# THE PREPARATION AND BIOLOGICAL PROPERTIES OF CERTAIN AMIDINES

A thesis presented in fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Glasgow.

by

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## Notes regarding the text.

1. References to the literature are indicated by ordinary numerals enclosed in brackets. These refer to the Bibliography, where references are given in full, and which is in alphabetical order according to the authors' surnames.
2. Relevant references are grouped before the description of procedure in each preparation.
3. Structural formulae are indicated by Roman numerals.
4. All melting and boiling points are uncorrected.
5. Where the literature gives a melting or boiling point for a compound, it is quoted after the value found.

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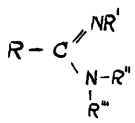
# INTRODUCTION

## HISTORICAL SURVEY

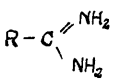
A review of the chemistry of the amidines has recently been made by Shriner and Neumann (161).

### I. DEFINITION

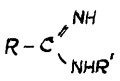
Amidines are strong monoacid bases, alkaline in aqueous solution and forming stable crystalline salts. The general formula is:



where R,R',R'',R''' is hydrogen, an alkyl, or aryl radicle, or a substitution product. The experimental work of this thesis is concerned with unsubstituted (I) and mono-N--substituted (II) amidines.



(I)



(II)

### II. NOMENCLATURE

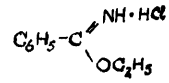
Generally, the amidine is named after the acid produced on hydrolysis (this is the method adopted by "Chemical Abstracts"). Thus, $C_6H_5C(:NH)NH_2$ is named "benzamidine".

In some instances it is difficult or inconvenient to name the amidine after the carboxylic acid and Shriner and Neumann (161) recommend that the amidine group in such cases should be referred to as "carboxamidine"; for example, $H_2NC(:NH)(CH_2)_{11}C(:NH)NH_2$ would be called "undecane-1:11-di carboxamidine". However, it is more frequently referred to as "undecane-1:11-diamidine" or "1:11-diamidinoundecane". Some authors (33,178) have referred to amidines as carbazylic acids, ammonocarboxylic acids, amimides or imidoamides, but the terminology "amidine" is almost universal.

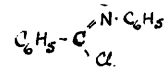
The two nitrogen atoms of the amidine grouping are usually designated N and N', but, as is shown below, no differentiation in property can be made between the two atoms in unsubstituted or monosubstituted derivatives. The carbon atoms adjacent to the amidine grouping are termed $\alpha$, $\beta$, $\gamma$, etc. in the normal manner.

In the preparation of an amidine from a nitrile, the intermediate addition product of the nitrile with an alcohol is most correctly termed an imidoester (i.e. an ester of the aci-form of the amide). Sidgwick (162) and most other authors, however, have named these compounds "iminoethers".

For example, (III) is called benzimino ethyl ether hydro--chloride (or ethyl benzimidate hydrochloride).



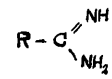
(III)



(IV)

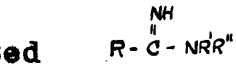
Similarly, the chloro derivative of an N-substituted amide is generally called an iminochloride but can also be referred to as an imidyl chloride. (IV) is N-phenyl--benziminochloride or N-phenylbenzimidyl chloride.

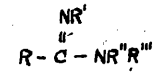
Amidines have been classified into five types, based on the number and position of the substituents on the nitrogen atoms:

A. Unsubstituted 

B. Monosubstituted 

C. Symmetrical disubstituted 

D. Unsymmetrical disubstituted 

E. Trisubstituted 

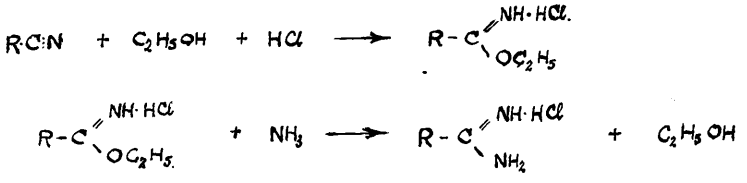
It will be noticed that the monosubstituted and symmetrical disubstituted amidines can each exist in two tautomeric forms.

III. PREPARATION

A. Unsubstituted Amidines

1. From nitriles through imino-ethers

The original work on the preparation of amidines was done by Pinner in 1877 (132,139) and his method is still the most useful laboratory procedure. A detailed description of a typical preparation can be found in Organic Syntheses (44). The nitrile is dissolved or suspended in anhydrous alcohol (generally ethyl alcohol) into which dry hydrogen chloride is passed under cooling. The iminoether hydrochloride formed is treated with dry alcoholic ammonia to give the amidine:



Depending on the relative basicities of the amidine and ammonia, the amidine is produced as hydrochloride or as free base.

The method is of very general application and both aliphatic and aromatic nitriles readily undergo the reaction Ashley and others (5,52) have prepared the amidines from a large number of aromatic dinitriles by the method, while King, Lourie and Yorke (86) have synthesised some diamidinoalkanes.

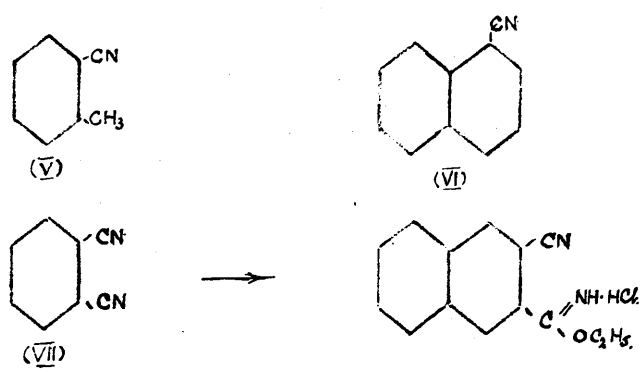
Other alcohols may replace ethanol (139) and hydrogen bromide can be used instead of hydrogen chloride. The optimum conditions for the formation of the iminoether from a nitrile have been defined (5a) as the presence of 2.5 to 3 equivalents of alcohol for each (aromatic) nitrile group and saturation with dry hydrogen chloride under anhydrous conditions.

An exception to the general procedure was found necessary for the formation of the iminoethers from chloro derivatives of phenoxyacetonitrile (p. 145 ). The reaction with alcohol in the presence of hydrogen chloride took place so readily in this instance that the proportions of reagents had to be reduced to the theoretical to prevent the formation of undesired products. Pinner (139) suggested that unstable chlorides of the type R.CCl(OR')$NH_2$.HCl may be formed when excess hydrogen chloride is

used, and that these chlorides decompose into esters and ammonium chloride. These two products were, in fact, isolated from the action of excess alcoholic hydrogen chloride on o-chloro-phenoxyacetonitrile (p.143), but Ashley (5a) has found no evidence of Pinner's unstable chlorides.

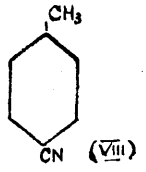
Steric factors - the "ortho effect".

Steric effects enter into the iminoether formation by Pinner's method. Some ortho-substituted aromatic nitriles will not react at all with ethyl alcohol and hydrogen chloride and it was concluded (135,136) that an ortho group interferes if it contains carbon attached directly to the nucleus. Thus, o-tolunitrile (V), 2:6-dimethylbenzonitrile and $\alpha$-naphthonitrile (VI) (41) did not react at all, while phthalonitrile (VII) formed only a mono-iminoether:

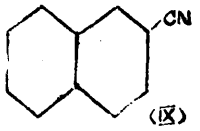



In addition, 2-nitro-4-methylbenzonitrile did not react. In contrast, p-tolunitrile (VIII) and $\beta$-naphthonitrile (IX)

formed iminoethers normally, while the other two isomeric dicyanobenzenes (m and p) gave di-iminoethers (106).



(VIII)



(IX)

This "ortho effect" was extended when it was found (98) that o-chlorobenzonitrile also would not react, but it has been shown that o-hydroxy-, o-ethoxy-, and 2:5-dimethoxybenzamidine can be made in the usual way (46,138). It was not found possible, however, to secure 2:4-dihydroxybenz--amidine directly from the nitrile.

Conditions for iminoether formation.

The manipulative difficulty of the method lies partly in the sensitivity of the iminoether to moisture. The decomposition with water mainly follows the course:

$$R\text{-}C\overset{NH}{\underset{OR'}{}} + H_2O \longrightarrow R\text{-}C\overset{O}{\underset{OR'}{}} + NH_3$$

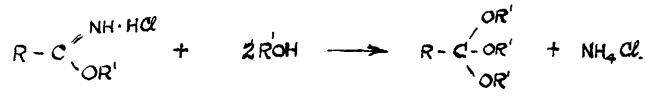
The action is accelerated by hydrogen ions and, since it is almost invariably as the hydrochloride that the iminoether is produced, the need for anhydrous conditions is obvious. The iminoether also decomposes in aqueous solution to some extent according to the equation:

$$R\text{-}C\overset{NH}{\underset{OR'}{}} \longrightarrow RCN + R'OH$$

The thermal decomposition of iminoether hydrochlorides is formulated as (162):

and it was frequently found that the salts decomposed at their melting points with the evolution of gas, the residue showing a new melting point close to that of the corresponding amide. This is a further reason for cooling during the iminoether formation.

A reaction of importance in finding the optimum conditions for amidine formation is that between an iminoether hydrochloride and alcohol to form an ortho ester (146a):

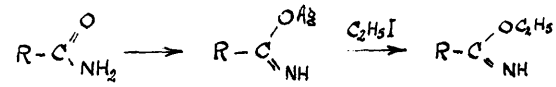


Since the ammonolysis of an iminoether to the amidine is generally carried out in alcoholic solution, this side-reaction is of special significance.

<u>Alternative imino-ether synthesis</u>.

Mention should also be made of an alternative method of preparing iminoethers (169) by acting on a

primary amide with silver nitrate and sodium hydroxide, producing a silver salt of the amide. Ethyl iodide on this compound gave the iminoethyl ether.



The procedure has been modified (98) by refluxing the amide, silver oxide and ethyl iodide in ether solution.

Ammonolysis.

The action of ammonia on the iminoether hydrochloride to give the amidine can be most simply formulated as:

$$R.C(:NH)OC_2H_5.HCl + NH_3 \longrightarrow R.C(:NH)NH_2.HCl + C_2H_5OH$$

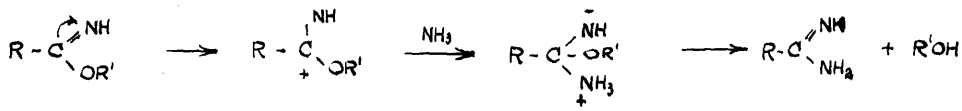
Ashley (5a), however, has pointed out the necessity of a large excess of ammonia to avoid the formation of non-basic by-products. This suggests that the reaction takes place between the benziminoether base and the excess ammonia and Ashley did obtain benzamidine from benziminoethyl ether base with alcoholic ammonia; but it has been shown (89a) that the reaction also occurs with the iminoether base and ammonium chloride. It is probable that the excess ammonia serves to displace the equilibrium:

$$R.C(:NH)OR'.HCl + NH_3 \rightleftharpoons R.C(:NH)OR' + NH_4Cl$$

as far as possible in favour of the free base. This

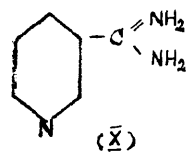
reacts less readily with alcohol than does the hydrochloride which forms the ortho ester.

The ammonolysis of an iminoether (i.e.imidoester) may be analogous to the hydrolysis of an ester (161):



Iminoether hydrochloride is added to a solution of dry alcoholic ammonia: 10% ammoniacal alcohol is recommended and also ten moles of ammonia for each iminoether group (5a). The mixture is heated under pressure, usually at 40 to 45° for ten to fourteen hours and the amidine isolated as a salt (nitrate, sulphate, hydrochloride) or as the free base.

A recent exception to the use of anhydrous media has been described (9) in the preparation of nicotinamidine (X).



(X)

The iminoether hydrochloride, formed in the usual manner, was treated with cold 50% aqueous potassium hydroxide, the iminoether base extracted with chloroform and added to

ammonium chloride in aqueous alcohol.

2. From Nitriles with Alkali Metal Amides.

This method originated in the work of Walther and Grossman (181) who investigated the reaction of aromatic amines with nitriles in the presence of sodium, to produce sodium derivatives of the amidines.

A close analogy can be drawn between amidines and carboxylic acids (see p.33) and amidines can be considered as acids on the ammonia system (33,178). On this basis, a nitrile is equivalent to the anammonide of the amidine, and, when treated with a metallic amide, the salt of the amidine is formed (33,53,177,185,186):

$$RC{\equiv}N + KNH_2 \longrightarrow R-C\begin{matrix}\diagup NK\\ \diagdown NH_2\end{matrix} + KOH$$

The process is analogous to the formation of the alkali salt of a carboxylic acid by action of alkali hydroxide on the anhydride.

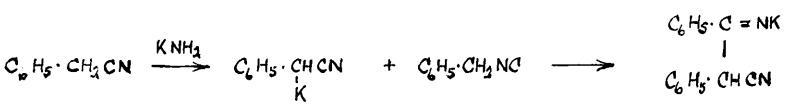
The reaction of the nitrile with the metal amide may be conducted in an inert solvent such as benzene, toluene, diphenyl or anisole, or at low temperature in liquid ammonia. The latter procedure is especially useful for lower aliphatic nitriles which undergo polymerisation on

treatment with metallic amides at 60 to 70°C. The potassium amide is often preferable (33) but the sodium and calcium derivatives are also used.

The alkali metal salt is transformed into the amidine by treatment with water under good cooling (185,186):

or the amidine hydrochlorides are obtained by the use of alcoholic hydrogen chloride (33).

The reaction fails entirely where the nitrile contains a reactive methylene group (161). With phenylacetonitrile, for example, salt formation occurs, followed by addition to another molecule, and a dimer results:



It has been stated (125) that the method generally is satisfactory only for aromatic nitriles, and tertiary aliphatic nitriles of the type $CR_3CN$.

3. <u>From Nitriles with Ammonium Salts</u>.

Some text-books state that it is possible to prepare unsubstituted amidines by heating a nitrile with ammonium

chloride. This has been accomplished in some instances (15), but the yields were very poor, the method being more suited to the preparation of monosubstituted amidines by use of amine salts instead of ammonium chloride. A few unsubstituted amidines (including benzamidine hydrochloride) have been prepared in low yield by heating the nitrile with ammonium chloride in liquid ammonia in a sealed tube (33):

$$RCN + NH_4Cl \longrightarrow R\text{-}C(=NH\cdot HCl)NH_2$$

Oxley and Short (124,125) have described a general method for the preparation of unsubstituted amidines which consists of fusing the nitrile with the ammonium salt of an aromatic or aliphatic sulphonic acid. They suggest as the mechanism of the reaction:

$$R\text{-}C\overset{\curvearrowright}{\equiv}N \quad (\delta+\ \delta-) + R'SO_3^-\ NH_4^+ \rightleftharpoons \left[R\text{-}C(=N^-)OSO_2R'\right]^-\ NH_4^+ \rightleftharpoons \left[R\text{-}C(=NH)NH_2\right]^+\ R'SO_3^-$$

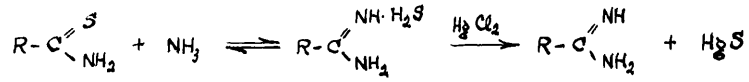
The nitrile and ammonium salt (generally the benzene--sulphonate) are fused and the amidine obtained as the sulphonic acid salt (yield 60%). The fact that ammonium chloride does not give the reaction is ascribed to its infusibility and inability to form homogeneous melts.

A point of particular interest is that this method

may be used to obtain ortho substituted aromatic amidines which are not accessible through the iminoethers.

4. From Thioamides.

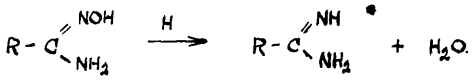
A thioamide reacts with concentrated aqueous ammonia and an equilibrium is set up:



Mercuric chloride is added to drive the reaction in favour of amidine formation by precipitation of sulphide as mercuric sulphide (14,15).

5. By Reduction of Amidoximes.

A novel synthesis of amidines consists of the reduction of an amidoxime by hydrogen over a nickel catalyst or electrolytically (8).



6. Miscellaneous

Methods 1 to 5 are the preparations useful in practice, but the following also occur in the literature.

Acetamidine hydrochloride was formed with other products when hydrogen chloride gas was passed into molten acetamide

(167).

Several amidines were obtained by heating the corresponding acids with benzenesulphonamide. The amidines were obtained as benzenesulphonates, the reaction being applied to acetic and benzoic acids and some derivatives (150).

$$R\text{-}CO_2H + 2\,C_6H_5SO_2NH_2 \longrightarrow R\text{-}C(=NH)\text{-}NH_2\cdot C_6H_5SO_3H + C_6H_5SO_3H.$$

Ortho esters can react with ammonia to form amidines:

$$R\text{-}C(OC_2H_5) + 2\,NH_3 \longrightarrow R\text{-}C(=NH)\text{-}NH_2 + 3\,C_2H_5OH.$$

## B. Monosubstituted Amidines.

### 1. From Nitriles through Iminoethers.

Pinner's method can be extended by acting on the iminoether with a primary amine instead of ammonia (72,101,139,182):

$$R\text{-}C(=NH\cdot HCl)\text{-}OC_2H_5 + R'NH_2 \longrightarrow R\text{-}C(=NH\cdot HCl)\text{-}NHR' + C_2H_5OH$$

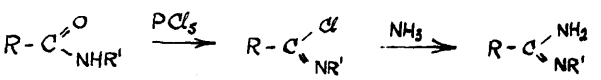
If, however, the temperature or time of the reaction is increased, disubstituted amidines result, probably owing to the equilibrium (139):

$$R\text{-}C(=NH)\text{-}NHR' + R'NH_2 \rightleftharpoons R\text{-}C(=NR')\text{-}NHR' + NH_3.$$

As was indicated for unsubstituted amidines, this method of obtaining the amidine through the iminoether requires a large excess (ten times) of the amine. Some little practical difficulty may arise in the separation of the two bases, the amidine, which is the desired product, and the excess amine.

2. From Substituted Amides.

A convenient method of preparation of monosubstituted amidines is from the corresponding N-substituted amides. When acted on by phosphorus pentachloride, a mono-N-substituted amide gives an iminochloride (162), the chlorine of which is reactive. On treatment with aqueous ammonia an amidine is formed:



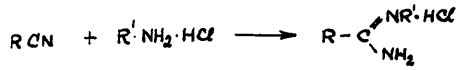
Yields are good and use of anhydrous solvents is obviated (101,179).

Although cases have been reported of similar action with primary amides (181), low yields were invariably obtained, the existence of the intermediate iminochlorides being doubtful.

3. From Nitriles with Primary Amine Salts

Nitriles of both the aliphatic and aromatic series, when heated with primary amine hydrochlorides, form mono-substituted

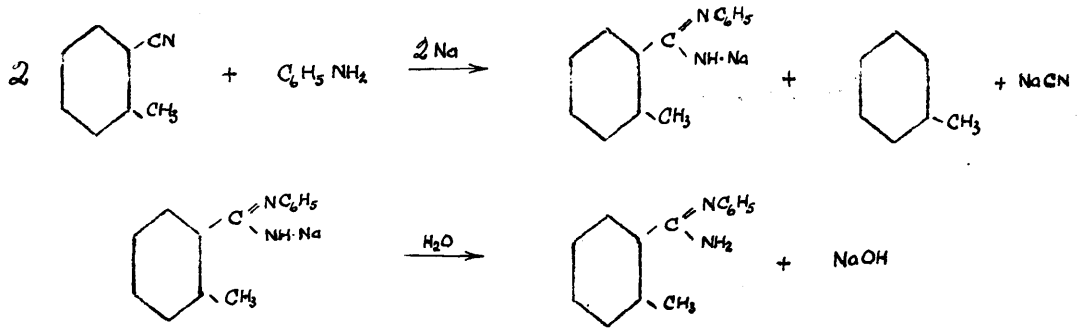
amidines (14,15,156,181) although higher temperatures may favour the formation of di-substituted products.



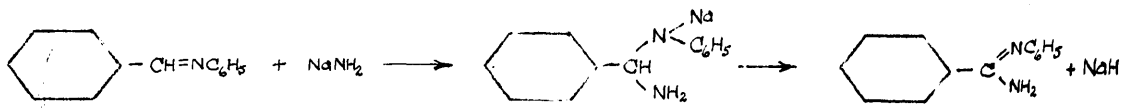
The method of Oxley and Short (124,125) is also applicable and consists in fusing a nitrile with an amine sulphonate. Details have already been given under the heading of unsubstituted amidines (p. 13 ).

4. From Nitriles with Primary Amines in Presence of Sodium and From Anils with Alkali Metal Amides

Connected with the action of alkali amides on nitriles is that of a primary amine on a nitrile in the presence of powdered sodium (103,180). Dry toluene or benzene is used as the reaction medium. The reaction appears to be more satisfactory for aromatic nitriles, but phenylacetonitrile has also been used. A substituent ortho to an aromatic cyano group does not hinder the course of the reaction, e.g. o-tolunitrile:

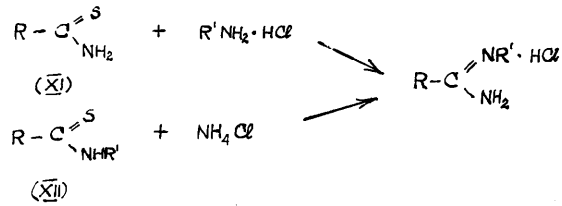


Connected with this method and analegous to the formation of unsubstituted amidines from nitriles with alkali metal amides is the reaction of an anil with sodium amide (88). Monosubstituted amidines are obtained in rather low yield, along with other products:



5. From Thioamides

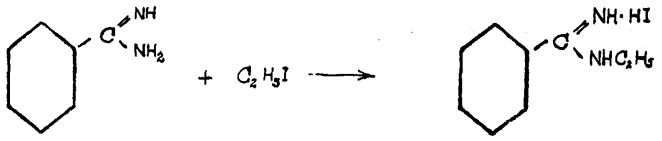
The method described for unsubstituted amidines may be extended to the mono-substituted series, either by reacting a primary thioamide (XI) with a primary amine hydrochloride, or by using an N-substituted thioamide (XII) and ammonium chloride, the same product resulting in each case (14,15):



6. From Unsubstituted Amidines

An alkyl iodide can be used to introduce a substituent into an unsubstituted amidine (133), e.g. benzamidine and

ethyl iodide at 100°C:



## C. Disubstituted Amidines

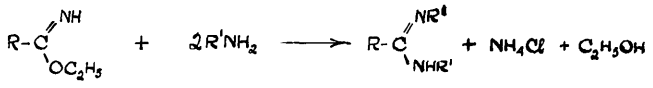
Two isomeric disubstituted amidines can exist:

Symmetrical Unsymmetrical

### Symmetrical Disubstituted Amidines

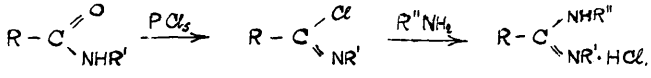
#### 1. From Nitriles through Iminoethers

Pinner's method has been extended to this series by utilising higher temperature and longer reaction times (72,105,134,139,181):



#### 2. From Substituted Amides

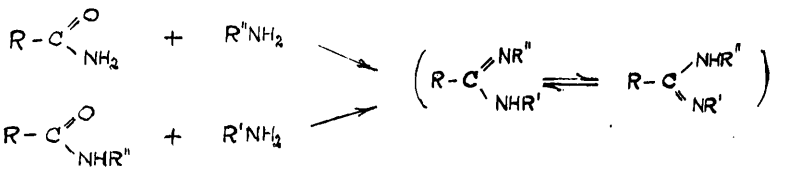
As described above (p. 16 ), phosphorus pentachloride acts on a secondary amide, to give an iminochloride (XIII). If this is reacted with a primary amine, a symmetrically disubstituted amidine results (67,129,130,179):



Other techniques described include heating the mixture

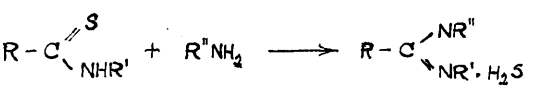
of amide and amine with phosphorus trichloride (72,159), phosphorus pentachloride, phosphorus oxychloride (26) or phosphorus pentoxide (157). The isolation of the intermediate iminochloride is thus avoided.

The same product results if the substituents in the two reactants are reversed (26,128,129,130):



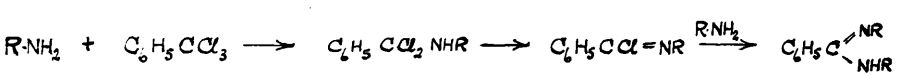
3. From Thioamides

This general method (15) can be used by reacting an N-alkylthioamide with a primary amine:



4. From Benzotrichloride

Benzotrichloride can react with aromatic primary amines in the presence of nitrobenzene to form a symmetrically disubstituted amidine (43,100). The reaction has been formulated (82):



This involves substantially the same mechanism as the preparation from substituted amides, above.

5. Miscellaneous

Many other methods are described in the literature but their scope has not been indicated. Such methods include the reaction of symmetrical dialkyl or diaryl ureas with acid chlorides (37,38) and that of anils with alkyl hypochlorites (65). Some symmetrically disubstituted amidine was also found among the products of alkylation of N-phenylbenzamidine with methyl iodide, but the unsymmetrical compound predominated (145). The reaction of nitriles with organic ammonium salts (124,125) can also be extended to this series.

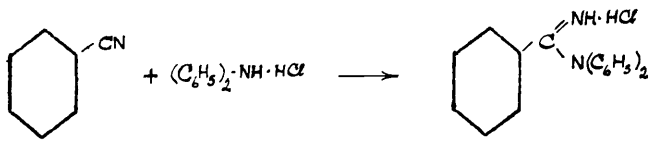
Unsymmetrical Disubstituted Amidines

1. From Nitriles through Iminoethers

Pinner's method is applicable by acting on the iminoether hydrochloride with excess of a secondary amine (105,134,137, 139):

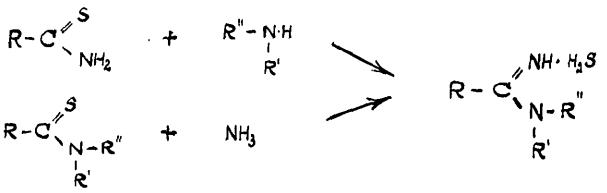
2. From Nitriles and Secondary Amines

Benzonitrile and diphenylamine hydrochloride give N,N-diphenylbenzamidine when heated together (15):



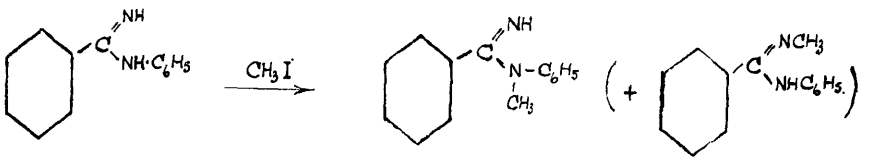
### 3. From Thioamides

The method, as previously described, may be used by choice of the appropriate secondary amine and primary thioamide (15); alternatively, a tertiary thioamide may be treated with ammonia:



### 4. From Monosubstituted Amidines

N-phenylbenzamidine, methylated with alkyl iodide, gave, principally, N-methyl-N-phenylbenzamidine (130). It was shown (145) that this was accompanied by some N'-methyl-N-phenyl compound, but that the unsymmetrically disubstituted compound was the principal product:

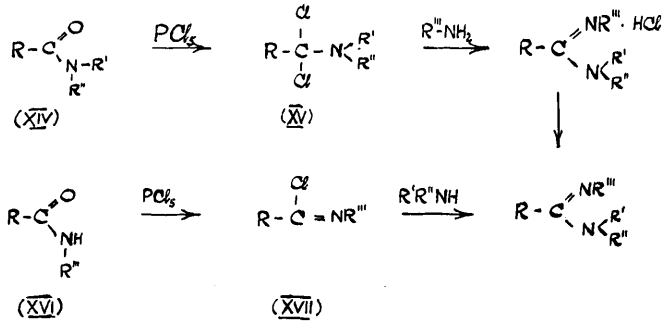


## D. Trisubstituted Amidines

### 1. From Substituted Amides

There are two possible routes from N-substituted amides to the trisubstituted amidines. A di-N-substituted amide (XIV) is treated with phosphorus

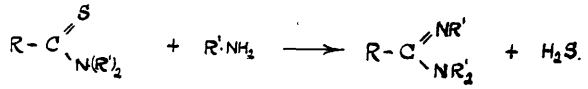
pentachloride to give the dichloro derivative (XV) which with a primary amine forms the trisubstituted amidine (21). Alternatively, a mono-substituted amide (XVI) is acted on by phosphorus pentachloride and the resulting iminochloride (XVII) treated with a secondary amine, to give the same product (11,22,72):



The intermediate chloro compound need not be isolated, the amide and amine being heated together with phosphorus trichloride (72). The use of various ortho-substituted aromatic secondary amines in the second method has been investigated (22).

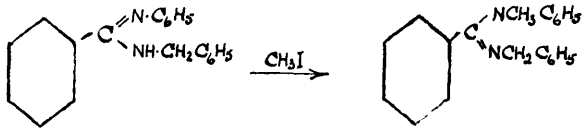
2. From Thioamides

A di-N-substituted thioamide reacts with a primary amine (14):

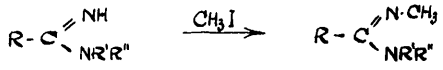


### 3. From Disubstituted Amidines

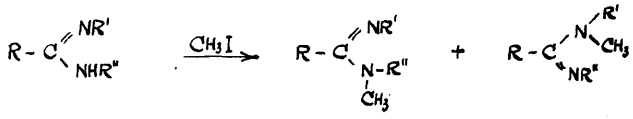
Benzylphenylbenzamidine treated with methyl iodide gave a trisubstitution product (11,108):



With an unsymmetrically disubstituted amidine, alkylation can manifestly follow only one course:



However, it has been shown (145) that alkylation of a symmetrically disubstituted amidine gives a mixture of two products:



The subject of alkylation is bound up with amidine tautomerism and is more fully dealt with under that heading.

## IV. PROPERTIES OF AMIDINES

### 1. Physical Properties

The amidines and their salts form well crystallised products, sometimes containing water of crystallisation, which may not be removed even in vacuo over concentrated sulphuric acid (5a). No generalisation can be made regarding the solubilities of the amidine bases and

salts, but the bases are usually soluble to some extent in the lipoid solvents; the salts are insoluble in the non-polar solvents, benzene, ether, acetone etc., but usually somewhat soluble in alcohol and water, in which salts of the weaker bases are appreciably hydrolysed.

The nitrate is often found to be less water-soluble than other salts and is sometimes convenient for isolation of the amidine. Other groups in the molecule, however, have a great effect, hydroxyl or amino increasing the solubility, nitro having the reverse effect.

Density and refractivity data have been listed for a number of amidines (6).

2. <u>The Basic Function</u>

Amidines are alkaline in aqueous solution and are mono-acid bases of which the unsubstituted amidines are the strongest. The basicity varies considerably and is affected by other groups in the molecule. For example, p-methoxybenzamidine is a stronger base than ammonia (as is also benzamidine) and is not precipitated by ammonia from a solution of its hydrochloride; on the other hand, p-hydroxybenzamidine and p-nitrobenzamidine bases are precipitated by ammonia (45). Alkali hydroxide is used to liberate the free bases of those amidines that are

stronger than ammonia.

Salts of amidines that have been prepared include hydrochlorides, nitrates, sulphates, carbonates, nitrites, formates, acetates, oxalates, picrates, chloroplatinates etc.

Resonance of amidinium cation

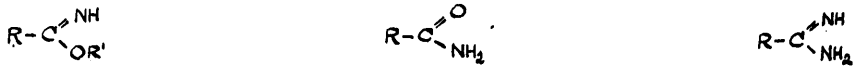
The amidinium cation is formulated by Sidgwick (162) as a resonance hybrid:

It is thus analogous to the nitro group and the carboxylate anion, in neither of which can any distinction be made between the oxygen atoms:

Like these two groups, when it is attached to an aromatic ring, the amidine grouping is exclusively meta directing.

Burtles and Pyman (27), in 1923, advanced a view of the amidinium cation which is in agreement with modern resonance theory. They assign the basic character of the amidine grouping to the imino nitrogen. The evidence for

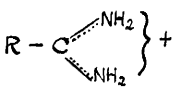
this is the basic character of iminoethers which readily form salts, in contradistinction to the almost complete lack of basic properties shown by amides.



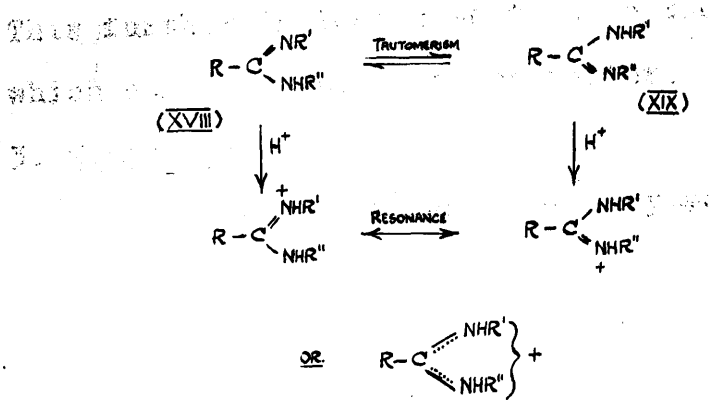
Iminoether (basic) Amide (non-basic) Amidine (basic)

Since the amidine, which contains both the imino and amido nitrogens, has strong basic properties, the basic characteristic can be ascribed to the doubly bound nitrogen atom.

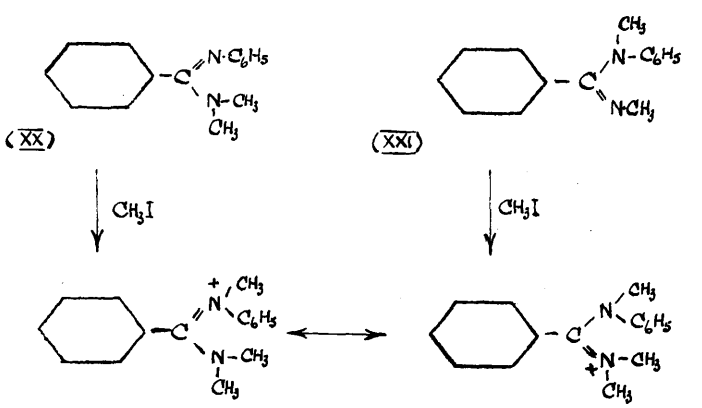
According to the resonance concept, when a proton adds to the imino nitrogen to form the amidinium cation, the conditions for resonance are fulfilled and the two nitrogen atoms are completely equivalent, the positive charge being distributed between them. This is sometimes represented as:



This argument, extended for example to the symmetrically disubstituted amidines, explains why, though chemical evidence indicates the tautomeric existence of isomers (XVIII) and (XIX), both give the same cation on salt formation:




From this argument on the resonance of the amidinium ion, it becomes clear how two independent compounds, N:N-dimethyl-N'-phenyl-benzamidine (XX) and N:N'-dimethyl--N-phenylbenzamidine (XXI) both give the same quaternary ammonium salt with methyl iodide (145):

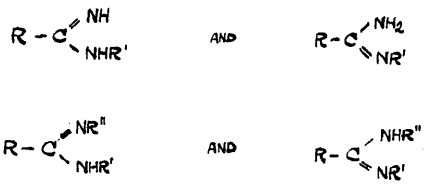



Since the only difference between the two formulations of the quaternary salt lies in the electron distribution, resonance takes place, and one product is obtained.

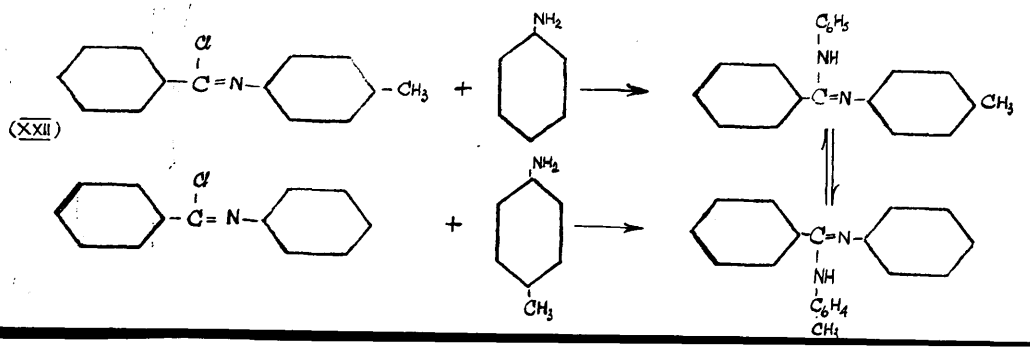
This further indicates that it is the doubly bonded nitrogen which contains the basic character.

3. Tautomerism

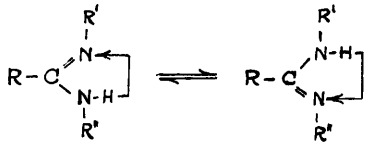
For a monosubstituted or a symmetrically disubstituted amidine, two formulae can be written in each case, differing only in the position of the double bond and of a hydrogen atom:



However, repeated attempts to synthesise the two individual isomers in a specific case have resulted in identical product (26,27,32,128,129,130). The type of alternative syntheses attempted is exemplified by the work of Pechmann (128) who obtained the same product from the treatment of benzoyl-p--toluidide iminochloride (XXII) with aniline as from the interaction of benzanilide iminochloride (XXIII) with p-toluidine:

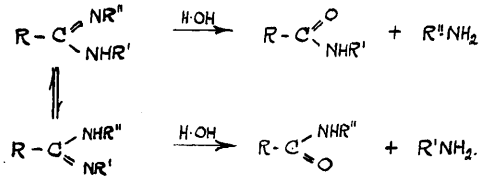


This suggests that a prototropic change occurs between the two amidine isomers and that a tautomeric equilibrium is set up:



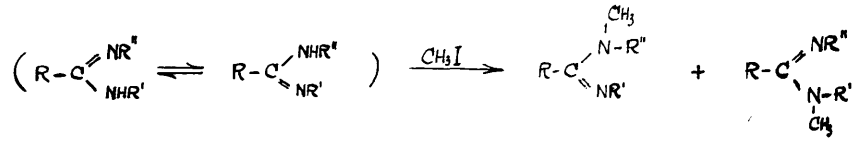
(R' = H for a monosubstituted product)

Corroboration of the presence of the two forms is provided by the hydrolysis of symmetrically disubstituted amidines which gives four products, each tautomer apparently giving rise to an amide and an amine (111):



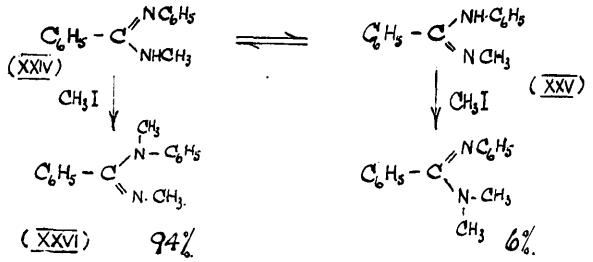
This evidence is not conclusive, however, as an alternative mechanism of hydrolysis can be shown (p. 35 ).

More concrete evidence for the formulation of tautomerism in mono or symmetrically disubstituted amidines is provided by treatment of these compounds with methyl iodide which always gives two separable products (145):

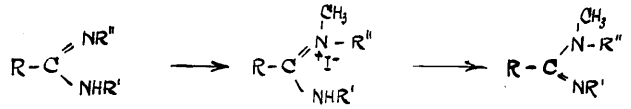


(R' = H for a monosubstituted amidine)

In an unsubstituted amidine (R' = R'' = H), the two tautomers are equivalent and so are the methylated products; this is also the case when R' = R''. If the two subsequent groups, R' and R'', are fairly similar (e.g. phenyl and p-tolyl) the two methylation products are formed in almost equal amounts. However, if the two groups are widely dissimilar, one tautomer preponderates in the equilibrium mixture. So, when treated with methyl iodide, a symmetrically disubstituted amidine such as N-phenyl-N'-methylbenzamidine (tautomers (XXIV) and (XXV)), where the two substituents differ greatly, gives principally (XXVI), though two methylated products are produced (144,145).

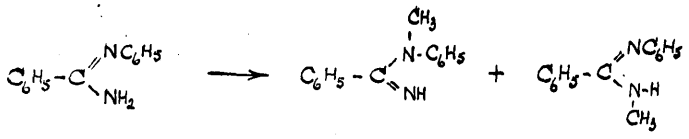


Assuming that it is the doubly-bound nitrogen which has the basic property (27) the methylation follows the mechanism:

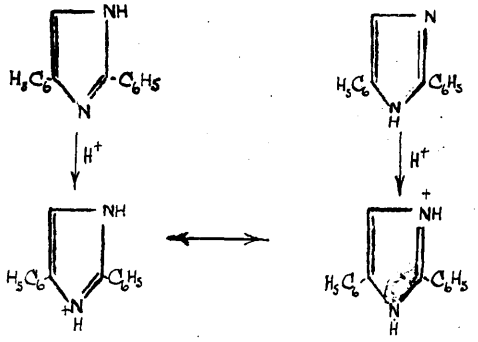


The conclusion is that, in the case quoted, tautomer (XXIV) is the principal constituent of the equilibrium mixture.

It is therefore an error to say that only one product is formed in the methylation of a compound such as N-phenyl-benzamidine (130). Both possible isomers are produced, but the N:N-disubstituted compound predominates (145):



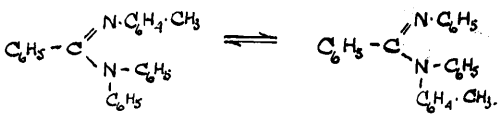
One instance where the two isomeric forms have been isolated is reported (27) in the case of the cyclic amidine diphenylimidazole. Addition of a proton to each, however, forms the same cation in both cases - a resonance hybrid:



So any attempt to resolve amidine tautomers which involves salt formation is bound to be unsuccessful.

An interesting extension to the tautomerism of amidines is provided by the demonstration of a mobile hydrocarbon radicle in the thermally stable compound

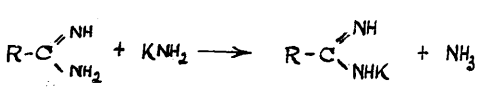
N,N-diphenyl-N'-p-tolylbenzamidine (29,30). At 340°C, an equilibrium is set up:



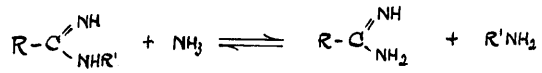
4. Analogy to Carboxylic Acids

Amidines have been shown to be completely analogous to carboxylic acids (33,178), and, as acids on the ammonia system, may be termed carbazylic acids; the substitution products are regarded as esters. In support of this view, the authors cite various reactions and show their analogy to those of carboxylic acids. Thus, an unsubstituted amidine reacts with metallic potassium in liquid ammonia to give the potassium salt and hydrogen:

The reaction with potassium amide produces the salt and ammonia:



The ammonolysis of a substituted amidine (ester) in liquid ammonia is catalysed by ammonium chloride (acid), exactly as in carboxylic ester hydrolysis:

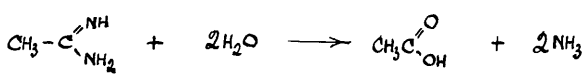


An even more striking analogy is provided by the decomposition of an alkali amidine salt when heated with sodamide. The hydrocarbon is produced:

It has also been shown that in an amidine which is not completely substituted, i.e. containing a hydrogen attached to nitrogen, hydrogen bonding takes place giving associated forms similar to the carboxylic acids (76).

5. Hydrolysis and Ammonolysis

Amidines are generally readily hydrolysed, the ease of hydrolysis decreasing from the unsubstituted to the trisubstituted compounds. Some unsubstituted amidines are hydrolysed merely by warming the aqueous solution (139,167):

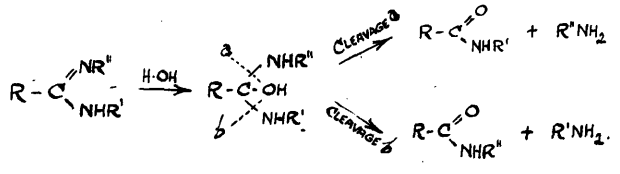


Generally acid or alkali is used to catalyse the hydrolysis

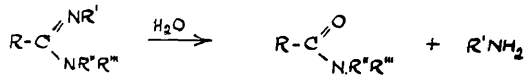
and an organic solvent may be added for water-insoluble compounds. Trisubstituted amidines generally and others with substituents of high molecular weight require more drastic hydrolysis.

In spite of the reported ease of hydrolysis of the unsubstituted amidine grouping, normal alcoholic hydro--chloric acid has been used at boiling temperature to split out the acetyl grouping in acetoxybenzamidines (35), and 57% aqueous hydriodic acid to demethylate various methoxy benzamidines (pp.73, 113 ).

The following mechanism has been proposed to explain the mixture of amides and amines formed from symmetrically disubstituted amidines (111):




However, tautomerism of the amidine offers an equally good explanation (p.30 ) and covers the case of a trisubstituted amidine, for which the above mechanism fails, since this compound gives only one amide and one primary amine on hydrolysis (161):

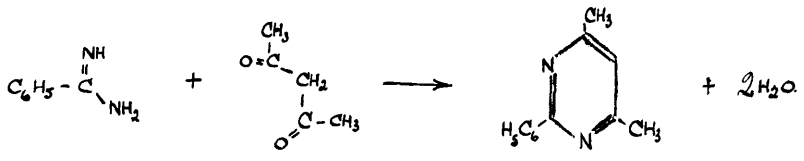


**The ammonolysis of amidines has already been mentioned in the section drawing the analogy to carboxylic acids (p.34 ):**

**On a mass action basis, excess of ammonia drives the reaction in favour of the lower substituted product, excess amine in the opposite direction (14). The action, if conducted in liquid ammonia, is catalysed by ammonium chloride.**

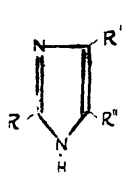
**6. Heterocyclics**

**Mention should be made of the heterocyclic derivatives of amidines, of which the most important are the pyrimidines. Unsubstituted amidines can react with β-ketonic esters or compounds containing a β-dicarbonyl grouping to form pyrimidines, e.g.:**

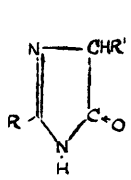


**Similar reactions occur with the α,β -unsaturated carbonyl grouping, with cyano esters etc.**

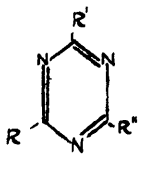
**Other heterocyclic amidines include imidazoles (XXVI), imidazolones (XXVII) and triazines (XXVIII):**



(XXVI)



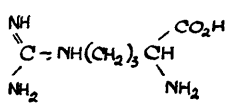
(XXVII)



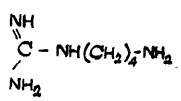
(XXVIII)

## V. PHARMACOLOGY OF THE AMIDINES

The modern use of amidines as trypanocidal agents has resulted from a series of coincidences and erroneous conclusions (55a,71). It was observed (90) that the urine of animals suffering from parathyroid tetany, or after removal of the parathyroid glands, contained abnormally large amounts of methylguanidine, $NH_2.C(:NH)NHCH_3$. When this compound was administered to healthy animals, it was later found that not only were the tetany symptoms reproduced, but there was also a fall in blood-sugar (hypoglycemic action). This latter property appeared potentially useful in the treatment of diabetes mellitus, whose symptom is a high concentration of sugar in the blood and various workers (60,69,160) conducted investigations to discover compounds of similar biological action but with reduced toxicity. Guanidine itself, though toxic, showed the hypoglycemic action, and arginine (XXIX) and its decarboxylation product, agmatine (XXX), were found to show similar property.

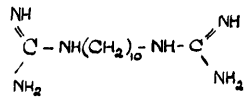


(XXIX)



(XXX)

This led to the synthesis of polymethylenediguanidines of which those with the highest therapeutic ratios (maximum tolerated dose/minimum effective dose) proved to be decamethylene diguanidine ("Synthalin") (XXXI) and dodecamethylene diguanidine ("Synthalin B").



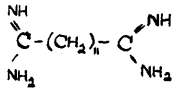
(XXXI)

The last-named compounds have been used to some extent since 1929 for oral treatment of diabetes, but they are dangerously toxic since they do not act, as does insulin, by replacement of the body product, but exert their hypoglycemic action at the expense of damage to the liver (24).

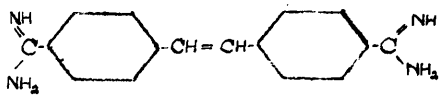
In 1930, the observation was made that trypanosomes, when grown in artificial culture, consumed considerable amounts of glucose (146), and this led to the investigation of the possibility of the use of hypoglycemic compounds as anti-trypanosome agents (78). Decamethylene diguanidine tested in vitro and in vivo (in mice) against various pathogenic trypanosomes, was, in fact, found extremely effective as an anti-trypanosome, but not due to lowering of blood sugar, since in vitro it showed marked trypanocidal

action in a concentration as low as 1 in 200 million, which is quite inadequate to produce appreciable hypoglycemia. Further, insulin shows no trypanocidal action either in vitro or in vivo. The conclusion which must be drawn is that the action of synthalin is direct (97,104).

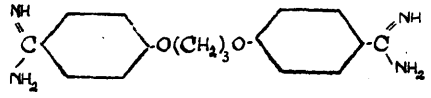
Research into similar compounds was stimulated and King, Lourie and Yorke (86) replaced the guanidine groups by amidine, preparing a series of diamidines of which the most effective was 1:11-diamidinoundecane (XXXII). Ashley and his collaborators (5) extended the series to aromatic diamidines and found therapeutic properties enhanced in many, notably 4:4'-diamidinostilbene ("stilbamidine") (XXXIII), 4:4'diamidino- $\alpha,\gamma$ -diphenoxypropane ("propamid--ine") (XXXIV) and 4:4'-diamidino- $\alpha,\epsilon$ -diphenoxypentane ("pentamidine") (XXXV).



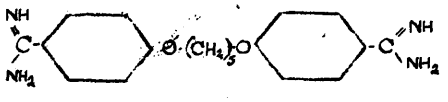
(XXXII)



(XXXIII)



(XXXIV)



(XXXV)

It has been recently shown that 2-halogen derivatives of 4:4'-diamidinostilbene show a higher chemotherapeutic

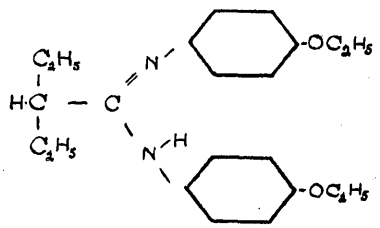
ratio than the parent compound (5d,55b).

Ewins (55a) has summarised the findings concerning the trypanocidal activity of the diamidines. Two amidine groups are essential for activity and they must be separated by a chain of molecular complexity such as indicated above (63). Alkyl or aryl substitution in the amidine groups usually (but not always) depresses the trypanocidal activity; replacement of a methylene group in the aromatic diamidines by oxygen (e.g. (XXXIV) and (XXXV)) increases the activity, while replacement by other radicles ( -NH- , -CO- , etc.) or atoms (-S-) causes a diminution.

Stilbamidine, propamidine and pentamidine have been given clinical trial with some success in African human trypanosomiasis (sleeping sickness), in kala-azar and in B.canis, a tropical infection of dogs. In spite of some unfavourable toxic reactions, these drugs appear to be of genuine value, especially pentamidine.

More recently, amidines have been used in treating other tropical infections such as babesia and leishmania (5). Some use has also been made of these compounds to clear up wound infection by external application. Amidines here have an advantage in that, unlike sulphonamides, their action is not opposed by pus or p-aminobenzoic acid.

Other physiological actions of amidines have been demonstrated in the pressor effects of some diamidines and a relationship has been proposed between this effect and the basic dissociation constants (56): in another direction, N:N'-bis(p-ethoxyphenyl)-$\alpha_1\alpha$-diethylacetamidine (XXXVI) and other similar derivatives show local anaesthetic properties (85):



(XXXVI)

## DRYING of SOLVENTS.

**Alcohol.**

**References:-** Lund, Bjerrum (107); Tepentiev (170); Evans, Fetsch (51).

| | |
|---|---|
| Ethyl alcohol (s.g.0.74 ) | 1000ml. |
| Sodium | 15gms. |
| Magnesium turnings | 5gms. |
| Iodine | 1gm. |

The specific gravity of the alcohol, found by hydrometer, was equivalent to 98.6% by weight. The sodium was added and, when it was completely dissolved, 800ml. of the alcohol were distilled, showing specific gravity 0.795 (= 99.6% alcohol) (Note I). The magnesium (Note II) was dried at 120°C and 70ml. of the sodium-dried alcohol added along with the iodine. Hydrogen was evolved and the mixture was refluxed on the water-bath until all the magnesium was converted to ethoxide. 600ml. of the sodium-dried alcohol was added and, after an hour's reflux, the mixture was distilled, the first 500ml. being collected. Dry ground-glass apparatus was used throughout, with precautions for exclusion of moisture, and

the final product was stored in a dark bottle, sealed with wax.

Notes I. The sodium-dried alcohol was used in the ammonolysis of the iminoethers.

II. Magnesium ribbon was equally satisfactory if first cleaned with emery paper and cut into ½" lengths.

ETHER

Ether was given four consecutive treatments with anhydrous calcium chloride, and, finally, dried with sodium wire under cooling. The ether was distilled over fresh sodium and stored in a dark bottle, whose stopper was sealed with wax. Ground-glass apparatus was used.

CHLOROFORM

Chloroform was treated at twenty-four-hourly intervals with four quantities of calcium chloride, and the final drying performed with phosphorus pentoxide. The chloroform was distilled in ground glass apparatus and stored in a dark bottle whose stopper was sealed with wax.

**PART I**

# DERIVATIVES OF 4-HYDROXYBENZAMIDINE

**Theoretical**

**Experimental:-**

**Preparation of Compounds**

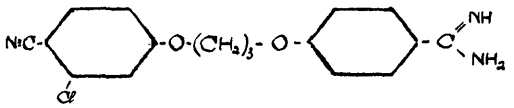
**Biological Tests.**

## PART I DERIVATIVES OF p-HYDROXYBENZAMIDINE

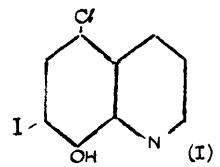
### THEORETICAL

### Reasons underlying Investigation

Following the discovery of the trypanocidal activity of the long-chain di-amidines (Introduction, p. 38 ) and, in particular, that of 4:4'-diamidino-$\alpha,\gamma$-diphenoxy-propane, it was observed that the compound:



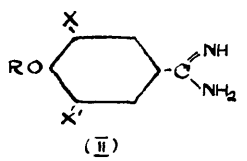
had considerable amoebicidal action in vitro (35). Compounds that have been successfully used in vivo in treatment of amoebiasis include 5-chloro-7-iodo-8-hydroxy-quinoline ("Vioform") (I):



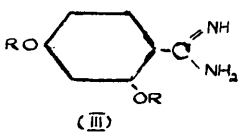
(I)

and the similar di-iodo-compound. These two quinoline derivatives are substituted phenols containing a basic centre and this general description applies also to the long-chain mono-amidine noted above.

Two series of compounds were therefore prepared of the general formulae:



(II)



(III)

where R = H, alkyl, halogenoalkyl, phenyl or substituted phenyl; X and X' = H, halogen or alkoxyl. These compounds were then tested for amoebicidal action.

Chemical Notes

Since, in all cases except 2:4-dihydroxybenzonitrile, the nitriles were readily converted to the amidines by Pinner's method, the chemical problem generally was the preparation of the requisite nitriles.

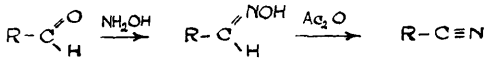
For the simpler compounds, such as p-ethoxybenzamidine (II), X = X' = H; R = $C_2H_5$) and vanillamidine ((II), X = R = H; X' = $CH_3O$-), diazotisation of the appropriate amine and addition to cuprocyanide solution (Sandmeyer reaction) offered a convenient route to the nitriles.

The conditions for replacement of the diazo group by cyano cannot be generalised and each compound had to be treated individually. It was not possible to forecast the optimum temperature, pH, etc., and, in some cases, the reaction failed completely. So, for example, it was not found possible to make the nitrile 4-cyano-4'-hydroxy-diphenylether from the amine. The hydroxy-amine was made but, although it was readily diazotised, it gave no yield of nitrile on following the Sandmeyer process or on modification of the usual procedure. The same applied to the 2'-hydroxy-compound. The methoxycyanodiphenylether was finally

prepared by condensing the potassium salt of the methoxyphenol with 4-bromobenzonitrile in the presence of copper catalyst:

The preparation of 4-cyano-2'- and 4'-methoxydiphenylethers by this procedure is simpler than the methods in the literature (166,174). The nitriles were converted to the amidines and it was found possible to demethylate these to the hydroxy-compounds by cautious treatment with 57% aqueous hydriodic acid.

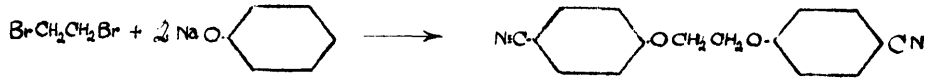
Another useful route to the nitrile, sometimes giving superior yields, was from the aldehyde through the aldoxime which was dehydrated with acetic anhydride.



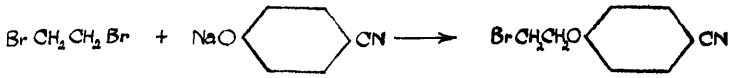
This method was especially suitable for 2:4-dimethoxybenzonitrile for which the syntheses in the literature are less satisfactory. The dimethoxybenzaldehyde was synthesised in very good yield from resorcinol dimethyl ether by the action of anhydrous hydrogen cyanide in presence of zinc chloride and hydrogen chloride (Gatterman's method).

The brominated hydroxybenzonitriles were most readily made by direct halogenation of 4-hydroxybenzonitrile, which

was produced in some quantity from 4-amino-phenol since it was also the starting material for 4-(β -bromethoxy)-benzonitrile. For this latter nitrile, excess ethylene dibromide was refluxed with sodium 4-cyanophenate, but the yield was always low since the reaction proceeded further to some extent according to:



instead of following the desired course:



The general procedure for the conversion of a nitrile to an amidine consisted of treatment of the dry compound with alcohol and hydrogen chloride to form the iminoether hydrochloride which produced the amidine with alcoholic ammonia. Ethyl alcohol was used in every case and an excess can be used as solvent, but it was usually more convenient to use an inert diluent such as ether, chloroform, benzene, nitrobenzene or dioxan (87). In any event, both the alcohol and diluent had to be rigorously dried. The solution (or suspension) of nitrile in the alcohol and diluent was saturated at $0^{o}$ with hydrogen chloride, dried by passage through concentrated sulphuric acid. The reaction was complete after two days, the iminoether salt usually crystallising, although, occasionally, the hydrochloride remained in solution (e.g.4-nitrobenziminoethyl

ether hydrochloride). The violet colour which frequently developed in the preparation was due to impurities in the alcohol (44). The iminoether salt was not usually purified but was either filtered and washed with dry diluent or the solvent and excess hydrogen chloride removed under reduced pressure.

The ammonolysis of the iminoether hydrochloride was conducted by heating under pressure with saturated alcoholic ammonia for ten to fifteen hours at about 40°C (thermostat). The amidine was isolated as the free base or as a salt; the hydrochloride was prepared by saturating the aqueous or alcoholic solution with gaseous hydrogen chloride and the nitrate by addition of strong aqueous ammonium nitrate to a solution of the hydrochloride.

2:4-Dihydroxybenzonitrile did not undergo these reactions but the cause was not the "ortho effect " (Introduction, p. 6 ) since 2-hydroxybenzonitrile reacts normally (46). 2:4-Dimethoxybenzonitrile, however, gave the amidine smoothly and demethylation produced the dihydroxybenzamidine.

Biological Notes

The bacteriological in vitro tests on the above series of compounds were performed by Messrs. I.C.I. (Dyestuffs Division), Blackley, Manchester.

All the compounds showed only slight activity by comparison with emetine hydrochloride.

# PART I

## EXPERIMENTAL

### 4-Hydroxybenzamidine Derivatives:-

Preparation of Compounds

Biological Tests

---

PART I - DERIVATIVES of p-HYDROXYBENZAMIDINE.

## p-ETHOXYBENZAMIDINE.

### Outline of Preparation

p-Phenetidine was diazotised and converted to the nitrile which gave the amidine.

### p-ETHOXYBENZONITRILE

**Reference:-** Eberhardt (47).

| | | |
|---|---|---|
| **Diazotisation** | p-Phenetidine | 50gms. |
| | conc. HCl | 95ml. |
| | in water | 350ml. |
| | Sodium nitrite | 26gms. |
| | in water | 250ml. |

The amine was dissolved in the acid with warming and then cooled to $0^{\circ}C$ with stirring to ensure precipitation of the salt in a fine state of division. The nitrite solution was run in at $0-5^{\circ}$ until the end of the reaction was indicated by starch-KI paper. The filtered solution was clear and colourless.

| | | |
|---|---|---|
| **Sandmeyer** | Copper sulphate, $5H_2O$ | 46gms. |
| | in water | 200ml. |
| | Potassium cyanide | 70gms. |
| | in water | 100ml. |

The copper sulphate and cyanide solutions were mixed under a good draught and the brown solution maintained at 90°C as the diazonium solution was added over fifteen minutes with stirring. Nitrogen was freely evolved and, after heating for thirty minutes on the steam-bath, the mixture was steam distilled. The nitrile was filtered from the distillate, dried, and distilled under vacuum, the fraction boiling 180-90°C/15mm. being collected. M.P. 67-8°C (listed - 69°C). Yield 23gms. (43%).

p-ETHOXYBENZAMIDINE

Reference:- Eberhardt (47).

| | | |
|---|---|---|
| Iminoether | p-Ethoxybenzonitrile | 12gms. |
| | Ethyl alcohol (Mg dried) | 30ml. |
| | Ether (Na dried) | 30ml. |

The nitrile was dissolved in the alcohol and ether and the solution maintained at 0°C as hydrogen chloride, dried through concentrated sulphuric acid, was passed in to saturation with precautions for the exclusion of atmospheric moisture. The solution was allowed to stand overnight and, after re-saturation, left for another twenty four hours. The dark red solution was evaporated at 40°C under water-pump

vacuum and the crude iminoether hydrochloride obtained as a red powder.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | Ethyl alcohol | 350ml. |
| | Ammonia gas. | |

The alcohol was saturated with dry ammonia at 0°C, the iminoether salt added, and the solution heated in a sealed bottle at 40°C for twelve hours. After the solvent was removed at 40°C by water-pump, the yellow residue was dissolved in dilute hydrochloric acid and warmed with activated carbon. The process was repeated and the solution finally clarified by filtration through sintered glass.

To form the hydrochloride the solution was maintained at 0°C as it was saturated with hydrogen chloride and the salt so precipitated filtered and washed with hydrochloric acid, then dried in vacuo over sulphuric acid. M.P. 260°C (listed - 260°).

Part of the solution was basified with strong sodium hydroxide solution with cooling andthe resulting free base filtered on sintered glass and washed with water. The compound was recrystallised frombenzene. M.P. 104°C.

Total yield (as free base) 9gms. (67%).

## 4(β-BROMOETHOXY)-BENZAMIDINE

### Outline of Preparation

4-Hydroxybenzonitrile, prepared from p-aminophenol, is treated with ethylene dibromide in alkaline solution and the resulting 4(β-bromoethoxy)-benzonitrile converted to amidine.

### 4-HYDROXYBENZONITRILE

References:- Ashley (5b) from 4-aminophenol; isolation according to Ellingworth (49).

| | | |
|---|---|---|
| Diazotisation | 4-aminophenol hydrochloride | 75gms. |
| | Hydrochloric acid (conc.) | 110ml. |
| | in water | 400ml. |
| | Sodium nitrite | 40gms. |
| | in water | 400ml. |
| | Sodium acetate (hydrated) | 200gms. |

The aminophenol was dissolved in the hot acid, cooled with stirring and diazotised at 0°C over twenty minutes. When the reaction was complete to starch - KI paper. sodium acetate was added until the solution was neutral to congo red (pH 3-5) and the solution filtered.

| | | |
|---|---|---|
| Sandmeyer | Cupric sulphate, $5H_2O$ | 200gms. |
| | in water | 700ml. |
| | Potassium cyanide | 200gms. |
| | in water | 400ml. |
| | Hydrochloric acid (conc.) | 700ml. |

The cyanide solution was slowly added to the copper sulphate under a good draught, until a clear solution resulted. The temperature was maintained at 85-90°C as the diazonium solution was added over twenty minutes, with good stirring, nitrogen being briskly evolved. After a further fifteen minutes at 90°C and cooling to room temperature, excess hydrochloric acid was added, the precipitated solid filtered, and the filtrate extracted with ether. The solid material was exhaustively extracted with ether and the total ethereal solution washed three times with saturated sodium acetate solution. The compound precipitated, which contained copper, was filtered, and the ether solution washed with water, dried over sodium sulphate, and evaporated. The residue was refluxed for an hour with benzene (400ml.) and filtered hot from tarry material.

The solution was boiled for thirty minutes with 1gm. of decolorising carbon and the benzene evaporated. The residue was distilled in vacuo (B.P.158-65°/3mm.) and finally recrystallised from dilute alcohol. M.P.112°C (listed - 113°). Yield 29gms. (47%).

## 4(β-BROMOETHOXY)-BENZONITRILE

**References**:- Ashley (5c) modified by Ellingworth (49).

| | |
|---|---|
| 4-Hydroxybenzonitrile | 6gms. |
| Ethyl alcohol (absolute) | 65ml. |
| Sodium | 1.2gms. |
| Ethylene dibromide | 18.8gms. (8.7ml.) |

To the solution of hydroxybenzonitrile in alcoholic sodium ethoxide, ethylene dibromide was added and the mixture refluxed for five hours on the water-bath. After filtering the sodium bromide and precipitated 4:4'-dicyanodiphenoxy--ethane, the alcoholic solution was concentrated and diluted with water. Sodium hydroxide solution was added to pH 8 (Thymol blue) and the oil extracted with ether. After filtering more of the dinitrile, the extract was washed with alkali and water, dried over sodium sulphate, and the ether evaporated. The residue was dissolved in the minimum alcohol, boiled with decolorising carbon, filtered, and cooled in ice as a large excess of ice-water was added. The emulsion crystallised on standing to white needles which were filtered, washed with water, and recrystallised. M.P. 59°C (listed - 61°C). Yield 3.2gms. (30%).

4(β-BROMOETHOXY)-BENZAMIDINE

| | | |
|---|---|---|
| Iminoether | 4(β-Bromoethoxy)-benzonitrile | 10gms. |
| | Ethyl alcohol (Mg dried) | 20ml. |
| | Ether (Na dried) | 30ml. |

The solution of nitrile in alcohol-ether was saturated with dry hydrogen chloride at 0°C on two separate days and the solvents removed at 40°C at the water-pump on the third. The residue consisted of a white crystallise solid.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | Alcohol (Na dried) | 300ml. |
| | Ammonia gas. | |

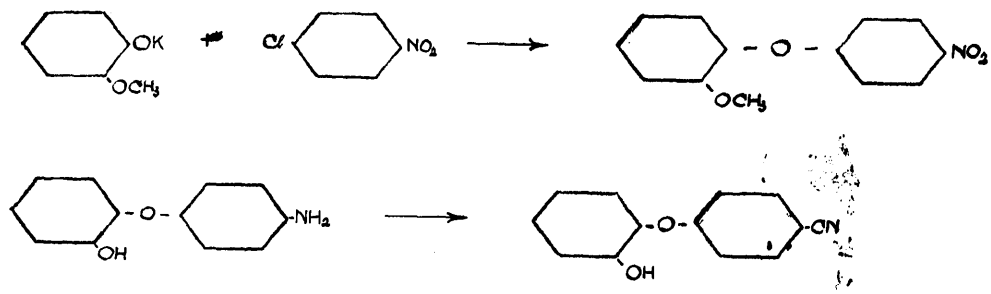
The iminoether hydrochloride was added to the alcohol saturated with dry ammonia at 0°C and heated under pressure at 40°C for fifteen hours, after which the solvent was removed at the water-pump at 40°C. The residue was found to be insoluble in alcohol, ether, benzene, etc., and only partially in water. From the aqueous extract, sodium hydroxide produced an unsatisfactory, oily precipitate. The substance (amidine hydrochloride) was finally recrystall--ised from hot nitrobenzene, in which it is slightly soluble, being deposited as small white needles, decomposing at 250°C. Yield 5.2gms. (42.6%).

| Analysis | Found | Required by $C_9H_{12}ON_2ClBr$ (hydrochloride) |
|---|---|---|
| C | 38.6% | 38.6% |
| H | 4.38 | 4.29 |
| N | 9.1 | 10.0 |
| Br + Cl | 40.5 | 41.3 |

## 4-(2'-HYDROXYPHENOXY-) BENZAMIDINE

Outline of Attempted Method A.

The potassium salt of guiacol was condensed with p-chloronitrobenzene and the resulting diphenyl ether derivative reduced and demethylated to the aminophenol. It was proposed to convert this to the nitrile by the Sandmeyer method and hence to amidine.




The nitrile could not be formed by this method.

4-NITRO-2'-METHOXYDIPHENYL ETHER

References:- (25) - for this compound, (23) and (166) for similar preparations of 4-nitrodiphenyl ether and 4-nitro-4'-methoxydiphenyl ether. Catalytic copper according to (23). General procedure - (59).

| Copper catalyst:- | | |
|---|---|---|
| | $CuSO_4.5H_2O$ | 10gms. |
| | Zn dust | 3.5gms. (excess) |
| | Water | 35ml. |

Zinc dust was added to the copper sulphate solution and the precipitated copper washed by decantation with 5% hydrochloric acid to remove excess zinc. When the evolution of hydrogen ceased, the copper powder was filtered, washed and stored under water.

Satisfactory results were also obtained with commercial copper bronze. This was treated with 30% nitric acid for one minute and the powder washed by decantation with water. It was filtered and washed with acetone, being sucked dry on the filter.

Note:- Copper bronze is not always a satisfactory substitute for the specially prepared catalyst (59).

**Nitromethoxydiphenyl ether**

| | |
|---|---|
| Guiacol | 100gms. (1.1mol.) |
| Stick KOH | 38gms. (1 mol.) |
| 4-Chloronitro--benzene | 120gms. (1 mol.) |
| Copper catalyst | 0.5gms. |

Enough water was added to the alkali to form a clear melt at 200°C, which was mixed with the guiacol when the temperature had fallen to 100°C. The catalyst and chloronitrobenzene were added (temperature 110°C) and, after five hours reflux on the oil-bath (internal temperature 210°C)

the mixture was cooled to incipient crystallisation and poured into 5% sodium hydroxide containing ice. After further treatment with alkali to remove excess guiacol, the residue was washed with water, dried in air and distilled under vacuum, the fraction boiling 230-4°C/25mm. being collected. Recrystallisation from alcohol gave pale yellow needles. M.P.104-5°C (listed - 106°). Yield 100gms. (56%).

Attempted Demethylation to 4-nitro-2'-hydroxydiphenyl ether.

Reference:- (25) - use of hydrobromic acid (48%) / acetic acid, but no details given.

| | |
|---|---|
| 4-nitro-2'-methoxydiphenyl ether | 5gms. |
| Hydrobromic acid (48%, s.g.1.4) | 10ml. |
| Glacial acetic acid | 10ml. |

The methoxy-compound was refluxed with the hydrobromic--acetic acid for six hours, but only starting material was obtained on dilution. Reference (25) gives no indication of quantities.

4-AMINO-2'-HYDROXYDIPHENYL ETHER

Reference:- This compound has not been previously prepared, but simultaneous demethylation and reduction of 2-nitro-2'-methoxydiphenyl ether by use of

57% hydriodic acid has been described (34).

| | |
|---|---|
| 4-Nitro-2'-methoxydiphenyl ether | 10gms. |
| Iodine | 12gms. |
| Red phosphorus | 12gms. |
| Water | 20 ml. |

The mixture was refluxed gently until all the nitro compound had gone into solution (60 minutes), filtered hot through acid-resistant paper, diluted and allowed to cool. A little material which separated was filtered and excess ammonia to the dark solution precipitated a solid which was filtered after fifteen minutes, washed with cold water and recrystall--ised from alcohol. Colourless needles, M.P.170°C (not listed). The compound is soluble in acids and alkalis, but not in ammonia; the acid solution, on treatment with nitrous acid, gives a solution which couples with alkaline $\beta$-naphthol to form a brilliant red dye. The free base is slightly soluble in water and the solution turns blue due to atmospheric oxidation. Yield, as free base, 5gms. (61%).

Note:- Hydriodic acid (57%, s.g.1.7) was used instead of phosphorus and iodine for some runs, giving almost the same yield, but the method described is more convenient.

Attempted Conversion to 4-cyano-2'-hydroxydiphenyl ether.

This compound has not been previously prepared.

Diazotisation

| | |
|---|---|
| 4-Amino-2'-hydroxydiphenyl ether | 8gms. |
| Hydrochloric acid (s.g.1.16) | 20ml. |
| in water | 200ml. |
| Sodium nitrite | 5.6gms. |
| in water | 50ml. |

The amine was dissolved in the hot dilute acid and cooled with stirring to $-5^{\circ}C$, when the nitrite solution was run in below the surface of the solution at such a speed that the temperature did not rise above $0^{\circ}$. The end-point was indicated by starch-KI paper and a little brown flocculent matter was filtered, giving a clear yellow diazo solution. A sample coupled with alkaline β-naphthol gave a brilliant scarlet dye. Excess nitrous acid was destroyed by urea, the solution neutralised (congo red paper) with hydrated sodium acetate and refiltered.

Sandmeyer

| | |
|---|---|
| Copper sulphate, $5H_2O$ | 10gms. |
| Potassium cyanide | 10gms. |
| Water | 100ml. |

A. The cupric sulphate was dissolved in the warm water and the solid KCN added under a good draught. A clear brown solution resulted which was maintained at 80° as the diazo solution was slowly run in with stirring. The solution was kept at 80-90° until no more nitrogen was evolved and then filtered leaving a considerable amount of tarry material, from which no product could be extracted. The filtrate was acidified and extracted with ether, which was dried over sodium sulphate and evaporated, but only tarry material remained, from which solvents failed to separate any nitrile.

B. The diazotisation and Sandmeyer were repeated, both solutions being cooled to 0° and mixed at that temperature. The solution was allowed to warm up to room temperature and left for four days, until no more nitrogen was evolved. Extraction as in (A) gave only tar.

C. The acetate buffered diazo solution was added to the cuprocyanide at 0° and the mixture warmed on a water bath to 80° during one hour. Result was as before.

D. The diazo solution was added to a solution of 10gms. KCN in 100ml. water and 3gms. copper powder stirred in. The solution was warmed on the water bath, but very little

nitrogen was evolved. Extraction as before gave no nitrile.

E. The diazo solution was run into the cuprocyanide on the surface of which there was a layer of 100cc ether, to extract the nitrile as produced. Good stirring was applied during the reaction. Evaporation of the ether gave only tar.

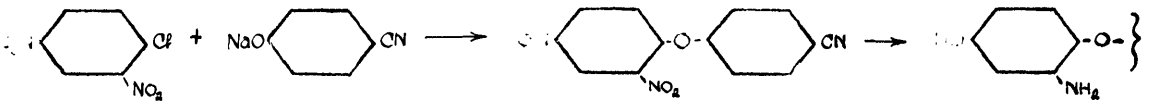
F. The diazonium solution was neutralised as before and added to a solution of potassium nickelocyanide which was warmed to 80°C. Ether extraction gave no product.

From the above results, it is seen that, although the aminohydroxydiphenyl ether diazotises quite smoothly, the conditions for the conversion to nitrile were not found.

## 4-(2'-HYDROXYPHENOXY-) BENZAMIDINE

Outline of Attempted Method B.

Sodium 4-cyanophenoxide was heated with chloronitro--benzene and it was proposed to reduce the nitro-cyano compound to the amine, which could be converted to the hydroxy-cyano derivative.




Attempted Preparation of 2-nitro-4'-cyanodiphenyl ether

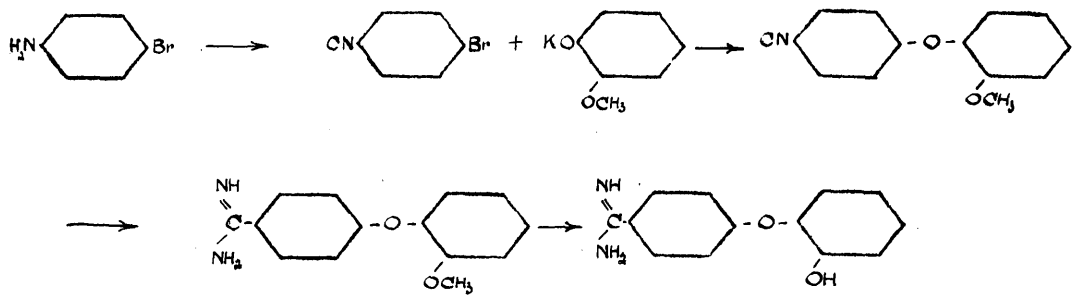
| | |
|---|---|
| 4-Hydroxybenzonitrile | 12gms. |
| Sodium | 2.3gms. |
| Alcohol | 50ml. |
| 4-Nitrochlorobenzene | 16gms. |
| Copper catalyst (p. 57 ) | 0.1gm. |

The hydroxybenzonitrile was dissolved in the alcoholic sodium ethoxide, the solution evaporated to dryness on the water bath and the resulting sodium salt dried at 120°C in the air oven. The nitrochlorobenzene was melted in an oil bath with the copper catalyst, the powdered phenate added with shaking and the mixture heated at 210° for 3½ hours. Much carbonisation ensued and extraction with boiling alcohol gave only unchanged nitro compound. No diphenyl ether derivative was found.

## 4-(2'-HYDROXYPHENOXY-) BENZAMIDINE

### Outline of Method C

4-Bromobenzonitrile, prepared from bromoaniline, was treated with the potassium salt of guiacol and the resulting cyanomethoxydiphenyl ether converted to amidine which was demethylated to the hydroxy compound.



### 4-BROMOANILINE

References:- (64)- by direct bromination of aniline;
(68,147)- bromination of acetanilide with subsequent hydrolysis (75).

Direct bromination of aniline in glacial acetic acid was found to be unsatisfactory since much dibromo compound was produced.

| | | |
|---|---|---|
| 4-Bromoacetanilide | Acetanilide | 270gms. (2mols.) |
| | in Glacial acetic acid | 500ml. |
| | Bromine | 320gms. (105ml.) |
| | in Glacial acetic acid | 700ml. |

The acetanilide in acetic acid was cooled to room temperature and the bromine solution introduced over thirty minutes, with good stirring. The temperature of the solution rose shortly after all the bromine had been added, the mixture became semi-solid and was stirred manually for a further thirty minutes, then added in portions to four litres of cold water, with which it was stirred for fifteen minutes. After settling, the white product was filtered with suction and washed with cold water, being pressed as dry as possible on the filter. It was immediately hydrolysed, but a small sample was dried between filter papers and consisted of fine white crystals, M.P. 167° (listed - 167°).

Hydrolysis to bromoaniline

| | |
|---|---|
| Bromoacetanilide | 2 mols. |
| Potassium hydroxide | 114 gms. |
| in water | 250ml. |
| Ethyl alcohol | 500ml. |

The potassium hydroxide solution was added to the suspension of bromoacetanilide in the alcohol, and, after thirty six hours reflux on the sand-bath, the mixture was poured into 4 litres of cold water. The precipitated oil was stirred and soon solidified, being then filtered and washed free of alkali. The crude amine was not purified but converted directly to the nitrile. Yield of crude p-bromoaniline (air-dried) 350gms. (99%). M.P. 58-61° (listed - 67°). The main impurity was (unchanged) p-bromoacetanilide.

4-BROMOBENZONITRILE.

References: (112) - from amine with cuprocyanide;
(91,92) - use of nickelocyanide.

| | | |
|---|---|---|
| Diazotisation | 4-Bromoaniline (crude) | 200gms. |
| | Hydrochloric acid (s.g.1.16) | 290ml. |
| | Sodium nitrite | 81gms. |
| | in water | 300ml. |

The amine was dissolved in the hot acid and cooled with stirring. When the mixture had reached room temperature the volume was made up to approximately 800ml. with crushed

ice, and the nitrite solution added over fifteen minutes, the temperature not being allowed to rise above 3°C. When the end-point was indicated by starch-KI paper, the solution was filtered from about 10gms. of bromoacetanilide, M.P. 167°C. The filtrate was clear yellow and a sample coupled with alkaline β-naphthol to give a red dye.

| | | |
|---|---|---|
| Sandmeyer | Nickel chloride, $6H_2O$ | 160gms. |
| | Potassium cyanide (technical) | 380gms. |
| | Water | 1000ml. |

The nickel chloride was dissolved in the water at 80°C and the powdered cyanide added with stirring. Heat was evolved and the nickel cyanide re-dissolved to a clear light brown solution which was maintained at 80° with stirring during the addition of the diazo solution and until the evolution of nitrogen ceased about fifteen minutes after the addition was complete. The solution was rendered acid with hydrochloric acid and the precipitated solid filtered. On cooling, the filtrate deposited some material which was added to the original residue and the whole distilled in a current of steam. The nitrile

which crystallised in the condenser and condensate as white needles was collected, washed with cold water, and dried in a vacuum desiccator over sulphuric acid. Yield 116gms. (55%) M.P. 113° (listed - 113°).

Note:- Cuprocyanide gives an inferior yield.

4-CYANO-2'-METHOXYDIPHENYL ETHER

References:- (174) - from the aminomethoxy compound by cuprocyanide solution (yield 30%) or through iodo compound (yield 41%). M.P. 93-4°C.
(5b) - 4-cyanodiphenyl ether from potassium phenate and 4-bromobenzonitrile (yield not stated) and 4:4'-dicyanodiphenyl ether from sodium 4-cyanophenate and 4-bromobenzonitrile (yield 37%).

| | |
|---|---|
| Guaiacol | 25gms. (4 mols.) |
| KOH (Stick, 85%) | 3,5gms. (1 mol.) |
| p-Bromobenzonitrile | 9.1gms. (1 mol.) |
| Copper bronze (p. 58) | 0.1gm. |

The alkali was dissolved in the molten guaiacol and the solution heated till the temperature immediately above the surface was about 130°C.

When the mixture had cooled to 100°C, the copper bronze and bromobenzonitrile were added and the flask heated in an oil bath under an air condenser. When the bath temperature reached 160°C, a reaction was initiated which continued spontaneously for five minutes after the flask was removed from the bath. Crystals of potassium bromide were seen to separate and the internal temperature rose to 210°C. After reaction had subsided, the mixture was refluxed for a further thirty minutes and the melt, after cooling to 109°, was poured into cold water and ground. The whole was extracted with ether and the bulked extracts washed with dilute sodium hydroxide solution until free from guaiacol. After washing with water and drying over anhydrous sodium sulphate, the ether was distilled off and the residue taken up in hot alcohol. The nitrile crystallised as short white rods, M.P.88-91° and, after purification by vacuum sublimation, (180°C/2mm.) showed M.P. 92-93° (listed - 93-4°C). Yield 4.5gms. (40%).

Notes: Copper catalyst is not mentioned in reference (5b) but its use decreases the time of heating from six to less than one hour, so that much less hydrolysis

of the nitrile group occurs.

The excess of guiacol acts as solvent and depresses hydrolysis of the nitrile.

The crude cyanomethoxydiphenyl ether contained no halogen.

Attempted demethylation of 4-cyano-2'-methoxydiphenyl ether.

| | |
|---|---|
| 4-Cyano-2'-methoxydiphenyl ether | 2gms. |
| Hydriodic acid (57% s.g. 1.7) | 10ml. |

The methoxy compound was refluxed with the hydriodic acid until a sample was completely soluble in cold dilute sodium hydroxide (fifteen minutes). An extract was made with ether and washed with dilute ammonia, but, on evaporation, gave only a small quantity of unchanged starting material.

Note: Hydriodic acid has been used to hydrolyse the cyanomethoxy compound to the hydroxycarboxylic acid (174).

4-(2'-METHOXYPHENOXY-)BENZAMIDINE

| | | |
|---|---|---|
| Iminoether | 4-Cyano-2'-methoxydiphenyl ether | 9gms. |
| | Ether (Na dried) | 40ml. |
| | Ethyl alcohol (Mg dried) | 10ml. |

The nitrile was dissolved in the ether-alcohol and dry

hydrogen chloride passed into the mixture at 0°C. The nitrile tended to crystallise on cooling, but a vigorous stream of gas prevented formation of large crystals and the suspended solid gradually passed into solution, forming a clear red liquid. With the usual precautions for the exclusion of moisture, hydrogen chloride was passed in on two days and on the third the ether was evaporated at 40°C under vacuum, leaving an almost white crystalline solid. This residue had a pleasant smell and melted at 155°C with evolution of gas, resolidifying almost immediately and remelting at 157°C (probably amide) After cooling, the compound remelted smoothly at 157°C.

Amidine

Iminoether hydrochloride (from above)
Ethyl alcohol (Na dried) 150ml.
Ammonia gas.

The alcohol was saturated at 0°C with dry ammonia, the iminoether hydrochloride added and the sealed pressure bottle placed in a bath thermostatically controlled at 40°C. Ammonolysis for fourteen hours gave a yellow solution which deposited a little crystalline material on

cooling. The solvent was removed under water-pump vacuum at 50°C and the residual brown oil taken up in 50ml. water containing a few drops of hydrochloric acid. After treatment with decolorising carbon at 40-50°C the solution was cooled to 0°C and excess ammonia (s.g.0.88) added. An oil which was thrown out solidified on stirring and the emulsion remaining crystallised after thirty minutes as long white needles which were filtered on sintered glass and washed with cold water. The base was dried in vacuo over sulphuric acid. M.P.148-51°. Yield 6gms. (62%). Base is alkaline to litmus.

| Analysis | Found | Required by $C_{14}H_{14}O_2N_2$ (base) |
|---|---|---|
| C | 69.2% | 69.4% |
| H | 5.69 | 5.77 |
| N | 11.8 | 11.6 |

Before attempting to demethylate this compound, experiments were made with 4-methoxy-benzamidine.

Demethylation of 4-Methoxybenzamidine.

No reference could be found to the demethylation of methoxy-benzamidine, so a number of small scale tests were carried out. The test substance was 4-methoxybenzamidine hydrochloride (prepared by G. Dunn) and a sample of

4-hydroxybenzamidine was also available for comparison. The demethylating reagents tested, in a qualitative manner, were aqueous hydrochloric, hydrobromic and hydriodic acids and hydrogen bromide in acetic acid. The only successful demethylating agent was the constant boiling hydriodic acid (57%, B.P.127°C).

Demethylation:- 4-Methoxybenzamidine hydrochloride 5gms.
Hydriodic acid (57%, s.g.1.7) 20ml.

The amidine hydrochloride and hydriodic acid, in an open conical flask (100ml.), were gradually heated to boiling and gentle boiling continued for five minutes. After dilution to 200ml. with water, the solution was filtered, a small brown crystalline residue discarded, and excess ammonia added to the clear, dark solution. After a few minutes, a considerable amount of a finely crystalline material separated and was filtered and dried. The crystals were white needles, M.P.229-231°C, while 4-methoxybenzamidine base is not precipitated by ammonis and melts at 112°C (102). Dunn (45) gives M.P. of 4-hydroxybenzamidine base as 229-230°C and a mixed melting point of the product of demethylation with authentic 4-hydroxybenzamidine base showed no depression. The demethylated base was soluble in dilute acid or alkali

and solutions of its hydrochloride gave an immediate white crystalline precipitate with ammonium nitrate, while the free base was precipitated by ammonia.

4-(2'-HYDROXYPHENOXY-)BENZAMIDINE

| | |
|---|---|
| 4-(2'-Methoxyphenoxy-)benzamidine base | 3gms. |
| Hydriodic acid (57%, s.g.1.7) | 30ml. |

The methoxy compound and the freshly distilled acid were slowly heated to boiling point in an open vessel and gently boiled for ten minutes. On dilution to about 100ml. with cold water, a brown crystalline hydriodide precipitated and was filtered and washed with water. Addition of ammonia to the filtrate formed a small discoloured precipitate. The hydriodide was suspended in 20ml. water and excess ammonia (s.g.0.88) added. The discoloured solid which precipitated was filtered and washed with dilute ammonia till the filtrate was free from iodide, then re-dissolved in dilute acid and treated with carbon at 60°C to give a clear solution from which the hydrochloride of the amidine separated as an oil on cooling. Sodium hydroxide solution gave a precipitate which redissolved in excess. Saturated ammonium nitrate was added to the solution of hydrochloride and the amidine nitrate

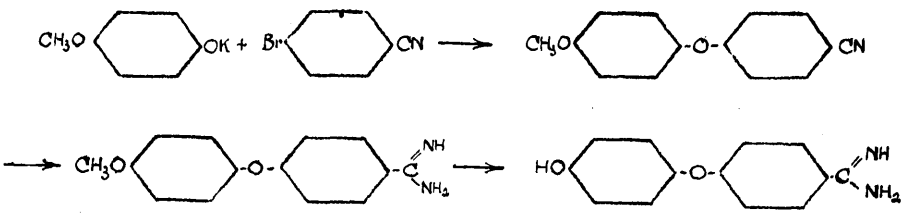
was precipitated as an oil which crystallised in a few minutes as small rectangular plates. The nitrate was recrystallised from water at 60°C and filtered on sintered glass, washed with water and dried over sulphuric acid under vacuum. The nitrate is slightly soluble in cold water and the solution gives the brown ring test for nitrate ion. Yield 1.5gms. (42%) M.P. 164-166°C.

| Analysis | Found | Required by $C_{13}H_{13}O_5N_3$ (nitrate) |
|---|---|---|
| C | 52.9% | 53.6% |
| H | 4.51 | 4.47 |
| N | 13.9 | 14.4 |

## 4-(4'HYDROXYPHENOXY-)BENZAMIDINE

### Outline of Preparation

The potassium salt of 4-methoxyphenol, prepared from hydroquinone, was condensed with 4-bromobenzonitrile and the resulting methoxycyanodiphenyl ether converted to amidine and demethylated.



4-METHOXYPHENOL

References: A (70) (172) - methyl iodide with mono-potassium salt of hydroquinone.

B (148) - sodium salt of hydroquinone treated with dimethyl sulphate in hydrogen atmosphere.

Method A.

| | |
|---|---|
| Hydroquinone | 56gms. |
| Potassium hydroxide | 25gms. |
| Methyl iodide | 28ml. (64.5gms.) |
| Methyl alcohol | 250ml. |

The methyl iodide was added in one quantity to the methyl alcoholic solution of the potassium phenate and,

after refluxing on the water bath until the mixture was no longer alkaline (3-4 hours) the alcohol was evaporated. Steam distillation of the residue removed the dimethyl ether (plates, M.P.56°C) and the non-volatile material was extracted with ether giving a brown oil which was digested with benzene, and the insoluble hydroquinone filtered. The benzene was evaporated and the product distilled at atmospheric pressure, B.P.240-5°C (listed - 242°C). M.P.49°C (listed - 53°C). Yield 17gms. (27%).

Method B.

| | | |
|---|---|---|
| Hydroquinone | 78gms. | |
| Sodium hydroxide | 71gms. | |
| Dimethyl sulphate | 85.5ml. | (112gms.) |
| Water | 500ml. | |
| Hydrogen gas. | | |

The aqueous solution of the alkali was cooled to 12°C and the flask filled with hydrogen from a cylinder. The hydroquinone dissolved to an almost colourless solution, which was immersed in crushed ice and stirred while the dimethyl sulphate was added in one quantity. After five

minutes, the temperature of the solution being kept below 15°, the dimethyl ether was filtered and washed. The filtrate was acidified and cooled in a freezing mixture for one hour, after which the precipitated monomethyl ether was filtered and washed with ice water. The filtrate was extracted repeatedly with ether, and, after drying over sodium sulphate, the solvent was evaporated. The yield of monomethyl ether was bulked and distilled at ordinary pressure, the fraction boiling 243-246°C being collected, and solidifying in the receiver. M.P.52-54°C (listed - 53°C). Yield 58gms. (66%). Dimethylether M.P.56°, yield 23gms. (24%).

Note:- An inert atmosphere of nitrogen functioned equally well.

4-CYANO-4'-METHOXYDIPHENYL ETHER

Reference:- (166) - two methods described. (a) 4-Methoxydiphenyl ether was converted to the 4'-aldehyde by the Gatterman method which was converted to nitrile through aldoxime. (b) Potassium 4-methoxyphenate and 4-bromonitro--benzene give the nitromethoxy diphenyl ether which is converted to nitrile through the amine. Overall yield - 12.5%. M.P.109°C. See also (5b) and p. 69.

The method described below is less involved than either of these two methods, but has not been previously described.

| | | |
|---|---|---|
| 4-Methoxyphenol | 25gms. | (4mols.) |
| Potassium hydroxide (85%) | 3.5gms. | (1mol.) |
| 4-Bromobenzonitrile (p. 67) | 9.1gms. | (1mol.) |
| Copper bronze (p. 58 ) | 0.1gm. | |

The solution of alkali hydroxide in the phenol was heated till the temperature immediately above the surface was 130°C, when the copper bronze and bromobenzonitrile was added, and the mixture heated under an air condenser on an oil bath. Frothing took place and some ammonia was evolved by hydrolysis of the nitrile and, when this side reaction has subsided, the internal temperature was raised to 210°C at which it was maintained for 90 minutes, during which potassium bromide was seen to separate. The melt, cooled below 100°C, was treated with cold dilute alkali with which it was triturated. The residue was extracted with ether which, after washing with alkali and water, was dried over sodium sulphate and evaporated. The residue was crystallised from alcohol after treatment with charcoal

and sublimed under reduced pressure (180°C/2mm.). White crystals, M.P.108°C (listed - 109°C). Yield 3gms. (27%).

4-(4'-METHOXYPHENOXY-)BENZAMIDINE.

Iminoether

| | |
|---|---|
| 4-Cyano-4'-methoxydiphenyl ether | 3gms. |
| Ether (dried over Na) | 30ml. |
| Ethyl alcohol (Mg dried) | 5ml. |

Dry hydrogen chloride was passed into the solution of the nitrile and alcohol in ether at 0°C, with precautions to exclude moisture. The mixture was saturated on two days, a dark red solution being formed, which, on evaporation at 40°C under vacuum, left a somewhat discoloured solid which melted with evolution of gas over the range 110-120°, resolidifying and re-melting 178-180°C.

Amidine

| | |
|---|---|
| Iminoether hydrochloride | (from above) |
| Ethyl alcohol (Na dried) | 150ml. |
| Ammonia gas. | |

The ammonia gas was dried by passing over caustic flake and passed into the alcohol at 0°C. The iminoether was added, the mixture sealed in a pressure bottle, and ammonolysis conducted for ten hours in a bath thermostatic-

-ally maintained at 45°. A small quantity of brown flocculent material was filtered from the yellow solution which was then evaporated to dryness under vacuum at 50-60°C. The discoloured crystalline solid remaining was taken up in dilute hydro--chloric acid and treated with activated charcoal at 60°, the warm solution being finally filtered through sintered glass to give a perfectly clear solution. On cooling, this deposited an oil which crystallised in fifteen minutes to plates of the amidine hydrochloride. After thirty minutes at 0°C, the product was filtered on sintered glass and washed with cold dilute hydrochloric acid. The filtrate gave a small precipitate with dilute sodium hydroxide, but none with ammonia. Amidine hydrochloride, $1H_2O$ (not removed by drying for forty eight hours in vacuum over concentrated sulphuric acid). M.P.158-60°. Yield 2.7gms. (68%).

| Analysis | Found | Required by $C_{14}H_{17}O_3N_2Cl$ (hydrochloride, $1H_2O$) |
|---|---|---|
| C | 56.8% | 56.6% |
| H | 5.72 | 5.72 |
| N | 9.88 | 9.45 |

Note:- This 4-methoxy amidine is apparently a stronger base than ammonia, not being precipitated from its salts

by aqueous ammonia. The 2-methoxy compound, however, is a weaker base than ammonia (p. 73).

4-(4'-HYDROXYPHENOXY-)BENZAMIDINE.

Demethylation

4-(4'-Methoxyphenoxy)-benzamidine hydrochloride 1.7gms.

57% Hydriodic acid 20ml.

Ammonia Solution (s.g.0.88) 20ml.

The Hydriodic acid was freshly distilled (B.P.127°) and added to the methoxy-amidine hydrochloride in a 100ml. beaker. The mixture was heated slowly to boiling point and boiled gently for ten minutes. On cooling, the amidine hydriodide was then, filtered, and washed with water. The hydriodide was stirred with the strong ammonia and the discoloured precipitate of free base filtered after five minutes and washed free of halide with cold water. 2N hydrochloric acid, heated to 70°C was poured through the filter to dissolve the amidine base, and the solution treated with decolourising carbon to give an almost colourless solution from which suspended matter was removed by filtration through sintered glass. Slow cooling from 70° gave plates of the hydrochloride which were filtered and washed with ice water. The filtrate

was cooled to 0°C in a freezing mixture and basified with ammonia (s.g.0.88). The precipitated amidine base was redissolved in hot hydrochloric acid and re-precipitated with ammonia to give pure white crystals. Total yield (as hydrochloride) 1.1gms. (68%). Hydrochloride M.P.228-31° is slightly soluble in cold water and from this solution ammonia precipitates the free base, M.P.218°. The base is also precipitated from its salts by sodium hydroxide, but dissolves in excess. The free base is alkaline to litmus, but is unstable and turns yellow on standing.

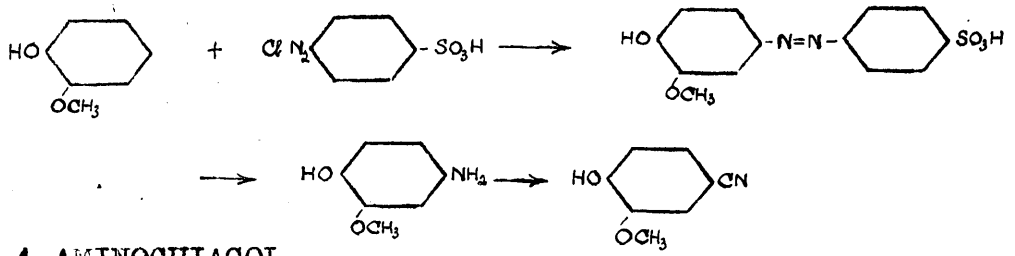
Both the free base and hydrochloride crystallise with one molecule of water of crystallisation which was not lost after forty eight hours in vacuo over concentrated sulphuric acid.

| Analysis | Found | Required by $C_{13}H_{15}O_3N_2Cl$ (hydrochloride, $1H_2O$) |
|---|---|---|
| C | 54.6% | 55.1% |
| H | 5.15 | 5.31 |
| N | 9.90 | 9.92 |
| Cl | 13.05 | 12.6 |

## 3-METHOXY-4-HYDROXYBENZAMIDINE (VANILLAMIDINE).

### Outline of Preparation

Sulphanilic acid was diazotised and the dye produced by coupling with guiacol reduced to give 4-aminoguiacol. This compound was converted to the nitrile from which the amidine was produced.




4-AMINOGUIACOL

**References**:- (152) - Guiacol coupled with diazotised sulphanilic acid and the dyestuff reduced with tin and acid: (109), (122) - use of phenylhydrazine as reducing agent: (77) - reduction by hydrogen sulphide.

The reductions by tin or phenylhydrazine were found to be very inferior to that by sulphide.

| | | |
|---|---|---|
| Diazotisation | Sulphanilic acid | 26gms. |
| | 2N Sodium hydroxide | 75ml. |
| | Sodium nitrite | 12gms. |
| | Hydrochloric acid (s.g.1.16) | 50ml. |
| | in water | 50ml. |

The sulphanilic acid and nitrite were dissolved in the alkali and the solution made up to 600ml. with crushed ice. The hydrochloric acid was run in below the surface with stirring and the temperature maintained below 5°C. The solution turned red at first and the diazo salt precipitated as a light yellow crystalline compound.

| | | |
|---|---|---|
| Coupling | Guiacol | 19.5gms. |
| | 2N Sodium hydroxide | 300ml. |
| | Hydrochloric acid (s.g.1.16) | 400ml. |

The solution of guiacol in the alkali was diluted with ice to 600ml. and the slurry of diazonium salt added over fifteen minutes with strong stirring. The mixture was turbined for forty-five minutes and the hydrochloric acid added to salt out the dye. Stirring was continued for thirty minutes and after the solid dye had settled for the same time, it was filtered with suction and washed with acetone

The product consisted of irridescent green crystals soluble in water and even more readily in alkali to an intensely red solution.

| | | |
|---|---|---|
| Reduction | Azo dye | (from above) |
| | Aqueous Ammonia (s.g.0.88) | 200ml. |
| | in water | 400ml. |
| | Hydrogen sulphide. | |

A rapid stream of hydrogen sulphide was passed into the solution of the dye in the diluted ammonia. After forty-five minutes the temperature rose and aminoguiacol began to separate as the red colour of the solution changed to yellow; the sulphanilic acid formed simultaneously was retained in solution as the ammonium salt. After a further fifteen minutes, the amine was filtered, washed with cold water, and dried in vacuo over sulphuric acid. Needles, M.P.181°C (listed - 182°C). Yield 21.5gms. (98%).

3-METHOXY-4-HYDROXYBENZONITRILE (VANILLONITRILE).

Reference: (152) - Diazotisation of aminoguiacol.

| | | |
|---|---|---|
| Diazotisation | 4-Aminoguaiacol | 15gms. |
| | Hydrochloric Acid (s.g.1.16) | 33ml. |
| | in water | 600ml. |
| | Sodium Nitrite | 7.5gms. |
| | in water | 75ml. |

The amine, in complete solution in the dilute acid, was cooled to -5°C during the addition of the nitrite, but some decomposition was apparent, since nitrogen was evolved and the solution became very dark. The end-point was indicated by starch-KI paper.

| | | |
|---|---|---|
| Sandmeyer | Copper sulphate, $5H_2O$ | 45gms. |
| | in water | 200ml. |
| | Potassium cyanide | 45gms. |
| | in water | 100ml. |

The cyanide was mixed with the copper solution under a good draught, and the clear brown solution maintained at 80-90°C with agitation during the addition of the diazo solution. The solution was heated on the water-bath until the evolution of nitrogen ceased. After acidification the mixture was filtered and the residue and filtrate extracted with ether. After drying over sodium sulphate, the ether

was removed leaving a dark oil which was refluxed with petroleum ether (fraction boiling 100-120°C) which was decanted from the tar. The process was repeated till all the product was extracted. The nitrile separated as a light yellow crystalline powder which was recrystallised from petroleum ether forming glistening white needles. M.P.90°C (listed - 89-90°C). Yield 2.5gms. (15%).

3-METHOXY-4-HYDROXYBENZAMIDINE (VANILLAMIDINE).

| | | |
|---|---|---|
| Iminoether | Vanillonitrile | 2.5gms. |
| | Ethyl alcohol (Mg dried) | 10ml. |
| | Ether (Na dried) | 20ml. |

The solution of nitrile in the mixed solvents was saturated for five hours with dry hydrogen chloride at 0°C, and, after standing overnight, for a further four hours. The crimson solution was evaporated under vacuum at 40°C leaving the somewhat red iminoether salt.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | Alcohol (Na dried) | 150ml. |
| | Ammonia gas. | |

The iminoether salt was added to the alcohol, saturated with dry ammonia at 0°C, and the mixture in a sealed pressure bottle maintained at 40°C (thermostat) in a water bath. On

cooling, the crystalline amidine base was deposited, but was not separated. The solvent was evaporated at 40°C under vacuum and the residue taken up in dilute hydrochloric acid. Warming the acid solution at 40°C with active charcoal gave water-clear solution which was cooled in ice as hydrogen chloride gas was led in. A large excess of hydrogen chloride was avoided as the amidine hydrochloride which separated tended to redissolve. The salt was collected on sintered glass and dried in vacuo over sulphuric acid. Hydrochloride. M.P.275°C (decomp). Yield 2.3gms. (68%).

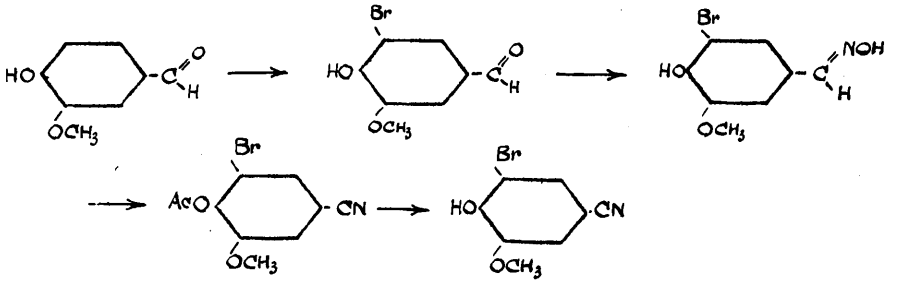
| Analysis | Found | Required by $C_8H_{11}O_2N_2Cl$. | |
|---|---|---|---|
| C | 47.5% | 47.2% | (hydrochloride) |
| H | 5.6 | 5.4 | |
| N | 14.0 | 13.7 | |

Note:- Vanillamidine is a weaker base than ammonia which precipitates the free base from hydrochloride solutions

## 3-METHOXY-4-HYDROXY-5-BROMOBENZAMIDINE (5-BROMOVANILL--AMIDINE.

### Outline of Preparation

Vanillin was monobrominated and the oxime with acetic anhydride gave an acetyl derivative which was hydrolysed to 5-bromovanillonitrile. The amidine was formed in the usual way.




### 5-BROMOVANILLIN

**References**:- (20), (39).

| | |
|---|---|
| Vanillin | 27gms. |
| in glacial acetic acid | 60mls. |
| Bromine | 9.6ml. (30gms.) |
| in glacial acetic acid | 40mls. |
| Sodium acetate (fused) | 10gms. |

After the vanillin and sodium acetate were dissolved with warming in the acetic acid, the solution

was cooled to room temperature and then in cold water as the bromine was added, with stirring, over thirty minutes. The cooling was removed, and, after stirring for a further thirty minutes, the product was precipitated by addition of much water, giving colourless cubes, M.P. 160°C (listed - 164°). Yield 41gms. (99%).

5-BROMOVANILLONITRILE

Reference:- (20).

| | | |
|---|---|---|
| Bromovanillinoxime | 5-Bromovanillin | 41gms. |
| | Hydroxylamine hydro--chloride | 16.2gms. |
| | Sodium carbonate (anhydrous) | 9.5gms. |
| | Alcohol | 200ml. |

The alcoholic suspension of the reactants was refluxed for sixty minutes and poured into 800mls. of water. The precipitated oxime was filtered, washed with water, and dried at 90°C. White needles, M.P.176°C (listed - 179°). Yield 35gms. (80%).

3-Methoxy-4-hydroxy-5-bromobenzonitrile

| | |
|---|---|
| 5-Bromovanillinoxime | 35gms. |
| Acetic anhydride | 150ml. |

The oxime was refluxed with the acetic anhydride for sixty minutes and poured with stirring into a large excess of ice-water containing some sodium hydroxide. The suspension was vigorously stirred until the acetyl derivative crystallised as white needles which were filtered and washed.

5-Bromovanillonitrile

3-Methoxy-4-acetoxy-5-bromobenzonitrile (from above)

2N Sodium hydroxide 100ml.

The acetate was boiled with the alkali for a few minutes until it had just dissolved. The solution was quickly cooled (when the sodium salt separated) and acidified with dilute acid, precipitating the nitrile which was filtered, washed with water and crystallised from alcohol. Colourless crystals. M.P.143°C (listed - 144°). Yield 12gms. (30%).

Note:- To minimise hydrolysis of the nitrile grouping, it was essential that the acetate be treated for the shortest time with the boiling hydroxide and cooled immediately.

5-BROMOVANILLAMIDINE

| | | |
|---|---|---|
| Iminoether | 5-Bromovanillonitrile | 8gms. |
| | Ethyl alcohol (Mg dried) | 10mls. |
| | Ether (Na dried) | 20mls. |

Dry hydrogen chloride was passed for five hours through the solution of nitrile, with precautions for the exclusion of moisture, and, after standing overnight, the liquid was re-saturated. The iminoether hydrochloride precipitated as a crystalline solid and the solvent was removed at 40°C under reduced pressure.

| | | |
|---|---|---|
| Amidine | Alcohol (Na dried) | 150mls. |
| | Dry ammonia gas. | |

The alcohol was saturated with ammonia at 0°C, the iminoether introduced, and the mixture heated under pressure at 40°C for fourteen hours. The solvent was removed at 50°C under reduced pressure and the crystalline residue taken up in dilute hydrochloric acid; this solution was clarified with activated carbon at 40°C and strong aqueous ammonia added in slight excess with cooling. The free base began to precipit--ate in five minutes and, after three hours, was filtered on sintered glass, washed with distilled water, and dried in a

vacuum desiccator over sulphuric acid. Free base M.P. 286°C.

The base was taken up in dilute hydrochloric acid and concentrated acid added to the warm solution. On cooling, the hydrochloride crystallised as white needles, was filtered and washed with cold dilute hydrochloric acid, and dried in vacuo over sulphuric acid. M.P.275-280°C (decomp). Total yield (as hydrochloride) 3.4gms. (35%).

| Analysis (free base). | Found. | Required by $C_9H_9O_2N_2Br$. |
|---|---|---|
| N | 11.3% | 11.4% |
| Br | 32.6 | 32.6 |

## 3-BROMO-4-HYDROXYBENZAMIDINE

Outline of preparation -

4-Hydroxybenzonitrile was monobrominated and the amidine made in the usual manner.

### 3-BROMO-4-HYDROXYBENZONITRILE

Reference:- (126) - bromination of 4-hydroxybenzalde--hyde in chloroform. A similar technique was employed for the nitrile.

| | | |
|---|---|---|
| Bromination:- | 4-Hydroxybenzonitrile (P.52) | 11.9gms. |
| | in chloroform | 300ml. |
| | Bromine | 5ml. |
| | in chloroform | 200ml. |

The hydroxybenzonitrile was dissolved with warming in the chloroform and the solution cooled in ice with stirring as the bromine was introduced below the surface of the liquid over thirty minutes. The temperature was allowed to rise to atmospheric and, after four hours, the chloroform was evaporated on the water-bath and the residue taken up in boiling water. The aqueous solution was filtered and deposited needles of the brominated nitrile on cooling, which were filtered and recrystallised from boiling water White needles, M.P.147-150°C (listed - 155°C). Yield 12gms. (60%).

Note:- Bromination was attempted with bromate-bromide mixture, but always resulted in the 3:5-dibromo--derivative along with starting material.

3-BROMO-4-HYDROXYBENZAMIDINE.

Iminoether:- 3-Bromo-4-hydroxybenzonitrile 2gms.
Ethyl alcohol (Mg dried) 5ml.
Ether (Na dried) 10ml.

Dry hydrogen chloride was passed for seven hours through the solution of the nitrile, which was maintained at 0°C, and for a similar period after standing overnight. The precipitated iminoether was filtered on sintered glass, washed with alcohol, and dried in a vacuum desiccator over concentrated sulphuric acid.

Amidine:- Iminoether hydrochloride (from above)
Alcohol (Na dried) 150ml.
Ammonia gas.

The alcohol was saturated with dry ammonia at 0°C, the iminoether added, and the mixture sealed in a pressure bottle. This was placed in a water bath, whose temperature was maintained at 40°C for fourteen hours. The liquid was then evaporated at 40° under vacuum, and the residue taken

up in warm dilute hydrochloric acid. Decolourisation was accomplished by warming with charcoal at 60°C. Concentrated hydrochloric acid with cooling gave crystals of the hydro--chloride, which was filtered and dried in a vacuum desiccator. M.P.270°C. Yield 2gms. (79%).

| Analysis:- | Found | Required by $C_7H_8ON_2BrCl$ (hydrochloride) |
|---|---|---|
| C | 33.2% | 33.42% |
| H | 3.28 | 3.18 |
| N | 10.75 | 11.12 |
| Cl + Br | 45.9 | 45.0 |

3:5-DIBROMO-4-HYDROXYBENZAMIDINE

Outline of Preparation

4-Hydroxybenzonitrile was dibrominated and the amidine made in the usual manner.

3:5-DIBROMO-4-HYDROXYBENZONITRILE

Reference:- (45) - Bromination using bromate-bromide.

Brominating mixture:- $KBrO_3 + 5KBr + 6HCl = 3Br_2 + 6KCl + 3H_2O$

167.02 595.1 480

Potassium Bromate A.R. 27.837gms.

Potassium Bromide A.R. 100gms.

Water to 1000ml.

The bromate was accurately weighed, dissolved along with the bromide with warming, cooled, and made up to 1000ml. 1 litre of this solution is equivalent to 1 gm-atom of bromine (1 ml. = 0.08gm. $Br_2$).

Note:- This solution tends to crystallise, but is quite satisfactory if warmed before use.

Bromination:- 4-Hydroxybenzonitrile (p.52) 11.9gms. (0.1 mol)

2N Sodium hydroxide solution 50ml.

Brominating mixture 400ml.

Concentrated hydrochloric acid.

The hydroxybenzonitrile was dissolved in the cold alkali, the standard brominating mixture added, and excess acid added with stirring. The colour of free bromine appeared but was discharged at once and the dibromo--hydroxybenzonitrile precipitated. Stirring was continued for a further thirty minutes and the solid filtered and washed with water, until free from acid. The product consisted of micro-needles. Recrystallisation from alcohol gave long, white, needles, M.P.186° (187°). Yield 17gms. (61%).

3:5-DIBROMO-4-HYDROXYBENZAMIDINE

Iminoether:- 3:5-Dibromo-4-hydroxybenzonitrile 17gms.

Iminoether (Contd.):-

| | |
|---|---|
| Ethyl alcohol (Mg dried) | 15ml. |
| Ether (Na dried) | 30ml. |

Observing the usual precautions for the exclusion of moisture, hydrogen chloride was passed through the mixture at 0°C. for five hours and, again, after standing overnight, for a further eight hours. The iminoether crystallised as almost white crystals which were filtered on sintered glass, washed with alcohol, and dried in a vacuum desicca--tor.

Amidine:- Iminoether hydrochloride (from above)

| | |
|---|---|
| Alcohol (Na dried) | 350ml. |
| Ammonia gas. | |

The alcohol was saturated with dry ammonia at 0°C, the iminoether hydrochloride introduced, and the mixture sealed in a pressure bottle, which was heated slowly to 40°C and maintained at that temperature for fourteen hours. On cooling, needles of the amidine were deposited, filtered on sintered glass and washed with water. The filtrate was evaporated under vacuum at 40° and the residue, which was slightly discoloured, taken up in warm dilute hydrochloric acid, and decolorised with charcoal at 60° to give a water

clear solution. Cooling was applied and strong ammonia added. Fine needles of the free base appeared after a short time. These were filtered and dried in a vacuum desiccator. M.P.308°C with decomposition. Yield 15gms. (65%).

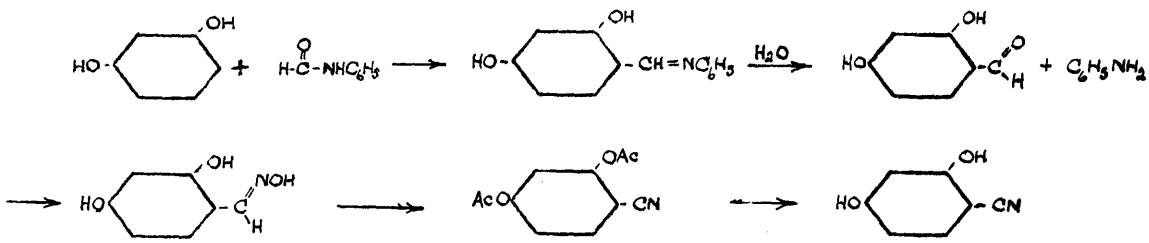
| Analysis:- | Found | Required | $C_7H_6ON_2Br_2$ (base) |
|---|---|---|---|
| C | 28.75% | 28.4% | |
| H | 2.30 | 2.04 | |
| N | 9.44 | 9.53 | |
| Br | 54. 0 | 54.4. | |

The hydrochloride was formed by taking up the free base in warm dilute hydrochloric acid, adding concentrated acid, and cooling in a freezing mixture. M.P.275-280°C.

## 2:4-DIHYDROXYBENZAMIDINE

Outline of Attempted Preparation A.

Resorcinol with formanilide, in the presence of phosphorus oxychloride, gave an anil which was hydrolysed to 2:4-dihydroxybenzaldehyde. The aldoxime was dehydrated with acetic anhydride to the diacetoxybenzonitrile which was hydrolysed to the dihydroxy-compound.



It was not found possible to convert either the diacetyl or the dihydroxy compound to the amidine by Pinner's method.

### 2:4-DIHYDROXYBENZALDEHYDE

References: (81) - modified from (42)

| | | |
|---|---|---|
| Anil | Resorcinol | 32gms. |
| | Formanilide | 35.2gms. |
| | Phosphorus oxychloride | 15.3gms. |
| | Ether (Na dried) | 95mls. |

The mixture, in ethereal solution, was warmed on the water bath to initiate the reaction which them proceeded spontaneously with evolution of hydrogen chloride. On standing overnight, the anil separated as a red solid from which the ether was evaporated.

| | | |
|---|---|---|
| Aldehyde | Sodium hydroxide | 66gms. |
| | in water | 600mls. |
| | Sulphuric acid (s.g.1.84) | 45mls. |
| | in water | 300mls. |

The anil was digested with the alkali for thirty minutes on the steam bath and the mixture distilled. The aniline formed in the hydrolysis was collected in 300mls. of distillate. The residual solution was diluted with 150mls. of water and cooled in ice as the ice-cold sulphuric acid was added with stirring. The yellow solid produced was filtered after three hours and the filtrate saturated with salt. The solid and liquid were exhaustively extracted with ether, which was dried over anhydrous sodium sulphate, and evaporated, The crude product was used without further purification. M.P. 129-31°C (listed -135-6°C). Yield 38gms. (90%).

2:4-DIHYDROXYBENZONITRILE (4-CYANORESORCINOL)

References:- (110) - Process outlined. (73) - Details for 3:4-dihydroxy compound.

| | | |
|---|---|---|
| Aldoxime | 2:4-Dihydroxybenzaldehyde | 38gms. |
| | Hydroxylamine hydrochloride | 22gms. |
| | 2N Sodium hydroxide | 160mls. |

To the solution of the aldehyde in alkali, the hydroxylamine salt was added in concentrated solution with

stirring. In fifteen minutes, a large amount of crystalline material separated and a further 50mls. of water were added. After two hours at room temperature, hydrochloric acid was added to slight acidity and the oxime extracted with ether. After evaporation of the solvent, the oxime crystallised from hot water as needles, m.p. 196°C (listed - 196-7°). Yield 20gms. (48%).

Diacetoxybenzonitrile 2:4-Dihydroxybenzaldoxime 14gms.
Acetic anhydride 105mls.

The anhydride solution of the oxime was raised very slowly to the boiling point (Note I) and refluxed for two and a half hours with stirring. After cooling, the mixture was poured into 1 l. of water from which the diacetate separated in crystalline form. A sample was re-crystallised once from alcohol and melted at 70°C (listed - 72°C). The main yield was immediately hydrolysed. Yield 18gms. (90%).

Dihydroxybenzonitrile 2:4-Diacetoxybenzonitrile 18gms.
2N Sodium hydroxide 200mls.

The diacetyl derivative was refluxed for two hours with the alkali (Note II) and acidified with hydrochloric acid on cooling. The nitrile was extracted with ether which was dried over anhydrous sodium sulphate and evaporated. The residue was taken up in alcohol and,

after treatment with activated charcoal, allowed to crystallise by spontaneous evaporation of the solution (Note III). In spite of repeated crystallisations, a white product could not be obtained. Yield 8gms. (72% on diacetyl derivative). M.P.173 °C (listed - 175 °C)

Notes, I. If the solution be raised to boiling too quickly, considerable decrease in yield ensues.

II. Even on prolonged boiling, the nitrile group is not appreciably hydrolysed by 2N alkali. This is apparently a steric effect.

III. The nitrile is very soluble in water and organic solvents and is crystallised only with difficulty.

Attempted conversion of 2:4-dihydroxybenzonitrile to 2:4-dihydroxybenzamidine

References:- (45) - Preparation of 4-hydroxybenzamidine
(46) - preparation of 2-hydroxybenzamidine.

| | |
|---|---|
| 2:4-Dihydroxybenzonitrile | 9gms. |
| Ethyl alcohol (Mg dried) | 10mls. |
| Ether (Na dried) | 20mls. |

The ether-alcohol solution of the nitrile was saturated with dry hydrogen chloride at 0°C for five hours. The strongly coloured solution was re-saturated on the following day and allowed to stand, after sealing, for twelve hours, when some solid matter had separated. Solvent was removed by water pump at 40°C leaving an amorphous red powder.

Alcohol (Na dried) 150mls.

Ammonia gas.

The above product was added to the alcohol saturated with ammonia at 0°C and the mixture heated in a closed vessel at 40°C for fifteen hours. The cloudy solution, which was dark red, was filtered and evaporated leaving a somewhat tarry residue which was taken up in hydrochloric acid (colour changed to yellow), filtered and the filtrate treated with charcoal. It was not found possible to obtain the free base, the nitrate or the hydrochloride of the amidine or to isolate any material with amidine properties.

Attempted conversion of 2:4-diacetoxybenzonitrile to 2:4-diacetoxybenzamidine

| | |
|---|---|
| 2:4-Diacetoxybenzonitrile (p. 104 ) | 9gms. |
| Ethyl alcohol (Mg dried) | 15mls. |
| Ether (Na dried) | 25mls. |

The same procedure as above was followed and the product obtained as a dark red solid from the first stage.

Alcohol (Na dried) 300mls.

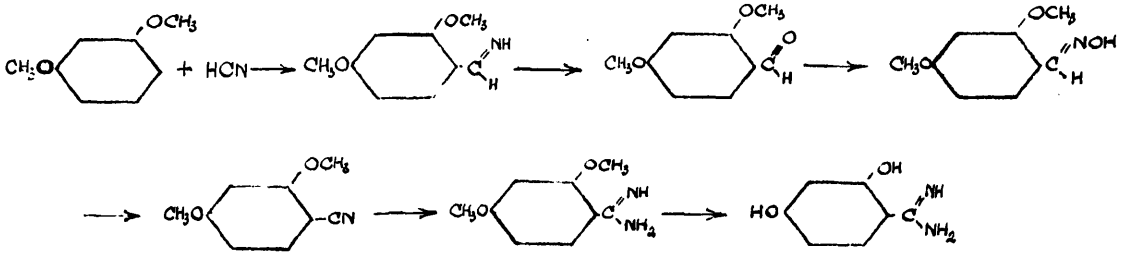
Ammonia gas.

After the ammonolysis, removal of solvent left a red syrup which could not be crystallised and showed no amidine properties.

## 2:4-DIHYDROXYBENZAMIDINE

Outline of Preparation B.

Resorcinol is converted to the dimethyl ether which, with hydrogen cyanide, gives the aldimine, hydrolysed to the aldehyde. The aldoxime is dehydrated to the nitrile which is converted to the dimethoxybenzamidine and demethylated.



### RESORCINOL DIMETHYL ETHER (3-METHOXYANISOLE)

Reference:- (176), (131).

Resorcinol 52gms.

Sodium hydroxide 40gms.
in water 300mls.

Dimethyl sulphate 64gms.

The dimethyl sulphate was added in small portions to the resorcinol, dissolved in sodium hydroxide. After the first vigorous action had subsided, and all the dimethyl sulphate had been added, the solution was refluxed for thirty minutes on the sand bath and poured into a separating funnel. The heavy oil was run off after settling and the remaining aqueous portion repeatedly extracted with ether. The oil, and the ether extracts were combined, and washed free of resorcinol

with dilute sodium hydroxide solution, followed by water to remove the alkali. After drying over calcium chloride, the ether was evaporated leaving a dark oil which was distilled at atmospheric pressure and boiled sharply at 217°C (listed - 216.5-217.7°C). Yield 40gms. (61.5%).

2:4-DIMETHOXYBENZALDEHYDE

References:- (66), (99).

Anhydrous Hydrogen Cyanide

| | |
|---|---|
| Potassium cyanide | 70gms. |
| 50% Sulphuric acid | 200mls. |

The powdered cyanide in a 250ml. distilling flask fitted with rubber stopper and tap funnel was placed in a water bath, the temperature of which was adjusted during the preparation to give a steady stream of the gas. The acid was dripped in slowly and the evolved gas dried through a calcium chloride U-tube suspended in a bath of water at 35°C. The gas was condensed in a wash-bottle immersed in freezing mixture. Yield 50gms. of a pale brown liquid (53%).

| | | |
|---|---|---|
| Dimethoxybenzaldehyde | Resorcinol dimethyl ether | 40gms. |
| | Anhydrous hydrogen cyanide | 40gms. |
| | Anhydrous zinc chloride | 20gms. (approx.) |
| | Ether (Na dried) | 300mls. |
| | Anhydrous hydrogen chloride. | |

The zinc chloride was powdered in a warm mortar and added to the ethereal solution containing the dimethoxy-

-compound and hydrogen cyanide. Hydrogen chloride was passed in with cooling, as quickly as possible without rise in temperature above 3° and the brown solution became deep red. The crystalline aldimine hydrochloride separated quickly and, after standing for thirty minutes, was hydrolysed by adding to 1200mls. of water at 70°C, with which it was agitated for twenty minutes. On cooling to 0°C and scratching the sides of the beaker, white needles separated, and were re-crystallised from aqueous alcohol. M.P.67°C (listed - 68°C). Yield 44gms. (92%). The product has a heliotrope odour.

2:4-DIMETHOXYBENZONITRILE

References:- Similar preparations for 2:5-dimethoxybenzonitrile - (84); for 2:6- compound - (113)

Dimethoxybenzaldoxime - (66)

Dimethoxybenzonitrile, M.P.89°C, by cyanogen bromide on resorcinol dimethyl ether - (83); M.P. 95-96°C, from β-resorcylic acid - (164).

This compound has not been previously prepared by the method described below.

| | | |
|---|---|---|
| Aldoxime | 2:4-Dimethoxybenzaldehyde | 35gms. |
| | Hydroxylamine hydrochloride | 14gms. |
| | Anhydrous sodium carbonate | 10.6gms. |
| | in water | 150mls. |

The aldehyde and hydroxylamine were added to the sodium carbonate solution and the mixture refluxed for one hour.

Pouring into cold water precipitated white needles of the oxime which were filtered, washed and re-crystallised from water. M.P.99°C (listed - 106°C). Yield 35gms. (92%).

Dimethoxybenzonitrile

| | |
|---|---|
| 2:4-Dimethoxybenzaldoxime | 20gms. |
| Acetic anhydride | 100ml. |

The oxime, which was perfectly dry, was added to the acetic anhydride, and the temperature gradually raised to boiling. The oxime dissolved readily and, after reflux for one hour, the solution was poured into ice water and stirred for fifteen minutes, when needles were deposited. The product was recrystallised three times from alcohol, with treatment with activated carbon and finally gave white needles melting sharply at 94°C.

This product was not impure oxime since a mixed melting point showed a depression of 15°. A proof of the nature of the product was furnished by hydrolysis to the acid. 1 gm. was refluxed with 10mls. 30% aqueous sodium hydroxide for eight hours, during which ammonia was evolved and the material passed completely into solution. Acidification gave a white solid which was recrystallised from hot water. The substance was acid in reaction and had M.P.108°C. 2:4-Dimethoxybenzoic acid melts at 109°C (149).

The nitrile is hardly soluble in water and only

sparingly in ether. It is soluble in cold chloroform and benzene and in hot alcohol. Yield of dimethoxybenzonitrile 15gms. (83%). M.P.94°C (listed - 95-96°).

Note: The yield of nitrile from oxime was greatly reduced if either the oxime was at all moist or the mixture was brought to reflux temperature too quickly. In the latter case, a vigorous reaction was initiated and continued spontaneously for about five minutes. Yield was reduced to about 50% of the theoretical.

2:4-DIMETHOXYBENZAMIDINE

| | | |
|---|---|---|
| Iminoether | 2:4-Dimethoxybenzonitrile | 6gms. |
| | Ethyl alcohol (Mg dried) | 10mls. |
| | Chloroform ($P_2O_5$ dried) (Note 1) | 30mls. |

Dry hydrogen chloride was passed into the solution of nitrile which was maintained at 0°C (Note 2). When saturated, the mixture was kept in a sealed vessel overnight and re-saturated. The dark red solution was evaporated after a further twenty four hours, the crystalline residue showing a very diffuse melting point and decomposing with evolution of gas at about 110°C.

| | | |
|---|---|---|
| Ammonolysis | Iminoether hydrochloride | (from above) |
| | Alcohol (Na dried) | 150mls. |
| | Ammonia gas. | |

The iminoether salt was added to the alcohol saturated with dry ammonia at 0°C and the mixture heated in a sealed

vessel at 41°C (thermostat) for twelve hours. The yellow solution deposited large white crystals on cooling, and, on evaporation under vacuum at 50°C a further crop was obtained. The compound which was the amidine hydrochloride was recrystallised from hydrochloric acid at 50-60°C after clarification with activated charcoal, precipitation being completed by saturation of the solution at 0°C with gaseous hydrogen chloride. Needles, M.P.258°C with decomposition. Yield as hydrochloride 5gms. (63%).

Notes: 1. Owing to the slight solubility of the nitrile in ether, chloroform had to be used as diluent.

2. If cooling was too strong, the nitrile crystallised.

| Analysis | Found | Required by $C_9H_{13}O_2N_2Cl$ (hydrochloride) |
|---|---|---|
| C | 49.7% | 49.8% |
| H | 6.09 | 6.00 |
| N | 13.1 | 12.9 |
| Cl | 15.9 | 16.4 |

2:4-DIHYDROXYBENZAMIDINE

References:- Preparation of amidine sulphate - (46).
Demethylation of methoxybenzamidines - see p. 73.

2:4-Dimethoxybenzamidine hydrochloride 3.5gms.
Hydriodic acid (57%, B.P. 127°C) 30mls.
Silver sulphate (Note 1) 10gms. (approx.)

The A.R. hydriodic acid was freshly distilled and slowly heated to boiling point with the dimethoxy compound. After ten minutes gentle reflux, the solution was cooled and tarry material which separated was filtered. The dark brown solution was evaporated to dryness under vacuum at 60°C and the residue taken up in 15mls. of water. The solution was warmed at 40°C with moist, freshly precipitated, silver sulphate (Note 1) and the mixture filtered. The clear yellow filtrate was freed from silver by addition of a few drops of concentrated hydrochloric acid and the solution treated with activated charcoal. The clear and almost colourless solution was evaporated to small bulk on the steam bath, gradually assuming a red colour. About 4 ml. of viscous material finally remained, and, after seven days at room temperature crystal nuclei appeared; after fourteen days, there were

needle crystals throughout the material which was now very viscous. On stirring the mixture with acetone (10ml.), the red material passed completely into solution and the amidine sulphate was filtered on sintered glass and thoroughly washed with the same solvent. White needles, M.P.276°C with decomposition. The salt is easily soluble in water and in alcohol. Yield 0.2gms. (6.2%).

Notes: 1. The silver sulphate was prepared by mixing neutral concentrated solution of silver nitrate and sodium sulphate. The precipitate was washed with distilled water and used moist.

2. The dihydroxybenzamidine base is probably water-soluble and is not precipitated by alkali from the salts. Ether extraction of the alkaline solution gave no material.

| Analysis | Found | Required by $(C_7H_8O_2N_2)_2 \cdot H_2SO_4$ |
|---|---|---|
| C | 42.0% | 41.8% |
| H | 4.50 | 4.47 |
| N | 14.5 | 13.9 |
| S | 8.04 | 7.96 |

## p-HYDROXYBENZAMIDINE DERIVATIVES

## BIOLOGICAL TESTS

The following test for amoebicidal property was carried out by Messrs. I.C.I. (Dyestuffs Division), Blackley, Manchester, on each of the amidines described in Part I.

4,000 to 6,000 amoebae from a three-day culture of Entamoeba histolytica were inoculated into a buffered medium containing a known concentration of the amidine under test. After two days' incubation at 37°C, the mixture was examined microscopically. Emetine hydrochloride is highly active by this test at 1:1,000,000.

Each amidine was tested at concentrations of 1:1,000, 1:10,000, 1:100,000 and 1:1,000,000.

4-Hydroxybenzamidine )
4-Methoxybenzamidine ) Prepared by G.Dunn (45)
4-Phenoxybenzamidine )
4-Ethoxybenzamidine hydrochloride
4-(β-Bromoethoxy)-benzamidine hydrochloride
4-(2'-Methoxyphenoxy)-benzamidine
4-(2'-Hydroxyphenoxy)-benzamidine nitrate
4-(4'-Methoxyphenoxy)-benzamidine hydrochloride) Slightly active at 1:10,000.
4-(4'-Hydroxyphenoxy)-benzamidine hydrochloride)
3-Methoxy-4-hydroxybenzamidine hydrochloride
3-Methoxy-4-hydroxy-5-bromobenzamidine hydrochloride
3-Bromo-4-hydroxybenzamidine hydrochloride
3:5-Dibromo-4-hydroxybenzamidine hydrochloride
2:4-Dimethoxybenzamidine hydrochloride
2:4-Dihydroxybenzamidine sulphate.

All the above compounds were inactive at the two lowest concentrations. Only 4-(4'-Methoxyphenoxy)-benzamidine and the corresponding hydroxy-compound showed any activity at 1:10,000; slight activity was shown by the others at 1:1000.

Similar tests were performed on some of the compounds described in Part II (p.182).

PART II

# AMIDINES RELATED TO BIOLOGICAL GROWTH FACTORS

Theoretical

Experimental:-

A. Phenoxyacetamidine Derivatives

B. 4-Aminobenzamidine Derivatives

## PART II AMIDINES RELATED TO BIOLOGICAL GROWTH FACTORS

### THEORETICAL

#### Reasons underlying Investigation

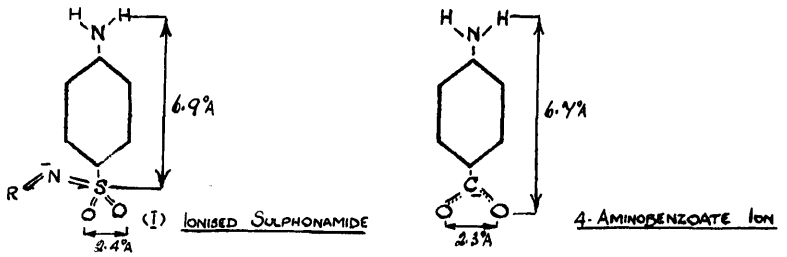
Much work has been done on the mode of action of chemotherapeutic agents, in particular that of the sulphonamide series of drugs. It is suggested here that the theory developed regarding the mechanism of sulphonamide action may be capable of extension to the amidine series.

1. Growth factors and drugs

It has been suggested (58,184) that sulphonamides compete for the enzyme system of the bacteria with 4-aminobenzoic acid, which is an essential bacterial growth factor. The sulphonamide being structurally similar to the carboxylic acid, substitutes for the acid in the metabolism of the organism, but, since it does not satisfy the requirements for growth and reproduction, bacteriostasis is caused. Thus, the addition of even small amounts of 4-aminobenzoic acid nullifies the action of the sulphonamide, since the bacteria can then satisfy their requirements. This action has been observed both in vivo and in vitro.

The theory has been further developed, in a more quantitative aspect, by Bell and Roblin (13) and others (89,96). These workers have related the acid dissociation

of the sulphonamide to its bacteriostatic activity. Comparison of the sulphonamide anion (I) with the 4-aminobenzoate ion reveals a striking similarity in physical dimensions.



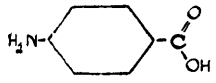

(I) Ionised Sulphonamide  4-Aminobenzoate Ion

The basis of the theory, the steric similarity of the sulphonamides to 4-aminobenzoic acid, appears well established, since the presence of the amino group in the 4 position is found to be essential for activity and it has been found possible to apply the analogy to other compounds. For example, Kumler and Daniels (96) quote tests made with 4-nitroaniline (II) which bears a strong resemblance to the 4-aminobenzoate ion (III):

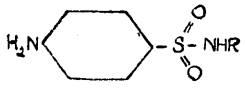
(II)  (III)

4-Nitroaniline was bacteriostatic against B.coli at a concentration of 1 in 4,000, and this activity was nullified by 4-aminobenzoic acid at 1 in 10,000.

There are numerous other factors to be taken into account as regards drugs, for example, solubility, absorption and toxicity; and the relationship shown above is derived from



(IV)



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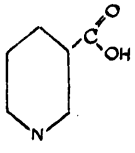
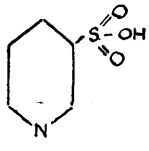
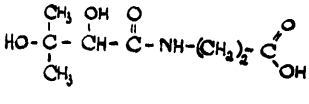


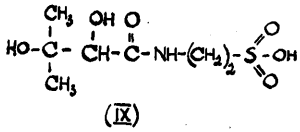
(VI)



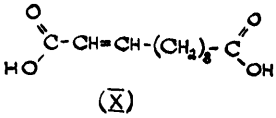
(VII)



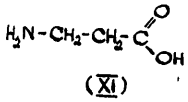
(VIII)



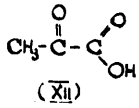
(IX)



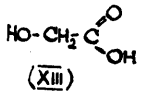
(X)



(XI)



(XII)



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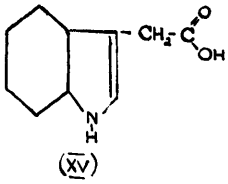
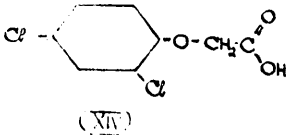


(XV)



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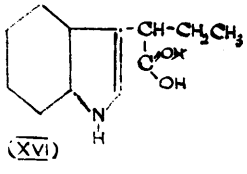


(XVI)

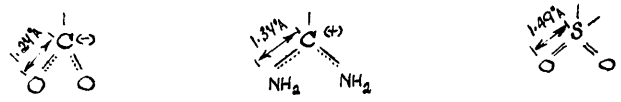
in vitro rather than in vivo activity. The interpretation as far as the latter is concerned is open to argument. Perhaps the most significant implication of the work on the relation of sulphonamides to 4-aminobenzoic acid is that study of the chemical nature of other essential growth factors may suggest new types of chemotherapeutic agents. In this connection, it is noteworthy that very many of the bacterial and plant growth factors that have been isolated or synthesised are carboxylic acids. Bacterial growth factors include 4-aminobenzoic acid (IV) (151); nicotinic acid (VI) (119); pantothenic acid (VIII) (94); traumatic acid (X) (50,123); $\beta$-alanine (XI) (93,120); mixed pyruvic (XII) and glycollic acids (XIII) (121). Plant growth factors, or auxins (erroneously called "plant hormones") include substances such as halogenated phenoxyacetic acids, e.g. 2:4-dichlorophenoxyacetic acid (XIV), indole-3-acetic acid (XV) and indole-3-butyric acid (XVI) etc. (188). On the other hand, compounds closely analogous in structure to the carboxylic acids (IV), (VI) and (VIII) have been shown to exert a bacteriostatic action. In particular, the sulphonamide series of drugs (V,R = hydrogen, pyridine, thiazole, guanidine, pyrimidine, etc. residues) are related to growth factor (IV); and the sulphonic acids, pyridine-3-sulphonic acid (VII) (114) and pantoyl-taurine (IX) (115), bearing similar relationship to (VI) and (VIII), also show bacteriostatic properties.

## 2. Amidines and carboxylic acids

The chemical analogy between amidines and carboxylic acids has already been mentioned (p.33 ) and the possibility is now considered that this analogy may be extended to biological action, the amidines acting as negative growth agents.

A comparison can be made of the carboxylate and amidinium ions, the physical dimensions underlining the similarity. Both ions are resonance hybrids, though of opposite charge:

The interatomic distances for the carboxylate ion (XVII) have been quoted by Pauling (127), while those for the amidinium ion (XVIII) can be approximately calculated from covalent radii data. Since the structure is a resonance hybrid, the C-N distances are somewhat less than the mean of the single and double bond values. The $-SO_2-$ group in the sulphonamides is shown for comparison:

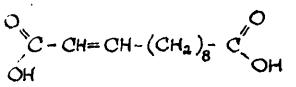


(XVII) (XVIII)

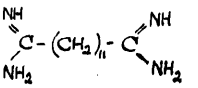
## 3. Amidines as growth inhibitors

There is a very little to be found in the literature concerning the mode of action of the therapeutically active amidines. The original theory that they interfere in vivo with bacterial metabolism by virtue of their hypoglycemic action has been disproved; in vitro tests show that these

amidines exert a direct effect on the bacteria. From the only generalisations that have yet emerged, it is clear that the maximum trypanocidal activity in the amidine series is associated with an $\alpha\omega$-diamidine, the two functional groups being separated by a chain of molecular complexity equivalent to ten to twelve methylene groups. This suggests a relationship between the active diamidines, of which undecam-1:11-diamidine (XIX) is a prototype and the growth factor traumatic acid (X):



(X)



(XIX)

The similarity is reminiscent of that of the sulphonamides to 4-aminobenzoic acid, of pyridine-3-sulphonic acid to nicotinic acid and of pantoyltaurine to pantothenic acid. In the same way, it was thought possible that the amidine might replace the carboxylic acid growth substance in the enzyme system of the organism, but act as a growth inhibitor.

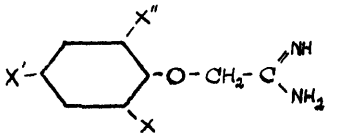
To test this hypothesis, a number of carboxylic acids of known growth promoting property were prepared along with the corresponding amidines, the growth factors being chosen from both bacterial and plant fields. The synthetic plant auxins offered a series of carboxylic acids of well-graded growth promoting action, and the method of testing also readily indicated growth inhibition. There is, moreover,

a definite relationship between plant and bacterial growth factors. Boysen Jensen (79) showed that bacteria produce a substance promoting plant growth while others (18,50,123, 171) have shown that certain compounds which act as growth promoters for micro-organisms perform the same function for higher plants. It seems probable that the mechanisms of growth promotion in both fields are connected.

Two series of amidines were accordingly prepared:-

Series A

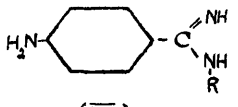
The phenoxyacetamidines (XX), related to the halogenated phenoxyacetic acids shown by Zimmermann (188) to be powerful plant auxins:



X, X', X'', = HYDROGEN or CHLORINE.

Series B

The aminobenzamidines (XXI), related to the well-known bacterial growth factor, 4-aminobenzoic acid:



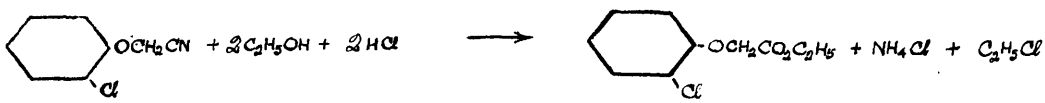
(XXI)

R = hydrogen, benzene, pyridine or thiazole residues.
The pyridine and thiazole substituents were chosen by analogy to the sulphonamide series.

## A. Phenoxyacetamidines

### Chemical Notes

The Pinner method of preparation from the nitriles was adopted, but required modification in some cases as the nitriles proved extremely reactive under the conditions of iminoether formation. Whereas, normally, a nitrile was treated with excess alcohol and hydrogen chloride, this procedure gave the iminoether only with phenoxyacetonitrile and the 4-chloro derivative and in poor yield. In the second case, the yield of amidine was only 15%. When the usual conditions for the reaction were applied to the 2-chlorophenoxyacetonitrile, it was found that the ethyl ester and ammonium chloride were produced, presumably according to the equation:




When the ammonolysis was carried out without isolating the intermediate product, the amide resulted. This is possibly the type of reaction observed by Pinner (139) when he suggested the formation of unstable chlorides of the iminoethers, which decomposed to esters and ammonium chloride.

Those nitriles which reacted in this manner were treated with the theoretical amounts of alcohol and hydrogen chloride to form the iminoether hydrochlorides. A solution of dry hydrogen chloride in dry ether was prepared and standardised,

the correct amount for the reaction being measured from a burette, the alcohol was similarly measured by volume. The reaction could be seen to commence almost immediately, and the iminoether hydrochloride precipitated as a crystalline compound. This procedure was followed for the 2-chloro-, 3-chloro-, 2:4-dichloro, 2-methyl-4-chloro- and 2:4:6-trichloro-phenoxyacetonitriles.

That this abnormal reactivity of the nitrile group was due to the ether linkage was demonstrated by the fact that phenylacetonitrile, treated with excess alcohol and hydrogen chloride gave the iminoether normally, and then the amidine in 70% yield. It is possible that the ethereal oxygen, being electrophilic, enhanced the positive character given to the carbon atom of the nitrile group by the electromeric shift, $R\text{-}C\overset{\curvearrowright}{\equiv}N$. The total result, expressed by $R\text{-}O\text{-}\leftarrow C\overset{\curvearrowright}{\equiv}N$, was that the reactivity of the nitrile towards nucleophilic reagents was increased, in the same way as in trichloroacetonitrile (36).

Since the nitrile group was removed some distance from the nucleus, the "ortho effect" (p. 6 ). disappeared. This was shown by the preparation of 2-chloro- and 2-methyl-4-chloro-phenoxyacetonitrile which reacted very readily to form the iminoethers, in contrast to the unreactivity of 2-chlorobenzonitrile.

The nitriles themselves were prepared from chloroaceto-

nitrile which was heated, in alcoholic solution, with the sodium salt of the appropriate phenol. The nitriles were found to be oils which were thermally unstable, and, with the exception of phenoxyacetonitrile, could not be distilled even under reduced pressure without decomposition. On some occasions, possibly due to traces of alkali, samples of the nitriles decomposed on standing at room temperature to tarry material. Since the nitriles were so reactive, they were also rather easily hydrolysed, and their preparation from chloroacetonitrile did not give good yields. The compounds were of pungent odour, and unpleasant lachrymatory properties.

The phenoxyacetic acids for comparison purposes were readily prepared from chloroacetic acid and the sodium salt of the appropriate phenol.

Biological Notes

The standard method of testing a substance for plant growth promotion or inhibition consists of application of the material unilaterally on coleoptiles of Avena sativa (oat seedlings). If a growth promoting substance is applied to one side of such a plant, it accelerates growth (actually cell elongation) on that side, and hence the plant curves away from the treated side. If a growth inhibitor is used, the curvature is in the opposite sense.(80).

The plants may be decapitated, and agar blocks containing the material under test applied to one side of the stump; alternatively, the material may be applied in lanolin to one side of the otherwise untreated coleoptile. This latter method was adopted, since only an indication of effect on growth was required, and the errors inherent in the decapitation technique were thereby avoided. The lanolin used was shown to have no action in itself.

Culture conditions necessitated constant temperature and humidity and the application of material had to be performed by phototropically inactive (red) light. A daylight technique has been described (163).

The phenoxyacetamidines showed growth inhibiting properties in those cases where the acid showed growth promotion, although the order of activity was not the same, 2:4:6-Trichlorophenoxyacetic acid and the corresponding amidine both caused growth inhibition. The acid has been previously noted (188) as showing no growth-promoting properties.

Some compounds of the series were also tested as amoebicides (Part I, p. 115 ) but showed only slight activity.

## B. Aminobenzamidines

### Chemical Notes

The aminobenzamidine series were prepared by reduction of the corresponding nitro compounds and the parent 4-nitrobenzamidine was formed in good yield from 4-nitrobenzonitrile through the iminoether with ammonia. Use of excess (ten moles) of 2-aminopyridine or 2-aminothiazole instead of ammonia was the best route to the amidine substituted compounds, but gave reduced yields. The N-phenyl derivative was easily accessible from 4-nitrobenzanilide, through the iminochloride and ammonia, but attempts at analogous synthesis for the other compounds confirmed that the nitrobenzoyl derivatives of aminopyridine and aminothiazole are not readily obtained (172a).

The N-phenyl compound was also made by fusion of 4-nitrobenzonitrile with aniline benzenesulphonate (125), but the product required greater purification.

The nitrobenzamidines were crystalline yellow bases, weaker than ammonia. Reduction by stannous chloride or, preferably, by hydrogen over platinum (except for the thiazole compound) produced the colourless aminobenzamidines which were isolated as the dihydrochlorides, the grouping

which occurs in the pyridyl and thiazyl derivatives, being mono-acidic. The bases and salts were water soluble.

Biological notes

The compounds were tested in vitro for bacteriostatic action against a representative selection of organisms which were known to utilise 4-aminobenzoic acid for growth. Standard procedures were followed, including the Oxford Cup method as in penicillin assay. In addition, 4-aminobenzamidine was tested in vivo on mice.

All results were negative.

**PART IIA**

## EXPERIMENTAL

### Phenoxyacetamidine Derivatives:-

**Preparation of Compounds**

**Biological Tests.**

## Part IIA. - PHENOXYACETAMIDINE DERIVATIVES.

### CHLOROACETONITRILE

This reagent was used in the synthesis of each amidine.

References:- All references indicate preparation of the nitrile by dehydration of the amide with phosphorus pentoxide. Scholl (155), 30-40% yield by distillation of chloroacetamide with phosphorus pentoxide at atmospheric pressure. Steinkopf (165), 70% yield by distillation under reduced pressure. He indicates that sublimation of the amide is troublesome.

| | | |
|---|---|---|
| Chloroacetonitrile | Chloroacetamide | 100gms. |
| | Phosphorus pentoxide | 160gms. |

The chloroacetamide was measured out into a dry 1000ml. flask and the phosphorus pentoxide added a little at a time, the contents of the flask being mixed by shaking after each addition. A luminous flame was played on the bottom of the flaskas a slight vacuum was applied, a pressure of 260mm. at the commencement of distillation being decreased slowly to 60mm. towards the end, and the nitrile distilled slowly

(approximately 45 minutes) as a clear, colourless liquid of rather pungent odour. It was redistilled at ordinary pressure, almost all boiling sharply at 124°C (listed - 124°C). Yield 80gms. (98.5%). No trouble was experienced with amide sublimation.

## PHENOXYACETAMIDINE

### Outline of Preparation

Chloroacetonitrile is refluxed in alcohol with sodium phenate and the resulting nitrile converted to the amidine.

### PHENOXYACETONITRILE

Reference:- Pomeranz (142), from the unstable aldehyde through the oxime with acetic anhydride. Fritzsche (62), from phenoxyacetamide with phosphorus pentoxide. Powell and Adam (143), modification of Fritzsche. No yield quoted. B.P.235°/745mm., 132°/30mm.

| Nitrile | | |
|---|---|---|
| | Phenol | 12gms. (1.2mol) |
| | Ethyl Alcohol | 50ml. |
| | Sodium | 2.3gms. (1 mol) |
| | Chloroacetonitrile | 6.7ml. (8gms.,1 mol) |

To a solution of sodium in alcohol containing phenol, chloroacetonitrile was added and the whole refluxed for thirty minutes on the water-bath. After cooling, 250ml. of water were added to dissolve the precipitated sodium chloride and the resulting oil separated.

The aqueous portion was extracted with ether, added to the oil, the whole shaken with 2N sodium hydroxide solution till free of phenol, and then washed with water. The ethereal solution was dried over anhydrous sodium sulphate and evaporated to give an almost colourless oil which distilled at 235-240°C at ordinary pressure, with some decomposition. Even under reduced pressure, the liquid darkened on distillation. The product was a clear colourless liquid of pungent odour, the vapour being distinctly lachrymatory. Yield 8 gms. (60%).

Note:- Powell and Adams obtained a 90% recovery after refluxing 25gms. for 15 hours, although the liquid darkened.(143).

PHENOXYACETAMIDINE

The method used in Part I was followed.

| | | |
|---|---|---|
| Iminoether | Phenoxyacetonitrile | 8 gms. |
| | Ethyl alcohol (Mg dried) | 20 ml. |
| | Ether (Na dried) | 30 ml. |

The solution of the nitrile in the alcohol-ether was saturated at 0°C with dry hydrogen chloride, the iminoether starting to crystallise after five hours. The mixture was re-saturated on the following day and after a further 24 hours the iminoether hydrochloride was filtered and washed with

ether, giving a pure white crystalline product.

Amidine — Iminoether hydrochloride (from above)

Ethyl alcohol (Na dried) 150ml.

Ammonia gas

The alcohol was saturated at 0° with ammonia gas dried by flake sodium hydroxide, the iminoether hydrochloride added and the pressure bottle placed in a bath thermostatically controlled at 40° for fourteen hours. The solution, now somewhat discoloured, was evaporated at 40° under water-pump vacuum, and the residue dissolved in 150ml. of 2N hydrochloric acid at 60°C. The solution was treated twice with decolorising carbon and finally filtered through sintered glass to remove suspended matter. The perfectly clear and colourless solution was cooled to 0° in crushed ice, and 10cc. of a 50% solution of ammonium nitrate added dropwise. Within a few minutes, white plates of phenoxyacetamidine nitrate separated, which, after standing for an hour, were filtered on sintered glass, washed with ice-water and dried over concentrated sulphuric acid under vacuum. Yield 4gms. (31%). M.P. 123°C.

| Analysis | Found | Required by $C_8H_{11}O_4N_3$. (nitrate) |
|---|---|---|
| C | 45.02% | 45.0% |
| H | 5.28 | 5.16 |
| N | 19. 5 | 19. 7 |

## PHENOXYACETIC ACID

Reference:- Minton and Stephen (116).

| | |
|---|---|
| Phenol | 9.5gms. |
| Sodium hydroxide | 4 gms. |
| in water | 40ml. |
| Chloroacetic acid | 10gms. |
| Sodium carbonate | 5.5gms. |
| in water | 50ml. |

The phenol was dissolved in the hydroxide solution and the chloroacetic acid in the carbonate. The two solutions were mixed and boiled under reflux for eight hours, when the original brown colour had lightened considerable. The hot solution was acidified with 2N hydrochloric acid, and the white solid precipitated was filtered after cooling. The phenoxyacetic acid was dissolved in 400ml. of boiling water and the solution boiled for a few minutes to remove unchanged phenol. On cooling, white, flat needles of the acid separated and these were filtered, washed with water, and dried in air. M.P.98°C (listed 96° or 99°). Yield 14gms. (92%).

## p-CHLOROPHENOXYACETAMIDINE

### Outline of Preparation

Chloroacetonitrile was treated with sodium p-chlorophenate and the resulting nitrile converted to amidine.

### p-CHLOROPHENOXYACETONITRILE

This nitrile is not listed.

| | |
|---|---|
| p-Chlorophenol | 28gms. |
| Ethyl alcohol | 80ml. |
| Sodium | 4.6gms. |
| Chloroacetonitrile | 13.4ml. (16gms.) |

The phenol in sodium ethoxide solution and the chloroacetonitrile were refluxed on a water bath for thirty minutes and the mixture poured into 500ml. of cold water. The precipitated oil was separated and the aqueous portion extracted three times with ether. The extracts were added to the oil, and washed free of phenol with 2N sodium hydroxide, followed by water. After drying over anhydrous sodium sulphate, the ether was removed on the water bath, leaving a pale brown oil which could not be distilled even under vacuum (10mm.) as the liquid decomposed if heated above 100°. The

Liquid did not solidify in a freezing mixture of -5°C, so the product was dried over sulphuric acid in a desiccator under vacuum. The nitrile had a pungent odour, and was very irritant to the eyes. Yield 30gms. (89%).

Note:- Powell and Adams (143) attempted to prepare a nitrile from p-bromophenoxyacetamide with phosphorus pentoxide. No yield was obtained, but deep-seated decomposition ensued.

p-CHLOROPHENOXYACETAMIDINE

| | | |
|---|---|---|
| Iminoether:- | p-Chlorophenoxyacetonitrile | 30gms. |
| | Ethyl alcohol (Mg dried) | 40ml. |
| | Ether (Na dried) | 20ml. |

The dry nitrile, alcohol and ether were kept at 0°C as dry hydrogen chloride was passed in for nine hours on the first day and for a further six hours on the day following. The iminoether hydrochloride separated as a very dark violet compound which was filtered, washed with ether and dry alcohol, and dried over concentrated sulphuric acid.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | Ethyl alcohol (Na dried) | 300ml. |
| | Ammonia gas. | |

The iminoether salt was added to alcohol, saturated with ammonia at 0°C in a pressure bottle which was placed in a bath of cold water and slowly heated to 40°C, this temperature being maintained by a thermostat for fourteen hours. The solution deposited a considerable tarry precipitate which was filtered and found to be insoluble in warm dilute acid. The dark filtrate was evaporated at 40°C under vacuum, leaving a very dark residue which smelt strongly of the original chlorophenol. It was taken up in 200ml. of 2N hydrochloric acid and treated repeatedly with

carbon at 60°C until the solution showed no oiliness. On cooling, this deposited crystals of the hydrochloride, so the solution was cooled to 0°C and hydrogen chloride gas passed in to complete the precipitation. The product was filtered, washed with ice-cold acid and recrystallised from hydrochloric acid at 60°C. The final product was dried over concentrated sulphuric acid at atmospheric pressure. The product, M.P.70°C, was analysed after drying for thirty six hours.

| Analysis | Found | Required by $C_8H_{10}ON_2Cl_2 \cdot 3H_2O$ (hydrochloride, $3H_2O$) |
|---|---|---|
| C | 37.3% | 35.0% |
| H | 5.54 | 5.8 |
| N | 10.15 | 10.18 |
| Cl | 25. 4 | 25. 8 |

The compound was further recrystallised and dried in vacuo over sulphuric acid for ninety six hours. M.P. 169-71°.

| Second Analysis | Found | Required by $C_8H_{10}ON_2Cl_2$ (hydrochloride) |
|---|---|---|
| C | 43.8% | 43.4% |
| H | 4.9 | 4.5 |

Final yield of anhydrous product 6gms. (15.1%).

Note:- Ashley (5a) describes amidine hydrochlorides with water of crystallisation.

## p-CHLOROPHENOXYACETIC ACID

Reference:- (116)

| | |
|---|---|
| p-Chlorophenol | 10gms. |
| Sodium Hydroxide | 3.5gms. |
| in water | 40mls. |
| Chloroacetic acid | 8gms. |
| Sodium carbonate | 4.5gms. |
| in water | 50mls. |

The chlorophenol was dissolved in the hydroxide, added to the chloroacetic acid dissolved in the carbonate, and the solution refluxed on the sand bath for eight hours. It was acidified with hydrochloric acid and the white solid which precipitated was filtered after cooling. The solid was added, a little at a time, to 500mls. of boiling water from which it crystallised as white needles, M.P.154-6°C (listed 155-6°C). The product was dried in air. Yield 12gms. (82%).

## o-CHLOROPHENOXYACETAMIDINE.

### Outline of Preparation

Chloroacetonitrile was heated with sodium o-chlorophenate in alcohol and the resulting nitrile converted to the amidine by a modification of Pinner's method. Other methods were also attempted.

### o-CHLOROPHENOXYACETONITRILE

This nitrile is not listed.

| | |
|---|---|
| o-Chlorophenol | 28gms. |
| Ethyl alcohol | 80ml. |
| Sodium | 4.6gms. |
| Chloroacetonitrile | 13.4ml. (16gms.) |

Chloroacetonitrile was added to the alcoholic solution of sodium o-chlorophenate and the mixture refluxed for 30 minutes on the water bath. The precipitated sodium chloride was dissolved by addition of 500ml. of cold water forming an emulsion, which partially separated on standing. The nitrile was extracted with ether, freed from chlorophenol by sodium hydroxide solution, and finally washed with water.

Drying. Method A.

The ethereal solution was dried over anhydrous sodium sulphate and the ether evaporated. The pale yellow oil remaining was kept in a shallow layer over sulphuric acid in a vacuum desiccator. The product had a pungent odour and was lachrymatory. Yield 22gms. (60%). The nitrile decomposes on attempted vacuum distillation.

Drying. Method B.

The ethereal extract was dried over three consecutive portions of sodium sulphate and finally over two quantities of phosphorus pentoxide. The ether was then evaporated and the nitrile used immediately. The yield by this method was 55%.

NOTES:- (1) The first sodium sulphate drying was usually overnight, when the solution sometimes became cloudy and discoloured, the yield being adversely affected.

(2) The yields varied greatly in different preparations, the lowest being 10%.

(3) In the preparations, even when a large excess of chlorophenol was added, ammonia was evolved during the reflux and amide was also formed.

o-CHLOROPHENOXYACETAMIDINE - Method I.

The nitrile was submitted to the procedure previously described, using excess of reagents.

| | | |
|---|---|---|
| Iminoether | o-Chlorophenoxyacetonitrile (dried by Method A) | 9gms. |
| | Ethyl alcohol (Mg. dried) | 13ml. |
| | Ether (Na. dried) | 20ml. |

The nitrile, alcohol and ether were maintained at 0°C while hydrogen chloride dried through sulphuric acid was passed in. The mixture was saturated for six hours on the first day and a further six hours on the second. On the third, the solvent was evaporated under vacuum at 50°C.

| | | |
|---|---|---|
| Ammonolysis | Ethyl alcohol (Na dried) | 150ml. |
| | Ammonia gas. | |

The alcohol was saturated at 0°C with dry ammonia gas, the above product added, and the sealed pressure bottle placed in a bath at 40°C (thermostat) for fourteen hours. The resulting solution, filtered from a little dark material, consisted of a clear yellow liquid which was evaporated under water-pump vacuum at 45°C. The residue crystallised from hot water as white plates which showed no amidine properties.

The substance had no basic character and melted at 150°C. o-Chlorophenoxyacetamide melts at 149.5°C (11ϕ).

| Analysis | Found | Required by $C_8H_8O_2NCl$ (Amide) |
|---|---|---|
| C | 51.9% | 51.7% |
| H | 4.65 | 4.32 |
| N | 7.65 | 7.55 |
| Cl | 19. 2 | 19. 2 |

The product was entirely amide.

REPEAT of PREPARATION

It was thought that the production of amide was due to unsatisfactory drying of the original nitrile, so the preparation was repeated using nitrile dried by Method B. Approximately the same quantities were used and similar conditions were adhered to, but o-chlorophenoxyacetamide was again the only product.

COURSE of REACTION

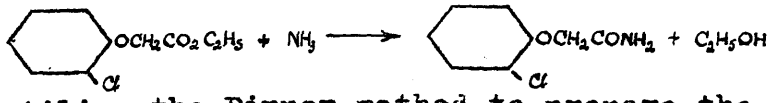
The actual course of the reaction was investigated as follows:-

| | |
|---|---|
| o-Chlorophenoxyacetonitrile | 9gms. |
| Ethyl alcohol (Mg dried) | 30ml. |
| HCl gas | |

The nitrile, which had been dried by Method B, was mixed with the alcohol and cooled in a freezing mixture as hydrogen chloride, dried through concentrated sulphuric acid was passed in. When the solution was saturated, the bottle was sealed and allowed to stand overnight. On the following day, the solution, which was still clear and pale yellow, was re-saturated. During the next four days, a crystalline solid separated and proved to be ammonium chloride. The alcoholic solution was evaporated to small bulk under vacuum at 50°C and the large crystals which were deposited on cooling were filtered and washed with alcohol. The product had a pleasant smell and melted at 32°C. Ethyl o-chloro--phenoxyacetate melts at 32°C (116).

The reaction apparently followed the course:-

The ethyl o-chlorophenoxyacetate gave the amide with alcoholic ammonia:-



To utilise the Pinner method to prepare the iminoether, it is necessary to control the amount of alcohol, or HCl, or both.

o-CHLOROPHENOXYACETAMIDINE - Method II

From the foregoing it is seen that the formation of iminoether from o-chlorophenoxyacetonitrile does not take place in the presence of excess alcohol and hydrogen chloride. A modification was made by adding these two reactants only in the proportions required by the equation:

$$RCN + C_2H_5OH + HCl \longrightarrow R\text{-}C(=NH\cdot HCl)(OC_2H_5)$$

Ethereal Hydrogen Chloride

The simplest method of adding a specific small quantity of hydrogen chloride was by measuring a volume of a standardised solution of the gas in dry ether. The ether was dried and distilled over sodium, and the absorption carried out in a dry vessel, the exit from which was protected by a calcium chloride tube. The hydrogen chloride gas was generated by dropping concentrated sulphuric acid on sodium chloride, and dried through concentrated sulphuric acid. The ether was saturated with hydrogen chloride at about 20°C, and then an equal quantity of dry ether added. The final solution was stored in a tightly stoppered bottle.

For standardisation a volume was run out under the surface of about 50ml. of water and titrated with standard alkali. The ethereal hydrogen chloride was measured for use from a 10ml. burette, dried and fitted with a calcium chloride tube.

The strength of the ethereal hydrogen chloride was found to be 1ml. = 0.164gm. HCl.

o-CHLOROPHENOXYACETAMIDINE

| | | |
|---|---|---|
| Iminoether | o-Chlorophenoxyacetonitrile | 10.65gms. |
| | Ethyl alcohol (Mg dried) | 3.7ml. (2.9gms.) |
| | Ethereal HCl (see above) | 14.1ml.(=2.33gm.HCl) |
| | Ether (Na dried) | 20ml. |

The nitrile, dried by Method B, was mixed with the alcohol and ether and the ethereal hydrogen chloride added from a dry burette, after which the vessel was sealed with a well-fitting ground stopper. A crystalline precipitate appeared in 15-30 minutes, with the evolution of some heat, and the mixture was allowed to stand for two days at room temperature, when the solid material was filtered and washed with dry ether. The residue consisted of pure white crystals of pleasant odour, which showed chloride ion

when shaken up with water. M.P. 100°C with evolution of gas, re-solidifying and remelting at 148-50°C (o-chlorophenoxyacetamide melts at 149.5°C).

Ammonolysis - Procedure A

Iminoether hydrochloride (from above)
Ethyl alcohol (Na dried) 150ml.
Ammonia gas.

The iminoether salt was added to the alcohol, saturated at 0°C with dry ammonia gas and heated for fifteen hours at 40°C under pressure. The solution, originally yellow, was now very dark and on removing excess ammonia, a pronounced odour of the original chlorophenol was apparent. Evaporation of the solution to dryness at 40°C under vacuum left a very dark residue smelling strongly of the phenol. This was taken up in dilute hydrochloric acid at 70°C and treated with decolorising carbon to give crystals on cooling which were filtered and washed with cold water. The melting point of this compound was 144-6°C and it showed no ionisable chlorine or other amidine properties. It did not melt below 145°C when mixed with a sample of o-chlorophenoxy--acetamide (M.P.149.5°C).

The filtrate was given further treatment, but heating appeared to cause decomposition and darkening. Addition of strong ammonium nitrate solution did not give any precipitate, nor did saturation with hydrogen chloride or treatment with sodium hydroxide or ammonia.

Ammonolysis - Procedure

The iminoether hydrochloride was made as before and added to the alcoholic ammonia. The mixture was shaken at room temperature for two hours, allowed to stand overnight, and then heated for one hour at 30°C giving a clear yellow solution, the colour darkening during the final heating. A slight smell of free phenol was noticed and a little crystalline material (amide, M.P.149°C) was filtered. The solvent was evaporated under water-pump vacuum at 40°C and the residue taken up in dilute hydrochloric acid at 50°C filtered from tarry material, which yielded some amide on recrystallisation, and the hydrochloride solution treated twice with charcoal at 50-60°C. The clear, pale yellow solution was clarified by filtration through sintered glass and a 50% solution of ammonium nitrate added dropwise. A crystalline precipitate formed immediately and was filtered after thirty minutes and washed with ice-water. It was recrystallised from

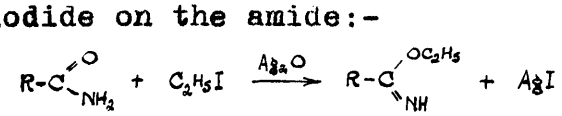
water at 60°C, giving needles and plates of the amidine nitrate. This salt is only sparingly soluble in cold water and the solution shows nitrate ion in the brown ring test. M.P.154°C with decomposition. Yield 9gms. (53%)

| Analysis | Found | Required by $C_8H_{10}O_4N_3Cl$ (nitrate) |
|---|---|---|
| C | 39.1% | 38.8% |
| H | 4.10 | 4.04 |
| N | 16.7 | 16.96 |
| Cl | 14.5 | 14.3 |

NOTE:- In another preparation, ammonolysis for 10 hours at 30°C also yielded amidine, but in smaller yield (35%) and with greater quantities of chlorophenol and chlorophenoxyacetamide.

o-CHLOROPHENOXYACETAMIDINE - Method III

Reference:- (98) o-Chlorobenzonitrile did not form the iminoether by the Pinner method, but this compound was made by action of silver oxide and ethyl iodide on the amide:-



Iminoether

| | |
|---|---|
| o-Chlorophenoxyacetamide | 1gm. |
| Ethyl iodide | 6gms,(3.1ml.) |
| Silver oxide | 5gms. |
| Ether (Na dried) | 50ml. |

The ethyl iodide was dried over calcium chloride and freshly distilled and the silver oxide was prepared by addition of sodium hydroxide solution to silver nitrate solution the oxide being washed and dried at 90°C.

The amide, ethyl iodide and ether were heated on the water bath, the silver oxide added and the mixture refluxed for 3 hours, when the brown colour had disappeared. The solution was filtered, but evaporation of the ether gave only unchanged amide, M.P.150°C., showing no depression when mixed melting point was taken with known amide.

o-CHLOROPHENOXYACETAMIDINE - METHOD IV.

Reference:- (54) A nitrile is heated with an alkali amide to give the metallic derivative of the amidine. The reactants are heated alone or in an inert solvent.

| | |
|---|---|
| o-Chlorophenoxyacetonitrile | 10gms. |
| Sodamide | 2.9gms. |
| Toluene (Na dried) | 50ml. |

The toluene was dried and distilled over sodium and added to the nitrile which was dried by method B. The sodamide was powdered in a warm mortar, quickly weighed and transferred to the flask which was heated gently under reflux. After a short period of heating, however,

decomposition of the nitrile was obvious and no amidine was isolated.

Similar results were obtained using benzene as diluent.

NOTE:- The difficulty of thermal instability might be obviated by the use of liquid ammonia at reduced temperatures (54)

## o-CHLOROPHENOXYACETIC ACID

Reference:- (116)

| | |
|---|---|
| o-Chlorophenol | 26gms. |
| Sodium hydroxide | 9.1gms. |
| in water | 100ml. |
| Chloroacetic acid | 20.8gms. |
| Sodium carbonate | 11.7gms. |
| in water | 150ml. |

The chlorophenol, dissolved in the hydroxide, was added to the solution of the chloracetic acid in the sodium carbonate and the mixture refluxed on the sand-bath for eight hours. The hot solution was acidified with hydrochloric acid, cooled and the white solid filtered and recrystallised by adding it a little at a time to a litre of boiling water. The acid crystallised on cooling as white plates. M.P.144-5°C. (listed - 145-6°C) Yield 31gms. (84%).

## 2:4-DICHLOROPHENOXYACETAMIDINE

### Outline of Preparation

Phenol was chlorinated to give the 2:4-dichloro compound, treated in alcoholic alkaline solution with chloroacetonitrile to give the nitrile and converted to the amidine.

### 2:4-DICHLOROPHENOL

Reference:- (74) Chlorine passed into phenol until the required increase in weight is observed.

Chlorination — Phenol 47gms.

Chlorine

Chlorine was generated by dropping concentrated hydrochloric acid (s.g.1.16) on to solid potassium permanganate in a distilling flask, and dried through sulphuric acid. The phenol was kept molten on a boiling water-bath and the chlorine passed in until the increase in weight was 35 gms., when the molten material was transferred to a 250ml. flask and distilled at atmospheric pressure under a good draught. The fraction boiling 209-210°C was collected (listed - 209-10° ) and the material solidified as a white crystalline solid of unpleasant and distinctive odour, of M.P.43-44° (listed - 45°). Yield 61gms. (75%).

Note:- On storage, the dichlorophenol turned slightly pink and sublimed as fine needles.

2:4-DICHLOROPHENOXYACETONITRILE

This nitrile is not listed.

| | |
|---|---|
| 2:4-Dichlorophenol | 18gms. |
| Ethyl alcohol | 50ml. |
| Sodium | 2.3gms. |
| Chloroacetonitrile | 6.7ml. (8gms.) |

The sodium was dissolved in the alcohol, the dichloro--phenol added, followed by the chloroacetonitrile and the mixture heated on the water-bath for thirty minutes under reflux. The solution was diluted with 300ml. cold water and extracted repeatedly with ether. The extract was washed with 2N sodium hydroxide followed by water and, after drying twice over sodium sulphate, then twice over phosphorus pentoxide, the ether was evaporated to give a clear yellow oil, which did not show a boiling point at ordinary pressure, but became dark and tarry when heated. Yield 11gms. (55%).

NOTE:- In a second preparation, the chloroacetonitrile was refluxed with 10ml. of alcohol while the alcoholic alkaline solution of the phenol was run in over twenty minutes. The yield, however, was almost identical

2:4-DICHLOROPHENOXYACETAMIDINE

Controlled amount of reagents for iminoether formation and mild ammonolysis are necessary.

| | | |
|---|---|---|
| Iminoether | 2:4-Dichlorophenoxyacetonitrile | 10gms. |
| | Ethyl alcohol (Mg dried) | 2.9ml. (2.3gms) |
| | Ethereal HCl (p.145) | 11.1ml. (= 1.83gms. HCl) |
| | Ether (Na dried) | 15ml. |

The nitrile was weighed into a dry bottle and the ether added followed by the alcohol and ethereal HCl. After about sixty minutes, the iminoether hydrochloride started to crystallise and this was completed overnight giving long white needles which were filtered on sintered glass and washed with dry ether. It had a pleasant smell and showed chloride ion when shaken with cold water. M.P. 108-110°C with evolution of gas, resolidifying and remelting at 199-201°C.

| | | |
|---|---|---|
| Ammonolysis | Iminoether hydrochloride | (from above) |
| | Ethyl alcohol (Na dried) | 150ml. |
| | Ammonia gas | |

The alcohol was saturated with dry ammonia gas at 0°C and the iminoether hydrochloride added. An immediate reaction was apparent, and a clear colourless solution resulted, although the solid material turned yellow at the moment of contact with

the ammonia. After twenty hours at room temperature, the mixture was heated for one hour at 40°C and the solution, after evaporation under water-pump vacuum at 40°C, left a rather dark residue which had a distinct odour of the dichlorophenol. The solid was taken up in 100ml. of 2N hydrochloric acid at 50°C, filtered from a little dark material and treated with decolorising carbon till all oiliness had disappeared, though a slight brown colour remained. The liquid was clarified by filtration through sintered glass and chilled in a freezing mixture as 50% ammonium nitrate was added dropwise. The nitrate crystallised immediately as small needles, which were filtered after one hour on sintered glass and washed with ice-water. The product was dried in vacuo over sulphuric acid for 96 hours. M.P.181°C with decomposition. Yield (as nitrate) 8 gms. (57%).

| Analysis | Found | Required by $C_8H_9O_4N_3Cl_2$ (nitrate) |
|---|---|---|
| C | 34.3% | 34.0% |
| H | 3.5 | 3.2 |
| N | 14.3 | 14.8 |
| Cl | 24.7 | 25.2 |

## 2:4-DICHLOROPHENOXYACETIC ACID

References:- (116) (141)

| | |
|---|---|
| 2:4-Dichlorophenol | 8.5gms. |
| Sodium hydroxide | 2gms. |
| in water | 50ml. |
| Chloroacetic acid | 5gms. |
| Sodium carbonate | 3gms. |
| in water | 50ml. |

The chlorophenol was dissolved in the alkali hydroxide and added to the solution of theacid in the carbonate. The mixture was refluxed for eight hours on the sand-bath and the sodium dichlorophenoxyacetate, which crystallised on cooling, was redissolved and the solution acidified with concentrated hydrochloric acid. The white solid which precipitated was collected when cold, washed, and recrystallised by adding it a little at a time to 500ml. of boiling water. To prevent the formation of an oil, the solution was cooled slowly and the acid formed fine white crystals which were filtered and washed with water. The product was air dried. M.P. 136-7°C (listed - 138°C) Yield 9 gms. (82%).

## 2:4:6-TRICHLOROPHENOXYACETAMIDINE

### Outline of Preparation

Phenol was trichlorinated and the sodium salt reacted in alcohol solution with chloroacetonitrile. The trichlorophenoxyacetonitrile was converted to the amidine. Yields from the trichlorophenol stage were poor.

### 2:4:6-TRICHLOROPHENOL

References:- Faust (57) direct chlorination of molten phenol. Datta and Mitter (40) sulphonated phenol, then passed chlorine into the aqueous solution.

### Method I

Phenol 47gms.

Chlorine gas.

The phenol was kept molten in a boiling water-bath and chlorine, generated by dropping hydrochloric acid on to solid potassium permanganate, was passed in until an increase of weight of 54gms. was noted. The contents were transferred to a 250ml. flask and distilled at ordinary pressure under a good draught. The fraction boiling 243-46° was collected (listed - 243-244.5°C) and solidified to a lightly coloured solid of extremely unpleasant odour. M.P.67-8°C (listed - 69.5°C). Yield 66gms. (65%) after recrystallisation from aqueous alcohol.

Method II

| | |
|---|---|
| Phenol | 24gms. |
| Sulphuric acid (S.G.1.84) | 75ml. |
| Chlorine gas | |

The phenol was heated with the sulphuric acid on the water bath for four hours at 100°C and then diluted with 400ml. of water. Chlorine, generated as before, was passed into the clear, faintly coloured, solution which slowly turned cloudy and deposited crystalline material. The product was filtered, washed with water and recrystallised from aqueous alcohol. M.P.68°C. Yield 18gms. (36%).

2:4:6-TRICHLOROPHENOXYACETONITRILE

This nitrile is not listed.

| | |
|---|---|
| 2:4:6-Trichlorophenol | 25gms. |
| Ethyl alcohol | 50ml. |
| Sodium | 2.3gms. |
| Chloroacetonitrile | 6.7ml. (8gms.) |

The chloroacetonitrile was added to the alcoholic solution of sodium trichlorophenate and the mixture refluxed on the water bath for thirty minutes. The solution was diluted to 400ml. with cold water, an oil being thrown out along with some solid material. The solid was filtered and

was found to be impure unreacted trichlorophenol. The filtrate was repeatedly extracted with ether and the extract washed with dilute sodium hydroxide solution, followed by water. After drying twice over anhydrous sodium sulphate, then twice over phosphorus pentoxide, the ether was evaporated and dark oil remaining filtered from a very little crystalline material (M.P. about 190°C probably amide). The filtered nitrile was taken up in dry ether, treated again with phosphorus pentoxide and the ether evaporated. The final residue consisted of a rather dark brown oil, of very pungent odour, and lachrymatory effect. The nitrile darkened and decomposed if heated above 100°C. Yield 2.8gms. (11.9%).

NOTE:- The preparation was repeated a number of times with almost identical yield. The hydrolysis of the nitrile group was obvious by evolution of ammonia and was not diminished by increasing the proportion of trichlorophenol, or by adding the solution of trichlorophenate slowly to the chloroacetonitrile.

2:4:6-TRICHLOROPHENOXYACETAMIDINE

| | | | |
|---|---|---|---|
| **Iminoether** | 2:4:6-Trichlorophenoxyacetonitrile | | 2.5gms. |
| | Ethyl alcohol (Magnesium dried) | | 0.6ml. |
| | Ethereal HCl (P.145 ) | 2.3ml. | (=0.38gms.HCl) |
| | Ether (Sodium dried) | | 10ml. |

The nitrile, ether and alcohol were mixed in a dry bottle, the ethereal hydrogen chloride added, and the bottle tightly stoppered. A white precipitate appeared almost immediately and the mixture was shaken and left overnight, when the solid material was filtered and washed with ether. M.P.128°C with evolution of gas, resolidifying and re-melting at 188-190°C.

Ammonolysis Iminoether hydrochloride (from above)
Ethyl alcohol (Na dried) 150ml.
Ammonia gas

The solution of the iminoether hydrochloride in the alcohol, saturated with ammonia at 0°C, was shaken for thirty minutes, then allowed to stand for twenty four hours at room temperature and finally heated at 35°C for one hour. The mixture had darkened somewhat even at room temperature and the final heating gave a very dark solution, which smelt strongly of the trichlorophenol. The excess ammonia and alcohol were removed at 40° under the water-pump vacuum, after a little dark tarry material had been filtered, and the residue from the evaporation consisted of dark material with a pronounced odour of trichlorophenol. This was taken up in 80ml. dilute hydrochloric acid at 50°C and undissolved material filtered and discarded. The solution was treated with decolorising carbon

until all oiliness had been removed, though repeated heating apparently caused further decomposition. 50% ammonium nitrate solution was added dropwise to the cold solution and a crystalline precipitate appeared at once. This was filtered after thirty minutes and washed with ice-water. Needles, M.P. 165-6°C with decomposition. Yield 0.8gms. as nitrate (24%).

| Analysis | Found | Required by $C_8H_8O_4N_3Cl_3$ (nitrate) |
|---|---|---|
| C | 30.3% | 30.3% |
| H | 2.57 | 2.53 |
| N | 13.0 | 13.26 |
| Cl | 33.3 | 33.6 |

## 2:4:6-TRICHLOROPHENOXYACETIC ACID

References:- (116) - method of preparation. (16) - M.P.177°C.

| | |
|---|---|
| 2:4:6-Trichlorophenol | 10gms. |
| Sodium hydroxide | 2gms. |
| in water | 50ml. |
| Chloroacetic acid | 5gms. |
| Sodium carbonate | 3gms. |
| in water | 50ml. |

The trichlorophenol was dissolved in the aqueous alkali, the acid in the carbonate and the solutions mixed and refluxed

on the sand-bath for ten hours. The solid sodium salt which separated on cooling was redissolved and the solution acidified with hydrochloric acid. The precipitate was filtered when cold and repeatedly recrystallised from dilute alcohol, giving glistening white needles. Trouble was experienced in removing unchanged trichlorophenol which caused the product to come out as an oil and materially reduced the yield, but the final product had no odour of the phenol. M.P.177°C. Yield 5gms. (39%).

## m-CHLOROPHENOXYACETAMIDINE

### Outline of synthesis

m-Chloronitrobenzene is reduced to the amine, which is diazotised and converted to the phenol. This, in alcoholic sodium ethoxide, is heated with chloroacetonitrile to give the nitrile readily converted to amidine.

### m-CHLOROANILINE

Reference:- Beilstein, Kurbatow (12)

| | |
|---|---|
| m-Chloronitrobenzene | 46gms. |
| Ethyl alcohol | 100ml. |
| HCl (s.g.1.14) | 200ml. |
| Tin foil | 55gms. |

The chloronitrobenzene was dissolved in the alcohol with warming, the acid added and the solution heated to boiling as the tin foil, torn into strips, was added at a rate sufficient to maintain the temperature. Between additions of the tin, the flask was connected to a reflux condenser and, at the end of the addition, which took about thirty minutes, the mixture was refluxed for ten minutes. The solution was rendered alkaline with solid sodium hydroxide, sufficient being added to redissolve the stannic hydroxide, and the mixture steam distilled until no more amine passed over. The condensate was allowed to settle and the oily

layer separated. The aqueous portion was extracted with ether, and the extract added to the main yield. The solution was dried over solid sodium hydroxide, the ether removed and the residual oil distilled at atmospheric pressure. The fraction boiling 225-30°C was collected (listed b.p. 230°C). Yield 30gms.(81%).

m-CHLOROPHENOL

References:- Varnholt (175), Beilstein and Kurbatow (12) and Uhlemann (173).

| Diazotisation | | |
|---|---|---|
| | m-Chloroaniline | 30gms. |
| | Sulphuric acid (s.g.1.84) | 13ml. (23.5gms.) |
| | in water | 1800ml. |
| | Sodium nitrite | 16.5gms. |
| | in water | 150ml. |

The amine was dissolved in the diluted sulphuric acid and the solution cooled in a freezing mixture as the nitrite solution was introduced with good stirring, the temperature being maintained below 5°C. The end-point was indicated in the usual way with starch-KI paper. A considerable amount of an orange compound was precipitated and filtered, the clear solution coupling with β-naphthol to give a red dye. This solution was allowed to warm up to room temperature during ninety minutes, then heated on the steam bath with good stirring.

Nitrogen was slowly evolved as the temperature rose and the clear solution gradually grew cloudy, a strong odour of chlorophenol also being apparent. The final temperature was 60°C, at which the mixture was maintained until nitrogen ceased to be evolved. The final mixture was reddish-brown and there was also some tar formed. The tarry material was filtered and extracted with ether, which was used to extract the aqueous portion, and the total dried over anhydrous sodium sulphate. The dark oil remaining after removal of the ether was distilled at atmospheric pressure and the material passing over at 210-14°C was collected (listed b.p. 214°). The product consisted of a brown oil of strong phenolic odour which solidified on standing to needles of low melting point. Yield 7.5gms. (25%).

Note:- Varnholt (175) indicates the use of only two equivalents of acid, so the orange compound is possibly the diazo-amino compound.

m-CHLOROPHENOXYACETONITRILE

This nitrile is not listed.

| | |
|---|---|
| m-Chlorophenol | 7.5gms. |
| Ethyl alcohol | 30ml. |
| Sodium | 1.2gms. |
| Chloroacetonitrile | 3.4ml. (4gms.) |

The chlorophenol, dissolved in the alcoholic ethoxide solution, was mixed with the chloroacetonitrile and the mixture was refluxed for thirty minutes on the water bath, when sodium chloride was seen to separate. After dilution with about 400ml. of cold water, the nitrile was extracted with ether and the extract washed with dilute sodium hydroxide solution to remove unchanged phenol. After washing with water, the ethereal solution was dried twice over anhydrous sodium sulphate, then twice over phosphorus pentoxide. The solvent was evaporated on the water bath, leaving a pungent brown oil which could not be distilled even under reduced pressure and did not freeze at $-5^{\circ}C$. Initial yield was 4.3gms. (44%). The compound was stored in vacuo over sulphuric acid, but, after two days, had become very dark and deposited tarry material. The nitrile was extracted with dry ether, leaving 2.6gms. of decomposed material. Final yield 1.7gms.(17.5%).

m-CHLOROPHENOXYACETAMIDINE

| | | | |
|---|---|---|---|
| Iminoether | m-Chlorophenoxyacetonitrile | 3.2gms. | |
| | Ethyl alcohol (Mg. dried) | 1.1ml. | |
| | Ethereal HCl (p.145) | 4.5ml. | (=0.74gm.HCl) |
| | Ether (Na dried) | 20ml. | |

The reagents were mixed in a well-stoppered bottle and there was an immediate reaction, an oil being thrown out. The

stoppered bottle was shaken for thirty minutes, when the material crystallised, and the whole action appeared complete after sixty minutes. After standing overnight, the somewhat discoloured iminoether hydrochloride was filtered on sintered glass and washed with dry ether.

Amidine — Iminoether hydrochloride (from above)
Ethyl alcohol (Na dried) 150ml.
Ammonia gas.

The iminoether was added to saturated alcoholic ammonia and kept at room temperature for twenty hours, then for a further hour at 30°C. The solution was now very dark and, after evaporation of the solvent under vacuum at 40°C, the dark residue had the odour of the chlorophenol. The material was taken up in dilute hydrochloric acid at 50°, and filtered from a little tarry solid. After treatment with charcoal, the solution, which showed no oiliness, was cooled to 0° and 50% ammonium nitrate added dropwise. Almost immediately, a crystalline precipitate appeared and was filtered after thirty minutes. Small white plates M.P.139-41°C. Yield 2.5gms. (47%).

| Analysis | Found | Required by $C_8H_{10}O_4N_3Cl$ (nitrate) |
|---|---|---|
| N | 17.0 | 17.0 |
| Cl | 14.5 | 14.3 |

## 2-METHYL-4-CHLOROPHENOXYACETAMIDINE

### Outline of Preparation

o-Cresol is chlorinated to 2-methyl-4-chlorophenol, converted to the phenoxyacetonitrile by treatment with chloroacetonitrile, and hence to the amidine.

### 2-METHYL-4-CHLOROPHENOL

References:- Claus, Jackson (31) direct chlorination of o-cresol. B.P. 222-5°C (40).

| | |
|---|---|
| o-Cresol | 25gms. |
| Glacial acetic acid | 50ml. |
| Iron wire | |
| Chlorine gas. | |

The cresol was dissolved in the glacial acetic acid and a short length of iron wire placed in the solution, which was cooled in crushed ice as a slow stream of dry chlorine gas was passed in. The process was continued until the weight has increased by 8.5gms., when the solution was poured into 300ml. of water and the product extracted with ether. The ethereal solution was washed free of acetic acid with aqueous sodium bicarbonate, followed by water, and then dried over anhydrous sodium sulphate. The ether was evaporated on the water bath and the residual brown oil distilled at atmospheric pressure,

the fraction boiling at 220-25°C being collected. The product was a pale brown liquid, solidifying on cooling to room temperature, and of typical phenolic odour. Yield 18gms. (54%).

2-METHYL-4-CHLOROPHENOXYACETONITRILE

This nitrile is not listed

| | |
|---|---|
| 2-Methyl-4-chlorophenol | 13gms. |
| Ethyl alcohol | 50ml. |
| Sodium | 2.1gms. |
| Chloroacetonitrile | 6.2ml. (7.3gms.) |

The phenol was dissolved in the alcoholic sodium ethoxide, the chloroacetonitrile added, and the mixture refluxed on the water bath for thirty minutes. On pouring into 250ml. of cold water, a little solid material separated and was filtered and the filtrate extracted with ether. The extract was washed with dilute sodium hydroxide solution, followed by water, and after drying (twice over anhydrous sodium sulphate, then twice over phosphorus pentoxide) the ether was evaporated and the residue filtered, giving the product as a clear pale yellow oil of pungent odour. Yield 6gms. (33.3%). Decomposes above 100°C.

2-METHYL-4-CHLOROPHENOXYACETAMIDINE

Iminoether -

| | |
|---|---|
| 2-Methyl-4-chlorophenoxyacetonitrile | 6gms. |
| Ethyl alcohol (Mg dried) | 1.9ml. |
| Ethereal HCl (p.145) | 7.2ml.(=1.18gms.) |
| Ether (Na dried) | 50ml. |

The nitrile was mixed with the ether and alcohol and the ethereal hydrogen chloride added. After fifteen minutes shaking, the iminoether hydrochloride started to crystallise. The product was filtered after standing for seventy-two hours and washed with ether, giving white crystals of pleasant smell, showing chloride ion when shaken with water. M.P.115°C with evolution of gas, re-solidifying and re-melting at 185-90°.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | Ethyl alcohol | 150ml. |
| | Ammonia gas | |

The iminoether was added to alcohol saturated with ammonia at 0°C, the mixture was shaken in a pressure vessel and left overnight at room temperature. The solution which had darkened from the original light yellow was finally heated for one hour at 35° and the solvent removed at 50° under vacuum, leaving a dark residue with a strong smell of the chlorophenol. The solid was taken up in dilute

hydrochloric acid at 65° and treated with decolorising carbon to give a clear yellow solution. This was cooled and 50% ammonium nitrate added dropwise. The nitrate which separated was filtered after thirty minutes, washed with ice-water, and recrystallised from water at 60°. M.P. 180°C with decomposition. Yield 2.5gms. (28.9%).

| Analysis | Found | Required by $C_9H_{12}O_4N_3Cl$ (nitrate) |
|---|---|---|
| C | 40.0% | 41.3% |
| H | 4.46 | 4.58 |
| N | 15.4 | 16.1 |

Note:- The alcohol for the ammonolysis was not specially dried.

## o-CHLOROBENZONITRILE.

o-Chlorobenzonitrile was prepared to compare its reactivity with that of the o-substituted phenoxyacetonitriles.

Outline of Preparation

o-Chloronitrobenzene was reduced to the amine, which was diazotised and converted to the nitrile.

o-CHLOROANILINE

Reference:- Beilstein and Kurbatow (12).

| | |
|---|---|
| o-Chloronitrobenzene | 32gms. |
| Tin foil | 36gms. |
| HCl (s.g.1.16) | 130ml. |
| Ethyl alcohol | 100ml. |

The nitro-compound was dissolved in the hot alcohol, the hydrochloric acid added, and the tin foil in strips at a rate sufficient to maintain the mixture at boiling point. The reflux was continued for ten minutes after the last of the tin had dissolved and the whole treated with solid sodium hydroxide until all the tin had redissolved. The amine was steam distilled and separated from the distillate: the aqueous portion was extracted with ether which was combined with the main yield and dried over anhydrous sodium sulphate. Evaporation of the ether gave a brown oil solidifying to

needles. The crude product was utilised for the preparation of the nitrile. Yield 20gms. (80%)

o-CHLOROBENZONITRILE

References:- Korczynski (92); Montagne (117).

| | | |
|---|---|---|
| Diazotisation | o-Chloroaniline | 20gms. |
| | HCl (s.g.1.16) | 40ml. |
| | Sodium nitrite | 11gms. |
| | in water | 100ml. |

The solution of amine in the acid was diluted to 300ml. and maintained at 0-5°C while the nitrite solution was run in under the surface with good stirring. The amine diazotised smoothly, the end-point being indicated with starch-KI paper and was filtered from a very little suspended material, giving a clear solution a little of which coupled readily with β-naphthol to form a red dye.

| | | |
|---|---|---|
| Sandmeyer | Nickel chloride ($6H_2O$) | 35gms. |
| | in water | 200ml. |
| | Potassium cyanide | 52gms. |

The nickel chloride was dissolved in warm water and the powdered KCN added with stirring, giving a light brown solution which was maintained at 80-90° while the diazonium solution was added with good stirring. When the addition

was complete, the reaction mixture was heated on a boiling water-bath until no more nitrogen was evolved and, after being rendered acid, was distilled in a current of steam. The nitrile was separated from the distillate and the aqueous portion extracted with ether. The total yield was dried over sodium sulphate and the ether evaporated leaving a brown oil which solidified to discoloured needles. The nitrile was dissolved in alcohol, treated with decolorising carbon at the boiling point, and precipitated by dropwise addition of ice-water to the cold solution. The precipitated product was filtered and washed with ice-water and dried over sulphuric acid. M.P.40-42° (listed - 42-43°). Yield 5.5gms.(25%).

REACTION OF o-CHLOROBENZONITRILE WITH ALCOHOL AND HCl.

Reference:- According to Lander and Jewson (98) o-chlorobenzonitrile does not form an iminoether by the normal Pinner method.

Pinner procedure

o-Chlorobenzonitrile 1gm.
Ethyl alcohol (Mg.dried) 10ml.
HCl gas.

The dry nitrile was dissolved in the alcohol and dry HCl passed in to saturation at 0°C. This was repeated on

the next day, but, although the solution turned red, no iminoether was isolated from the mixture, only impure nitrile. The reaction, if taking place at all, did so in negligible yield.

Modified Procedure

| | |
|---|---|
| o-chlorobenzonitrile | 2.5gms. |
| Ethyl alcohol (mg dried) | 1.1ml. |
| Ethereal HCl (p.145) | 4.5ml. (= 0.74gms.HCl) |
| Ether (Na dried) | 25ml. |

The mixture was shaken for one hour with no appreciable action and, after twenty-four hours, the only change visible was that the colour had become red. Removal of the solvent left only discoloured o-chlorobenzonitrile.

## PHENYLACETAMIDINE.

Reference:- Luckenboch (105).

Iminoether

| | |
|---|---|
| Phenylacetonitrile | 25.4gms. |
| Ethyl alcohol (Mg dried) | 25ml. |
| Ether (Na dried) | 30ml. |

The freshly distilled nitrile was mixed with the alcohol and ether and saturated at 0°C with dry hydrogen chloride. After standing for two days, the solvent was removed at 40°C under water-pump vacuum and the iminoether hydrochloride remained as a light yellow powder, which was not purified.

Amidine

| | |
|---|---|
| Iminoether hydrochloride | (from above) |
| Ethyl alcohol (Na dried) | 300ml. |
| Ammonia gas | . |

The iminoether salt was added to the alcohol, which had been saturated with dry ammonia at 0°C, and heated in a sealed bottle at 40°C for fifteen hours. The clear yellow solution was evaporated at 40°C at the water-pump, leaving a syrupy residue, which was taken up in water at 40°C, treated with activated charcoal and filtered. To the filtrate, which was clear and colourless, 50% ammonium nitrate was added dropwise giving white plates of the nitrate. The salt was filtered on

sintered glass, washed with distilled water, and dried in vacuo over sulphuric acid. M.P.170°C. Yield 25gms. (70%).

The free base was prepared by adding sodium hydroxide solution to the nitrate, evaporating to dryness over sulphuric acid in vacuo, and extracting with boiling benzene (125ml. for 2 gms. of phenylacetamidine) (105). The base was recrystallised from benzene. M.P. 106-8°C (listed - 108-112°C).

## PHENOXYACETAMIDINE DERIVATIVES

### BIOLOGICAL TESTS

**References:** Techniques of testing for plant growth promotion or inhibition are described by Jensen (80). A daylight procedure has been developed by Soding (163).

Growth of test seedlings (coleoptiles).

The seedlings were grown from "Ayr Bounty" seed oats (Avena sativa). After soaking for two hours in distilled water, the seeds were spread on moist filter paper and exposed to bright daylight for two hours (Note 1). After thirty-six hours in darkness the seeds were planted in tubes, 60mm.x 15mm. filled with sterile silver sand (Note 2), each containing one seed. The sand was well moistened with distilled water and the tubes, held together by rubber bands, incubated at 26°C (± 0.5°) under a dark cover (Note 3). After 36 to 48 hours, those plants were selected which were 15 to 25mm. in length and were straight for their whole length (a high proportion showed some curvature). This operation and the application of growth substance, were performed in the dark-room by photo-tropically inactive light (wavelength greater than 5,500Å).

Notes
1. This exposure decreased the length of the first internode of the coleoptile.
2. The sand was sterilised by ignition, the tubes by heating to 150°C for thirty minutes.
3. The dark cover inverted over the plants also maintained the necessary humidity.

Preparation of growth substances

A 1:100 (weight/volume) solution in ethanol was made of each of the acids and amidines under test (Note 1) and 1ml. of this solution mixed with 10gms. molten lanolin (Note 2). The mixture was suspended in a boiling water-bath for a few minutes and then allowed to set. A blank was also prepared with only alcohol and lanolin.

Notes 1. The solution of an amidine salt was made up to contain 1:100 of the amidine base.

2. A series of ranging tests with 2:4-dichlorophenoxy-acetic acid showed that a concentration of 1:1000 was suitable for the technique described.

Application of growth substances

Using a small glass spatula, the lanolin paste containing the substance under test was applied to one side of each seedling for about 20mm. from the tip. This operation was conducted by photropically inactive light and the plants replaced under the dark cover before incubation at 26°C. Each compound was applied unilaterally to nine to twelve seedlings (Note 1) which were accompanied by three controls. After 200 to 300 minutes, the curvatures were photographed.

Notes 1. Six to eight test objects are considered sufficient to demonstrate growth action in a compound; ten to twelve are preferable for semi-quantitative work (80).

2. Twelve plants, treated with "Blank" lanolin paste, showed no curvatures.

Results - An approximation to the relative growth promoting and inhibiting properties of the compounds tested was provided by measuring the average angle of curvature, $\alpha$, produced by a compound at a concentration of 1:1000.


Photographs of typical curvatures induced by acids and the corresponding amidines are shown, the preparations being applied on the left of the plants, as seen in the photographs. Details are given in the table.

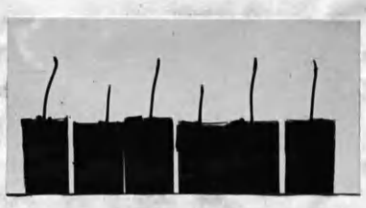


Fig.1. 4-Chlorophenoxyacetic acid. Slight growth promotion

Fig.2. 4-Chlorophenoxyacetamidine hydrochloride. Powerful growth inhibition.

Fig.3. 2:4-Dichlorophenoxyacetic acid. Powerful growth promotion.

Fig.4. 2:4-Dichlorophenoxyacetamidine nitrate. Moderate growth inhibition.

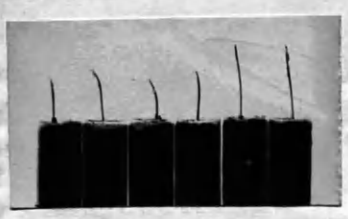


Fig.5. 2:4:6-Trichlorophenoxyacetic acid. Moderate growth inhibition.

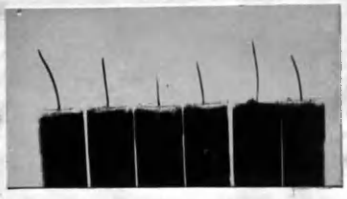


Fig.6. 2:4:6-Trichlorophenoxyacetamidine nitrate. Moderate growth inhibition.

The following table summarises the results. The concentration of each compound was 1:1000 (amidines as bases) and there were three controls for each compound tested. According to convention (80), $-\alpha$ indicates growth promotion, $+\alpha$ inhibition.

| Compound | Photo-graph reference | Time of applcn. in mins. | No. of test objects | Average curvature $\alpha$, in degrees |
|---|---|---|---|---|
| Phenoxyacetic acid | - | 210 | 9 | -4 |
| Phenoxyacetamidine nitrate | - | 210 | 9 | +3 |
| 2-Chlorophenoxy-acetic acid | - | 210 | 10 | -15 |
| 2-Chlorophenoxy-acetamidine nitrate | - | 230 | 11 | +6 |
| 4-Chlorophenoxy-acetic acid | Fig. 1. | 280 | 9 | -8 |
| 4-Chlorophenoxy-acetamidine hydrochloride | Fig. 2. | 260 | 12 | +34 |
| 2:4-Dichlorophen-oxyacetic acid | Fig. 3. | 300 | 11 | -22 |
| 2:4-Dichlorophen-oxyacetamidine nitrate | Fig. 4. | 200 | 12 | +12 |
| 2:4:6-Trichloro-phenoxyacetic acid | Fig. 5. | 280 | 12 | +11 |
| 2:4:6-Trichloro-phenoxyacetamid-ine nitrate | Fig. 6. | 280 | 10 | +14 |

Note: Maximum curvature in these tests occurred after about 200 minutes.

Amoebicidal tests

The following compounds were also tested as amoebicidal agents. The technique is described in Part I, p. 115

Phenoxyacetamidine nitrate
2-Chlorophenoxyacetamidine nitrate
4-Chlorophenoxyacetamidine hydrochloride
2:4-Dichlorophenoxyacetamidine nitrate and also
Phenylacetamidine nitrate.

All compounds showed slight activity at a concentration of 1:1000 and none at 1:100,000. p-Chlorophenoxyacetamidine nitrate showed some activity at 1:10,000 but was inactive in vivo against Entamoeba histolytica infection in rats at a dose of 250mg./Kgm. body weight.

**PART IIB**

## EXPERIMENTAL

### 4-Aminobenzamidine Derivatives:-

**Preparation of Compounds**

**Biological Tests.**

---

Part IIB. - 4-AMINOBENZAMIDINE DERIVATIVES

## 4-AMINOBENZAMIDINE

### Outline of Preparation

4-Nitrobenzonitrile, made from p-nitroaniline, was converted to 4-nitrobenzamidine and reduced to the amino compound.

### 4-NITROBENZONITRILE

References:- Diazotisation of amine and addition to cuprocyanide - (17,153): use of nickelcyanide solution - (92): nitrile, M.P.149°C - (19).

Diazotisation

| | |
|---|---|
| 4-Nitroaniline | 70gms. |
| Hydrochloric acid (s.g.1.16) | 180mls. |
| in water | 180mls. |
| Sodium nitrite | 38gms. |
| in water | 250mls. |
| Crushed ice | 250gms. |

The amine was dissolved in hot dilute acid and allowed to cool to room temperature with good stirring. The crushed ice was added, followed by 200mls. of the nitrite solution in one quantity, the temperature rising to 8°C. After stirring for fifteen minutes, the diazotisation was completed by careful addition of nitrite and the result was a clear yellow solution which coupled with alkaline β-naphthol to give a red dye.

Sandmeyer

| | |
|---|---|
| Nickel sulphate | 78gms. |
| in water | 1000mls. |
| Potassium cyanide (technical) | 180gms. |

The nickel sulphate was dissolved in hot water and the powdered cyanide added. The light brown solution resulting was maintained at 80-90°C while the diazonium solution was added over forty five minutes with good stirring. The solution was then rendered acid with hydrochloric acid and kept at 90° till gas ceased to be evolved. The mixture was steam distilled and the nitrile obtained as plates which were filtered from the condensate (10 litres), washed and dried in air. M.P.148°C (listed - 149°). Yield 28gms. (38%).

4-NITROBENZAMIDINE

References:- M.P. of iminoether hydrochloride 197°C (140): M.P. of amidine hydrochloride 295-6°C - (46).

| | | |
|---|---|---|
| Iminoether | 4-Nitrobenzonitrile | 8gms. |
| | Ethyl alcohol (Mg dried) | 10mls. |
| | Chloroform ($P_2O_5$ dried) | 40mls. |

The ether-alcohol solution of the nitrile was saturated with dry hydrogen chloride at 0°C (Note 1.) and re-saturated after twelve hours. After a further eighteen hours, the dark red liquid was evaporated at 45°C under reduced pressure (Note 2.) and the crystalline residue melted at 196-8°C with decomposition (listed - 197°C).

Notes 1. Cooling was not intensive since the nitrile tended to crystallise.

2. The iminoether hydrochloride was readily soluble in chloroform.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | Ethyl alcohol (Na dried) | 150mls. |
| | Ammonia gas | |

The alcohol was saturated at 0°C with dry ammonia, the iminoether hydrochloride added, and the pressure container sealed. The mixture was heated for ten hours in a water bath, thermostatically maintained at 41°, forming a clear and lightly coloured solution, which, on cooling, deposited a crop of light yellow rhombs. M.P.294°C with decomposition (listed - 294-6° for 4-nitrobenzamidine hydrochloride). This product, when shaken up with warm water, showed chloride ion. The mother liquor was evaporated at 45°C and gave a further crop of the hydrochloride. Total yield of hydrochloride 8.9gms. (82%).

4-AMINOBENZAMIDINE

Reference:- (46)

| | |
|---|---|
| 4-Nitrobenzamidine hydrochloride | 6.5gms. |
| Stannous chloride, $2H_2O$ | 20 gms. |
| Hydrochloric acid (s.g.1.16) | 35 ml. |
| Tin foil | 10 gms. |

The amidine hydrochloride was dissolved in the solution of stannous chloride in the hydrochloric acid and the tin foil, torn into small strips, added over a short time. After gentle reflux for one hour most of the acid

was removed under reduced pressure and the solution diluted to about 150ml. The tin was removed as sulphide and the clear solution evaporated on the steam bath to crystallisation point. On cooling, the dihydrochloride separated as long needles, which were filtered and washed with the minimum of cold hydrochloric acid. M.P.317-20°C with decomposition (listed - about 320°C). This salt is very soluble in water or alcohol and sodium hydroxide does not precipitate the amidine from an aqueous solution.

The monohydrochloride was prepared from the dihydrochloride by treating a solution with the theoretical amount of standard sodium hydroxide solution and evaporating to dryness. The amidine was extracted with boiling alcohol and gave crystals which were re-crystallised from alcohol. M.P.220-22°C (listed - 225-6°) after drying in vacuo over sulphuric acid. Total yield (as dihydrochloride) 2 gms. (30%).

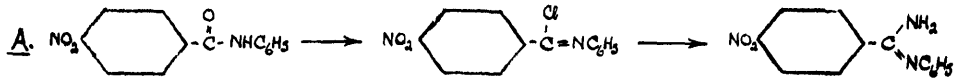
## 4-AMINO-N¹-PHENYLBENZAMIDINE

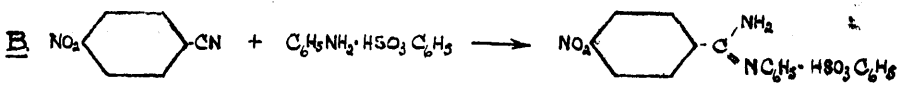
### Outline of Preparation

The 4-nitro-N¹-phenylbenzamidine was prepared by two methods:-

(A) 4-Nitrobenzoyl chloride and aniline gave 4-nitrobenzanilide which was converted to 4-nitro-N¹--phenylbenzamidine via the iminochloride.

(B) 4-Nitrobenzonitrile was fused with aniline benzenesulphonate to give the amidine benzenesulphonate.





In both cases, the nitro compound was catalytically reduced to the amine.

### Preparation A.

#### 4-NITROBENZOYL CHLORIDE

**References**:- (3), (61).

| | |
|---|---|
| 4-Nitrobenzoic acid | 50gms. |
| Phosphorus pentachloride | 65gms. |

The acid was added with shaking to the phosphorus pentachloride and the mixture warmed on the water bath to

initiate the reaction. When this had subsided, the reaction mass was heated for thirty minutes and volatile matter distilled off at 100°C under reduced pressure. The residue was exhaustively extracted with boiling petroleum ether (boiling 60-80°C) which was filtered and, on cooling, deposited pale yellow needles of the acid chloride. M.P. 74°C (listed - 75°). Yield 49gms. (88.5%).

4-NITROBENZANILIDE

Reference: (10).

| | |
|---|---|
| 4-Nitrobenzoyl chloride | 8gms. |
| Aniline | 3.7gms. (3.6ml.) |
| 2N Sodium hydroxide | 30ml. (excess). |

The aniline was shaken up in a glass-stoppered bottle with the aqueous alkali and the nitrobenzoyl chloride added in one quantity. There was an immediate reaction and, after shaking for twenty minutes, the yellow crystalline product was filtered and washed thoroughly with water. Crude material, M.P.209° (listed - 216°). Yield 9gms. (93%).

4-NITRO-$N^1$-PHENYLBENZAMIDINE

References:- (101) - 3-Nitro-$N^1$-phenylbenzamidine;
(179) - N-phenylbenzamidine.

Iminochloride 4-Nitrobenzanilide 9gms.
Phosphorus pentachloride 8gms.

The anilide was treated with the phosphorus pentachloride at 100°C for thirty minutes with shaking and volatile matter

removed under vacuum. The dark residue was extracted three times with boiling petroleum ether (B.P.60-80°) which deposited on cooling lemon yellow plates of the iminochloride, M.P.118°. Product was easily soluble in ether.

| | | |
|---|---|---|
| Amidine | Iminochloride | (from above) |
| | Ether | 75ml. |
| | Aqueous Ammonia (s.g.0.88) | 25ml. |

The iminochloride was transferred to a glass-stoppered bottle containing the ether and the aqueous ammonia added. The bottle was shaken for a short time, when the solid passed into solution, and it was noticed that the ether layer was coloured yellow, while the aqueous portion was colourless. After standing overnight, the yield of bright yellow solid was filtered and the ether layer evaporated to give more of the same crystalline product. Bright yellow needles (base) M.P.185°C. When shaken with concentrated hydrochloric acid, this compound formed a sparingly soluble hydrochloride of a paler yellow colour and M.P.240°C with preliminary darkening at about 230°C. Yield (as base) 4.2gms. (47%).

Preparation B.

ANILINE BENZENESULPHONATE

| | |
|---|---|
| Aniline sulphate | 15gms. |
| Barium benzenesulphonate | 20gms. |

The two salts were dissolved in the minimum of water, heated to boiling point and mixed. After settling on the steam bath, the barium sulphate was filtered and the filtrate evaporated to dryness. The residue which was not purified, was dried at 100°C.

4-NITRO-N¹-PHENYLBENZAMIDINE

References: (124,125).

| | |
|---|---|
| 4-Nitrobenzonitrile (p.183) | 7gms. |
| Aniline benzenesulphonate | 30gms. |

The two components were intimately mixed and heated on an oil-bath in a hard-glass tube. The temperature was slowly raised until a homogeneous melt was obtained (200-210°C, internal temperature) and the fusion maintained for fifteen minutes. After cooling, the tube was broken and the product ground and triturated with acetone. From the residue, after filtration, hot dilute hydrochloric acid extracted all basic material and, after treatment with charcoal, this was precipitated with sodium hydroxide. The yellow amidine base was filtered, washed and re-crystallised from alcohol to give yellow needles, M.P.185°C and showing no depression of melting point when mixed with the product from Preparation A.

4gms. of 4-nitrobenzonitrile were obtained from the acetone extract.

Yield of amidine base, 2.3gms. (20% on 4-nitrobenzonitrile). Recovered 4-nitrobenzonitrile, 4gms.(57%).

4-AMINO-$N^1$-PHENYLBENZAMIDINE

References:- Platinum oxide hydrogenation catalyst - (2)

| | |
|---|---|
| 4-Nitro-$N^1$-Phenylbenzamidine | 3gms. |
| in ethyl alcohol | 30mls. |
| Platinum oxide catalyst | 0.1gm. |

The platinum oxide catalyst was prepared according to reference (2). The nitro-compound was dissolved in hot alcohol, the platinum oxide added and hydrogen passed in at slightly above atmospheric pressure (fifteen inches of water). With constant shaking, hydrogen was readily absorbed and 840mls. were taken up after allowing 70mls. for reduction of the catalyst. When hydrogen absorption was complete, the platinum was filtered and the alcohol evaporated under reduced pressure. The residue was dissolved in 15mls.2N hydrochloric acid, treated with charcoal and the volume reduced to 5 mls. Addition of excess acetone (200mls.) precipitated the white dihydrochloride as a microcrystalline powder which was filtered and washed with acetone. M.P.254-6°C. This salt, on exposure to air, assumed a light brown colour. Yield 2.2gms. (62%).

| Analysis | Found | Required by $C_{13}H_{15}N_3Cl_2 \cdot H_2O$ (dihydrochloride, $1H_2O$) |
|---|---|---|
| C | 52.8% | 51.7% |
| H | 5.66 | 5.63 |
| N | 13.7 | 13.9 |

## 4-AMINO-$N^1$-(2'-PYRIDYL)-BENZAMIDINE

Outline of Preparation

4-Nitrobenziminoethyl ether, prepared from the nitrile, was treated with excess 2-aminopyridine to give 4-nitro-$N^1$-(2'-pyridyl)-benzamidine which was catalytically reduced to the amine.

4-NITRO-N'-(2'-PYRIDYL)-BENZAMIDINE

| | | |
|---|---|---|
| Iminoether | 4-Nitrobenzonitrile | 3.4gms. |
| | Ethyl alcohol (Mg dried) | 5ml. |
| | Chloroform ($P_2O_5$ dried) | 30mls. |

The iminoether was prepared as previously described (p.184). Yield of hydrochloride, 4.2gms. (79%).

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride (1 mol.) | 4.2gms. |
| | 2-Aminopyridine (10 mols.) | 17gms. |
| | Ethyl alcohol (Na dried) | 150mls. |

The iminoether hydrochloride was added to the alcoholic aminopyridine and the solution heated in a sealed bottle for ten hours at 40°C (thermostat). The volume was reduced, under decreased pressure, to less than 100mls., and the yellow crystalline product which deposited on cooling was filtered and washed with cold alcohol. A further crop was obtained on evaporation and the total yield was recrystallised from aqueous alcohol. Bright yellow plates, sparingly soluble in cold water, and not alkaline to litmus. Free base, M.P.184°C. Concentrated hydrochloric acid produces the hydrochloride as white needles, M.P.226-8°C, soluble in

water to a colourless solution from which ammonium nitrate precipitates the amidine nitrate as white needles. M.P. 212°C. Both these salts were filtered, washed with ice-water and dried over concentrated sulphuric acid. Ammonia precipitates the base from solutions of the salts.

Yield (as base) 3.1gms. ( 70 %).

| Analysis | Found | Required by $C_{12}H_{10}O_2N_4$ (base) |
|---|---|---|
| C | 60.1% | 59.5% |
| H | 4.32 | 4.14 |
| N | 23.6 | 23.1 |

4-AMINO-$N^1$-(2'-PYRIDYL)-BENZAMIDINE

Reference:- Platinum oxide hydrogenation catalyst - (2).

| | |
|---|---|
| 4-Nitro-$N^1$-(2'-pyridyl)-benzamidine | 2gms. |
| in Ethyl alcohol | 30mls. |
| Platinum oxide catalyst | 0.1gms. |

The platinum oxide catalyst, prepared according to the above reference, was added to the solution of nitro compound which was agitated as hydrogen was passed in under slight pressure (fifteen inches of water). A total of 600mls. of hydrogen were absorbed in five minutes with the evolution of heat and the colourless solution, after filtration, was evaporated to about 15mls. under reduced pressure. Three mls. concentrated hydrochloric acid were added and the solution clarified by warming to 40°C with charcoal. The solution was evaporated to crystallising point and the

product obtained on cooling filtered and washed with acetone. Similar re-crystallisation from alcohol gave the dihydrochloride as a micro-crystalline powder. M.P.284-6°C with decomposition. Yield 2.0gms. (85%).

| Analysis | Found | Required by $C_{12}H_{14}N_4Cl_2$ (dihydrochloride) |
|---|---|---|
| | C 50.3% | 50.5% |
| | H 5.03 | 4.91 |
| | N 20.15 | 19.7 |

## 4-AMINO-$N^1$-(2'-THIAZYL)-BENZAMIDINE

**Outline of Preparation**

The iminoether of 4-nitrobenzonitrile was treated with excess 2-aminothiazole and the resulting nitrothiazylbenzamidine reduced to the amine,

4-NITRO-$N^1$-(2'-THIAZYL)-BENZAMIDINE

| | | |
|---|---|---|
| Iminoether | 4-Nitrobenzonitrile (p.183) | 6gms. |
| | Ethyl alcohol (Mg dried) | 10mls. |
| | Chloroform ($P_2O_5$ dried) | 40mls. |

4-Nitrobenziminoethyl ether hydrochloride was prepared as described on p.184.

| | | |
|---|---|---|
| Amidine | Iminoether hydrochloride | (from above) |
| | 2-Aminothiazole | 42gms. |
| | Ethyl alcohol (Na dried) | 150mls. |

The iminoether was added to the alcoholic solution of the amine and the mixture heated at 41°C for eighteen hours. The volume was reduced to 100mls. at the water-pump and 150 mls. 2N hydrochloric acid added. The solution, after clarification by treatment at 70°C with charcoal, was cooled to 0°C and non-basic material filtered. Aqueous ammonia (s.g.0.88) precipitated the amidine base which was recrystallised from dilute alcohol. Yellow plates, M.P.166-7°C. Yield 4.4gms. (44%).

| Analysis | Found | Required by $C_{10}H_8O_2N_4S$ (base) |
|---|---|---|
| C | 47.2% | 48.4% |
| H | 3.88 | 3.23 |
| N | 22.2 | 22.4 |

## 4-AMINO-$N^1$-(2'-THIAZYL)-BENZAMIDINE

**Reference:-** Reduction by alcoholic stannous chloride - (152a)

| | |
|---|---|
| 4-Nitro-$N^1$-(2'-thiazyl)-benzamidine | 1.5gms. |
| in alcohol | 15mls. |
| Stannous chloride, $2H_2O$ | 5.3gms. |
| in alcohol | 6mls. |

The stannous chloride was suspended in the alcohol and dissolved by saturation with dry hydrogen chloride. The solution was cooled and the passage of gas continued during the dropwise addition of the alcoholic solution of the nitro compound. The temperature rose, and the yellow colour was discharged immediately with the separation of the white crystalline amine stannichloride. After standing for twelve hours, the solid was filtered and the filtrate evaporated to dryness under reduced pressure. The two residues were dissolved in 50mls. water and the tin removed by hydrogen sulphide. The clear solution was evaporated to about 5mls. under reduced pressure and the crystalline solid obtained on cooling filtered on sintered glass and washed with acetone. Dihydrochloride, needles, M.P.235-8°C (decomposes) with darkening at 230°C. Yield, 0.4gms. (23%).

| Analysis | Found | Required by $C_{10}H_{12}N_4SCl_2$ (dihydrochloride) |
|---|---|---|
| C | 40.3% | 41.3% |
| H | 4.16 | 4.13 |
| N | 18.6 | 19.3 |
| S | 11.2 | 11.0 |
| Cl | - | 24.4 |

**Note** - **The nitro compound could not be hydrogenated over platinum oxide (2) while excess Raney nickel (118) caused desulphurisation of the compound.**

# AMINOBENZAMIDINE DERIVATIVES

## BIOLOGICAL TESTS

### In Vitro Tests

### Preparation of Solutions

1:1000 solutions of the dihydrochlorides of 4-aminobenzamidine, 4-amino-$N^1$-phenylbenzamidine, 4-amino-$N^1$-(2'-pyridyl)-benzamidine, and 4-amino-$N^1$-(2'-thiazyl)-benzamidine were prepared in sterile distilled water and serial dilutions made from these to give concentrations of 1:2000, 1:4000 and 1:8000. By following the usual bacteriological precautions in the preparation of the dilutions, it was found possible to dispense with sterilisation of the solutions by Seitz filtration.

### Testing of Compounds

The test organisms were Bacterium coli, Bacterium typhosum, Staphylococcus pyogenes (aureus) and Proteus vulgaris, all of which are known to utilise 4-aminobenzoic acid in their life processes.

A channel, 3-6mm. wide was cut from one side of an agar plate 6-8mm. deep and 100mm. in diameter. A streak was made of each organism from the edge of the channel across the plate, using a platinum loop and a suspension in sterile water and the channel filled with the solution under test. Similar plates were prepared for each concentration and a control with water was included for each compound. After 20-24 hours at 37°C,

growth on the treated plates was compared with that on the controls.

A further series of tests were conducted by first seeding an agar plate by flooding it with a water suspension of Staphylococcus pyogenes, draining the excess, and placing the plate in the incubator for one hour. Four porcelain rings, 9mm. long and 6mm. internal diameter, were pressed lightly into the surface of the agar, and the resulting cups filled with the various concentrations under test. Similar plates were prepared for each compound and incubated at 37°C for 20 hours.

Results

Inspection of the plates in both series of tests and comparison with the controls showed that, in the concentration 1:1000 to 1:8000, the four amidines did not inhibit the growth of the organisms tested.

In Vivo Test

Professor C.H. Browning, of Glasgow University, made subcutaneous injections of 4-aminobenzamidine dihydrochloride in mice. The maximum tolerated dose, 600mg/Kgm. body weight, showed no chemotherapeutic effect on Trypanosoma brucei or Trypanosoma congolense.

## APPENDIX

## COMPOUNDS CLAIMED AS NEW.

The following compounds are not described in the literature up to that covered by Chemical Abstracts of October 1946.

"d" indicates decomposition at temperature quoted.

| | Melting point °C |
|---|---|
| 4-Amino-2'-hydroxydiphenyl ether | 170° |
| 4-Amino-$N^1$-phenylbenzamidine dihydrochloride | 254-6° |
| 4-Amino-$N^1$-(2'-pyridyl)benzamidine dihydrochloride | 284-6° |
| 4-Amino-$N^1$-(2'-thiazolyl)benzamidine dihydrochloride | 235-8°d |
| 4-(β-Bromoethoxy)benzamidine hydrochloride | 250°d. |
| 3-Bromo-4-hydroxybenzamidine hydrochloride | 270° |
| 2-Chlorophenoxyacetamidine nitrate | 154°d. |
| 3-Chlorophenoxyacetamidine nitrate | 139-41° |
| 4-Chlorophenoxyacetamidine hydrochloride | 169-71° |
| $3H_2O$ | 70° |
| 2-Chlorophenoxyacetonitrile | Unstable oil |
| 3-Chlorophenoxyacetonitrile | Unstable oil |
| 4-Chlorophenoxyacetonitrile | Unstable oil |
| 3:5-Dibromo-4-hydroxybenzamidine base | 308°d |
| hydrochloride | 275-80° |
| 2:4-Dichlorophenoxyacetamidine nitrate | 181°d |
| 2:4-Dichlorophenoxyacetonitrile | Unstable oil |
| 2:4-Dihydroxybenzamidine sulphate | 276°d |
| 2:4-Dimethoxybenzamidine hydrochloride | 258°d |
| 4-Ethoxybenzamidine base | 104° |
| 4-(2'-Hydroxyphenoxy)benzamidine nitrate | 164-6° |
| 4-(4'-Hydroxyphenoxy)benzamidine base, $1H_2O$ | 218° |
| hydrochloride, $1H_2O$ | 228-31° |
| 3-Methoxy-4-hydroxybenzamidine hydrochloride | 275°d |
| 3-Methoxy-4-hydroxy-5-bromobenzamidine base | 286° |
| hydrochloride | 275-80°d |
| 4-(2'-Methoxyphenoxy)benzamidine base | 148-51° |
| 4-(4'-Methoxyphenoxy) benzamidine hydrochloride | 158-60° |
| 2-Methyl-4-chlorophenoxyacetonitrile | Unstable oil |
| 2-Methyl-4-chlorophenoxyacetamidine nitrate | 180°d |
| 4-Nitro-$N^1$-phenylbenzamidine base | 185° |
| hydrochloride | 240°d |
| 4-Nitro-$N^1$-(2'-pyridyl)benzamidine base | 184° |
| hydrochloride | 226-8° |
| nitrate | 212° |
| 4-Nitro-$N^1$-(2'-thiazolyl)benzamidine base | 166-7° |
| Phenoxyacetamidine nitrate | 123° |
| Phenylacetamidine nitrate | 170° |
| 2:4:6-Trichlorophenoxyacetonitrile | Unstable oil |
| 2:4:6-Trichlorophenoxyacetamidine nitrate | 165-6°d |

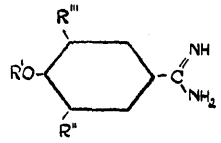
## SUMMARY AND CONCLUSIONS

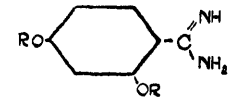
### Suggestions for further work.

## SUMMARY AND CONCLUSIONS

### Part I

The preparation is described of twelve derivatives of 4-hydroxybenzamidine of the general formulae:-





R' = H-: $C_2H_5$-: $BrCH_2CH_2$-:
o-$CH_3OC_6H_4$-: p-$CH_3OC_6H_4$-:
o-$HOC_6H_4$-: p-$HOC_6H_4$-.

R = H-: $CH_3$-

R'',R''' = H-: Br-: $CH_3O$-

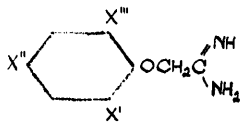
The method of preparation was from the nitrile through the iminoether hydrochloride (Pinner's method) applying modifications as required, e.g. as regards solvent, method of isolation of product, etc. Owing to the failure of the methods attempted for the synthesis of 2- and 4-hydroxy-4'-cyanodiphenylether, the corresponding amidines were prepared by demethylation of the methoxy compounds with 57% hydriodic acid. Similarly, demethylation of 2:4-dimethoxybenzamidine produced the dihydroxy derivative which was not available through the nitrile. A full list of the compounds is given on p.115.

The compounds were submitted to Messrs. I.C.I., Dyestuffs Division, Blackley, Manchester for test as

amoebicidal agents, Emetine hydrochloride being used as a comparative standard. A few of the compounds showed slight activity in concentrations of 1:10,000: the remainder were slightly active at 1:1,000. None of the compounds showed any promise as a therapeutic agent. The tests are fully reported on p.115.

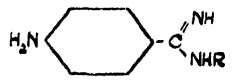
Part II

Two series of amidines were prepared, the phenoxyacetamid--ines (A), and the aminobenzamidines (B).




A.

X',X'',X''' = H-:Cl-.




B.

R = H-: phenyl: 2-pyridyl: 2-thiazolyl.

Both series of compounds are analogous to carboxylic acid growth factors, A to the phenoxyacetic acid derivatives which are synthetic plant growth factors, and B to the natural bacterial growth factor 4-aminobenzoic acid.

Part IIA.

Phenoxyacetamidine and four chlorinated derivatives were prepared from the corresponding nitriles, of which only phenoxyacetonitrile has been previously described. These nitriles, which were thermally unstable oils, showed abnormal reactivity towards alcoholic hydrogen chloride, and it was found necessary to modify the conditions of

reaction in order to obtain the iminoether hydrochloride. Using the conditions for iminoether formation developed in Part I, a nitrile such as 2-chloro-phenoxyacetonitrile gave the ester and ammonium chloride.

It was shown that the reactivity could be ascribed to the ethereal oxygen atom, and was not affected by ortho substitu--tion in the aromatic nucleus.

Concurrent biological tests of each amidine and its carboxylic acid analogue were carried out by the recognised technique on oat seedlings (p.178). The amidines were found to show distinct growth inhibitory properties, though in varying degree, 4-chlorophenoxyacetamidine being the most powerful. The carboxylic acids have already beenlisted as plant growth promoting agents.

Part IIB.

Four amidine analogues of 4-aminobenzoic acid were prepared, 4-amino-benzamidine itself, and three amidine substituted derivatives, in each case by reduction of the corresponding nitro compound. The parent unsubstituted amidine and the 2-pyridyl and 2-thiazolyl compounds were prepared from 4-nitrobenzonitrile, through the iminoether with excess ammonia or primary amine; the phenyl derivative was most easily made from 4-nitrobenzanilide through the iminochloride and another method consisted in fusion of 4-nitrobenzonitrile with aniline benzenesulphonate. The

amines were prepared from the nitroamidines by reduction with stannous chloride or, where applicable, with hydrogen over platinum.

The biological tests were carried out using four bacterial organisms which are known to require 4-aminobenzoic acid in their metabolism. The techniques used are standard and are described on p.198. The organisms were cultured on ordinary agar media and exposed during growth to concentrations of the amidines varying from 1:1,000 to 1:8,000. No indication could be found of interference with growth by any of the four amidines tested.

Suggestions for further work.

The uniformly negative results of the 4-hydroxybenzamidine series indicate that there is little to be hoped for from the investigation of mono-amidines derived from this compound, at any rate as regards amoebicidal activity.

The growth inhibitory properties demonstrated in the phenoxyacetamidine derivatives are of biological significance and it would be of interest to prepare the amidines corresponding to other plant growth factors, many of which are carboxylic acids, e.g. indole-3-acetic acid, naphthoxyacetic acid, the chlorophenoxypropionic and butyric acids, etc.

Such biological acid-amidine antithesis suggests possible extension to the carboxylic acid bacterial growth factors, even though the 4-aminobenzoic acid analogues gave negative

results; for example, amidines related to traumatic acid, nicotinic acid, pantothenic acid, and other naturally occurring growth factors might be prepared. Other examples are quoted on p.118. A necessary prerequisite would be a strictly controlled and standardised method of in vitro testing. Culture of the organisms on an inorganic substrate (silica gel) with synthetic nutrient and growth substance added in known amounts would be preferable, and should be supplemented by in vivo tests.

The effect of substitution in the amidine grouping of active compounds could be investigated, in the first place by systematic substitution with alkyl, aryl and heterocyclic groups to show whether significant alteration of activity could be expected, finally narrowing down the type of substituent to achieve maximum biological activity. For the compounds intended as anti-bacterial agents the type of substituent shown to be active in the sulphonamide series of drugs would be of particular interest, viz. pyridine, thiazole, pyrimidine, guanidine, etc.

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