STUDIES OF THE CHEMISTRY OF DITHIOCARBAMATES AND THEIR METAL COMPLEXES

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ABSTRACT

The studies of the chemistry of dithiocarbamates and its related compounds have been undertaken. It is hoped that such studies would shed light on the interaction between such compounds and the thiol-enzyme, aldehyde dehydrogenase. The facts of previous publication revealed that metabolism of alcohol occurs chiefly in the liver and involves several different enzyme systems. The major pathway, however, is oxidation of ethanol to acetaldehyde, catalysed by alcohol dehydrogenase, followed by oxidation of acetaldehyde to acetate, catalysed by aldehyde dehydrogenase. This normal pathway can be disrupted by the ingestion of certain compound, the famous of which is disulfiram or Antabuse, prior to the drinking of alcohol. The compound 4-nitro-phenyl di-methyldithiocarbamate has a close structural similarity to both the inactivator, disulfiram, and the substrate, 4-nitro-phenyl acetate. It turns out that the dithiocarbamate ester is in fact an inactivator of aldehyde dehydrogenase. The chemical reaction resulted in the formation of an inactivated enzyme. The extent of the inhibition can be measured by the release of 4-nitrothiophenoxide ion and upon the treatment of di-methyldithiocarbamate ion with acid to form carbon disulphide gas. However, the analysis is not fully understood, due to further complex reactions occured in the system. Two other suggestions have been put forward to account for the gap. Nevertheless, the study of the chemistry of dithiocarbamate is a step further towards understanding.

The study of metal complexes of some substituted dithiocarbamates has found considerable use in analytical methods for heavy metals. The complexes gave approximately the correct metal analysis based on the expected stoichiometry of a 2:1 ratio of dithiocarbamate to metal.

ACKNOWLEDGEMENTS

For a painful task, I am very grateful to Dr T.M. KITSON for the guidance and assistance he provided. I would also like to thank Professor R. Hodges for carrying out the Mass Spectra analyses, Mr R. McKenzie for the Atomic Absorption analyses and Professor G.N. Malcolm for his kind advice. The result of Micro analyses was carried out by the Chemistry Staff of University of Otago, New Zealand. To my wife, Selina Christine Wong, for her encouragement and understanding. Lastly, my Parents, Brothers and Sisters who have supported me financially throughout my study career.

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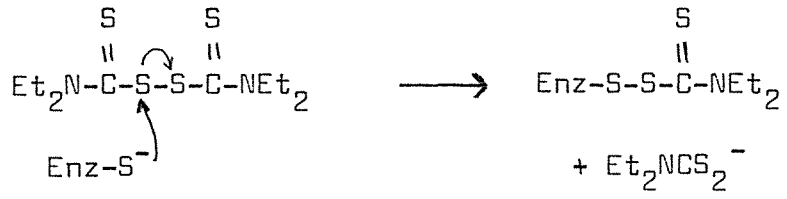
1. INTRODUCTION

The main aim of the work described in this thesis was to investigate the reaction between certain dithiocarbamate esters (such as 4-nitrophenyl dimethyldithiocarbamate) and the amino acid, cysteine. It was hoped that such studies would shed light on the interaction between such compounds and the thiol-enzyme, aldehyde dehydrogenase. I will give a brief description of some of the properties of aldehyde dehydrogenase explaining why it is important, and what the connection is between this enzyme and dithiocarbamate compounds.

1.1 The mechanism of action of aldehyde dehydrogenase, and its inactivation by disulfiram

The metabolism of alcohol occurs chiefly in the liver and involves several different enzyme systems. The major pathway, however is oxidation of ethanol to acetaldehyde, catalysed by alcohol dehydrogenase, followed by oxidation of acetaldehyde to acetate, catalysed by aldehyde dehydrogenase. This normal pathway can be disrupted by the ingestion of certain compounds, the most famous of which is disulfiram or Antabuse, prior to the drinking of alcohol. Under these cirsumstances the oxidation of acetaldehyde is blocked, the blood concentration of this toxic substance rises, and characteristic unpleasant symptoms (nausea, low blood pressure, flushing, dizziness) ensue. This phenomenon, known as the 'disulfiram - ehtanol reaction' was discovered accidentally in 1948 by Hald and Jacobsen (1), who quickly recognised the therapeutic potential of the reaction in treating alcoholism. Since that time, disulfiram has been widely used throughout the world in efforts to dissuade alcoholics from drinking; the idea is that they remain sober in fear of the unpleasant consequences which they know they would experience if they were to drink.

The biochemical basis of the disulfiram-ethanol reaction has been the subject of much research (which has been reviewed (2,3)). Initially, it was claimed that disulfiram merely competes for the active site of aldehyde dehydrogenase with the coenzyme, nicotinamide adenine dinucleotide (NAD^+), but this has been shown to be incorrect. Instead, it appears that aldehyde dehydrogenase has an active-site thiol group which reacts avidly with disulfiram (tetraethylthiuram disulphide) in a disulphide-exchange reaction, according to Scheme 1:

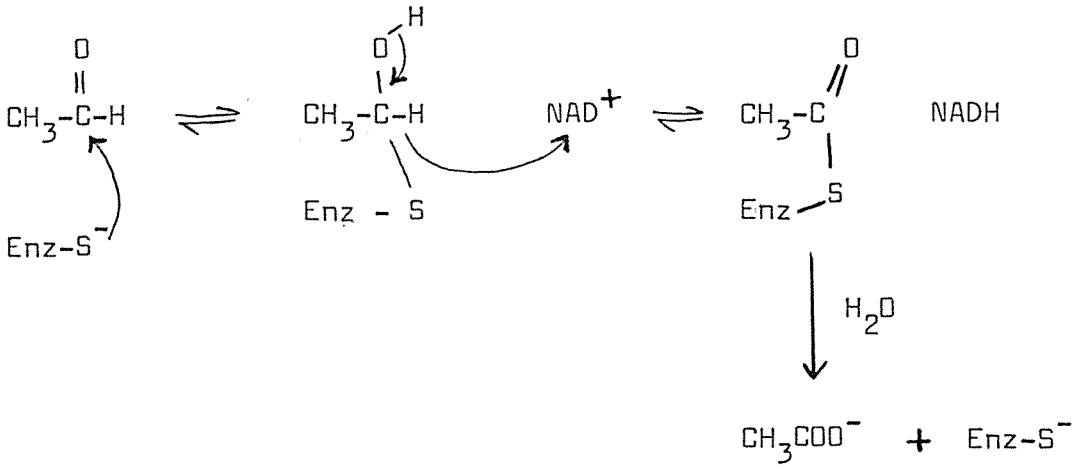


SCHEME 1

This results in covalent modification of the enzyme's essential thiol group and consequently an inactive enzyme and (during ethanol metabolism) a high blood level of acetaldehyde. In most mammalian species studied (eg man, horse, sheep) it is the cytoplasmic form of aldehyde dehydrogenase, rather than the mitochondrial form, which has this pronounced sensitivity to disulfiram.

That this process cannot be the whole explanation for the disulfiram-ethanol reaction is shown by the fact that infusion of acetaldehyde itself into the bloodstream causes a rise in blood pressure whereas one of the main symptoms of the disulfiram-ethanol reaction is low blood pressure. This paradox has been explained in terms of the inhibition by disulfiram (or its metabolites) of enzymes in the body other than solely aldehyde dehydrogenase - for example, it is claimed that the activity of dopamine-β-hydroxylase is reduced in patients on disulfiram (4) (see later).

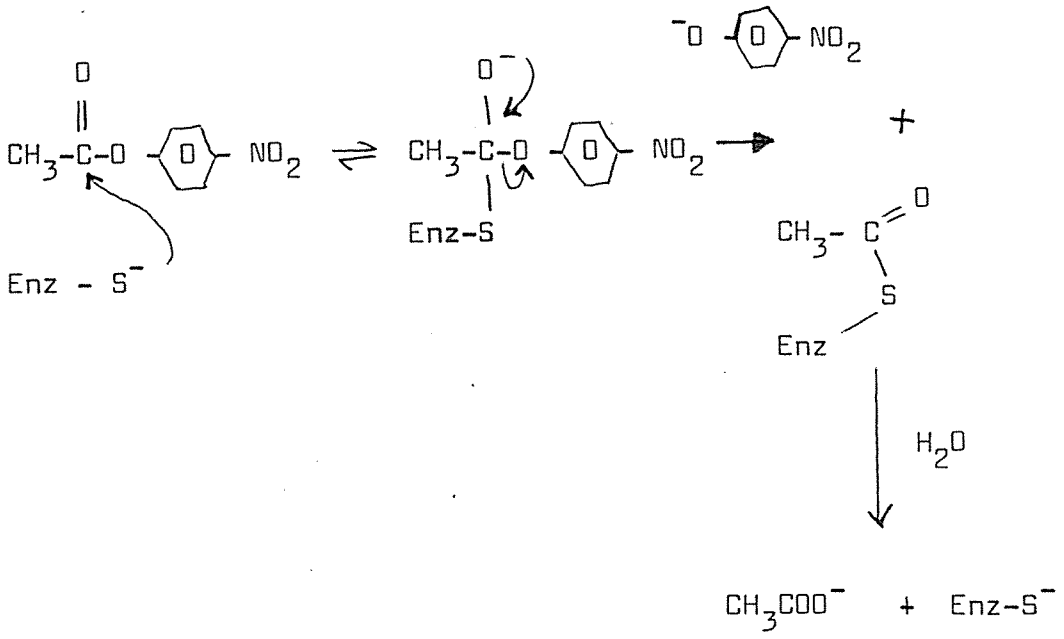
The mechanism of the reaction which aldehyde dehydrogenase normally catalyses is thought to be as shown in Scheme 2:



SCHEME 2

In this scheme, we see the enzyme's reactive thiol group (the existence of which has been mainly deduced from the enzyme's susceptibility to compounds such as disulfiram, 4-chloromercuribenzoate, etc.) attack acetaldehyde nucleophilically to give a thiohemiacetal. In the oxidation-reduction step of the pathway this thiohemiacetal is converted to a thioester whilst simultaneously NAD^+ (the enzyme's required cofactor) is reduced to NADH. Finally the acyl-enzyme intermediate is hydrolysed, releasing acetate ion and free enzyme.(5) Many other aldehydes, as well as acetaldehyde itself, can be utilised as substrates by this enzyme.

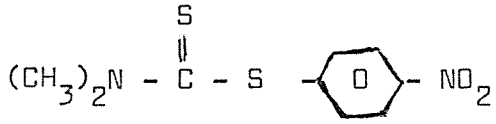
The same enzymic thiol group as discussed above can apparently also act as a nucleophile towards activated esters, since aldehyde dehydrogenase has been shown to rapidly hydrolyse 4-nitrophenyl acetate. (6) (Many other enzymes show similar versatility; eg trypsin, the function of which is to act as a protease, will also cleave esters such as bensoyl-L-arginine ethyl ester). The mechanism of aldehyde dehydrogenase-catalysed hydrolysis of 4-nitrophenyl acetate is presumably as shown in Scheme 3:



SCHEME 3

The mechanism involves a common intermediate (the thioester or acyl-enzyme) with that shown in Scheme 2.

In his continuing study of the action of compounds related to disulfiram on aldehyde dehydrogenase (7,8), Kitson decided to investigate 4-nitrophenyl dimethyldithiocarbamate :



A glance at Schemes 1 and 3 will show that this compound has a close structural similarity to both the inactivator, disulfiram, and the substrate, 4-nitrophenyl acetate. It turned out that the dithiocarbamate ester is in fact an inactivator of aldehyde dehydrogenase as can be seen from Figure 1, which is taken from Kitson's publication. (9).

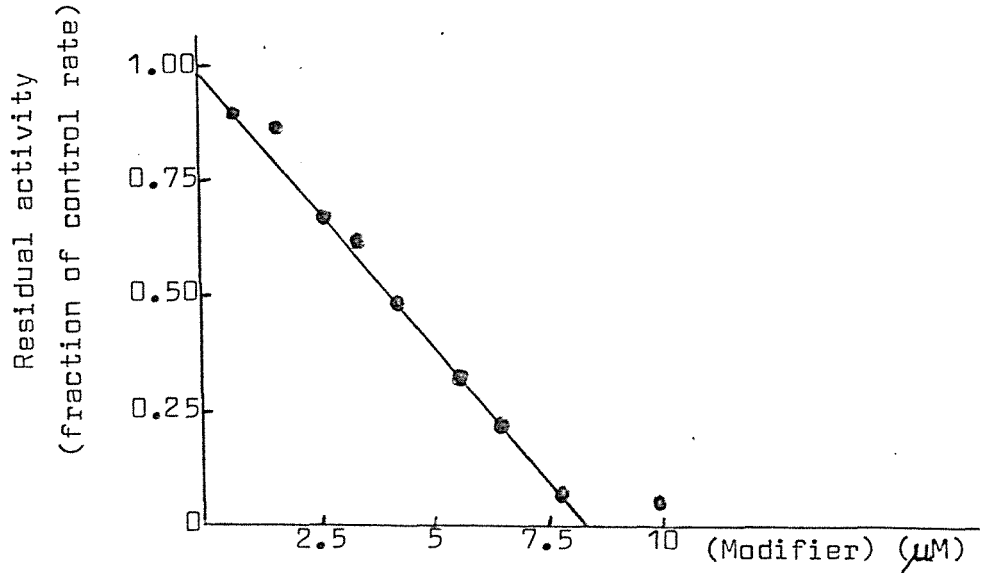
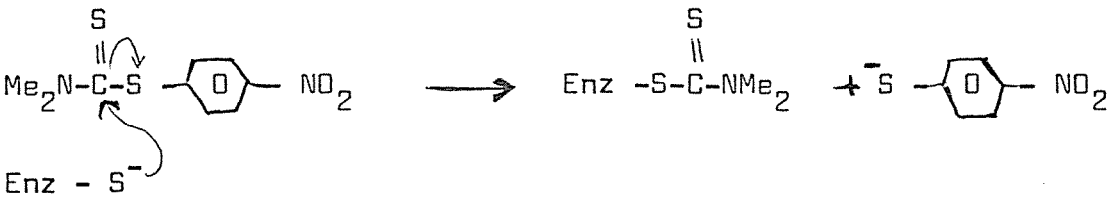


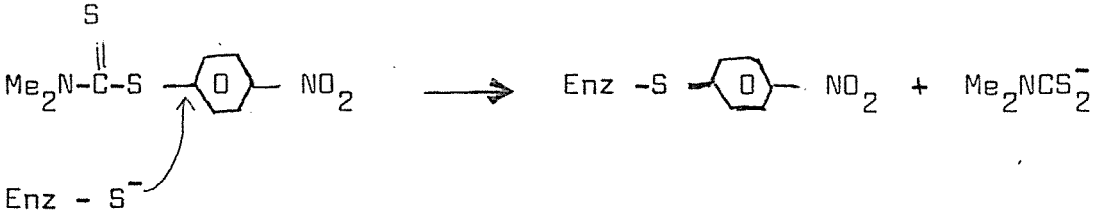
Figure 1. Effect of 4-nitrophenyl NN-dimethyl-dithiocarbamate on cytoplasmic aldehyde dehydrogenase.

The concentration of this compound necessary to cause a certain loss of activity is approximately ten times the concentration of disulfiram required for the same effect, suggesting perhaps that much of the dithiocarbamate ester reacts non-specifically with other, non-essential groups on the enzyme molecule.

Two mechanisms suggest themselves for the interaction between aldehyde dehydrogenase and 4-nitrophenyl dimethyldithiocarbamate (see Schemes 4 and 5) :



SCHEME 4



SCHEME 5

In the first, which intuitively seems the more likely, the mechanism is essentially the same to start with as that in Scheme 3 for the hydrolysis of 4-nitrophenyl acetate. Presumably however, unlike the acyl-enzyme in Scheme 3, the species Enz-S-CS-NMe_2 is not susceptible to hydrolysis, resulting in an inactivated enzyme. (Dithiocarbamate esters are known to be unusually resistant to hydrolysis, compared to simple carboxylic esters and thioesters. (10) Kitson attempted to measure the release of 4-nitrothiophenoxide ion quantitatively, but since the concentration of enzyme used was so small, this was difficult. It did appear however, that the amount of 4-nitrothiophenoxide released was less than stoichiometrically expected, suggesting that maybe some other mechanism was also operating. This led to the consideration of Scheme 5. Here the enzymic thiol group nucliophilically displaces dimethyldithiocarbamate ion from the benzene ring, aided by the activating effect of the 4-nitro group, leading to an enzyme thioether derivative, which again would be inert to hydrolysis. In this mechanism, the leaving group is dithiocarbamate ion, as it is in the case of disulfiram (see Scheme 1).

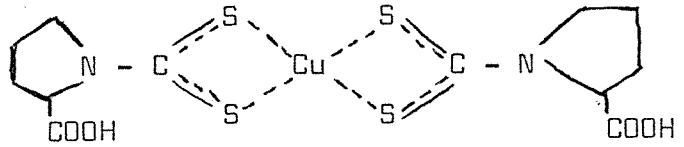
Before further work with the enzyme was attempted, it was decided to examine in detail the reaction between 4-nitrophenyl dimethyldithiocarbamate and the simple thiol compound, cysteine, in aqueous solution at pH 7.3, in an attempt to understand more fully the likely effect of the compound on aldehyde dehydrogenase. This then, as stated in the first paragraph, was the main aim of my research project. The procedure was basically to react the dithiocarbamate ester with cysteine and estimate quantitatively the amounts of 4-nitrothiophenoxide (from Scheme 4) and dimethyldithiocarbamate ion (from Scheme 5, after its conversion to carbon disulphide) which were produced.

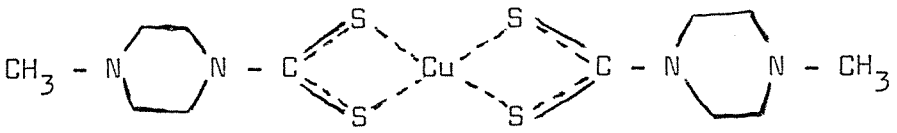
1.2 Metal complexes of dithiocarbamates

A second investigation carried out in the course of this work was into the properties of some dithiocarbamate metal complexes. It is well known (10) that simple dithiocarbamates (such as the dimethyl and diethyl species) form very stable covalent complexes with divalent metal ions, especially Cu (II).

It is the avidity of diethyldithiocarbamate (a metabolite of disulfiram) for the Cu (II) of the metalloenzyme, metalloenzyme, dopamine-β-hydroxylase, which results in the inhibition of this enzyme *in vivo* referred to above.

Kitson (8) has synthesised dithiocarbamates and thiuram disulphides based on proline and N-methylpiperazine in order to study how negatively or positively charged analogues of disulfiram interact with aldehyde dehydrogenase. I decided to synthesise and examine the properties of the corresponding metal complexes of such dithiocarbamates. By analogy with the dimethyldithiocarbamate case, these complexes would have the structures :





Simple complexes such as that of dimethyldithiocarbamate and copper are soluble in many organic solvents but completely insoluble in water. However, because of the presence of the carboxyl and tertiary amino groups respectively in the complexes

drawn above, it was expected that such compounds would exhibit selective solubility in either aqueous or organic solvents depending upon the pH, and might prove to be of use in the extraction and estimation of metals such as copper.

2. RESULTS

2.1 Reaction between cysteine and some dithiocarbamate esters

4-Nitrophenyl dimethyldithiocarbamate was allowed to react with a large excess of L-cysteine in aqueous solution, pH 7.3, at room temperature, as described in Capter 4. Liberation of 4-nitrothiophenoxide ion was evident immediately upon mixing, but the amount released was less than the theoretical maximum expected if Scheme 4 (Chapter 1) is operative. There was no further release of 4-nitrothiophenoxide over a period of time.

The results of these experiments with 4-nitrophenyl dimethyldithiocarbamate and some other related compounds are presented in Table 1. During these same experiments the liberation of dithiocarbamate ion was also quantitatively studied. Initially, it was attempted to do this by forming the copper complex of the dithiocarbamate *in situ*, extracting it into an organic solvent and measuring the absorbance spectrophotometrically. However, non-reproducible results were obtained using this method. An alternative procedure (involving acid decomposition of the dithiocarbamate and trapping of the liberated carbon disulphide in McKee's solution, as described in Chapter 4) was developed and proved to be more reliable. The results of these experiments are shown in Table 2.

TABLE 1. Release of substituted thiophenoxide ion in the reaction between cysteine and some dithiocarbamate esters.

Ester	Product	Percentage Yield ¥
4-nitrophenyl dimethyldithiocarbamate	4-nitrothiophenoxide	63 67 66
4-nitrophenyl diethyldithiocarbamate	4-nitrothiophenoxide	65 64 65
2,4-dinitrophenyl dimethyldithiocarbamate	2,4-dinitrothiophenoxide	75 74
2,4-dinitrophenyl diethyldithiocarbamate	2,4-dinitrothiophenoxide	73 74

¥ The yield is calculated as the percentage of the maximum on the basis that only Scheme 4 (Chapter 1) occurs.

TABLE 2. Release of substituted dithiocarbamate ion in the reaction between cysteine and some dithiocarbamate esters.

Ester	Product	Percentage Yield ¥
4-nitrophenyl dimethyldithiocarbamate	$(CH_3)_2NCS_2^-$	5.4 6.0 6.1
4-nitrophenyl diethyldithiocarbamate	$(C_2H_5)_2NCS_2^-$	6.9 5.7 6.3
2,4-dinitrophenyl dimethyldithiocarbamate	$(CH_3)_2NCS_2^-$	14.4 16.7
2,4-dinitrophenyl diethyldithiocarbamate	$(C_2H_5)_2NCS_2^-$	16.5 24.5

¥ The yield is calculated as the percentage of the maximum on the basis that only Scheme 5 (Chapter 1) occurs.

2.2 Result of study on dimethyldithiocarbamate metal complexes

The absorption spectra of copper, cobalt and nickel di-methyl dithiocarbamate extracted into ethyl acetate are shown in Figure 2. These spectra are similar to those of J.M. Chilton (11) which were determined in carbon tetrachloride solution. Copper has only one absorption peak above 300 nm. at 432 nm. Cobalt has two peaks, one at 380 nm and one at 320 nm, Nickel has three peaks at 420 nm, 383 nm and 320 nm. The absorbance of the sample, using the blank (ethyl acetate) as a reference, is read at 432, 380, 320 nm. Comparison is made with calibration curves made under the same condition. The values of the molar extinction coefficients used are listed in Table 3.

Figure 2.

Absorption spectra of copper, cobalt and nickel di-methyl-dithiocarbamate in ethyl acetate.

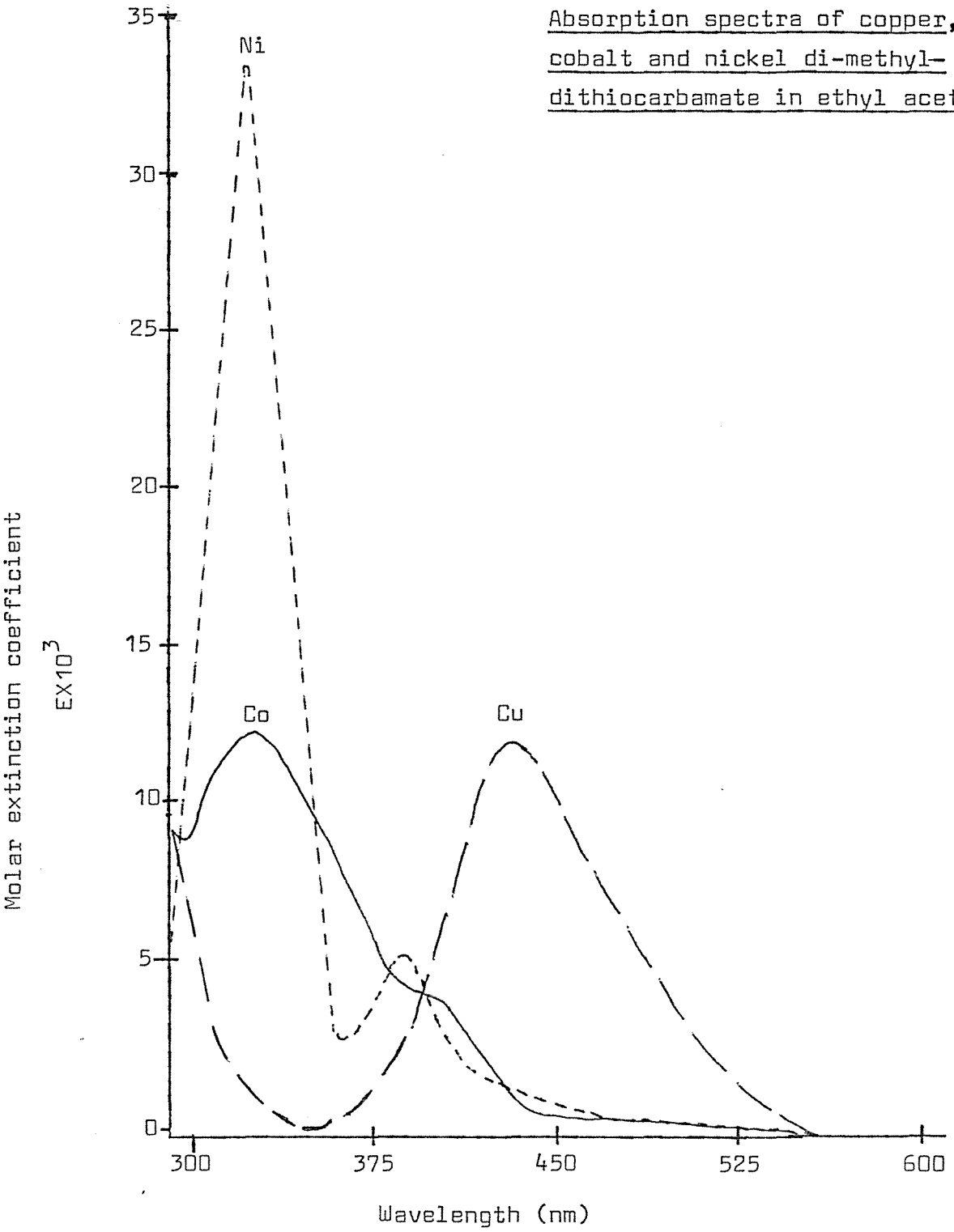


TABLE 3. Molar extinction coefficients of metal complexes of di-methyldithiocarbamate at pH: 8.9

Complexes	432 nm	380 nm	320 nm
ECu	12699	3950	11635
ECo	410	5002	12505
ENi	243	5306	34629

2.3 Result of study on L-proline dithiocarbamate metal complexes

The absorption spectra of copper, cobalt and nickel L-proline dithiocarbamate in ethyl acetate solution were shown to be very similar to those of the di-methyldithiocarbamate. Copper has one absorption peak above 300 nm at 432 nm. Cobalt has two peaks, one at 380 nm and one at 320 nm. Nickel has three peaks at 420, 380 and 320 nm. The absorption spectra using blank (ethyl acetate) as a reference, was read at 432, 380 and 320 nm. The value of the molar extinction coefficient used are listed in Table 4.

TABLE 4. Molar extinction coefficient of metal complexes of L-proline dithiocarbamate at pH: 3.5

Complexes	432 nm	380 nm	320 nm
ECu	9594	1025	1100
ECo	1640	4756	12218
ENi	1025	3157	18983

2.4 Result of study on N-methylpiperazine dithiocarbamate metal complexes.

The absorption spectra of copper, cobalt and nickel N-methylpiperazine dithiocarbamate were also very similar to those of the di-methyl dithiocarbamate and L-proline dithiocarbamate. The absorbance of the sample, using the blank (ethyl acetate) as a reference was read at 432, 380 and 320 nm. The value of the molar extinction coefficients used are listed in the table 5.

TABLE 5. Molar extinction coefficient of N-methylpiperazine dithiocarbamate metal complexes at pH 8.5

Complexes	432 nm	380 nm	320 nm
ECu	13120	410	615
ECo	2255	6027	12505
ENi	2706	5576	32964

2.5 The solubility behaviour of dithiocarbamates

The solubility behaviour of the L-proline dithiocarbamate complexes of copper, cobalt and nickel is illustrated in Figures 3, 4 and 5. In these Figures the absorbance of the complex in aqueous and in organic solution is plotted as a function of pH. In each case the complex is to be found in the aqueous phase above about pH 5.

The solubility behaviour of the corresponding complexes of N-methylpiperazine dithiocarbamate is shown in Figures 6, 7, and 8. In this case, each complex is preferentially soluble in the organic phase above a pH of approximately 5.5 to 6.

Figures 9, 10 and 11 record the distribution of copper, cobalt and nickel respectively over organic and aqueous phases as a function of pH in the presence of both dimethyldithiocarbamate and L-proline dithiocarbamate. (The reagents were mixed in a ratio such that there was sufficient metal ion to complex with one or other of the dithiocarbamates but not both.) From the figures it is evident that copper and nickel have a greater tendency to complex with dimethyldithiocarbamate than with proline dithiocarbamate (and thus remain in the organic phase throughout the pH range), whereas cobalt apparently has a high affinity for proline dithiocarbamate (and occurs in the aqueous phase at high pH).

Finally, Figure 12 shows the results of a control experiment designed to test the stability of simple dithiocarbamate complexes over a range of pH. The dimethyldithiocarbamate complexes of copper and cobalt, prepared in aqueous solution at a variety of pH's and then extracted into ethyl acetate, show a reasonably constant absorbance. That of nickel, however, seems not to form at pH around 3; this agrees with the previous results with nickel (see Figures 5 and 8). In fact there is a tendency for many of the complexes to show lowered absorbance when prepared at low pH. This is probably associated with the instability of dithiocarbamic acids, e.g:

$$Me_2NCS_2^- + H^+ \longrightarrow Me_2NCS_2H \longrightarrow Me_2NH + CS_2 \uparrow$$

Figure 3.

Plot of absorbance versus pH for copper-L-proline dithiocarbamate at 432 nm.

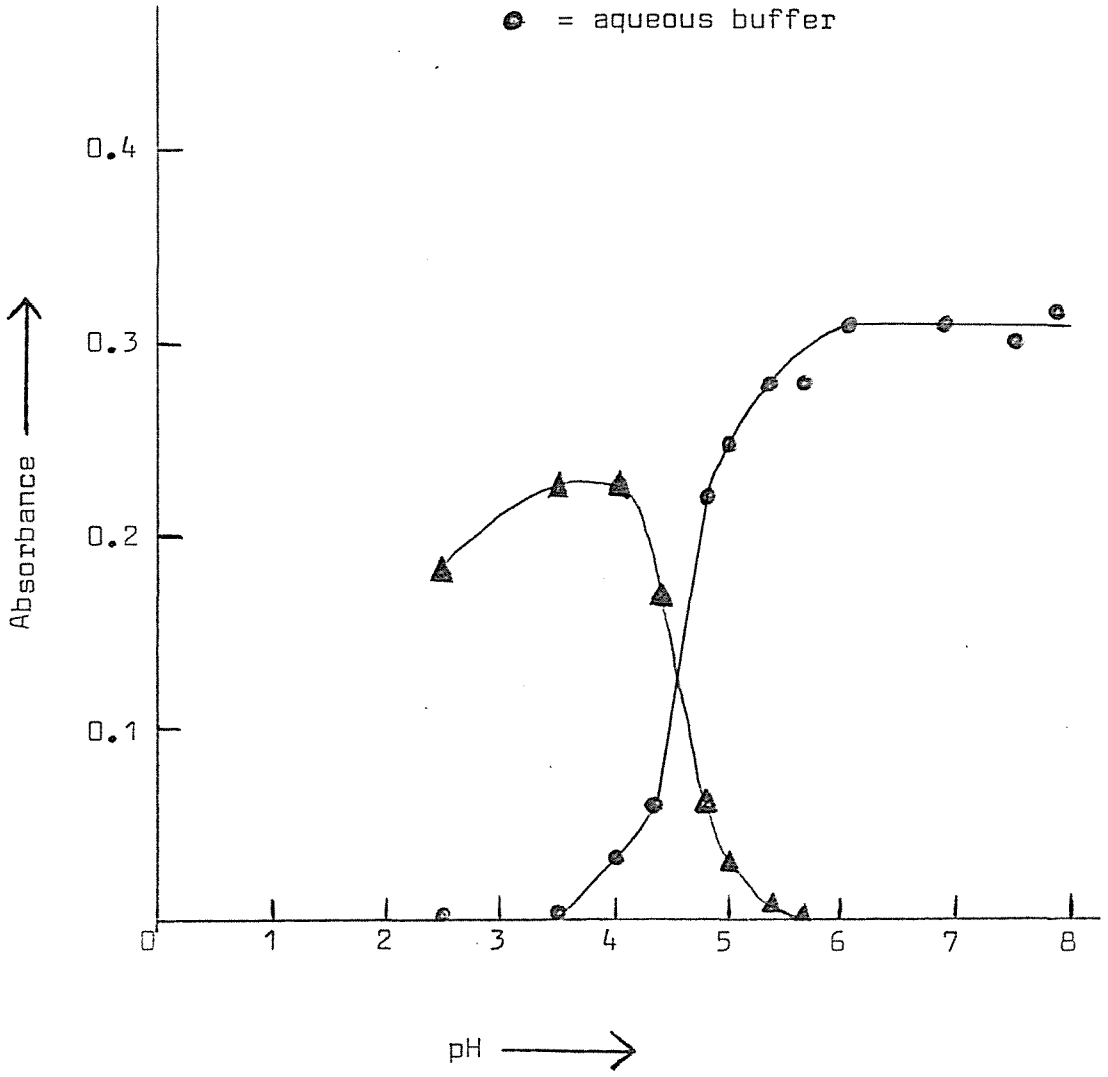


Figure 4.

Plot of absorbance versus pH for Cobalt-L-proline dithiocarbamate at 320 nm.

▲ = ethyl acetate

● = aqueous buffer

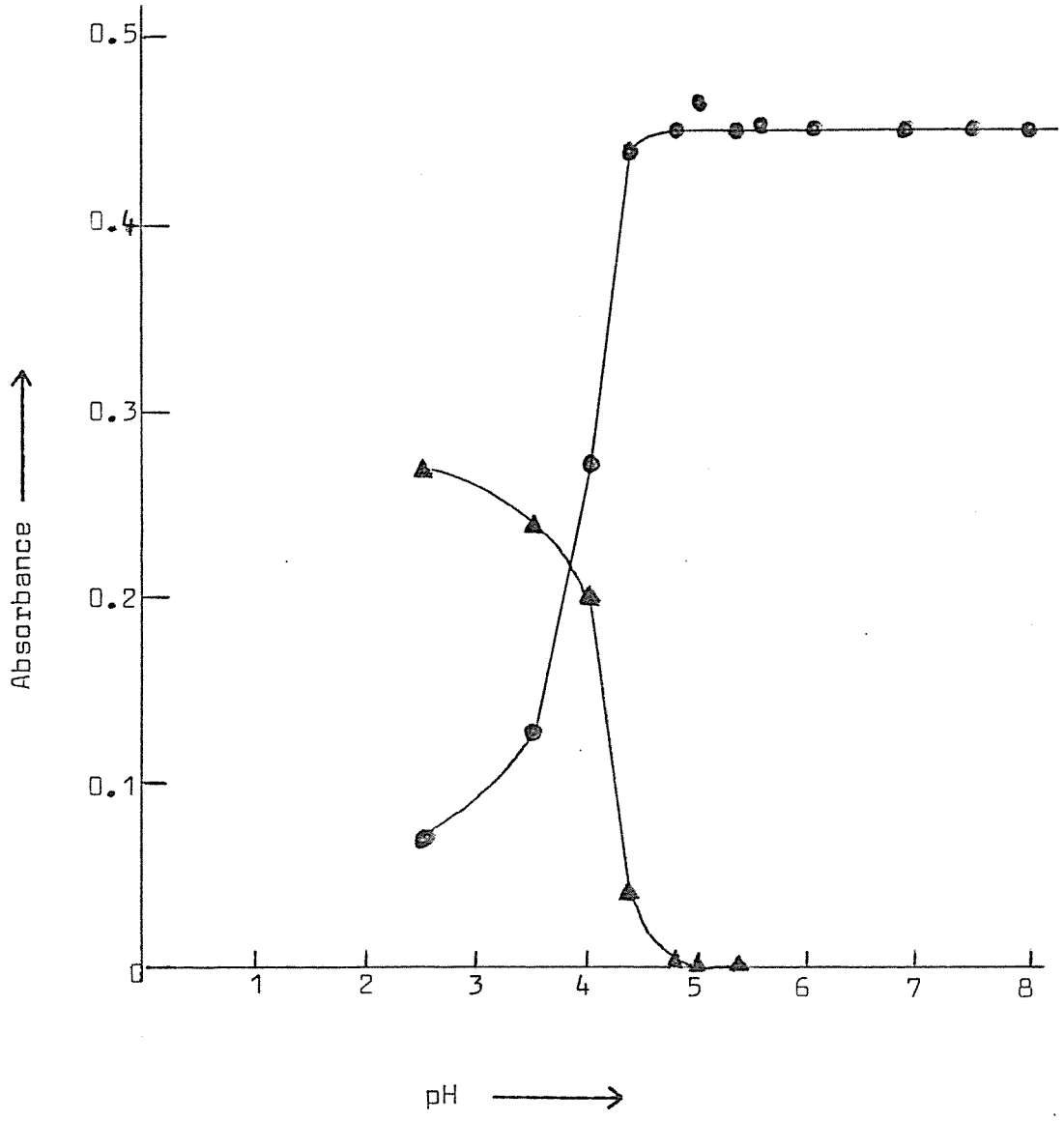


Figure 5.

Plot of absorbance versus pH for nickel-L-proline dithiocarbamate at 320 nm.

▲ = ethyl acetate

● = aqueous buffer

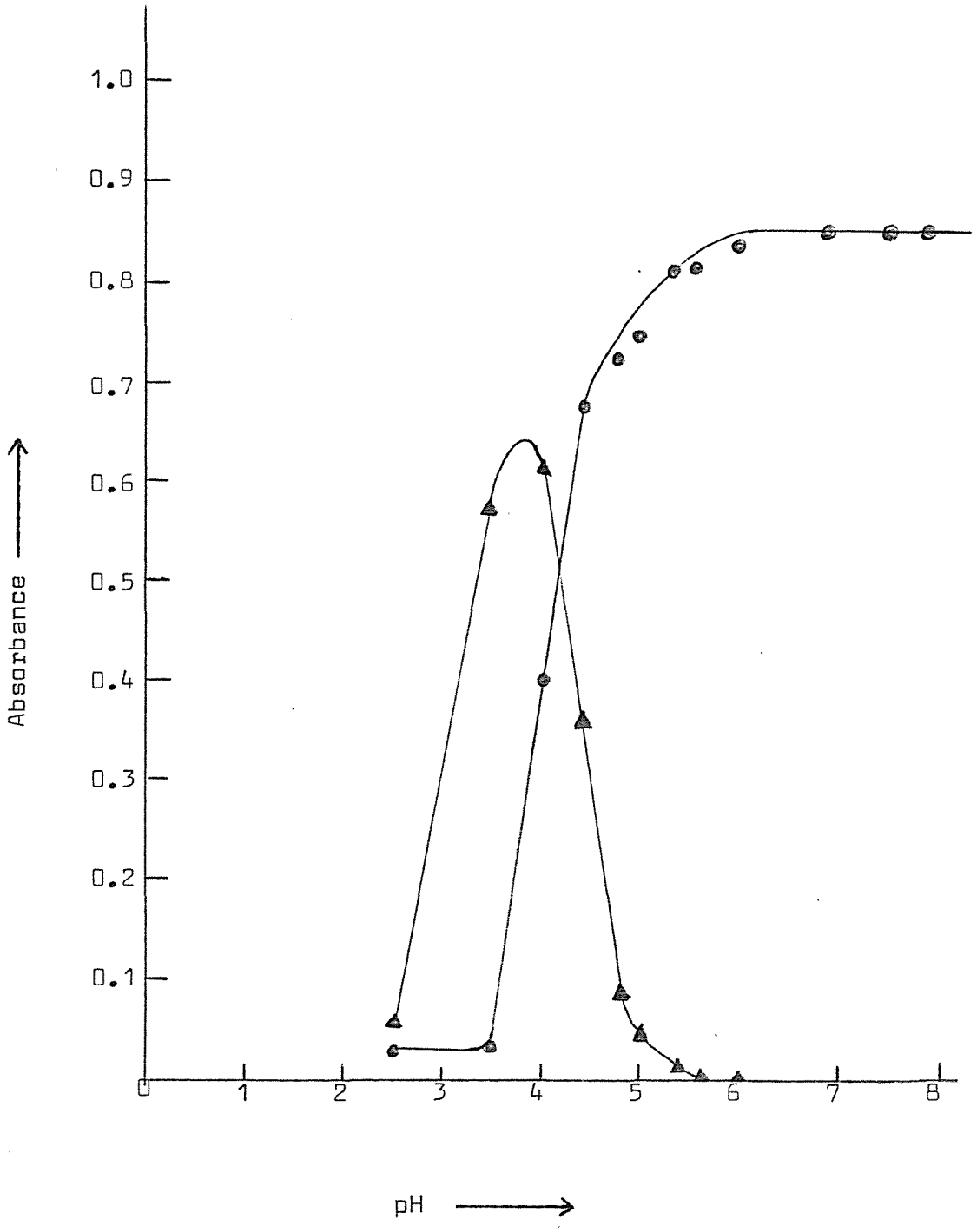


Figure 6.

Plot of absorbance versus pH for copper N-methylpiperazine dithiocarbamate at 432 nm.

▲ = ethyl acetate

● = aqueous buffer

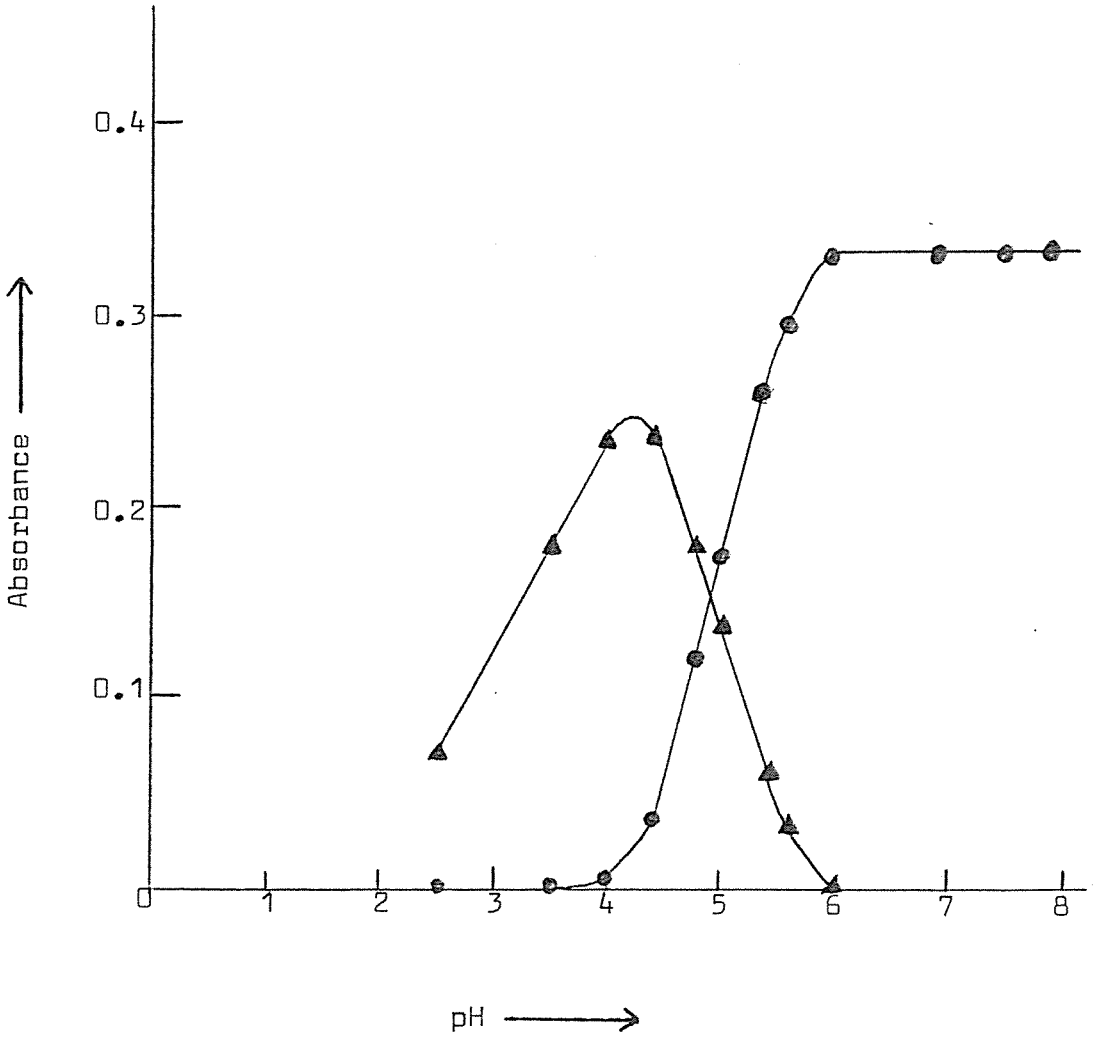


Figure 7.

Plot of absorbance versus pH for Cobalt-N-methyl piperazine dithiocarbamate at 320 nm.

▲ = ethyl acetate

● = aqueous buffer

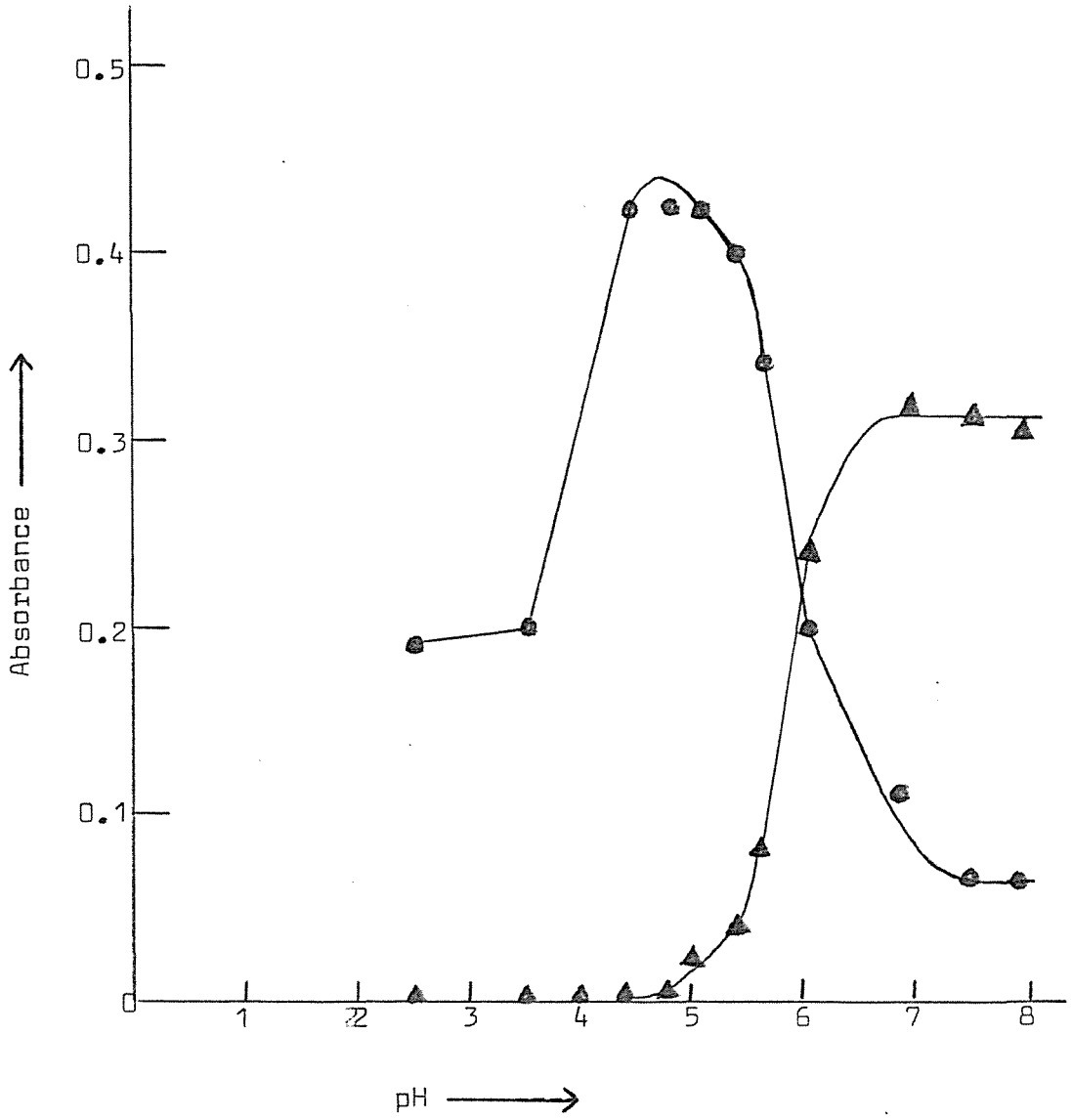


Figure 8.

Plot of absorbance versus pH for Nickel-N-methyl piperazine dithiocarbamate at 320 nm

▲ = ethyl acetate

● = aqueous buffer

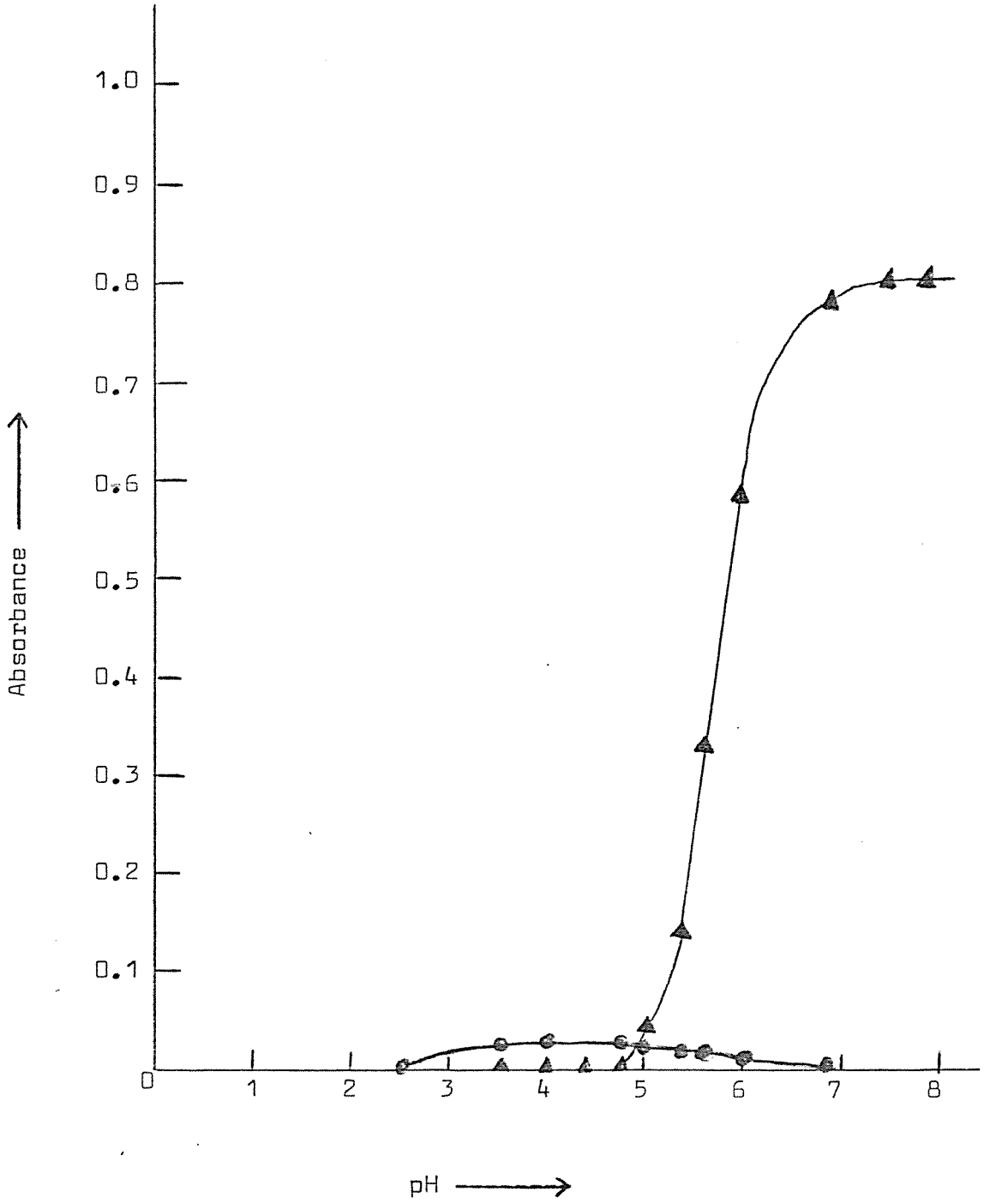


Figure 9.

Plot of absorbance versus pH for copper in the presence of di-methyldithiocarbamate and L-proline dithiocarbamate.

▲ = ethyl acetate

● = aqueous buffer

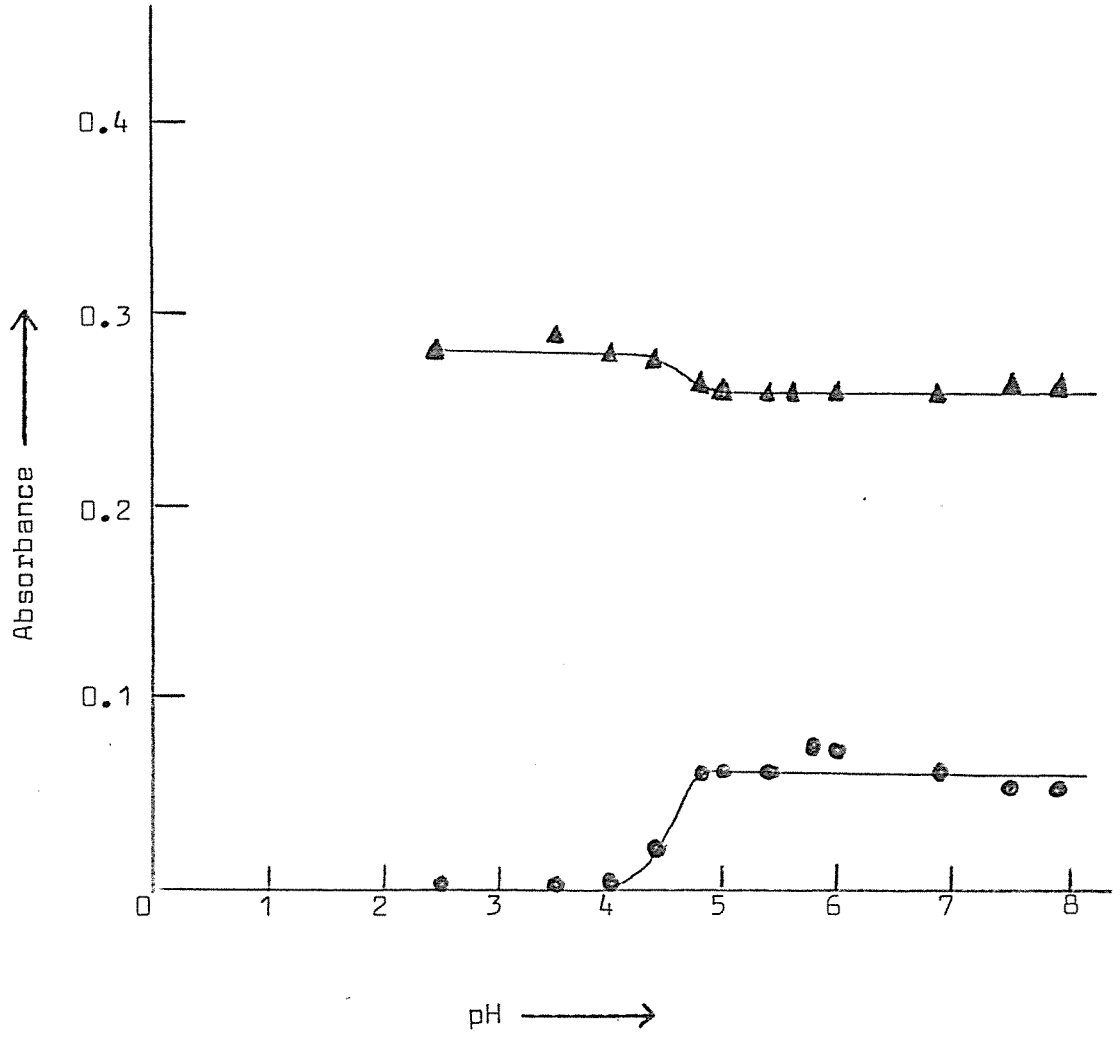


Figure 10.

Plot of absorbance versus pH for cobalt in the presence of di-methyldithiocarbamate and L-proline dithiocarbamate

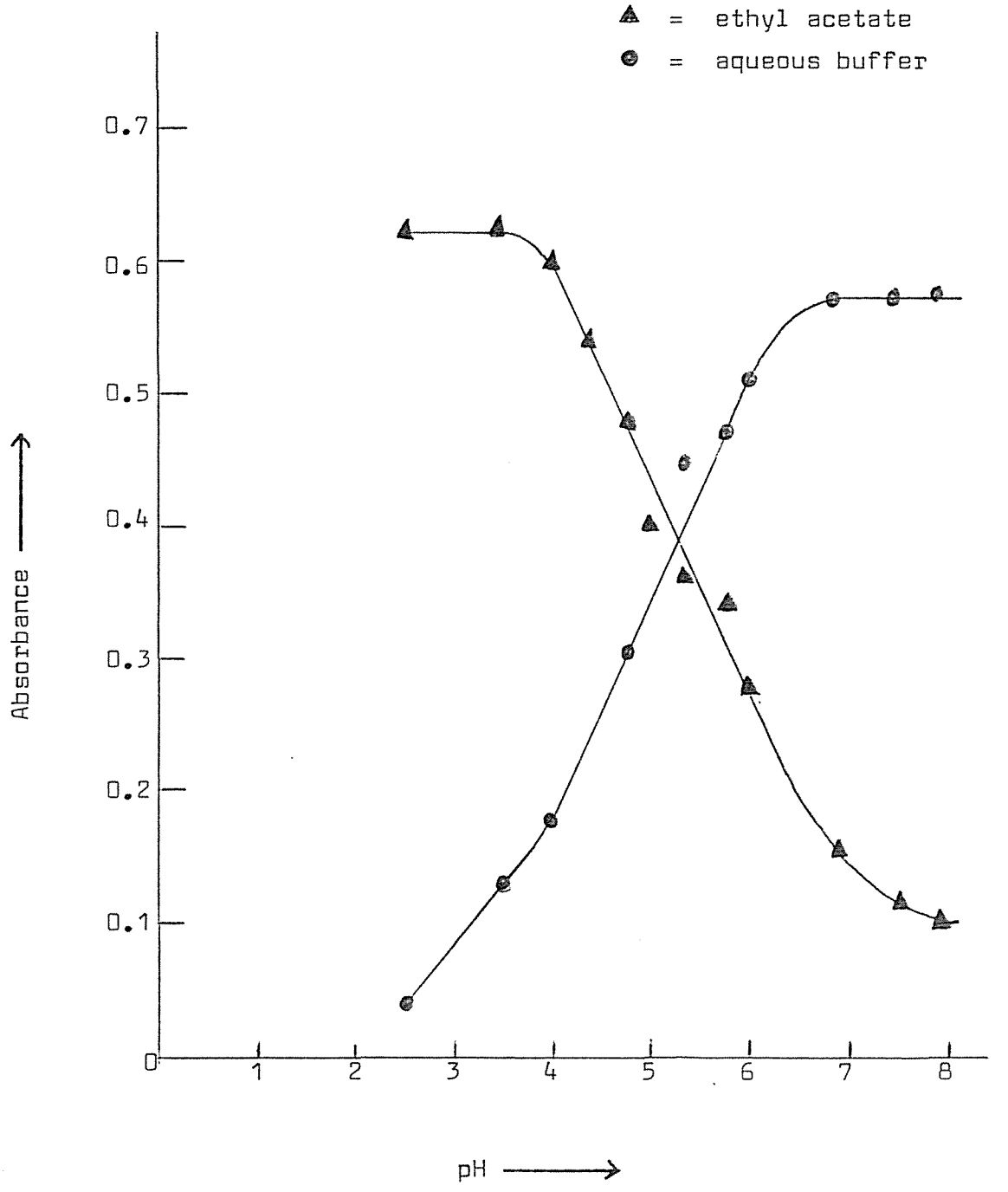


Figure 11.

Plot of absorbance versus pH for Nickel in the presence of di-methyldithio-carbamate and L-proline dithiocarbamate.

▲ = ethyl acetate

● = aqueous buffer

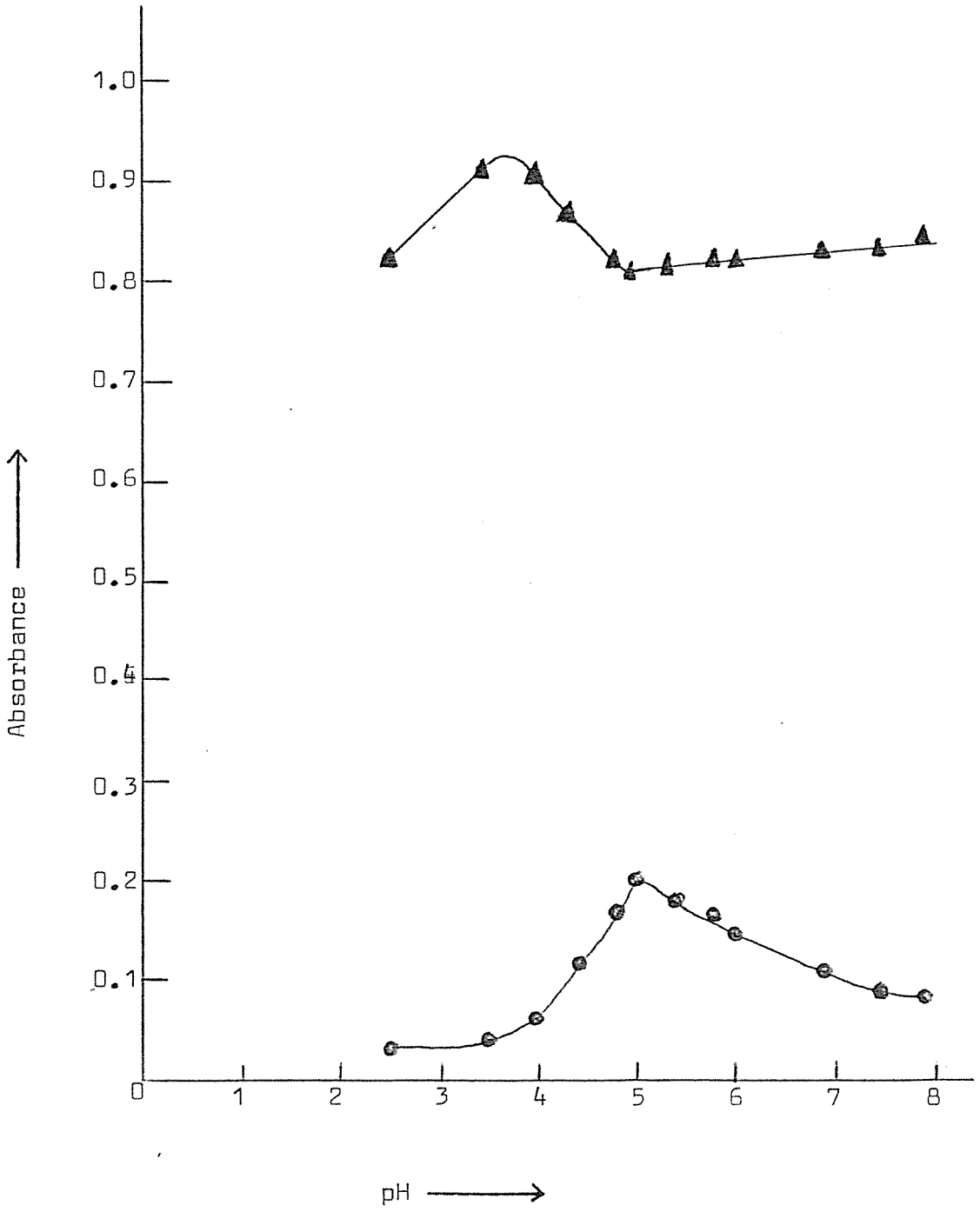


Figure 12.

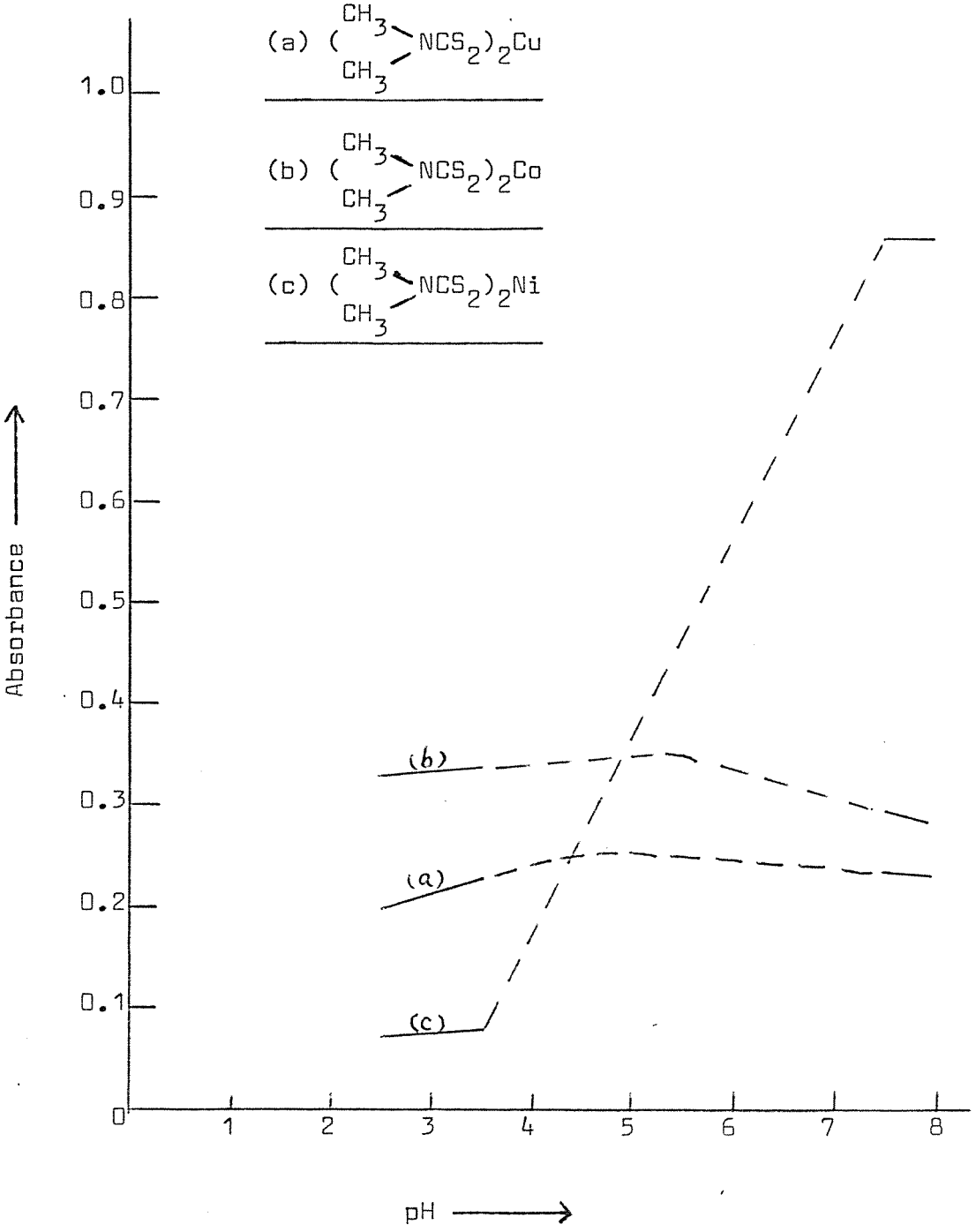
Plot of absorbance versus pH for varies complexes in ethyl acetate solution.

(The complexes were prepared in buffer solution at the pH's indicated and then extracted into ethyl acetate.)

(a) $(CH_3)_2NCS_2)_2Cu$

(b) $(CH_3)_2NCS_2)_2Co$

(c) $(CH_3)_2NCS_2)_2Ni$

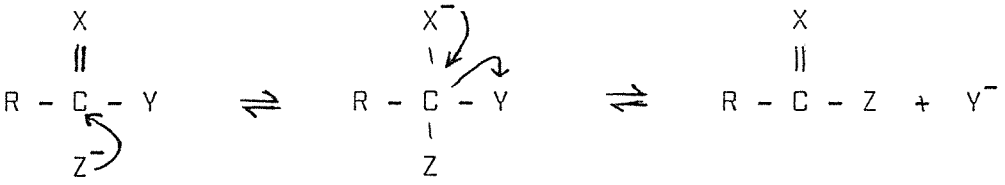


3. DISCUSSION

3.1 The reaction of cysteine with some dithiocarbamate esters

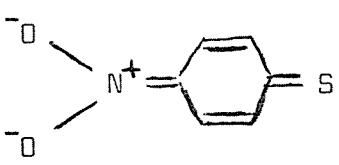
As mentioned in Chapter 1, Kitson (9) has shown that 4-nitrophenyl dimethyldithiocarbamate, which is structurally related to both a substrate (4-nitrophenyl acetate) and an inactivator (disulfiram) of aldehyde dehydrogenase, is itself an inactivator of the enzyme. I was interested in gaining evidence towards the possible mechanism of this inactivation.

The most obvious possibility for the mechanism is that discussed in Chapter 1 under the label 'Scheme 4'. This proposed mechanism is a specific example of the general nucleophilic displacement reaction shown in Scheme 6.

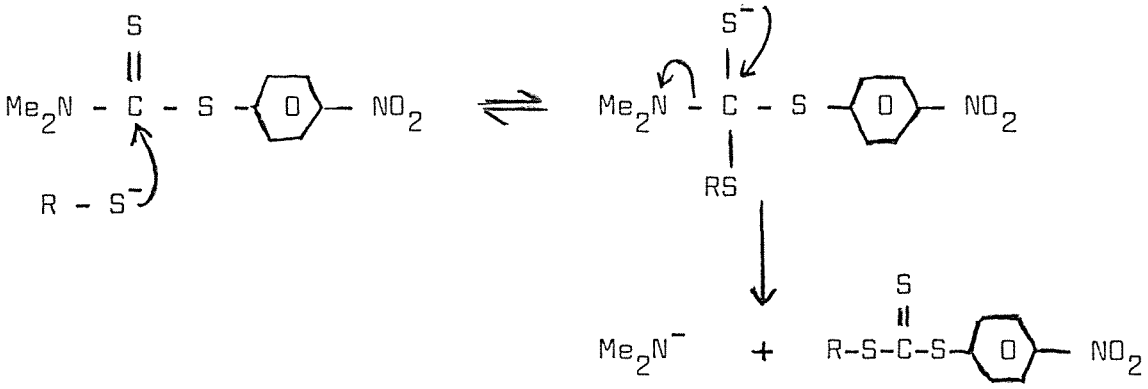


SCHEME 6

In this general reaction C = X represents a carbonyl or thiocarbouyl group, Z^- represents an attacking nucliophile and Y represents a leaving group. Whether or not the reaction proceeds in the forward direction depends upon whether Y^- is a better leaving group than Z^- (i.e. whether HY is a stronger acid than HZ). On this basis, Scheme 4 is intrinsically a likely reaction since the 4-nitrothiophenoxide ion is a much better leaving group than the thiolate ion of cysteine. The former ion is, of course, stabilised by contributing resonance structures such as:

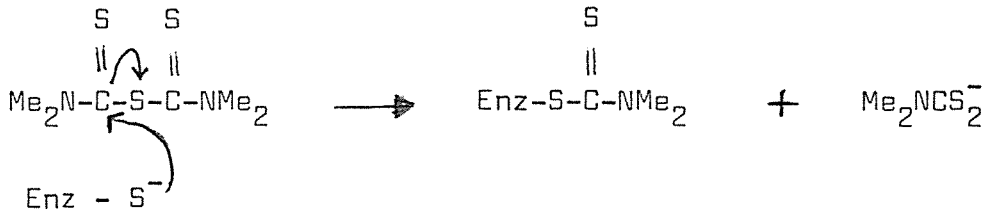


It is also obvious why the mechanism in Scheme 7 has not been considered as a possibility; the anion of dimethylamine is a very much poorer leaving group than 4-nitrothiophenoxide.



SCHEME 7

There are two other specific versions of Scheme 6 which are relevant here. The first has already been discussed in Chapter 1 as Scheme 3 - the action of 4-nitrophenyl acetate as a substrate for aldehyde dehydrogenase. The second is the proposal which Kitson (8) has put forward to explain the inactivatory effect of tetramethylthiuram monosulphide on sheep liver cytoplasmic aldehyde dehydrogenase (see Scheme 8).



SCHEME 8

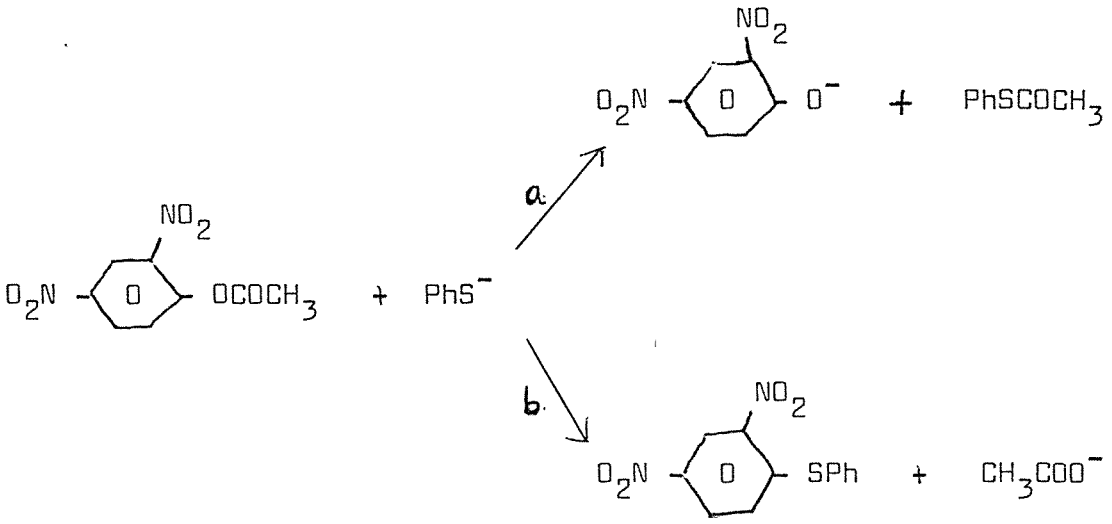
(Note this is not the same reaction as is thought to happen with a thiuram <u>di</u>sulphide such as disulfiram, see later.)

There is an obvious close similarity between Schemes 4 and 8; the only difference is in the identity of the leaving group, either 4-nitrothiophenoxide or dimethyldithiocarbamate, respectively. (It is interesting that tetramethylthiuram monosulphide inactivates aldehyde dehydrogenase much more slowly than does 4-nitrophenyl dimethyldithiocarbamate. Presumably under the conditions used, dimethyldithiocarbamate ion is a poorer leaving group than 4-nitrothiophenoxide). Kitson (12) has shown that tetramethylthiuram monosulphide does indeed react in the way shown in Scheme 8 with a variety of nucleophiles, including thiolate ions. For example, it reacts with sodium ethanethiolate to give a high yield of S-ethyl dimethyldithiocarbamate.

Thus there seems to be plenty of precedent for the suggestion that 4-nitrophenyl dimethyldithiocarbamate would react with cysteine (either free or combined within aldehyde dehydrogenase) according to Scheme 4. The results presented above (Chapter 2) show that in practice this suggestion is certainly justified but that it seems to be only partially true, in that less than a stoichiometric amount of 4-nitrothiophenoxide is produced in the reaction (see Table 1). Similar results were obtained with the other dithiocarbamate esters studied. It is unlikely that the reason for this is that Scheme 4 represents an equilibrium rather than a reaction going to completion. As already stated, 4-nitrothiophenoxide is expected to be a very good leaving group, and moreover, a large excess of cysteine (33-fold) is used in the experimental procedure.

I therefore concluded that a fraction of the 4-nitrophenyl dimethyldithiocarbamate was reacting by a different pathway which did not involve the release of 4-nitrothiophenoxide. A possibility for such a pathway has been discussed in Chapter 1. This is Scheme 5, an aromatic nucliophilic displacement reaction, which is superficially similar to the mechanism of action of disulfiram on aldehyde dehydrogenase (Scheme 1) in that both processes involve the release of dithiocarbamate ion as the leaving group.

Again, previous work with similar compounds provides support for the suggestion embodied in Scheme 5. Thus Guanti et al (13) report that a number of nitrophenyl esters of acetic and benzoic acid have been found to undergo both carbonyl C-O and aryl C-O scission in reaction with sodium benzenethiolate in ethanol (see Scheme 9).

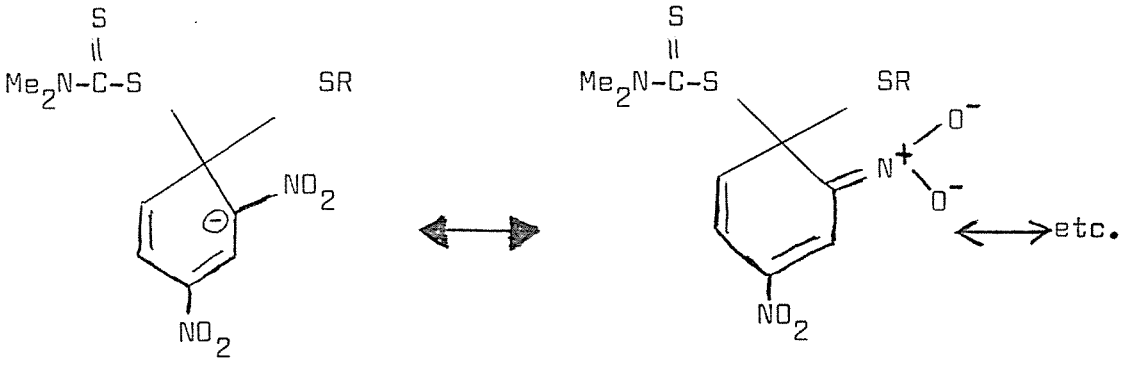


SCHEME 9

These workers found that both pathways a and b in Scheme 9 were operative with the dinitro compounds. With only one nitro group only reaction a occurred, whereas with the 2, 4, 6 - trinitro compound pathway b was exclusively followed. In some of the compounds I studied there is only one nitro group, but it may be that my reaction conditions (aqueous solution, pH 7.3, a different nucliophile and different substrate) tend to favour more of the aryl C - S scission pathway. Guanti et al quote other cases in which competition arises between two electrophilic centres in the same ester substrate, including nitrophenyl sulphonates, sulphates and phosphates.

The results of my experiments to detect the occurrence of aromatic nucleophilic displacement in the reaction between cysteine and 4-nitrophenyl dimethyldithiocarbamate (and related compounds) are presented in Table 2, Chapter 2. In short, this process does occur, it occurs to a somewhat greater extent with the dinitro compounds studied than the mononitro ones, but it is insufficient to account for all of that fraction of the reaction which does not follow the first pathway discussed above (nucleophilic displacement at the thiocarbonyl group). In other words, the corresponding data in Tables 1 and 2 do not add up to 100%.

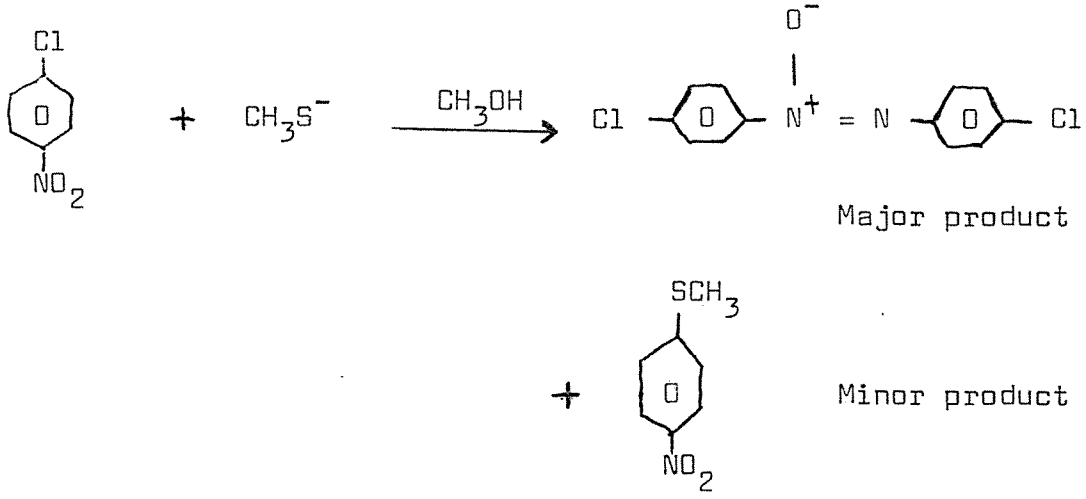
It is to be expected that the aromatic displacement reaction should be favoured with the dinitro compounds. The mechanism involves intermediates such as the following:



The more nitro groups present (in the ortho and para positions), the more important is the resonance stabilisation depicted and the easier the reaction becomes. In fact, the amount of dithiocarbamate ion released from the dinitro compounds when reacted with cysteine is perhaps surprisingly small. Guanti et al found that 2,4-dinitrophenyl acetate and 2,4-dinitrophenyl benzoate react by aryl oxygen scission with benzenethiolate to the extent of 51.7% and 84.2%, respectively.

Why do the data in Tables 1 and 2 not add up to 100%? Experimental error is one answer, but the estimation of 4-nitrothiophenoxide (to give the data in Table 1) is a simple

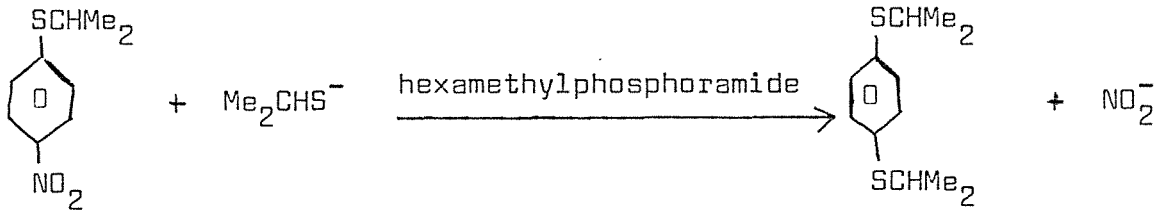
straightforward procedure, and the apparatus used to trap carbon disulphide (to give the data in Table 2) was checked to give a quantitative recovery of carbon disulphide from standard dithiocarbamate solutions. Presumably, therefore, there must be other possibilities for the reaction between cysteine and these dithiocarbamate esters which have not as yet been considered. A mechanism which springs to mind is one involving oxidation-reduction. Gold et al (14) have reported a procedure whereby the nucleophilic substitution of halonitrobenzenes by thiols can be achieved in high yield, but under other reaction conditions, reduction of the nitro group giving rise to azoxy compounds occurs instead. For example, addition of 4-chloronitrobenzene to a methanolic solution of sodium methanethiolate affords mainly 4,4-dichloroazoxybenzene and only a very small amount of the corresponding thioanisole (see Scheme 10).



SCHEME 10

In my experiments, similar reactions may have occurred to some extent. (Cysteine is a good reducing agent, being easily oxidised to the dimer, cystine.) It might be possible, in future experiments, to look for evidence on this point.

It has recently been shown that an alkyl thioether group in the para position to a nitro group activates the latter to displacement by a thiolate ion (15). For example :



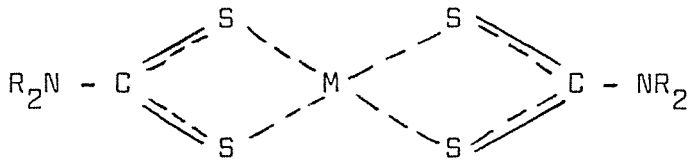
If the dimethyldithiocarbamate group can similarly activate the benzene ring towards nucleophilic displacement, then possibly an analogous reaction may have occurred to some extent in the course of my experiments. Again, at the moment, I have no evidence in support of this suggestion.

In conclusion, it has been shown that both Schemes 4 and 5 introduced in Chapter 1 are quite feasible as a major and minor route respectively for the reaction of 4-nitrophenyl dimethyl-dithiocarbamate with aldehyde dehydrogenase. There may be, in addition, other pathways which the reaction might follow. Further examination of the model system involving cysteine at this point does not seem as worthwhile as further experiements with the enzyme itself. Attempts should be made to isolate the enzyme in sufficient quantity that reliable quantitative estimation of 4-nitrothiophenoxide can be made upon reaction of the enzyme with the dithiocarbamate ester. The interaction could be studied over a range of pH, as the relative proportions of reaction by Schemes 4 and 5 might change under these circumstances. The possibility of reaction occurring between 4-nitrophenyl dimethyldithiocarbamate and aldehyde dehydrogenase previously modified with disulfiram should be looked into, to see whether the dithiocarbamate ester does indeed react with non-essential groups on the enzyme molecule as suggested in Chapter 1.

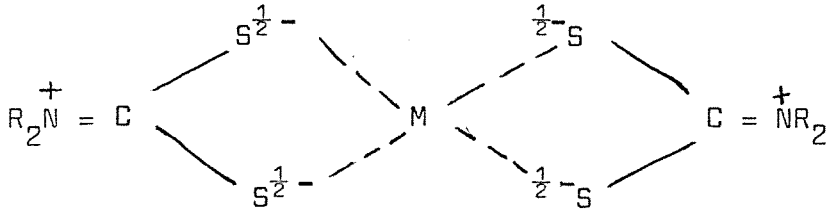
3.2 Metal complexes of some substituted dithiocarbamates

The N-substituted dithiocarbamates have found considerable use in analytical methods for heavy metals. (10) They have been used particularly for the detection and quantitative determination of traces of copper in various materials, but in recent years it has been demonstrated that the usefulness of the dithiocarbamates is by no means limited to copper analysis. A list of metals in order of increasing strength of complex formation with diethyldithiocarbamate at pH 5.6 is : Mn, As (III), Zn, Sn (II), Fe (III), Cd, Pb, Bi, Co (II), Ni, Cu, Ag, Hg. These heavy metal salts are sparingly soluble in water, being more soluble in organic solvents such as chloroform, carbon tetrachloride, ether and ethyl acetate.

The structure of a metal dithiocarbamate complex is usually represented as :



(where M is a divalent metal, in this case.) This formulation emphasises the covalent rather than ionic bonding within the complex, and the consequent solubility characteristics referred to above. It is also considered that the following structure makes a considerable contribution to the overall resonance hybrid :

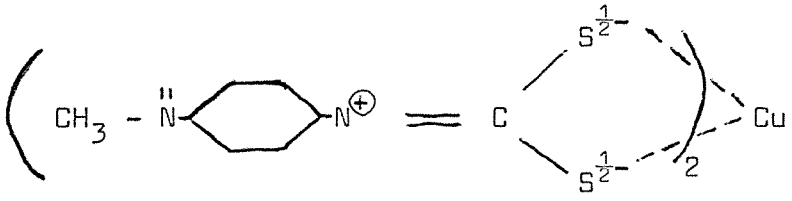


In support of this, Chatt et al (16) consider that the only possible assignment to fit the thioureide band in the infra-red spectrum (approx. 1500 cm^{-1}) is a polar C to N partial double bond.

The usual procedure in using a dithiocarbamate to estimate the concentration of a heavy metal is to mix aqueous solutions of the two species and extract the resulting insoluble complex into an organic solvent. The solution is then analysed quantitatively by spectrophotometry. By using a relatively small volume of the organic solvent, a considerable increase in concentration can be achieved at the extraction stage. However, in some circumstances it is advantageous to use a water-soluble complex, eliminating the need for extraction with a non-polar solvent. For example, in this connection bis-(2-hydroxyethyl) dithiocarbamate has received considerable attention. (17, 18) I thought it likely that dithiocarbamates carrying an ionisable group might be of more usefulness for this purpose and so I undertook a study of heavy metal complexes of L-proline dithiocarbamate and N-methylpiperazine dithiocarbamate.

The results shown in Figures 3, 4 and 5 in Chapter 2 show that proline dithiocarbamate does form intensely-absorbing complexes with copper, cobalt and nickel over the pH range of approximately 3 — 8. (Below pH 3 the situation is complicated by the instability of dithiocarbamate salts to acid). Above a pH of approximately 5 the complexes are soluble in aqueous solution, whereas at pH 3 to 4 they are soluble in ethyl acetate. Thus proline dithiocarbamate appears to be a useful, versatile reagent for the estimation of metals. By controlling the pH, it could either be used directly to determine copper, etc, in aqueous solution, or (in circumstances when a concentration step is necessary) after extraction into ethyl acetate.

The solubility characteristics of the metal complexes of proline dithiocarbamate are just what one would expect for species carrying a carboxyl group, which normally has a pKa of approximately 4 to 5. A similar statement cannot be made about the complexes of N-methylpiperazine dithiocarbamate. (See Figures 6, 7 and 8). Here the complexes remain preferentially soluble in ethyl acetate right down to pH 6 to 6.5, although the pKa of a simple tertiary amine is 10 - 11. The reason for this is not known. The complexes gave approximately the correct metal analysis based on the expected stoichiometry of a 2 : 1 ratio of dithiocarbamate to metal. (See Chapter 4. In all cases the amount of metal found upon analysis was 1 - 2% lower than expected. This may be a systematic error in the method used - atomic absorption spectroscopy carried out in this Department, by Mr R. McKenzie.) One might speculate that the appreciable positive charge on the dithiocarbamate nitrogen atom of the complex in a structure such as :



might interact across the ring with the other nitrogen atom, reducing the availability of its lone pair of electrons. However, it seems unlikely that this effect could have such a dramatic effect on the pKa of the tertiary amine group. Moreover, there is no similar perturbation of the pKa of the carboxyl group in the proline dithiocarbamate complexes.

Returning to the more straightforward case of proline dithiocarbamate, Figures 9, 10 and 11 in Chapter 2 portray the competition between this species and dimethyldithiocarbamate for copper, cobalt and nickel. It is evident that copper and nickel have a greater affinity for dimethyldithiocarbamate since the metal remains in the organic phase throughout the pH range studied, whereas cobalt occurs preferentially in the aqueous phase at pH's above 6, in the form of the negatively charged proline dithiocarbamate complex.

Thus it seems that proline dithiocarbamate, used in conjunction with dimethyldithiocarbamate may be a useful reagent for the separation of cobalt from copper and nickel, or for the quantitative estimation of cobalt in the presence of the other metals. Further development of this suggestion might prove to be a profitable project.

Finally, it should be pointed out that several metal complexes of dithiocarbamates have had widespread use in agriculture as fungicides. (10) Water soluble analogues, based on proline and N-methylpiperazine dithiocarbamates might prove to have useful applications in this field.

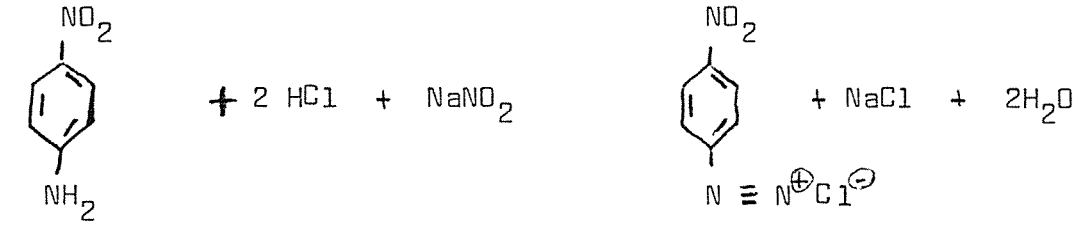
4. EXPERIMENTAL

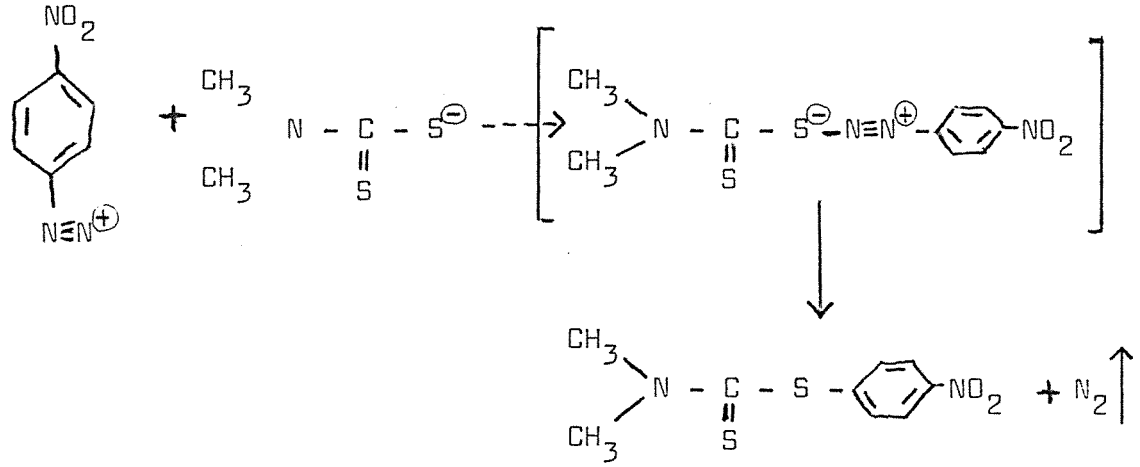
4.1 Materials

The following compounds were obtained from the sources indicated. Sodium di-methyldithiocarbamate, Fluka, A.G. (Buchs, Switzerland); Para-nitroaniline, Riedel-De-Haën (Germany); Sodium nitrite, May and Baker (Dagenham, England); Hydrochloric acid (One Molar), Berk, Spencer, (England). To the following compounds; Sodium di-ethyldithiocarbamate; 2,4-dinitro—fluorobenzene; Sodium tetraborax: Potassium hydrogen phthalate; Potassium dihydrogen phosphate; Copper sulphate; Cobalt Chloride and Nickel Chloride, they are obtained from British Drug House, Chemical (Poole, England).

4.2 Preparation of para-nitrophenyl dimethyldithiocarbamate

Para-nitrophenyl dimethyldithiocarbamate was prepared from paranitrophenyl diazonium chloride with sodium dimethyl-dithiocarbamate according to the synthetic scheme :





4.2.1 Preparation of diazoniam salt

Para-nitroaniline, 6.9065gm (0.05 mole) was dissolved in an organic solvent, acetone (25 mls) to give a dark brown solution. This was then added into 100mls of hydrochloric acid solution (0.1 molar) maintained at $0^{o}C$. A yellow precipitate was formed. To this was added 0.051 mole (2% excess) of sodium nitrite dissolved in 30mls water. This resulted in the

formation of para-nitrophenyl diazonium salt. At the end of the addition of the sodium nitrite, some precipitate was formed, as well as dark brown solution. While this was not desirable, it was unavoidable in as much as excess of acid would decompose the dithiocarbamate during coupling process.

4.2.2 Coupling Process

Sodium di-methyldithiocarbamate, 8.962gm (0.05 mole) was dissolved in 200mls of distilled water. The cold aqueous solution of the diazoniam salt was added in equi-molecular proportion, drop wise, to the well-stirred aqueous dithiocarbamate solution at room temperature. Each drop of the diazoniam salt solution added caused an immediate, violent evolution of nitrogen with only a small liberation of heat. The mixture was left to stir for half an hour. The result was the formation of yellow precipitate. The yellow precipitate was collected and re-crystallised from absolute ethanol. Bright orange crystals were formed together with some contaminating oil. This product was filtered off and washed with a small amount of di-ethyl either (50 mls) to remove adhering oil. The crystalline product was redissolved in absolute ethanol and boiled for ten minutes. At this stage, 2 gms of activated charcoal powder was added to absorb the coloured impurities. The solution was then filtered hot and set aside to crystalline. Pale yellow, well defined crystals (5 mm across) were obtained. The yield was 79%; melting point 125^{o}C.

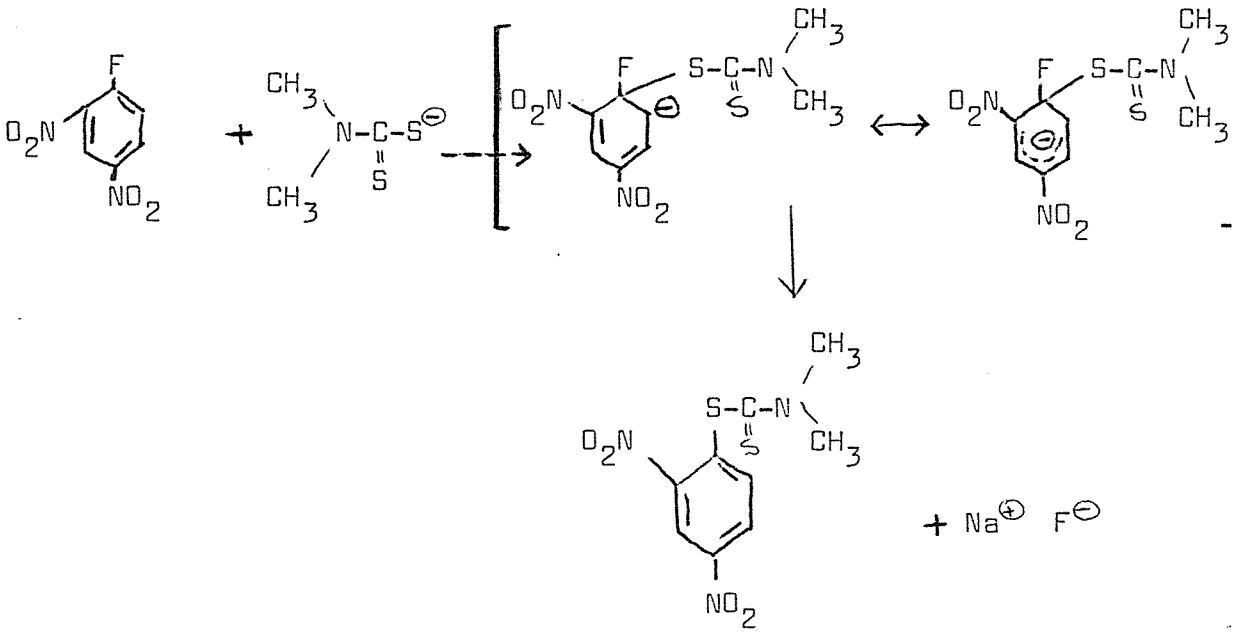
Although this melting point was considerably lower than the literature (18) value (154^{o}C), the product was shown to be authentic para-nitrophenyl di-methyldithiocarbamate by mass spectrometry (peaks at 242, $Me_2NCS_2C_6H_4NO_2$; 198, $O_2N\text{-}C_6H_4\text{-}S\text{-}\overset{\oplus}{C}S$; 88, $Me_2\overset{\oplus}{N} = C = S$) and microanalysis (Found: C, 44.41; H 3.99; N, 10.73; S, 26.72. Calculated for $C_9H_{10}N_2O_2S_2$: C, 44.61; H, 4.16; N, 11.56; S, 26.46%).

4.3 Preparation of para-nitrophenyl diethyldithiocarbamate

This compound was prepared in the same way as para-nitrophenyl di-methyldithiocarbamate. The product consisted of pale yellow crystals 3-5mm across. Melting point 115°C. It was shown to be $Et_2NCS_2C_6H_4NO_2$ by mass spectrometry and micro-analysis (Found: C, 48.20; H, 4.86; N, 9.82; S, 22.98. Calculated for $C_{11}H_{14}N_2O_2S_2$: C, 48.86; H, 5.22; N, 10.36; S, 23.72%).

4.4 Preparation of 2, 4-dinitrophenyl dimethyldithiocarbamate

The synthesis of this compound from 2, 4-dinitrophenyl diazanium salt and sodium di-methyldithiocarbamate was attempted, but a mixture of products, including 2,4-dinitrophenyl dimethyldithio-carbamate, bis-(2, 4-dinitrophenyl) disulphide and unchanged 2,4-dinitroaniline was obtained. An alternative route utilised their reactions:

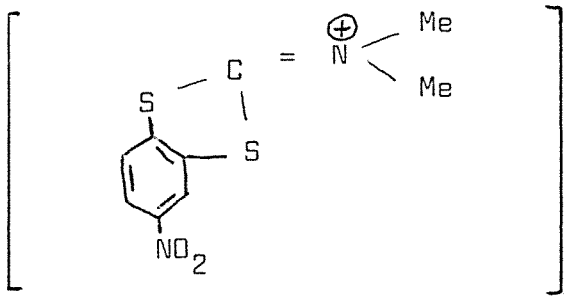


Sodium di-methyldithiocarbamate, 2.9663gm (0.0166 mole) was initially dissolved in absolute ethanol. Then, 2,4-dinitrofluro-benzene, 2mls, (0.0166 mole) was added. Immediately, an orange

solution together with a yellow precipitate resulted. The reaction mixture was stirred for an hour. The solution was then evaporated under reduced pressure. The residue was re-dissolved in chloroform-water mixture. The organic layer was dried over magnesium sulphate and evaporated to dryness, yielding the crude product, which was re-crystallised from absolute ethanol.

Two apparently different crystalline materials were obtained, one yellow and one orange. However, these had the same melting point, (147-149^{0}C), the same U.V. spectram (λmax = 325 nm), and mass spectrometry showed both samples to be the required $Me_2NCS_2C_6H_3(NO_2)_2$.

A major peak in the mass spectram was due to:



This ion has been implicated as an intermediate in the reactions of some substituted dithiocarbamates (19).

Microanalysis of $Me_2NCS_2C_6H_3(NO_2)_2$ gave C, 37.62; H, 3.74; N, 14.33; S, 22.45. Calculated for $C_9H_9N_3O4S_2$: C, 37.62; H, 3.16; N, 14.63; S, 22.32%.

4.5 Preparation of 2,4-dinitrophenyl diethyldithiocarbamate

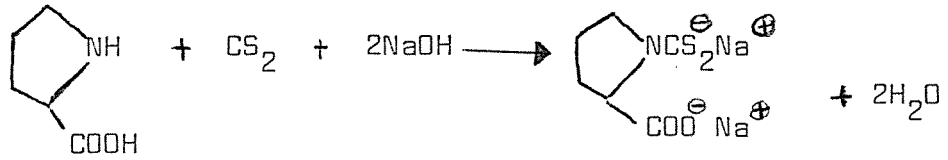
This compound was prepared from 2,4-dinitro flurobenzene and sodium diethyldithiocarbamate in the same way as the di-methyl compound. Bright yellow crystals (3-5 mm across) were obtained. Yield = 75%. Melting point, 82-83^{0}C. (Found = C, 42.57; H, 4.36; N, 12.90; S, 20.74.

Calculated for $C_{11}H_{13}N_3O_4S_2$: C, 41.89; H, 4.15; N, 13.32; S, 20.33%).

The mass spectrum confirmed the identity of the compound to be $Et_2NCS_2C_6H_3(NO_2)_2$.

4.6 Preparation of L-Proline dithiocarbamate sodium salt.

The preparation of L-proline dithiocarbamate sodium salt follows the scheme indicated.



Initially, carbon disulphide 0.30ml (5m Mole) was dissolved in distilled water by vigorously stirring for half an hour. L-proline, 0.5755gm (5m Mole) was added, followed by 20ml of one molar sodium hydroxide solution. The resultant solution was then raised to a volume of 500 mls (Conc = 10m Molar) and it was stirred for ten minutes.

4.7 Preparation of N-Methylpiperazine dithiocarbamate sodium salt

The preparation of N-methylpiperazine dithiocarbamate sodium salt follows the scheme indicated.

Carbon disulphide 0.3ml (5m Mole) was dissolved in distilled water, by vigorously stirring for half an hour. N-methylpiperazine, 0.55 ml (5 m Mole) was then added, followed by sodium hydroxide,

5ml (5 m Mole). The resultant solution was then raised to a volume of 500 mls (Conc : 10m Molar) and stirred for half an hour.

4.8 Preparation of metal complexes of L-proline dithiocarbamates

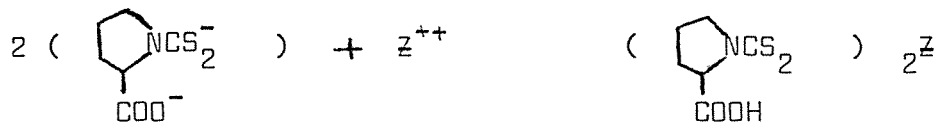
The diluted salt solution of L-proline dithiocarbamate, $2X10^{-3}$m Mole, (5 mls) was added to a 250 mls volumeric flask. It was then followed by $1X10^{-3}$ m Mole (5 mls) of copper sulphate solution. After shaking for two minutes, the solution was mixed with 100 mls of potassium hydrogen phthalate buffer solution (0.1 Molar) of pH: 2.5. The resultant solution was vigorously shaken for 5 minutes on the shaking machine, and subsequent extraction of the coloured complex with 50 mls of ethyl acetate solution was undertaken. This process was carried out analogously with cobalt and nickel.

4.9 Preparation of metal complexes of N-methylpiperazine dithiocarbamates

Diluted metal salt solutions of copper sulphate, cobalt chloride and nickel chloride, 5mls ($1X10^{-3}$ m Mole), were each added to 250 mls volumoric flask. To each of these solutions was added 5 ml ($2X10^{-3}$ m Mole) of N-methylpiperazine dithiocarbamate solution. The combined solution was then shaken for five minutes, assisted by a further shaking machine. 100 mls of potassium di-hydrogen phosphate and sodium hydroxide buffer solution of pH : 8.5 (0.1 Molar) was added. The solution was then vigorously shaken for five minutes, and subsequently extracted into 50 mls of ethyl acetate for analysis.

4.10 Metal analyses of dithiocarbamate complexes

Each individual complex of metal-L-proline dithiocarbamate was further obtained in small quantity to analyse for its metal component, by the following reaction scheme at a pH range of 2.5 to 3.0.



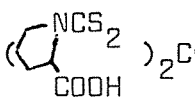
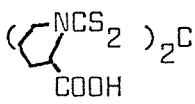
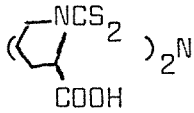
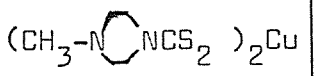
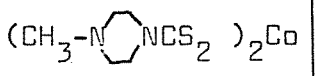
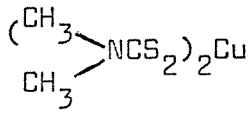
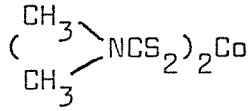
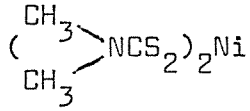
where Z^{++} = Cu^{++}, Co^{++}, Ni^{++}.

The ethyl acetate solution of the metal complex was dried over magnesium sulphate and evaporated under reduced pressure for analysis.

Samples of the N-methylpiperazine dithiocarbamate complexes were similarly prepared (at pH 8.5 - 9.0) for analysis. For purposes of comparison, the metal complexes of di-methyl-dithiocarbamate were also analysed.

Analysis was carried out using atomic absorption spectroscopy. The results are given in table 6.

TABLE 6 Results of metal analysis of dithiocarbamate complexes.

Compound	Yield	Theoretical	Found	Colour & Texture
	89.6%	14.31%	13.6%	Chocolate Powder
	80.5%	14.32%	12.8%	Pink Powder
	75.0%	14.28%	13.6%	Light Green Powder
	83.0%	15.34%	13.6%	Dark Chocolate Powder
	62.0%	14.34%	12.9%	Light Green Powder
	65.0%	14.39%	13.7%	Dark Green Powder
	89.4%	20.90%	18.8% 19.0%	Chocolate Powder
	85.5%	19.68%	18.0%	Purple Powder
	75.0%	19.62%	18.8%	Light Green Powder

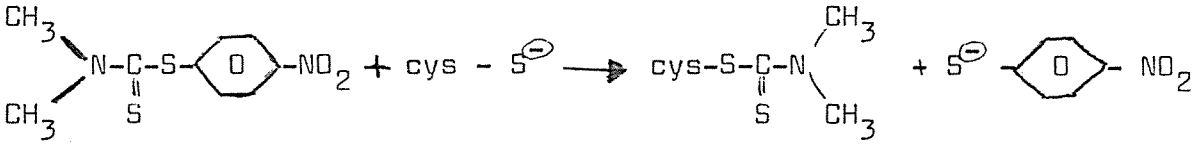
4.11 Reaction of para-nitrophenyl dimethyldithiocarbamate with cysteine.

The analysis of the products of reaction of para-nitrophenyl dimethyldithiocarbamate with cysteine is outlined below. The method was also applied to other compounds, such as para-nitrophenyldiethyldithiocarbamate; 2,4di-nitrophenyldimethyl-dithiocarbamate and 2,4-di-nitrophenyldiethyl dithiocarbamate.

4.11.1 Colorimetric determination of the para-nitrothiophenoxide ion

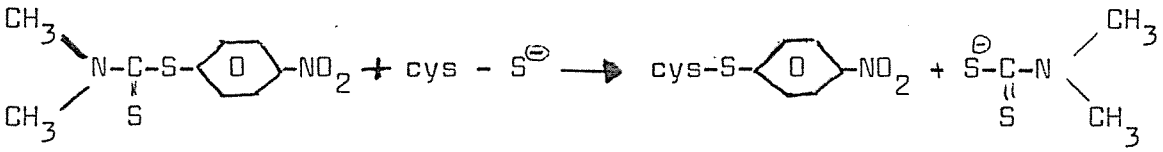
Two possibilities for the reaction between para-nitrophenyl dimethyldithiocarbamate and cysteine are as follows:

Either:



SCHEME 6

Or:



SCHEME 7

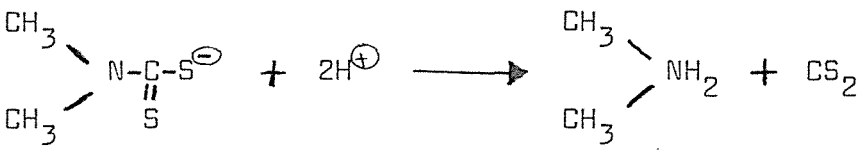
This is a simple mole to mole ratio reaction, but in my work, I used a great excess of L-cysteine solution in order to ensure that the para-nitrophenyldimethyldithiocarbamate is totally converted.

To a 100 mils flask, one ml of 3 m molar para-nitrophenyl dimethyldithiocarbamate in acetone solution was added, followed by 50 mls of 2 m Molar L-cysteine solution (in phosphate buffer pH = 7.3). Immediately, a yellow coloured solution was formed. This results from the release of the para-nitrothiophenoxide ion. Calculation showed that as a Mole of para-nitrophenyl dimethyldithiocarbamate compound was mixed with 100 Mole of L-cysteine compound. Thus, L-cysteine was in 33.3 - fold molar excess over other reactant. The resultant solution was stirred well and then analysed on the U.V./Vis spectrometer. The para-nitrothiophenoxide ion was found to absorb at the wavelength of 407 nm. The amount of this species liberated was calculated as a percentage of the theoretical maximum of only Scheme 6 above occurred.

4.11.2 Colorimetric determination of carbon disulphide

The extent of occurence of Scheme 7 above was estimated by acid decomposition of the liberated dithiodicarbamate ion to give carbon disulphide Scheme 8, which was trapped in McKee's solution (20) as shown in Scheme 9, using the apparatus illustrated in figure 13.

McKee's solution was prepared as follows: An ethanolic solution of 1% di-ethylamine, 1% of triethanolamine and 0.006% of cupric acetate.



SCHEME 8

$$2CS_2 + Et_2NH + Cu^{++} \longrightarrow (Et_2NCS_2)_2Cu + 2H^{\oplus}$$

SCHEME 9

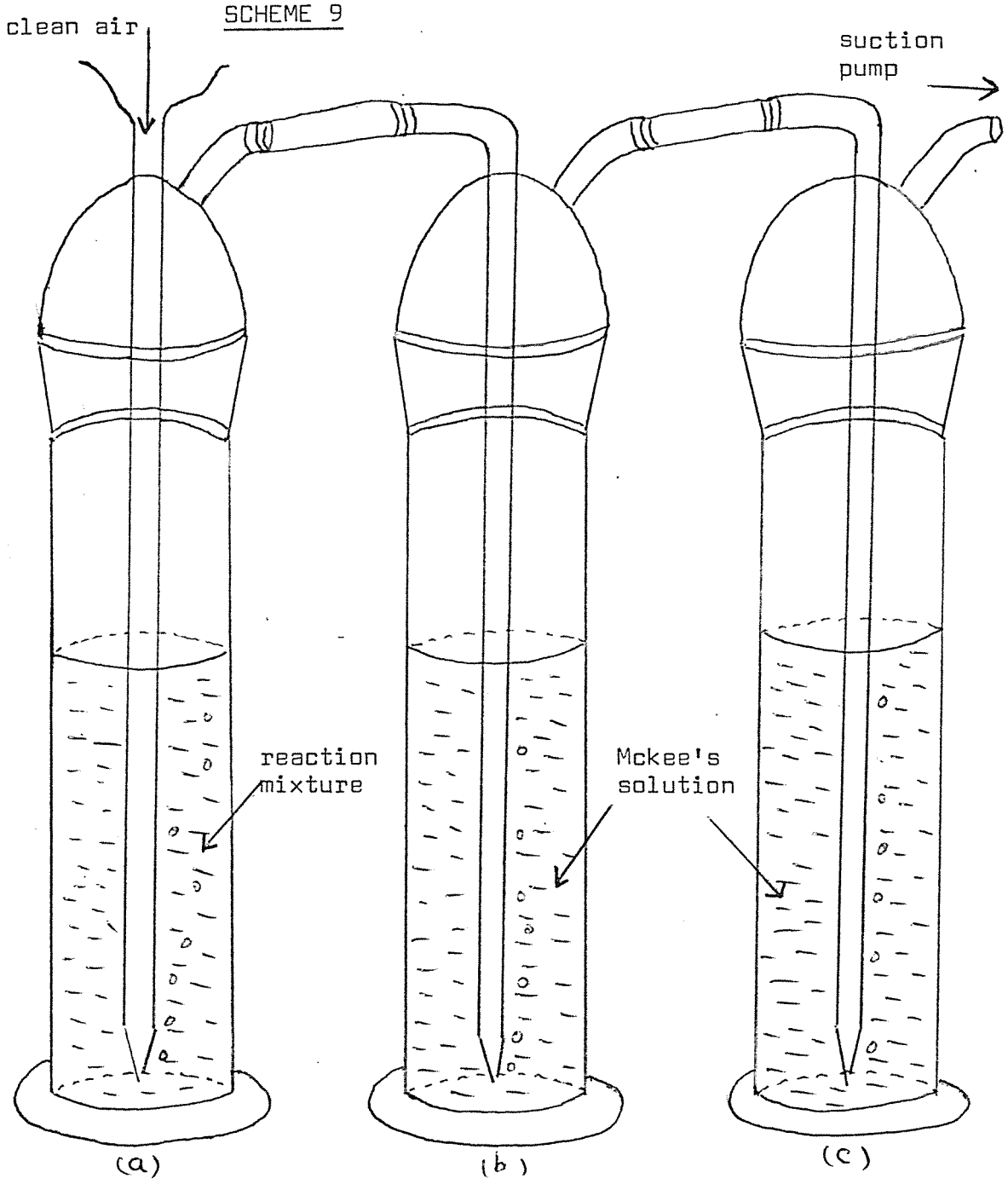


FIGURE 13 Apparatus for trapping carbon disulphide gas produced.

To the wash-bottle (a), one ml of para-nitrophenyldimethyl-dithiocarbamate solution (3 m Mole in acetone) was added, followed by 50 ml of L-cysteine solution of 2 m Molar in phosphate buffer of pH = 7.3. The resultant solution is yellow. It is allowed to stir for 5 minutes, then 50 mls of 2N solution of hydro-chloric acid was added, which was delivered from the special built mouth-piece, by drawing clean air through it. After the addition of hydrochloric acid, the solution became clear and carbon disulphide was liberated. The carbon disulphide was drawn into the McKee's solution which traps the gas by coupling with it in the wash-bottle (b). The third bottle (c) here merely acts as a control-bottle, such that any gas that is not trapped in the second bottle will be trapped in this bottle. The reaction was allowed to proceed for $1\frac{1}{2}$ to 2 hours. The resultant McKee's solution was collected in a separating funnel (500 ml). To this was added 25 mls of N-hexane solution. The mixture was shaken well to ensure the complex, copper di-ethyl-dithiocarbamate, was abstracted into the N-hexane layer. It was then followed by 400 mls of distilled water, to separate the N-hexane solution from the ethanolic layer. The N-hexane layer was collected and then centifuged to remove the air-bubbles. The clear yellow solution was then analysed in the same way in U.V./Vis spectrometer at 432 nm to determine the amount of carbon-disulphide trapped, expressed as a percentage of the maximum if only Scheme 9 above occurred.

The apparatus was shown to be a reliable; quantitative method for trapping carbon disulphide by decomposing known standard amounts of sodium di-methyldithiocarbamate by acid in the first wash-bottle.

4.12 Colorimetric investigation of copper, cobalt and nickel as di-methyldithiocarbamates

The colorimetric determination of small amount of copper, cobalt and nickel with sodium di-methyldithiocarbamate was carried out by a method essentially the same as that published by J.M. Chilton (11). The method involves the formation of the dithiocarbamate metal salt at a pH of 8.5 to 9.0 in an aqueous solution to which borax and sodium hydroxide have been added and subsequent extraction of the coloured complexes with ethyl acetate.

The sodium di-methyldithiocarbamate, 1.7924 gm (10 m Mole) was dissolved in distilled water, and subsequently brought up to a volume of 1000 ml, (Conc 10 m Molar). Each of the metal salts, (copper sulphate, nickel chloride, cobalt chloride), 2.5 m Mole was dissolved in distilled water and subsequently the volume was raised to 500 mls, (Conc 5 m Molar). As the concentration was still high, it was further subjected to dilution, by a factor of 25.

Initially, 5 mls ($2X10^{-3}$ m Mole) of the dithiocarbamate salt was added to a 250 mls volumeric flask. It was followed by 5 ml ($1X10^{-3}$ m Mole) of copper sulphate solution. After shaking for two minutes, the solution was mixed with 100 mls of borax buffer solution, pH = 9.5. The resultant solution was vigorously shaken for five minutes and subsequently extracted with ethyl acetate (50 mls). This process was also carried out with cobalt and nickel salt individually. The complex containing ethyl acetate solutions were analysed for their absorption in the ultra-violet and visible regions of the spectrum.

4.13 Colorimetric investigation of copper cobalt and nickel as L-proline dithiocarbamate under various pH conditions

Copper sulphate solution, $1X10^{-3}$ m Mole (5 mls) was added to 100 mls of various buffer solution (0.1 Molar) in 250 mls volumeric flask. (The buffer solutions consist of 50 mls of (0.1 Molar) potassium hydrogen phthalate and x mls of hydrochloric acid (0.1 Molar) for pH between 2 to 4; and 50 mls of 0.1 Molar potassium hydrogen phthalate and x mls of sodium hydroxide (0.1 Molar) for pH between 4 to 6; and 50 mls of 0.1 Moler potassium di-hydrogen phosphate and x mls of 0.1 Molar sodium hydroxide for pH between 6 to 8.)
It was shaken well before the addition of 5 mls ($2X10^{-3}$ m Mole) of L-proline dithiocarbamate solution. A yellow coloured solution resulted, which was extracted with 50 mls of ethyl acetate. The extraction process was assisted by a bottle shaking machine. Both the aqueous phase and the ethyl acetate extract were analysed for their U.V./visible absorbance.
The complexes of cobalt chloride and nickel chloride with L-proline dithiocarbamate were investigated in a similar way.

It was noted that the ethyl acetate solution was slightly soluble in distilled water thus, the final concentration of each complex was determined by accounting the actual final volume of ethyl acetate solution and also the final volume of a mixture of ethyl acetate solution and distilled water.

4.13.1 Colorimetric investigation of copper, cobalt and nickel as N-methylpiperazine dithiocarbamate under various pH conditions

These experiments were carried out in the same way as above, using N-methylpiperazine dithiocarbamate.

4.14 Competition between dimethyldithiocarbamate and L-proline dithiocarbamate for copper, cobalt and nickel under various pH conditions

Dimethyldithiocarbamate, 5 mls ($2X10^{-3}$ m Mole) and L-proline dithiocarbamate, 5 mls ($2X10^{-3}$ m Mole) were added to a 250 mls volumeric flask. The solution was thoroughly mixed before the addition of copper sulphate, 5 mls ($1X10^{-3}$ m Mole). The whole solution was then shaken for five minutes before the addition of 100 mls of buffer solution (0.1 Molar) and subsequent extraction of the solution with ethyl acetate (50 mls). Both the aqueous phase and the ethyl acetate solution were analysed for U.V./visible absorbance. The process was repeated using buffers over a range of pH.

Similar experiments were also carried out with cobalt chloride and nickel chloride solution.

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REFERENCES

1. Hald, J and Jacobsen, E. Lancet, 255, 1001-1004, (1948).

2. Kitson, T.M. J. Stud Alc. 38, 96-113, (1977).

3. Kitson, T.M. New Scientist, 79, 195-197, (1978).

4. Truitt, E.B. and Walsh, M.J. In 'The Biology of Alcoholism', Ed. by Kissin, B. and Begleiter, H. Vol. 1, 161-195, Plenum Press, (1971).

5. Jakoby, W.J. In 'The Enzymes'. Ed. by Boyer, P.D. Lardy, H. and Myrback, K. Vol. 7, 203-221, Academic Press, (1963).

6. Feldman, R.I. and Weiner, H. J. Biol. Chem. 247, 267-272, (1972).

7. Kitson, T.M. Biochem. J. 151, 407-412, (1975).

8. Kitson, T.M. Biochem. J. 155, 445-448, (1976).

9. Kitson, T.M. Biochem. J. 175, 83-90, (1978).

10. Thorn G.D. and Ludwig, R.A. 'The Dithiocarbamates', Elsevier Publishing Co., (1962).

11. Chilton, J.M. Analytical Chemistry 1274, Vol 25, No. 8 August (1953).

12. Kitson, T.M. J. Chem Soc. (Perkin I) 565-566, (1977).

13. Guanti, G. Dell'Erba, C. Pero, F. and Leandri, G. J. Chem. Soc. (Perkin II) 966-970 (1977).

14. Gold, E.H., Piotrowski, V. and Weiner, B.Z. J. Org. Chem, 42 554-556, (1977).

15. Cogalli, P. Testaferri, L. Tingoli, M. and Tiecco, M. J. Org. Chem, 44 2636-2642 (1979).

16. Chatt, J. Duncauson, L.A. and Venangi, L.M. Nature, 177 1042 (1956).

17. Fritz, J.A. and Sutton, S.A. Anal. Chem. 28, 1300, (1956)

18. Geiger, E. and Müller, H.G. Helv. Chem Acta 26, 996, (1943)

19. Clifford, L.M. and Lighty, J.G. J. Amec.Chem.Soc. 54, 1163-1166 (1932).

20. Rashead, K. and Warkentin, D. J.Org.Chem. 44, No:2 (1979).

21. McKee' R.W. A Quantitative Microchemical Colorimetric Determination of Carbon Disulphide in Air, Water and Biological Fluids. J. Ind. Hyd. Toxicol, 23, 151-158, (1941).

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