in the ratio 4:1, assigned to the four equatorial cyanides and the single axial cyanide (*trans* to the NO group). The chemical shifts of the peaks corresponded to 139.0 (4) and 137.0 (1) ppm from TMS.

The natural abundance of carbon-13 is so low (1%) that the probability of there being two ^{13}C atoms in one nitroprusside ion is negligible. Hence the spectrum observed is that of one ^{13}C in the presence of four ^{12}Cs. The case of the 90% labelled material is rather more complicated. Firstly there will be a chemical shift difference due to the presence of additional ^{13}C rather than ^{12}C; secondly, because the proportion of ^{12}C is relatively high the fraction of the ions containing one and even two ^{12}C atoms will not be negligible.

The relative proportions of the fully labelled (five ^{13}C), singly unlabelled (four ^{13}C) and doubly unlabelled (three ^{13}C) species present can be found from the binomial expansion $(9 + 1)^5$, i.e. 9 ^{13}C atoms for every 1 ^{12}C atom to be distributed over 5 possible sites. The first three terms of the expansion are $9^5 = 59049$, $5 \times 9^4 = 32805$ and $10 \times 9^3 = 7290$. These account for over 99% of the total and so further species need not be considered. The 90% labelled sodium nitroprusside

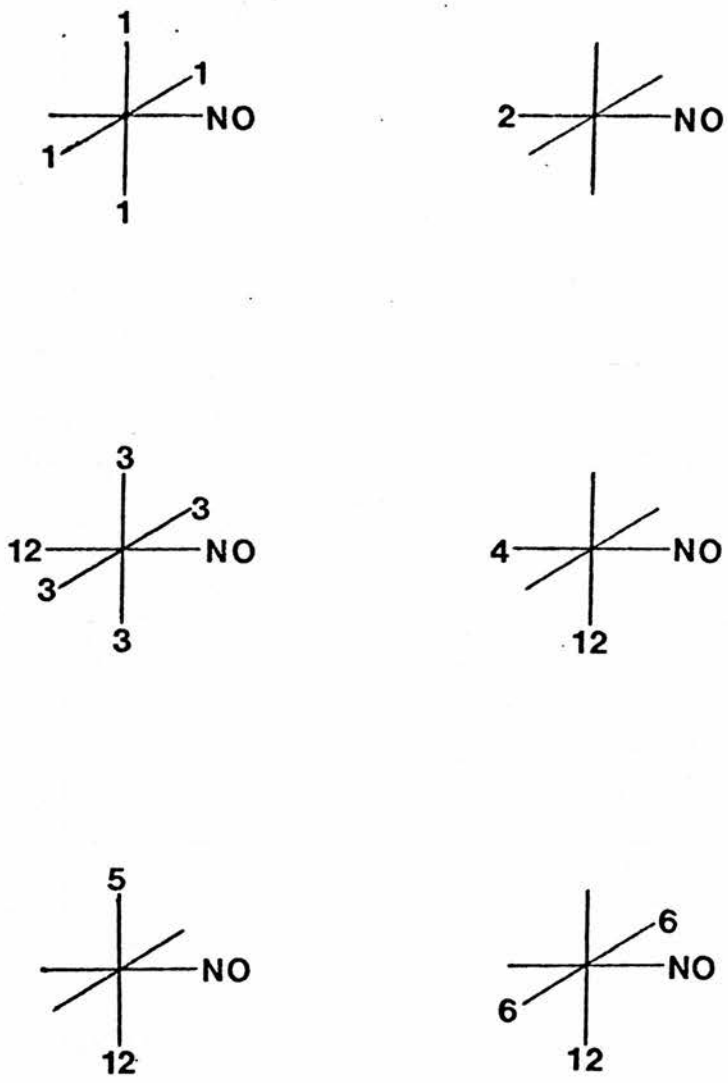

Fig. 5.3. Non-equivalent ^{13}C environments in totally labelled and singly unlabelled C-13 nitroprusside.
The environments are numbered 1-6; each atom in a particular environment is indicated by the same number. "12" represents ^{12}C.

thus consists of 59% $Fe(^{13}CN)_5NO^{2-}$, 32.8% $Fe(^{13}CN)_4(^{12}CN)NO^{2-}$ and 7.3% $Fe(^{13}CN)_3(^{12}CN)_2NO^{2-}$. The n.m.r. spectrum should show signals for all of these species. The position is further complicated by the fact that the placement of the ^{12}C atoms gives rise to different isomers, and within each isomer there are different magnetically non-equivalent ^{13}C environments. Thus for the totally labelled species there is only one isomer, which has two ^{13}C environments, axial (in which there is one carbon) and equatorial (in which there are four). The singly unlabelled species has two isomers, one with ^{12}C axial and the other with ^{12}C equatorial, in the relative ratio of 1:4. The isomer with axial ^{12}C has only one ^{13}C environment, in which there are four atoms. The isomer with ^{12}C equatorial has three ^{13}C environments: trans to NO (one carbon), cis to NO and trans to ^{12}C (one carbon) and cis to both NO and ^{12}C (two carbons). See Fig. 5.3.

The doubly unlabelled species gives rise to three isomers in which there are a further six non-equivalent environments. Each distinct ^{13}C environment will give rise to a separate signal in the n.m.r. spectrum; further complications arise when the possibility of coupling between ^{13}C atoms in different environments is considered.

A completely rigorous "theoretical spectrum" of 90% labelled sodium nitroprusside could be constructed by extending the above arguments, but would not be relevant to the present problem. The observed spectra can be explained by the ideas already

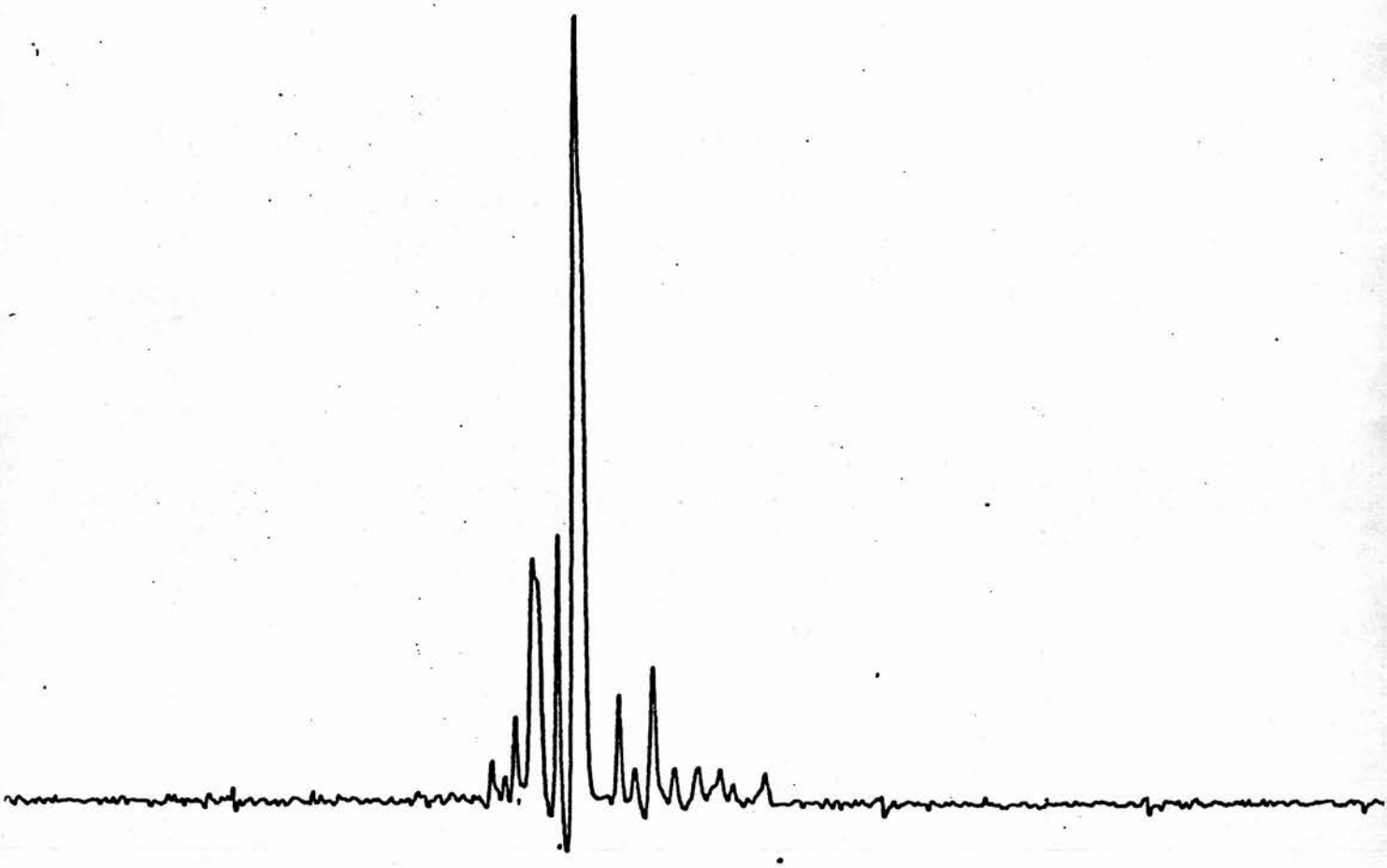

Fig. 5.4 20 MHz ^{13}C n.m.r. spectrum of 90% labelled sodium nitroprusside.

Major peak at δ136.6; scale approx. 10.4 Hz/cm.

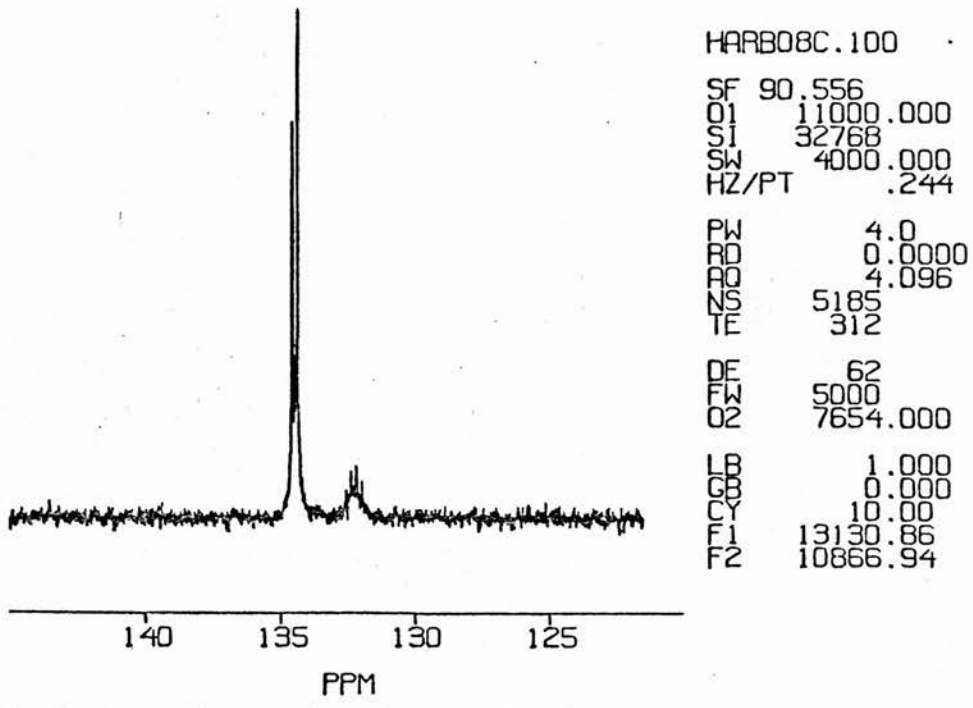

Fig. 5.5 90 MHz ^{13}C n.m.r. spectrum of 90% labelled sodium nitroprusside.

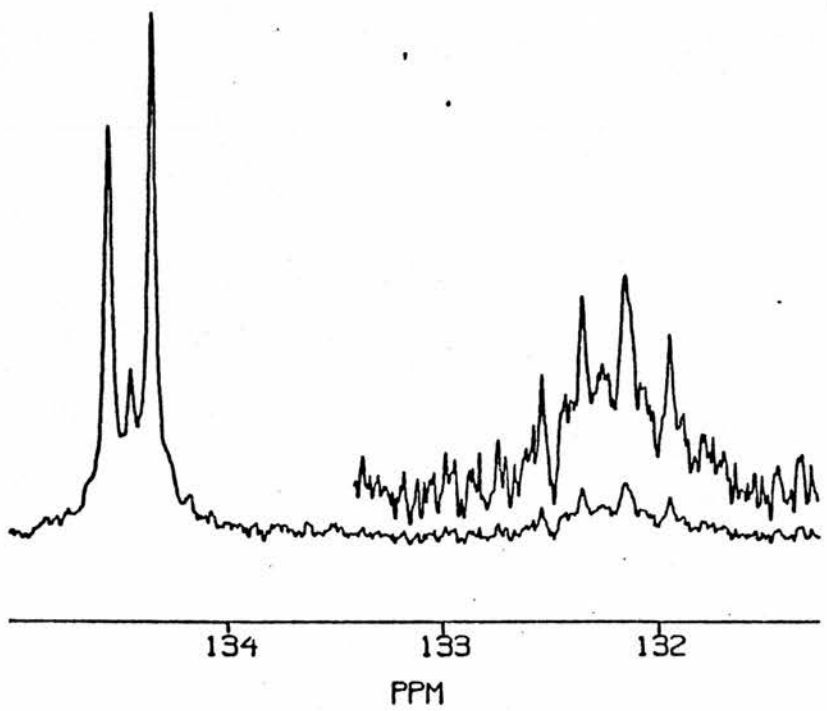

Fig. 5.6 90 MHz ^{13}C n.m.r. spectrum of 90% labelled sodium nitroprusside (expanded).

presented.

The 20 MHz ^{13}C n.m.r. spectrum of 90% labelled sodium nitroprusside is shown in Fig. 5.4; the 90 MHz spectrum is shown in Fig. 5.5, expanded in Fig. 5.6. For simplicity the 90 MHz spectrum will be considered first.

The discernible features of the spectrum are predominantly due to the totally labelled species. This gives two signals, that for the four equatorial carbons split into a doublet by the single axial carbon, and that for the axial carbon split into a quintet by the four equatorial carbons. The separation between the signals, $\Delta\nu$, is ~2 ppm as observed in the natural abundance spectrum, and the coupling constant J = 18 Hz. In the expanded spectrum (Fig. 5.6) a third line is clearly visible between the two lines of the doublet; this is thought to be due to the isomer of the singly unlabelled species with the ^{12}C axial. The four ^{13}C atoms in this isomer are all equivalent (environment 3 of Fig. 5.3) and should give a singlet spectrum. The signals due to the carbons in the other equatorial environments are of low intensity further diminished by coupling; they appear only as the broad non-resolved area at the base of the doublet. Similarly the signals from the other axial carbons make up the broad area above which the quintet can just be discerned.

The 20 MHz spectrum appears completely different. Fourteen lines are discernible, one of which is much more intense than the rest. At 20 MHz the separation of the axial and equatorial signals (2 ppm) is 40 Hz; the coupling constant J remains 18 Hz, hence $\Delta\nu << 10J$ and a highly distorted non-first order spectrum results. The pattern of the peaks is quite characteristic.

The chemical shift of the major peak in the 20 MHz spectrum is 136.6 ppm from 3-(trimethylsilyl)propanesulphonate. The two sets of signals in the 90 MHz spectrum appear at 134.5 and 132.3 ppm from external TMS.

There was no n.m.r. evidence for the presence of free cyanide as an impurity in the labelled compound. In particular a 20 MHz spectrum of the 0.054 M solution used in the blood experiments, acquired the following day, showed no peaks not attributable to nitroprusside.

5.3.3 The reaction of nitroprusside with blood.

The present series of n.m.r. experiments has produced no evidence for the release of cyanide from nitroprusside on incubation with whole blood. This is in agreement with earlier findings in this department[10-12], but in contradiction to many reports in the medical literature. These results are summarised here before discussing the n.m.r. work.

The results of Butler and co-workers[10-12] are as follows:

i) No cyanide could be detected in mixtures of nitroprusside with whole blood or any blood fraction when measured with a cyanide-specific electrode.

ii) Cyanide was detected when a different method of analysis was used. In this technique the solution to be analysed was acidified and a stream of nitrogen passed through it. Any cyanide present was swept out as volatile HCN and trapped in NaOH solution for assay. Cyanide was detected only when the solution under analysis was exposed to light; the presence or absence of blood made no difference.

iii) Very little cyanide release from nitroprusside, in the presence or absence of blood, in the light or dark, was observed using the diffusion technique of Spiegel and Kucera[6].

iv) Mixtures of nitroprusside and oxy- or deoxyhaemoglobin showed evidence for the release of cyanide only in the light.

These observations were explained on the basis of the known photochemistry of the nitroprusside ion. Wolfe and Swinehart[13] have shown that photolysis of nitroprusside, independent of pH and wavelength (above 400 nm), yields the aqua-pentacyanoferrate(III) ion, $Fe(CN)_5OH_2^{2-}$. This species is thermodynamically stable but contains d^5 iron(III) and is labile to ligand substitution. The analytical procedure used to isolate and concentrate the cyanide for assay is a non-equilibrium, entropy-driven process. Any labile cyanide is protonated and removed from the solution, so eventually all the

aquapentacyanoferrate(III) is converted into Fe^{3+} and HCN. When preformed aquapentacyanoferrate(III) was subjected to this procedure, its behaviour in the light or dark was found to be similar to that of nitroprusside in the light.

Considering now the medical reports, Vesey, Cole, Simpson and co-workers have reported increased concentrations of cyanide and thiocyanate in the blood of both humans[3,4] and dogs[26] after treatment with nitroprusside. They also report an increase in the HCN content of the expired air of persons undergoing nitroprusside therapy[4]. In vitro incubation of various human tissues and body fluids with nitroprusside is also said to result in cyanide release[5,27-29]. Smith and Kruszyna[5] state that blood is by far the most active biological preparation in this respect, and suggest that the free cyanide found in vivo after nitroprusside treatment arises primarily as a result of a reaction of nitroprusside with haemoglobin. Spiegel and Kucera[6] report a quantitative release of cyanide from nitroprusside in the presence of whole blood; however they fail to detect cyanide in the blood of patients undergoing nitroprusside therapy when analysing samples taken within 2 minutes after infusion had stopped.

In none of these papers is any mention made of whether or not precautions were taken against exposure of nitroprusside-containing solutions to light; hence the possibility exists that the reported cyanide concentrations could have arisen as a result of photolysis with the production of

labile cyanide ligands.

If there is a reaction of nitroprusside with blood which produces free cyanide, there are several points which can be considered even without knowing the detailed chemistry of the process. Firstly there is the question of the approximate reaction rate. The hypotensive action of nitroprusside is known to be fast, but the cyanide release on incubation with blood is said by Vesey and co-workers to be "slow", with 50% decomposition in 20 minutes and over 90% in two hours[16a,27]. In a later paper[30] the same workers report only 46% decomposition of nitroprusside after three hours' incubation. However Spiegel and Kucera[5] state that cyanide release is "rapid", without actually quoting any data relevant to the rate beyond the observation of 100% decomposition over the course of a 2 hour diffusion period during analysis. These authors also attribute their failure to detect cyanide after <u>in vivo</u> nitroprusside treatment to a phenomenally rapid detoxification process in the liver.

The next question concerns the nature of the reacting species causing the breakdown. Smith and Kruszyna[5] state specifically that nitroprusside reacts with haemoglobin, and propose a plausible mechanism whereby one haem iron(II) transfers an electron to nitroprusside, giving haem Fe(III) and a reduced form of nitroprusside which then releases its cyanides. One of these is picked up by the oxidised haem iron, resulting ultimately in the formation of metcyanhaemoglobin, while the others are released into the system. However this does not

account for the observations of cyanide release from nitroprusside on contact with various other biological materials, such as liver and kidney[5] and also plasma dialysates and urine[27], which obviously do not contain haemoglobin. Free thiol groups have been suggested as being responsible for these effects.

Attention has been focussed on haemoglobin, however, because of the fact that blood was by far the most active material and the red cells were much more active than the plasma. This leads to the crucial question of whether or not the nitroprusside ion can penetrate the red cell membrane. The membrane is known to be permeable to small ions such as cyanide, but it is also known that ferricyanide $Fe(CN)_6^{3-}$, which is of comparable size to nitroprusside[18], will not penetrate the membrane[31,32]. Smith and Kruszyna[5] investigated this point to a certain extent, and found that very high external nitroprusside concentrations (8 mM) were necessary to convert even 30% of the haemoglobin in human red cells to metcyanhaemoglobin. Vesey, Krapez and Cole, however, using much lower nitroprusside concentrations, interpreted their results on the basis of cell penetration by nitroprusside occurring prior to breakdown[30].

There is thus general agreement in the medical literature that sodium nitroprusside will release cyanide in the presence of blood; evidence pertaining to the nature of the reaction is rather confused and occasionally contradictory. Investigations from a chemical viewpoint have failed to detect a reaction, and have cast doubt on the suitability of the analytical procedures

used to determine cyanide.

The n.m.r. experiments described here do not settle the matter completely; they do provide further information on which an eventual conclusion can be based.

The concentrations of nitroprusside used in these experiments were far higher than anything which would be achieved in the body during nitroprusside therapy. The recommended maximum total dose is 1.5 mg/kg[8] (though prior to this recommendation higher, and in some cases much higher, doses were used), which for a 70-kg person containing 6 litres of blood amounts to a maximum possible concentration of 6×10^{-5} M. This is some two orders of magnitude below the concentration of 5 mM used for the 90 MHz n.m.r. experiments. However this concentration is meaningful on the basis of Smith and Kruszyna's proposal for the breakdown of nitroprusside by reaction with haemoglobin. The concentration of iron in blood is about 42-50 mg/100ml, almost all in the haemoglobin[31]. Taking 40 mg/100 ml as a reasonable lower limit for haemoglobin iron, this represents a concentration of 7.2 mM. Thus if there was a reaction between one haem iron and one nitroprusside ion, there would be enough haem present to decompose nitroprusside totally at a concentration of 5 mM.

This argument is valid only if any cyanide which may be released does not cause oxidation of the haem iron leading to formation of metcyanhaemoglobin. If this was the case, then decomposition of one fifth of the nitroprusside would release enough cyanide to oxidise all the haemoglobin and the remaining 80% would be undecomposed (in the limiting case of nitroprusside and haem iron concentrations being equal; the experimental situation was rather better than this as far as detection of nitroprusside decomposition is concerned since there was always a definite excess of haem present). However oxidation of iron(II) by cyanide is chemically highly unlikely; indeed cyanide is known to reduce iron(III) in at least one case[18]. The action of nitrites and various other compounds as cyanide antidotes depends on their ability to oxidise haemoglobin (which has a low affinity for cyanide) to methaemoglobin (which has a high affinity)[33]; if cyanide itself were able to bring about this reaction then it would to a large extent function as its own antidote. For these reasons oxidation of haem iron by cyanide is not thought to be a problem.

Another possibility for the formation of metcyanhaemoglobin has been suggested by Vesey et al.[30]. This involves the trapping by cyanide of methaemoglobin formed by spontaneous oxidation. However fully oxygenated haemoglobin, as is present on exposure of blood to air, is known to be resistant to oxidation[34]; a high percentage conversion into methaemoglobin seems unlikely, and in any case could only limit the percentage decomposition of

nitroprusside to a level which would still be expected to be detectable in these experiments.

It should be pointed out here that metcyanhaemoglobin is paramagnetic and would not be expected to be detectable by n.m.r.; a reaction leading to its formation could be detected only by the decrease in intensity of the nitroprusside signal.

A similar reduction in the observable decomposition of nitroprusside could occur if the oxidation of one haem iron(II) to iron(III) by nitroprusside affected the ability of the other three iron atoms in the haemoglobin molecule to undergo the same reaction. In this case up to 75% of the nitroprusside would be undecomposed. Although the effects by which the oxygen affinity of the haem irons is altered according to the number of O_2 molecules already bound are well known, similar effects would not be expected to be so important for an electron transfer process. If they were the complete rapid oxidation of haemoglobin by ferricyanide[5] could not occur. Again this is not expected to be a drawback to the present experiments.

Thus on the basis of the mechanism proposed by Smith and Kruszyna the n.m.r. signal due to nitroprusside in blood at a concentration of 5 mM would be expected to disappear completely with the appearance of a signal due to free cyanide (which at blood pH would be expected to be present largely as HCN; this is confirmed by the observation of an n.m.r. signal in the region associated with organic nitriles).

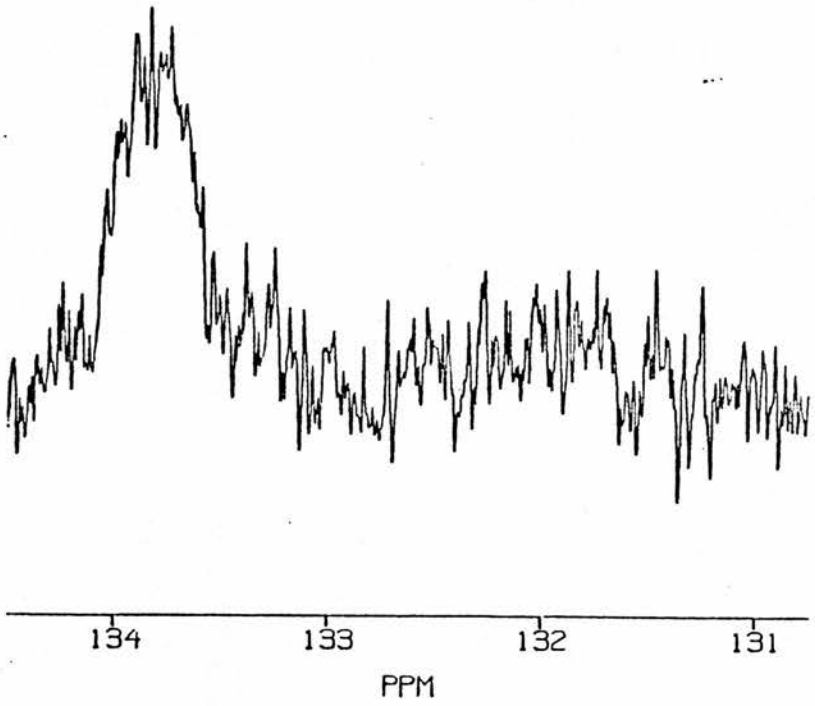

Fig. 5.8 Nitroprusside in blood after 15 minutes.

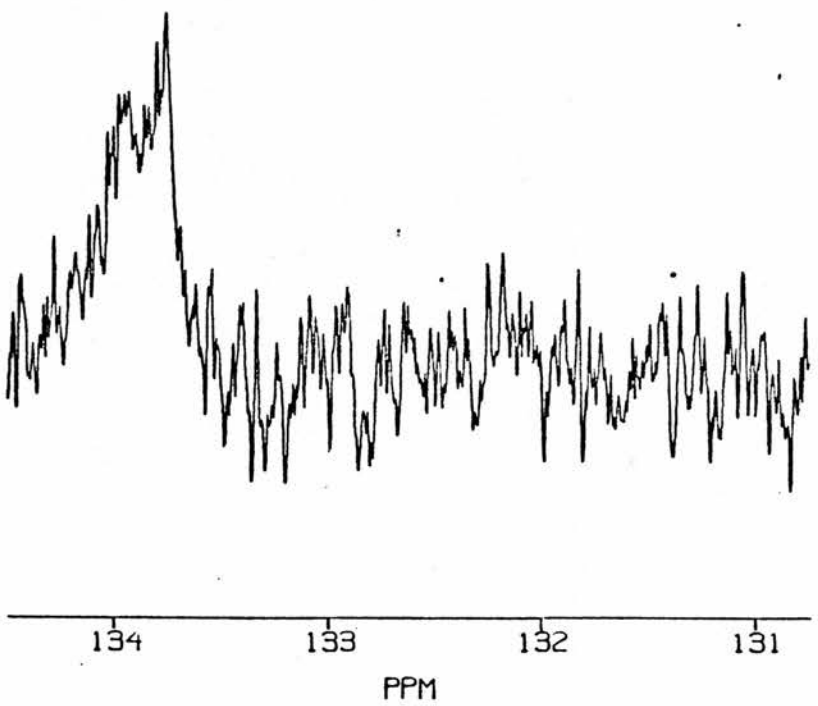

Fig. 5.9 Nitroprusside in blood after 2 hours.

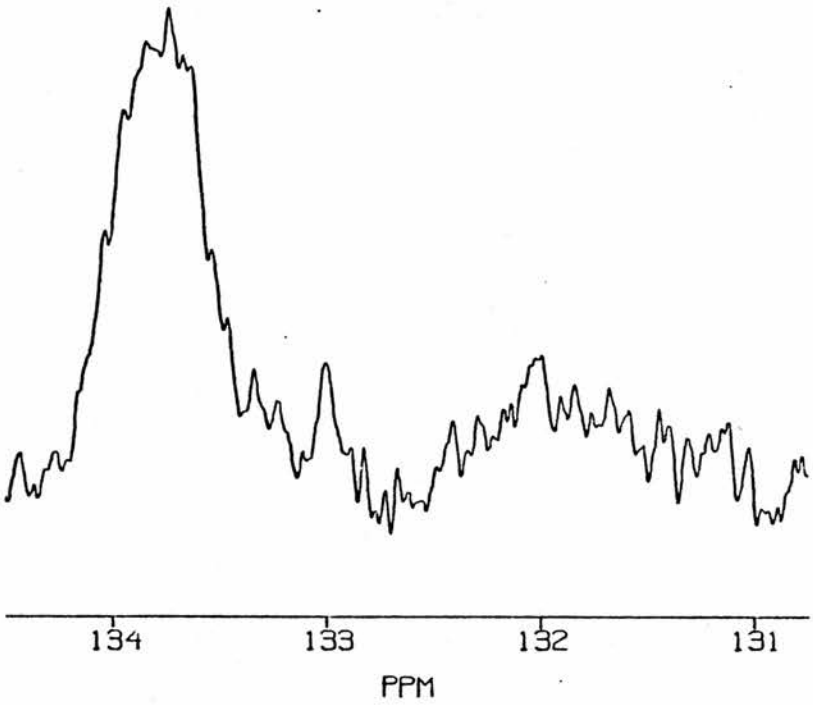

Fig. 5.7 Labelled nitroprusside 0.005 M in blood; spectrum acquired immediately after mixing.

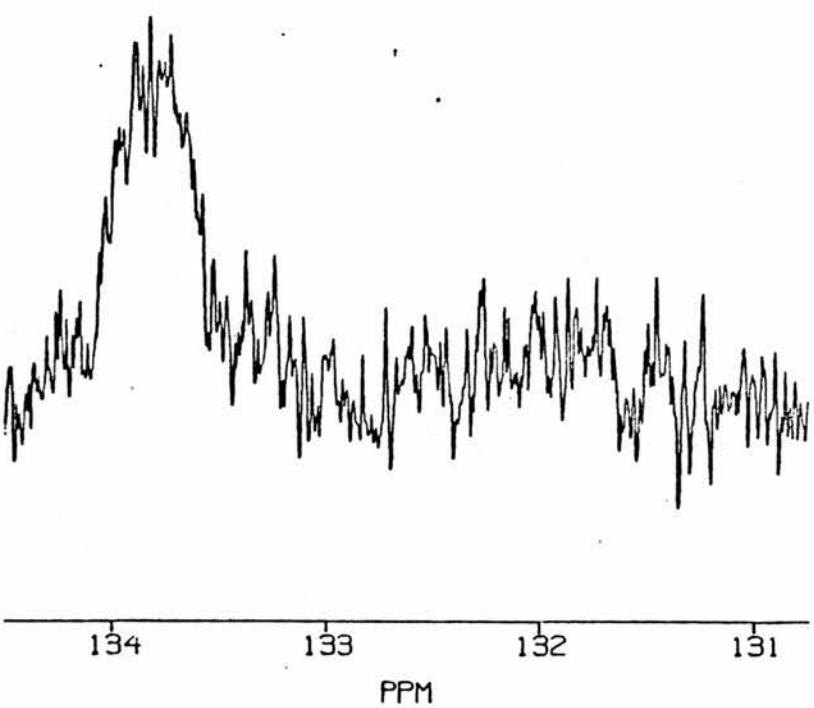

Fig. 5.8 Nitroprusside in blood after 15 minutes.

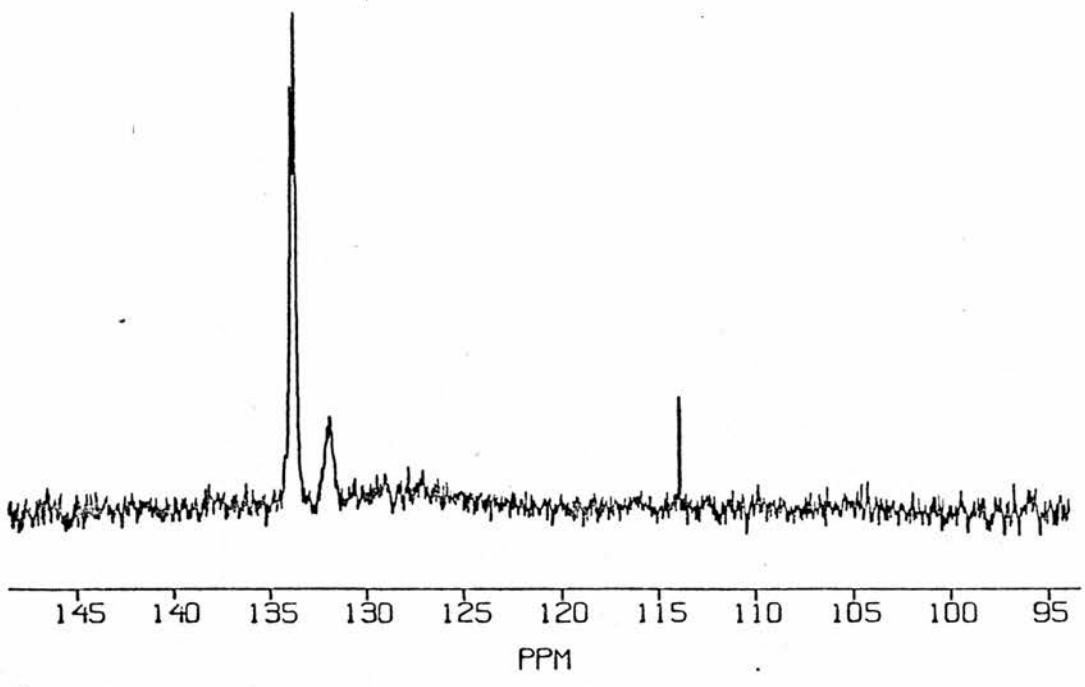

Fig. 5.10 Labelled nitroprusside 0.005 M in blood;

spectrum accumulated for 1 hour.

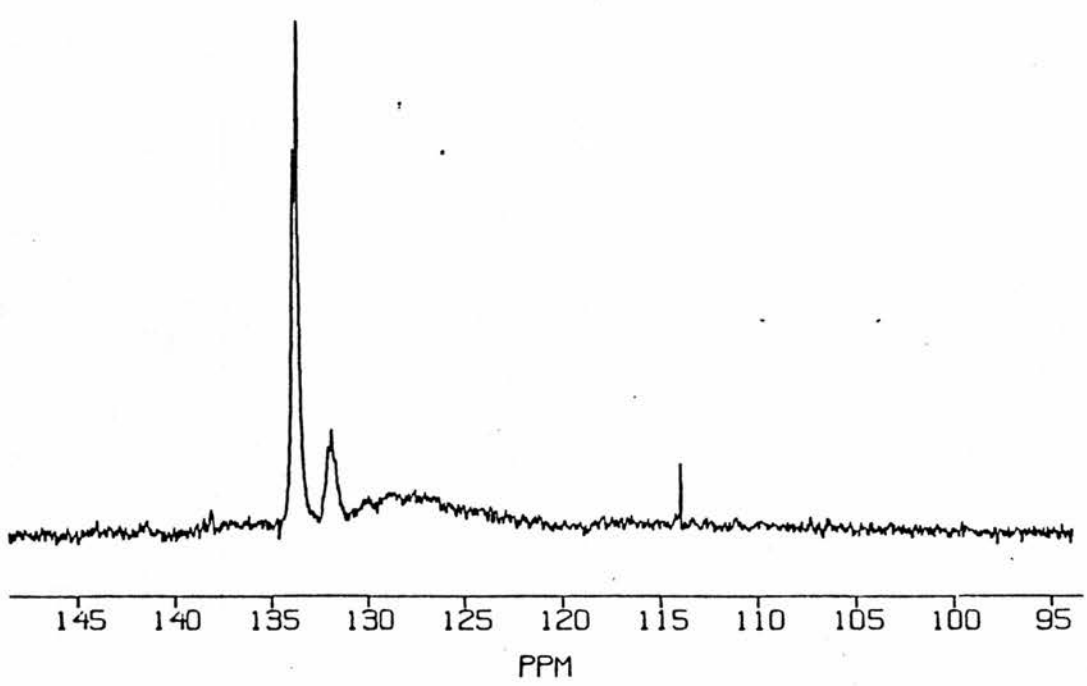

Fig. 5.11 Labelled nitroprusside 0.005 M in blood;

spectrum accumulated for 14 hours.

Figure 5.7 shows the spectrum of 5 mM nitroprusside in blood soon after mixing, Fig. 5.8 that after 15 minutes and Fig. 5.9 that after two hours. The quality of the later spectra is poor due to separation of the red cells from the plasma in the n.m.r. tube; nevertheless it can be seen that the nitroprusside signal between 15 minutes and two hours is apparently undiminished. There does appear to be a small decrease over the first 15-minute period but this could equally well be due to the separation of the red cells as to any reaction. If there is a reaction, it is complete in 15 minutes, leaves about 80% of the nitroprusside undecomposed and does not result in the release of free HCN. This can be seen from the overnight spectra. Fig. 5.10 shows the spectrum of 5 mM nitroprusside in blood after accumulation for one hour; Fig. 5.11 is the spectrum of the same sample after fourteen hours' accumulation. There is no peak at 121 ppm even after accumulation for this length of time; thus there has been no release of HCN.

Two further features of these spectra require explanation. Firstly there is a broad hump in the 125-130 ppm region. This may be due to some paramagnetic ^{13}CN-containing species resulting from a small percentage decomposition of the nitroprusside, but could equally be a natural abundance signal of some component of the blood showing up after the long acquisition time. Secondly there is a peak at about 114 ppm; this is an instrumental spike as was demonstrated by adjusting the line broadening on the instrument. The peak disappeared which could not happen with a

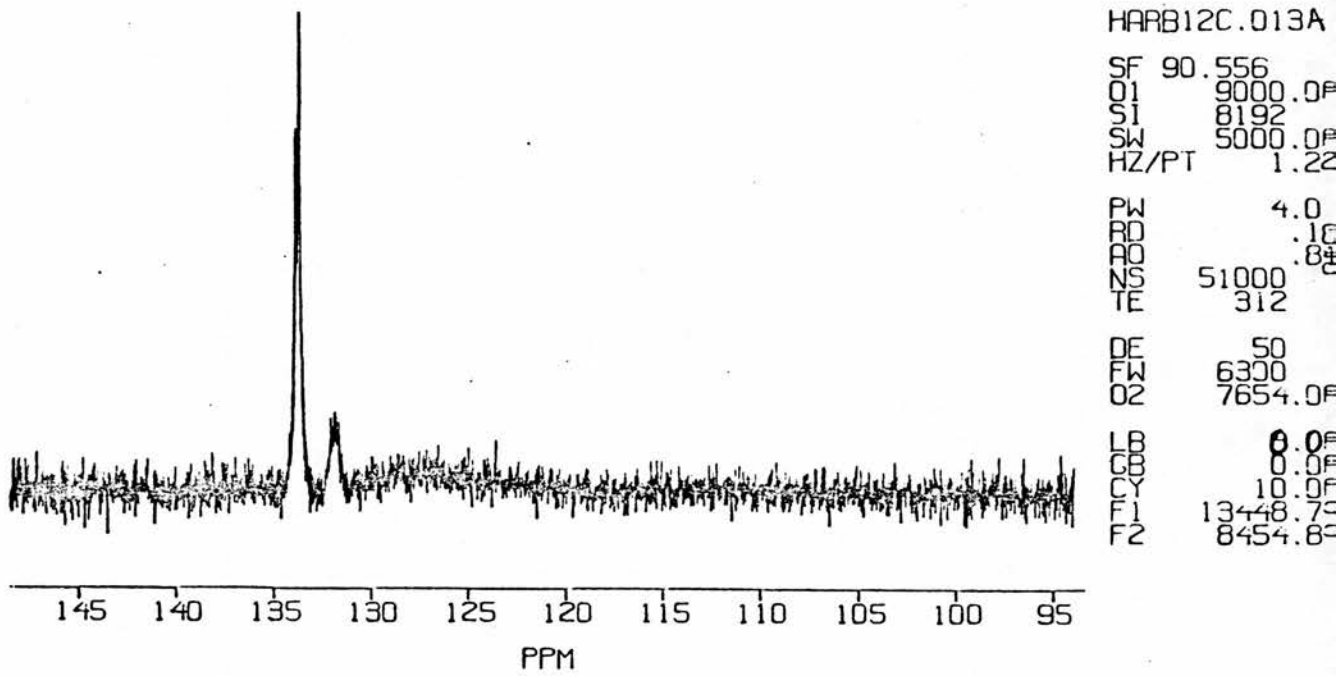

Fig. 5.12 Spectrum of Fig. 5.11 with line broadening adjusted; note disappearance of spike at δ114.

real signal (Fig. 5.12).

These results are consistent with several possible interpretations:

i) There is no reaction between nitroprusside and haemoglobin, or any other blood component present at concentrations of the order of 5 mM.

ii) There is a reaction which is complete in 15 minutes, decomposes no more than ~20% of the nitroprusside and does not result in the release of free HCN. Instead the cyanide is present in some form which does not give an n.m.r. spectrum, such as a paramagnetic species.

iii) There is a reaction of nitroprusside with haemoglobin, but nitroprusside does not penetrate the red cells to a sufficient extent to render the reaction detectable by the n.m.r. method, even at 5 mM concentration. If this is true, the reported *in vivo* cyanide release cannot be due to this reaction. Alternatively, nitroprusside does penetrate the membrane but the maximum concentration attainable in the cells is too low to permit detectable decomposition in these experiments, though high enough to allow for complete decomposition *in vivo*. Even in this case, decomposition of nitroprusside within the red cell would be expected to set up a concentration gradient across the membrane whereby more nitroprusside could diffuse in, eventually allowing the limit of the stoicheiometric reaction to be reached, unless the decomposition product also saturated the cell and prevented penetration of more nitroprusside.

iv) There is a reaction of nitroprusside which results in the

release of cyanide, but the other reacting species is present in too low a concentration to effect detectable cyanide release in these experiments. For example a species at 5×10^{-5} M could decompose almost all of the nitroprusside at the concentrations used clinically, but only 1% of that used here. It is estimated that if 5% of the cyanide in these experiments had been released as HCN, it would have been detectable in the spectrum of Fig. 5.11.

Of these possibilities, only the alternative explanation (iii) is consistent with the idea that a stoicheiometric reaction of nitroprusside with haemoglobin by the mechanism proposed by Smith and Kruszyna is primarily responsible for the reported <u>in vivo</u> cyanide release.

Further work is required to differentiate between the other possibilities. Explanation (ii) is unlikely; the apparent decrease in intensity of the nitroprusside signal over the first 15 minutes is thought to be due to the separation of the blood components. This explanation could be ruled out completely by incubating blood with labelled nitroprusside at a concentration of 5×10^{-4} M for 15 minutes (or preferably up to two hours) and accumulating the n.m.r. spectrum overnight. This could demonstrate the presence of intact nitroprusside at ten-fold lower concentration than was used here.

The question of the permeability of red cells to nitroprusside is very important and requires investigation. One possible method of doing so would be to carry out a similar experiment to that proposed above but to separate the red cells from the plasma and accumulate the spectra of the two phases separately. The effect of using the relatively high nitroprusside concentrations required to obtain an n.m.r. spectrum in reasonable time would again have to be considered carefully. It is unfortunately not yet practical to carry out n.m.r. experiments on nitroprusside at the concentrations used clinically.

The matter of red cell permeability and saturation levels would also seem to be related to the observation by Vesey and co-workers[30] that red cell:plasma cyanide ratios are very much higher after incubating blood with nitroprusside than after incubating with KCN.

A technique whereby nitroprusside at clinical levels can be studied is to have the compound labelled with carbon-14. The low concentrations can be compensated for by taking sufficiently long time periods to count the radioactivity. This technique cannot distinguish between intact nitroprusside and free cyanide without further chemical manipulation. A study of the nitroprusside/blood interaction using carbon-14 has now been undertaken in this department. An interesting <u>in vivo</u> experiment has also been proposed[35] involving infusion of carbon-13 labelled

nitroprusside into patients and examining the HCN in the expired air by mass spectrometry. There are other metabolic sources of cyanide; if these were responsible in some way for the increase in expired HCN, then only $H^{12}CN$ would be detected.

The possibility that free thiol groups are responsible for the hypothetical cyanide-releasing reaction should not be forgotten. The hypotensive effect of sodium nitroprusside is believed to be due to a reaction with thiol groups in cell receptor sites in the smooth muscle membrane[28,36]; it could be that cyanide release is a necessary consequence of the reaction producing the beneficial effects, though this is not generally thought to be the case.

Vesey, Cole and Simpson[27] have reported liberation of cyanide from nitroprusside on incubation with cysteine. This reaction has been studied from a chemical viewpoint by Mulvey and Waters[37] and by Blesa and co-workers[38]. An initial very fast attack on nitroprusside by RS^- anion is proposed, followed by decomposition of the transient red intermediate with the formation of the reduced species $Fe(CN)_5NO^{3-}$. Blesa reports that this species is reoxidised to nitroprusside in the presence of air and that nitroprusside can effect a catalytic oxidation of cysteine to cystine by cycling through the species $Fe(CN)_5NO^{3-}$.

It is known[39] that $Fe(CN)_5NO^{3-}$ can release one cyanide with the formation of $Fe(CN)_4NO^{2-}$ or $Fe(CN)_4NO^{3-}$; further reduction steps are possible and these may lead to the formation of cyanide-labile species[37].

It is emphasised at this point that the cyanide assay procedure involving acidification and flushing with nitrogen cannot distinguish between free cyanide and a stable but labile cyanoferrate, however the latter species may be formed. With this in mind there is a possible explanation for the nitroprusside/blood reactions which appears capable of reconciling many of the observations. This is as follows: there is a reaction of nitroprusside, possibly with thiol groups, which produces not free cyanide but a cyanoferrate species with labile cyanide ligands, such as $Fe(CN)_5NO^{3-}$ or its possible decomposition products. Such a species would release cyanide during analysis even if the light levels were such that significant photolysis of nitroprusside did not occur. If it was produced only at the 10^{-4} - 10^{-5} M level, it would not have been detectable in the n.m.r. experiments. Electrode and colorimetric tests on blood fractions[10-12] would be negative as there would be no free cyanide present in the blood. The upper limit on the amount of nitroprusside decomposing could be due to a low concentration of the other reacting species or to a low maximum concentration of nitroprusside in the red cells at saturation.

It should be possible to test this idea to some extent by carrying out the cyanide analysis procedure, in the dark, on incubation mixtures of nitroprusside and blood in which the nitroprusside concentration is varied in stages from say 10^{-6} M to 10^{-4} M. There should be a maximum total amount of cyanide released corresponding to the upper limit for nitroprusside decomposition. The method used for cyanide determination would have to be sufficiently sensitive - the colorimetric procedure[4,10], or perhaps radioactive counting using ^{14}C labelled material. The results obtained should be compared with direct colorimetric analysis of the blood fractions[12], which would not be expected to contain free cyanide. (The question of cyanide partition between red cells and plasma or between separated red cells and isotonic buffer would have to be considered carefully here; if the red cells are able to concentrate and retain cyanide up to a certain saturation level greater than the total amount which could be released, then there would be no possibility of direct colorimetric determination of free cyanide as the colour of the red cells themselves would prevent this. In this case differentiation between free cyanide and labile cyanoferrate would still be impossible. However since plasma is also said to decompose nitroprusside it should be possible to carry out a similar experiment in the absence of red cells.)

This explanation accounts for the fate of the nitroprusside iron atom, which has been largely ignored in the medical literature. In fact if free cyanide were to be released from nitroprusside it would seem that the iron atom would have to be sequestered into a complex with a formation constant of the order of 10^{30} to make the process energetically favourable. When investigation of the purported cyanide release from nitroprusside was begun in this department such a mechanism was considered a plausible explanation, but it was shown[12] that none of the likely biological iron complexes had a high enough formation constant. Conversion of nitroprusside into a different cyanoferrate complex deals with this problem and is in accord with the known chemistry of the nitroprusside ion.

5.3.4 Summary.

The ^{13}C n.m.r. experiments provided no evidence for the release of cyanide from nitroprusside in the presence of whole blood; however because of the high concentration of nitroprusside employed, care must be taken in interpreting these results. A reaction at a low enough concentration level would not have been detected. The upper limit on this reaction could be imposed by a low concentration of the other reactant or by a low maximum concentration of nitroprusside in red cells. The question of the permeability of red cells to nitroprusside requires further investigation. The possibility that the product of the reaction

is not free HCN but a cyanoferrate complex with labile ligands is also suggested as being worthy of study.

5.4 References - Chapter Five.

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