STUDIES IN CHEMOTHERAPY

PREPARATION OF CERTAIN ACRIDINE DERIVATIVES

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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The author wishes to acknowledge the assistance and encouragement given by the late Professor F.J. Wilson, Dr. M.M.J. Sutherland and Professor W.M. Cumming.

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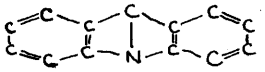
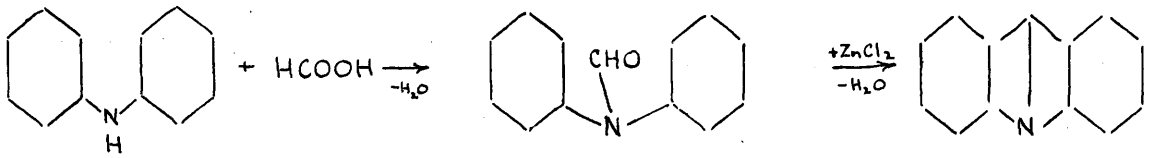
INTRODUCTION

I N T R O D U C T I O N

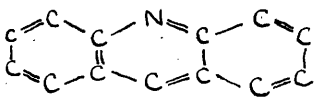
Representation of the Acridine Molecule

The structural formula of acridine was formerly thought to be 

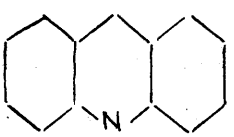
due to the compound's formation from diphenylamine and formic acid in the presence of zinc chloride (29).



It was shown, however, by Ramart-Lucas, Grumez and Martynoff (139) that the absorption spectra of acridine in visible and U.V. light are almost the same as those for anthracene, indicating that the nitrogen atom is spectrographically similar to -C=. This, in conjunction with the chemical properties of the molecule has led to the adoption of an anthracene-like structure for the acridine molecule.



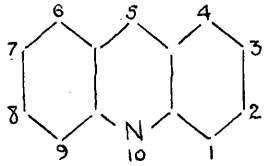
In this thesis, however, the following contracted form will represent the acridine molecule:-



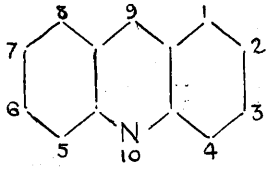
Nomenclature

There are two methods of nomenclature of the acridine series in common use at the present time. There is the

system employed in "The Ring Index" (143) which numbers according to a given set of rules irrespective of the source or components of the system and is used in British publications. This is the system adopted throughout this thesis.



In American and German publications the numbering of the acridine system follows that for anthracene which was numbered before the introduction of "The Ring Index" (143) to emphasize that the reactivity of the 9,10 positions was distinctly different from those at other positions.



Recently, a new system of notation has been proposed by Dyson (51) for all organic compounds. On this system acridine itself is denoted by T.10ZN. Acridone,  becomes T.10ZN.9,10H.9EQ., while 5-chloroacridine (British numbering) becomes T.10ZN.9C1 and 3-cyanoacridine becomes T.10ZN.2C.11E3N.

Preparation of Acridine Derivatives

As the acridine molecule is a chromophore, much attention has been devoted to methods of preparation of acridine derivatives by dyestuffs chemists since the end of last century and in this century by pharmaceutical chemists. Despite this,

only a few types of syntheses have been evolved since, in all cases, ring-closure of the meso-ring (i.e. the central pyridine ring) has had to be effected.

The methods of synthesis can be grouped under three headings:-

1) From diphenyl-methane derivatives,

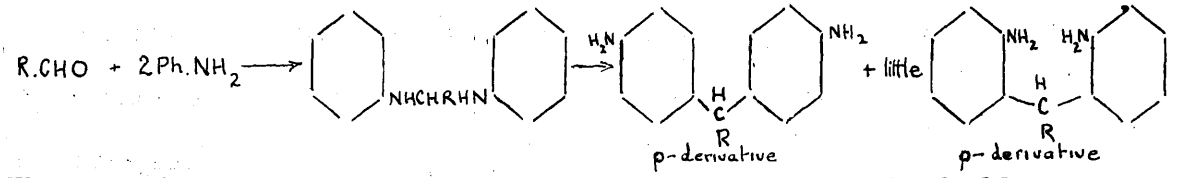
2) From diphenylamine derivatives,

3) From benzyl- and benzal- anilines.

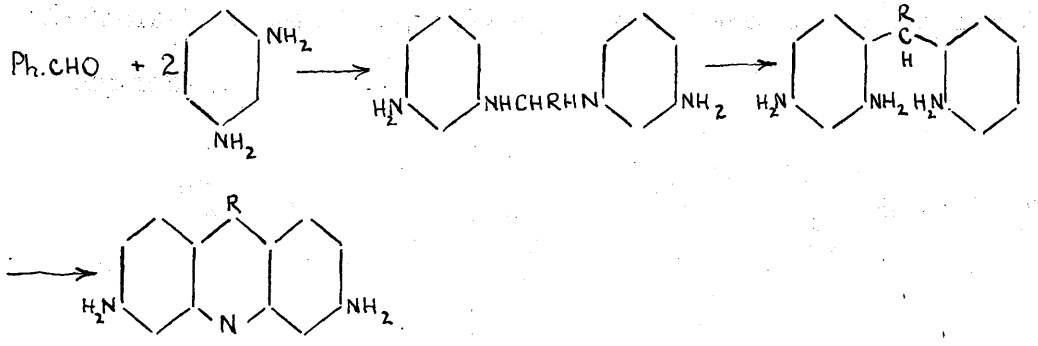
1) FROM DIPHENYL-METHANE DERIVATIVES

(a) From diamines and aldehydes

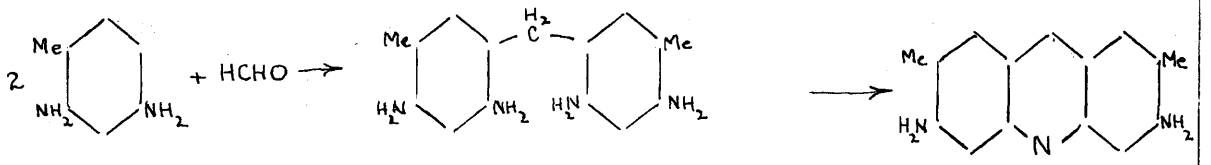
When an aldehyde is condensed with an amine of the benzene series, there is little tendency for the methylene group to take up the ortho position, the product being almost entirely the para derivative.



The simplest method of ensuring that the product shall contain amino-groups ortho to the aldehyde residue is to use a meta diamine (118).

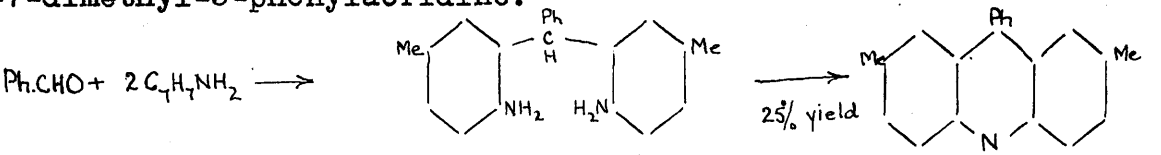


By the use of 2-4-diaminotoluene, 3-aminodimethylaniline and many other substituted m-diamines a series of 2-8-substituted acridines can readily be obtained (155).



(b) From p-substituted amines and aldehydes

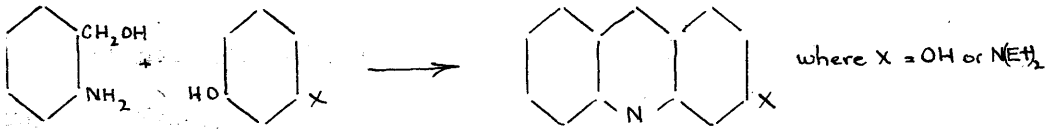
If the para position is occupied, the entrant group is forced into the ortho-position and the product can be converted into an acridine (156). Thus Ullman prepared 3-7-dimethyl-5-phenylacridine.



This method is general and can be used with any para-substituted aromatic amine. The methane base which is formed as an intermediate may be acridinated without the use of zinc chloride by simply heating to 200° with p-toluidine hydrochloride.

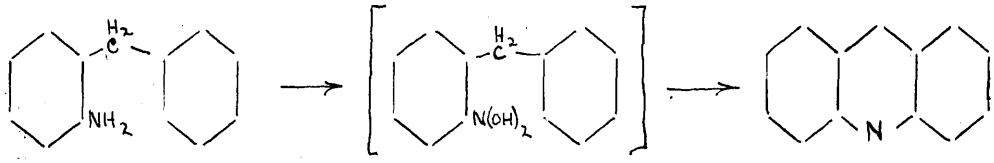
(c) From o-nitrobenzyl chloride

Ullman and Baezner (157) obtained acridines by condensing o-aminobenzyl alcohol with β-naphthol, resorcinol or diethyl--m-aminophenyl.

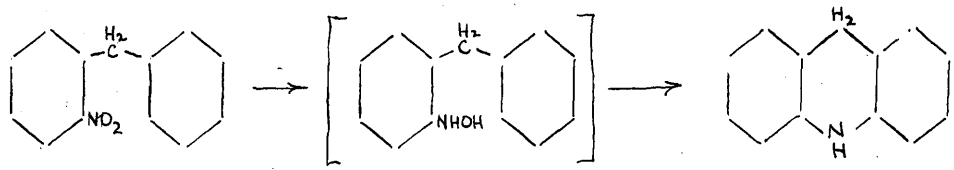


(d) By oxidation of o-amino-diphenylmethanes or reduction of o-nitrophenylmethanes.

Fischer and Schutte (62) synthesised acridine by oxidation of o-aminodiphenylmethane with lead oxide.



The hydroxylated nitrogen compound which may be regarded as an intermediate in the above synthesis is also obtainable by reduction of o-dinitrophenylmethane derivatives. Thus Fischer (63) prepared dihydroacridine.



By using substituted diphenylamines as starting materials many substituted acridines can be obtained.

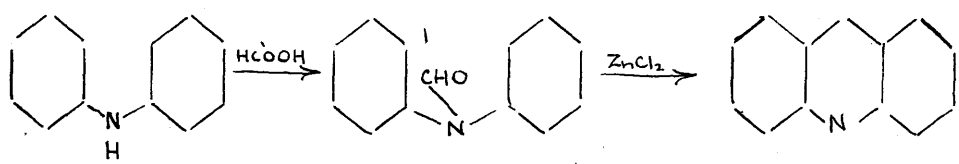
(e) From anthranilic ester and phenyl magnesium bromide

The tricarbinol obtained by the action of excess phenyl magnesium bromide on methyl anthranilate is converted to 5-phenyl-acridine when heated (19) (150).

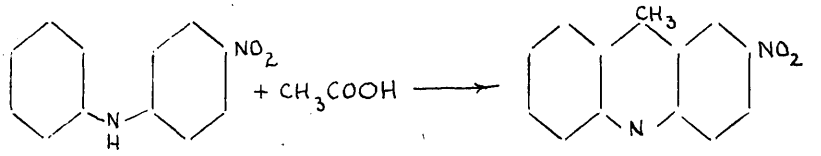
2) FROM DIPHENYLAMINE DERIVATIVES

(a) From diphenylamine derivatives

Method first adopted by Bernthsen (29) who prepared acridine by heating diphenylamine and formic acid in the presence of $ZnCl_2$

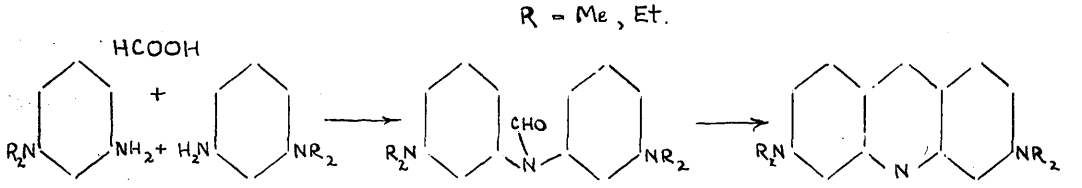


Using substituted diphenylamines and acetic, propionic benzoic acids etc., series of substituted acridines can readily be prepared -

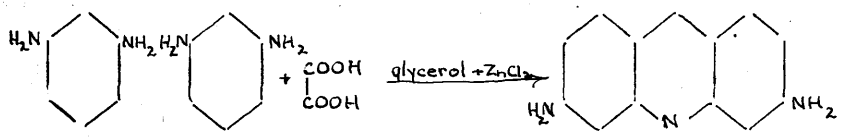


(b) From m-diamines and m-substituted amines with formic acid

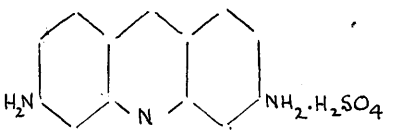
The earliest reaction of this type was the preparation of acridine dyestuffs by heating the diamines with formic acid and zinc chloride (52)



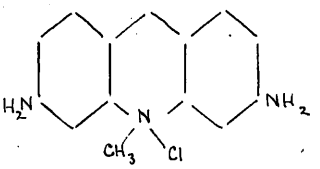
Albert and Large (2a, 2b) and Thomson (154) showed that a variety of substituted m-di-amines react in this way giving up to 72% yield of aminoacridines. They found that aniline itself does not react but that the following m-substituted anilines did in order of decreasing reactivity: NH_2 NMe_2, CH_3, Cl, NO_2, SO_3H, COOH.



This method has lately been used in the large-scale production of acridine pharmaceuticals, especially proflavine and acriflavine.



PROFLAVINE



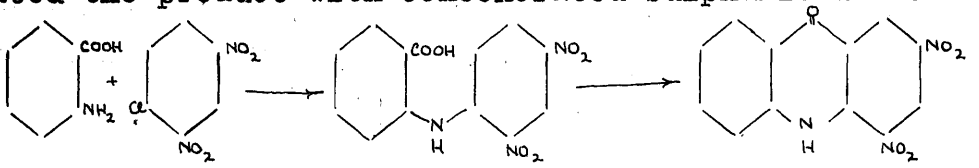
+ 2:8 diaminoacridine hydrochloride

ACRIFLAVINE

The use of this method is limited however by the fact that only substitution in the 2- and 8- positions can be effected and that good yields are only obtained with nucleophilic substituents.

(c) From aryl-o-aminoacids

This method was first used by Jourdan (91) who condensed anthranilic acid with 2-4-dinitrochlorobenzene and heated the product with concentrated sulphuric acid.



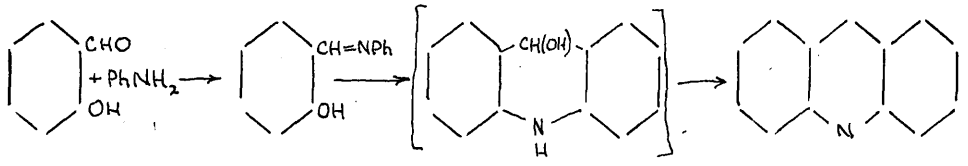
The ring-closure occurs readily in most cases, the chief difficulty being the preparation of the anthranilic acids.

With the development of Ullman's reaction of an arylamine and an o-chlorobenzoic acid in the presence of copper powder and potassium carbonate to form a diphenylamine derivative, this difficulty was overcome and this is now the method generally adopted in the laboratory preparation of substituted acridine derivatives. The method works equally well whether the amino-group is on the acid molecule and the halogen on the benzene ring or the chloro-group is on the acid molecule and the amino group on the benzene ring.

Ring-closure to the acridone, however, will not take place if the carboxyl group is di-ortho substituted.

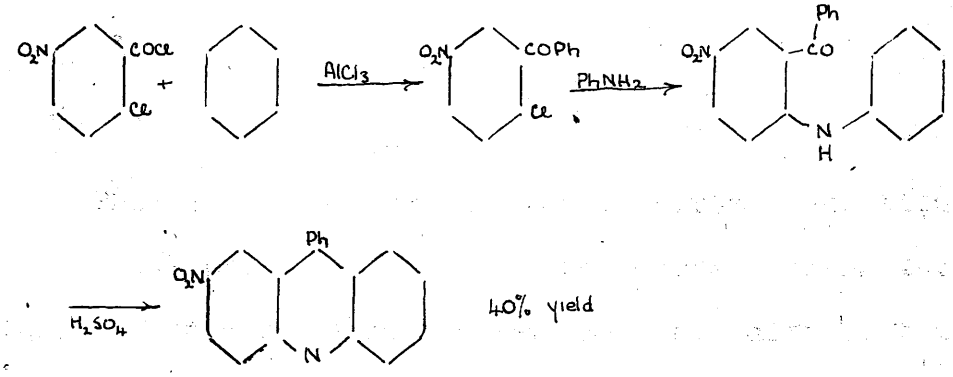
(d) From aryl-o-aminoaldehydes and aryl-o-aminoketones.

This is closely related to method (b). Just as salicylic acid and aniline give acridone, salicaldehyde and aniline yield acridine(122).



As many aniline derivatives, however, when condensed with o-chlorobenzaldehyde only give azomethines, this method is much more restricted in use than method (c).

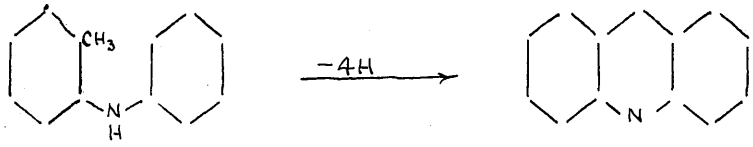
Similarly, Ullmann and Ernst (158) prepared substituted acridines easily and in good yield by the action of aluminium chloride on the o-chlorobenzoyl chloride derivative and subsequent treatment with concentrated acid.



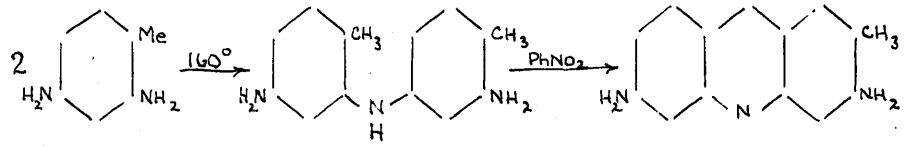
(e) From o-methyldiphenylamine derivatives.

This method is mainly of historical importance since

Graebe (75) obtained considerable quantities of acridine by passing the vapours of o-tolylaniline through a hot tube.



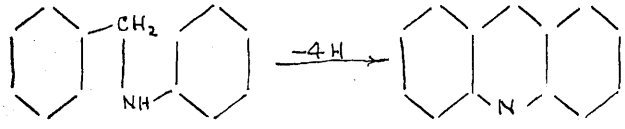
More recently 2-4-tolylenediamine has been converted to an acridine nitrobenzene being used as an oxidant (53).



The 2-4-compound may be replaced by the 2-6-isomer or by 4-6-diaminoxylene. Alternative constitutions for the products are possible in each case.

3) FROM BENZYL-AND BENZAL-ANILINES

Method 3) is of restricted use and consists of passing benzyl-aniline through a hot tube resulting in the formation of considerable quantities of acridine (119).



This method can be applied to certain substituted benzyl and benzal-anilines to obtain the corresponding acridines. These aniline derivatives are, however, difficult to prepare and an alternative method of preparation is generally to be preferred.

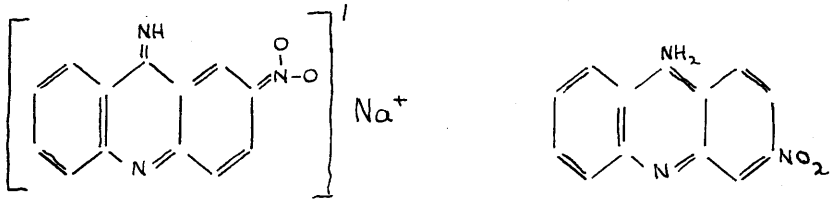
PREPARATION OF CYANO-ACRIDINES

Two cyanoacridines have so far been prepared. 3-Methoxy-8-cyano-5-chloroacridine was prepared by Yu Magidson and Travin (163) and 3-cyano-5-aminoacridine by Albert and Gedhill (9). Both compounds were prepared by method 2c.

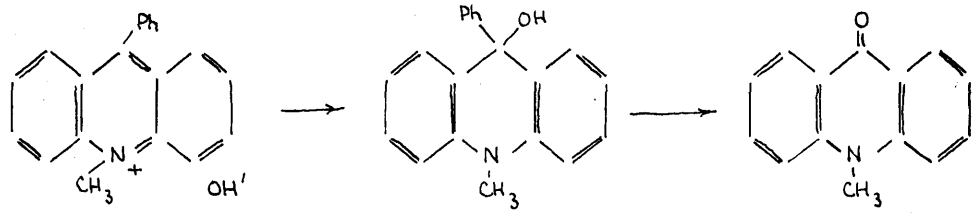
General Properties of Acridine Derivatives

The general properties of the acridines are those predicted from the chemistry of pyridine and quinoline coupled with some anthracene-like behaviour. Acridine itself is a base ($K_b=3\times10^{-10}$) slightly stronger than aniline ($K_b=2.7\times10^{-10}$) and weaker than pyridine ($K_b=2.3\times10^{-9}$) and in its derivatives this basicity is modified by the various substituents in the nucleus. Acridines can readily be reduced to 5,10-dihydroacridines (which are non-basic) and oxidised to acridones, extreme conditions being necessary before rupture of the acridine skeleton is effected.

Resonance can exist between substituents in the different rings, e.g. 3-nitro-5-amino but not 2-nitro-5-aminoacridine gives a purple nitronate with alcoholic alkali (4). Re-arrangement of bonds is possible in the former but not in the latter



Acridine forms alkyl acridinium salts which form unstable -inium hydroxides, the hydroxide radical of which migrates from the 10- to the 5-position, where conversion of the 5-hydroxy-5, 10-dihydro product to an acridone, analogous to the pyridones, takes place with mild oxidising agents (18).



Acridine does not methylate when it is refluxed with methyl iodide in methyl alcohol, however, though 5-amino-acridine gives an almost quantitative yield of 5-amino-10-methylacridinium iodide (4) due to increased basicity of the molecule.

The activity of substituents in the 5-position is shown by the ready formation of 5-cyanoacridine (98) when acridine is treated with potassium cyanide and the ease with which 5-chloroacridine can be converted to 5-alkoxy (163) and hence to 5-amino- or any 5-substituted-aminoacridine.

5-Chloroacridine is readily hydrolysed to acridone, the ketonic form of 5-hydroxyacridine (5) with the elimination of hydrochloric acid.

The activity of substituents in positions other than 5- and 10- is similar to that of benzenoid structures.

Nitration occurs in the benzene rather than the pyridine ring (89) and perhalide formation occurs with bromine. An amino group in the 5-position suffers hydrolysis more readily than in other positions and forms no diazonium salt under normal conditions, although it will under highly acid conditions (4).

Like anthracene, acridine-5-carboxylic acid is resistant to esterification by alcohol and mineral acid catalyst, due to steric hindrance (ortho effect) and it is necessary to prepare the ester from the acid chloride (90).

Preparation of Aromatic Nitriles - General Methods.

1. Treatment of an aromatic halide with potassium cyanide and a catalyst at 200°. This method works most satisfactorily when a nitro or a sulphonate group is present and can be further modified by replacing potassium cyanide by cuprous cyanide (144).

$$ArX + KCN \xrightarrow[200^\circ]{\text{catalyst}} ArCN + KX.$$

The potassium salt of a sulphonic acid may replace the halogen group.

2. Ammonium salts or amides can be converted to nitriles with the loss of water by the action of phosphorus trichloride or phosphorus oxychloride and pyridine, p-toluene-sulphonyl chloride or trichlorophenylmethane (95).

$$Ar\,COONH_4 + PCl_3 \xrightarrow{\text{pyridine}} ArCN + H_3PO_4 + 3HCl$$

3. The diazotisation of aromatic primary amines followed by a Sandmeyer reaction gives, in general, about a 60% yield of the corresponding nitrile and is one of the most general methods of preparation (129).

$$ArNH_2 + HNO_2 \longrightarrow ArN_2Cl \longrightarrow ArCN$$

4. The Grignard reaction can also give rise to nitriles by treatment of the aryl magnesium halide with cyanogen or cyanuryl chloride.

$$ArX + RMgX \longrightarrow ArMgX \longrightarrow ArCN.$$

5. When aldoximes are treated with acetic anhydride, nitriles are formed in 90% yield with the loss of a molecule of water (162).

$$ArCHO \xrightarrow{H_2NOH} ArCH=NOH \xrightarrow{Ac_2O} ArCN$$

6. Mustard oils (phenyl isocyanates) on treatment with copper or zinc dust yield nitriles.

$$ArNCO \xrightarrow{Zn} ArCN$$

Methods 3 and 5 are of most general application and are those generally adopted in this thesis.

THERAPEUTIC ACTIVITY

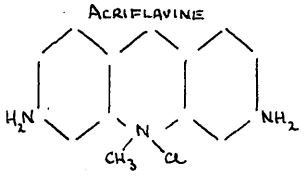
The trypanocidal activity of acridine compounds (27) was discovered by Ehrlich in 1912 during studies with the triphenylmethane dyes. It was found that crude dichloro-ortho-para-fuchsin was less toxic and more trypanocidal than pure dichloro-ortho-para-fuchsin, the difference being traced to an impurity, an acridine compound. Acriflavine, the first of this group and one of the most widely investigated acridine therapeutics, was found by Ehrlich to be especially effective against nagana infections in mice.

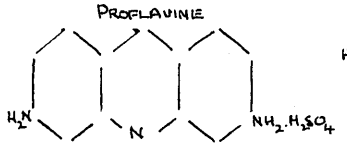
In 1914, Browning and Gilmour (36) discovered the antiseptic action of acridine derivatives when they included in their investigation of the bactericidal properties of organic bases, some of the aminoacridines which Ehrlich had synthesised for his trypanocidal studies. It was found that while acridine itself has only slight activity, is toxic and irritant, the aminoacridines, especially proflavine, acriflavine and 2,7-diaminoacridine are very active (126) and this prompted further study of acridine derivatives, especially when it was shown by Browning and co-workers (37) that the antiseptic properties of acridines were due to the acridine nucleus as a whole and were not shown by the corresponding derivatives of pyridine, quinoline or phenazine.

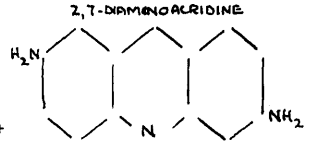
Acridines are effective antibacterial agents against Gram-positive organisms, have a moderate action against Gram-negative organisms and are in no way adversely affected by the presence of dissolved protein (serum). Browning and

Gulbransen (38) claimed enhanced activity for acridine derivatives in the presence of serum but this was disproved by Eggerth (57) who demonstrated that the potentiating effects of serum in vitro was due to the fact that this fluid becomes quite alkaline as carbon dioxide is evolved. Inasmuch as the acridines belong to the basic group of antiseptics, an alkaline medium greatly favours its action.

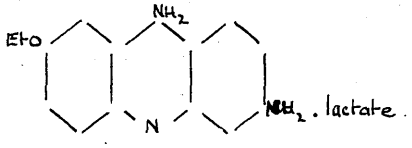
The most active acridine antibacterials are acriflavine , proflavine and 2,7-diaminoacridine (the last of which has the same order of antiseptic activity as proflavine and acriflavine but is only two-fifths as toxic as the former and one-tenth as toxic as the latter) and are especially active against gonococci, streptococci and anaerobic bacteria in general. They are active against T.B. in vitro (1:10,000) but not in vivo (68).





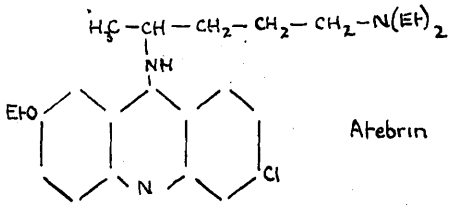


Another acridine derivative, which attracted considerable attention though it has now largely fallen into disuse due to the discovery of the sulpha drugs, is "rivanol", the lactate of 2,5-diamino-7-ethoxyacridine, which is very active against streptococci (in vivo dose 1:40,000)(mice).

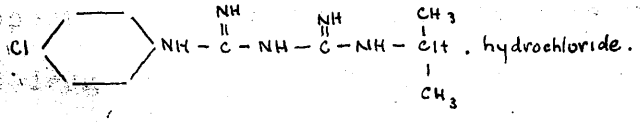


RIVANOL

In the search for a synthetic antimalarial, Mietzsch and Mauss (1215) discovered "atebrin" (known as "mepacrin"), 2-chloro-7-ethoxy-5-(δ-diethylamino-α-methylbutylamino) acridine. This compound, as well as its successor paludine, has been manufactured on a large scale in Scotland since 1943.



Atebrin



Paludrine

(N1 - p - chlorophenyl - N5 - isopropyl biguanide hydrochloride)

It was found to combine general antibacterial character with great antimalarial activity, being four times more active than quinine against plasmodium praecox. It was found that whereas quinine attacks the gamete stage of the infection, atebrin kills the schizont form.

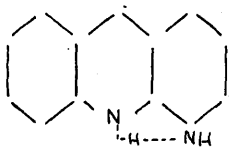
Relationship between Structure and Activity

Many substituted acridines have been prepared where halogen, hydroxy, nitro, methyl, ethoxy, carboxy,

sulphonamido radicles have been substituted in the molecule in differing combinations, numbers and positions in the acridine nucleus. These compounds were all found to have antibacterial activity to a greater or less degree but no relationship between structure and activity has yet been satisfactorily formulated.

There is:—
Reduction of activity with introduction of NO_2 group in position 7, halogen in position 8, Br for Cl, OH for OMe, morpholine for $N(Et)_2$ in position 5. (111)

Within the series of the aminoacridines, Albert and co-workers (6) have shown that the basicity and hydrophilic properties of the aminoacridines vary as the activity of the drug (except in the case of 1-aminoacridine which though more active than acridine is less basic due to hydrogen bonding).

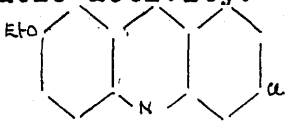


It was found that all compounds containing a 1-NH_2 substituent are without antiseptic effect, whereas 4,5,3,2, -NH_2 groups greatly increase the activity (11b). Further, Bradbury and Linnell (23b) showed that none of the chloroaminoacridines had a bacterial activity equal to that of the parent amino compound.

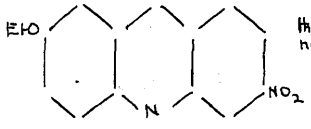
A study of the reduction potentials of the amino-acridines, however, led Beyer (30) to the conclusion that

the antibacterial activity in this series of compounds is not connected with a single physical or chemical property but is the result of the sum total of properties. Other workers (153) have also been unable to show any relationship between chemical structure and pharmacological action.

The discovery of atebrin led Magidson and Grigorovskii (111) to prepare many similar compounds altering both the nuclear and the side-chain substituents. They, and other workers, were unable to prepare any compound of superior activity to atebrin and found that the absence of a substituent in positions 6 or 7 completely annulled the therapeutic activity.



Ate



this has no therapeutic activity (I = 0)

If $NO_2 \rightarrow Cl$, I = 8, 15, 20, 6 when n (no of C atoms inside chain) = 2, 3, 4, 5 respy.

If $NO_2 \rightarrow Cl$, I = 8, 15, 20, 6 when n (no of C atoms inside chain) = 2, 3, 4, 5 respy.

As can be seen, empirical relationships have been demonstrated in specific cases but no real understanding exists of the fundamental relation between therapeutic activity and chemical structure.

Mode of Action of Acridines. I Trypanocidal Action.

Hawking (81) has shown that the trypanocidal action of acriflavine and similar derivatives resembles closely that of the trivalent arsenicals. From his experiments,

he came to the conclusion that the reaction between the drug and the trypanosome occurs in three stages - viz. fixation of the drug, that is absorption of the drug by the trypanosome which results in a condition resembling bacteriostasis, secondary chemical reactions and eventual death of the organism. The fixation occurs rapidly, being complete in a few minutes and is reversible .

II Bacterial Action.

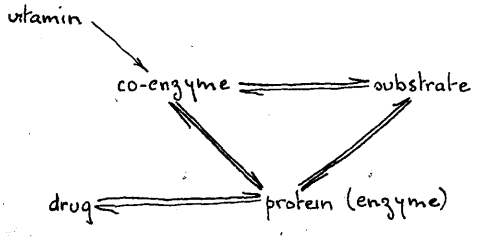
Acridines or normal cationics as they are sometimes called are bacteriostatic from the moment they are applied and within two hours are exerting a profound bactericidal action (77).

McIlwain (121a) found that B.coli and streptococcus haemolyticus, inhibited by acriflavine, required for further growth two types of compound not normally required for growth of the organism. The most effective of type I compounds were nucleotides, and of type II a concentrate of aminoacids, phenylalanine alone being fairly active. Type I compounds formed complex salts with acriflavine and it was hence thought that acriflavine inhibited the enzyme systems of which type I compounds were essential parts and of which type II compounds were substrates of products.

This is in agreement with the general theory of the action of chemotherapeutic agents propounded by Sir Henry Dale (46) who suggested that for successful chemotherapy

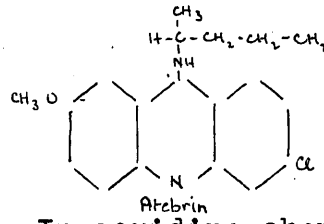
of an infection due to an intracellular parasite there may be sought a metabolic inhibitor or inhibitors which selectively inhibit a reaction or reactions essential to the intracellular multiplication of the parasite but, at least temporarily, inessential to the survival of the host cells (124).

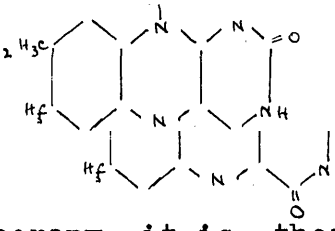
These critical sites of chemotherapeutic action have, in all cases so far examined, been found to be within the system of respiratory enzymes which mediate the aerobic and anaerobic oxidation of glucose and its derivatives. The respiratory enzymes are proteins reversibly combined with co-enzymes; the co-enzymes contain as essential structural units certain components of the B vitamin complex, notably nicotinic acid amide, riboflavin and thiamine



The drug-protein or drug – protein-co-enzyme complex are supposed to be inactive enzymatically. Quantitative antagonism between drug and co-enzyme thus becomes understandable and similarly antagonism between drug and vitamin, since the vitamin is a precursor of the corresponding co-enzyme.

Haas (80), working with isolated enzyme systems of yeast, showed that atebrin inhibited oxidation mediated by flaveoprotein and that the inhibition is antagonised by the co-enzyme. He further showed that cyto-chrome-oxidase (another enzyme system) is inhibited by atebrin.





Riboflavine phosphoric acid (alloxazine mononucleotide)

Riboflavine phosphoric acid (alloxazine mononucleotide)

In acridine chemotherapy, it is, therefore, thought that the cation (or basic portion) of the antiseptic combines with an anion (or acidic portion) in the bacterium to form a non-ionised complex. This reaction immobilises these vital anions, which would appear from McIlwain's work to be those of nucleic acids. When it is realised that streptococci contain 20% of their dry weight of nucleic acid, it can be seen that cationics can be powerful antibacterial agents.

In the acridine compounds this theory would account for the immediate onset of bacteriostasis but does not explain the later bactericidal effects unless it can be shown that interruption of the respiratory enzyme system can cause the death of the organism as well as inhibiting its division and propagation.

Effect on Host

Acridine derivatives, though non-toxic in therapeutic

doses, are not without systemic effects. The toxic intravenous dose of proflavine for mammals is approximately 30mgms./Kgm. body weight and causes central paralysis progressing from the cortex of the brain to the medulla and spinal cord. Continued administration of subtoxic doses leads to pathological changes in the liver and kidneys (82) though in antiseptic concentrations it is not toxic to the tissues. Acridines are toxic to human leucocytes (7) proflavine and 2:7-diaminoacridine being least toxic and exhibiting no toxic effects when applied locally.

Uses

The main use of acridines is in the chemotherapy of wounds with gross sepsis, where, due to the large amounts of pus (and hence p-aminobenzoic acid) present, sulpha drugs are inactivated. With systemic infections, the sulpha drugs are generally better though acridines are especially active against gonococci, streptococci etc., and may be used in the presence of sulpha-resistant bacteria. They may also be used effectively in the prophylaxis of gas-gangrene, being more active than sulpha drugs against Clostridium welchii.

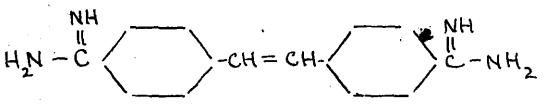
Acriflavine, like most antiseptics, causes haemorrhage and necrosis of the brain but proflavine and 2:7-diamino-acridine are little more harmful than isotonic saline solution and have been much used in brain surgery (8).

Though penicillin has a greater action against all cocci it is very costly to produce and is inactive against many Gram-negative organisms. The use of the more active acridines is therefore to be preferred in many cases.

Atebrin is still much used as an antimalarial, though its unrivalled position in this field of chemotherapy has been challenged by a new drug "paludrine."

Therapeutic Action of Amidines

Amidines, especially diamidines, have been found (61) to possess trypanocidal character. It was shown that two amidine groups were essential for activity and that they had to be separated by a chain of molecular complexity such as



(stilbamidine)

Alkyl or aryl substitution depressed the trypanocidal activity; replacement of a methylene group in the aromatic diamidines by oxygen increased the activity, while replacement by other radicles (-NH-, -CO-) or atoms (-S-) caused diminution.

Stilbamidine, propamidine and pentamidine have been given clinical trials with some success in African human trypanosomiasis (sleeping sickness), kala azar, B.canis and more recently in other tropical infections such as

babesia and leishmania (15) despite some unfavourable toxic reactions.

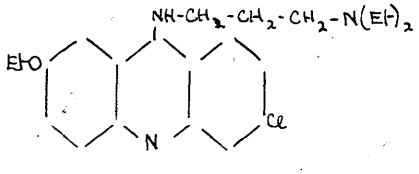
Investigation into Preparation of Cyanoacridines.

From the foregoing considerations of the therapeutic activity of acridines and amidines it was decided to prepare a series of cyanoacridines as precursors of amidinoacridines with special reference to 2 -8-diamidino-acridine due to the activity of proflavine and the configuration of the active diamidines to which it is structurally similar.

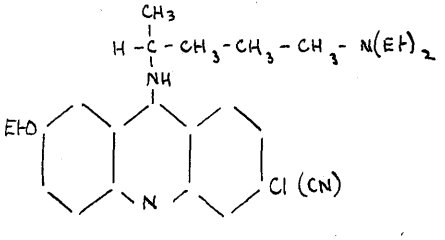
It was also borne in mind that the cyanoacridines themselves might have considerable activity as Yu, Magidson and Travin (163) have shown that the therapeutic index (I),

$$\left[\text{Therapeutic Index I} = \frac{\text{max. tolerated dose}}{\text{min. curative dose}} = \frac{\text{D.M.T}}{\text{D.M.C.}}\right]$$

for 7-methoxy-2-chloro-5-(γ -diethylaminopropylamino) acridine is 15, while that for 7-methoxy-2-cyano-5-(γ -diethylaminopropylamino)acridine is 10. The 5-(δ -diethylamino- α -methylbutylamino) homologue has I=23.5 in both cases.



I = 15



I = 23.5

The cyanogen group (CN), therefore, produces no specific toxic effects and the compounds behave biologically like stable organic nitriles.

Albert and Gedhill (9) prepared 3-cyano-5-aminoacridine and found it also to have antibacterial activity.

Two methods of approach to these preparations were adopted. The first was to start with a preformed acridine nucleus and then prepare the cyano derivative from some suitable precursor and the second was to reverse this process and prepare aromatic nitriles which were then ring-closed to form the acridine derivative.

The thesis is accordingly divided into two parts. Part I deals with the preformed acridine nucleus and the subsequent nitrile substitution and Part II with the formation of diphenylamine derivatives from benzonitriles followed by ring-closure to cyanoacridines.

THEORETICAL

PART I

THEORETICAL

Cyanoacridines from 2-8-diaminoacridine

As 2-8-diaminoacridine is the most readily obtained acridine derivative it was thought to be the most suitable starting material for the preparation of 2-amino-8-cyanoacridine and 2-8-dicyanoacridine by diazotisation of the amine and subsequent treatment by the Sandmeyer reaction.

The treatment of 2-8-diaminoacridine with sodium nitrite and dilute hydrochloric acid was carried out and the boiling potassium copper complex Sandmeyer solution added to the cold diazo-solution. After purification, an oxygen-containing acridine derivative was obtained. As the most likely cause of this was hydrolysis, the experiment was repeated, the Sandmeyer solution being added at 0° and the resultant mixture allowed to come to room temperature. Similar results were obtained. The Gatterman modification with copper powder, both at 100° and 0°, gave the same results, the product containing about 15% oxygen.

No formula could be found to agree with the analysis, though the fact that consistent results were obtained with different preparations indicated that a definite compound had been obtained.

As the substance was insoluble in alkaline solution, the oxygen could not be present in a carboxyl or amide grouping (probably decomposition products of a nitrile)

and it was therefore thought that the diazo-group was decomposed by the Sandmeyer solution before the nitrile group could be attached. Though a primary amino-group was found to be still present, no ammonia was evolved on treatment with hot alkali indicating the absence of a cyano-group. The compound was, therefore, not further investigated.

The structure of this compound was not ascertained. In an attempt to overcome this difficulty, it was decided to prepare 2-amino-8-iodoacridine by treating the diazo-solution with potassium iodide and converting to the nitrile by the Grignard reaction. It was found, however, that the iodoaminoacridine could not be prepared, as an oxygen-containing compound which did not contain halogen was formed.

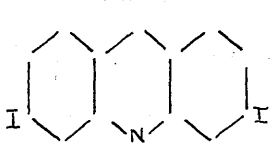
As the Sandmeyer reaction must be performed in an aqueous medium, no way of overcoming this difficulty could be suggested and it was found impossible to prepare 2-cyano-8-aminoacridine from 2-8-diaminoacridine.

Matsumuru (117) heated 2-8-diaminoacridine which had been tetrazotised to form the corresponding hydroxy compound. Much black matter with less than 1% hydroxy-acridine was obtained. Spalding, Moersch, Mosher and Whitmore (151) applied Matsumuru's method to 2-8-diamino-acridone and obtained 2-8-dichloroacridone.

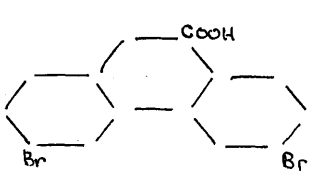
The tetrazo solution was prepared by dissolving the amine in concentrated sulphuric acid and treating with excess nitrosylsulphuric acid, the resultant solution being added to excess copper-potassium complex Sandmeyer solution at 100°. This yielded a product containing 23% oxygen which did not contain a primary amino-group. As the golden plates so obtained were insoluble in alkali and did not evolve ammonia on warming the alkaline suspension, neither a cyano-group nor its hydrolysis products appeared to be present. The nature of the compound was not further investigated.

As the 2-8-diiodo compound can be prepared (Grandmougin and Smirous (76)) it was decided to attempt the preparation of the nitrile from this by the general method of refluxing with dry cuprous cyanide (Ungnade (159)).

Further, Barber and Stickings (23a) have prepared 3-6-dicyanophenanthrene from 3-6-dibromophenanthrene-9-carboxylic acid by refluxing it with cuprous cyanide and quinoline, the quinoline acting as a decarboxylating agent. As acridines are, apart from basic character and especially with regard to substitution in the benzenoid rings, chemically similar to phenanthrenes it was thought that this method might be successfully applied to 2-8-diiodoacridine.



2,8-diiodoacridine



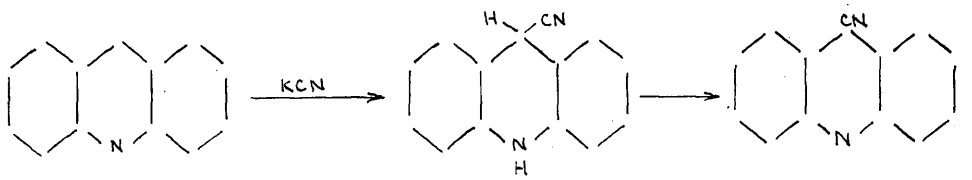
3,6-dibromophenanthrene-9-COOH.

Accordingly, 2-8-diiodoacridine was prepared. In this case, however, it was found that the halogen could not be removed and unchanged starting material was obtained.

No further attempts to prepare 2-8-dicyanoacridine with a preformed acridine nucleus were made.

Preparation of 5-cyanoacridine

The most convenient method for preparing 5-cyanoacridine described in the literature is that adopted by Lehmstedt and Dostal (99) who refluxed acridine with potassium cyanide with glacial acetic acid and obtained the cyanocompound in good yield.



Two other methods of approach were possible:

1) The preparation of a 5-substituted acridine which could be readily converted to the 5-cyano compound.
2) The preparation of 5-cyanoacridine from 5-chloroacridine.

Method I

Aromatic nitriles can best be prepared from primary amines or from aldehydes. Albert and Ritchie (4), however, found that 5-aminoacridine could only be diazotised with difficulty under highly acid conditions due to imine formation and since a Sandmeyer conversion to nitrile had already proved unsuccessful in the case of the

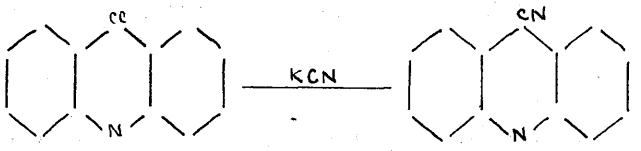
2-8-diaminoacridine, no attempt to prepare the nitrile from the corresponding amine was made.

Acridine-5-aldehyde was prepared from 5-methyl-acridine and the aldehyde converted to the nitrile through the oxime. The product was found to give no depression in m.p. with a sample of 5-cyanoacridine prepared by the method of Lehmstedt and Dostal (99).

The conversion of aldehyde to nitrile can be accomplished in 80-90% yield so this method is convenient for production in quantity.

Method II

Eisleb (58) described the preparation of 5-cyanoacridine from 5-chloroacridine by treating it with sodium cyanide in methyl alcohol at 140° for six hours.



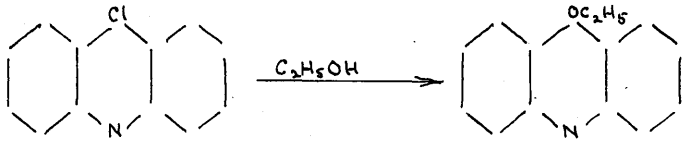
Braz and Gortinskaya (35) also described the preparation of 5-cyanoacridine by this method.

It was thought that due to the reactivity of the 5-position and the ready preparation of 5-cyanoacridine from acridine itself at normal pressures, it might be possible to convert the chloroacridine similarly to the cyano compound.

5-Chloroacridine was prepared by the method of Graebe and Lagodzinski (74) by treating acridone with phosphorus oxychloride and phosphorus pentachloride at 130°.

It was found that when freshly prepared 5-chloroacridine was refluxed with potassium cyanide in sodium dried methyl, ethyl or amyl alcohol only the starting material was obtained at the end of the experiment.

If the chloroacridine were not freshly prepared and undried alcohol used, a product was obtained which did not contain halogen nor a cyano-group and was found on analysis to be 5-ethoxyacridine formed by the reaction between the chloroacridine and the solvent



This is in agreement with the findings of Drozdov (50), who claimed that on standing, particularly under the influence of daylight, that 5-chloroacridine is converted to the reactive complex

$$(C_{13}H_9ONC_{13}H_8NCl)HCl$$

which is readily converted into the ethoxy compound. Thus the conflicting data in the literature concerning the relative reactivity of 5-chloroacridine and its derivatives can be explained by the use of products which have undergone differing degrees of decomposition.

To elevate the temperature of the reaction a dry fusion of 5-chloroacridine and potassium cyanide wascarried out at 140° but again only starting material was recovered.

The 5-chloroacridine was then converted to a quaternary salt, the methyl-p-toluene-sulphonate, as it was hoped this might activate the molecule sufficiently to allow the formation of the 5-cyano compound. Only unchanged 5-chloroacridine resulted.

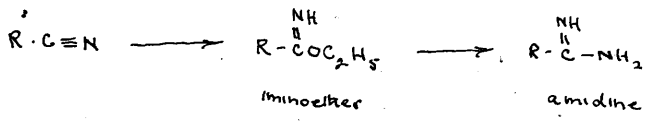
Preparation of 5-amidinoacridine

The conversion of nitriles to unsubstituted amidines can be accomplished in the following ways -

1) Conversion to imino-ether and hence to amidine,
2) Treatment with alkali metal amides,
3) Heating with ammonium salts.

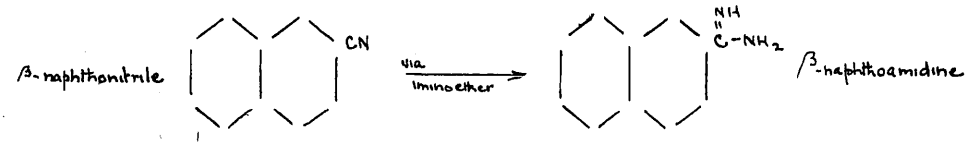
Method I

This is the original method of preparing amidines employed by Pinner (134) and is still the most widely-used. Details of a typical procedure can be found in Organic Syntheses (127). The nitrile, dissolved in sodium dried ether and magnesium dried alcohol and into which dry hydrogen chloride is passed with cooling, forms an imino-ether hydrochloride, which, on treatment with dry alcoholic ammonia under pressure gives the amidine

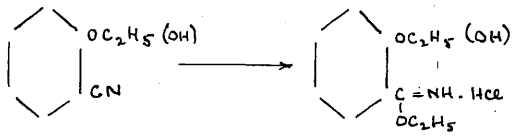


The method is of general application to aliphatic compounds and to aromatic provided that certain ortho substituents are not present, when steric effects interfere with imino-ether formation. Some ortho-substituted aromatic nitriles will not react with ethyl alcohol and hydrogen chloride (Pinner (135)).

Thus o-tolunitrile and α-naphthonitrile do not form iminoethers (Dietz (47)) though p-tolunitrile and β-naphthonitrile do so readily (Luchenbach (110))



Later, however, it was found that some o-substituted nitriles could form imino-ethers. Dietz and Pinner (48) prepared the imino-ether of o-ethoxyphenyl cyanide in poor yield and Easson and Pyman (56) obtained a 40% yield of o-hydroxybenziminoethyl ether hydrochloride from o-hydroxyphenyl cyanide.



No case of successful imino-ether formation from an α-naphthonitrile has been reported and as the steric hindrance present in this nitrile is similar to that in 5-cyanoacridine, it was doubtful whether this method could be applied to the latter compound successfully.

Experimentally, it was found that treatment of 5-cyanoacridine with dry ether, dry alcohol and dry hydrogen chloride resulted in unchanged starting material.

Modification of the experiment by altering solvents, concentrations and temperature gave the same result.

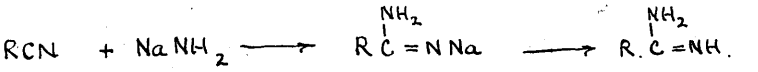
Note - On attempting to prepare 5-cyanoacridine methiodide by refluxing the two substances it was found that only unchanged 5-cyanoacridine was recovered at the end of the reaction. In this connection, it should be noted that, under these conditions, acridine itself does not form a methiodide though the 5-amino compound readily does, due to its increased basicity. Treatment of the methosulphate with dry alcohol and hydrogen chloride resulted again in unchanged starting material.

Method II

This method originated in the work of Walther and Grossman (160) who investigated the reaction of aromatic amines with nitriles in the presence of sodium to produce sodium derivatives of the amidines.

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 sodium derivative is the one generally employed, though both the potassium and calcium amides are also used.

The alkali metal salt is transformed into the amidine by treatment with water or to the amidine hydrochloride with hydrogen chloride.

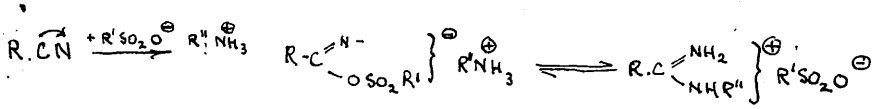


When 5-cyanoacridine was treated with sodamide under toluene, however, only unchanged starting material was obtained at the end of the reaction.

Increase of temperature by substituting xylene for toluene also led to the recovery of the starting material at the end of the reflux. Dry fusion of the nitrile with sodamide led to the production of a charred mass from which no acridine derivative could be isolated.

Method III

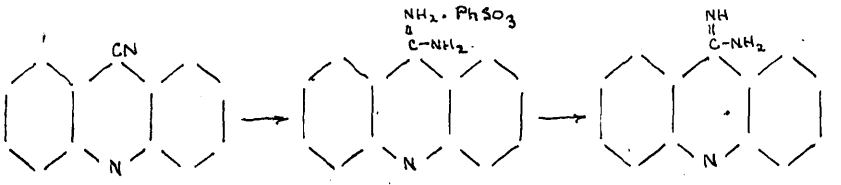
Oxley and Short (133) have described a general method for the preparation of unsubstituted amidines which consists of fusing the nitrile with the ammonium salt of an aromatic or aliphatic sulphonic acid. They suggest as the mechanism of the reaction:



The nitrile and the ammonium salt, generally the benzene sulphonate, are fused and the amidine obtained as the sulphonic acid salt. They further state that this

method may be applied to ortho substituted nitriles to give ortho substituted aromatic amidines which are not accessible through the imino-ethers.

By this method it was found possible to obtain 5-amidino-acridine from 5-cyanoacridine in good yield through the intermediate benzene sulphonate derivative.



No further attempts to prepare cyanoacridines with a preformed acridine nucleus were made.

EXPERIMENTAL

PART I

PREPARATION OF 2-AMINO-8-CYANOACRIDINE

Reference - The diazotisation of 2-8-diaminoacridine was mentioned by Benda (28) and Grandmougin and Smirous (76), but no details were given.

Method I

3gms. 2-8-diaminoacridine sulphate were added to 2c.c. of concentrated hydrochloric acid in 50c.c. water. The mixture was heated to 80° when complete solution was effected and the finely-divided crystalline precipitate obtained on rapid cooling was diazotised by slowly adding a solution containing 1gm. sodium nitrite (slight excess) in 10c.c. water, the temperature being maintained below 0°. The end-point of the reaction was somewhat difficult to determine by starch-iodide paper due to the strong violet colour of the diazo solution, but by careful observation of the outspread on the filter paper, the end-point could be obtained.

A solution for the Sandmeyer reaction was prepared by dissolving 3.5gms. hydrated copper sulphate in 20c.c. water and adding to this 2gms. potassium cyanide dissolved in 20c.c. water, the whole being warmed till a clear solution was obtained.

After keeping at 0° for 15 minutes, the diazonium solution was added to the boiling Sandmeyer solution. There was a vigorous evolution of nitrogen. The product

was filtered hot and a chocolate-brown precipitate and an orange-yellow solution (fluorescing yellow-green on dilution) obtained.

The residue, which was inorganic, was discarded and the remaining copper removed from the filtrate by the passage of hydrogen sulphide. The filtrate was then made alkaline with sodium hydroxide. The deposited base was purified by redissolving with charcoal in hot dilute hydrochloric acid. Addition of ammonia to the filtrate gave the base as clustersof small brown needles, which decomposed at 130-150° to a dark brown residue but did not melt below 360°.

Yield - 1.6gms.

Analysis	Calculated (for $C_{14}H_9N_3$)	Found
%C	76.7	66.5
%H	4.1	4.6
%N.	19.2	13.6
Total	100.0	84.7

There was no residue. Repetition of the experiment yielded consistent results. Halogen and sulphur were both absent and the remaining 15.3% was concluded to be oxygen.

The reaction was repeated with variations in temperature and concentrations but gave no cyano-aminoacridine.

Note - 1) It was noticed that if the temperature was

allowed to rise above 5° during the diazotisation a dark brown solid, insoluble in organic solvents, was rapidly formed. Prolonged standing at 0° also resulted in the slow formation of this substance.

2) The Gatterman modification of the above reaction using copper powder was performed and similar results obtained.

3) The substance was insoluble in alkaline solution and on boiling no ammonia was evolved.

4) The substance could be further diazotised with hydrochloric acid and sodium nitrite to give a pink dye with alkaline β-naphthol showing that a primary amino group was still present.

Method II

Preparation of 2-iodo-8-aminoacridine

The violet diazo solution was prepared as in the previous experiment (p. 40). A slight excess of the equivalent quantity of potassium iodide was slowly added at 0° and the dark brown precipitate which resulted, neutralised with sodium hydroxide and washed with sodium thiosulphate solution and water. The residue, crystallised from nitrobenzene as brown needles, did not melt below 300°.

The compound did not contain inorganic matter, halogen

or sulphur and on analysis was found to contain 67.1%C, 4.4%H, 13.9%N, leaving 14.6% unaccounted for, which must represent oxygen.

On diazotisation of the product it was found that a primary amino group was still present.

PREPARATION OF 2-8-DICYANOACRIDINE

Reference - The tetrazotisation of 2-8-diaminoacridine was mentioned by Grandmougin and Smirous (76) but no details were given.

Method I

2gms. 2-8-diaminoacridine sulphate were dissolved in 20c.c. concentrated sulphuric acid and added slowly with stirring to 2gms. sodium nitrite (excess) dissolved in 10c.c. concentrated sulphuric acid, below 20°. The strong green-yellow fluorescence exhibited in concentrated sulphuric acid disappeared on addition to the nitrite solution.

After standing for 10 minutes below 20°, the tetrazo solution was added to a solution containing 1gm. hydrated copper sulphate and 2gms. potassium cyanide in 20c.c. water, the temperature being kept below 40°. There was a brisk evolution of nitrogen.

After the final addition, the solution was boiled for five minutes, almost neutralised with solid ammonium carbonate and filtered to remove the inorganic residue, the

remaining copper being removed from the filtrate by the passage of hydrogen sulphide.

The filtrate was then neutralised with sodium hydroxide and the base which was deposited, recrystallised from boiling chloroform. On cooling, lustrous, orange-gold plates separated, m.p.198°. On further recrystallisation no elevation of m.p. was obtained.

Analysis	Calculated (for $C_{15}H_7N_3$)	Found
%C	78.6	54.2
%H	3.1	4.3
%N	18.3	19.2

Sulphur and halogen were both absent and the test for a primary amine was negative.

The experiment was repeated, the solution being kept at a lower acidity by repeated additions of solid sodium carbonate during the addition of the tetrazo solution to the Sandmeyer solution. The product was purified as before and the same compound obtained.

Method II

Preparation of 2-8-diiodoacridine

Reference - (76)

2gms. 2-8-diaminoacridine sulphate were dissolved in 20c.c. concentrated sulphuric acid and slowly added with stirring below 20° to 2gms. sodium nitrite (excess)

dissolved in 10c.c. concentrated sulphuric acid as in the previous experiment (p. 43). After 10 minutes, 2gms. potassium iodide were added in small amounts. Considerable frothing occurred with a brisk evolution of nitrogen peroxide and a black precipitate was formed. The mixture was boiled for five minutes and the residue washed with sodium thiosulphate solution followed by water. The residue was recrystallised from nitrobenzene as lustrous, light brown plates, m.p. 285° (m.p.Lit. - 286°).
Yield - 1.9gms.

Preparation of 2-8-dicyanoacridine

1gm. 2-8-diiodoacridine was mixed with 2.5gms. dry cuprous cyanide and the whole added to 5c.c. boiling pyridine over a period of five minutes and the solution boiled for a further thirty minutes. A dark brown solution resulted, from which needles crystallised on cooling. Excess ether was added and the residual mixture of organic matter and copper salts precipitated and filtered. The residue was warmed with dilute hydrochloric acid and hydrogen sulphide passed through the suspension till no further precipitation of copper sulphide occurred. The suspension was shaken with chloroform and the chloroform layer evaporated to small bulk. On cooling, lustrous, golden plates were formed containing halogen and having m.p. 285°.

A mixed m.p. with some of the starting material gave no depression.

The aqueous layer was found to contain no organic material.

PREPARATION OF 5-CYANOACRIDINE

Method I

1. Preparation of 5-cyano acridine

Reference - (98)

4.5gms. acridine and 1.6gms. glacial acetic acid were dissolved in 20c.c. alcohol and mixed at room temperature with a solution containing 2.5gms. potassium cyanide in 3.5c.c. water. After thirty minutes gentle refluxing the mixture was cooled and filtered. The filtrate was evaporated to dryness and the residue together with the original precipitate were spread out in a thin layer so that the air would oxidise the dihydro compound which had been formed to the cyanoacridine. The oxidation was judged complete when a test sample dissolved in alcohol with a trace ofconcentrated hydrochloric acid gave no green dye. The dihydro compound which is a meriquinoidal salt gives this green dye, whereas the 5-cyanoacridine being benzenoid gives no colour.

The crude 5-cyanoacridine (5gms.) was purified by

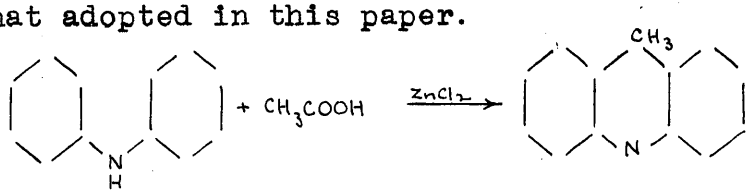
dissolving it in the minimum quantity of hot alcohol and filtering whereby a small amount of 5-5'-diacridyl was left behind undissolved. The nitrile was allowed to crystallise from the alcohol on cooling. Light brown needles, m.p. 182° (m.p.Lit. 182°, 183°) were obtained.

Yield - 3gms.

Method II - I. Preparation of 5-methylacridine

Reference - (29)

Product was extracted from melt by a different procedure than that adopted in this paper.



350gms. diphenylamine, 595gms. anhydrous zinc chloride and 210c.c. glacial acetic acid were mixed in a large flask fitted with an air condenser followed by a water condenser at its extreme end. A 360° thermometer was suspended in the flask through the two condensers.

On heating gently, the reagents dissolved and the temperature was gradually raised to 180-200° for the first hour. During the first six hours reflux, the temperature of the melt, which was greenish in colour, did not rise above 200°. During the next eight hours, the temperature gradually rose to 220° and the mixture lost its greenish colour becoming brown. Finally the temperature was allowed to rise to 220-240° for a further four hours.

After cooling to 100° the mixture was slowly poured into three litres of cold water. The lumpy product was broken into small pieces and allowed to stand for forty-eight hours, to remove most of the zinc chloride, the methyl acridine remaining undissolved. The water was then decanted and the product ground in a mortar and extracted with tepid water. The residue was extracted eight times with warm 15% sulphuric acid and the resultant red solution made alkaline with ammonia.

The precipitated base after settling was filtered, washed with water and dried giving a brown powder, m.p. 114-116° (m.p.Lit. 118°). Further purification was unnecessary for the next stage of the synthesis.
Yield - 320gms. (80% theor.).

2. Preparation of acridine-5-aldehyde

Reference - (92)

a) Condensation of 5-methyl acridine with 4-nitrosodimethylaniline

96.5gms. 5-methyl acridine and 75gms.4-nitrosodimethylaniline were intimately mixed in a large evaporating basin and gently heated with vigorous stirring.

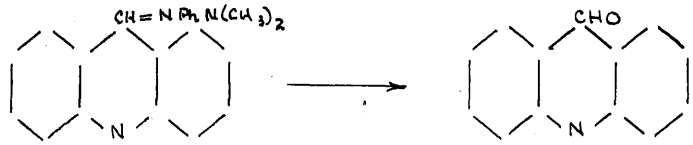
A dark green melt was obtained and when the temperature reached 110° the reaction commenced and heating was discontinued. The temperature rose spontaneously and there was a brisk evolution of steam. Throughout this, stirringhad been continued but the melt now began to

solidify and became too stiff to stir. A dark red cake was obtained which was cooled, powdered and refluxed with 200c.c. alcohol to remove impurities. The mixture was filtered hot and the insoluble anil washed with hot alcohol.

The anil was obtained as a brick red powder, m.p.236° (m.p.Lit. 239°).

Yield - 61.5% theor.

b) Hydrolysis of anil to acridine-5-aldehyde



The anil was made into a paste with water and 5N HCl added. The mixture first became black in colour but on warming and adding a little concentrated hydrochloric acid a bulky greenish-yellow precipitate was obtained which gradually became yellow.

The mixture was cooled rapidly and the yellow acridine-5-aldehyde hydrochloride filtered and washed with a little water. The hydrochloride was dissolved in hot water, the solution filtered and made alkaline with sodium hydroxide. The bulky yellow precipitate of the aldehyde was filtered, washed with water and dried, giving a yellow powder, m.p. 146° (m.p.Lit. 148°).

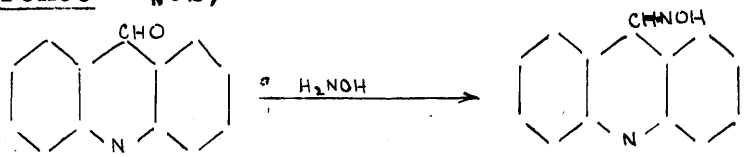
Yield - 85% theor.

Note - It was essential to wash the aldehyde with hot water till it was completely free from alkali.

The aldehyde was readily soluble in alcohol, ether, nitrobenzene and ethylene glycol monoethyl ether, both alcohol and ethylene glycol monoethyl ether being suitable solvents for recrystallisation purposes.

3) Preparation of acridine-5-aldoxime

Reference - (92)



50gms. acridine-5-aldehyde were dissolved in 250c.c. alcohol and the theoretical quantity (22gms.) hydroxylamine hydrochloride added. After refluxing for two hours, the oxime separated out as yellow needles, m.p. 240-245°.

The impure product was refluxed with charcoal and hydrochloric acid, filtered hot and the base reprecipitated with ammonia to give a bright yellow product, m.p.243-246° with decomp. (m.p.Lit. 247°).

Yield - 53gms.

4) Preparation of 5-cyanoacridine

10gms. acridine-5-aldoxime were refluxed for an hour with 300c.c. acetic anhydride giving an amber solution.

This was poured with vigorous stirring into much ice-water containing a little sodium hydroxide and stirring continued till the oil globules dissolved and a yellow-brown precipitate was deposited. After an hour the product was filtered and dried to give a yellow-brown powder, m.p.175-177°.

Yield - 8.0gms.

The crude nitrile was dissolved in the minimum amount of hot alcohol, filtered and the filtrate cooled to give fine, feathery yellow-brown needles, m.p.181° (m.p.Lit.182°).

A portion of the product was dissolved in the minimum amount of boiling alcohol, boiled with charcoal and filtered hot. On cooling, a crop of pale ochre coloured needles were obtained from the filtrate, m.p.182°.

A small amount of this compound was mixed with some 5-cyanoacridine prepared by method I and the whole gave a mixed m.p.182°.

Analysis	Calculated (for $C_{14}H_8N_2$)	Found
%C	82.3	82.1
%H	3.9	4.0
%N	13.7	13.8

Note - The nitrile was insoluble in water, ether, benzene and carbon tetrachloride. It was sparingly soluble in cold alcohol and acetone, this solubility being greatly increased on heating. It was readily

soluble in cold chloroform. The solutions in alcohol, acetone and chloroform showed pink-blue fluorescence which greatly increased at high dilutions. The base dissolved in conc.sulphuric acid and with hydrochloric acid formed a bright yellow hydrochlorode which was sparingly soluble in cold water but readily soluble in hot water. The nitrile could only be hydrolysed with considerable difficulty, refluxing with 50% sodium hydroxide for three hours being necessary before solution occurred. The acid was reprecipitated with conc. hydrochloric acid as buff-coloured needles which decomposed at 300° to acridine, thereafter melting at 110°.

Method III

5-chloroacridine was prepared according to the method of Graebe and Ladozinski (74). 5gms. acridine were refluxed with 5gms. phosphorus pentachloride and 50c.c. phosphorus oxychloride on a water-bath for half an hour. The phosphorus oxychloride was then evaporated above an oil-bath at 180° and the well-cooled residue added to ice-water. The insoluble acridone was removed by filtration, the filtrate made just alkaline and the 5-chloroacridine precipitated. The base was filtered and recrystallised from acetone as pale yellow needles, m.p.118° (m.p.Lit.=119°).

0.5gms. 5-chloroacridine were refluxed with dry alcohol and the equivalent amount of potassium cyanide. After one hour, the alcohol was distilled, the residue being recrystallised from aqueous alcohol. It was found to contain halogen and melted at 118°. A mixed m.p. with 5-chloroacridine gave no depression.

The experiment was repeated using anhydrous amyl alcohol to obtain a higher temperature of reflux but only unchanged starting material was obtained.

A fusion of 5-chloroacridine with excess potassium cyanide was then performed on an oil-bath at 140°. After half an hour, the residue was extracted with boiling alcohol and the base precipitated with water. It was again found to consist entirely of unchanged 5-chloroacridine.

On refluxing 5-chloroacridine which had been prepared some time previously with undried ethyl alcohol for half an hour, the alcohol being distilled at the end of this period, a product which did not contain halogen and melted at 126° after purification from aqueous alcohol was obtained.

On analysis the compound was found to contain 80.4% C,5.8%H,6.6%N which corresponds to 5-ethoxyacridine (80.9%C,5.8%H,6.3%N).

Preparation of methyl 4-toluene sulphonate of 5-chloro-acridine

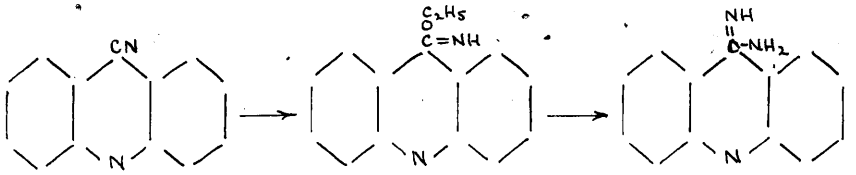
Molecular proportions of 5-chloroacridine and methyl-4-toluene sulphonate were heated at 140° for two hours. A dark oil resulted from which dark brown crystals separated on cooling. The crystals had a slight solubility in water giving a yellow solution. They were readily soluble in alcohol with a yellow-pink fluorescence and were recrystallised from aqueous alcohol to give pale yellow needles, m.p.82°.

Analysis	Calculated (for $C_{21}H_{18}O_3NSCl$)	Found
%C	63.0	62.8
%H	4.5	4.2
%N	3.5	3.6
%S	8.0	7.9

0.5gms. of the above salt were dissolved in the minimum amount of hot dry alcohol and the equivalent quantity of potassium cyanide added. After refluxing for an hour and distilling the excess alcohol, the residue was crystallised from aqueous alcohol and found to consist of unchanged starting material.

PREPARATION OF 5-AMIDINOACRIDINE

Method I



2gms. dry 5-cyanoacridine were placed in wash-bottle with 50c.c. magnesium dried alcohol and 25c.c. sodium dried ether. Dry hydrogen chloride was passed through the apparatus for ten hours, the temperature being kept at 0° by immersion of the wash-bottle in an ice-hydrochloric acid freezing mixture.

After two hours the nitrile had all dissolved to give a deep amber solution from which bright yellow needles crystallised out on further passage of hydrogen chloride. After ten hours the reaction was stopped and the ends of the reaction vessel sealed with screw clips and left for 48 hours.

The residue was then filtered & placed in a pressure bottle containing 100c.c. sodium dried alcohol which had previously been saturated at 0° with dry ammonia gas.

The pressure bottle was sealed and heated in a water-bath at 40° for ten hours. It was noticed that whenever the bright yellow powder was added to the ammoniacal solution it became pale yellow. After

several hours heating in the water-bath, the residue dissolved. On allowing to stand overnight, however, at room temperature a yellow-brown crystalline product was obtained. This was removed by filtration, after which the filtrate was evaporated to dryness under reduced pressure at 45°. Both these residues melted at 180° and were found to be unchanged 5-cyanoacridine, the bright yellow intermediate being the hydrochloride of the base.

Modification - a)

The temperature of the reaction for the imino-ether formation was raised to 50° and a large excess of dry alcohol was added. No imino-ether formation resulted.

Modification - b)

The 25c.c. dry ether originally used was replaced by 25c.c. dry chloroform as the nitrile is much more soluble in this solvent. Again, no imino-ether formation resulted.

Modification - c)

0.5gms. 5-cyanoacridine were refluxed with excess methyl iodide for one hour. After purification the residue was obtained as yellow needles, m.p.180° and was found to be unchanged 5-cyanoacridine.

Modification - d)

0.5gms. 5-cyanoacridine were dissolved in a slight excess methyl sulphate and gently warmed. There was

considerable frothing as the nitrile dissolved to give a deep brown solution. After one hour, the solution was cooled when an orange-yellow precipitate was formed which was purified by dissolving in alcohol and reprecipitating with ether to give orange-yellow needles, m.p.156°.

Analysis	Calculated (for $C_{16}H_{14}O_4N_2S$) (5-cyanoacridine methosulphate)	Found
%N	8.8	9.0
%S	10.1	10.1

The preparation of the imino-ether was attempted as previously described using the methosulphate instead of the free base but again only unchanged starting material was obtained.

Method II

Some sodamide was powdered in a mortar under toluene and excess refluxed with 1.0gms. of 5-cyanoacridine and 50c.c. toluene for six hours. On cooling the mixture was filtered, washed with ether and the residue dropped into dilute hydrochloric acid. The solution was warmed till the effervescence ceased and then filtered to remove a small amount of dark brown impurity. Concentrated hydrochloric acid was added to the filtrate and on dilution a yellow-orange precipitate was formed, m.p.240° (m.p.Lit. for 5-cyano-acridine hydrochloride - 240°). The orange product was basified with ammonia to give a pale yellow precipitate,

m.p.180° confirming that unchanged nitrile was present.

The method was repeated using xylene as an inert solvent to obtain a higher temperature of reflux but only unchanged starting material resulted.

On omitting any solvent and performing a dry fusion at 180°, charring resulted and no product could be separated from the resultant mixture.

Note - It was noted that, when the residue was dropped into the dilute hydrochloric acid after it had been washed with ether, nitrogen peroxide was evolved in considerable amounts. No mention of this could be found in any of the references relevant to this reaction.

Method III

0.5gms. 5-cyanoacridine and 0.8gms. (2mols.) ammonium benzene sulphonate (obtained by heating barium benzene sulphonate with ammonium sulphate, filtering the barium sulphate, evaporating the filtrate to dryness and drying the residue in a vacuum desiccator) were heated in a test-tube in an oil-bath at 250-260° for two hours.

Some of the cyanoacridine sublimed up the sides of the test-tube and had to be replaced in the dark brown melt from time to time with a glass rod. After two hours the melt was cooled and the contents and glass ground in a mortar to a fine powder. Any unchanged cyanoacridine was extracted with chloroform in which it was readily soluble in the cold but

which should not dissolve either the amidine benzene sulphonate or any excess ammonium benzene sulphonate.

On evaporating this filtrate to dryness, yellow needles were obtained, m.p.178° which showed no depression on mixing with 5-cyanoacridine; the residue could, therefore, be taken as being only unchanged starting material.

The residue from the chloroform was extracted with cold alcohol, giving a solution with a deep amber-blue fluorescence. A small amount of black material which formed on standing was filtered and the filtrate evaporated to dryness at room temperature. Deep brown needles, m.p. about 260° were obtained. These needles were presumed to be the amidine sulphonate, together with ammonium benzene sulphonate and it was decided to separate them by dissolving the whole in water and precipitating the amidine by the addition of sodium hydroxide, the ammonium benzene sulphonate remaining in solution.

Accordingly, the residue was dissolved in water, much shaking and three extractions of about 50c.c. each being necessary. The solution was filtered to remove a small brown amorphous residue.

The filtrate was bright yellow with a faint fluorescence. It was made alkaline with sodium hydroxide and a bright yellow amorphous precipitate obtained. This was filtered and the residue washed with water till the washings were neutral to

litmus. The filtrate showed a strong yellow-green fluorescence. The residue was dried in a vacuum desiccator and was found to soften at 160° to a darker mass which then melted at 216-219° with decomposition.

The yellow powder was recrystallised from aqueous alcohol to give fine yellow needles, m.p.226°. Yield -.45gms.

Analysis	Calculated (for $C_{14}H_{11}N_3$) (5-amidino-acridine)	Found
%C	76.0	75.8
%H	5.0	5.2
%N	19.0	18.9

Note - The amidine was readily soluble in alcohol, moderately soluble in chloroform, water and acetone and sparingly soluble in benzene and ether at room temperature. On warming the aqueous solution, ammonia was evolved. The free base fluoresced strongly yellow-green in all solvents. Addition of excess acid produced a pale yellow solution with a faint blue-yellow fluorescence. On treating a saturated solution of the amidine with solid ammonium nitrate, yellow needles, insoluble in water were formed. The amidine nitrate thus formed did not melt below 360° but charred above 300°. It could readily be converted to the free base by the addition of sodium hydroxide. The solubility of the chloride and the sulphate was slightly greater than that of the free base. This formation of insoluble nitrates was characteristic of the amidine group.

THEORETICAL

PART II

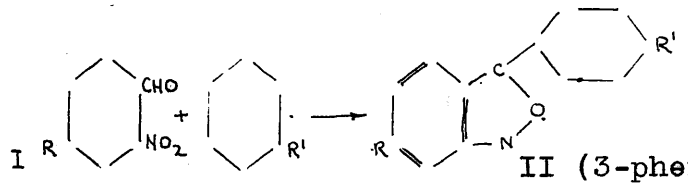
THEORETICAL

PART II

Of the known methods of synthesis of acridine derivatives, all but three are unsuitable for the preparation of cyano-acridines due to the difficulty which would be encountered in the preparation of the necessary starting materials.

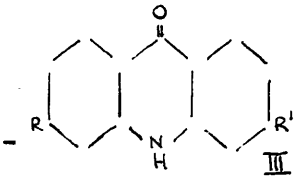
The first of these three methods, that of treating substituted 3-amino compounds with formaldehyde or formic acid to give the corresponding acridine or acridone respectively, works best in the presence of strong ortho-para directing groupings, giving very low yields in the presence of meta-directing groups like -COOH and NO_2. As the cyano group is strongly meta-directing and deactivating, good yields of cyanoacridines would not be obtained by this method.

Spalding, Moersch, Mosher and Whitmore (151) obtained a 2:8-disubstituted acridone by the following synthesis:-



I II (3-phenylanthranil)

II - on strong heating or on treatment with nitrous acid at room temperature -



III

For the formation of the anthranil, however, R' must be an ortho-para directing group so that a) the molecule is

sufficiently activated to permit condensation of the two molecules at the aldehydic carbon atom and b) R′ is sufficiently directional to ensure that linkage occurs in the position para to R′.

As neither of these conditions is satisfied by the cyano group, this method could not be applied successfully to the cyanoacridines.

Further, both the above-mentioned methods are limited by the fact that only 2:8 derivatives can be prepared by these syntheses.

The third method, that of treating a 2-chlorobenzoic acid derivative with an arylamine, or an anthranilic acid with a chlorobenzene derivative to give a diphenylamine-2-carboxylic acid which could be ring-closed to the corresponding acridone, appeared to be applicable to cyanoacridines, two having already been prepared by this method. It had the added advantage that acridines substituted in any position could be prepared.

Accordingly, the three aminobenzonitriles and 2-chloro-5-cyanobenzoic acid, 2-bromo-4-cyanobenzoic acid and 2-bromo-3-cyanobenzoic acid were prepared.

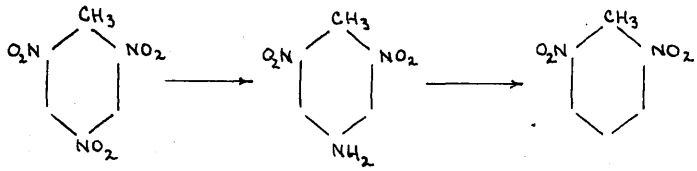
Preparation of 2-chloro-6-cyanobenzoic acid

It appeared from the literature that this compound could best be prepared from 2-chloro-6-nitrotoluene by oxidation, reduction and subsequent Sandmeyer.

Three methods of preparation of 2-chloro-6-nitrotoluene were mentioned in the literature.

1) By nitrating 2-chlorotoluene, Wibaut (161) obtained 2-chloro-6-nitrotoluene along with all the other 2-chloro-nitrotoluenes. He isolated the 2:6-derivative in 20% yield by a lengthy and complicated system of fractional crystallisation. 2) Wibaut (161) also prepared 2-chloro-6-nitrotoluene from 2:6-dinitrotoluene, by reduction of one nitro group followed by a Sandmeyer reaction.

As no 2:6-dinitrotoluene was available it would first have to be prepared from trinitrotoluene by reduction of the 4-nitro-group (5% yield) followed by deamination (70% yield).



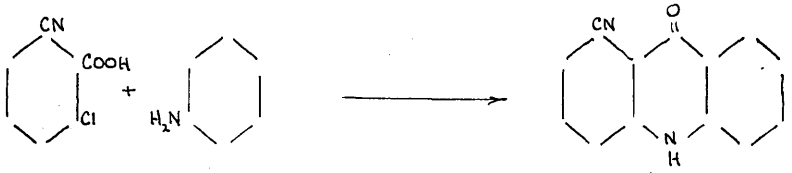
3) Jansen (55), was able to prepare 2-chloro-nitrotoluene by the direct chlorination of 2-nitrotoluene. Normally, 2-nitro-4-chlorotoluene is obtained by this operation but under certain conditions the author claimed that, contrary to theoretical assumptions, he could obtain the 2:6-derivative by direct chlorination. For this preparation he used the theoretical quantity of chlorine and reacted it with pure nitrotoluene i.e. free from p-nitro and nitro-thiotoluene, in the presence of a chlorine carrier such as iodine, ferric chloride, antimony pentachloride etc. He found that

the temperature always rose to about 100° and that a yield one third of the theoretical could be obtained, though higher yields could be obtained by maintaining the temperature between 20-50°.

Method 3) was attempted but despite taking all the precautions mentioned in the patent only a mixture of isomers could be obtained.

As the other two methods for the preparation of this compound were lengthy and only gave the product in low yield, an alternative procedure was adopted.

If 2-chloro-6-cyanobenzoic acid were treated with aniline and ring-closed to an acridone, the product would be 4-cyano-acridone.



This compound is obtained along with 2-cyanoacridone from 2-chlorobenzoic acid and 3-aminobenzonitrile and it was to confirm the presence of the isomer obtained by this separation that the attempt to prepare 2-chloro-6-cyanobenzoic acid was made.

4-Carboxyacridone could, theoretically at least, be prepared from 3-chlorophthalic acid which is easily prepared and compared with the 4-carboxyacridone obtained by hydrolysing the 4-cyanoacridone from the separation of the isomers.

Accordingly, 3-chlorophthalic acid was prepared instead of 2-chloro-3-cyanobenzoic acid.

Preparation of diphenylamine derivatives

Many modifications of the Ullman reaction with copper powder have been described in the literature. Albert and Linnell (11b), Matsumuru (116), Drozdov and Bekhli (49), Glen and Nitzsche (72), Knunyanto and Benevolenskaya (93), Magidson and Travin (113), Albert and Gedhill (13) and Samant (147).

For the formation of cyano-diphenylamine-2-carboxylic acid derivatives from aminobenzonitriles and 2-chlorobenzoic acid, the method of Albert and Gedhill (9) was adopted, consisting of the removal of most of the water by evaporation and the addition of anhydrous sodium acetate before the addition of the nitrile and the catalytic copper and then refluxing with cyclohexanol at 180°.

Reflux at a lower temperature resulted in much unchanged starting material, while raising the temperature above 200° caused much tarring with a concomitant lowering in the yield of diphenylamine derivative obtained.

On treating the cyano-2-halogenbenzoic acids with aniline by Albert and Gedhill's method (loc.cit.) at 180°, much charring occurred and some decomposition of the nitrile was observed before the addition of the aniline.

Lowering the temperature of reflux to 130-160° (depending on the compound) and heating with amyl alcohol as described by Goldberg and Kelly (78), for four hours, decreased the amount of charring and a better yield of the diphenylamine-2-carboxylic acids could be obtained. Some decomposition of the cyano group was still observed, however, probably due to the water, produced by the action of the potassium carbonate present, on the acid.

To overcome this hydrolysis effect, it was decided to prepare the sodium salt of the acid and treat this with the dry nitrile in the presence of anhydrous sodium carbonate and dry amyl alcohol thus eliminating all water except the small amount formed in the reaction.

It was found, however, that, under these conditions, no diphenylamine formation was effected, the starting materials being obtained in almost quantitative amounts at the end of the experiment. This is in agreement with the findings of Goldberg and Kelly (78), who stated that the physical state of the salt of the acid had much bearing on the quantity of diphenylamine-2-carboxylic acid produced. They further stated that on occasions when preformed anhydrous potassium 2-chloro-4-nitrobenzoate, ignited potassium carbonate and anhydrous amyl alcohol were treated with p-phenylenediamine, no reaction took place, the whole of the starting material being recovered unchanged. From this they deduced that some

water must be present for this reaction to take place. Other workers, Albert and Gedhill (loc.cit.) however were able to prepare diphenylamine derivatives from potassium salts in the presence of anhydrous sodium carbonate andanhydrous sodium acetate.

A further modification, that of Yu, Magidson and Travin (163), who treated 2-chloro-4-cyanobenzoic acid, p-anisidine and potassium carbonate in amyl alcohol with catalytic copper powder and heated with stirring to gentle boiling to give the diphenylamine compound, was adopted and a 60% yield of the cyano-diphenylamine derivative obtained.

Boiling the cyano-chlorobenzoic acid with potassium carbonate in amyl alcohol to remove water before the addition of copper and aniline causes some hydrolysis, the presence of water not being so critical once the diphenylamine compound has been formed as the cyano-diphenylamine-2-carboxylic acids appear to be more stable.

The general literature relating to the Ullmann condensation between negatively substituted arylamines and 2-halogenbenzoic acids (Ullmann (158b), Tuttle (154b), Bogert and Hirschfelder (33b), Magidson and Travin (112), Albert and Linnell (11a), Lehmstedt and Schrader (104), Goldberg and Kelly (78)) indicates that the presence of a negative substituent on either of the reactants, particularly in the arylamine nucleus, exerts an inhibiting influence upon

the course of the condensation.

Goldberg and Kelly (78) further found that treatment of p-phenylenediamine with 2-chloro-4-nitrobenzoic acid at 120° in amyl alcohol effected reductive dehalogenation of the chloronitrobenzoic acid in almost quantitative yield. By lowering the temperature to 80°, the normal Ullmann reaction took place smoothly with the production of 5-nitro-4'-amino-diphenylamine-2-carboxylic acid in satisfactory yield.

Similarly, at 140°, p-amino-dimethylaniline reacted vigorously with 2-chloro-4-nitrobenzoic acid with the formation of 2-nitrobenzoic acid, though at 120° 5-nitro-4'-dimethylaminodiphenylamine-2-carboxylic acid was obtained in high yield.

With a cyano group in either the aryl amine or carboxylated nucleus, no reductive dehalogenation was observed in the formation of the cyano-diphenylamine-2-carboxylic acids.

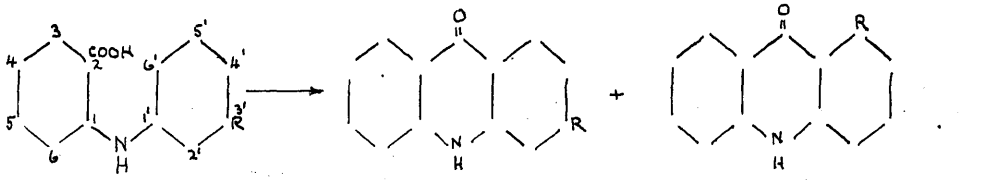
The position of the cyano group in the ring did not appear to be critical in the formation of monocyanoacridines.

By condensing 2-bromo-4-cyanobenzoic acid with 3-amino-benzonitrile at 130° a 30% yield of 3'-5-dicyano-diphenyl-amine-2-carboxylic acid was obtained though on treating 2-chloro-5-cyanobenzoic acid with 4-aminobenzonitrile no diphenylamine-2-carboxylic acid was produced, unchanged starting materials being the only product at 130°. At

high temperatures ammonia was evolved but no diphenylamine compounds could be isolated from the reaction mixture.

Preparation of Acridones

While 6-,5-,4-,3-substituted diphenylamine-2-carboxylic acids and 2′-,4′-substituted diphenylamine carboxylic acids can undergo cyclisation with formation in each case of only one product, the 3′-substituted diphenylamine-2-carboxylic acids may give rise to 4- and 2-substituted acridones, according as to whether the acridone closes on the 2′-position (ortho-closure) or the 4′-position (para-closure) respectively.



Lehmstedt and Schrader (101) made quantitative measurements upon the directional cyclisation of a series of 3′-substituted diphenylamine-2-carboxylic acids. They further showed that the ratio of the isomers produced was independent of the cyclisation agent by performing parallel experiments with concentrated sulphuric acid and phosphorus oxychloride.

They obtained the following ratios:

	4-substitution	2-substitution
Me	18	82
MeO	50	50
Cl	75	25
NO_2	75	25
NH_2	75	25

Albert and Ritchie (12) later showed that these figures for the aminoacridones were incorrect as they obtained 2- and 4-aminoacridones in the ratio of ca, 8:1 by the cyclisation of 3′-amino diphenylamine-2-carboxylic acid, i.e. para-closure predominated. Goldberg and Kelly (78) investigated the cyclodehydrogenation of 3′-nitro and 3′-aminodiphenylamine-2-carboxylic acids which had another substituent in the carboxylated nucleus and showed that, in all cases, while with the 3′-nitro series ortho-closures largely predominated, with the 3′-amino series para-closure took place almost exclusively.

It was thus shown to be the group present on the arylamine nucleus which controls the ratio of isomers produced on cyclisation.

Lehmstedt (101) separated the isomers he obtained by fractional crystallisation of the corresponding 5-chloro-compounds which, after separation, were reconverted to the acridones. This was a tedious process. A distinctly

quicker and simpler method was employed by Albert and Ritchie (loc.cit.) who separated the amino isomers by the relative insolubility of the 2- (compared with the 4-) amino-acridine hydrochloride.

Lehmstedt's method of separation was applied to the isomeric chlorocyanoacridines but only one isomer, the 2-cyano could be obtained pure. Vacuum sublimation of the chlorocyanoacridines did not effect a separation of the isomers.

Chromatographic methods have been applied in the separation of isomers (152) and this method was applied with considerable modifications due to the insolubility of the cyanoacridones. Separation was effected and it was found that the ratio of ortho to para closure was approximately 2:7, i.e. 2-cyano acridone predominated.

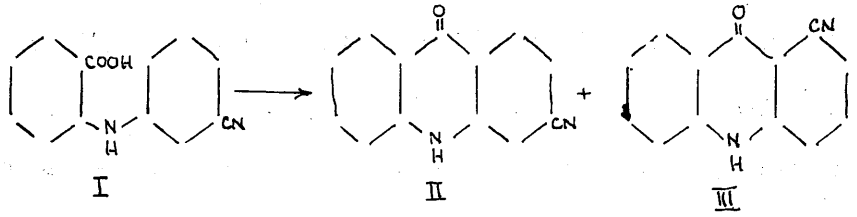
The ratio of isomers produced thus appears to be independent of the positive or negative groups on the arylamine nucleus as ortho-closure predominates with 3′-nitro-diphenylamine-2-carboxylic acids (negative) and 3′-chloro (positive) and para-closure with 3′-cyano (negative) and 3′-amino (positive).

It was found that on ring-closing diphenylamine-2:3-dicarboxylic acid, no 4-carboxyacridone resulted, the exact nature of the product not being further investigated.

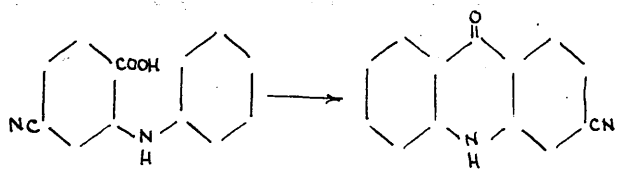
Lehmstedt and Schrader were similarly unable to ring-close 3-nitro-diphenylamine-2-carboxylic acid using either concentrated sulphuric acid, phosphorus oxychloride or Ullmann's acid chloride method. Some 3-substituted diphenylamine-2-carboxylic acids have been ring-closed to the corresponding acridones, but only where the 3-substituent has been nucleophilic in character, e.g. amino or methyl (Glen and Nitzsche (72)).

As 3-carboxy and 3-nitro-diphenylamine 2-carboxylic acids did not ring-close, it was anticipated that 3-cyanodiphenylamine-2-carboxylic acid would not as the cyano group has similar deactivating, electrophilic character.

The cyclisation of 3'-cyanodiphenylamine-2-carboxylic acid (I) resulted in a mixture of two isomers (II) and (III), separated by chromatography. The 2-cyanoacridone (II) was identified by comparison with 2-cyanoacridone prepared by cyclisation of 5-cyanodiphenylamine-2-carboxylic acid (IV) where no isomerism is possible. The orther compound (III) must, therefore, be 4-cyanoacridone.



I II III



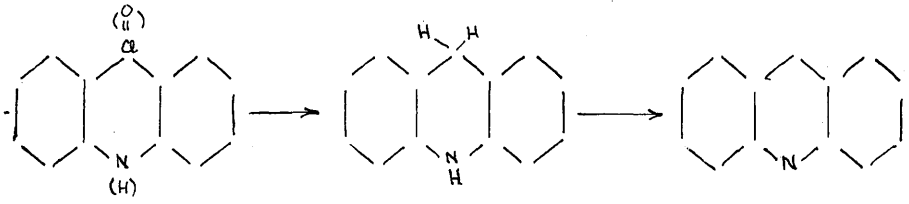
IV II

Preparation of chloroacridines

All the acridones prepared could be readily converted to the corresponding chloroacridines. These chloroacridines were even more unstable to air and sunlight than 5-chloroacridine itself, the 2-cyano isomer being most readily decomposed and the 1-cyano compound least attacked (see theoretical section, part I, page 33).

Preparation of cyanoacridines

Acridine derivatives can be prepared from the corresponding acridones and chloroacridines by reduction to the dihydro compounds and subsequent mild oxidation to the acridine.



Reduction of acridones has been effected by Perkin and Clemo (136b), using sodium and alcohol, Lehmstedt and Hundertmark (103) with sodium amalgam and alcohol, and by Sherlin and Braz (150b), using amyl alcohol and sodium amalgam, the product being oxidised by potassium dichromate, sodium nitrite or ferric chloride. Reed (141) modified this last method and the reduction of 1-cyanoacridone by this modification was attempted.

Reduction of the acridone occurred but the cyano

group also appeared to be attacked by this vigorous treatment and the odoriferous oil obtained on extracting the product with alcohol could not be purified to give any definite crystalline product.

Adkins and Coonradt (1) reduced acridone to dihydroacridine with Raney nickel but due to the insolubility of the cyanoacridones it was thought that catalytic hydrogenation of the 5-chloro compounds could more easily be effected.

Albert and Willis (14) reduced 5-chloroacridine by shaking under hydrogen with Raney nickel in alcoholic potassium hydroxide-benzene solution. The acridan so obtained was oxidised to acridine by potassium dichromate following the method of Graebe and Caro (73).

On applying this method to the cyano-5-chloroacridines, great difficulty was found in dissolving the chloroacridine in the alcoholic potassium hydroxide. On warming the suspension, formation of the corresponding 5-ethoxyacridine resulted with the deposition of potassium chloride. At room temperature, hydrogen was absorbed only very slowly, 24 hours often being necessary for the absorption of the theoretical quantity. This may be due to the deactivating properties of the cyano group as Albert and Willis (loc.cit.) have shown that this method did not work well with nitro-

5-chloroacridines.

Only poor yields of the acridines were obtained by this method.

By treating the alcoholic solution of the cyano-5-chloroacridine with benzene and shaking for two to three hours a yellow solution of the chloroacridine was obtained. On adding palladium-calcium carbonate catalyst and shaking under hydrogen, more rapid reduction was obtained (about two hours).

In all cases, the acridan was readily oxidised to the corresponding acridine by potassium dichromate.

The cyanoacridines so obtained were not unstable like the cyano-5-chloroacridines and could be purified by vacuum sublimation as yellow needles, which fluoresced yellow-green in alcoholic solution.

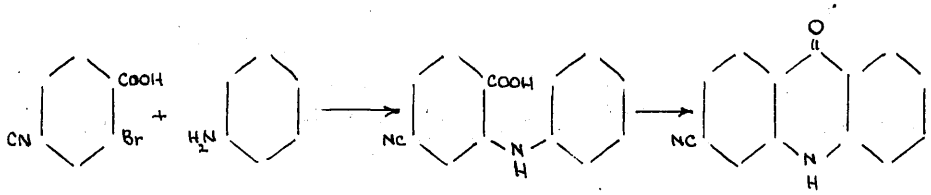
Separation of dicyanoacridones

By condensing 2-bromo-4-cyanobenzoic acid with 3-aminobenzonitrile and cyclising the 5-3′-dicyanodiphenylamine-2-carboxylic acid so obtained, an isomeric mixture of 2:6- and 2:8-dicyanoacridones was produced in 25% yield.

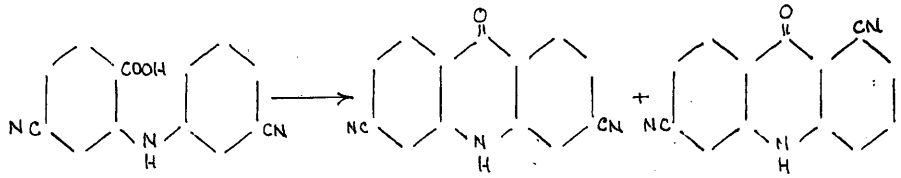
The dicyanoacridones were separated by chromatographic means.

With the isomeric monocyanoacridones, identification of the 2-cyano isomer had been possible as this isomer could be synthesised from 2-bromo-4-cyanobenzoic acid and aniline where no isomerism is possible. This method of identifica-

tion was not applicable,however, to the dicyano-isomers.



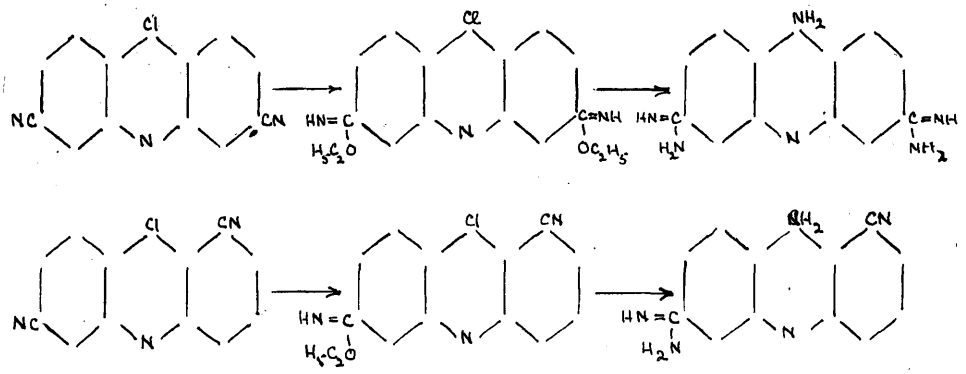
It had been found that while 2- and 3-cyano5-chloro-acridines formed amidines by Pinner's method (135) through the imino-ethers, 1- and 4-cyano-5-chloroacridines were sterically hindered due to the ortho effect (cf. 5-cyano-acridine, page 34.) and no iminoether formation resulted on the passage of dry hydrogen chloride into the dry alcoholic solution of the nitrile.



One of the isomers, 2:8-dicyanoacridone should thus form a diamidine by Pinner's method while the other isomer, 2:6-dicyanoacridone would form a cyano-amidino- derivative.

The separated isomers were accordingly converted to their respective 5-chloroacridines and converted to the amidino compounds. The isomeric fraction which passed through the chromatographic column with nitrobenzene gave a diamidine by this treatment and must, therefore, be 2:8-dicyanoacridone, whereas the isomer which remained on the column formed a cyano-amidine and must, therefore, be

2:6-dicyanoacridone.

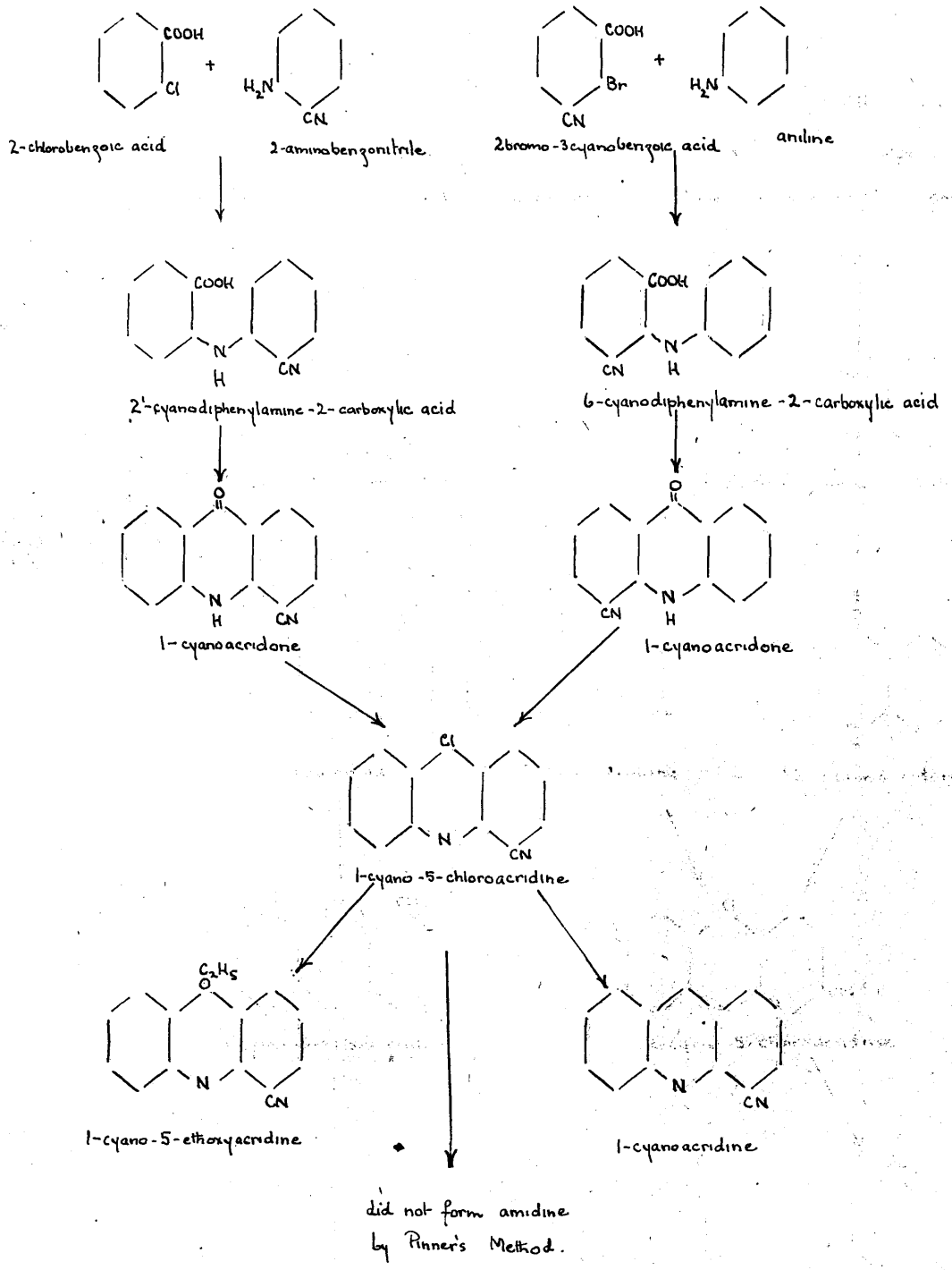


The isomers were obtained in the ratio of ortho to para closure of about 1:3, i.e. 2:8-dicyanoacridone predominated, as was the case with the mono-cyanoacridones.

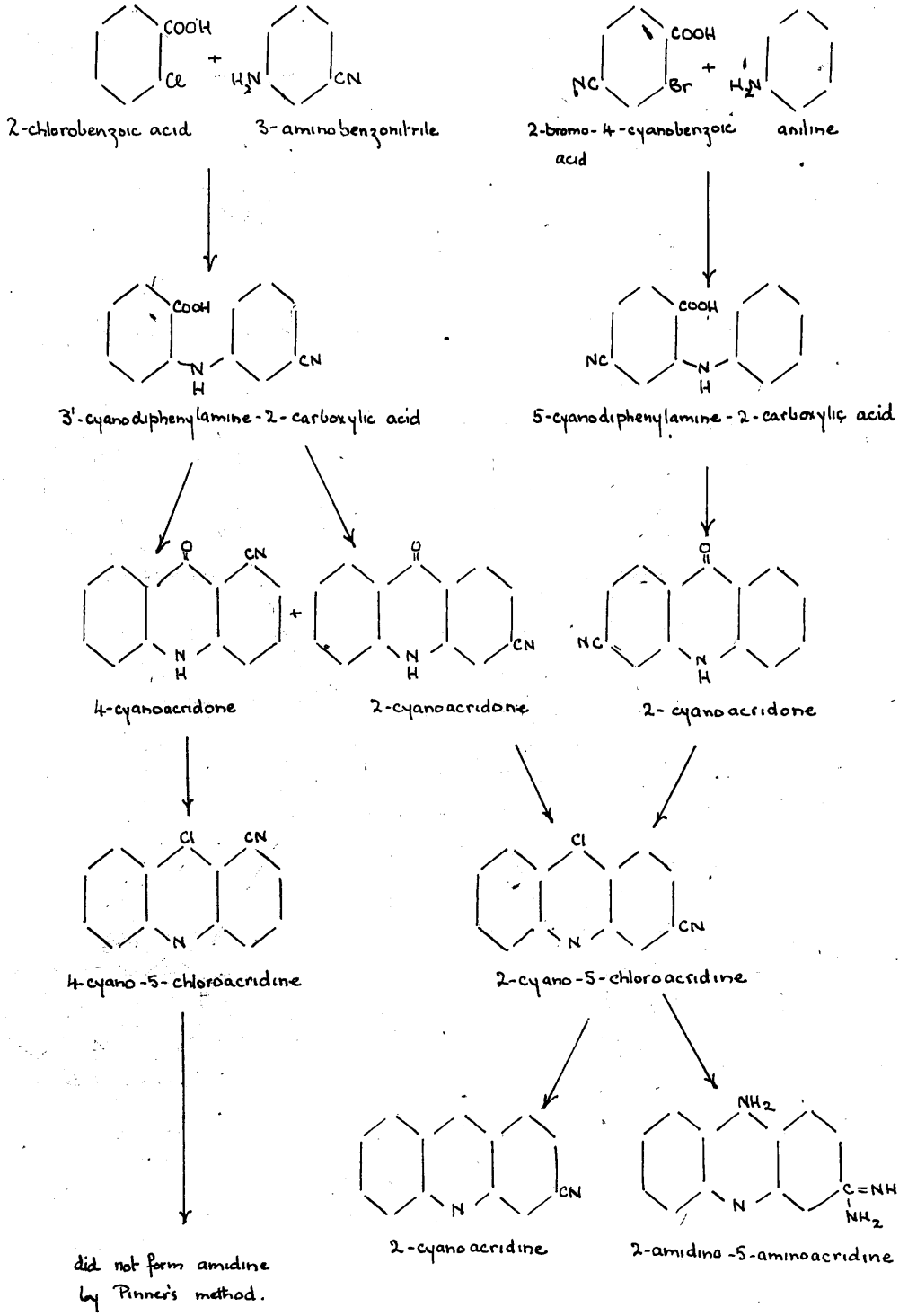
EXPERIMENTAL

PART II

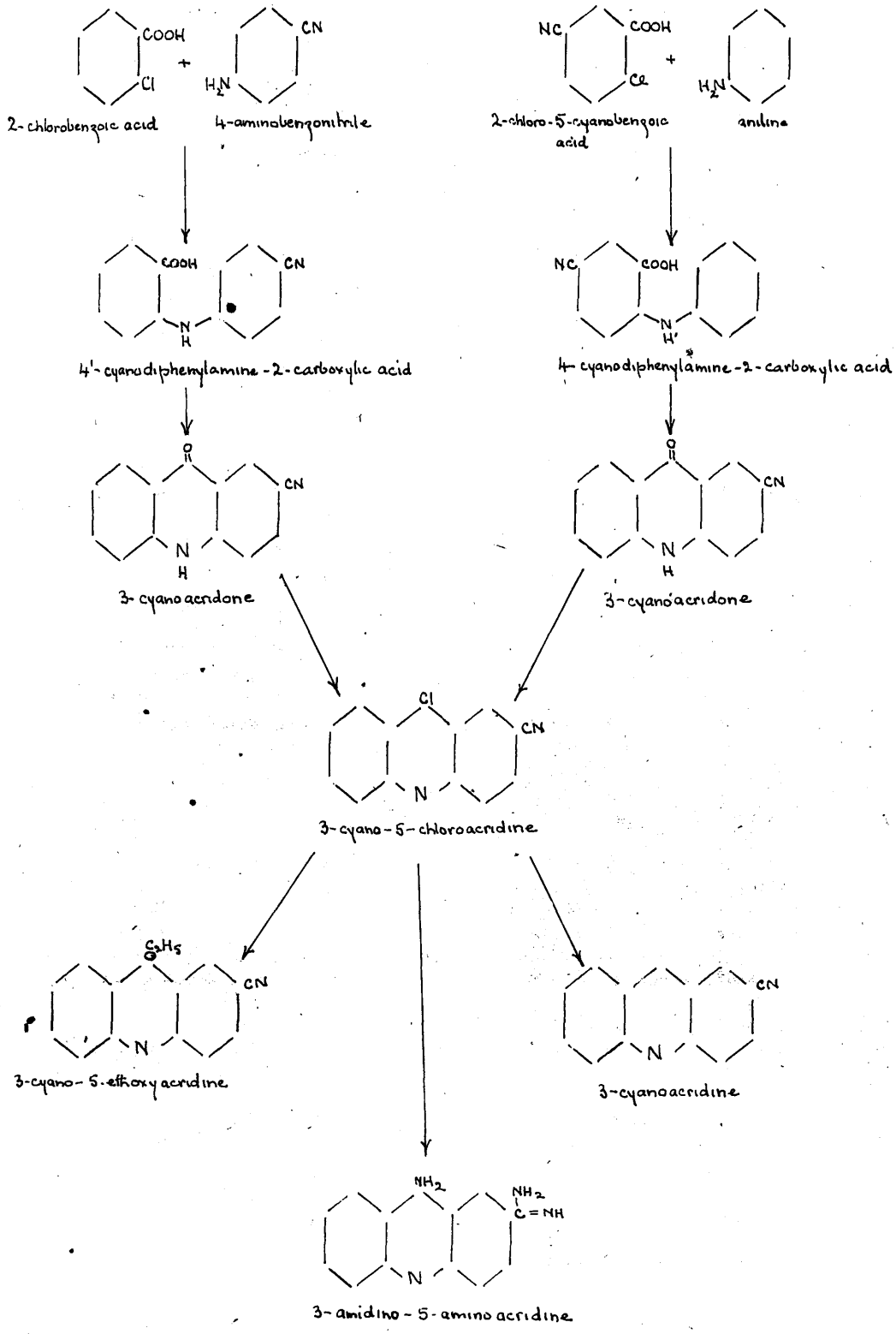
Syntheses of 1-cyanoacridine Derivatives



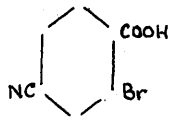
Syntheses of 2- and 4-cyanoacridine Derivatives



Syntheses of 3-cyanoacridine Derivatives

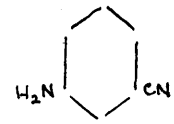


Syntheses of 2,6- and 2,8-dicyanoacridine Derivatives

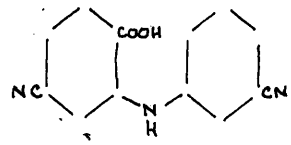


2-bromo-4-cyanobenzoic acid

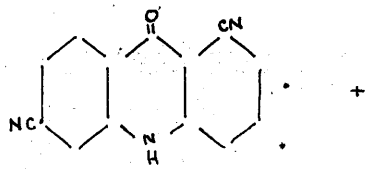
+



3-aminobenzonitrile



5,3'-dicyanodiphenylamine-2-carboxylic acid



2,6-dicyanoacridone

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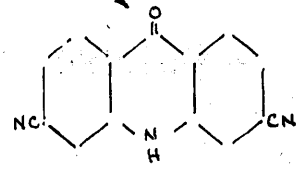
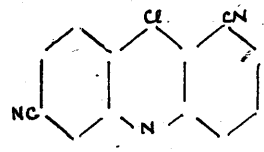
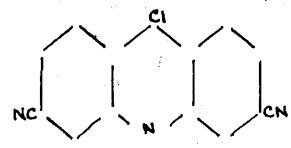


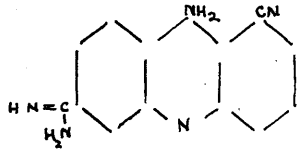
2,8-dicyanoacridone



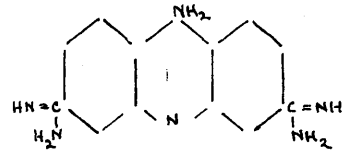
2,6-dicyano-5-chloroacridine



2,8-dicyano-5-chloroacridine



2-amidino-5-amino-6-cyanoacridine



2,8 diamidino-5amino acridine

SYNTHESES OF 1-CYANOACRIDINES

SYNTHESES OF 1-CYANOACRIDINE DERIVATIVES FROM 2-AMINOBENZONITRILE

1. PREPARATION OF 2-AMINOBENZONITRILE

Reference - Two methods described (a) 2-nitrobenzaldehyde was reduced to 2-aminobenzaldehyde by ferrous sulphate and ammonia at 100° (Bamberger (20)) then converted to the oxime (Bamberger and Demuth (21)) and thence on treatment with formamide to the nitrile (Gabriel (66))
(b) 2-nitraniline on diazotisation and Sandmeyer was converted to 2—nitrobenzonitrile (Bogert and Hand (32)) (Pinnow and Muller (136)) which was reduced to 2-aminobenzonitrile (Bogert and Hand (32), Leissert and Graebe (107)).
Method (b) was adopted (Bogert and Hand's modification).

Preparation of 2-nitrobenzonitrile

55.2gms. powdered 2-nitraniline were treated with 70c.c. concentrated hydrochloric acid (sp.gr.1.178) and the mass stirred until all was changed to the buff-coloured hydrochloride. 1200c.c. water were then added all at once and the mixture thoroughly stirred. The free base was thus formed in fine needles which diazotised much more readily than the powdered nitraniline.

The suspended nitraniline was diazotised by adding a solution of 28gms. sodium nitrite in water to the well-stirred solution, at least an hour being taken for this operation. At the end of this time, all but a small amount of the orange nitraniline had changed to the light-coloured flocculent diazo compound and potassium iodide paper still showed the presence of excess nitrite. No external cooling was necessary as the diazo compound was remarkably stable at ordinary temperatures.

The flocculent diazo compound was then decanted, any unchanged nitraniline remaining behind due to its much greater density.

The suspended diazo body was slowly poured into a potassium cuprocyanide solution prepared from 100gms. hydrated copper sulphate, 600c.c. water and 112gms. potassium cyanide. The cuprocyanide solution was maintained above 90° and stirred constantly during the addition of the diazo solution. The mixture was then allowed to boil for five minutes and filtered through glass-wool in a large filter-funnel. On cooling, the nitronitrile crystallised from the filtrate in long yellow needles. By boiling the tar which remained on the glass-wool with 10 litres water and again filtering, considerable quantities of almost pure nitro-nitrile were obtained on cooling. Only a small amount of

tar was left at the end of these operations.

The nitrile was purified from the minimum amount of boiling carbon tetrachloride by treatment with animal charcoal. After filtration and cooling, golden-yellow scales of the nitrile separated, m.p. 108° (m.p.Lit. - 109°). Yield - 39gms. (theor. - 58gms.).

Preparation of 2-aminobenzonitrile

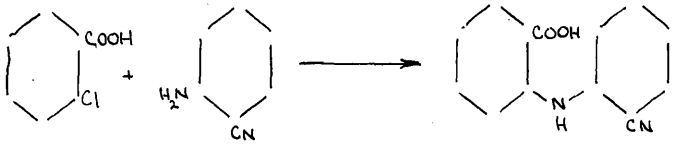
125gms. stannous chloride were dissolved in 106c.c. concentrated hydrochloric acid, diluted with 2c.c. water and the solution vigorously stirred, while 25gms. 2-nitrobenzonitrile were gradually added at such a rate that the temperature of the solution remained at 20-30°, occasional cooling with ice being necessary. Below 20° the reaction proceeded very slowly. As the action progressed, the nitronitrile gradually dissolved and the reaction was complete in a few hours. When all the nitronitrile had been reduced a large volume of concentrated hydrochloric acid was added and the mixture left at 0° for 12 hours. The aminonitrile was thus precipitated as the hydrochloride in fine white granular needles which were filtered through sintered glass.

The precipitated chloride was washed free from tin with concentrated hydrochloric acid, sucked as dry as possible and the free base liberated by addition to an excess of moderately dilute ammonia solution, the precipitated

base being washed with water till free from ammonium chloride, dried and crystallised from carbon disulphide, m.p.49° (m.p.Lit.- 50°).

Yield - 17gms. (theor. - 20gms.).

2. PREPARATION OF 2'-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID



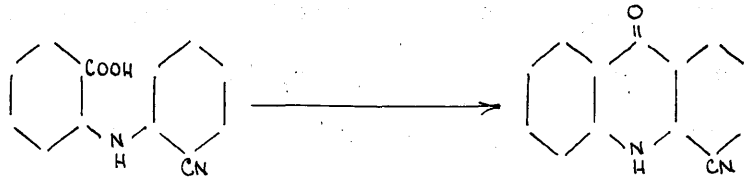
28.8gms. 2-chlorobenzoic acid, 8gms. potassium carbonate, 7.2gms. sodium acetate and 30c.c. cyclohexanol were heated in an oil-bath till a thermometer held above the solution registered a temperature of 160°.

The solution was allowed to cool to 100° and 13gms. 2-aminobenzonitrile and 0.2gms. catalytic copper powder added. This mixture was refluxed for six hours in the oil bath at 180°, at the end of which time the contents of the flask were steam-distilled to remove the cyclohexanol. The residue was then made alkaline with ammonia, diluted to 200c.c., boiled with animal charcoal and filtered. Some black tar remained on the filter funnel. The filtrate was dark brown in colour and was allowed to cool to room temperature after which time 30c.c. acetone were added and the solution acidified with concentrated hydrochloric acid. A buff-coloured precipitate was formed. After standing

for 15 minutes this was filtered from the unchanged 2-chlorobenzoic acid which remained in solution. After filtration, the precipitate was dissolved in 80c.c. water with diluted ammonia and treated with 10c.c. acetone. Acidification with concentrated hydrochloric acid gave cream-coloured needles, which, on recrystallisation, from aqueous acetone, formed creamy-white needles, m.p.215-216°. Yield - 10gms. (theor.17gms.)

Analysis	Calculated (for $C_{14}H_{10}O_2N_2$)	Found
%C	70.58	70.36
%H	4.20	4.27
%N	11.78	11.80

3. PREPARATION OF 1-CYANOACRIDONE



3gms. 2'-cyanophenylamine-2-carboxylic acid and 6c.c. phosphorus oxychloride were refluxed for 30 minutes, the excess reagent distilled off under vacuum and the residue boiled for half-an-hour with 100c.c. water. There was considerable frothing when refluxing commenced. Towards the end of the experiment the solution became deep orange with a faint greenish fluorescence. On boiling, an orange solution was formed, from which a dark green precipitate separated on further boiling.

The solution was allowed to cool and filtered, the residue being dried at 120°. On heating this green precipitate on a sandbath, a yellow sublimate was deposited on a cold watch-glass, which had a m.p.266° in a sealed tube. Yield - 2.5gms. (theor. 2.8).

Analysis	Calculated (for $C_{14}H_8ON_2$)	Found
%C	76.7	76.5
%H	3.6	3.3
%N	12.8	12.9

4. PREPARATION OF 1-CYANO-5-CHLOROACRIDINE

2.5gms. 1-cyanoacridone (.16 moles) and 8c.c. (.88 moles) phosphorus oxychloride were slowly heated to 90° on a water-bath, when considerable frothing occurred, the temperature not being allowed to rise any higher. When all the solid had dissolved and the frothing subsided a dark brown solution resulted. The flask was then transferred to an oil-bath and the temperature raised to 135-140° where it was maintained for two hours.

The excess phosphorus oxychloride was removed by vacuum distillation and the residue cooled to 0°. To this a well-stirred mixture of 10c.c. concentrated ammonia solution, 25gms. ice and 10c.c. chloroform was added and the whole shaken for half-an-hour. The liquid was then removed and the remaining solid shaken for a further half-

hour with 10c.c. concentrated ammonia and 20c.c. chloroform. At the end of this time only a very small amount of material remained undissolved.

The two filtrates were combined and the chloroform layer, which was reddish brown in colour, separated. The aqueous portion was shaken with a further 20c.c. chloroform and this was added to the first chloroform extract. These were dried over 5gms. calcium chloride, filtered and a light brown filtrate obtained. A small amount of a dark residue remained on the filter with the calcium chloride.

The filtrate was allowed to evaporate to dryness at room temperature and a yellow-brown residue obtained, m.p.153-156°,

Yield - 2gms. (theor. 2.7gms.).

Purification of product

On treating the freshly prepared chloroacridine with boiling alcohol, no solid separated out on cooling. On the addition of water to the alcoholic solution a light-brown irregular crystalline product was obtained, m.p.153-156°. If the chloroacridine had been prepared some time previously, on heating with alcohol, hydrogen chloride was evolved and insoluble 1-cyanoacridone was precipitated. The chloro-acridine was soluble in acetone, giving a yellow solution with a violet fluorescence. Water was added to the hot acetone solution till it became opalescent and the solution

allowed to come to room temperature when yellow needles, m.p.158°, were deposited.

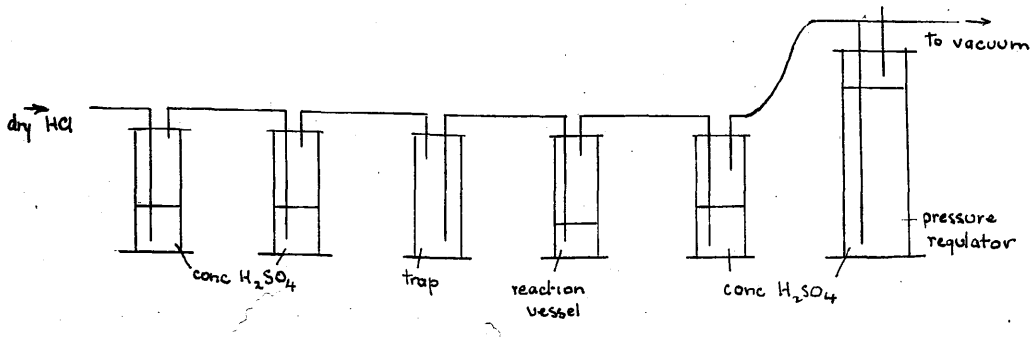
Treatment with hot acetone did not affect the chloro-compound even if it had been prepared some time previously.

It was also found possible to purify the chloroacridine by vacuum distillation at 160°/4mm. to obtain fine yellow needles, m.p.158°.

Analysis	Calculated (for $C_{14}H_7N_2Cl$)	Found
%C	70.4	7.0.1
%H	2.9	3.1
%N	11.7	11.8
%Cl	14.9	15.1

5. PREPARATION OF 1-AMIDINO-5-AMINOACRIDINE

A solution containing 0.5gms. 1-cyano-5-chloroacridine in 7c.c. magnesium dried ethyl alcohol and 15 c.c. dry chloroform was saturated with dry hydrogen chloride passed into the ice-cooled solution as in diagram.



The solution became bright yellow in colour and a bright yellow precipitate was formed. After eighteen hours the passage of hydrochloric acid was stopped and the solution was evaporated to dryness under vacuum at 45°. The bright yellow needles so obtained did not melt below 300° (sealed tube).

Analysis	Calculated (for $C_{16}H_{15}ON_2Cl_3$)	Found
%C	53.8	60.9
%H	4.2	3.1
%N	7.9	10.1
%Cl	29.7	26.0

The material was sent for analysis in a sealed tube to prevent decomposition of the iminoether hydrochloride. The analysis calculated above for 5-chloro-1-iminoether-acridine dihydrochloride did not agree with that obtained experimentally. The experimental results, however, gave good agreement with the values calculated for 1-cyano-5--chloroacridine hydrochloride (C-61.1%, H-2.9%, N-10.2%, Cl—25.8%).

6. PREPARATION OF 1-CYANOACRIDINE

Method I. 1.0gms. 1-cyano-5-chloroacridine were dissolved with shaking for two hours in 30c.c. alcohol and 10c.c. sulphur-free benzene and 1gm. palladium-calcium carbonate catalyst added.

On shaking under hydrogen the gas was slowly absorbed, several hours being necessary for the absorption of the theoretical amount. At the end of this time, the solution was filtered and the residue washed with hot alcohol. The deep rose filtrate, fluorescing red-blue, was evaporated to dryness above a water-bath and the deep red solid so obtained washed with warm water to remove inorganic material and added to 113c.c. boiling water containing 6c.c. 25% sulphuric acid. Mechanical stirring was begun and as soon as even dispersion of 1-cyanoacridan was attained, 0.8gms. potassium dichromate (1 oxygen equivalent) dissolved in 6c.c. boiling water was added in two equal portions five minutes apart.

After five minutes further boiling and stirring, 1.55gms. potassium dichromate in 11c.c. boiling water were added to precipitate the acridine as its dichromate; boiling was continued for five minutes more and the mixture cooled and refrigerated over-night.

The orange precipitate so obtained was filtered, suspended in 30c.c. hot water, treated with 7c.c. concentrated ammonia solution, brought just to the boil, cooled quickly to room temperature, filtered and washed with cold water. The original filtrate was made alkaline with sodium hydroxide but no precipitate was depositied indicating that all the cyanoacridine had been precipitated as the insoluble chromate.

The precipitate was dissolved in 5 c.c. hot dilute hydrochloric acid and filtered from a trace of cyanoacridan. The cyanoacridine was precipitated with ammonia solution, filtered and dried in a vacuum desiccator.

The product was purified by vacuum sublimation at 180° and pale yellow needles, m.p.156°, obtained.

Analysis	Calculated (for $C_{14}H_8N_2$)	Found
%C	82.3	82.2
%H	3.9	4.1
%N	13.7	13.8

Yield - 0.74gms. (80% theor.).

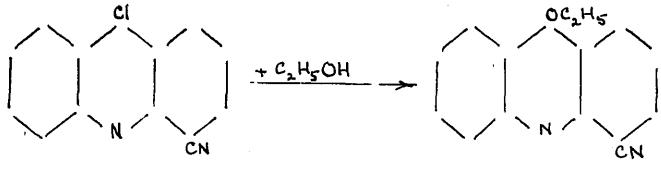
Method II. 0.5gms. 1-cyanoacridone was heated with 15c.c. amyl alcohol and treated with 1.0gms. sodium. The mixture was refluxed until the sodium had dissolved and all greenish fluorescence had vanished. The liquid was then steam-distilled to remove the amyl alcohol, the cooled residue of cyanoacridan dissolved in warm alcohol and the cooled solution treated dropwise with aqueous ferric chloride until the green colour changed to yellow. Excess ammonia was added to precipitate the ferrous hydroxide with the cyanoacridine. After the cold suspension had been filtered by gravity, the damp solid was extracted with boiling methyl alcohol, filtered hot and the filtrate evaporated to small bulk.

On cooling, no crystals were deposited, but on

evaporating the amber solution (fluorescing pink-yellow) to dryness, a resinous product was obtained. It was found impossible to crystallise the cyanoacridine from this material, though on treating the alcoholic solution with picric acid, orange rhombic crystals which did not melt below 360° were obtained in small quantity. These crystals did not analyse for 1-cyanoacridine picrate and on reconversion to the free base again yielded a resinous product.

The resinous product had a distinct odour, unlike any of the cyanoacridines described elsewhere in this thesis.

7. PREPARATION OF 1-CYANO-5-ETHOXYACRIDINE



1.0gms. 1-cyano-5-chloroacridine were gently warmed with 30c.c. alcohol and 0.5c.c. alcoholic sodium hydroxide solution. As the acridine derivative dissolved a white residue was deposited and the solution became bright yellow.

The solution was filtered and the residue found to be sodium chloride. The filtrate was evaporated to small bulk, excess water added and the yellow precipitate so formed, filtered after half an hour. The residue was dissolved in the minimum amount of hot acetone, hot water

added till the solution became opalescent and allowed to come to room temperature when long, yellow needles were obtained, m.p.130°, with decomposition.

Yield - 0.82gms. (75% theor.).

Analysis	Calculated (for $C_{16}H_{12}ON_2$)	Found
%C	77.2	76.9
%H	4.8	4.8
%N	11.3	11.4

SYNTHESES OF 1-CYANOACRIDINE DERIVATIVES FROM 3-CYANO-2-CHLOROBENZOIC ACID

1. PREPARATION OF 3-CYANO-2-BROMO-BENZOIC ACID

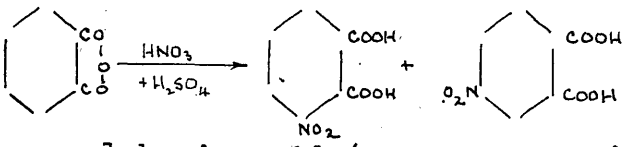
Reference - Two methods described. (a) Method of Bamberger (23) who converted 2-chloro-3-amino-toluene to 2-chloro-3-acetamidotoluene which he oxidised to 2-chloro-3-acetamidobenzoic acid and thence prepared 2-chloro-3-amino benzoic acid (b) Phthalic anhydride was nitrated to 3-nitro-phthalic acid which, on treatment with mercuric acetate gave anhydro-2-hydroxymercuri-3-nitro-benzoic acid. This, on bromination, was converted to 2-bromo-3-nitrobenzoic acid (Organic Syntheses (131b)).

No reference to the preparation of 2-bromo-3-cyanobenzoic acid could be found in the literature.

Method (b) was adopted.

Preparation of 3-nitrophthalic acid

Reference - Organic Syntheses (130).



650c.c. sulphuric acid (sp.gr. - 1.84) and 500gms. phthalic anhydride were placed in a beaker and heated to 80°

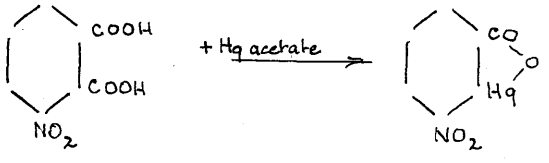
in an oil-bath. 210c.c. fuming nitric acid (sp.gr. - 1.15) were slowly added at such a rate as to maintain the temperature of the stirred mixture at 100-110°. After two hours, all the nitric acid had been added and 90c.c. concentrated nitric acid were added as rapidly as possible without causing the temperature to rise above 110°. The mixture was maintained at this temperature with constant stirring for a further two hours.

The mixture was allowed to stand overnight and poured into 1500c.c. water. After cooling, the solid mixture of 3- and 4-nitrophthalic acids was filtered through sintered glass and the wet cake stirred with 200c.c. water which dissolved most of the 4-nitrophthalic acid. After filtration, the wet cake was dissolved by boiling with 300c.c. water, the solution filtered hot and the filtrate stirred mechanically until crystallisation started and pale yellow needles, m.p.205-210° (m.p.Lit. - 218°) in a sealed tube, were obtained.

Yield - 226gms. (33% theor.).

<u>Preparation of anhydro-2-hydroxymercuri-3-nitrobenzoic acid</u>

<u>Reference</u> - Organic Syntheses (131a).



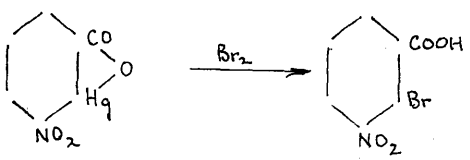
40gms. sodium hydroxide dissolved in 400c.c. water was added to 106gms. 3-nitrophthalic acid and the whole warmed. A small amount of insoluble material was removed by filtration. To the filtrate a solution of 175gms. mercuric acetate in a mixture of 50c.c. glacial acetic acid and 700c.c. water was added and the mixture heated in an oil-bath (over a period of one hour) to 170°. Refluxing at 165-170° was continued for seventy hours, the end of the reaction being determined by the stoppage of the evolution of carbon dioxide.

The product was allowed to settle and the hot liquid removed by decantation after which the residue was shaken with several 50c.c. portions of water and filtered. 100c.c. alcohol were then poured over the material on the filter. The product was an almost white powder which dissolved in sodium hydroxide with only slight turbidity.

As the product could be used wet for the next stage in the synthesis it was not dried and no yield could therefore be calculated.

Preparation of 2-bromo-3-nitrobenzoic acid

Reference - Organic Syntheses (131b).



A boiling solution of 25gms. sodium hydroxide in

750c.c. water was prepared and 200gms. damp anhydro-2-hydroxymercuri-3-nitrobenzoic acid added in small quantities.

The material was heated to boiling and stirred vigorously and 85c.c. concentrated hydrochloric acid (sp.gr.-1.19) added. Heating was discontinued at this point and 30c.c. glacial acetic acid slowly added when a curdy precipitate formed.

A solution of bromine was prepared by dissolving 52gms. of sodium bromide and 80gms. bromine in 75c.c. water. The mixture was stirred and the bromine solution added as rapidly as possible through the shaft of the stirrer. The precipitate dissolved. The solution was boiled for five minutes after the last addition of bromine. It was then made slightly alkaline, filtered and the filtrate made acid to Congo red using about 75c.c. concentrated hydrochloric acid. The precipitated 2-bromo-3-nitrobenzoic acid was filtered and recrystallised from 500c.c. boiling 30% alcohol to give almost white needles, m.p.186° (m.p.Lit. - 191°).
Yield - 56gms. (56% theor. based on 3-nitrophthalic acid).

Preparation of 2-bromo-3-aminobenzoic acid

Method I

5.0gms. 2-bromo-3-nitrobenzoic acid were added to a slight excess of stannous chloride dissolved in concentrated hydrochloric acid, the temperature being maintained below 45°.

A white precipitate of a tin complex was rapidly precipitated and was decomposed by the passage of hydrogen sulphide. 3gms. of the aminoacid hydrochloride were finally obtained as white needles. The precipitation of the tin as sulphide was a tedious procedure.

Method II

3.0gms. 2-bromonitrobenzoic acid were dissolved in sodium hydroxide and sodium hydrosulphite added in small quantities till the solution no longer darkened to ruby red on the addition of hydrosulphite but remained pale yellow. The solution was then made acid with concentrated hydrochloric acid, boiled and the precipitated sulphur removed by filtration. On cooling, cream-coloured needles were obtained, which on diazotisation gave a red dye with β-naphthol. It was found very difficult to remove all the sulphur from the amino-acid.

Method III

7.0gms. 2-bromo-3-nitrobenzoic acid were dissolved in the minimum amount of dilute ammonia with warming and poured into a boiling solution of 7 molecular equivalents of hydrated ferrous sulphate in 100c.c. water. The solution was treated with small portions of concentrated ammonia, each addition being followed by vigorous agitation of the mixture. When the solution was distinctly alkaline and a

brown residue had formed, it was boiled for five minutes, care being taken that the mixture was still alkaline at the end of the five minutes. If not, more ammonia was added and the suspension was filtered hot.

After filtration, an amber solution, free from iron, was obtained which, on diazotisation, gave a red dye with β-napthol. The solution was evaporated to small bulk and on the addition of concentrated hydrochloric acid a white precipitate was formed which was filtered and found to be the hydrochloride of the aminoacid.

Yield - 6gms.

It was not found possible to obtain the free amino-benzoic acid except in very low yield. No mention of this compound could be found in the literature. It was obtained as cream-coloured needles. M.P.226-233°. This material was not further purified at this stage.

Method III was repeated on a larger scale using 70gms. nitrocompound and 60gms. 2-bromo-3-aminobenzoic acid hydrochloride were obtained.

Preparation of 2-bromo-3-cyanobenzoic acid

60gms. 2-bromo-3-aminobenzoic acid hydrochloride were added to a solution of 60c.c. concentrated hydrochloric acid in 200c.c. water and the suspension cooled to 0°. 50gms. of ice were added and 24gms. of sodium nitrite in 70c.c. water slowly added with constant stirring, the end-point

being obtained with starch-potassium iodide paper. After the last addition of nitrite, the solution was allowed to stand for fifteen minutes at 0°.

80gms. hydrated copper sulphate and 20gms. sodium chloride were heated to boiling and a solution containing 16gms. sodium bisulphite and 12gms. sodium hydroxide in 100c.c. boiling water, added over five minutes. The cuprous chloride was allowed to settle and washed by decantation several times after which excess (70gms.) potassium cyanide was added and the solution (300c.c.) boiled.

The diazo solution was gradually added to this well-stirred solution, the temperature being maintained above 90°. A dark brown solution was obtained with a brisk evolution of nitrogen. Owing to the large excess of potassium cyanide the solution remained alkaline throughout and no precipitation of the acid occurred. After the final addition of the diazo solution, the hot solution was just acidified with concentrated hydrochloric acid and the solution filtered hot when the cuprous salts remained insoluble. The yellowish-green filtrate was cooled to 0° when a pale brown residue was precipitated. This was removed by filtration and the filtrate extracted with ether, the ether extract shaken with sodium hydroxide, which was then acidified with concentrated hydrochloric acid when a

further brown precipitate was obtained.

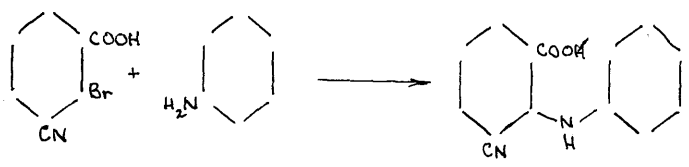
As the precipitate was found to still contain copper, it was warmed with sodium carbonate solution and animal charcoal; on boiling ammonia was evolved, indicating the decomposition of the nitrile. A deep brown filtrate resulted, which, on acidification with concentrated hydrochloric acid till an opalescence developed and then cooling, yielded small buff-coloured needles, m.p.180-182°.
Yield - 40gms. (theor. - 54gms.).

On purification with animal charcoal and boiling water, buff-coloured needles, m.p.184°, resulted.

Analysis	Calculated (for $C_8H_4O_2NBr$) 2-bromo-3-cyanobenzoic acid	Found
%C	42.5	42.9
%H	1.8	2.1
%N	6.2	6.4
%Br	35.4	35.1

The acid was sparingly soluble in cold water, readily soluble in hot. It dissolved readily in ammonia and sodium hydroxide solutions and dissolved in sodium carbonate solution with the evolution of CO_2. It was readily soluble in ether and acetone and dissolved in alcohol on warming. It was insoluble in benzene.

PREPARATION OF 6-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID.



8.5gms. (1 equivalent) 2-bromo-3-cyanobenzoic acid, 3.3gms. sodium carbonate, 3.0gms. anhydrous sodium acetate and 13c.c. cyclohexanol were heated till a thermometer in the vapour read 160°.

The temperature was then allowed to drop to 100° when 4.5gms. aniline and 0.6gms. copper powder were added and the mixture refluxed on an oil-bath at 180° for six hours. After removal of the cyclohexanol by vacuum distillation, the residue was made alkaline with ammonia, warmed with animal charcoal and filtered. Some black tar remained on the filter-paper. The filtrate was dark brown and on acidification with concentrated hydrochloric acid a dark tar, which solidified in standing, was formed.

The tar was boiled in the minimum amount of acetone with animal charcoal, filtered and hot water added to the filtrate till it became opalescent. On cooling, yellow needles, m.p.184°, were obtained.

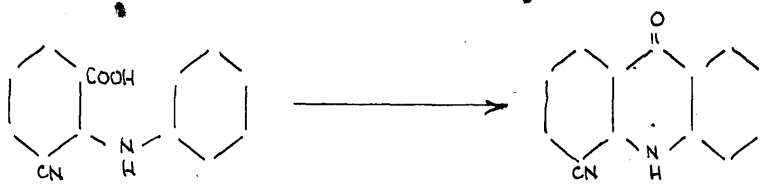
Yield - 5gms. (45%).

Analysis	Calculated (for $C_{14}H_{10}O_2N_2$)	Found
%C	70.6	70.3
%H	4.2	4.25
%N	11.8	11.75

The experiment was repeated using amyl alcohol as solvent and maintaining the temperature of the reflux at

130° for four hours. Less tar was produced by the method and a 55% yield of the diphenylamine carboxylic acid was obtained.

PREPARATION of 1-CYANOACRIDONE



2gms. 6-cyanodiphenylamine-2-carboxylic acid were refluxed at 135-140° with 4c.c. phosphorus oxychloride as described on p.88 and the product poured into water and boiled for twenty minutes. The dried product was sublimed at 300°, giving yellow needles, m.p.265° (m.p. of 1-cyanoacridone prepared from 2-aminobenzonitrile and 2-chlorobenzoic acid - 266°. A mixed m.p. of the two products showed no depression. Yield 1.5gms. (theor. 1.9gms.).

Analysis	Calculated (for $C_{14}H_8ON_2$)	Found
%C	76.7	76.4
%H	3.6	3.6
%N	12.8	12.7

PREPARATION of 5-CHLORO-1-CYANOACRIDINE

0.5gms. 1-cyanoacridone were added to 3c.c. phosphorus oxychloride as on p.89 and the ice-cold product extracted with ammonia and chloroform. After the chloroform extract had been evaporated to dryness at room temperature, the

residue was purified by vacuum sublimation (4mm. Hg) at 160° to give yellow needles, m.p.158° (by other method of synthesis (page 89) m.p.158°). A mixed m.p. caused no depression. Yield - 0.4gms. (theor. - 0.55gms.).

Analysis	Calculated (for $C_{14}H_7N_2Cl$)	Found
%C	70.4	70.2
%H	2.9	2.7
%N	11.7	12.0
%Cl	14.9	15.0

PREPARATION OF SODIUM 2-BROMO-3-CYANOBENZOATE

5.0gms. of 2-bromo-3-cyanobenzoic acid were dissolved in the minimum quantity of sodium dried ethyl alcohol and a slight excess of sodium ethoxide in the minimum amount of dry ethyl alcohol added. A brown precipitate immediately formed in the deep amber solution. The solution was heated on the water-bath and the alcohol distilled off under vacuum. A strong smell of ammonia was noted on testing the distilled alcohol. Heating was, therefore, stopped and the brown residue filtered off, washed with dry alcohol and dried in a vacuum desiccator overnight.

PREPARATION OF 6-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID

2.0gms. of sodium 2-bromo-3-cyanobenzoate were added to 20c.c. sodium dried amyl alcohol with 1gm. of potassium carbonate. To this was added at 100° 1.5gms. of aniline and 0.1gms. of copper bronze and the whole refluxed on an oil-bath at 140° for 4.5 hours.

At the end of this time no tar had been formed and no change in the constituents was observed. The amyl alcohol was steam distilled. The solution was rendered alkaline with ammonia, animal charcoal added and the mixture filtered

hot. When the filtrate was cold, 10c.c. of acetone were added and the solution acidified with concentrated hydrochloric acid. The pale buff precipitate was filtered and dried in a vacuum desiccator. The substance had a m.p. 178° and was found to contain nitrogen and halogen. It was therefore unchanged 2-bromo-3-cyanobenzoic acid.

PREPARATION OF 6-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID

2.5gms. 2-bromo-3-cyanobenzoic acid, 2gms. aniline and 2gms. dry powdered potassium carbonate were warmed with stirring to gentle boiling in the presence of 0.5gm. copper powder in 15c.c. amyl alcohol. During this small amounts of water and alcohol distilled over, the former of which was formed during the reaction. After four hours the reaction mixture was filtered hot. The amyl alcohol was removed by steam-distillation and the aqueous solution of the potassium salt filtered hot to remove tar, boiled with charcoal, filtered and the filtrate treated with concentrated hydrochloric acid at 90°.

A light brown residue was obtained. The product was purified by aqueous acetone to give cream-coloured needles, m.p.184°.

Yield - 2.5gms. (60% theor.).

SYNTHESES OF 2- and 4-CYANOACRIDINES

SYNTHESES OF 2-CYANO-AND 4-CYANOACRIDINE DERIVATIVES FROM 3-AMINO BENZONITRILE

1. PREPARATION OF 3-AMINOBENZONITRILE

Reference - Four methods of preparing 3-nitrobenzonitrile.

(a) By nitration of benzonitrile with fuming nitric acid (Beilstein and Kuhlberg (26))

(b) By nitrating benzonitrile with potassium nitrate and concentrated sulphuric acid (Schopff (149)) (Bogert and Beans (33)).

(c) By treating 3-nitrobenzamide with phosphorus pentachloride (26) or with phosphorus pentoxide (Engler (60)). (d) From 3-nitraniline by diazotisation and Sandmeyer (Sandmeyer (146)(33)).

Method (b) was adopted.

3-nitrobenzonitrile could be reduced to 3-amino-benzonitrile in three ways -

(a) with zinc and alcoholic hydrogen chloride (Hofmann (86))

(b) with tin and glacial acetic acid (Fricke (64)

(c) with stannous chloride and concentrated hydrochloric acid (Bogert and Beans (33)).

Method (a) was adopted.

Preparation of 3-nitrobenzonitrile

51.0gms. potassium nitrate were dissolved in 200c.c. concentrated sulphuric acid in the cold and run in over an

hour below the surface of 52.0gms. benzonitrile dissolved in 150c.c. concentrated sulphuric acid. Good stirring was maintained throughout the nitration, the temperature being kept below 25°. Cooling was applied as necessary by a bath of ice-water. The mixture was allowed to stand for a further 30 minutes, then poured on to 400gms. crushed ice. About 1000c.c. water were added and the precipitated solid filtered to give an almost white powder on drying, m.p.117° (m.p.Lit. - 118°).

Yield - 68gms. (92% theor.).

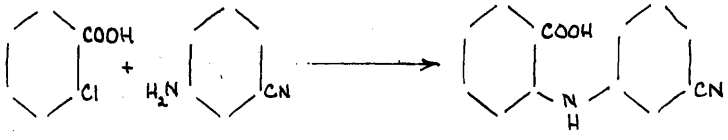
Preparation of 3-aminobenzonitrile

60gms. 3-nitrobenzonitrile were dissolved in 300c.c. hot alcohol and 300c.c. concentrated hydrochloric acid added. Zinc was added gradually to the resultant emulsion with constant stirring. The emulsion was soon dispersed but the reduction was continued till a sample gave no precipitation on dilution with water. No heating was necessary for this reduction. The unattacked zinc was filtered and excess sodium hydroxide solution added with cooling till the zinc redissolved. The precipitated base was separated and the liquid extracted with ether. The base was added to this ethereal solution and the whole was washed free from alkali and dried over anhydrous sodium sulphate. The ether was removed by distillation and the residual oil heated to

300°, the fraction boiling at 278-285° being collected. This was a dark oil which solidified after some time to pale brown needles, b.p.285° (b.p.Lit.- 288°).

Yield - 28gms. (59% theor.).

2. PREPARATION OF 3'-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID



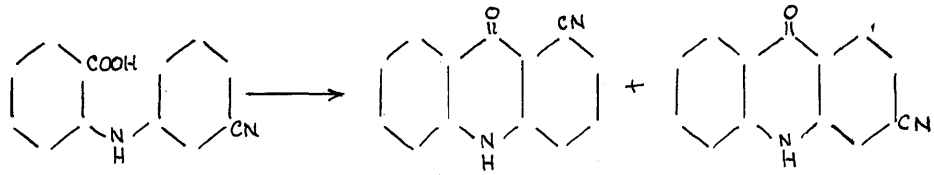
14.4gms. 2-chlorobenzoic acid, 4gms. potassium carbonate, 3.6gms. sodium acetate and 15c.c. cyclohexanol were treated in the same manner as in the preparation of 2'-cyanodiphenylamine-2-carboxylic acid (p. 87) with 6.5gms. 3-aminobenzonitrile and 0.1gm. catalytic copper. After steam-distillation the residue was made alkaline with ammonia, diluted to 150c.c., treated with charcoal and filtered. 30c.c. acetone were added to the cold filtrate which was then acidified with concentrated hydrochloric acid. The precipitate was filtered from any unchanged 2-chlorobenzoic acid which remained in solution and crystallised from the minimum amount of boiling alcohol to give creamy-white needles, m.p.205°.

Yield - 3gms. (50% theor.).

Analysis	Calculated (for $C_{14}H_{10}O_2N_2$)	Found
%C	70.6	70.5
%H	4.20	4.3
%N	11.80	11.6

Note - If the temperature of the oil-bath was raised above 200° during the refluxing of the mixture, the amount of tar produced was increased with a concomitant lowering in amount of product obtained. If the temperature of reflux was about 130°, amyl alcohol being used as solvent, very little tar was produced but a large amount of unchanged 2-chlorobenzoic acid was obtained and the yield of diphenylamine carboxylic acid was only about 30% the theoretical value.

3. PREPARATION OF 2-CYANO and 4-CYANOACRIDONE



3.0gms. 3′-cyanodiphenylamine-2-carboxylic acid and 6c.c. phosphorus oxychloride were refluxed at 140° for half an hour, the excess reagent was distilled under vacuum and the residue boiled for half an hour with water when a green insoluble precipitate was obtained. After filtration, the residue was dried at 120° giving a pale green powder. On dissolving this powder in cold alcoholic sodium hydroxide and reprecipitating the base with concentrated hydrochloric

acid a deep green slimy precipitate was obtained which could only be filtered with great difficulty. The solid was, therefore, purified by sublimation, when orange-yellow needles, m.p.340-350° (in sealed tube) with charring were obtained.

Yield - 2.5gms. (85% theor.).

Analysis	Calculated (for $C_{14}H_8ON_2$)	Found
%C	76.7	76.4
%H	3.6	3.7
%N	12.8	12.9

4. PREPARATION OF 2-CYANO AND 4-CYANO-5-CHLOROACRIDINE

2.0gms. of the isomeric mixture of 2-cyano and 4-cyanoacridones prepared in the previous experiment were refluxed with 6c.c. phosphorus oxychloride at 135°, the excess reagent distilled under vacuum and the product treated with 8c.c. concentrated ammonia, 20gms. ice and 10c.c. chloroform. After shaking for half an hour the liquid was poured off and the residue shaken with 8c.c. concentrated ammonia and 10c.c. chloroform till all the solid had dissolved.

The aqueous layer was shaken with a further 20c.c. chloroform and the combined chloroform extracts dried over calcium chloride. After filtration, the amber-coloured filtrate was evaporated to dryness at room temperature and the residue dissolved in the minimum amount of hot acetone.

Hot water was added till the solution became opalescent and the mixture allowed to come to room temperature, when yellow-green needles were precipitated, m.p.140-160°.

Analysis	Calculated (for $C_{14}H_7N_2Cl$)	Found
%C	70.4	69.9
%H	2.9	3.1
%N	11.7	11.8
%Cl	14.9	14.8

Note - This mixture of isomers appeared to decompose even more readily than the other two cyano-5-chloroacridines, as after about a fortnight, even in the dark, the yellow-green needles had become reddish and the material no longer melted below 300°.

5. CHROMATOGRAPHIC SEPARATION OF 2-CYANOACRIDONE AND 4-CYANOACRIDONE

The solubility of the acridones in various solvents was examined as in the following table:

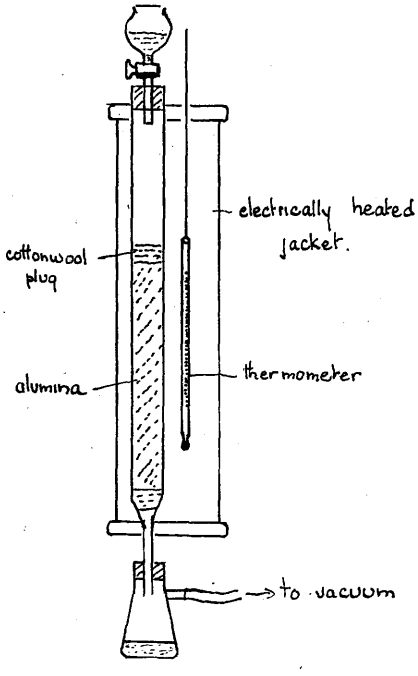
Solubility	Solvent
Sparingly soluble	Ether
Insoluble	Benzene
Sparingly soluble	Carbon tetrachloride
Moderately soluble	Chloroform
Soluble in hot, sp.sol.in cold	Alcohol
As alcohol but less so	Acetone
Readily soluble on warming	Nitrobenzene

Accordingly, nitrobenzene was used as solvent for the separation.

Some nitrobenzene was allowed to stand overnight over some sodium wire and the dried product distilled under vacuum. A column was filled with chromatographic alumina to a depth of 4-5 inches.

The tube of activated alumina was surrounded by an electrically heated jacket and maintained at a temperature of 75°.

A hot solution containing 0.1gms. acridone isomers in 20c.c. nitrobenzene was slowly added through a dropping funnel. Under slight vacuum, a deep orange band was formed at the surface of the alumina and a broader yellow band below it. On the addition of further quantities of dry, hot nitrobenzene, the yellow band moved down the column, the orange band only moving very slowly at the top of the column.



When the yellow band neared the foot of the column the washings were collected in a flask and were bright yellow in colour (A). Washing was continued until the yellow band had been discharged from the tube and the liquid coming through was practically colourless. Meanwhile, the orange band at the top of the tube had increased in length to about an inch.

A fresh flask was placed under the column and hot nitrobenzene passed through until the washings were about 70c.c. in volume. By this time, the orange band had moved about half-way down the column. Passage of nitrobenzene was then stopped, the column allowed to drain and the filtrate (second fraction) (B) removed.

The alumina was carefully pushed out of the top of the tube on to a watch-glass and cut half-an-inch beyond the orange band to obtain a third fraction (C). This was placed in an air-oven at 120° to remove the nitrobenzene.

The fraction (A) was allowed to stand overnight in a refrigerator and in the morning it was observed that yellow needles had crystallised out. The solution was filtered through a sintered glass crucible, washed with benzene and dried in a vacuum desiccator. The dried product consisted of yellow needles, m.p.350-360° with charring in a sealed tube.

The filtrate from the above was evaporated to dryness

under vacuum and the residue dried in an air-oven at 120° for half-an-hour. This material also melted at 350-360° with charring.

The fraction (B), from which nothing crystallised on refrigeration, was similarly evaporated to dryness.

The fraction (C), still adsorbed on the alumina, was diluted with boiling alcohol (96%) and filtered. The orange, alcoholic filtrate was evaporated to dryness and the m.p. of the residual orange needles was 300-310° (with charring).

Residue from first crystallisation.....	- .03gms)	
Residue from evaporation of first filtrate...	- .04gms)	)(A)
Residue from evaporation of second filtrate..	- .01gms	(B)
Residue adsorbed in orange band.....	- .01gms	(C)
Total.....	- .09gms.	

The separation was repeated using 0.5gms. of the acridones and similar results obtained.

6. PREPARATION OF 2-CYANO-5-CHLOROACRIDINE

The residue from the first crystallisation and the residue from evaporation of the first filtrate were added to one another (A) and refluxed with phosphorus oxychloride for half-an-hour, the excess solvent being distilled off under vacuum at the end of the reaction. The dry residue was extracted with chloroform-ice 0.88 ammonia mixture as

described on page the chloroform layer being preserved, dried with anhydrous calcium chloride overnight, filtered and the filtrate evaporated to dryness at room temperature to obtain the corresponding chloroacridine.

A portion of the residue was purified by sublimation at 180° under vacuum and the melting point of the product found to be 192°. 2-cyano-5-chloro-acridine prepared from 3-cyano-2-chloro-benzoic acid, where no isomerism can occur (as shown on page) has a m.p.of 193°. A mixed melting point was then taken of these two compounds, the mixture melting at 192° showing that only 2-cyano-5-chloroacridine was present and that a separation of the isomers had been effected.

Note- On conversion of the residue from evaporation of the second fraction to the chloroacridine and subsequent purification by sublimation, the product melted over about 20° (from 140-160°) and must, therefore, be considered as still being a mixture of the two isomers.

7. PREPARATION OF 4-CYANO-5-CHLOROACRIDINE

The residue obtained from the orange band (C) was converted into the corresponding chloro-acridine by the action of phosphorus oxychloride as before and purified by vacuum sublimation at 180° to give a yellow-orange sublimate, m.p.175-178°.

8. PREPARATION OF 4-AMIDINO-5-AMINOACRIDINE

0.2gms. 4-cyano-5-chloroacridine were placed in a flask with 5c.c. magnesium dried alcohol and 10c.c. dry chloroform and dry hydrogen chloride passed into the ice-cooled solution as in diagram on page 91.

The solution became bright yellow and a bright yellow precipitate was depositied on further passage of the gas. After eighteen hours the passage of hydrogen chloride was stopped and the solution evaporated to dryness at 45° under vacuum. The orange-yellow needles so obtained did not melt below 300° in a sealed tube. A sample was sent for analysis in a sealed tube to prevent decomposition of the iminoether-hydrochloride.

Analysis	Calculated (for $C_{16}H_{15}ON_2Cl_3$)	Found
%C	53.8	60.7
%H	4.2	2.7
%N	7.9	10.5

These results, however, gave good agreement with the values calculated for 2-cyano-5-chloroacridine hydrochloride (61.1%C, 2.9%H, 10.2%N).

9. SEPARATION OF ISOMERIC MIXTURE OF 2-CYANO- AND 4-CYANO-5-CHLOROACRIDINE

Method I

Separation by vacuum sublimation

0.05gms. of the isomeric mixture (see page 114) were

sublimed at 140°/10^{-4}mm. for thirty minutes. It was observed that some yellow product had sublimed but this material melted over 15° from 145-160°. The residue left from the sublimation also melted over a range, from 150-165°.

Method II

Separation by fractional crystallisation

1.0gm. of the chloroacridines was dissolved in the minimum quantity of hot acetone and the product allowed to crystallise slowly. It was then filtered and the whole process repeated six times to give a yellow crystalline solid, m.p.168-176°. The yield of this product was .01gms.

The filtrate from the first crystallisation was evaporated to dryness and the resultant solid dissolved in the minimum quantity of hot acetone. This was repeated twice and the final filtrate evaporated to dryness to give a yellow solid, m.p.189-191° which showed no depression of melting point with some pure 2-cyano-5-chloroacridine. It was obtained in a yield of almost .02gms.

SYNTHESES OF 2-CYANOACRIDINE DERIVATIVES FROM 2-BROMO-4-CYANOBENZOIC ACID

1. PREPARATION OF 2-BROMO-4-CYANOBENZOIC ACID

Reference - 2-Chloro-4-nitrobenzoic has been prepared by Albert and Linnell (10), 2-chloro-4-amino-benzoic acid by Blanksma (31) and Kunchell, Richartz (96) and 2-chloro-4-cyanobenzoic acid by Yu, Magidson and Travin (163) from 2-chloro-4-toluidine.

2-Bromo-4-aminotoluene has been prepared by nitrating 4-nitrotoluene and reducing this to the amino-compound with iron and hydrochloric acid (Higginbottam, Hill and Short (84)).

Preparation of 2-bromo-4-nitrotoluene

The method described in the reference, consisted of heating 140gms. 4-nitrotoluene and 5gms. iron powder on a boiling water-bath after which 50c.c. bromine were added over a period of two hours. The mixture was then heated for a further 45 minutes till the evolution of hydrogen bromide had almost ceased. After standing 12 hours, the solution was poured into a litre of hot water when a dark oil separated. To this

a) a hot solution of sodium bisulphite

b) a hot solution of dilute hydrochloric acid

were added seriatim. Cold water was then added and the oil solidified out as a grey-brown residue.

On purification it was found that only a small amount (15gms.) of material, m.p.75° (m.p.Lit.- 76°) was obtained, most of the nitrotoluene having been converted to 4-nitrobenzyl bromide, m.p.98° (m.p.Lit.- 100°). By modifying the method, however, a good yield of 2-bromo-4-nitrotoluene could be obtained.

The following was the method finally adopted:

280gms. 4-nitrotoluene and 10gms. iron powder were heated in absence of light above a water-bath and vigorous stirring commenced. 100c.c. bromine were slowly added over three hours, at the end of which time heating was continued till the evolution of hydrogen bromide had almost ceased.

After 12 hours the hot solution was poured into a litre of hot water when a dark oil separated. To this

a) a hot solution containing 30gms. sodium bisulphite

b) a hot solution of dilute hydrochloric acid

were added seratim. Cold water was then added and the oil solidified out as a greyish residue, m.p.70-73°. The residue was dissolved in the minimum amount of boiling alcohol and filtered. On cooling pale brown needles separated, m.p. 76° (N-6.50%, theor.N-6.45%) m.p.Lit.- 76°. Yield - 380gms. (theor.440gms.).

Preparation of 2-bromo-4-aminobenzoic acid

Method I

a) Preparation of 2-bromo-4-aminotoluene

Reference - (84).

Method - 200gms. 2-bromo-4-nitrotoluene were refluxed above a water-bath with 10c.c. concentrated hydrochloric acid, 500c.c. alcohol and 170gms. iron filings. The filings were added gradually as considerable frothing accompanied their addition. The mixture was kept at vigorous ebullition to prevent the iron from caking and the heating continued for nine hours after the last addition of iron.

The hot solution was made neutral with alcoholic sodium hydroxide and filtered, the residue being washed with boiling alcohol. The bulk of the alcohol was removed by distillation and the hydrochloride of the base precipitated with concentrated hydrochloric acid. The hydrochloride was basified with sodium hydroxide solution and the layer of oil which separated washed with water. The product was a dark brown oil which solidified on cooling and had the properties of a primary aromatic amine. A sample was dried over solid sodium hydroxide and had a b.p. 250-255° (b.p.Lit.- 254-257°).

Yield - 180gms. (theor.- 206gms.).

Note - Iron Powder. The iron powder for reduction of the nitro group was prepared by etching ordinary iron

filings with dilute sodium hydroxide, washing the product with water, followed by alcohol, acetone and, finally, ether. The iron powder was dried at 110° and stored in a well-stoppered bottle.

b) Preparation of 2-bromo-4-acetamidotoluene

100gms. 2-bromo-4-aminotoluene were refluxed with excess acetic anhydride, the solution cooled and a few drops of concentrated sulphuric added. The solution was then warmed to 40°. On pouring the solution into much cold water, white needles, m.p.111°, were obtained. Yield - 102gms. (theor. - 122gms.) (m.p.Lit. - 113°).

Note - On refluxing the amine with glacial acetic and sodium acetate, some diacetyl formation resulted and the product was difficult to obtain crystalline.

c) Preparation of 2-bromo-4-acetamidobenzoic acid

50gms. 2-bromo-4-acetamidotoluene were heated over a water-bath at 65° in 1.5 litres water and 60gms. potassium permanganate added in six batches of 10gms. at twenty minute intervals. Some frothing occurred but the reaction was not very vigorous. When all the potassium permanganate had been added, the flask was transferred to an oil-bath and the temperature raised to 120°. After six hours, the reflux was discontinued and the mixture filtered hot, the acid passing into the filtrate.

A second 50gms. 3-bromo-4-acetamidotoluene were

heated to 95° in 1.5 litres water and the potassium permanganate gradually added over two hours till a permanent pink colour remained. After the last addition of permanganate, the mixture was allowed to boil gently above an oil-bath for six hours, the mixture then being filtered hot. The filtrates, which were alkaline, were shaken with ether to remove a small amount of tarry material and the ether layer discarded. Concentrated sulphuric acid was added dropwise till a permanent precipitate remained leaving pale pink solid, m.p.180-200°.

The substance was purified by dissolving the acid in sodium carbonate, boiling with animal charcoal, filtering and acidifying the filtrate with concentrated hydrochloric acid, when cream-coloured needles were formed, m.p.180-210° (m.p.Lit.- 206°).

No further purification was attempted at this stage.

Yield - First method, 19gms.

Second method, 32gms.

d) Preparation of 2-bromo-4-aminobenzoic acid

24gms. dry acetyl derivative prepared above was refluxed with 100c.c. alcohol till all the substance had dissolved. 100c.c. concentrated hydrochloric acid were added and refluxing continued for a further two hours. During this time, some solid separated out. The alcohol was then distilled, the residue cooled and filtered after the addition of 50c.c. cold water. The 2-bromo-4-amino-

benzoic acid hydrochloride was obtained as almost white needles. The free base was not isolated due to its solubility in most solvents.

Yield - 18gms. (theor. - 24gms.).

Method II

a) Preparation of 2-bromo-4-nitrobenzoic acid

Reference - J.A.Turnbull, private communication.

50gms. 2-bromo-4-nitrotoluene were dissolved in 500gms. glacial acetic acid and 70gms. (excess) chromium trioxide added. To this solution, 120gms. acetyl chloride were slowly added from a dropping funnel with constant stirring, and cooling of the reaction vessel with ice to keep the temperature below 20°. Stirring was continued till all the chromic oxide had dissolved and then for a further thirty minutes at room temperature. The mixture was allowed to stand for twenty four hours at room temperature and then poured into five times its volume of water. This was extracted with one third its volume of ether and the aqueous portion discarded as it was found on examination to contain no nitro-bromo-benzoic acid. The ether layer was shaken with an equal volume of 5N sodium hydroxide and the aqueous portion collected.

The sodium hydroxide extract was acidified with concentrated hydrochloric acid and a white crystalline precipitate, m.p.163-165°, obtained.

The acid was recrystallised from boiling water to give long white needles, m.p.167° (m.p.Lit. - 167°).
Yield - 34gms. (theor. - 56gms.).

Preparation of 2-bromo-4-aminobenzoic acid

34gms. 2-bromo-4-nitrobenzoic acid were dissolved in the minimum amount of dilute ammonia with warming and poured into a boiling solution of 7 molecular equivalents of hydrated ferrous sulphate (242gms.) dissolved in 500c.c. water. The solution was treated with small portions of concentrated ammonia at the boil, each addition being followed by vigorous agitation of the mixture. When the solution was distinctly alkaline, it was boiled for five minutes, care being taken that the solution was still alkaline at the end of this time. The solution was filtered hot and evaporated to one third its original volume. Concentrated hydrochloric acid was added to the concentrate till no further precipitation occurred and the hydrochloride of the aminoacid collected as white needles. A small amount of the free base was prepared by treating the concentrated ammonia solution with acetic acid, when white needles, m.p.187-191°, were obtained. (m.p.Lit.192°). No further purification was attempted at this stage.
Yield - 29gms. (theor. - 35gms.) as hydrochloride.

Preparation of 2-bromo-4-cyanobenzoic acid

18gms. 2-bromo-4-aminobenzoic acid were added to

15c.c. concentrated hydrochloric acid and 100gms. crushed ice. 7gms. sodium nitrite dissolved in 50c.c. water was rapidly run in, the temperature being maintained below 5° with vigorous stirring. The excess nitrite was destroyed with urea and the diazo solution allowed to stand at 0° for 15 minutes.

23gms. copper sulphate were dissolved with heating with 7gms. sodium chloride in 100c.c. water and a solution of 5gms. sodium bisulphite and 4gms. sodium hydroxide in 50c.c. water added with stirring over five minutes. The cuprous chloride so formed was allowed to cool to room temperature and settle and was then washed by decantation three times. 200c.c. water were finally added, the solution warmed and 20gms. potassium cyanide stirred till solution was effected.

This gave a colourless copper cyanide solution which was heated above 90° and the diazo solution introduced slowly with vigorous stirring. There was much evolution of nitrogen. The temperature of the Sandmeyer solution was maintained above 90° during the addition and the final solution was deep brown in colour.

The hot solution was acidified with concentrated hydrochloric acid and filtered. On cooling, a light brown powder was precipitated from the filtrate. As it was thought the acid would have some solubility in water, the

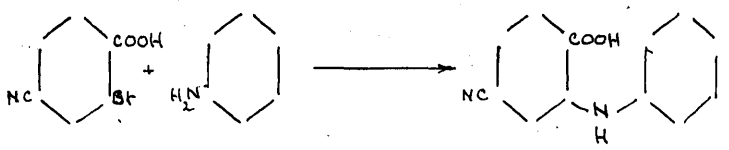
filtrate was shaken with ether, the ether extract shaken with sodium hydroxide and this added to concentrated hydrochloric acid, when pale brown needles, m.p.180-185°, were obtained.

On recrystallisation from boiling water, cream coloured needles, m.p.193°, were obtained.

Yield - 10gms. Analysis for 2-bromo-4-cyanobenzoic acid, %N - calculated 6.2, found 6.5
%Br. - " 35.4, " 34.8.

2. PREPARATION OF 5-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID

Method -



8.56gms. (1 equivalent) 2-bromo-4-cyanobenzoic acid, .66gms. (.25 equivalent) + 2.61gms. potassium carbonate, 3gms. of anhydrous sodium acetate and 12.5c.c. of cyclohexanol were heated till a thermometer in the vapour read 160°. During this some water was evolved due to the interaction of acid and carbonate and it was feared that this might cause decomposition of the nitrile. No smell of ammonia was discernable but this might have been masked by the strong smell of the cyclohexanol. The vapour, however, turned red litmus blue.

Heating was, therefore, discontinued and when the temperature had dropped to 100°, 4.43gms. of aniline and

.66gms. of copper powder were added and the whole refluxed on an oil-bath for six hours at 180°. A black tarry mass resulted from which the cyclohexanol was removed by steam-distillation. The residue while still hot was made alkaline with ammonia, treated with charcoal and filtered.

30c.c. of acetone were added when the solution had cooled and the mixture was acidified with concentrated hydrochloric acid. A yellow precipitate turning almost immediately to a black tarry mass resulted. On standing overnight, a further yellow crystalline product appeared. The total residue was filtered off and treated with 40c.c. ammonia solution and 10c.c. acetone. The tar did not completely dissolve so the solution was filtered and the residue was seen to consist of two distinct substances, one ochre-coloured and the other sea-green. On performing a physical separation of part of this residue it was found that the ochre portion gave a m.p.about 184°, but that the green portion (on standing on a porous plate) turned into a dark tar, from which no m.p. could be obtained. On adding more ammonia and acetone mixture to the residue complete solution could be obtained. It was thought that the difficulty encountered in dissolving the residue was due to its physical state and not to any difference in chemical composition from the filtrate, so both filtrates were combined and acidified with concentrated hydrochloric acid.

Again a yellow precipitate was formed which quickly became a dark tar, solidifying on standing. A further yellow solid crystallised out on standing which could physically easily be removed from the tar. This gave a m.p.180-190°.

The total residue was filtered, dried and dissolved in the minimum quantity of acetone. The solution was allowed to cool and water gradually added. A small quantity of a black tar was first formed and this was filtered, leaving an opaque yellow solution to which more water was added and the solution allowed to stand overnight. In the morning, a bulky yellow crystalline precipitate was present. This was filtered off, dried and gave a m.p.200°-204°.

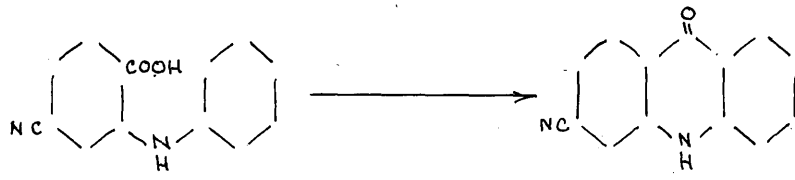
On acidifying, the filtrate, a further crop of small yellow needles were obtained. Some of these were examined under the microscope and were seen to consist of clusters of yellow needles. These were filtered and dried in a vacuum desiccator. The bright yellow needles had a m.p. 218° and did not contain halogen.

Yield (from both fractions melting at 200° and 218°) - 4gms. (46% theor.). No further purification of the tar was attempted.

Analysis	Calculated (for $C_{14}H_{10}O_2N_2$)	Found
%C	70.6	70.9
%H	4.2	4.3
%N	11.8	11.9

The experiment was repeated using amyl alcohol and maintaining the temperature of the reflux at 130° for four hours. Less tar was produced by the method, a 60% yield of the diphenylamine carboxylic acid being obtained.

3. PREPARATION OF 2-CYANOACRIDONE



2.0gms. 5-cyanodiphenylamine-2-carboxylic acid were refluxed with 4 c.c. phosphorus oxychloride at 140° as described on page 88 . A dark tar was formed. The excess phosphorus oxychloride was removed by vacuum distillation at 120° and the resultant black solid shaken with water till all the solid had been removed from the sides of the flask. The brownish suspension so obtained was boiled for twenty minutes and the pale yellow solid filtered and dried at 120°.

The powder was purified by sublimation to give yellow needles. The needles charred at 330° but did not melt below 360°.

Yield - 1.5gms.

Analysis	Calculated (for $C_{14}H_8ON_2$)	Found
%C	76.7	76.6
%H	3.6	3.7
%N	12.8	12.6

4. PREPARATION OF 2-CYANO-5-CHLOROACRIDINE

1.0gms. 2-cyanoacridone was refluxed with 2c.c. of phosphorus oxychloride for thirty minutes, after which the excess phosphorus oxychloride was removed by vacuum distillation. To the residue a well-stirred mixture of 5c.c. concentrated ammonia solution, 13gms. of ice and 5c.c. of chloroform was added. The flask was shaken on a mechanical shaker for thirty minutes and the solution decanted. A further portion of 5c.c. concentrated ammonia solution, 13gms. of ice and 5c.c. of chloroform was added and the flask shaken till all the solid had dissolved.

The two filtrates were then placed in a separating funnel and the chloroform layer removed. The aqueous layer was further shaken with 10c.c. chloroform and this was added to the first chloroform extract. This was dried over-night over calcium chloride and filtered, an amber filtrate being obtained. The filtrate was allowed to evaporate to dryness on a watch-glass at room temperature. An elements test showed the presence of both halogen and nitrogen.

A sample was purified by sublimation under vacuum at 180°/4mm. and the sublimed product analysed. The purified product melted at 193° in a sealed tube.

Note - It was found that the chloroacridine could also be satisfactorily purified by crystallisation from hot acetone.

Yield - 1.0gm. (90%).

Analysis	Calculated (for $C_{14}H_7N_2Cl$)	Found
%C	70.4	70.2
%H	2.9	3.1
%N	11.7	11.6
%Cl	14.9	14.9

5. PREPARATION OF 2-AMIDINO-5-AMINOACRIDINE

0.5gms. 2-cyano-5-chloroacridine were placed in a flask with 7c.c. magnesium dried alcohol and 15c.c. dry chloroform and dry hydrogen chloride passed into the ice-cooled solution (diagram on page 91).

The solution became bright yellow and a bright yellow precipitate was formed which redissolved on further passage of hydrogen chloride. After twelve hours some long shining needles were deposited. After a further nine hours the apparatus was dismantled and the excess alcohol and chloroform distilled under vacuum below 45°. The bright yellow solid so obtained did not melt below 360° in a sealed tube though it charred above 300°.

The above product was added to 80c.c. magnesium dried alcohol which had previously been saturated with dry ammonia at 0° in a pressure bottle. The pressure bottle was sealed and placed in a water-bath at 45° for fifteen hours. At the end of this time, yellow crystals were seen to have been deposited. The mixture was evaporated to dryness under

vacuum at 45°. The crystals were dissolved in cold water with continued shaking and solid ammonium nitrate added. The resultant yellow-green precipitate was filtered, dried and pale yellow needles which did not melt below 360° obtained.

Yield - 0.8gms.

Analysis	Calculated (for $C_{14}H_{14}O_6N_6$)	Found
%C	46.4	45.9
%H	3.9	4.1
%N	23.2	23.6

0.5gms. of the above nitrate were shaken with 30c.c. alcoholic potassium hydroxide till complete solution had been effected.

The white inorganic residue was removed by filtration and benzene added to the filtrate. After twenty four hours, orange needles were deposited, m.p.304°.

Yield - 0.2gms.

Analysis	Calculated (for $C_{14}H_{12}N_4$)	Found
%C	71.2	71.0
%H	5.1	4.8
%N	23.7	23.5

6. PREPARATION OF 2-CYANOACRIDINE

1.0gms. 2-cyano-5-chloroacridine were dissolved with shaking for two hours in 30c.c. alcohol and 10c.c. sulphur-free benzene and 1gm. palladium-calcium carbonate catalyst

added. On shaking under hydrogen the gas was slowly absorbed. The reduced compound was filtered, the residue washed with hot alcohol and the deep red solution, fluorescing red-blue, evaporated to dryness. The red solid so obtained, the dihydrocompound was washed with water to remove all inorganic material and added to 115c.c. boiling water containing 6c.c. 25% sulphuric acid. The compound was then oxidised to 2-cyanoacridine and isolated from the resultant mixture by a method identical with that employed in the preparation of 1-cyanoacridine (see page 92).

The compound was purified by sublimation under vacuum at 200° to give yellow needles, m.p.191° which did not contain halogen.

Yield - 0.6gms. (70% theor.)

Analysis	Calculated (for $C_{14}H_8N_2$)	Found
%C	82.3	82.1
%H	3.9	4.1
%N	13.7	13.4

SYNTHESES OF 4-CYANOACRIDINE DERIVATIVES

1. PREPARATION OF 2-CHLORO-6-NITROTOLUENE

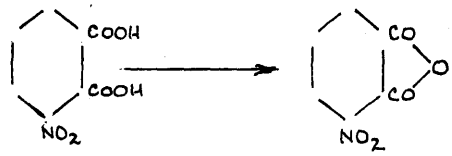
Reference - (54)

50gms. completely dry and pure as possible 2-nitrotoluene were treated with 10gms. antimony pentachloride and dry chlorine passed until the increase in weight of the reaction mixture amounted to about 13gms. The reaction mixture was cooled from the start of the reaction in ice-water mixture and the stream of chlorine so arranged that the temperature of the reaction mixture was kept at 30-40°.

The reaction product was washed with dilute hydrochloric acid, water and sodium hydroxide and distilled in superheated steam. The yellow oil passing over was separated from the aqueous layer, but on standing did not give a solid and liquid fraction as Jansen (D.R.P.107505) was able to obtain. The liquid contained halogen and boiled about 220°. (b.p.Lit., 2-chloro-6-nitrotoluene, - 238°). A small portion of the oil was oxidised with chromic acid, glacial acetic acid and acetyl chloride and white needles of chloronitrobenzoic acid obtained, m.p. 145-155°. M.P.Lit., 2-chloro-6-nitrobenzoic acid - 161; m.p.Lit., 2-chloro-4-nitrobenzoic acid - 140°.

2. PREPARATION OF 3-NITROPHTHALIC ANHYDRIDE

Reference - (34).



Laurent (97), Faust (64b) and Miller (120b) all stated that the anhydride is formed by the action of heat on the acid. Lipschitz (109) reported that by careful heating of the acid at 220°, even in a stream of CO_2 he obtained only a brown melt containing decomposition products with the odour of NO_2 and benzaldehyde. The authors of the above paper were in agreement with the earlier workers however.

Accordingly, 15gms. of 3-nitro-phthalic acid (prepared as shown on page 97) were heated in a small flask in an oil-bath at 235-240° until water vapour ceased to be given off (about three hours were required in the present case though the above authors using similar quantities required six to eight hours heating). On cooling, a light brown crystalline solid appeared, which was nearly pure anhydride and after a single recrystallisation from boiling glacial acetic acid, cooling and filtering, almost colourless needle melting 160-163° were obtained. The above authors claimed that the pure anhydride is colourless and melts at 163° but no further purification was attempted at this stage.

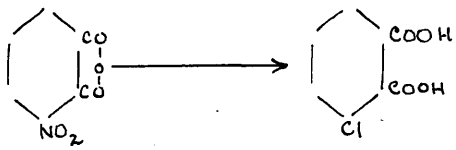
Note - The above authors suggested that if there was any indication of the presence of unchanged acid the product should be recrystallised from acetyl chloride which like-wise converts the acid to the anhydride (Leupold, Lipschitz (109)).

The anhydride was easily soluble in acetyl chloride or hot glacial acetic acid, moderately so in acetone or hot alcohol and very sparingly soluble in benzene.

Yield - 10gms. (theor. - 13.6gms.).

3. PREPARATION OF 3-CHLOROPHTHALIC ACID

Reference - Bogert and Boroschek (34).

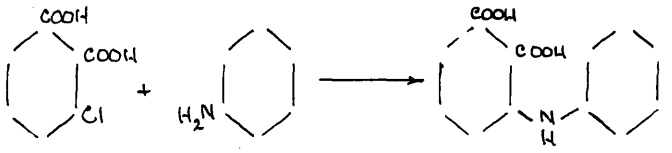


10gms. of 3-nitro-phthalic anhydride and 11gms. of PCl_5 were heated together in a Carius tube for six hours at 175°. From the contents of the tube, yellow-brown crystals were separated, which after crystallisation from a mixture of benzene and naphtha, formed nearly colourless needles, m.p.122°.

On boiling, the anhydride obtained above for several hours with dilute hydrochloric acid, the corresponding 3-chlorophthalic acid was obtained. This was filtered off. The acid dissolved in hot water and crystallised in almost colourless needles on cooling, m.p.186°(m.p.Lit.- 180°

Yield - 5.4gms. (some material was lost in opening the Carius tube).

4. PREPARATION OF DIPHENYLAMINE-2-3-DICARBOXYLIC ACID



2.5gms. 3-chlorophthalic acid, 2gms. of aniline and 2.0gms. of dry powdered potassium carbonate were warmed in the presence of 0.5gms. of copper powder in 15 c.c. iso-amylalcohol with stirring till weak ebullition of the alcohol was observed. During this, small amounts of water and alcohol distilled over, the former of which was formed during the reaction. After four hours the reaction mixture was filtered hot. The amyl alcohol was removed by steam distillation and the aqueous solution of the potassium salt filtered hot to remove tars, boiled with charcoal, filtered and the filtrate treated with concentrated hydrochloric acid at 90°. A dark brown residue, somewhat tarry was formed. Purification was attempted by using aqueous acetone but the product was still precipitated as a tar. Treatment of a small portion of the tar with chromic acid resulted in the formation of a deep purple colour, indicating the presence of a diphenylamine.

On warming the tar with benzene, a yellow filtrate was obtained and a small amount of dark brown solid remained

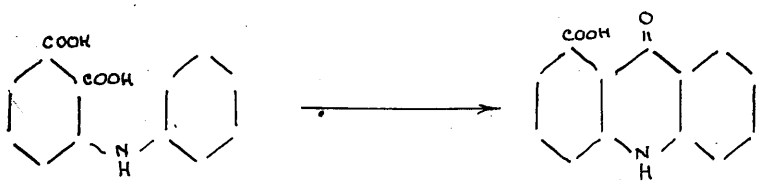
undissolved. This was removed by filtration at 80°. On cooling, yellow-green needles were precipitated from the filtrate and after standing in the refrigerator overnight, were filtered off and dried in a vacuum desiccator. The filtrate was evaporated to dryness on a steam-bath and more yellowish substance (amorphous) obtained.

The residue was redissolved in the minimum quantity of benzene and boiled with a little animal charcoal, filtered and the filtrate left overnight in the refrigerator. Yellow needles were obtained and these were filtered through a sintered-glass crucible, m.p.178°.

Analysis	Calculated (for $C_{14}H_{11}O_4N$)	Found
%C	65.9	65.6
%H	4.2	4.5
%N	5.4	5.7

5. PREPARATION OF 4-CARBOXYACRIDONE

Method -



0.5gms. of the above acid and 1c.c. phosphorus oxychloride were refluxed for half-an-hour, the excess reagent distilled off under vacuum and the residue boiled with water

for half-an-hour and dried at 120°. The product was yellow in colour and dissolved in alcohol readily with no fluorescence even under the U.V. lamp. It had a m.p. from 150-160° and showed none of the other characteristics of an acridone.

SYNTHESES OF 2-CYANOACRIDINES

SYNTHESES OF 3-CYANOACRIDINES

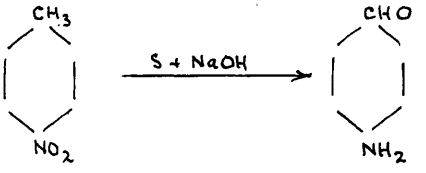
SYNTHESES OF 3-CYANOACRIDINE DERIVATIVES FROM 4-AMINOBENZONITRILE

1. PREPARATION OF 4-AMINOBENZONITRILE

Reference - 4-Nitrotoluene was converted to 4-aminobenzaldehyde (54) with sodium hydroxide and sulphur. The 4-aminobenzaldehyde was treated with acetic anhydride to give 4-acetamidobenzaldehyde (Hodgson and Beard (85)) which was converted to the oxime (Gabriel and Herzberg (87)). The oxime on refluxing with acetic anhydride gave 4-acetamidobenzonitrile which on refluxing with 2N hydrochloric acid gave 4-aminobenzonitrile (Ashley etc. (17)).

Method I

Preparation of 4-aminobenzaldehyde



50gms. sulphur were refluxed with 80gms. sodium hydroxide and 200c.c. water until all the sulphur had dissolved. 40gms. 4-nitrotoluene dissolved in 200c.c. alcohol were then added and the whole refluxed for half-an-hour. The resultant liquor was steam-distilled to remove most of the alcohol and any 4-toluidine which had been

formed, after which it was extracted with ether and the ether extract evaporated to dryness. A yellow powder of 4-aminobenzaldehyde, m.p.70° (m.p.Lit.= 71°) was formed which, on standing, formed an insoluble orange polymerisation product.

Yield - 25gms. (theor.36gms.).

Note - By adopting a modification of Beard and Hodgson (25) and, after steam-distillation, converting the non-volatile 4-aminobenzaldehyde to the sulphate thus preventing polymerisation, a 75% yield of 4-aminobenzaldehyde was obtained.

Preparation of 4-acetamidobenzaldehyde

32gms. 4-aminobenzaldehyde were suspended in 50c.c. water and 50c.c. glacial acetic acid added. The base gradually turned red. To this 40c.c. acetic anhydride were gradually added and the solution boiled for five minutes after it became clear. The solution when cool was then poured into much ice-water and the acetamidobenzaldehyde allowed to crystallise out as pale yellow needles, m.p.150° (m.p.Lit.- 153°).

Yield - 43gms. (theor. - 45gms.).

Preparation of 4-acetamidobenzaldoxime

43gms. 4-acetamidobenzaldehyde were dissolved in the minimum amount of alcohol and 30gms. hydroxylamine hydro-

chloride in sodium hydroxide solution added and the whole refluxed for an hour. To this hydrochloric acid was added until the solution was acid. On cooling, the oxime crystallised out as cream coloured needles which were filtered and dried at 110°. M.P. - 192° (m.p.Lit. - 194°). Yield - 47gms. (theor. - 48gms.).

Preparation of 4-aminobenzonitrile

The dried oxime (47gms.) was boiled for one hour with 50c.c. acetic anhydride. After stirring into 500c.c. ice-water, the solution was neutralised and the resulting 4-acetamidobenzonitrile collected and added to 400c.c. boiling 2N hydrochloric acid. Boiling was continued for twenty minutes. The solution was filtered hot to remove insoluble impurities and the resultant clear solution cooled in ice, neutralised and the precipitated 4-amino-benzonitrile collected as a light brown powder. On recrystallisation from boiling alcohol with animal charcoal, brown needles were obtained, m.p. 84° (m.p.Lit. - 86°). Yield - 26gms. (theor.- 32gms.).

Method II

Preparation of 4-nitrobenzonitrile

90gms. powdered 4-nitraniline were treated with 120c.c. concentrated hydrochloric acid and the mass stirred for half-an-hour until completely changed to the pale yellow

hydrochloride. 1500c.c. water were then added and the mixture thoroughly stirred.

The suspended nitraniline was diazotised by slowly adding a solution of 42gms. sodium nitrite in water to the well-stirred solution. At the end of an hour all but a little of the orange nitraniline had changed to the dark green flocculent diazo compound. No external cooling was necessary as the diazo compound was very stable at room temperatures.

The flocculent diazo compound was then decanted, any unchanged nitraniline remaining behind due to its much greater density. The suspended diazo-body was slowly poured into a potassium cuprocyanide solution prepared from 180gms. hydrated copper sulphate, 1000c.c. water and 200gms. potassium cyanide. The cuprocyanide solution was maintained above 90° during this reaction which was accompanied by vigorous stirring during the addition of the diazo compound. After the last addition, the solution was boiled for five minutes and the boiling solution filtered through glass-wool On cooling, orange needles of 4-nitrobenzonitrile were deposited from the filtrate. On boiling the tar which remained on the glass-wool with 15 litres of water and filtering, considerable quantities of almost pure nitrile were obtained on cooling. Only a small amount of tar was left at the end of these extractions.

As the product so obtained had m.p.135-140°, it was further purified from boiling carbon tetrachloride to give golden scales, m.p. 148° (m.p.Lit. - 149°).

Yield - 71gms. (theor. - 95gms.).

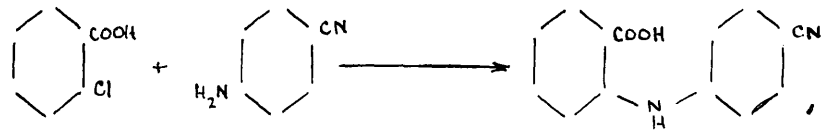
Preparation of 4-aminobenzonitrile

250gms. stannous chloride were dissolved in 210c.c. concentrated hydrochloric acid and the solution vigorously stirred while 50gms. 4-nitrobenzonitrile were added at such a rate that the temperature of the solution remained at 30-40°. As the action progressed, the nitrobenzonitrile gradually dissolved and the reaction was complete in a few hours. Towards the end of the reaction, white needles of the hydrochloride were precipitated. The precipitation of the hydrochloride was completed by the addition of 400c.c. concentrated hydrochloric acid after which the mixture was allowed to stand at 0° for 12 hours. The hydrochloride was then filtered through sintered glass and the residue washed with concentrated hydrochloric acid to remove any tin, sucked as dry as possible and the free base liberated by addition to an excess of moderately dilute ammonia solution, the precipitated base being washed with water till free from ammonium chloride, dried and crystallised from carbon tetrachloride, m.p.85° (m.p.Lit. - 86°).

Yield - 37gms. (theor. - 40gms.).

2. PREPARATION OF 4′-CYANODIPHENYLAMINE-2-CARBOXYLIC ACID

Reference - Albert and Gedhill (9).

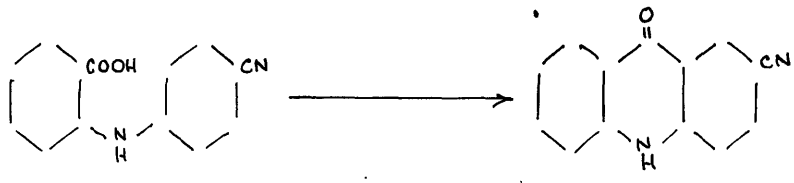


28gms. 2-chlorobenzoic acid, 8gms. potassium carbonate, 7.2gms. sodium acetate and 50c.c. cyclohexanol were heated until a thermometer in the vapour registered 160° (to remove all the water). The mixture was cooled to 100° and 15gms. 4-aminobenzonitrile added with 0.2gms. catalytic copper powder and the whole refluxed on an oil-bath at 180° for six hours. After steam-distillation to remove the cyclohexanol, the residue was made alkaline with ammonia, diluted to 500c.c., treated with charcoal and filtered. 60c.c. acetone were added when the solution had cooled to room temperature and the mixture acidified with concentrated hydrochloric acid. The precipitate was filtered from the colution of 2-chlorobenzoic acid, dissolved in 80c.c. water with ammonia and treated with 20c.c. acetone. Acidification with concentrated hydrochloric acid gave pale brown crystals, m.p.210°, which were recrystallised from 16 parts of boiling alcohol giving a 50% yield of 4′-cyanodiphenylamine-2-carboxylic acid as creamy white crystals, m.p.225° (m.p.Lit. 225°).

Yield - 12gms. (theor. - 25gms.).

3. PREPARATION OF 3-CYANOACRIDONE

Reference - Albert and Gedhill (9).



6gms. 4'-cyanodiphenylamine-2-carboxylic acid and 12c.c. phosphorus oxychloride were refluxed for half-an-hour. At the end of this time the excess reagent was distilled and the residue boiled with water for thirty minutes and dried at 120°. The acridone was purified by dissolving it in cold alcoholic sodium hydroxide, filtering and reprecipitating with acid to give a pale greenish-yellow product which did not melt below 300° and was almost insoluble in organic solvents. The acridone was purified by sublimation to give yellow needles.

Yield (after sublimation) from 6gms. starting material - 4.5gms. (theor. - 5.6gms.).

4. PREPARATION OF 5-CHLORO-3-CYANOACRIDINE

5gms. 3-cyanoacridone (.32moles) were mixed with 10c.c. (1.76 moles) phosphorus oxychloride giving a green fluorescence. The mixture was slowly heated to 90° when considerable frothing occurred and the temperature was not allowed to rise any higher. When all the solid had dissolved and the frothing subsided a dark brown solution resulted. The temperature was then raised to 140° where it was maintained

for two hours, after which time the excess phosphorus oxychloride was removed by vacuum distillation. The flask was cooled and a well stirred mixture of 20c.c. concentrated ammonia, 50gms. ice and 20c.c. chloroform added and the whole shaken for half-an-hour. The liquid was then decanted and the residue shaken with a further 20c.c. ammonia and 20c.c. chloroform for half-an-hour. At the end of this time only a small amount of solid remained undissolved and the two filtrates were placed in a separating funnel and the chloroform layer removed. The aqueous portion was shaken with a further 20c.c. chloroform and this added to the first chloroform extract. These were dried with 10gms. calcium chloride, filtered and a brown filtrate obtained. A small amount of a dark residue was left behind on the filter-paper with the calcium chloride.

The filtrate was allowed to evaporate to dryness at room temperature and a yellowish-brown crystalline residue obtained, m.p.178-181°.

Yield - 4gms. (theor. - 5.8gms.).

Purification of 3-cyano-5-chloroacridine

Freshly prepared chloroacridine had some solubility in alcohol but could not be satisfactorily crystallised from it. Material which had been standing decomposed on warming with alcohol, liberating hydrogen chloride gas, to the acridone, no m.p. below 360°.

Analysis	Calculated (for $C_{14}H_8ON_2$)	Found
%C	76.72	76.9
%H	3.64	4.0
%N	12.78	12.6

The chloroacridine was dissolved in the minimum amount of hot acetone, filtered to remove a little insoluble matter, and cooled, when a fine crystalline meal of yellow needles was obtained, m.p.183°, at which temperature a red liquid was formed from which, on cooling, a red solid, not melting below 300° on reheating, was formed.

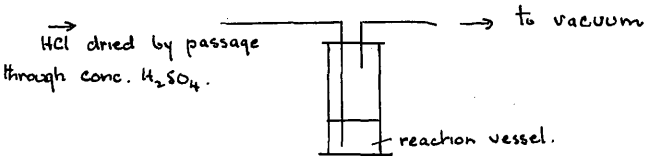
Yield - 1.5gms. (from 2gms. crude product).

Analysis	Calculated (for $C_{14}H_7N_2Cl$) 3-cyano-5-chloroacridine	Found
%C	70.4	70.3
%H	2.9	2.8
%N	11.7	11.4
%Cl	14.9	14.9

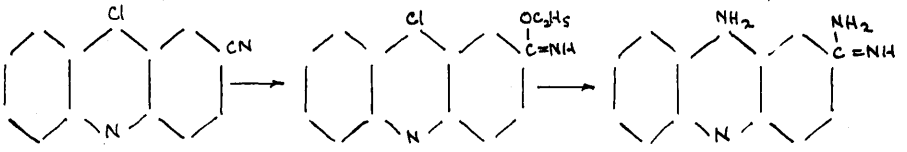
Note - It was found that, on standing, especially exposed to sunlight, the chloroacridine decomposed to a reddish-brown solid which was almost insoluble in organic solvents and did not melt below 300°. The purity of the compound did not appear to affect the rate of this decomposition.

5. PREPARATION OF 5-AMIDINO-5-AMINOACRIDINE

Method -



0.75gms. of 5-cyano-5-chloroacridine were placed in a flask with bubbler (as in diagram) with 7c.c. Mg.dried ethyl alcohol and 15 c.c. dry chloroform. The chloroacridine was first dissolved in the chloroform to give a bright yellow solution and the alcohol added.



The flask was placed in the apparatus shown above and immersed in a bath at 15° below which temperature the nitrile tended to crystallise. Suction was applied and dry hydrogen chloride passed through the apparatus. The solution became brighter in colour and a bright yellow precipitate was formed which redissolved on further passage of hydrogen chloride. After seven hours, the hydrogen chloride was stopped and the apparatus sealed at both ends with clips to prevent the entrance of moisture. After twelve hours, it was observed that a small quantity of shining needles had been formed and on starting the passage

of the hydrogen chloride again the precipitate increased in size. Hydrogen chloride was passed for a further nine hours and the apparatus dismantled.

The excess alcohol and chloroform were distilled under vacuum at 40-45° and the dry hydrochloride of the imino-ether which was bright yellow in colour obtained. It was found that the compound did not melt below 300° in a sealed tube though it charred above 300°. On exposure to air, the dry yellow powder became sticky and bright orange in colour. On further standing this orange powder also became dry. This behaviour is in agreement with imino-ether formation since these are in general unstable in air being decomposed by the slightest traces of water. A sample was sent for analysis in a sealed tube (after drying under vacuum over sodium hydroxide). Due to the compound's instability no purification was attempted.

Analysis	Calculated (for $C_{16}H_{15}ON_2Cl_3$)	Found
%C	53.8	55.8
%H	4.2	4.1
%N	7.9	8.3
%Cl	29.7	29.5

The above product was added to 80c.c. of Mg.-dried alcohol which had previously been saturated with dry ammonia at 0° in a pressure bottle. The bright yellow solid immediately turned pale yellow on addition (due

probably to the formation of the free base) and soon dissolved to give a bright yellow solution fluorescing yellow-green. The pressure bottle was tightly sealed and maintained at 45° for fifteen hours. At the end of this time, large yellow crystals were seen to have been deposited. The pressure bottle was allowed to come to room temperature and opened. The crystals were filtered off and when dry weighed 0.5gms. The compound did not melt below 300° but became dull brown about this temperature.

The filtrate was evaporated to dryness at 45-50° under reduced pressure and gave a yield of 0.5gms. material, which also did not melt below 300° but became duller in colour, Both portions were insoluble in chloroform, ether, acetone and moderately soluble in alcohol and water. Since the product was completely insoluble in chloroform none of the original cyanoacridine remained. On the addition of nitric acid and silver nitrate, a yellow precipitate, insoluble in ammonia, as well as a white one of silver halide, soluble in ammonia was obtained (i,e. ionisable halogen was present). On the addition of hydrochloric acid, glacial acetic acid or sulphuric acid, the colour of aqueous solution decreased and a blue-yellow fluorescence developed. The solid was sparingly soluble in acid solution. On the addition of sodium hydroxide or ammonium hydroxide to the aqueous solution, a bright yellow solution was obtained with

a strong yellow-green fluorescence. The material was more soluble in alkali than in acid.

On the addition of ammonium nitrate to acid or neutral solutions a flocculent, amorphous yellow precipitate was obtained. The chromate was also very insoluble. On boiling with water, ammonia was evolved and the cold aqueous solution turned red litmus blue. From the above evidence, it was deduced that the product consisted of a mixture of ammonium chloride and the amidine hydrochloride, the chloro in position 5- having been converted to an amino group by the action of ammonia in alcohol under pressure.

Accordingly, 0.5gms. of the yellow crystals were shaken with 50c.c. water for 30 minutes and filtered as all the solid had not dissolved. To the filtrate, solid ammonium nitrate was added and a yellow precipitate obtained. This was filtered and washed with water till the washings were free from halogen. The residue was dried in a vacuum desiccator and halogen was found to be absent. The yellow-orange precipitate decomposed at 260°, the residue starting to melt with charring at 280°. It did not dissolve in water, but dissolved slightly in ammonium hydroxide and completely in sodium hydroxide on gentle warming to give a bright yellow solution in low concentration. The substance was soluble in alcoholic sodium hydroxide giving a green-yellow fluorescence.

Yield - 0.4gms.

Analysis	Calculated (for $C_{14}H_{14}O_6N_6$) 3-amidino-5-aminoacridine dinitrate	Found
%C	46.2	45.9
%H	3.9	4.2
%N	23.2	23.0

0.1gm. of the material was dissolved in alcohol and the solution treated with a saturated solution of picric acid in alcohol and the resultant solution allowed to stand overnight. Cubic orange crystals separated out. The crystals darkened at 260° but did not melt below 360°.

0.3gms. of the nitrate were dissolved in 50c.c. alcoholic potassium hydroxide, the solution filtered and evaporated to half its bulk at room temperature. 50c.c. benzene were added and the mixture allowed to stand for twenty four hours. Orange needles were deposited, m.p.306°, with decomposition.

Yield - 0.2gms.

Analysis	Calculated (for $C_{14}H_{12}N_4$) 3-amidino-5-aminoacridine	Found
%C	71.2	71.4
%H	5.1	5.3
%N	23.7	23.4

Note - It was found that no red dye could be obtained on treating the diazotised amino-amidinoacridine with β-naphthol under normal conditions.

6. PREPARATION OF 3-CYANOACRIDINE

0.3gms. potassium hydroxide were dissolved in 0.5c.c. water and diluted with 20c.c. alcohol. This solution was added to a suspension of 1.0gm. 3-cyano-5-chloroacridine in 5c.c. benzene and the solution shaken till a clear yellow solution was obtained. This solution took several hours to accomplish. About 0.7gms. Raney-nickel catalyst were added and the air in the flask replaced by hydrogen. Shaking was resumed until no more hydrogen was taken up (about twenty four hours). Air was admitted and the mixture warmed on a water-bath to dissolve the precipitate of acridan; the pyrophoric nickel was then filtered and extracted with 3 volumes of 5c.c. boiling alcohol.

The combined filtrates were taken to dryness, the residue washed twice with 10c.c. water at 80° to remove inorganic material and the residue added to 120c.c. boiling water containing 5c.c. 20% sulphuric acid. Mechanical stirring was begun and 0.8gms. potassium dichromate (one equivalent) dissolved in 6c.c. boiling water were added in two equal portions, five minutes apart. After ten minutes further boiling and stirring, 1.6gms. potassium dichromate in 11c.c. water were added to precipitate the cyanoacridine as its insoluble dichromate; boiling was continued for five minutes more and the mixture cooled and refrigerated overnight.

The precipitate was then filtered, suspended in 30c.c. hot water, treated with 7c.c. concentrated ammonia solution, brought just to the boil, cooled, filtered and the residue washed with water. The precipitate was dissolved in two equivalents of hot dilute hydrochloric acid and filtered from a trace of acridan. The acridine was precipitated with ammonia and dried.

The product was purified by vacuum sublimation at 200° and yellow needles, m.p.204°, were obtained.
Yield - 0.1gm. (12% theor.).

Analysis	Calculated (for $C_{14}H_8N_2$)	Found
%C	82.3	82.3
%H	3.9	4.0
%N	13.7	13.7

The experiment was repeated using palladium-calcium carbonate catalyst as described for 1-cyanoacridine on page 92 . By this method, a 75% yield of 3-cyanoacridine was obtained.

7. PREPARATION OF 3-CYANO-5-ETHOXYACRIDINE

0.5gm. 3-cyano-5-chloroacridine were added to a solution containing 0.3gm. potassium hydroxide in 20c.c. alcohol and the solution gently warmed. The solution became bright yellow and a white crystalline precipitate was deposited which was filtered and found to be potassium chloride. The filtrate was evaporated to dryness and recrystallised from

aqueous alcohol as yellow needles, m.p.200°.

Yield - 0.35gms.

Analysis	Calculated (for $C_{16}H_{12}ON_2$)	Found
%C	77.2	76.8
%H	4.8	4.8
%N	11.3	11.5

[illegible] sulphuric acid and a solution of [illegible] ...p.gr.1.5) in sulphuric acid (...c.c. ...ng at such a speed that the reaction ... ...s 50-55°). After some hours, the ... ...ess of ice and the pale pink pre... ... and dissolved in three litres of boil... ...solution was allowed to cool to 40° and the ... ...-chloro-5-nitrobenzoic acid, m.p.164° (...). ...ed and washed with water. ...Yield ...gms.

Preparation of 2-chloro-5-nitrobenzoic acid

...gms. of 2-chloro-5-nitrobenzoic acid were dissolved ... ...minimum quantity of hot dilute ammonia and poured ... ...boiling solution of seven molecular equivalents of ...

SYNTHESIS OF 3-CYANOACRIDINE DERIVATIVES FROM 2-CHLORO-5-CYANOBENZOIC ACID

1. PREPARATION OF 2-CHLORO-5-CYANOBENZOIC ACID

Preparation of 2-chloro-5-nitrobenzoic acid

Reference - Goldberg & Kelly (78)

(cf. Hubner, Annalen (88)).

100gms. of o-chlorobenzoic acid were dissolved in 300c.c. sulphuric acid and a solution of nitric acid (32c.c; sp.gr.1.5) in sulphuric acid (68c.c.) added with stirring at such a speed that the reaction mixture maintained itself at 50-55°. After some hours, the mixture was added to an excess of ice and the pale pink precipitate collected, washed and dissolved in three litres of boiling water. The solution was allowed to come to 40° and the crystals of 2-chloro-5-nitrobenzoic acid, m.p.164° (m.p.Lit.- 165°) collected and washed with water.

Yield = 90gms.

Preparation of 2-chloro-5-cyanobenzoic acid

56gms.of 2-chloro-5-nitrobenzoic acid were dissolved in the minimum quantity of hot dilute ammonia and poured into a boiling solution of seven molecular equivalents of ferrous sulphate (485gms.) in one litre of water.

The solution was treated with small portions of concentrated ammonia, each addition being followed by

vigorous agitation of the mixture. When the solution was distinctly alkaline and a deep brown residue had formed it was boiled for five minutes, care being taken that the solution was still alkaline at the end of that time, and the product filtered hot to give an amber-coloured solution.

30c.c. concentrated hydrochloric acid were added to the above solution and the whole cooled to 0°. 50gms. of ice were added and 12gms. sodium nitrite in 50c.c. water run in with stirring, the end-point being obtained with starch potassium iodide paper and the solution allowed to stand for fifteen minutes at 0°. A small brown precipitate had formed and this was filtered to give an amber-coloured filtrate which gave a red dye with alkaline /β-naphthol.

40gms. hydrated copper sulphate and 10gms. sodium chloride were heated to boiling and a solution containing 8gms. sodium bisulphite and 6gms. sodium hydroxide in 100c.c. boiling water added over five minutes. The chloride was allowed to settle to room temperature and washed by decantation after which excess (55gms.) potassium cyanide was added and the solution (300c.c.) heated to boiling. The diazo solution was gradually added to this, the temperature being kept above 90°, when a very dark brown solution was obtained with a brisk evolution of nitrogen.

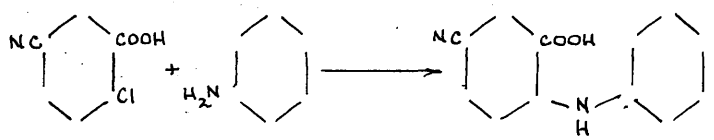
The cooled alkaline solution was treated with excess

concentrated hydrochloric acid and the residue filtered off. The filtrate was extracted with ether and the dried ethereal extract evaporated to dryness. The combined residues were dissolved in the minimum quantity of boiling water to remove any copper salts and the whole filtered. The cyano-chloro-benzoic acid crystallised out on cooling and was filtered off. As the acid has some solubility in water, the filtrate was extracted with ether and the ether extract evaporated to dryness. Cream coloured needles were obtained, m.p.176°

Analysis	Calculated (for $C_8H_4O_2NCl$)	Found
%C	53.0	52.5
%H	2.2	2.4
%N	7.8	7.9
%Cl	19.3	19.0

Yield - 29gms.

2. PREPARATION OF 4-CYANO-DIPHENYLAMINE-2-CARBOXYLIC ACID



4.3gms. of 2-chloro-5-cyanobenzoic acid and 1.0gms. potassium carbonate were refluxed in 20c.c. amyl alcohol till a thermometer held in the vapour read 130° and all the water formed had been removed. The temperature of

the solution was then allowed to drop to 100° and 5.5gms. of aniline added with 0.1gm. of copper. The whole was allowed to reflux in an oil-bath at 180° for four and a half hours and the product steam-distilled to remove the amyl alcohol and any excess aniline.

The solution was then made alkaline with ammonia, animal charcoal added and the whole filtered. The charcoal and some black tar remained on the filter-paper, the filtrate being deep-brown in colour. The filtrate was allowed to cool, 5c.c. of acetone were added, followed by concentrated hydrochloric acid to distinct acidity and a dark brown tar was deposited. This solidified on standing and was filtered. The residue was dissolved in sodium carbonate solution, boiled with a little animal charcoal, filtered and the filtrate re-acidified with concentrated hydrochloric acid. A dark brown tarry solid was depositied, which became quite hard on standing. This was filtered off and dissolved in the minimum quantity of hot acetone. Hot water was added to the hot solution till it became opalescent and the whole cooled in a refrigerator. Small yellow needles were obtained, m.p.170-173°.

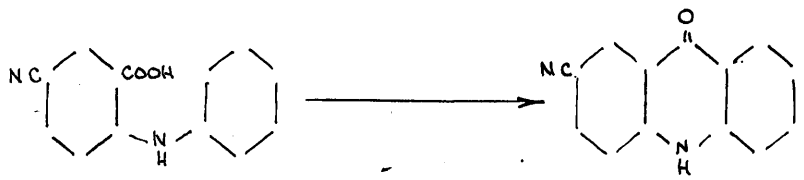
The crystals were redissolved in the minimum amount of acetone, boiled with a little animal charcoal, filtered and hot water added to the filtrate. On standing, yellow

needles were formed which melted sharply at 170°.

Analysis	Calculated (for $C_{14}H_{10}O_2N_2$)	Found
%C	70.6	70.4
%H	4.2	4.3
%N	11.8	11.7

Yield - 2gms.

3. PREPARATION OF 3-CYANOACRIDONE



1gm. 4-cyano-diphenylamine-2-carboxylic acid was refluxed with 2c.c. of phosphorus oxychloride for half-an-hour and the excess phosphorus oxychloride then removed by vacuum distillation. The product was boiled with water for half-an-hour and a dark green precipitate obtained which was filtered, dried and purified by sublimation at atmospheric pressure, to give a bright yellow sublimate which did not melt below 360° but charred about this temperature. It was sparingly soluble in most organic solvents but imparted a green-yellow fluorescence to the alcoholic solution.

Analysis	Calculated (for $C_{14}H_8ON_2$)	Found
%C	76.7	77.0
%H	3.6	3.7
%N	12.8	12.8

Yield -.7gms.

4. PREPARATION OF 3-CYANO-5-CHLOROACRIDINE

0.5gms. 3-cyanoacridone were treated with 2c.c. phosphorus oxychloride as described on page 89 . The yellow solid obtained on evaporating the chloroform extract to dryness was purified by vacuum sublimation at 200° to give yellow needles, m.p.183°. A mixed m.p. with 3-cyano-5-chloroacridine prepared from 4-aminobenzonitrile showed no depression.

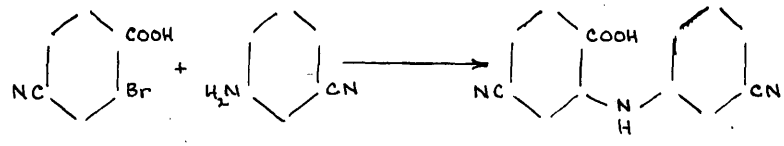
Yield - 0.45gms.

Analysis	Calculated (for $C_{14}H_7N_2Cl$)	Found
%C	70.4	70.2
%H	2.9	3.1
%N	11.7	11.6
%Cl	14.9	15.0

SYNTHESES OF 2,6- and 2,8-DICYANOACRIDINES

SYNTHESES OF 2-8-DICYANO- AND 2-6-DICYANOACRIDINE DERIVATIVES FROM 2-BROMO-4-CYANOBENZOIC ACID AND 3-AMINO-BENZONITRILE

1. PREPARATION OF 2'-5-DICYANO-DIPHENYLAMINE-2-CARBOXYLIC ACID.



5.0gms. 2-bromo-4-cyanobenzoic acid, 30c.c. amyl alcohol, 5.0gms. potassium carbonate and 5.0gms. 3-amino-benzonitrile were heated to 100°. 0.2gms. copper powder were then added and the mixture refluxed on an oil-bath at 100° for five hours. At the end of this time, the amyl alcohol was removed by steam-distillation and the residue made alkaline with ammonia, boiled with animal charcoal, filtered and 5c.c. acetone added to the cooled amber filtrate.

On acidifying the filtrate with concentrated hydrochloric acid, a green-yellow solid was obtained. The product was purified by dissolving it in the minimum amount of hot acetone with animal charcoal, filtering the hot solution and adding hot water to the filtrate till it became opalescent. On cooling, greenish-yellow needles, m.p.228° were obtained.

Yield - 2.8gms.

Analysis	Calculated (for $C_{15}H_9O_2N_3$)	Found
%C	68.4	68.2
%H	3.4	3.8
%N	16.0	15.7

2. PREPARATION OF 2-8- AND 2-6-DICYANOACRIDONE

2.0gms. 2'-5-dicyano-diphenylamine-2-carboxylic acid were refluxed with 4.0c.c. phosphorus oxychloride for thirty minutes after which the excess phosphorus oxychloride was removed by vacuum distillation. The product was boiled with water for thirty minutes and a dark residue obtained. On sublimation yellow-orange needles, m.p.290-310° with charring in a sealed tube were obtained.

Analysis	Calculated (for $C_{15}H_7ON_3$)	Found
%C	72.1	72.2
%H	2.9	3.0
%N	18.0	17.9

Yield - before sublimation - 1.5gms.

- after sublimation - 1.1gms.

Note - 0.5gms. 2'-5-dicyanodiphenylamine-2-carboxylic acid were treated with hydrofluoric acid in a platinum basin and the hydrofluoric acid allowed to evaporate at room temperature. A bright yellow product, m.p.248-249°, was obtained, soluble in hot alcohol and acetone. The material did not fluoresce even under U.V. light.

Analysis	Calculated (for $C_{15}H_7ON_3$)	Found
%C	72.1	70.9
%H	2.9	3.7
%N	18.0	16.8

The nature of the product was not investigated.

3. PREPARATION OF 2.8- AND 2.6-DICYANO-5-CHLOROACRIDINE

1.0gm. of the above mixture of isomeric dicyano acridones was refluxed with 4c.c. phosphorus oxychloride for thirty minutes at 145° and the product extracted as on page 89 . The product was purified by vacuum sublimation at 200° to give yellow needles, m.p.245-265°.

Yield - 0.7gms.

Analysis	Calculated (for $C_{15}H_6N_3Cl$)	Found
%C	68.4	68.3
%H	2.3	2.5
%N	16.0	15.7
%Cl	13.5	13.7

4. SEPARATION OF 2-8-DICYANO AND 2-6-DICYANOACRIDONE ISOMERS

Chromatographic separation

Some nitrobenzene was allowed to stand over some sodium and the dried product vacuum distilled. A column was filled with chromatographic alumina to a depth of 4-5 inches.

0.5gms. of the acridones were dissolved in 30c.c. dried nitrobenzene and the solution slowly added to the heated column (diagram of apparatus on page 116) under slight

vacuum. An orange band was formed at the surface of the alumina and a broad yellow band below it. On the addition of a further 50c.c. hot nitrobenzene the yellow band moved half way down the column, the orange band widening to about half an inch.

Dry, hot nitrobenzene was added till the yellow band neared the foot of the column when the washings were collected in a flask till the yellow band had been discharged from the column.

The yellow filtrate was removed and a fresh flask placed under the column, and hot nitrobenzene passed through till the washings were about 50c.c. in volume. By this time the orange band was two inches long.

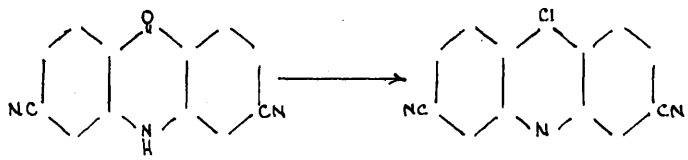
Both filtrates were evaporated to dryness and the alumina carefully pushed out of the tube on to watch-glass and cut half an inch below the beginning of the orange band to obtain a third fraction. The alumina containing the orange material was dried at 120° and the product eluted, boiled with 15c.c. boiling alcohol, giving an orange solution.

The residue from the first fraction charred above 300° but did not melt below 360°. The second fraction gave similar results.

The third fraction melted with charring at 300°.

Residue from first evaporation.....0.35gms.
Residue from second evaporation.....0.05gms.
Residue adsorbed in orange band.....0.07gms.

5. PREPARATION OF CYANO-5-CHLOROACRIDINE FROM FIRST FRACTION

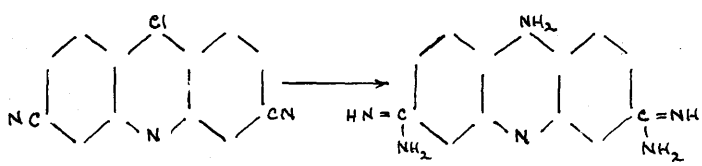


0.3gms. of the acridone obtained from the first fraction were refluxed with 5c.c. phosphorus oxychloride at 145° for half-an-hour. The product was extracted as described on page 89 , and purified by vacuum sublimation at 200°, giving yellow needles, m.p.271°.

Analysis	Calculated (for $C_{15}H_6N_3Cl$)	Found
%C	68.4	68.2
%H	2.3	2.0
%N	16.0	16.2
%Cl	13.5	13.8

Yield - 0.25gms.

6. PREPARATION OF DIAMIDINO-5-AMINOACRIDINE FROM FIRST FRACTION (i.e.from 2:8-dicyano isomer).



0.2gms. of the above isomer were placed in a flask with 5c.c. magnesium dried alcohol and 10c.c. dry chloroform and dry hydrogen chloride passed into the ice-cooled solution as in diagram on page 91 .

The solution became bright yellow and a bright yellow precipitate was deposited. On further passage of the gas, the precipitate redissolved. After eighteen hours the apparatus was dismantled and the reaction vessel sealed with screw-clips and allowed to stand at room temperature for twenty four hours.

At the end of this time it was observed that some needles had been deposited. The mixture was evaporated to dryness under vacuum at 45° and the product added to a solution containing 50c.c. sodium dried alcohol which had been saturated with dry ammonia at 0°. The whole was placed in a pressure bottle, sealed and heated to 45° in a water-bath for twenty four hours.

Orange-yellow needles were deposited at the end of this time. The pressure bottle was removed from the water-bath and allowed to cool to room temperature. It was then opened and the contents transferred to a flask where they were evaporated to dryness under vacuum at 45°.

The residue was shaken with 100c.c. water till almost complete solution had been effected and filtered. Excess solid ammonium nitrate was added to the filtrate and yellow needles were precipitated. These were filtered and the product dried at room temperature, giving a yellow powder which did not melt below 300°.

The nitrate obtained above was shaken with excess alcoholic potassium hydroxide, the solution filtered from a

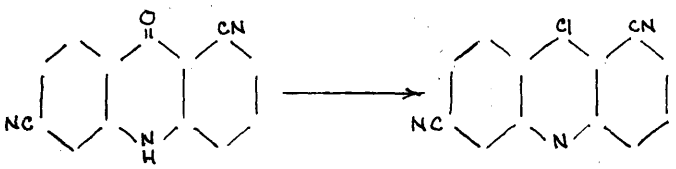
small amount of undissolved material and the bright yellow solution, fluorescing yellow-green, evaporated to half its bulk in a vacuum desiccator at room temperature. 50c.c. benzene were added and the orange needles deposited on standing were filtered and the dry residue washed with two 20c.c. portions of water to remove inorganic material. Orange needles, m.p.336°, with charring (in a sealed tube) were obtained.

Yield - 0.1gms.

Analysis	Calculated (for $C_{15}H_{14}N_6$)	Found
%C	64.9	65.1
%H	5.0	5.3
%N	30.2	29.9

The analysis calculated for cyano-amidino-aminoacridine was 68.9%C, 4.2%H, 26.8%N.

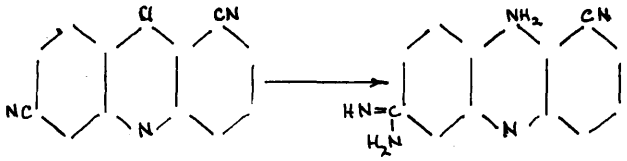
7. PREPARATION OF CYANO-CHLOROACRIDINE FROM THIRD FRACTION



0.07gms. of the fraction obtained from the orange band in the isomeric separation were treated with 2c.c. phosphorus oxychloride, refluxed for thirty minutes, the excess solvent removed by vacuum distillation and the product extracted as described on page 89 . The yellow-orange needles obtained on vacuum sublimation at 220° had a m.p.249°.

Yield - 0.05gms.

8. PREPARATION OF AMIDINO-CYANO-AMINOACRIDINE FROM THIRD FRACTION (i.e.2:6-dicyano isomer)



0.05gms. of the above compound were placed in a flask with 2c.c. magnesium dried alcohol and 5c.c. dry chloroform and dry hydrogen chloride passed into the ice-cooled solution as described for the other isomer.

The product again was precipitated as its insoluble nitrate from which the free base was liberated by alcoholic potassium hydroxide and precipitated by benzene as orange needles, m.p.320-325°, in a sealed tube, with decomposition.

Analysis	Calculated (for $C_{15}H_{11}N_5$)	Found
%C	68.9	68.6
%H	4.2	4.4
%N	26.8	26.4

A P P E N D I X

PREPARATION OF CATALYSTS

PREPARATION OF RANEY NICKEL

Reference - Gilman (70).

Method - The starting material for this preparation is an alloy containing equal weights of nickel and aluminium corresponding to the formula $NiAl_2$. A suitable procedure for the preparation of the catalyst from the alloy is described by Mozingo, Org. Synthesis, 21 (1941), p.15.

A solution containing 38gms. sodium hydroxide in 150c.c. of distilled water contained in a 500c.c. beaker equipped with a stirrer was cooled in an ice-bath to 10° and 30gms. $NiAl_2$ added in small portions such that the temperature did not rise above 25°, the beaker being allowed to remain in the ice-bath. When all the alloy had been added stirring was stopped and the beaker allowed to come to room temperature. After the evolution of hydrogen became slow the reaction mixture was allowed to stand on a steam-bath till the evolution of hydrogen again becomes slow. Meanwhile, the volume of the solution was maintained constant by the addition of distilled water.

After heating, the nickel was allowed to settle and most of the liquid decanted. Distilled water was added to the original volume and the process was repeated. This was again repeated. A solution of 5gms. sodium hydroxide in

50c.c. water (distilled) was then added and the catalyst suspended and allowed to settle. The alkali was then decanted. The nickel was washed by suspension in distilled water till the washings were neutral to litmus and then ten times more to remove the alkali completely. The washing process was then repeated three times with 20c.c. 90% alcohol and three times with absolute alcohol and the catalyst was then stored under alcohol in a bottle which was completely filled with absolute alcohol and tightly closed. The product was highly pyrophoric. The Raney nickel contained in the suspension weighed 15gms. Raney nickel in alcohol contains about 0.6gms. of catalyst per c.c. of settled material. The catalyst so prepared can be kept for up to six months.

PREPARATION OF PALLADIUM-CALCIUM CARBONATE CATALYST

Reference - Gilman, Org.Synthesis (71) and Busch and Stove (39).

Method - According to the method of Busch and Stove, calcium carbonate prepared by treatment of a hot solution of calcium chloride with sodium carbonate, is suspended in water and a solution of palladous chloride added. The mixture is gently warmed until the palladium is deposited on the carbonate as palladous hydroxide and the catalyst is washed a few times with distilled water by decantation. It is then filtered, washed on the filter until free of chloride, using as little water as possible. Reduction of the palladous hydroxide takes place during hydrogenation.

DRYING OF SOLVENTS

Alcohol

References - Lund, Bjerrum (110b), Terentiev (154C), Evans, Fetsch (61b).

The specific gravity of 1000c.c. alcohol was found by hydrometer to be equivalent to 98.6% by weight. 15gms. sodium were added to the alcohol and when complete solution had been effected, 800c.c. of the alcohol were distilled, sp.gr.0.795 (equivalent to 99.6% alcohol). This alcohol was sufficiently dry for the ammonolysis of the iminoethers.

5gms. magnesium ribbon was cleaned with emery paper and cut into half-inch lengths. After drying at 120°, it was placed in a flask with 1.0gms. iodine and 70c.c. sodium dried alcohol. Hydrogen was evolved and the mixture was refluxed on a water-bath till all the magnesium had been converted to the ethoxide. 600c.c. of sodium dried alcohol was then added and, after an hour's reflux, the mixture was distilled and the first 500c.c. collected.

Dry ground-glass apparatus was used throughout, precautions being taken for the exclusion of moisture.

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

Ether

Ether was treated consecutively with four 30gm. samples of anhydrous calcium chloride. The solvent so obtained was further dried by the addition of 20gms. sodium wire, the solution being cooled in an ice-hydrochloric acid freezing mixture. The ether was distilled over fresh sodium and stored in a dark bottle and the stopper sealed with wax. Ground-glass apparatus was used.

Peroxides were eliminated with ferrous sulphate.

Chloroform

Chloroform was treated at intervals of twenty-four hours with four quantities of anhydrous calcium chloride and, finally, dried with phosphorus pentoxide. It was then distilled in ground-glass apparatus and stored in a dark bottle whose stopper was sealed with wax.

SUMMARY and CONCLUSIONS

In the course of the fore-going experimental preparations, several new compounds were prepared. These are listed below.

Since this work was completed, a paper has been published by Goldberg and Kelly (J.C.S.,1947,637) wherein some of these compounds were prepared. The m.ps. obtained by these workers are given in brackets after those obtained by the author.

Compound	M.P. (uncorrected)
5-chloroacridine metho-4-toluene sulphonate	82°
5-cyanoacridine methosulphate	156°
5-amidinoacridine	226°
2'-cyanodiphenylamine-2-carboxylic acid	215-216°
1-cyanoacridone	266°
1-cyano-5-chloroacridine	158°
1-cyanoacridine	156°
1-cyano-5-ethoxyacridine	130°
2-bromo-3-cyanobenzoic acid	184°
6-cyanodiphenylamine-2-carboxylic acid	184°
3'-cyanodiphenylamine-2-carboxylic acid	205°(204-205°)
2-cyano-5-chloroacridine	193° (193°)
4-cyano-5-chloroacridine	175-178°
2-bromo-4-cyanobenzoic acid	193°
5-cyanodiphenylamine-2-carboxylic acid	218°
2-cyanoacridone	charred 330°
2-amidino-5-aminoacridine	304° (305°)
2-cyanoacridine	191°
diphenylamine-2,3-dicarboxylic acid	178°
3-cyano-5-chloroacridine	183° (186°)
3-amidino-5-aminoacridine	306° (313°)
3-cyanoacridine	204°
3-cyano-5-ethoxyacridine	200°
2-chloro-5-cyanobenzoic acid	176° (178°)
4-cyanodiphenylamine-2-carboxylic acid	176° (220°)
3-cyanoacridone	charred 330°
2'-5-dicyanodiphenylamine-2-carboxylic acid	228°
2,8-dicyano-5-chloroacridine	271°
2,8-diamidino-5-aminoacridine	336°
2,6-dicyano-5-chloroacridine	249°
2-amidino-5-amino-6-cyanoacridine	320-325°

In the preparation of cyanoacridines from compounds containing a preformed acridine nucleus, it is apparent that although diazotisation of 2,8-diaminoacridine can readily be accomplished, the conversion of the diazo compound to the nitrile by the Sandmeyer reaction is not possible under the conditions investigated. Only an oxygen-containing compound is obtained in each case.

On diazotisation in dilute mineral acid only one amino group is attacked. Tetrazotisation can be effected however in concentrated acid solution.

5-Cyanoacridine, owing to the unique properties of a substituent in this position, can be prepared using a preformed acridine nucleus, either from acridine itself of its 5-methyl homologue.

It does not appear possible, however, to prepare 5-cyanoacridine from 5-chloroacridine without recourse to pressure. In the conversion of acridine to 5-cyanoacridine, an intermediate dihydroacridine is first formed and the failure of the 5-chloro compound to be similarly converted to the cyanoacridine may be due to the fact that the intermediate dihydrochloro compound is not formed under these conditions.

The cyanoacridines, with the exception of the 5-cyano isomer, can readily be prepared from cyano-halogenobenzoic acids and aniline or halogenobenzoic acids and aminobenzo-

nitriles, which undergo the Ullmann condensation to the corresponding cyanodiphenylamine-2-carboxylic acids which can then be cyclised to cyanoacridones.

Better yields of cyanodiphenylamine derivatives are obtained from aminobenzonitriles and 2-chlorobenzoic acid rather than from cyano-halogenobenzoic acids and aniline, the latter acids showing considerable tendency to hydrolyse to tarry products during the condensation. These results are in disagreement with the findings of Goldberg and Kelly (loc.cit.) who were unable to prepare cyanodiphenylamine derivatives from 2- and 4-aminobenzonitriles by this method, though their general method of preparation appears to have been similar to that employed in this thesis. They did prepare a cyanodiphenylamine-2-carboxylic acid from 3-amino-benzonitrile, but only in very low yield.

In this condensation, the position of the substituents in the rings does not appear to be critical. For optimum yields, however, a higher temperature of reflux is desirable for the condensation of halogeno-benzoic acid and amino-benzonitriles.

The deactivating properties of the mono-cyano group do not appear to be sufficiently strong to inhibit this condensation though in the formation of dicyanodiphenylamines a lower yield was obtained, probably due to inactivation of the nuclei.

These diphenylamine derivatives give intense crimson or violet colours with chromic acid and their possible use as redox indicators perhaps merits further study.

Cyclisation of the diphenylamine derivative obtained from 3-aminobenzonitrile gives rise to two isomers, 2- and 4-cyano-acridones.

These are most readily separated using chromatographic techniques by passing a hot nitrobenzene solution through an alumina column. The 4-isomer is absorbed on the column much more strongly than the 2-isomer which passes down the column fairly rapidly.

It should be noted that the ratio of isomers produced appears to be independent of the positive or negative groupings on the arylamine nucleus. Ortho closure (i.e.4-substituted acridones) predominates with nitro derivatives (negative) and chloro an methyl derivatives (positive) while para closure (i.e. 2-substituted acridones) predominates with cyano derivatives (negative) and amino derivatives (positive).

The cyano-5-chloroacridines are very unstable on keeping, decomposing to reddish-brown compounds from which only cyano-acridones can be isolated. Light accelerates this decomposition which appears to be independent of the purity of the sample. The position of the cyano groups in the acridine nucleus does appear to affect the rate of decomposition.

The cyano-5-chloroacridines can be converted to cyano-5-ethoxy compounds by the action of alcohol and potassium hydroxide. Freshly prepared 5-chloroacridine and alcohol, however, do not react, though material which has been kept for some time can be converted to 5-ethoxyacridine in moderate yield by refluxing with alcohol.

It is thought that the presence of potassium hydroxide catalyses the formation of a reactive complex which is also formed by the slow decomposition of the 5-chloro compound on keeping. This complex is converted to the 5-ethoxy compound. It is noted that the formation of these ethoxyacridines is always accompanied by some acridone formation as might be expected from a reaction of this type.

Reduction of the insoluble cyanoacridones to cyano-acridines is not possible since the treatment necessary (sodium and amyl alcohol) appears to attack the cyano group. More gentle reduction, however, of the cyano-5-chloroacridines with hydrogen and Raney nickel or better palladium calcium carbonate catalysts leaves the cyano group unattacked. Gentle oxidation of the dihydro compounds so obtained with potassium dichromate readily forms the cyanoacridines. The cyano-5-chloroacridines are much less readily reduced by this method than 5-chloroacridine itself, probably due to the deactivating nature of the cyano groups.

While 2- and 3-amidinoacridines can be prepared by the Pinner method via the imino-ether, it was found impossible to prepare 5-, 4- and 1-amidinoacridines by this method. As position 1- is, electronically, similar to position 3- and position 2- similar to position 4-, this selective amidine formation cannot be due to the differing activities at these loci but must rather be a manifestation of the steric hindrance obtained in the conversion of nitriles to imino-ethers when another substituent, here a benzene or pyridine nucleus, is located ortho to the cyano group. This hindrance is supposedly purely geometrical in origin and according to Huckel can be overcome if sufficient activation energy is supplied to allow of orientation of the hindered grouping. The extent of the orientation measured by a (where K, the velocity constant, $=ae^{-q/RT}$ where q is the activation energy) depends as a function of temperature upon the energy of orientation which may rest upon various causes.

In the case of imino ether formation, however, since the temperature had to be maintained below 80° to avoid decomposition of the product, sufficient activation energy in the form of heat could not be supplied to overcome the steric hindrance, i.e. to reorientate the nitrile grouping sufficiently for reaction to ensue.

Pinner's original postulation of the ortho effect ,

i.e. that ortho-substituted nitriles will not form iminoethers, appears to have been accepted by later workers without further investigation into the mechanism of this process.

Huckel's expression is a quantitative statement of the phenomenon rather than an elucidation of its nature and the mechanism of the inhibition is obviously one which requires further fundamental investigation.

This preferential amidine formation was used as a means of identifying the two isomers obtained from 2-bromo-4--cyanobenzoic acid and 3-aminobenzonitrile, i.e.2,6- and 2,8-dicyanoacridones, as 2,8-dicyanoacridone forms a diamidine by this method whereas the 2,6-isomer forms a monoamidine.

Chemotherapeutic Action

Bacteriological tests on 3-amidino-5-aminoacridine and 2-amidino-5-aminoacridine were performed by Goldberg and Kelly (loc.cit.) who showed that the introduction of the amidine group in these positions conferred no increased bacteriostatic effect on the acridine nucleus.

This lack of enhanced activity is analogous to the results obtained with sulphanilamide-acridines. The antibacterial activity of a molecule does not appear to be a summation of the activities of the individual groupings in the molecule

but is rather a specific function of the molecule as an entity.

This is in accord with the modern theory (Sevag and co-workers) that antibacterial action depends on the ability of the drug, due to its particular characteristics, to interfere with the prosthetic groups of a specific respiratory enzyme system.

B I B L I O G R A P H Y

(1) Adkins and Coonradt, 1941,63,1563.
(2) Albert and Large, 1940,142,435 and J.C.S.,1941,121.
(3) Albert and Linnell, J.S.C.I., 1945,64,171.
(4) Albert and Ritchie, J.C.S., 1943,458.
(5) Albert and Goldacre, J.C.S., 1943,454.
(6) Albert and co-workers, J.C.S., 1943,484.
(7) Albert, Brit.J.Exptl.Path., 1938,19,41.
(8) Albert, Med.J.Australia, 1944,245.
(9) Albert and Gedhill,J.S.C.I., 1945,64,177.
(10) Albert and Linnell, J.S.C.I., 1936,55,54.
(11) Albert and Linnell, J.C.S., 1936,89.
(11b) Albert and Linnell, J.C.S., 1938,22.
(12) Albert and Ritchie, J.S.C.I., 1941,60,120.
(13) Albert and Gedhill, J.S.C.I., 1942,61,159.
(14) Albert and Willis, J.S.C.I., 1946,65,26.
(15) Ashley and co-workers, J.C.S., 1944,103.
(16) Ashley and Ewins, Brit.Patent,545,708 (1943).
(17) Ashley and co-workers, J.C.S., 1942,103.
(18) Aston, J.A.C.S., 1931,53,1448.
(19) Baeyer and Villiger, Ber., 1904,37,3200.
(20) Bamberger, Ber., 1927,60,319.
(21) Bamberger and Demuth, Ber., 1901,34,1330.
(22) Bamberger, Ber., 1902,35,3706.
(23) Bamberger, Ber., 1902,35,3719.
(24) Barber and Stickings, J.C.S., 1945,167.
(24b) Bradbury and Linnell, Quart.J.Phar.Pharmacol., 1941,15,31.
(25) Beard and Hodgson, J.C.S., 1944,4.
(26) Beilstein and Kuhlberg, Ann., 1868,146,336.
(27) Beilstein and Wilbrand, Ann., 1863,128,256.
(28) Benda, Ber., 1912,45,1789.
(29) Bernthsen, Ann., 1884,224,1.
(30) Beyer, J.C.S., 1944,360.
(31) Blanksma, Chem.Centr., 1910,I,260.
(32) Bogert and Hand, J.A.C.S., 1902,24,1035.
(33) Bogert and Beans, J.A.C.S., 1904,26,468.
(33b) Bogert and Hirschfelder, Coll.Trav.Chim.Czeckoslav., 1930,5/6,382.
(34) Bogert and Boroschek, J.A.C.S., 1901,23,746.
(35) Braz and Gortinskaya, J.Gen.Chem., U.S.S.R., 1940,10,1151.
(36) Browning and Gilmour, J.Path.Bact., 1914,18,144.
(37) Browning and co-workers, Proc.Roy.Soc.,Ser.B.,1922, 93,329.
(38) Browning and Gulbransen, Proc.Roy.Soc., London (B), 1918,90,136.

(39) Busch and Stove, Ber.,1916,49,1063.
(40) Cherouis and Koeck, J.Chem.Ed.,1943,488,20.
(41) Christiansen, J.A.C.S.,1923,45,2193.
(42) Clar, Ber.,1939,72,1645.
(43) Clemo,Perkin and Robinson, J.C.S.,1924,1751.
(44) Cohen and McCandlish, J.C.S.,1905,87,1271.
(45) Cohen, Monatsh.,1901,22,473.
(46) Dale, Brit.Med.J.,1943,2,411.
(47) Dietz, Ber.,1878,11,1485.
(48) Dietz and Pinner, Ber.,1890,23,2942.
(49) Drozdov, J.Gen.Chem., U.S.S.R.,1938,8,1505.
(50) Drozdov, J.Gen.Chem., U.S.S.R.,1939,9,1373.
(51) Dyson, A New Notation and Enumeration System for Organic Compounds , Longmans,1947.
(52) D.R.P.67126.
(53) D.R.P.258560.
(54) D.R.P.86874 (Geigy's Patent).
(55) D.R.P.107505 (Jansen's Patent).
(56) Easson and Pyman, J.C.S.,1931,2999.
(57) Eggerth, J.Infect.Dis.,1926,38,440.
(58) Eisleb, Chem.Centr.,1937,I,604.
(59) Eisleb, Med. u Chem., Abhandl.med-chem.Forschungsstatten, I.G. Farbenind.,1936,3,41.
(60) Engler, Ann.,1869,149,297.
(61) Ewins, J.C.S., 1944,352.
(61b) Evans and Fetsch, J.A.C.S.,1904,26,1158.
(62) Fischer and Schutte, Ber., 1893,26,3085.
(63) Fischer, Ber.,1895,28,1335.
(64) Fricke, Ber.,1874,7,1321.
(65) Friedlander, Ber.,1882,15,2573.
(65b) Faust, Ztschr.Chem.,1869,108.
(66) Gabriel, Ber.,1903,36,804.
(67) Gabriel and Herzberg, Ber.,1883,16,2004.
(68) Le Guyon, Comp.rend.soc.biol.,1937,126,1213.
(69) Gerchuk, Russ.,1938,53,169.
(70) Gilman, An Advanced Treatise on Organic Chemistry , 2nd Edition, Volume I, p.788.
(71) Gilman, as above, p.787.
(72) Glev and Nitzsche, J.prakt.Chem.,1939,153,200.
(73) Graebe and Caro, Ann.,1871,158,265.
(74) Graebe and Lagodzinski, Ann.,1893,276,48.
(75) Graebe, Ber.,1884,17,1370.
(76) Grandmougin and Smirous, Ber.,1913,46,3427.
(77) Goetchius and Lawrence, J.Lab.Clin.Med.,29,134.
(78) Goldberg and Kelly, J.C.S.,1946,107.
(79) Goldschmidt and Ingebrechtsen, Zeit.fur Phys.Chem., 48,455.
(80) Haas, J.Biol.Chem.,1944,55,321.
(81) Hawking, Ann.Trop.Med., 1938,32,313.

(82) Heathcote and Urquhart, J.Phar., and Exper.Therap., 1930,38,145.
(83) Hickinbottam, Reactions of Organic Chemistry , Longmans, 1936,317.
(84) Higginbotham, Hill and Short, J.C.S.,1937,264.
(85) Hodgson and Beard, J.C.S.,1927,20.
(86) Hofmann, Ber., 1868,1,1321.
(87) Hubner and Burghard, Ber., 1875,8,560.
(88) Hubner, Ann.,1883,222,195.
(89) Jensen and Friedrich, J.A.C.S.,1927,49,1049.
(90) Jensen and Rethwisch, J.A.C.S.,1928,50,1144.
(91) Jourdan, Ber.,1885,18,1444.
(92) Kaufmann and Vallette, Ber.,1912,45,1740.
(93) Knunyanto and Benevolenskaya, J.Gen.Chem., U.S.S.R., 1940,10,1415.
(94) Kranzlein, Ber.,1937,70,1785.
(95) Krafft and Stauffner, Ber.,1882,18,1729.
(96) Kunchell and Richartz, Ber.,1907,40,3394.
(97) Laurent, Ann.Chem.(Liebig),1842,41,110.
(98) Lemstedt and Dostal, Ber.,1939,72,806.
(99) Lehmstedt and Dostal, Ber.,1939,72,721.
(100) Lehmstedt, Ber.,1931,64,2384.
(101) Lehmstedt and Schrader, Ber.,1937,70,847.
(102) Lehmstedt and Wirth, Ber.,1928,61,2044.
(103) Lehmstedt and Hundertmark, Ber.,1929,62,414.
(104) Lehmstedt and Schrader, Ber.,1937,70,1526.
(105) Lehmstedt, Ber.,1938,71,1609.
(106) Lehmstedt, Ber.,1931,64,210.
(107) Leissert and Graebe, Ber.,1909,42,3712.
(108) Lund and Bjerrum, Ber.,1931,64,210.
(109) Lipschitz, Monatsch.Chem.,1900,21,787.
(110) Luchenbach, Ber.,1884,17,1428.
(111) Magidson and Grigorovskii, Ber.,1939,69,396.
(112) Magidson and Travin, Ber.,1939,69,537.
(113) Magidson and Travin, J.Gen.Chem.,U.S.S.R.,1941,11,243.
(114) Magidson, Ber.,1933,66,869.
(115) Matsumuru, J.A.C.S.,1939,61,2247.
(116) Matsumuru, J.A.C.S.,1938,60,591.
(117) Matsumuru, J.A.C.S.,1929,51,816.
(118) Meyer and Gross,Ber.,1902,32,2352.
(119) Meyer and Hofmann, Monatsh.,1916,37,681.
(120) Minaev and Ripper, Chem.Centr.,1924,I,905.
(120b) Miller, Ann.Chem.(Liebig),1881,208,223.
(121) McIlwain, Biochem.J.,1941,35,1311.
(121b) Mietzsch and Mauss, U.S.Patent,208217.
(122) Mohlau, Ber.,1883,19,2451.
(123) Mozingo, Organic Syntheses,1941,21,15.
(124) Mudd, J.Bact.,1945,49,527.
(125) Narishkin, Ukrainskii,Khem.Zhur.4,Sci.Pt.,1929,525.
(126) v.Oettingen, Therapeutic Agents of the Quinoline Group

(127) Organic Syntheses,Coll.Vol.I,2nd Ed.,New York(1941)p.5
(128) p.107.
(129) p.514.
(130) p.408.
(131) p. 56.
(131b) p.125.
(132) Organic Syntheses, Vol.22,p.5.
(133) Oxley and Short, J.C.S.,1946,147.
(134) Pinner, Ber.,1877,10,1889.
(135) Pinner, Ber.,1890,23,161.
(136) Pinnow and Muller, Ber.,1895,28,151.
(136b) Perkin and Clemo, Brit.Patent 214756.
(137) Porai-Koshitz, Auschkap and Amsler,J.Russ.Phy-Chem. Soc.,43,524.
(138) Raiford, Am.Chem.J.,1912,46,417.
(139) Ramart-Lucas, Grumez and Martynoff,Bull.Soc.Chim.8,228.
(140) Reed, J.C.S.,1945,186.
(141) Reed, J.C.S.,1944,676.
(142) Reed, J.C.S.,1944,425.
(143) Ring Index , Patterson and Capell, Reinhold Pub.Corp., New York,1940.
(144) Rosenmund and Struck, Ber.,1919,52,1749.
(145) Rupe, Ber.,1897,30,1099.
(146) Sandmeyer, Ber.,1885,18,1494.
(147) Samant, Ber.,1942,75,1008.
(148) Schiff, Ann.,1857,101,94.
(149) Schopff, Ber.,1885,18,1063.
(150) Semon and Craig, J.A.C.S.,1936,58,1278.
(151) Spalding, Moersch, Mosher and Whitmore, J.A.C.S., 1946,68,1596.
(152) Strain, Chromatographic Absorption Analysis , p.93, Interscience Pub.Inc., New York, 1942.
(153) Earl of Suffolk and Berkshire, Quart.J.Exptl.Physiol., 1939,29,1.
(154) Thomson, Brit.Patent 137214.
(154b) Tuttle, J.A.C.S,1923,45,1906.
(154c) Terentiev, Z.anorg.Chem.,1927,162,350.
(155) Ullmann and Marie, Ber.,1901,34,4307.
(156) Ullmann, Ber.,1903,36,1017.
(157) Ullmann and Baezner, Ber.,1902,35,2670.
(158) Ullmann and Ernst, Ber.,1906,39,298.
(158b) Ullmann, Ann.,1907,355,312.
(159) Ungnade, J.A.C.S.,1941,63,2091.
(160) Walther and Grossman, J.prakt.Chem.,1908,(2),78,478.
(161) Wibaut, Rec.trav.chim.,1913,32,452.
(162) Wohl, Ber.,1893,26,732.
(163) Yu,Magidson and Travin, Ber.,1936,69,537.
(164) Zinin,Berzelius Jahresber, 26,450.