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

A Thesis

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by

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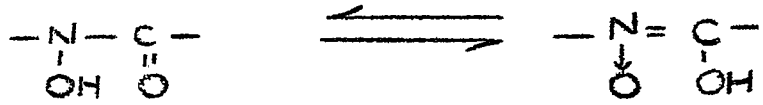
I should sincerely like to thank Dr. R.T. Coutts for his guidance and helpful advice concerning this thesis.

TABLE OF CONTENTS

I. INTRODUCTION

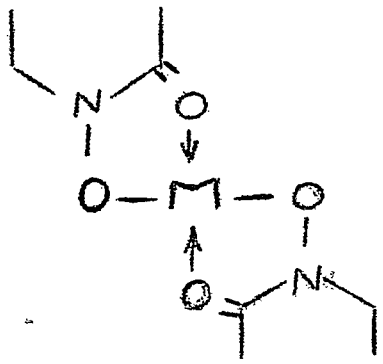
A variety of reasons exist for making an investigation into the chemistry of cyclic hydroxamic acids, especially those possessing a 2H-1,4-benzothiazine nucleus. These reasons are discussed below.

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



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).



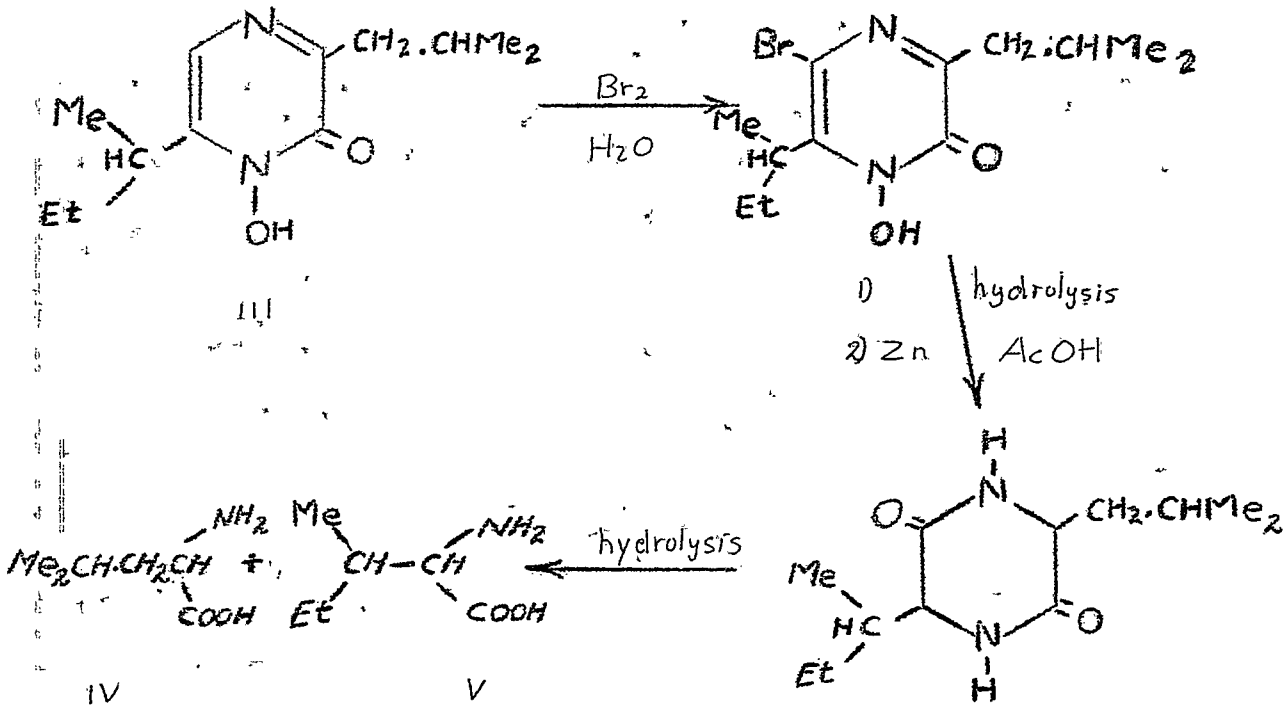
II

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.

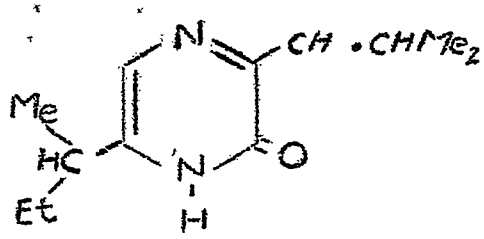
There are many compounds that contain a hydroxamic acid grouping, which are reported to possess antimicrobial activity (1-8).

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 α-amino acids, DL-leucine (IV) and DL-isoleucine (V).



III IV V

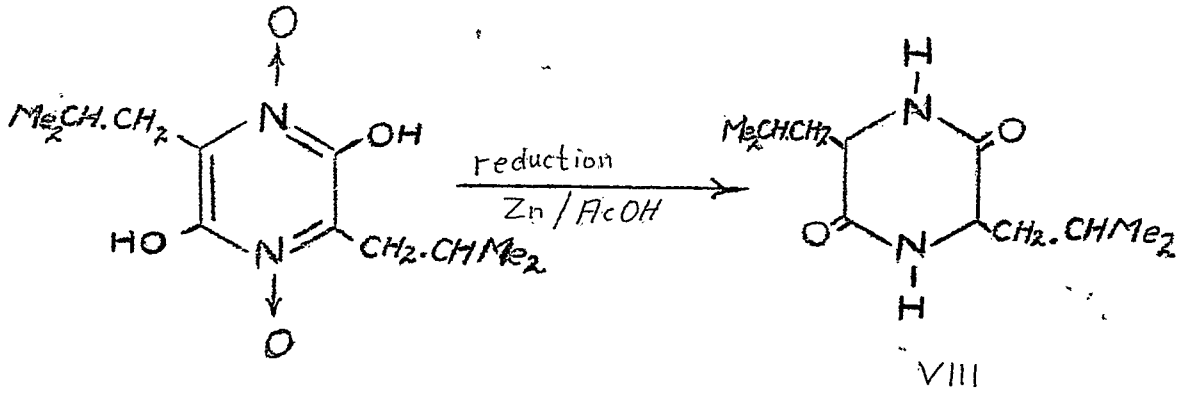
Further evidence for the structure of aspergillic acid occurred when its reduction product, deoxyaspergillic acid (VI) was examined. Degradative studies of deoxyaspergillic acid indicated its structure to be either 2-hydroxy-3-isobutyl-6-sec-butylpyrazine (VI) or 2-hydroxy-3-sec-butyl-6-isobutylpyrazine (11).



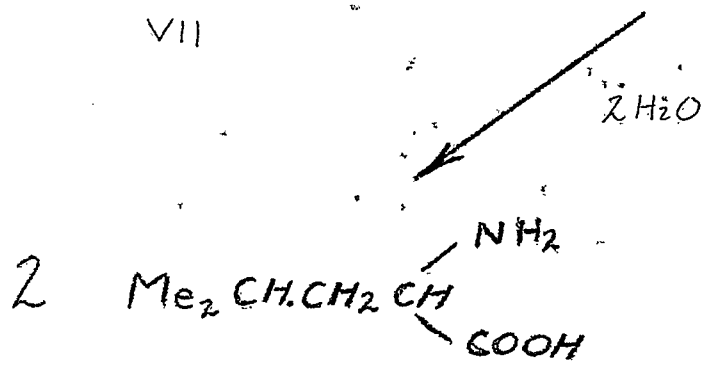
VI

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-dioxopiperazine (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.

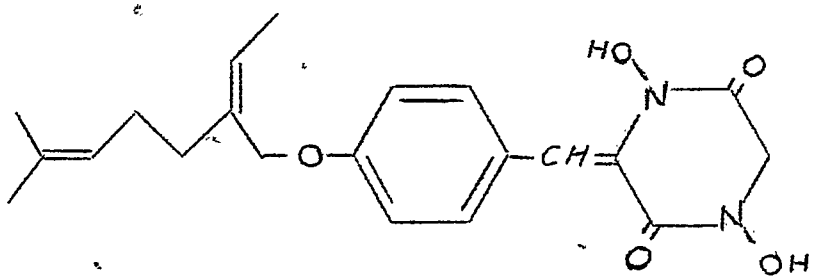


VII VIII



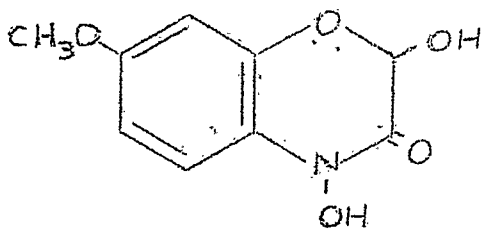
IX

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).



(X)

The compound 3,4-dihydro-2,4-dihydroxy-7-methoxy-3-oxo-2H-1,4-benzoxazine (XI) (Dimboa), which has been isolated from corn seedlings and other grasses, exhibits antimetabolic activity (16). Dimboa has two unique structural features of interest; it contains a benzoxazine ring system and it is a cyclic hydroxamate.



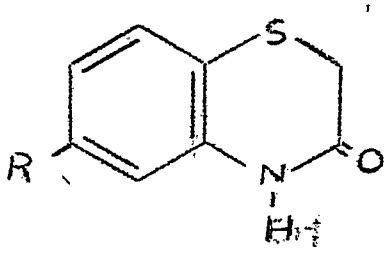
XI

The examples of cyclic hydroxamic acids quoted possess two heteroatoms 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 sulfonamides. 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-

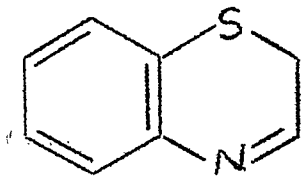
vestigational treatment of leprosy (17) and tuberculosis (18).

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 medicinal importance, and many compounds of potential importance are structurally related to 2H-1,4-benzothiazine, and some of its simple derivatives are known to possess anthelmintic 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-sulfonomido. Other derivatives prepared are substituted in position 7.

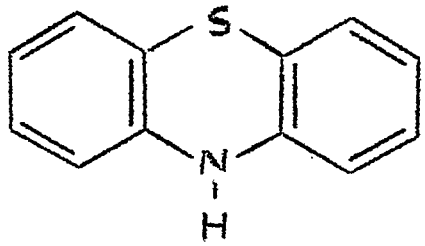


XII

The 2H-1,4-benzothiazine (XIII) structure is chemically related to the now very important group of drugs, the phenothiazines. This group of drugs is made up of relatively simple derivatives of the parent compound, phenothiazine (XIV).



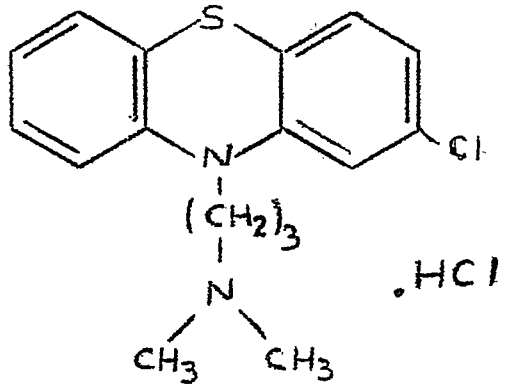
XIII



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 dimethylaminoalkyl type. An example of a phenothiazine derivative is Chlorpromazine Hydrochloride (U.S.P.) (XV).

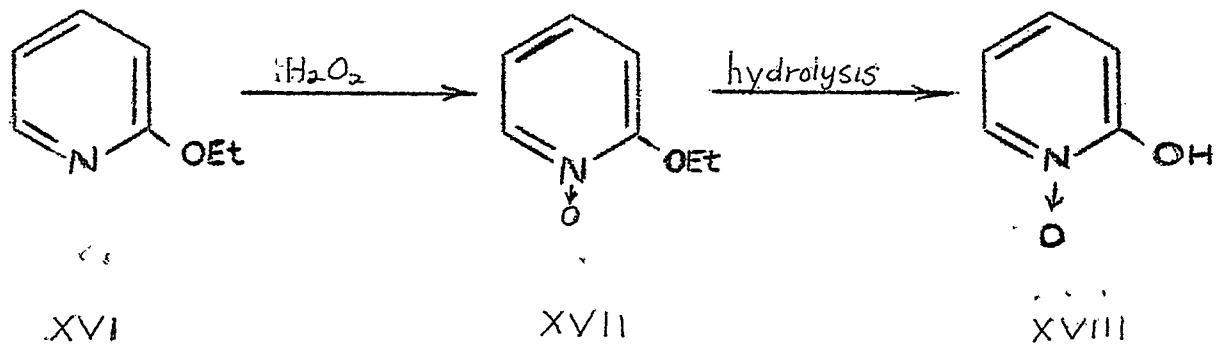


XV

Recently, a great deal of interest has been focused on phenothiazine derivatives substituted in position 2 of the nucleus. For example, Trifluprομazine Hydrochloride (Vesprin) has a trifluoromethyl group, $-CF_3$, 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:

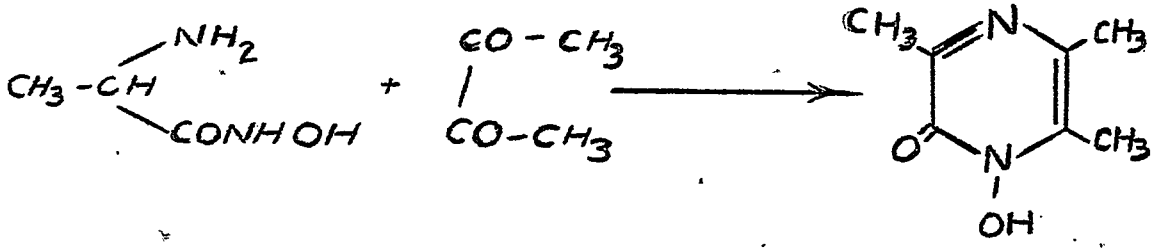
1) oxidation of pyridine and quinoline derivatives (22). An example is the oxidation of 2-ethoxypyridine (XVI) with hydrogen peroxide to 2-ethoxypyridine-1-oxide (XVII) which upon hydrolysis with dilute hydrochloric acid gave 2-hydroxypyridine-1-oxide (XVIII), possessing the properties of hydroxamic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



XVI XVII XVIII

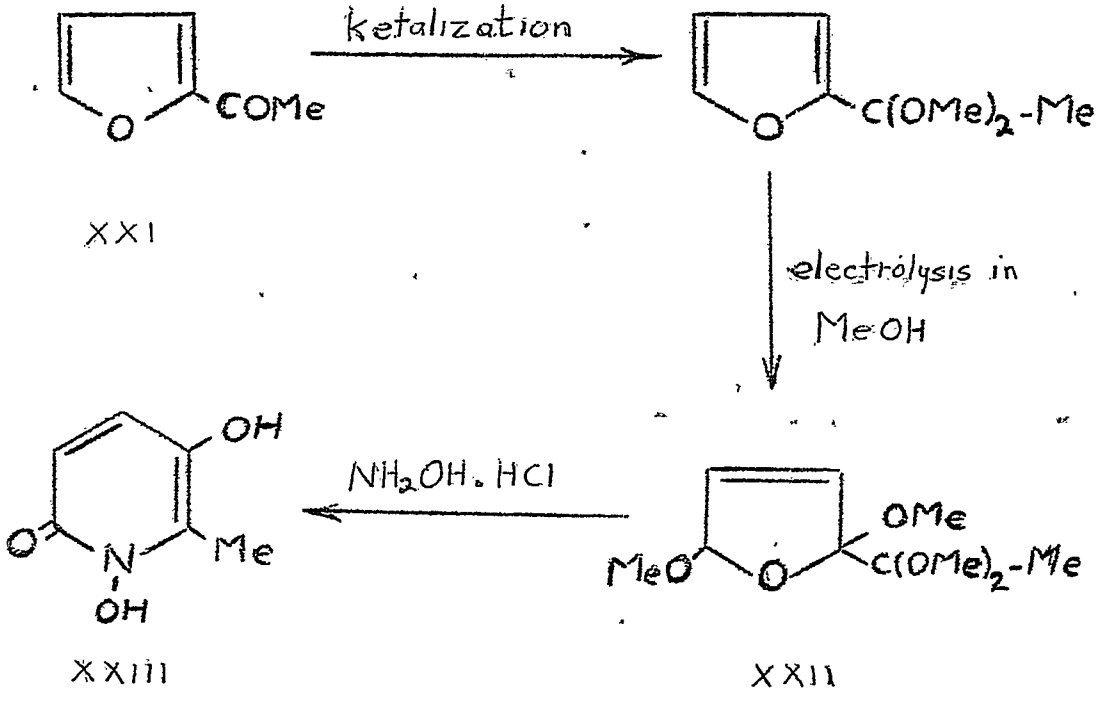
Further synthesis of pyridine and quinoline derivatives of cyclic hydroxamic acids have been described by Cunningham et al. (23).

2) condensation of an α-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).



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-(α,α-dimethoxyethyl)-2,5-dihydrofuran (XXII), was reacted with an aqueous solution of hydroxylamine hydrochloride and yielded 1,5-dihydroxy-6-methyl-2-pyridone (XXIII).



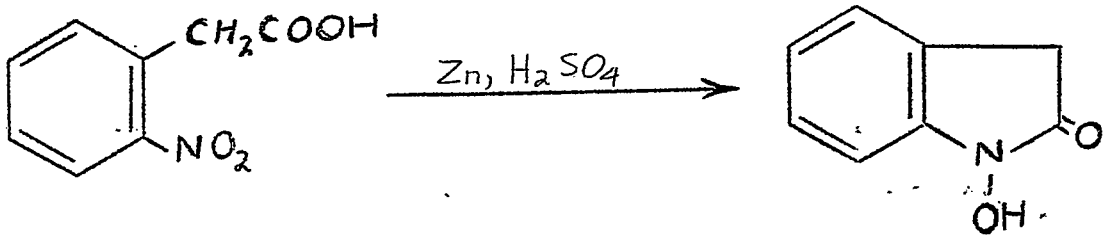
XXI XXIII XXII

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.

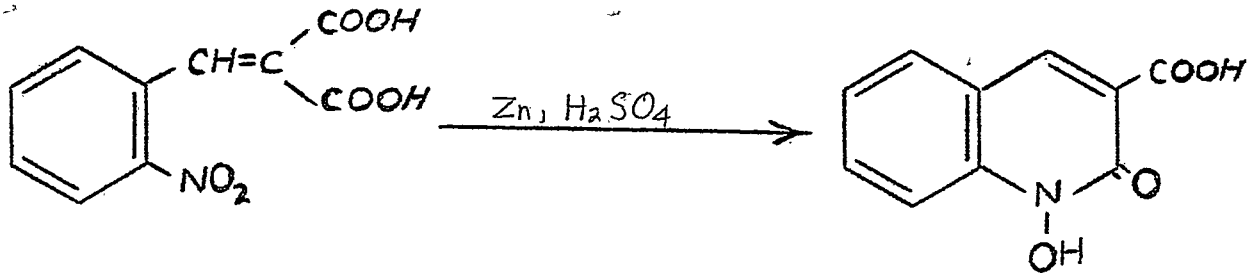
a) Reduction of suitable α-(o-nitrophenyl) acids using zinc and sulfuric acid;

Reissert (26) obtained 1,2-dioxindole (XXIV) by the reduction of o-nitrophenylacetic acid with zinc and sulfuric acid.



XXIV

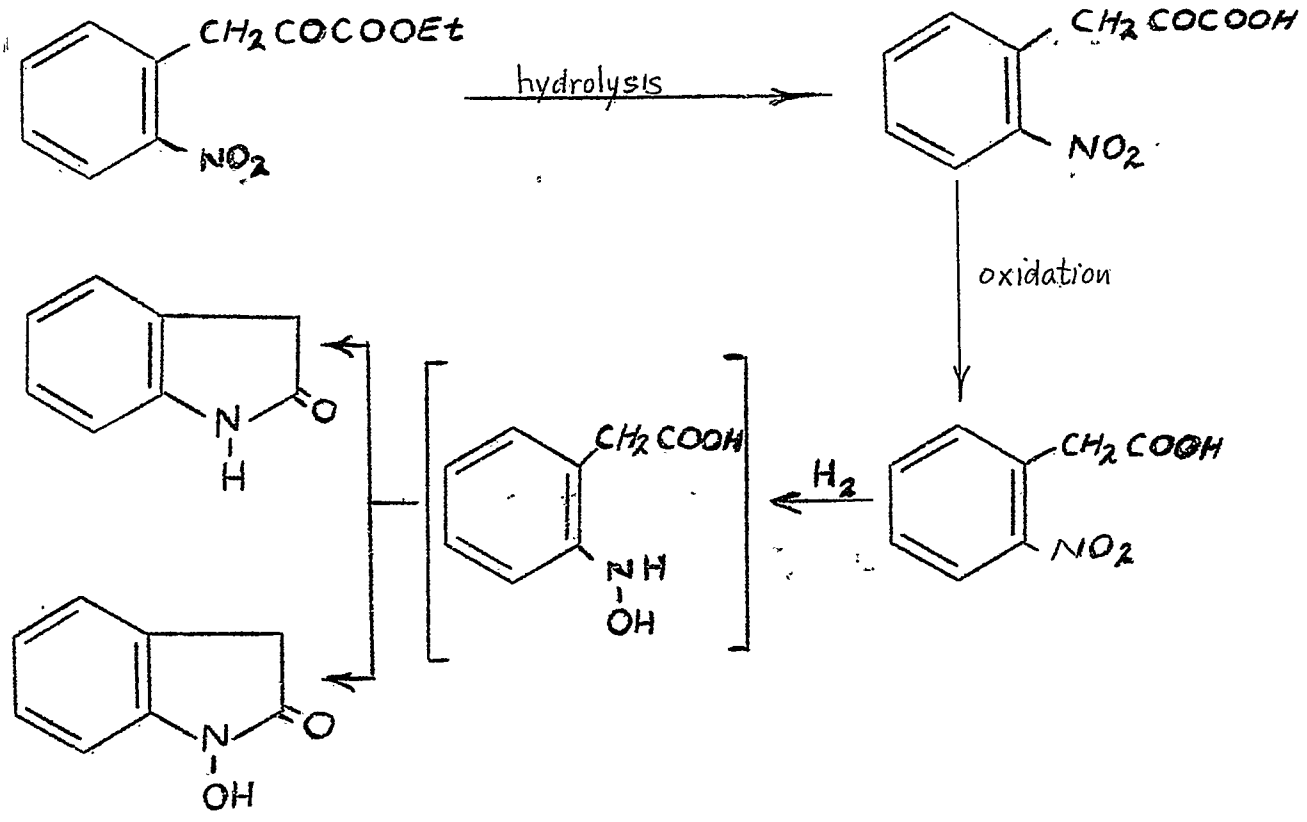
Heller and Wunderlich (27) obtained 1,2-dihydro-1-hydroxy-2-oxoquinoline-3-carboxylic acid (XXV) by reduction of o-nitrobenzylidene malonic acid with zinc and sulfuric acid.



XXV

b) Reduction of suitable α-(o-nitrophenyl) acids using catalytic hydrogenation.

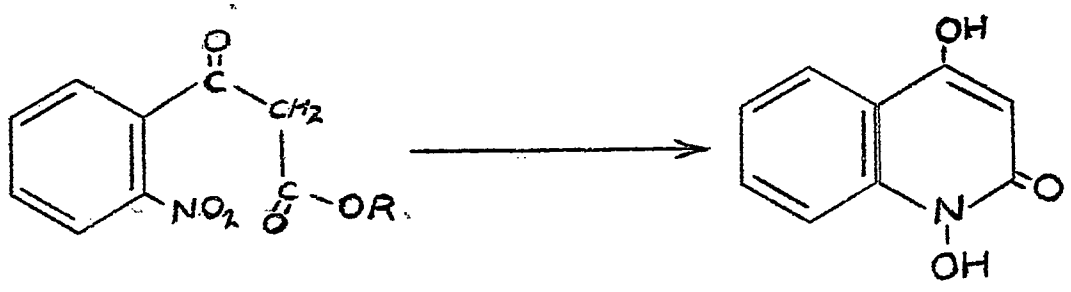
Dicarlo (28), following initial oxidation of o-nitrophenylpyruvate to o-nitrophenylacetic acid, isolated 1,2-dioxindole (XXIV) in appreciable amounts by catalytic hydrogenation of the latter compound with platinum oxide.



XXIV

c) Reduction of suitable α-(o-nitrophenyl) esters using hydrazine hydrate in the presence of the catalyst palladium (10%)-on-charcoal.

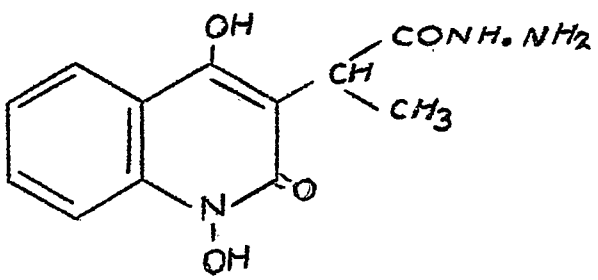
The synthesis of 1,2-dihydro-1,4-dihydroxy-2-oxo-quinoline (XXVI) by the action of this reducing system on ethyl or methyl o-nitrobenzoylacetate was described by Coutts et al. (29).



R= Me or Et

XXVI

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).



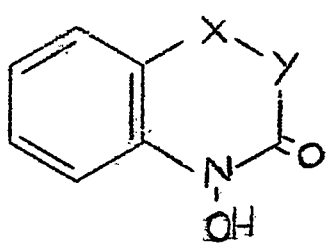
XXVII

d) Reductive cyclization of a series of similar α-(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 α-(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 = CH_2-CH_2), 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinazoline (XXVIII; x-y =CH_2-NH), 1,2-dihydro-1-hydroxy-2-oxo-4H-3,1-benzoxazine (XXVIII; x-y = CH_2-O), and 3,4-dihydro-4-hydroxy-3-oxo-1,4-benzothiazine (XXVIII; x-y = S-CH_2).

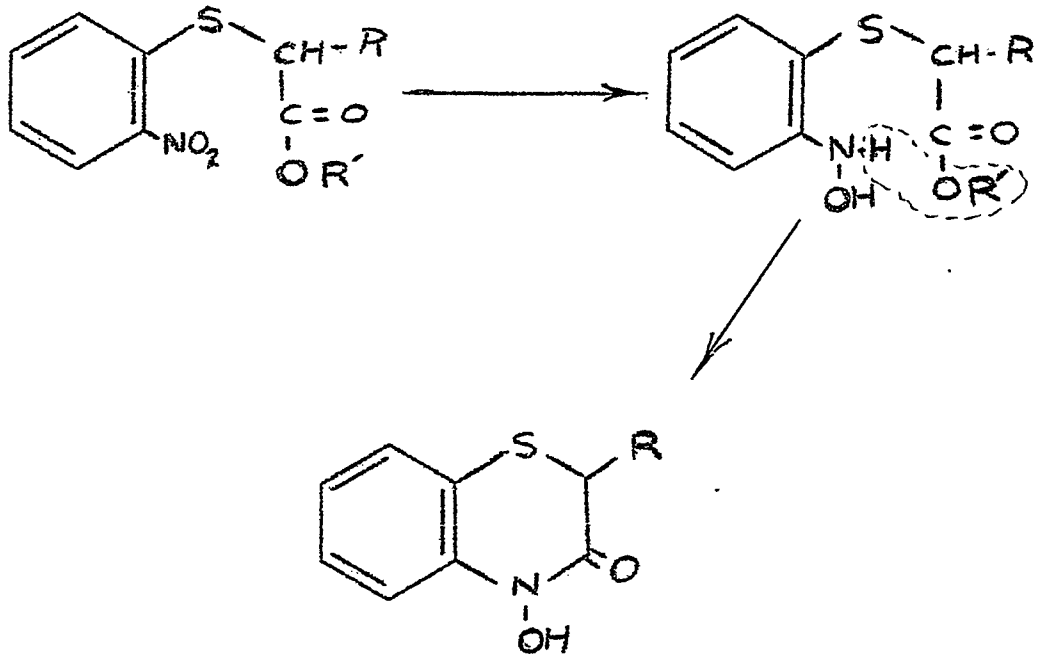


XXVIII

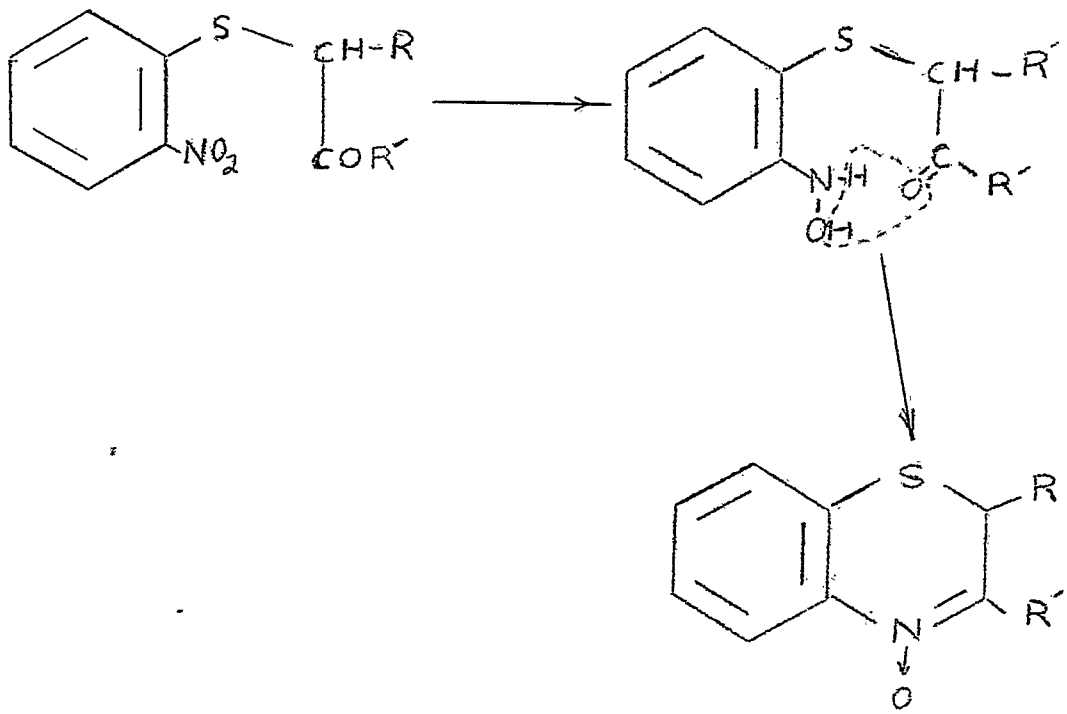
There are a large number of o-nitro-ester intermediates that are suitable for reductive cyclization. Because of the reasons mentioned before, suitable o-nitrophenylthio-ester intermediates were prepared which, on reductive cyclization, would yield 2H,1,4-benzothiazine hydroxamate derivatives. The only 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).



XXIX



XXX

Thus, suitable o-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-(o-nitrophenylthio)-keto derivatives using sodium borohydride and palladium charcoal catalyst. The production of an N-oxide was reported by Coutts (32), having reduced o-nitrobenzoylacetone with sodium borohydride and palladium charcoal. 4-Hydroxy-2-methylquinoline-1-oxide was the product in this case.

II. DISCUSSION

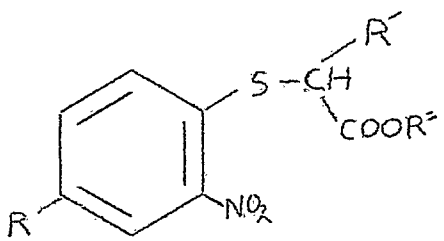
The problems that were investigated in this thesis were as follows:

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

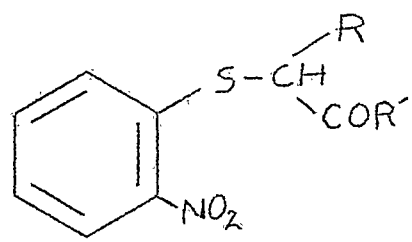
b) the preparation of suitable intermediates for reductive cyclization to N-oxy or N-hydroxy compounds.

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 spacially for cyclization.

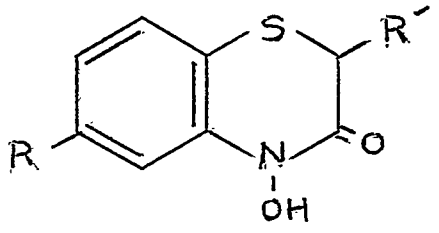


XXXI

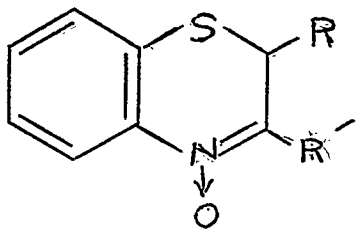


XXXII

Reduction of compounds of types XXXI and XXXII were expected to yield compounds XXXIII and XXX respectively.



XXXIII



XXX

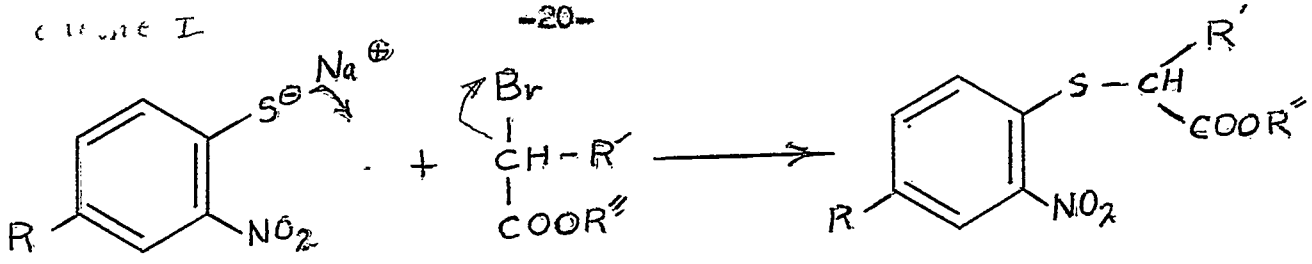
The problem can be conveniently subdivided. The first section deals with the preparation of intermediates of types XXXI and XXXII, and the second will consider the reduction of these intermediates.

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

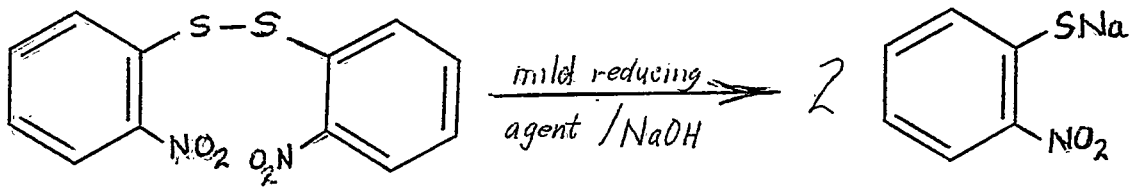
The first method of synthesis attempted was the condensation of sodium o-nitrothiophenolate with suitable α-bromoesters (scheme I).



scheme I

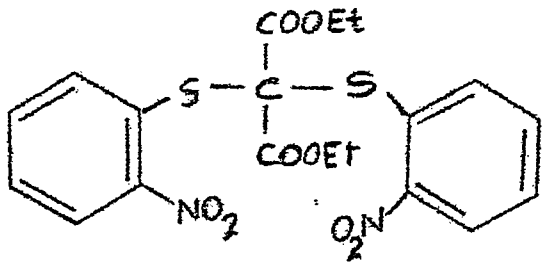
This apparently simple reaction gave rise to an unexpected product in significant amounts, and proved to be unsatisfactory for a number of reasons. In the first place, preparation of pure o-nitrothiophenol obtained by mild reduction of bis(o-nitrophenyl) disulfide (XXXIV) according to the methods of 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



XXXIV

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.



(XXXV)

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)-