SYNTHESIS OF 2H-1,4-BENZOTHIAZINES
AND RELATED COMPOUNDS

A Thesis
Submitted to the
Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science
in the College of Pharmacy
University of Saskatchewan
by

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Saskatoon, Saskatchewan. October, 1965.

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ACKNOWLEDGEMENT

I should sincerely like to thank Dr. R.T. Coutts for his guidance and helpful advice concerning this thesis.
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I. INTRODUCTION

A variety of reasons exist for making an investigation into the chemistry of cyclic hydroxamic acids, especially those possessing a 2H-1,4-benzothiazine nucleus. These reasons are discussed below.

Cyclic hydroxamic acids, as the name suggests, possess a hydroxamate group which exists in an equilibrium between the two forms, as shown (I).

\[
\text{OH} \quad \overset{\text{N}}{\vdash} \quad \text{O} \quad \text{OH}
\]

I

The group is strongly acidic in nature, being soluble in relatively weak bases such as sodium bicarbonate. The hydroxamic acid group is able to form chelates with metals. The structure of a divalent metal chelate is thought to be as shown (II).

\[
\text{O} \quad \overset{\text{N}}{\vdash} \quad \text{O} \quad \text{O} \quad \overset{\text{M}}{\vdash} \quad \text{O}
\]

II

The ferric chelate, formed by the interaction of
ferric salts and hydroxamic acids, is usually brilliant purple in color. This distinctive color, and their solubility in sodium carbonate solution are qualitative methods of identifying hydroxamic acids.

There are many compounds that contain a hydroxamic acid grouping, which are reported to possess antimicrobial activity (1-8).

The importance of cyclic hydroxamic acids as antibacterial compounds has been shown by the work done on aspergillic acid. In 1940, White discovered that a strain of Aspergillus flavus, growing on a surface culture of tryptone salt medium, produced a highly bacteriocidal filtrate (9). The active component was found to be aspergillic acid (III) and was best isolated by extraction of the acidified culture filtrate with chloroform, concentration to a small volume, re-extraction from the chloroform portion with sodium bicarbonate and precipitation from the aqueous portion upon acidification (10). Treatment of aspergillic acid (III) with bromine, followed by reduction of the bromo-substituted product with zinc and acetic acid (10), gave 3-isobutyl-6-sec-butyl-2,5-diketopiperazine which on hydrolysis (11) gave two $\alpha$-amino acids, DL-leucine (IV) and DL-isoleucine (V).
Further evidence for the structure of aspergillic acid occurred when its reduction product, deoxyaspergillic acid (VI) was examined. Degradative studies of deoxyaspergillic acid indicated its structure to be either 2-hydroxy-3-isobutyl-6-sec-butylpyrazine (VI) or 2-hydroxy-3-sec-butyl-6-isobutylpyrazine (VII).
The synthesis of VI by Newbold et al. (12), which was identical to deoxyaspergillic acid, established the structure of aspergillic acid as 3-isobutyl-6-sec-butyl-2-hydroxypyrazine-1-oxide (III). A 1:25,000 solution of aspergillic acid was bactericidal for a number of Gram positive and Gram negative organisms. Aspergillic acid is very toxic to animals however, and is not used therapeutically.

A similar compound to aspergillic acid which has recently been found to have antibacterial activity (8) is pulcherriminic acid. Pulcherrimin, the red pigment of Candida pulcherrima, was found to be a ferric chelate of a dibasic acid (13). It was shown that pulcherrimin could be converted to the dibasic acid, pulcherriminic acid. The structure of pulcherriminic acid (VII) was proven by MacDonald (14) using a similar method to that used for the elucidation of the structure of aspergillic acid. Pulcherriminic acid (VII) was extracted from the culture, isolated, and reduced to 2,5-diisobutyl-3,6-dioxopiperezine (VIII) with zinc and acetic acid, which in turn gave L-leucine (IX) on hydrolysis. Investigations of the reduced product led to the assignment of structure (VII) to pulcherriminic acid.
Another example of a cyclic hydroxamic acid found in nature is mycelianamide (X). It is a metabolic byproduct of *Penicillium griseofulvum*. This substance has no effect on Gram negative organisms, but completely inhibits the *in vitro* growth of a number of Gram positive organisms (15).
The compound 3,4-dihydro-2,4-dihydroxy-7-methoxy-3-oxo-2H-1,4-benzoazaine (XI) (Dimboa), which has been isolated from corn seedlings and other grasses, exhibits anti-metabolic activity (16). Dimboa has two unique structural features of interest; it contains a benzoazaine ring system and it is a cyclic hydroxamate.

![Chemical Structure](image)

The examples of cyclic hydroxamic acids quoted possess two heterocatoms in the ring. One type of cyclic hydroxamic acid not investigated thoroughly is that in which the heterocycle contains nitrogen and sulfur. Compounds of this type may be potentially important because many known medicinal compounds contain sulfur and nitrogen.

There are many antibacterial compounds which contain both sulfur and nitrogen in their structure. An example of this is the sulphonamides. They are a large group of compounds which have a wide range of activity and were used widely in therapeutic treatment of bacterial infection. Another drug possessing the sulfone group is Dapsone, bis (4-aminophenyl) sulfone. Dapsone has been used in in-
vestigational treatment of leprosy (17) and tuberculosis
(18).

As was mentioned initially, cyclic hydroxamic acids
and related compounds, which have a 2H-1,4-benzothiazine
nucleus, were considered to be sufficiently interesting to
warrant a systematic investigation. Various drugs of med-
icinal importance, and many compounds of potential im-
portance are structurally related to 2H-1,4-benzothiazine, and
some of its simple derivatives are known to possess anthel-
mintic properties (19,20). Examples of such derivatives are
6-substituted 3,4-dihydro-3-oxo-2H-1,4-benzothiazine (XII)
where R equals H, Cl, I, SCN, Br, or toluene-p-sulfonamido.
Other derivatives prepared are substituted in position 7.

![Diagram](image)

The 2H-1,4-benzothiazine (XIII) structure is chemically
related to the now very important group of drugs, the phen-
othiazines. This group of drugs is made up of relatively
simple derivatives of the parent compound, phenothiazine
(XIV).
The derivatives of phenothiazine give rise to an extremely wide variety of drugs with diverse pharmacological application, and at present there are about 14 drugs of the series in use (21). Originally, phenothiazine itself was considered important only for its anthelmintic properties, but in recent years much attention has been given to its derivatives, many of which are excellent tranquillizers and sedatives. Most of them exhibit an anti-emetic effect and an antihistaminic effect.

The phenothiazine derivatives consist of the phenothiazine nucleus and a side chain of the dimethylamine-alkyl type. An example of a phenothiazine derivative is Chlorpromazine Hydrochloride (U.S.P.) (XV).
Recently, a great deal of interest has been focused on phenothiazine derivatives substituted in position 2 of the nucleus. For example, Trifluromazine Hydrochloride (Vesprin) has a trifluoromethyl group, -CF₃, substituted on the 2 position of the chlorpromazine molecule in place of the chlorine atom, which causes the potency to be increased. In synthesizing compounds in this project, similar substituents were considered in the corresponding position of the benzothiazine nucleus. For example, various 2H-1,4-benzothiazine derivatives were prepared which were substituted in the position 6 with such groups as a bromo-group, a methyl-group, and a trifluoromethyl-group.

Four general methods for preparing cyclic hydroxamic acids are known. Briefly they are:

1) oxidation of pyridine and quinoline derivatives (22). An example is the oxidation of 2-ethoxypyridine (XVI) with hydrogen peroxide to 2-ethoxypyridine-1-oxide (XVII) which upon hydrolysis with dilute hydrochloric acid gave 2-hydroxy2-pyridine-1-oxide (XVIII), possessing the properties of hydroxamic acid.

\[
\begin{align*}
\text{XVI} & \xrightarrow{\text{H}_2\text{O}_2} \text{XVII} \xrightarrow{\text{hydrolysis}} \text{XVIII}
\end{align*}
\]
Further synthesis of pyridine and quinoline derivatives of cyclic hydroxamic acids have been described by Cunningham et al. (23).

2) condensation of an \( \alpha \) -amino hydroxamic acid with 1,2-dicarbonyl compounds (24). An example is the condensation of DL-alanine hydroxamic acid (XIX) and diacetyl in methanol and aqueous sodium hydroxide solution to yield the cyclic hydroxamic acid 1-hydroxy-2-keto-3,5,6-trimethyl-1,2-dihydropyrazine (XX).

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{NH}_2 \\
\text{CONH} & \quad \text{OH} \\
\text{CH}_3\text{CH} & \quad \text{CO-CH}_3
\end{align*}
\]

XIX

XX

3) a ring expansion using 2-acetylfuran (25), an example of which is the methoxylation of 2-acetylfuran (XXI) in methanol. The product, 2,5-dimethoxy-2-(\( \alpha \),\( \alpha \)-dimethoxyethyl)-2,5-dihydrofuran (XXII), was reacted with an aqueous solution of hydroxylamine hydrochloride and yielded 1,5-dihydroxy-6-methyl-2-pyridone (XXIII).
4) reductive cyclization of suitably substituted aromatic o-nitro-compounds using various reducing systems. With the last method, a variety of reducing systems have been used to prepare cyclic hydroxamic acids.

There are five such methods of reductive cyclization that have been used to prepare cyclic hydroxamic acids.

a) Reduction of suitable \( \alpha \)-(o-nitrophenyl) acids using zinc and sulfuric acid.

Reissert (26) obtained 1,2-dioxindole (XXIV) by the reduction of o-nitrophenylacetic acid with zinc and sulfuric acid.
Heller and Wunderlich (27) obtained 1,2-dihydro-1-hydroxy-2-oxoquinoline-3-carboxylic acid (XXV) by reduction of o-nitrobenzylidene malonic acid with zinc and sulfuric acid.

b) Reduction of suitable $\alpha$-(o-nitrophenyl) acids using catalytic hydrogenation.

Dicarlo (28), following initial oxidation of o-nitrophenylpyruvate to o-nitrophenylacetic acid, isolated 1,2-dioxindole (XXIV) in appreciable amounts by catalytic hydrogenation of the latter compound with platinum oxide.
c) Reduction of suitable α-(o-nitrophenyl) esters using hydrazine hydrate in the presence of the catalyst palladium (10%)-on-charcoal.

The synthesis of 1,2-dihydro-1,4-dihydroxy-2-oxo-quinoline (XXVI) by the action of this reducing system on ethyl or methyl o-nitrobenzoylacetate was described by Coutts et al. (29).
Attempts to prepare 3-substituted analogues, other than 3-alkyl derivatives of XXVI, were unsuccessful due to competitive reactions involving the hydrazine hydrate. Later investigations by Coutts (30) extended the reaction to a limited extent, and some 3-substituted derivatives were obtained, such as 2-(1,2-dihydro-1,4-dihydroxy-2-oxo-3-quinolyl)-propionic acid hydrazide (XXVII).

\[
\begin{align*}
\text{CONH}_2\text{NH}_2 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{OH} \\
\end{align*}
\]

XXVII

\(d\) Reductive cyclization of a series of similar \(\alpha\)-(o-nitrophenyl)-esters with cyclohexene and palladium charcoal resulted in the formation of their N-oxides, but no quinolines and cyclic hydroxamic acids were obtained (31).

\(e\) Reduction of suitable \(\alpha\)-(o-nitrophenyl) esters and ketones using sodium borohydride and palladium charcoal catalyst produces cyclic hydroxamic acids and N-oxide derivatives in good yields (32).

The reduction using sodium borohydride and palladium charcoal appeared to be the most interesting method for the present investigation because of the indication of good yields, and also because of the limited amount of work.
done with this system. The variety of applications of the sodium borohydride and palladium charcoal reducing system to yield cyclic hydroxamic acids was shown by Coutts et al. (73). Using this method, they prepared certain quinolines, quinazolines, quinoxalines, benzoxazines and benzothiazines containing the cyclic hydroxamic grouping, such as 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline (XXVIII; x-y = CH2-CH2), 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinazoline (XXVIII; x-y = CH2-NH), 1,2-dihydro-1-hydroxy-2-oxo-4H-3,1-benzoazetine (XXVIII; x-y = CH2-0), and 3,4-dihydro-4-hydroxy-3-oxo-1,4-benzothiazine (XXVIII; x-y = S-CH2).

![XXVIII]

There are a large number of o-nitro-ester intermediates that are suitable for reductive cyclization. Because of the reasons mentioned before, suitable o-nitro-phenylthio-ester intermediates were prepared which, on reductive cyclization, would yield 2H,1,4-benzothiazine derivatives. The only 2H,1,4-benzothiazine hydroxamates that have been reported in the literature are 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine and 3,4-dihydro-4-hydroxy-
3-oxo-2H-1,4-benzothiazine-1,1-dioxide (32).

Coutts and Wibberley have indicated that the intermediate used for reductive cyclization must be an \(o\)-nitro-ester, in which the ester group is suitably orientated with respect to the \(o\)-nitrophenyl group in order to give the cyclic hydroxamate (32). The reductive cyclization of the \(o\)-nitro-ester occurs by an initial reduction of the nitro-group to a hydroxyamino-group, at which stage cyclization occurs. Hydroxylamines condense most readily with ketones and esters, but not with acids. Therefore, reductive cyclization on suitable \(o\)-nitro-esters would yield cyclic hydroxamic acids (XXIX), \(o\)-nitro-ketones would give cyclic \(N\)-oxides (XXX).
Thus, suitable α-nitrophenylthio-ketones were also prepared as intermediates for reductive cyclization in anticipation of obtaining N-oxide products. Hereto, there has been no reaction of this type reported with α-(α-nitrophenylthio)-keto derivatives using sodium borohydride and palladium charcoal catalyst. The production of an N-oxide was reported by Coutts (32), having reduced α-nitrobenzoylaceton with sodium borohydride and palladium charcoal. 4-Hydroxy-2-methylquinoline-1-oxide was the product in this case.
II. DISCUSSION

The problems that were investigated in this thesis were as follows:

a) a further investigation of the usefulness of sodium borohydride and palladium charcoal as a reducing system for aromatic nitro groups.

b) the preparation of suitable intermediates for reductive cyclization to N-oxy or N-hydroxy compounds.

c) the preparation of N-oxy and N-hydroxy compounds related to 2H-1,4-benzothiazine for evaluation as anti-bacterial compounds and enzyme inhibitors.

The envisaged intermediates were suitably substituted o-nitro-esters or o-nitro-ketones, of types (XXXI) and (XXXII), in which the nitro group and the ester, or ketone group, were ideally situated spatially for cyclization.

\[
\text{XXXI}
\]

\[
\text{XXXII}
\]

Reduction of compounds of types XXXI and XXXII were expected to yield compounds XXXIII and XXX respectively.
The problem can be conveniently subdivided. The first section deals with the preparation of intermediates of types XXXI and XXXII, and the second will consider the reduction of these intermediates.

**Preparation of Intermediates of Types XXXI and XXXII**

The preparation of intermediates suitable for reduction to compounds XXXIII and XXX was carried out using three different methods. In each method, the intermediate prepared had an o-nitrophenylthio group attached to a side chain containing an ester or keto group in a suitable position for cyclization.

**Method I**

The first method of synthesis attempted was the condensation of sodium o-nitrothiophenolate with suitable α-bromoesters (scheme I).
This apparently simple reaction gave rise to an unexpected product in significant amounts, and proved to be unsatisfactory for a number of reasons. In the first place, preparation of pure o-nitrothiophenol obtained by mild reduction of bis(o-nitrophenyl) disulfide (XXXIV) according to the methods of Claass (33), and Mills and Whitworth (34) was difficult (equation I). The yields were poor and an impure product resulted. A slight modification to the procedure was made that resulted in a better yield; this was achieved by using acetic acid rather than hydrochloric acid in the final precipitation of the o-nitrothiophenol from a solution of its sodium salt.

equation I
Using this method of preparing intermediates, ethyl 2-(o-nitrophenylthio)butyrate ($XXXI; R = H, R' = R'' = Et$), ethyl 2-(o-nitrophenylthio)propionate ($XXXI; R = H, R' = Me, R'' = Et$), and diethyl bis(o-nitrophenylthio)malonate ($XXXV$) were prepared by condensing sodium o-nitrothiophenolate with ethyl α-bromobutyrate, ethyl α-bromopropionate and diethyl dibromomalonate respectively.

\[
\begin{align*}
\text{(XXXV)}
\end{align*}
\]

In the reactions done using method I, bis(o-nitrophenyl) disulfide ($XXXIV$) was produced in each case in significant amounts. It was observed to be formed as a precipitate very quickly after the two reactants were mixed together.

The interaction of sodium o-nitrothiophenolate with ethyl 2-bromobutyrate was carried out as described below. o-Nitrothiophenol was dissolved in a solution of sodium hydroxide and aqueous ethanol, forming sodium o-nitrothiophenolate. When ethyl 2-bromobutyrate was added to this solution, a precipitate of bis(o-nitrophenyl) disulfide formed almost immediately in 36% yield. Ethyl 2-(o-nitrophenylthio)-
butyrate was obtained as an oil, in 37% yield, by evaporating the mother liquors, flooding with water, extracting the aqueous mixture with ether, and evaporating the ether extract.

Condensation of sodium o-nitrothiophenolate with diethyl bromomalonate gave no diethyl (o-nitrophenylthio)malonate as expected. Instead, a greater than 80% yield of the disulfide (XXXIV) resulted immediately on the addition of the bromomalonate to an aqueous ethanolic solution of sodium o-nitrothiophenolate. The filtrate of this reaction on standing and concentration deposited a small amount of diethyl bis(o-nitrophenylthio)malonate (XXXV) whose structure was indicated by elemental analysis, which included a molecular weight determination, and by an infrared spectrum which indicated the presence of the ester group, and the nitro group.

A similar condensation of sodium o-nitrothiophenolate and diethyl dibromomalonate, in aqueous ethanolic solution, resulted in formation of the disulfide (XXXIV) in a greater than 80% yield. Only a small quantity of the diethyl malonate derivative (XXXV) was isolated from the mother liquors after concentration and extraction of the solution. When the condensation of sodium o-nitrothiophenolate and diethyl dibromomalonate was repeated in a solution of sodium ethoxide and absolute ethanol, the yield of the diethyl mal-
onate derivative (XXXV) was increased to about 10%; the yield of disulfide (XXXIV) was 67%. This reaction requires further investigation.

The formation of a similar product was reported by Honkanen and Virtanen (35) who reacted sodium o-nitrophenolate and diethyl bromomalonate and obtained as a minor product of the reaction, a substance they indicated was diethyl-bis(o-nitrophenoxy)malonate (XXXV, O replacing S).

In the attempted synthesis of 2-(o-nitrophenylthio)benzaldehyde, sodium o-nitrothiophenolate and 2-chloro-benzaldehyde were reacted in an aqueous ethanolic solution of sodium hydroxide. The only product recovered from the reaction mixture was the disulfide (XXXIV).

Because of the undesired formation of bis(o-nitrophenyl) disulfide in this group of reactions, a brief investigation of the reasons for its facile formation was carried out. The significance of the ortho nitro group in formation of the disulfide (XXXIV) was shown by achieving similar results when sodium p-nitrothiophenolate was condensed with diethyl chloromalonate. The reaction was carried out as before in an aqueous alcoholic solution of sodium hydroxide. Bis(p-nitrophenyl) disulfide was recovered from the reaction mixture in a yield of 67%. When sodium p-nitrothiophenolate and diethyl chloromalonate were refluxed in anhydrous xylene, bis(p-nitrophenyl) disulfide was recovered again in a yield of about 80%.
The effect of a strong electron withdrawing group in the ortho or para position in aiding the formation of the disulfide is shown by substituting a methoxycarbonyl group in place of the nitro group in sodium o-nitrothio-phenolate and condensing it with \(-\)bromoesters. The methoxycarbonyl group is a weaker electron withdrawing group than the nitro group (36). When methyl thiosalicylate, prepared by esterifying thiosalicylic acid by the Fischer-Speier method, was condensed with ethyl 2-bromobutyrate in a solution of sodium hydroxide in aqueous ethanol, no solid product was obtained. The product was an oil, assumed to be ethyl 2-(o-carbomethoxyphenylthio)isobutyrate. Di-o-carbomethoxyphenyl disulfide is reported to be a solid with a melting point 131-132\(^{\circ}\) (37). The fact that this disulfide derivative was not obtained indicates that the strong electron withdrawing power of the nitro group aids in the formation of the disulfide in these types of reactions.

In an attempt to increase the yields of the intermediates XXXI and XXXII, and to reduce the occurrence of disulfide (XXXIV), some condensations involving sodium o-nitrothiophenolate and ethyl \(-\)bromoesters were attempted in various types of solvents.

Condensations were attempted in an aqueous polar solvent, an anhydrous polar solvent, and a non-polar solvent. Bis(o-nitrophenyl) disulfide was the major product when
sodium o-nitrothiophenolate and ethyl bromomalonate were reacted in aqueous ethanol; it was also the major product when sodium o-nitrothiophenolate and diethyl dibromomalonate were reacted in anhydrous ethanol. If acetone was used as a solvent, there was no observed reaction between sodium o-nitrothiophenolate and the dibromomalonate. As mentioned before, bis(p-nitrophenyl) disulfide was the major product when the non-polar solvent, xylene, was used in the reaction of sodium p-nitrothiophenolate and diethyl dibromomalonate.

The unsatisfactory yields of the required intermediates, and the occurrence of the disulfide (XXXIV) in undesirable quantities prompted an investigation of other methods for preparing suitably orientated intermediates for reductive cyclization.

**Method II**

The second method used to prepare intermediates of types XXXI and XXXII was a nucleophilic substitution reaction involving the interaction of a substituted o-chloro-(or bromo-) nitrobenzene and suitable α-mercapto acids (scheme II).

![Chemical Reaction Diagram](attachment:reaction_diagram.png)
This reaction was used to a limited extent to prepare derivatives of 2-nitrophenylthioacetic acid by Badger et al. (38). Substituted o-chloro-(or bromo-) nitrobenzenes derivatives were condensed with α-mercaptoacetic acid in the presence of sodium bicarbonate to give the required derivatives.

The possibility of extending this preparative method was investigated. Attempts to condense various o-chloro-(or bromo-) nitrobenzenes with α-mercapto succinic acid and α-mercapto propionic acid were successful and a variety of acids of type XXXVI were prepared. The following α-(o-nitrophenylthio)acids were obtained in this way (table I).

Table I: α-(o-Nitrophenylthio)Acids Prepared by Method II.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure XXXVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) α-(o-nitrophenylthio)-</td>
<td>( R = ) H ( R' = ) ( \text{CH}_2\text{COOH} )</td>
</tr>
</tbody>
</table>
Table I (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure XXXVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>c) ( \alpha-(4\text{-bromo-2-nitrophenylthio})-) succinic acid</td>
<td>R = Br, R' = CH₂COOH</td>
</tr>
<tr>
<td>d) ( \alpha-(4\text{-bromo-2-nitrophenylthio})-) propionic acid</td>
<td>R = Br, R' = CH₃</td>
</tr>
<tr>
<td>e) ( \alpha-(4\text{-methyl-2-nitrophenylthio})-) succinic acid</td>
<td>R = CH₃, R' = CH₂COOH</td>
</tr>
<tr>
<td>f) ( \alpha-(4\text{-methyl-2-nitrophenylthio})-) propionic acid</td>
<td>R = CH₃, R' = CH₃</td>
</tr>
<tr>
<td>g) ( \alpha-(4\text{-trifluoromethyl-2-nitrophenylthio})) succinic acid</td>
<td>R = CF₃, R' = CH₂COOH</td>
</tr>
<tr>
<td>h) ( \alpha-(4\text{-trifluoromethyl-2-nitrophenylthio})) propionic acid</td>
<td>R = CF₃, R' = CH₃</td>
</tr>
</tbody>
</table>

\( \alpha \)-Mercaptosuccinic acid was condensed with 2-chloro-nitrobenzene, 2,5-dibromo-nitrobenzene, 4-chloro-3-nitrotoluene and 4-chloro-\( \alpha, \alpha, \alpha \)-trifluoro-3-nitrotoluene to give the acids XXXVI a, c, e, and g respectively. When \( \alpha \)-mercaptopropionic acid was condensed with 2-chloronitrobenzene, 2,5-dibromo-nitrobenzene, 4-chloro-3-nitrotoluene and 4-chloro-\( \alpha, \alpha, \alpha \)-trifluoro-3-nitrotoluene, the acids XXXVI b, d, f, and h were obtained respectively. The various acids were isolated, usually in good yields, purified and characterized before proceeding to prepare the methyl esters, which were readily obtained by the Fischer-Speier esterification procedure. This resulted in the formation of the following inter-
mediates of type XXXI (table II).

**Table II. Substituted α-(o-nitrophenylthio) Esters**

![Structure XXXI](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure XXXI</th>
<th>R</th>
<th>R¹</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) dimethyl α-(o-nitrophenylthio) succinate</td>
<td>H</td>
<td>(\text{CH}_2\text{COOMe})</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>b) methyl α-(o-nitrophenylthio) propionate</td>
<td>H</td>
<td>(\text{CH}_3)</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>c) dimethyl α-(4-bromo-2-nitrophenylthio) succinate</td>
<td>Br</td>
<td>(\text{CH}_2\text{COOMe})</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>d) methyl α-(4-bromo-2-nitrophenylthio) propionate</td>
<td>Br</td>
<td>(\text{CH}_3)</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>e) dimethyl α-(4-methyl-2-nitrophenylthio) succinate</td>
<td>(\text{CH}_3)</td>
<td>(\text{CH}_2\text{COOMe})</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>f) methyl α-(4-methyl-2-nitrophenylthio) propionate</td>
<td>(\text{CH}_3)</td>
<td>(\text{CH}_3)</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>g) dimethyl α-(4-trifluoromethyl-2-nitrophenylthio) succinate</td>
<td>(\text{CF}_3)</td>
<td>(\text{CH}_2\text{COOMe})</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>h) methyl α-(4-trifluoromethyl-2-nitrophenylthio) propionate</td>
<td>(\text{CF}_3)</td>
<td>(\text{CH}_3)</td>
<td>Me</td>
<td></td>
</tr>
</tbody>
</table>
In preparing the substituted \( \alpha \)-mercaptosuccinic acids and \( \alpha \)-mercaptopropionic acids, conditions were important if good yields of the acids were to be obtained. Generally, longer reaction times were required than those reported by Badger (38) for similar reactions using \( \alpha \)-mercaptoacetic acid. The reaction time was extended from 4 hours to 18 hours in order to obtain better yields. An excess \( \alpha \)-amount of sodium bicarbonate was necessary for the condensation to proceed.

This method of preparing intermediates of type XXXI is capable of further extension. There are many substituted \( \alpha \)-chloro- (or bromo-) nitrobenzene compounds available. It would appear that the availability of \( \alpha \)-mercapto acids would be the only limitation in preparing various types of \( \alpha \)-(\( \alpha \)-nitrophenylthio) acids.

In carrying out this group of reactions using \( \alpha \)-mercaptosuccinic acid and \( \alpha \)-mercaptopropionic acid, a difference in the reactivity of the substituted \( \alpha \)-chloro- (or bromo-) nitrobenzene to nucleophilic substitution was observed. In condensations involving \( \alpha \)-chloro-3-nitrotoluene with either \( \alpha \)-mercaptoc acid, there was a definite reduction in the yields of \( \alpha \)-(4-methyl-2-nitrophenylthio) acids (XXXVI, e and f) obtained, whereas the \( \alpha \)-(\( \alpha \)-nitrophenylthio) acids, the \( \alpha \)-(4-bromo-2-nitrophenylthio) acids and the \( \alpha \)-(4-trifluoromethyl-2-nitrophenylthio) acids were obtained in larger yields. This would indicate the methyl
group of 4-chloro-3-nitrotoluene had a deactivating effect on the o-nitrophetyl nucleus towards nucleophilic reaction with the \(\alpha\)-mercapto acid.

It has been observed by Coutts (39) that the reactivity of the thiol group of the \(\alpha\)-mercapto acid is significant also. In condensations between \(\alpha\)-mercaptoisobutyric acid and 4-chloro-\(\alpha\),\(\alpha\)-trifluoro-3-nitrotoluene or 2,5-dibromonitrobenzene, the nucleophilic reaction is typical; \(\alpha\)-(4-trifluoromethyl-2-nitrophenylthio)isobutyric acid and \(\alpha\)-(4-bromo-2-nitrophenylthio)isobutyric acid are readily obtainable in good yield. However, if o-chloronitrobenzene or 4-chloro-3-nitrotoluene were reacted with \(\alpha\)-mercaptoisobutyric acid, no \(\alpha\)-(o-nitrophenylthio)isobutyric acids were obtained. Under the conditions used, neither the thiol group of the \(\alpha\)-mercapto acid nor the ortho chlorine atom of the nitrobenzene derivative employed in the reaction were sufficiently activated to promote a nucleophilic substitution reaction. In both cases, the same product, \(\alpha\),\(\alpha\)-dithiodiisobutyric acid was the only acid material isolated. Thus, the reactivity of the thiol group of the \(\alpha\)-mercapto acid would appear also to limit the scope of this preparative reaction.

**Method III**

The third method of synthesis attempted in the preparation of intermediates was the condensation of \(\alpha\)-nitrobenzenesulfenyl chloride (XXXVII) with suitable com-
pounds containing an active hydrogen in the position alpha to a carbonyl group (XXXVIII).

\[
\begin{align*}
\text{XXXVII} & \quad \text{XXXVIII} \\
\end{align*}
\]

Kharasch et al. (40, 41, 42) have done extensive work in the field of nitrobenzenesulphenyl derivatives, employing mainly 2,4-dinitrobenzenesulfenyl chloride as reagent. He used o-nitrobenzenesulfenyl chloride only occasionally.

Barltrop and Horgan (43) have investigated numerous reactions of nitrobenzenesulfenyl chlorides with ketones that were applicable to method III. They indicated that the best solvent system to be used for this condensation was acetonitrile. This agrees with Kharasch's previous investigations where he indicated that the reactions involving nitrobenzenesulfenyl chlorides were best done in anhydrous polar solvents (41).

The work of these investigators appeared to be important in the preparation of intermediates of types XXXI and XXXII for a variety of reasons. First, it could be envisaged that o-nitrobenzenesulfenyl chloride could be condensed with either esters or ketones in which there
is a methylene group in the position alpha to the carbonyl group, thus making the hydrogens more acidic in nature and more reactive. Secondly, a similar reaction should occur more readily when o-nitrobenzenesulfenyl chloride is reacted with compounds containing both an ester and keto group which are separated by a methylene group. For example, o-nitrobenzenesulfenyl chloride could be condensed with ethyl benzoylacetate to give ethyl α-benzoyl-α(α-nitrophenylthio)acetate (XXXIIe).

\[
\begin{align*}
\text{Ph} & \quad \text{CH}_2 \\
\text{N} & \quad \text{C-Ph} \\
\text{C} & \quad \text{OEt} \\
\text{NO}_2 & \quad \text{S-CH} \\
\text{C} & \quad \text{OEt}
\end{align*}
\]

XXXIIe

This product contains an ester group, suitably orientated with respect to the nitro group, so that upon reductive cyclization a cyclic hydroxamic acid (XXXIII k) was a possible product. The keto portion of the benzoyl group is reduced to a secondary alcohol. As mentioned previously, reductive cyclization using sodium borohydride and palladium charcoal system, occurs by initial reduction of the nitro group to the hydroxyamino-stage. An adjacent ester or keto group, if suitably orientated, would react to form the hydroxamate or N-oxide respectively. The intermediate (XXXII e) also contains a suitably situated keto group, so that the
formation of the N-oxide product XXXIX might also be expected.

Thus, by reacting o-nitrobenzenesulfonyl chloride with suitable esters and ketones, \(-\alpha\)-(o-nitrophenylthio) ester and ketone intermediates were prepared in the hope that reductive cyclization would give rise to cyclic hydroxamic acids and N-oxide derivatives.

Following the method of Barltrop and Morgan (43), the following compounds (XXXII a - XXXII e, table III) were prepared by condensing o-nitrobenzenesulfonyl chloride with acetone, acetophenone, 2,4-pentadione, ethyl acetoacetate, and ethyl benzoylacetate respectively.

\textbf{Table III: \(-\alpha\)-(o-Nitrophenylthio) Ketones.}
Because of the success achieved using this reaction, it was anticipated that o-nitrobenzenesulfenyl chloride should similarly condense with other systems which contain an active methylene group in the position alpha to the carbonyl. To investigate this possibility, o-nitrobenzenesulfenyl chloride was condensed with 3-methyl-1-phenyl-2-pyrazolin-5-one (XL) to give 3-methyl-4-(o-nitrophenylthio)-1-phenyl-2-pyrazolin-5-one (XLI).

The infrared spectrum of XLI indicated the presence of a nitro group as expected, but there was no carbonyl absorption; instead, there was present a strongly chelated hydroxyl group, which would suggest an enol structure. In addition,
XLI was soluble in sodium hydroxide solution and reprecipitated on making acid. Such properties are consistent with the compound being in the enol (XLI a) form in the solid state. Elemental analysis of the product (XLI) indicated the structure to be correct.

This method of preparing intermediates of types XXXI and XXXII, using o-nitrobenzenesulfonyl chloride gave good yields of product. A wide variety of products are possible. Further products might be achieved by using substituted o-nitrobenzenesulfonyl chloride derivatives. Indeed, Bartrop and Morgan (43) have done similar reactions using 2,4-dinitrobenzenesulfonyl chloride.

Two reactions, which did not occur in the expected manner, were the attempted condensations of o-nitrobenzenesulfonyl chloride with ethyl chloroacetate and pyruvic acid. The only product isolated from each of these reactions was bis(o-nitrophenyl) disulfide. Both condensations were attempted using acetonitrile as solvent. The reaction using pyruvic acid was also attempted in an aqueous ethanol solution of sodium bicarbonate. Bis(o-nitrophenyl) disulfide was the
product. These reactions should be investigated further using different solvent systems.
Reduction of Intermediates of Types XXXI and XXXII.

Reductive cyclization on the intermediates of types XXXI and XXXII was attempted using sodium borohydride and palladium (10%)-on-charcoal catalyst. Except for minor differences in reduction and quantities of solvent used, all the reductions were carried out in a similar manner. In general, sodium borohydride (0.011 mole) and palladium charcoal (0.050 g.) were used to reduce 1 gram of the intermediate. In all cases, the mixture was basic during reduction, and effervesced upon the addition of acid at the completion of the reduction, indicating the presence of excess sodium borohydride.

The reductions involving intermediates of type XXXI proceeded as expected and cyclic hydroxamic acids of type XXXIII were obtained in good yields. All the cyclic hydroxamic acids prepared were soluble in sodium carbonate solution, and gave a purple color with ferric chloride solution.

Table IV: Cyclic Hydroxamic Acids.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure XXXIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine</td>
<td>H</td>
</tr>
<tr>
<td>b) 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine</td>
<td>H</td>
</tr>
<tr>
<td>c) methyl (3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate</td>
<td>H</td>
</tr>
<tr>
<td>d) methyl (6-bromo-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate</td>
<td>Br</td>
</tr>
<tr>
<td>e) 6-bromo-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine</td>
<td>Br</td>
</tr>
<tr>
<td>f) methyl (3,4-dihydro-4-hydroxy-6-methyl-3-oxo-2H-1,4-benzothiazinyl)acetate</td>
<td>Me</td>
</tr>
<tr>
<td>g) 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzothiazine</td>
<td>Me</td>
</tr>
<tr>
<td>h) methyl (6-trifluoromethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate</td>
<td>CF$_3$</td>
</tr>
<tr>
<td>i) 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine</td>
<td>CF$_3$</td>
</tr>
<tr>
<td>j) 1-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)ethanol</td>
<td>H</td>
</tr>
<tr>
<td>k) α-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)benzyl alcohol</td>
<td>H</td>
</tr>
</tbody>
</table>
The reduction time was found to be significant in determining the yields of the products. Illustrative of this was the reduction of methyl α-(4-trifluoromethyl-2-nitrophenoxythio)propionate (XXXI h). When reduced over a period of 30 minutes, a 60% yield of 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H,1,4-benzothiazine (XXXIII i) was obtained; an increased reduction time of 60 minutes (i.e. a slower addition of the intermediate to the reduction mixture) resulted in a yield of 34%. As the importance of the reduction time was not discovered until experimental investigations had almost terminated, the majority of the reductions were done over a period of 30 minutes.

The reduction of diethyl bis(o-nitrophenythio)malonate (XXXV) gave an unexpected final product of bis(o-nitrophenyl) disulfide (XXXIV). The portion of the reduction mixture, which was soluble in sodium carbonate solution, was an oil and gave a purple color with ferric chloride solution, indicating the presence of a cyclic hydroxamic acid. However, on standing, the oil slowly solidified to yield a yellow solid, which was identified as the disulfide (XXXIV).

This interesting reaction was not investigated further except to confirm the purity of the starting material. It may be that partial reduction occurred only (hence the positive ferric chloride test) and the disulfide (XXXIV) slowly formed by decomposition of the malonate (XXXV).
In the case of ethyl \( \alpha-(\text{o-nitrophenylthio}) \text{acetoacetate} \) (XXXII d) and ethyl \( \alpha \)-benzoyl-\( \alpha-(\text{o-nitrophenylthio}) \text{acetoacetate} \) (XXXII e), two reduction products were theoretically possible. A hydroxamic acid could result if the ester group cyclized with the resulting hydroxyamino-group. Alternatively, the ketone group might be expected to cyclize with the hydroxyamino-group to yield a cyclic N-oxide derivative. An examination of the yields of the two reductions indicates that the formation of the cyclic hydroxamate is about \( \frac{1}{2} \) that of the other product.

The ketone carbonyl groups of compounds XXXIIId and XXIIie appear to have been reduced to secondary alcohols in the cyclized products (XXXIII). This was indicated when the ferrous chelate of \( \alpha-(3,4\)-dihydro-4-hydroxy-3-oxo-2H-1,8-benzothiaziny1)benzyl alcohol (XXXII k) was prepared, and its infrared spectrum examined. The carbonyl band of the hydroxamate (XXXII k) was depressed from 1640 cm\(^{-1}\) to 1535 cm\(^{-1}\) in the chelated form. Since there were no other bands ascribable to a carbonyl group in the spectrum of XXXIIIk, the benzoyl group of ethyl \( \alpha \)-benzoyl-\( \alpha-(\text{o-nitrophenylthio}) \text{acetate} \) appears to have been reduced during reductive cyclization to a secondary alcohol. Depression of the carbonyl band between a cyclic hydroxamate and its chelate was observed by MacDonald (14) in his investigation of pulcherriminic acid and its chelate product, pulcherrimin.
As was mentioned earlier, reduction of intermediates of type XXXII was expected to yield cyclic N-oxides of type XXX. This appeared to be a correct assumption for two reasons. First, the hydroxyamino group is known to react with keto groups to form N-oxides. Secondly, this type of reaction has been reported previously when 4-hydroxy-2-methylquinoline-1-oxide was obtained by the reduction of o-nitrobenzoylacetonone using sodium borohydride and palladium charcoal catalyst (32).

However, it was soon evident that reduction of intermediates of type XXXII had not occurred as expected and the products were not the expected N-oxide derivatives. Five compounds of type XXXII were reduced with sodium borohydride and palladium charcoal. The infrared spectra of the reduction products of o-nitrophenylthiopropan-2-one (XXXII a), \(\omega\)-o-nitrophenylthioacetophenone (XXXII b) and 3-o-nitrophenoxyphenylthio)pentan-2,4-dione (XXXII c) indicated the presence of a nitro group, the absence of a keto group and the presence of a hydroxyl group. A comparison of the infrared spectra of these \(\alpha\)-o-nitrophenoxyketones and their reduced products showed that the nitro group had not been involved in the reduction; only the keto group was reduced to an alcohol. The reduction of a ketone group to an alcohol would have been the expected reaction if sodium borohydride had been used in the absence of palladium charcoal catalyst. This would suggest that the palladium charcoal catalyst
was in some way poisoned by the \(\alpha\)-(o-nitrophenylthio)-ketones or by the initial reduction product of the reduction.

The other two ketones of type XXXII that were reduced were ethyl \(\alpha\)-(o-nitrophenylthio)acetoacetate (XXXII d) and ethyl \(\alpha\)-benzoyl-\(\alpha\)-(o-nitrophenylthio)acetate (XXXII e), each of which gave the expected reduction products, the cyclic hydroxamic acids, 1-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)ethanol (XXXIII j) and \(\alpha\)-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)benzyl alcohol (XXXIII k), respectively. No N-oxides were isolated. In contrast, reduction of ethyl (o-nitrophenylthio)acetoacetate also gave a product shown by infrared study to no longer possess a nitro group, though the ketone was reduced to the alcohol. Similarly, reduction of the related ethyl \(\alpha\)-benzoyl-\(\alpha\)-(o-nitrophenylthio)acetate yielded a product which showed no strong absorption in the 1500-1560 cm\(^{-1}\) regions indicating in this case reduction of the nitro group (as well as the ketone group) had occurred. This reduction requires further investigation.

The infrared spectra of ethyl \(\alpha\)-(o-nitrophenylthio)acetoacetate (XXXII d) and ethyl \(\alpha\)-benzoyl-\(\alpha\)-(o-nitrophenylthio)acetate (XXXII e) indicated that these two \(\beta\)-keto esters exist in the enolic form. The infrared spectra showed no absorption in the 1700 cm\(^{-1}\) region; rather a peak around the 1600 to 1625 cm\(^{-1}\) region was ascribable
to an ester carbonyl group that is depressed due to conjugation with an enolic double bond and to chelation of the enolic hydroxyl group.

\[
\begin{align*}
\text{Reduction of } &\text{o-nitrophenylthiopropan-2-one (XXX a), } \\
&\text{ω-(o-nitrophenylthio)acetophenone (XXXII b) and } 3-(\text{o-nitrophenylthio})\text{pentan-2,4-dione (XXXII c) gave the alcohols XLII a, XLII b, and XLII c, respectively.}
\end{align*}
\]

Table IV: Substituted 2-(o-Nitrophenylthio)Alcohols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure XLII</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 1-methyl-2-(o-nitrophenylthio)ethanol</td>
<td>R = H, R' = Me</td>
</tr>
<tr>
<td>b) 2-(o-nitrophenylthio)-1-phenylethanol</td>
<td>R = H, R' = Ph</td>
</tr>
<tr>
<td>c) 3-(o-nitrophenylthio)pentan-2,4-diol</td>
<td>R = CHOHMe, R' = Me</td>
</tr>
</tbody>
</table>
The 2-(o-nitrophenylthio)alcohols, XLII a, b, and c were examined in some detail in order to prove their structure. A study of the infrared spectra of the 2-(o-nitrophenylthio)ketones XXXII a, b, and c and these reduced products XLII (see table V) revealed that the nitro groups had apparently not been affected by the reduction. Only the keto group was reduced as indicated by the appearance of a hydroxyl group in the spectrum of the reduced product.

Table V: Infrared Spectra of 2-(o-Nitrophenylthio)Ketones and Their Derivatives.
The p-nitrobenzoates (XLIII) of compounds XLII a, b, and c were prepared in order to further prove the presence of an alcohol group in the reduced compounds. Hydroxyl absorption was absent from the infrared spectra of these derivatives, as expected, having been replaced by an absorption band ascribable to an ester carbonyl group (see table V).

A product typical of this series of reductions was 2-(o-nitrophenylthio)-1-phenylethanol (XLII b). The structure of this product was confirmed by elemental analysis and infrared spectrum studies. 2-(o-Nitrophenylthio)-1-phenylethanol was also obtained when (o)-(o-nitrophenylthio)acetophenone (XXXII b) was reduced with sodium borohydride alone. The fact that the same product resulted with sodium borohydride in the presence and absence of palladium charcoal catalyst, would tend to confirm the idea of catalyst poisoning.

The compounds obtained from the reduction of XXXII a and c were not analysed, but evidence from infrared
absorption studies as described in table V indicates that they too were 2-(o-nitrophenylthio)alcohols.

The reduction of 3-methyl-4-(o-nitrophenylthio)-1-phenyl-2-pyrazolin-5-one (XL\textsubscript{I}) was of interest because of the proximity of the amide carbonyl group to the nitro group. It was anticipated that reduction, accompanied by cyclization, might occur. Reduction with sodium borohydride and palladium charcoal catalyst gave rise to a compound which analysed correctly for C\textsubscript{16}H\textsubscript{15}N\textsubscript{3}OS. Examination of the infrared spectrum of the product indicated that reductive cyclization had occurred and 9,9a-dihydro-9-hydroxy-3-methyl-1-phenyl-1H-pyrazolo-[4,3-b](1,4)benzothiazine (XL\textsubscript{IV}), was the product. The infrared spectra indicated the absence of a nitro group or an amino group, the absence of a carbonyl group, the presence of a hydroxyl group and a band at 1590 cm\textsuperscript{-1}, ascribed to \textsuperscript{1}C=N-grouping (\textsubscript{44}).
The compound (XLIV) was acidic in nature, being soluble in aqueous sodium hydroxide solution and re-precipitated on making acid. The reduced product was only slightly soluble in many of the common organic solvents, such as ethanol, chloroform, benzene, ether and petroleum. This product (XLIV) is reminiscent of the work of Coutts, Edwards and Jeffrey (45) who reduced 3-methyl-4-(o-nitrobenzylidene)-1-phenyl-2-pyrazoline-5-one (XLV) with sodium borohydride and palladium charcoal catalyst, and obtained 3a,4,9,9a-tetrahydro-3-methyl-1-phenyl-1H-pyrazolo-[3,4-b]quinoline (XLVI).

![Chemical structure](image)

The ability of hydroxamic acids to form chelates was used to characterize a product isolated as a viscous oil. The cyclic hydroxamate $\alpha$-(3,4-dihydro-4-hydroxy-3-
oxo-2H-1,4-benzothiazinyl)benzyl alcohol (XXXIII k) was an oil, and because it was available in a very small quantity, the formation of the ferrous chelate was attempted in anticipation that the product would be a solid substance, suitable for elemental analysis. This method of isolating cyclic hydroxamates by chelate formation was used by MacDonald (14), in his work with pulcherriminic acid. The chelate of α-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)benzyl alcohol (XXXIII k) was prepared by adding ferrous chloride to a solution of the hydroxamate in glacial acetic acid. The mixture was flooded with water, precipitating the ferrous chelate, which was purified by washing alternatively with sodium carbonate solution, drying, and washing with petroleum ether. Elemental analysis of the chelate indicated the structure, α-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)benzyl alcohol, to be correct. The ferrous chelate of 1-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)ethanol was also prepared. It could not be purified, and therefore was considered unsuitable for characterization.

Attempts were made to prepare other chelates by reacting zinc chloride and lead acetate with known hydroxamic acids. These chelates did not form well and are therefore unsuitable derivatives for characterization purposes.
Methyl 2-(o-nitrophenylthio)benzoate (XLVII) was easily prepared in good yield by condensing o-chloronitrobenzene and sodium (o-methoxycarbonyl)thiophenolate. It was anticipated that reduction of this product (XLVII) with sodium borohydride and palladium charcoal would produce a cyclic hydroxamic acid with a seven-membered ring system. The reduction product was neither an N-hydroxy compound nor a simple N-oxy compound, but was an azoxy compound, 2,2'-bis[(o-methoxycarbonyl)phenylthio]azoxybenzene (XLVIII).

When sodium (o-methoxycarbonyl)thiophenolate was reduced with zinc and ammonium chloride, the azoxy compound was also isolated, but in a poor yield.

The azoxy structure was assigned to the compound
for the following reasons: elemental analysis and a molecular weight determination indicated the molecular formula \( C_{28}H_{22}N_{2}O_{2}S_{2} \); the infrared spectrum of the starting material, sodium (o-methoxycarbonyl)thiophenolate, showed a strong nitro group absorption, which was absent from the reduced product (XLVIII), having been replaced by a strong peak at 1474 cm\(^{-1}\). This peak, and another at 1300 cm\(^{-1}\) are characteristic of azoxy groups (16). The presence of the ester group was indicated by a strong peak at 1705 cm\(^{-1}\). Another factor which tended to substantiate the presence of an azoxy group was the bright yellow colour of the product, similar to that of azoxybenzene itself.

Reduction of methyl 2-(o-nitrophenylthio)benzoate using sodium borohydride and palladium charcoal was also carried out in an ethanol-methanol mixture rather than dioxan. The product of this reduction was obtained in a much poorer yield. The infrared spectrum of this compound was identical to that of the azoxy compound (XLVIII) prepared using dioxan as a solvent.

As was mentioned in the Introduction, there was good evidence to indicate that the 2H-1,4-benzothiazine derivatives which contain a cyclic hydroxamate group would have some anti-microbial activity and anti-enzymatic activity. For these reasons, a number of new cyclic hydroxamic acids mentioned in this thesis were tested, in order to evaluate their anti-bacterial properties.
The bacterial testing was carried out under the direction of Dr. A.H. Holden, Department of Bacteriology, University Hospital, Saskatoon, Saskatchewan.

The cyclic hydroxamic acids were screened at a concentration of 40 mg/ml against Staphylococcus aureus, a Gram positive organism, and Escherichia coli, a Gram negative organism. None of the cyclic hydroxamic acids prepared in this project was active against the organisms at this concentration.

A number of cyclic hydroxamic acids prepared in this laboratory have been tested at the Food and Drug Directorate, Ottawa, through the kindness of Dr. W.J. Johnson, for their action on selected enzymes. Included in the screening were the compounds 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (XXXIII b), methyl (6-bromo-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate (XXXIII d), 3,4-dihydro-4-hydroxy-3-oxo-1,4-benzothiazine (32), and 3,4-dihydro-4-hydroxy-3-oxo-1,4-benzothiazine-1,1-dioxide (32). These compounds were tested as reactivators of difluorophosphate (DFP) inhibited acetylcholinesterase. It was found that their potencies ranged from 0.44 to 2.25 times the activity of pyridine-2-aldoxime methiodide (PAM), a well known reactivator of organophosphate-inhibited cholinesterase, and an effective antidote against insecticide poisoning. While PAM suffers
from the defect of being itself an inhibitor of cholinesterase, two of these new compounds failed to inhibit the enzyme at the concentration required for reactivation, and the remainder were less inhibitory than PAM.
III. EXPERIMENTAL

Notes

All temperatures are in centigrade units and were recorded on a thermometer which was previously calibrated using appropriate reference compounds. The infrared spectra were recorded on either a Beckman IR-8 Infrared Spectrophotometer or a Perkin Elmer, Model 21, Recording Spectrophotometer. Elemental analysis was performed by Drs. F. Pascher and E. Pascher, Bonn, West Germany. The molecular weight determinations were obtained by vapour pressure measurements. Neutralization equivalents were determined by direct titration of the acid in aqueous methanol using standard sodium hydroxide solution, with phenolphthalein as indicator. The product yields were calculated on the basis of the weight of the crude product.
Preparation of Intermediates of Types XXXI and XXXII

Method I; Condensation of sodium \(\alpha\)-nitrothiophenolate and suitable \(\alpha\)-bromoesters.

1) Ethyl \(\alpha\)-(\(\alpha\)-nitrophenylthio)butyrate (XXXI; R=H, R'=R''=Et)

\(\alpha\)-Nitrothiophenol (3.9 g) was dissolved in an aqueous ethanolic solution of sodium hydroxide \([\text{NaOH} (1 \text{ g}), \text{H}_2\text{O} (2 \text{ ml}), \text{ethanol} (18 \text{ ml})]\). Ethyl 2-bromobutyrate (5.1 g) in ethanol (15 ml) was added to the red colored alkaline solution and the mixture was stirred for about one hour. A precipitate of bis (\(\alpha\)-nitrophenyl)disulfide was filtered off. The filtrate was evaporated down to about quarter volume. A precipitate of bis(\(\alpha\)-nitrophenyl) disulfide and sodium bromide was filtered off; then the filtrate was flooded with water and extracted twice with ether. The ether extract was dried (\(\text{Na}_2\text{SO}_4\)), and evaporated to yield an orange oil, ethyl \(\alpha\)-(\(\alpha\)-nitrophenylthio)butyrate (37%), b.p. 195-205\(^\circ\) at 25 mm.

\(\nu\) max 1723 (C=O), 1510 and 1335 cm\(^{-1}\) (NO\(_2\)).

Found: C, 53.21; H, 5.89; N, 4.96; S, 12.07.

Calc. for \(\text{C}_1\text{2H}_\text{15N}_\text{2O}_\text{5S}\): C, 53.52; H, 5.61; N, 5.20; S, 11.91.

The other product of the reaction, bis(\(\alpha\)-nitrophenyl) disulfide (36%), m.p. 191-195\(^\circ\)-reported m.p. 192-195\(^\circ\) (47), was isolated. The infrared spectrum of this compound was identical to the infrared spectrum of an authentic sample.
ii) Ethyl \((\alpha\text{-nitrophenylthio})\)propionate (XXXI; \(R = H, R' = Me, R'' = Et\))

Bis(\(\alpha\text{-nitrophenyl}\)) disulfide (10 g) was converted to sodium \(\alpha\text{-nitrothiophenolate}\), as reported by Claass (33), and the red solution was filtered. Ethyl 2-bromopropionate (12.2 g) was added to the filtrate and shaken. The mixture was extracted with ether, the ether layer was dried (MgSO\(_4\)), then the ether and excess ethyl 2-bromopropionate were fractionally distilled off prior to the oil, ethyl \((\alpha\text{-nitrophenylthio})\)propionate (21%), which boiled at 184-188° at 10-15 mm.

\(\nu\) max 1735 (C = O), 1510 and 1320 cm\(^{-1}\) (NO\(_2\)).

Found: C, 51.82; H, 4.86; N, 5.67; S, 12.38.
Calc. for C\(_{11}\)H\(_{13}\)NO\(_5\)S: C, 51.71; H, 5.13; N, 5.52; S, 12.55.

iii) Attempted condensation of sodium \(\alpha\text{-nitrothiophenolate}\) and diethyl dibromomalonate

a) Reaction in aqueous ethanol solution

\(\alpha\text{-Nitrothiophenol}\) (3.9 g) was dissolved in a solution of sodium hydroxide (1.0 g), water (2 ml), and ethanol (18 ml). Diethyl dibromomalonate (3.9 g) was added to this red solution and stirred. A yellow precipitate occurred immediately which was identified as bis(\(\alpha\text{-nitrophenyl}\)) disulfide (3.5 g), m.p. 192-196°. The infrared spectrum of this product was identical to an authentic sample. After concentrating and cooling the filtrate, diethyl bis(\(\alpha\text{-nitrophenylthio})\)malonate (2.9%) was precipitated,
m.p. 101-102° (methanol), as a yellow solid.

$\nu$ max 1775 (C=O); 1555, 1355, 855 cm$^{-1}$ (NO$_2$).

Found: C, 48.72; H, 4.05; N, 5.84; S, 13.69; mol. wt. 464.

Calc. as C$_{19}$H$_8$N$_2$O$_8$S$_2$: C, 48.92; H, 3.86; N, 6.01; S, 13.73; mol. wt. 466.5.

b) Reaction in anhydrous ethanolic solution

$\alpha$-Nitrothiophenol (3.0 g) was dissolved in a solution of sodium ethoxide in absolute ethanol sodium (0.8 g) in absolute ethanol (75 ml). Diethyl dibromo-
malonate (3.1 g) was added to the solution of sodium
$\alpha$-nitrothiophenolate and stirred for 15 minutes. A precipit-
ate of sodium bromide and bis($\alpha$-nitrophenyl) disulfide was
filtered off and the filtrate concentrated to about half
volume, when more disulfide and sodium bromide were removed.
The filtrate was flooded with water and extracted twice
with ether. The ether extract was dried (Na$_2$SO$_4$) and evap-
orated to a brown oil, which upon trituration with ethanol
yielded a yellow solid, diethyl bis($\alpha$-nitrophenylthio)-
malonate (10%), m.p. 101-102° (from methanol). The infra-
red spectrum of this product was the same as that obtained in iii a) (above). The other product of the
reaction was bis($\alpha$-nitrophenyl) disulfide (67%), m.p.
192-198° (crude). The infrared spectrum of this product
was identical to the spectrum of an authentic sample.
iv) Condensation of sodium o-nitrothiophenolate and diethyl bromomalonate

\[ \text{o-Nitrothiophenol (3.9 g) was added to a solution of sodium hydroxide (1.0 g) in water (4 ml) and ethanol (80 ml). Diethyl bromomalonate (6.0 g) was added, with stirring, to this solution which caused an immediate precipitation of bis(o-nitrophenyl) disulfide (2.7 g), m.p. 192-196°. The infrared spectrum of this product was identical with that of an authentic sample. After standing overnight, a yellow precipitate was isolated, diethyl bis(o-nitrophenylthio)malonate (XXXV) (0.59 g), m.p. 101-102° (from methanol).} \]

\[ \text{\textit{j) max 1775 (C = O); 1555, 1355, 855 cm}^{-1} (\text{NO}_2).} \]

v) Attempted synthesis of 2-(o-nitrophenylthio)benzaldehyde

\[ \text{A solution of 2-chlorobenzaldehyde (1.4 g) in 50% aqueous ethanol (10 ml) was added to a solution of o-nitrothiophenol (1.6 g) in 50% aqueous ethanol (10 ml) which contained sodium hydroxide (0.4 g). After standing for four days, a yellow solid was filtered off and found to be bis(o-nitrophenyl) disulfide (0.8 g), m.p. 183-190° (not purified). The infrared spectrum of the product was identical to a known sample of bis(o-nitrophenyl) disulfide. No other product was isolated.} \]
Method II: Condensation of substituted o-chloro- (or bromo-)nitrobenzene with suitable \(\delta\)-mercapto acids.

General Method

The substituted o-chloro- (or bromo-)nitrobenzene (1/20 mole) and the appropriate \(\delta\)-mercapto acid (1/20 mole) were refluxed for 18 to 24 hours in 50% aqueous ethanol (80 ml), in which was suspended an excess of sodium bicarbonate. Approximately 20 milliliters of ethanol were distilled off before the basic mixture was flooded with water and extracted twice with ether. The basic aqueous mixture was acidified with hydrochloric acid. The precipitate which formed was filtered off and washed with aqueous methanol.

i) \(\delta\)-(o-Nitrophenylthio)succinic acid (XXXVI a)

Using the general method, 2-chloronitrobenzene (7.9 g) and mercaptosuccinic acid (7.5 g) were condensed to give \(\delta\)-(o-nitrophenylthio)succinic acid (54%) as a yellow solid, m.p. 187-189° (from water).

\(\nu\) max 3000 (OH), 1695 (C = O), 1515 and 1345 cm\(^{-1}\) (NO\(_2\)).

Found: C, 44.21; H, 3.39; eq. wt. 140.
Calc. for C\(_{10}\)H\(_9\)NO\(_6\)S: C, 44.28; H, 3.34; eq. wt. 135.6.

ii) \(\delta\)-(4-Bromo-2-nitrophenylthio)succinic acid (XXXVI c)

Using the general method, 2,5-dibromonitrobenzene
(13.9 g) and mercaptosuccinic acid (7.5 g) were condensed to give \(\alpha\)-(4-bromo-2-nitrophenylthio)succinic acid (69%) as a yellow solid, m.p. 218-220° (from ethanol).

\[\text{max } 3000 \text{ (OH), 1710 } (C = 0), 1528 \text{ and } 1345 \text{ cm}^{-1} (\text{NO}_2).\]

Found: C, 34.62; H, 2.26; eq.wt. 176.
Calc. for C\(_{10}\)H\(_8\)BrN\(_6\)O\(_6\): C, 34.30; H, 2.29; eq.wt. 175.0.

(iii) \(\alpha\)-(4-Methyl-2-nitrophenylthio)succinic acid (XXXVI e)
Using the general method, 4-chloro-3-nitrotoluene (8.5 g) and mercaptosuccinic acid (7.5 g) were condensed to give \(\alpha\)-(4-methyl-2-nitrophenylthio)succinic acid (28%) as a yellow solid, m.p. 215-218° (from ethanol).

\[\text{max } 3100 \text{ to } 2750 \text{ (OH), 1700 } (C = 0), 1530 \text{ and } 1340 \text{ cm}^{-1} (\text{NO}_2).\]

Found: C, 46.32; H, 3.86; eq.wt. 150.
Calc. for C\(_{11}\)H\(_{11}\)N\(_6\)O\(_6\): C, 46.32; H, 3.86; eq.wt. 142.6.

(iv) \(\alpha\)-(4-Trifluoromethyl-2-nitrophenylthio)succinic acid (XXXVI g)
Using the general method, 2-chloro-5-trifluoromethylnitrobenzene (10.9 g) and mercaptosuccinic acid (7.5 g) were condensed to give \(\alpha\)-(4-trifluoromethyl-2-nitrophenylthio)succinic acid (60%) as a pale brown solid, m.p. 196-198° (from 50% aqueous ethanol).

\[\text{max } 3180 \text{ to } 2740 \text{ (OH), 1715 } (C = 0), 1540 \text{ and } 1340 \text{ cm}^{-1} (\text{NO}_2).\]

Found: C, 39.32; H, 2.58; eq.wt. 174.
Calc. for C\(_{11}\)H\(_8\)F\(_3\)N\(_6\)O\(_6\): C, 39.94; H, 2.38; eq.wt. 169.6.
(v) $\alpha$-(2-Nitrophenylthio)propionic acid (XXXVI b)

Using the general method, 2-chloronitrobenzene
(7.9 g) and $\alpha$-mercaptopropionic acid (5.3 g) were con-
densed to give $\alpha$-(2-nitrophenylthio)propionic acid (76%)
as a yellow solid, m.p. 107-109° (from aqueous ethanol).

$\nu$ max 3100 to 2780 (OH), 1710 (C = O), 1505 and 1328
em$^{-1}$ (NO$_2$).

Found : C, 47.71; H, 4.12; eq.wt. 224.
Calc. for C$_9$H$_7$NO$_4$S : C, 47.56; H, 3.99; eq.wt. 227.3.

(vi) $\alpha$-(4-Bromo-2-nitrophenylthio)propionic acid
(XXXVI d)

Using the general method, 2,5-dibromonitrobenzene
(13.9 g) and $\alpha$-mercaptopropionic acid (5.3 g) were con-
densed to give $\alpha$-(4-bromo-2-nitrophenylthio)propionic acid
(65%) as a yellow solid, m.p. 149-150° (from aqueous
ethanol).

$\nu$ max 3200 to 2800 (OH), 1695 (C = O), 1529 and 1337
em$^{-1}$ (NO$_2$).

Found : C, 35.36; H, 2.53; eq.wt. 310.
Calc. for C$_{9}$H$_{8}$BrNO$_{4}$S : C, 35.31; H, 2.36; eq.wt. 305.9.

(vii) $\alpha$-(4-Methyl-2-nitrophenylthio)propionic acid
(XXXVI f)

Using the general method, 2-chloro-5-methyl-
nitrobenzene (8.5 g) and $\alpha$-mercaptopropionic acid (5.3 g)
were condensed to give $\alpha$-(4-methyl-2-nitrophenylthio)-
propionic acid (47%) as a yellow solid, m.p. 141-143° (from aqueous ethanol).

1) max 3200 to 2750 (OH), 1690 (C = O), 1508 and 1326 cm⁻¹ (NO₂).

Found: C, 49.71; H, 4.78; eq.wt. 244.
Calc. for C₁₀H₁₁NO₄S: C, 49.78; H, 4.60; eq.wt. 241.3.

(viii) α-(4-Trifluoromethyl-2-nitrophenylthio)propionic acid (XXXVI h)

Using the general method, 2-chloro-5-trifluoromethylnitrobenzene (10.9 g) and α-mercaptopropionic acid (5.3 g) were condensed to give α-(4-trifluoromethyl-2-(α-nitrophenylthio)propionic acid (60%) as a buff coloured solid, m.p. 196-198° (from aqueous ethanol).

1) max 3115 to 3080 (OH), 1720 (C = O), 1520 and 1331 cm⁻¹ (NO₂).

Found: C, 40.82; H, 2.93; eq.wt. 287.
Calc. for C₁₀H₆F₃NO₄S: C, 40.68; H, 2.73; eq.wt. 295.3.

Preparation of methyl α-(α-nitrophenylthio)esters

The α-(α-nitrophenylthio)acids were esterified by the Fischer-Speier esterification procedure. The esters were not analysed prior to reduction.

General Method

The α-(α-nitrophenylthio)acid (5 g) was refluxed
in a mixture of methanol (40 ml) and sulfuric acid (4 ml) for 24 hours. The mixture was flooded with water. If a solid product occurred, it was filtered off and washed with water. If an oil resulted, the mixture was extracted twice with ether. The ethereal extract was washed with water, dried (Na$_2$SO$_4$) and evaporated.

(i) Dimethyl \(\alpha\)-(o-nitrophenylthio)succinate (XXXI a)

Using the general method, the acid (XXXVI a) was esterified to yield this ester as an orange oil, in 89% yield.

(ii) Dimethyl \(\alpha\)-(4-bromo-2-nitrophenylthio)succinate (XXXI c)

Using the general method, the acid (XXXVI c) was esterified to yield this ester as a yellow crystalline solid, m.p. 70-72\(^\circ\) (from ethanol) in an 89% yield.

(iii) Dimethyl \(\alpha\)-(4-methyl-2-nitrophenylthio)succinate (XXXI e)

Using the general method, the acid (XXXVI e) was esterified to yield this ester as an orange oil in a 70% yield.

(iv) Dimethyl \(\alpha\)-(4-trifluoromethyl-2-nitrophenylthio)-succinate (XXXI g)

Using the general method, the acid (XXXVI g) was esterified to yield this ester as a brown oil in 92% yield.
(v) Methyl \(-\alpha-(2\text{-nitrophenylthio})\text{propionate (XXXI b)\}

Using the general method, the acid (XXXVI b) was
esterified to yield this ester as a yellow solid, m.p. 54°
(from ethanol) in 83% yield.

(vi) Methyl \(-\alpha-(4\text{-bromo-2-nitrophenylthio})\text{propionate (XXXI d)\}

Using the general method, the acid (XXXVI d) was
esterified to yield this ester as a yellow solid, m.p. 62-
64° (from ethanol) in 95% yield.

(vii) Methyl \(-\alpha-(4\text{-methyl-2-nitrophenylthio})\text{propionate (XXXI f)\}

Using the general method, the acid (XXXVI f) was
esterified to yield this ester as a yellow solid, m.p. 42-
43° (from aqueous ethanol) in 90% yield.

(viii) Methyl \(-\alpha-(4\text{-trifluoromethyl-2-nitrophenylthio})\text{propionate (XXXI h)\}

Using the general method, the acid (XXXVI h) was
esterified to yield this ester as a yellow solid, m.p.
79-80° (from aqueous methanol) in 93% yield.
Method III: Condensation of o-nitrobenzenesulfonyl chloride and suitable keto compounds (43)

General Method

o-Nitrobenzenesulfonyl chloride, prepared according to the procedure described in Organic Synthesis (48), and the appropriate keto compound (an excessive amount) were refluxed either alone or in acetonitrile (40 ml) for 2.5 to 5 hours. If acetonitrile was used as solvent, about 30 millilitres of it was distilled off. The product precipitated when the mixture was cooled, and was filtered off and washed with cold methanol. The infrared spectra of some of the products are described in table V.

(i) o-Nitrophenylthiopropan-2-one (XXXII a)

Using the general method, o-nitrobenzenesulfonyl chloride (5 g) and acetone (10 ml) were condensed to give o-nitrophenylthiopropan-2-one (37%) as a yellow solid, m.p. 75–77° (from ethanol) — reported m.p. 81° (43). The positions of the main infrared peaks of this compound are recorded in table V.

(ii) ∞ -(o-Nitrophenylthio)acetophenone (XXXII b)

Using the general method, o-nitrobenzenesulfonyl chloride (5.0 g) and acetophenone (3.0 g) in acetonitrile (50 ml) were condensed to give ∞ -(o-nitrophenylthio)-acetophenone (85%) as a yellow solid, m.p. 143–146° (from
ethanol) - reported m.p. 147° (43). The positions of the main infrared peaks of this compound are recorded in table V.

(iii) 3-(o-Nitrophenylthio)pentan-2,4-dione (XXXII c)

Using the general method, o-nitrobenzenesulfonyl chloride (5.0 g) and 2,4-pentadione (10.0 g) in acetonitrile (40 ml) were condensed to give this ketone as a yellow solid (64%), m.p. 134-136° (from ethanol) - reported m.p. 136-137° (43). The positions of the main infrared peaks of this compound are recorded in table V.

(iv) Ethyl \( \alpha \)-(o-nitrophenylthio)acetoacetate (XXXII d)

Using the general method, o-nitrobenzenesulfenyl chloride (5.0 g) and ethyl acetoacetate (10.0 g) in acetonitrile (40 ml) were condensed to give this keto ester as a yellow solid (51%), m.p. 75-76° (from ethanol) - reported m.p. 74-75° (43).

\( \nu \) max 3340 (OH), 1628 (C = O, ester), 1505, 1330 cm\(^{-1}\) (NO\(_2\)).

(v) Ethyl \( \alpha \)-benzoyl-\( \alpha \)-(o-nitrophenylthio)acetate (XXXII e)

Using the general method, o-nitrobenzenesulfenyl chloride (5.0 g) and ethyl benzoylacetate (10.0 g) in acetonitrile (40 ml) were condensed to give this keto ester as a yellow solid (61%), m.p. 123-125° (from ethanol).

\( \nu \) max 1600 (C = O, ester), 1505, 1330 cm\(^{-1}\) (NO\(_2\)).

Found: C, 59.25; H, 4.40; N, 4.00; S, 9.34.

Calc. for C\(_{17}\)H\(_{15}\)NO\(_5\)S: C, 59.12; H, 4.38; N, 4.06; S, 9.28.
(vi) 3-Methyl-4-(o-nitrophenylthio)-1-phenyl-2-pyrazolin-5-one (XL)

Using the general method, o-nitrobenzenesulfenyl chloride (5.0 g) and 3-methyl-1-phenyl-2-pyrazolin-5-one (10.0 g) in acetonitrile (40 ml) were condensed to give this product as a yellow solid (86%), m.p. 207° (from aqueous ethanol) - reported m.p. 207° (49).

\[ \text{max } 3000-2400 \text{ (chelated OH), 1589 } (C=O), 1505 \text{ and} \]
\[ 1330 \text{ cm}^{-1} (\text{NO}_2). \]

Found: C, 58.68; H, 4.20; N, 12.08; S, 10.21.
Calc. for C\(_{16}\)H\(_{13}\)N\(_3\)O\(_3\)S: C, 58.70; H, 4.00; N, 12.84; S, 9.80.

(vii) Attempted preparation of ethyl 2-chloro-2-(o-nitrophenylthio)acetate

o-Nitrobenzenesulfenyl chloride (2.0 g) and ethyl chloroacetate (5 ml) were refluxed in acetonitrile (25 ml) for 3.5 hours, after which about 15 millilitres of acetonitrile were distilled off. A yellow solid was obtained and found to be bis(o-nitrophenyl) disulfide (XXXIV) (54%), m.p. 195-199° (crude). The infrared spectrum of this product was identical to the spectrum of a known sample of bis(o-nitrophenyl) disulfide. This was the only product obtained from the reaction.

(viii) Attempted reaction of o-nitrobenzenesulfenyl chloride and pyruvic acid
(a) α-Nitrobenzenesulfenyl chloride (1.5 g) and pyruvic acid (0.8 g) were stirred in acetonitrile (20 ml) for 12 hours. The mixture was flooded with water and a yellow solid filtered off which was identified as bis(α-nitrophenyl) disulfide (XXXIV) (41%), m.p. 182° (crude). The infrared spectrum of this product was identical to the spectrum of a known sample of bis(α-nitrophenyl) disulfide. No other product was isolated from this reaction.

(b) α-Nitrobenzenesulfenyl chloride (7.8 g), pyruvic acid (4.5 g) and sodium bicarbonate (12.0 g) were refluxed in 50% aqueous ethanol (80 ml) for 4 hours. Upon cooling, a yellow solid was obtained that was identified as bis(α-nitrophenyl) disulfide (60%), m.p. 199° (crude). The infrared spectrum of this product was identical to a known sample of bis(α-nitrophenyl) disulfide. There was no other product obtained when the reaction mixture was acidified.
Reduction of Intermediates of General Formulae XXXI and XXXII

The substituted \( \alpha-(o\text{-nitrophenylthio}) \) esters (XXXI) and \( \alpha-(o\text{-nitrophenylthio}) \) ketones (XXXII) were reduced by a general method using sodium borohydride and palladium charcoal catalyst (7).

General Method

The substituted \( \alpha-(o\text{-nitrophenylthio}) \) ester or -ketone (2.0 g) was dissolved in dioxan (20–30 ml). A suspension of sodium borohydride (0.02 mole) and palladium (10%) on charcoal (0.10 g) in water (8 ml) was prepared and nitrogen bubbled through it. Dioxan, (10 ml) and 20% sodium hydroxide solution (3 drops) were added to the reducing mixture. The solution of the nitro compound was added slowly to the reducing mixture over a period of 10 minutes and the passage of nitrogen continued for a further 20 minutes. The mixture was filtered and washed with dioxan and then with water. The combined filtrate and washings were flooded with water and acidified with dilute hydrochloric acid.

If a solid hydroxamate product resulted, it was filtered off and dissolved in sodium carbonate solution (5%) or sodium hydroxide solution (10%) and filtered. This alkaline filtrate was acidified with
dilute hydrochloric acid, which reprecipitated the solid hydroxamate. This was filtered off and washed with aqueous methanol.

If an oil resulted on acidification of the filtrate of the reduction mixture, the filtrate was extracted twice with ether. The ethereal extract (A) was washed twice with water, then extracted with sodium carbonate solution (5%) or sodium hydroxide solution (10%) to extract any hydroxamate product. The basic aqueous extract was then acidified with dilute hydrochloric acid which yielded the crude hydroxamate as an oil. The oily hydroxamate was isolated by extracting the acid mixture with ether, washing the ethereal extract with water, drying it (Na$_2$SO$_4$), and evaporating the ether off to yield the hydroxamate as an oil.

If a secondary alcohol was anticipated as a product of the reduction - (i.e. reduction product of intermediate XXXII) - the ether extract corresponding to extract A above, was washed with water, dried (Na$_2$SO$_4$) and evaporated to yield the product.

(i) 2-Ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine (XXXIII a)

Using the general method, ethyl α-(o-nitrophenylthio)butyrate was reduced to give this hydroxamic
acid as a yellow solid (37%), m.p. 104-106° (from aqueous ethanol). The product gave a purple colour with ferric chloride solution.

\[ \text{max } 3240 \text{ to } 3140 \text{ (OH), } 1635 \text{ cm}^{-1} \text{ (O = O).} \]

Found : N, 6.61; S, 15.26.

Calc. for \( \text{C}_{10}\text{H}_{11}\text{NOS} \): N, 6.70; S, 15.33.

The other product obtained from the reaction was bis(o-nitrophenyl) disulfide (36%), m.p. 195-200° (crude). The disulfide precipitated out of the basic solution after reduction, and was identified by comparing its infrared spectrum with a known sample of bis(o-nitrophenyl) disulfide.

(ii) Reduction of diethyl \( \alpha, \alpha'\)-di(o-nitrophosphythio)malonate (XXXV)

Using the general method, diethyl \( \alpha, \alpha'\)-di(o-nitrophosphythio)malonate was reduced to give an orange oil (0.6 g), out of which precipitated a yellow solid identified as bis(o-nitrophenylthio) disulfide, m.p. 185-190° (crude). The infrared spectrum of the yellow solid was identical to a known sample of bis(o-nitrophenyl) disulfide. The oil gave a purple colour with ferric chloride, but was not identified.
(iii) Methyl (3,4-dihydro-4-hydroxy-3-oxo-2H,1,4-
benzothiazinyl)acetate (XXXIII c)

Using the general method, dimethyl \( \alpha \)-(o-nitro-
phenylthio)succinate was reduced to give an oil, which
upon trituration with ethanol yielded the hydroxamate
as a yellow solid (71%), m.p. 122–124° (from aqueous
ethanol). This product gave a purple colour with ferric
chloride solution.

\( \nu \) max 3340 to 3180 (OH), 1728 (C = O, ester), 1640
\( \text{cm}^{-1} \) (C = O, hydroxamate).

Found: C, 52.08; H, 4.57; N, 5.42; S, 12.72.
Calc. for C\(_{11}\)H\(_7\)NO\(_4\)S: C, 52.16; H, 4.40; N, 5.53;
S, 12.62.

(iv) Methyl (6-bromo-3,4-dihydro-4-hydroxy-3-oxo-2H-
1,4-benzothiazinyl)acetate (XXXIII d)

Using the general method, dimethyl \( \alpha \)-(4-
bromo-2-nitrophenylthio)succinate was reduced to give
this hydroxamate as a white solid (83%), m.p. 152–
154° (from aqueous ethanol). This product gave a purple
colour with ferric chloride solution.

\( \nu \) max 3310 to 3140 (OH), 1730 (C = O, ester), 1655
\( \text{cm}^{-1} \) (C = O, hydroxamate).

Found: C, 39.33; H, 2.92; N, 4.36; S, 9.66.
Calc. for C₁₁H₁₀BrNO₄S : C, 39.77; H, 3.03; N, 4.22; S, 9.75.

(v) Methyl (3,4-dihydro-4-hydroxy-6-methyl-3-oxo-2H-1,4-benzothiazinyl)acetate (XXXIII f)

Using the general method, dimethyl (4-methyl-2-nitrophenylthio)succinate was reduced to give this hydroxamate as a pale pink solid (41%), m.p. 96-97° (from ethanol). This product gave a purple colour with ferric chloride solution.

\[
\text{max} \quad 3300 \text{ to } 3100 \text{ (OH)}, \quad 1715 \text{ (C = O, ester)}, \quad 1660 \text{ cm}^{-1} \text{ (C = O, hydroxamate)}.
\]

Found : C, 54.05; H, 4.95; N, 5.53; S, 12.21.
Calc. for C₁₂H₁₃NO₄S : C, 53.92; H, 4.90; N, 5.24; S, 11.99.

(vi) Methyl (6-trifluoromethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate (XXXIII h)

Using the general method, dimethyl (4-trifluoromethyl-2-nitrophenylthio)succinate was reduced to give this hydroxamate as a white solid (29%), m.p. 122-125° (from aqueous ethanol). This product gave a purple colour with ferric chloride solution.

\[
\text{max} \quad 3310 \text{ to } 3100 \text{ (OH)}, \quad 1720 \text{ (C = O, ester)}, \quad 1670 \text{ cm}^{-1} \text{ (C = O, hydroxamate)}.
\]
Found: C, 45.07; H, 3.30; N, 4.47; S, 9.96.
Calc. for C₁₂H₁₀F₃N₂O₄S: C, 44.86; H, 3.14; N, 4.36; S, 9.98.

(vii) 3,4-Dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (XXXII b)

Using the general method, methyl α-(2-nitrophenylthio)propionate was reduced to give this hydroxamate as a white solid (33%), m.p. 149-151°C (from aqueous ethanol). This product gave a purple colour with ferric chloride solution.

!\( \text{max} \quad 3210 \text{ (OH), } 1655 \text{ cm}^{-1} (\text{C} = 0). \)

Found: C, 55.28; H, 4.47; N, 7.12; S, 16.24.
Calc. for C₉H₉NO₂S: C, 55.37; H, 4.65; N, 7.18; S, 16.42.

(viii) 6-Bromo-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (XXXII c)

Using the general method, methyl α-(4-bromo-2-nitrophenylthio)propionate was reduced to give this hydroxaminate as a white solid (41%), m.p. 157-158°C (from aqueous ethanol). This product gave a purple colour with ferric chloride solution.

!\( \text{max} \quad 3100 \text{ (OH), } 1655 \text{ cm}^{-1} (\text{C} = 0). \)
Pound :
0, 39.44; H, 2.96; N, 4.72; S, 11.49.

Calc. for C_{9}H_{8}BrNO_{2}S : O, 39.43; H, 2.94; N, 5.11; S, 11.70.

(ix) 3,4-Dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzothiazine (XXXII g)

Using the general method, methyl α-(4-methyl-2-nitrophenylthio)propionate was reduced to give this hydroxamate as a pale pink solid (72%), m.p. 135-137° (from aqueous ethanol). This product gave a purple colour with ferric chloride solution.

\[ \text{max } 3205 \text{ (OH), } 1650 \text{ cm}^{-1} \text{ (C = 0).} \]

Found : C, 57.25; H, 5.31; N, 7.24; S, 15.45.
Calc. for C_{10}H_{11}NO_{2}S : C, 57.39; H, 5.30; N, 6.69; S, 15.32.

(x) 6-Trifluoromethyl-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (XXXIII i)

Using the general method, methyl α-(4-trifluoromethyl-2-nitrophenylthio)propionate was reduced to give this hydroxamate as a white solid (60%), m.p. 126-128° (from aqueous ethanol). This product gave a purple colour with ferric chloride solution.

\[ \text{max } 3310 \text{ to } 3010 \text{ (OH), } 1655 \text{ cm}^{-1} \text{ (C = 0).} \]
Found: C, 45.77; H, 4.12; N, 5.11; S, 12.45.
Calc. for C₁₀H₈F₃N₂O₈: C, 45.63; H, 3.16; N, 5.32; S, 12.16.

The reduction of methyl α-(4-trifluoromethyl-2-nitrophenylthio)propionate was repeated in a similar manner, except that the reduction time was extended to one hour. The hydroxamate was obtained in an 82% yield.

(xi) 1-Methyl-2-(o-nitrophanylthio)ethanol (XLII a)

Using the general method, o-nitrophenylthio-propan-2-one was reduced to give this alcohol as a viscous brown oil (59%). This product was reported to be a liquid, b₁,5 170° (50). The positions of the main infrared peaks of this compound are recorded in table V.

(xii) 2-(o-Nitrophenylthio)-1-phenylethanol (XLII b)

Using the general method, ω-(o-nitrophenylthio)acetophenone was reduced to give this alcohol as an orange oil, which on standing, yielded a yellow solid (35%), m.p. 97-98° (from aqueous ethanol). The positions of the main infrared peaks of this compound are recorded in table V.

Found: C, 61.03; H, 4.60; N, 4.92; S, 11.52.
Calc. for $C_{14}H_{13}NO_2S$: C, 61.07; H, 4.76; N, 5.09; S, 11.65.

(xiii) 3-($o$-Nitrophenylthio)pentan-2,4-diol (XLII c)

Using the general method, 3-($o$-nitrophenylthio)pentan-2,4-dione was reduced to give this alcohol as a brown oil, which on standing yielded a yellow solid (29%), m.p. 138-141° (from methanol). The positions of the main infrared peaks of this compound are recorded in table V.

(xiv) 1-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)ethanol (XXXIII j)

Using the general method, ethyl $\alpha$-(o-nitrophenylthio)acetoacetate was reduced to give this hydroxamate as a viscous yellow oil (10%). The product gave a purple colour with ferric chloride solution.

$\nu$ max 3410-3280 (OH), 1640 cm$^{-1}$ (C = O, hydroxamate).

The other product of the reduction which was isolated from the ether extract A was not identified. The product, which was insoluble in sodium carbonate solution, was an orange oil (0.55 g), b.p. 212° at 29 mm.

$\nu$ max 3340 (OH), 1720 cm$^{-1}$ (C = O, ester).
(xv) \((3,4\text{-Dihydro-4-hydroxy-3-oxo-2H-1,4-benzo-thiazinyl})\text{benzyl alcohol (XXXIII k)}\)

Using the general method, ethyl \(-\text{benzoyl-}\-
\(-\text{(o-nitrophenylthio)acetate was reduced to give this}
\text{hydroxamate as a viscous yellow oil (22\%). The product}
gave a purple colour with ferric chloride solution.
\(\nu\) max 3300 (OH), 1640 cm\(^{-1}\) (C = O, hydroxamate).

The ferrous chelate of this hydroxamate was
prepared, in order to determine the elemental analysis.
The hydroxamate (50 mgm) was dissolved in glacial
acetic acid (1 ml). One milliliter of ferrous chloride
solution 30 mgm FeCl\(_2\) in glacial acetic acid (2 ml)
was added to the hydroxamate solution and mixed. Water
(6 ml) was added to the chelate solution, the solution
cooled, and the solid chelate product filtered off. The
chelate product was washed alternatively with sodium
hydroxide solution (10\%) and petroleum ether, and then
dried in a vacuum desiccator for one week at room
temperature. The ferrous chelate had a melting point
of 140-145\°.

Found : C, 57.42; H, 3.61; Fe, 8.94.
Calc. for C\(_{30}\)H\(_{20}\)N\(_2\)O\(_6\)S\(_2\)Fe : C, 57.70; H, 3.23; Fe, 9.64.

The other product of the reduction which was
isolated from the ether extract A was not identified. The product isolated was a pale oil, 0.7 g, which had a b.p. 170°C at 23 mm.

\[ \text{\text{max 3360 (OH), 1718 cm}^{-1} (C = O, ester).} \]

(xvi) 9,9a-Dihydro-9-hydroxy-3-methyl-1-phenyl-1H-
pyrazole- [4,3-b](1,4)benzothiazine (XLIV)

Using the general method, 3-methyl-4-(o-nitro-
phenylthio)-1-phenyl-2-pyrazolin-5-one was reduced to
give this product as a brown solid (58%), m.p. 165-
170°C. The product was purified by dissolving in sodium
hydroxide solution (10%), extracting with ether, and
repurifying the product by acidifying with acetic
acid. This process was repeated twice. The product was
purified in this manner because it was insoluble in
common organic solvents.

\[ \text{\text{max 2890 (OH), 1590 cm}^{-1} (>C = N-) (44).} \]

Found: C, 64.15; H, 4.82; N, 13.85; S, 10.56.
Calc. for C\textsubscript{16}H\textsubscript{15}N\textsubscript{3}OS: C, 64.62; H, 5.08; N, 14.13;
S, 10.78.
Miscellaneous Reactions

(1) Methyl 2-(o-nitrophenylthio)benzoate (XLVII)

Methyl o-mercaptopbenzoate was prepared by esterifying o-mercaptopbenzoic acid in methanol solution by the Fischer-Speier method, as described by Gatterman (51). Sodium hydroxide (1.0 g) was dissolved in water (2 ml) and added to a solution of methyl o-mercaptopbenzoate (4.2 g) in methanol (50 ml). A solution of o-chloronitrobenzene (4.8 g) in methanol (20 ml) was added to the solution of sodium (o-methoxycarbonyl)thiophenolate and stirred. After standing for two days the mixture was evaporated to about one-half volume; the precipitate was filtered off and washed thoroughly with water. The product was identified as methyl 2-(o-nitrophenylthio)benzoate (70%), m.p. 92-93° (from ethanol) - reported m.p. 92-93° (52).

\[
\text{max } 1725 (\text{C} = 0), 1562 \text{ and } 1330 \text{ cm}^{-1} (\text{NO}_2).
\]

Found: C, 58.02; H, 3.78; N, 4.58; S, 10.99.

Calc. for C_{14}H_{11}NO_4S: C, 58.12; H, 3.83; N, 4.84; S, 11.08.

(ii) 2,2′-Bis [o-(methoxycarbonyl)phenylthio]azoxy-
bensene (XLVIII)

a) A suspension of sodium borohydride (0.8 g) and palladium (10%) on charcoal (0.2 g) in water (8 ml) was prepared and nitrogen bubbled through it. Three drops of 20% sodium hydroxide solution were added to the reducing mixture. Methyl 2-(o-nitrophenylthio)benzoate (2.0 g) dissolved in dioxan (20 ml) was added slowly to the reducing mixture; the mixture was allowed to react for 30 minutes under nitrogen. The mixture was filtered and washed with dioxan, and then with water. The combined filtrate and washings were flooded with water and acidified with dilute hydrochloric acid. The acid aqueous solution was extracted with ether; the ether extract was washed with sodium hydroxide solution (20%), washed with water, dried (Na₂SO₄), and evaporated to yield a yellow solid (69%), m.p. 113-115° (from ethanol).

\( \nu \) max 1710 (C = O), 1474 and 1300 cm⁻¹ (N = N⁻) (46).

Found : C, 63.12; H, 4.24; N, 5.23; S, 12.16; mol. wt. 511.

Calc. for C₂₈H₂₂N₂O₅S₂ : C, 63.24; H, 4.17; N, 5.26; S, 12.28; mol. wt. 531.79.
b) Methyl 2-(α-nitrophenylthio)benzoate (2.0 g) dissolved in a mixture of ethanol: methanol (3:1) (40 ml), was reduced as described in ii a) (above), except that dioxan was replaced with the mixed solvent. The acidified filtrate yielded a viscous orange oil (1.3 g). After trituration with ethanol, the azoxybenzene (XLVIII) was isolated as orange crystals (0.19 g), m.p. 145-149°, from this oil. The infra-red spectrum of this azoxybenzene product was identical to the spectrum of the compound obtained in the previous reduction.

c) Reduction using zinc and ammonium chloride

Methyl 2-(α-nitrophenylthio)benzoate (2.0 g) was dissolved in ethanol, (95%) (50 ml). Ammonium chloride (2.0 g) in water (20 ml) and zinc dust (2.0 g) were added separately to the ethanol solution. The mixture was stirred for 2 hours and the inorganic solid components filtered off. A red oil (1.5 g) was obtained on acidification of the filtrate with hydrochloric acid. The red oil was dissolved into ether solution and washed with sodium carbonate solution (5%), washed with water, dried (Na2SO4), and evaporated. The purified
red oil, on standing, yielded yellow crystals (0.2 g), m.p. 113-115 ° (from ethanol). The infrared spectrum of this solid product was identical to 2,2′-bis[6-(methoxycarbonyl)phenylthio]azoxybenzene, produced above. The remaining red oil was not identified.

(iii) Preparation of sodium α- (and p-)nitrothiophenolate

a) Sodium α-nitrothiophenolate

α-Nitrothiophenol (2.0 g), as prepared by Claass (33), was added to a solution of sodium (1.0 g) in absolute ethanol (60 ml) and shaken. The solution was evaporated at room temperature. The red solid which remained, sodium α-nitrothiophenol (63%), was washed with ether.

b) Sodium p-nitrothiophenolate

p-Nitrothiophenol (3.8 g) was added to a solution of sodium (1.0 g) in absolute ethanol (60 ml) and shaken. The solution was evaporated at room temperature to yield an orange solid, sodium p-nitrothiophenolate (86%).
(iv) Attempted preparation of diethyl (p-nitrophenyl-thio)malonate

a) A solution of sodium p-nitrothiophenolate (1.8 g) in ethanol (50 ml) was added to a solution of diethyl chloromalonate (1.9 g) in ethanol (5 ml) and stirred for 15 minutes. A pale yellow solid occurred which was identified as bis(p-nitrophenyl) disulfide (67%), m.p. 180-183° - reported m.p. 181° (53).

b) Sodium p-nitrothiophenolate (1.8 g) was refluxed in a solution of diethyl chloromalonate (1.9 g) in xylene (15 ml) for 14 hours. A yellow precipitate was filtered off, which was found to be bis(p-nitrophenyl) disulfide (81.5%), m.p. 180-185° (crude). The infrared spectrum of this product was identical to the spectra of a known sample of bis(p-nitrophenyl) disulfide.

(v) Preparation of p-nitrobenzoates (XLIII)

General Method

The secondary alcohol (100 mgm) was refluxed for 30 minutes with p-nitrobenzoyl chloride (200 mgm)
in pyridine (2 ml). The reaction mixture was cooled, and flooded with chloroform. The mixture was extracted twice with dilute hydrochloric acid to remove the pyridine, washed with water, extracted with sodium hydroxide (10%) solution to remove excess p-nitrobenzoyl chloride, washed with water, dried (Na₂SO₄) and filtered. The p-nitrobenzoate derivative was isolated when the chloroform was evaporated off. The infrared spectra of these derivatives are described in table V.

a) 1-(o-Nitrophenylthio)-2-propyl p-nitrobenzoate (XLIII a)

Using the general method, 1-methyl-2-(o-nitrophenylthio)ethanol (XLII a) was esterified to yield this derivative as a yellow solid, m.p. 95-100°.

b) 1-(o-Nitrophenylthio)-2-ethyl p-nitrobenzoate (XLIII b)

Using the general method, 2-(o-nitrophenylthio)-1-phenylethanol was esterified to yield this derivative as a yellow solid, m.p. 134-135° (from methanol).

c) 3-(o-Nitrophenylthio)-2,4-pentyl di(p-nitrobenzoate) (XLIII c)
Using the general method, 3-(o-nitrophenylthio)pentan-2,4-diol was esterified to yield this derivative as a viscous oil, which when triturated with ethanol, produced brown crystals, m.p. 143-146\(^\circ\) (from aqueous methanol).

(vi) Reduction of \(\omega\)-(o-nitrophenylthio)acetophenone (XXXII b)

\(\omega\)-(o-Nitrophenylthio)acetophenone (0.4 g) was dissolved in dioxan (15 ml), and mixed with a solution of sodium borohydride (0.1 g) in ethanol (5 ml). The mixture stood for 30 minutes, and then was flooded with water, and acidified with hydrochloric acid. The acidic solution was extracted twice with ether and the ether extract washed with water, dried (\(\text{Na}_2\text{SO}_4\)), and evaporated to yield an orange oil. The infrared spectrum of this crude product was identical to the infrared spectrum of 2-(o-nitrophenylthio)-1-phenylethanol (XLII b).
BIBLIOGRAPHY

2. F.A. BARCLAY, E.V. BROWN, F.E. ANDERSON and D.N. GREEN. Antibiotics and Chemotherapy, 6, 261 (1956).
(1914).
Soc. 2518 (1962).
(1962).
(1963).
(1927).
1214 (1960).
36. E.S. Gould. Mechanism and Structure in Organic
10, 374 (1945).
38. G.M. Badger, D.J. Clark, W. Davies, K.T.R. Farrer and
39. R.T. COUTTS, H.W. PEEL and E.M. SMITH. To be published.


A number of substituted 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine derivatives were prepared in anticipation that they may have some antibacterial properties. The substituted 2H-1,4-benzothiazine hydroxamic acids were prepared by reducing suitably substituted (o-nitrophenylthio)esters by means of sodium borohydride and palladium charcoal. The ester precursors were obtained using three different methods. They are as follows:

a) condensation of sodium o-nitrothiophenolate and appropriate α-bromoesters.

b) condensation of substituted o-chloro- (or bromo-)nitrobenzene and suitable α-mercapto acids, which gave the corresponding α-(o-nitrophenylthio)acid. They were esterified by means of the Fischer-Speier method to yield the α-(o-nitrophenylthio)esters.

c) condensation of o-nitrobenzenesulfenyl chloride with suitable compounds containing an active hydrogen in the position alpha to the carbonyl group.

The substituted (o-nitrophenylthio)esters prepared in this project, which have not been reported previously are as follows: ethyl 2-(o-nitrophenylthio)butyrate; ethyl 2-(o-nitrophenylthio)propionate; diethyl bis(o-nitrophenylthio)malonate; dimethyl α-(o-nitrophenylthio)succinate;
dimethyl \( \alpha \)-(4-bromo-2-nitrophenylthio)succinate; methyl \( \alpha \)-(4-bromo-2-nitrophenylthio)propionate; dimethyl \( \alpha \)-(4-methyl-2-nitrophenylthio)succinate; methyl \( \alpha \)-(4-methyl-2-nitrophenylthio)propionate; dimethyl \( \alpha \)-(4-trifluoromethyl-2-nitrophenylthio)succinate; methyl \( \alpha \)-(4-trifluoromethyl-2-nitrophenylthio)propionate; ethyl \( \alpha \)-benzoyl-\( \alpha \)-(o-nitrophenylthio)acetate. The following \( \alpha \)-(o-nitrophenylthio)acids have not been previously reported: \( \alpha \)-(o-nitrophenylthio)succinic acid; \( \alpha \)-(o-nitrophenylthio)propionic acid; \( \alpha \)-(4-bromo-2-nitrophenylthio)succinic acid; \( \alpha \)-(4-bromo-2-nitrophenylthio)propionic acid; \( \alpha \)-(4-methyl-2-nitrophenylthio)succinic acid; \( \alpha \)-(4-trifluoromethyl-2-nitrophenylthio)succinic acid; \( \alpha \)-(4-trifluoromethyl-2-nitrophenylthio)propionic acid.

A number of \( \alpha \)-(o-nitrophenylthio)ketones were prepared by interacting o-nitrobenzenesulfonyl chloride with an appropriate carbonyl compound. The resulting ketones were reduced with sodium borohydride and palladium charcoal.

A brief investigation of the facile formation of bis(o-nitrophenyl) disulfide in reactions involving sodium o-nitrothiophenolate was carried out.
Reduction of the substituted \(\alpha\)-(o-nitrophenyl-thio)esters, in almost every case, produced the corresponding 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine derivative. In ester intermediates containing a ketone group, reduction of the latter to a secondary alcohol occurred in addition to reductive cyclization to the hydroxamate. The substituted 2H-1,4-benzothiazines prepared in this manner, that have not been previously reported in the literature, are as follows: 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine; 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine; methyl (3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate; methyl (6-bromo-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate; 6-bromo-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine; methyl (3,4-dihydro-4-hydroxy-6-methyl-3-oxo-2H-1,4-benzothiazinyl)acetate; 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzothiazine; methyl (6-trifluoromethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)acetate; 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine; \(\alpha\)-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)ethanol; \(\alpha\)-(3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazinyl)benzyl alcohol.
Reduction of the \(\alpha\)-(o-nitrophenylthio)ketones produced the corresponding secondary alcohols. The following secondary alcohols, or their \(\alpha\)-nitrobenzoate derivatives, have not been previously reported: 1-methyl-2-(o-nitrophenylthio)ethanol; 2-(o-nitrophenylthio)-1-phenylethanol and 3-(o-nitrophenylthio)pentan-2,4-diol. Reduction of 3-methyl-4-(o-nitrophenylthio)-1-phenyl-2-pyrazolin-5-one resulted in the formation of 9,9a-dihydro-9-hydroxy-3-methyl-1-phenyl-1H-pyrazolo-[4,3-b](1,4)benzothiazine, an example of a new ring system.

Reduction of methyl 2-(o-nitrophenylthio)benzoate, using sodium borohydride and palladium charcoal, produced an azoxy compound 2,2-bis[(o-methoxycarbonyl)phenylthio]-azoxybenzene.

A variety of the benzothiazine cyclic hydroxamic acids synthesized in this project were tested for antibacterial activity, and their action on enzyme systems. The compounds showed no antibacterial activity against the organisms tested. This is surprising in that there are a large number of cyclic hydroxamates which do possess definite activity. The inactivity of the benzothiazine cyclic hydroxamic acids would appear to indicate that these molecules do not have the correct geometric or
aromatic character, possessed for example, by aspergillic acid which is a very active antibacterial substance, containing a cyclic hydroxamic acid group. Two synthetic cyclic hydroxamic acids which exhibit extreme antibacterial activity (8) are 1,2-dihydro-1-hydroxy-3-methyl-2-oxoquinoline and 3-ethyl-1,2-dihydro-1-hydroxy-2-oxoquinoline. Each of these compounds are completely aromatic in character.

Some of the cyclic hydroxamic acids that were tested on enzymes were found to reactivate difluorophosphate inhibited acetylcholinesterase.

* The bacterial testing was carried out under the direction of Dr. A.H. Holden, University Hospital, Saskatoon. Dr. W.J. Johnson, of the Food and Drug Directorate in Ottawa, has tested a number of the cyclic hydroxamic acids on certain enzymes.