STUDIES OF THE CHEMISTRY OF DITHIOCARBAMATES AND THEIR METAL COMPLEXES

A thesis presented in partial fulfilment of
the requirements for the degree of
Master of Science with Honours
in Chemistry at
Massey University
New Zealand.

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1980

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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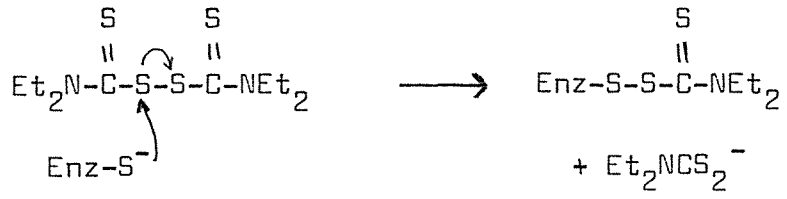
1. INTRODUCTION

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:

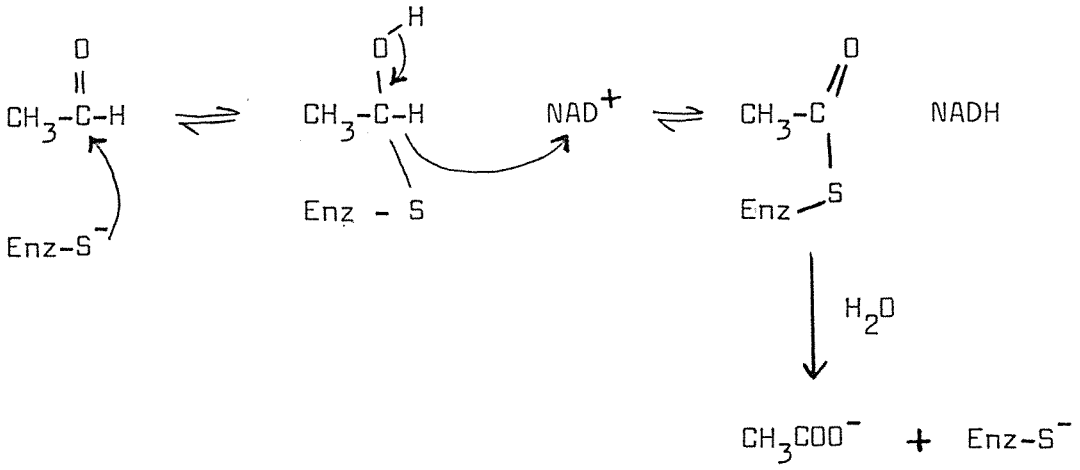


SCHEME 1

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).

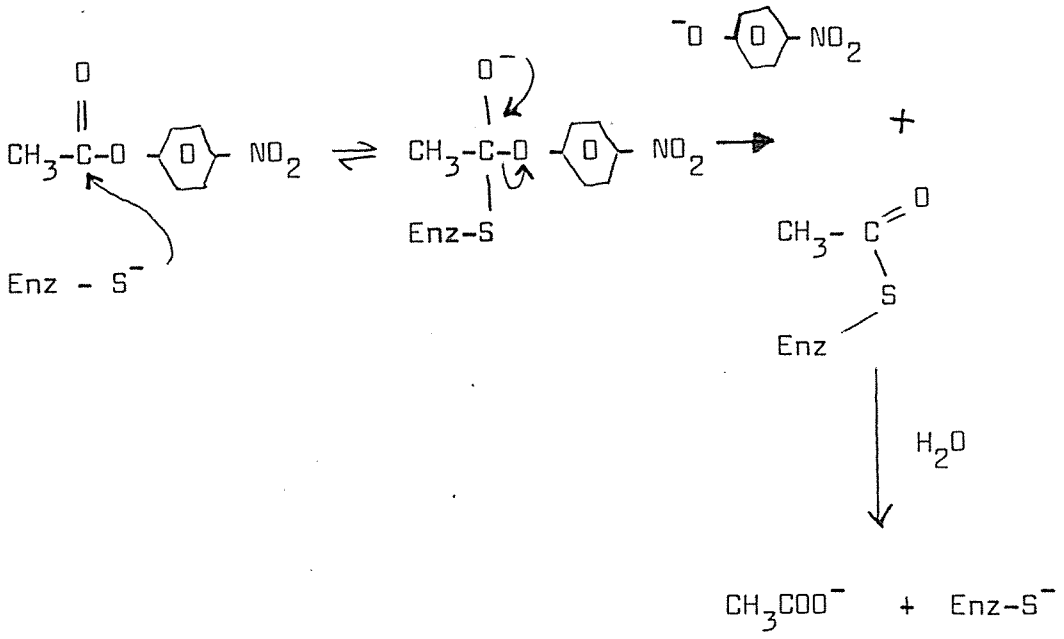
The mechanism of the reaction which aldehyde dehydrogenase normally catalyses is thought to be as shown in Scheme 2:



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.

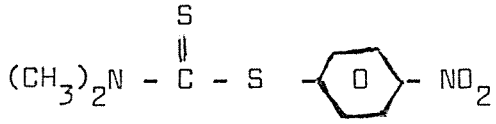
The same enzymic thiol group as discussed above can apparently also act as a nucleophile towards activated esters, since aldehyde dehydrogenase has been shown to rapidly hydrolyse 4-nitrophenyl acetate. (6) (Many other enzymes show similar versatility; eg trypsin, the function of which is to act as a 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).

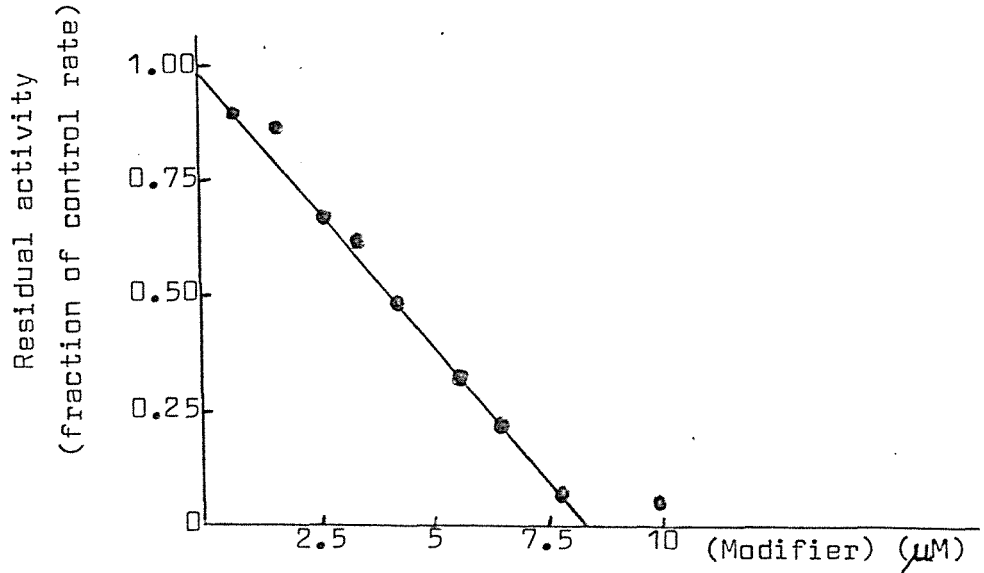
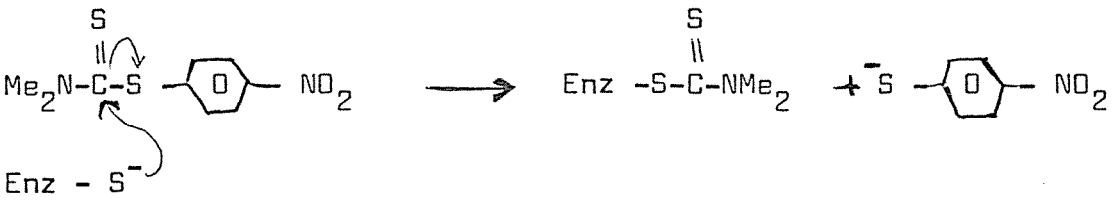


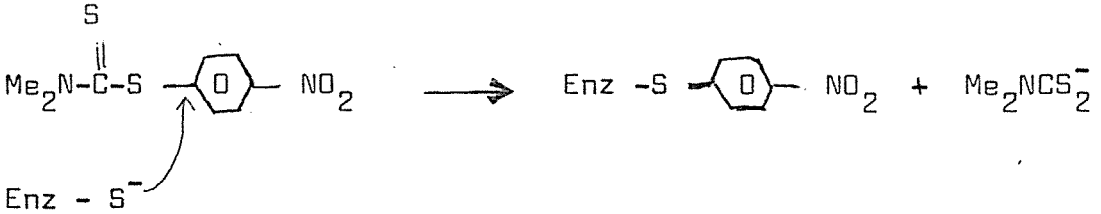
Figure 1. Effect of 4-nitrophenyl NN-dimethyl-dithiocarbamate on cytoplasmic aldehyde dehydrogenase.

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).

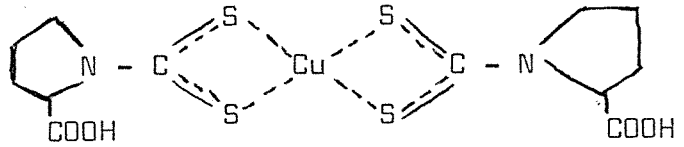
Before further work with the enzyme was attempted, it was decided to examine in detail the reaction between 4-nitrophenyl dimethyldithiocarbamate and the simple thiol compound, cysteine, in aqueous solution at pH 7.3, in an attempt to understand more fully the likely effect of the compound on aldehyde 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.

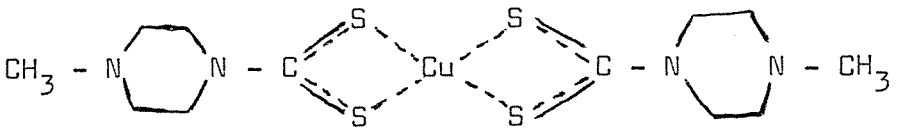
1.2 Metal complexes of dithiocarbamates

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.