THE SYNTHESIS OF NEW ANTIMALARIAL DRUGS -
PYRIDOACRIDINES DERIVED FROM
5-, 6-, AND 7-AMINOQUINOLINES.

by

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The acridine molecule has been numbered in accordance with the method adopted by the Journal of the Chemical Society, viz.:

All new compounds which have been analysed, or of which a derivative has been analysed, are underlined in ink, wherever their names occur in the text.
I. INTRODUCTION AND GENERAL SURVEY OF THE LITERATURE

Malaria is a very ancient disease, but the history of rational attempts to cure it is comparatively modern. In degree of incidence it is exceeded only by helminthiasis, which is considerably less lethal, so that the discovery of an effective therapy for the disease is a matter of great social urgency.

In 1880 Laveran discovered that malaria was caused by a parasite, and in 1898 Ross demonstrated that these parasites are sporozoans which have become adapted to an asexual existence in the red blood cells of certain vertebrates and to a sexual mode of life in the anopheline mosquito. When an infected mosquito bites the intermediate vertebrate host sporozoites, present in the salivary glands of the mosquito, are liberated into the victim's blood, where, possibly after an existence as exoerythrocytic forms, they invade the red blood cells becoming trophozoites. Whether the parasites always exist as exoerythrocytic forms before they enter the red blood cells has not been settled. They have been demonstrated in the fowl (James and Tate, Nature, 1937, 139, 545; Parasitology, 1938, 30, 123), but not yet in other species, though it has been suggested that they are responsible for relapses in human malaria. Upon the division of the nuclear chromatin material the schizont stage is/
is reached. When the red blood corpuscle bursts numerous small bodies called merozoites are liberated into the blood stream, and the characteristic attacks of rigor and fever occur at this stage. The merozoites re-enter the red blood corpuscles and become trophozoites again or else differentiate into male and female forms known as gametocytes which finally break out of the red cells but do not divide and cause no further symptoms. It is these gametocytes, however, which are infective for mosquitoes, in which they undergo a sexual reproductive cycle with subsequent formation of sporozoites in the salivary glands of the mosquito.

The earliest attempts to cure malaria seem to date from the seventeenth century, when an extract of the bark of the cinchona tree was used in Peru, and very soon introduced into Europe. However it was not until almost two centuries later that Pelletier and Caventou in 1820 isolated quinine (I) from cinchona. In 1908 Rabe postulated the now

![Chemical Structure](image)
accepted structure of quinine, recently confirmed by synthesis (Woodward and Doering, J. Amer. Chem. Soc., 1945, 67, 860). Apart from quinine, cinchonine, cinchonidine and quinidine, the alkaloids which are most abundant in nature, many derivatives of these alkaloids have been synthesised and tested for antimalarial activity.

The synthesis of pamaquin and mepacrine, which, until the recent work leading to the discovery of paludrine, were the main antimalarial drugs besides quinine, came as the result of the researches of Schulemann and co-workers (Proc. Roy. Soc. Med., 1932, 26, 897) whose work was based on earlier work of Ehrlich and Guttmann on the antimalarial activity of methylene blue (II).

![Methylene Blue](image)

This activity was enhanced when one of the methyl groups was replaced by a $\beta$-diethylaminoethyl group (III). The introduction of similar groups into the quinoline nucleus led to the production of pamaquin (originally "plasmoquine") which is 8-($\delta$-diethylamino-$\alpha$-methylbutylamino)-6-methoxyquinoline (IV). The chief value of pamaquin is that it acts on the gametocytes/
gametocytes; its general antimalarial activity is high, the dose for humans being of the order of centigrams as compared with decigrams for mepacrine and grams for quinine, but at the same time it is highly toxic, and this limits its therapeutic use.

With a view to reducing the toxic effects associated with quinoline other heterocyclic ring systems were tried, and this led Mauss and Mietzch (Klin. Wschr., 1933, 12, 1276) to the synthesis of mepacrine/
mepacrine (originally "atebrine") or 8-chloro-8-methoxy-5-(\(\delta\)-diethylamino-\(\alpha\)-methylbutylamino)acridine (V) which is schizonticidal in action.

\[
\begin{align*}
\text{C}_2\text{H}_5 & \quad \text{N} \quad \text{C}_2\text{H}_5 \\
\quad \text{(CH)}_3 \quad \text{CH(CH)}_3 \quad \text{NH} \\
\quad \text{Cl} \quad \text{OCH}_3
\end{align*}
\]

(V)

It has recently been claimed (Hamilton-Fairley, Trans. Roy. Soc. Trop. Med. Hyg., 1945, 39, 511) that mepacrine is capable of almost complete clinical suppression against New Guinea strains of malaria. Mepacrine is not, however, a true prophylactic, as when treatment with the drug is discontinued malaria may develop, even although fresh infection has been avoided.

Much work has been done in an attempt to correlate therapeutic activity with chemical constitution. It was early discovered that cinchonine (VI) and dihydroquinine (VII) possessed antimalarial activity comparable with that of quinine itself, so that the methoxy and vinyl groups are not essential for therapeutic action. Inactivation was, however, produced by changes in the secondary alcoholic group, \(-\text{CHOH}^-,\) e.g. by oxidation to \(-\text{CO}^-,\) reduction/
reduction to \(-\text{CH}_2-\), acetylation to \(-\text{CH}_2\text{OAc}\)-, or conversion to \(-\text{CHCl}\)- (Cf. Giemsa and Oesterlin, Arch. Schiffs- u. Tropenhyg., 1933, 37, Beih. 4, 217). Replacement of the vinyl group by a carboxyl group also gives an inactive compound, but here activity can be restored by esterification, the activity at first increasing with the molecular weight of the alcohol used, reaching a maximum with butyl and amyl alcohols, higher homologues giving compounds of lower activity.

Ring and his co-workers have synthesised a number/
number of compounds resembling quinine closely in structure, and possessing activity against avian malaria. Examples of these compounds are 4-[(6-methoxyquinolyl)-α-piperidyl carbinol (VIII) (Ainley and King, Proc. Roy. Soc., 1938, 125 B, 60) and dibutyl-, diamyl-, and dihexyl-aminomethyl-6-methoxy-4-quinolyl carbinols (IX, R=C₆H₅-, C₃H₇-, or C₆H₁₃-) (King and Work, J.C.S., 1940, 1307).

It is interesting to note that the dimethyl, diethyl, and dipropyl compounds (IX, R=CH₃, C₂H₅, or C₃H₇) are inactive and that the methoxy group appears to be essential.
Variations of the pamaquin type of structure have been studied extensively in the last twenty years. The chief workers in this field have been Fourneau in France, Robinson in Britain, Magidson in Russia, and during the war a large team of chemists working under the auspices of the National Research Council of the U.S.A. A very large number of compounds have been prepared consisting of a quinoline nucleus substituted in various ways and carrying a basic side chain. Many variations of both the nuclear substituents and the side chain have been tried, but a review of the work is not contemplated here. None of the compounds tested has been found to be definitely superior to pamaquin in therapeutic qualities.

However, mention should perhaps be made of three quinoline derivatives prepared by I.G. Farbenindustrie. The first of these is certuna (X) in which the methoxy group of pamaquin is replaced by

\[
\begin{align*}
\text{N} & \quad \text{C}_7\text{H}_5 \\
\text{(CH}_3\text{)}_3 & \quad \text{I} \\
& \quad \text{CH(C}_3\text{H}_3) \quad \text{NH} \\
\text{HO} & \quad \text{N}
\end{align*}
\]

\( (X) \)

hydroxyl, and which is said to have a gametocidal activity of the same order as pamaquin, but to be less...
less toxic. The other two are derivatives of 4-aminoquinoline and have been given the names sonto chin (XI, $R=\text{NH} \cdot \text{CH}(\text{CH}_2) \cdot (\text{CH}_2)_3 \cdot N(\text{C}_6 \text{H}_5)_2$) and brachysan (XI, $R=\text{NH} \cdot (\text{CH}_2)_3 \cdot N(\text{C}_6 \text{H}_5)_2$).

![Chemical structure of XI](image)

These drugs have an activity of the same order as mepacrine, but are relatively non-toxic, and are colourless, one of the disadvantages of mepacrine being that it stains the skin yellow.

I.G. Farbenindustrie have produced another interesting quinoline compound. This is endochin (XII) which was prepared by Salzer in 1940 and which

![Chemical structure of XII](image)

has no basic side chain, being in fact 4-hydroxy-7-methoxy-3-$n$-heptylquinaldine. It has been found to possess activity as a causal prophylactic in avian malaria in which it can also affect clinical cures.
Much interesting work has been done in Russia initiated by Magidson in 1933. This work deals mainly with mepacrine analogues, thus Magidson and Grigorovskii (Chemico-Pharm. Ind. (USSR), 1933, No. 1) describe the preparation of several 8-nitro- and 8-chloro-3-alkoxy-5-(diethylaminoalkylamino)-acridines (XIII, R=NO₂ or Cl, R'=OCH₃ or OCS₂H₆) and also the 7-nitro- and 7-chloro-derivatives.

\[
\text{\begin{align*}
\text{C}_2\text{H}_5 & \quad \text{N} & \quad \text{C}_2\text{H}_5 \\
(\text{CH}_2)_n & \quad \text{NH}
\end{align*}}
\]

(XIII)

In the case of the 8-chloro-3-methoxy series (XIII, R=Cl, R'=OCH₃) they found that the chemotherapeutic activity was greatest when \(n=4\). Two of the compounds prepared, 8-chloro-3-methoxy-5-(\(\gamma\)-diethylamino-\(n\)-propylamino)-acridine (XIII, R=Cl, R'=OCH₃, n=3) with an activity of the same order as mepacrine, and the corresponding 5-diethylamino-\(n\)-butylamine compound (XIII, R=Cl, R'=OCH₃, n=4) with an activity even higher than mepacrine are used in Russia under the names of "Achrichin No. 5" and "Achrichin No. 8".

The chlorine atom in position 8 in the mepacrine molecule appears to be very important, for its removal results in an almost inactive compound. Feldmann and Kopeliovitsch (Arch. Pharm., 1935/
1935, 273, 486) found that transferring the chlorine atom to position 7 gave a product with a very slight activity, while the activity of the 7:8-dichloro compound was approximately half that of mepacrine. It does, however, seem to be possible to replace the 8-chlorine atom by a cyano group without loss of activity; indeed, 8-cyano-6-methoxy-5-(5-diethylamino-α-methylbutylamino)-acridine (XIV) is rather more active than mepacrine.

\[
\text{C}_2\text{H}_5\text{N}-\text{C}_2\text{H}_4\text{N}-
\]
\[
(\text{CH}_3)_3\text{N}-\text{CH}_2(\text{CH}_3)\text{NH}
\]

(XIV)

Removal of the chlorine in position 8 and introduction of a nitro group in position in position 7 gives a compound of low activity.

Magidson and Grigorovskii (Ber., 1936, 69, 396) investigated the effect of a second methoxy group in position 3 of the mepacrine molecule, but found that their product was inactive. Replacement of methoxy by ethoxy in position 3 reduced activity as in the case of pamaquin analogues, and introduction of methylmercapto for methoxy in position 3 resulted in reduced activity and increased toxicity.

Goodall and Kermack (J.C.S., 1936, 1546) have used dialkylaminodialkylamines to produce various/
various mepacrine analogues without a chlorine atom in position 8 and carrying the basic side chain in the 5-position as usual. The products were inactive.

A recent paper by Hall and Turner (J.G.S., 1945, 694) deals with the preparation of a number of acridine derivatives. It is of interest to note that 1-chloro-5-(5-diethylamino-α-methylbutylamino)-9-methylacridine (XV) prepared by these workers showed definite antimalarial activity.

Much recent work on the relationship between chemical structure and chemotherapeutic activity has been based on a consideration of the possibilities of tautomerism and related phenomena in the drug molecule. Thus Schönhöfer (Z. Physiol. Chem., 1943, 274, 1) has suggested that only such quinoline and acridine derivatives exhibit antimalarial activity as are convertible, even if only temporarily, into compounds possessing a γ-quinonoid structure. In the case of mepacrine we may have tautomerism of the type (XVI) \( \leftrightarrow \) (XVII).

It is worthwhile noting that in the case of the/
the hydrochloride of the base which is actually used in practice the two tautomeric forms become two mesomeric structures (XVIII and XIX) which are in fact the resonance components of the cation of the salt.

This theory of Schönhöfer does not explain all the facts. Thus it does not explain why so much of the activity of mepacrine seems to depend on the chlorine atom in position 8. Curd, Davey and Rose (Ann. Trop. Med. Parasitol., 1945, 39, 157) have attempted an explanation of this by suggesting that with the chlorine atom in the 8-position an electronic displacement is possible, giving a different type of quinonoid structure (XX). The carbon atom in position 5 possesses a lone pair of electrons which confer the negative charge. This type/
type of displacement, leading to what must be

![Chemical structure](image)

regarded as an excited form of the molecule, does not seem very probable.

Another argument against the Schönöfer hypothesis is provided by the compound Pa 8861, 8-chloro-3-methoxy-5-(3'-diethylaminopyrrolidino)-acridine (XXI) prepared during the war in Schönöfer's own laboratories at Elberfeld. In this

![Chemical structure](image)

compound because of the tertiary nature of the nitrogen in the pyrrolidine ring no tautomerism is possible, so that, according to the Schönöfer hypothesis the compound should be inactive. It was in fact found to be as potent as mepacrine. It is to/
to be noted, however, that although tautomerism in such a compound is impossible, the cation of the salt is still capable of mesomerism, its structure being represented by the two resonating forms (XXII) and (XXIII). It may be that it is the mesomerism

![Resonating Forms](image)

rather than the tautomerism of the base which is chemotherapeutically significant.

In spite of its failings, the Schönäfer theory must be considered to have played some part in stimulating the researches of Curd, Rose and co-workers which have led to the discovery of paludrine. In their search for a new type of antimalarial drug, Curd and Rose were led to a consideration of pyrimidine derivatives for a number of reasons. Thus pyrimidine derivatives are of fundamental biological importance and occur widely in enzyme systems, and it was thought that a compound might be found which would act by blocking some enzyme system essential to the metabolism of the malaria parasite. Secondly, though only a small amount/
amount of work had been done on pyrimidine derivatives as chemotherapeutic agents, it had produced two sulphonamide drugs - sulphadiazine and sulphanemazine - which were known to possess antimalarial properties, and indeed showed promise of true prophylactic activity. Lastly, according to the Schönhöfer hypothesis 4-dialkylaminalkylamino-pyrimidines might possess activity since they are capable of tautomerism analogous to that of mepacrine (XXIV = XXV).

The first active compound discovered was

\[
\begin{align*}
\text{(XXIV)} & \\
\text{(XXV)}
\end{align*}
\]

M-2666, 3-\(\beta\)-chloroanilino-4-(\(\beta\)-diethylaminoethyl-amino)-6-methylpyrimidine (XXVI), but an investigation of the positional isomers of this compound demonstrated/
demonstrated the untenability of the Schönhöfer hypothesis. Thus in 2-methyl-4-\(\eta\)-chloroanilino-6-(\(\beta\)-diethylaminoethylamino)-pyrimidine (XXVII) a tautomeric system of the Schönhöfer type is possible

\[
\text{XXVII}
\]

and yet the compound was found to be inactive; while activity was demonstrable in 2-(\(\beta\)-diethylaminoethylamino)-4-\(\eta\)-chloroanilino-6-methylpyrimidine (XXVIII)

\[
\text{XXVIII}
\]

in which no such tautomerism is possible.

A study of these three types (XXVI, XXVII and XXVIII) in order to determine if possible why the type (XXVII) was inactive, showed that in (XXVI) and in (XXVIII) but not in (XXVII) two independent linked/
linked amidine systems were present. This suggested that possibly the carbon atoms 5 and 6 in the pyrimidine ring of (XXVI) and (XXVIII) were unnecessary. Attention was therefore turned to arylbiguanide derivatives and this work culminated in the synthesis of paludrine (M.4688) which is N1-γ-chlorophenyl-N3-isopropylbiguanide (XXIX). Clinical trials of paludrine on human malaria have shown that not only is it much more active than mepacrine or quinine in the treatment of developed cases of the disease, but it also possesses considerable prophylactic action. It seems likely to supersede mepacrine and quinine for the general treatment of malaria.

In the course of their researches Curd and Rose prepared 2-(p-chlorophenylguanidino)-4-(β-di-ethylaminoethylamino)-6-methylpyrimidine (XXX, M.3349) which is interesting from the theoretical point of view since it possesses a pyrimidine ring system joined to the aromatic part of the molecule by a guanidino linkage. Though possessing a high degree of activity, this compound was found to be too toxic/
toxic for clinical use.

The antimalarial properties of a number of other heterocyclic ring systems have been studied. Thus Kermack and Weatherhead (J.C.S., 1940, 1164) and Kermack and Webster (J.C.S., 1942, 213) prepared derivatives of \( p \) - and \( m \) -phenanthrolines carrying dialkylaminoalkylamino side chains in positions 2 or 4. None of the compounds showed marked activity though 4-(\( \delta \)-diethylamino-\( \alpha \)-methylbutylamino)-2-methyl-\( m \) -phenanthroline (XXXI) showed doubtful

activity against \textit{Plasmodium relictum} in canaries.

Recently,
Recently Jacob and Kermack (J.C.S., 1946, 62) have synthesised 9-(γ-diethylaminopropylamino)-
α-phenanthroline (XXXII) but this too failed to

show any activity against *P. relictum* in canaries.

Böeseken and Bijloma (B.P. 454326) claim
activity for 9- and 10-amino-α-phenanthrolines, the
former against *Haemoproteus orizivora*, and the
latter against *P. relictum*. 10-(γ-Diethylaminopropylamino)-α-phenanthroline was also stated to
be active against *P. relictum*.

More recently Halcrow and Kermack (J.C.S.,
1946, 155) have prepared 2-dialkylaminoalkylamino-
α-phenanthrolines, but none of the compounds prepared
were active. (Cf. Burger, Bass, and Fredericksen,

It will be noticed that these phenanthrolines
can be regarded as derived from quinoline, which
is, of course, the basis of pamaquin, by fusing on
a pyridine ring. A natural extension of the investi-
gation of the antimalarial activity of various
types of chemical compounds is therefore a consider-
ation/
ation of pyridoacridine derivatives, which again we may regard as formed by fusing a pyridine ring to the acridine ring system, the basis of mepacrine. This type of compound has been prepared by Dobson and Kermack (J. C. S., 1946, 150), and the present research is an extension of their work. It is interesting to note that pyridoacridines may also be regarded as benzphenanthrolines.

Dobson and Kermack synthesised six different 5-chloropyridoacridines derived from the corresponding substituted quinolylanthranilic acids. These latter acids were synthesised by condensing a chlorobenzoic acid with an aminoquinoline. Thus condensation of a chlorobenzoic acid (XXXIII) with 8-aminoquinoline (XXXIV) yielded a substituted (8'-quinolyl)-anthranilic acid (XXXV). Cyclisation of this acid

\[
\begin{align*}
\text{(XXXIII)} & \quad \text{(XXXIV)} & \quad \text{(XXXV)} \\
\end{align*}
\]

with phosphorus oxychloride led to the formation of a substituted 5-chloro-1:2:2':3'-pyridoacridine (XXXVI) In this way four different 5-chloro-1:2:2':3'-pyridoacridines were prepared, using 8-aminoquinoline (XXXIV, \(R' = H\)) or 8-amino-6-methoxyquinoline (XXXIV, \(R' / \))
R' = OCH₃) on the one hand and o-chlorobenzoic acid (XXXIII, R = H) or 2:4-dichlorobenzoic acid (XXXIII, R = Cl) on the other.

\[ \text{(XXXV)} \quad \text{(XXXVI)} \]

Dobson and Kermack \cite{DOB} also prepared 3:4:2':3'-pyridoacridines in a similar fashion, starting with 6-amino-quinoline (XXXVII) and o-chloro- or 2:4-dichloro-benzoic acid (XXXIII, R = H or Cl) when a (6'-quinoly)-anthranilic acid (XXXVIII) was produced. This was converted by

\[ \text{(XXXIII)} \quad \text{(XXXVII)} \quad \text{(XXXVIII)} \]

phosphorus/
phosphorus oxychlorides to the 5-chloro-3:4:2':3'—
pyridoacridine (XXXIX).

All these 5-chloropyridoacridines were
condensed with dialkylaminoalkylamines and the
products tested for antimalarial activity against
P.gallinaceum in chicks. None of the derivatives
of 1:2:2':3'-pyridoacridine showed any activity,
but all the derivatives of 3:4:3':5'-pyridoacridine
tested were active, but this activity was very
weak when the chlorine atom in position 8 was
absent. The two most active compounds were
8-chloro-5-(γ-butylaminopropylamo)-3:4:2':3'-
pyridoacridine (XL, R = NH(CH₃)₃·NH·C₄H₉) and
8-chloro-5-(δ-diethylamino-α-methylbutylamino)-
3:4:2':3'-pyridoacridine (XL, R = NH·CH(CH₃)·(CH₃)₃·
N(C₆H₅)₃). The full significance of these biological

![Diagram of XL](image)

results when considered in conjunction with those
obtained for the pyridoacridines which have been
prepared in the present work is discussed in
Section II.

Before concluding this general introduction,
some reference may be made to the possible modes
of/
of action of antimalarial drugs. Some theories have been advanced postulating an immunological basis for the action of the drug, and others have assumed some special action on the surface of the red blood cells preventing entry of the parasites.

Magidson and his co-workers have suggested that in antimalarial drugs of the mepacrine and pamaquin types we have two distinct portions of the molecule with separate functions. The first of these, the basic side chain, they term the conductophoric part, which is concerned with the penetration of the drug into the parasite. The second or parasiticidal part is the substituted heterocyclic nucleus. They suggest further that in the case of mepacrine the basic side chain is split in vivo by enzymic hydrolysis accompanied by demethylation giving 3-hydroxy-8-chloroacridone (XLI) as the parasiticide. A certain amount of 3-hydroxy-8-chloroacridone can in fact be obtained by simply heating mepacrine with water. In the light of this theory the recently discovered activity of endochin (XII, vide supra) may be significant.

Mepacrine bears a general resemblance to riboflavin/
riboflavin (XLII), and it has been suggested that it may act by competing with riboflavin for an enzyme system essential to the metabolism of the malaria parasite. It has recently been demonstrated that mepacrine inhibits choline esterase (Waelsh and Nachmansohn, Proc. Soc. Exp. Biol. Med., 1943, 54, 336), \(d\)-amino-acid oxidase and yellow enzyme systems (Wright and Sabine, J. Biol. Chem., 1944, 155, 315).

A resemblance to riboflavin is also seen in the case of the series of 2-anilino-4-dialkylamino-alkylamino-6-methylpyrimidines prepared by Curd and Rose (Chem. and Ind., 1946, 75; Ann. Trop. Med. and Parasitol., 1945, 39, 157). In support of this
Madinaveitia (Biochem. J., in the press) has shown that the growth inhibitory action of mepacrine and 3-p-chloroaniline-4-(6-diethylaminoethylamino)-6-methylpyrimidine (XXVI) for L. casei is antagonised by riboflavin.

Though there is some evidence for this theory of riboflavin antagonism there is also evidence which indicates that it cannot be accepted without modification. Thus Curd and Rose (loc. cit.) found that 2-methyl-4-p-chloroanilino-6-(6-diethylaminoethylamino)-pyrimidine (XXVII) which resembles riboflavin more closely than the 3-p-chloroanilino-4-(6-diethylaminoethylamino)-6-methylpyrimidine (XXVI) which was tested by Madinaveitia was devoid of antimalarial activity. Also it seems difficult to understand why compounds in which the substituent in the anilino residue is in the p-position are more active than those in which we have m-substitution. The evidence for substrate competition cannot as yet be regarded as convincing.

Recently another avenue of approach to the problem/
problem of the mode of action of antimalarial drugs has been opened up with the isolation in crystalline form by Laser and Friedmann (Nature, 1945, 156, 507) of a haemolytic substance from normal blood, and of the subsequent discovery (ibid., 1946, 157, 301) that the rate of haemolysis produced by this substance in vitro was decreased by the addition of antimalarial drugs. A remarkable correlation was found among 117 compounds tested, those of known antimalarial activity delaying haemolysis and those with no antimalarial action being inactive in the test. The authors suggest that the malaria parasites may produce this haemolytic substance or a closely related compound and that the action of antimalarial drugs is really an inhibition of the haemolytic action of this compound. It may be, too, that by finding whether a given compound has any effect on the rate of haemolysis, we may have an in vitro test which may be helpful in screening potential antimalarial drugs. However, in view of the fact that the same compound may have different activities in different species, a simple in vitro test like this can never be a complete guide to the therapeutic possibilities of a particular compound, for a proper assessment of which in vivo tests and toxicity determinations will still be required.
II. GENERAL DISCUSSION.

The object of the present research was to extend in various directions the previous work of Dobson and Kermack (J.C.S., 1946, 150). In the first place attempts were made to introduce various substituents into the 3:4:2':3'-pyridoacridine nucleus. Secondly it was decided to synthesise pyridoacridines in which the pyridine ring is fused to the acridine nucleus in different positions. As Dobson and Kermack had dealt exclusively with pyridoacridines derived from 6- and 8-aminoquinolines the work was extended to pyridoacridines derived from 5- and 7-aminoquinolines. In all these series it was decided in general to adopt γ-diethylamino-propylamine as side chain.

The work falls naturally into three sections:

A. Pyridoacridines derived from 6-aminoquinolines.
B. Pyridoacridines derived from 5- and 7-aminoquinolines.
C. The results of tests for antimalarial activity.
A. The Synthesis of Pyridoacridines derived from 6-Aminoquinoline.

As explained above, this section deals with attempts to introduce various substituents into the 3:4:2':3'-pyridoacridine nucleus, by using as starting material various 6-aminoquinolines carrying different substituents in the 8-position. Thus there are described below the preparations of 2:8-dichloro-, 2-chloro- and 2-methyl-8-chloro-3:4:2':3'-pyridoacridine derivatives, and the attempted preparation of a 2-bromo-8-chloro-3:4:2':3'-pyridoacridine.

2-Chloro-4-nitroaniline (IV) prepared originally by nitration of o-chloroacetanilide (II) (Cf. Chattaway, Orton and Evans, Ber., 1900, 33, 3057) but later supplied in bulk by Imperial Chemical Industries, Ltd., was submitted to a

```
\begin{align*}
(I) & \xrightarrow{(CH_2CO)_2O} (II) & \xrightarrow{HNO_3} (III) & \xrightarrow{H_2O} (IV) \\
\end{align*}
```

\[ \text{NH}_3 \quad \text{Cl} \quad \xrightarrow{(CH_2CO)_2O} \quad \text{NH}_3 \cdot CO \cdot CH_3 \quad \xrightarrow{HNO_3} \quad \text{NH}_3 \cdot CO \cdot CH_3 \quad \xrightarrow{H_2O} \quad \text{NH}_3 \quad \text{Cl} \]

```
\begin{align*}
(IV) & \xrightarrow{\text{Skraup}} (V) & \xrightarrow{\text{West, Reduction}} (VI) \\
\end{align*}
```

\[ \text{NH}_3 \quad \text{Cl} \quad \text{NO}_2 \quad \xrightarrow{\text{Skraup}} \quad \text{Cl} \quad \text{N} \quad \text{Cl} \quad \text{N} \quad \xrightarrow{\text{West, Reduction}} \quad \text{Cl} \quad \text{N} \quad \text{Cl} \]

Verifying text via diagrams.
Skraup reaction as modified in E.P. 394416, arsenic acid being used as the oxidising agent. This modification of the Skraup reaction uses 69% sulphuric acid in place of the usual concentrated acid, and has been found to give good results with a wide range of aniline derivatives. The resulting 6-nitro-3-chloroquinoline (V) was reduced to 6-amino-3-chloroquinoline (VI) by West's method (J.C.S., 1925, 127, 494) and the product, m.p. 154°, condensed with potassium 2,4-dichlorobenzoate (VII) in amyl alcohol using copper bronze as a catalyst (Cf. Ullmann, Ann., 1907, 355, 312). This yielded

\[
\begin{align*}
\text{(VII)} &\quad \text{(VI)} &\quad \text{(VIII)} \\
\text{4-chloro-}(3'-\text{chlore}-6'-\text{quinoly})\text{-anthranilic acid (VIII). The crude product was freed from amyl alcohol by washing with acetone and extracted with a large volume of hot dilute ammonia. This extract yielded the acid on acidification with acetic acid. There was, however, a considerable residue which was insoluble in the ammonia and this was extracted with hot 2N hydrochloric acid and filtered hot. Again there was a residue, but this seemed to be largely the relatively insoluble hydrochloride of/}
\end{align*}
\]
of the desired acid, since on treating it with ammonia crude 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid was produced. The hydrochloride of the acid also separated on cooling from the filtrate from the hydrochloric acid extraction and was filtered off and treated with ammonia to yield the acid. The reason for the failure of the ammonia to extract the 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid completely is obscure.

4-Chloro-(8'-chloro-6'-quinolyl)-anthranilic acid crystallised from ethyl alcohol as white needles, m.p. 300°.

It has been found that, in general, these quinolylanthranilic acids are very difficult to crystallise, and later examples were usually purified either through the potassium salt or else the ethyl ester was made.

The cyclisation of 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid was carried out by refluxing it with excess phosphorus oxychloride at 150° for 6 hours. Dobson and Kermack (loc. cit.) found that the 5-chloropyridoacridines obtained by
this method were readily hydrolysed by acid to the corresponding pyridoacridone. The precautions recommended by these authors were therefore observed. The excess phosphorus oxychloride was distilled off under reduced pressure and the residue triturated with 20% sodium hydroxide solution and ice, care being taken to avoid local heating. The insoluble material was filtered off, washed till neutral, and dried in a desiccator. It was then boiled with dry benzene in the presence of a pellet of potassium hydroxide when a small quantity of brownish material m.p. 395° was left. This was presumed to consist largely of 2:8-dichloro-3:4:2':3'-pyridoacridone (X), but an attempt to convert it to the 5-chloro compound by refluxing it at 150° with phosphorus oxychloride and phosphorus pentachloride gave a poor yield; it appears therefore to contain only a small amount of 2:8-dichloro-3:4:2':3'-pyridoacridone the rest being of unknown composition. The benzene filtrate on cooling deposited 2:5:8-trichloro-3:4:2':3'-pyridoacridine as buff coloured needles, which when recrystallised from dry benzene melted at 286°.

It has been found very difficult to obtain these 5-chloropyridoacridines in a pure condition. Analysis usually indicated a slight degree of hydrolysis to the pyridoacridone, but experience showed that it was better to carry on with the condensation with the dialkylaminoalkylamine, the final base so obtained being relatively easily purified. The
ease with which these 5-chloro compounds are hydrolys ed in neutral solvents such as alcohol or even moist benzene is not surprising since each molecule which hydrolyses produces one molecule of HCl which catalyses further hydrolysis. The facility of such chloro compounds to undergo acid hydrolysis is in conformity with the sensitiveness of many 5-substituted acridines to acids (Cf. Magidson, Ber., 1933, 66, 866; Albert and Linnell, J.C.S., 1936, 91).

Three samples of 2:5:8-trichloro-3:4:2':3'-pyridoacridine were heated respectively with 2N sodium hydroxide solution, 2N hydrochloric acid and ethyl alcohol for 1 hour on the water bath. In the first case the original material was recovered unchanged, there being no change in appearance or melting point, but in the case of the hydrochloric acid and the alcohol the colour changed to yellow. On treatment with ammonia the colour became greyish brown, and the filtrate gave a reaction for chloride ions. It is probable that the/
the yellow compound is the hydrochloride of the pyridoacridone. The greyish brown material was recrystallised from alcohol, when it melted just over 400°. This product purified by sublimation yielded 2:8-dichloro-3:4:2':3'-pyridoacridone (X) as yellow crystals, m.p. 385°.

\[
\begin{align*}
\text{(IX)} \\
\text{(XI)} \\
\end{align*}
\]

2:5:8-Trichloro-3:4:2':3'-pyridoacridine was condensed with \( \gamma \)-diethylaminopropylamine. The method was almost identical with that of Dobson and Kermack (loc. cit.) differing only slightly in the details of working up. Redistilled phenol was dried at 100° in a vacuum for 2 hours, a slight excess of \( \gamma \)-diethylaminopropylamine introduced and the mixture dried in a vacuum for a further hour, the object of this being to remove the last traces of water. 2:5:8-Trichloro-3:4:2':3'-pyridoacridine dried overnight in a vacuum dessicator over calcium chloride/
chloride was added, and the mixture heated at 100° under an air condenser for 2 hours with occasional shaking. Most of the pyridoacridine dissolved yielding a dark brown solution, but a small yellow residue remained, which probably consisted of the pyridoacridine. The cooled phenol mixture was poured into 2N sodium hydroxide solution when a yellow precipitate was obtained. This was filtered off, washed, dissolved in 5% acetic acid and reprecipitated with ammonia with the object of removing any pyridoacridine. This precipitate was filtered off, washed and dried in a desiccator. Crystallisation from dry ligroin yielded 2:8-dichloro-5-(γ-diethylamino- propylamino)-3:4:2':3'-pyridoacridine as yellow needles, m.p. 144°.

In view of the fact that Dobson and Kermack (loc. cit.) found that with 5-diarylaminooalkylamino-8-chloro-3:4:2':3'-pyridoacridines the most active members were those in which the side chains were γ-monobutylaminopropylamino and 5-diethylamino-α-methylbutylamino, an attempt was now made to prepare compounds of the present series carrying these side chains.

2:8-Dichloro-5-(γ-monobutylaminopropylamino)-
3:4:2':3'-pyridoacridine was prepared without
difficulty from 2:5:8-trichloro-3:4:2':3'-pyrido-
acridine and γ-monobutylaminopropylamine by
condensation in phenol. It crystallised as yellow
needles from dry ligroin, and had m.p. 131°.

This/
This compound was also prepared by a second method (Cf. Drosdov, J. Gen. Chem. (U.S.S.R.), 1938, 8, 1192). The quinolylanthranilic acid was converted to the acid chloride which was converted to the amide by condensation with the dialkylaminoalkylamine. This gave the final base directly on cyclisation.

4-Chloro-3\(^\prime\)-chloro-6\(^\prime\)-quinonyl)-anthranilic acid (VIII) was treated with thionyl chloride in dry chloroform to form 4-chloro-3\(^\prime\)-chloro-6\(^\prime\)-quinonyl)-anthranilic acid chloride (XIII). This
was converted to 4-chloro-(8'-chloro-6'-quinolyl)-anthranil-(γ-monobutylaminopropyl)-amide hydrochloride (XIV) by treatment with γ-monobutylaminopropylamine also in dry chloroform solution. The chloroform was removed and cyclisation of the residual amide hydrochloride was effected by refluxing with excess phosphorus oxychloride. The residue after removal of the excess phosphorus oxychloride by distillation under reduced pressure was dissolved in ice water. The brown solution so obtained was filtered, and the filtrate basified with ammonia yielding 2:8-dichloro-5-(γ-monobutylaminopropylamino)-3:4:2':3'-pyridoacridine (XII) as a yellow solid which crystallised from dry ligroin, and melted at 130°. The yield was very poor. A mixed melting point with the sample prepared by the previous method showed no depression.

An attempt was made to condense 2:5:8-tri-chloro-3:4:2':3'-pyridoacridine with 5-diethylamino-α-methylbutylamine in phenol in the usual way. This yielded a yellow crystalline base which was recrystallised/
allised from dry ligroin, when it melted at 187°.
The analytical results, which are duplicate deter-
minations, are as follows: Found: C, 65.75; H, 4.7;
N, 8.45; Cl, 15.45%. 2,3-Dichloro-5-((5-diethylamino-
α-methylbutylamino)-3:4:2':5'-pyridoacridine requires
C, 65.9; H, 6.15; N, 12.3; Cl, 15.6%. It is difficult
to reconcile these figures with any obvious formula-
tion of the compound, but it is worth-while noting
that a mixture of 2,3-dichloro-5-((5-diethylamino-α-
methylbutylamino)-3:4:2':5'-pyridoacridine (XV) and
the 5-phenoxy compound in equimolecular proportions
and containing a small amount of water would analyse
as/
follows - Found for compound, m.p. 187°, C, 65.75; H, 4.7; N, 8.45; Cl, 15.45%. Ca₂H₈N₄Cl₂Ca₁H₂Cl₃ON₂Cl₆. H₂O requires C, 65.3; H, 4.9; N 9.7; Cl, 16.4%. The same substance was obtained from three separate experiments. This material was tested for biological activity and found to have only doubtful activity at all the dosages employed. There is little doubt that 2,5-dichloro-5-(5-diethylamino-3 methylbutylamino)-3,4,2',3'-pyridoacridine would possess high antimarial activity, so that the lack of activity in this product confirms the conclusion drawn from the analytical results that the compound was not that expected, though it has not been found possible to elucidate its structure.

It will be seen in Section II (c) that the derivatives of 2,5-dichloro-3,4,2',3'-pyridoacridine showed a high measure of activity. Therefore, as Dobson and Kermack (loc. cit.) found that in the case of 3,4,2',3'-pyridoacridines derived from 6-aminoquinoline the introduction of a chlorine atom in position 8 led to greatly enhanced activity, it was clearly of considerable theoretical interest to prepare a 3,4,2',3'-pyridoacridine carrying a chlorine atom in position 2 but unsubstituted in position 8. Such a compound can obviously be obtained from 6-amino-8-chloroquinoline and 8-chlorobenzoic acid (XVII) according to the scheme shown on the next page.

6-Amino-8-chloroquinoline (VI) was condensed with/
with potassium \( g \)-chlorobenzoate in amyl alcohol in the presence of copper bronze.  \( \text{(C' \text{-Chloro-C' -quinolyl)} \)} \)
quinolyl)-anthranilic acid (XVIII) was isolated as described for the analogous 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid. It had m.p. 256°. The ethyl ester was prepared by refluxing the acid with ethyl alcohol and concentrated sulphuric acid, pouring the cooled solution into ice water, and, after filtering, basifying the filtrate with ammonia. This produced a yellow oily precipitate which was extracted with ether, and the residue after the ether had been distilled off from the dried extract was shaken with warm sodium bicarbonate solution. The solid ester so obtained was crystallised from alcohol as white needles, m.p. 118°.

(8'-Chloro-6'-quinolyl)-anthranilic acid (XVIII) was cyclised in the usual way with phosphorus oxychloride yielding 2:5-dichloro-3:4:2':3'-pyridoacridine (XIX), m.p. 208°. This was obtained in good yield, the crude product being almost completely soluble in dry benzene. The compound crystallised readily from dry benzene, and gave a better analysis than was obtained for most of the analogous 5-chloro-3:4:2':3'-pyridoacridines. It therefore appears to be less easily hydrolysable. However, heating with 2N hydrochloric acid or ethyl alcohol yielded 2-chloro-3:4:2':3'-pyridoacridone (XXI), m.p. over 400°.

2:5-Dichloro-3:4:2':3'-pyridoacridine was condensed with β-diethylaminopropylamine in phenol to yield 2-chloro-5-(β-diethylaminopropylamino)-3:4:2':3'-pyridoacridine (XX). In this case the product/
product on pouring the phenol mixture into sodium hydroxide solution was a sticky yellow solid which

was extracted with ether, the ether extract being in turn extracted with 5% acetic acid. The base was precipitated from the acetic acid solution by ammonia and the yellow sticky precipitate extracted once more with ether. This ether extract was dried, the ether distilled off and the excess $\gamma$-diethylaminopropylamine removed \textit{in vacuo}. The residue was crystallised from dry ligroin as yellow needles, m.p. 115°.

Attention was now turned to the preparation of a 2-methyl-3:4:2':3'-pyridoacridine. For this purpose it was necessary to synthesise 6-amino-8-methylquinoline.

5-Nitro-$\alpha$-toluidine was prepared by nitration of 2-methylacetanilide and hydrolysis of the product. Latterly this preparation was not necessary, as 5-nitro-$\alpha$-toluidine was supplied in bulk by Imperial Chemical Industries, Ltd. 6-Nitro-8-methylquinoline was/
was prepared from 5-nitro-α-toluidine by a Skraup reaction modified as in E.P. 394416, arsenic acid being used as the oxidising agent. 6-Nitro-8-methylquinoline crystallised from alcohol in greyish white needles, m.p. 128°. (Lellmann and Ziemsen, Ber., 1891, 24, 2116 give m.p. 129°.) It was reduced by West's method to 6-amino-8-methylquinoline. Some difficulty was encountered in obtaining the 6-amino-8-methylquinoline in a pure condition. In the first experiment the product was purified by conversion into the hydrochloride, from which the base was recovered and crystallised from benzene when it melted at 128°. A second crystallisation from benzene raised the melting point to 129°, the compound consisting of buff coloured crystals. This gave the correct analysis, and appeared satisfactory, but in a second experiment, in which the base was re-crystallised from benzene without previous conversion to the hydrochloride, a crop of reddish brown crystals was produced, which melted at 109°, a mixed melting point with the buff coloured material being 103-107°. Two or three recrystallisations of the reddish brown material did not raise the melting point. It was found that the difference in melting point between the two samples persisted when the acetyl derivatives were made, that from the reddish brown crystals melting at 128-129°, and that from the buff coloured crystals at 173°. Here, however, the mixed melting point was intermediate between the melting points of the individual acetyl compounds being 158-164°.
On the other hand, the hydrochlorides of both substances appeared to be identical, that from the reddish brown material melting at 277-279°, and that from the buff coloured material melting at 276-278°, the mixed melting point being 277-279°. Also from both hydrochlorides the base recovered melted at 128-129°. Further, although the reddish brown material could be recrystallised from benzene without apparent change, when recrystallised from alcohol it melted in the neighbourhood of 129°. There seems little doubt from these observations that the two substances are essentially the same compound, the reddish brown material containing some impurity accounting for the colour and the lower melting point. The fact that it recrystallises unchanged from benzene, and that its melting point is depressed by the pure compound may indicate that the compound and the impurity form some kind of intermolecular complex, which separates unchanged from benzene, but is decomposed in alcohol. In later reductions it was found that in almost every case, when the product was recrystallised from benzene, the first material which separated was buff coloured and melted at 129°, but on concentrating the mother liquors crystalline material separated, which on inspection was obviously a mixture of two types of crystals, one buff coloured and the other reddish brown. Such mixtures usually melted indefinitely in the neighbourhood of 105-107°. An attempted mechanical separation gave for the melting/
melting point of the reddish brown crystals 101-106°, and for that of the buff coloured crystals 109-112°. In only one experiment was a large amount of the reddish brown material, m.p. 109°, obtained in a relatively pure condition.

The possibility was considered that the contaminant of the 6-amino-8-methylquinoline, m.p. 129°, which was present in the reddish brown material melting at 109° was the isomeric 5-amino-8-methylquinoline derived from 4-nitro-2-toluidine, possibly present in the 5-nitro-2-toluidine. It was noted that the melting point of the 5-nitro-2-toluidine used was somewhat low, being 121-122° after previous softening at 110°, instead of 129°, an observation consistent with the presence of the contaminating isomer, 4-nitro-2-toluidine melting at 105°. Some pure 4-nitro-2-toluidine was therefore submitted to the Skraup reaction, and the 5-nitro-8-methylquinoline obtained reduced by West's method. This yielded yellow crystals of 5-amino-8-methylquinoline, m.p. 140.5-142.5° (lit. 143°). Both these 5-substituted quinolines are described in the literature (Noelting and Trautmann, Ber., 1890, 23, 3673). The 5-amino-8-methylquinoline depressed the melting points of both the reddish brown material melting at 109°, and the pure 6-amino-8-methylquinoline melting at 129°, the mixed melting point with the former being 99-102° and that with the latter 122-136°.

Furthermore the addition of one drop of a 1% aqueous solution of ferric chloride to a dilute alcoholic/
alcoholic solution of 5-amino-3-methylquinoline gave
an intense blood red colour. With 6-amino-3-methyl-
quinoline a canary yellow colour was obtained. This
canary yellow colour with no trace of red was also
obtained with the material melting at 109°. When a
mixture of the 6-amino-3-methylquinoline (m.p. 129°)
and 5-amino-3-methylquinoline (m.p. 140.5-142.5°)
was submitted to the test, the characteristic red
colour developed. This seemed to exclude the
possibility that the contaminating compound in the
reddish brown material melting at 109° was the
isomer 5-amino-3-methylquinoline. It is possible
that the contaminant is an azoquinoline, since azo-
quinolines are known to be formed in the reduction
of 6-nitroquinoline to 6-aminoquinoline (Kneppel,
Ann., 1900, 310, 75).

The ferric chloride colour test as described
above was carried out on a series of quinoline
derivatives with the following results.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Amino-8-methylquinoline</td>
<td>Blood red</td>
</tr>
<tr>
<td>5-Amino-4-hydroxyquinoline</td>
<td>Green</td>
</tr>
<tr>
<td>6-Aminoquinoline</td>
<td>Canary yellow</td>
</tr>
<tr>
<td>6-Aminoquinaldine</td>
<td>Canary yellow</td>
</tr>
<tr>
<td>6-Amino-8-chloroquinoline</td>
<td>Canary yellow</td>
</tr>
<tr>
<td>6-Amino-8-methylquinoline</td>
<td>Canary yellow</td>
</tr>
<tr>
<td>6-Amino-8-methoxyquinoline</td>
<td>Canary yellow</td>
</tr>
<tr>
<td>8-Aminoquinoline</td>
<td>Brown</td>
</tr>
<tr>
<td>8-Amino-6-methoxyquinoline</td>
<td>Brown</td>
</tr>
<tr>
<td>8-Amino-4-chloroquinoline</td>
<td></td>
</tr>
</tbody>
</table>
It will be seen that only the 5-amino-8-methylquinoline gave the blood red colour, and that all the 6-aminoquinolines gave a canary yellow colour.

As indicated above, the amount of the reddish brown material seemed to vary in different experiments. In most of the later reductions, which were worked up by recrystallising from benzene the residue left on distilling off the alcohol, approximately pure buff-coloured 6-amino-8-methylquinoline, m.p. 129°, separated without difficulty, and even the residue from the benzene mother liquors was often apparently free from any admixture of reddish brown crystals.

6-Amino-8-methylquinoline, m.p. 129°, was condensed with potassium 2:4-dichlorobenzoate. In the earlier experiments the 6-amino-8-methylquinoline and an equivalent quantity of the potassium salt were mixed in amyl alcohol and a trace of copper bronze added to act as catalyst. The mixture was refluxed for 6 hours at 150°, allowed to cool, the amyl alcohol filtered off, and the residue washed with acetone. The residue was dissolved in a large volume of hot dilute ammonia, filtered, and 4-chloro-(8'-methyl-6'-quinolyl)-anthranilic acid (XXII) precipitated from the filtrate by acetic acid (8% yield).
(XXII)

yield. When the residue which was insoluble in ammonia was treated with dilute hydrochloric acid in the hot, however, a bright yellow compound was obtained which separated more completely on cooling. This was filtered off, dissolved in hot dilute hydrochloric acid, and ammonia added to precipitate a pale yellow compound which proved to be 4-chloro-(8'-methyl-6'-quinolyl)-anthranilic acid, melting at 275°. By direct ammonia extraction a yield of 8% of the theoretical was obtained and 72% by recovery from the insoluble residue, so that the total yield was 80% of the theoretical. As this acid, like analogous acids of the series, was rather difficult to recrystallise, the ethyl ester was prepared by refluxing the acid with ethyl alcohol and concentrated sulphuric acid and making the solution alkaline with ammonia. Ethyl 4-chloro-(8'-methyl-6'-quinolyl)-anthranilate crystallised from/
from ethyl alcohol as light brown needles, m.p. 74°.

At first it was not realised that the reason why the yield of acid in the Ullmann condensations was poor was because of the failure to work up the portion insoluble in ammonia. Attempts were therefore made to improve the yield in other ways, and it was, in fact, as a result of these experiments that the working up of the insoluble residue was adopted as a routine. It was thought that the poor yield might be due to the fact that the mixture was too basic, the aminomethylquinoline being itself basic. Accordingly an experiment was carried out using one half molecular quantity potassium salt and one half molecular quantity free acid. These were dissolved in alcohol along with one molecular quantity of 6-amino-8-methylquinoline, a trace of copper bronze being added, boiled under reflux, and to the boiling solution after 1 hour one half molecular quantity potassium carbonate was added, the addition taking half an hour. The total time of refluxing was 6 hours. The subsequent treatment of the mixture was as before. In this case the yield obtained by direct ammonia extraction was improved, but the total yield of acid, including the additional amount obtained from the residue insoluble in ammonia, was of the order of 50 - 60%, so was not so good as that obtained in the experiment with the full amount of potassium salt. Another condensation was carried out in which equal molecular amounts of the dichlorobenzoic acid and 6-amino-8-methylquinoline/
quinoline were dissolved in amyl alcohol with a trace of copper bronze, and one molecular amount of potassium bicarbonate added to the boiling mixture after 1 hour, the addition taking half an hour. Refluxing was continued for a further 4½ hours bringing the total time of refluxing up to 6 hours as before. The total yield was again 50 - 60%, but in this experiment the bulk of the yield was obtained from the residue insoluble in ammonia. From these experiments it seems best to use one molecular amount of potassium 2:4-dichlorobenzoate and one molecular amount of 6-amino-8-methylquinoline as originally employed.

An experiment was carried out condensing potassium 2:4-dichlorobenzoate with the reddish brown material, m.p. 109°, described above under the preparation of 6-amino-8-methylquinoline. This was done at a time when the importance of working up the residue insoluble in ammonia had not yet been realised. An acid melting at 235-237° was obtained in 20% yield. The mixed melting point with the acid from the pure 6-amino-8-methylquinoline was intermediate, being 257-260°. It was found that the acid could be purified by repeated solution in ammonia and precipitation by acetic acid, the melting point being raised by this process to 271-272°. This is consistent with the explanation that the reddish brown material consists of 6-amino-8-methylquinoline along with some impurity.

Several attempts were made to carry out the cyclisation of 4-chloro-(2'-methyl-6'-quinoly1)-anthranilic/
-si-

anthranilic acid (XXII) using phosphorus oxychloride. The compounds obtained had melting points ranging from 195° to 295°. The melting point of one sample remained constant through two recrystallisations from dry benzene, and this sample, m.p. 250-252°, was analysed. The analytical results, however, indicated partial hydrolysis of the 2-methyl-5:8-
dichloro-3:4:2:3'-pyridoacridine (XXIII) to 2-methyl-8-chloro-3:4:2:3'-pyridoacridone (XXIV). In view

![Chemical structure of XXIII]

(XXIII)

![Chemical structure of XXIV]

(XXIV)

of the small yield, and of the obvious impurity of the compound as judged by the analytical results it appeared/
appeared unsuitable for condensation with γ-diethylaminopropylamine. It should perhaps be stated here that it has been found with 5-chloropyridoacridine derivatives of this type, the melting point is sometimes raised by partial hydrolysis of the 5-chlorine atom, so that the sample with the highest melting point is not necessarily the purest.

In view of the difficulty of obtaining 2-methyl-5:8-dichloro-3:4:2':3'-pyridoacridine (XXIII) by the use of phosphorus oxychloride as cyclising agent, it was decided to attempt the synthesis of 2-methyl-8-chloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine (XXVII) by another route. The scheme proposed was that shown below, being analogous to that used in the preparation of 2:8-dichloro-5-(γ-monobutylaminopropylamino)-3:4:2':3'-pyridoacridine on page 56.

\[
\text{Scheme:} \quad \text{XXII} \quad \xrightarrow{\text{SOCl}_2} \quad \text{XXV}
\]
4-Chloro-(8'-methyl-6'-quinolyl)-anthranilic acid (XXII) was treated with thionyl chloride in dry chloroform, and the resulting acid chloride condensed with \(\gamma\)-diethylaminopropylamine in dry chloroform solution. The chloroform was distilled off, and the residual dark red oil refluxed with phosphorus oxychloride; the intermediate compound was not isolated. After removal of the phosphorus oxychloride the residue was dissolved in water from which/
which a base was precipitated by the addition of ammonia. After purification by solution in dilute acetic acid and reprecipitation by ammonia the product was dried and recrystallised from dry ligroin as yellow micro-needles, m.p. 125°. The same product was obtained in two other experiments using chloroform as solvent and one using nitrobenzene. Analysis indicated, however, that the compound had not the expected constitution. Found: C, 74·0; H, 7·55; N, 10·0%. 2-Methyl-2-chloro-5-((γ-diethylaminopropylamino)-3:4:2′:3′-pyridoacridine requires C, 70·9; H, 6·6; N, 13·8%. The formula which fits these figures is C₉H₈N₅Cl instead of C₈H₇N₄Cl, and the only explanation of the results which can be offered is that in the formation of the amide from the quinolylanthranilic acid chloride and γ-diethylaminopropylamine two -CO·Cl groups had reacted with one molecule of γ-diethylaminopropylamine giving rise to the compound (XXVIII) and that this had

![Chemical structure](image)

(XXVIII)
undergone a double cyclisation on treatment with phosphorus oxychloride forming the compound (XXIX).

\[
\begin{align*}
\text{CH}_3 & \quad \text{Cl} \\
\text{N} \cdot \text{(CH}_3)_3 \cdot \text{N} & \quad \text{C}_2\text{H}_5 \\
\text{Cl} & \quad \text{CH}_3
\end{align*}
\]

(XXIX)

It is also necessary to assume that this product (XXIX) had crystallised with one molecule of nonane which might presumably have come from the ligroin used in recrystallisation. The substance m.p. 123° would then have the formula C_{41}H_{90}NeCl_{12}, C_{6}H_{2}O. Found: C, 74.0; H, 7.55; N, 10.0%. C_{41}H_{90}NeCl_{12}, C_{6}H_{2}O requires C, 74.0; H, 6.9; N, 10.4%. The fact that this base is not 2-methyl-8-chloro-5-(\text{\textgamma}-diethylaminopropylamino)-3:4:2':3'-pyridoacridine was later confirmed by the synthesis of the latter base, m.p. 110°, by another method.

Following a suggestion of Dr Curd of Imperial Chemical Industries Ltd., a further attempt to prepare/
prepare 2-methyl-3:8-dichloro-3:4:2':3'-pyridoacridine was made, this time successfully. The idea was to cyclise the quinolylanthranilic acid with concentrated sulphuric acid and treat the resulting pyridoacridone with phosphorus oxychloride and phosphorus pentachloride to give the 5-chloro compound.

When 4-chloro-(8'-methyl-6'-quinolyi)-anthranilic acid (XXII) was heated with concentrated sulphuric acid at 100° for 4 hours and the cooled solution poured on to ice 2-methyl-8-chloro-3:4:2':3'-pyridoacridone (XXIV) was obtained after addition of ammonia; it was crystallised from alcohol when it melted at 383° with decomposition. The pyridoacridone was then converted to 2-methyl-5:8-dichloro-3:4:2':3'-pyridoacridine (XXIII) by refluxing at 150° for 6 hours with phosphorus oxychloride containing phosphorus pentachloride. The product was worked up as in the case of cyclisations with phosphorus oxychloride, but as compared with such experiments a considerably larger portion of the product was soluble in dry benzene, and the recrystallised material showed a sharp melting point at 232°. Analysis indicated that it was, in fact, 2-methyl-5:8-dichloro-3:4:2':3'-pyridoacridine.

This compound was then condensed without difficulty in phenol with γ-diethylaminopropylamine yielding 2-methyl-8-chloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine (XXVII), m.p. 110°.

The last part of this section deals with an attempt to prepare a 2-bromo-3:4:2':3'-pyridoacridine/
acridine carrying a chlorine atom in position 8 and a basic side chain in position 5 as before. For reasons which are not yet apparent this compound has so far resisted all attempts to synthesise it.

In the first place it was necessary to prepare 6-amino-8-bromoquinoline (XXX). 2-Bromo-4-nitroaniline was made by direct bromination of p-nitraniline hydrochloride according to the method of Nicolet and Ray (J. Amer. Chem. Soc., 1927, 49, 1803). Yield 80%, m.p. 104°. (Nicolet and Ray give a yield of 81%, and m.p. 104.5°.) 2-Bromo-4-nitroaniline was then submitted to the Skraup reaction to yield 6-nitro-8-bromoquinoline, m.p. 167° (Claus and Hartman, J. pr. Chem., 1896, 52, 207, give 164°), and this was reduced by West's method to 6-amino-8-bromoquinoline (XXX), m.p. 148° (Haworth and Sykes, J.C.S., 1944, 311, give 148°).

6-Amino-8-bromoquinoline (XXX) was condensed with potassium 2:4-dichlorobenzoate in amyl alcohol copper bronze being used as catalyst. After removal of the amyl alcohol by steam distillation, the crude
product was filtered off and extracted with 2N potassium hydroxide solution, the insoluble residue being treated with hydrochloric acid according to the method usually adopted in condensations of this type. It was found to be very difficult to recrystallise this acid satisfactorily, so it was converted to the potassium salt by adding concentrated potassium hydroxide solution to a solution of the acid in hot alcohol. The potassium salt separated on cooling and was filtered off. It was extracted with boiling alcohol, the insoluble residue consisting of the pure potassium salt. Treatment of this with 2N hydrochloric acid converted it to pure 4-chloro-(8'-bromo-6'-quinolyl)-anthranilic acid (XXXI) which was well washed with water and dried, m.p. 293°.

An attempt was made to cyclise 4-chloro-(8'-bromo-6'-quinolyl)-anthranilic acid in the usual way with phosphorus oxychloride. This yielded a product (A), m.p. 273°, which on heating with 2N hydrochloric acid for 2 hours in the boiling water bath yielded a second compound (B), m.p. over 400°, which resembled a pyridoacridone in general properties. Exact details of these two preparations are given in Section III. The analyses of both compounds were unsatisfactory, being respectively as follows. Found for (A): C, 55.95; H, 2.3; Ag halides = 1 mg. substance, 1.250 mg. 2-Bromo-5:8-dichloro-3:4:2':3'-pyridoacridine requires C, 50.8; H, 1.85%; Ag halides = 1 mg. substance, 1.257 mg. Found for (B): C, 57.9; H, 2.7; Ag halides = 1 mg. substance, 0.905 mg. 2-Bromo-/
2-Bromo-8-chloro-3:4:2':3'-pyridoacridone requires C, 53.4; H, 2.3%; Ag halides = 1 mg. substance, 0.922 mg. An explanation of these results which deserves consideration is that during the treatment with phosphorus oxychloride some of the bromine had been replaced by chlorine. Although this reaction seems intrinsically unlikely, it is at least consistent with the following figures:

Found for (A): C, 55.95; H, 2.3%; Ag halides = 1 mg. substance, 1.250 mg. Required for 80% 2:5:8-trichloro-3:4:2':3'-pyridoacridine + 20% 2-bromo-5:8-dichloro-3:4:2':3'-pyridoacridine: C, 56.15; H, 2.0%; Ag halides = 1 mg. substance, 1.250 mg.

Found for (B): C, 57.9; H, 2.7%; Ag halides = 1 mg. substance, 0.905 mg. Required for 60% 2:8-dichloro-3:4:2':3'-pyridoacridone + 40% 2-bromo-3-chloro-3:4:2':3'-pyridoacridone: C, 58.0; H, 2.4%; Ag halides = 1 mg. substance, 0.915 mg.

The enrichment of the proportion of bromo compound from one fifth to two fifths might well have occurred during the recrystallisation of (B), which was, of course, made from (A). It is interesting to note that the melting point of (A) is 273°, that of 2:5:8-trichloro-3:4:2':3'-pyridoacridine being 286°. A mixed melting point, however, was much depressed, being 213–218°, and this seems to be impossible to reconcile with the idea that the analytical results are to be explained by the replacement of bromine by chlorine.

Before these analytical results were available
two experiments had been carried out to condense compound (A) with \(\gamma\)-diethylaminopropylamine in dry phenol. The first experiment yielded a yellow crystalline compound, m.p. 231°, which also analysed incorrectly. In the second experiment the product was a sticky yellow solid which after some months in the refrigerator was found to have crystallised in large yellow prisms, m.p. 185°. The analytical figures obtained for this substance were also unsatisfactory. Full details are given in Section III. In the case of the product, m.p. 231°, from the first experiment the high C is again consistent with the partial replacement of bromine by chlorine. The following figures for a 30% replacement are suggestive.

Found: C, 60.3; H, 3.85; N, 12.0%. Required for 70% 2-bromo-8-chloro-8-(\(\gamma\)-diethylaminopropylamino)-3:4:2':3'-pyridoacridine + 30% 2:8-dichloro-5-(\(\gamma\)-diethylaminopropylamino)-3:4:2':3'-pyridoacridine:

C, 60.3; H, 5.2; N, 12.3%

It will be seen, however, that the H value fails to fit in with this assumption. As this represents a duplicate analysis like most of the other analytical results given, it provides further evidence against the theory that the bromine was being replaced by chlorine. The fact that the melting point of the pure 2:8-dichloro-5-(\(\gamma\)-diethylaminopropylamino)-3:4:2':3'-pyridoacridine is 144° is also against the theory of the partial replacement of bromine by chlorine. No explanation has so far been found for the/
the results for the product, m.p. 135°, obtained from the second experiment.

Attention was now turned to the possibility of obtaining 2-bromo-3-chloro-3:4:3':3'-pyrido-
acridone (XXXII) by cyclising 4-chloro-(8'-bromo-6'-
quinoyl)-anthranilic acid (XXXI) with concentrated
sulphuric acid. The product obtained by this method
crystallised with difficulty from alcohol and melted
over 400°. The analytical figures agreed in C and
H for, probably, 2-bromo-3-chloro-3:4:3':3'-pyrido-
acridone, but diverged in N and halogen to the extent
of about 10%. Recrystallisation left these figures
unchanged. In view of the discrepancy it was not
considered desirable to attempt to convert this
compound to the 5-chloro compound by treatment with
phosphorus oxychloride and phosphorus pentachloride,
but instead other possibilities of obtaining the
desired compounds were explored.

Because of the suggestion mentioned above
that the bromine might be partially replaced by
chlorine during the treatment with phosphorus oxy-
chloride/
chloride, an attempt was made to effect ring-closure using phosphorus oxybromide.

4-Chloro-(8'-bromo-8'-quinolyl)-anthranilic acid (XXXI) was heated with nitrobenzene containing phosphorus oxybromide for 6 hours in an oil bath at 150°. The cooled mixture was poured into 4N sodium hydroxide solution with stirring, and the nitrobenzene removed by steam distillation. The residual solid was separated and dried. It crystallised from dry ligroin, when it melted at 121-122°. The analytical results again diverged from the expected values. Found: C, 55.2; H, 3.1; N, 9.5%; Ag halides = 1 mg. substance, 0.756 mg. 2:5-Dibromo-8-chloro-3:4:2'-3'-pyridoacridine requires C, 45.4; H, 1.7; N, 6.6%; Ag halides = 1 mg. substance, 1.230 mg.

On repeating this experiment on a larger scale the same product was obtained, but it was also noticed that the filtrate after filtering off the solid separating during the steam distillation deposited a greyish crystalline compound on standing over the weekend. This substance was found to melt at 375° and to resemble a pyridoacridone in general properties. It was recrystallised from alcohol when the melting point was unchanged at 375°. The following analysis was obtained. Found: C, 47.1; H, 2.4%; Ag halides = 1 mg. substance, 0.411 mg. 2-Bromo-3-chloro-3:4:2':3'-pyridoacridone requires C, 53.4; H, 2.3%; Ag halides = 1 mg. substance, 0.922 mg.

An attempt was made to effect the cyclisation with/
with phosphorus oxychloride in nitrobenzene as solvent. This experiment gave a product which crystallised from dry benzene and melted at 232-233^\circ. The analytical figures again showed discrepancies as compared with the theoretical values. Found: C, 57.13, H, 2.95%; Ag halides = 1 mg. substance, 1.065 mg.

2-Bromo-5:8-dichloro-5:4:2':3'-pyridoacridine requires C, 53.4, H, 2.9%; Ag halides = 1 mg. substance, 1.257 mg. If it is assumed that the product had crystallised along with one molecule of benzene we get a better agreement - Required C, 57.9; H, 2.95%; Ag halides = 1 mg. substance, 1.043 mg. Whether this is the actual explanation of the discrepancies is doubtful, because when the product was hydrolysed to the acridone deviations in the same sense from the theoretical values were obtained, and here the possibility of the compound having crystallised containing benzene of crystallisation is non-existent, as the solvent employed was alcohol. A sample of the material, m.p. 232-233^\circ, heated at 100^\circ for 2 hours with 3N hydrochloric acid yielded a yellow product which after treatment with ammonia crystallised from alcohol, m.p. past 400^\circ. Found: C, 53.5; H, 3.0%; Ag halides = 1 mg. substance, 0.391 mg. 2-Bromo-8-chloro-5:4:2':3'-pyridoacridone requires C, 55.4; H, 2.3%; Ag halides = 1 mg. substance, 0.932 mg.

It will be noted that in all the above analyses the halogen estimated is lower and the C higher than that required theoretically. This suggested that possibly the original bromination of p-nitraniline/
p-nitraniline had been incomplete, with the result that some unbrominated material would be present in all the intermediates, and was perhaps being concentrated in varying degrees in the various attempts at cyclisation. A careful examination of the melting points of the intermediates used, however, failed to reveal any possibility of impurity in this direction. It will be observed that 4-chloro-(8'-bromo-6'-quinoyl)-anthranilic acid did in fact give a very good analysis, and the sample used for the cyclisation experiments had the same melting point. Furthermore, the mixed melting point was not depressed.

In view of the unsatisfactory nature of the products obtained from these efforts to cyclise 4-chloro-(8'-bromo-6'-quinoyl)-anthranilic acid, the attempt to prepare a 2-bromo-8-chloro-3:4:2':3'-pyridoacridine carrying a basic side chain in position 5 was abandoned for the time being.
B. The Synthesis of Pyridoacridines derived from 5- and 7-Aminoquinolines.

This section deals with the preparation of 1:2:3′:2′-pyridoacridines derived from 5-aminoquinoline and 2:4-dichlorobenzoic acid, and with an attempt to prepare the isomeric 3:4:3′:2′-pyridoacridines from 7-aminoquinoline and 2:4-dichlorobenzoic acid. The schemes of synthesis involved are as follows.

1:2:3′:2′-Pyridoacridine Series
Both 5- and 7-aminoquinolines (XXXIV and XXXVIII) can be obtained by reduction of the corresponding nitro compounds, which are produced as a mixture when m-nitraniline is submitted to the Skraup reaction, the 5-nitroquinoline predominating. The method of separation is outlined below, and then the two series of pyridoacridines are considered separately.

A modified Skraup reaction was carried out on/
on m-nitraniline, the reaction being complete in 1½ hours. The mixture was basified with sodium hydroxide solution, ice being added to keep the temperature low because of the low melting point of the mixed nitroquinolines. The precipitate was filtered off. It contained a certain amount of inorganic matter to remove which was difficult by washing, and the crude material owing to its low melting point was not easily dried. It was therefore extracted with boiling chloroform and treated with charcoal. The residue after the removal of the chloroform was essentially a mixture of 5- and 7-nitroquinolines. The method of separation, which is described in a thesis of the University of Leeds by T.J. Elliott, depends on two properties - the relative insolubility of 5-nitroquinoline nitrate in N nitric acid, and secondly, the greater solubility of 5-nitroquinoline in light petroleum as compared with 7-nitroquinoline.

The mixture was dissolved in hot N nitric acid and the orange solution allowed to cool to 20°, the 5-nitroquinoline nitrate separating as orange crystals, m.p. 191°. It was filtered off and suspended in water, when treatment with 10N sodium hydroxide solution yielded 5-nitroquinoline, m.p. 71°. The mother liquor from the 5-nitroquinoline nitrate which contained all the 7-nitroquinoline nitrate and some 5-nitroquinoline nitrate was also basified with 10N sodium hydroxide solution and the precipitate filtered off and dried. The 5-nitroquinoline was dissolved out by light petroleum (b.p./
(b.p. 40-60°), the residue consisting of 7-nitroquinoline which was crystallised from benzene and melted at 131°. Some 7-nitroquinoline was, however, extracted by the light petroleum, and the residue obtained after removal of the light petroleum by distillation was further submitted to purification through the nitrates.

In carrying out the separation with nitric acid it was found best to treat the original mixture of nitroquinolines in portions of not more than 55 g. at a time, since with larger quantities a good separation was not obtained, the 5-nitroquinoline nitrate being contaminated with some 7-nitroquinoline nitrate, small quantities of which depress the melting point of the 5-isomer considerably. Presumably this difficulty experienced with larger quantities arises from the different rate of cooling of the larger amount of solution.

5-Nitroquinoline was reduced by West’s method to 5-aminquinoline (XXXIV), which was condensed with potassium 2:4-dichlorobenzoate in amyl alcohol, copper bronze being present as catalyst. The amyl alcohol was removed by steam distillation, and the crude product found to be completely soluble in hot 2N potassium hydroxide solution from which the acid was precipitated by acetic acid. 4-chloro-5'-quinolylantranilic acid (XXXV) crystallised from alcohol as yellow brown needles, m.p. 244°.

Cyclisation of this acid was effected by refluxing with phosphorus oxychloride containing a small/
small amount of phosphorus pentachloride for 6 hours in an oil bath at 150°. A good product was obtained almost completely soluble in dry benzene, and it is possible that earlier preparations of this type might have given better results if this modification involving the addition of phosphorus pentachloride had been used. 5:8-Dichloro-1:2:3:3'-pyridoacridine (XXXVI) crystallised from dry benzene as buff coloured needles, m.p. 244°.

Three samples of 5:8-dichloro-1:2:3:3'-pyridoacridine were heated for 2 hours in the boiling water bath with 2N sodium hydroxide solution, 2N hydrochloric acid and ethyl alcohol respectively. As is usual with 5-chloropyridoacridines the compound was stable to alkali, but in the case of the hydrochloric acid and ethyl alcohol 8-chloro-1:2:3:3'-pyridoacridone (XLII) was formed, m.p. 398°.

\[ \begin{align*}
\text{5:8-Dichloro-1:2:3:3'-pyridoacridine (XXXVI)}
\end{align*} \]

was condensed with \( \gamma \)-diethylaminopropylamine in dry molten phenol. On pouring the cooled phenol solution into sodium hydroxide solution, a sticky yellow solid/
solid was obtained which was extracted with ether. The ether extract was in turn extracted with 5% acetic acid from which the base was precipitated by ammonia and re-extracted with ether. The ether extract was dried, the ether distilled off and the residual oil crystallised from dry ligroin, yielding 8-chloro-5-(β-diethylaminopropylamino)-1:2:3:3'-pyridoacridine (XXXVII) as yellow needles, m.p. 98-99°.

An attempt was made to prepare a 3:4:3:3'-pyridoacridine by submitting 7-aminoquinoline to a similar series of reactions. 7-Nitroquinoline was reduced by West's method to 7-aminoquinoline (XXXVIII), m.p. 73°, and this was refluxed with potassium 2:4-dichlorobenzoate in amyl alcohol for 6 hours in an oil bath at 150°, copper bronze being present as catalyst. The crude product after removal of the amyl alcohol by steam distillation was completely soluble in hot potassium hydroxide solution (2N), as in the case of the acid obtained from the isomeric 5-aminoquinoline. The product was rather dark in colour and various attempts to crystallise it failed to yield a satisfactory product, but a brownish material was obtained from a fairly concentrated alcoholic solution which melted at 289°, and an attempt is being made to prepare a crystalline ester suitable for analysis.

On the assumption that the compound, m.p. 289°, was 4-chloro-7'-quinolylanthranilic acid (XXXIX) a sample, previously well dried in a desiccator, was refluxed for 6 hours in an oil bath at 150° with phosphorus/
phosphorus oxychloride containing phosphorus pentachloride as in the case of 4-chloro-5'-quinolylanthranilic acid. The product after removal of excess phosphorus oxychloride by distillation under reduced pressure was triturated with sodium hydroxide solution and ice, and the solid residue filtered off and dried. It was extracted with dry benzene in the presence of a pellet of potassium hydroxide and found to leave almost no residue, the benzene extract depositing light brown needles on cooling. The yield from benzene was relatively good, and a sample was recrystallised without difficulty successively from dry benzene and dry ligroin as light brown needles, m.p. 209°; the melting point remaining unchanged after further recrystallisation from dry ligroin. The compound appeared pure and homogeneous, but, somewhat surprisingly, the analysis did not agree with that expected for 5:8-dichloro-3:4:3':2'-pyridoacridine (XL), but agreed closely with a molecular complex of three molecules of 5:8-dichloro-3:4:3':2'-pyridoacridine and one molecule of 8-chloro-3:4:3':2'-pyridoacridine (XLIII). Found: C, 65·2; H, 2·95; N, 9·2; Cl, 21·2%. C_{16}H_{23}N_{3}Cl_{2} requires C, 64·2; H, 2·7; N, 9·4; Cl, 23·7%. 3C_{16}H_{23}N_{3}Cl_{2} · C_{16}H_{20}O_{2}N_{2}Cl requires C, 65·2; H, 2·8; N, 9·5; Cl, 21·1%.

When this material was condensed with /β/-diethylaminopropylamine in dry phenol, a very good yield of a base was obtained which separated as a solid on pouring the phenol mixture into sodium hydroxide solution. This solid readily dissolved almost/
almost completely in 5% acetic acid from which it was reprecipitated by ammonia. It was then recrystallised three times from dry ligroin, the melting points of the products being respectively 97-99°, 99-100°, 99-100°. The final orange yellow compound was obviously crystalline and to all appearances homogeneous. The analytical figures, however, did not agree with those required for the expected base, but indicated the presence of the base in a hydrated form along with a certain amount of pyridoacridone.

Found: C, 67.8; H, 6.0; N, 12.5; Cl, 9.55%.

C₃₆H₂₈N₄Cl requires C, 70.3; H, 6.4; N, 14.3; Cl, 9.0%.

3C₃₆H₂₈N₄Cl.C₄H₁₀O₂HCl.4H₂O requires C, 67.5; H, 5.9; N, 13.0; Cl, 9.4%. It may not be without significance that there is apparently one molecule of pyridoacridone/
acridone present to three molecules of 8-chloro-5-
(\text{\text{-diethylaminopropylamino}})-3:4:3':2'-pyridoacridine
(XLI), just as there was one molecule of pyridoacridone
present to three molecules of 5:8-dichloro-3:4:3':2'-
pyridoacridine.

When in a second experiment the base was
prepared on a larger scale, exactly the same compound
was obtained, m.p. 99-100°. The melting point of
this compound happens to be almost exactly the same
as that of 8-chloro-5-(\text{\text{-diethylaminopropylamino}})-1:2:3':2'-pyridoacridine (XXXVII), but a mixed melting
point showed a considerable depression, being 75-85°.

The unsatisfactory nature of these results
is being further investigated. It will be seen that
the two well-defined compounds in this series, viz.
5:8-dichloro-3:4:3':2'-pyridoacridine and 8-chloro-
5-(\text{\text{-diethylaminopropylamino}})-3:4:3':2'-pyridoacridine,
apparently contain a certain amount of pyridoacridone,
which, in view of the seeming purity of the compounds,
must be regarded as existing as some sort of molecular
complex which appears to be relatively stable because
of the extensive purification of the compounds which
has been carried out. The nature of this complex
clearly requires elucidation, and an attempt is
being made to obtain these compounds completely
free of pyridoacridone.

In the above attempt to prepare 5:8-dichloro-
3:4:3':2'-pyridoacridine, it is assumed that in the
cyclisation of 4-chloro-7'-quinolylanthranilic acid
the carboxyl group reacts with the carbon atom in position 8 of the quinoline nucleus and not that in position 6, so that it forms the angular 3:4:3':2'-pyridoacridine and not the linear 3:3:2':5'-pyridoacridine (XLIV). The *a priori* likelihood of this occurring is very great. Thus it has been shown that a Skraup reaction on 7-aminoquinoline yields m-phenanthroline (Skraup, Monats., 5, 532), the same product being derived by a double Skraup on m-phenylenediamine (Skraup and Vortmann, Monats., 3, 571). However, a striking exception to the general rule that when the two possibilities exist the angular type is in fact formed, has been found by Johnson and Mathews (*J. Amer. Chem. Soc.*, 1944, 66, 210) who prepared 4-(3-naphthylimino)-pentanone-2 (XLV) from β-naphthylamine and acetylacetone in the presence of anhydrous calcium sulphate at 100° and found that this compound in concentrated sulphuric acid at 100° cyclises mainly to 2:4-dimethyl-6:7-benzquinoline (XLVI), the linear derivative, the same product being also obtained in better yield when the cyclising agent was anhydrous hydrogen fluoride.
fluoride. This is, of course, a highly special case,

![Chemical Structures](image)

(XLV) (XLVI)

and does not seriously reduce the probability that the pyridoacridines have the angular structure, but it indicates the desirability of obtaining positive evidence to support this view.

An attempt was therefore made to explore an alternative route to the 3:4:3':2'-pyridoacridine system. It was thought that if m-nitraniline could be condensed with 2:4-dichlorobenzoic acid and the product cyclised, we should get 4-nitro-3-chloroacridone (XLVII). This expectation is based on the

(XLVII)
fact that 3'-nitrodiphenylamine-2-carboxylic acid (XLVIII) on cyclisation with concentrated sulphuric acid gives almost exclusively 4-nitroacridone and practically none of the 2-nitro isomer (Ullmann and Bader, Ann., 1907, 355, 332). Also Goldberg and Kelly (J. C.S., 1946, 102) have recently found that in the cyclisation of 3'-nitro- and 3'-aminodiphenylamine-2-carboxylic acids which have another substituent in the carboxylated nucleus (XLIX and LI), 4-substituted acridones (L) are obtained in the case of the 3'-nitro series, and 2-substituted acridones (LII) in the case of the 3'-amino series. Reduction of
of the 4-nitro-8-chloroaacidone followed by a Skraup reaction should yield 8-chloro-3:4:3':2'-pyrido-acridone (XLI). It will be noticed that, assuming that the cyclisation occurs so as to yield 4-nitro-8-chloroaacidone, the second cyclisation is unambiguous, and must result in an angular pyridoacridine nucleus.

The condensation of m-nitraniline and 2:4-dichlorobenzoic acid has been carried out under various conditions. In the first place, the m-nitraniline was allowed to react with the potassium salt of the acid in amyl alcohol in the usual manner. There was only a very small yield of product, m.p. 230-232°, most of the m-nitraniline and 2:4-dichlorobenzoic acid being recovered unchanged. In the second experiment the mixture was made more alkaline by the addition of excess potassium carbonate, and the yield here was better, though still extremely poor. In further experiments the mixture was refluxed for periods up to 72 hours but with no larger/
larger yields. A better yield was obtained using cyclohexanol as the solvent. The reaction was carried out under reflux at the boiling point, viz. 161.5°, all other conditions being as before except that the time of refluxing was 15 hours. The product after removal of the cyclohexanol by steam distillation was a black rubbery tar which was extracted repeatedly with hot 2N hydrochloric acid to remove unchanged m-nitraniline, with hot water to remove unchanged 2:4-dichlorobenzoic acid and with hot 2N potassium hydroxide solution to remove the diphenylamine carboxylic acid. Addition of acetic acid to the potassium hydroxide extract precipitated 3'-nitro-3-chlorodiphenylamine-6-carboxylic acid which was crystallised from alcohol as yellow needles, m.p. 249-250°. A mixed melting point determination showed this to be the same compound as that obtained in smaller yield when the condensation was carried out in amyl alcohol. The yield in this experiment with cyclohexanol as solvent though improved was still poor.

An attempt to cyclise the product from the condensation in amyl alcohol with concentrated sulphuric acid yielded 4(?)-nitro-8-chloroaesridone (XLVII) as yellow needles crystallisable from alcohol, m.p. over 330°. When this experiment was repeated on a larger scale using the product from the cyclohexanol condensation a different product was obtained. In this case it behaved as an acid, being soluble in alkali and precipitated by acid.

The/
The melting point was 245-246°, that of the diphenylamino carboxylic acid being 249°. A mixed melting point, however, was 214-217°. A sample of this product recrystallised from alcohol and melting at 247° analysed as follows. Found: C, 52.05; H, 3.5; N, 1.7; Cl, 32.95%. 4-Nitro-8-chloroacridone requires C, 56.8; H, 2.55; N, 10.2; Cl, 12.6%. It seems impossible to fit these figures to any compound which could possibly have been formed.

An extensive investigation of this problem was made difficult by the poor yield in the condensation of m-nitraniline and 2,4-dichlorobenzoic acid. It seems probable, however, that if the modification of the Ullmann synthesis very recently described by Goldberg and Kelly (loc. cit.) proves to give better yields, then this series of reactions would constitute a feasible alternative route to the synthesis of 4-nitro-8-chloroacridone.
C. The Results of Tests for Antimalarial Activity.

As it is out of the question to test new compounds for antimalarial activity on human subjects other methods must be adopted. One of the earlier methods was devised by Roehl at Elberfeld and published some years later in 1926. The potential antimalarial was given orally to canaries which had previously been infected with *P. relictum*. If there was a delay in the appearance of parasites in the blood as compared with control birds, the compound was considered active. This technique is still used, but has been largely replaced by other methods using other species of parasites and other avian hosts. One of these uses an acute infection of *P. gallinaceum* in six day old chicks. These chicks are infected by the intravenous injection of an approximately standard number of parasitised red cells and are then treated twice daily with the compound under examination, this being administered orally through a catheter tube. This course is continued until the number of parasites in a random sample of 500 red blood cells reaches a maximum. By this method it is possible to effect a quantitative determination of the activity of the compound, and also of its critical dose.

An unsatisfactory feature of the use of birds as test animals is that a compound which is active in avian malaria is not necessarily active in/
In human malaria, and what is more important in practice, a compound which fails to show activity against avian malaria, and which is therefore rejected may show activity in human malaria.

Preliminary tests for curative action against *P. gallinaceum* infections of chicks have been carried out on pyridoacridines by the staff of the Biological Laboratories of Imperial Chemical Industries, Ltd., to whom I am indebted for the results which follow.

The activities of the compounds tested are shown in Table I, the activity at various doses being indicated as negative (-), slight (+) or marked (++).

<table>
<thead>
<tr>
<th>Reference No. and Name of Compound</th>
<th>Dose mg/kg</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M.4602</strong>&lt;br&gt;2:3-Dichloro-5-((\gamma)-diethylaminopropyl)-amino-5:4:2':3'-pyridoacridine</td>
<td>120</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>++</td>
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<tr>
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<td>40</td>
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<td>20</td>
<td>+</td>
</tr>
<tr>
<td><strong>M.5071</strong>&lt;br&gt;Product from 2:5:8-trichloro-3:4:2:13'-pyridoacridine and 5-diethylamino-(\alpha)-methylbutylamine (See p.37)</td>
<td>340</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td><strong>M.4774</strong>&lt;br&gt;2-Chloro-5-((\gamma)-diethylaminopropylamino)-3:4:2:13'-pyridoacridine</td>
<td>120</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td><strong>M.5754</strong>&lt;br&gt;3-Methyl-8-chloro-5-((\gamma)-diethylaminopropylamino)-3:4:2:13'-pyridoacridine</td>
<td>30</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>+</td>
</tr>
</tbody>
</table>
Before discussing these results, it is convenient to give in tabular form the results obtained for the 3:4:2':3'-pyridoacridines prepared by Dobson and Kermack (loc. cit.). These are given in Table II.

Table II.

<table>
<thead>
<tr>
<th>Reference No. and Name of Compound</th>
<th>Dose mg/kg</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M.3302</strong> 5-(β-diethylaminoethylamino)-3:4:2':3'-pyridoacridine</td>
<td>125</td>
<td>+</td>
</tr>
<tr>
<td><strong>M.3561</strong> 5-(5-diethylamino-α-methylbutylamino)-3:4:2':3'-pyridoacridine</td>
<td>125</td>
<td>+</td>
</tr>
<tr>
<td><strong>M.4562</strong> 8-Chloro-5-(β-diethylamino-butylamino)-3:4:2':3'-pyridoacridine</td>
<td>120</td>
<td>+</td>
</tr>
<tr>
<td><strong>M.3652</strong> 8-Chloro-5-(β-diethylamino-propylamino)-3:4:2':3'-pyridoacridine</td>
<td>120</td>
<td>++</td>
</tr>
<tr>
<td><strong>M.3502</strong> 8-Chloro-5-(β-dimethylamino-propylamino)-3:4:2':3'-pyridoacridine</td>
<td>120</td>
<td>++</td>
</tr>
<tr>
<td><strong>M.3304</strong> 8-Chloro-5-(β-diethylamino-ethylamino)-3:4:2':3'-pyridoacridine</td>
<td>125</td>
<td>++</td>
</tr>
<tr>
<td><strong>M.3555</strong> 8-Chloro-5-(5-diethylamino-α-methylbutylamino)-3:4:2':3'-pyridoacridine</td>
<td>120</td>
<td>+</td>
</tr>
</tbody>
</table>
It was found (Dobson and Kermack, loc. cit.) that when the pyridine ring is fused on to the 1:2-position of the acridine nucleus so as to form 1:2:2':3'-pyridoacridines, the resulting compounds were devoid of activity. In the active series it will be noticed that the nitrogen atom of the fused-on pyridine ring is attached to the same carbon atom of the acridine nucleus as is the methoxy group in mepacrine, and indeed the series may be regarded as derived from the mepacrine series by replacement of the methoxy group by this pyridine ring. In this connection it may be significant to notice that 9-(\gamma\text{-diethylaminopropylamino})-\beta\text{-phenanthroline prepared by Jacomb and Kermack (J.C.S., 1946, 62), which may be regarded as derived similarly from pamaquin by replacement of the methoxy group by a pyridine ring, was inactive. As 3:4-benzacridines are active and 1:2-benzacridines are inactive (Dobson and Kermack, private communication), it appears likely that the geometrical form of the heterocyclic nucleus as a whole is an important factor/

| M.8555 | 8-Chloro-5-(\text{i}-diethylamino-\alpha-methylbutylamino)-3:4:2':3'-pyridoacridine | 125 | ++ |
|        |                                                                                 | 60  | ++ |
|        |                                                                                 | 40  | +  |

| M.8544 | 8-Chloro-5-(\text{\gamma}-butylamino-propylamino)-3:4:2':3'-pyridoacridine | 125 | ++ |
|        |                                                                                 | 40  | +  |
|        |                                                                                 | 20  | +  |
factor in determining antimalarial activity. Unfortunately, results for tests for antimalarial activity on 1:3:3';2'-pyridoacridines and 3:4:3':2'-pyridoacridines are not available.

It was found by Dobson and Kermack (loc. cit.) that activity was most marked in the case of those 3:4:2';3'-pyridoacridines carrying a chlorine atom in position 8. In view of the fact that this is the position occupied by the chlorine atom in mepacrine, such enhanced activity is not perhaps surprising.

In the results obtained in the present research it will be seen that 2-chloro-5-(γ-diethylaminopropylamino)-3:4:2';3'-pyridoacridine exhibits activity at the higher doses, but is inactive at lower doses at which the 2:8-dichloro-5-(γ-diethylaminopropylamino)-3:4:3';3'-pyridoacridine and mepacrine are still active. These results, in conjunction with those for M.8302, M.8561, and M.8652 prepared by Dobson and Kermack, show that the activity with a chlorine atom at position 2 is greater than with no substituents at all, and even somewhat greater than with a chlorine atom at position 8. Thus we may conclude that while the chlorine atom at position 8 is important, that at position 2 is even more potent, and 2:8-dichloro-5-(γ-diethylamino- propylamino)-3:4:3';3'-pyridoacridine which has chlorine atoms at both positions 2 and 8 possesses the highest activity. In view of this it would be interesting to prepare the analogue of mepacrine carrying an additional chlorine atom in the 2-position.

The/
The activity of 2-methyl-8-chloro-5-(\(\gamma\)-diethylaminopropylamino)-3:4:2\':3\'-pyridoacridine is interesting. If we compare it with 8-chloro-5-(\(\gamma\)-diethylaminopropylamino)-3:4:2\':3\'-pyridoacridine, it is seen to possess slightly higher activity than the latter compound, retaining its activity at a lower dose. The methyl group therefore appears to have a slightly beneficial action. At the same time, however, the 2-methyl-8-chloro-5-(\(\gamma\)-diethylaminopropylamino)-3:4:2\':3\'-pyridoacridine exhibited high toxicity.

3-Chloro-5-(\(\gamma\)-diethylaminopropylamino)-1:2:3\':2\'-pyridoacridine and 3:8-dichloro-5-(\(\gamma\)-monobutylaminopropylamino)-3:4:3\':3\'-pyridoacridine have also been sent for biological testing, but the results have not yet been received. They will be published in due course along with those given above, when it is also hoped that 8-chloro-5-(\(\gamma\)-diethylaminopropylamino)-3:4:3\':3\'-pyridoacridine will have been successfully prepared and tested for antimalarial activity.

In conclusion, it might perhaps be mentioned that several of the 3:4:3\':3\'-pyridoacridines listed above have been tested for antisporeozoite activity, which, of course, determines their possible prophylactic action. None of the compounds showed any activity in this test.
III. EXPERIMENTAL•
β-Diethylaminoethylamine

(Cf. Ristenpart, Ber., 1896, 29, 2536)

β-Diethylaminoethylchloride hydrochloride (8 g.) was slowly added with shaking to a mixture of ammonia (25 cc, sp.gr. 0.880) and alcohol (25 cc.) and the mixture gently heated under reflux for 2 hours. The alcohol was distilled off on the water bath, the residue made strongly alkaline with sodium hydroxide solution, and the β-diethylaminoethylamine separated by several extractions with ether. The ethereal extract was dried over anhydrous potassium carbonate, filtered and the ether distilled off. The residue was distilled, the β-diethylaminoethylamine being collected as a clear liquid, b.p. 148-155°. Yield 5.9 g.

Note: It was unnecessary to prepare the dialkylaminoalkylamines used as side chains, as these were supplied by Imperial Chemical Industries, Ltd., from whom further supplies of β-diethylaminoethylamine were also obtained.
o-Chloroacetanilide.

o-Chloraniline (107 cc.) and acetic anhydride (190 cc.) were refluxed on the water bath for 2 to 3 hours. The mixture was then poured into water and allowed to stand for some hours after which the crystalline o-chloroacetanilide was filtered off and dried. On basifying the filtrate with sodium hydroxide solution another crop of crystals was obtained. o-Chloroacetanilide was recrystallised from ether, m.p. 86°. Yield 103 g.
2-Chloro-4-nitraniline.


Chattaway, Orton and Evans, Ber., 1900, 33, 3057)

Fuming nitric acid (20 cc., 94% HNO₃ w/w) was placed in a conical flask and cooled in a freezing mixture to below 0°. 2-Chloroacetanilide was then added slowly with stirring (4 g.) care being taken that the temperature did not rise much above 0°. The product was poured on to ice when 2-chloro-4-nitroacetanilide separated and was filtered off and dried, m.p. 122-123°. Yield 3.5 g. Hydrolysis to 2-chloro-4-nitraniline was effected by refluxing with 2N sulphuric acid (25 cc.) for 1 hour and allowing to cool before filtering. The product was recrystallised from dilute acetic acid, m.p. 115° (lit. 119°). Small amounts of water depress the melting point considerably.
6-Nitro-8-chloroquinoline.

(Cf. E.P. 394416)

2-Chloro-4-nitraniline (86 g.) was added to a mixture of concentrated sulphuric acid (333.3 cc.) and water (260 cc.) in a 2-litre bolhead flask. Glycerin (135 cc.) and arsenic acid (148 cc., 65% As₂O₅ solution) were added and the mixture gradually heated on a gauze to the boil. After refluxing gently for 3 to 4 hours (i.e. until the diazo test for unchanged amine was negative), it was poured into its own volume of water and basified with 10N sodium hydroxide solution with stirring. The addition of the sodium hydroxide solution caused first the precipitation of bright yellow 6-nitro-8-chloroquinoline sulphate, but as the medium became alkaline the colour changed to brownish grey. The 6-nitro-8-chloroquinoline was filtered off, washed till neutral and recrystallised from 65% aqueous alcohol, m.p. 153-154°. Yield 56 g. (E.P. 394416 gives m.p. 149-150°)
6-Amino-8-chloroquinoline.

(Cf. West, J.C.S., 1925, 127, 494)

6-Nitro-8-chloroquinoline (83·4 g.) was heated to boiling with methylated spirit (225 cc.) and concentrated hydrochloric acid (5 cc.). Iron filings (69 g.) were added in portions over ½ hour. The mixture was kept boiling vigorously to prevent caking. After 2 hours the acid was neutralised with 10N sodium hydroxide solution, filtered hot and the residue well washed with hot spirit. The alcohol was distilled off on the water bath, and the residue recrystallised from benzene, m.p. 154°. Yield 67 g.

A sample prepared by Imperial Chemical Industries, Ltd. (private communication) melted at 156-157° and gave the following analysis. Found: C, 60·55; H, 4·15; Cl, 20·15%. C₆H₇N₂Cl requires C, 60·5; H, 3·9; Cl, 19·9%.

6-Amino-8-chloroquinoline is soluble in dilute acids, in alcohol, ether, benzene and acetone, and insoluble in dilute alkali.
4-Chloro-(8'-chloro-6'-quinolyl)-anthranilic acid.

Dobson and Kermack, J.C.S., 1946, 150.)

6-Amino-8-chloroquinoline (12 g.), potassium 2:4-dichlorobenzoate (15.3 g.), amyl alcohol (16.7 cc.) and copper bronze (0.1 g.) were mixed together in a round bottomed flask and heated under reflux for 6 hours in an oil bath at 150°. The amyl alcohol was filtered off, the residue well washed with acetone and extracted with a large volume of hot dilute ammonia. After filtration the filtrate was acidified with acetic acid giving a brownish yellow precipitate of 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid in relatively small yield. A further quantity was obtained by extracting the residue with 2N hydrochloric acid on the boiling water bath and filtering hot. The hydrochloride of the acid separated on cooling, was suspended in water and carefully made alkaline with ammonia. The yellow residue insoluble in dilute hydrochloric acid evidently consisted of the relatively insoluble hydrochloride of the acid, for on treatment with ammonia it yielded crude 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid. The acid (12.3 g.) recrystallised from alcohol as white needles, m.p. 300°. (Found: C, 56.6; H, 3.35; N, 8.65; Ash 0.5% (approx). C₁₆H₁₀O₆N₄Cl₂ • ½H₂O requires C, 56.1; H, 3.2; N, 8.2%).

4-Chloro-(8'-chloro-6'-quinolyl)-anthranilic acid.
acid is soluble in dilute alkali and hot alcohol. The insoluble sodium or potassium salt is produced when the acid is treated with relatively concentrated sodium or potassium hydroxide solution. The acid is sparingly soluble in cold alcohol, dilute acid, and insoluble in water.
2:5:8-Trichloro-3:4:2':3'-pyridoacridine.

(Cf. Magidson, Ber., 1933, 66, 866.
Drosdov, Chimitecheski, 1934, 4, 117.
Albert and Linnell, J.C.S., 1936, 88.
Dobson and Kermack, J.C.S., 1946, 150.)

4-Chloro-(8'-chboro-6'-quinolyl)-anthranilic acid (6 g.) and phosphorus oxychloride (30 cc.) were refluxed together for 6 hours in an oil bath at 150°. The excess phosphorus oxychloride was removed by distillation under reduced pressure, and the residue triturated with 2N sodium hydroxide solution and ice. After filtering and washing, the residue was dried in a desiccator over calcium chloride and then extracted in the presence of a pellet of potassium hydroxide with benzene which had been dried over sodium. Recrystallisation from benzene yielded 3 g. of 2:5:8-trichloro-3:4:2':3'-pyridoacridine as brown yellow needles, m.p. 286°. (Found: C, 57.5; H, 2.3; N, 9.4%. C_{16}H_{7}N_{5}Cl_{3} requires C, 57.6; H, 2.1; N, 8.4%).

2:5:8-Trichloro-3:4:2':3'-pyridoacridine was also prepared by refluxing 2:8-dichloro-3:4:2':3'-pyridoacridone (5 g.), phosphorus oxychloride (25 cc.) and phosphorus pentachloride (3.3 g.) for 6 hours in an oil bath at 150° and working up as described above. Yield 1 g., m.p. 268-269°.

2:5:8-Trichloro-3:4:2':3'-pyridoacridine was found to be soluble in dilute acid and alcohol in the cold/
cold, and in benzene and light petroleum in the hot. It is insoluble in dilute alkali. A marked green fluorescence is exhibited in acid and alcohol solutions.
2:8-Dichloro-3:4:2':3'-pyridoacridone.

(Cf. Dobson and Kermack, J.C.S., 1946, 150)

2:5:8-Trichloro-3:4:2':3'-pyridoacridine

was heated at 100° for 2 hours with N hydrochloric acid when a pale yellow crystalline compound was formed. This was filtered off and dried. It was presumably the hydrochloride of 2:8-dichloro-3:4:2':3'-pyridoacridone, for on treatment with ammonia the colour changed to greyish brown and the filtrate gave a reaction for chloride ions. 2:8-Dichloro-3:4:2':3'-pyridoacridine was crystallised from alcohol as brown yellow needles, m.p. over 400°. This material was purified by sublimation, yielding a yellow crystalline product, which melted at 385°. Found: C, 59.45; H, 3.9; N, 9.5%. C_{16}H_{15}NO_{3}Cl_{2}·\frac{1}{2}H_{2}O requires C, 59.35; H, 3.8; N, 8.6%.

2:8-Dichloro-3:4:2':3'-pyridoacridone hydrochloride was also produced more slowly by refluxing the 5-chloro compound with ethyl alcohol.

2:8-Dichloro-3:4:2':3'-pyridoacridone is soluble in concentrated acid and hot alcohol, in both of which solvents it exhibits a green fluorescence.
2:8-Dichloro-5-[(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine.

(Cf. E.P. 363392.
Dobson and Kermack, J.C.S., 1946, 150.)

Redistilled phenol (10 g.) was dried in a vacuum at 100° for 2 hours, γ-diethylaminopropylamine (1 g.) added, and the mixture dried for a further hour. 2:5:8-Trichloro-3:4:2':3'-pyridoacridine (1 g.) which had been dried in a dessicator was introduced and the dark reddish brown solution heated at 100° with an air condenser for 2 hours, when a marked reaction for chloride ions was given. The cooled phenol mixture was poured into 2N sodium hydroxide solution (30 cc.) and the brown precipitate filtered off and washed. It was then extracted with 5% acetic acid, filtered, and the filtrate basified with ammonia giving a yellow precipitate which was filtered off, washed and dried in a dessicator over calcium chloride. The dry material was crystallised from dry ligroin yielding 2:8-dichloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine (0.6 g.) as yellow needles, m.p. 144°. (Found: C, 64.45; H, 5.7; N, 13.1%. C_{23}H_{24}N_{4}Cl_{2} requires C, 64.65; H, 5.6; N, 13.1%).

2:8-Dichloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine is soluble in most organic solvents and fluoresces green in alcohol and blue in ether solutions. It is soluble in dilute acids with the formation of the corresponding salts which exhibit/
exhibit a green fluorescence in solution.

Unless otherwise stated all the succeeding
5-(dialkylaminoalkylamino)-pyridoacridines possess
similar properties.
2:5:8-Dichloro-5-(γ-monobutylaminopropylamino)-3:4:2':3'-pyridoacridine.

Method I - From 2:5:8-trichloro-3:4:2':3'-pyridoacridine and γ-monobutylaminopropylamine.

(Cf. E.P. 363392.
Dobson and Kermack, J.C.S., 1946, 150.)

2:5:8-Trichloro-3:4:2':3'-pyridoacridine (0.5 g.) was condensed with γ-monobutylaminopropylamine (0.25 g.) in dry molten phenol (5 g.) as described under 2:6-dichloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine. 2:6-Dichloro-5-(γ-monobutylaminopropylamino)-3:4:2':3'-pyridoacridine crystallised from dry ligroin as yellow needles, m.p. 131°. Yield 0.2 g. (Found: C, 64.55; H, 5.7; N, 13.05%. C₆H₄N₄Cl₂ requires C, 64.6; H, 5.6; N, 13.1%.)

Method II - By cyclisation of 4-chloro-(8'-chloro-6'-quinolyl)-anthranil-(γ-monobutylaminopropyl)amide.

(Cf. Drosdov, J. Gen. Chem. (U.S.S.R.), 1938, 8, 1192.)

Thionyl chloride (2.4 g.) was dissolved in chloroform (10 cc.) which had been dried over phosphorus pentoxide, and the solution added to a suspension of 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid (6 g.) in dry chloroform (80 cc.). The mixture was maintained at room temperature for 3½/
3½ hours and then boiled under reflux for a further hour. The chloroform and excess thionyl chloride were then removed by distillation and the bright yellow residue, presumably 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid chloride, was dissolved in dry chloroform (34 cc.). A solution of \( \gamma \)-monobutylaminopropylamine (2.6 g.) in dry chloroform (10 cc.) was added, and the mixture heated at 100° under reflux for 1 hour. At this stage a small amount of white sublimate was formed in the condenser and was found to have m.p. 325° but its identity was not known. The chloroform solution was filtered and the chloroform removed from the filtrate by distillation leaving a dark reddish brown oil, probably 4-chloro-(8'-chloro-6'-quinolyl)-anthranil-(\( \gamma \)-monobutylaminopropyl)-amide hydrochloride. This was refluxed with phosphorus oxychloride (20 cc.) for two hours in an oil bath at 150°, when hydrogen chloride was evolved. The excess phosphorus oxychloride was removed by distillation under reduced pressure and the thick dark red oil remaining was dissolved in ice water. The brown solution so obtained was filtered and the filtrate made alkaline with ammonia when crude \( 2:8 \)-dichloro-5-(\( \gamma \)-monobutylaminopropylamo)-3:4:2':3'-pyridoacridine separated as a yellowish solid which was recrystallised from dry ligroin. The yield was very poor, there being just sufficient for a melting point which was 130°. A mixed melting point with a sample prepared by Method I showed no depression.
Attempt to prepare 2:3-Dichloro-5-([S]-diethylamino-$\alpha$-methylbutylamino)-3:4:2':3'-pyridoacridine.

(Cf. E.P. 363392.
Dobson and Kermack, J.C.S., 1946, 150.)

2:5:3-Trichloro-3:4:3':3'-pyridoacridine (1 g.) was condensed with $\beta$-diethylamino-$\alpha$-methylbutylamine (0.5 g.) in dry molten phenol (10 g.), the procedure and method of working up the product being as described for 2:3-dichloro-5-([$\beta$]-diethylaminopropylamino)-3:4:2':3'-pyridoacridine. As in that case the product on pouring the mixture into sodium hydroxide solution was a yellow solid, and this, after purification, was recrystallised three times from dry ligroin, when it melted at 167°. (Found: C, 65.75; H, 4.7; N, 8.45; Cl, 15.45%. C$_{24}$H$_{28}$N$_4$Cl$_4$ requires C, 65.9; H, 6.15; N, 13.3; Cl, 15.6%. C$_{24}$H$_{28}$N$_4$Cl$_4$. C$_{24}$H$_{28}$O$_2$N$_4$Cl$_4$.H$_2$O requires C, 65.3; H, 4.9; N, 9.7; Cl, 16.4%.)

The same product was obtained in three separate experiments. The significance of the results has already been discussed on page 38.
(8'-Chloro-6'-quinolyl)-anthranilic acid.

Dobson and Kermack, J.C.S., 1946, 150.)

6-Amino-8-chloroquinoline (21.5 g.), potassium 2:4-dichlorobenzoate (24.5 g.), copper bronze (0.1 g.) and amyl alcohol (40 cc.) were refluxed together for 6 hours in an oil bath at 150°. The product was worked up as described under 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid. (8'-Chloro-6'-quinolyl)-anthranilic acid crystallised from alcohol as grey brown needles, m.p. 256°. Yield 20.5 g.

(8'-Chloro-6'-quinolyl)-anthranilic acid resembles 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid in general properties.
Ethyl (3'-chloro-6'-quinolyl)-anthranilate.

Ethyl (3'-chloro-6'-quinolyl)-anthranilate was prepared by refluxing (3'-chloro-6'-quinolyl)-anthranilic acid (1 g.) with ethyl alcohol (10 cc.) and concentrated sulphuric acid (3 cc.) for 2 hours. The cooled solution was poured into ice water, filtered and the filtrate made faintly alkaline with ammonia, when a yellow oily precipitate was produced. This was extracted with ether and the ethereal extract dried over anhydrous sodium sulphate. The ether was removed by distillation and the residue shaken up with a warm solution of sodium bicarbonate when the solid ester was obtained on cooling. It was filtered off, washed, dried and recrystallised from alcohol as white needles, m.p. 118°. (Found: C, 66·35; H, 4·5; N, 8·6%. C₁₉H₁₃O₂N₂Cl requires C, 66·2; H, 4·6; N, 8·6%.

The ester is soluble in dilute acid and hot alcohol, slightly soluble in cold alcohol and benzene, and insoluble in dilute alkali.
2:5-Dichloro-3:4:2':3'-pyridoacridine.

(Cf. Magidson, Ber., 1933, 66, 866.
Drosdov, Chimitscheski, 1934, 4, 117.
Albert and Linnell, J.C.S., 1936, 88.
Dobson and Kermack, J.C.S., 1946, 150.)

(3'-Chloro-6'-quinoloyl)-anthranilic acid (6 g.)
and phosphorus oxychloride (30 cc.) were refluxed
together for 6 hours in an oil bath at 150°. The
2:5-dichloro-3:4:2':3'-pyridoacridine was worked up
as described for 2:5:8-trichloro-3:4:2':3'-pyrido-
acridine. It crystallised easily from dry benzene
as yellow needles, m.p. 208°. Yield 4.7 g. (Found:
C, 64.75; H, 2.6; N, 9.6; Cl, 23.5%. C_{18}H_{12}N_{2}Cl_{2}
requires C, 64.2; H, 2.7; N, 9.4; Cl, 23.7%.)

2:5-Dichloro-3:4:2':3'-pyridoacridine resembles
2:5:8-trichloro-3:4:2':3'-pyridoacridine in general
properties, but tends to be rather more soluble in
organic solvents.
2-Chloro-3:4:2':3'-pyridoacridone.

(Cf. Dobson and Kermack, J.C.S., 1946, 150.)

2:5-Dichloro-3:4:2':3'-pyridoacridine was heated for 2 hours at 100° with N hydrochloric acid. The bright yellow compound formed was filtered off and dried. It was found to melt over 400°. On treatment with ammonia the colour changed to a lighter yellowish brown, and the filtrate gave a reaction for chloride ions. The melting point was still over 400°. The bright yellow compound is presumably the hydrochloride of the pyridoacridone, which possibly loses hydrogen chloride before melting. 2-Chloro-3:4:2':3'-pyridoacridone crystallised from alcohol as yellow needles, m.p. over 400°. (Found: C, 67.4; H, 3.6; N, 9.9%. C₁₀H₆On₂Cl₁.1/₄H₂O requires C, 67.4; H, 3.3; N, 9.8%.)

2-Chloro-3:4:2':3'-pyridoacridone hydrochloride was also produced by refluxing 2:5-dichloro-3:4:2':3'-pyridoacridine with absolute ethyl alcohol.

2-Chloro-3:4:2':3'-pyridoacridone resembles 2:3-dichloro-3:4:2':3'-pyridoacridone in general properties.
2-Chloro-5-\(\{-\text{diethylaminopropylamino}\}\)-3:4:2':3'-pyridoacridine.

(Cf. E.P. 363392.)

Dobson and Kermack, J.C.S., 1946, 150.)

2:5-Dichloro-3:4:2':3'-pyridoacridine (1 g.) and \(\{-\text{diethylaminopropylamine}\}\) (1 g.) were heated in dry molten phenol at 100° for 2 hours. The cooled phenol mixture was poured into sodium hydroxide solution and the yellow oily precipitate extracted with ether. The base was purified by shaking the ethereal extract with 5% acetic acid, reprecipitating with ammonia and re-extracting with ether. This ethereal extract was dried over anhydrous potassium carbonate and the ether distilled off. The residual brownish red oily solid was heated at 100° in a vacuum to remove excess \(\{-\text{diethylaminopropylamine}\}\), and the sticky solid which remained was dissolved in hot dry ligroin and filtered. The sticky solid which separated on cooling crystallised after two days at room temperature and melted at 109°. Two further recrystallisations raised this to 115°.

Yield 0.7 g. (Found: C, 69.9; H, 6.4; N, 13.95%. 
\(\text{C}_{28}\text{H}_{36}\text{N}_{4}\text{Cl}\) requires C, 70.3; H, 6.4; N, 14.3%.)
5-Nitro-o-toluidine.

Chattaway, Orton and Evans, Ber., 1900, 33, 3057)

5-Nitro-o-toluidine was prepared from o-toluidine by acetylation followed by nitration and hydrolysis in a manner analogous to that used for 2-chloro-4-nitraniline. The product after recrystallisation from hot water and benzene melted at 127° (lit. 129°).

This compound was later supplied in bulk by Imperial Chemical Industries, Ltd., and this material was used for most of the following work.
6-Nitro-8-methylquinoline.

(Cf. Lellmann and Ziemssen, Ber., 1891, 24, 2116. E.P. 394416.)

Concentrated sulphuric acid (440 cc.) was added to water (343 cc.) to give 69% sulphuric acid. The diluted acid was placed in a 2 litre flask fitted with a reflux condenser. 5-Nitro-o-toluidine (100 g.) was added followed by glycerin (176 cc.) and arsenic acid (194.5 cc., 65% As2O3 solution). The mixture was gradually heated on a gauze to the boil, and allowed to reflux gently for 8 hours, at the end of which time no unchanged amine could be detected by the diazo test. After cooling it was poured into its own volume of water, and this solution was made alkaline with 10N sodium hydroxide solution, which caused first the precipitation of bright yellow sulphate, but as the mixture became alkaline the colour changed to a brownish grey. The mixture was allowed to cool to about 40° (lower temperatures cause crystallisation of sodium sulphate), filtered and washed till neutral. The grey precipitate of 6-nitro-8-methylquinoline was dried at 70° and crystallised in greyish white needles from alcohol (1200 cc.), m.p. 128° (lit. 129°). Yield 97 g.

(Found: C, 63.75; H, 4.25%. Calculated for C19H10O4N4 C, 63.8; H, 4.3%.)
6-Amino-8-methylquinoline.

(Cf. West, J.C.S., 1925, 127, 494.)

6-Nitro-8-methylquinoline \((30.4 \text{ g.})\) was dissolved in boiling methylated spirit \((100 \text{ cc.})\) containing concentrated hydrochloric acid \((5 \text{ cc.})\). Iron filings \((34 \text{ g.})\) were added in four portions at five minute intervals. The product was worked up as described for 6-amino-8-chloroquinoline.

6-Amino-8-methylquinoline crystallised from benzene as buff coloured needles, m.p. 129°. Yield 20 g. Alternatively the compound may be crystallised from alcohol.

6-Amino-8-methylquinoline is soluble in most organic solvents and in dilute acid. It is insoluble in dilute alkali.

6-Acetamido-8-methylquinoline was prepared by heating 6-amino-8-methylquinoline \((1 \text{ g.})\) with acetic anhydride \((10 \text{ cc.})\) under an air condenser for 2 hours in the water bath. The mixture was poured into cold water when a blue fluorescence was obtained. Addition of ammonia gave a cream precipitate which was filtered, washed and dried at 70°. 6-Acetamido-8-methylquinoline was recrystallised from hot water in glistening white plates, m.p. 174-174.5°. (Found: C, 71.95; H, 5.75%. \(\text{C}_12\text{H}_8\text{ON}_2\) requires C, 72.0; H, 6.0%).
4-Chloro-(8'-methyl-6'-quinolyl)-anthranilic acid.


Dobson and Kermack, J.C.S., 1946, 150.)

6-Amino-8-methylquinoline (31.8 g.), potassium 2:4-dichlorobenzoate (46.3 g.), copper bronze (0.1 g.) and amyl alcohol (60 cc.) were mixed together in a round bottomed flask and heated for 6 hours under reflux in an oil bath at 150°C. The amyl alcohol was filtered off, the residue well washed with acetone and extracted with a large volume of hot dilute ammonia. Alternatively the amyl alcohol may be removed by steam distillation and the residue filtered off and extracted with ammonia as before. Acidification of the ammoniacal extract with acetic acid yielded a small amount of 4-chloro-(8'-methyl-6'-quinolyl)-anthranilic acid (8%). A further yield (72%) was obtained from the residue insoluble in ammonia. This was found to be completely soluble in hot 2N hydrochloric acid. On cooling, the hydrochloride of the acid separated and was filtered off. It was dissolved in hot 2N hydrochloric acid and ammonia added to precipitate 4-chloro-(8'-methyl-6'-quinolyl)-anthranilic acid as a pale yellow compound which crystallised from alcohol as buff coloured needles, m.p. 275°C. Yield 48 g.

The acid is soluble in hot alcohol, but only sparingly soluble in cold alcohol, dilute alkali or dilute acid, and insoluble in water. With concentrated sodium or potassium hydroxides the acid is converted into the sparingly soluble alkali salt.
Ethyl 4-chloro-(8'-methyl-6'-quinolyl)-anthranilate.

4-Chloro-(8'-methyl-6'-quinolyl)-anthranilic acid (0.5 g.), ethyl alcohol (10 cc.) and concentrated sulphuric acid (4 cc.) were refluxed gently for 1 hour. The mixture was allowed to cool somewhat and then poured on to ice. After standing for about ½ hour it was made alkaline with ammonia when a grey precipitate was produced. This recrystallised from alcohol in light brown needles, m.p. 74°. (Found: C, 66.8; H, 5.1; N, 8.1%. C₁₉H₁₇O₂N₂Cl requires C, 67.0; H, 5.0; N, 8.2%).

The ester is soluble in hot ethyl alcohol, hot benzene and dilute acids, slightly soluble in cold alcohol and benzene, and insoluble in dilute alkali.
Attempt to cyclise 4-chloro-(3'-methyl-6'-quinolyl)-anthranilic acid with phosphorus oxychloride.

(Cf. Magidson, Ber., 1933, 66, 866.
Drosdov, Chimtschoaki, 1934, 4, 117.
Albert and Linnell, J.C.S., 1936, 38.
Dobson and Kermack, J.C.S., 1946, 150.)

4-Chloro-(3'-methyl-6'-quinolyl)-anthranilic acid (6 g.) was refluxed with phosphorus oxychloride (30 cc.) for 6 hours in an oil bath at 150°. After removal of the excess phosphorus oxychloride by distillation under reduced pressure, the product was triturated with sodium hydroxide solution and ice, and the residue filtered off and dried. The dry material was boiled with dry benzene in the presence of a pellet of potassium hydroxide and filtered hot. There was a large black insoluble residue (4.8 g.). The filtrate on cooling deposited a brownish material whose melting point remained constant at 250-252° being unchanged after two further recrystallisations from dry benzene. (Found: N, 10.9; Cl, 16.4%.
C_{17}H_{10}N_{2}Cl_{2} requires N, 8.95; Cl, 22.7%.)

In other experiments carried out in the same manner the products were found to have varied melting points ranging from 195° to 295°. The significance of these results has already been discussed on page 51.
Attempts to condense 4-chloro-(2'-methyl-6'-quinolyl)-anthranilic acid with γ-diethylaminopropylamine and cyclise the resulting amide.

(Cf. Drosdov, J. Gen. Chem. (U.S.S.R.), 1938, 8, 1192)

Thionyl chloride (2.4 g.) dissolved in dry chloroform (10 cc.) was added to a suspension of 4-chloro-(2'-methyl-6'-quinolyl)-anthranilic acid (6.25 g.) in dry chloroform (80 cc.). The mixture was maintained at room temperature for 3½ hours and then boiled under reflux for a further hour. The small brownish yellow residue was filtered off and found to be unmelted at 400°. The chloroform and excess thionyl chloride were then removed from the filtrate by distillation. The bright yellow residue, m.p. 338° (decomp.), was dissolved in dry chloroform (34 cc.) and a solution of γ-diethylaminopropylamine (2.6 g.) in dry chloroform (10 cc.) was added. The mixture was heated at 100° for 1 hour and the chloroform distilled off, leaving a dark reddish brown oil which was then refluxed for 2 hours at 150° with phosphorus oxychloride (20 cc.) when hydrogen chloride was evolved. The excess phosphorus oxychloride was distilled off at 100° under reduced pressure, and the residual thick dark red oil was dissolved in ice water. The brown solution so obtained was filtered, and the filtrate made alkaline with ammonia when a yellowish solid separated. This was filtered off and dried when it melted at 80-83°. It was dissolved in 5% acetic acid and reprecipitated with ammonia.
ammonia. It now melted at 95-97° and recrystallisation from dry ligroin raised the melting point to 123°. (Found: C, 74.0; H, 7.55; N, 10.0%. C₉H₇N₄Cl requires C, 70.9; H, 6.6; N, 13.8%. C₉H₇N₄Cl₂·C₃H₆O requires C, 74.0; H, 6.9; N, 10.4%).

The same product was obtained from two other identical experiments and also from one in which the chloroform used as solvent was replaced by nitrobenzene. The possible significance of the result has been discussed in Section II (page 54).
2-Methyl-3-chloro-3:4:2':3'-pyridoacridone.

(Cf. Dobson and Kermack, J.C.S., 1946, 150.)

4-Chloro-(3'-methyl-6'-quinolyl)-anthranilic acid (5 g.) and concentrated sulphuric acid (50 cc.) were mixed in a round bottomed flask, maintained at 100° for 4 hours, allowed to cool and poured on to ice. The yellow brown precipitate formed was filtered off. The filtrate was basified with sodium hydroxide solution and brought back to neutrality with acetic acid but without yielding any further precipitate. The original precipitate was presumably the sulphate of 2-methyl-3-chloro-3:4:2':3'-pyridoacridone. It was therefore suspended in water and excess ammonia added when the precipitate became lighter in colour and flocculent. It was filtered off, the filtrate giving a marked reaction for sulphate ions. The residue was washed, dried and recrystallised from alcohol as yellow needles, m.p. 383° (decomp.).

(Found: C, 67.6; H, 3.9; N, 9.6%. C_{17}H_{10}ON_4Cl_2 requires C, 67.2; H, 4.0; N, 9.2%.)

2-Methyl-3-chloro-3:4:2':3'-pyridoacridone is soluble in strong acid and hot alcohol and in both of these solvents it exhibits a marked green fluorescence.
2-Methyl-5:8-dichloro-3:4:2':3'-pyridoacridine.

2-Methyl-8-chloro-3:4:2':3'-pyridoacridone (10 g.), phosphorus pentachloride (4 g.) and phosphorus oxychloride (60 cc.) were refluxed together for 6 hours in an oil bath at 150°. The product was worked up as described for 2:5:8-trichloro-3:4:2':3'-pyridoacridine. The crude product (10 g., m.p. 217-218°) crystallised from dry benzene as yellow needles, m.p. 232°. Yield 6 g. (Found: C, 65.7; H, 3.5; N, 8.7; Cl, 21.5%. C_{17}H_{10}N_{a}Cl_{4} requires C, 65.2; H, 3.2; N, 8.95; Cl, 22.7%.)

2-Methyl-8-chloro-3:4:2':3'-pyridoacridine is soluble in cold dilute acid, alcohol, hot benzene and light petroleum and insoluble in dilute alkali. It exhibits a green fluorescence in aqueous acids and alcohol solution.
2-Methyl-8-chloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine.

(Cf. E.P. 363392.

Dobson and Kermack, J.C.S., 1946, 150.)

2-Methyl-8-chloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine was prepared from 2-methyl-5,8-dichloro-3:4:2':3'-pyridoacridine (1 g.) and γ-diethylaminopropylamine (0.5 g.) in dry phenol (10 g.) as described under the preparation of 2,8-dichloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine. Yield 0.4 g., m.p. 110°. (Found: C, 70.3; H, 6.45; N, 13.9%. C_{24}H_{27}N_{4}Cl requires C, 70.9; H, 6.6; N, 13.8%.)

2-Methyl-8-chloro-5-(γ-diethylaminopropylamino)-3:4:2':3'-pyridoacridine crystallises from ligroin as orange yellow needles.
2-Bromo-4-nitraniline.


p-Nitraniline (75 g.) was dissolved by heating in concentrated hydrochloric acid (1 l.) and the solution rapidly cooled to room temperature. The aspiration of bromine (87 g.) through the solution was begun promptly and continued for 2 to 3 hours until all the bromine had passed over, the solution being frequently shaken. When the bromination was complete, the solid was filtered off and stirred well with a large volume of water to hydrolyse the salt. The 2-bromo-4-nitraniline was filtered off and crystallised from 65% aqueous alcohol (600 cc.) as yellow needles, m.p. 104.5°. Yield 118 g.
6-Nitro-8-bromoquinoline.

(Cf. Claus and Hartman, J. pr. Chem., 1896, 52, 207.)

Concentrated sulphuric acid (333.3 cc.) was added to water (260 cc.) to give 69% sulphuric acid, and to this diluted acid was added 2-bromo-4-nitraniline (109 g.) followed by glycerin (135 cc.) and arsenic acid (148 cc., 65% As₂O₃ solution). The mixture was boiled gently under reflux for 3 to 4 hours, until no unchanged amine could be detected, and the product worked up as described for 8-chloro-6-nitroquinoline. 6-Nitro-8-bromoquinoline crystallised from 65% aqueous alcohol as yellow needles, m.p. 167°. Yield 83 g.
6-Amino-8-bromoquinoline.

(Cf. Haworth and Sykes, J.C.S., 1944, 311.)

6-Nitro-8-bromoquinoline (62.3 g.) was heated to boiling with methylated spirit (125 cc.) and concentrated hydrochloric acid (5 cc.). Iron filings (42.5 g.) were added in four portions at 5 minute intervals, and the mixture kept at vigorous ebullition for 2 hours. The product was worked up as already described for 6-amino-8-chloroquinoline, the residue obtained after removal of the alcohol being crystallised from benzene. 6-Amino-8-bromoquinoline crystallised from benzene as yellow brown needles, m.p. 148°. Yield 47.5 g.
4-Chloro-(8'-bromo-6'-quinolyl)-anthranilic acid.

Dobson and Kermack, J.C.S., 1946, 150.)

6-Amino-8-bromoquinoline (44.5 g.), potassium 2,4-dichlorobenzoate (46.2 g.), copper bronze (0.1 g.) and amyl alcohol (60 cc.) were heated under reflux for 6 hours in an oil bath at 150°. The amyl alcohol was removed by distillation in steam. The solid residue was filtered off and extracted with boiling 2N potassium hydroxide solution. This extract yielded a small amount of 4-chloro-(8'-bromo-6'-quinolyl)-anthranilic acid on acidification with acetic acid. There was, however, a large residue insoluble in the potassium hydroxide solution and this was treated with hydrochloric acid as described for 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid. 4-Chloro-(8'-bromo-6'-quinolyl)-anthranilic acid proved very difficult to crystallise, so it was converted to the potassium salt by adding concentrated potassium hydroxide solution to an alcoholic solution of the crude acid. This precipitated the potassium salt which was filtered off and extracted with boiling alcohol. The residue consisted of pure potassium 4-chloro-(8'-bromo-6'-quinolyl)-anthranilate and was converted to the acid by treatment with 2N hydrochloric acid followed by dilute ammonia solution. The acid was filtered off, well washed with water and dried. Yield 58 g., m.p. 293°. (Found: C, 50.7; H, 2.75; N, 7.55%. C₁₆H₁₀N₂Cl₂Br requires C, 50.9; H, /
H, 2.65; N, 7.4\%)

4-Chloro-(8'-bromo-6'-quinolyl)-anthranilic acid resembles 4-chloro-(8'-chloro-6'-quinolyl)-anthranilic acid in general properties, but was found to be more difficult to purify by crystallisation.
Attempts to cyclise 4-chloro-(8'-bromo-6'-quinolyl)-anthranilic acid

I. With phosphorus oxychloride.

4-Chloro-(8'-bromo-6'-quinolyl)-anthranilic acid (5 g.) was refluxed with phosphorus oxychloride (25 cc.) for 6 hours in an oil bath at 150°. The excess phosphorus oxychloride was removed by distillation under reduced pressure and the residue triturated with sodium hydroxide solution and ice. The insoluble material was filtered off, dried and extracted with hot dry benzene. The extract deposited a brownish substance on cooling and this was recrystallised from dry benzene yielding light brown microcrystals, m.p. 273°. Yield 2.7 g. (Found: C, 55.95; H, 2.3; Ag halides = 1 mg. substance, 1.250 mg.

C₁₄H₁₇N₂Cl₂Br requires C, 50.8; H, 1.85; Ag halides = 1 mg. substance, 1.267 mg.)

A sample of this compound was heated with 2N hydrochloric acid to convert it into the pyridoacridone. The bright yellow product was crystallised from alcohol and melted over 400°. (Found: C, 57.9; H, 2.7%; Ag halides = 1 mg. substance, 0.905 mg.

C₁₆H₁₆ON₂Cl₂Br requires C, 53.4; H, 2.2; Ag halides = 1 mg. substance, 0.922 mg.)

Before the analytical results for these two compounds had been received two attempts were made to condense the product of the reaction with phosphorus oxychloride with β-diethylaminopropylamine, in dry phenol in the usual manner. The first of these experiments yielded a yellow crystalline compound/
compound, m.p. 231°. (Found: C, 60.3; H, 3.85; N, 12.0%. C₂₆H₁₄N₄ClBr requires C, 58.5; H, 5.1; N, 11.9%). In the second experiment difficulty was experienced in crystallising the product, but the sticky material was found to have deposited large yellow prisms after four months in the refrigerator. This material melted at 125°. (Found: C, 65.55; H, 5.5; N, 10.9%; Ag halides = 1 mg. substance, 0.601 mg. C₂₆H₁₄N₄ClBr requires C, 58.5; H, 5.1; N, 11.9%; Ag halides = 1 mg. substance, 0.703 mg.)

II. With phosphorus oxychloride in nitrobenzene.

4-Chloro-(8'-bromo-6'-quinoly)-anthranilic acid (5 g.) was refluxed with phosphorus oxychloride (5 cc.) in nitrobenzene (25 cc.) for 6 hours in an oil bath at 150°. The mixture was cooled, poured into 4N sodium hydroxide solution (100 cc.) with vigorous stirring, and the nitrobenzene removed from the alkaline liquid by distillation in steam. The residual solid was filtered off, washed, dried in a dessicator, and extracted with hot dry benzene in presence of a pellet of potassium hydroxide. The extract on cooling deposited a brownish material which was crystallised from dry benzene. Yield 1.2 g., m.p. 222-223°. (Found: C, 57.15; H, 2.95%; Ag halides = 1 mg. substance, 1.065 mg. C₂₆H₁₄N₄Cl₂Br requires C, 50.8; H, 1.85%; Ag halides = 1 mg. substance, 1.257 mg.)

A sample of this compound was heated with 2N hydrochloric acid to convert it to the pyrido-acridone. The bright yellow product was crystallised from/
from alcohol and melted over 400°. (Found: C, 58.5; H, 3.0%; Ag halides = 1 mg. substance, 0.891 mg. 
C₆H₇NOClBr requires C, 53.4; H, 2.2%; Ag halides = 1 mg. substance, 0.922 mg.)

III. With phosphorus oxybromide in nitrobenzene.

An attempt was made to prepare 2:5-dibromo-8-chloro-3:4:2':3'-pyridoacridine by refluxing 4-chloro-(8'-bromo-6'-quinolyl)-anthranilic acid (5 g.) with phosphorus oxybromide in nitrobenzene (5 g. POBr₃ in 25 cc.) for 6 hours in an oil bath at 200°. The product was worked up as described under attempt II, the solid which remained after steam distillation being filtered, dried and extracted with dry benzene in the presence of a pellet of potassium hydroxide. Brown micro-crystals were obtained from dry ligroin in relatively poor yield (0.8 g.), m.p. 121-122°. (Found: C, 55.2; H, 3.1; N, 9.3%; Ag halides = 1 mg. substance, 0.758 mg. 
C₆H₇NOClBr requires C, 45.4; H, 1.7; N, 6.6%; Ag halides = 1 mg. substance, 1.250 mg.)

On repeating this experiment with four times the quantities given above the same product was obtained, but in this case it was noticed that the filtrate after filtering off the solid which had separated during the steam distillation to remove nitrobenzene deposited a greyish crystalline material on standing over the weekend. This was filtered off and dried. It melted at 375° and in general properties resembled a pyridoacridone, being soluble in concentrated mineral acid but insoluble in alkali. In alcohol/
alcohol solution it fluoresced green in white light, blue in blue light and bright red in purple light. Recrystallisation from alcohol left the melting point unchanged. (Found: C, 47·1; H, 2·4%; Ag halides = 1 mg. substance, 0·411 mg. \( \text{C}_4\text{H}_6\text{ON}_2\text{ClBr} \) requires C, 53·4; H, 2·2%; Ag halides = 1 mg. substance, 0·922 mg.)

IV. With concentrated sulphuric acid.

4-Chloro-(8'-bromo-6'-quinolyl)-anthranilic acid (5 g.) was heated at 100° for 4 hours with concentrated sulphuric acid (50 cc.), and the product worked up as already described for 2-methyl-8-chloro-3:4:2':3'-pyridoacridone. The final material was obtained as brownish microcrystals from alcohol, m.p. over 400°. (Found: C, 52·0; H, 2·6; N, 8·55%. \( \text{C}_4\text{H}_6\text{ON}_2\text{ClBr} \) requires C, 53·4; H, 2·2; N, 7·8%).

As this analysis seemed to indicate that the compound was substantially 2-bromo-8-chloro-3:4:2':3'-pyridoacridone, the sample was recrystallised from alcohol, after which it still gave brown micro-crystals, m.p. over 400°. (Found: C, 51·95; H, 2·55%; Ag halides = 1 mg. substance, 0·828 mg. \( \text{C}_4\text{H}_6\text{ON}_2\text{ClBr} \cdot 3/4\text{H}_2\text{O} \) requires C, 51·5; H, 2·55; Ag halides = 1 mg. substance, 0·889 mg.)

The possible significance of all these results has already been discussed in Section II (pp 58-64).
5- and 7-Nitroquinolines.

(Cf. T.J. Elliott, Thesis, University of Leeds.)

Concentrated sulphuric acid (533.3 cc.) was added to water (200 cc.) to give 69% sulphuric acid. To this was added m-nitraniline (60 g.) followed by glycerin (134.4 cc.) and arsenic acid (147.4 cc.) (65% As2O3 solution), and the mixture boiled gently under reflux for 1½ hours when no unchanged amine could be detected by the diazo reaction. After cooling, the mixture was made alkaline with 10N sodium hydroxide solution, ice (6 kg. in all) being added to keep the temperature from rising. The precipitate which was filtered off consisted of a mixture of 5- and 7-nitroquinolines mixed with a certain amount of inorganic matter. It was extracted with chloroform (600 cc.), charcoal being added, filtered hot, and the chloroform layer separated off from the filtrate and evaporated to dryness, leaving a pale brown solid (70 g.). This mixture of 5- and 7-nitroquinolines was divided into two lots of 35 g. each of which was dissolved in 300 cc. boiling water containing concentrated nitric acid (25 cc.) to give a pale orange solution. On allowing this solution to cool to 20° an orange precipitate of 5-nitroquinoline nitrate separated and was filtered off, m.p. 191°. The two lots of nitrate were combined and suspended in 2 litres of water, warmed to about 60° and basified with 10N sodium hydroxide solution. The 5-nitroquinoline precipitated as/
as white needles, was filtered off, dried at 40° and then in a dessicator, m.p. 71°. Yield 36 g. The mother liquors from the 5-nitroquinoline nitrate which contained all the 7-nitroquinoline and some residual 5-nitroquinoline were also combined and basified with 10N sodium hydroxide solution and the precipitate filtered off and dried in a dessicator. The 5-nitroquinoline was dissolved out by light petroleum (b.p. 40-60°) by soxhlet extraction. The insoluble material consisted of the 7-nitroquinoline and this was crystallised from benzene as brown yellow needles, m.p.131°. Yield 12.5 g. The residue (12 g.) obtained on distilling off the light petroleum melted at 59-61° and was evidently a mixture of the two isomers. It was therefore separated through the nitrates followed by light petroleum extraction to purify the 7-nitroquinoline as described above, yielding 5-nitroquinoline (9.6 g.), m.p. 69° and 7-nitroquinoline (1.9 g.), m.p. 131°. Total yield of 5-nitroquinoline, 45.6 g. Total yield of 7-nitroquinoline, 14.4 g.
5-Aminoquinoline.

(Cf. West, J.C.S., 1925, L27, 494.)

5-Nitroquinoline (34.8 g.) was dissolved in methylated spirit (134 cc.) and concentrated hydrochloric acid (5 cc.) at the boil and iron filings (46 g.) added in small portions over ½ hour. The product was worked up as described for 6-amino-8-chloroquinoline. 5-Aminoquinoline crystallised from benzene as dark brown needles, m.p. 106°. Yield 22 g.
4-Chloro-5'-quinolylantranilic acid.

Dobson and Kermack, J.C.S., 1946, 150.)

5-Aminoquinoline (7.4 g.) and potassium 2,4-dichlorobenzoate (11.4 g.) were condensed in amyl alcohol (10 cc.) in the presence of copper bronze (0.1 g.) as already described for 4-chloro-(3'-methyl-6'-quinoyl)-anthranilic acid. The product after removal of the amyl alcohol by steam distillation was filtered off and extracted with 2N potassium hydroxide solution on the boiling water bath. It dissolved completely and the alkali extract was acidified with acetic acid, the precipitate after filtering and drying, crystallising from alcohol as yellow brown needles, m.p. 244°. Yield 8.2 g. (Found: C, 61.4; H, 3.8; N, 9.25%.

C_{16}H_{10}O_{3}Cl.NaCl.3H2O requires C, 61.5; H, 4.0; N, 9.0%.)

4-Chloro-5'-quinolylantranilic acid is soluble in dilute alkali and hot alcohol and sparingly soluble in warm dilute acid. It forms sodium and potassium salts which separate with concentrated sodium or potassium hydroxide solutions.
5:8-Dichloro-1:2:3':3'-pyridoacridine

(Cf. Magidson, Ber., 1933, 66, 866.
Drosov, Chimistheski, 1934, 4, 117.
Albert and Linnell, J.C.S., 1936, 89.
Dobson and Kermack, J.C.S., 1946, 150.)

4-Chloro-5'-quinolylanthranilic acid (5 g.) was refluxed with phosphorus oxychloride (30 cc.) and phosphorus pentachloride (0.5 g.) for 6 hours in an oil bath at 150°. After removal of excess phosphorus oxychloride by distillation under reduced pressure, the residue was triturated with sodium hydroxide solution and ice, the insoluble material being filtered off and dried. It almost completely dissolved in boiling dry benzene, a pellet of potassium hydroxide being added to prevent hydrolysis to the pyridoacridone. Crude 5:8-dichloro-1:2:3':3'-pyridoacridine separated from the benzene extract on cooling, and crystallised from dry benzene in buff coloured needles, m.p. 244°. Yield 5.7 g. 
(Found: C, 63.9; H, 2.85; OI, 24.0%. C₁₆H₁₈N₂Cl₂ requires C, 64.2; H, 2.7; OI, 23.7%) 

5:8-Dichloro-1:2:3':3'-pyridoacridine resembles 2:5:8-trichloro-3:4:2':3'-pyridoacridine in general properties.
5:8-Dichloro-1:2:3':2'-pyridoacridine

(Dobson and Kermack, J.C.S., 1946, 150.)

5:8-Dichloro-1:2:3':2'-pyridoacridine (1 g.)

and (γ-diethylaminopropylamine (1 g.) were heated
together in dry molten phenol at 100° for 2 hours.
The cooled phenol mixture was poured into sodium
hydroxide solution and the sticky yellow precipitate
extracted with ether. The ethereal solution was
extracted in turn with 5% acetic acid from which the
base was precipitated with ammonia and re-extracted
with ether. This ethereal extract was dried over
anhydrous potassium carbonate and the ether distilled
off. The residual reddish brown oil after two
recrystallisations from dry ligroin formed bright
yellow needles, m.p. 98-99°. (Found: C, 70.5;
H, 6.35; N, 14.1; Cl, 9.15%. C₃₆H₃₄N₄Cl requires
C, 70.3; H, 6.4; N, 14.3; Cl, 9.0%.)

8-Chloro-5-(γ-diethylaminopropylamino)-
1:2:3':2'-pyridoacridine resembles 2:8-dichloro-5-
(γ-diethylaminopropylamino)-3:4:2':5'-pyridoacridine
in general properties, but gives an unusually vivid
light blue fluorescence in ether solution.
7-Aminoquinoline.

(Cf. West, J.C.S., 1925, 127, 494.)

7-Nitroquinoline (3.7 g.) was dissolved in methylated spirit (33.3 cc.) and concentrated hydrochloric acid (5 cc.) at the boil, and iron filings (11.5 g.) were added in four portions at 5 minute intervals. The product was worked up as described for 6-amino-8-chloroquinoline. 7-Aminoquinoline crystallised from benzene as dark brown needles, m.p. 73°. Yield 5g.

7-Aminoquinoline exhibits a strong green fluorescence in alcohol solution and has a powerful yellow dyeing action on the skin. Otherwise, it resembles other aminoquinolines in general properties.
Condensation of 7-Aminoquinoline and Potassium 2:4-
dichlorobenzoate.

Dobson and Kermack, J.C.S., 1946, 150.)

7-Aminoquinoline (14.8 g.) and potassium
2:4-dichlorobenzoate (22.8 g.) were condensed in
amyl alcohol (20 cc.) in the presence of copper
bronze (0.1 g.) as already described for 4-chloro-
(8'-methyl-6'-quinolyl)-anthranilic acid. The
product after removal of the amyl alcohol by steam
distillation was filtered off and extracted with
2N potassium hydroxide solution on the boiling water
bath. It dissolved completely and the alkali extract
was acidified with acetic acid, the precipitate
being filtered and dried. It was found very diffi-
cult to crystallise this product, which was presum-
ably 4-chloro-7'-quinolylantrhanilic acid. A
brown compound was, however, obtained from a fairly
concentrated alcohol solution on cooling, and this
had m.p. 389°.
The product from the condensation of 7-aminoquinoline and potassium 2:4-dichlorobenzoate which was presumably 4-chloro-7'-quinolylanthranilic acid (15 g.) was refluxed with phosphorus oxychloride (90 cc.) containing phosphorus pentachloride (1.5 g.) for 6 hours in an oil bath at 150°. After removal of excess phosphorus oxychloride by distillation under reduced pressure, the residue was triturated with sodium hydroxide solution and ice, the insoluble material being filtered off and dried in a desiccator. It was extracted with boiling dry benzene in the presence of a pellet of potassium hydroxide, only a very small residue of insoluble material remaining unextracted. The extract on cooling deposited light brown needles which were filtered off and recrystallised further from dry benzene, giving a final melting point of 209°. Yield 9.2 g.

(Found: C, 65.2; H, 2.95; N, 9.2; Cl, 31.3%).

C₁₆H₁₇N₂Cl₄ requires C, 64.2; H, 2.7; N, 9.4; Cl, 23.7%. 3C₁₆H₁₇N₂Cl₄·C₁₆H₂O₆Cl requires C, 65.2; H, 2.8; N, 9.5; Cl, 21.1%).

The significance of the analytical results has already been explained on page 71.
Attempt to prepare 6-chloro-8-(2-diethylaminopropylamino)-3:4:5:6'-pyridoacridine.

(Cf. E.P. 363392.

Dobson and Kermack, J.C.S., 1946, 156.)

5:6-Dichloro-5:4:3:2'-pyridoacridine (1 g.)

(somewhat hydrolysed as the analytical results ultimately indicated) and 9-diethylaminopropylamine (1 g.) were heated together in dry molten phenol at 100° for 2 hours. The cooled phenol mixture was poured into sodium hydroxide solution when a solid yellow compound was obtained. This was dissolved readily in 5% acetic acid in the cold, and reprecipitated from acid solution by ammonia. This precipitate was filtered off and dried in a dessicator.

It was recrystallised from dry ligroin raising the melting point from 97-99° to 99-100°. A further recrystallisation from dry ligroin left this m.p. unchanged. The compound consisted of orange yellow needles. Yield 0.8 g. (Found: C, 67.8; H, 6.0; N, 12.5; Cl, 9.55%. C20H26N4Cl requires C, 70.3; H, 6.4; N, 14.3; Cl, 9.0%. 3C20H26N4Cl·Cr2K10M2Cl·3H2O requires C, 67.5; H, 5.9; N, 13.0; Cl, 9.4%.)

These results have been discussed on page 78.
3'-Nitro-3-chlorodiphenylamine-6-carboxylic acid.

(Cf. Ullmann and Bader, Ann., 1907, 355, 332.
Goldberg and Kelly, J.C.S., 1946, 102.)

m-Nitraniline (13.8 g.), potassium 2:4-dichlorobenzoate (22.9 g.), copper bronze (0.1 g.) and cyclohexanol (30 cc.) were heated together under reflux for 15 hours in an oil bath at 170°. The cyclohexanol was removed by distillation in steam and the residue, a black rubbery tar, was extracted several times, first with hot 3N hydrochloric acid to remove unchanged m-nitraniline, and then with hot water to remove unchanged 2:4-dichlorobenzoic acid. The black residue consisted of crude 3'-nitro-3-chlorodiphenylamine-6-carboxylic acid. It was extracted with hot 2N potassium hydroxide solution, precipitated by acetic acid and crystallised from alcohol as yellow needles, m.p. 249-250°. Yield 4.4 g. (Found: C, 52.85; H, 3.45; N, 9.7%.
C₁₈H₁₀O₄N₂Cl requires C, 53.3; H, 3.1; N, 9.6%.)

3'-Nitro-3-chlorodiphenylamine-6-carboxylic acid is soluble in dilute alkali and hot alcohol. It is insoluble in dilute acid and water.
4(?)-Nitro-8-chloroacridone.

(Cf. Ullmann and Bader, Ann., 1907, 355, 332.
Goldberg and Kelly, J.C.S., 1946, 102.)

3'-Nitro-8-chlorodiphenylamine-6-carboxylic acid (2 g.) was heated at 100° with concentrated sulphuric acid for 4 hours, allowed to cool and poured on to ice. The yellow brown precipitate was filtered off, suspended in water and treated with ammonia to produce yellow 4(?)-nitro-8-chloroacridone. Crystallisation from alcohol yielded yellow needles unmelted at 330°. (Found: C, 55.7; H, 2.15; N, 10.6%; Ash, trace. C_{18}H_{7}ClN_{5} requires C, 56.8; H, 2.55; N, 10.2%. C_{18}H_{7}ClN_{5}Cl.1/4H_{2}O requires C, 55.9; H, 2.7; N, 10.0%.)

4(?)-Nitro-8-chloroacridone is soluble in concentrated sulphuric acid and hot alcohol and insoluble in dilute alkali. It gives a faint yellow green fluorescence in alcoholic solution.
IV. SUMMARY.

1. A survey of various types of antimalarial compounds is given, and some aspects of the relation between chemical constitution and antimalarial activity are discussed.

2. The preparation is described of 5-chloro-3:4:2':3'-pyridoacridine derivatives from 6-amino-8-chloroquinoline and 2:4-dichlorobenzoic acid or 8-chlorobenzoic acid from 6-amino-8-methylquinoline and 2:4-dichlorobenzoic acid. An attempt to prepare 2-bromo-5:8-dichloro-3:4:2':3'-pyridoacridine from 6-amino-8-bromoquinoline and 2:4-dichlorobenzoic acid is also described. In the case of 2:5:8-trichloro-3:4:2':3'-pyridoacridine the 8-chlorine atom has been replaced by \( \gamma \)-diethylaminopropylamino and \( \gamma \)-monobutylaminopropylamino side chains and an attempt has been made to replace it by a \( \varepsilon \)-diethylamino-\( \alpha \)-methylbutylamino side chain. 2-Methyl-3-chloro-5-(\( \gamma \)-diethylaminopropylamino)-3:4:2':3'-pyridoacridine has been successfully prepared from 2-methyl-5:8-dichloro-3:4:2':3'-pyridoacridine and \( \gamma \)-diethylaminopropylamine.

3. 2:8-Dichloro-5-(\( \gamma \)-monobutylaminopropylamino)-3:4:2':3'-pyridoacridine, in addition to being prepared from 2:5:8-trichloro-3:4:2':3'-pyridoacridine/
acridine and γ-monobutylaminopropylamine, has also been synthesised by the cyclisation of the amide prepared from 4-chloro-(3'-chloro-6'-quinolyl)-anthranilic acid chloride and γ-monobutylaminopropylamine.

4. 5:8-Dichloro-1:2:3':2'-pyridoacridine has been prepared by condensation of 2:4-dichlorobenzoic acid and 5-aminoquinoline and subsequent cyclisation of the product with phosphorus oxychloride containing phosphorus pentachloride. This compound has been condensed with γ-diethylaminopropylamine to yield 8-chloro-5-(γ-diethylaminopropylamino)-1:2:3':2'-pyridoacridine. A similar series of reactions has been carried out in which 5-aminoquinoline has been replaced by 7-aminoquinoline. The final compound obtained appeared pure and resembled the expected 8-chloro-5-(γ-diethylaminopropylamino)-3:4:3':2'-pyridoacridine in its properties, but the analysis suggests that it contains some 8-chloro-3:4:3':2'-pyridoacridones in a relatively stable molecular complex.

5. All the new derivatives of 3:4:2':3'-pyridoacridine tested show antimalarial activity against _Plasmodium gallinaceum_ in chicks, and the biological activities are compared with those obtained for the other pyridoacridines previously prepared by Dobson and Kermack (J.C.S., 1946, 150). It is shown that a chlorine atom in position 2 of the 3:4:2':3'-pyridoacridine/
pyridoacridine nucleus appears to be even more important than the chlorine atom in position 8, while 2:8-dichloro-5-(γ-diethylaminopropylamino)-3:4:2′:3′-pyridoacridine which possesses chlorine atoms in both positions exhibits the highest activity, being not less active than mepacrine.

6. Quantities of 2:8-dichloro-5-(γ-monobutylaminopropylamino)-3:4:2′:3′-pyridoacridine and 8-chloro-5-(γ-diethylaminopropylamino)-1:2:3′:2′-pyridoacridine have been submitted for biological testing, but the results are not yet available.

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I should also like to place on record my gratitude to my supervisor, Dr W.O. Kernack, F.R.S., for much helpful encouragement and advice.

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I. INTRODUCTION

Malariologists are agreed that there are two distinct problems in the chemotherapy of malaria, one concerning the treatment of the disease, and the other its complete and certain prevention in persons who are exposed to the bite of infected mosquitoes. How distinct the two problems are can be understood if certain aspects of the life cycle of the malarial parasite are emphasised.

Every textbook of parasitology describes the salient characters of this life cycle, the development of the parasite in the mosquito, the inoculation of sporozoites into the vertebrate host when an infected mosquito bites it, and the asexual reproduction of the parasite which subsequently occurs in the red blood cells of the vertebrate, but few of these texts as yet draw attention to two very important points in this life history, firstly that Schaudinn's observation that the injected sporozoites penetrate the red blood cells directly has never been confirmed, and secondly that there is a period of days or weeks, following the bite of a mosquito before parasites can be demonstrated in the blood. The immediate fate of the injected sporozoites was, until recently, uncertain, but now it is known that at least in bird malaria, a period of development of the parasite occurs in the tissues of the vertebrate before the red blood cells are invaded, and it is the duration of this "tissue phase" which constitutes the incubation period or, more strictly, the prepatent period of the infection (Raffaele, 1936; Kikuth and Mudrow, 1939; Huff, Coulston and Cantrell, 1943; personal observations). It is not proposed, at this time, to quote all the evidence for the existence of the tissue phase which follows the injection of the sporozoites, because it is hoped to publish separately a full account of the research which has been done in these laboratories on the biology of the malarial parasite in the vertebrate host. A simple experiment may be described, however, to show that at least the sporozoite quickly leaves the blood stream, and that no blood infection can be subsequently demonstrated for several days. Thus, if sporozoites of *Plasmodium gallinaceum* are injected intravenously into chicks, subinoculation of the injected chicks between a period of fifteen minutes and about four days afterwards yields negative results.
contributed to the perturbation. In the first place, certain malarialogists, among whom James is particularly noteworthy, were emphasising that the urgent problem awaiting chemotherapy solution was not a substitute for quinine, but the discovery of a substance which, taken by people exposed to the bite of the infected mosquitoes, would prevent parasites developing as far as the blood phase, an accomplishment which quinine did not possess. In other words, it was argued that quinine, and for that matter mepacrine, were satisfactory suppressive or clinical prophylactic drugs; what was needed now was a casual prophylactic drug, a drug which would kill either the sporozoite or the phase intervening between the sporozoite and the blood phase, and clearly the search for such a drug would involve the use in the laboratory of a mosquito-borne infection. These malarialogists were not, of course, arguing against the Roehl test as such, but they were objecting to the exclusion of other tests from the laboratory.

The second factor contributing to the desire for an overhaul of laboratory methods was bound up with the specificity of action of drugs. Commencing in 1937 reports began to be published showing that certain of the sulphonamide drugs were active in human malaria. True their action was not so good as that of quinine or mepacrine, but nevertheless it was definite. On the other hand none of the sulphonamides could be shown to have any measurable effect when tested by Roehl's method against P. relictum, a fact which was disturbing. The sulphonamides might or might not constitute an important lead towards new types of antimalarial substances, but clearly they could not be ignored completely, and a laboratory infection had to be sought which was sensitive to them. Preferably the new infection, as well as being sensitive to sulphonamides, should also be sensitive to all the other types of substances known to be efficacious in human malaria. It happened that, almost coincident with the height of the perturbation concerning the Roehl test, two new species of bird malarial parasites were discovered, both of which were potential laboratory infections. The first was P. gallinaceum, described by Brumpt in 1935, and the second was P. lophurae, described by Coggeshall in 1938. In the same year Coggeshall showed that P. lophurae was sensitive to sulphonamides.

We in these laboratories entered into the field of malarial chemotherapy just early enough to feel the air of doubt about the satisfactoriness of the Roehl test. We had used it with increasing disquiet for some time, and then, about eighteen months ago, after much discussion, we decided to overhaul completely our laboratory methods. The exigencies of the war situation had prompted us to make research on the chemotherapy of malaria one of our major problems, and we planned our attack according to the following conclusions:

(a) While the great malarial problem awaiting a satisfactory solution might be the discovery of a substance exerting true casual prophylactic action, we believed that the loss of Java, the possible shortage of quinine after the war, and several drawbacks to mepacrine - its effect in yellowing the skin, the many stages in its synthesis, etc. - made it imperative that we conduct an intensive search for a suppressive drug as well as a casual prophylactic one.
was given by Huff and Bloom (1935) who had shown that *P. elongatum* in canaries infected lymphocytes and monocytes as well as erythrocytes, and also blood forming cells in the bone marrow and spleen. The tendency was to regard *P. elongatum* as specialised in the sense that it had extended its parasitising activities to include blood cells other than red blood cells, but the work of James and Tate raised larger issues. The stage in *P. gallinaceum* which they described occurs in endothelial cells in all the tissues of the body - hence their description exoerythrocytic stage. It is plentiful in the brain and spleen, and can be found almost as easily in the liver, lungs and kidneys. It will develop following the simple inoculation of infected red cells or, in much larger numbers, following the injection of sporozoites (James, 1939).

James and Tate suggested that an exoerythrocytic stage, similar to that in *P. gallinaceum*, might exist in human malaria, and might explain the problem of relapses. This was the larger issue which they raised, and in it lies the core of the controversy which is still waged. It is, however, unnecessary to discuss the details of the controversy here, and it will be sufficient if we point out that, up to the present, exoerythrocytic forms have been described definitely only in bird malaria, and it appears to us that more than one type of exoerythrocytic form exists. It was mentioned that the sporozoite after it has been inoculated into the vertebrate host, develops first in the tissues and it is only at the end of the tissue phase that the red blood corpuscles are invaded. We have evidence that this tissue phase is different from the exoerythrocytic stage described by James and Tate, the first being a developmental phase, while the second is a schizogonic phase which is capable of growth and reproduction in the tissues for, it seems, an indefinite period. (Davey, 1944). The course of development of *P. gallinaceum* in the chicken, according to this view, can be illustrated diagrammatically as follows:

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Blood phase (reproduces itself by continual schizogony).

Sporozoite primary tissue phase (developmental)

Secondary tissue phase (reproduces itself by continual schizogony).
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The secondary tissue phase of this scheme is the original exoerythrocytic stage of James and Tate, the name having been changed because exoerythrocytic has to wide a meaning. It appears probable that the primary tissue phase is common to all species of malarial parasites, while the secondary tissue phase might be a specialised feature present in only a limited number of species. However, more research is needed on this point.

The presence of exoerythrocytic forms (i.e., a secondary tissue phase in the nomenclature used in this paper) in *P. gallinaceum* has been considered by some workers to be a drawback to the use of the species as a laboratory test infection. It is true that no drug is yet known which attacks this phase and no chick can be cured of
(ii) and (iii) The inoculum and the density of the infection. The inoculum consists of blood drawn from heavily parasitised chicks whose infection is not more than five days old. The blood is partly heparinized and partly citrated, and, again to make the infection acute and to make it possible to commence immediate treatment against an established blood infection, it is given intravenously to the experimental animals, usually into the right jugular vein. An infection initiated in this way climbs rapidly to a peak, and then, if it has not been too heavy, a spontaneous recovery from the acute infection occurs, or at least is attempted, and the number of parasites in the blood commences to diminish. It is clear that in assessing the action of a drug blood smears must be read before the spontaneous recovery begins, and preferably at or near the peak of the parasitaemia curve. We wished this peak to occur on the Friday following the Monday when a test was started, and we wanted rather more than half the corpuscles to be parasitised at this time. We found that an inoculum containing about 40 to 50 million parasitised corpuscles achieved this end; we found, too, that an estimation of the number of parasitised corpuscles in a random sample of 500 red blood cells was a satisfactory method of computing the density of the infection.

Some of the data on which these conclusions are based are shown in Graph I which illustrates the course of infection in ten untreated chicks each of which receive approximately 45 million parasitised corpuscles; it shows the type of infection we aim at, and gives a concise idea of what is meant by the term "peak of the parasitaemia curve".

(iv) The frequency of dosing. Malaria is such a ubiquitous disease that treatment of the majority of cases cannot possibly be under medical supervision continually; consequently an antimalarial drug must be efficacious when given orally and in our search for new antimalarial substances it is our customary measure to administer all speculative substances by this route. In exceptional cases when we suspect that a substance is not absorbed very well from the alimentary tract, and when we are particularly interested in its possibly intrinsic activity, we administer it intraperitoneally.

How often a dose should be repeated is a matter for some debate. Substances will vary in the rate at which they are excreted, and active drugs will vary in the time taken for them to exhibit activity. It is clearly difficult to lay down a regime of dosing which will cover all eventualities and, at the same time, be practicable for testing on a big scale. Some workers, e.g., Marshall, Lichfield and
and because, in the *P. gallinaceum* test, we see every gradation between no parasites and perhaps 400 or more in 500 corpuscles we foresaw the same difficulties in our work. Fortunately, however, we were allowed to conduct a fair amount of testing before it became necessary for us to make a decision on the point, and by that time we had found an answer to the problem.

The answer came from the large amount of work which we did on the effect of mepacrine on *P. gallinaceum*. We used this drug to establish a method for assessing activity, and in so doing we found the criterion which we think indicates slight activity of a substance. Briefly, at the border-line dose where mepacrine begins to lose its activity the variation in the parasitic density in different birds is very great; some of the estimates overlap with those obtained in a control group, while others are quite far removed from the controls. In other words, the results of treatment with a drug which, under a particular dosage regime, has only slight activity are such that they exhibit a greater variation than is to be expected. In practice this works out that if the results in two or more treated birds are out of line with the controls the test is repeated; if the original results are confirmed they are taken to indicate slight activity of the drug. The extent of the variation is reflected, of course, in the average figure for the group, and statistical analysis has shown that when the control average is about 300 a difference of about 90 in the chemotherapeutic effect is significant as an indication of chemotherapeutic effect. The explanation of the variation, more than probably, is bound up with the absorption of the drug; some birds absorbing better than others and maintaining an effective concentration for a longer time. If the dose can be increased, so that more drug is absorbed, results can be made uniform, as in the case of mepacrine. The figures quoted in table 1 (in each instance they represent the proportion of a random sample of 500 red blood corpuscles which, at the peak of the parasitaemia curve, is parasitized) illustrate this fact, and give an adequate idea of the gradations of activity encountered in the test.

One more point in connection with the test for drugs with suppressive activity needs to be mentioned. The activity of a drug is judged, for the most part, from the readings of the blood smears made at the peak of the parasitaemia curve. Smears, however, are also taken on the two previous days, although they are read only if the results in any particular birds on the supposed peak day are out of line with the controls. In this way it can be ascertained whether the lack of alignment is due to a slowly rising infection, which sometimes does occur in an unusually refractory chick or perhaps in a chick which has not received the full inoculum, or is due to a rapidly rising infection, in which case the peak may have been passed (a rare occurrence).

The contrast between the Roehl test and the chick test will now be clear. The canaries used in the Roehl test are adult birds, and the route of inoculation of parasites (the blood phase drawn from donor canaries) is intramuscular. Under these conditions the infection is almost invariably benign; parasites cannot easily be found in the blood for about five days, and they may commence to disappear three or four days later as the immunity of the bird develops. Treatment of
The timetable of the *P. gallinaceum* test is as follows -

**Monday** (i) 9 a.m. Donor birds with a four day old infection, and showing at least half the corpuscles parasitised, are bled. The blood is mixed with an equal volume of a citrate-heparin mixture, and an estimate is made of the corpuscular content of the suspension, and of the proportion of the corpuscles which are parasitized. The blood is then further diluted with citrated saline to give a mixture containing approximately 45 million parasitized corpuscles in each 0.2 ml.

(ii) The chicks selected by weight for the experiment are inoculated into the right jugular vein with 0.2 ml of the blood mixture. A practised worker will inoculate two to three chicks per minute in this way.

(iii) The birds are weighed and numbered, and arranged in groups of six. Six birds are used for the trial of each dose of each drug, and for each control group.

(iv) 4 p.m. The first dose of drug is given. The dose is calculated for a 50 gramme chick and dissolved or dispersed in 1 ml of water; it is then administered by a catheter tube passing into the gizzard.

**Tuesday.** Dosing is repeated at 9 a.m. and 5 p.m.

**Wednesday.** At 9 a.m. all birds are dosed; at 10 a.m. smears are made; at 5 p.m. dosing is repeated.

**Thursday.** The procedure is the same as Wednesday.

**Friday.** At 9 a.m. smears of the control birds are made, stained, and read. If the parasite level indicates that the peak has been reached (as it should have been with the inoculum that was used), smears are made of all birds. They are stained with Giemsa, read, and the test ends.

III THE ASSAY OF SUBSTANCES FOUND TO HAVE A SUPPRESSIVE ACTION AND THE CHOICE OF DRUGS FOR CLINICAL TRIAL.

In the account which was just given of the use of *P. gallinaceum* in chicks as a test for substances with suppressive activity the difficulty of drawing an arbitrary line between slight activity and inactivity was emphasised. In the same way it is equally as difficult to make an arbitrary definition for what constitutes marked activity. No drug known to us will eradicate the parasites from an infected chick, an achievement which, of course, would constitute marked activity, and there are no clear symptoms which might serve as criteria of a clinical cure. It might be thought that time of death could be used, but this is too variable a factor, and in any event it is complicated by the presence of the secondary tissue phase in the infection. The only course left to us, therefore, was to determine what a known antimalarial drug would accomplish in our test, and then to compare any active substances with this standard.

Our choice of a standard drug lay between quinine and mepacrine. Purely from the point of view of efficacy in malarial infections there is little to choose between them; both are effective against the same parasites, and both attack the same forms in the life cycle, so that which is used as a standard is entirely a matter
under those conditions, a dose of 2 mg/50 gm. will accomplish as much
as 10 mg/50 gm., but if we change our dosage schedule this fact no
longer holds good; if, for example, we administer the drugs only once,
say four hours after injection, a dose of 2 mg/50 gm. will not
accomplish what a dose of 10 mg/50 gm. will, and the critical dose
then becomes about 6 mg/50 gm. to 8 mg/50 gm.

We have not tried to delimit the critical dose of mepacrine
against P.gallinaceum with any great accuracy; we know that it is
approximately 2 mg/50 gm., and we are satisfied with such an approx-
imation because, instead of quoting a critical dose, we prefer to
quote a critical dosage region. Thus we can say, in the case of
mepacrine, that a dose of 2 mg/50 gm., administered twice daily in
the acute attack, accomplishes virtually everything of which the drug
is capable, whereas at 1 mg/50 gm. much of the activity is lost, how
much being dependent on the absorptive powers of the bird. The region
1 mg/50 gm. to 2 mg/50 gm. is therefore the critical dosage region for
mepacrine, and in our assay of a new active drug we find its critical
region and compare it with that for mepacrine. What happens afterwards
depends upon the constitution of the new drug, the nearness of its
critical region to that of mepacrine, and its toxicity in a mammal.
The procedure will become clear if we consider a concrete example.

The first new antimalarial drug unrelated to any known drug
which we discovered was M.2666, which has the constitution

\[
\begin{align*}
\text{Cl} & \quad \text{NH} \\
\text{NH} & \quad \text{CH}_2\text{CH}_2\text{N.}(\text{C}_2\text{H}_5)_2\text{.2HCl}
\end{align*}
\]

Its critical dosage region was found to be 2 mg/50 gm. to 4 mg/50 gm.,
and its chronic toxicity for mice was two and a half to three times
that of mepacrine. If we assume that activity against P.gallinaceum
is a fair indication of activity in human malaria, we could say from
these figures that the chances of M.2666 exhibiting activity in human
malaria were less by five or six times than those of mepacrine.
However, since M.2666 was an entirely new type of antimalarial drug,
and there was always the possibility of striking a favourable example
of specificity of action, we decided to give it a clinical trial in
human malaria. More than this, M.2666 represented for us a test of
our laboratory work, and a guide to the value of P.gallinaceum in
chemotherapeutic research on malaria. As far as we knew it was the
first new type of antimalarial substance discovered through the use
of P.gallinaceum and, frankly, we were anxious to determine if it
exerted any activity at all in human malaria.

The drug was tried by Dr A.R.D. Adams of the Liverpool
School of Tropical Medicine in two cases of malignant tertian malaria,
in two cases of benign tertian, and in one case of quartan, and in all
cases the results were negative. The maximum dose administered by
Dr Adams was 40 mg. three times daily (the standard mepacrine dose is
100 mg. t.d.s.), and even at this small dose toxic symptoms were
encountered and he did not feel justified in going higher. We
it, an air which to say the least can be very misleading. We have already mentioned that he used as his criterion of activity delay in the appearance of parasites in the blood-stream until the tenth day; that is to say, if parasites were not found easily in the blood of a treated canary by the tenth day then the drug administered to the canary was regarded as active. (Easily is usually interpreted to mean within three minutes examination under the oil immersion lens). His next step was to determine the minimum dose of the drug which would achieve this delay, a dose which he described as the minimum effective dose. He then computed his chemotherapeutic index, which was the minimum effective dose divided by the maximum tolerated dose. This index, for pamaquin, has been worked out as 1:30, and for mepacrine as 1:30, and for quinine as 1:4.

As a criterion for the choice of a substance in the treatment of canary malaria Roehl's index is admirable; for the rest, its value is doubtful. Its magnitude depends as much on the toxicity of a drug for canaries as it does on the antimalarial activity of the drug, and we are not primarily interested in the bird toxicity figures. We should be if the toxicity of a substance for birds were the same as it is for mammals, but every body who has worked with substances in both birds and mammals knows that frequently this is not so.

It is an easy matter to point out the disadvantages and misleading nature of Roehl's index; but admittedly it is difficult to substitute something in its place. Ideally, of course, we should compute the antimalarial activity of a drug in terms of its concentrations in the blood constituents, both plasma and erythrocytes, but since many of the speculative drugs submitted for test would demand much research to obtain a method for estimating them, the ideal is far from being attained, particularly in large scale testing. The alternative is to make what seemed to be reasonable assumptions, and to remember that many of them, if not all of them, may be erroneous. The assumptions which we make in following our method for the assay of antimalarial substances can be understood if we reiterate the steps we take in evaluating a compound.

(a) Toxicity figures in mammals and birds give some idea of the degree of absorption of a substance; at least, if the toxicity is suspiciously low we administer the substance intraperitoneally as a cross check.

(b) If the substance is found to have antimalarial action it is tested at length until its critical dosage region is known with fair accuracy.

(c) Its chronic toxicity for mice is determined.

(d) The activity of the substance against other species of bird malaria, e.g., against *P.lophurae* and *P.cathemerium*, is measured.

(e) The figures for the critical dosage region and for the chronic toxicity are compared with the corresponding figures for mepacrine. The divergence between the two sets is taken to give a measure of the chances of the substance being active in human malaria.
Our results, both for activity and toxicity, in terms of blood concentrations the method of assay outlined above will not be altered in any essential way as far as we can see; we will continue to determine for a speculative drug its critical dosage region in chicks and its chronic toxicity in mice, but instead of blindly comparing the values obtained with the corresponding ones for mepacrine we will first translate oral doses into blood level concentrations, and then make our comparison.

The two major assumptions which were just defined comprise the main drawbacks to our method of assay of new antimalarial substances; it remains now to stress its advantages, of which there are three important ones. Firstly, it will be clear that there is nothing arbitrary about the concept of a critical dose; it is the lowest dose of a drug which, under a particular dosage regime, exerts what is materially the maximum effect of the drug. We prefer to use a critical dosage region, rather than the critical dose itself, merely because it does not require a great deal of work to define it fairly accurately. Secondly, a comparison of two drugs at the critical dosage region of one allows what seems a very sensitive comparison between them. Thirdly, once the critical dosage region for the type of a family of substances has been established, the other members of the family can be easily compared with the type, and much information can be gleaned even from the results of one experiment. A few concrete examples, all of them drawn from the "2666 story", will serve to make these points clear. The examples are set out in Table III; the results quoted as usual in terms of the proportion of 500 red blood corpuscles which are parasitised, show the progressive approach of the critical dosage region of our speculative substance to that of mepacrine. More than one experiment is included in the table and for this reason all the results are expressed as a ratio x/y where x is the average figure for the treated birds, and y is the average figure for the control birds.

Table III

<table>
<thead>
<tr>
<th>Compound</th>
<th>Doses /50 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 mg.</td>
</tr>
<tr>
<td>M.2666</td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com" alt="Structural formula" /></td>
<td>17/331</td>
</tr>
<tr>
<td>M.3666</td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com" alt="Structural formula" /></td>
<td>12/319</td>
</tr>
<tr>
<td>M.3349</td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com" alt="Structural formula" /></td>
<td>3/265</td>
</tr>
<tr>
<td>M.3502</td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com" alt="Structural formula" /></td>
<td>4/329</td>
</tr>
<tr>
<td>Mepacrine</td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com" alt="Structural formula" /></td>
<td>2/302</td>
</tr>
</tbody>
</table>
diazine, sulphamethazine, sulphathiazole, etc., are efficaceous in this infection and their activity is easily recognised.

3. The method of testing allows for a reasonably strict definition of what constitutes slight activity of a substance so that a lead to a possibly practical antimalarial drug can be recognised in the test.

4. The assay of drugs, using this infection, is described. The most important feature of the assay is the concept of a critical dose, which is defined as the minimum dose of a substance which, administered according to a particular dosage schedule, exerts what is materially the maximum effect of the drug. In using the concept of a critical dose it is essential that both the effect being measured and the particular dosage schedule should be defined.

5. It is described how the use of this infection has enabled an antimalarial drug to be found. The clinical results obtained with this drug are described by Adams and Sanderson in a later paper in this series. It is of interest to note that Collier and Lourie (personal communication) have shown that prolonged exposure of Trypanosoma rhodesiensi to 3349 does not produce arsenic fastness of this organism.
The researches being conducted in these laboratories on the chemotherapy of malaria have as their two objectives the discovery of a true prophylactic drug on the one hand and, on the other, of improved drugs for the treatment of clinical malaria.

While it is realised that a true prophylactic drug which will destroy the sporozoites or the subsequent tissue stage as defined by Curd, Davey, and Rose (1944) and which will in consequence prevent the appearance of detectable infection in the human host would remove the necessity for, or at least render of secondary importance, any other type of therapy, our greatest effort has so far been devoted to the problem of suppressive therapy. This paper gives an account of one approach we have made to this latter problem from the chemical point of view.

The large amount of chemical synthetic work which has been carried out all over the world following the introduction of pamaquin and mepacrine has been mainly concerned with derivatives of either acridine or quinoline, but no material advance has resulted. Complication rather than simplification has been the keynote in most of this work. A number of attempts have been made by various workers to build up antimalarial structures on other heterocyclic ring systems. Thus, phenanthridine, 5:6-, 6:7-, and 7:8-benzoquinolines, the corresponding pyridoquinolines, quinazoline, benziminazole and carbazole, to mention only a few ring systems, have all received some attention, and in certain cases some degree of activity appears to have been achieved but the position of mepacrine as the outstanding synthetic substitute for quinine has never been in doubt.

The guiding principle in most of this work was the fundamental knowledge which emerged from the pioneer researches of Schulemann, Schönhöfer and Wingler and which led to the synthesis of pamaquin, namely, that by linking a basic residue of a certain type to an amino group of a heterocyclic ring system such as quinoline, acridine, benzoxazine, etc., and in some cases of a non-heterocyclic structure such as triphenylmethane, antimalarial activity against avian malaria resulted.

With the lapse of time it became increasingly clear that no fresh advance was to be expected unless a completely new lead was obtained and our investigations were undertaken with that object in view.

At the start of our researches to find a new synthetic substitute for quinine without the disadvantages of mepacrine we therefore decided to break away from the conventional type of
with growth occur, they take part in a number of fundamental life processes. It might therefore be anticipated that pyrimidine would possess important intrinsic chemotherapeutic properties and further that these would include activity against the parasites of malaria.

It is postulated, however, that in order to produce an effective chemotherapeutic substance on the basis of an intrinsically active nucleus it is necessary to attach to it certain pendant groupings which may, among other functions, confer the correct pharmacological properties on the compound. In the case of the synthetic antimalarials mepacrine and pamaquin the important pendant grouping is the basic alkylamino group. The investigation of pyrimidine derivatives containing similar basic alkylamino groups was thus indicated as the first step.

Schönhöfer (1942) has suggested that the antimalarial action of mepacrine is connected with the possibility of tautomerism of the type

\[
\text{III} \rightleftharpoons \text{IV}
\]

and it is obvious that in a 4-dialkylaminoalkylaminopyrimidine a similar type of tautomerism is possible

Instead of starting with the simple pyrimidines we decided to commence with the investigation of compounds of the general formula

\[
\text{V} \rightleftharpoons \text{VI}
\]

where \(X\) is p-chloro or m- and p-methoxy, because the molecular weight of such compounds would approximate more closely to those of the well known antimalarials which all lie between 300 and 400. Such compounds were, however, found to be inactive against \(P. gallinaceum\) in chicks.

Attention was next directed towards similar derivatives of the anilino pyrimidines originally investigated and here activity was immediately encountered in the compound \(M.2666\) which has the formula
towards another linkage between the aryl group and the pyrimidine nucleus offering similar possibilities of tautomerism, namely the guanidine group and the compound M.3349

was prepared and tested against P.gallinaceum in chicks. The result was increased activity as compared with the original M.2666.

A clinical trial of this compound in human malaria carried out by Dr A.R.D.Adams has shown that it is active against P.vivax, P.malariae and P.falciparum so that an indication of the method used for its preparation and a brief account of its chemical properties will be given. Full details will be described elsewhere. p-Chloroaniline hydrochloride when reacted with dicyandiamide in aqueous solution gives p-chlorophenyl biguanide hydrochloride which on condensation with ethyl acetoacetate in presence of caustic soda in aqueous ethanol gives 2-p-chlorophenylguanidino-4-hydroxy-6-methylpyrimidine. When this compound is heated with phosphorus oxychloride the hydroxy group is replaced by chlorine giving 2-p-chlorophenylguanidino-4-chloro-6-methylpyrimidine. Reaction of this with diethylaminoethylamine gives 2-p-chlorophenylguanidino-4-β-diethylaminoethylamino-6-methylpyrimidine. The complete reaction scheme is as follows:

M.3349 (2-p-chlorophenylguanidino-4-β-diethylaminoethylamino-6-methylpyrimidine) is a colourless substance which crystallises from petroleum ether in needles, m.p. 154-155°C. It is insoluble in water but readily soluble in hot ethanol, acetone and toluene and somewhat soluble in these solvents in the cold. It forms three series of salts mono-, di- and tri-acid salts with inorganic acids and also with organic acids like acetic acid. The salts are colourless like the parent base. For the laboratory biological investigation it was used as the dihydrochloride, and the initial clinical trials were carried out using tablets made from this salt. The dihydrochloride was chosen for this purpose because it is readily soluble in water. The monohydrochloride is only very sparingly soluble in water while the trihydrochloride is hygroscopic.

The structure of M.3349 presents another point of interest.
While the similarity might not at first sight appear very striking another consideration was introduced.

In the antimalarial drugs of the acridine and quinoline type the Russian workers (Magidson, Delektorskaya and Lipowitch, 1934; Magidson and Grigorowsky, 1936) differentiate two distinct portions of the molecule with separate functions. Firstly the conductophoric part, identified with the basic side chain, which aids absorption of the molecule into the body of the parasite carrier, thence into the parasite and to the point at which the toxic effect can best be exerted. Secondly the parasiticidal part, identified with the substituted quinoline or acridine nucleus, which brings about the real parasiticidal effect. It is further suggested that the basic side chain is split off in vivo by enzymic hydrolysis, demethylation also occurring, so that the ultimate parasiticidal substance is α-hydroxy-7-chloroacridine. In support of this Magidson and Grigorowsky (loc. cit,) have shown that by simply heating with water mepacrine decomposes in the following manner

\[
\begin{align*}
\text{1} & \quad \text{2} \\
\begin{array}{c}
\text{NHCH(CH}_2\text{)}_3\text{N(C}_2\text{H}_5\text{)}_2 \\
\text{Cl} \\
\text{OCH}_3 \\
\end{array} & \quad \Rightarrow \\
\begin{array}{c}
\text{Cl} \\
\text{NH} \\
\text{OCH}_3 \\
\text{NH}_2\text{CH(CH}_2\text{)}_3\text{N(C}_2\text{H}_5\text{)}_2 \\
\text{CH}_3 \\
\end{array}
\end{align*}
\]

giving 2-methoxy-7-chloroacridone, a certain amount of demethylation also occurring giving the corresponding hydroxyacridone.

If the same in vivo degradation of the pyrimidine antimalarials occurs and this does not appear unlikely as enzyme systems are known which can degrade aminopyrimidine derivatives, the compounds produced exhibit marked structural similarities with riboflavin. This is illustrated below in the case of M.2666 and M.3349

\[\text{Riboflavin} (V) \quad \text{Hypothetical decomposition product of M.2666} \quad \text{Hypothetical decomposition product of M.3349}\]

The similarity in structure is even more marked in the case of an isomer of M.2666

\[\text{Since malaria parasites have not so far been successfully cultivated in vitro an investigation of inhibitors through nutritional studies with a view to indicating the substrate or type of enzyme}\]
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Marshall emphasised that the safety and pharma of comparatively few organic compounds were well known and that "at least the preliminary studies of malaria chemotherapy be carried on animals" before the drug could be administered to man. With this aim in mind he submitted his "memorandum on Research in Malarial Chemotherapy". Mainly concerned with animal infections Several questions Most direct method might be via discovery of the mechanism of action of known antimalarial and correlation with life cycle of malarial parasite. Study of biology of plasmodia might help. Properties of organic compounds such as fluorescence, dissociation constants etc. should be classified and correlated with antimalarial activity. Three fundamental problems (1) Does there exist a specificity of drugs for sporozoites, gametocytes and trophozoites (2) what experimental infection resembles most closely human malaria (3) things like schedule of dosage, optimal duration of treatment, response to be used as criterion of activity and development of accurate methods of comparison. A solution of these problems was a prerequisite for an intelligent effort to synthesise and test a large number of organic compounds.

- Bull. Mal. Res. pp 24-26 -

Plasmoquin did not protect against relapses.

Dihydroquinine no more active and no more toxic than quinine

Summary of the relative merits of atabrine quinine and tosiquinine
(Bull. Mal. Res. pp331-332)

On the basis of controlled quantitative studies in civilian Army and Navy establishments, the evidence at hand justifies the following statement -

(1) In the Suppressive Therapy: Quinacrine has proved to have all the antimalarial properties ascribed to quinine in the suppression of malaria during and subsequent to exposure to infected mosquitoes. Effective suppression can be accomplished over long periods of time by proper use of quinacrine. Available evidence indicates that this end may be achieved without danger to the individual.

Earlier reports indicated a significant incidence of gastrointestinal disturbances in certain groups receiving suppressive quinacrine therapy. For practical purposes, these adverse reactions can be avoided by proper administration of the drug. Quinine in doses adequate to ensure suppression of malaria equivalent to that produced by quinacrine in the dosage currently used by the Armed Forces, is frequently attended by symptoms of cinchonism.

Quinacrine has been demonstrated to prevent consistently the development of falciparum malaria when the drug is administered in proper dosage before, during, and after exposure.

(2) In the therapy of the Acute Attack: Experience in the past two years has demonstrated conclusively that quinacrine when properly administered is fully as effective as quinine in the
Principles and Methods Guiding the Search for New Antimalarial Drugs

The drugs which have not yet found a definite place in the treatment of malaria will not be listed in the present report, since their number is great and is increasing almost daily. On September 1, 1942, the Survey of Antimalarial Drugs listed 127 drugs, on July 1, 1943, the total had risen to 4,141, and in January, 1944, to approximately 7,000 compounds, about 15% of which had exhibited some antiplasmodial action in birds.


Life Cycle of Plasmodia:
Huff and Coulston worked with P. gallinaceum (Mal. Rep. Nos. 91 & 112) inoculated into skin of chicks. Entered cells of lymphoid-macrophage system within 30 minutes, then forming first generation of parasites ("cryptozoites") in the vertebrate host. After 6 hours no sporozoites in intercellular spaces. Cryptozoites completed schizogony in about 42 hours, and resulting merozoites entered into new cells and formed 2nd generation ("Metacryptozoites") completed in 70 to 84 hours. Erythrocytic parasites observed after 75 hours from inoculation. After 90 hours these increased in number after the 6th day precipitously. The degree of natural immunity to cryptozoite infections with P. gallinaceum varied in the chick, goose, duck, and canary. Whereas the chick apparently possessed little immunity, that of the canary was complete. Duck and goose intermediate.


Definitions

Suppressive A drug which keeps infection below the level of clinical expression.
Prophylactic A drug which prevents infection.
Causal Prophylactic A drug which prevents sporozoite infection.

Since a satisfactory in vitro test for antimalarial activity still does not exist, the testing of drugs for antiplasmodial action has to be performed mainly in birds, and to a limited extent in monkeys. For preliminary studies of toxicity, however, mammals should be used. It is not possible to say exactly what avian malarial tests mean for the selection of a compound for trial in man. There are three main reasons why drugs may exhibit different activity in avian and human infections. According to Marshall these are (Mal. Rep. 71)
(1) a difference in susceptibility of different species of parasite
(2) a difference on absorption, excretion and/or distribution in the avian host as opposed to man, resulting in a different blood concentration of the drug.
logical explanation. "The sulfonamides, for example, are highly active in the duck infected with P. lophurae but are 1/30 as active in the same host infected with P. catemerium and they are completely inactive against the P. cathemerium in the canary." The difficulties which arise when one attempts to translate the activity of a drug against one developmental form of a parasite to other developmental forms of the same parasite, or to other parasites are illustrated in the following examples. "Against P. gallinaceum sulfadiazine is a true causal prophylactic in the chick, but is not a cure for either sporozoite or trophozoite infections. In P. knowlesi in monkeys sulfadiazine completely cures the infection. In P. vivax in man sulfadiazine is not a causal prophylactic and has a very low grade activity. In P. falciparum in man sulfadiazine is a true suppressive but again has low curative activity. Atabrine against P. gallinaceum is effective against the trophozoites but is not a causal prophylactic. Against P. knowlesi it acts as it does in humans but is much less active than the sulfonamides. Against P. vivax it is curative in trophozoite infections. Against P. falciparum it is a practical prophylactic and curative.

Differences in the behaviour of a drug, both as to its anti-malarial and its toxic action, in birds, lower animals and man may be due to different degradation and other factors which the study of the special pharmacology of a new compound is able to explain. But such studies require much and prolonged work. In Marshall's opinion, therefore, a new compound that shows activity in avian malaria "should be promptly cleared for toxicity, given a preliminary trial in man, and then restudied in animals for special pharmacological and chronic toxicity."

The clinical testing program was discussed by Shannon with regard to the aim guiding the development of a new drug. Such a drug should either do things which atabrine cannot do, i.e. affect the tissue phases in P. vivax infections, or be sufficiently more active than atabrine to exert a chemotherapeutic action on these phases through the increased intensity of activity. "The latter view assumes that relapses of P. vivax malaria are due to repeated sporulations of a tissue phase, and that, although atabrine has some action against these tissue forms, the intensity of the action is insufficient to produce a cure. A new drug should be so much more potent than atabrine that the area of its activity would include a lethal affect on these tissue phases. In the search for such a drug acting by the latter mechanism the trophozoite induced infection is an adequate test object. If however a drug is to be found which affects tissue forms without at the same time having an action on trophozoites, sporozoite induced relapsing vivax infections must be used as preliminary test objects. The sporozoite testing facilities of this general type have been increased at Atlanta under the direction of the National Institute of Health and studies to be made will include a short and long prophylactic test. Three kinds of responses can be anticipated (1) all or none (2) partial without complete protection, (3) long-term suppressive effect. Drugs which do not prevent the incremental inception of the disease can be retested against the clinical attack when it appears.