# ANTITUBERCULAR AND ANTIBACTERIAL AGENTS

A Thesis

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# Part I

# INTRODUCTION

A wide-spread search for new antibiotics was initiated by the discovery of therapeutic properties of penicillin in 1940. Since then several anti-bacterial substances, produced by a large number of micro-organisms, have been isolated. Following the discovery of streptomycin, the antimicrobial properties of actinomycetis have probably been investigated more thoroughly than any other groups of micro-organisms. These investigations have led to the discovery of three powerful chemotherapeutic agents - Chloramphenicol, Aureomycin and Terramycin. Among these, chloramphenicol - better known as Chloromycetin - is remarkable in several respects. It has been found to be most effective against certain virus and ricketisal diseases. It is widely used against typhoid, paratyphoid, undulent fever, ornithosis, etc., though this by no means exhausts its therapeutic range. It shows promise against some important diseases like whopping cough and pneumonia. It is administered orally and is well tolerated. Chemically also, chloramphenicol is remarkable being a naturally occurring compound that

contains a nitro and a dichloroacetyl group and has a rather simple structure. No other naturally occurring compound of this type had been found before. Synthetic chloramphenicol has been marketed also.

The isolation of chloramphenicol from "Streptomyces venezuela" was first reported in 1947[1] by Paul Burkholder and others, working in conjunction with Parke Davis and Co. Intensive work on this substance was carried out in the laboratories of this company and within two years its structure was elucidated, both by degradation and synthesis.[2,3] Chloramphenicol has been synthesized by several methods.[3,4,5,6] It represents the first antibiotic produced synthetically on a commercial scale and at present both the synthetic and fermentation processes are employed in its manufacture.

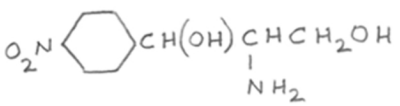
Constitution of chloramphenicol :

Chloramphenicol is a relatively stable, neutral compound, which crystallizes in colourless needles, m.p. 150°. It is quite stable in acid or neutral aqueous solutions but is inactivated in alkali. It is soluble in many organic solvents but is sparingly

soluble in water. Chloramphenicol can be sublimed in high vacuum without decomposition. It contains carbon, hydrogen, nitrogen and non-ionic chlorine, and has the molecular formula $C_{11}H_{12}Cl_2N_2O_5$.[2] It does not give a thiosemicarbazone or other derivatives characteristic of a carbonyl function, indicating its absence in the molecule. Primary amino group is also absent in chloramphenicol. Ultraviolet absorption spectra suggested that it is a nitrobenzene derivative. The presence of a nitro group in the molecule is confirmed by its reduction with tin and hydrochloric acid, followed by diazotization and coupling with β-naphthol, when it gives a red precipitate.

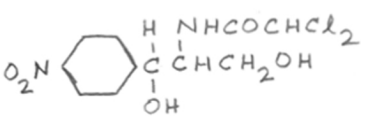
The degradation studies of chloramphenicol were carried out by Rebstock *et al.*[2], which finally led to the establishment of its structure. They found that on alkali or acid hydrolysis, chloramphenicol gives dichloroacetic acid and an optically active base (A), with the molecular formula $C_9H_{12}N_2O_4$. This base when treated with methyl dichloroacetate gave chloramphenicol. The presence of an amino group and two hydroxyl groups in the base (A) was proved by formation of acetyl derivatives, followed

by their estimation. When treated with periodic acid, the base gave ammonia, formaldehyde and p-nitrobenzenealdehyde, all of which were estimated quantitatively. The base thus appeared to have the following structure.



(A)

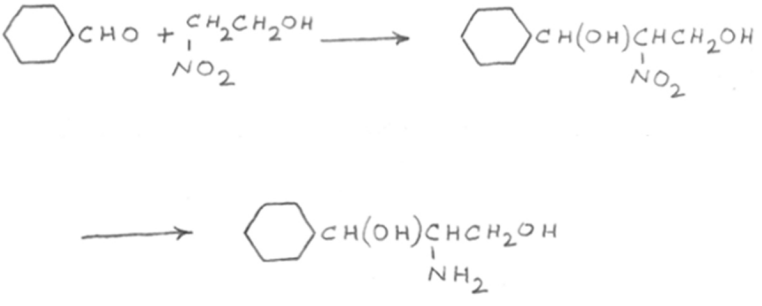
On the above facts chloramphenicol was constituted as D-(-)-threo-2-dichloracetamido-1-p-nitrophenyl-1:3-propanediol. The threo configuration was arrived at by analogy with l-nor-pseudo-ephedrine. The structure was consequently proved by synthesis.



CHLORAMPHENICOL

The degradation studies of chloramphenicol had shown that l-p-nitrophenyl-2-amino-1:3-propanediol was the key intermediate for the synthesis of the antibiotic, as it could be readily converted to chloramphenicol. In the first synthesis of chloramphenicol, by Controulis et al.,[3] l-p-nitrophenyl-2-

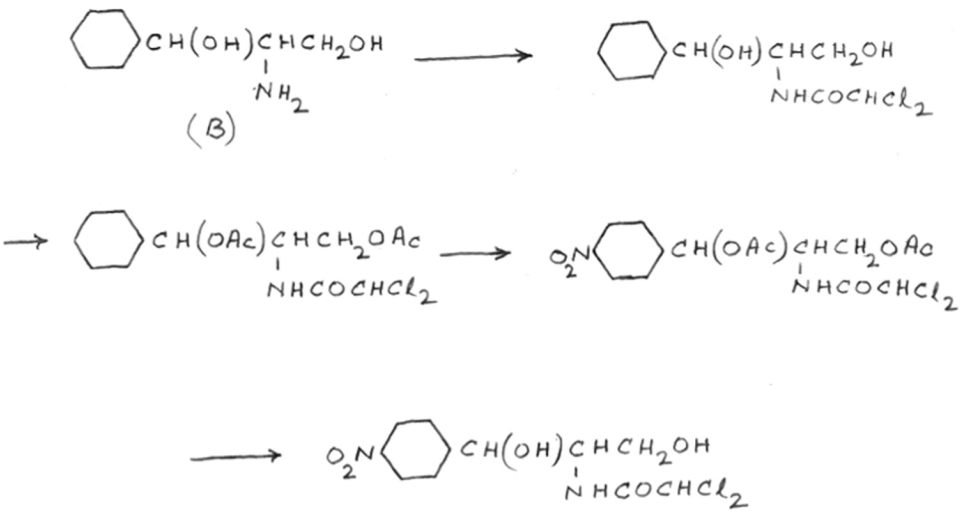
amino-1:3-propanediol was obtained starting from benzaldehyde. Condensation of benzaldehyde with nitroethanol, followed by reduction, gave a recemic mixture of 1-phenyl-2-amino-1:3-propanediol. The stereoisomers were separated by using chloroform, when a crystalline compound (B) and an amorphous substance (C) were obtained. Fraction (B) was



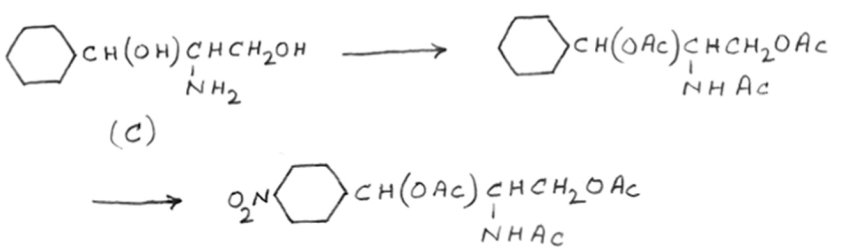
(B + C)

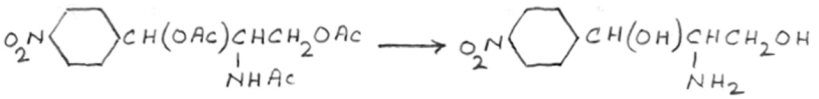
converted first to 1-phenyl-2-dichloracetamido-1:3-propanediol by treating it with methyl dichloroacetate. The former compound on acetylation followed by nitration gave 1-p-nitrophenyl-2-dichloracetamido-1:3-diacetoxypropane. Partial hydrolysis of the latter gave 1-p-nitrophenyl-2-dichloroacetamido-1:3-propanediol, corresponding in ultraviolet absorption to natural chloramphenicol. However, it was found to be inactive in microbiological assay, which

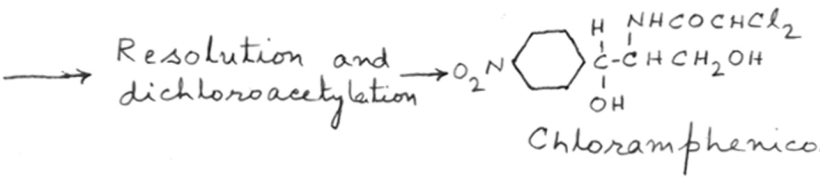
proved that it belongs to <u>erythro</u> series.



Fraction (C) on acetylation followed by nitration gave 1-<u>p</u>-nitrophenyl-2-acetamido-1:3-diacetoxypropane. The latter on hydrolysis gave 1-<u>p</u>-nitrophenyl-2-amino-1:3-propanediol. This base was resolved by crystallization of its camphor sulphonic acid salt from isopropyl alcohol. The two salts were hydrolysed with ammonia. The free base from the (-) salt on

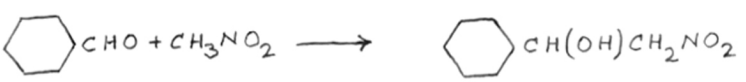


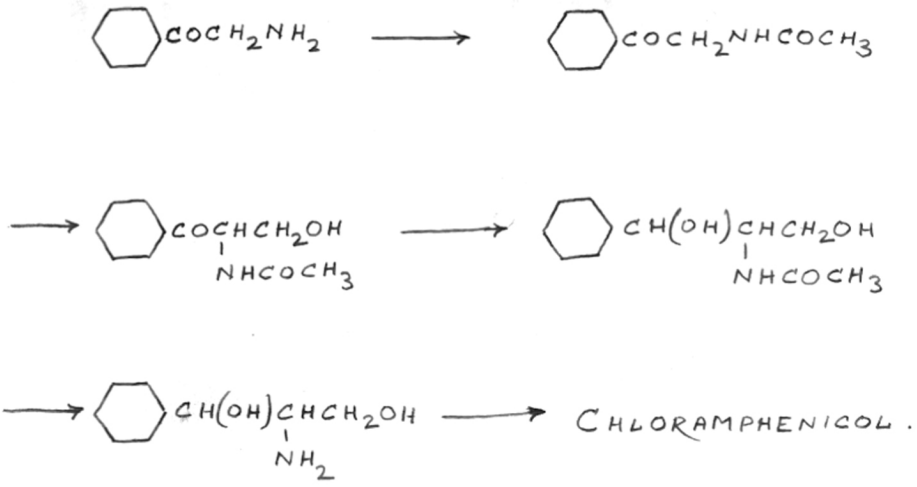




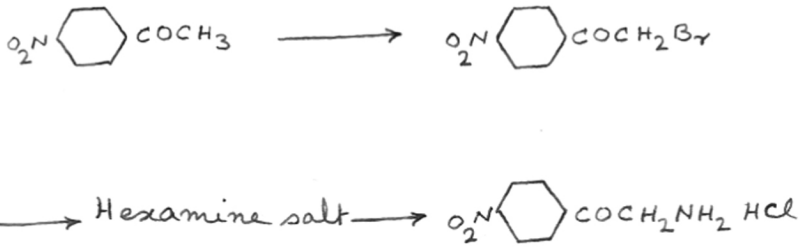

treatment with methyl dichloroacetate gave 1-p-nitrophenyl-2-dichloroacetamido-1:3-propanediol which was shown to be identical in chemical, physical and antibacterial properties with natural chloramphenicol prepared by fermentation.

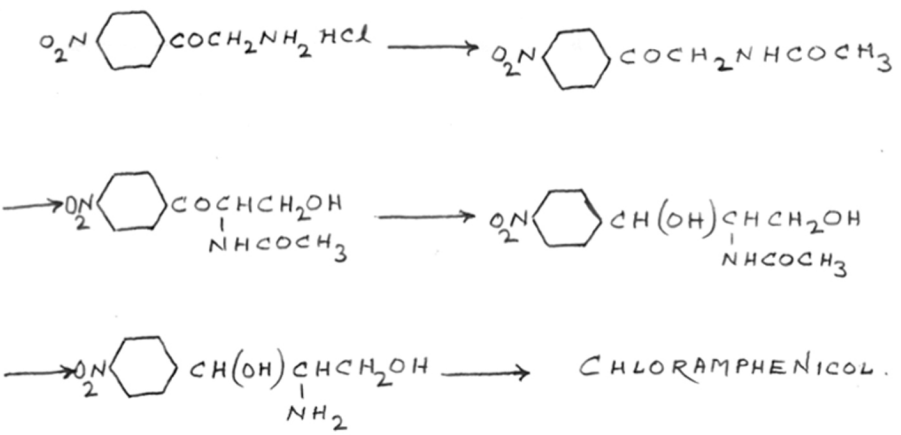
In the synthesis described by Long and Troutmann,[4] 1-phenyl-2-amino-1:3-propanediol was obtained starting from α-nitroacetophenone. The latter compound was obtained by condensation of benzaldehyde with nitromethane followed by oxidation. The main steps in the synthesis were as follows :-





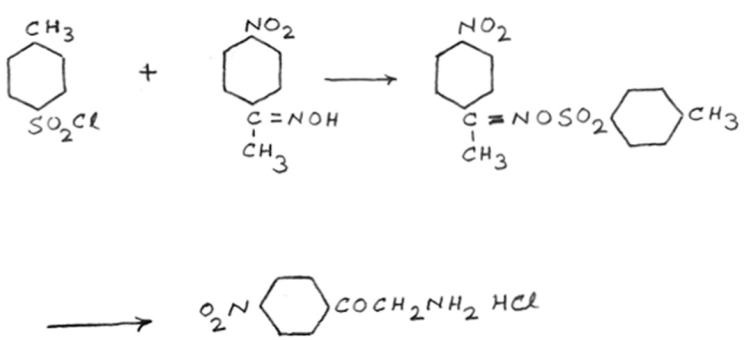

In the alternative synthesis described by Long and Troutmann,[5] 1-p-nitrophenyl-2-amino-1:3-propanediol was obtained starting from p-nitroacetophenone. The latter compound was converted to p-nitro-ω-acetamidoacetophenone through p-nitrophenacyl bromide and its hexamine salt. p-Nitro-ω-acetamidoacetophenone on hydroxymethylation gave α-acetamido-β-hydroxy-p-nitropropiophenone. The latter compound on





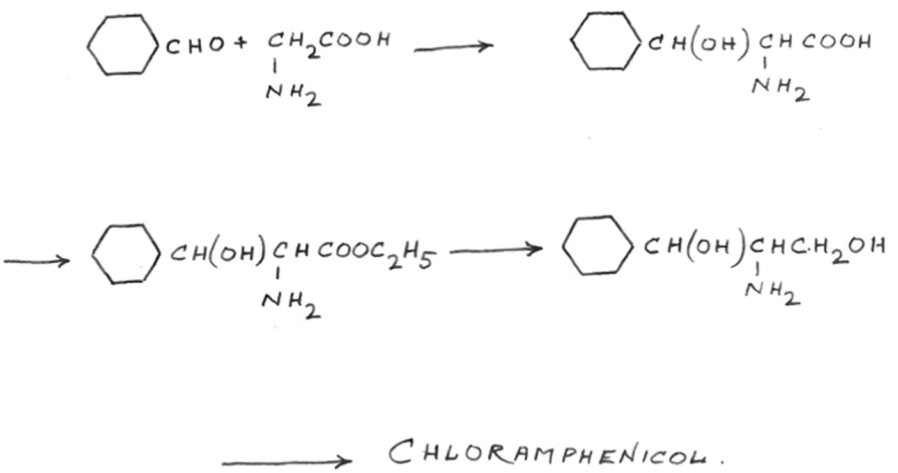
reduction and hydrolysis gave 1-p-nitrophenyl-2-amino-1:3-propanediol.

p-Nitro- ω -aminoacetophenone, an intermediate in the above synthesis by Long and Troutmann, was obtained by Sueo Tatsuoka[7] by a newer method. p-Tolylsulphonyl chloride was condensed with p-nitro-acetophenone oxime in pyridine. The resulting compound on treatment with sodium ethoxide followed

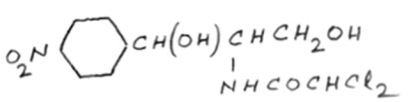


by hydrochloric acid gave p-nitro- -aminoacetophenone hydrochloride.

Several other routes for the synthesis of chloramphenicol have since been described. All these aim at getting either 1-p-nitrophenyl-2-amino-1:3-propanediol or 1-phenyl-2-amino-1:3-propanediol, starting from different materials. Carrara and Weitnauer[8] have, for example, obtained the latter compound starting from benzaldehyde and α-aminoacetic acid. The main steps in the synthesis were as follows:



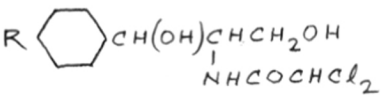
The relatively simple structure of chloramphenicol and the several routes available for its synthesis starting from simple organic compounds, have led to the preparation of a very large number of derivatives and analogues of chloramphenicol. In order to study the significance of the four functional groups present in chloramphenicol, a large number of analogues of chloramphenicol wherein one or more of these functional groups were altered, have been reported by several workers. The functional groups in the molecule are the p-nitrophenyl group, primary alcoholic group, secondary alcoholic group, and the dichloracetamido group. Following are some of the changes made in the functional groupings of chloramphenicol. All the different routes available for the synthesis of chloramphenicol had been used for the synthesis of these compounds:



CHLORAMPHENICOL.

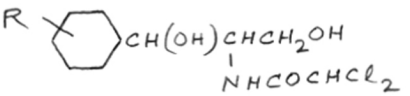
1. Changes in p-nitrophenyl grouping :

(a) Ring halogenated compounds :



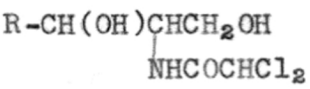
R=F, Br, Cl or I.[9,10,11]

(b) Change in the position of nitro group:



R=o or m nitro[12,12A]

(c) p-Nitrophenyl group had been replaced by the following groups:



R = 2:4-$Me(NO_2)C_6H_3$- ;[13-16]

= 3:4-$MeO(NO_2)C_6H_3$- ;

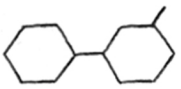
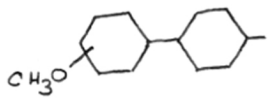
= 3:5-$NO_2(Cl)C_6H_3$- ;

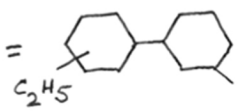
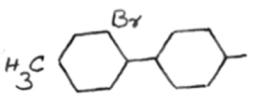
= 2:6:4-$Me(Cl)NO_2C_6H_2$- ;

= 4:5:2-$Me_2(NO_2)C_6H_2$- ;

= 2:5-$Cl(NO_2)C_6H_3$- ;

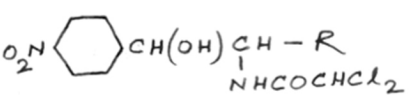
= $2\text{-}MeC_6H_4$- ; $3\text{-}MeOC_6H_4$- ;

R =  ;  ;

=  ;  ;

= 4-$CNC_6H_4$- ;

2. Changes in the primary alcoholic groups :



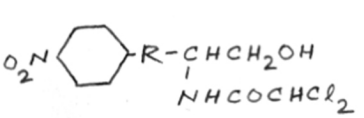
R = -$COOC_2H_5$ ; COOH [8,17,18]

= -$C_2H_5$ [19] ;

= -$COOCH_3$ ; [16]

= -$CH_3$ [20]

3. Changes in the secondary alcoholic group :



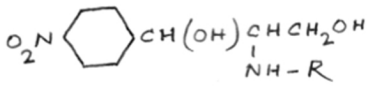
R = -$CH_2$ [19,21]

= -$(CH_2)_2$

= -$CH(CH_3)$- ;

Compounds without the secondary alcoholic groups have also been prepared.[19]

4. Changes in the dichloroacetamido group :[8,12a,20,22-25]



R = $-COCHClBr$; $-COCH_2Cl$ ; $-COCH(Me)Br$

= $-COCHBr_2$ ; $-COCCl_3$; $-COCH_2C\equiv N$

= $-COCH_2C_6H_5$ ; $-SO_2C_6H_5$ ; $-COCH_2I$

= $-COCH_2CH_2COOH$ ; $-COC_6H_5$ ; $-SO_2C_6H_4Cl$

= $-COC_6H_4COOH$(p) ; $-COC_6H_4NO_2$(p)

= $-SO_2C_6H_4CH_3$(p) ; $-COC_6H_4Cl$(p)

= $-COC_6H_4NH_2$

= $-COC_6H_3I_2$(3:5)

= $-COCHBr_2$; $-COCHBrCH_2Br$;

= $-COC(Br)(CH_3)_2$; $-COCH(Br)CH_2CH_3$

= $-COCBr=CH_2$

= ~~$-COCH_2Cl$~~; $-COCHClCH_3$ ; $-COCH(Cl)CH_2CH_3$

= ~~$-COCCl_3$~~; $-CO-C(Cl)=CH_2$

= $-COCH_2CH_3$ ; $-CO(CH)_2CH_3$ ; $-COCH(CH_3)_2$

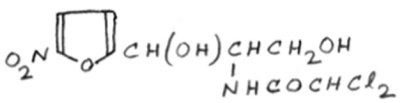
= $-CO-C(CH_3)_3$ ; $-CO-CH=CH-CH_3$

= $-COCH_2F$ ; $-COCHF_2$ ; $-COCF_3$

= ~~$-COCH_2Cl$~~; $-COCHFCl$ ; $-COCF_2Cl$

Among the analogues in which the ring system is entirely changed include the following :[26,27]

1.



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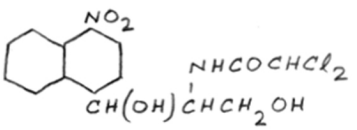

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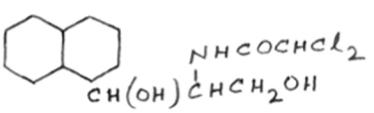
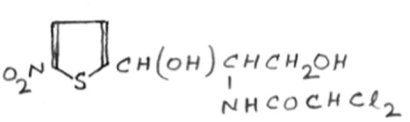


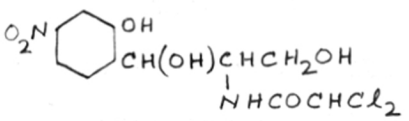
Partial synthesis of the thiophene analogue has also been reported.[30]



The changes listed before are, however, not all that have been made in the functional groups of chloramphenicol. A very large number of compounds were also prepared by changing more than one of the functional groups in chloramphenicol, as well as by other changes not listed ~~before~~ here.

# PRESENT WORK

Since the publications of Long, Troutmann, Rebstock and others on the constitution and synthesis of chloramphenicol (Chloromycetin),[2,3,4] numerous attempts have been made to modify and if possible improve its therapeutic activity by alterations in its structure. A study of the antibacterial activity of the large number of compounds prepared in this connection leads to the conclusion that any change in the functional groupings of chloramphenicol molecule results in a loss or dimunition of its antibacterial activity. The present work deals with the attempts to synthesize a hydroxy derivative (I) of chloramphenicol. Compound (I) contains all the structural features of chloramphenicol, and in addition, contains a hydroxyl group in the ortho position to the alkyl gr side chain, so that (I) is also partly related to the well known antitubercular drug, 4-aminosalicylic acid (PAS).



(I)

The different routes by which chloramphenicol has been synthesized can be roughly grouped as follows (vide Introduction):

(1) Starting from simple compounds like benzaldehyde or acetophenone, the alkyl side-chain of the chloramphenicol molecule is progressively built up. Most of these routes involve a reduction of a $-NO_2$- or $-COOC_2H_5$- groups in the side chain, followed by nitration in the ring, so as to get p-nitrophenyl alkyl compounds which are used as intermediates in the synthesis of chloramphenicol.

(2) Starting from p-nitroacetophenone, the side chain is built up to give chloramphenicol. In following this route, care is taken to see that no reagent which will affect the nitro group in the phenyl nucleus is employed.

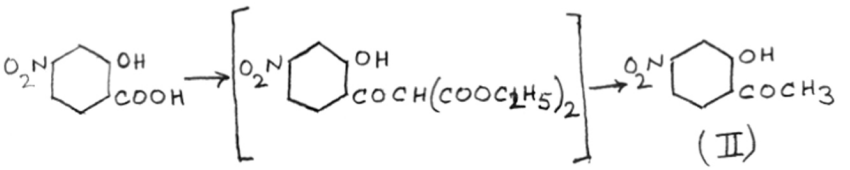
The choice of the route for the synthesis of the hydroxy derivatives (I) of chloramphenicol was governed by the following factors.

It was necessary to start with compounds with hydroxyl or methoxyl groups oriented ortho to the side chain and meta to the nitro group. This is necessary because nitration of the ring system containing a hydroxyl function, at a later stage, would give a

compound with a nitro group in para or ortho position to the hydroxyl function and not in the meta position as desired. It was also necessary to see that during the introduction of the alkyl side chain in (I), no reaction was employed which would affect the nitro group in the ring system.

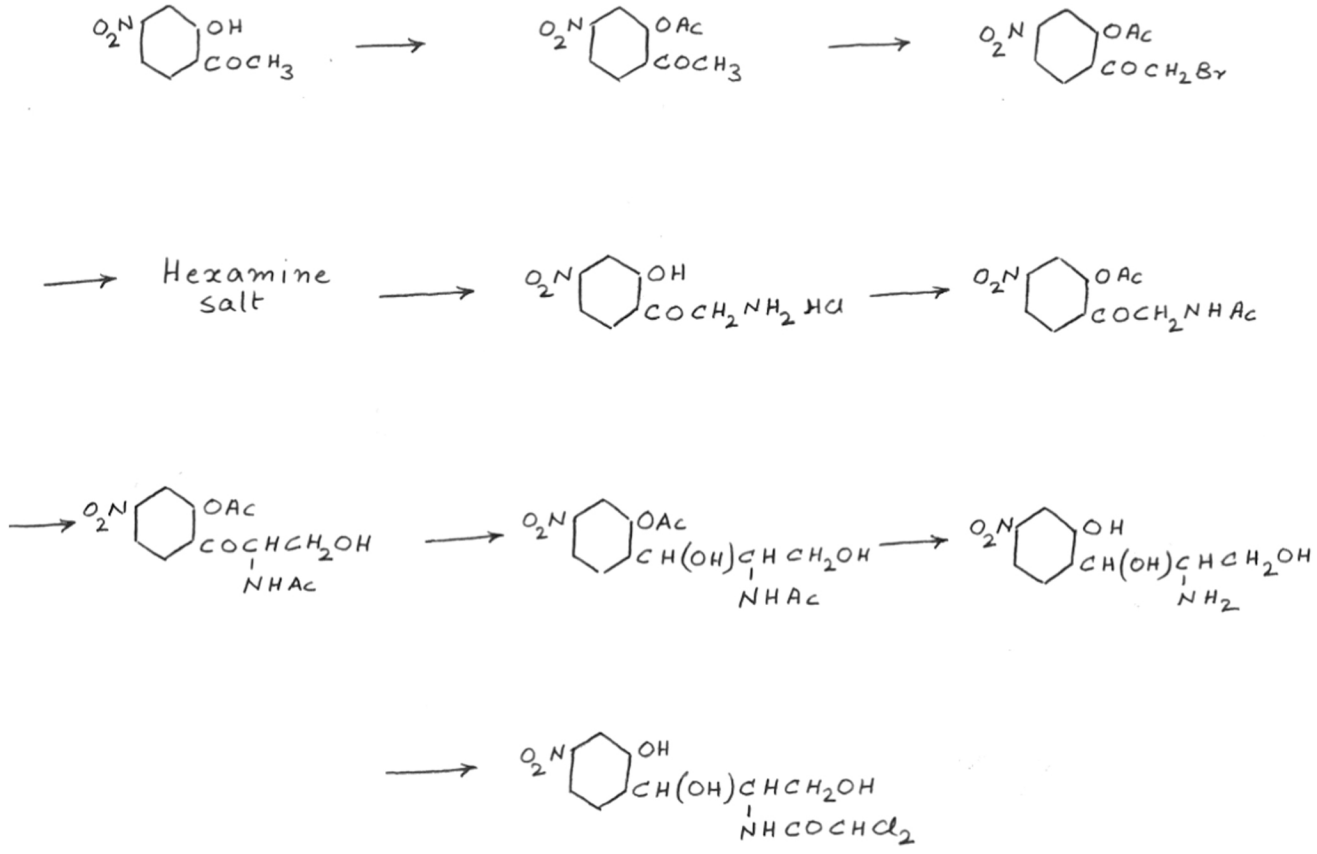
Keeping in view the above considerations, synthesis of (I) according to the scheme shown in Chart I was attempted. This scheme is analogous to that followed by Long and Troutmann[5] for the synthesis of chloramphenicol from p-nitroacetophenone.

A synthesis of 2-hydroxy-4-nitroacetophenone (II) was attempted in a manner analogus to the synthesis of p-nitroacetophenone described by Long and Troutmann.[5] But conversion of 4-nitrosalicylic acid to its acid chloride and condensation of the later with ethyl malonate and subsequent de-esterification and decarboxylation did not give the desired compound (II). Instead of (II), a small quantity of ethyl 4-nitro-




(II)

salicylate and a crystalline carboxylic acid were

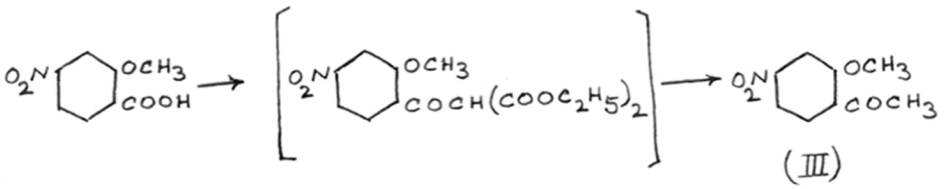

O2N
OH
COCH3
OAc
COCH2Br
Hexamine salt
COCH2NH2 HCl
COCH2NHAc
COCHCH2OH
NHAc
CH(OH)CHCH2OH
NH2
NHCOCHCl2

(I)

CHART I.

obtained. The acid still remains to be identified.

Protection of the hydroxyl group in 4-nitrosalicylic acid by acetylation, followed by its condensation with ethyl malonate also failed to give (II). The same two compounds, as obtained from 4-nitrosalicylic acid were again obtained. However, 2-methoxy-4-nitrobenzoic acid gave 2-methoxy-4-nitroacetophenone (III) in good yield. Demethylation of (III) with aluminium chloride gave (II). Further

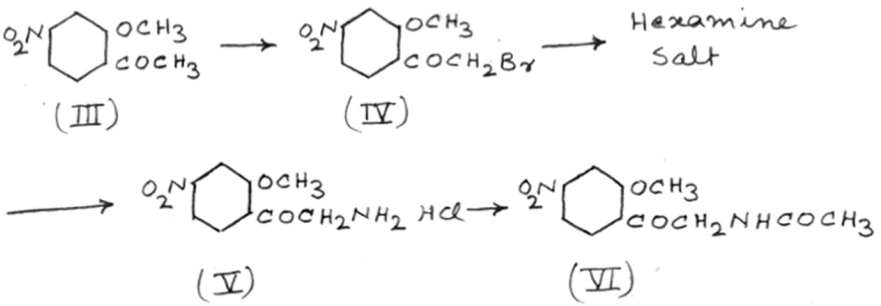


(III)

steps in the synthesis were, however, carried out starting from (III) instead of (II), as the hydroxyl group needs protection in the proposed synthesis.

2-Methoxy-4-nitroacetophenone (III) on bromination in acetic acid gave 2-methoxy-4-nitro-ω-bromoacetophenone (IV) in good yields. Addition of a solution of (IV) in chloroform to a stirred solution of hexamethylenetetramine in chloroform, gave a crystalline colourless compounds. The hexamine salt on hydrolysis with dilute hydrochloric acid in presence of alcohol gave 2-methoxy-4-nitro-ω-aminoacetophenone hydrochloride (V). Attempts

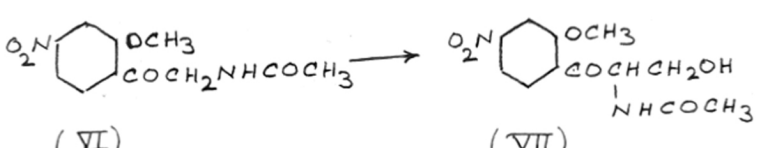
for purification of (V) by crystallization led to its decomposition. Acetylation of (V) gave 2-methoxy-4-nitro-ω-acetamidoacetophenone (VI).




(III) (IV) (V) (VI)

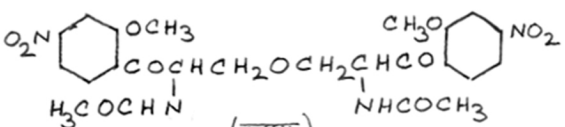
The yield of (VI) from (IV) was rather low. It is interesting to note that the yield of p-methoxy- and p-phenoxy-ω-acetamidoacetophenones from the corresponding phenacyl bromide is less than 40%,[29] whereas the yield of p-nitro-ω-acetamidoacetophenone from p-nitrophenacyl bromide is 67%.[5]

Hydroxymethylation of (VI) to give 2-methoxy-4-nitro-β-hydroxy-α-acetamidopropiophenone (VII) was next attempted. Although these attempts were unsuccessful, they however led to some interesting results. When (VI) was treated with aqueous

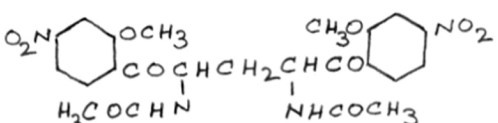


(VI) (VII)

formaldehyde in presence of ethyl alcohol and sodium bicarbonate for 3 1/2 hours, a compound melting at 75-90° was obtained. This compound could not be purified. But when the reaction was carried out for 5 1/2 hours, a compound melting at 170-80° (Compound A) was obtained. Compound (A) could not be purified by crystallization from usual solvents such as alcohol, acetic acid, chloroform, benzene, etc. It was therefore purified by dissolving in hot aqueous alcohol, from which it separated on cooling as an amorphous powder. After several purifications it melted at 225°. Elementary analysis of (A) however indicated that it was different from (VII). The analysis agreed with structures such as (VIII) and (IX). Formation of compounds similar to (IX) during hydroxy methylation



(VIII)

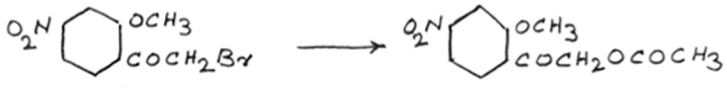


(IX)

of p-nitrophenyl acetamidomethyl ketone has also been reported earlier.[4] Definite identity of (A) has not been established as yet.

Treatment of (VI) with paraformaldehyde in methyl alcohol in presence of sodium bicarbonate led to a compound (B), m.p. 87? Elementary analysis of (B) indicated that it was different from the desired compound (VII). The analysis and the low melting point also indicated that it was different from (VIII) or (IX). Compound (B) analysed for C, 51.6; H, 4.4; N, 6.8%. The possible identity of (B) with 2-methoxy-4-nitro-ω-hydroxyacetophenone (X) was considered. The latter was therefore synthesized as follows.

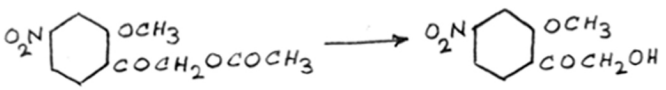
2-Methoxy-4-nitro-ω-bromoacetophenone (IV) when treated with potassium acetate in glacial acetic acid



(IV) (XI)

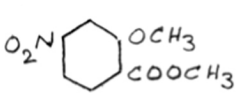
gave 2-methoxy-4-nitro-ω-acetoxyacetophenone (XI). The latter on hydrolysis gave (X), m.p. 140-41°. Compound (B), m.p. 87°, was thus different from (X). Compound (B) also did not give tests for either hydroxyl or

carbonyl functions. The elementary analysis of (B)



(XI) (X)

agreed with methyl 2-methoxy-4-nitrobenzoate (XII). The latter, prepared from 4-nitrosalicylic acid melted at 87°. The melting point of (B) was not lowered

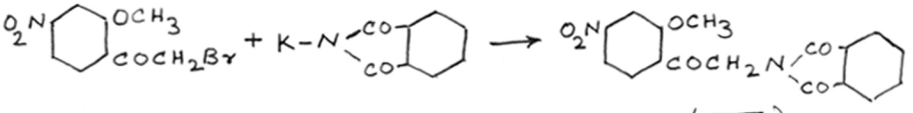


(XII)

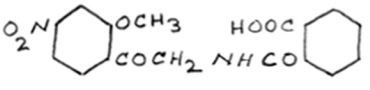
by admixture with (XII), proving the identity of the two compounds. The formation of (XII) from (VI) is remarkable as it shows that oxidation of carbon side chain in (VI) to a carbonyl group and esterification of the latter group has occurred even under the mild

conditions employed. All attempts to hydroxymethylate (VI) under variegated experimental conditions proved unsuccessful.

An alternative route to (VII) was to employ 2-methoxy-4-nitrophenyl phthalimidomethyl ketone (XIII) instead of (VI) in the hydroxymethylation reaction. Condensation of (IV) with potassium phthalimide gave, instead of the expected (XIII), a phthalamic acid (XIV) in low yields. As the yield of (XIV) was very low, the route was not followed further.



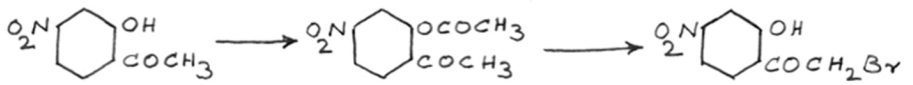
(XIII)



(XIV)

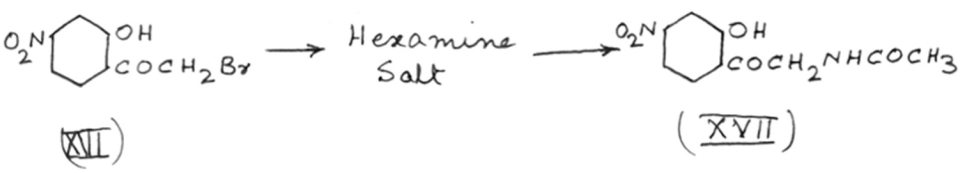
The synthesis of 2-methoxy-4-nitro-β-hydroxy-α-acetamidopropiophenone (VII) from (VI) having proved unsuccessful, attempts was then made to prepare 2-hydroxy-4-nitro-β-hydroxy-α-acetamidopropiophenone (XVIII), starting from 2-hydroxy-4-nitroacetophenone (II). Acetylation of (II) gave 2-acetoxy-4-nitroacetophenone

(XV). The latter on bromination gave 2-hydroxy-4-nitro- ω -bromoacetophenone (XVI), the acetyl group being cleaved during bromination.

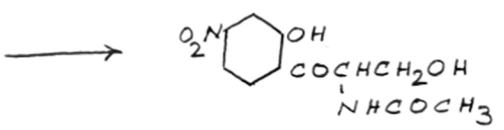


(II) (XV) (XVI)

Treatment of (XVI) with hexamine in chloroform led to a crystalline solid. Hydrolysis of the hexamine salt gave a very small quantity of a colourless solid, from which 2-hydroxy-4-nitro- ω-acetamidoacetophenone (XVII) could not be obtained by acetylation. Attempts to obtain (XVII) by acetylation of the mother liquor after removal of the solid from hydrolysis, were also unsuccessful.




(XVI) (XVII)

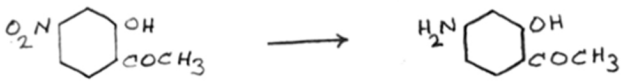


(XVIII)

Due to failure in obtaining the necessary intermediates (VII) and (XVIII), the proposed synthesis of the hydroxy derivative (I) of chloramphenicol could not be achieved. Other routes, which have been used for the synthesis of chloramphenicol, could not be employed for the reasons discussed earlier. It may be of interest to record that, whereas the synthesis of methoxy derivative of chloramphenicol in which this group is in the meta position with respect to the propanediol side chain has been reported, synthesis of the hydroxy and methoxy derivatives which has been unsuccessfully attempted in the present work has not been reported so far. Although (I) could not be synthesized, a number of interesting, hitherto unreported compounds related to 4-nitrosalicylic acid and 4-aminosalicylic acid, have been prepared. Biological activity of some of these compounds is described later.

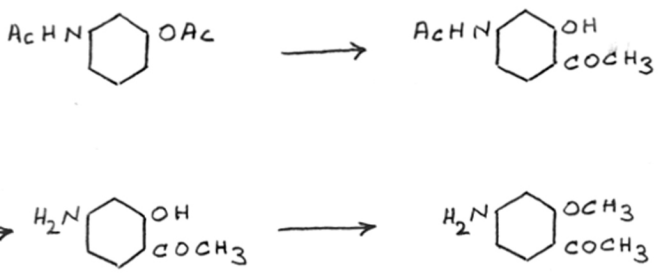
2-Methoxy-4-aminoacetophenone (XIX) and 2-hydroxy-4-aminoacetophenone (XX) were obtained by reduction of (III) and (II) respectively. These compounds are particularly interesting because of their close similarity with p-aminosalicylic acid (PAS). Compounds (XIX) and (XX) have also been prepared by

(XII) (XIX)



(II) (XX)

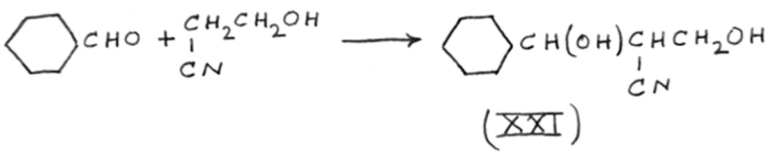
Marc Julia,[28] but by a different route starting from m-acetoxyacetanilide or m-methoxyacetanilide as follows:-



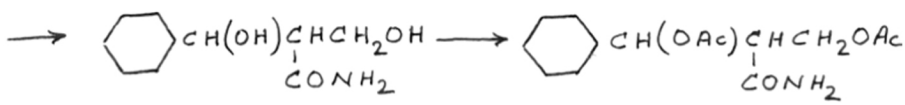
New approaches to chloramphenicol :

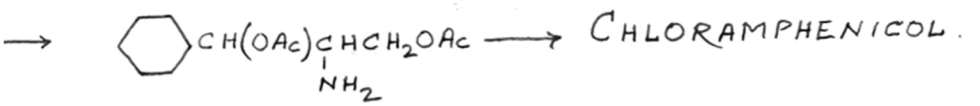
Two new routes for the synthesis of chloramphenicol have also been investigated. In view of the condensation of benzaldehyde with nitromethane and nitroethanol which ultimately leads to chloramphenicol, condensation of benzaldehyde with ethylene cyanohydrin was also investigated. Use of cyanohydrin may have advantages

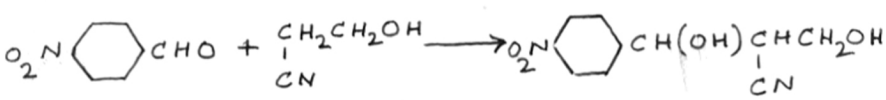
over nitro alkanes, as with the latter type of compounds, bis-condensation products are also formed. Reaction of ethylene cyanohydrin with benzaldehyde in alkali, however, did not give the expected 1-phenyl-2-cyano-1:3-propanediol (XXI), but benzoic acid was obtained by Cannizaro reaction. Reaction of ethylene cyanohydrin with p-nitrobenzaldehyde also, under similar conditions, gave p-nitrobenzoic acid instead of (XXII).



(XXI)

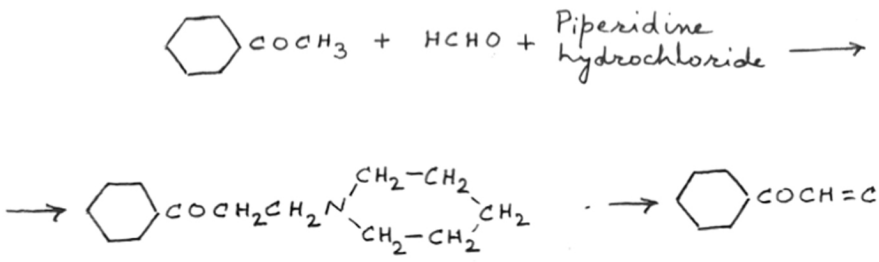






(XXII)

Vinyl phenyl ketone was the starting point in the second synthesis of chloramphenicol which was attempted. Condensation of acetophenone with




Vinyl phenyl ketone

paraformaldehyde and piperidine hydrochloride gave the "Mannich base", which on decomposition ~~g~~ by steam distillation gave vinyl phenyl ketone. The different steps in the proposed synthesis starting from vinyl phenyl ketone are outlined in Chart II.

Addition of a solution of ~~bri~~ bromine in water to vinyl phenyl ketone gave a pale yellow oil which distilled at 105-15°/2 mm. with partial decomposition. It has not been found possible to prepare an analytically pure sample from this oil. On the other hand, repeated distillations of this product ultimately led to a colourless liquid which analysed for phenylethylene oxide, and not for the desired α-bromo-β-hydroxy~~p~~propiophenone (XXIII).

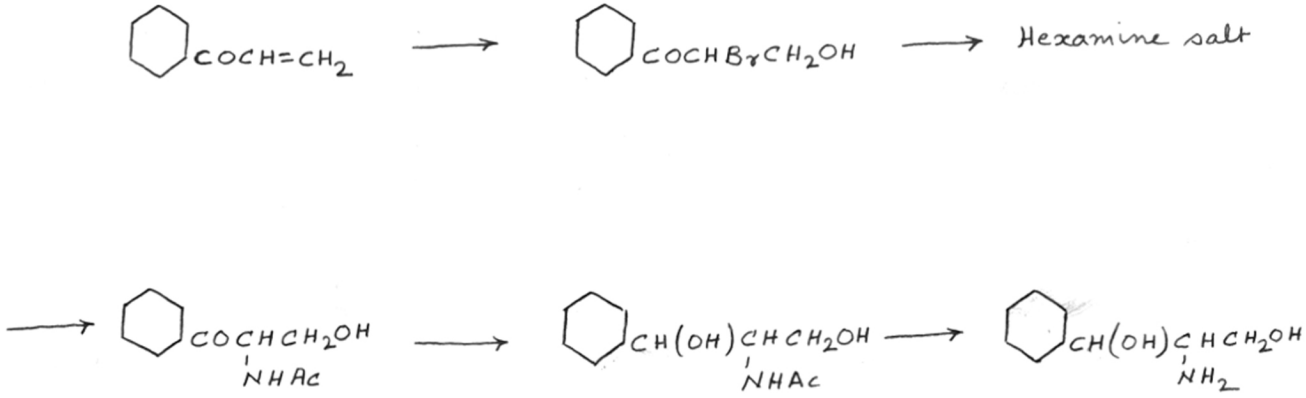
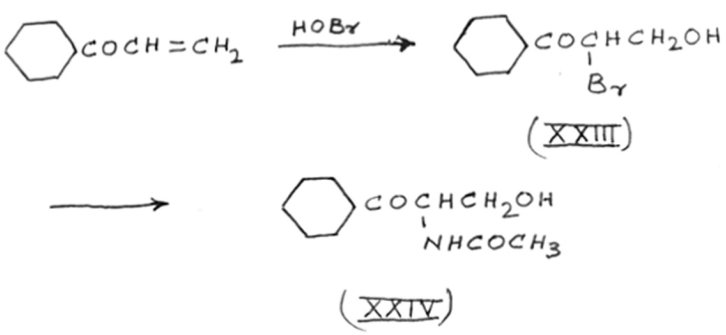

COCH=CH2
COCHBrCH2OH
Hexamine salt
COCHCH2OH
NHAc
CH(OH)CHCH2OH
NHAc
CH(OH)CHCH2OH
NH2
Further steps as in the synthesis of chloramphenicol by Controulis et al.3
Chloramphenicol

CHART II

The identity of this compound is not yet fully established. The crude product obtained by the addition of "HOBr" to vinyl phenyl ketone was therefore used as such in subsequent steps. On treatment with hexamethylene tetramine in chloroform solution, it gave a colourless hexamine salt. Crystallization of this product did not lead to an analytically pure product. On hydrolysis of the hexamine salt a small quantity of a colourless solid was obtained. Acetylation of this solid with acetic anhydride and sodium acetate, either under anhydrous conditions or in aqueous solution failed to give the desired α-acetamido-β-hydroxypropiophenone (XXIV).




Another approach to α-bromo-β-hydroxypropiophenone (XXIII) was to partially hydrolyse α:β-dibromopropiophenone. This was also unsuccessful.

# E X P E R I M E N T A L

2-Hydroxy-4-nitroacetophenone (II):

(a) From p-nitrosalicylic acid: p-Nitrosalicylic acid (14.64 g., 0.08 mole) was converted to its acid chloride by treating it with excess of thionyl chloride (15 cc.) in benzene (15 cc.), After refluxing for 4 hours, excess of thionyl chloride and benzene were removed under vacuum.

Magnesium (3.14 g., 0.088 mole) was placed in a three-necked flask fitted with a dropping funnel, condenser, thermometer and stirrer. It was covered with a mixture of absolute alcohol (2 cc.), carbon tetrachloride (2 cc.) and benzene (15 cc.).A small portion of a mixture of ethyl malonate (14.08 g., 0.088 mole), absolute alcohol (8 cc.) and benzene (30 cc.) was then added and the mixture heated to 50°. A small crystal of iodine was then added to initiate the reaction. When a vigorous reaction set in the remainder of the mixture was added slowly. After complete addition, the mixture was refluxed for 30 minutes and then cooled to 30°. The acid chloride prepared initially was dissolved in benzene (50 cc.) and was added slowly to above mixture under stirring.After about half the acid chloride was added, a gelatenious precipitate separated and stirring became difficult.After complete

addition, during which the temperature was maintained at 35°, the mixture was refluxed for 1/2 hour and then cooled to 10°. Dilute sulphuric acid (5 cc. of conc. acid in 30 cc. water) was then added slowly with stirring and external cooling. When all the unreacted magnesium was dissolved, the water layer was separated and rejected. Benzene was then removed under reduced pressure.

The residue was mixed with acetic acid (24 cc.) sulphuric acid (3 cc.) and water (16 cc.) and refluxed for 8 hours. The resulting mixture was cooled and poured on crushed ice. The solid which precipitated was filtered and treated with 5% sodium bicarbonate solution (50 cc.). The residue (0.9 g.) was filtered. It crystallized from aqueous alcohol (30%) in yellow needles, m.p. 83° (Found: C, 51.4; H, 4.0; N, 6.7. $C_8H_7NO_4$ (II) requires C, 53.03; H, 3.86; N, 7.7%). This compound was identified as ethyl-4-nitrosalicylate. The melting point of this compound was undepressed when mixed with an authentic sample of ethyl-4-nitrosalicylate (calculated for $C_9H_9NO_5$: C, 51.1; H, 4.2; N, 6.6%).

The bicarbonate solution on acidification gave a compound which crystallized from acetic acid in colourless plates, m.p. 197° (Found: N, 5.1%).

b) From O-acetyl-p-nitrosalicylic acid: When the above experiment was repeated using the acid chloride of O-acetyl-p-nitrosalicylic acid, the same two products were obtained.

2-Methoxy-4-nitroacetophenone (III):

2-Methoxy-4-nitrobenzoic acid (15.0 g.) was converted to its acid chloride by treatment with excess of thionyl chloride in benzene. The method described before for the attempted preparation of 2-hydroxy-4-nitroacetophenone (II) from 4-nitrosalicylic acid was then followed. The reaction gave a good yield (14.0 g.) of a substance insoluble in sodium bicarbonate solution. It crystallized from aqueous alcohol in yellow needles, m.p. 93° (Found: C, 55.4; H, 4.7; N, 6.7. $C_9H_9NO_4$ requires C, 55.4; H,4.6; N, 7.1%).

Semicarbazone of (III) was prepared by treating (III) with semicarbazide hydrochloride in pyridine. It crystallized from alcohol in yellow needles, m.p. 225-26° (Found: N,22.8; $C_{10}H_{12}N_4O_4$ requires N, 22.2%).

2-Hydroxy-4-nitroacetophenone (II) from (III):

a) A mixture of 2-methoxy-4-nitroacetophenone (III)(0.5 g.), aluminium chloride (0.5 g.) and dry nitrobenzene (10 cc.) was heated at 100° for 1 hour, cooled and poured in ice water containing hydrochloric

acid. It was extracted with ether and the ether layer washed several times with acidulated water.The ether extract was then extracted with 3% aqueous sodium hydroxide solution (30 cc.). On acidification, the alkaline extract gave a yellow compound (0.4 g.), which crystallized from aqueous alcohol in yellow needles, m.p. 66° (Found: C, 53.8; H, 3.9; N, 7.5. $C_8H_7NO_4$ requires C, 53.1; H, 3.9; N, 7.7%).

b) A mixture of 2-methoxy-4-nitroacetophenone (III)(0.5 g.), hydrobromic acid (3 cc., d.1.4) and acetic acid (4 cc.) was refluxed for 1 hour. It was then poured in ice water and the solid which precipitated was filtered. It crystallized from aqueous alcohol in yellow needles (0.3 g.), m.p.66°. It was found to be identical with (II) obtained before.

2-Methoxy-4-nitro-ω-bromoacetophenone (IV):

Bromine (0.2 cc.) was added to 2-methoxy-4-nitroacetophenone (III)(0.5 g.) in acetic acid (15 cc.) and the solution refluxed for 2 hours.Acetic acid was then partly removed by distillation under reduced pressure, and the residue was poured on crushed ice. The solid which precipitated was filtered and washed with water. It crystallized from n-hexane in light yellow needles,m.p.94-95° (Found: C, 39.5; H,3.1; N,5.4.

$C_9H_8BrNO_4$ requires C, 39.4; H, 2.9; N, 5.1%).

Hexamine salt of 2-methoxy-4-nitro-ω-bromoacetophenone (XV):

Hexamethylene tetramine (4.2 g., 0.03 mole) was dissolved in chloroform (20 cc.), and a solution of (II) (5.48 g., 0.02 mole) in chloroform (15 cc.) was added to it under stirring. The hexamine salt separated immediately as a colourless precipitate. It was stirred at room temperature (28°) for 3 hours and filtered. The solid was washed with chloroform and dried under vacuum (yield 7.1 g.). Attempts to purify led to its decomposition.

Hydrolysis of the hexamine salt of (IV):

a) Hexamine salt from 5.48 g. of (IV) was suspended in a mixture of conc. hydrochloric acid (15 cc.) and alcohol (7 cc.), and the mixture stirred for 16 hours at 30°. The hexamine salt first dissolved and later a yellow crystalline solid separated. It was filtered and washed with a small quantity of ice cold water and dried under vacuum (2.1 g.).

b) The hexamine salt from 5.48 g. of (IV) was suspended in 25 cc. of alcohol and dry hydrochloric acid gas was passed through it. On saturation a yellow product separated, which was filtered and washed with little ice-cold water (1.8 g.)

The yellow product (2-methoxy-4-nitro-ω-amino-acetophenone hydrochloride, V), could not be purified because of its decomposition on heating. Concentration of the mother liquors from (a) and (b) under vacuum also led to decomposition of the product.

2-Methoxy-4-nitro-ω-acetamidoacetophenone (VI):

The amine hydrochloride (V)(0.5 g.) was suspended in ice water (10 cc.) and acetic anhydride (1.5 g.) was slowly added to it under stirring. The temperature was maintained below 5° by external cooling. A solution of sodium acetate (2 g.) in water (10 cc.) was then added with stirring. The mixture was stirred for 1/2 hour during which the temperature was allowed to rise to 20°. It was then acidified with hydrochloric acid. The solid which separated (0.45 g.) was filtered and washed with water. It crystallized from aqueous alcohol in pale yellow needles, m.p. 165° (Found: C, 52.3; H, 5.1; N, 11.2. $C_{11}H_{12}N_2O_5$ requires C, 52.3; H, 4.6; N, 11.1%).

Hydroxymethylation of 2-methoxy-4-nitro-ω-acetamido-acetophenone (VI):

(a) Formaldehyde (36%)(0.4 cc.) and sodium bicarbonate (50 mgm.) were added to a suspension of (VI) in

alcohol (5 cc.). The mixture was stirred at 35-40° for 3 1/2 hours. The pale red coloured mixture was then filtered to remove sodium bicarbonate. The filtrate was cooled to 5° and as there was no separation of a solid, it was diluted with water and extracted with ether. The ether extract was dried and the ether removed. The pale yellow product (0.15 g.) m.p. 75-90° could not be purified.

The aqueous solution, left after the removal of the ether soluble product, was evaporated to dryness, when a dark sticky product was obtained which could not be purified.

(b) A mixture of (VI)(0.5 g.), formaldehyde (36%) (0.4 cc.), sodium bicarbonate (50 mgm.) and alcohol (5 cc.) was stirred at 35-40° for 5 1/2 hours. A pale yellow product, m.p. 170-80°, which separated on dilution of the reaction mixture, could not be crystallized from solvents such as alcohol, acetic acid, chloroform, etc. It was purified by repeated extractions with hot water. On cooling the aqueous solution, it separated as an amorphous powder. (This (compound A) on several such purifications melted at 225° (Found: C, 53.1; H, 4.7. $C_{12}H_{14}N_2O_6$ (VII) requires C, 51.1; H, 4.9%. $C_{24}H_{26}N_4O_{11}$ (VIII) requires C, 52.7; H, 4.7% and $C_{23}H_{24}N_4O_{10}$ (IX) requires C, 53.5; H, 4.6%).

(c) Use of sodium carbonate and methyl alcohol instead of sodium bicarbonate and ethyl alcohol in the above reaction also gave the same compound A. If the reaction was carried out at 60° or if large excess of formaldehyde (4 equivalents) was used, a sticky product resulted which could not be purified.

(d) A mixture of (VI)(1 g.), paraformaldehyde (0.120 g.), sodium bicarbonate (0.1 g.) and methyl alcohol (7 cc.) was stirred at 35-40° for 3 hours. Dilution of the yellow solution with water gave a crystalline yellow compound (B)(0.6 g.), m.p.81-85°. It crystallized from aqueous methanol in pale yellow needles, m.p. 87° (Found: C, 51.5; H, 4.3; N, 6.8. $C_{12}H_{14}N_2O_6$ (VII) requires C, 51.1; H, 4.9; N, 9.6%).

Compound B did not give tests for carbonyl or hydroxyl functions. It did not form either a p-nitrobenzoyl derivative or a semicarbazone. It was later identified as methyl-2-methoxy-4-nitrobenzoate (XII) m.p. 87°. The melting point of compound B was undepressed when mixed with an authentic sample of (XII)(Calc. for $C_9H_9NO_5$ (XII); C, 51.2; H, 4.3; N, 6.6%).

2-Methoxy-4-nitro-ω-acetoxyacetophenone (XI):

A mixture of 2-methoxy-4-nitro-ω-bromoacetophenone (IV)(1 g.), anhydrous potassium acetate (2 g.) and acetic

acid (10 cc.) was heated at 100° for 5 hours. It was then cooled and poured in ice cold water. The yellow solid (1.1 g.) which separated was filtered and washed with water. It crystallized from alcohol in yellow needles, m.p. 97° (Found: C, 52.3; H, 4.6; N, 5.2. $C_{11}H_{11}NO_6$ requires C, 52.2; H, 4.3; N, 5.5%).

Semicarbazone of (XI) crystallized from alcohol in yellow needles, m.p. 193° (Found: N, 18.7. $C_{12}H_{14}N_4O_6$ requires N, 18.1%).

2-Methoxy-4-nitro-ω-hydroxyacetophenone (X):

A suspension of (XI)(1 g.) in acetic acid (5 cc.) and water (20 cc.) was refluxed for 4 1/2 hours. After cooling, the mixture was neutralized with sodium bicarbonate. The yellow solid was filtered and crystallized first from aqueous alcohol and then from benzene-n-hexane mixture, when it gave yellow needles (0.7 g.), m.p. 140-41° (Found: C, 51.2; H, 4.9; N, 7.1. $C_9H_9NO_5$ requires C, 51.2; H, 4.3; N, 6.7%).

2-Methoxy-4-nitrophenyl phthalimidomethyl ketone (XIII):

A mixture of potassium phthalimide (5.5 g.), 2-methoxy-4-nitro-ω-bromoacetophenone (IV)(5.48 g.) and acetone (50 cc.) was refluxed for 6 hours. Acetone was then removed by distillation and water was added to the residue. The solid was filtered and washed. It

crystallized from benzene in yellow needles (0.7 g.), m.p. 185° (Found: C, 56.9; H, 4.1; N, 7.9. $C_{27}H_{12}N_2O_6$ (XIII) requires C, 60.0; H, 3.5; N, 8.2%); $C_{17}H_{14}N_2O_7$ (XIV, the phthalamic acid) requires C, 56.9; H,3.9; N,7.8%).

2-Acetoxy-4-nitroacetophenone (XV):

2-Hydroxy-4-nitroacetophenone (II)(1 g.), acetyl chloride (5 cc.) and a drop of pyridine were mixed together and refluxed for one hour. It was then cooled and poured in ice water. The solid which precipitated was filtered, washed with water and dried (0.9 g.). It crystallized from ether-n-hexane mixture in pale yellow needles, m.p. 66° (Found: C, 54.4; H, 3.6; N,6.3 $C_{10}H_9NO_5$ requires C, 53.8; H, 4.0; N, 6.3%).

2-Hydroxy-4-nitro-ω-bromoacetophenone (XVI):

Bromine (0.25 cc.) was added to a mixture of (XV)(1 g.) and glacial acetic acid (7 cc.). The mixture was refluxed for 3 hours, cooled and poured in ice water. The solid was filtered and dried (1.1 g.). It crystallized from ether-n-hexane mixture in yellow needles, m.p. 114-15° (Found: C, 37.1; H, 2.4; N, 5.2. $C_8H_6BrNO_4$ requires C, 36.9; H, 2.3; N, 5.4%).

Hexamine salt of 2-hydroxy-4-nitro-ω-bromoacetophenone (XVI):

Hexamethylenetetramine (2.1 g.) was dissolved in chloroform (15 cc.) and a solution of (XVI)(2.8 g.) in chloroform (10 cc.) was added to it with stirring. The colourless hexamine salt separated immediately. It was stirred at 30° for 3 hours and filtered.The solid was washed with chloroform and dried (3.2 g.) The salt could not be purified by crystallization.

Hydrolysis of hexamine salt of (XVI):

Hexamine salt from 2.8 g. of (XVI) was suspended in a mixture of conc. hydrochloric acid (8 cc.) and alcohol (5 cc.) and the mixture was stirred for 16 hours. at 30°. A small amount of a crystalline solid separated, which was filtered and washed with a small quantity of ice cold water. It was dried under vacuum (0.9 g.)

Acetylation of the product obtained by hydrolysis of hexamine salt of (XVI):

The product obtained by the hydrolysis (0.5 g.) fused sodium acetate (0.5 g.) and acetic anhydride (3 cc.) were mixed and heated at 100° for 3 hours. It was then cooled and poured in ice water. No solid separated. The solution was then extracted with ether, the ether layer was washed with sodium bicarbonate solution, and dried. On removal of ether, a sticky compound

was obtained which could not be purified.

(b) The hydrolysis product (0.5 g.) was suspended in water (5 cc.) and acetic anhydride (2 cc.) was slowly added with stirring. The temperature was maintained at 5° by cooling. A solution of 0.5 g. sodium acetate in water was then added to it with stirring. The mixture was stirred for 1/2 hour and then acidified with hydrochloric acid. As a solid compound did not separate, the mixture was extracted with ether. The ether extract was washed with sodium bicarbonate solution, then with water and dried. On removal of ether, a very small quantity of oil was obtained which could not be purified.

Attempts to obtain 2-hydroxy-4-nitro-ω-acetamido-acetophenone (XVII) by acetylation of the mother liquor after removal of the solid from hydrolysis by the second method (b) used before, were also unsuccessful.

2-Methoxy-4-aminoacetophenone (XIX):

2-Methoxy-4-nitroacetophenone (III)(1 g.) in alcohol (50 cc.) was reduced in presence of Raney nickel catalyst at 42 lbs./sq.in. hydrogen pressure for 6 hrs. The catalyst was then filtered off, and alcohol was removed by distillation. The resulting solid was crystallized from aqueous alcohol, when it gave colourless needles (0.85 g.), m.p. 121-22° (m.p. 121.5°

reported by Marc Julia[28]) (Found: C, 66.1; H, 6.7; N,7.8. Calc. for $C_9H_{11}NO_2$, C, 65.5; H, 6.7; N, 8.4%).

2-Hydroxy-4-aminoacetophenone (XX):

2-Hydroxy-4-nitroacetophenone (II)(1 g.) was dissolved in alcohol (35 cc.) and reduced in presence of Raney nickel catalyst under 42 lbs./sq.in. pressure for 8 hours. The catalyst was removed by filteration and washed with alcohol. The alcoholic solution on removal of alcohol gave a solid, which crystallized from aqueous alcohol in colourless needles (0.84 g.), m.p. 131° (m.p. reported by Marc Julia[28] 130°)(Found: C, 63.6; H, 6.0; N,9.2. Calc. for $C_8H_9NO_2$ ~~requires~~ C, 63.6; H, 5.9; N, 9.2%).

p
1-Phenyl-2-cyano-1:3-propanediol (XXI):

Benzaldehyde (10.6 g., 0.1 mole) and ethylene cyanohydrin (7.1 g., 0.1 mole) were dissolved in methyl alcohol (40 cc.) and cooled to -10°. A solution of sodium methoxide (2.7 g., 0.12 mole of sodium) in methanol (20 cc.) was added to it with stirring. Temperature was maintained at -10° and stirring continued for 1/2 hour. The white solid which separated was filtered and washed with methanol (yield 8.1 g.). It crystallized from aqueous alcohol in colourless needles

m.p. 122°. It was identified as benzoic acid. Its melting point was undepressed when mixed with pure benzoic acid.

1-p-Nitrophenyl-2-cyano-1:3-propanediol (XXII):

Reaction of p-nitrobenzaldehyde and ethylene cyanohydrin under the same conditions as in the foregoing experiment gave p-nitrobenzoic acid.

Vinyl phenyl ketone:

Piperidine hydrochloride [prepared by passing dry hydrochloric acid gas in piperidine (8.5 g.)], acetophenone (12 g.) and paraformaldehyde (4.5 g.) were added to absolute alcohol (30 cc.). The mixture was refluxed for 1 hour and paraformaldehyde (3 g.) was added to it. It was again refluxed for 2 hours.The hot solution was then poured in boiling acetone (250 cc.). The acetone solution on cooling gave the colourless crystalline "Mannich base" (phenyl-β-piperidinoethyl ketone hydrochloride), m.p. 183-87°. It was dissolved in 30 cc. water and steam distilled when vinyl phenyl ketone distilled over as an oil. It was separated by extraction with ether. On evaporation of ether, vinyl phenyl ketone was obtained as a pungent smelling oil (3.5 g.) This was used as such in next reactions as attempts

for its purification by distillation under vacuum led to a colourless solid polymer.

ϕ α-Bromo-β-hydroxypropiophenone (XXIII):

Vinyl phenyl ketone (3 g.) was suspended in water (200 cc.) and bromine (1.3 cc.) in water (100 cc.) was added to it dropwise under stirring. The temperature was maintained below 10° and a stream of carbon dioxide was passed through the solution. After the complete addition of the bromine solution, the temperature was allowed to rise to the room temperature. It was then extracted with ether and the ether extract dried. On removal of the ether, a pale yellow oil was obtained, which distilled at 105-15°/2 mm. with partial decomposition, and preparation of an analytically pure sample was not possible. On repeated distillation it gave a pale coloured oil, b.p. 85-90°/2 mm. (Found: C, 72.1; H, 5.8. $C_9H_9BrO_2$ (XXIII) requires C, 47.1; H, 3.9%).

Hexamine salt :

The oil (4.3 g.) obtained by bromination of vinyl phenyl ketone in water was dissolved in chloroform (15 cc.) and was added to a stirred solution of hexamethylene tetramine (2.9 g.) in chloroform (15 cc.)

The mixture was stirred for 1/2 hour and the solid which separated was filtered and washed with chloroform (yield 3 g.).

Hydrolysis of the hexamine salt and acetylation:

The hexamine salt (3 g.) was suspended in a mixture of alcohol (7 cc.) and hydrochloric acid (10 cc.), and the suspension was stirred at room temperature for 8 hours. A colourless cyrstalline solid separated which was filtered and washed with little cold water (yield 0.8 g.).

The solid was dissolved in water (10 cc.), the solution was cooled to 5° and acetic anhydride (3 cc.) was slowly added to it with stirring. A solution of sodium acetate (1.5 g.) in water was then added to it. The stirring was continued for 1/2 hour and temperature was maintained at 10°. It was then acidified with hydrochloric acid. No solid separated.The solution was then extracted with ether, the extract washed with sodium bicarbonate solution and dried. On removal of ether, a very small quantity of a sticky compound was obtained. It could not be purified.

The attempt for acetylation of the solid from hydrolysis with acetic anhydride and sodium acetate at

100° also failed to give the desired compound (XXIV).

α:β-Dibromopropiophenone:

Bromine (2.4 cc.) was added to a solution of vinyl phenyl ketone (6 g.) in carbon tetrachloride (75 cc.) It was refluxed for 1/2 hour and carbon tetrachloride was removed by distillation.The residue was crystallized from petroleum ether when it gave colourless plates of α:β-dibromopropiophenone, m.p. 56°.

Hydrolysis of α:β-dibromopropiophenone:

α:β-Dibromopropiophenone (1 g.) was suspended in water (15 cc.) and boiled under reflux for 7 hours. The brown oil obtained was separated by extraction with ether. It could not be purified as distillation under vacuum led to its decomposition.

The oil obtained formed a salt with hexamethylene tetramine in chloroform, but its hydrolysis with hydrochloric acid followed by acetylation failed to give (XXIV).

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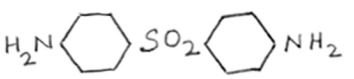
# Part II

# INTRODUCTION

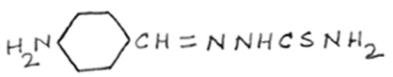
An intensive search for chemotherapeutic agents, which will be effective in the treatment of tuberculosis has been in progress for the past several years. Hundreds of compounds of various type have been examined for their antitubercular properties. Although many of them inhibit the growth of Myco. tuberculosis, at high dilution in various media, the activity was often considerably reduced in presence of blood or serum. Only few of these compounds have been found to retain their antitubercular activity in experimental animals. These researches have led to the discovery of some very valuable antitubercular substances. Among these, the more important agents, which are used in treatment of tuberculosis in man, are: (1) derivatives of diaminodiphenyl sulphone, (2) p-aminobenzaldehyde thiosemicarbazone, (3) p-aminosalicylic acid, (4) isonicotinic acid hydrazide, (5) the phenazine dye, B.283, and (6) the natural antibiotics streptomycin, neomycin, viomycin and terramycin.

Diaminodiphenyl sulphone was developed as a natural corrolary to the use of sulphanilamides in chemotherapy. Sulphathiazole and sulphathiadiazoles initiated the search which resulted in the finding of

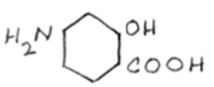
antitubercular properties of p-aminobenzaldehyde thiosemicarbazone. The attempts to find a compound which would antagonise the effect of salicylic acid in increasing the respiration of tubercle bacillus, led to the discovery of p-aminosalicylic acid (PAS)[1], although the mechanism of the action of PAS is different from that expected. On the basis of PABA-sulphanilamide relationship, antitubercular properties of isonicotinic acid hydrazide were discovered during a routine check of a number of compounds by



DIAMINODIPHENYL SULPHONE.

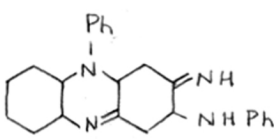


p-AMINOBENZALDEHYDE THIOSEMICARBAZONE.



PAS.

ISONICOTINIC ACID HYDRAZIDE.



B.283.

pharmaceutical firms.[2] Successive modifications of the structure of the lichen product, diplocin, resulted in the discovery of the phenazine dye B.283.[3] Waksman

discovered streptomycin during a routine examination of soil organisms.[4] A systematic search of various soil organism led to the discovery of the other antibiotics-neomycin, viomycin and terramycin.

4-Aminosalicylic acid (PAS) has, due to its low toxicity and usefulness in the treatment of tuberculosis, attracted wide attention and considerable efforts have been directed towards its economic synthesis and manufacture. It has also led to the preparation and study of the antitubercular properties of its derivatives and compounds analogous to it.

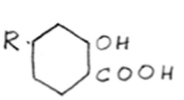
The two main methods for the preparation of PAS are: (1) carboxylation of m-aminophenol and (2) reduction of 4-nitrosalicylic acid. Numerous patents describe the manufacture of PAS.[5] The method, in general, consists in heating m-aminophenol, aqueous potassium carbonate and carbon dioxide under pressure. A British Patent[6] claims a yield of 92% by heating m-aminophenol, solid carbon dioxide and potassium carbonate without the use of any solvents at 150° for 12 hours.

Martin prepared PAS by the carboxylation of m-aminophenol using modified Kolbe conditions.[7] Siedel and Bittner[8] had obtained PAS by reduction of

4-nitrosalicylic acid by tin and hydrochloric acid. Bhate et al.[9] have developed a procedure for the preparation of 4-nitrosalicylic acid starting from 4-nitro-o-toluidine, a commercially available dye-stuff intermediate (Fast Scarlet G Base). Preparation of derivatives from PAS is difficult because of the instability of PAS towards heat. Several derivatives of PAS are accessible from 4-nitrosalicylic acid which is more stable.

The simple structure of PAS and the presence of three functional groups in the molecule have led to the preparation of numerous derivatives of PAS and compounds analogous to it. Some of the derivatives of PAS and other compounds analogous to PAS, which have been prepared and examined for their antitubercular properties, are listed below. This list is by no means complete as hundreds of PAS analogues have been prepared and the purpose of giving the list is to illustrate the scope of structural variation which is possible in PAS.

Monofunctional derivatives of PAS

a) 

R = ~~XXXXXXXX~~ $-NHCOCH_2CH_3$; [10-12]

$-NHCOCH_2CH_2CH_3$ ; $-NHCOCH(CH_3)_2$ ;

$-NHCOCH(CH_2CH_3)_2$ ; $-NHCO(CH_2)_8CH_3$ ;

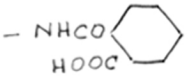
$-NHCO(CH_2)_{12}CH_3$ ; $-NHCO(CH_2)_{16}CH_3$ ;

$-NHCOCH_2N(CH_3)_2$ ; $-NHCOCH_2N(CH_2CH_3)_2$ ;

$-NHCOC(CH_3)HN(CH_3)_2$ ; $-NHCOCH = CHCH_3$ ;

$-NHCOPh$; [10,13] $-NHCO-C_6H_4-O-CH_2CH_3$

- $NHCOC_6H_4NH_2$ (p);
- $NHCOC_6H_4NH_2$ (m);
- $NHCOCH_2Ph$ ;
- $NHCOCH_2CH_2Ph$;
- $NHCOCH = CHPh$ ;
- $NHCOCH_2CH_2COOH$;
- $NHCOCH=CHCOOH$ ;



;

- $NHCOCH_2SO_3Na$;

11,12,14

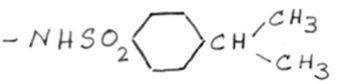
- $NHCH_3$ ; $-NHCH_2Ph$ ; - $N(CH_2CH=CH_2)_2$

- $NHCONH_2$ ; $-NHCONHPh$;

- $NHCOOC_2H_5$; $-NHCOO(CH_2)_3CH_3$ ;

$-NHCOO(CH_2)_{11}CH_3$ ;

- $NHSO_2CH_3$ ; $-NHSO_2Ph$ ; $-NHSO_2C_6H_4CH_3$ ;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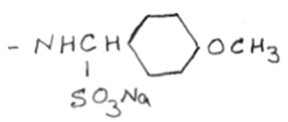
;

- $NHSO_2CH_2Ph$ ;

- $NHCH_2SO_3Na$;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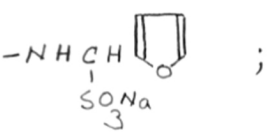
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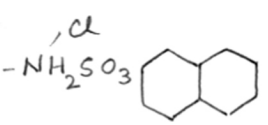
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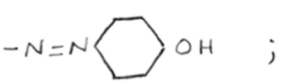
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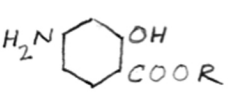
(b)



R = Methyl; ethyl; propyl; isopropyl;[13,14,15,17]

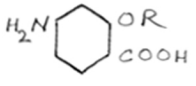
n-amyl;

$-NH_2$; [redacted]

$-NHCH_3$ ; $-NHCH_2H_5$; $-NHC_3H_7$;[18]

$-N(Et)(Et)$ ; $-N(C_3H_7)(C_3H_7)$ ; $-NHCH_2Ph$ ;

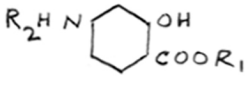
c)



R = methyl, ethyl; [11,17]

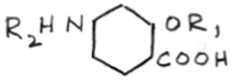
Difunctional derivatives of PAS[10]

a)



1. $R_1 = C_2H_5$; $R_2 = -COCH_3$;
2. = " = -COPh;
3. = " = $-COCH_2Ph$;
4. = " = -COCH=CH-COOH;
5. = $C_3H_7$ = $-COCH_3$
6 = $CH_3$ = -COPh
7. = $CH_3$ = $-COCH_3$
8. = $CH_3$ = $-NHSO_2C_6H_4NHCOCH_3$

b)



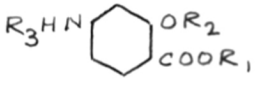
$R_1 = COCH_3$ ; $R_2 = COCH_3$ [10]

c)

$R_1 = CH_3$ ; $R_2 = CH_3$; $C_2H_5$

$= COCH_3$ ; $R_2 = CH_3$; $C_2H_5$

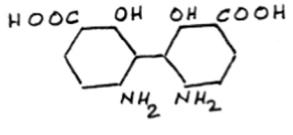
## Trifunctional derivatives of PAS [10]



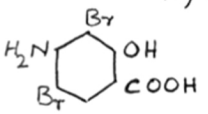
1. $R_1 = CH_3$; $R_2 = COCH_3$; $R_3 = COCH_3$
2. $R_1 = CH_3$; $R_2 = COC_6H_5$; $R_3 = COC_6H_5$

## Nuclear substituted derivatives of PAS

II.



10, 15.

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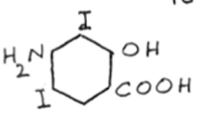
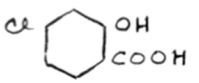
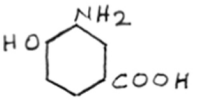
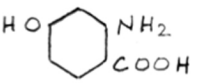
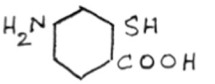


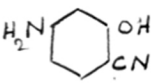

## Compounds analogous to PAS



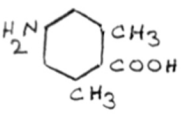



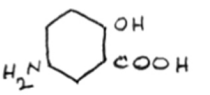







Most of the compounds listed here have been tested for antitubercular activity. Whereas no definite conclusions can be arrived at as regards the relation between chemical constitution and antitubercular activity of PAS-type of compounds, nevertheless the following broad generalizations can be made:-

1. Among the monosubstituted derivatives of PAS, compounds in which the amino group was substituted were less active than PAS.

2. Substitution in the hydroxy group also reduces the activity.

3. Many esters of PAS have nearly the same tuberculostatic activity as the free acid, but the amides are less active.

4. Among derivatives of PAS, wherein two of the three functional groups in PAS were substituted, only a few of those in which the $NH_2$ and COOH groups were substituted, had tuberculostatic activity comparable to ~~p~~ PAS.

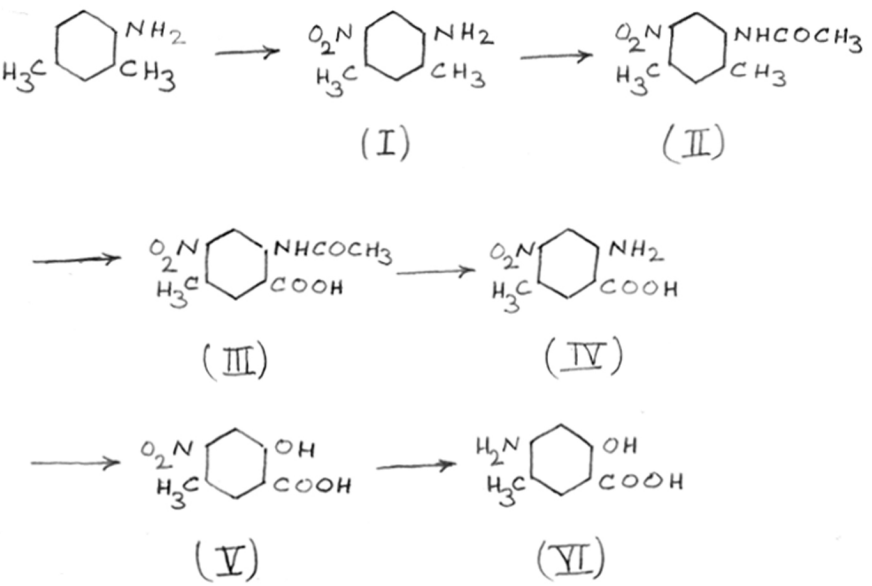
5. Most of the analogues and compounds similar to PAS are inactive.

## Present Work

Since the discovery of its high antitubercular properties and low toxicity, p-aminosalicylic acid (PAS) has been widely used in the treatment of tuberculosis. A few other chemotherapeuticals are also being used in the treatment of tuberculosis. The study of the chemical structure of these antitubercular drugs shows, that no strict relation between antitubercular activity and chemical structure can be established. The antitubercular activity of many of these compounds was discovered during routine check. A large number of derivatives of PAS and compounds analogous to it have been prepared and similarly tested. Though none of the derivatives of PAS or its analogues are as useful as PAS, many of them possess high antitubercular properties. The possibility of a PAS derivative or its analogue having high antitubercular property, therefore, always exists. It was, therefore, proposed to prepare some derivatives and analogues of PAS during the present work.

Though a large number of derivatives of PAS have been prepared, comparatively few nuclear substituted analogues of PAS have been prepared. 5-Methyl-4-aminosalicylic acid, a C-methyl derivative of PAS, was synthesized. The starting material for this compound

was m-4-xylidine, which on nitration gave m-6-nitro-4-xylidine (I). The latter compound on acetylation gave 4-acetamido-6-nitro m-xylene (II). Oxidation of (II) with potassium permanganate gave 2-acetamido-4-nitro-5-methylbenzoic acid (III). 2-Amino-4-nitro-5-methylbenzoic acid (IV) was obtained by deacetylation of (III). Diazotization of (IV) followed by



hydroxylation gave 4-nitro-5-methylsalicylic acid (V). When reduced in presence of Raney nickel catalyst, (V) gave 5-methyl-4-aminosalicylic acid (VI).

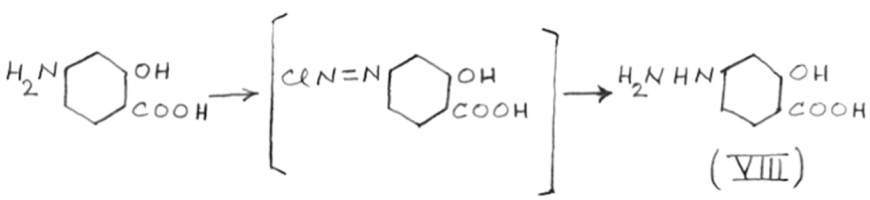
An attempt to synthesize 6-carboxy-7-hydroxyquinoline (VII), an analogue of PAS, was unsuccessful. 4-Aminosalicylic acid when subjected to Skraup

reaction failed to give (VII). A dark product which could not be ~~probably~~ purified was obtained. This was probably due to the high ~~temperature~~ temperature involved during the reaction at which temperature PAS is unstable. An attempt to prepare (VII) from

(VII)

methyl-4-aminosalicylate was also unsuccessful.

Instability of PAS towards heat was probably also responsible for the failure in getting 3-hydroxy-4-carboxyphenylhydrazine (VIII) from PAS. 4-Aminosalicylic acid on diazotization followed by reduction with

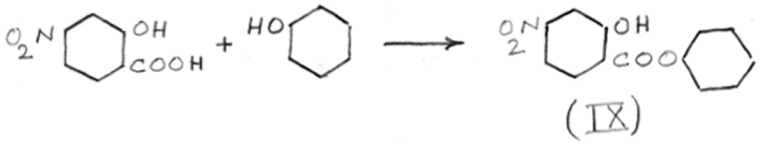


(VIII)

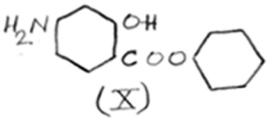
sodium bisulphite did not give (VIII).

Some esters and ethers of PAS were also prepared starting from 4-nitrosalicylic acid. 4-Nitrosalicylic ~~acid~~ acid and phenol when heated together in presence of phosphorus oxychloride at 140° gave phenyl 4-nitrosalicylate (IX). Reduction of (IX) with

hydrogen in presence of Raney nickel gave phenyl-4-



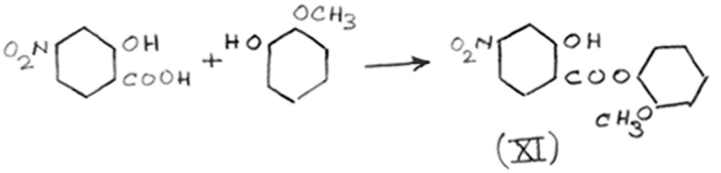
(IX)



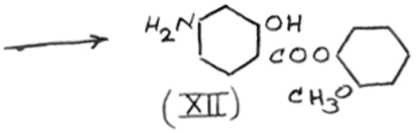
(X)

aminosalicylate (X).

Reaction of 4-nitrosalicylic acid and guaiacol as above gave 2-methoxyphenyl 4-nitrosalicylate (XI). 2-Methoxyphenyl 4-aminosalicylate (XII) was



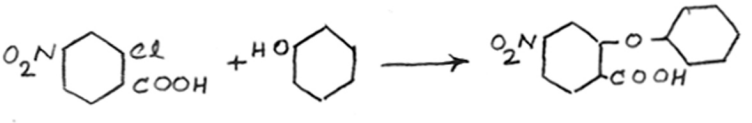
(XI)



(XII)

obtained by reduction of (XI).

2-Chloro-4-nitrobenzoic acid was used as the starting material for the preparation of ethers of PAS. Phenol and 2-chloro-4-nitrobenzoic acid, when heated in nitrobenzene in presence of copper and

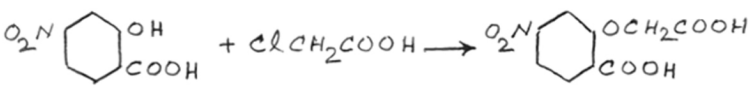


(XIII)

anhydrous potassium carbonate, gave 2-phenoxy-4-nitrobenzoic acid (XIII). On reduction of (XIII) ~~I~~ 2-phenoxy-4-aminobenzoic acid (XIV) was obtained.

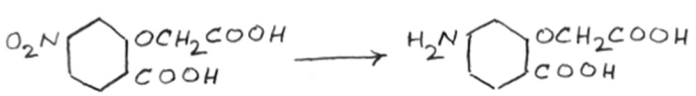
(XIII) (XIV)

4-Nitrosalicylic acid and chloroacetic acid, when heated in nitrobenzene in presence of copper and anhydrous sodium carbonate, did not give O-carboxymethyl-4-nitrosalicylic acid (XV), but when 4-nitro-



(XV)

salicylic acid and chloroacetic acid were dissolved in sodium hydroxide solution and refluxed for 6 hours, (XV) was obtained. O-Carboxymethyl-4-aminosalicylic acid (XVI) was obtained from (XV) by reduction with hydrogen in presence of Raney nickel. The compound (XVI), however, does not melt sharply.



(XV) (XVI)

It may be mentioned that although 4-nitrosalicylic acid has a sharp melting point, 4-amino-salicylic acid (PAS) starts decomposing before its melting point.

# EXPERIMENTAL

m-6-Nitro-4-xylidine (I):

m-4-Xylidine (10 g.) was dissolved in sulphuric acid (54 cc.) and the solution was cooled to 5°. A mixture of nitric acid (70%, 5.2 cc.) and sulphuric acid (5.3 cc.) was added slowly under stirring. Temperature was kept below 8° by external cooling. The solution was then stirred for 5 hours, and kept overnight in a refrigerator. The solution was then poured into crushed ice and neutralized with 50% aqueous sodium hydroxide. The orange product which separated on crystallization from alcohol gave needles, m.p. 122°.

4-Acetamido-6-nitro-m-xylene (II):

m-6-Nitro-4-xylidine (I)(5 g.), acetic anhydride (7 cc.) and fused sodium acetate (5 g.) were mixed together and heated at 120° for 1/2 hour. The mixture was poured in ince water and the solid which separated was collected and crystallized from dilute alcohol when it gave needles, m.p. 158°.

2-Acetamido-4-nitro-5-methylbenzoic acid (III):

4-Acetamido-6-nitro-m-xylene (II)(5 g.) was suspended in a solution of magnesium sulphate (12 g.) in water. The mixture was boiled and a solution of

potassium permanganate (15 g., 4%) was added dropwise with stirring, keeping the mixture at the boil. The mixture was filtered hot and the cooled filtrate acidified with hydrochloric acid. The product which separated was collected and extracted with aqueous sodium bicarbonate solution. The sodium bicarbonate solution on acidification gave a product which crystallized from alcohol in pale yellow needles, m.p. 220°. Purification of the bicarbonate insoluble part gave back (II)(1.8 g.)

2-Amino-4-nitro-5-methylbenzoic acid (IV):

A mixture of 2-acetamido-4-nitro-5-methylbenzoic acid (III)(5 g.) and hydrochloric acid (10%, 50 cc.) was refluxed for 2 hours, then cooled and carefully neutralized to litmas by addition of solid sodium carbonate. The product which separated wascrystallized from dilute alcohol when it gave orange yellow plates, m.p. 238°.

4-Nitro-5-methylsalicylic acid (V):

2-Amino-4-nitro-5-methylbenzoic acid (IV)(1 g.) was dissolved in sulphuric acid (40%, 15 cc.), cooled to 0° and diazotized with sodium nitrite solution at 0-5°. The diazonium solution was then poured into 40% boiling sulphuric acid in a thin stream. The

mixture was boiled further till a test portion did not give a red precipitate when mixed with an alkaline solution of β-naphthol. The reaction mixture was cooled and diluted when a dark product separated. It crystallized from dilute alcohol in pale yellow needles, m.p. 198° (Found: N, 6.7. $C_8H_7NO_5$ requires N, 7.1%).

5-Methyl-4-aminosalicylic acid (VI):

4-Nitro-5-methylsalicylic acid (1 g.) was dissolved in alcohol (10 cc.) and reduced in presence of Raney nickel under hydrogen pressure (42 lbs. per sq.inch) for 6 hours in a Parr hydrogenator. The solution was filtered and alcohol removed by distillation. The residue crystallized from alcohol in pale brown needles, m.p. 164° (Found: C, 57.7; H, 5.2; N, 8.2 $C_8H_9NO_3$ requires C, 57.5; H, 5.4; N, 8.3%).

Attempts to synthesise 6-carboxy-7-hydroxyquinoline (VII):

Finely powdered ferrous sulphate (0.2 g.), glycerine (1.8 g.), 4-aminosalicylic acid (0.8 g.) and 4-nitrosalicylic acid were mixed together. Sulphuric acid ~~were mixed together~~ (1.5 cc.) was then added and the mixture was heated at 135-40° for 1 1/2 hours. The mixture was cooled and poured into water and the precipitate which separated was collected

and purified by extraction with aqueous sodium bicarbonate solution and ~~xdifixx~~ acidification of the alkaline solution. The dark compound obtained could not be purified.

The above reaction when carried out with 50% sulphuric acid also failed to give the desired compound. A sticky product which could not be purified was obtained. Use of methyl-4-aminosalicylate and methyl 4-nitrosalicylate did not give the desired compound either.

Attempts to prepare 3-hydroxy-4-carboxyphenylhydrazine (VIII):

4-Aminosalicylic acid (5 g.) was dissolved in hydrochloric acid (15 cc.), cooled to 0° and diazotized with sodium nitrite solution at 0-5°. The diazonium solution was added to a cooled solution of sodium bisulphite (15 g. in 100 cc. of water). It was then slowly warmed to 60°, acidified with hydrochloric acid, and heated at 60° for 5 hours. It was then cooled to 0°, but no compound separated. The solution was then neutralized and extracted with ether. The ether extract on removal of ether gave a little sticky compound which could not be purified.

Phenyl 4-nitrosalicylate (IX):

4-Nitrosalicylic acid (6.1 g.) and phenol (3.7 g.)

were mixed and heated at 130° for 15 minutes. The mixture was cooled, phosphorous oxychloride (3.5 cc.) added, and the contents were then heated at 130-40° for 3 1/2 hours.After cooling, the mixture was poured into water and the solid which separated was filtered, washed first with water and then with dilute sodium bicarbonate solution.The undissolved solid was crystallized from alcohol when it gave pale yellow needles, m.p. 142° (Found: N, 5.4. ~~C₁₃H₉NO₅~~ requires $C_{13}H_9NO_5$:N, 5.4%).

Phenyl 4-aminosalicylate (X):

Phenyl 4-nitrosalicylate (IX)(1 g.) was dissolved in alcohol and reduced inpresence of Raney nickel under hydrogen pressure (42 lbs. per sq.inch.) for 6 hours. The mixture was then filtered, alcohol removed by distillation and the residue crystallized from dilute alcohol when it gave a colourless needles, m.p. 142° (Found: C, 68.7; H, 4.7; N, 6.2. $C_{13}H_{11}NO_3$ requires C, 68.2; H, 4.8; N, 6.1%).

2-Methoxyphenyl 4-nitrosalicylate (XI):

A mixture of 4-nitrosalicylic acid (3.6 g.) and guaiacol (2.5 g.) was heated at 130° for 15 mins. After cooling phosphorus oxychloride (2 cc.) was added and

the mixture was again heated at 140° for 3 1/2 hours. The product was extracted with water and the residue filtered washed with water and sodium bicarbonate solution and finally crystallized from alcohol when it gave pale yellow plates, m.p. 106° (Found: N, 5.1. $C_{14}H_{11}NO_6$ requires N, 4.8%).

2-Methoxyphenyl 4-aminosalicylate (XII):

2-Methoxyphenyl 4-nitrosalicylate (1 g.) was dissolved in benzene and reduced in presence of Raney nickel under hydrogen pressure for 6 hours. After separation of catalyst by filtration benzene was removed by distillation and the residue crystallized from alcohol when it gave colourless needles, m.p. 139-40° (Found: C, 65.3; H, 5.2; N, 5.4. $C_{14}H_{13}N_4O_4$ requires C, 65.2; H, 5.0; N, 5.3%).

2-Phenoxy-4-nitrobenzoic acid (XIII):

A mixture of 2-chloro-4-nitrobenzoic acid (5 g.) phenol (5 g.), anhydrous potassium carbonate (5 g.) and copper powder (0.5 g.) was heated at 150° for 5 hours. The reaction mixture was diluted with water and phenol was removed by steam distillation. The solution was filtered hot, cooled and the filtrate acidified. The precipitate obtained was collected and

and crystallized from dilute alcohol when it gave needles, m.p. 157°.

2-Phenoxy-4-aminobenzoic acid (XIV):

2-Phenoxy-4-nitrobenzoic acid (XIII)(2 g.) was dissolved in alcohol and reduced in presence of Raney nickel under hydrogenpressure (42 lbs.per sq.inch). The catalyst was filtered off and alcohol removed by distillation.The residue crystallized from dilute alcohol in colourless needles, m.p. 142-43° (Found: C, 68.7; H, 4.7; N, 6.3. $C_{13}H_{11}NO_3$ requires C, 68.2; H, 4.8; N, 6.1%).

O-Carboxymethyl-4-nitrosalicylic acid (XV):

a) 4-Nitrosalicylic acid (5 g.), chloroacetic acid (5 g.), anhydrous sodium bicarbonate (5.5 g.),copper (0.5 g.) and nitrobenzene (25 cc.) were refluxed together for 1 hour. After removal of nitrobenzene by steam distillation, the mixture was filtered to remove copper and the filtrate acidified.The product which separated was crystallized from dilute alcohol when it gave pale yellow needles, m.p. 230-34°.The latter was identified as unreacted 4-nitrosalicylic acid.

b) 4-Nitrosalicylic acid (5 g.) and chloroacetic

acid (2.7 g.) were dissolved in an aqueous solution of sodium hydroxide (4 g. in 25 cc.) and the solution was refluxed for 6 hours. After cooling, the solution was acidified.The product which separated was boiled with 400 cc. of water and filtered.The filtrate on concentration gave a product which crystallized from water in yellow needles, m.p. 179-80° (Found: N, 5.8. $C_9H_7NO_7$ requires N, 5.8%).

O-Carboxymethyl-4-aminosalicylic acid (XVI):

The above phenoxyacetic acid (XV)(0.5 g.) was dissolved in alcohol (10 cc.) and treated with Raney nickel under hydrogen pressure for 6 hours. The reduction product, isolated as usual, crystallized from water in pale brown plates, which melted with decomposition between 172-80° (Found: C, 51.8; H, 4.3; N, 6.6. $C_9H_9NO_5$ requires C, 51.14; H,4.2; N,6.6% ).

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# Part III

Some of the compounds prepared during the present work (Part I and II) have been tested for their antitubercular, antibacterial and anthelmintic properties. The results obtained are discussed below.

1. Antitubercular activity:

5-Methyl-4-aminosalicylic acid and O-phenyl-4-aminosalicylic acid were tested for tuberculostatic properties by Mr.J.C.Puri of Central Research Institute, Kasauli. These were found to be inactive (Puri, Ind. J. Med. Research, 1952, 40, 1). 2-Methoxy-4-nitroacetophenone, 2-hydroxy-4-nitroacetophenone, 2-methoxy-4-nitro-ω-bromoacetophenone and 2-methoxy-4-nitro-ω-acetamidoacetophenone were tested by Prof. B.V.Bhide of the S.P.College, Poona. None of these compounds have appreciable tuberculostatic activity. Antitubercular activity of other compounds are being studied.

2. Antibacterial activity:

Tests for the antibacterial properties of some compounds were carried out by Mrs.S.R.Shah and Mr. M.L. Khorana of this Department.The tests were carried out by the broth dilution method against two strains, viz. Staphylococcus aureus and E.typhus. Table I shows the

Table I

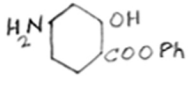
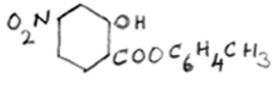
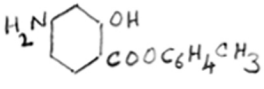
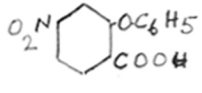
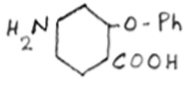
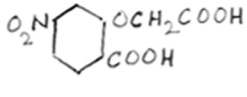
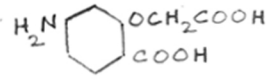
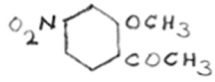
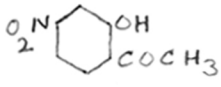
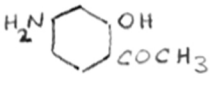
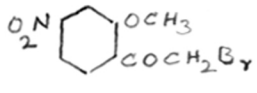
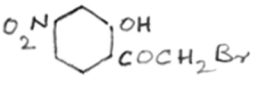
| | Compound | St. aureus | | | E. typhus | | |
|---|---|---|---|---|---|---|---|
| | | 1:10,000 | 1:20,000 | 1:40,000 | 1:10,000 | 1:20,000 | 1:40,000 |
| 1 |  | + | + | + | + | + | + |
| 2 |  | + | + | + | + | + | + |
| 3 |  | + | + | + | + | + | + |
| 4 |  | - | + | + | - | + | + |
| 5 |  | + | + | + | + | + | + |
| 6 |  | + | + | + | + | + | + |
| 7 |  | + | + | + | + | + | + |

Table I (contd.)

| | Compound | St. aureus | | | E. typhus | | |
|---|---|---|---|---|---|---|---|
| | | 1:10,000 | 1:20,000 | 1:40,000 | 1:10,000 | 1:20,000 | 1:40,000 |
| 8 |  | + | + | + | + | + | + |
| 9 |  | + | + | + | + | + | + |
| 10 |  | - | + | + | - | + | + |
| 11 |  | - | + | + | - | + | + |
| 12 |  | - | - | - | - | - | - |
| 13 |  | - | - | - | - | - | - |

results of these tests. The positive sign indicates growth of the organism at the particular dilution and the negative sign indicates absence of growth.

These tests have shown that 2-hydroxy-4-nitroacetophenone, 2-hydroxy-4-aminoacetophenone and 2-methoxyphenyl-4-aminosalicylic acid prevented the growth of St.aureus and E.typhus at a dilution of 1:10000, but were inactive at further dilution. 2-Methoxy-4-nitro-ω-bromoacetophenone and 2-hydroxy-4-nitro-ω-bromoacetophenone however prevented the growth of these organisms at a dilution of 1:40000. All other compounds were found to be inactive.

3. Anthelmintic activity:

The anthelmintic tests were carried out by Mr.L.R. Gunay and Mr.M.L.Khorana of this Department. The following general procedure, which was found to be more suitable, was used for the testing, in which earthworms were used as test worms (Gunay, M.Sc.Tech. Thesis, Bombay University, 1954). Hexyl resorcinol was used as standard anthelmintic for comparison.

Six earthworms were placed in a beaker containing 100 cc. of test solution of a particular concentration. Two worms were removed at different intervals, washed with

Table II

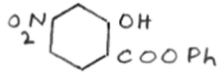
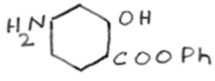
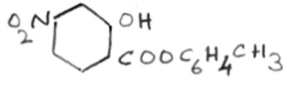
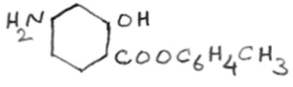
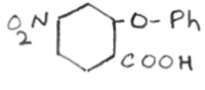
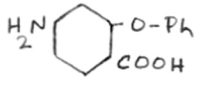
| Compound | 1/200 | | | 1/1000 | | | 1/5000 | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C |
| Hexylresorcinol | .... Instantaneous death ..... | | | | | | 1 | 2 | 3 |
| 1.  | 5 | 8 | 10 | 10 | 15 | 20 | 30 | 40 | 50 |
| 2.  | - | - | 2 | 3 | 4 | 5 | 10 | 15 | 20 |
| 3.  | 6 | 8 | 10 | 12 | 17 | 25 | 35 | 45 | 60 |
| 4.  | 4 | 5 | 6 | 8 | 9 | 10 | 15 | 21 | 29 |
| 5.  | 6 | 7 | 8 | 11 | 13 | 18 | 25 | 35 | 40 |
| 6  | 5 | 6 | 7 | 10 | 12 | 15 | 20 | 25 | 35 |

Table II (contd.)

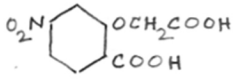
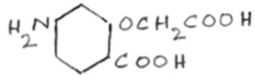
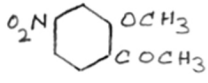
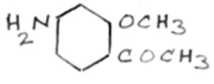
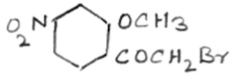
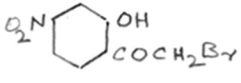
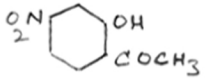
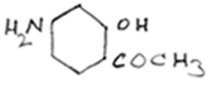
| | Compound | 1/200 | | | 1/1000 | | | 1/5000 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C | A | B | C |
| 7. |  | 5 | 6 | 7 | 10 | 12 | 15 | 20 | 25 | 35 |
| 8 |  | 7 | 8 | 9 | 12 | 15 | 20 | 30 | 40 | 50 |
| 9 |  | 25 | 30 | 35 | 40 | 45 | 50 | 72 | 100 | 110 |
| 10 |  | 1 | 2 | 3 | 4 | 5 | 6 | 10 | 20 | 30 |
| 11 |  | - | - | 1 | - | - | 2 | 3 | 4 | 5 |
| 12 |  | ..... Instantaneous death ..... | | | | | | 1 | 2 | 3 |

Table II (contd.)

| | Compound | 1/200 | | | 1/1000 | | | 1/5000 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C | A | B | C |
| 13 |  | - | - | 1 | 4 | 5 | 6 | 8 | 11 | 16 |
| 14 |  | - | - | 1 | 6 | 7 | 8 | 10 | 15 | 20 |

distilled water, and placed in fresh water. Their condition was observed ~~in fresh water~~ from time to time for 24 hours and the results for each were recorded as follows:

(A) Time when all worms became fatally injured - both worms when taken out were living, but either died in 24 hours or remained benumbed for the period of observation.

(B) Time when 50% of the worms died - when one of the worms taken out was found dead and of the rest some were dead and some were alive.

(C) Time when all worms died - when both worms taken out were ~~dire~~ dead as also the rest.

All the compounds were tested at three concentrations: 1/200, 1/1000 and 1/5000. The results obtained are recorded in Table II. It shows for different concentrations, the time in minutes, required for the worms to attain the condition given above in (A), (B) and (C).

The anthelmintic tests have shown that 2-hydroxy-4-nitro-ω-bromoacetophenone has activity comparable to hexylresorcinol. 2-Methoxy-4-nitro-ω-bromoacetophenone is the only other compound with comparative anthelmintic properties. These two compounds need further investigation as regards their usefulness as antibacterial and anthelmintic agents.

# SUMMARY

The thesis consists of three parts. Part I deals with the attempts to synthesize a hydroxy derivative of chloramphenicol, which is partly related to PAS. Preparation of some esters and ethers of 4-aminosalicylic acid and 4-nitrosalicylic acid have been described in Part II. The results of the antitubercular, antibacterial and anthelmintic tests of compounds prepared during the present work (Part I and II) constitute Part III.

## Part I

Synthesis of the hydroxy derivative, 1-(θ-hydroxy-p-nitro)phenyl-2-dichloroacetamido-1:3-propanediol (I) of chloramphenicol, which is also partly related to PAS, was attempted. 2-Methoxy-4-nitroacetophenone (III) was obtained from 2-methoxy-4-nitrobenzoic acid. 2-Methoxy-4-nitro-ω-acetamidoacetophenone (VI) was prepared from (III) through 2-methoxy-4-nitro-ω-bromoacetophenone (IV) and its hexamine salt. All attempts to prepare 2-methoxy-4-nitro-α-acetamido-β-hydroxypropiophenone (VII) by hydroxymethylation of (VI) were unsuccessful. When (VI) was treated with aqueous formaldehyde in presence of ethyl alcohol and sodium bicarbonate, a compound (A) melting at 170-80°, was obtained. Definite identity of this compound has not been established. Treatment of

(VI) with paraformaldehyde in presence of methyl alcohol and sodium bicarbonate gave a compound (B) melting at 87°. The possible identity of compound (B) with 2-methoxy-4-nitro-ω-hydroxyacetophenone (X) was considered to be possible because of the close similarity of the elementary analysis of (B) with (X). Compound (X) was, therefore, synthesized starting from (IV) but was found to be different from (B). Compound (B) was later identified as methyl-2-methoxy-4-nitrobenzoate. The formation of the latter compound (identical with B) from (VI) is remarkable in view of the fact that oxidation of (VI) to 4-nitro-2-methoxybenzoic acid has taken place even under the mild conditions of experiment, followed by methylation of the latter acid.

The preparation of (VII) through 2-methoxy-4-nitrophenyl phthalimidomethyl ketone (XIII) was also attempted. Condensation of (IV) with potassium phthalimide gave a phthalamic acid (XIV) instead of (XIII). The route was not followed further because of very low yields.

2-Hydroxy-4-nitroacetophenone (II) was obtained from (III). Acetylation of (II) followed by bromination gave 2-hydroxy-4-nitro-ω-bromoacetophenone (XVI). 2-Hydroxy-4-nitro-ω-acetamidoacetophenone (XVII) could

not be obtained from (XVI), through the hexamine salt of the latter compound.

2-Methoxy-4-aminoacetophenone (XIX) and 2-hydroxy-4-aminoacetophenone (XX) were obtained by reduction of (III) and (II) respectively.

Two new routes for the synthesis of chloramphenicol were also investigated. Condensation of benzaldehyde and p-nitrobenzaldehyde with ethylene cyanohydrin in alkali did not give the desired compounds, but benzoic acid and p-nitrobenzoic acid were obtained. Attempts to obtain α-amino-β-hydroxypropiophenone, an intermediate in the synthesis of chloramphenicol, from vinyl phenyl ketone, and also from α:β-dibromopropiophenone were unsuccessful.

## Part II

5-Methyl-4-aminosalicylic acid (VI), a C-methyl derivative of 4-aminosalicylic acid (PAS), was synthesized starting from m-4-xylidine. Attempts to synthesize 6-carboxy-7-hydroxyquinoline and 3-hydroxy-4-carboxyphenyl-hydrazine from 4-aminosalicylic acid were unsuccessful.

Some ethers and esters of 4-nitrosalicylic acid were also prepared. These include phenyl-4-nitrosalicylate

(IX), 2-methoxyphenyl-4-nitrosalicylate (XI), 2-phenoxy-4-nitrobenzoic acid (XIII) and O-carboxymethyl-4-nitrosalicylic acid (XV). The corresponding derivatives of 4-aminosalicylic acid were obtained by reduction of the nitro compounds mentioned above.

## Part III

5-Methyl-4-aminosalicylic acid, O-phenyl-4-aminosalicylic acid and some derivatives of p-nitroacetophenone and p-aminoacetophenone were tested for tuberculostatic properties, but none was found to be active. Among the compounds tested for antibacterial and anthelmintic activity, 2-hydroxy-4-nitro-ω-bromoacetophenone and 2-methoxy-4-nitro-ω-bromoacetophenone have good antibacterial as well as anthelmintic activity. 2-Hydroxy-4-nitro-ω-bromoacetophenone has anthelmintic activity comparable to hexylresorcinol.

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M. R. Paranjape

(Candidate)

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