THE PREPARATION AND PROPERTIES OF CERTAIN ACRIDINE DERIVATIVES OF CHEMOTHERAPEUTIC INTEREST.

by

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C O N T E N T S.

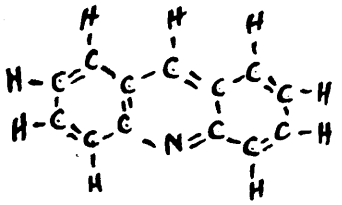
Page.

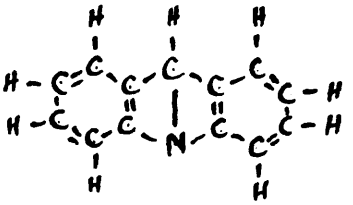
IV Preparation of Acridinium Compounds.

GENERAL NOTES.

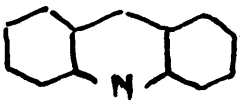
(1) Representation of the Acridine Molecule.

The full graphic formula of acridine is usually given in one or other of the following ways:-



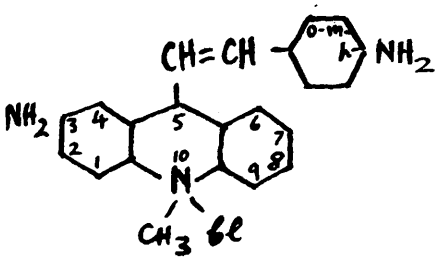


In general, however, throughout this thesis, the following contracted form has been used to represent the acridine molecule:-



(2) Nomenclature.

The following system has been adopted for naming derivatives of acridine and 5-styrylacridine:-



A quaternary acridinium salt has been referred to as (e.g.) a "methochloride" rather than a 10-methyl-acridinium

chloride. Thus the above compound has been named 3-amino-5-(p-aminostyryl)-acridine methochloride.

(3) References.

References to the literature are given by a number following the author's name e.g. Bernthsen (Ref.8), the key to the numbering being given in the List of References in the Preface.

Where an author's name appears without a reference number, this means that the number has already been given a little earlier in the text.

The following abbreviations have been used in the List of References:-

Ann.	Leibig's Annalen der Chemie.
Ber.	Berichte der Deutschen Chemischen Gesellschaft.
Brit. J. Exp. Path. ...	British Journal of Experimental Pathology.
J. Am. C.S.	Journal of the American Chemical Society.
J. C. S.	Journal of the Chemical Society.
J. Roy. Soc. Arts . ..	Journal of the Royal Society of Arts.
Proc. Roy. Soc.	Proceedings of the Royal Society.
Quart. J. Pharm.	Quarterly Journal of Pharmacy and Pharmacology.
Zeit. Farb. Ind.	Zeitschrift für Farben-Industrie.

The year of the publication, in brackets, follows the

abbreviated name of the journal.

(4) Preparations from the literature.

Three preparations involving little or no deviation from the description already given in the literature have been included for the sake of convenience, in the Experimental Section. The preparations in question are:-

Anhydrous stannous chloride reagent (page 114)
5-Methylacridine (page 135)
o-Chloroacetophenone (page 83)

The preparation and properties of a few other substances, already described in the literature, have been explored, improvements made, and/or new facts recorded. The substances in question are:-

5-Nitro-2-chloroacetophenone (page 88)
3-Nitro-5-methylacridine (page 93)
5-(p-Nitrostyryl)-acridine (page 137)
5-(p-Aminostyryl)-acridine (page 148)
5-(m-Nitrostyryl)-acridine (page 152)
5-(m-Aminostyryl)-acridine (page 158)
5-(p-Dimethylaminostyryl)-acridine (page 164).

The extent to which work on the above substances is claimed as original is clearly indicated in the text.

All other acridine compounds prepared and described in the Experimental Section are claimed as new.

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1. Thorp and Brunskill, J. Am. C. S., (1915), 37, 1258.
2. Ullmann and Ernst, Ber., (1906), 39, 298.
3. Aston and Ramsay, J. C. S. (1894), 167.
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21. Benda, Ber., (1912), 45, 1787.
22. Browning, Cohen, et al., Proc.Roy.Soc., B, (1926), 100, 293.
23. " " " " " " " " (1929), 105, 99.

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24. Browning, Cohen, et al., Proc.Roy.Soc., B, (1931), 109, 51.

25. " " " " " " " " (1932), 110, 249.

26. " " " " " " " " (1933), 113, 293.

27. " " " " " " " " (1933), 113, 300.

28. " " " " " " " " (1934), 115, 1.

29. Albert and Linnell, J. C. S., (1936), 89.

30. " " " J. C. S., (1936), 1614.

31. " " " J. C. S., (1938), 22.

32. Albert, Dyer, and Linnell, Quart.J.Pharm., (1937), 10, 649.

33. Albert, Francis, Garrod, and Linnell, Brit.J.Exp.Path., (1938), 19, 41.

34. Albert, Rubbo, and Goldacre, Nature, (1941), 147, 332.

INTRODUCTION.

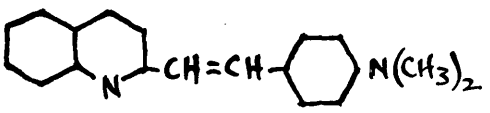
The relationship between the chemical structure of a compound and its therapeutic properties was not studied on a scientific basis until a little over thirty years ago when Ehrlich introduced the term "chemotherapy" to describe this new field of study and formulated its first broad hypotheses. Since the early work of Ehrlich a vast amount of research has been carried out in chemotherapy. In spite of this, the relationship between chemical structure and therapeutic properties, except for a few generalisations, still remains obscure, the chief difficulty being the lack of knowledge concerning the exact mechamism by which known drugs act. Nevertheless research on chemotherapy has produced many new drugs of outstanding value in the treatment of disease and will doubtless produce many more before it has unravelled even a fraction of the mysteries which at present prevent a complete understanding of the mode of action of drugs.

It is not intended to review in this Introduction the rise of chemotherapy from the experiments of Ehrlich on dye-stuffs and arsenicals, effective chiefly against trypanosomes and spirochaetes, to the triumphs of the last few years in the introduction of the sulphonamides, effective against bacterial infections, since this has been done on numerous occasions lately by lecturers before the various learned societies. In this connection the author has found the two lectures delivered

by Dr. G.S. Whitby (Ref.11) before the Royal Society of Arts, very useful and instructive. It is intended here, however, to refer, very briefly, only to those aspects of chemotherapy which form the basis of this research.

It is well-known that many compounds in the quinoline group are valuable therapeutic agents. A series of styryl (I) and anil (II) derivatives of quinoline has within recent years been studied by Browning and co-workers (Refs. 22/8):

I



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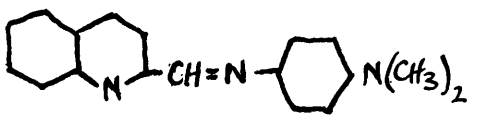
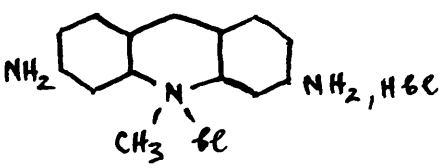


These workers examined the effect on therapeutic activity of substituent groups (chiefly primary amino, or tertiary amino) in the benzene and quinoline nuclei. They not only arrived at certain generalisations of theoretical interest, but found that many of the derivatives examined possessed antiseptic and trypanocidal properties.

Derivatives of acridine (which itself may be regarded as a derivative of quinoline) have also been the subject of much chemotherapeutic investigation. Acriflavine (III) is well-known as a powerful antiseptic; it also possesses some trypanocidal action:

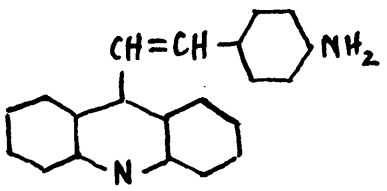
III



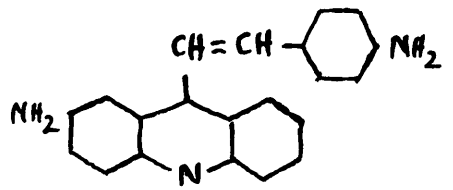
The acridine nucleus appears to be capable of great antiseptic action on the introduction of amino-groups in certain positions, and the relationship between antiseptic properties and position of substituent amino groups has been investigated recently by Albert and co-workers (Refs. 29/34).

The research undertaken by the author evolved from a consideration of the above facts. The activity of the styryl-quinolines suggested that styryl-acridines (e.g. IV) might also be active, and that the presence of amino-groups in the acridine nucleus might further enhance activity (V).

IV



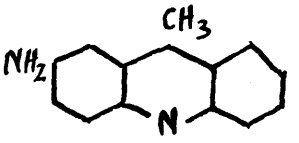
V



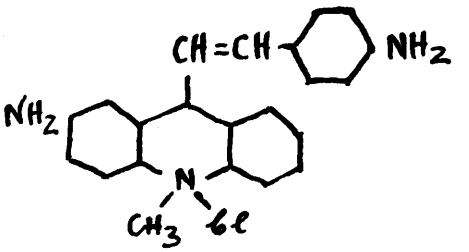
Hence a series of derivatives of 5-styrylacridine was prepared and the influence on therapeutic activity of various substituent groups (chiefly amino) in the benzene and acridine nuclei studied. The preparation of these styryl derivatives led to the isolation, as intermediates, of certain amino-5-methylacridines (e.g. VI) which were also submitted to therapeutic tests and, incidentally, permitted the therapeutic effect of replacing the 5-styryl group by methyl to be observed. In all cases the corresponding acridinium salts, usually methochlorides (e.g. VII), were also prepared and their

therapeutic properties tested, since the change from trivalent to pentavalent* nitrogen in quincline and acridine compounds generally enhances activity.

VI



VII

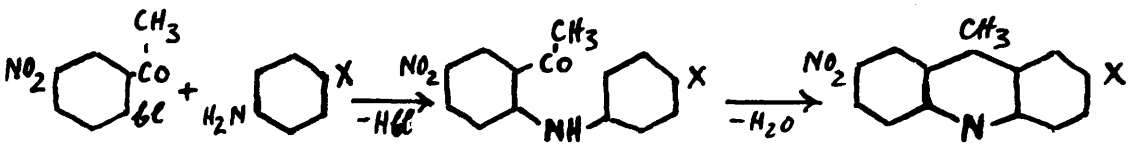


The arrangement of the subject matter in the thesis follows the order of the chemical work, and is broadly divided into two sections, theoretical and practical. The Theoretical Section is divided into five parts, the first four of which deal chiefly with chemical considerations, the results of the therapeutic tests on the compounds prepared being tabulated without comment at the end of each of the four parts. In the fifth part of the Theoretical Section the results of the therapeutic tests are discussed in relation to the chemical structures concerned. The Experimental Section contains the practical details of the chemical work and is divided into four parts which correspond to the first four parts of the Theoretical Section.

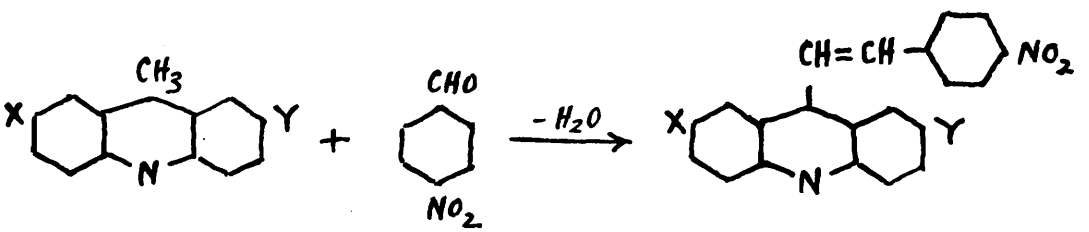
Parts I - IV of both Theoretical and Experimental Sections have been compiled according to the principal general chemical reactions employed. Thus Part I deals with the

* Strictly 4-covalent

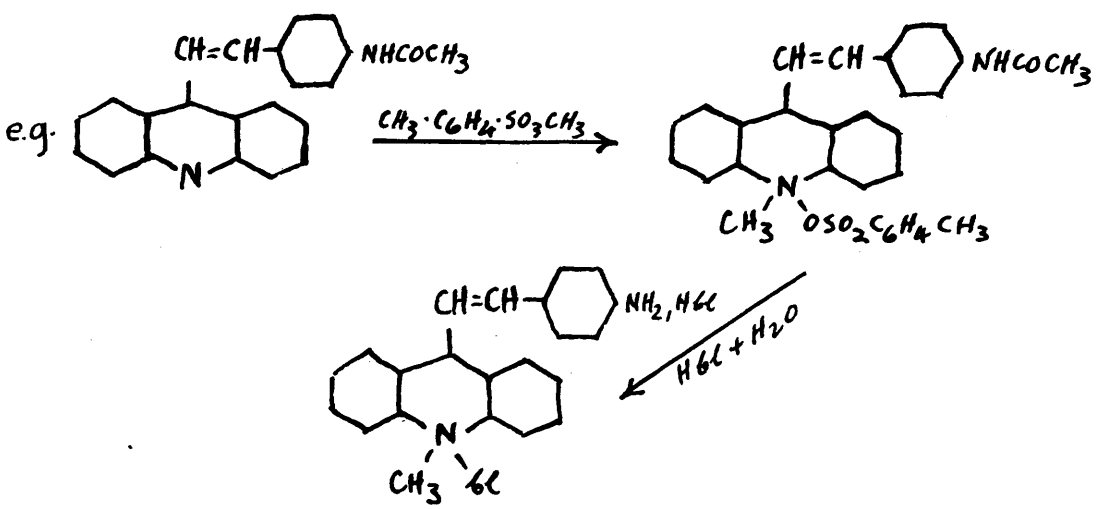
synthesis of derivatives of 5-methylacridine from 5-nitro-2-chloro-acetophenone and substituted anilines:-



Part II deals with the condensation of 5-methylacridine with substituted benzaldehydes to form styryl-acridines, and, in Part III this work is extended to the derivatives of 5-methylacridine:-



Part IV deals with the preparation of acridinium salts from suitable compounds prepared in Parts I - III, generally by combining such compounds with methyl p-toluenesulphonate and hydrolysing the products with hydrochloric acid:-



Whilst the work throughout Parts I - IV has had as its chief object the preparation of compounds suitable for therapeutic testing, several other related new compounds have been isolated and described. Original work on the fundamental chemical reactions involved has also been carried out.

The division of the thesis into Theoretical and Experimental Sections is not entirely rigid; some overlapping in function between these two Sections may have occurred occasionally. It has been the aim throughout, however, to deal in the Theoretical Section with the development of the work as a whole, including connections with the literature, co-ordination of the practical work, and discussion of problems arising in the practical work; the Experimental Section deals with the practical details of the separate pieces of chemical work, which contain, however, sufficient references to the theoretical matter to prevent them being a mere catalogue of experiments, preparations, and properties.

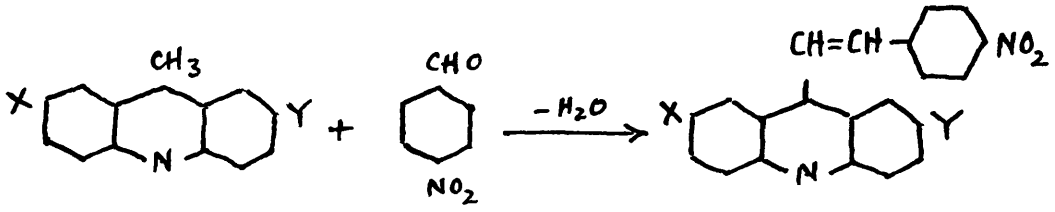
THEORETICAL SECTION

PART I

DERIVATIVES OF 5-METHYLACRIDINE PREPARED FROM 5-NITRO-2-CHLOROACETOPHENONE.

General Considerations.

As pointed out in the Introduction, one of the primary aims of this research has been the preparation of styryl-acridines with amino-groups in the acridine nucleus. Only 5-styryl-acridines have been considered in this work and these have been prepared from the corresponding 5-methylacridines (see Parts II and III) by condensing the latter with certain substituted benzaldehydes, e.g.:-

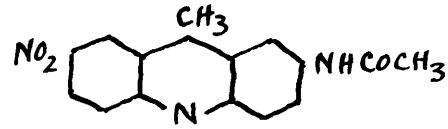


The first necessity in preparing styryl-acridines with amino-groups in the acridine nucleus is therefore the preparation of suitable derivatives of 5-methylacridine. It will be seen from the above example that substituent groups X and Y must not, at this stage, be free amino-groups since these are themselves capable of reacting with the aldehyde to form anils. Thus X and Y must be groups such as nitro or acetylamino, which do not react with the aldehyde, yet are capable of ready conversion to the amino-group once the styryl-acridine has been formed.

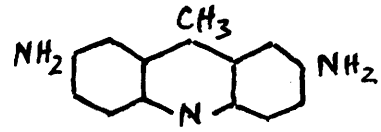
In addition to the requirements for styryl-formation however, certain substituted 5-methylacridines were also prepared for therapeutic testing themselves, the substituent groups being chiefly amino-groups, and the compounds generally submitted for the tests as hydrochlorides. These amino-methylacridines were also converted, for therapeutic testing, to acridinium salts, usually methochlorides (see Part IV); this required the preparation of the corresponding acetylaminomethylacridines, since free amino-groups might have been methylated in the process of acridinium salt formation with methyl p-toluenesulphonate.

Hence the 5-methylacridine derivatives prepared in this part of the work were of three principal types (examples chosen from the 3:7-substituted series):-

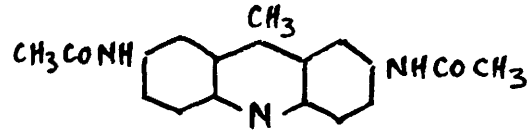
(i) Those suitable for conversion to styryl-acridines as in Part III:

e.g. 

(ii) Those immediately suitable for therapeutic testing themselves:

e.g. 

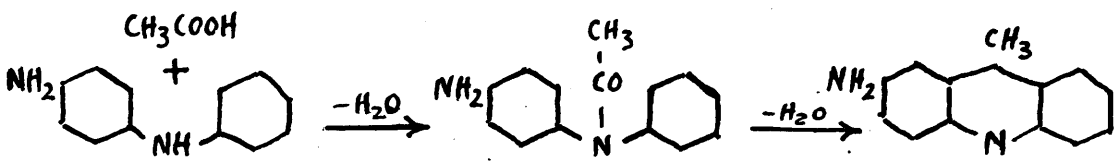
(iii) Those suitable for conversion to acridinium salts as in Part IV:

e.g. 

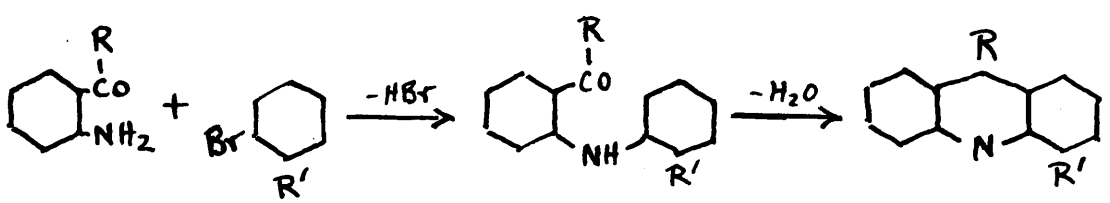
The problem of preparing these nitro, amino, and acetylamino

derivatives of 5-methylacridine will now be considered. It will be evident that 5-methylacridine itself is not a suitable starting material for this work since the direct introduction of a substituent group would raise the difficulty of determining the position taken up by such a group entering the molecule. Further, when 5-methylacridine is nitrated, the methyl group is oxidised to carboxyl (Bernthsen, Ref. 6). Hence a method was sought for synthesising these 5-methylacridines from suitable aromatic compounds.

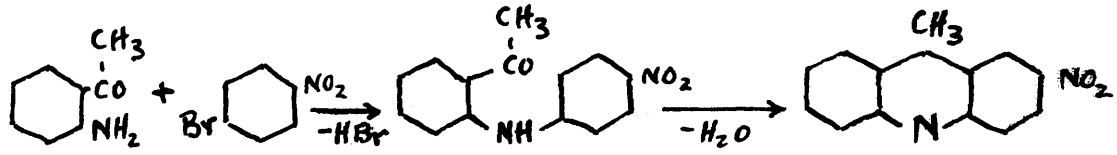
Although no amino-5-methylacridines have hitherto been described, an unsuccessful attempt to synthesise 3-amino-5-methylacridine was made by Bernthsen and Hess (Ref. 4). These workers used as starting materials p-amino-diphenylamine and acetic acid, the process recalling that for preparing 5-methyl-acridine itself (see page 135):-



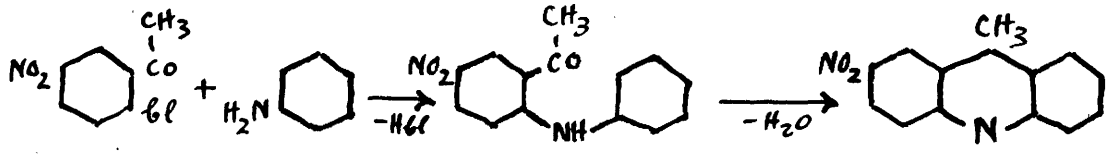
More recently Jensen and Rathwisch (Ref. 5) have described the preparation of various acridine derivatives, including 5-methylacridines, by the following general reaction (R = H, CH_3-, or C_6H_5-; R' = CH_3-, NO_2-, or alkoxy; R' being either o - or p - to Br):-



Thus for the preparation of 3-nitro-5-methylacridine, these workers condensed o-amino-acetophenone with p-nitro-bromobenzene:-



In the same paper Jensen and Rethwisch also describe an alternative method for preparing 3-nitro-5-methylacridine, starting from 5-nitro-2-chloroacetophenone and aniline:-



Regarding the general application of this last reaction Jensen and Rethwisch merely state that the first stage of the condensation also occurs using o- and p-nitranilines in place of aniline, without, however, describing the products, which they state "would give dinitromethylacridines in the final step of the reactions".

The relative merits of the above two methods of preparing 3-nitro-5-methylacridine were now considered in the light of their possible use as general processes for preparing the type of 5-methylacridine derivative required in this research.

In the first method the only suitable 5-methylacridines obtainable by the general reaction as it stands are 1-nitro-5-methylacridine and 3-nitro-5-methylacridine (using respectively o- and p-nitrobromobenzene with the o-amino-acetophenone;

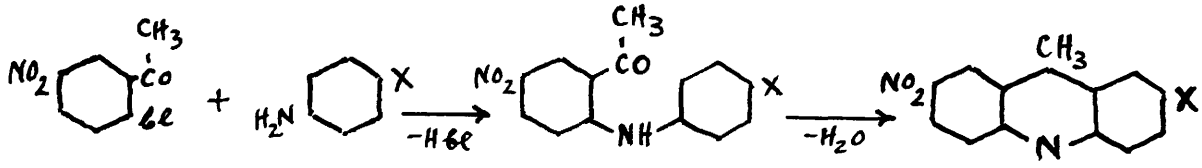
the use of m-nitrobromobenzene would probably result in a mixture of 2- and 4-nitro-5-methylacridines difficult to separate.). Further extension of the reaction is, of course, possible, by using either

(i) Nitro or acetylamino derivatives of o-aminoacetophenone or

(ii) Dinitro-bromobenzenes.

The objection to item (i) is that such derivatives would be tedious to prepare; item (ii) would result in di-nitro-methylacridines being produced which yield on reduction diamino-methylacridines having both amino-groups in the same benzene nucleus of the acridine molecule and this might be a disadvantage from the therapeutic point of view, since Albert and Linnell* (Ref.3) have shown that the diamino-acridines with both amino-groups in the same ring are either too toxic or too unstable for use.

The second method of synthesis, starting from 5-nitro-2-chloroacetophenone, seemed to offer better possibilities and was the method adopted in this work, for many substituted anilines could be tried in place of aniline, the resultant acridines always having a nitro-group in position 3 in one ring, and the new substituent group in the other ring, thus:

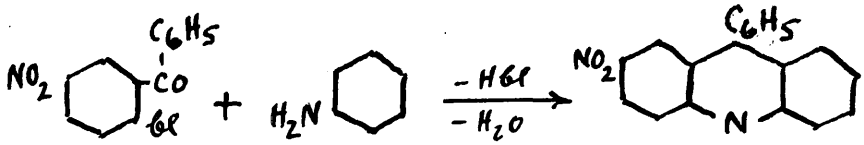


* et al.

Since the nitro-group in the 5-nitro-2-chloro-acetophenone molecule activates the chlorine atom, its presence in the ketone is essential for the first stage in the above condensation, and the resultant acridine derivatives therefore all have a nitro-group in position 3. (Time did not permit of an investigation into the preparation and use of other nitro-2-chloroacetophenones in this connection). Although this means that variations in the nature and position of other substituent groups have to be confined to positions 6, 7, 8, or 9, the constant presence of a 3-nitro-group in the 5-methylacridines synthesised this way is of advantage from the therapeutic point of view, for on reduction an amino-group is thus obtained in position 3; Albert and Linnell (Ref. 31) have shown that the amino-group in position 3 in the acridine molecule is only slightly less active and much less toxic than the amino-group in position 2, which causes marked antiseptic properties to appear in the acridine molecule.

The possibility of preparing 3-nitro-5-methylacridine from 5-nitro-2-chloroacetophenone and aniline in one stage, instead of in the two stages described by Jensen and Rethwisch was suggested by the work of Ullmann and Ernst (Ref. 2) who prepared 3-nitro-5-phenylacridine from 5-nitro-2-chlorobenzo-phenone and aniline not only by the analogous two-stage method used by Jensen and Rethwisch (involving isolation of the intermediate diphenylamine derivative), but also in one stage,

as follows:-



If this one-stage method could be extended to the general preparation of 5-methylacridines contemplated here, time might be saved and better yields obtained. Hence it was decided to investigate fully this possibility in the case of 5-nitro-2-chloroacetophenone and aniline before proceeding with the experiments on the substituted anilines.

Having reviewed the types of 5-methylacridine derivatives required here, and the means whereby they can best be prepared, the various points arising in the practical work will now be considered. The order in which this work developed was as follows:-

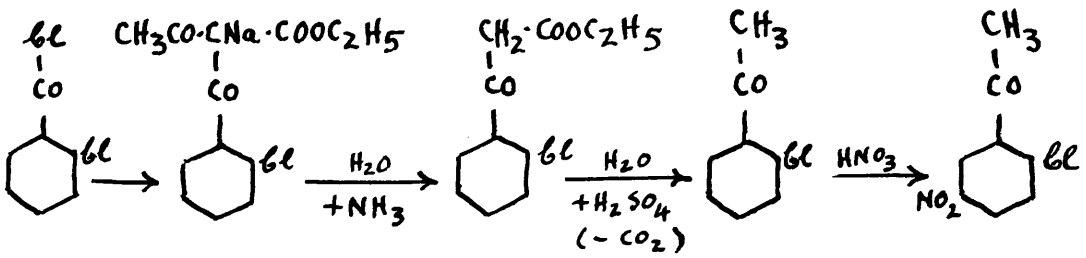
The synthesis of 5-nitro-2-chloroacetophenone was first carried out, as described in the literature, and some new facts established, including the isolation of a new substance as a by-product in the nitration of o-chloroacetophenone. Next the condensation between 5-nitro-2-chloroacetophenone and aniline was fully explored; it was shown that although the one-stage method of preparing the acridine derivative, suggested earlier, was unsuccessful, the two-stage method of Jensen and Rethwisch required modification for reasonable yields of product to be obtained. Having then converted the 3-nitro-5-methylacridine into other new related methylacridines

for the purposes outlined earlier, the reaction between 5-nitro-2-chloroacetophenone and the nitranilines was next explored, but no condensation products could be isolated from this reaction. This failure, as will be explained later, led to experiments on the condensation of 5-nitro-2-chloroacetophenone with the aminoacetanilides, which proved successful and provided several new 5-methylacridines of the type required. Lastly, the reaction between 5-nitro-2-chloroacetophenone and the toluidines was explored, with some success in the case of p-toluidine, as a result of which certain other new methylacridines were obtained.

Full practical details of the work outlined in the above paragraph appear in Part I of the Experimental Section in the above order. The various points arising from this work will now be discussed, following the same order.

Preparation of 5-nitro-2-chloroacetophenone.

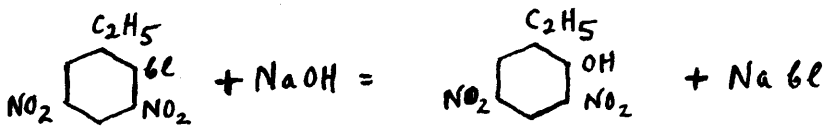
The method used for preparing 5-nitro-2-chloroacetophenone was that of Thorp and Brunskill (Ref. 1), starting from o-chlorobenzoyl chloride, acetoacetic ester and sodium ethoxide:



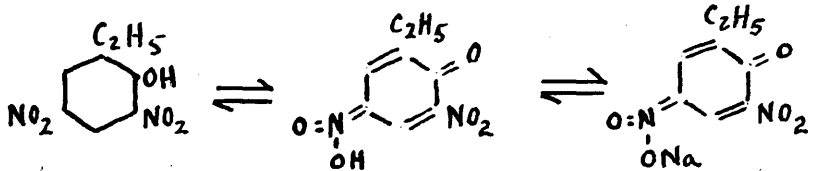
The properties of the various substances obtained in the course of the above series of reactions were found to agree with those described in the literature. Owing to the high cost of the starting material, o-chlorobenzoyl chloride, a careful record was kept of the yields obtained each time the process was carried out. The yields were found to compare favourably with those obtained by Thorp and Brunskill, except in the final stage, when the yield obtained in the nitration of o-chloroacetophenone was considerably less than that claimed by these workers. On investigation this loss was shown to occur on crystallising the crude 5-nitro-2-chloroacetophenone from alcohol, when about a quarter of the substance was lost in the mother liquor as an uncrystallisable oil. The nature of this oil was not investigated fully, although it was shown to be neither 5-nitro-2-chloroacetophenone, too impure to crystallise, nor simply unchanged o-chloroacetophenone.

Another substance, insoluble in alcohol, was isolated in the crystallisation of the crude 5-nitro-2-chloroacetophenone, and was readily obtained crystalline from benzene. The amount of this substance obtained varied with the batch of o-chlorobenzoyl chloride used initially, ranging from almost nil to about 3% of the crude 5-nitro-2-chloroacetophenone. It was thought at first that this might be a di-nitro, or another mono-nitro, derivative of o-chloroacetophenone which might then be of use in extending the general reaction whereby the

methylacridines required for this research were prepared. Analysis and molecular weight determination have, however, indicated that the formula of the substance is $C_8H_7O_4N_2Cl$. Thus the substance is thought to be one of the dinitrochloroethyl-benzenes, none of which is, however, described in the literature*. It was considered to be outside the scope of this thesis to investigate the actual orientation of the various groups in this new substance, since it did not appear to be of use in the preparation of the types of compounds required. The properties described in the Experimental Section (page 91), however, strongly support the view that it is a dinitrochloroethylbenzene. Thus although insoluble in cold sodium hydroxide solution, the substance dissolved on heating, producing a red solution which on cooling and treating with a slight excess of dilute hydrochloric acid became yellow, reverting to bright red on the addition of a slight excess of sodium hydroxide solution. Assuming, for the sake of argument the following constitution for the dinitrochloroethylbenzene, the effect of heating with sodium hydroxide solution would be:

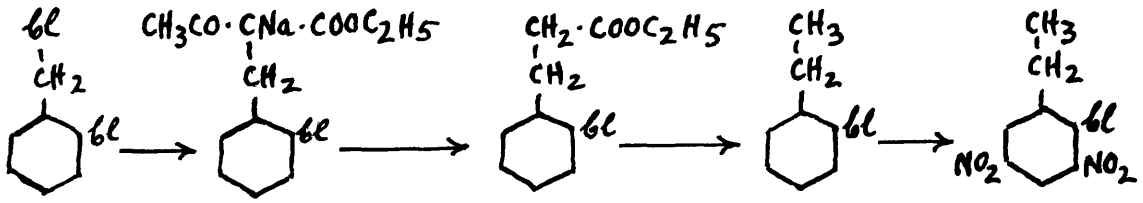


and the indicator phenomenon would be explained thus:-



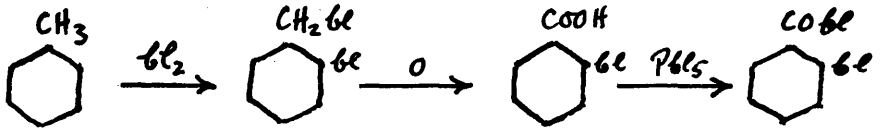
* at least not with the chlorine atom in the nucleus.

A possible explanation of the occurrence of a dinitrochloroethylbenzene in the crude 5-nitro-2-chloroacetophenone may be as follows. It will be seen from the series of reactions representing the preparation of 5-nitro-2-chloroacetophenone from o-chlorobenzoyl chloride that if the -CO- group of the acid chloride is replaced by a $-CH_2-$ group, and if in the final nitration a dinitro compound is formed, the product will be a dinitrochloroethylbenzene:-



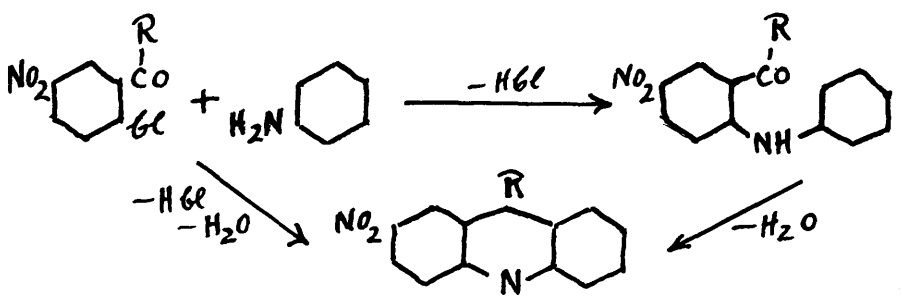
Thus the formation of the dinitrochloroethylbenzene can be explained if the presence initially of a little o-chlorobenzyl chloride is assumed, since the products appearing in the above series of reactions would separate out with the corresponding principal products at each stage, and would not be separated from them until the last stage, since only at this stage was any rigorous purification employed. Since the amount of the dinitrochloroethylbenzene obtained varied with the batch of o-chlorobenzoyl chloride purchased, and was sometimes almost nil, it was thought that the o-chlorobenzoyl chloride used may occasionally have been contaminated with a little o-chlorobenzyl chloride. Considering the method commonly employed for preparing o-chlorobenzoyl chloride it will be seen that o-chlorobenzyl chloride is a possible

intermediate in the process and therefore a possible impurity:



5-Methylacridines from the reaction between 5-nitro-2-chloro-acetophenone and aniline.

Before proceeding with the preparation of 3-nitro-5-methylacridime by this reaction, using the two-stage method of Jensen and Rethwisch (Ref. 5), it was decided, as explained earlier (page 12), to investigate the possibility of preparing the compound from the same reactants in a single stage analogous to the preparation of 3-nitro-5-phenylacridine by Ullmann and Ernst (Ref.2). The relationship between the two methods will be clear from the following scheme:-



In each of the cases described in the literature the ketone is boiled for 2 - 3 hours with an excess of aniline in presence of a dehydrating agent which also combines with hydrochloric acid. When anhydrous potassium carbonate is used for the latter purpose both Ullmann and Ernst ($R = C_6H_5$ -) and Jensen and Rethwisch ($R = CH_3$ -) report that the intermediate

diphenylamine derivative is the product; this can then be ring-closed in boiling glacial acetic acid by means of a little concentrated sulphuric acid, to give the corresponding acridine derivative. Only Ullmann and Ernst ($R = C_6H_5$ -) have shown that by replacing the potassium carbonate in the initial reaction mixture by anhydrous sodium acetate, the acridine derivative is then produced directly.

Experiments were therefore undertaken by the author (see Experimental Section, page 93) to see if this last finding of Ullmann and Ernst would apply in the preparation of 3-nitro-5-methylacridine ($R = CH_3$ -), in each case heating the reactants in presence of anhydrous sodium acetate. In the first experiment, following closely the analogous method of Ullmann and Ernst (excess aniline; 2½ hours heating at the boil, 180° - 190° C) no crystalline product was isolated from the tarry reaction mass. In subsequent experiments modifications were introduced in an attempt to prevent tarring. No improvement resulted on reducing the time of heating to one hour. By studying the effect of gradually increasing the temperature of the reaction mass above 100°C, it was observed that tarring only set in seriously above 130° C. As a result of this observation an experiment was carried out in which the temperature was kept at 125° C; a crystalline product was this time isolated but proved on examination to be the intermediate diphenylamine derivative, no acridine derivative being detected.

The tarring produced in the earlier experiments is probably due not only to the higher temperature employed but also to the presence of excess aniline, for on repeating the first experiment at 180° - 190° C with only one molecular proportion of aniline, the intermediate diphenylamine derivative could be isolated crystalline from the reaction mass. In none of the above cases, however, could the acridine derivative be isolated from the reaction mass.

Hence the attempt to prepare 3-nitro-5-methylacridine in one stage, as above, proved unsuccessful. Since the yields of diphenylamine derivative obtained in these experiments were much smaller than those obtained in the improved Jensen and Rethwisch method about to be discussed, the method using sodium acetate in the reaction mass was not examined further.

The preparation of 3-nitro-5-methylacridine by the two-stage method of Jensen and Rethwisch in the first stage of which the reactants are heated with anhydrous potassium carbonate (3 hours at 170° - 180° C) was next attempted. No crystals of the intermediate 2-acetyl-4-nitro-diphenylamine were isolated however from the tarry product. Since the work on the unsuccessful one-stage method above has shown that tarring is greatly reduced by lowering the temperature of reaction to 125° C, a second experiment was carried out in presence of potassium carbonate at 125° C. An excellent

yield of the diphenylamine derivative was obtained this time, with only slight tarring. Details of the process, which introduced other minor modifications, are given in the Experimental Section (page 102) as an improvement on the method of Jensen and Rethwisch.

The second stage of the process, namely, ring-closure of the 2-acetyl-4-nitro-diphenylamine, was found to take place readily as described in the literature, using concentrated sulphuric acid in glacial acetic acid. Concentrated hydrochloric acid was also observed to effect this ring-closure, though not so readily as concentrated sulphuric acid.

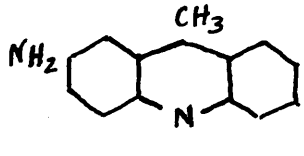
The yields obtained in the preparation of 3-nitro-5-methylacridine above were carefully noted in relation to the purity of the starting and intermediate materials on account of the high cost of these materials. If the latter were too impure, crystallisation of the final product became difficult owing to the larger amount of associated resinous matter, and much product was lost in the mother liquor. If on the other hand the starting and intermediate materials were purified by crystallisation, the losses encountered in doing so also considerably reduced the yield of final product. It was shown however, that by using starting and intermediate materials, which had been separated and washed (without crystallising) as described in the Experimental Section, the yield of final product, based on a given weight of washed 5-nitro-2-chloroacetophenone, was 12% better than that obtained

in the preparation involving crystallisation at each stage.

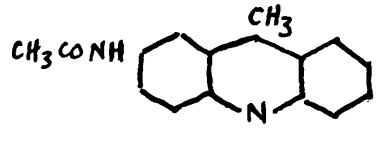
Jensen and Rethwisch have not stated the yields obtained in their work, and have not described the properties of 2-acetyl-4-nitro-diphenylamine or 3-nitro-5-methylacridine very fully. A fuller account of the properties of these substances is now given in the Experimental Section . In this connection it was shown that the addition of a trace of the acridine derivative to chloroform imparted a brilliant yellow-green fluorescence to this solvent in ultra violet light, no such phenomenon being associated with the diphenylamine derivative. A similar phenomenon was observed later in the experiments with the substituted anilines, when the diphenyl-amine derivatives again showed no fluorescent properties, but on ring-closure the resultant acridine derivatives imparted fluorescence to chloroform or benzene in ultra violet light.

The use of 3-nitro-5-methylacridine in the preparation of compounds suitable for therapeutic testing will now be outlined. The compound is itself suitable for converting to styryl-acridines, and these are described in Part III. 3-Amino-5-methylacridine (I) was prepared from it by reduction, and was submitted to therapeutic testing, as well as converted to 3-acetylamino-5-methylacridine (II) for the preparation of acridinium salts (see Part IV):-

I



II

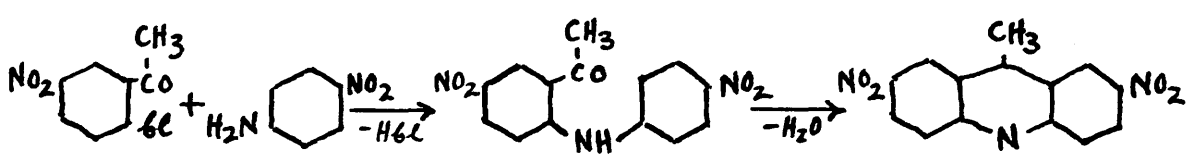


The preparation of these two new compounds from 3-nitro-5-methylacridine does not call for special comment in this Theoretical Section. The amino-compound in the freshly precipitated form appears to resinify in air, and is difficult to obtain crystalline, a property common to most of the acridines which have an amino group in the acridine nucleus and are considered in this thesis. A solution of the amino-compound in ether shows bright greenish fluorescence in daylight, a property not shown by the corresponding nitro- or acetylamino-compounds. This phenomenon has often been noticed with other methylacridines, prepared in this work, the amino-derivatives alone giving fluorescing solutions in ether in daylight. The bright green fluorescence given by the acetylamino-compound in glacial acetic acid in daylight is also generally given by other acetylamino-acridines prepared in this work.

Both 3-amino-5-methylacridine and 3-acetylamino-5-methylacridine have many properties in common with the corresponding 5-phenylacridines described by Ullmann and Ernst (Ref.2).

The Reaction between 5-nitro-2-chloroacetophenone and the nitranilines.

The following products are theoretically possible in this reaction (considering p-nitraniline):-

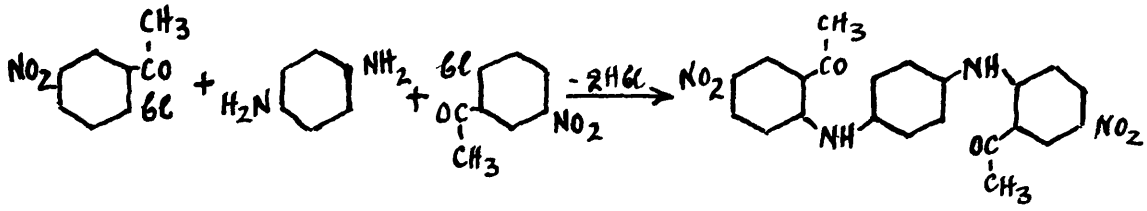


As mentioned earlier, Jensen and Rethwisch state that the first stage in this reaction takes place as with aniline, but do not describe the product. This was investigated, but the experiment proved unsuccessful, no crystalline substance being isolated from the tarry product. Further experiments were carried out, in the light of the experiments with the analogous reaction using aniline, with the object of reducing tarring. Thus the temperature of reaction was lowered from 175°C to 125°C, then to 100°C, and the time of heating reduced to 1 hour, but in every case a tarry product was formed which yielded no crystalline substance on examination. Similar results were obtained using o- and m-nitranilines in place of p-nitraniline.

The object of the above experiments was to obtain di-nitromethylacridines, and so various acridines for therapeutic testing having two free amino-groups in the acridine nucleus. The same end can, however, be achieved theoretically by using the amino-acetanilides in place of the nitranilines, and the successful work in this connection is discussed below. It is concluded from the success of the latter experiments that the failure with the nitranilines is due to the nitro-group in the substituted aniline causing excessive tarring, probably by oxidation, for replacement of the nitro-group by acetylamino, (and later by methyl) as will be seen below, results in a much less tarry product which easily crystallises from alcohol.

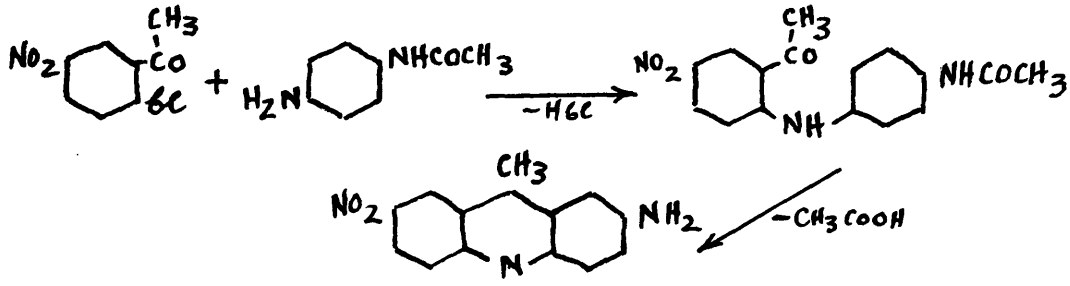
It should be mentioned here that the use of the

phenylenediamines, for achieving the same end as the nitranilines or aminoacetanilides, was avoided in this work owing to the possibility of two molecules of the ketone reacting with one of the diamine thus (considering p-phenylenediamine):-



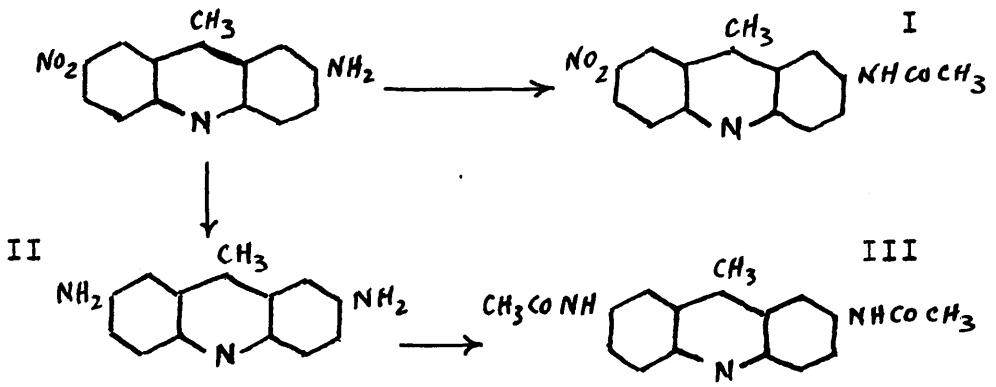
5-Methylacridines from the reaction between 5-nitro-2-chloroacetophenone and the aminoacetanilides:

The reaction with p-aminoacetanilide was investigated first. The experimental conditions previously shown to be best for the corresponding reaction using aniline were applied here with success. Thus in the first stage of the reaction the crystalline diphenylamine derivative was easily isolated. In the second stage of the reaction ring-closure was found to be accompanied by hydrolysis of the acetylamino-group, so that the final product was 3-nitro-7-amino-5-methylacridine:

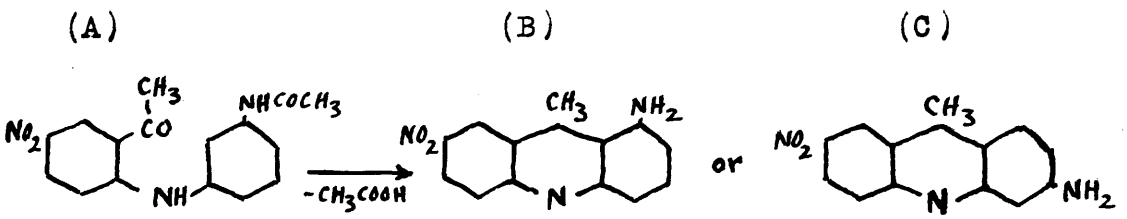


The use of the latter in the production of acridines for therapeutic testing was next considered. For the preparation of styryl compounds (see Part III) the

nitro-aminomethylacridine was converted to the acetyl-derivative (I). By reduction the diaminomethylacridine (II) was obtained. This was submitted to therapeutic testing and also converted to the di-acetylamino compound (III) for use in acridinium salt formation (see Part IV):-



Similar work on the reaction between 5-nitro-2-chloro-acetophenone and m-aminoacetanilide has shown that the di-phenylamine derivative (A) is readily obtained crystalline in the first stage, and is easily ring-closed in the second stage. The final product, which resembles in general properties the 3-nitro-7-amino-5-methylacridine obtained above is, however, probably a mixture of the two isomeric nitroaminomethyl-acridines (B and C) possible on the combined ring-closure and hydrolysis of the diphenylamine derivative:-

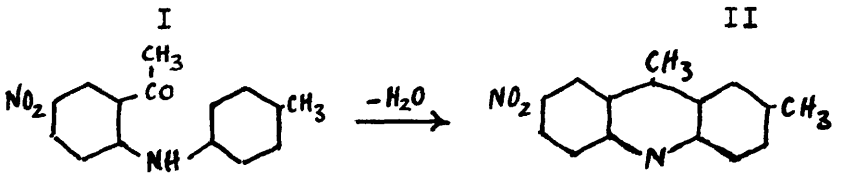


The separation of these two isomers was not attempted, the product simply being washed, not recrystallised, for the preparation, by reduction, of the corresponding diamino product which was submitted for therapeutic testing. No further acridines were prepared from this source for therapeutic testing owing to the possibility of the products being mixtures of isomers; further, the diamino-product proved to be only moderately antiseptic.

The reaction between 5-nitro-2-chloroacetophenone and o-aminoacetanilide (to obtain eventually 3:9 diamino-5-methyl-acridine) was not investigated since o-aminoacetanilide was not available and its preparation, according to Leuchs (Ref.12), is tedious. Further, it appears to be unstable towards heat, and therefore may not have been suitable for this reaction.

5-Methylacridines from the reaction between 5-nitro-2-chloro-acetophenone and the toluidines.

The reaction with p-toluidine was investigated first, and was found to follow the same lines as the corresponding experiment with aniline when the improved conditions used there were applied. Both the intermediate diphenylamine derivative (I), and acridine derivative (II) from it by ring-closure, closely resemble the corresponding products obtained in the experiments with aniline.



The use of 3-nitro-5:7-dimethylacridine in preparing acridines suitable for therapeutic testing was next considered. The compound is itself suitable for the preparation of styryl-compounds as in Part III (the possibility of a di-styryl derivative being obtained from this di-methyl-derivative is discussed there). On reduction, 3-amino-5:7-dimethyl acridine was obtained, and this was submitted to therapeutic tests as well as converted to 3-acetylamino-5:7-dimethylacridine for the preparation of acridinium salts (see Part III). Both the amino- and acetylamino-compounds closely resembled, in general properties, the corresponding compounds obtained from 3-nitro-5-methylacridine.

The reaction between 5-nitro-2-chloroacetophenone and m-toluidine was not investigated owing to the possibility of a mixture of isomers being produced at the ring-closure stage of the reaction (compare m-aminoacetanilide, page 26).

An attempt to condense 5-nitro-2-chloroacetophenone with o-toluidine proved unsuccessful, the reaction taking the same course as with the nitranilines, no crystalline product being isolated..

TABLE I.

Antiseptic Properties of 5-methylacridines prepared in Part I.

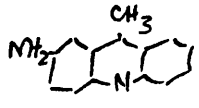
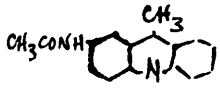
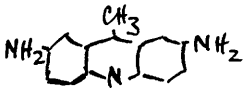
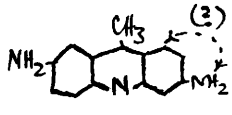
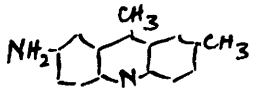
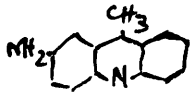
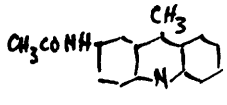
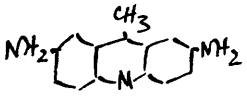
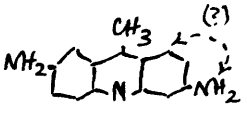
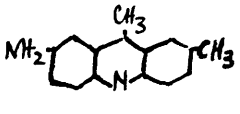
COMPOUND		STERILISING DILUTION			
		Staph. aureus		B. coli	
No.	Formula	Peptone water	Ox serum	Peptone water	Ox serum
1		-	-	-	-
2	" hydrochloride	1:10,000	<1:10,000	<1:10,000	<1:10,000
3		-	-	-	-
		(practically insoluble at neutral point)			
4		1:20,000	1:4,000	1:10,000	1:10,000
5		1:20,000 to 1:10,000	1:40,000	1:20,000	1;20,000
6		<1:10,000	<1:10,000	<1:10,000	<1:10,000

TABLE II

Trypanocidal Action and Toxicity of the 5-methyl-acridines prepared in Part I.

Note: Doses are expressed in terms of the strength of the solution used, 1 c.c. of the stated dilution being given per 20 g. mouse.

COMPOUND		TOXICITY		TRYPANOCIDAL ACTION (T. Brucei)	
No.	Formula	Lethal dose	Tolerated dose	Dose	Result
1		1:125	1:200	1:200	Nil
2	" hydrochloride	-	-	-	-
3		-	1:125	1:200	Nil
4		-	1:100	1:300	Nil
5		-	1:100	1:200	Nil
6		-	1:100	1:200	Nil

PART II

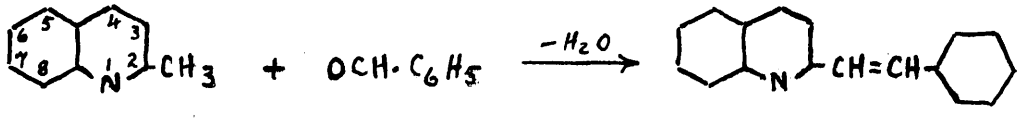
STYRYL-ACRIDINES PREPARED FROM 5-METHYLACRIDINE.

General Considerations.

The reasons for undertaking the preparation of the styryl-acridines have already been referred to in the Introduction. The styryl-acridines considered in this part of the Theoretical Section are all derived from 5-methylacridine itself and therefore have no substituent groups in the acridine nucleus. The object in preparing such compounds was two-fold. In the first place suitable compounds could be submitted to therapeutic testing, and the results compared with those of the corresponding styryl-acridines having substituent groups in the acridine nucleus (as prepared in Part III). The therapeutic effect of introducing these substituent groups could therefore be directly observed, not only in the case of the acridines prepared in Parts II and III, but also in the case of the corresponding acridinium salts prepared in Part IV. In the second place, by exploring the process of styryl-formation with 5-methylacridine itself, information would thereby be obtained which would prove of value when the process was extended to the substituted 5-methylacridines in Part III.

Before considering the work on the preparation of the 5-styrylacridines it may be well to review briefly the analogous reactions in the pyridine and quinoline series. It

is well known that certain methyl-pyridines and methyl-quinolines react with benzaldehyde or its derivatives to form styryl compounds. Thus 2-methylquinoline reacts with benzaldehyde to form 2-styrylquinoline:-



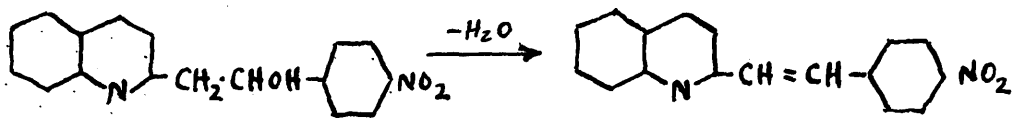
Similar reactions occur with 4-methylquinoline, 2-methylpyridine, and 4-methylpyridine. If the methyl group occupies a position other than 2 or 4 in the pyridine or quinoline nucleus it does not appear to be capable of reacting with the aldehyde as above.

Considering now the methylacridines in the light of the above facts it is evident that reactivity towards benzaldehyde and its derivatives can be expected only in the case of 5-methylacridine, since the 5-position in acridine corresponds to the 4-position in pyridine or quinoline, and no position in the acridine molecule capable of substitution corresponds to the 2-position in pyridine or quinoline. It was decided therefore to concentrate on the 5-position of acridine for styryl-formation; accordingly all the styrylacridines described in this thesis are 5-styrylacridines.

The condensation of pyridine and quinoline compounds, containing reactive methyl groups, with benzaldehyde or its derivatives to form styryl compounds may be effected in a

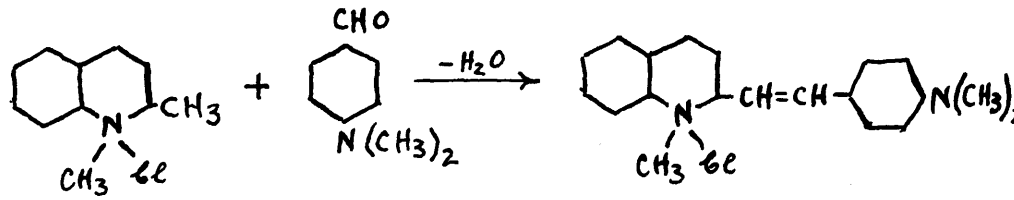
variety of ways. Occasionally the styryl compound is formed by simply heating the reactants together, but this method is sometimes liable to produce an addition product (a substituted ethanol, e.g. I), which may subsequently be convertible, however, to the styryl derivative (II) by heating with acetic anhydride:-

I II



The usual method of preparing such styryl compounds is therefore to heat together the corresponding methyl compound with the aldehyde in presence of a condensing agent, usually zinc chloride or acetic anhydride. The presence of a little piperidine often exerts a catalytic effect in styryl-formation, particularly in the case of quaternary salts of the methyl compound (in which the methyl group is generally more reactive than in the free base*). Thus Browning and his co-workers (Refs. 22/28) prepared their styryl quinolines simply by heating together the appropriate quaternary salts and the aldehyde in alcohol containing a little piperidine:-

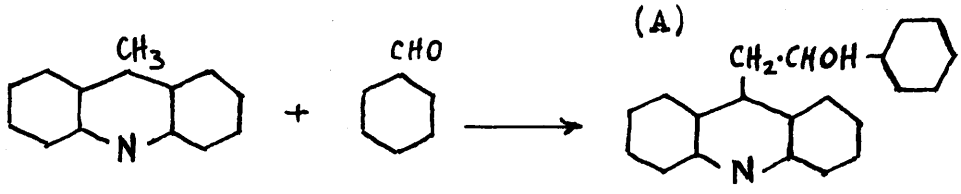
e.g.



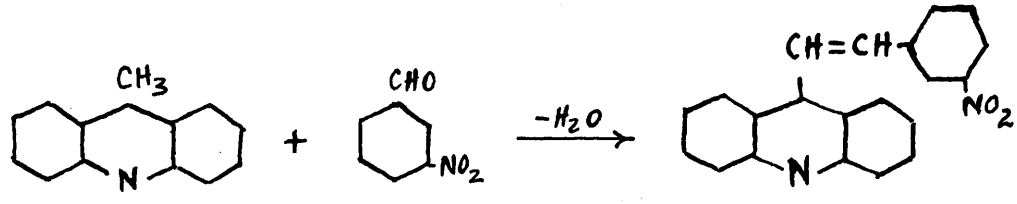
The reactivity of the methyl group in 5-methylacridine towards benzaldehydes was first explored by Friedlander (Ref.13),

* Mills and Smith (Ref.9)

who showed that the ethanol derivative (A) was obtained by heating 5-methylacridine with benzaldehyde; he failed however, to convert this product to the styryl derivative.



Using m-nitrobenzaldehyde in place of benzaldehyde, however, Friedlander obtained a styryl derivative after 24 hours heating at 100°C:



This styryl compound was later obtained by Porai-Koschitz (Ref.8) who condensed the reactants at 145°C in presence of zinc chloride, only 3½ hours heating being required. Porai-Koschitz also describes the corresponding styryl compounds obtained using first p-nitrobenzaldehyde, then p-dimethylamino-benzaldehyde, in place of m-nitrobenzaldehyde, the reaction in the case of the p-dimethylamino-benzaldehyde being slower than for the nitrobenzaldehydes.

Thus the reactivity of 5-methylacridine towards aldehydes seems to depend on the nature of the aldehyde used. It will be seen that the aldehydes used by Porai-Koschitz

produce the type of compounds required in this research, for the p-dimethylamino-styryl compound is suitable for therapeutic testing, and the nitro-styryl compounds on reduction (described by Porai-Koschitz) yield the corresponding amino-styryl compounds which are also suitable for therapeutic testing. The acetyl-derivatives of the latter (not previously prepared) are suitable for the conversion of these acridine compounds to the corresponding acridinium salts as in Part IV.

On repeating the work of Porai-Koschitz all the products described by that worker were obtained, but very poor yields resulted in the case of the two nitro-benzaldehydes; the p-nitro and p-amino-styryl compounds obtained by the author differed in several important respects from those described by Porai-Koschitz. Further, on examining the method of preparing the m-nitro-styryl compound described by Friedlander, the corresponding ethanol compound (not previously described) was isolated, as well as the styryl compound. The author therefore felt that the whole question of the reaction between 5-methylacridine and the derivatives of benzaldehyde capable of producing styryl compounds of the type required, should be more fully explored.

The practical work in this part of the research therefore developed along these lines, the reaction between 5-methylacridine and suitable benzaldehydes being investigated first with p-nitro-benzaldehyde, then in turn with m-nitro-benzaldehyde, o-nitro-benzaldehyde, and p-dimethylamino-

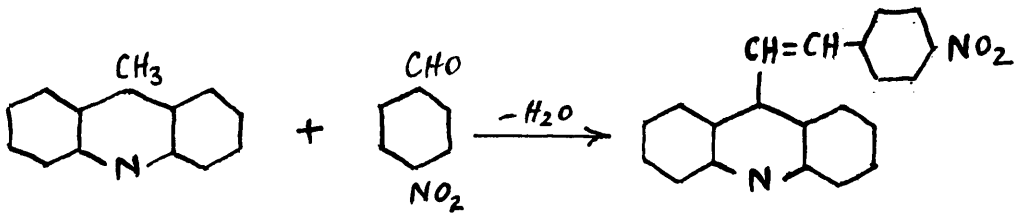
benzaldehyde. In each case the reaction was studied using various condensing agents, and also without such agents. The chief objects throughout were to find the best methods of preparing the compounds suitable for therapeutic testing and to obtain information for extending the reaction to the substituted 5-methylacridines in Part III. Since it was desired to study the therapeutic properties of the styryl derivatives both before and after conversion to acridinium salts, only styryl-formation with the free acridine bases has been explored in this thesis, conversion to acridinium salts being carried out subsequent to styryl-formation. Thus the method of Browning and co-workers (Refs. 22/28) of preparing the acridinium salt first then converting it to the styryl compound by heating it with the benzaldehyde derivative in alcohol, using piperidine as catalyst, was not used in this work.

Full details of the practical work indicated above are given in Part II of the Experimental Section. The various points arising in this work will now be discussed following the same order.

Compounds from the reaction between 5-methylacridine and p-nitrobenzaldehyde.

The first experiment in the investigation of this reaction followed closely the conditions described by Porai-Koschitz (Ref. 8) for preparing the styryl compound, the reactants being melted together at 145°C for 3½ hours in

presence of zinc chloride, and the crude product purified through its sparingly soluble hydrochloride:-



The yield of styryl-compound obtained, however, was very small (10% theoretical), considerable tarring occurring during the reaction. Another experiment was therefore carried out as above, but lowering the temperature of reaction to 130°C. This had the desired effect of greatly reducing tarring, and the styryl compound was isolated in good yield (90% theoretical) omitting this time the formation of the hydrochloride and simply crystallising the crude product (after removing the zinc chloride) from pyridine.

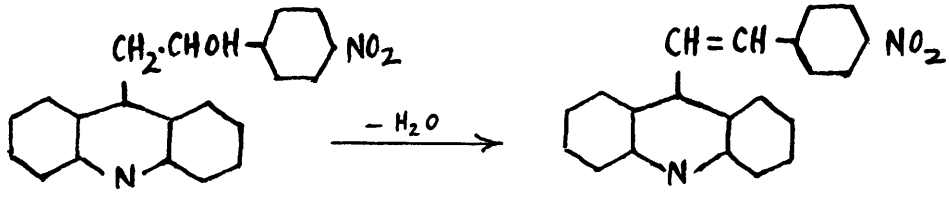
Although the improved method using zinc chloride as above is entirely satisfactory for the preparation of the styryl compound, further experiments were undertaken to obtain information on the production of the styryl compound by heating together the reactants in a suitable solvent (the application of the results of these experiments is described in Part III). Thus the reactants were first heated together in acetic anhydride, when it was found that the styryl compound was produced, but only in small yield (33% theoretical). A small yield (14% theoretical) of the styryl compound was also obtained by heating together the reactants in pyridine

containing a little piperidine as catalyst. No styryl compound was produced by heating the reactants together in alcohol containing a little piperidine, although a little of the corresponding ethanol derivative was isolated in this experiment.

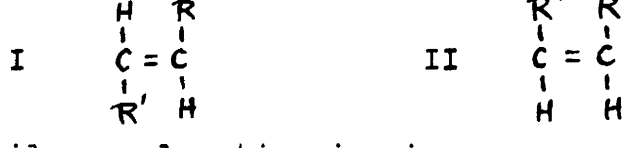
By melting the reactants together at 100°C in absence of condensing agents the chief product of the reaction is the ethanol derivative, although a small amount of the styryl compound is also formed. The relationship between the ethanol derivative, the styryl compound obtained by the author, and the styryl compound described by Porai-Koschitz, may now be discussed.

The styryl compound according to Porai-Koschitz is obtained crystalline with one molecule of water of crystallisation, and melts at 212°C. The styryl compound obtained by the author in all the different experiments above (including the method of Porai-Koschitz) was always obtained crystalline with no water of crystallisation, and melted at 293°C. These two styryl compounds, however, otherwise closely resemble each other in general properties. Although the corresponding ethanol derivative obtained by the author gives of course the same analysis as the hydrated styryl compound described by Porai-Koschitz, these two substances are otherwise quite different. Thus the ethanol derivative melts at 174°C, and does not lose a molecule of water on heating at 110°C as does the hydrated styryl compound of Porai-Koschitz. The ethanol

derivative, however, loses the elements of water on heating with acetic anhydride in the same way as the corresponding quinolyl-ethanol described by Bulach (Ref.14), the styryl compound being formed; the latter again was obtained free from water of crystallisation and melted at 293°C:-



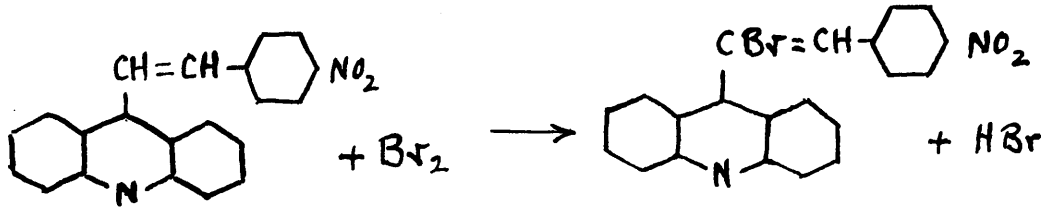
A careful inquiry into the method of isolating and crystallising the styryl and ethanol derivatives has failed to produce any trace of a styryl compound melting at 212°C. The difference in properties between the styryl derivative obtained by the author and that of Porai-Koschitz is further manifest in the corresponding amino-derivatives (obtained from the substances in question by reduction) which differ considerably in melting point though not substantially in other properties. An explanation of the existence of two such compounds corresponding to 5-(p-nitrostyryl)-acridine may be that the structure is capable of showing geometric isomerism, and that the compounds in question may therefore be geometric isomers (I and II; where R = p-nitrophenyl, and R' = acridyl-(5)):-



A similar explanation is given on page 46 to account for the existence of the two isomers obtained in analogous

experiments using m-nitrobenzaldehyde (i.e. R = m-nitrophenyl); both of these isomers, however, were actually obtained by the author.

An interesting derivative of 5-(p-nitrostyryl)-acridine was obtained by treating a solution of the compound in chloroform with a solution of bromine in chloroform. The orange-red bromo-derivative which separated was shown on analysis to be a mono-bromo-substitution product, and not the more likely di-bromo-addition product. A similar phenomenon is recorded by Loew (Ref.15), who has shown that 4-(o-nitrostyryl)-quinoline reacts with bromine in carbon disulphide to form a mono-bromo-substitution product, one or other of the hydrogen atoms of the -CH=CH- grouping being replaced by bromine. By analogy the reaction observed by the author in the case of 5-(p-nitro-styryl)-acridine might be represented thus:-



A possible explanation of the colour reaction observed with a similar experiment using the corresponding amino-styryl compound has been given (page 43) in the light of the above reaction.

The ethanol derivative isolated in the course of the experiments in the preparation of 5-(p-nitrostyryl)-acridine was not investigated for possible therapeutic properties, since

on attempted reduction to the corresponding amino-compound, it appeared to decompose into the methylacridine and aldehyde from which it is derived. This decomposition was noticed to occur when the compound was boiled with dilute hydrochloric acid for a time.

For the purpose of therapeutic testing 5-(p-nitrostyryl)-acridine was reduced to the corresponding amino-compound. This was submitted to therapeutic tests itself, and also converted to the corresponding acetylamino-compound for use in the preparation of acridinium salts as in Part IV.

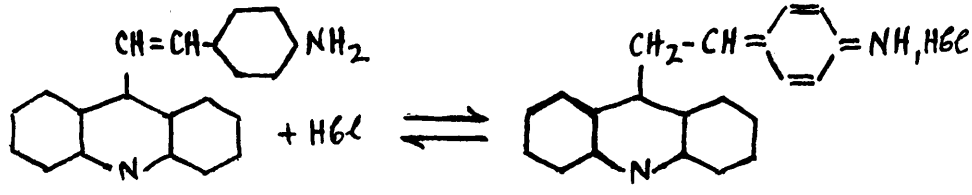
The discrepancy between the melting points of the 5-(p-aminostyryl)-acridine obtained by the author (242°C) and that obtained by Porai-Koschitz (209°C) has already been mentioned. Like the corresponding nitro-compounds, these amino-compounds may be geometric isomers. The amino-compound obtained by the author was found to possess, like the compound described by Porai-Koschitz, the property of dyeing wool violet. The colour phenomenon associated with the compound has been more fully explored in this work. Thus it was observed that the violet colour produced by the compound in weakly acid solution is most intense when the hydrogen ion concentration is very low; for example the compound dissolves in glacial acetic acid with a deep violet colour, which changes to red on the addition of water as the acetic acid becomes more ionised, and finally to yellow on the addition of a drop of dilute hydrochloric acid. Further, the solution of the

hydrochloride of the compound in water is reddish brown, but developes the violet shade on dilution, the violet colour again changing to yellow on the addition of a drop of dilute hydrochloric acid, On adding dilute alkali to the yellow solution of the compound in dilute mineral acid a transient reddish-violet colour is obtained near the neutral point, and finally a yellow precipitate of the free base separates when the alkali is in excess.

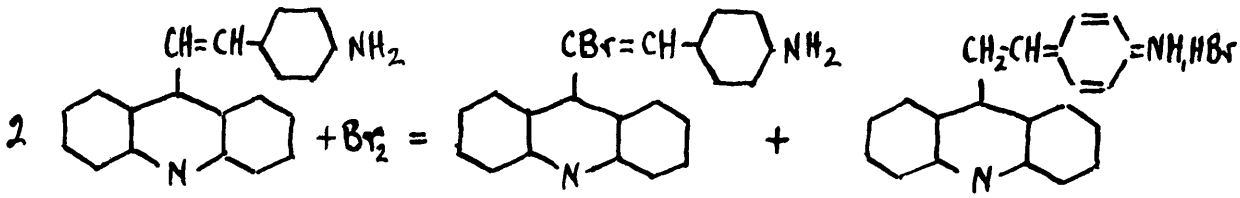
Similar observations were made with the corresponding p-dimethylamino-styryl compound (page 49), which was also found to give the most intense colour (deep prussian blue) in feebly ionised acids such as tartaric acid dissolved in absolute alcohol, or glacial acetic acid, the colour changing to green on the addition of water, and to yellow on the addition of a drop of dilute hydrochloric acid.

An explanation of the above colour phenomena, which are absent in the corresponding m-aminostyryl-compound, has been suggested from the work of Rupe and Porai-Koschitz (Ref. 16) on the amino-benzylidene-derivatives of acetone and acetophenone. The coloured salts of p-amino-benzylidene-acetone were considered by these workers to be produced by a transition of the benzene nucleus into the quinonoid form, and they have supported this hypothesis by preparing the amino-benzylidene-derivatives of acetophenone, and showing that the m-amino compound and its salts are colourless whereas the p-amino compound and p-dimethylamino-compound give rise to

coloured salts. Thus the explanation of the colour change observed with the aminostyrylacridines, based on these facts, may be as follows:



A possible explanation of the colour reaction given by the p-aminostyryl-compound with bromine in chloroform solution (not previously described) may now be given. When the corresponding nitro-styryl compound is treated with bromine in chloroform a bromo-derivative formed by substitution is obtained (see page 40). Assuming that a similar reaction occurs with the p-aminostyryl compound, hydrobromic acid will necessarily be produced. As observed above, a trace of acid causes the p-aminostyryl compound to become violet. Since a violet colour is produced in the reaction in question on the addition of the first drop of bromine in chloroform, later disappearing, with separation of a brown precipitate of the bromo-derivative, it is believed that the first drop of bromine in chloroform effects substitution in some of the p-aminostyryl compound, the hydrobromic acid thereby liberated producing the colour change in the remainder of the p-aminostyryl compound, thus:-



As more bromine in chloroform is added the whole of the original p-aminostyryl derivative is converted to the insoluble bromo-derivative. Hence the violet colour disappears and a brown precipitate of the bromo-derivative separates.

A similar reaction was observed with the corresponding p-dimethylamino-styryl compound (page 49) and may be explained in the same way.

The acetyl derivative of the above p-aminostyryl compound does not call for special comment here; it did not give the violet colour reaction in glacial acetic acid so characteristic of the free amino-compound, probably due to the inability of the acetylamino-group to form a salt.

Compounds from the reaction between 5-methylacridine and m-nitrobenzaldehyde.

The investigation of this reaction was carried out in much the same way (and with similar objects in view) as the investigation of the corresponding reaction using p-nitro-benzaldehyde.

The first experiment in preparing the styryl derivative was carried out according to the method of Porai-Koschitz (Ref. 8), melting the reactants together at 145° C for 3½ hours in presence of zinc chloride. So much tarring occurred in this

reaction that no styryl compound could be isolated. On repeating the experiment with the temperature of reaction reduced to 130°C, a satisfactory yield (64% theoretical) of the styryl derivative was obtained, although more tarring occurred in this experiment than in the corresponding experiment using p-nitrobenzaldehyde.

When the reactants were heated together in acetic anhydride, the styryl compound (identical with the product above) was obtained but only in very poor yield (9% theoretical), much tarring occurring. The possibility of carrying out the condensation in a suitable solvent with piperidine as catalyst was not explored on account of the poor results obtained in this connection with the experiments using p-nitrobenzaldehyde. The method of Porai-Koschitz, modified as indicated above, therefore remains the most satisfactory means of preparing 5-(m-nitrostyryl)-acridine.

On repeating the experiment of Friedlander (Ref.13) who obtained the styryl derivative by simply melting together the reactants at 100°C. for 24 hours, it was observed that the melt began to solidify after about half an hour's heating. After 7 hour's heating the product was examined and found to consist largely of the corresponding ethanol derivative (not previously described), together with a small amount of the nitrostyryl derivative. The latter, however, differed in appearance (pale cream coloured solid) and melting point

(207°C) from the product (a bright yellow solid melting at 210°C) obtained by the modified method of Porai-Koschitz above. The product obtained by Friedlander is described as a yellow solid melting at 206 - 208°C; Porai-Koschitz in his work did not isolate the substance in the pure state but simply converted it to the corresponding amino-compound. Since the two m-nitrostyryl compounds obtained by the author otherwise resemble each other in general properties they are thought to be geometric isomers, as already pointed out in the discussion on the corresponding p-nitrostyryl compound (see page 39). Both m-nitrostyryl compounds obtained by the author gave a reddish precipitate of a bromo-derivative on treatment with bromine in chloroform, but the compositions of these bromo-derivatives were not further investigated.

The ethanol derivative isolated in the investigation of the Friedlander method above, was not used further in this work, for reasons similar to those given for the corresponding p-compound (page 40) which it closely resembled in general properties.

For the purpose of preparing compounds suitable for therapeutic testing the m-nitrostyryl compound obtained by the modified Porai-Koschitz method was used. On reduction 5-(m-aminostyryl)-acridine was obtained; this was then submitted to therapeutic testing, and also converted to the corresponding acetylamino-styryl compound for the preparation of acridinium salts as in Part IV.

The properties of the m-amino styryl compound prepared were found to agree with the description of the compound given by Porai-Koschitz. The colour reactions described by the author for the corresponding p-amino and p-dimethylamino compounds were not given by the m-aminostyryl compound, (see page 42). In the case of the reaction with bromine in chloroform, the compound gave an orange precipitate of the bromo-derivative which was not preceded by a violet colouration. The absence of intense colour-reactions was also noted with the m-acetylamino-styryl compound (not previously prepared), which resembled the corresponding p-acetylamino-styryl compound.

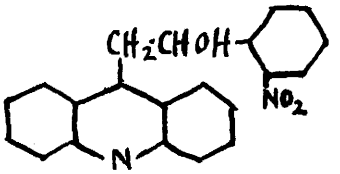
The reaction between 5-methylacridine and o-nitrobenzaldehyde.

The above reaction does not appear to have been previously investigated. With the same objects in view as in the corresponding reactions with m- and p-nitrobenzaldehydes, a series of experiments was again carried out in which the reactants were heated together with and without the addition of condensing agents. The experiments using zinc chloride as condensing agent failed to produce the desired styryl derivative, much tarring occurring even when the temperature of reaction was reduced to 110°C. Similarly no styryl derivative was isolated in the experiment using acetic anhydride as condensing agent.

On melting the reactants together at 100°C and continuing heating at this temperature the melt began to

solidify after 10 minutes owing to the formation of the ethanol derivative. The product obtained after 7 hours heating was still the ethanol derivative, no corresponding styryl compound being isolated. The general properties of this ethanol derivative were similar to those of the corresponding m- and p- compounds, but it resinified and did not yield the styryl compound on heating with acetic anhydride.

The stability of these three isomeric ethanol derivatives towards heating at 110°C decreases in the following order:- p-; m-; o-. This observation maybe correlated with the amount of tarring produced in the preparation (attempted preparation in the case of o-) of the corresponding styryl derivatives using zinc chloride as condensing agent, when tarring was found to increase in the same order (p-; m-; o-). Since from the evidence obtained it is reasonable to assume that styryl formation is preceded by ethanol formation it may be that the close proximity of the nitro-group in the molecule to the "ethane" carbon atoms in the case of the o-compound (I), either hinders the loss of the elements of water from these atoms, by steric effect, or results in resinification by self-oxidation.

I 

Thus the reaction between 5-methylacridine and o-nitrobenzaldehyde has not produced any compounds of the type

required.

The reaction between 5-methylacridine and p-dimethylaminobenzaldehyde.

Experiments were carried out on this reaction along the same lines as the previous experiments using the nitrobenzaldehydes. The method of Porai-Koschitz (Ref.8) of heating together the reactants for 6 hours at 135°C in presence of zinc chloride was found to be the most satisfactory means of preparing the styryl compound.

On heating the reactants together at 100°C, little or no reaction was observed to occur, no ethanol derivative was isolated from the product, and only qualitative evidence of a trace of styryl derivative was obtained. Piperidine showed no appreciable catalytic effect on the styryl-formation either on heating the reactants alone or in alcohol. This is in keeping with the findings of Hamer (Ref.17) who has shown that even the methiodide of 5-methylacridine does not react with p-dimethylaminobenzaldehyde in alcoholic solution containing piperidine.

The properties of 5-(p-dimethylaminostyryl)-acridine were found to agree with those recorded by Porai-Koschitz. A fuller account of the colour phenomenon associated with the compound has been given by the author in the Experimental Section. In this connection the compound closely resembles 5-(p-aminostyryl)-acridine, and the colour phenomena of the two

compounds, including their reactions with bromine in chloroform(not previously recorded), have been discussed earlier (page 42).

The p-dimethylamino-styryl compound was submitted to therapeutic testing, and also converted to the corresponding acridinium compound as in Part IV.

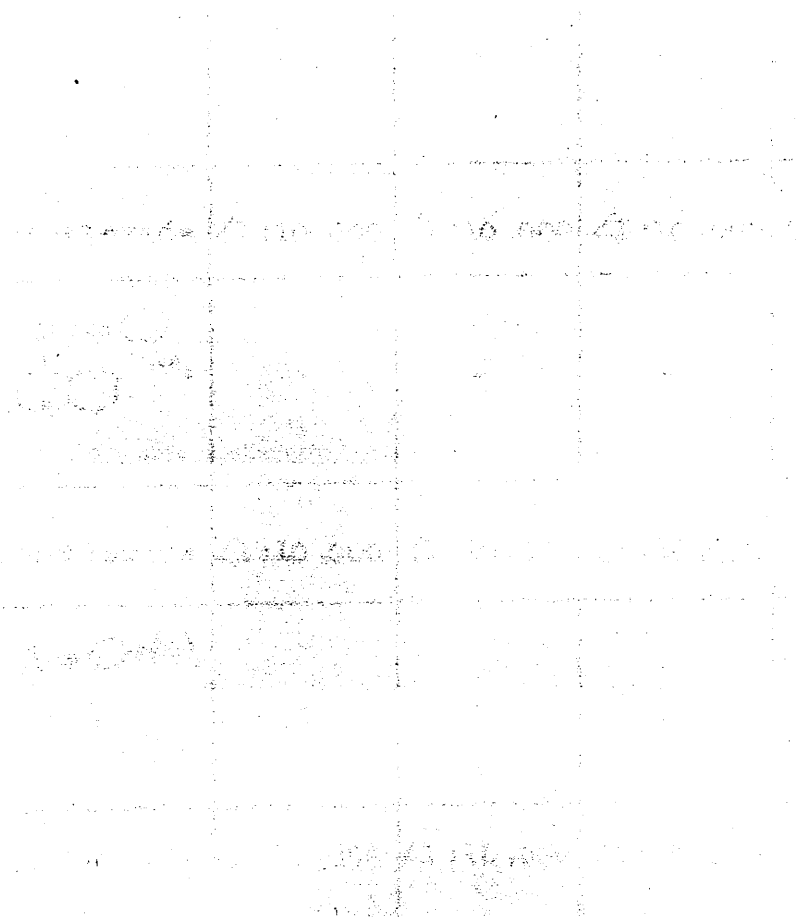


TABLE III.

Antiseptic Properties of 5-styrylacridines prepared in Part II.

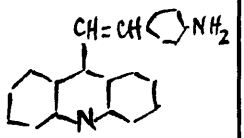
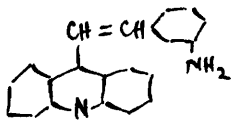
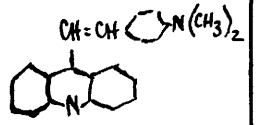
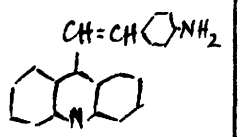
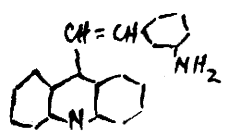
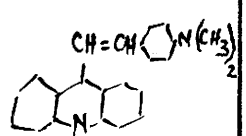
COMPOUND		STERILISING DILUTION			
		Staph. aureus		B. coli	
No.	Formula	Peptone water	Ox serum	Peptone water	Ox serum
7		-	-	-	-
8	" hydrochloride	<1:10,000	<1:10,000	<1:10,000	<1:10,000
9		-	-	-	-
10	" hydrochloride	<1:10,000	<1:10,000	<1:10,000	<1:10,000
11		-	-	-	-
12	" hydrochloride	<1:10,000	<1:10,000	<1:10,000	<1:10,000

TABLE IV

Trypanocidal Action and Toxicity of the 5-styryl-acridines prepared in Part II.

Note: Doses are expressed in terms of the strength of the solution used, 1 c.c. of the stated dilution being given per 20 g. mouse.

COMPOUND		TOXICITY		TRYPANOCIDAL ACTION (T. Brucei)	
No.	Formula	Lethal dose	Tolerated dose	Dose	Result
7		1:125	1:200	1:200	Nil
8	" hydrochloride	-	-	-	-
9		-	1:125	1:200	Nil
10	" hydrochloride	-	-	-	-
11		-	1:125	1:200	Nil
12	" hydrochloride	-	-	-	-

PART III

STYRYL-ACRIDINES PREPARED FROM DERIVATIVES OF 5-METHYLACRIDINE.

General Considerations.

In this part of the Theoretical Section the work on styryl-formation with 5-methylacridine itself, described in Part II, is extended to suitable substituted 5-methylacridines, with the object of preparing, for therapeutic testing, styryl-acridines having amino-groups in the acridine nucleus. The process of styryl-formation with substituted 5-methylacridines does not appear to have been carried out before, so preliminary experiments were undertaken to find out the best conditions for the process; in this connection the findings in the experimental work of Part II were applied with advantage.

The type of substituted 5-methylacridine required for styryl-formation in this work has already been discussed in Part I (see page 7). The following substances represent the principal types of methylacridines prepared in Part I (with the exception of the type obtainable from the reaction between 5-nitro-2-chloroacetophenone and m-aminoacetanilide, which is not considered here, for reasons given on page 27), and are suitable for styryl-formation:-

3-nitro-5-methylacridine.
3-nitro-7-acetylamino-5-methylacridine.
3-nitro-5:7-dimethylacridine.

In attempting to condense these substituted 5-methylacridines with nitrobenzaldehydes by heating in presence of zinc chloride, as described for 5-methylacridine in Part I, difficulty was experienced on account of the fact that these substituted methylacridines do not melt below 360°C. Thus although 5-methylacridine (melting point 115°C) and p-nitrobenzaldehyde(melting point 106°C) in molecular proportions readily form a melt during the heating with zinc chloride at 130°C, no satisfactory melt was formed in the case of the substituted 5-methylacridines; this prevents, or at any rate greatly hinders, styryl-formation.

To overcome this difficulty the use of a suitable solvent for the reactants was considered. It has been shown in Part II, that the most satisfactory substance in this connection is acetic anhydride, which fulfils the two-fold purpose of solvent and condensing agent. Although it was shown there that much poorer yields result using acetic anhydride as condensing agent instead of zinc chloride, a reasonable amount of the styryl compound was obtained from 5-methylacridine and p-nitrobenzaldehyde with acetic anhydride. Since it appeared from this and other work in Part II that the most satisfactory aldehyde as regards reactivity for styryl-formation is p-nitrobenzaldehyde, and time did not permit of an investigation into the use of other substituted benzaldehydes, only the reactions with p-nitrobenzaldehyde have been used for producing the required styryl compounds from the

substituted 5-methylacridines.

All these styryl-acridines, prepared using acetic anhydride therefore give on reduction, a p-amino-group in the benzene nucleus. This is an advantage from the therapeutic point of view, as Browning and his co-workers (Refs. 22/28) have shown that an amino-group in the benzene nucleus in the styryl-quinolines exerts its greatest therapeutic effect when occupying a p-position.

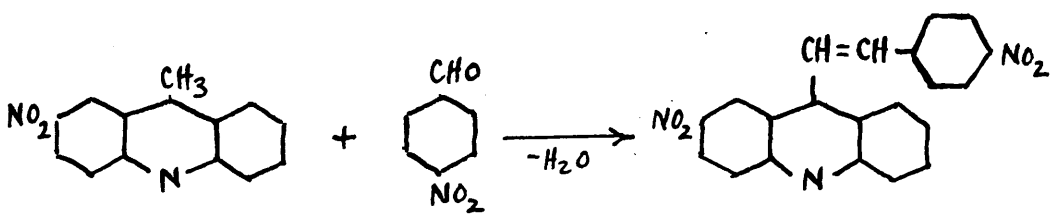
Details of the practical work involved in the preparation of these styryl-acridines suitable for therapeutic testing are given in Part III of the Experimental Section. The various points arising in the course of this work will now be discussed following the same order.

Compounds from the reaction between 3-nitro-5-methylacridine and the nitrobenzaldehydes.

Preliminary experiments on the above reaction were first carried out at 100°C in absence of condensing agents. In the case of p-nitrobenzaldehyde, the difficulty mentioned earlier was encountered - the substances did not melt together and no condensation products were isolated. With m- and o-nitrobenzaldehydes under these circumstances however, a pasty melt was produced which hardened during heating, the ethanol derivative being isolated from the product in each case. These ethanol compounds were not examined further for the preparation of compounds suitable for therapeutic testing,

since the corresponding compounds obtained in Part II were found to be unsuitable either for reduction or for satisfactory conversion to the corresponding styryl compounds. In any case, it was decided for reasons given earlier (page 54), to concentrate on the condensation of the substituted 5-methyl-acridines with p-nitrobenzaldehyde.

Attempts to prepare the styryl derivative by heating together 3-nitro-5-methylacridine and p-nitrobenzaldehyde in presence of zinc chloride, first at 130° C then at 110° C, failed since the reactants did not melt together satisfactorily and tarring occurred at the higher temperature. By using acetic anhydride as condensing agent in place of zinc chloride, however, the reactants went into solution on heating and crystals of the styryl derivative separated:-



For the production of compounds suitable for therapeutic testing, this styryl derivative was reduced to the corresponding diamino-compound. This was submitted to therapeutic testing and also converted to the di-acetylamino-compound for the preparation of quaternary salts as in Part IV.

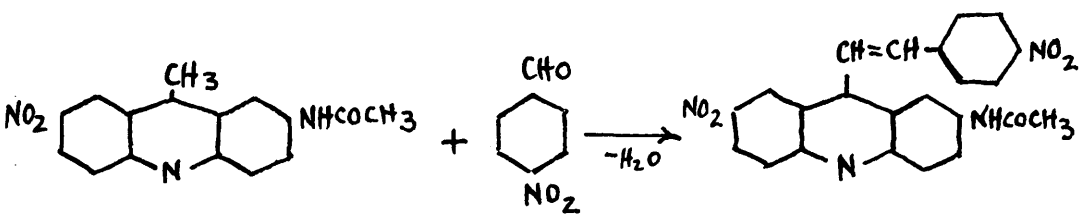
The diamino-compound obtained as above showed properties which resembled on the one hand those of 5-(p-aminostyryl)-acridine, and on the other those of 3-amino-5-

methylacridine; these facts are in keeping with the structure of the compound. Thus on treatment with glacial acetic acid the compound gave a colour reaction similar to that observed for 5-(p-aminostyryl)-acridine, whereas the compound itself tended to be resinous and difficult to crystallise, and its solution in ether showed greenish fluorescence in daylight, properties which recall those of 3-amino-5-methylacridine.

Similarly the di-acetylamino-compound resembled 3-acetylamino-5-methylacridine in its general solubility in the common solvents; by giving a reddish colour with glacial acetic acid it also resembles 5-(p-acetylaminostyryl)-acridine.

Compounds from the reaction between 3-nitro-7-acetylamino--5-methylacridine and p-nitrobenzaldehyde.

The method of condensing the reactants by heating them together in acetic anhydride was again applied here and found to produce a satisfactory yield of the styryl-derivative:-

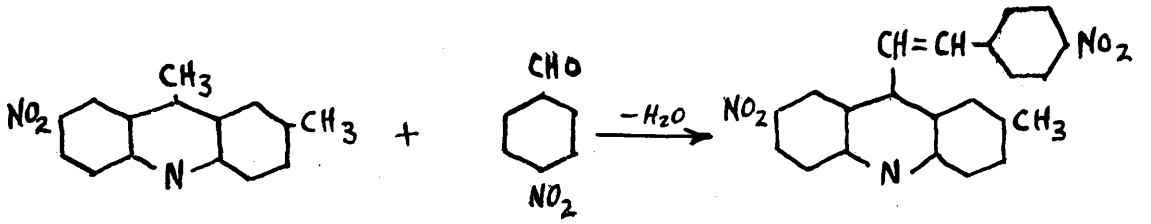


The styryl compound was reduced and hydrolysed to give the corresponding tri-amino-compound, which was submitted to therapeutic testing and also converted to the corresponding tri-acetylamino-compound for use in the preparation of acridinium salts as in Part IV. Like the corresponding

products obtained from 3-nitro-5-methylacridine and discussed above, the tri-amino-and tri-acetylamino compounds obtained here possessed properties intermediate between those of the corresponding di-substituted methylacridines on the one hand, and the corresponding p-substituted styrylacridines on the other.

Compounds from the reaction between 3-nitro-5:7-dimethyl-acridine and p-nitrobenzaldehyde.

Again a satisfactory yield of the styryl derivative was obtained by heating together the reactants in acetic anhydride:-



The styryl derivative obtained in this reaction was the mono-styryl derivative; in order to test the theory advanced in Part II (page 32) that a methyl group in the acridine nucleus will only be reactive towards aldehydes when it occupies the 5-position, two molecular proportions of p-nitrobenzaldehyde were used in this reaction. The fact that the mono-styryl compound has been obtained supports this theory for, since the 5-methyl-group throughout this work has been shown to be reactive, it is extremely unlikely that styryl-formation has occurred at position 7 in this case.

The styryl-compound was reduced to the corresponding

di-amino-compound, which was submitted to therapeutic testing and also converted to the di-acetylamino compound for use in the preparation of acridinium salts as in Part IV.

The products obtained above from 3-nitro-5:7-dimethyl-acridine closely resemble the corresponding products obtained from 3-nitro-5-methylacridine, and need not be further discussed here.

TABLE V

Antiseptic Properties of 5-styrylacridines prepared in Part III.

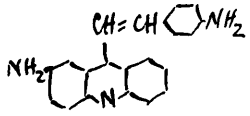
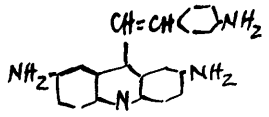
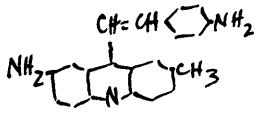
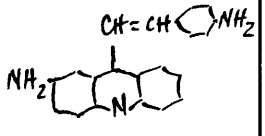
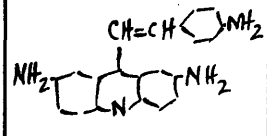
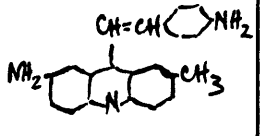
COMPOUND		STERILISING DILUTION			
		Staph. aureus		B. coli	
No.	Formula	Peptone water	Ox serum	Peptone water	Ox serum
13	 hydrochloride	< 1:1,000	< 1:1,000	< 1:1,000	< 1:1,000
14	 hydrochloride	< 1:1,000	< 1:1,000	< 1:1,000	< 1:1,000
15	 hydrochloride	< 1:10,000	< 1:10,000	< 1:10,000	< 1:10,000

TABLE VI

Trypanocidal Action and Toxicity of the 5-styryl-acridines prepared in Part III.

Note: Doses are expressed in terms of the strength of the solution used, 1 c.c. of the stated dilution being given per 20 g. mouse.

COMPOUND		TOXICITY		TRYPANOCIDAL ACTION (T. Brucei)	
No.	Formula	Lethal dose	Tolerated dose	Dose	Result
13	 hydrochloride	-	1:100	1:200	Nil
14	 hydrochloride	-	1:100	1:200	Nil
15	 hydrochloride	-	1:100	1:200	Nil

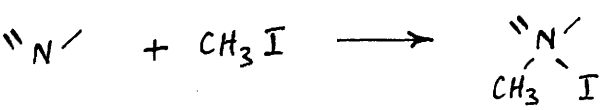
PART IV

ACRIDINIUM SALTS.

General Considerations.

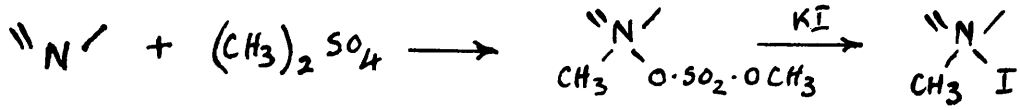
The various amino-derivatives of 5-methylacridine and 5-styrylacridine prepared in Parts I - III, are themselves insoluble in water, and were generally submitted to therapeutic testing as soluble hydrochlorides; occasionally in the case of "in vivo" tests, however, the free bases were injected as dispersions. Two advantages are therefore to be gained by converting these compounds into the corresponding acridinium salts. First, such salts, particularly the methochlorides, are easily soluble in water; secondly the change in therapeutic activity on forming the quaternary salts may be observed - it is known that the formation of these salts usually enhances the activity of quinoline and acridine compounds.

The method of preparing these acridinium salts may now be considered. 5-Methylacridine itself has been converted to its methiodide in two different ways. In the first of these the acridine base is simply heated with methyl iodide (Bernthsen (Ref.6); Hamer (Ref,17)):-



In the second method (Kaufmann and Albertini (Ref.18)) the acridine base is heated with dimethyl sulphate; from the

aqueous solution of the product the methiodide is precipitated by the addition of potassium iodide:-



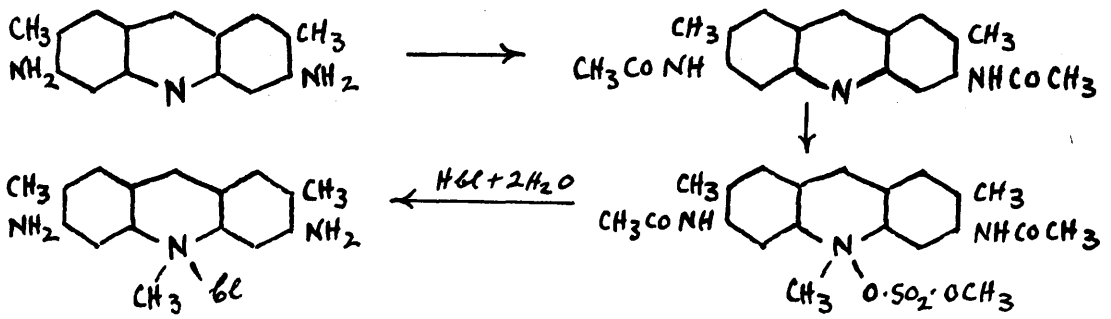
By using sodium chloride in place of potassium iodide in the second stage of this process the corresponding methochloride is precipitated.

Of these two methods the second seemed the more convenient as the first, using low-boiling methyl iodide, requires the reactants to be heated together in a sealed tube. Further, methochlorides were preferred to methiodides for therapeutic testing on account of their greater solubility in water.

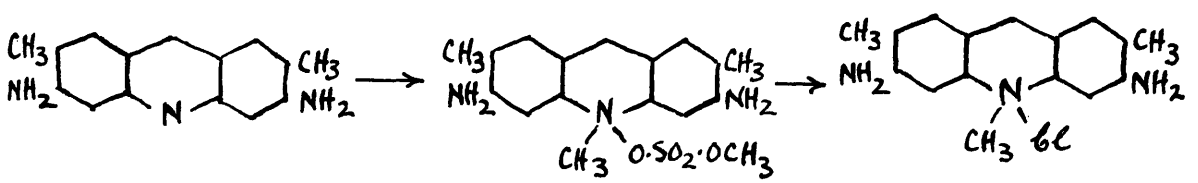
The use of methyl p-toluenesulphonate in place of dimethyl sulphate in the second method above does not appear to have been tried previously in the case of 5-methylacridine, although it has been used with success by Ullmann and Wenner (Ref.19) in the case of 5-phenylacridine, the metho-p-toluenesulphonate crystallising out on heating the reactants together in nitrobenzene. With a view to its possible general application in this work, the extension of this reaction to 5-methylacridine will be discussed after the following points from the literature, dealing with acridinium salt formation in the case of certain amino-acridines, have been considered.

Ullmann and Maric (Ref.20) have described the preparation of 2:8-diamino-3:7-dimethylacridine methochloride from the

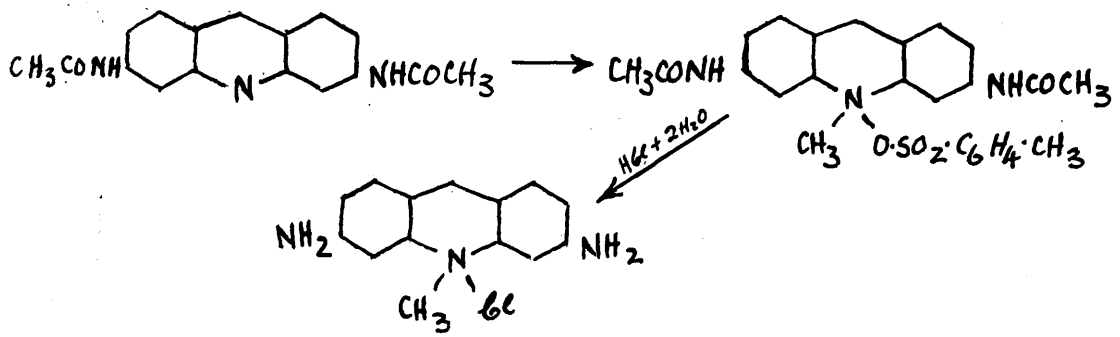
base of acridine yellow by heating its di-acetyl derivative with dimethyl sulphate in nitrobenzene, and hydrolising the product with hydrochloric acid:-



The acetylation of the free amino-groups, in order to prevent their methylation during the heating with dimethyl sulphate, apparently is unnecessary here for these workers also point out that the same methochloride was obtained by heating the 2:8-diamino-3:7-dimethylacridine itself with dimethyl sulphate in nitrobenzene, and treating the aqueous solution of the product with sodium chloride:-



In his synthesis of 2:8-diaminoacridine methochloride, Benda (Ref.21) has followed the first method of Ullmann and Maric, using, however, methyl p-toluenesulphonate in place of dimethyl sulphate:-



Benda appears to have considered it necessary to protect the amino-groups by acetylation to prevent their methylation. The author therefore considered it advisable, since all the compounds to be converted to acridinium salts in this work contain free amino-groups, to acetylate the compounds prior to quaternary salt formation; the preparation of these acetyl-derivatives has already been dealt with in Parts I - III.

Using 5-methylacridine itself, preliminary experiments were carried out on the use, in the first stage of metho-chloride formation, of methyl p-toluenesulphonate in place of dimethyl sulphate, since the former according to Benda is almost as reactive as the latter and much more convenient to work with. In the first experiment the reactants were heated together in nitrobenzene, but only a little of the metho-p-toluenesulphonate separated on cooling, the remainder of the yield remaining in the nitrobenzene, from which it could not be satisfactorily separated. It was therefore decided to try dispensing with the nitrobenzene and simply to melt the reactants together, in molecular proportions, at 145°C. This

resulted in a satisfactory yield of the metho-p-toluene-sulphonate, which resembled the corresponding methosulphate (described in the literature) by dissolving readily in water, the solution yielding a precipitate of the methiodide on treatment with potassium iodide, and, if the solutions were sufficiently concentrated, a precipitate of the more soluble methochloride with sodium chloride.

Considering now the various acetylamino-compounds from which it is proposed to prepare acridinium compounds suitable for therapeutic testing, it was found that the method of fusing with methyl p-toluenesulphonate at 145°C to obtain the metho-p-toluenesulphonates was of general application, although in a few cases the proportion of ester used had to be more than molecular, in order to obtain a satisfactory melt. As a general rule these metho-p-toluenesulphonates were difficult to isolate on account of their high solubility in water and in alcohol. Bearing in mind that these salts are the metho-p-toluenesulphonates of the acetylamino-compounds, two methods (suggested from the work of Ullmann and Maric´ and of Benda) are theoretically possible for converting them to the methochlorides of the corresponding amino-compounds. In the first method the aqueous solution of the metho-p-toluene-sulphonate is treated with sodium chloride to precipitate the methochloride of the acetylamino-compound; the acetylamino-groups are subsequently hydrolysed. In no case in this work,

however, did the methochloride separate as above with sodium chloride. In the second method the metho-p-toluenesulphonate is boiled with a mixture of equal volumes of water and concentrated hydrochloric acid; the methochloride of the amino-compound should separate crystalline from this hydrolysis mixture, but only in one case in this work was this found to occur.

The problem therefore resolved itself into finding a method of isolating the methochloride after boiling the metho-p-toluenesulphonate with hydrochloric acid. The residue remaining after evaporating the hydrolysis mixture contains p-toluenesulphonic acid as well as the methochloride; attempts to effect a separation of these two substances by means of solvents proved unsuccessful. The methochloride had eventually to be isolated through the less soluble methiodide, by dissolving the residue, obtained on evaporating the hydrolysis mixture, in water, neutralising the solution, and adding potassium iodide. The methiodide was then converted to the methochloride by boiling with excess silver chloride in aqueous methyl alcohol.

The description of the practical work given in the Experimental Section (Part IV) is arranged according to the types of acridine derivatives being converted to acridinium salts, in the following order:-

5-methylacridines prepared in Part I
5-styrylacridines prepared in Part II
5-styrylacridines prepared in Part III

The various points arising in the course of this work will now be considered, following the same order.

Compounds obtained from 5-methylacridines prepared in Part I.

The starting materials in question, prepared in Part I, are:-

3-acetylamino-5-methylacridine.
3:7-di-(acetylamino)-5-methylacridine.
3-acetylamino-5:7-dimethylacridine.

In all three cases the methochlorides of the corresponding amino-compounds were prepared by the general process discussed earlier, and submitted to therapeutic testing. Only in the case of 3-acetylamino-5-methylacridine was the metho-p-toluenesulphonate isolated crystalline and submitted to therapeutic testing. It dissolved in water with intense greenish fluorescence; the methochloride of the corresponding amino-compound dissolved in water with a deep red colour. These colour phenomena recall those of the corresponding acridine bases when dissolved in glacial acetic acid (see Part I).

It has been observed here, and generally throughout Part IV, that the aqueous solutions of the methochlorides possess the same colour as the hydrochlorides of the corresponding bases prepared in Part I. These solutions may be distinguished, however, by treating with a slight excess of dilute alkali; under these conditions the colour is discharged and a precipitate of the free base, usually yellow

or brown, immediately separates in the case of the hydrochlorides, whereas with the methochlorides the colour only gradually fades with slow precipitation.

The methiodide obtained as an intermediate in the isolation of 3-amino-5-methylacridine methochloride was submitted to therapeutic testing. Like all the methiodides of 3-aminoacridines prepared in Part IV, this compound was obtained as a purplish red solid which dissolved in alcohol with a beautiful reddish-pink shade. The methiodides in the case of 3:7-diamino-acridines were reddish solids forming orange-red solutions in alcohol. In the case of the methiodides prepared from acridines having no amino-groups in the acridine nucleus these were yellow-brown solids forming yellow brown solutions in alcohol. The methochlorides of all the compounds having amino-groups in the acridine nucleus were reddish solids forming deep red aqueous solutions.

Compounds obtained from 5-styrylacridines prepared in Part II.

The starting materials in question, prepared in Part II, are:-

5-(p-acetylaminostyryl)-acridine.
5-(m-acetylaminostyryl)-acridine.
5-(p-dimethylaminostyryl)-acridine.

The methochloride of the amino-compound corresponding to the first substance above, separated crystalline from the mixture in the hydrolysis of the metho-p-toluenesulphonate, and therefore the general procedure of isolation through the

methiodide was unnecessary. The compound was submitted to therapeutic testing.

The required methochlorides were prepared from the second and third starting materials above by the general procedure, and were submitted to therapeutic testing. In the case of the p-dimethylamino-compound only the ring-nitrogen has been affected during the quaternary salt formation, as shown by analysis and taking into account the fact that the ring-nitrogen has been reactive in this connection in all other acridines considered here. The fact that the quaternary salt of the dimethylamino-group has not been formed under the conditions of the reaction here is not surprising, for Ullmann and Maric (Ref.20) found that in the case of a dimethylamino-acridine treated with dimethylsulphate and subsequently converted to the methochloride, only the ring-nitrogen was affected.

Compounds obtained from 5-styrylacridines prepared in Part III.

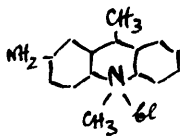
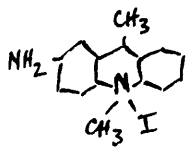
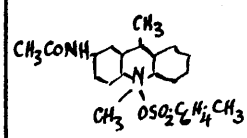
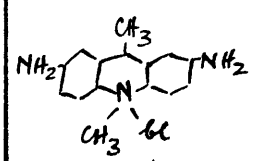
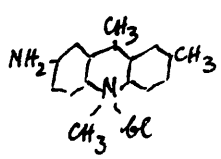
The starting materials in question, prepared in Part III, are:-

3-acetylamino-5-(p-acetylaminostyryl)-acridine.
3:7-di-(acetylamino)-5-(p-acetylaminostyryl)-acridine,
3-acetylamino-5-(p-acetylaminostyryl)-7-methylacridine.

The methochlorides of the corresponding amino-compounds were all obtained by the general procedure and do not call for special comment here. They were all submitted to therapeutic testing.

TABLE VII

Antiseptic Properties of Acridinium Salts prepared in Part IV.

COMPOUND		STERILISING DILUTION			
		Staph. aureus		B. coli	
No.	Formula	Peptone water	Ox serum	Peptone water	Ox serum
16		1:40,000	1:40,000	1:20,000	1:20,000
17		1:40,000	1:10,000	<1:10,000	1:20,000
18		1:10,000	1:20,000	<1:10,000	1:10,000
19		1:100,000	1:40,000	1:20,000	1:100,000
20		1:100,000	1:100,000	1:10,000	1:10,000

(continued on next page)

TABLE VII (contd.)

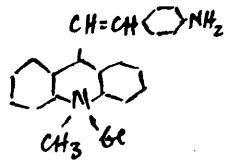
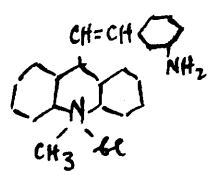
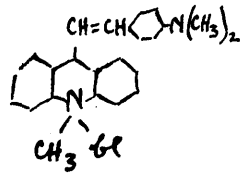
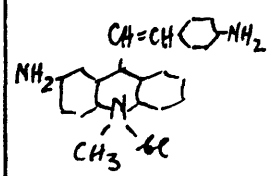
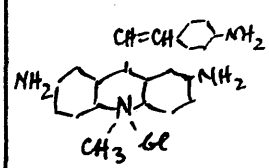
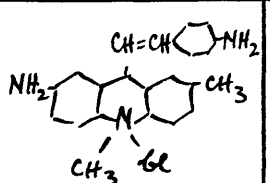
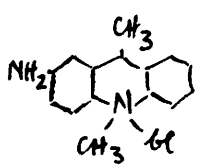
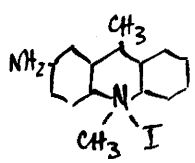
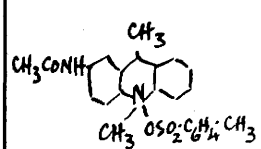
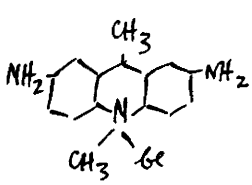
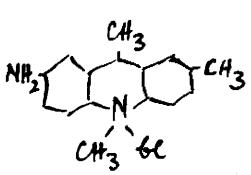
COMPOUND		STERILISING DILUTION			
		Staph. aureus		B. coli	
No.	Formula	Peptone water	Ox serum	Peptone water	Ox serum
21		1:40,000	1:20,000	1:20,000	1:40,000
22		1:20,000	< 1:10,000	< 1:10,000	< 1:10,000
23		1:100,000	1:10,000	1:10,000	< 1:10,000
24		1:40,000	1:40,000	1:40,000	1:10,000
25		1:10,000	1:10,000	1:20,000	1:10,000
26		1:40,000	1:40,000	1:2,000	1:100,000

TABLE VIII

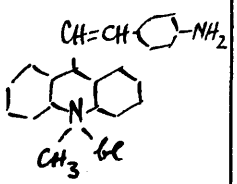
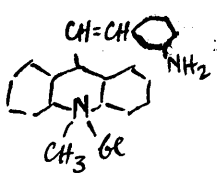
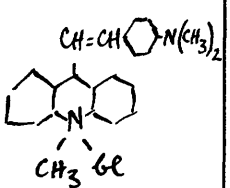
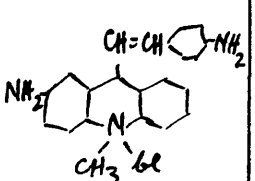
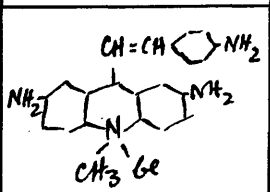
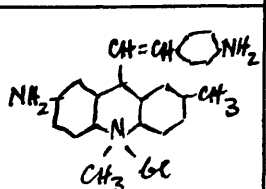
Trypanocidal Action and Toxicity of the Acridinium Salts prepared in Part IV.

Note: Doses are expressed in terms of the strength of the solution used, 1 c.c. of the stated dilution being given per 20 g. mouse.

COMPOUND		TOXICITY		TRYPANOCIDAL ACTION (T. Brucei)	
No.	Formula	Lethal dose	Tolerated dose	Dose	Result
16		1:100	1:250	1:500	Nil
17		-	1:100	1:100	Slight action
18		-	1:100	1:200	Nil
19		1:250	1:500	1:500	Action
20		1:100	1:250	1:500	Nil

(continued on next page)

TABLE VIII (contd.)

COMPOUND		TOXICITY		TRYPANOCIDAL ACTION (T. Brucei)	
No.	Formula	Lethal dose	Tolerated dose	Dose	Result
21		1:100	1:500	1:500	Very slight action
22		-	1:350	1:350	Nil
23		1:5,000	1:10,000	1:10,000	Nil
24		1:250	1:500	1:500	Action
25		-	1:100	1:350	Action
26		1:500	1:750	1:1000	Action

PART V.

DISCUSSION ON THE RESULTS OF THE THERAPEUTIC TESTS.

The results of the therapeutic tests on the various compounds prepared in this work have already been tabulated (Tables I - VIII) at the end of each of the foregoing Parts of the Theoretical Section. It is the object in this Part of the Theoretical Section to consider these results more fully and to discuss the therapeutic action of the compounds tested, in the light of their chemical structure. Before proceeding with this, however, it is necessary to elucidate certain points in connection with Tables I - VIII.

Considering first the antiseptic tests (Tables I, III, V, and VII), the sterilising dilutions of acriflavine under similar conditions may now be given, for the sake of comparison (pH of the peptone water in all cases was 7.2 to 7.8):-

Staph. aureus.		B. coli.	
Peptone water	Ox serum	Peptone water	Ox serum
1:200,000	1:200,000	1:20,000	1:100,000

Where, in these Tables, the sign $<$ appears before a dilution, it means that this dilution failed to sterilise, and that less dilution (greater concentration) would have been necessary to effect sterilisation.

Regarding trypanocidal action (Tables II, IV, VI, and VIII) the terms "very slight action", "slight action", and

"action" indicate that when the dose of the compound shown is injected subcutaneously in the amount of 1 c.c. per 20 g. body-weight to a mouse inoculated 24 hours previously with T. Brucei, and at the time of treating showing scanty parasites in the blood, life is prolonged for several days or longer beyond that of the control untreated animals which die regularly 3 to 4 days after inoculation. This prolongation of life in the case of:-

"very slight action"	means for	1 - 3	days
"slight action"	" "	3 - 7	days
"action"	" "	7 -10	days, or more.

None of the substances examined in this work, however, proved to be curative, i.e. in every instance the parasites, if ever absent from the blood, returned again and death from the infection occurred.

.....

Most of the compounds prepared for therapeutic testing have been tested both before (see Tables I - VI) and after (see Tables VII and VIII) conversion to acridinium salts. The results have shown that these acridinium salts are in general much more active therapeutically, though occasionally slightly more toxic, than the corresponding acridines. The latter, in fact, have shown no trypanocidal action at all, and only in the case of the diamino-methyl-acridines (Compounds 4 and 5) have they displayed even moderate antiseptic properties.

The acridines prepared in Parts I - III (i.e. before conversion to acridinium salts) will first be considered. The introduction of an amino-group into position 3, which greatly increases antiseptic action in the case of acridine itself (Albert and Linnell (Ref.31)), has not been found to confer even moderate antiseptic action in the case of 5-methylacridine, although the introduction of an additional amino-group causes moderate antiseptic properties to appear (see Compounds 4 and 5); the introduction of the 3-amino-group into 5:7-dimethyl-acridine was also found to have little or no effect on antiseptic action. The introduction of the aminostyryl-group into position 5 in the acridine molecule has failed to confer marked antiseptic properties, even on the introduction of amino-groups into the acridine nucleus; this failure will be discussed when dealing with the corresponding methochlorides.

Turning now to the acridinium salts prepared in Part IV it will be seen that with one exception (Compound 22) these are all moderately antiseptic, a few being strongly antiseptic; several, in addition, have shown trypanocidal action. The generally accepted theory that quaternary salt formation enhances the therapeutic properties of quinoline and acridine compounds is therefore fully borne out by the results obtained in this work.

Thus in the case of the amino-5-methylacridines it was found that the methochloride and methiodide of the

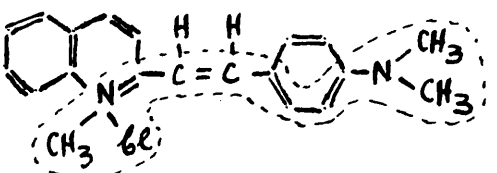
3-amino-compound were both moderately antiseptic; the methiodide shows slight trypanocidal activity and is less toxic than the methochloride, probably on account of the fact that the former is less soluble in water than the latter, and therefore is more slowly absorbed by the mouse. The 3-acetylamino-compound was tested as its metho-p-toluenesulphonate (Compound 18) and was found to be rather less active as an antiseptic than the 3-amino-compounds, but was less toxic than the methochloride of the 3-amino-compound (compare also the toxicities of the corresponding acridines prepared in Part I (Compounds 1 and 3) which show a similar reduction in toxicity on acetylation). These facts are as would be expected, for the effect of acetylation on an amino-group is usually to reduce both therapeutic activity and toxicity. The effect of the introduction of an additional amino-group, as in the methochloride of the 3:7-diamino-compound (Compound 19), is to produce a substance strongly antiseptic against both Staph. aureus and B. coli; this substance also possesses trypanocidal action, but is rather toxic. On introducing a second methyl group, as in the methochloride of 3-amino-5:7-dimethylacridine, strong antiseptic action against Staph. aureus results, there being no increase in toxicity over the corresponding mono-methyl-compound.

In the case of the methochlorides of the aminostyryl-acridines having no substituent groups in the acridine nucleus,

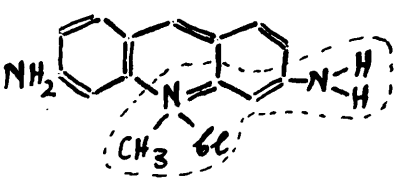
results have been obtained which can be explained in terms of the assumption made by Browning and co-workers (Refs. 22 - 28) that the following grouping, in which a chain of alternating single and double linkages joining two nitrogen atoms, the one being of a basic nature and trivalent, the other pentavalent (strictly 4-covalent), has an important bearing on therapeutic activity in the quinoline dyes and in acriflavine:-

Thus in the styryl-quinolines (I) and in acriflavine (II) the above type of grouping is present:-

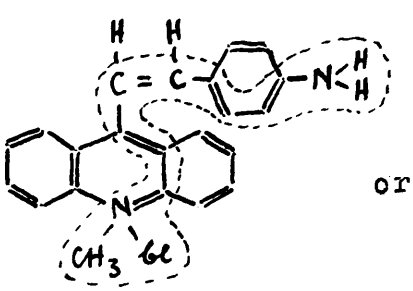
I



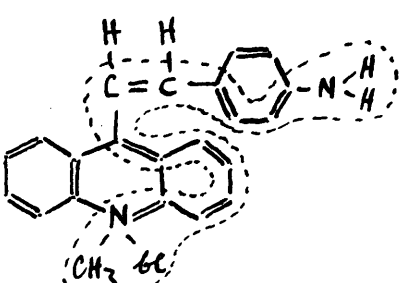
II



This grouping is also present in the methochlorides of some of the 5-styrylacridines prepared by the author; in these cases there are two alternating single and double linkage systems joining the nitrogen atoms: e.g. with 5-(p-aminostyryl)-acridine methochloride:-



or



Thus the results of the tests on 5-(p-aminostyryl)-acridine methochloride have shown that the compound is

moderately antiseptic, and possesses slight trypanocidal action. Similarly 5-(p-dimethylaminostyryl)-acridine methochloride shows some therapeutic activity, being strongly antiseptic against Staph. aureus; this compound is highly toxic however. It has been shown in two different ways, in this work on the 5-styrylacridines, that departure from the type of system including the above important grouping results in considerable reduction in therapeutic activity. In the first place 5-(m-aminostyryl)-acridine methochloride, in which the alternating single and double linkage system joining the nitrogen atoms is upset, possesses relatively poor antiseptic properties; the toxicity of the compound has not been fully tested although in the case of the corresponding acridines (Compounds 7 and 9) the m-amino-compound shows a lower toxicity than the p-isomer. In the second place, as already stated, the styryl-acridines prepared in Parts II and III do not possess even moderate antiseptic properties; these compounds are not acridinium salts and therefore both nitrogen atoms in the grouping are trivalent.

Considering now the methochlorides of the amino-styryl-acridines having substituent groups in the acridine nucleus, these compounds are all p-aminostyryl derivatives, and therefore possess the characteristic groupings present, as shown above in 5-(p-aminostyryl)-acridine methochloride; it is therefore to be expected that they will be at least as

active therapeutically as the latter. This has actually been borne out by the results, which have shown that the compounds are in fact more active than 5-(p-aminostyryl)-acridine methochloride, which may be regarded as the basic structure in these compounds. Thus on introducing the 3-amino-group into such a structure (as in Compound 24), the general antiseptic properties are slightly improved, and the trypanocidal action considerably improved; the toxicity, however, is slightly increased. The introduction of a second amino-group as in the 3:7-diamino-compound (Compound 25) did not improve the general antiseptic properties, but has improved the trypanocidal action. The effect of introducing both a 3-amino- and a 7-methyl-group into the basic structure(as in Compound 26) is to increase both antiseptic and trypanocidal properties; the compound is strongly antiseptic against B. coli, but is more toxic than either the basic structure or the 3-amino derivative of the basic structure.

The difference in therapeutic properties between the methochlorides of the 5-methylacridines (Compounds 16, 19, and 20) and those of the corresponding 5-styryl-acridines (Compounds 24, 25, and 26) is not very marked, although the latter appeared to be rather more active than the former. Thus, while the antiseptic properties of corresponding compounds in these two classes are not on the whole greatly different, trypanocidal action is shown by all three of the

styryl-acridines, but only by one (the diamino-derivative) of the methyl-acridines. No generalisation concerning the toxicity in these two classes of compounds can be made, for in the case of the 3-amino- and 3-amino-7-methyl-derivatives the styryl-acridines were the more toxic, whereas in the case of the 3:7-diamino-derivatives the methyl-acridine was the more toxic.

EXPERIMENTAL SECTION

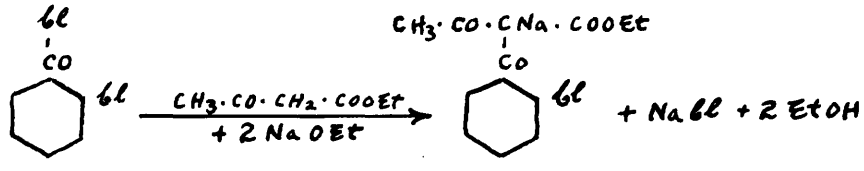
PART I

PREPARATION OF DERIVATIVES OF 5-METHYLACRIDINE FROM 5-NITRO-2-CHLOROACETOPHENONE.

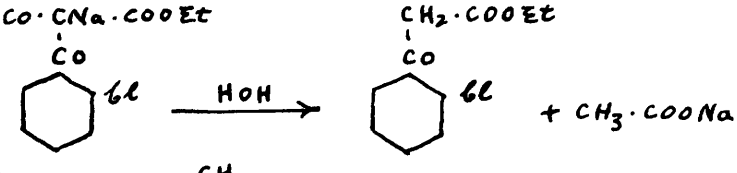
PREPARATION OF o-CHLOROACETOPHENONE.

This compound was prepared as described by Thorp and Brunskill (Ref.1) starting from o-chlorobenzoyl chloride:-

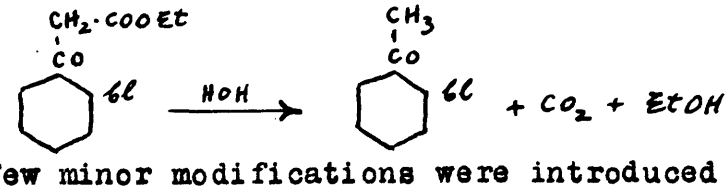
Stage(i)



Stage(ii)



Stage(iii)



A few minor modifications were introduced with a view to obtaining the maximum yield of ketone pure enough for the preparation of 5-nitro-2-chloroacetophenone, the starting material for some of the acridine derivatives prepared in this section.

Owing to the high cost of o-chlorobenzoylchloride, the synthesis of the ketone was carried out in small batches as required, using 50 g. of the acid chloride each time. Some six such syntheses were carried out in all, the following

experimental details being typical of one of these:-

Stage(i) Ethyl o-chlorobenzoylacetoacetate (as sodium derivative):-

13.95 g. sodium
215 ccs. absolute alcohol
39.4 g. ethyl acetoacetate
38 ccs. o-chlorobenzoyl chloride

The sodium was dissolved in the alcohol and to half of this solution the ethyl acetoacetate was added. This mixture was cooled to 5°C. and half of the o-chlorobenzoyl chloride slowly run in from a burette, stirring constantly and keeping the temperature below 12°C. After half an hour at 0° to 5°C. half the remaining sodium ethoxide solution was added and the temperature allowed to fall to 5°C. again. Half the remaining o-chlorobenzoyl chloride was slowly added as before, and after half an hour at 0° to 5°C. the process was repeated in this fashion, using half the remaining sodium ethoxide solution and o-chlorobenzoyl chloride, so as to complete the additions in five successive stages. The reaction mixture had by this time become very thick due to the separation of the sodium derivative of ethyl o-chlorobenzoylacetoacetate. To obtain the maximum possible yield of the latter, the reaction mixture was placed in a refrigerator at 0° to 5°C. for 3 or

4 days in place of the 24 hours suggested by Thorp and Brunskill.

The product was then filtered with suction and washed with dry ether, and was obtained as a white finely-divided powder. Yield: 77.5 g.

Stage(ii) Ethyl o-chlorobenzoylacetate:-

77.5 g. ethyl o-chlorobenzoylacetoacetate (sodium derivative)
389 ccs .water
34.9 g. ammonium chloride
38.9 ccs .strong ammonia solution

The sodium derivative of ethyl o-chlorobenzoylaceto-:acetate was dissolved in the water, the ammonium chloride and ammonia added, then the liquid heated on the water-bath to between 40°C and 50°C. The mixture was kept at this temp-:erature, and after a short time the solution became oily due to the separation of ethyl o-chlorobenzoylacetate. The reaction mixture was occasionally shaken during the period of heating, which was increased to 6 hours in place of the 2 to 3 hours recommended by Thorp and Brunskill, since it was found that the ester was still separating after 3 hours. On cooling, the ester was found to separate well from the clear aqueous layer, and appeared as a heavy yellow oil. The extraction of the ester with ether (Thorp and Brunskill's procedure) was therefore omitted, and the lower layer of

ester simply run off, washed with a little dilute sulphuric acid, separated, and used as such(without drying) for the next stage of the synthesis, which entails boiling with aqueous sulphuric acid anyway. Yield: 41 g.

Stage(iii) o-chloroacetophenone.

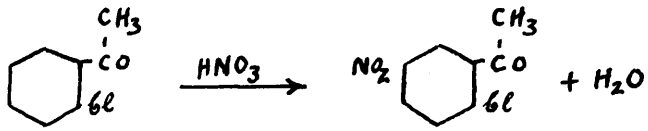
41 g. ethyl o-chlorobenzoylacetate
182 ccs. water
45.6 ccs. concentrated sulphuric acid

The sulphuric acid was added to the water, the ester added and the mixture boiled under reflux until carbon dioxide was no longer evolved. This was generally the case after 12 hours if the boiling was brisk enough to keep the oily ester well dispersed throughout the acid. On cooling, the o-chloroacetophenone floated to the top of the acid, and was extracted with ether. The ethereal solution was shaken with 10% potassium hydroxide solution to remove any unchanged ester, then washed with water and dried over anhydrous calcium chloride. The ether was distilled off from the dry solution and the ketone distilled, collecting the high boiling distillate up to 230°C. (when the small tarry residue in the distilling flask began to show signs of decomposing). The product was then sufficiently pure for nitration to 5-nitro-2-chloro-:acetophenone (page 88). Yield: 23.8 g.

Purity of the o-chloroacetophenone prepared: During the distillation of the ketone above, the distillate came over almost entirely between 228°C and 229°C at 758 mm. (Thorp and Brunskill give 227°C to 228°C at 738 mm.) The compound was therefore regarded as pure enough for nitration, omitting redistilling with fractionation. Omitting the fractionation of the ketone has incidently given rise to an interesting and unexpected by-product on nitration (page 15 and page 90).

The yield of ketone obtained represents 54% of that theoretically possible based on the amount of o-chlorobenzoyl chloride taken initially. It was a colourless liquid, not miscible with water, possessing an aromatic odour. In addition to the boiling point given above, the ketone was further characterised by preparing its oxime as described by Thorp and Brunskill. This was found to crystallise from 50% alcohol in silky needles melting at 103°C. as stated by these workers.

NITRATION OF o-CHLOROACETOPHENONE.



The nitration was carried out as described by Thorp and Brunskill (Ref.1), the product obtained being 5-nitro-2-chloroacetophenone. In crystallising the crude product a noticeable loss in yield, not mentioned by Thorp and Brunskill, always occurred, and this was investigated. During this investigation an interesting by-product was isolated and characterised.

The Nitration Process:

20 g. o-chloroacetophenone.

200 g. nitric acid (sp. gr. 1.52)

Nitric acid of sp. gr. 1.52 contains 99.7% HNO_3. The strongest acid conveniently obtainable was "fuming" nitric acid (sp. gr. 1.50, corresponding to about 95% HNO_3). By distilling nitric acid with concentrated sulphuric acid, mixing the distillate with phosphorus pentoxide and distilling over the water-bath, Aston and Ramsay (Ref.3) obtained nitric acid containing 99.8% HNO_3. It was found, however, that the distillation with phosphorus pentoxide could be omitted,

provided the distillate obtained by distilling "fuming" nitric acid with an equal volume of concentrated sulphuric acid was used straight away for the nitration.

The nitric acid (sp. gr. 1.52) so obtained was cooled to 0°C, and the ketone gradually added from a burette, with stirring, keeping the temperature below 5°C. When the ketone had all been added, the reaction mixture was allowed to stand for 3 hours at 0°C to 5°C, then poured into about 1000 ccs. ice-water. The nitro-compound separated as a white flocculent precipitate, which was filtered off, washed well with water, then with a little 50% alcohol, and allowed to dry in the air. Yield: 22.1 g.

This washed product was generally pure enough for use in a synthesis once the experimental details of the latter had been established using the crystallised product.

Crystallisation of the Nitration Product: The product was crystallised from alcohol. The Melting Point (62°C.) and other characteristics given by Thorp and Brunskill were tested and found to apply to the compound. An inquiry into the losses associated with the crystallisation, undertaken because of the high cost of preparing the compound, revealed the following figures: 10 g. washed product yielded:-

7.25 g. crystals (Main crop and two crops from mother liquor)
2.31 g. yellow residual oil (on removing alcohol from final mother liquor)
0.27 g. white solid insoluble in hot alcohol (the "by-product")

The "residual oil" was thought at first to be simply a residue of 5-nitro-2-chloroacetophenone too impure to crystallise, but the work on 2-acetyl-4-nitro-diphenylamine has shown (page 106) that this is probably not the case, for 10 g. of the washed 5-nitro-2-chloroacetophenone gave about the same yield of the diphenylamine derivative as 7.25 g. of the crystalline ketone. That this "residual oil" probably contains some un-nitrated o-chloroacetophenone was shown by distilling it, when a little liquid distilled between 220° and 230°C. At higher temperatures however the contents of the distilling flask became tarry, and at 260°C a violent reaction took place, dense clouds of white smoke being evolved leaving behind a spongy charred mass in the distilling flask.

The "residual oil" was not examined further than this for its nature appeared to be complex and its exploration of little value to the work as a whole. The "by-product" (alcohol-insoluble solid), on the other hand, was fully investigated for the reasons given on page 15, and the

following results recorded:-

By-Product from the Nitration: The substance insoluble in hot alcohol was obtained as a white crystalline powder by crystallising from benzene. Melting Point 178°C. It was insoluble in water, alcohol, ether, and petroleum ether, slightly soluble in glacial acetic acid, chloroform, and benzene, and soluble in hot benzene and in acetone.

The substance was neutral to moist litmus paper, and insoluble in cold sodium hydroxide solution. On heating the substance with sodium hydroxide solution, however, it dissolved forming a reddish solution which changed to yellow (no precipitate) on the addition of a slight excess of dilute hydrochloric acid, reverting to a brighter red colour on the addition of a slight excess of sodium hydroxide solution. This indicator effect is discussed on page 16.

Qualitative analysis showed that the compound contained nitrogen and chlorine. The presence of the nitro-group was shown by reducing with anhydrous stannous chloride reagent (page 114) to the primary amine group, which then gave the usual diazotisation and coupling reaction.

Quantitative analysis of the compound gave the following

results (Oxygen by difference):-

C ,42 .0%; H ,2 .7%; N ,12 .0%; Cl ,15 .4%; O ,27 .9%

Empirical formula indicated is $C_8H_7O_4N_2Cl$ which requires:-

C ,41 .7%; H .3 .0%; N .12 .1%; Cl .15 .4%; O .27 .8%

Molecular Weight determination (Rast's Camphor Method .) gave 236

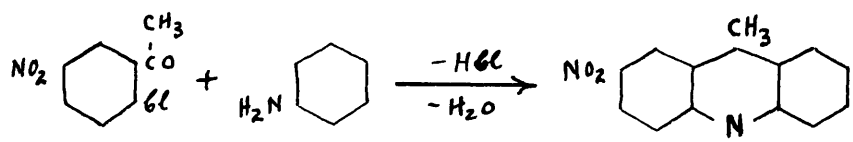
Molecular Weight of $C_8H_7O_4N_2Cl$ is 231

In the theoretical section (page 16) it is shown that the constitution of a dinitro-chloro-ethylbenzene $C_8H_7O_4N_2Cl$, a new substance, is in keeping with the properties of the above compound. An explanation for its occurrence is also given, in the light of such a constitution.

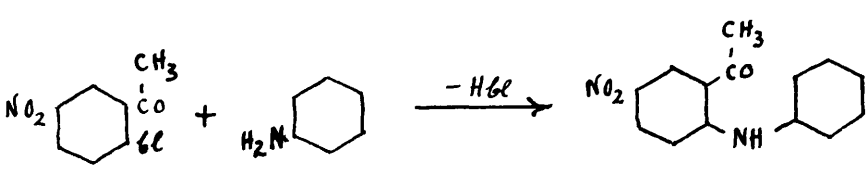
INVESTIGATION OF THE CONDITIONS UNDER WHICH 5-NITRO-2-CHLORO-ACETOPHENONE CONDENSES WITH ANILINE.

The object of this investigation, together with a discussion of the experimental results, is set out in the theoretical section (pages 12, and 18).

The experiments carried out fall into two groups. In the first of these an attempt was made to prepare 3-nitro-5-methylacridine directly from 5-nitro-2-chloroacetophenone and aniline:



In the second group of experiments the method of Jensen and Rethwisch (Ref.5) for producing the intermediate 2-acetyl-4-nitrodiphenylamine by the following reaction was examined and improved upon:



GROUP I: Attempts to prepare 3-nitro-5-methylacridine directly.

Experiment No.1 The method of Ullmann and Ernst (Ref. 2) for preparing 3-nitro-5-phenylacridine directly was applied

here:

1 g. 5-nitro-2-chloroacetophenone.
3 ccs.freshly redistilled aniline (large excess)
0.5 g.anhydrous sodium acetate.

The ketone was dissolved in the warm aniline. The orange-red solution so produced was treated with the sodium acetate and gently boiled (b.pt. 180-190°C) under reflux for 2½ hours. The colour of the mixture soon changed from orange-red to brown, then finally almost black. The tarry-:looking product was stirred up whilst warm with 10 ccs. warm alcohol, cooled, allowed to stand for a time, then filtered:-

Residue: This was washed, first with 50% alcohol then with water, and allowed to dry. The black amorphous powder so obtained failed to crystallise from the common solvents, and in no way resembled 2-acetyl-4-nitrodiphenylamine or 3-nitro-5-methylacridine, which are described later (pages 103 & 105) The acridine derivative would have been obtained at this stage had the reaction proceeded as for 3-nitro-5-phenylacridine (Ullmann and Ernst). The aqueous-alcohol washings from the black residue however, were found to contain a considerable amount of chloride, which appeared to indicate that condens-:ation (HCl elimination) had occurred, only to be followed by decomposition of the condensation product. It was thought that this decomposition might be avoided by altering one or all

of the following experimental conditions:

(1) Temperature employed.
(ii) Time of heating.
(iii) Proportion of aniline.

The effect of altering these conditions was investigated in Experiments Nos.2,3,4, and 5 which follow.

Filtrate: The alcoholic filtrate from the reaction mass appeared to contain only resinous matter and unchanged aniline, as the following examination showed.

The filtrate was poured into a mixture of 40 ccs. concentrated hydrochloric acid and 200 ccs. water, with the object of separating the aniline and any acridine derivative from any intermediate diphenylamine derivative, the latter being only feebly basic (page 103). The resinous precipitate so formed was filtered, but could not be obtained crystalline. The acid filtrate from this was made alkaline with ammonia and extracted with chloroform. On removing the solvent from the chloroform extract a brown viscous liquid containing aniline remained which failed to yield any solid product after treatment with alcohol and "Norite" decolourising carbon.

Experiment No.2 The time of heating in this Experiment was reduced to 1 hour, but in other respects the experiment was carried out exactly as for Experiment No.1

Again a tarry product was obtained from which no crystalline substance could be isolated. Decomposition to resinous matter appeared to start after only 15 minutes at the boil.

Experiment No.3: This experiment was carried out with the object of studying the effect of temperature on the re-:action mass.

Time of heating (hours)	Temperature of mass	Appearance of mass.
0	100°C	Orange-coloured and clear.
1/3	110°C	do.
2/3	120°C	do.
1	130°C	do.
1.1/3	140°C	Slightly darker.
1.2/3	150°C	Darkening continued.
2	160°C	Dark brown.
2.1/3	170°C	Dark brown and viscous.
2.2/3	180°C	do.
3	180°C	do
3.1/3	180°C	do.
3.2/3	180°C	do.
4	180°C	do.

The reaction mixture was prepared as in Experiment

No.1, but the heating was conducted in a bath of liquid paraffin. The temperature (internal) was raised from 100°C by increments of 10° every 20 minutes and observations of the appearance of the mass recorded as in accompanying table.

The product obtained at the conclusion of this experiment was examined as in Experiment No.1, but yielded no crystalline product. It was noted from the table, however, that no appreciable resinification occurred below 130°C, but that rapid tarring set in above 150°C.

Experiment No.4: In this experiment the temperature of reaction was reduced to 125°C.

The reaction mixture (quantities as in Experiment No.1) was heated in an oil bath to 125°C (±5°). After 3 hours at this temperature the mixture was deep orange-brown in colour but showed no signs of tarring, so the heating was continued for a further 3 hours at 125°C to compensate for the reduced temperature of reaction. The brown product while still warm was examined by the method described in Experiment No.1, i.e. stirred up with 10 ccs. warm alcohol, allowed to cool, then filtered after standing for several hours:-

Residue: No condensation product was obtained here for the residue dissolved completely on attempting to wash with water. The solution of the residue was shown to contain a

considerable amount of chloride, however, which seemed to indicate that some condensation (HCl elimination) had occurred.

Filtrate: This deep brown alcoholic solution was treated as in Experiment No.1, by adding it to a mixture of hydrochloric acid and water, when a brown resinous-looking precipitate was formed. After filtration the filtrate was examined as in Experiment No.1 but yielded no crystalline product. The residue, however, was extracted with ether, rejecting the small amount of insoluble matter, and found, after removing the ether, to crystallise readily from alcohol. The crystals so obtained were recrystallised from alcohol, treating with "Norite" decolourising carbon in the process. The bright yellow crystals so obtained melted at 130°C, and proved on examination to be 2-acetyl-4-nitrodiphenylamine (page 103). Yield: 0.3g. (23% theoretical).

Experiment No.5. The quantity of aniline was here reduced to the theoretical amount. The quantities used were therefore:

1g. 5-nitro-2-chloroacetophenone
0.46cc. freshly redistilled aniline
0.5g. anhydrous sodium acetate

Two separate reaction mixtures ("a" and "b") were prepared using the above quantities for each.

Reaction mixture "a": This was heated at 180°C for 3

hours in an oil bath. The dark brown tarry product was examined as in Experiment No.4. In contrast to the previous experiments conducted at 180°C, this examination produced results similar to those obtained in Experiment No.4, a yield of 2-acetyl-4-nitrodiphenylamine being obtained (0.4 g; 31% theoretical).

Reaction mixture "b": This was heated at 125°C (±5°) for 6 hours in an oil bath. The product, which showed no signs of tarring, was examined as in Experiment No.4. A notable difference, attributed to the absence of tarry matter, was observed this time when the mass was treated with alcohol. The alcohol-insoluble solid formed a bulky yellow residue which only slightly diminished when washed with water. On drying and crystallising from alcohol, the substance melted at 130°C and proved to be 2-acetyl-4-nitrodiphenylamine (page 103). Yield: 0.4 g. (31% theoretical). The filtrate from the re-:action mass in alcohol was examined as in Experiment No.4, but yielded no further crystalline product.

GROUP II: Examination of the Jensen and Rethwisch method for producing 2-acetyl-4-nitrodiphenylamine.

Experiment No.1 The method of Jensen and Rethwisch (Ref.5) was followed here:

1.g. 5-nitro-2-chloroacetophenone
1.25 ccs. freshly redistilled aniline
1.g. anhydrous potassium carbonate.

The reaction mixture, orange-coloured at first, was heated for 3 hours at 170°C - 180°C in an oil bath. The mixture rapidly became darker in colour, and at the end of the heating period had become dark brown and tarry. The product was divided into two portions, "a" and "b", and examined as under:-

Portion "a": This was extracted with 4 ccs. boiling alcohol as described by Jensen and Rethwisch, but yielded no crystals of 2-acetyl-4-nitrodiphenylamine, even after treating the deep-brown extract with "Norite" decolourising carbon.

Portion "b": This was examined by the method adopted in Experiment No.1 of Group I, and showed properties identical with those of the reaction mass in that experiment, no crystalline product being isolated.

Experiment No.2. The temperature of reaction was reduced here to 125°C.

The reaction mass was prepared as in the previous experiment using the same quantities, then heated at 125°C (±5°) in an oil bath. After 3 hours at this temperature no appreciable tarring was noticed, so the heating was continued at the same temperature for a further 3 hours to compensate for the lower temperature employed. The product was treated whilst warm with 6 ccs. alcohol and stirred up, when the liquid became thick due to separation of condensation product.

This mixture was allowed to stand overnight so that the maximum amount of product would separate, then filtered:-

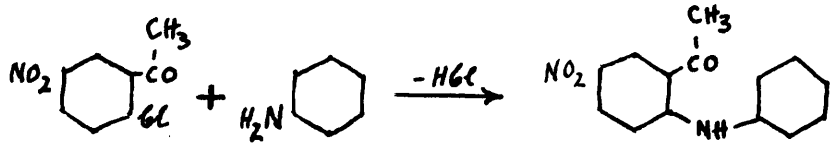
Residue: This was washed with a little cold alcohol, then with water until free from potassium carbonate and chloride. The yellow residue so obtained was allowed to dry, then crystallised from alcohol. The crystals so obtained melted at 130 °C and proved to be 2-acetyl-4-nitrodiphenylamine. Yield: 0.92 g. (72% theoretical)

Filtrate: This alcoholic solution was deep brown in colour and appeared to contain a little resinous matter. On further examination as in Experiment No.1 of Group I, no further yield of crystalline product could be isolated.

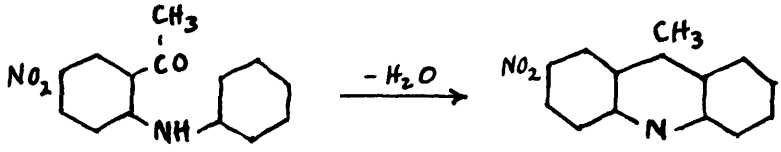
PREPARATION OF 3-NITRO-5-METHYLACRIDINE.

The preceding experimental work (pages 93 - 101) has shown that the best yields of 3-nitro-5-methylacridine are obtained by a modification of the two-stage method of Jensen and Rethwisch (Ref.5):-

Stage I



Stage II



Practical details of the process are now described, the yield of 3-nitro-5-methylacridine in relation to the purity of the starting and intermediate materials investigated, and some hitherto unrecorded properties of 2-acetyl-4-nitrodiphenylamine and of 3-nitro-5-methylacridine noted.

Stage I: Preparation of 2-acetyl-4-nitrodiphenylamine.

7 g. 5-nitro-2-chloroacetophenone
7 ccs. freshly redistilled aniline
7 g. anhydrous potassium carbonate

The ketone was dissolved in the warm aniline and the orange-red solution treated with the potassium carbonate. The mixture was then heated, with occasional stirring, in an oil

bath at 125°C (±5°) for 6 hours, when it became darker and more viscous. While still warm the product was stirred up with 20 ccs. alcohol, the mixture becoming thick owing to separation of the diphenylamine derivative. In order to obtain the maximum yield of the latter, the mixture at this stage was allowed to stand overnight, then filtered, discarding the filtrate which contained most of the resinous matter produced in the reaction. The yellow solid residue so obtained was washed with a little cold alcohol, then with water until free from inorganic salts, and allowed to dry in air. The product was then crystallised from alcohol using decolourising carbon. Yield: See Note on Yields (after Stage II).

Properties of 2-acetyl-4-nitrodiphenylamine:-

The compound formed light yellow needles from alcohol, Melting Point 130°C (Jensen and Rethwisch give 125°C). Analysis gave 65.7% C, 4.67% H, 10.89% N, thus confirming $C_{14}H_{12}O_3N_2$, which requires 65.6% C, 4.69% H, 10.93% N.

The compound is insoluble in water, dilute hydrochloric acid (difference from 3-nitro-5-methylacridine, which is more basic) and petroleum ether; soluble in benzene, ether, chloroform, glacial acetic acid, and hot alcohol; sparingly soluble in cold alcohol. The solution of the compound in chloroform or in glacial acetic acid does not fluoresce in ultra violet light (difference from 3-nitro-5-methylacridine).

Cold concentrated sulphuric acid dissolves the compound, forming an orange coloured solution, which becomes greenish yellow on heating, ring-closure occurring (compare Stage II). Ring-closure also occurs to some extent on boiling the compound with concentrated hydrochloric acid for a few minutes, since the product (precipitated from the yellow acid solution with ammonia) dissolved in chloroform to produce a solution which showed green fluorescence in ultra violet light.

Stage II: Ring-closure of 2-acetyl-4-nitrodiphenylamine.

5 g. 2-acetyl-4-nitrodiphenylamine
50 ccs. glacial acetic acid
2.5 ccs. concentrated sulphuric acid

The diphenylamine derivative was dissolved in the boiling glacial acetic acid, and the yellowish-brown solution treated with the sulphuric acid, drop by drop. Heating was continued with occasional shaking under reflux for 2 hours in an oil bath at 130°C, the solution becoming greenish in colour, and exhibiting bright green fluorescence in ultra violet light, showing that ring-closure had occurred. The warm reaction mixture was then added to 350 ccs. water, and the acridine base precipitated with a slight excess of ammonia. The thick yellow gelatinous precipitate was filtered off, washed with water and allowed to dry in air. The product was then

crystallised from benzene using decolourising carbon. Yield: See Note on Yields (after Stage II).

Note: The proportion of sulphuric acid to acetic acid used in the above preparation was that recommended by Ullmann and Ernst (Ref.2). It was found that this gave slightly better yields than the method of Jensen and Rethwisch, who used a higher concentration of sulphuric acid and heated for a shorter time.

Properties of 3-nitro-5-methylacridine:-

The compound formed yellow needles from benzene. It did not melt at 360°C (Jensen and Rethwisch found that it did not melt at 300°C), although it shrunk and appeared to decompose to a dark solid mass at about 220°C. The identity of the compound was confirmed by analysis which showed 70.5% C, 4.21% H, 11.5% N ($C_{14}H_{10}O_2N_2$ requires 70.6%C, 4.20% H, 11.75% N).

The compound is insoluble in water; soluble in dilute and concentrated hydrochloric acid, concentrated sulphuric acid, chloroform, ether, glacial acetic acid, hot alcohol, and hot benzene; slightly soluble in petroleum ether, cold alcohol and cold benzene. Benzene was found to be superior to alcohol in crystallising the crude base.

The solution of the compound in chloroform exhibits a brilliant yellowish green fluorescence in ultra violet light

but not in daylight. The fluorescence in ultra violet light disappears instantly on the addition of a drop of aniline to the chloroform solution.

Note on Yields:

10 g. washed 5-nitro-2-chloroacetophenone yielded 5.58g. crystalline 3-nitro-5-methylacridine, omitting the crystallisation of the intermediate 2-acetyl-4-nitrodiphenylamine.

7.25 g. crystalline 5-nitro-2-chloroacetophenone yielded 6.78 g. crystalline 2-acetyl-4-nitrodiphenylamine, which yielded 4.98 g. crystalline 3-nitro-5-methylacridine.

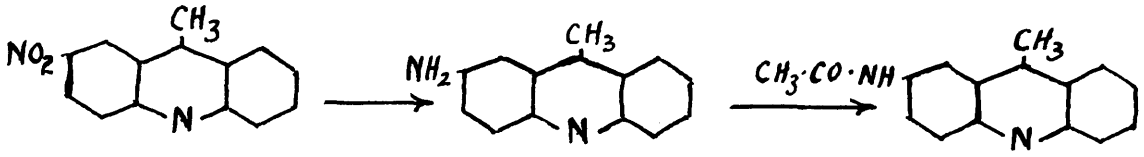
Since 10 g. of the sample of washed 5-nitro-2-chloroacetophenone on crystallisation yield 7.25 g. crystalline ketone (page 89), it follows that (5.58 - 4.98 =) 0.60 g. more (i.e. 12%) crystalline 3-nitro-5-methylacridine results if crystallisation of starting and intermediate materials is omitted. This is also discussed in the theoretical section (page 21). The best recorded overall yield of crystalline 3-nitro-5-methylacridine (based on the weight of crystalline 5-nitro-2-chloroacetophenone represented in the amount of washed ketone used) is therefore 64.5% theoretical. Starting from crystalline 5-nitro-2-chloroacetophenone and crystallising at the intermediate stage, the individual yields are: 73% theoretical for Stage I; 79% theoretical for Stage II.

10 g. washed 5-nitro-2-chloroacetophenone yielded 7.5g.

washed 2-acetyl-4-nitrodiphenylamine, whereas the corresponding amount (7.25 g.) of crystalline 5-nitro-2-chloroacetophenone yielded 7.1 g. washed 2-acetyl-4-nitrodiphenylamine. Since these two yields of diphenylamine derivative are not greatly different, it follows that the uncrystallisable matter in the washed ketone contributes little to the yield of 2-acetyl-4-nitro-diphenylamine and therefore contains very little 5-nitro-2-chloroacetophenone (see also page 90).

PREPARATION AND PROPERTIES OF 3-AMINO-5-METHYLACRIDINE AND 3-ACETYLAMINO-5-METHYLACRIDINE.

These compounds are new. The amino-compound was prepared by reducing 3-nitro-5-methylacridine, and the acetylamino-compound by heating the amino-compound with acetic anhydride and anhydrous sodium acetate:



3-amino-5-methylacridine:

The reduction process described here is essentially that of Ullmann and Ernst (Ref.2) for preparing 3-amino-5-phenylacridine, although the method of isolating the product had to be altered to suit the special properties of 3-amino-5-methylacridine:

2 g. 3-nitro-5-methylacridine (finely powdered)
5.7 g. stannous chloride crystals
10 ccs. alcohol
10 ccs. hydrochloric acid (concentrated)

The stannous chloride was dissolved in the boiling alcohol and half of the yellow nitro-compound gradually added. Reduction occurred at once, the solution becoming deep wine-red in colour. 4 ccs. of the concentrated hydrochloric acid were then added, causing a yellow solid to separate. The second half of the nitro-compound was now slowly added, with stirring, followed by the remainder (6 ccs.) of the hydrochloric acid,

and the yellow reduction mixture heated for 15 minutes on the water-bath. The alcohol was then removed by evaporation and the yellow salt filtered off, washed with a little concentrated hydrochloric acid, and dissolved in 250 ccs. water in which it formed a red solution. This was boiled for 20 minutes and filtered from the gelatinous precipitate of tin hydroxide which separated by hydrolysis. The red filtrate, however, did not deposit crystals of the hydrochloride on cooling or on concentrating, (difference from 3-amino-5-phenylacridine), so the following procedure was adopted:

The red solution of the hydrochloride, after removing tin as above, was treated with a slight excess of ammonia, when a yellow precipitate of the free base was obtained. As the latter was difficult to filter and tended to become brown and resinous on standing, it was best isolated by extraction with ether, any remaining tin (as hydroxide) being left suspended in the aqueous layer. The yellow ethereal extract, which showed bright greenish-yellow fluorescence, was washed with water, and separated. On removing the ether, the base remained as a reddish-brown solid, which would not crystallise satisfactorily from the common solvents.

The most satisfactory product was obtained by dissolving the crude base in warm alcohol, adding an equal volume of warm water, boiling for a few minutes with decolourising carbon,

filtering hot, and allowing some of the alcohol to evaporate slowly from the filtrate over the water-bath (simply allowing the hot filtrate to cool produced a resinous product). At a certain concentration of alcohol, as evaporation slowly proceeded, the base separated in reddish-brown crystalline tufts, which were filtered off, washed with a little aqueous alcohol and allowed to dry. Yield: 1.46 g. (84% theoretical).

The product was a reddish-brown crystalline solid, associated with a little resinous matter, as microscopic inspection showed. It melted with decomposition at about 200°C. Analysis of the compound was not attempted owing to the difficulty in purifying it sufficiently. The compound has been characterised, however, through its crystalline acetyl-derivative (page 111). The use of a cold reducing agent in preparing the compound was tried (anhydrous stannous chloride reagent, page 114, general procedure) but the base so obtained showed the same tendency to resinify as before.

The compound is insoluble in water, but dissolves in dilute hydrochloric acid with a red colour. This acid solution on treatment with excess ammonia, gives a yellow precipitate of the free base, which turns brown and resinous on standing. The compound is soluble in alcohol, ether, chloroform, acetone and concentrated sulphuric acid, the yellow solution in each case showing greenish fluorescence, which, in the case of the sulphuric acid, disappears on dilution with water. In glacial

acetic acid the compound dissolves forming a red solution which remains clear on dilution with water. In benzene and petroleum ether the compound is only sparingly soluble, showing bluish-green fluorescence in the former, and purplish fluorescence in the latter.

The hydrochloride does not separate on treating the solution of the base in dilute hydrochloric acid with concentrated hydrochloric acid, although the colour changes from red to yellow. The product obtained by passing dry hydrogen chloride into a solution of the base in dry ether is brown and resinous. On evaporating a solution of the base in dilute hydrochloric acid over the water-bath, a reddish-brown resinous residue remains (probably the mono-hydrochloride). When this is treated with a little concentrated hydrochloric acid and again evaporated, a brown residue, crystalline in parts, remains (probably the di-hydrochloride). Both salts dissolve readily in water with a red colour.

3-acetylamino-5-methylacridine:

1 g. 3-amino-5-methylacridine
5 ccs. acetic anhydride
1 g. anhydrous sodium acetate.

The above substances were mixed (slight heat being evolved) and heated for an hour in an oil-bath at 120°C. The deep brown liquid so produced was poured, with stirring, into water to which was added a slight excess of ammonia to liberate

the free base from any acetate formed. The yellow-brown precipitate of the acetyl-derivative which separated was allowed to stand for an hour, then filtered off, washed with water, and allowed to dry. The product was then purified by crystallisation from aqueous alcohol using decolourising carbon, and was obtained as a pale yellow crystalline powder. Yield: 1.04 g. (87% theoretical).

Analysis showed 76.8% C, 5.52% H, 11.1% N, ($C_{16}H_{14}ON_2$ requires 76.8% C, 5.60% H, 11.2% N). The substance gave no definite melting point, for it appeared to decompose at about 260°C, becoming darker and charring, but remaining unmelted at 360°C.

The compound is insoluble in water and petroleum ether, sparingly soluble with a yellow colour in benzene, ether (no fluorescence), and chloroform (no fluorescence; but in ultra-violet light the solution shows a bluish fluorescence), more soluble with a yellow colour in alcohol (bluish-green fluor-escence), glacial acetic acid (bright greenish fluorescence, more marked on dilution with water), and dilute hydrochloric acid. The addition of ammonia to a solution of the compound in cold dilute hydrochloric acid liberates the compound again as a pale yellow precipitate.

Hydrolysis of the acetylamino-compound occurs on boiling it with concentrated hydrochloric acid for a few

minutes. This was shown by liberating the free base with ammonia, after hydrolysis, and shaking with ether. The yellow ethereal solution now showed the bright greenish-yellow fluorescence characteristic of the un-acetylated amino-compound

ANHYDROUS STANNOUS CHLORIDE REAGENT.

This reagent is described by Albert and Linnell (Ref.30) who state that it is "a gentle, but effective, cold reducing agent for nitro-groups."

Preparation:

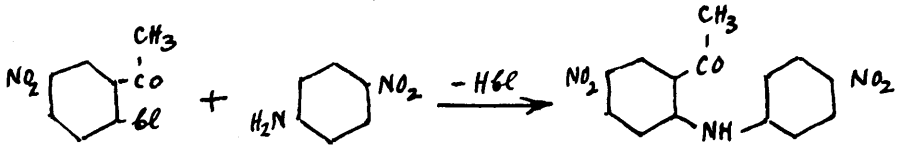
225 g. (1 gram-molecule) powdered stannous chloride crystals ($SnCl_2 \cdot 2H_2O$) were treated with 200 g. acetic anhydride. Heat was evolved as the anhydride reacted with the water of crystallisation, a white suspension of the anhydrous tin salt being obtained. This was diluted to 1 litre with glacial acetic acid, then saturated with dry hydrogen chloride, the suspended tin salt dissolving as saturation was approached.

The reagent is a clear, colourless liquid, fuming in air. It was found to retain its potency for several months when stored in an amber-coloured glass-stoppered bottle.

General Procedure for using the Reagent:

The powdered nitro-compound was shaken with a slight excess of the anhydrous stannous chloride reagent, allowed to stand with occasional shaking during the day, and finally allowed to stand overnight. The nitro-compound dissolved and the stannichloride of the amine separated. The latter was filtered off, dissolved in water, the solution filtered, then treated with an excess of sodium hydroxide solution. The precipitate of the amino-compound which separated was filtered off, washed with water, and allowed to dry.

ATTEMPTS TO CONDENSE 5-NITRO-2-CHLOROACETOPHENONE WITH THE NITRANILINES.



The work of Jensen and Rethwisch (Ref.5) on the above condensation was repeated but no crystalline condensation product was isolated. Experiments were also conducted in which modifications of the conditions employed by Jensen and Rethwisch were introduced, but these were also unsuccessful. In each case considerable tarring occurred although evidence of HCl elimination was obtained. Only the experiments using p-nitraniline are described here, since the experiments using o- and m-nitranilines gave similar results.

Experiment No.1: The method of Jensen and Rethwisch (Ref.5) was followed here:-

1 g. 5-nitro-2-chloroacetophenone.
1 g. recrystallised p-nitraniline (excess).
1 g. anhydrous potassium carbonate.

The above substances were mixed and heated for 3 hours at 170 - 180°C. A brown melt was formed at first, and this rapidly became darker and more viscous. The dark brown tarry product was divided into two portions "a" and "b", and examined by the method given in the corresponding experiment

with aniline (page 100). Similar results were obtained, no crystalline product being isolated, although the presence of chloride seemed to indicate that some condensation (HCl elimination) had occurred.

Experiment No.2: This was carried out as for Experiment No.1, but the temperature was reduced to 125°C (±5°) and the time of heating increased to 6 hours. Again rapid tarring occurred and the product failed to yield any crystalline substance when examined as in Experiment No.1

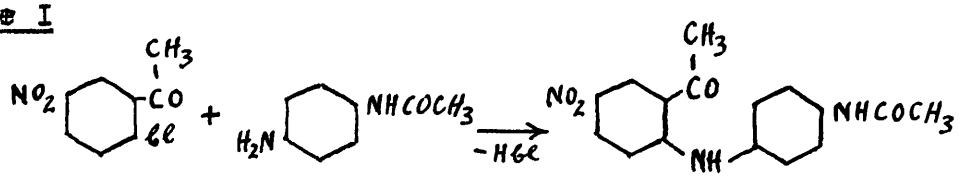
Experiment No.3: This was carried out as for Experiment No.2, but the time of heating was reduced to 1 hour. The product however was again tarry and showed the same properties on examination as the product obtained in Experiment No.2, no crystalline substance being isolated.

Experiment No.4: This was carried out as for Experiment No.1, but the temperature was reduced to 100°C. Again a tarry product was obtained, which failed to yield any crystalline substance on examination as in Experiment No.1

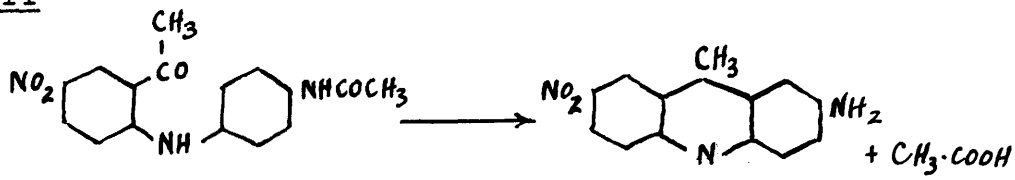
PREPARATION AND PROPERTIES OF 3-NITRO-5-METHYL-7-AMINOACRIDINE.

This new compound was prepared by the two-stage method described on page 102 for 3-nitro-5-methylacridine, combining the 5-nitro-2-chloroacetophenone with p-aminoacetanilide in place of aniline. Hydrolysis of the acetylamino-group occurs at the same time as ring-closure in Stage II. The intermediate 2-acetyl-4-nitro-4'-acetylamino-diphenylamine obtained at Stage I is also new:

Stage I



Stage II



Stage I: 2-Acetyl-4-nitro-4'-acetylamino-diphenylamine:

5 g. 5-nitro-2-chloroacetophenone
5 g. p-aminoacetanilide
5 g. anhydrous potassium carbonate.

The above substances were mixed and heated together at 125°C for 3 hours. A dark brown melt was first formed, which became stiffer and finally hardened as the heating continued. The brown product was powdered, then washed, first with water (to remove inorganic salts; chloride demonstrated in the washings), then with a little cold alcohol, which removed most of the tarry matter produced in the reaction. The

residue was then crystallised from alcohol, using decolourising carbon, and was obtained in the form of beautiful golden-yellow plates. Yield: 5.5 g. (72% theoretical).

The compound melted at 207°C (turning red at about 120°C). Analysis showed 61.3% C, 4.90% H, 13.2% N ($C_{16}H_{15}O_4N_3$ requires 61.3% C, 4.79% H, 13.4% N).

The compound is insoluble in water and petroleum ether, sparingly soluble in hydrochloric acid (dilute and concentrated) cold alcohol, ether, benzene, soluble in warm alcohol, chloroform, acetone and glacial acetic acid. Examined in ultra-violet light the solutions of the compound in ether, benzene, and chloroform showed no fluorescence. The compound became red when treated with concentrated sulphuric acid, forming a red solution on gentle heating. On further heating, this solution became temporarily dark brown in colour and finally orange-yellow, ring-closure occurring. This was shown by cooling the liquid, diluting and making alkaline with ammonia, when a red precipitate was obtained, a solution of which in benzene showed the bright golden fluorescence in ultra-violet light characteristic of the acridine derivative obtained at Stage II below. Heating for a few minutes with concentrated hydrochloric acid was also found to effect ring-closure.

Stage II: 3-Nitro-5-methyl-7-aminoacridine:

5 g. 2-acetyl-4-nitro-4'-acetylamino-diphenylamine
50 ccs. glacial acetic acid
2.5 ccs. concentrated sulphuric acid.

The above substances were heated together under reflux for 2½ hours in an oil-bath at 125°C. The dark greenish liquid was poured into 500 ccs. water containing a slight excess of ammonia. The acridine derivative separated as a dark red precipitate, which was filtered off, washed with water and allowed to dry. Yield: 3.8 g. (94% theoretical). The product was crystallised twice from benzene (1 in 300; 40% recovery), and was obtained as a brick-red crystalline powder, which appeared to decompose at about 270°C (darkened and shrunk) but remained unmelted at 380°C.

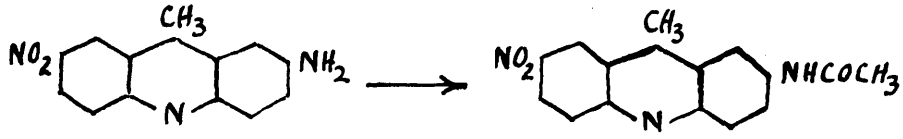
Analysis showed 66.0% C, 4.16% H, 16.4% N ($C_{14}H_{11}O_2N_3$ requires 66.4% C, 4.35% H, 16.6% N).

The presence of the free amino-group in the compound (showing that hydrolysis has in fact accompanied the ring-closure during the above preparation) is confirmed by this analysis and by the fact that the compound is readily acetylated (preparation and properties of the acetylamino-compound are given on page 120).

The compound is insoluble in water and petroleum ether, slightly soluble in alcohol, benzene, ether, and chloroform, soluble with a yellow colour in hydrochloric acid (dilute and concentrated), concentrated sulphuric acid, and acetone. The compound dissolves in glacial acetic acid with a red colour remaining clear on dilution with water. The solution of the compound in benzene shows bright golden-yellow fluorescence in ultra-violet light.

PREPARATION AND PROPERTIES OF 3-NITRO-5-METHYL-7--ACETYLAMINOACRIDINE.

This new compound was prepared by acetylating the corresponding amino-compound:-



1 g. 3-nitro-5-methyl-7-aminoacridine
5 ccs. acetic anhydride
1 g. anhydrous sodium acetate.

The above substances were mixed and the reddish mixture heated at 120°C for half an hour. The yellow acetyl-derivative began to separate soon after the commencement of heating. The reaction mixture was poured into water to which was added a slight excess of ammonia, stirred, allowed to stand for an hour then filtered. The acetyl-derivative was obtained as a yellow brown residue, which was washed with water, allowed to dry in air, then crystallised from aqueous alcohol using decolourising carbon. Yield: 1.05 g. (90% theoretical).

The compound was obtained as a yellow crystalline powder, which appeared to decompose (darkened) at about 280°C, but remained unmelted at about 360°C.

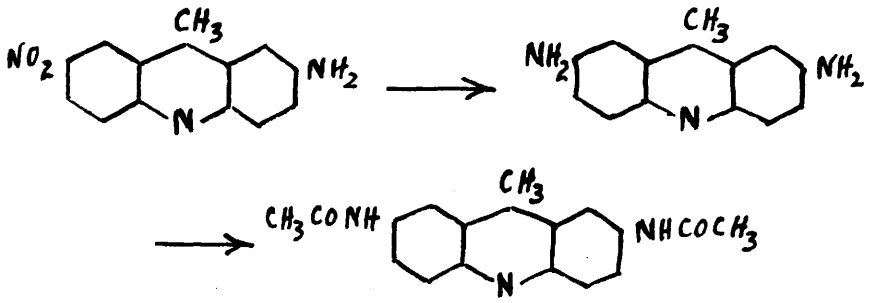
Analysis showed 65.7% C, 4.50% H, 14.20% N ($C_{16}H_{13}O_3N_3$ requires 65.1% C, 4.41% H, 14.23% N).

The compound is insoluble in water, sparingly soluble in benzene (solution showing only faint greenish fluorescence in ultra-violet light) and cold alcohol, more soluble in warm

alcohol and glacial acetic acid. On boiling it for a few minutes with concentrated hydrochloric acid, the free amino-compound was obtained. This was shown by diluting the liquid after heating, and adding an excess of ammonia, when a red precipitate was formed, which on shaking with benzene was found to impart a bright golden fluorescence to that solvent when examined in ultra-violet light.

PREPARATION AND PROPERTIES OF 3:7-DIAMINO-5-METHYL--ACRIDINE AND 3:7-DI-(ACETYLAMINO)-5-METHYLACRIDINE.

These new compounds were prepared from 3-nitro-5-methyl--7-aminoacridine according to the following scheme:



3;7-Diamino-5-methylacridine:

2 g. 3-nitro-5-methyl-7-aminoacridine were reduced with anhydrous stannous chloride reagent (general procedure, page 114) The intermediate stannichloride was greenish-brown in colour and dissolved in water with a deep red colour. The free base was precipitated from this solution as a brown precipitate by the addition of excess sodium hydroxide solution. It was filtered off, washed with water and allowed to dry (1.2 g.).

The product so obtained was a brown solid which would not crystallise satisfactorily from the common solvents. Owing to the difficulty in obtaining the substance free from resinous matter its analysis was not carried out. The compound was, however, characterised through its acetyl-derivative (page 123). The substance sintered with decomposition at about 200°C.

It dissolved easily in glacial acetic acid with a deep red colour, and slightly in ether, imparting a yellow-green

fluorescence to the latter solvent.

The hydrochloride was obtained as a purplish-black crystalline solid on evaporating a solution of the base in 15% hydrochloric acid over the water-bath. This salt was hygroscopic, and dissolved readily in water with an orange-red colour the solution yielding a brown precipitate of the free base with ammonia.

3:7-Di-(acetylamino)-5-methylacridine:

1 g. 3:7-diamino-5-methylacridine
7 ccs. acetic anhydride
1 g. anhydrous sodium acetate.

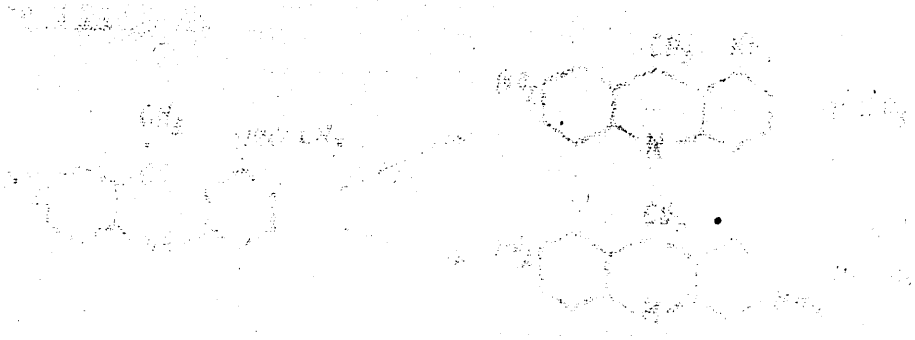
The mixture of the above substances was heated at 120° C for 1 hour. The yellow acetyl-derivative was observed to separate from the reaction mixture, which at the end of the heating was poured with stirring into water, to which was added a slight excess of ammonia to liberate the free base from any acetate formed. The yellow-brown precipitate was filtered off, washed with water and allowed to dry. It was then purified by crystallisation from aqueous alcohol, using decolourising carbon.

Yield: 1.0 g.

The substance was obtained as a yellow-brown crystalline powder, which remained unmelted at 360° C although probably some decomposition occurred above 250° C. Analysis showed 71.0% C., 5.40% H, 13.7% N ($C_{18}H_{17}O_2N_3$ requires 70.4% C, 5.54% H, 13.7% N).

In general properties the compound resembled 3-acetylamino-5-methylacridine (page 111), notably in the absence of

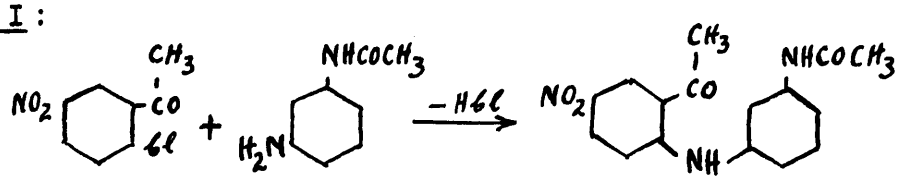
fluorescence in its solution in ether (difference from free di-amino-compound), the greenish fluorescence of its solution in glacial acetic acid, and its behaviour on hydrolysis, when the free di-amino-compound was formed.



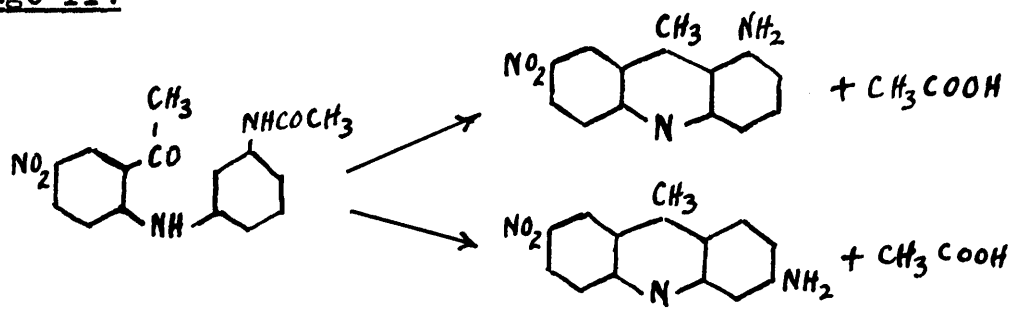
PREPARATION AND PROPERTIES OF 3-NITRO-5-METHYL-6(or 8?)-AMINOACRIDINE.

This substance was prepared by the two-stage method described on page 117 for 3-nitro-5-methyl-7-aminoacridine, using m-aminoacetanilide in place of its p-isomer:-

Stage I:



Stage II:



Again hydrolysis of the acetylamino-group was found to accompany ring-closure at Stage II. In this case, however, it will be evident that two isomeric acridine derivatives are possible on ring-closure, one with the amino-group in position 6, the other with the amino-group in position 8. Probably both are produced, although for reasons given in the theoretical section (page 27) the matter was not further explored. The intermediate 2-acetyl-4-nitro-3'-acetylamino-diphenylamine and the product from it by ring-closure are new.

Stage I: 2-Acetyl-4-nitro-3'-acetylamino-diphenylamine:

This compound was prepared in exactly the same way as

2-acetyl-4-nitro-4'-acetylamino-diphenylamine (page 117) using m-aminoacetanilide in place of its p-isomer. The substance, after crystallisation from alcohol, using decolourising carbon, was obtained in the form of yellow crystals, melting at 229°C. Yield: (from 2 g. m-aminoacetanilide) 1.88 g. (62% theoretical).

Analysis showed 61.6% C, 5.00% H, 13.25% N ($C_{16}H_{15}O_4N_3$ requires 61.3% C, 4.79% H, 13.4% N).

The compound closely resembles 2-acetyl-4-nitro-4'-acetylamino-diphenylamine (page 117) in its behaviour towards the common solvents and in the ease with which it is ring-closed to the acridine structure with concentrated sulphuric or hydrochloric acids, ring-closure again being manifest by the appearance of fluorescence phenomena in the resultant product (see also Stage II below).

Stage II: 3-Nitro-5-methyl-6(or 8?)-aminoacridine:

This product was obtained by ring-closure of the above diphenylamine derivative in glacial acetic acid with concentrated sulphuric acid by the method described for preparing 3-nitro-5-methyl-7-aminoacridine (page 118). The reddish-brown product, after precipitation with ammonia, washing, and drying, was not further purified. Yield: (from 1g. diphenylamine derivative) 0.7g.

The substance, which, as already pointed out, may be a mixture of two isomers, closely resembled 3-nitro-5-methyl-7-aminoacridine in general solubility, fluorescence phenomena, and ease of acetylation.

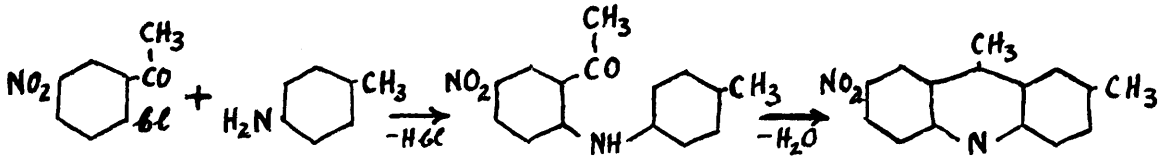
PREPARATION AND PROPERTIES OF 3:6(or 8?)-DIAMINO-5-METHYLACRIDINE.

This new substance was prepared from the product described as 3-nitro-5-methyl-6(or 8?)-amino-acridine (page 126) by reducing it with anhydrous stannous chloride reagent.

The preparation took the same course as described for 3:7-diamino-5-methylacridine (page 122). Again the stannichloride was dark greenish-brown in colour, dissolving in water with a deep red colour, but it was found better to extract the base, after adding excess sodium hydroxide to the stannichloride solution, with ether, in which solvent it formed an orange-yellow solution with golden-yellow fluorescence (becoming green on dilution with ether). The reddish-brown residue of the free base remaining after evaporation of the ether, was converted to its hydrochloride by dissolving it in warm 15% hydrochloric acid, and evaporating the filtered solution over the water-bath. This salt was obtained as a reddish-brown crystalline powder, easily soluble in water with a wine-red colour. This solution on treatment with ammonia gives an orange-red gelatinous precipitate of the free base.

PREPARATION AND PROPERTIES OF 3-NITRO-5:7-DIMETHYL--ACRIDINE.

This new compound was prepared by the two-stage method described for 3-nitro-5-methylacridine (page 102), combining the 5-nitro-2-chloroacetophenone with p-toluidine in place of aniline. The intermediate 2-acetyl-4-nitro-4'-methyl-diphenylamine obtained is also new:



Stage I: 2-acetyl-4-nitro-4'-methyl-diphenylamine:

5 g. 5-nitro-2-chloroacetophenone
5 g. p-toluidine
5 g. anhydrous potassium carbonate.

The above substances were heated together at 125°C for 3 hours. An orange-coloured melt was first formed but towards the end of the heating this had become brownish and more viscous. While still warm the product was treated with 20 ccs. alcohol, and stirred up, the mixture becoming thick owing to the separation of the yellow condensation product. The mixture was allowed to stand overnight then filtered, washing first with a little cold alcohol (filtrate and washings containing tarry matter were discarded), then with water to remove inorganic salts (washings gave positive test for chloride). The yellow diphenylamine derivative which remained was allowed

to dry, then crystallised from alcohol. Yield: 5.25 g. (77% theoretical).

The compound was obtained in the form of yellow crystals melting at 132°C. Analysis showed 66.8% C, 5.40% H, 10.25% N ($C_{15}H_{14}O_3N_2$ requires 66.6% C, 5.18% H, 10.37% N).

The compound is insoluble in water, and only sparingly soluble in dilute hydrochloric or sulphuric acids. Concentrated sulphuric acid dissolves the compound in the cold; on heating this orange solution for a minute, the colour deepened slightly and ring closure to the acridine derivative occurs. This was shown by cooling the solution, diluting with water, and adding an excess of ammonia. The yellow compound precipitated was now easily soluble in dilute hydrochloric acid and its solution in chloroform showed the bright green fluorescence in ultra-violet light characteristic of the acridine derivative (see Stage II below). Warming with concentrated hydrochloric acid also causes ring closure, though not so rapidly as sulphuric acid.

The diphenylamine derivative is sparingly soluble in cold alcohol and petroleum ether, soluble in ether, chloroform, benzene, acetone, glacial acetic acid, and hot alcohol, forming yellow solutions. The solution of the compound in chloroform shows no fluorescence in ultra-violet light (difference from ring-closed product).

Stage II: 3-nitro-5:7-dimethylacridine:

5 g. 2-acetyl-4-nitro-4'-methyl-diphenylamine
50 ccs. glacial acetic acid
2.5 ccs. concentrated sulphuric acid.

The above substances were heated together under reflux in an oil-bath at 125°C for 2½ hours. The yellow solution first formed became darker and greenish during heating. The warm liquid was then poured into 500 ccs. water containing a slight excess of ammonia, when the acridine derivative separated as a yellow gelatinous precipitate. This was filtered off, washed with water, allowed to dry, then crystallised from benzene. Yield: 4.20 g. (90% theoretical).

The compound was obtained in the form of yellow crystals, somewhat deeper in shade than the intermediate diphenylamine derivative. The compound did not melt at 360°C, but appeared to decompose at about 235°C, with charring.

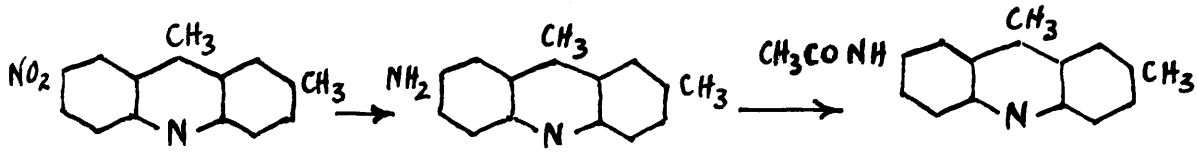
Analysis showed 72.0% C, 4.90% H, 11.0% N ($C_{15}H_{12}O_2N_2$ requires 71.4% C, 4.76% H, 11.12% N).

The compound is insoluble in water and soluble in dilute hydrochloric acid. The yellow solution in the latter gives a bulky yellow gelatinous precipitate of the free base with ammonia. The compound is almost insoluble in petroleum ether, and only sparingly soluble in ether. In alcohol, benzene, acetone, and glacial acetic acid the compound is slightly soluble in the cold, but more soluble in the hot

solvent, forming a yellow solution in each case. Chloroform dissolves the compound in the cold, the yellow solution fluorescing feebly in daylight, brightly (yellow-green) in ultra-violet light. The addition of water to a solution of the compound in glacial acetic acid results in the formation of a yellow gelatinous mass, presumably of the free base produced by hydrolysis of the acetate.

PREPARATION AND PROPERTIES OF 3-AMINO-5:7-DIMETHYL--ACRIDINE AND 3-ACETYLAMINO-5:7-DIMETHYLACRIDINE.

These new compounds were prepared from 3-nitro-5:7--dimethylacridine according to the following scheme:



The compounds were found to resemble closely in general properties the related mono-methyl compounds described on page 108.

3-Amino-5:7-dimethylacridine:

2 g. 3-nitro-5:7-dimethylacridine were reduced with anhydrous stannous chloride reagent (general procedure, page 114). The intermediate stannichloride was a yellow solid forming a wine-red solution in water. The amino-compound was separated from this solution by treating with excess sodium hydroxide solution, and was obtained as a yellow precipitate which became somewhat brown and resinous on standing. On account of this it was considered best to extract it with ether, in which it formed a yellow solution with greenish fluorescence. The product obtained, after removing the ether, was a brown solid, which would not crystallise satisfactorily from the common solvents. Like 3-amino-5-methylacridine (page 108) the most satisfactory product was obtained using aqueous alcohol with decolourising carbon, but this was again

a reddish-brown crystalline powder associated with a little resinous matter. Yield: 1.41 g. (80% theoretical).

The compound did not show a sharp melting point for it appeared to decompose with sintering at about 170°C. Owing to the difficulty in purifying it sufficiently, the substance was not analysed itself, but was characterised through its crystalline acetyl-derivative (see below).

The compound was found to resemble closely 3-amino--5-methylacridine(page 108) in its behaviour (including colour phenomena) towards the common solvents.

The hydrochloride did not separate from a solution of the base in dilute hydrochloric acid when treated with the concentrated acid, but was obtained as a dark brown crystalline solid on evaporating the resulting solution over the water bath. The salt so obtained (probably the di-hydrochloride) dissolved readily in water producing an orange-red solution, which was slightly acid to litmus.

3-Acetylamino-5:7-dimethylacridine:

1 g. 3-amino-5:7-dimethylacridine was acetylated with acetic anhydride in presence of sodium acetate following the procedure adopted for the related mono-methyl compound (page 111). The product after crystallisation from aqueous alcohol (using decolourising carbon) was obtained as a pale yellow crystalline powder. Yield: 1.0 g. (84% theoretical).

Like 3-acetylamino-5-methylacridine the compound gave no definite melting point, for it appeared to decompose about 250°C, becoming darker, but remaining unmelted at 360°C.

Analysis showed 77.0% C, 6.33% H, 10.7% N ($C_{17}H_{16}ON_2$ requires 77.3% C, 6.06% H, 10.6% N).

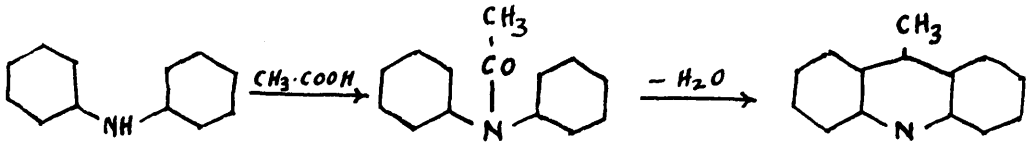
The compound closely resembled the related monomethyl derivative (page 111) in its solubility and fluorescent phenomena in the common solvents, and in the hydrolysis experiment with concentrated hydrochloric acid.

PART II

PREPARATION OF STYRYL-ACRIDINES FROM 5-METHYLACRIDINE.

PREPARATION OF 5-METHYLACRIDINE.

This compound was prepared by the method of Bernthsen (Ref.6) from diphenylamine and acetic acid, in presence of anhydrous zinc chloride:



The crude substance was then purified by the tartrate method of Koenigs (Ref.7).

280 g. diphenylamine
170 ccs. glacial acetic acid
480 g. anhydrous zinc chloride

The above substances were heated together under reflux and brought to the boil. Heating was continued for 14 hours. The temperature of the reaction mass was 200 - 220°C during the first ten hours, and 220 - 240°C during the last four hours.

The brown reaction mass was allowed to cool somewhat, then poured into 2 litres of water, and left in contact with the water for 3 days. By this means a large amount of zinc chloride dissolved and was removed on decantation. The product was then washed several times with warm water in order to remove more zinc chloride, then extracted with successive amounts of warm 15% sulphuric acid. The crude methylacridine was obtained from these acid extracts by precipitation with

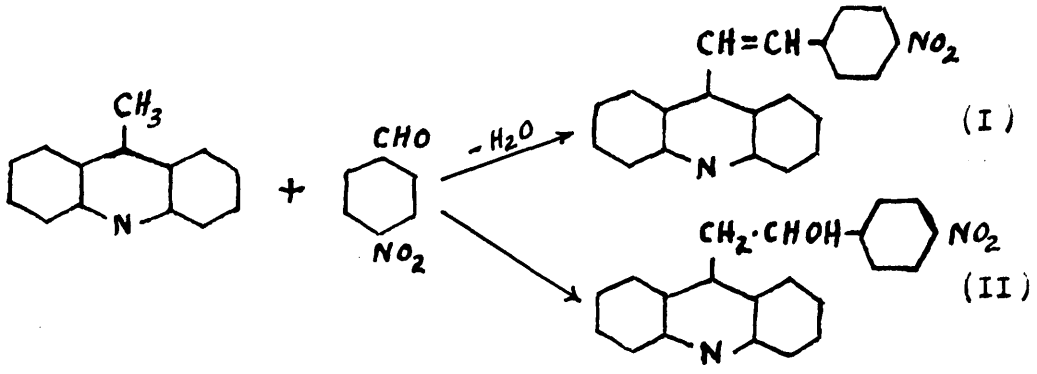
ammonia, and was filtered off and allowed to dry in air.

Purification: Equal weights of the crude base and tartaric acid were separately dissolved in about 4 times their weight of warm alcohol. The solutions were filtered, then the tartaric acid solution added to the solution of crude base. A heavy crystalline precipitate of 5-methylacridine tartrate separated, and after allowing to stand for 2 hours, was filtered off, washed with a little alcohol, then dried in air. The brownish-yellow tartrate was then dissolved in a mixture of hot water and dilute hydrochloric acid, the solution filtered, and treated with a slight excess of sodium hydroxide solution. The 5-methylacridine so precipitated was filtered off, washed well with water, and allowed to dry in air.

The purified substance was a light brown powder, melting sharply at 115°C (literature gives 114 - 115°C). The tartrate, prepared from the purified base by the method given above, and recrystallised from water, using decolourising carbon was obtained as a pale yellow crystalline powder, melting at 155°C (literature gives 153 - 154°C).

EXPERIMENTS ON THE CONDENSATION OF 5-METHYLACRIDINE WITH p-NITROBENZALDEHYDE.

The object of these experiments and the significance of the results obtained are set out in the theoretical section (page 35). Two products are possible when 5-methylacridine condenses with p-nitrobenzaldehyde, one an ethene (styryl) derivative (I), the other an ethanol derivative (II):



The experiments on the formation of the styryl derivative have shown that the best condensing agent is anhydrous zinc chloride, as used by Porai-Koschitz (Ref.8). The method used by this worker was improved by altering certain conditions. Further, the styryl derivative obtained in any of the experiments described here differed in many respects from that described by Porai-Koschitz. The ethanol derivative (a new compound) was found to be the chief product of the reaction at 100°C in absence of condensing agents.

Condensation in presence of zinc chloride:

Experiment No.1: In this experiment the conditions employed

by Porai-Koschitz (Ref.8) were closely followed:

5 g. 5-methylacridine
3.92 g. p-nitrobenzaldehyde
3.56 g. anhydrous zinc chloride (finely powdered).

The above substances (in molecular proportions) were mixed and heated together for 3 hours at 140-150°C. A dark brown viscous mass was produced, which solidified on cooling. The product was then powdered, and freed from zinc chloride and unchanged 5-methylacridine by stirring up with water containing dilute hydrochloric acid. On filtration, the hydrochloride of the styryl derivative, being sparingly soluble in cold water, remained. It was dissolved by boiling with a large volume of water containing dilute hydrochloric acid, filtered, and the free base precipitated from the hot filtrate with sodium carbonate solution. After filtering, washing, and drying, the product was crystallised from pyridine. The bright yellow crystals so obtained were washed with a little alcohol and allowed to dry in air. Yield: 0.85 g. (10% theoretical). The compound, recrystallised from alcohol and dried in a vacuum desiccator, was found to melt at 293°C (analysis and properties are given on page 143). The low yield obtained in this experiment is attributed to the formation of tarry matter at 140 - 150°C, and subsequent loss of product, on account of this, in the pyridine mother liquor, which failed to yield further crops of crystals on concentrating or on dilution with alcohol.

Experiment No.2. In this experiment the conditions employed

in Experiment No.1 were modified. By lowering the temperature of reaction to 130°C, thereby considerably reducing tarring, and by simplifying the process of working up the product, the yield of styryl derivative was improved to 7.6 g. (90% theoretical). Details of the method are given on page 142, where the styryl derivative is more fully described.

Condensation at 100°C in absence of condensing agents:

5 g. 5-methylacridine and 3.92 g. p-nitrobenzaldehyde were melted together at 100°C and kept at this temperature for 7 hours. After a few minutes heating, a pale yellow solid was observed to separate from the brown melt, causing it to solidify after 10 minutes. The product was powdered and freed from unchanged reactants by washing with a little warm alcohol in which the condensation product was only sparingly soluble. The product was then dried in the steam oven and its composition investigated by dividing it into two portions "a" and "b" (see also theoretical section, page 38):

Portion "a": This was crystallised twice from benzene, forming pale yellow crystals, melting at 174°C. It is shown on page 145 that this compound is the ethanol derivative, and more details of its preparation and properties are given there. The benzene was removed from the mother liquor from the above crystallisation, and the residue washed with a little alcohol, then crystallised from pyridine. 0.1 g. bright yellow crystals

of the styryl derivative, melting at 293°C, were obtained.

Portion "b": This was purified by the hydrochloride method described under Experiment No.1 of the condensations in presence of zinc chloride (page 138). The base obtained after treatment with sodium carbonate was divided into two parts. The first part was examined as under Portion "a" above and proved to be largely the ethanol derivative, although a small amount of styryl derivative was again demonstrated in the benzene mother liquor. The second portion was crystallised three times from small amounts of pyridine, and yielded 0.05 g. bright yellow crystals of the styryl derivative, melting at 293°C.

Condensation in presence of acetic anhydride:

0.63 g. 5-methylacridine
0.49 g. p-nitrobenzaldehyde
2 ccs. acetic anhydride.

The methylacridine and the aldehyde (molecular proportions) were melted together at 130°C and treated with the acetic anhydride. The greenish-brown solution was heated in an oil-bath at 130°C for three hours. Yellowish crystals made their appearance towards the end of the heating, and the liquid became dark-brown. The reaction mixture was then cooled, allowed to stand for two hours to allow more crystals to separate, then filtered. The bright yellow crystals were washed with a little alcohol, and allowed to dry. These were

found to be the styryl derivative, melting at 293°C. Little or no additional yield of the styryl derivative could be obtained by working up the tarry mother liquor from the crystals. Yield: 0.35 g. (33% theoretical)

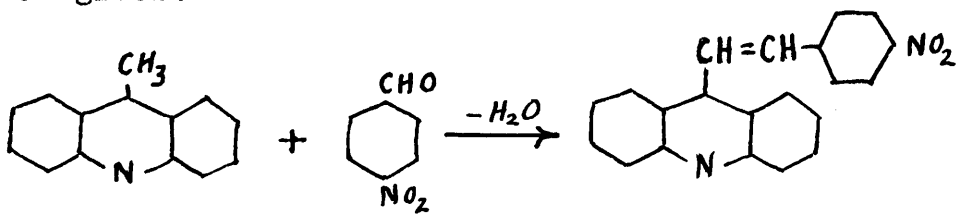
Condensation in presence of piperidine:

(a) Alcohol as solvent: 0.63 g. 5-methylacridine and 0.49 g. p-nitrobenzaldehyde were dissolved in 8 ccs. boiling alcohol containing 8 drops of piperidine, and heated under reflux for three hours. No crystals separated on cooling the liquid. The solvent was removed by evaporation and the residue crystallised twice from pyridine then alcohol but yielded only the ethanol derivative (0.15 g.) melting at 174°C.

(b) Pyridine as solvent: 0.63 g. 5-methylacridine and 0.49 g. p-nitrobenzaldehyde were melted together, treated with 2 ccs. pyridine and 10 drops piperidine, and heated in an oil bath at 130°C under reflux for three hours. The greenish-brown solution became darker during heating and deposited yellow crystals. The reaction mixture was cooled and allowed to stand for two hours to allow more crystals to separate. The bright yellow crystals were filtered off, washed with a little alcohol, and allowed to dry, then crystallised from pyridine. The crystals proved to be the styryl derivative melting at 293°C. Yield: 0.15 g. (14% theoretical). No further yield of the compound could be obtained from the tarry mother liquors from the crystals.

PREPARATION AND PROPERTIES OF 5-(p-NITROSTYRYL)-ACRIDINE.

The experimental work on the condensation of 5-methylacridine with p-nitrobenzaldehyde (page 137) has shown that 5-(p-nitrostyryl)-acridine is best prepared by a modification of the method of Porai-Koschitz (Ref.8), details of which are now given:



The properties of the styryl derivative were found to differ considerably from those of the compound described by Porai-Koschitz. A new bromo-derivative of the compound has been obtained.

5 g. 5-methylacridine
3.92 g. p-nitrobenzaldehyde
3.56 g. anhydrous zinc chloride

The above substances (in molecular proportions) were mixed and heated together at 130°C for three hours. The product obtained was a light brown solid mass, which, in contrast to the product obtained by adhering to the conditions in the literature (page 137), was not viscous or tarry when warm. When cold the product was powdered, washed with water to remove zinc chloride, and allowed to dry. It was then crystallised from 4 ccs. pyridine, the small amount of tarry matter produced during heating being removed in the mother liquor, which was

not further worked up. The bright yellow product so obtained was washed with a little alcohol and allowed to dry in air. Yield: 7.6 g. (90% theoretical). The product was further purified for investigation of its properties and for analysis by recrystallisation from pyridine then alcohol, and dried in a vacuum desiccator.

Analysis showed 77.6% C, 4.40% H, 8.56% N ($C_{21}H_{14}O_2N_2$ requires 77.3% C, 4.29%H, 8.59% N). Porai-Koschitz claims to have obtained the hydrate, $C_{21}H_{14}O_2N_2 \cdot H_2O$, which requires 73.2% C, 4.65% H, 8.14% N.

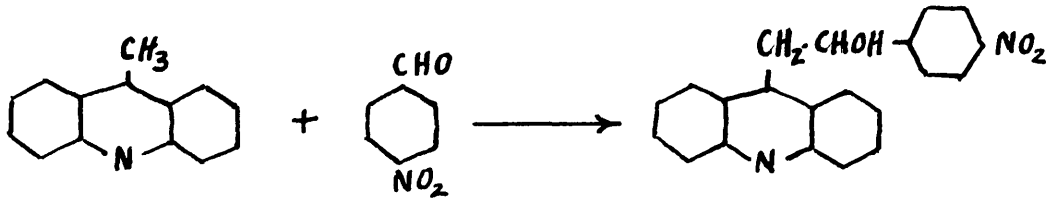
The compound obtained here is not the hydrate as shown by the above analysis, and by the fact that no loss in weight occurs when it is heated at 110°C. Further, the melting point of the compound obtained here is 293°C, whereas the product described by Porai-Koschitz is stated to melt at 212°C. The properties of the two compounds are in agreement, however, as regards colour (bright yellow) and general solubility (sparingly soluble in the common solvents except pyridine). The whole question of the relationship between the two compounds is dealt with more fully in the theoretical section (page 38).

The compound develops an orange-red colour when treated with concentrated hydrochloric acid or glacial acetic acid, and its solution in ether exhibits slight greenish-yellow fluorescence.

A solution of the compound in chloroform was found to yield an orange-red precipitate of a bromo-derivative on treatment with a solution of bromine in chloroform. The bromo-derivative was filtered off, washed with chloroform, and allowed to dry. It was found to be almost insoluble in the common solvents, except pyridine and hot glacial acetic acid. The compound did not melt at 360°C. Analysis of the bromo-derivative showed 20.2% Br (mono-bromo-substitution product, $C_{21}H_{13}O_2N_2Br$, requires 19.75% Br; di-bromo-addition product, $C_{21}H_{14}O_2N_2Br_2$, requires 32.9% Br). The bromo-derivative therefore appears to be the mono-bromo-substitution product (see theoretical section, page 40).

PREPARATION AND PROPERTIES OF α-[p-NITROPHENYL]-β-[ACRIDYL-(5)]-ETHANOL.

It has been shown in the experimental work (page 139) that this new compound is the chief product of the condensation between 5-methylacridine and p-nitrobenzaldehyde at 100°C in absence of condensing agents:-



Details of the preparation and properties of the new compound are now given, and its relationship to the corresponding ethene (styryl) derivative investigated. This relationship is discussed in the theoretical section (page 38).

5 g. 5-methylacridine
3.92g. p-nitrobenzaldehyde.

The above (molecular) quantities were melted together at 100°C and kept at this temperature for 6 hours. The brown melt produced at the commencement of heating soon began to deposit a pale yellow solid and became quite solid after about 10 minutes heating. The product was then ground up, washed with a little warm alcohol to remove unchanged reactants, and allowed to dry in air. Yield: 7.7 g. (87% theoretical). For analysis and investigation of its properties, the ethanol derivative was crystallised twice from benzene (1 in 300; 50% recovery), then dried in a vacuum desiccator.

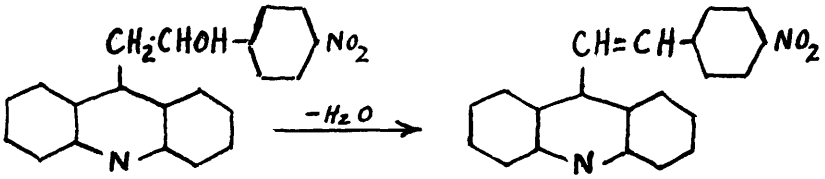
The compound so obtained was a pale yellow crystalline powder melting at 174°C. The melting point did not change on recrystallisation from pyridine or alcohol. Further, purification of the product through its hydrochloride by the method described for the corresponding styryl derivative (page 138), followed by crystallisation from pyridine or alcohol, did not alter the melting point. Analysis showed 73.11% C, 4.75% H, 8.10%N ($C_{21}H_{16}O_3N_2$ requires 73.2% C, 4.65% H, 8.14% N).

The compound resembled the corresponding styryl derivative in its general solubility, being sparingly soluble in the common solvents except pyridine and glacial acetic acid. It was more soluble in pyridine, but less soluble in benzene and in chloroform than the corresponding styryl derivative.

Attempts to reduce the ethanol derivative by warming with stannous chloride and hydrochloric acid were unsuccessful, the compound partly decomposing to 5-methylacridine, which was actually isolated through its tartrate. The odour of p-nitrobenzaldehyde was also apparent. This partial decomposition to 5-methylacridine was also noted in the experiment relating to the purification of the compound through its hydrochloride mentioned above, when the mother liquors from the crystallisation of the ethanol derivative were found to contain 5-methylacridine. This decomposition seems to be caused by warming with hydrochloric acid and is discussed in the theoretical section (page 40).

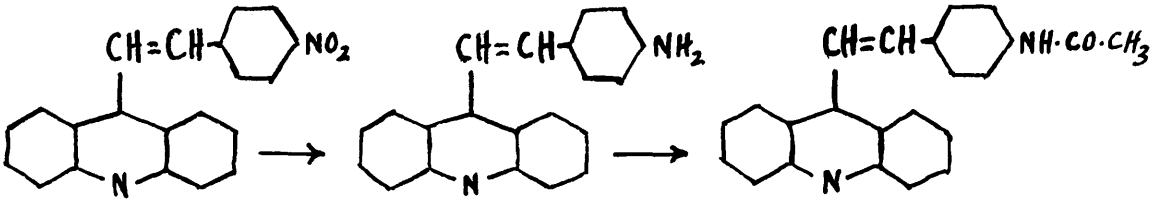
In contrast to the corresponding hydrated styryl derivative described by Porai-Koschitz (Ref.8), the ethanol derivative did not lose water at 110°C, to form the styryl derivative. The only effect of prolonged heating at 110°C was to cause slight decomposition, the bulk of the ethanol derivative, melting at 174°C, being recoverable.

The elements of water were, however, removed on heating the compound with acetic anhydride for 3 hours in an oil bath at 130°C, when bright yellow crystals of the styryl derivative, melting at 293°C, were obtained:



The experiment proceeded exactly as for the preparation of the styryl derivative from 5-methylacridine and p-nitrobenzaldehyde in presence of acetic anhydride (page 140).

THE PREPARATION AND PROPERTIES OF 5-(p-AMINOSTYRYL)-ACRIDINE AND 5-(p-ACETYLAMINOSTYRYL)-ACRIDINE.



The amino-compound was prepared by reducing the corresponding nitro-compound (page 142) according to the method of Porai-Koschitz (Ref.8). Like the nitro-compound from which it was obtained, the amino-compound was found to differ in some respects from the compound described by Porai-Koschitz (see theoretical section, page 39). Some hitherto unrecorded properties of the amino-compound are described.

The acetyl-derivative (a new compound) was obtained by heating the amino-compound with acetic anhydride and anhydrous sodium acetate.

5-(p-aminostyryl)-acridine:

5 g. 5-(p-nitrostyryl)-acridine
25 ccs. glacial acetic acid
15 g. stannous chloride crystals
25 ccs. concentrated hydrochloric acid.

The nitro-compound was treated with the glacial acetic acid, boiled, and the orange-red suspension poured into a solution of the stannous chloride in the concentrated hydrochloric acid. The mixture was brought to the boil, then heated on the water-bath for 3 hours. During the heating the

nitro-compound dissolved and the brown stannichloride of the amine separated. The latter was filtered off, after cooling, dissolved in warm water and the solution filtered. The brown solution (which developed a violet colour on dilution) was then treated with an excess of sodium hydroxide solution, when the free base separated as a yellow brown precipitate. This was filtered, washed with water, and allowed to dry in air. The compound was then purified by crystallisation from a mixture of pyridine and alcohol, washed with a little alcohol, and allowed to dry. Yield: 4.1 g. (90% theoretical).

Analysis showed 85.5% C, 5.50% H, 9.40% N ($C_{21}H_{16}N_2$ requires 85.1% C, 5.41% H, 9.46% N). The compound was obtained as a yellow crystalline powder, melting at 242°C (the compound obtained by Porai-Koschitz is stated to melt at 209°C). It was rather sparingly soluble in alcohol, ether, benzene, and petroleum ether (the compound obtained by Porai-Koschitz is stated to be readily soluble in these solvents), but was more soluble in pyridine and chloroform.

The most striking feature of the compound is the deep violet colour it shows in weakly acid solution. This is best shown by adding a few drops of glacial acetic acid to a little of the yellow crystals, when an intense violet colour is produced. This colour changes to red on dilution with water, and becomes yellow on the addition of a few drops of dilute hydrochloric acid. The properties of the hydrochloride further

illustrate this colour change phenomenon. This salt was obtained in the form of brown crystals by cooling a hot solution of the base in dilute hydrochloric acid, and was found to dissolve in water with a brownish-red colour. When this solution is diluted it developes a beautiful violet-red colour, which instantly changes to yellow on the addition of a drop of dilute hydrochloric acid. The colour of the violet-red solution is also immediately discharged on adding a drop of dilute ammonia solution, a yellow precipitate of the base separating. This colour change phenomenon is discussed in the theoretical section (page 42).

When the yellow solution of the compound in chloroform is treated drop by drop, with a solution of bromine in chloroform, a beautiful violet colour is first produced, but disappears as the addition of the bromine solution continues, a brown precipitate separating.

5-(p-acetylaminostyryl)-acridine:

3 g. 5-(p-aminostyryl)-acridine
10 ccs. acetic anhydride
3 g. anhydrous sodium acetate.

The above substances were mixed, some heat being evolved, and a yellow solid separating from the reddish liquid. The mixture was then heated for an hour at 130° C, and poured with stirring into water, to which was added a slight excess of ammonia to liberate the free base. The bright yellow precipitate

of the acetyl derivative so obtained was allowed to stand for an hour, then filtered, washed with water, and allowed to dry. The product was purified by crystallisation from aqueous alcohol using decolourising carbon, and was obtained as a yellow crystalline powder, melting at 236°C. Yield: 2.8 g. (82% theoretical).

Analysis showed 81.0% C, 5.50% H, 8.33% N ($C_{23}H_{18}ON_2$ requires 81.6% C, 5.33% H, 8.28% N).

The compound is insoluble in water, sparingly soluble in dilute hydrochloric acid (turning red), ether, benzene and petroleum ether, soluble in alcohol, chloroform, acetone, and glacial acetic acid. The solution of the compound in glacial acetic acid is deep red, a property which serves to distinguish it from the un-acetylated amino-compound, which gives a deep violet colour in that solvent. By boiling the acetyl-amino-compound for a few minutes with concentrated hydrochloric acid hydrolysis occurs with formation of the amino-compound. This was shown, after hydrolysis, by precipitating the base with ammonia, when the product now gave a violet colour with glacial acetic acid.

EXPERIMENTS ON THE CONDENSATION OF 5-METHYLACRIDINE WITH m-NITROBENZALDEHYDE.

Analogous experiments with p-nitrobenzaldehyde have already been described (see page 137). With m-nitrobenzaldehyde it was again shown that the best method of preparing the styryl derivative is by a modification of the method of Porai-Koschitz (Ref.8) using zinc chloride as condensing agent. The chief product of the condensation at 100°C in absence of condensing agents was again the ethanol derivative (a new compound), a small amount of styryl derivative also being formed. The latter compound, however, differed in some of its physical properties from the product obtained in the condensation with zinc chloride.

Condensation in presence of zinc chloride:

The experiments described under this heading for p-nitrobenzaldehyde (page 137) were repeated using m-nitrobenzaldehyde, with the following results:-

Experiment No.1: (Porai-Koschitz method). The mass obtained after heating the reactants together at 140 - 150°C for 3½ hours was again dark brown due to tarring. It was ground up and extracted with water then alcohol, the latter removing a large amount of tarry matter. No yield of styryl derivative was obtained from the residual brown solid on attempting to crystallise from a little pyridine, in which it

formed a brown tarry solution.

Experiment No.2: The temperature of reaction employed in Experiment No.1 was reduced to 130°C, when less tarring occurred, and the styryl derivative was obtained as a bright yellow crystalline solid (Yield: 64% theoretical), melting at 210°C. The preparation and properties of this compound are more fully described on page 155.

Condensation in presence of acetic anhydride:

The analogous experiment described on page 140 was repeated using m-nitrobenzaldehyde in place of its p-isomer. The reaction mixture after heating did not deposit crystals of the condensation product on cooling and standing, so the product was isolated by pouring the mixture, with stirring, into water, filtering off the yellow-brown solid and crystallising it from pyridine then alcohol. Yield (from 0.63 g. 5-methylacridine): 0.1 g. (9% theoretical). The product was a bright yellow solid melting at 210°C, and proved to be identical with the substance obtained above in the condensation with zinc chloride.

Condensation at 100°C in absence of condensing agents:

Using m-nitrobenzaldehyde in place of its p-isomer the analogous experiment described on page139 was repeated. The brown melt produced began to solidify after 30 minutes heating, and became quite solid after 45 minutes. The yellow-

brown product, after 7 hours heating, was powdered, washed with a little warm alcohol to remove any unchanged reactants, allowed to dry, then crystallised 3 times from benzene. The pale yellow crystals of the ethanol derivative so obtained melted at 145°C. Fuller details of the preparation and properties of this compound are given on page 157.

The benzene mother-liquors from the crystallisation of the ethanol derivative were evaporated, a resinous brown solid remaining. This was washed with a little cold pyridine (which removed tarry matter) then alcohol, the remaining solid (0.1g.) being very pale yellow in colour. On re-crystallising this solid from alcohol a pale cream coloured crystalline solid was obtained, melting at 207°C. Analysis and further properties of this compound are given on page 156 where it is shown to be a styryl derivative.

PREPARATION AND PROPERTIES OF 5-(m-NITROSTYRYL)-ACRIDINE.

The existence of two compounds corresponding to 5-(m-nitrostyryl)-acridine has already been shown in the preceding experimental work. It is shown in the theoretical section (page 46) that these are possibly geometric isomers.

I Compound from condensation in presence of zinc chloride.

NOTE: When 5-(m-nitrostyryl)-acridine is mentioned in this thesis without qualification, it refers to this compound, and not the isomer (II) described later.

This was prepared, by the method given for the corresponding p-compound (see page 142), from m-nitrobenzaldehyde and 5-methylacridine in presence of zinc chloride. Rather more tarring occurs in the case of the m-compound, however, so that the yield is only 64% theoretical.

The compound was recrystallised from pyridine then alcohol, and was obtained in the form of bright yellow crystals, melting at 210°C. Analysis showed 77.0% C, 4.40% H, 8.64% N ($C_{21}H_{14}O_2N_2$ requires 77.3% C, 4.29% H, 8.59% N).

The substance was insoluble in water and petroleum ether, sparingly soluble in dilute and concentrated hydrochloric acid, ether, acetone, and alcohol, more soluble in warm alcohol, glacial acetic acid, and chloroform. The yellow solution in the latter yields an orange-red precipitate of the bromo-derivative on the addition of a solution of bromine

in chloroform.

II Compound from condensation in absence of condensing agents.

The isolation of a small amount of styryl derivative from the product obtained by heating 5-methylacridine with m-nitrobenzaldehyde at 100°C for 7 hours is already described in the preceding experimental work (page 154).

Analysis of the compound showed 77.8% C, 4.10% H, 8.60% N ($C_{21}H_{14}O_2N_2$ requires 77.3% C, 4.29% H, 8.59% N). The colour of the compound (pale cream coloured) and its slightly lower melting point (207°C) are the chief points of difference from its isomer (I) described above. The mixed melting point of the two isomers showed considerable depression (180-190°C).

In general solubility the compound resembles its isomer (I) above. Its solution in chloroform is nearly colourless and gives a reddish precipitate of the bromo-derivative on treatment with a solution of bromine in chloroform.

PREPARATION AND PROPERTIES OF α-[m-NITROPHENYL]-β-[ACRIDYL-(5)]-ETHANOL.

It has already been shown (page 153) that this new compound is the chief product of the condensation between 5-methylacridine and m-nitrobenzaldehyde in absence of condensing agents. Further details of its preparation and properties are now given.

The compound was prepared by the method described on page 145 for the corresponding p-compound. Yield (from 5 g. 5-methylacridine): 6.76 g. (76% theoretical). The product was crystallised three times from benzene (1 in 150; 40% recovery), and was obtained as a pale yellow crystalline powder, melting at 145° C.

Analysis showed 73.5% C, 4.83% H, 8.10% N, ($C_{21}H_{16}O_3N_2$ requires 73.2% C, 4.65% H, 8.14% N).

The general solubility of the compound was found to be the same as the corresponding p-compound (page 146). It also resembled this compound in failing to lose water to form the corresponding styryl derivative on heating at 110° C, and by decomposing to 5-methylacridine on attempted reduction with stannous chloride and hydrochloric acid.

PREPARATION AND PROPERTIES OF 5-(m-AMINOSTYRYL)--ACRIDINE AND 5-(m-ACETYLAMINOSTYRYL)-ACRIDINE.

These two compounds were prepared by the method described for the corresponding p-compounds (page 148). The properties of the amino-compound obtained were found to agree with the description in the literature (Porai-Koschitz) (Ref.8), and a few additional properties are described. The acetylamino-compound is new.

5-(m-aminostyryl)-acridine:

5 g. 5-(m-nitrostyryl)-acridine were reduced exactly as described for the corresponding p-compound (page 148). The stannichloride obtained in this case was a bright orange-red solid, dissolving in water with an orange-yellow colour which remained yellow on dilution. The free base, separated from the stannichloride solution with sodium hydroxide in the usual way, and purified by crystallisation from a mixture of pyridine and alcohol, was obtained as a yellow crystalline solid, melting at 234°C (literature gives 232 - 234°C). Yield: 4.13g. (91% theoretical).

The solubility of the compound in the common solvents was found to be as stated in the literature. Analysis of the compound was not carried out since its properties agree with those given in the literature, and, further, its acetyl-derivative is characterised later (page 159).

The compound does not give the violet colour reactions so characteristic of its p-isomer (page 149), as the following tests showed:

(i) Treated with glacial acetic acid the compound dissolved with an orange colour, which became yellow on dilution.

(ii) The hydrochloride, obtained as an orange-yellow crystalline solid by cooling a solution of the base in warm 15% hydrochloric acid, was found to dissolve in water with a yellow colour which did not change on dilution.

(The addition of dilute ammonia to the yellow diluted acid solutions described in tests (i) and (ii) simply precipitated the yellow base)

(iii) The yellow solution of the compound in chloroform (in which solvent it is easily soluble) was treated drop by drop with a solution of bromine in chloroform. First an orange-red colour was produced then an orange precipitate.

5-(m-acetylaminostyryl)-acridine:

3 g. 5-(m-aminostyryl)-acridine were acetylated in exactly the same way as the corresponding p-compound (page 150). The product was obtained as a yellow crystalline solid, melting at 252°C, after purifying it by crystallisation from aqueous alcohol using decolourising carbon. Yield: 2.83 g. (83% theoretical).

Analysis showed 82.0% C, 5.20% H, 8.24% N, ($C_{23}H_{18}ON_2$ requires 81.6% C, 5.33% H, 8.28% N).

The solubility of the compound in the common solvents is similar to that of its p-isomer described on page 150. It further resembles the latter in turning red with cold dilute

hydrochloric acid (in which it is only slightly soluble), but differs in forming an orange-yellow solution in glacial acetic acid, in which solvent the p-isomer is red.

EXPERIMENTS ON THE CONDENSATION OF 5-METHYLACRIDINE WITH o-NITROBENZALDEHYDE.

Analogous experiments with p-nitrobenzaldehyde have already been described (see page 137). With o-nitrobenzaldehyde however, attempts to prepare the styryl derivative were unsuccessful, although the ethanol derivative (a new compound) was obtained by condensing the reactants at 100°C in the absence of condensing agents.

Condensation in presence of zinc chloride:

(a) At 140-150°C: This experiment was carried out exactly as for the corresponding experiment (No.1, page 152) described for m-nitrobenzaldehyde, using its o-isomer instead. The results obtained were similar, no yield of styryl derivative being obtained from the tarry product.

(b) At 130°C: This was carried out exactly as for experiment (a) above but reducing the temperature of reaction to 130°C. Again no styryl derivative was obtained due to tarring.

(c) At 110°C: Experiment (a) was again repeated, this time reducing the temperature of reaction to 110°C. Again no styryl derivative could be extracted from the tarry product.

Condensation in presence of acetic anhydride:

This experiment was carried out exactly as described under the corresponding experiment with m-nitrobenzaldehyde

(page 153), using the o-isomer instead, but failed to yield any crystalline product, considerable resinification occurring during heating.

Condensation at 100°C in absence of condensing agents:

Using o-nitrobenzaldehyde in place of its p-isomer the analogous experiment, described on page 139 was repeated. Again the reaction mass solidified after 10 minutes heating. On examination by the method described for the p-compound the product was shown to consist chiefly of the ethanol derivative, and no styryl derivative was isolated. Fuller details of the preparation and properties of the ethanol derivative are given on page 163.

PREPARATION AND PROPERTIES OF α-[o-NITROPHENYL]-β-[ACRIDYL-(5)]-ETHANOL.

This new compound, as already shown (page 162), is obtained by heating together 5-methylacridine and o-nitrobenzaldehyde in absence of condensing agents.

The compound was prepared by the method described for the corresponding p-compound (page 145). Yield: (from 5 g. 5-methylacridine) 7.2 g. (81% theoretical). The product was crystallised twice from benzene (1 in 250; 64% recovery), and was obtained as a pale yellow crystalline powder, melting at 177°C.

Analysis showed 73.2% C, 4.67% H, 8.18% N ($C_{21}H_{16}O_3N_2$ requires 73.2% C, 4.65% H, 8.14% N).

In general solubility the compound resembles its p-isomer (page 146). It further resembles this compound in decomposing to 5-methylacridine on attempted reduction with stannous chloride and hydrochloric acid. On heating at 110°C the compound did not lose water to form the styryl derivative, but decomposed, turning brown. In this connection it proved to be even less stable towards heat than its m- or p-isomers. An attempt to form the styryl derivative by heating with acetic anhydride (see corresponding experiment with the p-isomer, page 147) proved to be unsuccessful, much resinification occurring during heating and no crystalline substance being isolated.

EXPERIMENTS ON THE CONDENSATION OF 5-METHYLACRIDINE WITH p-DIMETHYLAMINOBENZALDEHYDE.

Analogous experiments with p-nitrobenzaldehyde have already been described (page 137). In this case it was found that the styryl derivative was best prepared by following closely the conditions employed by Porai-Koschitz (Ref.8) who used zinc chloride as the condensing agent. No corresponding ethanol derivative has been obtained.

Condensation in presence of zinc chloride:

In this experiment 5-methylacridine was condensed with p-dimethylaminobenzaldehyde in presence of zinc chloride as described in the literature (Porai-Koschitz, Ref.8), and a good yield of the styryl derivative obtained. Fuller details are given on page 167 where the compound is described.

Condensation at 100°C in absence of condensing agents:

1.93 g. 5-methylacridine
1.49 g. p-dimethylaminobenzaldehyde.

The above substances (in molecular proportions) were heated together at 100°C, when a dark brown melt was formed. In contrast to the corresponding experiments with the nitrobenzaldehydes, no solid separated as the heating continued, and even after 24 hours heating the product was still dark brown and fluid at 100°C. It was shown by the following tests to consist largely of unchanged reactants, together with

a small amount of the styryl derivative, although the latter was not actually isolated:-

(i) On submitting a portion of the product to steam-distillation the unchanged aldehyde was first removed from the above product, followed, on prolonged steam-distillation, by the unchanged 5-methylacridine which is only sparingly volatile in steam. The small residue was dark brown and tarry and failed to crystallise from the common solvents.

(ii) Another portion of the product was dissolved in alcohol and the brown solution treated with a solution of tartaric acid in alcohol, when the liquid assumed a greenish colour, a solid being precipitated. The green colour was shown, on filtering the liquid, to be due to a yellow solid suspended in a deep prussian-blue liquid. The solid proved on examination to be 5-methylacridine tartrate (page 136), produced from the unchanged 5-methylacridine in the reaction mass. The deep blue colour is characteristic of 5-(p-dimethylaminostyryl)--acridine in weakly acid solution. The colour changes of such a solution on treatment with water, dilute hydrochloric acid, and ammonia, described on page 168, were also found to be given by the deep blue solution obtained here, which would therefore appear to contain the styryl derivative.

Condensation in presence of piperidine:

(a) Alcohol as solvent: 1.93 g. 5-methylacridine and 1.49 g. p-dimethylaminobenzaldehyde were dissolved in 20 ccs. boiling alcohol containing 20 drops of piperidine and boiled under reflux for 3 hours. The brown solution on cooling gave no crystals so the solvent was removed by evaporation. The brown viscous residue was then examined in the same way as the product in the previous experiment at 100° C. Again the reaction mass consisted chiefly of unchanged reactants, but this time little or no styryl derivative was formed, for the

filtrate from the 5-methylacridine tartrate was green in place of the deep blue recorded at the corresponding stage in the previous experiment.

(b) No solvent used: 1.93 g. 5-methylacridine and 1.49 g. p-dimethylaminobenzaldehyde were melted together at 100°C, treated with 10 drops of piperidine, and heated at 100°C for 12 hours. The dark brown viscous product was examined by the steam-distillation and alcoholic tartaric acid tests described above in the condensation at 100°C in absence of condensing agents. Again the presence of a small amount of the styryl derivative was demonstrated but not isolated from the unchanged reactants which were the chief constituents of the reaction mass. It would therefore appear that the piperidine exerts no appreciable catalytic effect in this reaction.

PREPARATION AND PROPERTIES OF 5-(p-DIMETHYLAMINOSTYRYL)--ACRIDINE.

As shown in the preceding experimental work, this compound is best prepared by following closely the method of Porai-Koschitz (Ref.8). The properties of the compound obtained were found to agree with those described in the literature. A few hitherto unrecorded properties are also given.

5 g. 5-methylacridine
3.86 g. p-dimethylaminobenzaldehyde
3.56 g. anhydrous zinc chloride (finely powdered)

The above substances (in molecular proportions) were mixed and heated together at 135°C for 6 hours. The dark brown product was extracted with 15% hydrochloric acid, and the yellow acid solution filtered. On pouring the filtrate, with stirring, into an excess of ammonia solution the styryl derivative was obtained as an orange-red precipitate. This was filtered off, washed with water, allowed to dry, and purified by crystallisation from alcohol. Yield: 6.31 g. (75% theoretical).

The compound was obtained as an orange-brown crystalline powder, melting at 239°C (literature gives 238 to 239°C). It was found to be soluble in the common organic solvents and to exhibit colour change phenomena (new details regarding which are given below). Since all the above facts are in agreement with those given in the literature, the analysis of

the compound was not carried out.

The most striking method of demonstrating the colour change properties of this compound is to add glacial acetic acid to the compound, when an intense prussian-blue solution is produced. On dilution with water this becomes green. This green solution becomes yellow on the addition of dilute hydrochloric acid, and passes through blue to orange-yellow (with precipitate of the base) on the addition of ammonia. Similar results were obtained with a solution of tartaric acid in alcohol in place of glacial acetic acid.

The solution of the compound in ordinary dilute hydrochloric acid was found to be greenish-yellow (literature states deep blue), and in very dilute hydrochloric acid green.

The behaviour of the hydrochloride further illustrates the colour change phenomenon. This salt was prepared by dissolving the base in 15% hydrochloric acid and evaporating the solution over the water-bath. The greenish-brown resinous residue became dark blue and crystalline on drying at 100°C. On dissolving this blue salt in water an intense blue-black solution was formed, which developed a beautiful violet tint on dilution with water. The colour of this dilute solution was discharged on treatment with either a drop of dilute hydrochloric acid or ammonia, a yellow-green solution being formed in the case of the acid, an orange-yellow precipitate in the case of the ammonia.

The solution of the compound in chloroform is deep red. When this is treated drop by drop with a solution of bromine in chloroform a deep violet colour is first produced, then a reddish brown precipitate is formed.

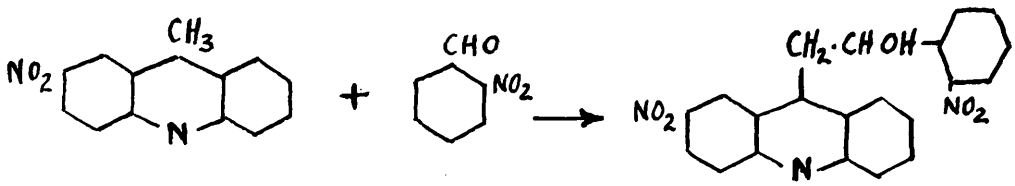
Thus in all the above colour changes the compound resembles 5-(p-aminostyryl)-acridine (page 149), and the whole question is discussed in the theoretical section (page 42).

PART III

PREPARATION OF STYRYL-ACRIDINES FROM DERIVATIVES OF 5-METHYLACRIDINE.

PREPARATION AND PROPERTIES OF α-[o-NITROPHENYL]-β-[3-NITROACRIDYL-(5)]-ETHANOL.

This new compound was produced by heating together 3-nitro-5-methylacridine and o-nitrobenzaldehyde at 100°C:



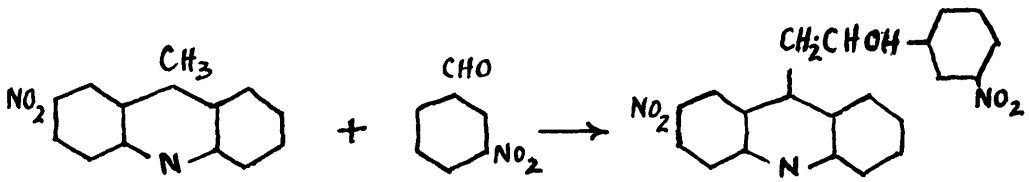
1.19 g. 3-nitro-5-methylacridine and 0.76 g. o-nitrobenzaldehyde (molecular proportions) were heated together at 100°C for 6 hours. The bright yellow melt at first produced became yellow-brown and harder during heating. The product was powdered, washed with a little alcohol to remove unchanged reactants, then with a little pyridine to remove tarry matter (washing out the pyridine with alcohol). The product was finally crystallised from benzene. Yield: 1.0 g.

The substance was obtained in small yellow crystals, which appeared to decompose, with charring, at 170°C, but remained unmelted at 360°C. Analysis showed 65.0% C, 3.80% H, 10.9% N ($C_{21}H_{15}O_5N_3$ requires 64.8% C, 3.86% H, 10.8% N).

The compound is insoluble in water and only sparingly soluble in hydrochloric acid (dilute or concentrated) and the common organic solvents. The general insolubility of the compound serves to distinguish it from 3-nitro-5-methyl-acridine.

PREPARATION AND PROPERTIES OF α-[m-NITROPHENYL]-β-[3-NITROACRIDYL-(5)]-ETHANOL.

This new compound was prepared by the method described on page 170 for its o-isomer, using m-nitrobenzaldehyde in place of o-nitrobenzaldehyde:-



The product was purified by washing with pyridine then alcohol, and finally crystallised from a mixture of pyridine and alcohol. Yield: 1.2 g. (from 1.19 g. 3-nitro-5-methyl-acridine).

The compound was obtained in the form of yellow crystals, which sintered with decomposition at 175° C. Analysis showed 65.0% C, 3.95% H, 10.8% N ($C_{21}H_{15}O_5N_3$ requires 64.8% C, 3.86%H, and 10.8% N).

The substance was found to resemble closely its o-isomer (page 170) in its general insolubility in the common solvents.

EXPERIMENTS ON THE CONDENSATION OF 3-NITRO-5-METHYL--ACRIDINE WITH p-NITROBENZALDEHYDE.

Analogous experiments with 5-methylacridine have already been described (page 137). It was found that the styryl derivative (a new compound) was best prepared by condensing the 3-nitro-5-methylacridine with p-nitrobenzaldehyde in presence of acetic anhydride. No corresponding ethanol derivative was obtained.

Experiment at 100°C in absence of condensing agents:

Molecular proportions of 3-nitro-5-methylacridine and p-nitrobenzaldehyde were mixed and heated together at 100°C.. In contrast to the corresponding experiments with o- and m-nitrobenzaldehydes (pages 170/1), the reactants did not form a melt but remained solid, since the melting-point of p-nitrobenzaldehyde is considerably higher than its o- or m-isomers. Thus no ethanol derivative was obtained.

Experiments in presence of zinc chloride:

(a) At 130°C:

1.19 g. 3-nitro-5-methylacridine
0.76 g. p-nitrobenzaldehyde
0.68 g. anhydrous zinc chloride (finely powdered)

The above substances (in molecular proportions) were mixed and heated together for 3 hours at 130°C. The substances sintered together, a dark brown product being obtained at the

end of the heating. This was ground up, washed with water to remove zinc chloride and allowed to dry. On attempting to crystallise the dark brown product from a little pyridine, however, no solid matter separated from the tarry dark brown solution in that solvent.

(b) At 110°C:

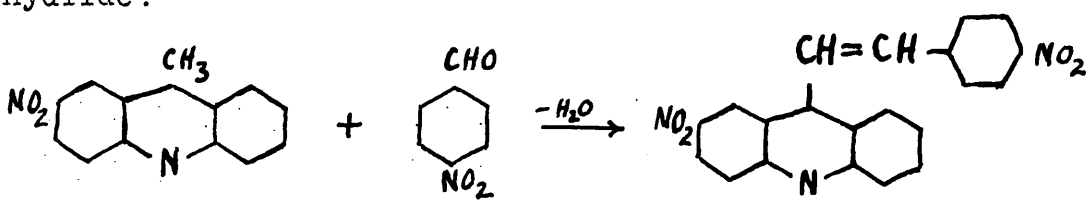
Experiment (a) above was repeated, the temperature of reaction being reduced to 110°C, however, in an attempt to reduce tarring. The substances remained unmelted however, on account of the lower temperature employed, the mixture simply becoming darker in colour, and no condensation occurring.

Condensation in presence of acetic anhydride:

Molecular proportions of the reactants heated together in presence of acetic anhydride were found to condense to form the styryl derivative. The preparation and properties of this compound are more fully described on page 174.

THE PREPARATION AND PROPERTIES OF 3-NITRO-5-(p-NITRO-STYRYL)-ACRIDINE.

As shown in the preceding experimental work (page 173) this compound is best prepared by condensing 3-nitro-5-methyl-acridine with p-nitrobenzaldehyde in presence of acetic anhydride:-



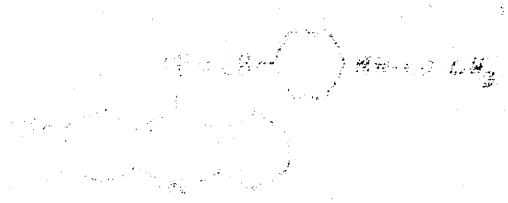
2.38 g. 3-nitro-5-methylacridine
1.51 g. p-nitrobenzaldehyde
6 ccs. acetic anhydride.

The above substances were heated together under reflux in an oil-bath at 130°C for 3 hours. The orange-coloured solution first formed began to deposit yellow crystals of the condensation product soon after the commencement of heating, the liquid becoming darker in colour. The mixture after heating was allowed to cool, then filtered, rejecting the dark brown filtrate which contained most of the tarry matter produced in the reaction. The yellow crystalline residue was washed with a little warm alcohol, and allowed to dry. The product was obtained almost pure in this way. Yield: 1.70 g. The product may be further purified by crystallisation from pyridine.

The substance did not melt at 360°C, although it appeared to decompose (darkened) above 250°C. Analysis showed

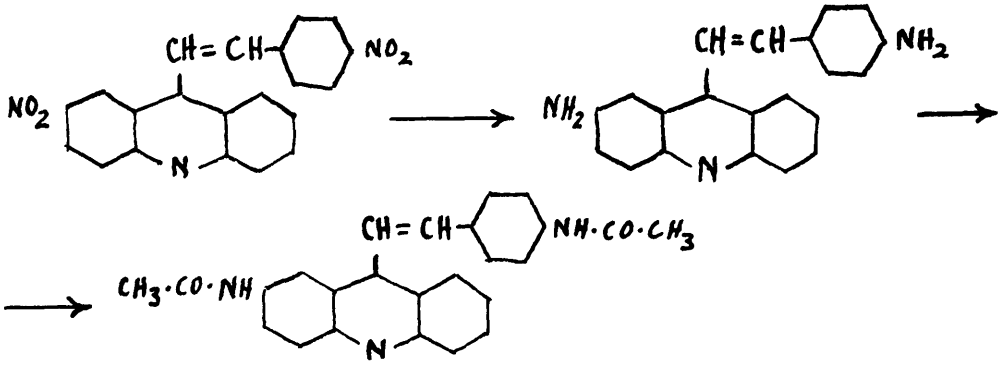
68.2% C, 3.60% H, 11.20% N ($C_{21}H_{13}O_4N_3$ requires 67.9% C, 3.51% H, 11.33% N,).

The general insolubility of the compound in the common solvents resembles that of the ethanol derivatives described on pages 170/1, and serves to distinguish the compound from 3-nitro-5-methylacridine.



PREPARATION AND PROPERTIES OF 3-AMINO-5-(p-AMINO--STYRYL)-ACRIDINE AND 3-ACETYLAMINO-5-(p-ACETYL--AMINOSTYRYL)-ACRIDINE.

These new compounds were prepared from 3-nitro-5--(p-nitrostyryl)-acridine according to the following scheme:



3-Amino-5-(p-aminostyryl)-acridine:

1.5 g. 3-nitro-5-(p-nitrostyryl)-acridine was reduced with anhydrous stannous chloride reagent (page 114, general procedure). The intermediate stannichloride was brown and formed a deep violet-red solution in water. The free base separated from this solution as a yellow-brown precipitate on treatment with excess sodium hydroxide solution. It was filtered off, washed with water and allowed to dry. Yield: 1.2g.

The yellow-brown powder so obtained could not be crystallised satisfactorily from the common solvents, but was characterised through its crystalline di-acetyl derivative (page 177). The substance gave no sharp melting point, decomposing with sintering above 200°C.

The general solubility of the substance in the common

solvents resembles that of 3-amino-5-methylacridine (page 109). In glacial acetic acid it forms a deep violet-red solution, which becomes pale red on dilution with water. This pale red solution becomes yellow on the addition of dilute hydrochloric acid, and gives a yellow precipitate of the free base on the addition of excess ammonia. The solution of the compound in ether is yellow and shows slight greenish fluorescence.

The hydrochloride did not separate from a hot solution of the compound in 15% hydrochloric acid on cooling, but was obtained as a dark purplish crystalline solid on evaporating such a solution over the water-bath. The salt was easily soluble in water with a violet-red colour.

3-Acetylamino-5-(p-acetylaminostyryl)-acridine:

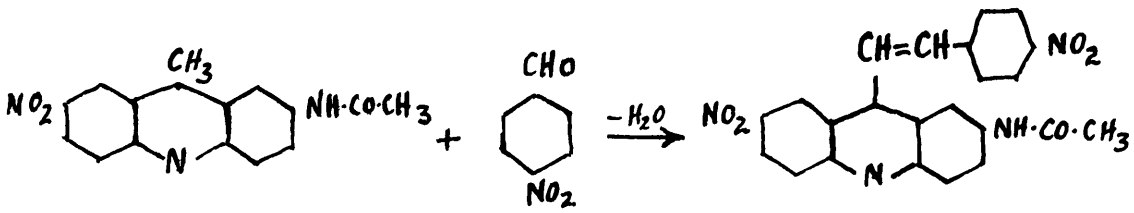
1 g. 3-amino-5-(p-aminostyryl)-acridine was acetylated in the same way as 3:7-diamino-5-methylacridine (page 123). The brown product was purified by crystallisation from aqueous alcohol using decolourising carbon, and was obtained as a yellow-brown crystalline powder. Yield: 1.0 g.

The substance remained unmelted at 360° C. Analysis showed 76.5% C, 5.20% H, 10.55% N ($C_{25}H_{21}O_2N_3$ requires 76.0% C, 5.32% H, 10.6% N).

The general solubility of the compound in the common solvents resembles that of 3-acetylamino-5-methylacridine (page 112). The compound dissolves in glacial acetic acid with a reddish-brown colour.

PREPARATION AND PROPERTIES OF 3-NITRO-5-(p-NITRO--STYRYL)-7-ACETYLAMINO-ACRIDINE.

This new compound was prepared by condensing 3-nitro--5-methyl-7-acetylamino-acridine with p-nitrobenzaldehyde in presence of acetic anhydride:-



1.48 g. 3-nitro-5-methyl-7-acetylamino-acridine.
0.76 g. p-nitrobenzaldehyde.
4 ccs. acetic anhydride.

The styryl derivative was prepared by the method already described (page 174) for 3-nitro-5-(p-nitrostyryl)-acridine, using the above quantities of reactants. Yield: 0.95g.

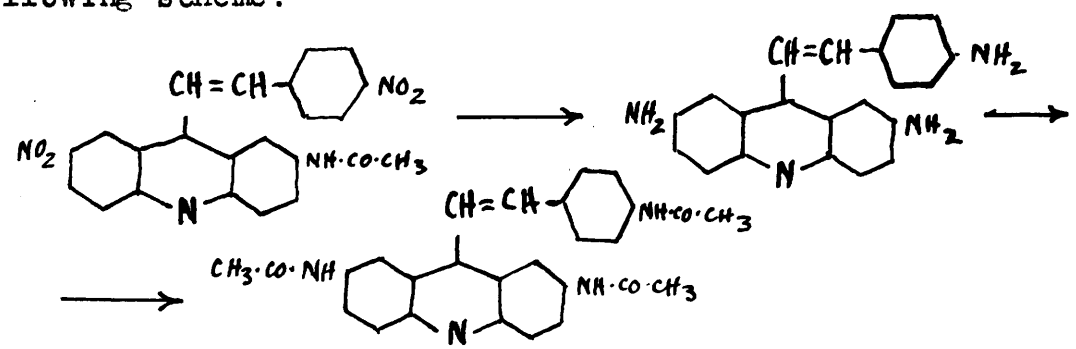
The substance was obtained as a yellow-brown crystalline powder after crystallisation from pyridine. It did not melt below 360° C; but darkened above 250° C, probably decomposing.

Analysis showed 64.0% C, 3.60% H, 13.1% N ($C_{23}H_{16}O_5N_4$ requires 64.5% C, 3.74% H, 13.1% N).

In its general insolubility in the common solvents, the compound resembles 3-nitro-5-(p-nitrostyryl)-acridine (page 175).

PREPARATION AND PROPERTIES OF 3:7-DIAMINO-5-(p-AMINO--STYRYL)-ACRIDINE, AND 3:7-DI-(ACETYLAMINO)-5-(p-ACETYL--AMINOSTYRYL)-ACRIDINE.

These new compounds were prepared from 3-nitro-5-(p-nitrostyryl)-7-acetylamino-acridine according to the following scheme:-



3:7-Diamino-5-(p-aminostyryl)-acridine:

1 g. 3-nitro-5-(p-nitrostyryl)-7-acetylamino-acridine was reduced with anhydrous stannous chloride reagant (general procedure, page 114). The brown stannichloride produced dissolved in water with a deep reddish-brown colour (deep wine-red on dilution). From this solution the reduction product was precipitated as a yellow-brown solid with excess sodium hydroxide solution. After filtering and drying, the product was heated for 1 hour with 15% hydrochloric acid at 100°C, to complete the hydrolysis of the acetylamino-group presumably already started during the acid-reduction. The free base was liberated from the acid solution after hydrolysis by adding excess sodium hydroxide solution, when it separated

as a brown precipitate. This was filtered off, washed with water and allowed to dry. Yield: 0.7 g.

The substance was obtained as a brown (somewhat resinous) solid which sintered with decomposition at about 180°C. It would not crystallise satisfactorily from the common solvents, but was characterised through its crystalline tri-acetylamino-derivative (see below).

In general solubility the compound resembles 3-amino-5-(p-aminostyryl)-acridine (page 176). In glacial acetic acid the compound forms a violet-red solution.

The hydrochloride was obtained by evaporating a solution of the free base in 15% hydrochloric acid (no crystals separated on cooling this solution) over the water-bath. The salt was obtained as a brownish-black crystalline solid, easily soluble in water with a deep yellow-brown colour, the solution yielding a brown precipitate of the free base on the addition of ammonia.

3:7-Di-(acetylamino)-5-(p-acetylaminostyryl)-acridine:

1 g. 3:7-diamino-5-(p-aminostyryl)-acridine was acetylated in the same way as 3:7-diamino-5-methylacridine (page 123), and the product purified by crystallisation from aqueous alcohol using decolourising carbon. Yield: 0.9 g.

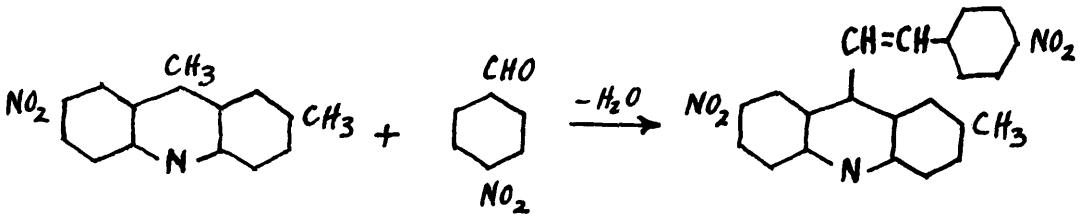
The substance was obtained as a yellow-brown crystalline powder which remained unmelted at 360°C. Analysis showed

71.4% C, 5.40% H, 12.3% N ($C_{27}H_{24}O_3N_4$ requires 71.6% C, 5.31%H, 12.4% N).

The general solubility of the compound in the common solvents resembles that of 3-acetylamino-5-methylacridine (page 112). The compound forms a deep reddish-brown solution in glacial acetic acid.

PREPARATION AND PROPERTIES OF 3-NITRO-5-(p-NITRO--STYRYL)-7-METHYLACRIDINE.

This new compound was prepared by condensing 3-nitro--5:7-dimethylacridine with p-nitrobenzaldehyde in presence of acetic anhydride:-



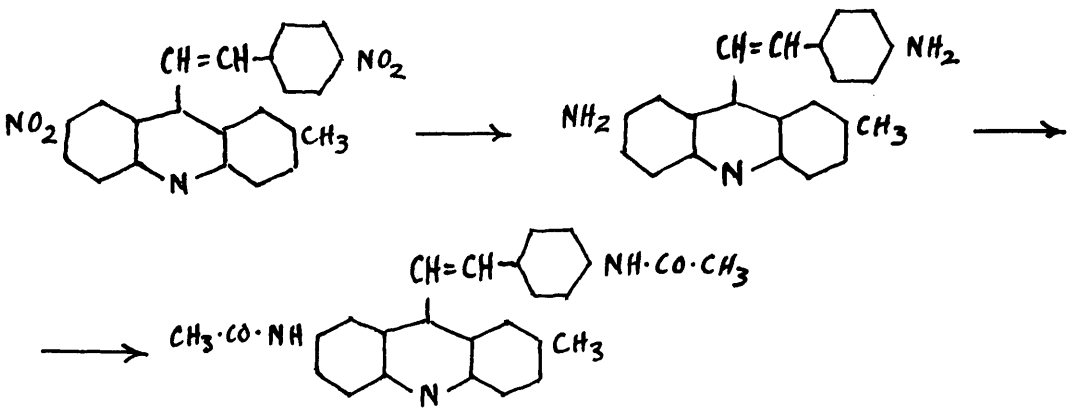
2.52 g. 3-nitro-5:7-dimethylacridine.
3.02 g. p-nitrobenzaldehyde (2 mols.)
7 ccs. acetic anhydride.

Using the above quantities of reactants the styryl derivative was prepared by the same method as described for 3-nitro-5-(p-nitrostyryl)-acridine (page 174). Although two molecular proportions of aldehyde were used, the product was a mono-styryl derivative, and since the 5-methyl group has previously proved reactive, it is concluded that this compound has the styryl group in position 5 (see also theoretical section, page 58). Yield: 2.0 g. The compound was purified by crystallisation from pyridine.

The substance was obtained as a brownish-yellow crystalline powder, which remained un-melted at 360°C. Analysis showed 68.3% C, 3.80% H, 10.9% N ($C_{22}H_{15}O_4N_3$ requires 68.6% C, 3.90% H, 10.9% N). In its general insolubility in the common solvents the compound resembles 3-nitro-5-(p-nitrostyryl)-acridine.

PREPARATION AND PROPERTIES OF 3-AMINO-5-(p-AMINO--STYRYL)-7-METHYLACRIDINE, AND 3-ACETYLAMINO-5-(p--ACETYLAMINOSTYRYL)-7-METHYLACRIDINE.

These new compounds were prepared from 3-nitro-5-(p--nitrostyryl)-7-methylacridine according to the following scheme:-



3-Amino-5-(p-aminostyryl)-7-methylacridine:

2 g. 3-nitro-5-(p-nitrostyryl)-7-methylacridine were reduced with anhydrous stannous chloride reagent (general procedure, page 114). The intermediate stannichloride was orange-red and dissolved in water with a deep violet-red colour. The free base was obtained as a yellow-brown precipitate from this solution on the addition of excess sodium hydroxide solution. After filtering and washing with water, the product was allowed to dry. Yield: 1.5 g.

The substance was obtained as a yellow-brown powder, which would not crystallise satisfactorily from the common solvents, and sintered with decomposition at about 200°C. The

substance was characterised through its crystalline di-acetyl derivative (see below).

The general solubility of the compound resembled 3-amino-5-(p-aminostyryl)-acridine (page 176). A deep violet-red solution is formed when the compound is treated with glacial acetic acid.

The compound dissolves in 15% hydrochloric acid with a yellow colour. The hydrochloride, which did not separate from a hot solution of the base in 15% hydrochloric acid on cooling, was obtained as a dark reddish-brown crystalline solid on evaporating such a solution over the water-bath. The salt was easily soluble in water with a deep wine-red colour. The addition of ammonia to an aqueous solution of the hydrochloride produced an orange-brown precipitate of the free base which on treatment with dilute acetic acid formed a deep violet-red solution which became yellower on the gradual addition of dilute hydrochloric acid.

3-Acetylamino-5-(p-acetylaminostyryl)-7-methylacridine:

1 g. 3-amino-5-(p-aminostyryl)-7-methylacridine was acetylated in the same way as 3:7-diamino-5-methylacridine (page 123). The yellow-brown product was purified by crystallisation from aqueous alcohol using decolourising carbon. Yield: 1.05 g.

The yellow-brown crystalline powder so obtained did not melt at 360° C. Analysis showed 76.5% C, 5.50% H, 10.2% N, ($C_{26}H_{23}O_2N_3$ requires 76.2% C, 5.62% H, 10.3% N).

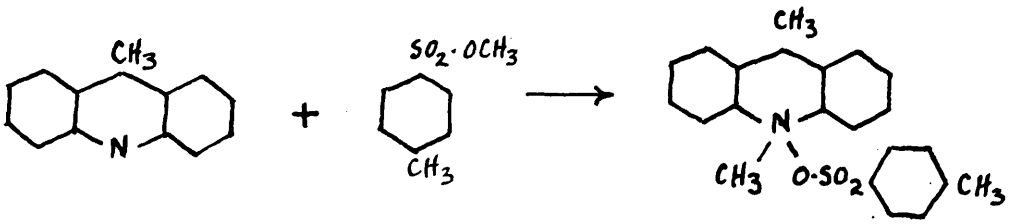
The general solubility of the compound in the common solvents resembles that of 3-acetylamino-5-methylacridine (page 112). A deep red solution is formed when the compound is treated with glacial acetic acid.

PART IV

PREPARATION OF ACRIDINIUM COMPOUNDS.

5-METHYLACRIDINE METHO-p-TOLUENESULPHONATE.

Experiments on the preparation of this compound, which does not appear to have been described before, were undertaken for reasons given in the theoretical section (page 63). The substance is easily produced by melting together 5-methylacridine and methyl p-toluenesulphonate at 145° C, without the use of nitrobenzene as solvent:



Experiment using Nitrobenzene as solvent:

The method used by Ullmann and Wenner (Ref.10) for preparing the corresponding phenylacridine metho-p-toluenesulphate was tried:-

1.93 g. 5-methylacridine
1.86 g. methyl p-toluenesulphonate (1 mol.)
25 ccs. nitrobenzene.

The 5-methylacridine was dissolved in the warm nitrobenzene, the brown solution brought to the boil, then treated with the ester. The boiling was continued for 5 minutes, during which time the liquid became deep red. On cooling and allowing to stand for a day with occasional scratching, no crystals separated. A small deposit of crystals was observed after standing for 5 days however. These were filtered off, setting aside the mothor liquor, and washing the yellow-green

residue with dry ether to remove nitrobenzene. The residue was found on examination to be identical with the product obtained in the experiment below without nitrobenzene, but only 0.2 g. was obtained.

The nitrobenzene mother liquor was divided into two portions. The first of these was mixed with an equal volume of alcohol and shaken with water in a separating funnel, removing the precipitated nitrobenzene by extraction with ether. The aqueous layer remaining was seen to contain the acridinium salt in solution since it showed greenish fluorescence and gave a precipitate of the methiodide (see page 189) on the addition of potassium iodide. An attempt was made to recover the acridinium salt from the second portion of the nitrobenzene mother liquor, by the careful addition of dry ether. The product which separated, however, was resinous, and could not be obtained crystalline from the common solvents.

In case the time allowed for methylation to occur in the above experiment had been too short, the whole experiment was repeated, boiling the reaction mixture for 30 minutes instead of for 5 minutes. This resulted in no improvement, however, the yield of product being, in fact, rather less than before, since more tarring occurred.

Experiment at 145°C in absence of nitrobenzene:

1.93 g. 5-methylacridine and 1.86 g. methyl-p-toluenesulphonate (1 mol.) were melted together in an oil-bath at

145°C, with occasional stirring, for 2 hours. The brown melt first produced became reddish and stiffer, and began to set solid after 20 minutes heating. At the end of the heating the product was quite hard. It was powdered and portions of it examined. In contrast to the starting materials, the product was almost completely soluble in water, a greenish-brown solution being formed which showed greenish fluorescence (more marked on dilution). On attempting to purify the product by washing with dry ether a sticky resinous mass was produced. A pure specimen of the acridinium salt was obtained however, by crystallising the crude product from a little alcohol. Since the compound is fairly soluble in cold alcohol considerable loss in yield occurs in purifying it this way (1 g. crude product yielded only 0.3 g. crystals); the careful addition of dry ether to the alcoholic mother liquor yielded only a sticky resinous precipitate, which however was largely soluble in water with green fluorescence.

5-Methylacridine metho-p-toluenesulphonate:

The substance was obtained as a yellow-brown crystalline powder after crystallisation from alcohol in the above experiment. Since most of the resinous matter was removed in the alcoholic mother liquor in that crystallisation, the substance could now be obtained crystalline from its solution in alcohol by carefully adding dry ether, the loss of product in the alcohol-ether mother liquor being small.

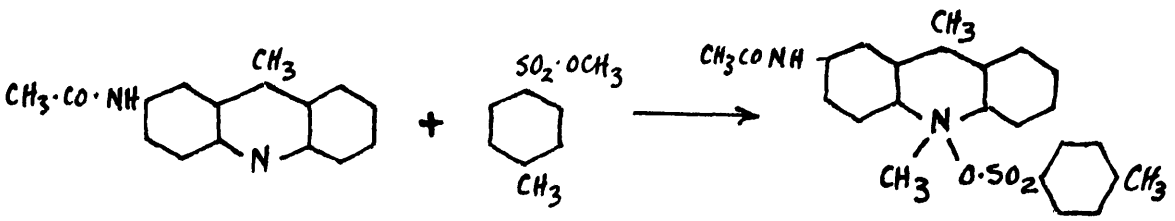
Recrystallised in the above way, the compound was obtained

as a pale yellow crystalline powder, which melted at 204° C. Analysis showed 3.65% N, 8.50% S ($C_{22}H_{21}O_3NS$ requires 3.69% N, 8.45% S).

The compound is readily soluble in water producing a greenish-yellow solution with intense greenish fluorescence which persists on dilution. The addition of potassium iodide to the aqueous solution produces a reddish-brown precipitate of the methiodide. Since the methochloride is more soluble in water than the methiodide, the addition of sodium chloride to an aqueous solution of the metho-p-toluenesulphonate only produces a yellow precipitate of the methochloride if the solution is concentrated enough. The addition of ammonia or sodium hydroxide solution to the aqueous solution produces a white precipitate, presumably of the acridinium base. The compound is insoluble in ether.

3-ACETYLAMINO-5-METHYLACRIDINE METHO-p-TOLUENESULPHONATE.

Like 5-methylacridine, 3-acetylamino-5-methylacridine readily combines with methyl p-toluenesulphonate at 145°C without the use of nitrobenzene as solvent:-



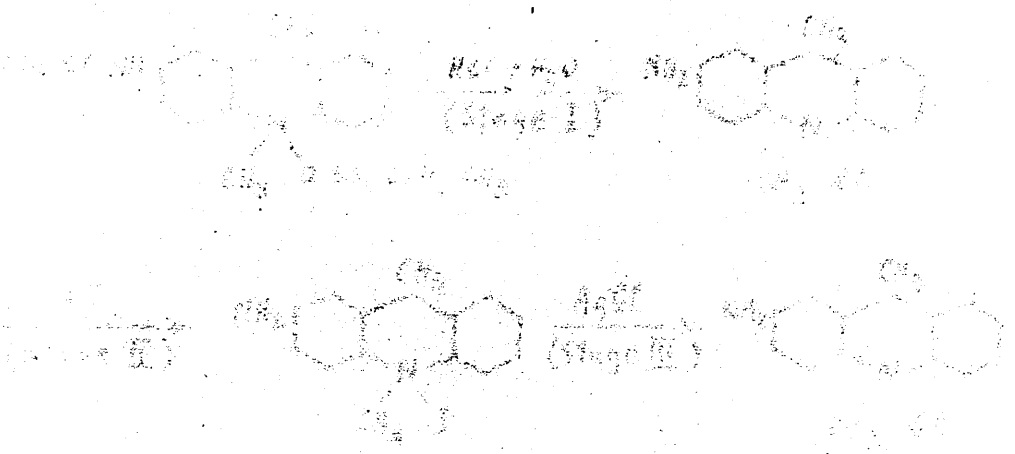
To prepare this new compound molecular proportions of 3-acetylamino-5-methylacridine and the ester were mixed and heated together in an oil-bath at 145°C with occasional stirring for 2 hours. The deep red melt produced became slowly stiffer during the heating, the final product being a hard dark-brown solid. This was allowed to cool, then powdered and crystallised from a little alcohol, when it was obtained as a yellow-brown crystalline solid melting at 226°C. Yield (from 1 g. acridine derivative): 0.7 g. The alcoholic mother liquor from the crystals contained the tarry matter produced during the reaction, and only yielded a sticky resinous solid on attempting to obtain a further yield of product from it by carefully adding dry ether.

The product obtained above could be further purified by dissolving in a little alcohol and treating the solution carefully with dry ether, when the compound separated in small yellow-brown crystals.

Analysis showed 6.50% N, 7.37% S ($C_{24}H_{24}O_4N_2S$ requires 6.42% N, 7.34% S).

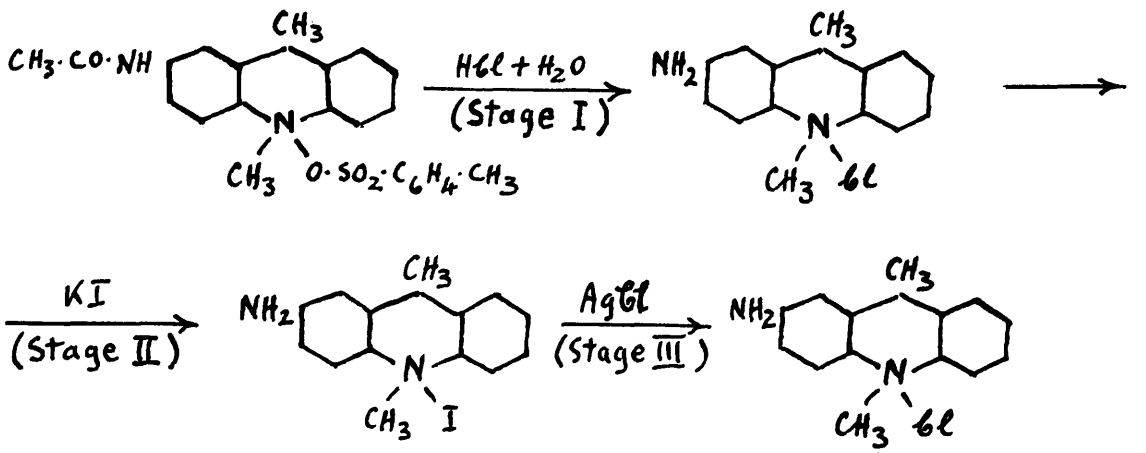
The compound is easily soluble in water producing a greenish-yellow solution with brilliant greenish fluorescence which persists on dilution with water. The aqueous solution on treatment with potassium iodide gives a yellow crystalline precipitate of the methiodide, but the methochloride appears to be too soluble in water to separate in this fashion with sodium chloride. The acridinium base separates from the aqueous solution as a blue-green precipitate on the addition of ammonia or sodium hydroxide solution. When the compound is boiled for a few minutes with concentrated hydrochloric acid hydrolysis occurs, the acetylamino-group becoming a free amino-group, and the p-toluenesulphonate radicle is replaced by chloride (see also preparation of 3-amino-5-methylacridine methochloride, page 193). Qualitative evidence of the change ($-NHCOCH_3$ to $-NH_2$) produced by boiling with hydrochloric acid was obtained by diluting the yellow acid solution and treating gradually with excess ammonia or sodium hydroxide solution. A deep red solution was now produced (compare the properties of 3-amino-5-methylacridine, page 195) at the neutral point, followed by the separation of a greenish-yellow precipitate with excess alkali (slow in the case of the ammonia). The addition of dilute hydrochloric acid to the alkaline solution reverses the colour changes.

The compound is easily soluble with a yellow colour in alcohol (the solution showing greenish fluorescence) and in glacial acetic acid. It does not, however, dissolve in ether.



3-AMINO-5-METHYLACRIDINE METHOCHLORIDE.

This new compound was prepared from 3-acetylamino-5-methylacridine metho-p-toluenesulphonate by hydrolysis with hydrochloric acid. Since the product did not separate as a solid from the hydrolysis mixture, it was found necessary to isolate it from its aqueous solution by precipitating (with potassium iodide) the corresponding methiodide, and converting the latter to the methochloride with silver chloride in aqueous methyl alcohol:-



Stage I: Hydrolysis with hydrochloric acid:

The 3-acetylamino-5-methylacridine metho-p-toluene-sulphonate was prepared as before (page 190), but the period of heating was shortened (1 hour) in order to reduce tarring, and the product simply powdered and not crystallised. 2 g. of the acridinium salt were dissolved in 3.5 ccs. water and the deep green solution treated with an equal volume of concentrated hydrochloric acid, the colour becoming deep yellow-brown.

The acid solution was gently boiled under reflux for 1 hour, then allowed to cool. No crystals separated from the solution however (even on allowing to stand overnight or on adding concentrated hydrochloric acid), so it was gently evaporated to dryness over the water-bath. The reddish-brown residue containing the acridinium salt (also hygroscopic p-toluene-sulphonic acid produced by hydrolysis) was extracted with water, the deep red aqueous solution filtered, and exactly neutralised by the careful addition of dilute potassium carbonate solution.

Stage II: Isolation of the methiodide:

The above neutral solution containing the methochloride of 3-amino-5-methylacridine was treated with 3 g. solid potassium iodide and shaken. An immediate purplish-red precipitate of the methiodide was formed. This was filtered off, washed with a little water, and allowed to dry. The purplish-red powder so obtained was slightly soluble in water with a pale red colour, and easily soluble in alcohol with a beautiful reddish-pink colour. The solution of the substance in dilute acetic acid is deep red, and remains deep red on the addition of a slight excess of ammonia, no immediate precipitate forming. Yield: 1.0 g.

Stage III: Conversion of the methiodide to methochloride:

1 g. of the methiodide, obtained as above, was dissolved

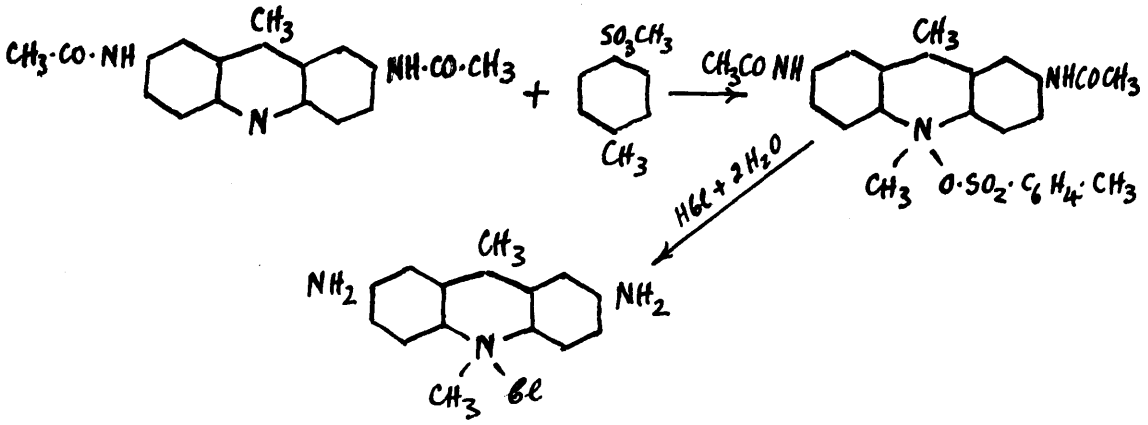
in a mixture of 20 ccs. methyl alcohol and 16.7 ccs. water, and the reddish solution gently boiled under reflux for 8 hours with excess freshly-precipitated and washed silver chloride. The mixture after cooling was filtered, and the residue of silver salts washed with a little hot aqueous methyl alcohol. The deep red filtrate and washings were then gently evaporated over the water-bath, leaving a dark violet-red crystalline residue of the methochloride (0.7g.).

The methochloride is slightly hygroscopic and is easily soluble in water with a deep red colour. The addition of a little ammonia to the aqueous solution causes the colour to become paler, but no immediate precipitate is formed. The compound can be purified by crystallisation from a mixture of absolute alcohol and dry ether.

The substance gives no sharp melting point, sintering with decomposition above 200° C. Analysis showed 10.65% N, 13.6% Cl ($C_{15}H_{15}N_2Cl$ requires 10.8% N, 13.75% Cl).

3:7-DIAMINO-5-METHYLACRIDINE METHOCHLORIDE.

This new compound was prepared from 3:7-di-(acetylamino)-5-methylacridine by combining it with methyl p-toluenesulphonate and hydrolysing the product with hydrochloric acid:



Since the methochloride did not separate as a solid from the hydrolysis mixture, it had to be isolated through the corresponding methiodide. The whole preparation was therefore carried out exactly as described for 3-amino-5-methylacridine methochloride (page **193**), with similar results (including yields). The following special points were observed:-

Stage I: Preparation and hydrolysis of the metho-p-toluenesulphonate: The deep yellow-brown hydrolysis mixture which again gave no crystals on cooling or standing, yielded a deep red residue on evaporation, which dissolved in water with a deep red colour.

Stage II: Isolation of the methiodide: In this case the methiodide was a reddish-brown solid, slightly soluble in water

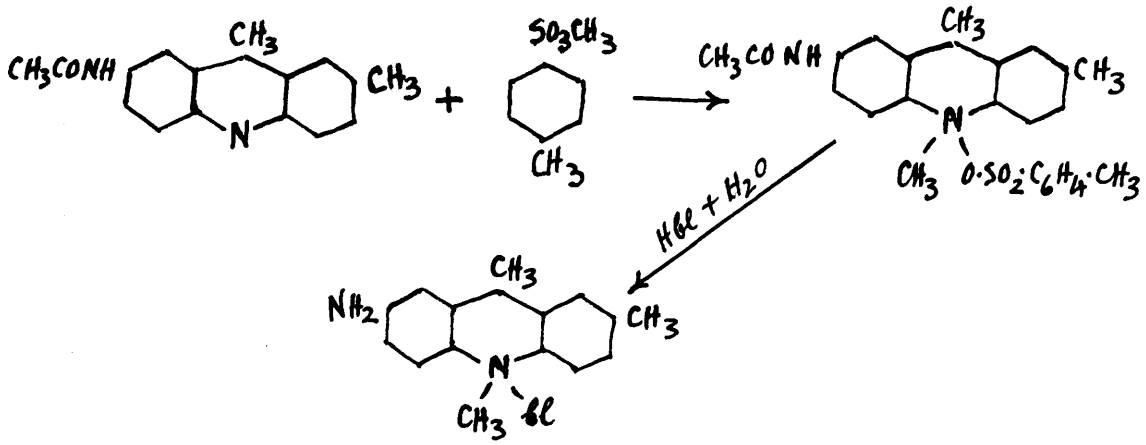
with a pale red colour, and easily soluble in alcohol with an orange-red colour.

Stage III: Conversion of methiodide to methochloride: The deep red filtrate from the reaction mixture obtained after refluxing the methiodide with silver chloride in aqueous methyl alcohol, in this case yielded a reddish-brown crystalline residue of the methochloride. This compound was hygroscopic and easily soluble in water with a deep reddish-brown colour. In other respects the compound resembled 3-amino-5-methyl-acridine methochloride, and was purified in the same way.

The compound gave no sharp melting point, sintering with decomposition above 200°C. Analysis showed 15.4% N, 13.1% Cl ($C_{15}H_{16}N_3Cl$ requires 15.35% N, 13.0% Cl).

3-AMINO-5:7-DIMETHYLACRIDINE METHOCHLORIDE.

This new compound was prepared from 3-acetylamino--5:7-dimethylacridine according to the following scheme:



The procedure adopted in its preparation was exactly the same as that already described for preparing 3-amino-5-methylacridine methochloride (page 193), it being again found necessary to isolate the methochloride through the corresponding methiodide.

The observations made as the preparation of the compound proceeded (e.g. yields, colour changes, and properties of intermediate substances) were found to be practically identical to those recorded at the corresponding stages in the preparation of 3-amino-5-methylacridine methochloride.

The methochloride finally obtained also closely resembled its mono-methyl analogue, although it was obtained as a reddish-brown (not violet-red) crystalline solid. It is

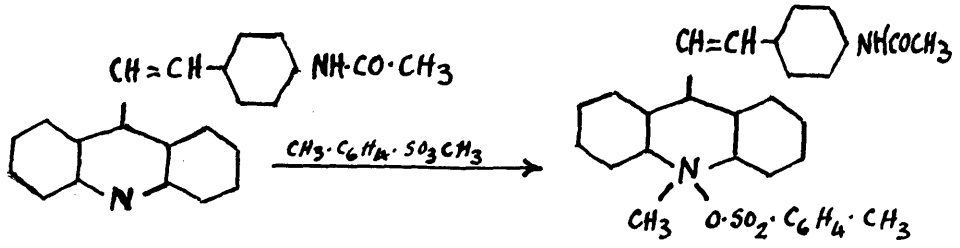
slightly hygroscopic and readily dissolves in water with a deep red colour, which becomes paler (no immediate precipitate) on the addition of a little ammonia solution.

The substance can be crystallised from a mixture of absolute alcohol and dry ether. It sinters with decomposition above 200° C. Analysis showed 10.4% N, 12.9% Cl ($C_{16}H_{17}N_2Cl$ requires 10.3% N, 13.0% Cl).

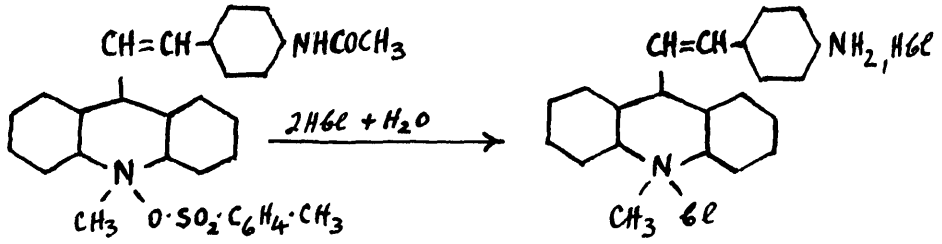
HYDROCHLORIDE OF 5-(p-AMINOSTYRYL)-ACRIDINE METHOCHLORIDE.

This new compound was prepared from 5-(p-acetylaminostyryl)-acridine by combining it with methyl p-toluenesulphonate and hydrolysing the product with hydrochloric acid. The compound separated in crystals from the hydrolysis mixture, and no further treatment was necessary:

Stage I:



Stage II:



Stage I: Preparation of the metho-p-toluenesulphonate:

Molecular proportions of 5-(p-acetylaminostyryl)-acridine and methyl p-toluenesulphonate were mixed and heated together at 145°C for 1 hour, with stirring. The reddish melt at first produced became stiffer during heating, finally yielding a hard reddish solid. This was cooled and powdered but could not be obtained crystalline from the common solvents. It dissolved readily in water and in alcohol, producing a reddish-brown solution in the former and a deep red solution in the latter. The addition of ether to the solution in alcohol precipitated a purplish resinous solid. It was

decided not to investigate the purification of the compound further, but simply to hydrolise it as in Stage II below.

Stage II: Hydrolysis of the metho-p-toluenesulphonate: 1 g. of the metho-p-toluenesulphonate prepared as above was dissolved in 3 ccs. water and the solution treated with an equal volume of concentrated hydrochloric acid, a yellow-brown crystalline precipitate separating from the reddish-brown solution. This was probably the methochloride of 5-(p-acetylaminostyryl)-acridine, since on examination it only feebly responded to the colour change test for the fully hydrolysed product i.e. 5-(p-aminostyryl)-acridine methochloride (see below). The hydrolysis mixture was therefore gently boiled under reflux (the yellow-brown precipitate dissolving on heating) for 1 hour in order to complete the hydrolysis. On cooling the solution after hydrolysis, the product separated in small dark reddish-brown crystals. After allowing to stand overnight in order that the maximum yield of product might separate, the crystals were filtered off and washed with a little 15% hydrochloric acid, and allowed to dry. Yield: 0.5 g. A small additional yield of less pure product was obtained by evaporating the deep brown mother-liquor (which stained the fingers brown, the stain becoming deep blue-black after a few minutes) over the water-bath, washing the residue with a little cold alcohol, and crystallising it from a little hot alcohol.

The compound was obtained as a reddish-brown crystalline

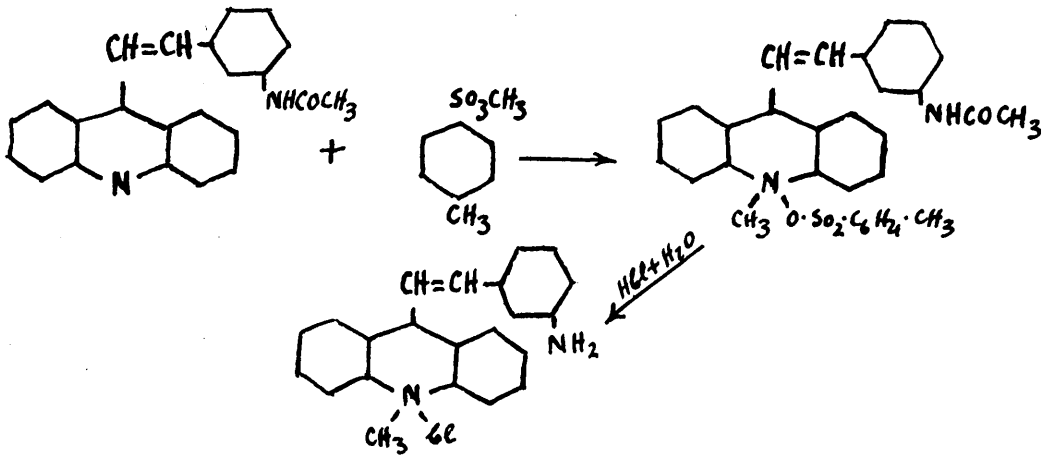
powder which becomes darker in colour on heating at 100°C. It is easily soluble in water producing a reddish-brown solution which on dilution develops a violet-red colour. The addition of a drop of dilute hydrochloric acid instantly changes this violet-red colour to yellow. In these colour phenomena the compound behaves like 5-(p-aminostyryl)-acridine hydrochloride (page 149), but may be distinguished from the latter by the fact that the dilute violet-red aqueous solution does not yield any immediate precipitate with a drop of dilute ammonia solution, the colour becoming somewhat more violet, but only slowly depositing a precipitate (greenish) on the addition of more ammonia. The addition of sodium hydroxide solution to the aqueous solution produces a deep violet-blue colour and a dark precipitate separates. The effect of alkali on an aqueous solution of 5-(p-aminostyryl)-acridine hydrochloride is simply to discharge the violet-red colour and produce a yellow precipitate of the free base.

Analysis showed 7.40% N, 18.5% Cl ($C_{22}H_{20}N_2Cl_2$ requires 7.31% N, 18.5% Cl). The compound did not melt, but decomposed with sintering about 250°C.

The aqueous solution of the compound on treatment with potassium iodide gives a yellow-brown precipitate of the methiodide.

5-(m-AMINOSTYRYL)-ACRIDINE METHOCHLORIDE:

The preparation of this new compound was carried out starting from 5-(m-acetylaminostyryl)-acridine according to the following scheme:



Since the product did not separate crystalline from the hydrolysis mixture, it had to be isolated through the corresponding methiodide. The preparation was thus carried out exactly as for 3-amino-5-methylacridine methochloride (page 193). The following special points were recorded:

Stage I: Preparation and hydrolysis of the metho-p-toluene-sulphonate: The deep yellow-brown hydrolysis mixture did not deposit crystals on cooling. On evaporation a sticky yellow-brown solid was left which dissolved in water with a yellow-brown colour.

StageII: Isolation of the methiodide: The methiodide was obtained as a yellow-brown solid, slightly soluble in water, and easily soluble in methyl alcohol, the solution in both cases being yellow.

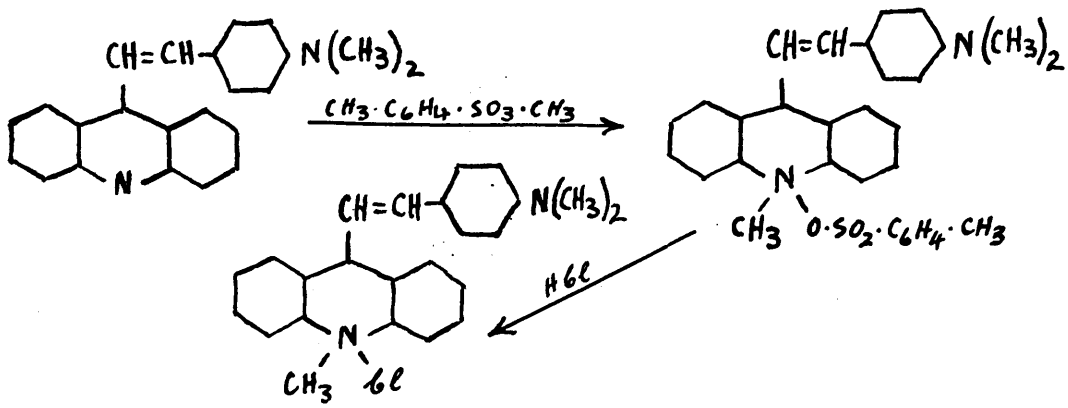
Stage III: Conversion of methiodide to methochloride: The deep yellow-brown filtrate from the reaction mixture after filtering off the silver salts gave a yellow-brown crystalline residue of the methochloride on evaporation. The product was recrystallised from absolute alcohol and dry ether. The substance was slightly hygroscopic and soluble with a deep yellow colour in water, giving no immediate precipitate with a drop of ammonia solution.

The substance did not melt but decomposed with sintering above 200°C. Analysis showed 7.99% N, 10.1% Cl, ($C_{22}H_{19}N_2Cl$ requires 8.08% N, 10.25% Cl).

Yield (from 1 g. metho-p-toluenesulphonate) 0.3 g.

5-(p-DIMETHYLAMINOSTYRYL)-ACRIDINE METHOCHLORIDE.

This new compound was prepared from 5-(p-dimethylaminostyryl)-acridine by combining it with methyl p-toluenesulphonate and converting the product to the methochloride with hydrochloric acid:-



Since the methochloride did not separate from the acid solution it had to be isolated through the corresponding methiodide. The preparation therefore followed precisely the same lines as that of 3-amino-5-methylacridine methochloride (page 193), the following special points being noted:-

Stage I: Preparation of the metho-p-toluenesulphonate and its conversion to metho-chloride. In preparing the metho-p-toluenesulphonate two molecular proportions of the ester were used (see theoretical section, page 70), and the blue-green melt only became slightly stiffer during heating. The melt was treated as before with hydrochloric acid but the deep yellow-brown solution produced gave no crystals on cooling or on standing. On evaporation a viscous brown residue was left

which dissolved completely in water with a deep yellow-brown colour and greenish fluorescence.

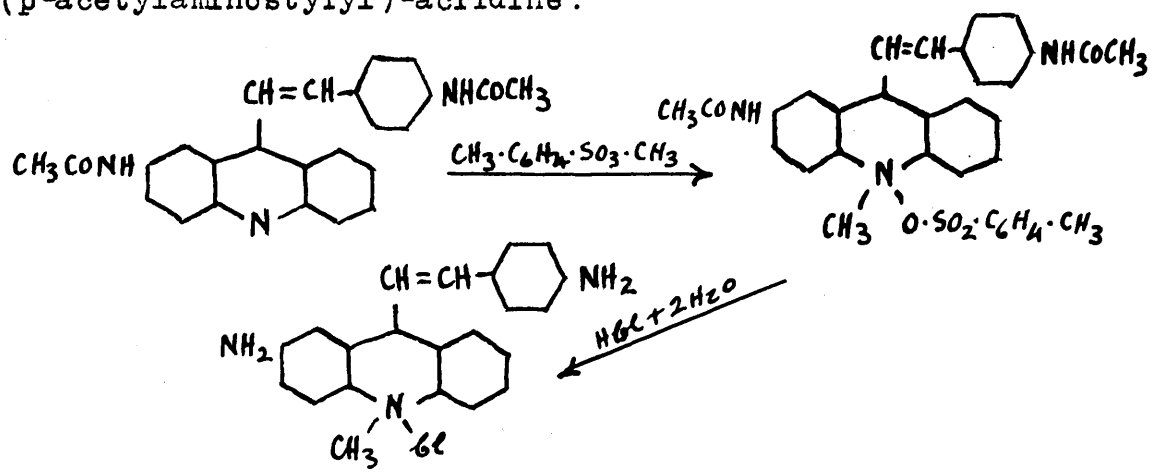
Stage II: Isolation of the methiodide. The methiodide was isolated as before and was obtained as a yellow-brown solid, slightly soluble in water with a yellow colour, and easily soluble with a deep yellow-brown colour in methyl alcohol.

Stage III: Conversion of methiodide to methochloride: The deep yellow-brown filtrate obtained after removing the silver salts from the reaction mixture gave a greenish-brown residue of the methochloride on evaporation. The compound was hygroscopic and easily soluble in water with a deep green colour, giving no immediate precipitate with a drop of ammonia solution. Yield: (from 1 g. 5-(p-dimethyl-aminostyryl)-acridine) 0.45 g.

The substance was obtained crystalline from a mixture of absolute alcohol and dry ether. It did not melt but decomposed, darkening in colour, above 200°C. Analysis showed 7.53% N, 9.50% Cl ($C_{24}H_{23}N_2Cl$ requires 7.48% N, 9.48% Cl).

3-AMINO-5-(p-AMINOSTYRYL)-ACRIDINE METHOCHLORIDE.

This new compound was prepared by exactly the same procedure as for 3-amino-5-methylacridine methochloride (page 193), starting in this case from 3-acetylamino-5--(p-acetylaminostyryl)-acridine:



The methochloride was again isolated through the corresponding methiodide. The practical details of the preparation have already been given for 3-amino-5-methyl-acridine methochloride so that only the following special points have been recorded:-

Stage I: Preparation and hydrolysis of the metho-p-toluene-sulphonate: In the preparation of the metho-p-toluene-sulphonate, molecular proportions of the acridine derivative and the ester did not form a melt on heating, so the proportion of ester was increased to 2½ mols. and the reddish-brown viscous product hydrolysed as before with hydrochloric acid, the deep yellow-brown hydrolysis mixture yielding no crystals on cooling. On evaporation, a deep brown residue remained

which was largely soluble in water with a deep red colour.

Stage II: Isolation of the methiodide:

The methiodide was isolated as before, and was obtained as a dark purplish-red solid, slightly soluble in water, and easily soluble in alcohol (ethyl or methyl), in all cases with a purplish-red colour.

Stage III: Conversion of methiodide to methochloride:

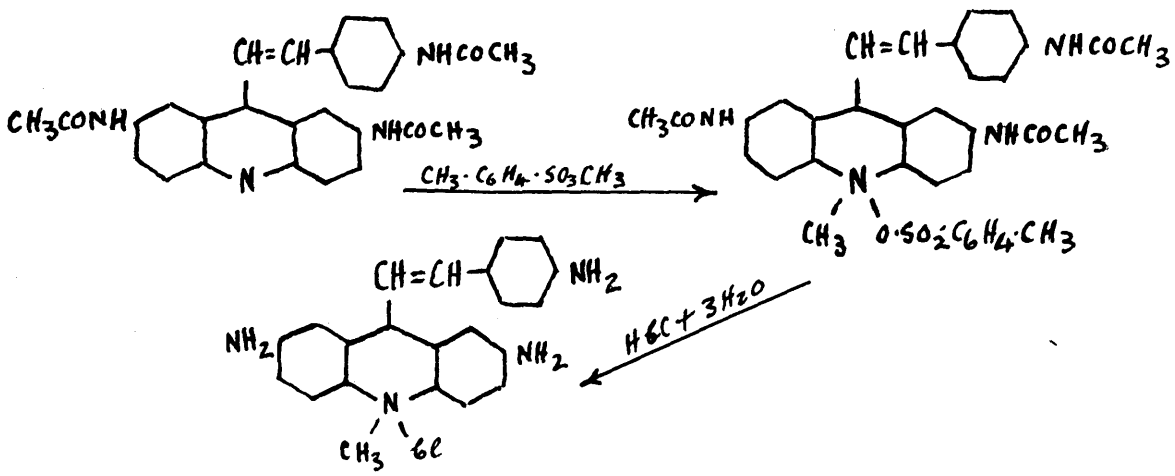
The deep purplish-red filtrate from the reaction mixture, after filtering to remove the insoluble silver salts, gave a dark purplish crystalline residue of the methochloride on evaporation. The compound was easily soluble in water with a violet-red colour, which did not change appreciably on the addition of a drop of dilute ammonia solution (no immediate precipitate).

The compound was obtained crystalline from alcohol and ether as before. It did not melt, but decomposed with sintering at about 250° C. Analysis showed 11.45% N, 9.85% Cl ($C_{22}H_{20}N_3Cl$ requires 11.6% N, 9.82% Cl).

Yield: (from 1 g. 3-acetylamino-5-(p-acetylamino-styryl)-acridine) 0.3 g.

3:7-DIAMINO-5-(p-AMINOSTYRYL)-ACRIDINE METHOCHLORIDE.

This new compound was prepared from 3:7-di-(acetylamino)-5-(p-acetylaminostyryl)-acridine by the same series of reactions as was employed in preparing 3-amino-5-(p-aminostyryl)-acridine (page 207):-



Practical details of the preparation which again involved isolation of the methochloride through the corresponding methiodide have already been given, so that only the following special points need be recorded:-

Stage I: Preparation and hydrolysis of the metho-p-toluenesulphonate: In the preparation of the metho-p-toluenesulphonate it was again found necessary to use 2½ mols. of the ester to produce a melt. The dark brown product was hydrolysed with hydrochloric acid as before, but no crystals separated from the reddish-brown hydrolysis mixture on cooling. The solid left on evaporation was brown and dissolved in water with a reddish-brown colour.

Stage II: Isolation of the methiodide: The methiodide was isolated as before and was obtained as a dark purplish-red solid, slightly soluble in water and easily soluble in alcohol forming a purplish-red solution in the former, and an orange-red solution in the latter.

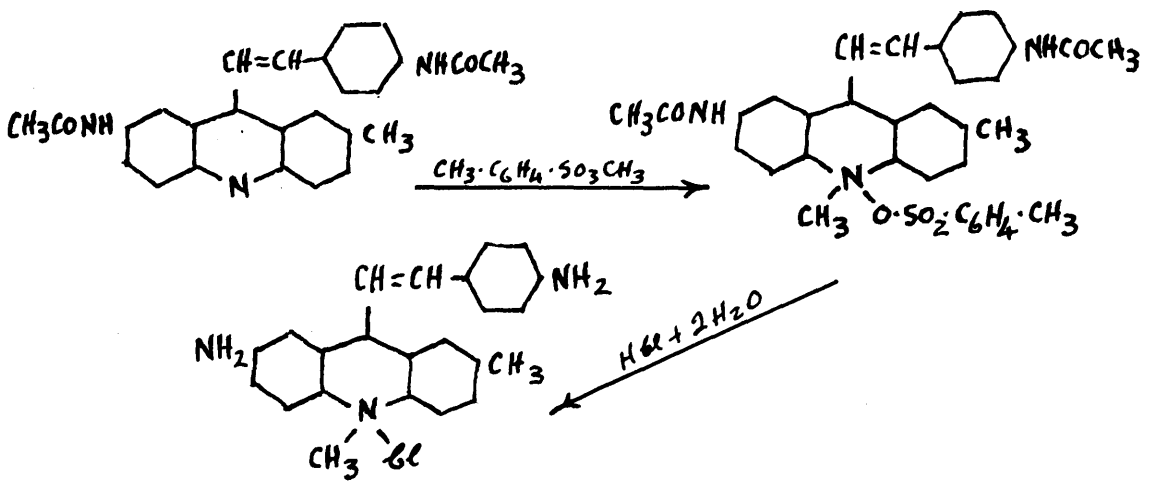
Stage III: Conversion of methiodide to methochloride: The deep brown filtrate obtained after filtering off the insoluble silver salts from the reaction mixture, on evaporation left a dark brown crystalline residue of the methochloride. The compound was readily soluble in water with a deep reddish-brown colour, the solution giving no immediate precipitate or colour change with a drop of dilute ammonia solution.

The compound was crystallised as before from alcohol and ether. Analysis showed 15.05% N, 9.33% Cl, ($C_{22}H_{21}N_4Cl$ requires 14.9% N, 9.42% Cl). The substance gave no sharp melting point, decomposing with sintering at about 200°C.

Yield: (from 1 g. 3:7-di-(acetylamino)-5-(p-acetylaminostyryl)-acridine) 0.3 g.

3-AMINO-5-(p-AMINOSTYRYL)-7-METHYLACRIDINE METHOCHLORIDE.

This new compound was prepared from 3-acetylamino-5--(p-acetylaminostyryl)-7-methylacridine by combining it with methyl p-toluenesulphonate and hydrolysing the product with hydrochloric acid:-



Since the methochloride did not separate from the hydrolysis mixture, isolation through the corresponding methiodide had again to be adopted. The whole preparation therefore closely resembles that of 3-amino-5-(p-aminostyryl)-acridine* (page 207) so that only the following special points need be recorded:-

Stage I: Preparation and hydrolysis of the metho-p-toluene-sulphonate: Again 2½ mols. of the ester were used to form a melt when preparing the acridinium salt, and the product was hydrolysed with hydrochloric acid as before. No crystals separated from the deep wine-red hydrolysis mixture on cooling. On evaporation a brown solid remained which was

* methochloride

largely soluble in water with a reddish-brown colour.

Stage II: Isolation of the methiodide: The methiodide was isolated as before and was obtained as a dark purplish-red solid, slightly soluble in water with a reddish-brown colour, and easily soluble in alcohol (methyl or ethyl) with a violet-red colour.

Stage III: Conversion of methiodide to methochloride: The deep purplish-red filtrate from the reaction mixture, after filtering off insoluble silver salts, gave a dark purplish-red crystalline residue of the methochloride on evaporation. The compound was slightly hygroscopic and easily soluble in water with a dark red solution, becoming somewhat brownish on the addition of a drop of ammonia solution and a slight precipitate slowly separating.

The substance was obtained crystalline from alcohol and ether as before. It did not melt, but appeared to decompose above 250°C. Analysis showed 11.05% N, 9.55% Cl ($C_{23}H_{22}N_3Cl$ requires 11.2% N, 9.45% Cl).

Yield: (from 1 g. 3-acetylamino-5-(p-acetylamino-styryl)-7-methylacridine) 0.35 g.