Abstract of thesis

Growth Factors and Growth Factor Antagonists,

submitted for

the Degree of

Doctor of Philosophy

by

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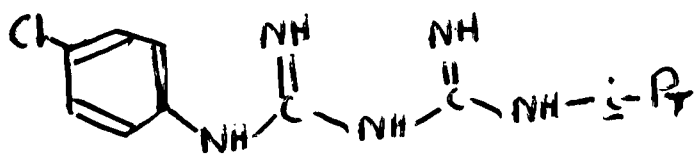
Growth Factors and Growth Factor Antagonists.

The introduction consists of a brief review of the subject with special reference to antimalarial agents and haematopoietic factors.

Part I. Some synthetic Biguanide derivatives.

It has been suggested (Curd and Rose, Nature, 1946, 158, 707) that the activity of paludrine as an antimalarial is due to the interference of a dimeric form with a porphyrin enzyme system essential for the growth of the plasmodia.

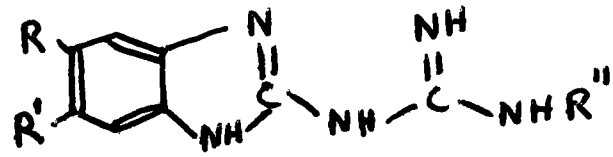


Paludrine.

Hawking (Nature, 1947, 159, 409) also showed that paludrine was inactive as an antimalarial in vitro, but was converted into an unidentified biologically active material in vivo. Oxidation to a benziminazole is one possibility, and a series of 2-guanidinobenziminazoles, which are structurally very similar to paludrine, was synthesised for biological examination.

The benziminazoles (1 to 15) were all made from the appropriate o-phenylenediamine dihydrochlorides and

dicyandiamide, or isopropyl- or n-butyldicyandiamides in aqueous solution, and were usually purified through the picrates or copper derivatives. The hitherto undescribed alkylated dicyandiamides were prepared from sodium dicyanimide and the appropriate amine hydrochlorides in boiling n-butanol.



	R.	R'.	R".		R.	R'.	R".		R.	R'.	R".
1.	H	H	H	6.	MeO	MeO	H	11.	Cl	H	i-Pr
2.	Me	H	H	7.	Cl	Cl	H	12.	Me	Me	i-Pr
3.*	MeO	H	H	8.	H	H	i-Pr	13.	MeO	MeO	i-Pr
4.*	Cl	H	H	9.	Me	H	i-Pr	14.	Cl	Cl	i-Pr
5.	Me	Me	H	10.	MeO	H	i-Pr	15.	MeO	MeO	n-Bu

These 2-guanidinobenziminazoles, as their hydrochlorides, were tested against P. gallinaceum in chicks, or P. relictum in canaries, but only two, 7 and 14, had even slight activity. These results showed that 11 could not be Hawking's biologically active metabolite and do not support Curd and Rose's theory. The bimolecular form of 11 is no less like the porphyrin ring system than the postulated paludrine dimer.

None of the 2-guanidinobenziminazoles showed large bacteriostatic action against B. coli, or S. aureus broth cultures.

Attempts to synthesise the 2-guanidinobenziminazoles

* Experiments by P.C. Spensley.

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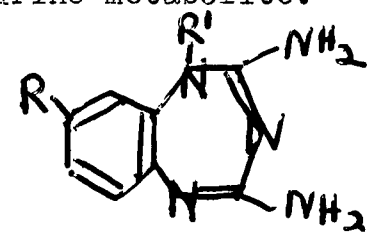
in the reverse direction failed. 2-Cyanamino-benziminazole was not formed from o-phenylenediamine and dicyanimide, and when prepared according to Pellizzari (Gazzetta, 1921, 51, I, 140) could not be induced to combine with isopropylamine.*

The product of the reaction of o-phenylenediamine with dicyanimide, 2:4-diamino-1:3:5-triazabenzepine (16), is a derivative of a new heterocyclic system. It was stable to dilute acid and alkali, and like its 1-methyl derivative (18) did not react with nitrous acid.

A series of these benzepines was prepared from the appropriate diamines and dicyanimide, and one (20) is a possible paludrine metabolite.



	R.	R'.		R.	R'.
16.	H	H	19.	Cl	Et.
17.	Cl	H	20.	Cl	i-Pr
18.	H	Me			

The compounds had no effect on the growth of B. coli or S. aureus broth cultures.

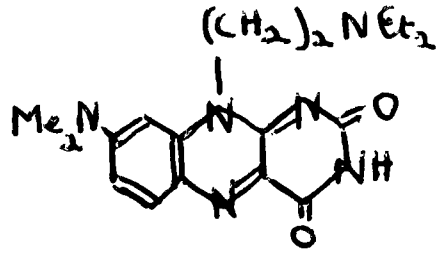
Part II. Alloxazines and isoAlloxazines.

This was a continuation of earlier work by the author and Prof. F.E. King (J., 1946, 681), on the

* Experiments by P.C. Spensley.

preparation of alloxazine derivatives for biological testing.

Piloty's synthesis of alloxazines (Annalen, 1904, 333, 44) was extended to the isoalloxazine series, and violuric acid was found to react with 3-dimethylamino-β-diethylamino-ethylaniline to give the corresponding isoalloxazine (21), isolated as the violurate. The constitution was proved by an independent synthesis from 2-amino-5-dimethylamino-β-diethylaminoethylaniline and alloxan, showing that the violuric acid condensation took place in the 4- position, rather than the 2- position of the benzene ring.



21.

The 3-dimethylamino-β-diethylaminoethylaniline was prepared from 3-dimethylamino-p-toluenesulphonanilide by alkylation and hydrolysis. The amine for the alloxan condensation was the reduction product of 2-nitro-5-dimethyl-amino-β-diethylaminoethylaniline, obtained from β-diethylamino-ethylamine and 3:4-dinitrodimethylaniline.

The reaction of violuric acid with 3-amino-N-methylaniline was investigated as this could give an

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alloxazine and/or an isoalloxazine, assuming that this condensation also takes place in ~~the 4-~~ α β- position of the benzene ring. A comparison of the ultra-violet absorption spectrum of the product with those of 7-dimethylaminoalloxazine, and 7-dimethylamino-9-methylisoalloxazine, prepared by Piloty's method, showed that it was inhomogeneous. Its sulphuric acid solution was therefore treated with sodium nitrite. The precipitated 7-(N-nitroso-N-methylamino)-alloxazine was collected and the filtrate gave an olive green azo-dye with β-naphthol in dilute alkali. From the respective yields of these products the ratio of alloxazine to isoalloxazine in the mixture was approximately 40:60. The first stage of the Piloty reaction is probably the attack of the 4-carbonyl of the violuric acid on an amino group of the diamine. If the first stage were the attack of the oximino group in violuric acid on the benzene nucleus the initial product would be an anil of a type that does not cyclise to an alloxazine derivative (Tischler, Wellman and Ladenburg, J. Amer. Chem. Soc., 1945, 67, 2165).

Part III. The synthesis of some Benziminazoles

The reaction of phenylacetiminomethylether with o-phenylenediamine to give 2-benzylbenziminazole was investigated. The free iminoether reacted with the

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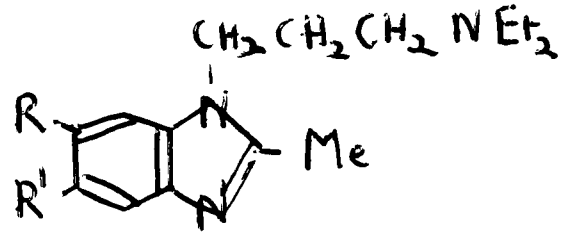
diamine only at elevated temperatures to give poor yields of the benziminazole, but the reaction was acid catalysed. In the presence of one, or two equivalents of acid good yields of the benziminazole were obtained, but if three equivalents were used the yield dropped. This was also found with phenylacetiminothiobenzylether, and the reaction mechanism has been interpreted as an attack of the iminoether cation on an uncharged aromatic amino group.

The reaction was extended to various mono-N-alkylated o-phenylenediamines with good results.

Three benziminazoles (22-24) were prepared, for antimalarial testing, from the appropriate diamines by the Phillips method; 22 and 23 were inactive against P. gallinaceum in chicks, and the results for 24 are awaited.

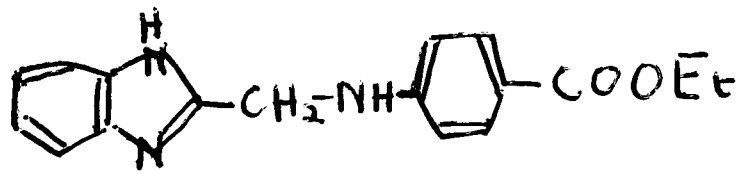


22. R = H, R' = Cl.
23. R = Cl, R' = H.
24. R = R' = Cl.

Acetiminothiobenzylether hydrochloride with 2-amino-5-chloro-γ-diethylaminopropylaniline also gave 23, but only small quantities of 22 were isolated from the

reaction of acetiminomethylether hydrochloride with 2-amino-4-chloro-γ-diethylaminopropylaniline under various conditions. The main product of the reaction was provisionally regarded as 2-(N-acetamidino)-4-chloro-γ-diethylaminopropylaniline. In support of the idea that the electrophilic chlorine atom para to the secondary amino group in the amine was reducing benziminazole formation the corresponding 2-amino-4-methoxy-γ-diethylaminopropylaniline gave a much larger yield of 1-(γ-diethylaminopropyl)-2-methyl-4-methoxy-benziminazole with acetiminomethylether hydrochloride than the chloro derivative.

Part IV. The synthesis of Pteroylglutamic acid and Related Compounds.

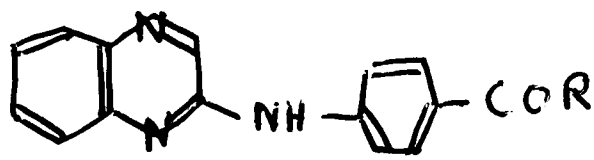


25.

The benziminazole analogue (25) of ethyl pteroate was prepared from chloroacet-2-nitroanilide, which reacted with ethyl p-aminobenzoate to give 4-carbethoxyphenylglycine-2-nitroanilide. On hydrogenation this gave the corresponding amine which cyclised to ethyl N-(2-benziminazole)-4-aminobenzoate (25) under acid conditions. Others

completed work in this series, and details are in press (King, Spensley, and Nimmo-Smith, Nature, 1948).

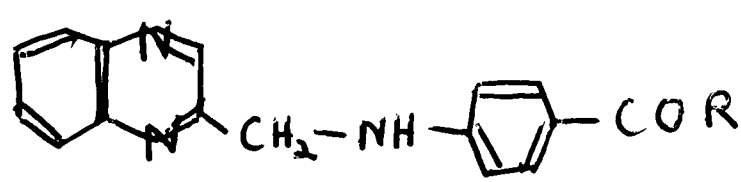
N-(2-quinoxaline)-4-aminobenzoic acid (26) was prepared from 2-chloroquinoxaline and p-aminobenzoic acid. It had little effect on the growth of S. lactis R.



26. R = OH.
27. R = l-, $NHCH(COOEt)CH_2CH_2COOEt$.
28. R = l-, $NHCH(COOH)CH_2CH_2COOH$.

The pteroylglutamic analogue (28) was prepared by the hydrolysis of ethyl N^4-(2-quinoxaline)-4-aminobenzoyl-l-glutamate (27), the condensate of 2-chloroquinoxaline and ethyl p-aminobenzoyl-l-glutamate. Its inhibitory action on the growth of S. lactis R was prevented by pteroylglutamic acid, and quantitative biological results are awaited.

Many attempts to synthesise N-(quinoxaline-2-methyl)-4-aminobenzoic acid (29) and some of its derivatives were made.



29, R = OH.
30, R = OEt.

It was not found possible to prepare 2-bromomethylquinoxaline by any route examined, but quinoxaline-2-aldehyde reacted with p-aminobenzoic acid, and its ethyl ester, to give the corresponding anils. Nothing homogeneous could be isolated from the reduction of N-(quinoxaline-2-methylene)-4-aminobenzoic acid, but a poor yield of ethyl N-(quinoxaline-2-methyl)-4-aminobenzoate (30) was obtained from the reduction of the corresponding ester. One attempt at hydrolysis failed.

Methylglyoxal on bromination gave monobromomethylglyoxal, which reacted with 2:4:5-triamino-6-hydroxypyrimidine and p-aminobenzoyl-l-glutamic acid to give a product containing about 5% of pteroylglutamic acid.

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Contents.

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

Growth Factors and Growth Factor Antagonists.

The necessity of the presence of certain materials for the normal growth of all living matter has long been recognised. Materials, without which a specific organism fails to grow or develop normally are known as "growth factors" for that organism but the term is often applied to materials only required in small quantity. The growth factor requirements of different organisms vary greatly. Some have the power of synthesising most, or all of these materials from simpler substances, while others have not. When an organism can synthesise one of these it becomes independent of an external source of supply, though the presence of the substance, usually termed an "essential metabolite" in the organism is necessary for normal growth.

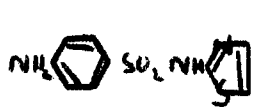
Fildes and others (1) have shown that the antibacterial action of various drugs may be due to three types of interference with essential metabolites, and these will now be considered.

The growth of certain anaerobic bacteria is stopped by the addition of various oxidizing agents, but only if these substances penetrate the cell wall (2). Growth restarts when all the oxidizing agent has been reduced by the organism, suggesting that the antibacterial action is due to the material oxidizing an essential metabolite involved in an oxidation reduction process.

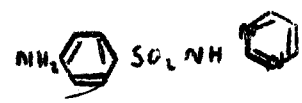
The second class of bacterial growth inhibition is caused by compounds which react with essential metabolites to form an inactive product. The antibacterial action of mercury on S. aureus, which requires compounds containing thiol groups for growth (3) is of this type. The growth inhibition, within limits, is specifically neutralised by -SH groups (4) and hydrogen sulphide (5), which preferentially combine with the mercury and release the essential metabolite. The toxic effect of arsenic in the blood of animals is similarly neutralised by treatment with 2:3-dithiopropanol, as the metal combines with this thiol in preference to the biologically essential compounds in the tissues (147).

The third type of interference was suggested from investigations into the mode of action of the very important antibacterials, the sulphonamides. The first theory of their mode of action, for which there was no evidence, was that of interference with a bacterial proteolytic enzyme system (6). McIntosh and Whitby later postulated interference with an enzyme system blocking a vital food supply (7), and Stamp (8), and Green (9) obtained bacterial extracts preventing the antibacterial action of sulphanilamide. This was the first time a sulphanilamide antagonist had been found, though Woods (10), assuming that sulphanilamides acted through chemical combination with an essential metabolite, had examined many compounds for this kind of activity. He also

found that yeast extracts had this property and showed that a proportionality existed between the quantities of extract required to antagonise the effect of various quantities of sulphanilamide. This led to the idea that the activity of the drug was due to its ability, because of similar chemical properties, to compete with an essential metabolite for an enzyme system in the bacteria. Various analagous compounds to sulphanilamide were then examined and p-aminobenzoic acid was found to have the required biological properties *in vitro*, one molecule antagonising the action of 25,000 molecules of sulphanilamide. This inhibition of sulphanilamide activity is also shown *in vivo* (30). Very many derivatives of sulphanilamide have been made in order to increase its usefulness as a drug (11), and some of these, e.g. sulphathiazole, (I), and sulphadiazine (II) have much greater bacteriocidal activity than the parent compound.



I.

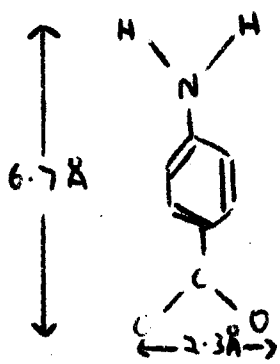


II.

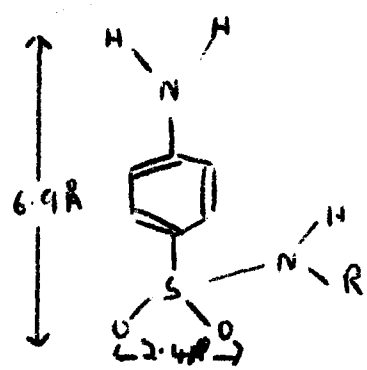
More detailed studies, suggesting that the activity of the sulphonamides was due to the action of the ion rather than that of the undissociated molecule, have been carried out by Bell and Roblin (12). They found that a plot of pKa against log $1/C_r$, where C_r is the minimum molar concentration necessary to

prevent growth, for a series of sulphonamides, gave a curve with the activity passing through a maximum. Up to a limit, the bacteriocidal activity of the sulphonamides increased with their acidity. This is stated by Bell and Roblin to be due to the electrophilic sulphonamide substituents increasing the negative charges on the SO_2 group and thus increasing the similarity to p-aminobenzoic acid.

More electrophilic sulphonamido substituents (R, in IV) will move this negative charge towards the nitrogen and away from the SO_2 group, decreasing the similarity to p-aminobenzoic acid (III) and explaining the fall in activity.



III.



IV.

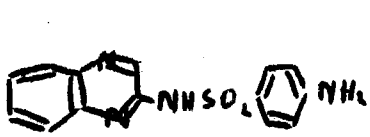
This correlation between a physical property and biological activity is very important, and an examination of the graph shows that the highest bacteriocidal activity probably attainable in this series is shown by sulphathiazole (I) and the pyrimidines. Other theories have also been put forward to explain the different activities of various sulphonamides (13) but they have no deciding evidence in their favour.

Henry (14) has stated that sulphonamides are bacteriostats, but it has been conclusively shown that they are

bacteriocidal in action (15). Wolff and Julius (16) have proved that they are bacteriocidal to the logarithmic phase of bacterial growth, and have no action on the resting condition.

O'Meara (17) has recently discussed the *in vitro* action of sulphonamides, and McIlwain (18) has shown that there is no bulk displacement of p-aminobenzoic acid from bacteria by sulphanilamide. This may be explained by assuming that the cell wall has a selective permeability, but this seems unlikely and the p-aminobenzoic acid is probably "bound" in some way in the organism. Numerous other substances apart from p-aminobenzoic acid have been found to antagonise sulphonamide action, e.g. methionine (19), adenine (20), urethane (21), and an unidentified substance, not p-aminobenzoic acid, isolated from yeast extract (22).

The metabolic products of sulphonamides in various animals has been investigated, and the isolated products, usually hydroxylated compounds, have little bacteriocidal activity. Sulphaquinoxaline (V), for example is converted into VI by rats (31) and sulphanilamide into VII by rabbits (32) and by man (33).



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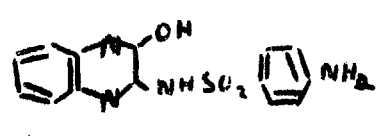
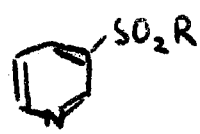


VI.

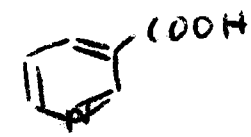
VII.

It is therefore clear that though the Pilaes - Woods theory is a very useful working hypothesis the evidence for it is not conclusive. The complexity of living organisms creates great difficulties in the interpretation of experimental results and there is no universally accepted theory of sulphonamide action at present.

Perhaps the most important consequence of this theory is that it indicates how the structure of new chemotherapeutic agents might be modelled on known growth factors. The first substances examined for growth inhibitory action on various bacteria because of structual resemblance to a growth factor were pyridine-3-sulphonic acid (VIII, R =OH) and its amide (VIII, R = NH_2). The inhibition produced by these compounds was prevented by the addition of nicotinic acid (IX) to the medium (23).



VIII.



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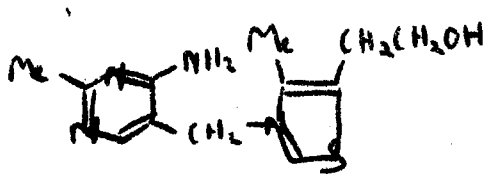
An analogue of thiamine, (X) pyrithiamine (XI), has been prepared (26) and its growth inhibitory effect on various organisms requiring thiamine is removed by further additions of the latter compound (27). The relationship



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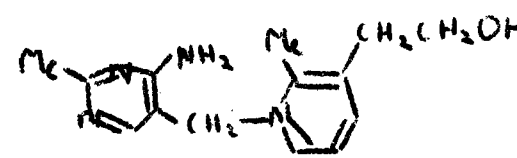


XI.

of the two compounds has been examined further (28), and in mice as little as 20 γ of pyrithiamine per day produced thiamine deficiency symptoms and the death of the animals. All the symptoms could be cured by the addition of appropriate quantities of thiamine.

α-Aminosulphonic acids inhibit the growth of bacteria requiring the corresponding α-aminocarboxylic acids. This inhibition is prevented by the addition of appropriate amounts of the corresponding α-aminocarboxylic acids (24). Similarly the inhibitory effect of pantoyltaurine on the growth of certain bacteria is prevented by the addition of pantothenic acid (25).

Very many compounds having structures comparable with essential metabolites have been prepared and tested for biological activity (29) but so far no useful chemotherapeutics have been discovered from this approach. Chemotherapeutical action only takes place in completely biological systems, and it is the differential action of the drug on the host and the parasite that is so important. Unless it is large, enabling the effective dose for the destruction of the parasite to be much less than the toxic dose for the host, the drug will be too dangerous to use. If more were known about the relative growth factor requirements of bacteria, other parasites, and their hosts, it should be possible to predict, with much more certainty than at present, the structure of effective chemotherapeuticals.

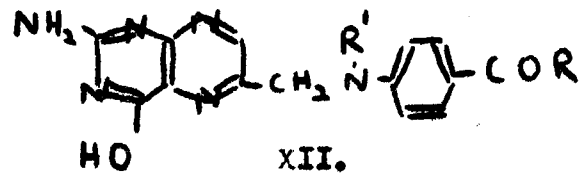
Most of the work reported in this thesis is concerned

with antianaemia factors and antimalarials, so these two subjects are now briefly discussed.

The important clinical effects of liver in the treatment of anaemias has long been recognised. Snell and Peterson (34) succeeded in concentrating a material (the "norite eluate factor") essential for the growth of L. casei from liver. This substance also occurred in yeast, and Hutchings showed it was a growth factor for other bacteria (35) and chicks (36). Mitchell, Snell and Williams (37) later obtained a spinach concentrate ("folic acid") required for the growth of S. lactis R. and also effective for L. casei. Pfiffner et al. in 1943 (38) isolated a crystalline material from liver, called vitamin B_c, which was a growth factor for chicks and highly active for L. casei. The materials from liver and yeast had approximately the same potency for L. casei, but the former was the more active for S. lactis R. Both these materials were different from a substance isolated by Kerenztesy (39), which was about 2,500 times as active for S. lactis R as for L. casei. Hutchings et al. (40) then isolated a crystalline material, 85-90% as active for L. casei as the liver factor, but only 6% as active for S. lactis R. Its u.v. absorption spectrum was similar to the compound from liver, and both materials had beneficial effects on chick nutrition.

Chemical investigation of the pure growth factors

has considerably clarified the position. Angier et al. (41) have shown, by degradation and synthesis, that the liver L. casei factor is XII (XII, R=l-, NHCH(COOH)CH_2 CH_2COOH, R' = H), called pteroylglutamic acid, and it is identical with Pfiffner's vitamin B_c (42). They have also shown that the L. casei growth factor obtained from yeast is pteroyl-triglutamic acid (43) but the mode of linkage in the peptide chain is not disclosed.



XII.

Pteroic acid (XII, R = OH, R' = H) has also been synthesised (41) and is a powerful growth factor for S. lactis R. It has little activity for L. casei, and is inactive in the chick.

A very similar growth factor for S. lactis R, having little growth factor activity for L. casei and none for the chick, has been isolated from the fermentation liquors of Rhizopus Nigricans (44). It has been identified and synthesised (45) and is formylpteroic acid (XII, R = OH, R' = CHO).

Lampen and Jones (46) investigated the effect of pteroylglutamic acid on the growth of bacteria prevented by sulphonamides, but only a small reversal of the growth inhibition was observed. Mahagen (47) found that p-aminobenzoyl-l-glutamic acid was 8-10 times as effective

as p-aminobenzoic acid in preventing the growth inhibitory effect of sulphanilamide on S. plantarum, and p-aminobenzene-sulphonyl-l-glutamic acid has little bacteriocidal activity (11). Little connection there appears to exist between the mode of action of sulphonamides and pteroylglutamic acid.

Other materials have been isolated from liver. Pfiffner et al. (49) obtained a crystalline vitamin B_c conjugate, shown to be pteroyl-hexaglutamylglutamic acid (50). This has only slight growth promoting activity for S. lactis R and L. casei, but is strongly active for the chick.

The clinical effect of synthetic pteroylglutamic acid on various types of anaemia has been compared with that of unpurified liver extract, and it is clear that pteroylglutamic acid is not the only haematopoietic factor of importance as the liver extract is often better, and acts in a rather different way. Hall (51), compared the Snell and Williams spinach concentrate microbiologically with pteroylglutamic acid.
His results indicated that the extract was a mixture. Later experiments showed that this spinach concentrate may contain two factors associated respectively with early and late acid production by S. lactis R during long incubation (52). Hall also suggested, though the evidence is scanty, that the compound associated with

early acid production was pterin-like, while the other material was non-pterin-like in character.

Smith (53) has recently isolated two red pigments in very small yield from liver. These compounds are highly active against pernicous anaemia, and may not contain a pterin nucleus. It is stated "the pigments are differing forms of the classical liver factor postulated by Minot and Murphy in 1926 (54), and not some incomplete substitute such as thymine of folic acid".

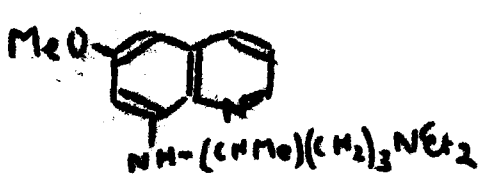
Rickes et al., (134) have also reported the isolation of a red crystalline material, vitamin B_{12}, from liver. It has an extremely powerful haematopoietic effect in cases of pernicous anaemia, but no chemical data has been reported so far.

The confusion existing in this field of research, now being clarified, has been mainly due to the attempted over-simplification of the problem. The indiscriminate use of the term "folic acid" to describe growth factors for L. casei regardless of source has not helped the situation, nor has the use of non-standardised biological tests for the examination of different concentrates. Much elucidation remains to be done and important advances are expected in the near future.

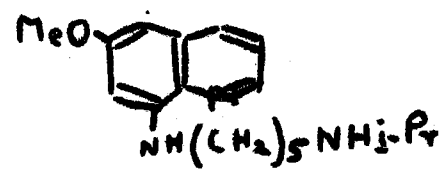
Quinine has been widely used since very early times for the treatment of malaria. It was unsatisfactory in many ways as an antimalarial and attempts to find a more

effective substitute have been in progress for a long time. The greatest efforts however took place during the last war, when the loss of Java deprived most of the world of its main source of quinine.

Methylene blue was the first synthetic drug found to show antimalarial activity, and Schulemann (55) made the first advance by showing that the activity increased with the introduction of a dialkylaminoalkyl side chain into the molecule. Many heterocyclic compounds were then examined, but the greatest activity was found in a series of quinoline derivatives (56). The most active was called "plasmoquin" (XIII). Similar compounds had the same order of activity (57) and Magidson et al. (58) found that the activity was greater when the number of carbon atoms in the side chain between the nitrogen atoms was odd than when it was even. Plasmoquin has the disadvantage of being rather toxic, but recently an equally active (59) less toxic analogue has been prepared (60), and named "pentaquine" (XIV). The relapse rate with patients treated with this drug was much lower than when plasmoquin was used (146).



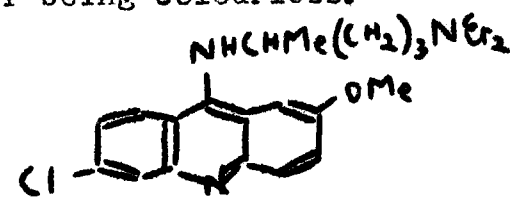
XIII.



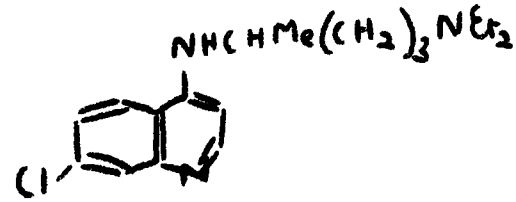
XIV.

Investigations into other ring systems (61) showed that certain acridine derivatives had powerful antimalarial properties, and "mepacrine" (XV) was one of the most effective. The d- and l- forms of this drug have been obtained (62) and show similar antimalarial and pharmacological properties (63).

Some other derivatives of quinoline (64), prepared first in Germany have been found to be more effective both in avian and human malaria than any known derivatives of quinoline, and one, "chloroquine" (XVI) has very similar biological properties to mepacrine. It has the advantage of being colourless.



XV.



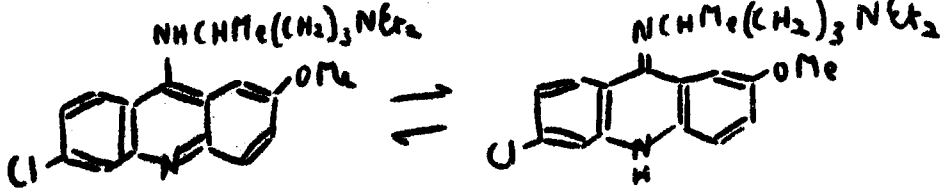
XVI.

Many theories have been put forward to explain the action of antimalarial drugs, but none are satisfactory. Magidson et al. (65) consider that the basic sidechain of mepacrine helps the drug to penetrate the cell wall, is split off inside, and that the residual demethylated acridone is the active plasmodicidal agent. Mepacrine is largely metabolised in the body, and Hammick and Mason (66) have shown that the corresponding 5-aminoacridine is one of the

products.

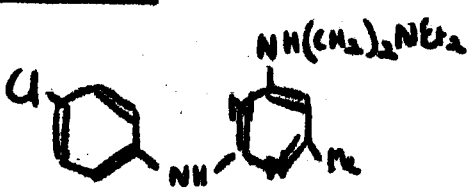
Oesterlin (67) has suggested interference with a riboflavine enzyme system involved in oxidation-reduction processes, as both acridine and plasmoquin can be reduced to dihydro forms. This is further discussed in Part II of this thesis.

Schonhofer (68) postulated that the antimalarial activity of mepacrin was due to the possibility of tautomerism of the type shown below, and it was with the help of this idea that "paludrine" was discovered.

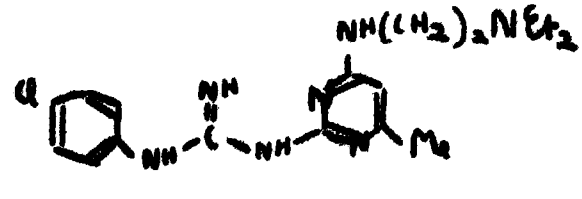


Curd, Davey and Rose (summary, 69) decided to investigate the antimalarial properties of some pyrimidines, as this ring system had considerable biological importance. They found that, of many compounds investigated, the 2-p-chloroanilino-pyrimidines were active, e.g. 2666 (XVII), and that the attachment of a benzene ring to either nuclei did not remove the activity. A number of tautomeric possibilities were discerned in 2666, and it was thought that the activity might be connected with the linking of a p-chloroanilino group to the aminoalkyl group through a pyrimidine containing a prototropic system. On this hypothesis a guanidinopyrimidine (XVIII), 3349,

was synthesised and was even more active against P. gallinaceum in the chick.

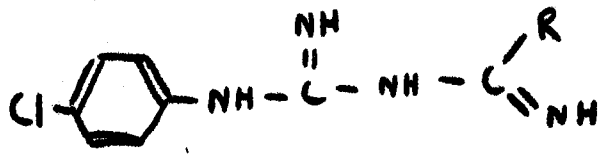


XVII.



XVIII.

As only those isomers of 2666 which had protropically independent amidine systems showed antimalarial activity, the necessity for the pyrimidine ring in 3349 was doubted and consequently XIX (R = NH $CH_2CH_2NEt_2$) was synthesised, but was devoid of activity. Substitution of the less basic diethylamine for β-diethylaminoethylamine in the synthesis led to a highly active material (XIX, R = NEt_2), and further investigation showed that the N^5-isopropylbiguanidine, "paludrine" (XIX, R = NHi-Pr) was the most potent antimalarial in this series.



XIX.

The methods of testing drugs for antimalarial activity have been reinvestigated (69) and improved, but the great specificity of antimalarial action has caused much anxiety. Many drugs active against bird malaria are inactive when

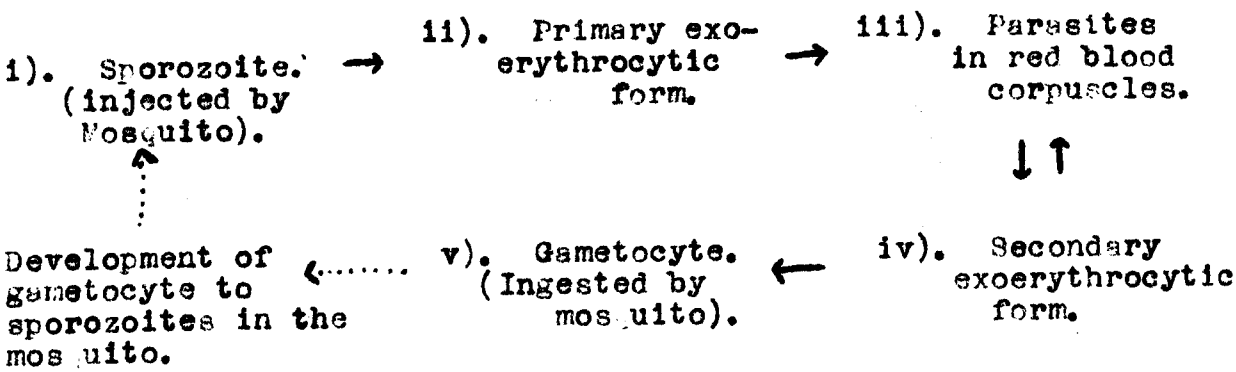
tested clinically, and some drugs act only on certain of the bird parasites. 3349 (XVIII), a development of 2666(XVII, active against P. gallinaceum and P. lophurae in chicks, but inactive in human malaria) is more active against P. gallinaceum, is active against P. falciparum, P. ovale, and P. vivax in man, but is inactive against P. lophurae in chicks. Another example is N^5-methyl-N^5-isopropyl-p-chlorophenylbiguanidine, 4430(XIX, R = NMe i-Pr) which is active against all types of human and bird malaria except P. cathemerium in canaries, while paludrine is active against this infection too. More detailed specificities are shown by paludrine, and the very similar compound 4430. Paludrine is an active prophylactic against all forms of bird malaria, and partially at least against the various types of human malaria. 4430, in contrast, acts as a prophylactic only in the case of P. gallinaceum (70). P. gallinaceum did not acquire resistance to mepacrine, quinine, sulphadiazine or 3349 after 2¼ years of intensive treatment, but acquired paludrine resistance after 3-4 months. This was transmissible through Aedes Aegypti, and the resistance was probably due to a mutant strain. A paludrine resistant strain was also resistant to the action of analagous biguanidines (including 4430) indicating a similar mode of action, but was not resistant to the action of quinine, mepacrine, sulphadiazine, 2666 or 3349 (71).

It is clear therefore, that a drug active in bird malaria

and inactive in human malaria will be discovered, but if the reverse is true it will be missed. This disadvantage is reduced by using many types of experimental plasmodia in their appropriate hosts for preliminary testing, but cannot be removed. Cultivation of malaria parasites in vitro is difficult. Preliminary testing on these cultures may result in active antimalarials remaining undiscovered as the effect of the host on the drug is eliminated.

The life cycle of the malaria parasite proceeds through two types of host, the mosquito, and birds and mammals, and in diagramatic form is shown below.

Malaria Cycle.



Stage "ii", the primary exoerythrocytic form, is assumed to exist in all types of malaria. It has been demonstrated in the avian variety, and recently in *P. cynomolgi* in monkeys, where it concentrates in the liver (72). Paludrine

(73) and mepacrine, are also largely concentrated in this organ. The only drug known to attack this stage of the life cycle in man is paludrine, which acts as a causal prophylactic with P. falciparum, and a partial prophylactic with P. vivax. Asexual reproduction of the parasites in the red blood corpuscles, followed by their disruption owing to the production of a haemolytic substance (74) by the parasite, causes the clinical symptoms, and this stage of the life cycle is attacked by paludrine (and other biguanidines), quinine and mepacrine. The secondary exoerythrocytic form is also attacked by paludrine and to some extent by plasmoquin, but not by the other antimalarials. The gametocytes, destroyed by plasmoquin still persist in the presence of paludrine, but do not develop in the stomach of the ingesting mosquito if the blood of the bitten host contains this drug.

Complete cures of P. falciparum infections can be achieved by one nontoxic dose of paludrine. P. vivax is much more resistant however, but the relapse rate of treated patients is much reduced if plasmoquin is used in conjunction with the paludrine.

Some suggestions concerning the mode of action of paludrine have been made, and these are discussed in part I of this thesis.

The completely different biological action of paludrine to its precursor, 3349, and the very similar biguanidine 4430,

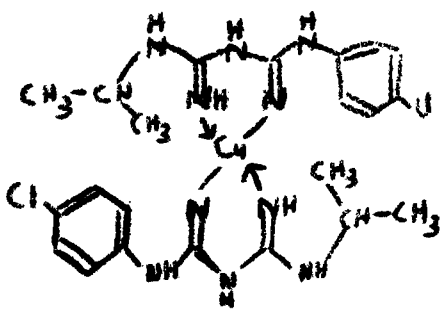
indicates that the discovery of this very important drug was fortuitous, though very well deserved. It is undoubtedly the best single drug used in malaria therapy, and further clinical results, especially in conjunction with "pentaquine" are awaited with interest.

In the future, as in the past, it seems that the search for antimalarial drugs will continue on an empirical basis, as so little is known about the metabolic processes of the parasites. Some investigations into the nature of the enzyme systems in the parasites have been carried out (75) but the results have not suggested the examination of any more materials for antimalarial activity.

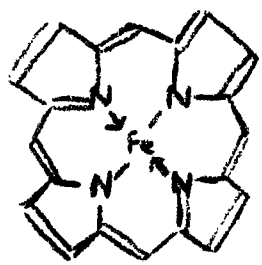
Part I. Some synthetic Biguanidine derivatives.

The mode of action of paludrine on the malarial parasite is clearly different from that of any other antimalarial. It does not appear to be due to interference with a riboflavine enzyme system because the effect of paludrine on L. casei, unlike that of mepacrine, quinine, 2666, and 3349, is not reversed by the addition of riboflavine.

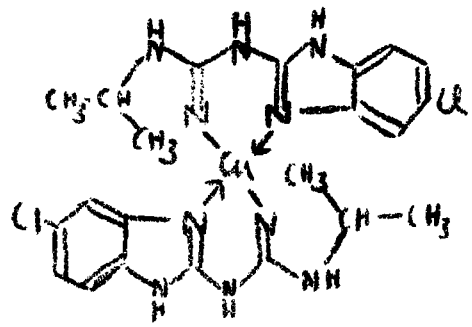
Curd and Rose (76) have suggested that the antiplasmodial action of paludrine is due to its interference with another growth factor required by the malarial parasite, and state that this may be a highly specific porphyrin. (It is coincidental that one stage of the organisms life cycle takes place within the red blood corpuscle). The evidence for this is the formation of a copper salt by paludrine containing one atom of copper to two molecules of the biguanidine. Assuming a planar structure the model of this compound (XIX) is remarkably like that of a porphyrin (XX). Curd and Rose point out that copper, or a metal, may not be implicated, but that hydrogen bonding may stabilise the porphyrin-like system. There is no evidence however for dimerisation in the free biguanidine.



XIX.

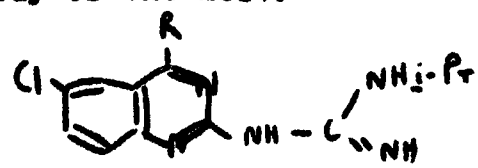


XX.



XXI.

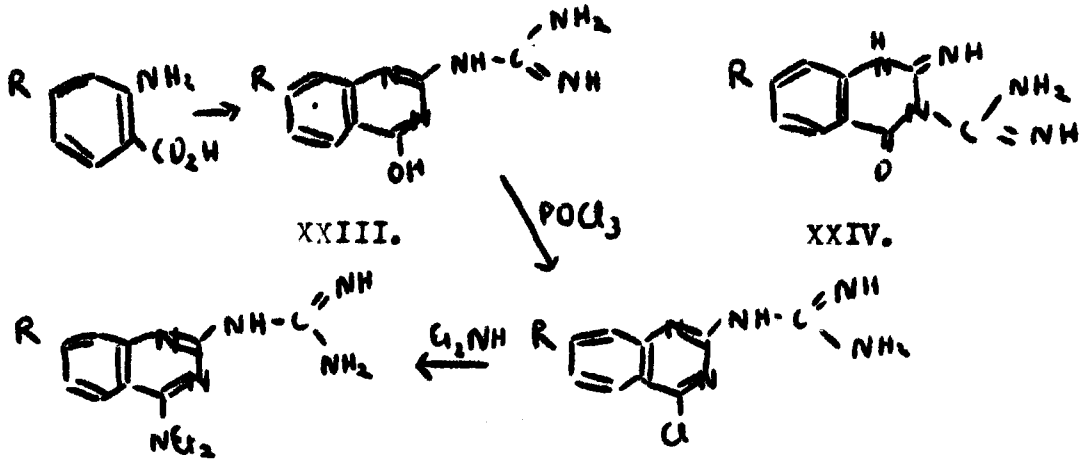
Hawking (77) has shown that paludrine itself has no action on in vitro malaria cultures, but that the serum of patients treated with the drug is highly active. Pharmacological studies (73) show that paludrine is largely metabolised in the host, supporting the implication of Hawlings work that the antimalarial activity is due to a metabolite produced from paludrine in the body of the host.



XXII.

There is no evidence as to the nature of this compound, and it was thought that it might possibly be of the quinazoline type (XXII) due to the interaction of an aminoacid or sugar residue with the N^3 atom and the benzene ring of paludrine. Some preliminary experiments on the synthesis of quinazolines, with the proposed preparation of XXV, (R = Cl), for biological testing, were carried out, but the project was dropped at an early stage.

The proposed scheme of synthesis was as follows (R = H, or Cl) :-



XXIII. XXIV.

XXV.

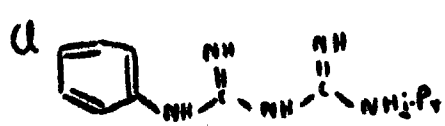
and preliminary experiments were carried out using anthranilic acid and dicyandiamide. These compounds reacted to give a product stated to be XXIV (78). The alternative structure, XXIII seemed as likely and the substance gives a colourless solution in alkali. Attempts were therefore made to synthesise XXIII by an unambiguous route from 2-thio-4-hydroxyquinazoline.

A small yield of a high melting compound was obtained when anthranilic acid and thio-urea were heated together (c.f. 79). It analysed for $C_{16}H_{12}ON_3S$ instead of the hoped for quinazoline and was not further investigated.

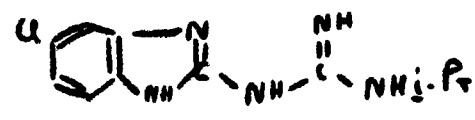
Another route was now tried and methyl anthranilate hydrochloride condensed with potassium thiocyanate to give N-(2-carbomethoxyphenyl)-thiourea , which lost methanol when heated above its melting point giving the quinazoline (c.f. 80).

Treatment with methyl sulphate and alkali gave 2-methylthio-4-hydroxyquinazoline, but though it condenses with aniline to give 2-phenylamino-4-hydroxyquinazoline (81) nothing homogeneous could be isolated from its reaction with guanidine.

Large volumes of hydrogen chloride were evolved when the hydrochloride of the anthranilic acid-dicyandiamide condensate was refluxed with phosphorous oxychloride, but the resulting chlorinated compound could not be characterised or isolated in a pure state. It reacted slowly with boiling diethylamine but nothing homogeneous could be isolated from the resulting mixture.



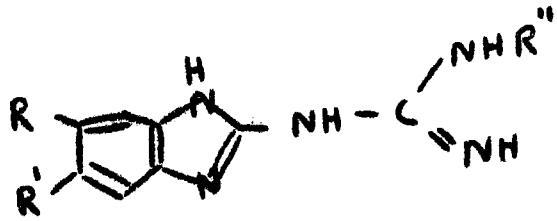
Paludrine



XXVI.

The relationship of paludrine to other possible growth factors required by the malaria parasite will now be considered. One biologically active material to which it is related is benziminazole. The exact analogue of paludrine in this series is XXVI and the two compounds are very similar. The formulation of XXVI from paludrine requires no rearrangement but only the loss of two hydrogen atoms and ring closure. Woolley has shown (82) that the growth of *L. casei*, inhibited by benziminazole, is restored by the addition of guanine or

adenine, so it was thought that paludrine might be acting through interference with a purine enzyme system. This benziminazole analogue of paludrine (XXVI), if dimerised (c.f. XXI) gives a compound even more like a porphyrin than the suggested paludrine dimer, and might also be a possible intermediate in the metabolism of paludrine. The synthesis of some 2-guanidinobenziminazoles, including XXVI, for biological examination therefore seemed well worth while and was immediately undertaken.



XXVII.

2-Guanidinobenziminazole (XXVII, R=R'=R"=H) was first prepared by Ziegelbauer (83) from dicyandiamide and o-phenylenediamine dihydrochloride in ethanol at 105°, although he assigned structure XXIX (R=R'=H) to the product. This was later corrected by Pellizzari (84) who also (85) improved the method of preparation by refluxing the reactants in boiling water. Reinvestigation has shown that a considerable reduction in reaction time is possible and better yields are obtained by using more concentrated solutions. The product was identified by means of the nitrate and picrate, and the other six compounds of type XXVII (R "=H), similarly prepared

from the substituted diamines, were characterised as their picrates, mono- and di-hydrochlorides (see table p. 31).

The intermediates required, apart from the diamines, for the preparation of N^5-alkylated-2-guanidinobenziminazoles are clearly the monoalkylated dicyandiamides, but as far as could be ascertained none had been described in the literature. Sodium dicyanimide, was known to react with two molecules of alkylamine hydrochloride to give biguanidines (86), so attempts were made to obtain the dicyandiamides by using one equivalent of amine hydrochloride. Nothing could be isolated from the attempted condensation of sodium dicyanimide and isopropylamine hydrochloride in the presence of aqueous copper sulphate (c.f. 87) but a small yield of dicyandiamide was obtained by condensing sodium dicyanimide with ammonium chloride in boiling n-butanol. Similar condensations with isopropylamine and n-butylamine hydrochlorides gave good yields of isopropyl- and n-butyldicyandiamides, which were obtained as glassy solids, the isopropyl derivative ultimately crystallising. (After the completion of this work the latter compound was prepared by May from isopropyl-isothiocyanate and reported as a glass, 88). It reacted vigorously with acetic anhydride giving a solid, which by analogy with the compound similarly prepared from dicyandiamide (89) is believed to be 2-isopropylamino-4-methyl-6-hydroxytriazine.

From isopropyldicyandiamide and the appropriate o-phenylene- diamine dihydrochlorides in aqueous solution the corresponding N^5-isopropyl-2-guanidinobenziminazoles were obtained. The hydrochlorides usually failed to separate on cooling, and the free bases, liberated by alkali, generally failed to crystallise at this stage, remaining as yellow gums. Purification through the copper derivatives, as in the paludrine series (87) was achieved with N^5-isopropyl-2-guanidinobenziminazole (XXVII, R = R' = H, R" = i-Pr), and its 5-chloro derivative (XXVI), and the benziminazoles were finally isolated as their highly crystalline dihydrochlorides.

Purification through the picrates was much better than via the copper derivatives, and the 5-methoxybenziminazole (XXVII, R = OMe, R' = H, R" = i-Pr) was isolated from the reaction mixture as the picrate which decomposed with aqueous hydrochloric acid. The 5:6-dimethoxybenziminazole picrate (XXVII R = R' = OMe, R" = i-Pr) was not affected by this treatment owing to its insolubility, but the addition of ether to its solution in hot β-ethoxyethanol in the presence of excess propanolic hydrogen chloride caused a quantitative precipitition of the dihydrochloride. This was undoubtedly the best method examined for the purification of the benziminazoles, and N^5-n-butyl-2-guanidino-5:6-dimethoxy- (XXVII, R = R' = OMe, R" = n-Bu), N^5-isopropyl-2-guanidino-5-methyl- (XXVII, R = Me, R' = H, R" = i-Pr), 5-chloro- (XXVI), and 5:6-dichloro-

benziminazoles(XXVII, R=R'=Cl, R"=i-Pr) were similarly purified and isolated as their dihydrochlorides, which were all colourless crystalline materials.

Attempts to synthesise the benziminazoles in the reverse direction were also made. It was hoped to obtain 2-cyanamino benziminazole (XXVIII) from o-phenylenediamine and dicyanimide, but an addition product, discussed later, was formed.

XXVIII.

2-Cyanaminobenziminazole (XXVIII) was however obtained from 2-guanidinobenziminazole by the action of nitrous acid (85), but could not be induced to combine with isopropylamine (experiments by P.C. Spensley).

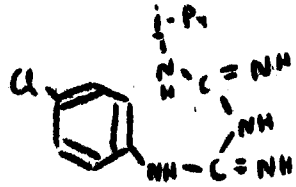
The benziminazoles were tested for antimalarial activity against either P. gallinaceum in chicks; or P. relictum in canaries, (see table, p.38). None were appreciably more toxic than paludrine, but at the maximum tolerated doses only two, 2-guanidino-5:6-dichlorobenziminazole (XXVII, R=R'=Cl, R"=H) and its N^5-isopropyl derivative (XXVII, R=R'=Cl, R"=i-Pr), showed even slight activity.

The absence of activity is particularly interesting in the case of N^5-isopropyl-2-guanidino-5-chlorobenziminazole

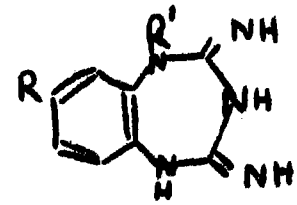
(XXVI) which has the close resemblance to paludrine. Both structures have the same capacity for tautomerism and prototropy, and the analogue forms a copper complex, like that of paludrine in which two molecules of the base combine with one atom of copper. The only definite conclusion that can be drawn from the inactivity of this analogue (XXVI) is that it cannot be the active metabolite of paludrine produced in vivo. Further conclusions are difficult to draw in view of the extreme specificity of biological action, but the porphyrin antagonism hypothesis is rendered unlikely. The inactivity of these compounds may also be considered to support Hawkings work in that they may be too condensed in structure to undergo the in vivo chemical change required to give the active compound.

The new amine obtained from boiling aqueous dicyanimide and o-phenylenediamine was isolated as the nitrate. It was further characterised as the picrate, and the free base, a brown powder, tenaciously retained water. Analytical results showed the amine to be an addition product of the reactants, isomeric with 2-guanidinobenziminazole. It slowly lost ammonia with hot 30% aqueous sodium hydroxide and was decomposed by hot concentrated hydrochloric acid. Nothing homogeneous could be isolated from these reactions. The amine failed to react with nitrous acid, unlike 2-guanidinobenziminazole, so the only likely structure for it is XXIX

(R=R'=H), 2:4-diamino-1:3:5-triazabenzepine.



Paludrine.



XXIX.

The formation of a 7-membered ring from o-phenylenediamine also takes place with ethyl malonate (90) and acetylacetone (91).

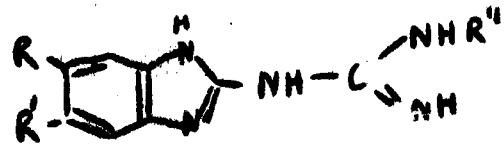
No compounds of this benzepine type, cyclic biguanidines, have hitherto been described, and in view of the chemotherapeutical importance of biguanidines it was decided to prepare a series for biological testing. The 7-chloro-derivative (XXIX, R=Cl, R'=H), prepared from 4-chloro-o-phenylenediamine as before, was isolated as the picrate and converted to the hydrated hemisulphate by the method used with the alkylated guanidinobenziminazoles.

An extension to the 1-alkylbenzepines was contemplated and it was found that N-methyl-o-phenylenediamine reacted with dicyanimide, as before, to give the benzepine (XXIX, R= H, R'=Me) isolated as the dipicrate. 1-Methyl-2-guanidinobenziminazole, obtained from the same diamine and dicyandiamide formed a monopicrate under the same conditions. The 1-methylbenzepine sulphate failed to react with nitrous acid, confirming its structure. The synthesis of 1-ethyl- and 1-isopropyl-8-chloro-2:4-diamine-1:3:5-triaza-benzepine

sulphates (XXIX R=Cl, R'=Et and i-Pr respectively) was achieved from the appropriate diamines by the same route; the latter compound is of interest in being a possible active metabolite of paludrine. It has however, a "blocked" amidine system, and the results of antimalarial tests are awaited (see table p 32). None of the benzepines showed any activity against S. aureus or B. coli broth cultures.

The diamines required for the preparation of the last two benzepines were obtained by catalytic reduction of 2-nitro-5-chloro-N-ethyl, and N-isopropyl-anilines, synthesised from 2:4-dichloronitrobenzene and the amines at 200°.

Biological Tests on the 2-guanidinobenziminazoles.

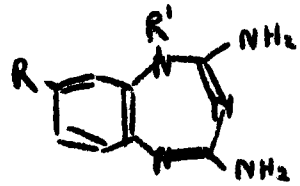


The ring size in mm., produced by the drug at a concentration of 1 mg./ml. when tested against broth cultures of B. coli (column A), S. aureus (column B) and S. aureus diluted 1:1,000 (column C), is recorded below, with the results of the antimalarial testing.

R.	R'.	R".	Salt.	A.	B.	C.	Tested against:	Activity.
H	H	H	2HCl	-		-	P. gallinaceum.	Nil.
Me	H	H	2HCl	-	-	-	P. relictum.	Nil.
* MeO	H	H	2HCl	-		-	P. gallinaceum.	Nil.
* Cl	H	H	2HCl	-		-	P. gallinaceum.	Nil.
Me	Me	H	HCl.1½H$_2$O	-	-	trace	P. relictum.	Nil.
MeO	MeO	H	HCl.H$_2$O	-	-	-	P. relictum.	Nil.
Cl	Cl	H	HCl.H$_2$O	-	-	-	P. relictum.	Slight.
H	H	i-Pr	2HCl	-		-	Not tested.	
Me	H	i-Pr	2HCl	-	-	-	P. relictum.	Nil.
MeO	H	i-Pr	2HCl	-		-	P. gallinaceum.	Nil.
Cl	H	i-Pr	2HCl	13		25	P. gallinaceum.	Nil.
Me	Me	i-Pr	HCl.H$_2$O	-	trace	13	P. relictum.	Nil.
MeO	MeO	i-Pr	2HCl	-	-	-	P. relictum.	Nil.
Cl	Cl	i-Pr	2HCl	-	-	10	P. relictum.	Slight.
MeO	MeO	n-Bu	2HCl	-	trace	-	P. relictum.	Nil.

* Prepared by P.C. Spensley, according to the method used for the unsubstituted derivative (XXVII, R=R'=R"=H).

Biological Tests on the Benzepines.



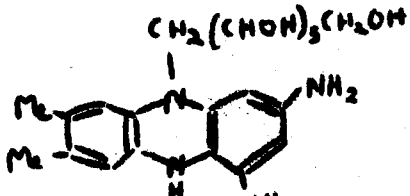
R.	R'.	Salt.	A.	B.	C.	Tested against:	Activity.
H	H	HNO_3	-		-		
Cl	H	$\frac{1}{2}H_2SO_4.\frac{1}{2}H_2O$	-		-		
H	Me	$H_2SO_4.2H_2O$	-		trace.		
Cl	Et	$H_2SO_4.H_2O$	-		-		
Cl	i-Pr	H_2SO_4	-		-		

Part II. Alloxazines and isoAlloxazines.

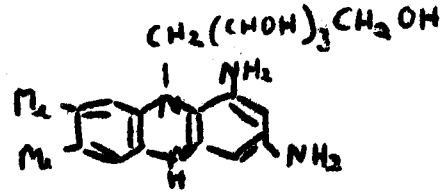
Some preliminary experiments on this subject were carried out by A.B. Yorke-Long (Part II thesis, Oxford University, June 1946). An independent investigation and extension of his work showed that most of it was misleading, and none of the results reported here are due to him.

Riboflavine is a growth factor of great importance and is a constituent of at least two coenzymes involved in carbohydrate and amino acid metabolism. Many very similar compounds have been prepared and examined for vitamin, and antagonistic activity. Riboflavine activity has been found in at least five isoalloxazines (99), but most of the compounds examined have been inactive, or weakly antagonistic towards the vitamin. 6:7-Dichloro-9-d-ribityl-isoalloxazine is however a powerful antagonist (100), and antagonises the growth of organisms both independent of and dependent on preformed riboflavine. The oxidation potential of this compound (E_0 = -0.095v, pH = 7) differs considerably from that of riboflavine (E_0 = -0.185v, pH = 7) and this may be the cause of the antagonism. Woolley (101) has also shown that the growth inhibitory action of a diamino-dihydrophenazine derivative, stated to be XXX, on certain organisms is reversed by riboflavine. There is not sufficient evidence in his paper to assign this structure to the phenazine which is synthesised by an ambiguous route

and structure XXXI is not excluded.

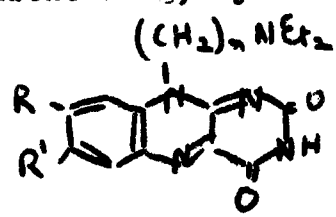


XXX.



XXXI.

Oesterlin (67) suggested that the antimalarial action of mepacrin was due to its interference with a riboflavine enzyme system. As it is impossible to alter appreciably the riboflavine concentration in the body of the host, the action of various drugs on L. casei, which requires riboflavine for normal growth, was examined (76 and 102). The growth inhibitory effect of mepacrin, propamidine, quinine, methylene blue, 2666 and 3349 was reversed by the addition of riboflavine, but the action of other bacteriocidal agents was not affected. This led to the preparation of a series of basically substituted isoalloxazines (XXXII) with the dialkylaminoalkyl groups of current antimalarials in position 9, and various substituents, e.g. R, R', = H, Cl, OMe, Me, in the benzene ring, by the author with



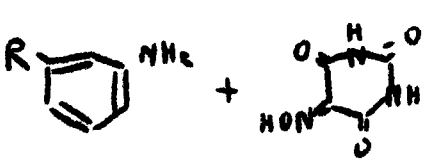
XXXII.

Prof. F.E. King (103), and others (104), but the compounds prepared had no large bacteriostatic or antimalarial properties.

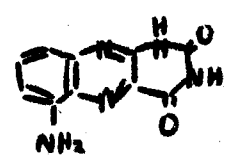
One part of the programme in this laboratory included the preparation of some 9-diethylaminoalkyl-*iso*alloxazines with amino groups in the benzene ring, for biological testing.

Some aminoalloxazines had already been prepared by Piloty (105) from the interaction of *m*-phenylenediamine and violuric acid, or its 1:3-dimethyl derivative. As 2:4-diamino-γ-diethylaminopropylaniline did not give the expected *iso*alloxazine with alloxan under the usual conditions (King and Acheson, 103.) it was decided to try and extend Piloty's synthesis to the *iso*alloxazine series.

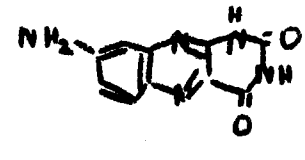
The formation of an alloxazine from *m*-phenylenediamine (XXXIII R=NH_2) and violuric acid requires an active position in the benzene ring *ortho*- to an amino group. Three such positions, two of which are equivalent, exist in this amine, and the product can be either the 5 (XXXIV) or the 7-amino-alloxazine (XXXV). Piloty however suggested that the 7-amino compound was formed.



XXXIII.



XXXIV.



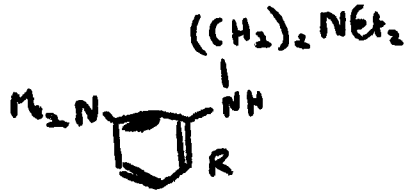
XXXV.

Ganapathi (106) while attempting to prove this, condensed 1:2:4-triaminobenzene with alloxan and obtained an amino-alloxazine, probably a mixture of the 6- and 7-isomers,

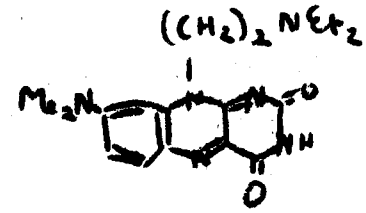
which he compared with the compound obtained by Filoty's method. Both materials were very sparingly soluble solids with high decomposition points, which rendered their comparison inconclusive.

The 7-amino structure for Filoty's compound is now confirmed by analogy with the results of the following experiments.

3-Dimethylamino-β-diethylaminoethylaniline (XXXVI, R = H) reacted with violuric acid in hot aqueous alcoholic solution to give 7-dimethylamino-9-(β-diethylaminoethyl)-isoalloxazine (XXXVII), which separated as the violurate tetrahydrate. This compound lost most (3½ mol.) of its water at 120°, the remainder at 150° in vacuo, and gave a crystalline picrate with aqueous sodium picrate. The constitution of the isoalloxazine was proved by a synthesis from 2-amino-5-dimethylamino-β-diethylaminoethylaniline (XXXVI, R = NH_2) and alloxan in boric-acetic acid solution, which can only give the 7-dimethylamino derivative. The complete identity of both products was established by a comparison of the violurate and picrate from both syntheses. In contrast to the high solubility of other 9-dialkylaminoalkyl-isoalloxazines in water (Kipnis et al., 104), the carmine 7-dimethylamino-9-(β-diethylaminoethyl)-isoalloxazine free base is only slightly soluble, but dissolves easily in dilute sodium hydroxide or acetic acid.



XXXVI.

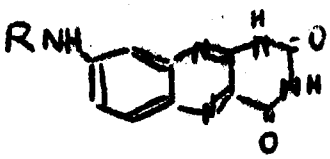


XXXVII.

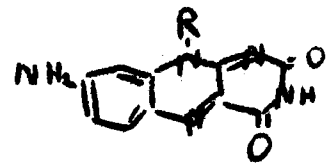
3-Dimethylamino-β-diethylaminoethylaniline, characterised as the mono- and di-picrates, was prepared from 3-dimethylamino-p-toluenesulphonanilide by alkylation with β-chlorethyldiethylamine, the resulting oily sulphonamide bing hydrolysed by diluted sulphuric acid. The tetraamine used in the alloxan synthesis was the reduction product of 2-nitro-5-dimethylamino-β-diethylaminoethylaniline, obtained by heating β-diethylaminoethylamine with 3:4-dinitrodimethylaniline, the labile 3-nitro group being displaced (c.f. 107). The preparation of the latter compound from 3-nitrodimethylaniline using nitrous acid (Hodgson and Smith, 108) was found to be unsatisfactory and gave a mixture of products. Direct nitration, mentioned by these authors and used earlier (109), gave much better results.

The use of a secondary-tertiary amine in the Piloty synthesis admits only of the formation of an isoalloxazine. If however a meta-primary-secondary amine, e.g. 3-amino-N-methylaniline, is used in the condensation, and this proceeds through the 6- position of the benzene ring as

before, the product may then be an alloxazine (XXXVIII, R = Me), and/or an isoalloxazine (XXXIX, R = Me).

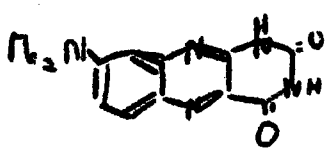


XXXVIII.

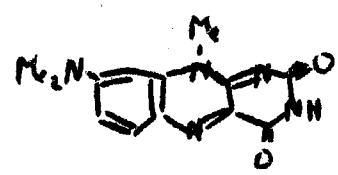


XXXIX.

The product from this condensation, a microcrystalline red-brown powder, was first thought to be homogeneous. It was hoped to determine its structure by a comparison of its ultra-violet absorption spectrum with the spectra of reference compounds of definite structure. For this reason the orange-red 7-dimethylaminoalloxazine (XL) was synthesised from 3-aminodimethylaniline and violuric acid, and the corresponding 7-dimethylamino-9-methyl-isoalloxazine (XLI), a carmine crystalline material, was similarly prepared from 3-methylamino-N-dimethylaniline. This was prepared from 3-dimethylamino-p-toluenesulphonanilide by alkylation and hydrolysis.

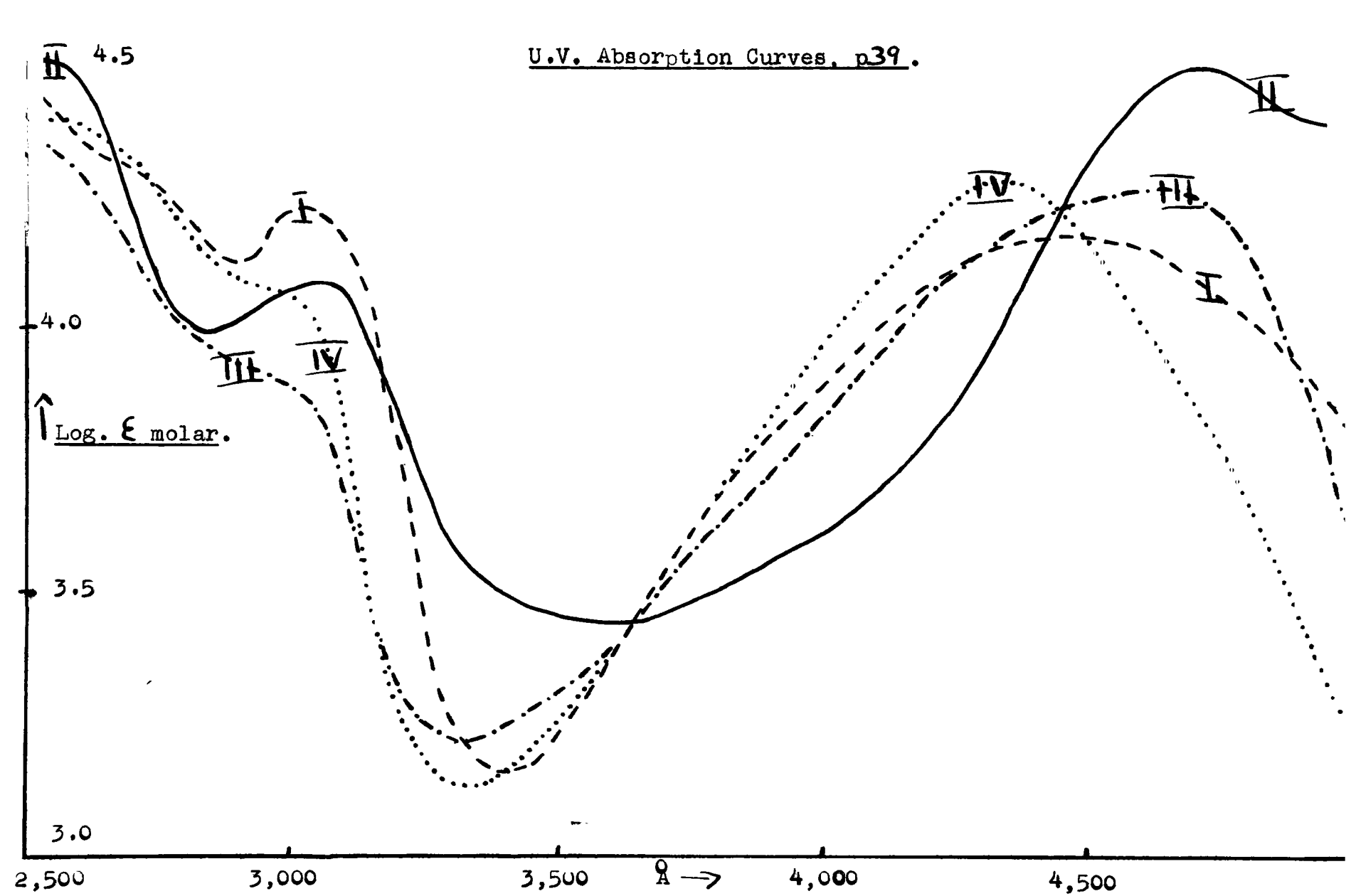


XL.



XLI.

The absorption spectra of these two compounds, the alloxazine (XL, p.39, curve 1), and the isoalloxazine (XLI, p. 39, curve 2), are similar, but there is an


U.V. Absorption Curves, p39.
II
4.5
I
IV
III
II
4.0
III
IV
I
Log. ε molar.
3.5
3.0
2,500
3,000
3,500
Å →
4,000
4,500

observable difference over the whole range examined. The absorption curve for the 3-aminomethylaniline - violuric acid condensate (p39 , curve 3) was found to lie between those of the reference compounds, and it was clear that the substance was heterogeneous. Repeated extraction of this red-brown condensate with dilute hydrochloric acid, which was designed to remove the more basic isoalloxazine, left an orange-red solid whose absorption (p.39 , curve 4) approximated closely to that of the alloxazine. The change in colour on acid extraction is significant in view of the different colours of the reference compounds.

The absorption spectra of 7-dimethylamino-9-methyl-isoalloxazine differed considerably from those of riboflavine and some 9-dialkylaminoalkyl-isoalloxazines (Adams et al., 104). The whole curve was shifted to the lower frequency end of the spectrum as might be expected from the introduction of an amino group, but the maxima present in riboflavine and in Adams's compounds at c.3500 Å had vanished.

Further proof that the material in question was a mixture was provided by the action of nitrous acid. Diazotisation in sulphuric acid solution gave the sparingly soluble 7-(N-nitrosomethylamino)-alloxazine, which was collected, and a solution of 9-methyl-isoalloxazine-7-diazonium sulphate. This coupled with alkaline β-naphthol to give a deep olive green azo-dye. Diazotisation of

7-aminoalloxazine under the same conditions gave a clear solution, and a larger yield of a similarly coloured azo-β-naphthol derivative. Assuming that the yields in the formation and coupling of the two diazonium sulphates are about the same, it is clear that the ratio of alloxazine to isoalloxazine in the material is about 40:60.

From the action of violuric acid on 3-amino-β-diethylaminoethylaniline, which was prepared from 3-nitro-p-toluenesulphon-(β-diethylaminoethyl)-anilide by hydrolysis and reduction of the resulting nitrosamine, a crystalline violurate was isolated. This may have contained alloxazine and/or isoalloxazine, but its constitution was not further investigated.

The higher proportion of isoalloxazine in the mixed product of the diamine - violuric acid condensation is to be expected from the probable course of the reaction, i.e., preliminary addition at the 4- or 6-carbonyl of the pyrimidine followed by ringclosure between the isonitroso group and the activated 6-position in the benzene ring. With amino groups of different basicities, e.g. NH_2 and NHMe, reaction with the more basic centre, NHMe, would predominate, leading to a larger proportion of isoalloxazine. Condensation of the isonitroso group with an activated position in the aromatic nucleus is very improbable, since the intermediate would be analogous to the so-called Kuhling products obtained when the condensation of o-diamines with

alloxan is carried out in the absence of acid, and these are known to be extremely resistant to cyclisation (110).

Attempts have been made, by Ganapathi (106), to extend the Piloty synthesis to other m-substituted anilines, but without success, from which he concludes that the m-amino group is specific for this reaction. It is evident, however, that the degree of activation in the aromatic nucleus is the important factor, since from 4-aminoveratrol and violuric acid, in acetic acid solution, it has been possible to synthesise, though not in a spectroscopically pure condition, 6:7-dimethoxyalloxazine. The use of water as a solvent gave a product containing only c. 9% of the alloxazine, whose constitution was proved by an independent synthesis from 4:5-diaminoveratrol and alloxan.

7-Dimethylamino-9-(β-diethylaminoethyl)-isoalloxazine hydrochloride (1 mg./ml.), 7-dimethylamino-9-methyl-iso-alloxazine and 7-dimethylaminoalloxazine (saturated aqueous alcoholic solutions) showed no activity when tested against B. coli and S. aureus broth cultures. The results of antimalarial testing are awaited.

Part III. The Synthesis of some Benziminazoles.

Most of the published methods for the synthesis of benziminazoles require fairly vigorous conditions, under which labile groups, such as peptide linkages, would be decomposed. The projected synthesis of some compounds with this type of substituent (see section 4) necessitated a mild reaction for the building up of the benziminazole nucleus, otherwise this part of the molecule would have to be synthesised first.

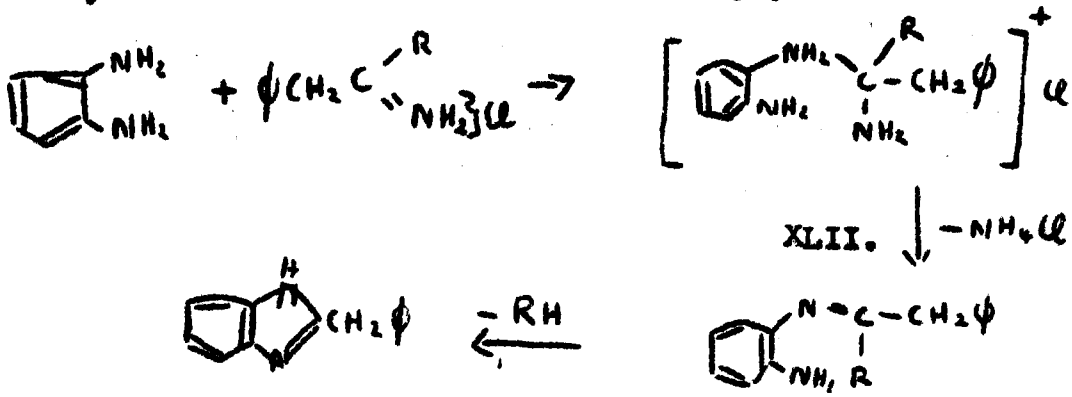
The reaction of iminoether hydrochlorides and ethylene diamine to give iminazolines (113) takes place under mild conditions, and its extension to the benziminazole series was contemplated.

Wheeler (114) obtained 2-phenylbenziminazole and the corresponding benzoxazole from benziminomethylether by heating with o-phenylenediamine or o-aminophenol without a solvent, and reported that little, if any, reaction took place in the cold. No yields are specified. The formation of 2-phenylbenziminazole by this method is not recorded in "Beilstein", and the method was rediscovered by the author. Wheelers observation has been confirmed by analogy as phenylacetiminomethylether did not appear to react with o-phenylenediamine in boiling methanol, but the addition of a crystal of iminoether hydrochloride caused a slow evolution of ammonia with the production of some

benziminazole. A mixture of the free iminoether and the diamine began to evolve ammonia at 115°, rapidly at about 130°, giving a 35% yield of the benziminazole. The iminoether hydrochloride in methanol, however, reacted vigorously with cold methanolic solutions of o-phenylenediamine and its N-methyl derivative, to give good yields of 2-benzyl-, and 1-methyl-2-benzylbenziminazole, and the reaction has been applied to the synthesis of other benziminazoles. The yield of 2-benzylbenziminazole was nearly as good when o-phenylenediamine hydrochloride replaced the amine in the reaction, but the use of the dihydrochloride reduced the yield by about 50%. Phenylacetiminothiobenzylether hydrochloride also gave good yields of the benziminazoles with both diamines, but the use of o-phenylenediamine dihydrochloride again gave a reduced yield.

This result follows if the first stage in the reaction is the attack of the iminoether cation on the aromatic diamine. If one equivalent of acid is present there will still be one uncharged amino group available for the condensation. A second equivalent of acid gives both aromatic amino groups positive charges, which will repel the approach of the iminoether cation, and so hinder reaction. The complex (XLII) produced by this reaction is then assumed to lose ammonium chloride (Schmidt, 116)

to give a substituted iminoether (XLIII), which can then react intramolecularly, losing methanol or benzyl thiol, to give the benziminazole. The alternative mechanism, loss of methanol or thiol followed by ringclosure of the resulting amidine, is less likely, as the Schmidt reaction is usually much faster than amidine formation.



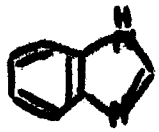
XLII.

R = OMe, or $SCH_2C_6H_5$. XLIII.

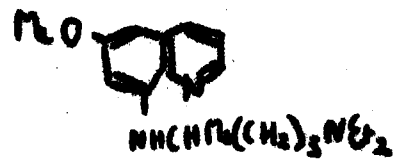
g-Aminophenol also reacted easily with acetiminomethylether, and acetiminothiobenzylether hydrochlorides to give 2-methylbenzoxazole, characterised as the picrate. In view of these two experiments the observation of Drozov and Bekhli (117) that palmitiminoethylether hydrochloride reacted with 2-hydroxy-3-piperidino-n-propylamine to give an iminazoline is unlikely. The compound was isolated from the reaction as the dipicrate, and analytical results were poor. It is provisionally regarded as 2-pentadecyl-5-(N-piperidinomethyl)-oxazoline dipicrate.

The structural similarity of the benziminazole nucleus

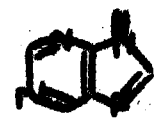
to that of plasmoquin and of the purine ring systems suggested that the synthesis of some benziminazoles for bacteriostatic and antimalarial testing might be worth while.



Benziminazole.



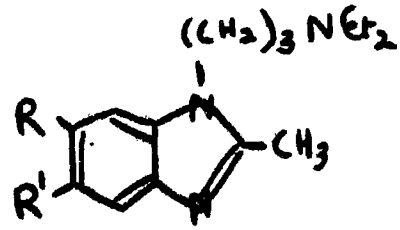
Plasmoquin.



Purine.

Some compounds in this series, containing dialkylaminoalkyl groups, with alkyl and alkoxyl substituents, had already been prepared, but were all devoid of antimalarial activity (summary, 115). None of the compounds described and tested contained chlorine as a substituent. This was surprising in view of its great importance in other antimalarials, and the preparation of some chlorobenziminazoles was undertaken.

5-, And 6-chloro-2-methyl-1-(γ-diethylaminopropyl)-benziminazole (XLIV, R = H, R' = Cl, and R = Cl, R' = H respectively) were prepared from the appropriate diamines, obtained from the corresponding 2-nitroanilines (103 and 121) by catalytic reduction, and acetic acid in 4N aqueous hydrochloric acid (c.f. 118). 5:6-Dichloro-2-methyl-1-(γ-diethylaminopropyl)-benziminazole (XLIV, R = R' = Cl) was similarly synthesised from 2-nitro-4:5-dichloro-γ-diethylaminopropylaniline, prepared from 1:2-dichloro-4:5-dinitrobenzene and γ-diethylaminopropylamine.



XLIV.

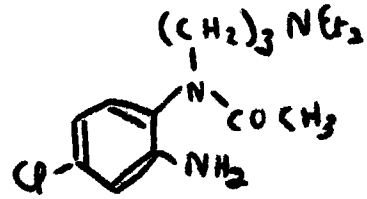
None of the benziminazoles showed any activity against S. aureus, or B. coli, at concentrations of 1 mg/ml. The monochloro derivatives (XLIV, R = H, R' = Cl and R = Cl, R' = H) were inactive against A. gallinaceum in chicks, and the results for the dichlorobenziminazole (XLIV, R = R' = Cl) are awaited.

The benziminazole bases were pale yellow oils, or low melting solids, which formed stable dipicrates and dipicrolonates.

Attempts to synthese these benziminazoles by the iminoether method were satisfactory in only one case; the 6-chloro-compound (XLIV, R = Cl, R' = H) was obtained in good yield from the diamine and acetiminothiobenzylether hydrochloride. Acetiminomethylether hydrochloride reacted easily with 2-amino-4-chloro-γ-diethylaminopropylaniline to give an amidine hydrochloride along with small quantities of the benziminazole (XLIV R = H, R' = Cl) isolated as the dipicrate. Similar results were obtained in the presence of one additional equivalent of hydrochloric acid, but in the presence of two equivalents no benziminazole was isolated.

The production of a little ammonium chloride in the last reaction indicated some hydrolysis, and this was borne out by the nitrogen content of the product. The free amidine, an oil rapidly blackening in air, was prepared by basifying the aqueous hydrochloride, and was further characterised by the preparation of mono- and dipicrates. Though the melting points of all these derivatives were sharp, the nitrogen values were invariably low, but not nearly low enough for the corresponding N-acetyl derivatives.

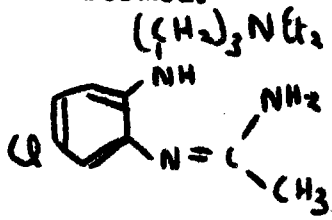
Attempts to synthesise XLV for comparison purposes from 2-nitro-4-chloro-γ-diethylaminopropylaniline by acetylation followed by reduction failed. Acetylation was not accomplished even by acetic anhydride in the presence of sulphuric acid at 200°, or by the action of acetyl chloride on a mixture of the amine and sodamide in boiling xylene.



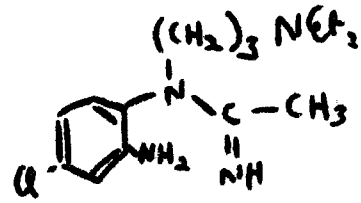
XLV.

There are two possible structures for this amidine, XLVI and XLVII, which is stable to distillation. Analogy to the formation of benziminazoles, or otherwise, from alkylated o-aminoacylanilides (119) is hardly justified as the amidino group is much more highly stabilised than the

acetamido group. The amidine, on refluxing with acetyl chloride, gave the benziminazole (XLIV R = H, R' = Cl) in nearly theoretical yield. Structure XLVII might be expected to acetylate on the primary amino group, while XLVI might acetylate either on the amidino group, or on the secondary nitrogen atom. The former seems more likely, and by cleavage of acetamide the benziminazole could be formed.



XLVI.



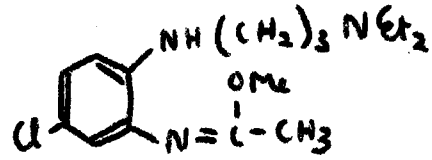
XLVII.

A solution of the amidine hydrochloride in N aqueous hydrochloric acid gave a deep cherry red colour with nitrous acid. Treatment with dilute sodium hydroxide gave a red-brown opalescence which gave back the red colour on reacidification. Alkaline β-naphthol solution gave the same colour with the "diazotised" solution as dilute sodium hydroxide. This suggests the absence rather than the presence of a primary amino group in the amidine, whose constitution is provisionally considered to be XLVI.

Acetiminomethylether hydrochloride and 2-amino-4:5-dichloro-γ-diethylaminopropylaniline, in the presence of two equivalents of hydrochloric acid or otherwise, likewise

failed to give the benziminazole, and the product analysed for the corresponding amidine.

A partial explanation of these results has been found. Steric hindrance seems eliminated because acetiminothiobenzylether hydrochloride reacts with 2-amino-5-chloro-γ-diethylaminopropylaniline to give the corresponding benziminazole, and the reaction gives benziminazoles with simple N-substituted diamines. Assuming that a Schmidt reaction takes place between the iminoether and the primary amino group of the triamine, the product will be XLVIII.



XLVIII.

If this is to give the benziminazole (XLIV, R = H, R' = Cl) intramolecular reaction involving the secondary amino group must take place. To obtain the amidine (XLVI) reaction must occur between the iminoether (XLVIII) and ammonium chloride in solution. The relative basicity of the secondary amino group, depressed by the p-chlorine atom, to that of ammonia, is therefore important, and the reaction was examined when the chlorine atom was replaced by a methoxyl group.

2-Nitro-4-methoxy-γ-diethylaminopropylaniline (103) was hydrogenated to the triamine, and i), converted into

the benziminazole (XLIV R = H, R' = OMe) by the Phillips method, for comparative purposes, ii), treated with acetiminomethylether hydrochloride. From this reaction a considerable quantity of benziminazole was isolated as the dipicrate.

The effect of p-substitution to the secondary amino-group is thus demonstrated, and it is hoped to investigate the reaction with substituted alkylated diamines to discover the effect of the γ-diethylaminopropyl sidechain on the reaction.

The author is indebted to Dr J.W. Cornforth for some helpful discussion on the iminoether reaction.

Part IV. The Synthesis of Pteroylglutamic acid and Related Compounds

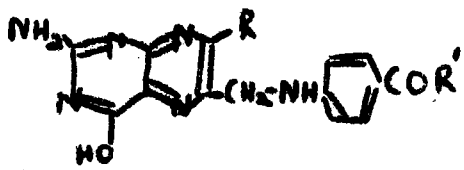
The main aim of this work was the preparation of some compounds designed to antagonise the growth promoting activity of pteroylglutamic acid (XLIX, R = H, R' = l-, NHCH(COOH) CH_2CH_2COOH).

A number of compounds with this property have been reported while this work was in progress, and are now summarised.

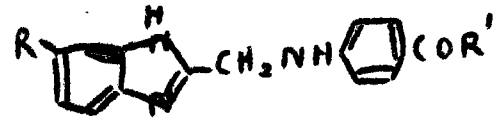
Compound XLIX (R = Me, R' = d-, NHCH(COOH)CH_2CH_2COOH), prepared in a crude state and not chemically characterised, reversibly antagonised the growth promoting action of pteroylglutamic acid on L. casei, and S. lactis R, and caused typical deficiency symptoms when administered to rats (123, 124). The bacteriostatic action of some pterins (125), N^4-(2:4-diaminopteridyl-6-methyl)-4-aminobenzoylglutamic acid (126), and pteroylaspartic acid (127) was also prevented by pteroylglutamic acid. The deleterious effects of pteroylaspartic acid on chick nutrition were also prevented by pteroylglutamic acid (127), and clinical examination of magnesium formylpteroate and formyl-pteroylglutamate, of pteroylaspartic, oxypteroic, oxypteroylglutamic, and N^4-(4-quinazoline)-4-aminobenzoylglutamic acids has revealed their inactivity against human anaemia (128).

L. casei and S. lactis R can develop in the absence of pteroylglutamic acid if certain purine and pyrimidine bases

are present (129), and the bacteriostatic effect of benziminazole on the growth of yeast and two other organisms is prevented by adenine (82). Though this is not true of E. coli (135) it was decided to prepare the benziminazole analogues (L, R = H, or Cl, and R' = OH or l-, NHCH(COOH) CH_2CH_2COOH) of pteroic and pteroylglutamic acids for biological testing.



XLIX.

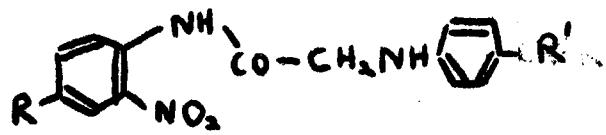


L.

Following the discovery of the iminoether benziminazole synthesis (part II), Spensley (this laboratory) tried to use the method to prepare these benziminazole analogues (L). He tried, as a model, to prepare some iminoetners of anilinoacetonitrile, for the o-phenylenediamine condensation. Great difficulty was experienced in their preparation and the route was abandoned.

Preliminary experiments, by the author, showed that chloracet-2-nitroanilide, and its 4-chloro-derivative condensed with aniline in boiling n-propanol to give the corresponding phenylglycineanilides (LI, R' = H, R = H and Cl respectively, c.f. 130). The use of pyridine as a solvent in the latter condensation gave mainly pyridiniumacet-2-nitro-4-chloroanilide chloride, with a little of the desired product (LI, R = Cl, R' = H). Catalytic reduction of phenylglycine-2-nitro-4-chloroanilide gave the corresponding

ringclosed to the benziminazole, isolated as the dihydrochloride and characterised as the picrate, in boiling aqueous methanolic hydrogen chloride.



LI.

This synthesis was now extended to the preparation of L (R = H, R' = OEt), and ethyl p-aminobenzoate condensed similarly with both chloracetanilides to give the corresponding p-carbethoxyphenylglycine anilides (LI, R'=COOEt, R = H and Cl) in poor yield, which was somewhat increased by the addition of pyridine. Catalytic reduction of p-carbethoxyphenylglycine-2-nitroanilide gave the corresponding amine, which ringclosed to the benziminazole in boiling alcoholic hydrochloric or picric acid. It was isolated as the dihydrochloride and picrate respectively, which proved identical with those prepared by Spensley from the 2-chloromethylbenziminazole - ethyl p-aminobenzoate condensate.

Further experiments in this series were carried out by Spensley, who finally prepared N^4-(benziminazole-2-methyl)-4-aminobenzoyl-l-glutamic acid (L, R = H, R' = l-, NHCH(COOH)CH_2CH_2COOH). Nimmo-Smith found that this compound did not replace pteroylglutamic acid for the growth of S. lactis R, and inhibited growth in the presence of suboptimal quantities

of this material. Essentially the same results were obtained with L. casei.

N-(Benziminazole-2-methyl)-4-aminobenzoic acid (1, R = H, R' = OH) was less inhibitory to the growth of S. lactis R than its glutamic derivative, and did not replace pteroylglutamic acid for the growth of this organism.

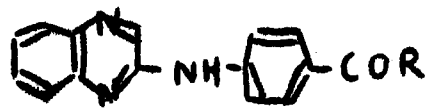
Edwards et al. (131) later reported the synthesis of L (R = H, R' = 1-, NHCH(COOH)CH_2CH_2COOH) and state that it replaced pteroylglutamic acid, though at a much higher concentration, for the growth of S. lactis R, and stimulated growth in the presence of suboptimal amounts of pteroylglutamic acid.

The corresponding benzene sulphonylglutamic acid (above) had only antagonistic properties.

These differing biological results are probably due to the slightly different culture solutions used by Nimmo-Smith and Edwards. This also explains the growth factor activity of N^4-(2-quinazoline)-4-aminobenzoylglutamic acid[132], examined in the medium used by Edwards, for L. casei, though the compound is useless in the treatment of anaemia (128).

The pterin ring system is much more closely related to quinoxaline than benziminazole, so investigations into the

preparation of some pteroic and pteroylglutamic analogues in this series were begun. Further interest was added to the project as Hall (133), while this work was in progress, reported that the growth of S. lactis R, inhibited by quinoxaline, was restored by excess pteroylglutamic acid.



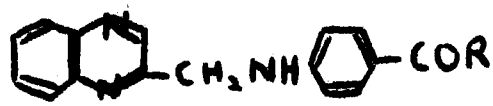
LII.

N-(2-quinoxaline)-4-aminobenzoic acid (LII, R = OH) was prepared from 2-chloroquinoxaline (prepared according to 136) by refluxing with p-aminobenzoic acid in n- propanol. It did not, however, appreciably affect the growth of S. lactis R.

2-Chloroquinoxaline and p-aminobenzoylglutamic acid did not give the pteroylglutamic analogue under the same conditions, but ethyl N^4-(2-quinoxaline)-4-aminobenzoyl-l-glutamate was obtained from the quinoxaline and the benzoylglutamic ester in ethanol. On alkaline hydrolysis it gave the desired acid (LII, R = l-, NHCH(COOH)CH_2CH_2 COOH).

This compound had a small inhibitory effect on the growth of L. casei, prevented by pteroylglutamic acid, and a more detailed biological investigation is being

carried out.



LIII.

Experiments on the synthesis of N-(quinoxaline-2-methyl)-4-aminobenzoic acid (LIII, R = OH) were now started, and it was hoped to extend them to the synthesis of the pteroylglutamic acid analogue (LIII, R = l-, $NHCH(COOH)CH_2CH_2COOH$).

The synthesis of 2-chloromethyl-, or 2-bromomethyl-quinoxaline (LIV) was the first object, and it was hoped to obtain this from 2-hydroxymethylquinoxaline. Norrish and Griffiths (137) reported the synthesis of this compound from a photodecomposition product of glyoxal and o-phenylenediamine, but there is little evidence for its constitution and attempts were made to prepare it from the tetrahydro series.

LIV.

The synthesis of 2-hydroxymethyl-1:2:3:4-tetrahydro-quinoxaline from glycide alcohol and o-phenylenediamine

was contemplated, but model experiments with ethylene oxide and the diamine showed that ringclosure to the quinoxaline did not take place. 2-Amino-β-hydroxyethylaniline, characterised as the mono- and dipicrates, was isolated from the reaction.

2:3-Dibromopropanol reacted with di-p-toluenesulphonyl-o-phenylenediamine in the presence of sodium ethoxide to give 1:4-di-p-toluenesulphonyl-2-hydroxymethyl-1:2:3:4-tetrahydroquinoxaline. Hydrolysis to the 2-hydroxymethylquinoxaline was first achieved using diluted sulphuric acid, but the yields were very variable and often very little product was obtained. Hydrochloric acid, at 160-170°, gave reproducible yields of the quinoxaline, characterised as the picrate. Oxidation with alkaline potassium ferricyanide, as for tetrahydroquinoxaline (138), gave a black tar, from which a little quinoxaline was isolated.

2-Methylquinoxaline was next prepared from o-phenylenediamine and isonitrosoacetone (151). Contrary to the statement of Kuster (139), confirming that of Freons (140), it was not found possible to nitrosate acetone in aqueous solution, and the isonitrosoacetone was made from acetoacetic ester (141).

Attempts to brominate 2-methylquinoxaline directly to 2-bromomethylquinoxaline gave an unstable black tar. 2-Tribromomethylquinoxaline was easily prepared by direct

bromination (142), but attempts to reduce it, using stannous bromide, to 2-bromomethylquinoxaline gave a black tar.

The tribromomethylquinoxaline with sodium methoxide gave methyl quinoxaline-2-orthocarboxylate. Attempts to prepare N-(quinoxaline-2-carbosy)-4-aminobenzoic acid by condensing this ortho-ester with p-aminobenzoic acid in boiling xylene gave a brown powder that could not be purified.

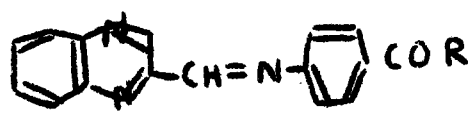
Oxidation of 2-methylquinoxaline with lead tetraacetate (c.f. 153) gave a black tar, from which some unchanged 2-methylquinoxaline was isolated.

It was thought that 3-bromo-1-isonitrosoacetone might react with o-phenylenediamine to give the required bromomethyl quinoxaline, but attempts to prepare this compound from 3-bromoacetoacetic ester by the method of Ceresole (141) gave black tars. The reaction of 1:3-dibromoacetone with o-phenylenediamine in boiling ethanol also gave a black intractable tar (c.f. 144).

The reaction of isonitrosoacetoacetic ester and o-phenylenediamine was examined in the hope that it would give 2-methyl-3-carbethoxyquinoxaline, and that the reaction might be extended to the use of 3-bromo-isonitrosoacetoacetic ester. The products of the reaction, in the presence or otherwise of acid, were 2-methylbenziminazole, characterised as the picrate, and 2-methyl-3-hydroxyquinoxaline.

Attempts were now made to obtain ~~ethyl~~ N-(4-carbethoxy-phenyl)-N-formyl-3-amino-1-isonitrosoacetone. The corresponding ethylacetoacetate was prepared, in very low yield, from the reaction of 3-bromoacetoacetic ester and the sodio derivative of ethyl N-formyl-p-aminobenzoate under certain conditions. Enough material for nitrosation and subsequent hydrolysis could not be obtained.

The next route examined started with quinoxaline-2-aldehyde, prepared in poor yield from 2-methylquinoxaline and selenium dioxide (143). It reacted with p-aminobenzoic acid, and its ethyl ester, to give N-(quinoxaline-2-methylene)-4-aminobenzoic acid (LV, R = OH) and the corresponding ester (LV, R = OEt).



LV.

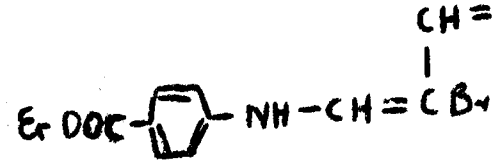
All attempts to reduce the acid (LV, R = OH) to the secondary amine in alkaline solution failed.

Better results were obtained using the ester (LV, R = OEt), which was not reduced by hydrogen over palladised charcoal. Some reduction (33%) took place over Raney nickel, the absorption ceased long before one double bond was saturated, but some of the desired product (LIII, R = OEt) was obtained. Hydrogenation over Adams catalyst gave the

best yield (36%) of this compound and the reaction was stopped after the theoretical adsorption of hydrogen had taken place.

The ethyl N-(quinoxaline-2-methyl)-4-aminobenzoate was very sparingly soluble in most solvents, but dissolved in pyridine. One attempt to hydrolyse it to the acid, by refluxing, under nitrogen, in pyridine containing a small excess of aqueous sodium hydroxide, gave an intractable product.

As the solubility properties of this ester (LIII, R = OEt) were now known attempts were made to prepare it from ethyl p-aminobenzoate, o-phenylenediamine and 2:3-dibromopropionaldehyde, under the conditions used in the synthesis of ethyl pteroate (41). None of the desired quinoxaline could be isolated from the product. The reaction was repeated using p-aminobenzoic acid, but the acid (LIII, R = OH) could not be isolated from the resulting mixture. Unsuccessful attempts to prepare the pteroyl-glutamic analogue (LIII, R = l-, $NHCH(COOH)CH_2CH_2COOH$) by this method from p-aminobenzoylglutamic acid were later reported (151).



LVI.

3-(4-Carbethoxyanilino)-2-bromoacrolein (LVI, R = O) was now prepared from 3-(4-carbethoxyanilino)-2-bromoacrolein 4-carbethoxyanil (or 1:3-bis-(4-carbethoxyphenylimino) 2-bromopropane, LVI, R = NC_6H_4COOEt) hydrobromide by hydrolysis. All attempts to condense the bromoaldehyde (LVI, R = O) with o-phenylenediamine gave black tars, from which nothing homogeneous could be isolated. The anilinobromoacrolein (LVI, R = NC_6H_4COOEt) was prepared from mucobromic acid (159) and ethyl p-aminobenzoate (c.f. 160).

It was found possible to brominate methylglyoxal to monobromomethylglyoxal, which distilled with a green vapour. No crystalline derivatives were made, and the analytical data was poor. The bromo compound reacted with 2:4:5-triamino-6-hydroxypyrimidine and p-aminobenzoyl-l-glutamic acid in aqueous ethanol at pH 8 to give a brown powder, containing c. 5% of pteroylglutamic acid (assayed using S. lactis R.).

Investigations using bromomethylglyoxal and bromomalondialdehyde derivatives are being continued.

Experimental Section.

Part I, Biguanidine Derivatives.

Anthranilic acid - dicyandiamide condensate (c.f. 78).

Anthranilic acid (6.85 g., 1 mol.) and dicyandiamide (4.2 g., 1 mol.) in water (35 ml.) were refluxed for about 2 hours. The product slowly separarated as a pale brown solid, and after cooling was collected, m.p. 289° (decomp.) (7.7 g., 76%). It crystallised from N aqueous sodium hydroxide, a much better solvent than water, in colourless hairlike needles, m.p. 306-8° (decomp.). Cohn (78) gives m.p. 245-6° (decomp.).

Found: N, 34.5.

Calc. for $C_9H_9ON_5$, N, 34.5%.

The hydrochloride separated from N aqueous hydrochloric acid in colourless prisms, m.p. 289° (decomp.).

Found: C, 44.9; H, 4.4; N, 29.0.

$C_9H_9ON_5.HCl$ requires, C, 45.1; H, 4.2; N, 29.2%.

The evolution of hydrogen chloride ceased when this compound (10 g.) was refluxed with phosphorus oxychloride (100 ml.) for 3 hours. Excess oxychloride was removed in vacuo, and the residual material treated with ice and water. The residual yellow solid (8 g.) was collected, but analyses on the crude material were unsatisfactory and solvents effecting crystallisation could not be found. The compound reacted with boiling diethylamine, but nothing homogeneous could be isolated from the reaction.

N-(2-Carbomethoxyphenyl)-thiourea (c.f. 80).

Methyl anthranilate (7.5 g., 1 mol.), potassium thiocyanate (5.0 g. , 1 mol.) and concentrated hydrochloric acid (5 ml., 1 mol.) in water (10 ml.) were warmed on a steam bath till all dissolved, and the solution was allowed to cool. The product (9.5 g., 91%) separated as a white solid, very soluble in methanol, acetone and hot water. It crystallised from benzene in small colourless prisms, m.p. 125-6°, resolidifying almost immediately and remelting at 303°.

Found: C, 51.4; H, 4.8; N, 12.9; S, 14.7.

$C_9H_{10}O_2N_2S$ requires, C 51.4; H, 4.8; N, 13.3; S, 15.2%.

2-Mercapto-4-hydroxyquinazoline.

i). The above ester (7.8 g.) was slowly heated to 200° (30 minutes) and the product (5.6 g., 85%) ground up with ethanol and collected, m.p. 302-4°. It crystallised from a very large volume of ethanol in pale yellow prisms, m.p, 305-6°. Rupe (80) gives m.p. 284°

Found: C, 54.4; H, 3.6; N, 15.3; S, 17.1.

Calc. for $C_8H_6ON_2S$, C, 53.9; H, 3.4; N, 15.7; S, 18.0%

ii). A mixture of anthranilic acid (15 g., 1 mol.) and thiourea (24 g., mol.) was heated slowly to 180-200° in an oilbath over 1 hour, and kept at this temperature for a further 4 hours.

Ammonia, and other fumes were evolved, and the residual brown glass was boiled with ethanol. The residual grey powder (0.9 g.) was collected and washed with ethanol and ether, m.p. 288-92°. It was insoluble in aqueous sodium bicarbonate but dissolved in dilute sodium hydroxide and was precipitated on acidification. It crystallised from ethanol in almost colourless plates, m.p. 289°, depressed on addition of 2-mercapto-4-hydroxyquinazoline from "i".

Found: C, 65.7); 65.2); H, 4.3); 4.0); N, 13.7; S, 10.7.

$C_{16}H_{12}ON_3S$ requires, C, 65.3; H, 4.1; N, 14.3; S, 10.9%.

2-Methylmercapto-4-hydroxyquinazoline (81).

Methyl sulphate (3.38 g., 1.05 mol.) was added, with vigorous shaking, to a solution of 2-mercapto-4-hydroxyquinazoline (4.55 g., 1 mol.) and sodium hydroxide (1.12 g., 1.1 mol.) in water (20 ml.). The crude product (4.4 g., 90%) separated almost immediately as a white solid, which crystallised from ethanol in colourless prisms, m.p. 218-9°.

Found: C, 56.8; H, 4.0.

Calc. for $C_9H_8ON_2S$, C, 56.3; H, 4.1%.

This compound (1.0 g., 1 mol.) and guanidine carbonate (0.6 to 3.0 g in several experiments), finely ground together, were heated in an oil bath to about 200° for three hours. The

product (0.85 g.), a yellow powder, was washed out with ethanol and had m.p. 296°. It dissolved in warm dilute sodium hydroxide, was precipitated by acid, but repetitions of this process failed to give a homogeneous material, and crystallisation from ethanol was unsatisfactory.

The 2-methylthioquinazoline also failed to react with ethanolic guanidine at 160° (6 hours) in a sealed tube; it was largely (90%) recovered unchanged.

2-Guanidinobenziminazole (XXVII; R=R'=R"=H. c.f. 85).
A mixture of o-phenylene diamine (2.16 g., 1 mol.), dicyandiamide (1.68 g., 1 mol.), concentrated hydrochloric acid (4 ml., 2 mol.) and water (15 ml.) were heated under reflux for one hour, when the solution was cooled and basified. 2-Guanidinobenziminazole (2.3 g., 66%) separated, m.p. 247°(decomp.), and crystallised from water in buff coloured plates or colourless needles, m.p. 251°(decomp.).

The mononitrate separated from water in small colourless needles, m.p. 235°(decomp.) (Pellizzari, 85, gives m.p. 216° (decomp.).

Found: C, 40.2; H, 4.2.

Calc. for $C_8H_9N_5.HNO_3$, C, 40.3; H, 4.2%

The picrate crystallised from a large volume of water in colourless needles, m.p. 274-5°(decomp.)

Found: C, 41.4; H, 2.9.

Calc. for $C_8H_9N_5.C_6H_3O_7N_3$, C, 41.2; H, 3.0%.

2-Guanidino-5-methylbenziminazole (XXVII, R = Me, R' = R" = H). 3-Nitro-4-aminotoluene (3.8 g., 1 mol.) was hydrogenated over Raney nickel in methanol to the diamine. The solution was filtered into concentrated hydrochloric acid (5 ml.) and the mixture evaporated to dryness in vacuo. The residual dihydrochloride was refluxed with dicyandiamide (2.1 g., 1 mol.) in water (15 ml.) for 45 minutes and the gum, precipitated by alkali, treated with alcoholic picric acid. The benziminazole picrate (6.0 g., 58%) m.p. 240°(decomp.) separated, and crystallised from aqueous ethanol in orange needles, m.p. 264°(decomp., after turning yellow at 240°)

Found: C, 43.4; H, 3.6; N, 26.7.

$C_9H_{11}N_5.C_6H_3O_7N_3$ requires C, 43.1; H, 3.3; N, 26.2%.

The crude picrate was converted into the dihydrochloride by the β-ethoxyethanol method (see XXVII, R = R' = OMe, R" = i-Pr). and it separated from n-propanol containing some hydrogen chloride in colourless prisms, m.p. 228-9°(decomp.).

Found: C, 41.0; H, 5.1.

$C_9H_{11}N_5.2HCl$ requires, C, 41.2; H, 5.0%.

2-Guanidino-5:6-dimethoxybenziminazole. (XXVII, R = R' = OMe; R" = H).

4:5-Dimethoxy-o-phenylenediamine dihydrochloride (3.38 g., 1 mol.) and dicyandiamide (1.2 g., 1 mol.) were refluxed in

water (10 ml.) for one hour, giving a deep red solution. After the addition of charcoal and filtering, pale pink prisms of the benziminazole mono-hydrochloride monohydrate (2.7 g., 66%) separated on cooling the solution and crystallised from water in colourless prisms, m.p. 285°(decomp.).

Found: C, 41.3; H, 5.6; N, 23.6; Cl, 12.3.

$C_{10}H_{13}O_2N_5.HCl.H_2O$ requires,

C, 41.4; H, 5.5; N, 24.2; Cl, 12.3%.

Found, on a sample dried at 120° in vacuo:

Found: loss, 3.11%; C, 42.4; H, 5.6.

$C_{10}H_{13}O_2N_5.HCl.\frac{1}{2}H_2O$ requires, loss, 3.11%, C, 42.8; H, 5.3%.

The free base separated from water in irregularly shaped colourless prisms, m.p. 163°(decomp.).

Found, when dried at room temperature over phosphorus pentoxide:

Found: C, 49.1; H, 5.8; N, 29.4.

$C_{10}H_{13}O_2N_5.\frac{1}{2}H_2O$ requires, C, 49.2; H, 5.7; N, 28.7%.

Found, after drying at 120° in vacuo, C, 50.9; H, 5.6.

$C_{10}H_{13}O_2N_5$ requires: C, 51.1; H, 5.6%.

The monopicrate monohydrate separated from aqueous ethanol in very fine orange needles, m.p. 280°(decomp.).

Found, after drying at 100° in vacuo:

C, 39.5; H, 3.6; N, 23.8.

$C_{10}H_{13}O_2N_5.C_6H_3O_7N_3.H_2O$ requires, C, 39.8; H, 3.7; N, 23.2%.

Found, after drying at 120° in vacuo: C, 40.4; H, 4.0.

$C_{10}H_{13}O_2N_5.C_6H_3O_7N_3.\frac{1}{2}H_2O$ requires, C, 40.6; H, 3.6%.

2-Guanidino-5:6-dichlorobenziminazole (XXVII, R=R'=Cl; R"=H).
1:2-Dichloro-4:5-dinitrobenzene (4.75g; 1 mol., prepared from o-dichlorobenzene by two successive nitrations, 92) was hydrogenated to the diamine over Raney nickel in methanol (20 ml.), filtered into concentrated hydrochloric acid (4.4 ml.) and the solution evaporated to dryness in vacuo. Dicyandiamide (1.68 g., 1 mol.) a nd water (12 ml.) were added to the residual solid and the thick grey sludge produced by boiling for 10 minutes heated on the steam bath for a further 50 minutes. After cooling the precipitated 2-guanidino-5:6-dichlorobenziminazole hydrochloride monohydrate (3.9 g., 66%) was collected, and crystallised from water (charcoal) in very fine colourless needles, m.p. 287-90° (decomp.)

Found: C, 32.3; H, 3.6; Cl. 35.0.

$C_8H_7N_5Cl_2.HCl.H_2O$ requires, C, 32.2; H, 3.4; Cl, 35.6%.

The free base separated from water in very pale brown needles, m.p. 244°C (decomp.).

Found, after drying at 100°C in vacuo; C, 39.2; H, 3.0.

$C_8H_7N_5Cl_2$ requires, C, 39.4; H, 2.9%.

The picrate separated from aqueous ethanol in very fine yellow prisms, m.p. 319°(decomp.).

Found: Cl, 14.8; N, 24.1.

$C_8H_7N_5Cl_2.C_6H_3O_7N_3$ requires, Cl, 15.0; N, 23.7%.

2-Guanidino-5:6-dimethylbenziminazole. (XXVII, R=R'=Me; R"=H)
This was similarly prepared from 1:2-dimethyl-4-amino-5-nitrobenzene (2.8 g., 1 mol.), obtained by nitrating the xylidine (93),

using concentrated hydrochloric acid (3.4 ml.) and dicyandiamide (1.42 g., 1 mol.). The hydrated hydrochloride separated on cooling the reaction mixture, and crystallised from water (charcoal) in very pale brown prisms, m.p. 265°(decomp.).

Found: C, 45.3; H, 6.4.

$C_{10}H_{13}N_5.HCl.\ 1\frac{1}{2}H_2O$ requires, C, 45.0; H, 6.4%.

The free base separated from aqueous ethanol as a hydrate, in long fawn coloured needles, m.p. 191°.

Found, after drying at 100° in vacuo; C, 56.4; H, 6.4.

$C_{10}H_{13}N_5.\frac{1}{2}H_2O$ requires, C, 56.6; H, 6.6%.

The picrate separated from aqueous ethanol in brick red prisms, m.p. 258°(decomp.).

Found: C, 44.7; H, 3.8.

$C_{10}H_{13}N_5.C_6H_3O_7N_3$ requires, C, 44.4; H, 3.7%.

isoPropyldicyandiamide.

Sodium dicyanimide (5.0 g., 1.12 mol.) and isopropylamine hydrochloride (4.8 g., 1 mol.) were refluxed in n-butanol (50 ml.) for 20 hours, after which the solution was filtered and evaporated to dryness in vacuo. The residual colourless glass, crude isopropyldicyandiamide (theoretical yield), was used directly in the syntheses of all the isopropylguanidinobenzimidazoles, later described.

A portion of the glass was redissolved in n-butanol, and the solution was filtered and evaporated as before. The resulting glass, on very long standing, solidified to a waxy solid, m.p. 84-6°.

Found, on a sample dried over phosphorus pentoxide at 115°/0.06 mm.

N, 44.8.

$C_5H_{10}N_4$ requires, N, 44.4%.

The alkyldicyandiamide was soluble in water and polar solvents, but was insoluble in light petroleum and benzene. It does not form a compound with picric acid.

A portion of the glass (5.9 g.) was heated to boiling with acetic anhydride (12 g.) when a vigor us reaction set in, which kept the mixture boiling for 10 minutes. After refluxing for a further 30 minutes the solution was set aside for 5 days. The solid, which separated, was collected and washed with a little acetic acid and ether. Further quantities were obtained by adding large volumes of ether to the filtrate. It crystallised from ethanol in very pale yellow prisms, m.p. 271°(slight decomp.), and analysed for 2-isopropylamino-4-methyl-6-hydroxytriazine, but was not further investigated.

Found: C, 49.6: H, 7.2: N, 33.9.

$C_7H_{12}ON_4$ requires, - C, 50.0; H, 7.1; N, 33.3%.

N^5-isopropyl-2-guanidinobenziminazole. (XXVII, R=R'=H; R"=i-Pr)

o-Phenylene-diamine (5.4 g., 1 mol.), concentrated hydrochloric acid (10 ml., 2 mol.), and isopropyldicyandiamide (6.3 g., 1 mol.) were refluxed in aqueous solution (20 ml.) for 1 hour. The addition of alkali gave a sticky yellow gum which dissolved in ethanol and was treated with an aqueous solution of copper sulphate pentahydrate (12.6 g., 1 mol.). The pale green copper derivative was collected, washed with ethanol, dried and dissolved

in concentrated hydrochloric acid (20 ml.) to give a deep red solution. Sodium sulphide (14 g.) in concentrated aqueous solution was added, the precipitated copper sulphide removed, and the filtrate strongly basified. A yellow gum, which slowly solidified, precipitated, and long colourless prisms of N^5-isopropyl-2-guanidinobenziminazole separated from its solution in aqueous ethanol, m.p. 168°.

	Found:	C, 60.4;	H, 6.8;	N, 32.6.
$C_{11}H_{15}N_5$	requires,	C, 60.8;	H, 6.9;	N, 32.3%.

The picrate separated from aqueous ethanol in fine yellow needles, m.p. 263-4° (decomp. after sintering at about 260°).

	Found:	C, 45.8;	H, 4.0;	N, 25.4.
$C_{11}H_{15}N_5.C_6H_3O_7N_3$	requires,	C, 45.7;	H, 4.0;	N, 25.1%.

The dihydrochloride separated in colourless prisms, m.p. 230-2° (decomp.) from ethanol containing a trace of hydrogen chloride.

	Found:	C, 45.8;	H, 6.0;	N, 24.0.
$C_{11}H_{15}N_5.2HCl$	requires,	C, 45.5;	H, 5.9;	N, 24.1%.

N^5-isoPropyl-2-guanidino-5-chlorobenziminazole. (XXVI)
4-Chloro-o-phenylene diamine dihydrochloride, prepared from 4-chloro-2-nitroaniline (8.65 g.,) by hydrogenation in the usual way with Raney nickel, was condensed with isopropyldicyandiamide (6.3 g.) by refluxing in water (20 ml.) for 45 minutes, and the product isolated as a very gummy red-green copper derivative. The free base (obtained from the copper derivative as with XXVII, R=R' =H, R"=i-Pr) was a yellow gum, which could not be induced to crystallise. Treatment with ethanolic hydrogen

chloride gave the benziminazole dihydrochloride which crystallised from n-propanol, containing a little hydrogen chloride, in colourless prisms, which were washed with ether, m.p. 215-7°(decomp.).

Found: C, 41.1; H, 4.8; Cl, 32.2.

$C_{11}H_{14}N_5Cl.2HCl$ requires, C, 40.7; H, 4.9; Cl, 32.8%.

The picrate separated from aqueous ethanol in fine yellow needles, m.p. 248°(decomp.).

Found: C, 42.5; H, 3.2; N, 23.0.

$C_{11}H_{14}N_5Cl.C_6H_3O_7N_3$ requires, C, 42.5; H, 3.5; N, 23.3%.

The copper derivative of the benziminazole was precipitated as a pink powder when a solution of the benziminazole dihydrochloride (1 g.) in hot water (5 ml.) was added to cupric nitrate hexahydrate (0.49 g.) in aqueous ammonia (2.5 ml., d. 0.880, in 2.5 ml. of water, c.f. 94). It crystallised from aqueous ethanol in deep red prisms, m.p. 265° (decomp.), easily soluble in chloroform. The chloroform solution was decomposed on shaking with dilute acids.

Found: C, 47.0; H, 4.9; Cl, 12.4.

$(C_{11}H_{13}N_5Cl)_2Cu$ requires, C, 46.8; H, 4.6; Cl, 12.6%.

N^5-isopropyl-2-guanidino-5-methoxybenziminazole (XXVII, R=H, R'=OMe, R"=i-Pr).

This was similarly made from 2-nitro-4-methoxyaniline (8.4 g., 1 mol.) and isopropylcyanodiamide (6.3 g.), but the yellow brown gum precipitated on basifying the deep blue reaction mixture was purified through the picrate as the copper salt method was unsatisfactory. The gum was dissolved in a little

hot ethanol, filtered and picric acid (11.5 g., 1 mol.) in hot ethanol (50 ml.) added. The picrate (10.7 g., 45%) rapidly precipitated, and after washing with ethanol and ether crystallised from a large volume of ethanol in very small orange prisms, m.p. 224-5^{0}(decomp.).

Found: C, 45.2: H, 4.1; N, 23.5.

$C_{12}H_{17}ON_5.C_6H_3O_7N_3$ requires C, 45.3: H, 4.2: N, 23.5%.

The finely ground picrate (8.0 g., 1 mol.) was shaken up with concentrated hydrochloric acid (5.1 ml., 3 mol.) and water, and the mixture extracted with ether till free from picric acid. The aqueous solution was then filtered basified, and the precipitated base collected. It separated as the monohydrate, in colourless prisms, from aqueous ethanol, m.p. 117-22^{0}(decomp.)

Found: C, 54.2: H, 7.0; N, 26.8.

$C_{12}H_{17}ON_5.H_2O$ requires, C, 54.3; H, 7.2; N, 26.4%

The anhydrous compound, m.p. 97°, was obtained as a pale brown glass on drying the hydrate at 100^{0} in vacuo.

Found: C, 58.0; H, 7.0.

$C_{12}H_{17}ON_5$ requires, C, 58.3; H, 6.9%.

The dihydrochloride, purified by dissolving in methanolic hydrogen chloride and precipitating by the slow addition of ether, was a white powder, m.p. 207^{0}(decomp.)

Found: C, 45.0; H, 5.9; Cl, 21.7.

$C_{12}H_{17}ON_5.2HCl$ requires, C, 45.0: H, 5.9: Cl, 22.2%.

N^5-isoPropyl-2-guanidino-5-methyl-benziminazole. (XXVII, R=Me;R'=H; R"=i-Pr)

The benziminazole was made by the above method from 3-nitro-4-aminotoluene (7.6 g.) and isopropyldicyandiamide (6.3 g.), and isolated as the picrate(11.0 g., 48%), which crystallised from aqueous ethanol in orange prisms, m.p. 217°.

Found, after drying at 100° in vacuo:

C, 47.2; H, 4.4; N, 23.6.

$C_{12}H_{17}N_5.C_6H_3O_7N_3$ requires, C, 46.9; H, 4.3; N, 24.3%.

It was decomposed by hydrogen chloride in β-ethoxyethanol (see XXVII, R=R'=OMe, R"=i-Pr) to give the dihydrochloride (5.5 g.) which separated from n-propanol, containing hydrogen chloride, as a white microcrystalline powder on the addition of ether, m.p. 214-7° (decomp.). It was not obtained analytically pure.

Found: C, 48.8; H, 6.2.

$C_{12}H_{17}N_5.2HCl$ requires, C, 47.4; H, 6.2%.

N^5-isoPropyl-2-guanidino-5:6-dimethoxybenziminazole (XXVII, R=R'=OMe, R"=i-Pr).

4:5-Dinitroveratrol (10.8 g.) was hydrogenated over Raney nickel in methanol (100 ml.). When half the theoretical volume of hydrogen had been absorbed an orange-brown powder precipitated from the solution, but the addition of more catalyst completed the hydrogenation. The diamine was converted to the dihydrochloride, and condensed with isopropyldicyandiamide (6.3 g.) as before. The benziminazole was isolated as the picrate, which crystallised from aqueous n-propanol in orange-brown needles, m.p. 278° (decomp.).

Found, after drying at 100° in vacuo: C, 44.9: H, 4.3: N, 21.7.

$C_{13}H_{19}O_2N_5.C_6H_3O_7N_3$ requires, C, 45.1; H, 4.3: N, 22.1%.

Decomposition of the picrate with aqueous hydrochloric acid was unsatisfactory owing to its insolubility, so a suspension of the crude picrate (1 g., 1 mol.) in boiling β-ethoxyethanol (10 ml.) was treated with hydrogen chloride (0.25 g., 3.5 mol.) in n-propanol (3 ml.). After 10 minutes on a steam bath dry ether was added to the crystalline sludge to dissolve the picric acid, the crude dihydrochloride being filtered off and washed with dry ether till free from picric acid, m.p. 240° (decomp. 0.65 g., 94% recovery). It crystallised from n-propanol, containing a little hydrogen chloride, in brushlike bunches of colourless needles, m.p. 214-6° (decomp.)

Found: C, 44.3; H, 6.3; N, 19.9; Cl, 20.5.

$C_{13}H_{19}O_2N_5.2HCl$ requires C, 44.6; H, 6.0; N, 20.0; Cl, 20.3%.

Attempts to isolate a monohydrochloride by the addition of the theoretical quantity of sodium hydroxide to a concentrated aqueous solution of the dihydrochloride failed to produce a precipitate, but addition of further quantities precipitated the base, which separated from aqueous ethanol in very pale brown nearly cubical prisms, m.p. 215° (decomp.)

Found, on a sample dried at 120° in vacuo:

C, 56.4; H, 6.8; N, 25.9.

$C_{13}H_{19}O_2N_5$ requires C, 56.3; H, 6.9; N, 25.3%.

The copper derivative, prepared in the same way as the copper derivative of XXVI, was slightly soluble in benzene, moderately in ethyl acetate, and easily in ethanol and chloroform. (The latter solution was decomposed by dilute hydrochloric acid). The hemihydrate crystallised from aqueous ethanol in purple red prisms, m.p. 227-8°C (decomp.).

Found: C, 49.7: H, 6.1; N, 22.2.

$(C_{13}H_{19}O_2N_5)_2Cu.\frac{1}{2}H_2O$ requires, C, 49.9: H, 6.2: N, 22.4 .

N^5-isoPropyl-2-guanidino-3:6-dichlorobenziminazole (XXVII, R=R'=Cl, R"=i-Pr).

This was prepared from 1:2-dichloro-4:5-dinitrobenzene (11.85 g.) and isopropyldicyandiamide (6.3 g.) in the usual way, and the picrate (11 g., 73%, m.p. 298° decomp.), after washing with a little ethanol, was dissolved in β-ethoxyethanol and converted to the dihydrochloride, as before, with 98% recovery, m.p. 282° (decomp.). It separated from n-propanolic hydrogen chloride in very small colourless prisms, m.p. 284-5° (decomp.).

Found: C, 37.1; H, 4.4; Cl, 31.1.

$C_{11}H_{13}N_5Cl_2.2HCl$ requires, C, 36.7: H, 4.2; Cl, 39.6%.

The picrate crystallised from a large volume of aqueous n-propanol in yellow prisms, m.p. 296° (decomp.).

Found: C, 39.9; H, 3.1; Cl, 13.6.

$C_{11}H_{13}N_5Cl_2.C_6H_3O_7N_3$ requires, C, 39.6: H, 3.1: Cl, 13.8%.

The dihydrochloride was decomposed by water to give the monohydrochloride monohydrate, separating in fine colourless needles, m.p. 157-60° (decomp. after sintering c. 145°).

Found: C, 38.5; H, 4.7; N, 20.6; Cl, 31.5.

$C_{11}H_{13}N_5Cl_2.HCl.H_2O$ requires,

C, 38.7; H, 4.7; N, 20.6; Cl, 31.3%.

The base crystallised from aqueous ethanol in very pale brown prisms, m.p. 204°.

Found, after drying at 100° in vacuo: C, 46.2; H, 4.6.

$C_{11}H_{13}N_5Cl_2$ requires, C, 46.2; H, 4.5%.

Attempts to form a copper derivative by the action of hot aqueous cupric chloride, and sulphate, on a boiling aqueous solution of the benziminazole dihydrochloride gave only the hydrated monohydrochloride, and hemisulphate hemihydrate on cooling. The latter separated from the solution in large buff prisms, m.p. 248-9° (decomp.).

Found: C, 38.4; H, 4.4; S, 4.9; Cl, 20.1.

$C_{11}H_{13}N_5Cl_2.\frac{1}{2}H_2SO_4.\frac{1}{2}H_2O$ requires,

C, 38.4; H, 4.4; S, 4.6; Cl, 20.6%.

N^5-isoPropyl-2-guanidino-5:6-dimethylbenziminazole (XXVII, R=R'=Me, R"=i-Pr).

1:2-Dimethyl-4-amino-5-nitrobenzene (6.1 g.) was converted to the diamine dihydrochloride as before, and condensed with isopropyldicyandiamide (4.38 g.) by refluxing for 45 minutes in aqueous solution (20 c.c.). On cooling the monohydrochloride monohydrate of the benziminazole separated as a brown solid (5.8 g., 53%, m.p. 137° decomp.), which crystallised from water (charcoal) in almost colourless prisms, m.p. 138-41° (decomp.).

Found: C, 52.2; H, 7.4; Cl, 11.6.

$C_{13}H_{19}N_5.HCl.H_2O$ requires, C, 52.1; H, 7.3; Cl, 11.9%.

The picrate separated in brushlike bunches of fine yellow needles from aqueous ethanol, m.p. 245° (decomp.).

Found: C, 47.8; H, 4.5.

$C_{13}H_{19}N_5.C_6H_3O_7N_3$ requires, C, 48.1; H, 4.6%.

N^5-n-Butyl-2-guanidino-5:6-dimethoxybenziminazole (XXVII, R=R'=OMe; R"=n-Bu).

n-Butylamine hydrochloride (7.7 g., 1 mol.) was refluxed with sodium dicyanimide (7.0 g., 1.12 mol.) in n-butanol (50 ml.) for 24 hours. The mixture was filtered hot and the residual solids washed with a little n-butanol. A portion (15 ml.) of the filtrate and washings (54 ml.) was evaporated to dryness (in vacuo), and the residual glass of n-butyldicyandiamide condensed with 4:5-dimethoxy-o-phenylenediamine dihydrochloride (prepared from 4:5-dinitroveratrole, 4.4 g., 1 mol. as before), under the conditions used for isopropyldicyandiamide. The product was isolated as the picrate (4.3 g., 43%), which separated from a large volume of aqueous n-propanol in fine hair-like red needles, m.p. 256° (decomp.).

Found, on a sample dried at 100° in vacuo:

C, 46.5; H, 4.6; N, 21.5.

$C_{14}H_{21}O_2N_5.C_6H_3O_7N_3$ requires C, 46.2; H, 4.6; N, 21.5%.

Decomposition with hydrogen chloride in β-ethoxyethanol and addition of ether gave the dihydrochloride, which crystallised from ethanolic hydrogen chloride in slender colourless needles,

m.p. 232° (decomp.).

Found: C, 46.1; H, 6.2.

$C_{14}H_{21}O_2N_5 2HCl$ requires, C, 46.2; H, 6.3%.

The base separated, as the sesquihydrate, from aqueous ethanol in stellate clusters of colourless prisms, m.p. 115-20° (decomp.).

Found: C, 52.9; H, 7.0.

$C_{14}H_{21}O_2N_5 \cdot 1\frac{1}{2}H_2O$ requires, C, 52.8; H, 7.5%.

Found, on a sample dried at 110° in vacuo:

C, 55.9; H, 7.0.

$C_{14}H_{21}O_2N_5 \cdot \frac{1}{2}H_2O$ requires, C, 56.0; H, 7.3%.

2:4-Diamino-1:3:5-triazabenzepine. (XXIX, R=R'=H)

o-Phenylenediamine (1.08 g., 1 mol.), sodium dicyanimide (0.89 g., 1 mol.) and water (10 ml.) containing concentrated hydrochloric acid (1 ml., 1 mol.) were heated to boiling and kept at 100° for 5 minutes. After cooling, nitric acid (0.9ml., d. 1.42, 1.04 mol.) was added, when the benzepine nitrate (1.40 g.) separated. It crystallised from water in colourless hairlike needles, m.p. 269°(decomp.).

Found: C, 40.3; H, 4.5; N, 35.1.

$C_8H_9N_5 \cdot HNO_3$ requires, C, 40.3; H, 4.2; N, 35.3%.

Increasing the amount of hydrochloric acid, or of diamine to 2 mol. in this preparation did not affect the nature of the product.

The picrate, prepared from aqueous solutions of the nitrate and sodium picrate, separated from aqueous ethanol in very fine yellow needles, m.p. 268°(decomp.).

Found: C, 41.8; H, 3.3; N, 27.0.

$C_8H_9N_5 \cdot C_6H_3O_7N_3$ requires, C, 41.6; H, 3.0; N, 27.7%.

The <u>base</u> crystallised from aqueous ethanol in pale brown prisms, which retained water very tenaciously, m.p. 191° (decomp.). Found, on a sample dried at 120° in vacuo:

C, 53.7; H, 5.5; N, 38.2.

$C_8H_9N_5 \cdot \frac{1}{4}H_2O$ requires, C, 53.5; H, 5.3; N, 39.0%.

The benzepine was stable to hot dilute alkali, but boiling 30% aqueous sodium hydroxide caused a slow evolution of ammonia. A small quantity of ammonia was also liberated when its solution in refluxing concentrated hydrochloric acid was basified after several hours, but nothing homogeneous could be isolated from these two degradations.

A suspension of the nitrate (0.29 g.) in N aqueous hydrochloric acid (4 ml.) was treated, at 0°, with sodium nitrite (0.093 g.) in a little water. No reaction was observed, and after 10 minutes a portion was treated with alkaline β-naphthol solution, but no red dye was produced. The main reaction mixture was therefore heated to boiling, when all the solid dissolved and nitrous fumes were evolved. A little aqueous nitric acid was added, and on cooling 0.215 g. (74% recovery) of the benzepine nitrate separated, m.p. and mixed m.p. 264° (decomp.).

<u>1-Methyl-2:4-diamino-1:3-5-triazabenzepine</u> (XXIX, R=Me; R'=H) 2-Nitro-methylaniline (1.02 g., 1 mol., 95) was hydrogenated to the diamine in methanol (20 ml.) over Raney nickel, filtered into concentrated hydrochloric acid (1.3 ml., 1 mol.) and the mixture

added to an aqueous (5 ml.) solution of sodium dicyanimide (1.17 g., 1 mol.). Most of the methanol evaporated after boiling on a steam bath for one hour, and the suspension of a black oil in the remaining solvent was treated with methanolic picric acid (6.0 g., 2 mol.). The yellow-brown benzepine dipicrate (5.9 g., 68%) rapidly separated, and crystallised from methanol (charcoal) in yellow needles, m.p. 216-7°(decomp.).

Found: C, 39.1; H, 2.8; N, 23.1.

$C_9H_{11}N_5.2C_6H_3O_7N_3$ requires, C, 38.9; H, 2.6; N, 23.8%.

Decomposition of the dipicrate in hot β-ethoxyethanol solution with alcoholic aqueous sulphuric acid gave the benzepine sulphate, precipitated as the dihydrate by addition of ether. After washing with ether to remove picric acid the sulphate crystallised from aqueous ethanol in colourless needles, m.p. 252°(decomp., after softening at c. 230°).

Found: C, 33.8; H, 5.9; N, 22.2; S, 9.5.

$C_9H_{11}N_5.H_2SO_4.2H_2O$ requires, C, 33.4; H, 5.3; N, 21.7; S, 9.9%.

The sulphate (0.2 g., 1 mol.), suspended in dilute sulphuric acid (0.053 ml., d. 1.842, in 2 ml. water) was treated with aqueous sodium nitrite (0.86 ml., 10%, 2 mol.). After two hours no visible reaction had taken place, and a sample of the solution gave no colour with alkaline β-naphthol solution. The mixture was heated to c.90° on a steam bath (1 hour) when all the solid dissolved, and an aqueous solution of sodium picrate (0.32 g., 2.25 mol.) was added. The dipicrate of the original benzepine precipitated (0.28 g., 70%), m.p. and mixed m.p. 216-7° (decomp.)

1-Methyl-2-guanidinobenziminazole.

2-Nitro-N-methylaniline (4.0 g. 1 mol.) was hydrogenated to the diamine in ethanol (20 ml.) over Raney nickel, and filtered into a mixture of concentrated hydrochloric acid (8.3 ml.) and dicyandiamide (2.3 g. 1.04 mol.) in water (20 ml.). After boiling the mixture under reflux for one hour, methanolic picric acid (12 g., 2 mol.) was added, and the benziminazole monopicrate separated. It crystallised from ethanol (charcoal) in orange yellow prisms, m.p. 193-5°.

Found: C, 43.3; H, 3.5; N, 26.0.

$C_9H_{11}N_5.C_6H_3O_7N_3$ requires, C. 43.1; H, 3.3; N, 26.8%.

7-Chloro-2:4-diamino-1:3:5-triazabenzepine. (XXIX, R=Cl, R'=H)

2-Nitro-5-chloroaniline (2.02 g., 1 mol.) was hydrogenated over Raney nickel to the diamine in methanol (20 ml.), filtered into concentrated hydrochloric acid (1.17 ml., 1 mol.) and after the addition of water (5 ml.) and sodium dicyanimide (1.04 g., 1 mol.) was boiled for one hour. The benzepine monopicrate (4.05 g., 79%) separated as a green powder on the addition of hot methanolic picric acid (2.7 g., 1 mol.), and crystallised from methanol in short yellow needles, m.p. 263-4° (decomp.).

Found: C, 38.6; H, 2.5: Cl, 8.3.

$C_8H_8N_5Cl.C_6H_3O_7N_3$ requires, C, 38.3; H, 2.6; Cl, 8.1%.

The hemisulphate hemihydrate, prepared from the picrate by the β-ethoxyethanol method (see XXIX, R=H, R'=Me), separated from a large volume of water in colourless needles, m.p. 274° (decomp.).

Found: C, 36.0; H, 4.1; S, 6.0.

$C_8H_8N_5Cl.\frac{1}{2}H_2SO_4.\frac{1}{2}H_2O$ requires, C, 35.9; H, 3.7; S, 6.0%.

2-Nitro-5-chloro-N-ethylaniline. (c.f. 96)

2:4-Dichloronitrobenzene (4.3 g., 1 mol.), ethylamine hydrochloride (4.8 g., 1 mol), sodium hydroxide (2.1 g., 1 mol.), ethanol (10 ml.) and water (10 ml.) were slowly heated to 200° (5 hours) in a sealed tube. The resulting semisolid mass was washed out with ethanol, diluted largely with water, and the amine filtered off. It crystallised from aqueous methanol in yellow prisms, m.p. 83° (4.2 g., 80%). This amine has been prepared by other methods (97, and 93), and the m.pts. recorded are 83-4° and 75.5-76.5° respectively.

1-Ethyl-2:4-diamino-8-chloro-1:3:5-triazabenzepine. (XXIX, R=Cl; R'=Et.)

This was prepared from 2-nitro-5-chloro-N-ethylaniline (1.90g., 1 mol.) in the same way as the 7-chlorobenzepine derivative (XXIX, R=Cl; R'=H) using sodium dicyanimide (0.85 g., 1 mol.) and concentrated hydrochloric acid (0.95 ml., 1 mol.). The dark coloured reaction mixture was treated with hot methanolic picric acid (4.6 g. 2 mol.) and the yellow-green benzepine dipicrate (3.8 g., 58%) separated. It crystallised from aqueous methanol in irregular yellow prisms, m.p. 195-6°(decomp., with sintering c. 190°).

Found: C, 38.1; H, 2.9; N, 23.0: Cl, 5.8.

$C_{10}H_{12}N_5Cl.2C_6H_3O_7N_3$ requires, C, 37.8; H, 2.6; N, 22.1; Cl, 5.1%

It was converted to a hydrated sulphate, by the usual method (see XXIX, R=Cl; R'=H), which separated from aqueous ethanol in long colourless needles, m.p. 107-115° (decomp., and the solvent evaporated. After resolidification the colourless residue decomposes c. 170-80°).

Found: loss at 100° in vacuo, 11.9%; on dried material, S, 10.3

$C_{10}H_{12}N_5Cl.H_2SO_4$ requires, S, 9.5%

2-Nitro-5-chloro-N-isopropylaniline.

2:4-Dichloronitrobenzene (8.4 g., 1 mol.) and isopropylamine (5.2 g., 2 mol.) in ethanol (20 ml.) were heated to 200° for 6 hours in a sealed tube. After cooling the dark brown sludge was washed out with water, and the isopropylaniline filtered off. It crystallised from aqueous methanol (charcoal) in fine hairlike yellow needles, m.p. 50-1° (8.3 g., 88%).

Found: C, 50.2; H, 5.5.

$C_9H_{11}O_2N_2Cl$ requires, C, 50.3: H, 5.1%.

1-isoPropyl-2:4-diamino-8-chloro-1:3:5-triazabenzepine.

(XXIX, R=Cl; R'=i-Pr)

2-Nitro-5-chloro-N-isopropylaniline (6.4 g., 1 mol.) was hydrogenated to the diamine in methanol (20 ml.) over Raney nickel, and converted into the benzepine dipicrate (17.2 g., 31%), as with the corresponding 1-ethyl derivative (XXIX, R=Cl; R'=Et), using concentrated hydrochloric acid (3.0 ml.)

and sodium dicyanimide (2.66 g., 1 mol.) in water (15 ml.), and picric acid (14.5 g., 2.1 mol.) in methanol. The dipicrate crystallised from ethanol as the ethanolate in long thin yellow prisms, m.p. 208-10°(decomp. after sintering at 180°).

Found: C, 39.7; 40.1) H, 3.4; 3.3) N, 20.0: Cl, 4.9.

$C_{11}H_{14}N_5Cl.2C_6H_3O_7N_3.C_2H_5OH$ requires,

C, 39.7; H, 3.3; N, 20.4; Cl, 4.7%.

Decomposition in β-ethoxyethanol to the sulphate was unsatisfactory, owing to the insolubility of the dipicrate, but its (11.7 g.) solution in hot cyclohexanol (30 ml.) on treatment with concentrated sulphuric acid (6.0 ml.) in ethanol (20 ml.) followed by ether gave a precipitate of the sulphate (5.0 g., 86%) which was washed with ether. It crystallised from aqueous ethanol in colourless plates, m.p. 175-7°(decomp.)

Found: C, 38.2; H, 4.6; S, 8.9.

$C_{11}H_{14}N_5Cl.H_2SO_4$ requires, C, 37.8; H, 4.6; S, 9.2%.

Part II. Alloxazines and isoAlloxazines.

3-Dimethylamino-p-toluenesulphonanilide.

3-Nitrodimethylaniline (15.3 g., 101) was hydrogenated to the diamine in methanol (50 ml.)., filtered from catalyst (Raney nickel), and p-toluenesulphonyl chloride (18.5 g.) added. The mixture was heated on a steam bath for 20 minutes, diluted with water, neutralised with ammonia, and the precipitated sulphonamide collected. It crystallised from aqueous ethanol in colourless prisms, m.p. 156° (18 g., 68%).

Found: C, 62.0; H, 5.8; S, 10.7.

$C_{15}H_{18}O_2N_2S$ requires, C, 62.1; H, 6.2; S, 11.0%.

3-Dimethylamino-β-diethylaminoethylaniline (XXXVI, R=H).

β-Chloroethyldiethylamine (4.2 g., 1 mol.) and 3-dimethylamino-p-toluenesulphonanilide (9.0 g., 1 mol.) were added to a solution of sodium (0.72 g., 1 mol.) in ethanol (50 ml.). After refluxing on a steam bath for 6 hours the solvent was evaporated, the residue treated with water, and extracted with chloroform. Evaporation of the extract gave the alkylated sulphonamide as an oil, which failed to solidify or form crystalline derivatives. It was therefore hydrolysed with diluted sulphuric acid (100g., of 94%) at room temperature. After 20 hours the brown solution was diluted with ice water, basified, the amine collected with ether and distilled. It was a very pale yellow oil, b.p. 115-7°

(bath temperature)/0.03 mm. (4.45 g., 61%).

Found: C, 71.5; H, 10.7.

$C_{14}H_{25}N_3$ requires, C, 71.5; H, 10.6%,

The mono-picrate separated from aqueous ethanol in very deep red prisms, m.p. 133°

Found: C, 51.4; H, 6.1; N, 18.6.

$C_{14}H_{25}N_3.C_6H_3O_7N_3$ requires, C, 51.7; H, 6.0; N, 18.1%.

The di-picrate separated from ethanol as a microcrystalline yellow powder, m.p. 143-4° (decomp.).

Found: C, 44.9; H, 4.6.

$C_{14}H_{25}N_3.2C_6H_3O_7N_3$ requires, C, 45.0; H, 4.5%.

3:4-Dinitro-N-dimethylaniline.

The nitration of 3-nitro-dimethylaniline with nitrous acid, according to the directions of Hodgson and Smith (108) gave only 26% of the desired dinitro compound with quantities of 3-nitro-N-nitroso-N-methylaniline. Direct nitration, mentioned by these authors and used much earlier (109) proved much better. The amine (4.0 g.), finely powdered, was added slowly to dilute nitric acid (80 ml., 90%). After vigorously shaking for 10 minutes the mixture was diluted with water (200 ml.), the precipitate collected and dissolved in boiling n-propanol. On cooling, 3:4-dinitrodimethylaniline separated in yellow needles, m.p. 178-9° (2.07 g., 41%).

Dilution of the mother liquor with water precipitated 2:5-dinitrodimethylaniline, crystallising from ethanol in scarlet prisms, (0.7 g., 14%) m.p. 112°

2-Nitro-5-dimethylamino-β-diethylaminoethylaniline (XXXVI, R=NO_2).

3:4-Dinitrodimethylaniline (3.84 g., 1 mol.), β-diethylamino-ethylamine (3.2 g., 1.5 mol.) and fused sodium acetate (4 g.) were heated in an oil bath when a vigorous reaction occurred. After 6 hours at 140-60° the mixture was cooled, dissolved in dilute acid and extracted with ether to remove unreacted nitro compound. The aqueous solution was basified, the precipitated nitroamine, a pale brown oil, being collected with ether and distilled, b.p. 195-205°(bath temperature)/0.1 mm. (3.9 g., 77%). On standing it solidified in yellow-brown prisms, m.p. 45-6°.

Found: N, 20.3.

$C_{14}H_{24}O_2N_4$ requires, N, 20.0%.

The picrate separated from ethanol in yellow diamond shaped plates, m.p. 171°(decomp.).

Found: C, 46.8; H, 5.4; N, 18.6.

$C_{14}H_{24}O_2N_4.C_6H_3O_7N_3$ requires, C, 47.1; H, 5.3; N, 19.3%.

7-Dimethylamino-9-β-diethylaminoethyl-isoalloxazine (XXXVII) i). On mixing hot solutions of 3-dimethylamino-β-diethyl-

aminoethylaniline (2.75 g., 1 mol.) in ethanol (10 ml.) and of violuric acid (1.84 g., 1 mol.) in water (20 ml.) a purple colour, rapidly changing to crimson, developed. After one hour on a steam bath the solution was evaporated to dryness under reduced pressure, the residual isoalloxazine violurate boiled with ethanol (20 ml.), and after cooling collected (2.35 g., 69% calculated on the violuric acid, m.p. 265° decomp.). The tetrahydrate separated from its deep crimson water solution in red-brown hair like needles, which crush to a crimson powder, m.p. 265° (decomp.).

Found: C, 45.4: H, 5.7; N, 22.4.

$C_{18}H_{24}O_2N_6.C_4H_3O_4N_3.4H_2O$ requires C, 45.1; H, 6.0; N, 21.5%.

Found, after drying at 120° in vacuo:

loss, 10.2%, C, 50.5: H, 5.3; N, 24.3.

$C_{18}H_{24}O_2N_6.C_4H_3O_4N_3.\frac{1}{2}H_2O$ requires,

loss, 10.8%, C, 50.6; H, 5.4; N, 25.1%.

Found, after drying at 150° in vacuo:

loss, 11.6%, C, 51.1; H, 5.5.

$C_{18}H_{24}O_2N_6.C_4H_3O_4N_3.$ requires,

loss, 12.3%, C, 51.5; H, 5.3%.

The hydrated hydrochloride, which was purified by precipitation with ether from methanolic hydrogen chloride, was a bright red micro-crystalline powder, very soluble in water, m.p. 290° (decomp.),

Found, after drying at 100° in vacuo:
C, 53.0; H, 6.5; N, 19.8, Cl, 8.3.

$C_{18}H_{24}O_2N_6.HCl.H_2O$ requires,
C, 52.6; H, 6.6; N, 20.5; Cl, 8.7%.

The picrate separated from aqueous ethanol in dull red microscopic needles, m.p. 242° (decomp.).
Found, on a sample dried at 120° in vacuo:
C, 48.9; H, 4.9; N, 21.2.

$C_{18}H_{24}O_2N_6.C_6H_3O_7N_3$ requires, C, 49.2: H, 4.6; N, 21.5%.

ii). 2-Nitro-5-dimethylamino-β-diethylaminoethylaniline (0.9 g., 1 mol.) was hydrogenated in acetic acid (25 ml.) over palladised charcoal catalyst, and the filtered solution of the tetra-amine added to warm acetic acid (30 ml.) containing alloxan monohydrate (0.6 g., 1.17 mol.) and boric acid (0.9 g.). A deep orange red colour developed, and after 30 minutes at 60° the solution was evaporated to dryness in vacuo. The residue was dissolved in hot water (15 ml.), filtered and neutralised with dilute sodium hydroxide when the isoalloxazine separated on cooling the deep red solution (0.47 g., 41%), m.p. 278° (decomp.). It was purified by dissolving in dilute sodium hydroxide, acidifying with acetic acid and neutralising with aqueous sodium carbonate, when carmine hairlike needles of the monohydrate separated, m.p. 298-300° (decomp.).

Found: C, 57.5; H, 6.8; N, 23.0.
$C_{18}H_{24}O_2N_6.H_2O$ requires, C, 57.8; H, 7.0; N, 22.5%.

Found, after drying at 200° in vacuo, (no loss at 150°);

loss, 2.37%, C, 59.3; H, 6.5.

$C_{18}H_{24}O_2N_6 \cdot \frac{1}{2}H_2O$ requires, loss, 2.41%, C. 59.2; H, 6.8%.

The picrate had m.p. 242° (decomp.), undepressed by addition of a sample from "1".

Found: C, 48.6; H, 4.8; N, 21.9%.

The violurate had m.p. 264° (decomp.), undepressed by addition of a sample from "1",

Found, on a sample dried at 120° in vacuo;

loss, 10.8%, C, 50.4; H, 5.5%.

7-Dimethylaminoalloxazine (XL).

3-Nitrodimethylaniline (1.0 g., 1 mol) in ethanol (20 ml.) was hydrogenated to the diamine over Raney nickel, and the filtered liquid added to a hot aqueous (10 ml.) solution of violuric acid (0.95 g., 1 mol.). The purple mixture, which rapidly went crimson, was boiled for one hour; the dark red solid (1.03 g., 66.5%) which separated was collected next day and washed with water and ethanol. The alloxazine was purified by dissolving in hot aqueous methanol containing sodium hydroxide (1%) and was precipitated by the addition of dilute hydrochloric acid to the boiling deep red solution. If the solution is not boiling during the precipitation a gel is formed. The orange-red powder then had m.p. 350-7° (decomp.).

Found, on a sample dried at 120° in vacuo:,

	C, 53.7; 54.2;	H, 4.3; 4.7;	N, 26.9. 26.7.
$C_{12}H_{11}O_2N_5 \cdot \frac{1}{2}H_2O$ requires	C, 54.1;	H, 4.5;	N, 26.3%.

The u.v. spectrum (p 34, curve 1) was examined in N/20 aqueous sodium hydroxide.

The sodio derivative separated in microscopic brick red prisms on cooling the alkali (1%) solution of the alloxazine.

Found, after drying at 120° in vacuo: C, 50.2; H, 4.2.

$C_{12}H_{10}O_2N_5Na \cdot \frac{1}{2}H_2O$ requires, C, 50.0; H, 3.8%.

3-Methylamino-N-dimethylaniline.

3-Dimethylamino-p-toluenesulphonamide (4.0 g., 1 mol.) and methyl iodide (2.0 g., 1 mol.) were added to a solution of sodium (0.32 g., 1 mol.) in ethanol (20 ml.) and the mixture refluxed for 7 hours. After evaporation of the alcohol the residue was treated with water. An oil separated, but solidified on treatment with dilute hydrochloric acid. The solid, 3-(p-toluenesulphonmethylamido)-N-dimethylaniline hydriodide (4.3 g., 72%) crystallised from water in short colourless needles, m.p. 163° (decomp.).

Found: C, 44.6; H, 5.0; I, 28.2.

$C_{16}H_{20}O_2N_2S \cdot HI$ requires C, 44.4; H, 4.9; I, 29.4%.

Attempts to prepare a solid free base or picrate failed.

and the hydriodide (3.85 g.) was hydrolysed by dissolving in sulphuric acid (15 ml., 98%). A vigorous reaction took place and after 24 hours the deep blue solution was diluted with ice water, filtered from iodine, basified, and the 3-methylamino-N-dimethylaniline collected with ether. It was a colourless oil,(0.7 g., 58%), and was characterised as the dipicrate which separated from alcoholic picric acid in yellow-brown prisms, m.p. 124° (decomp.).

Found: C, 41.4; H, 3.6.

$C_9H_{14}N_2.2C_6H_3O_7N_3$ requires, C, 41.4; H, 3.3%.

7-Dimethylamino-9-methyl-isoalloxazine (XLI).

3-Methylamino-dimethylaniline (0.45 g., 1 mol.) in ethanol (5 ml.) was added to a hot concentrated aqueous solution of violuric acid (0.47 g., 1 mol.), and the purple mixture heated on a steambath for 1½ hours. The colour rapidly changed to crimson, the isoalloxazine precipitated, and next day it was collected and washed with water and ethanol (0.60 g., 74%). It dissolved in hot dilute (1½%) aqueous sodium hydroxide to give a deep red solution. On neutralisation with hot dilute hydrochloric acid the isoalloxazine separated microscopic brick red needles, m.p. 360° (decomp.).

Found on a sample dried at 120° in vacuo:

Found: C, 57.8;: H, 5.1: N, 25.8

$C_{15}H_{13}O_2N_5$ requires C, 57.6; H, 4.8; N, 25.8%.

The u.v. absorption spectrum in N/7 aqueous sodium hydroxide is shown on p39, curve 2.

Condensation of 3-amino-methylaniline with violuric acid.

3-Nitro-methylaniline (2.0 g., 1 mol.) was hydrogenated to the diamine in methanol (20 ml., Raney nickel), and filtered into a boiling solution of violuric acid (2.07 g., 1 mol.) in water (20 ml.). The mixture, which rapidly darkened, deposited a red-brown solid, and was kept on a steambath for one hour. The solid was washed out with water and collected (2.06 g., 93%), m.p. 355-7° (decomp.). It dissolved almost completely in hot 1½% aqueous sodium hydroxide to give a deep red solution, from which it was precipitated in an amorphous condition by the addition of hot dilute hydrochloric acid. After several repetitions this process gave a red-brown solid, m.p. 340-5° (decomp.).

Found, after drying at 120° in vacuo,

Found: C, 52.4; H, 4.1; N, 27.2.

$C_{11}H_9O_2N_5.\frac{1}{2}H_2O$ requires, C, 52.4; H, 4.0; N, 27.8%.

The u.v. absorption of this compound (p39 curve 3) was

examined in N/10 aqueous sodium hydroxide.

The crude reaction product (0.3 g.) was extracted three times with boiling N/aqueous hydrochloric acid (70, 30, and 20 ml. respectively). The last extract was almost colourless, and the insoluble residue (0.055 g., 18%) collected. It dissolved completely in 1½% aqueous alkali, and after several precipitations with dilute hydrochloric acid was obtained as an orange red powder, lighter in colour than the original material, blackening 352-5°. From its u.v. absorption spectrum in N/10 aqueous alkali (p39 curve 4) it is believed to consist of 7-methyl-aminoalloxazine.

Found: C, 47.1; H, 4.8; N, 24.4.

$C_{11}H_9O_2N_5 \cdot 2H_2O$ requires, C, 47.3; H, 4.7; N, 25.1%.

Found, after drying at 120° in vacuo,

Found: loss, 9.2, C, 52.4; H, 3.9.

$C_{11}H_9O_2N_5 \cdot \frac{1}{2}H_2O$ requires loss, 9.7, C, 52.4; H, 4.0%

A further sample of the crude reaction product (0.2 g.) was dissolved in diluted sulphuric acid (4 ml., of 70%) by gentle warming, and after cooling to 0° was treated with 10% aqueous sodium nitrite (1 ml.), disregarding the salt which precipitated on cooling. After about 15 minutes the mixture was diluted with water (25 ml.), and the 7-N-nitroso-methylaminoalloxazine collected. It was washed with water till the washings

were free from SO_4", then with ethanol, and dried at room temperature in vacuo. It was a yellow powder (0.08 g., 37.2%), blackening c. 300° and slowly decomposing above this temperature.

Found: C, 46.4; H, 3.3; N, 28.8.

$C_{11}H_8O_3N_6.\frac{1}{2}H_2O$ requires C, 46.9; H, 3.2; N, 29.9%.

Found, after drying at 120° in vacuo, C, 19.4; H, 3.3.

$C_{11}H_8O_3N_6$ requires, C, 48.5; H, 2.9%.

The filtrate, and aqueous washings, from this substance were added to a solution of β-naphthol (0.12 g.) in aqueous sodium hydroxide (30 ml., of 15%), and after a short time the deep purple mixture was neutralised with acetic acid. The precipitated azo-compound (0.15 g., 41.8%), a deep olive green powder m.p. 310°(decomp.), was washed with water till free from SO_4", and then with ethanol. It dissolved in dilute alkali to give a purple solution. Found, after drying in vacuo at room temperature:

C, 56.1; H, 4.2; N, 17.9.

$C_{21}H_{14}O_3N_6.3H_2O$ requires, C, 56.7; H, 4.4; N, 18.6%.

Found, after drying at 150° in vacuo:

loss, 8.04, C, 60.1; H, 3.8.

$C_{21}H_{14}O_3N_6.H_2O$ requires, loss, 7.96%, C, 60.6; H, 3.8%.

7-Aminoalloxazine (0.2 g.) was diazotised under exactly the same conditions, and a clear solution of the diazonium salt was obtained. It gave, with alkaline

β-naphthol, a deep olive green azo-β-naphthol derivative (0.23 g., 62.7%), m.p. 335-8°(decomp., after sintering c. 275°), which dissolved in alkali to give a red solution.

Found: C, 57.4; H, 4.0; N, 19.1.

$C_{20}H_{12}O_3N_6 \cdot 2H_2O$ requires, C, 57.1; H, 3.8: N, 20.0%.

Found, after drying at 120° in vacuo:

Found: loss, 7.35%; C, 61.0; H, 3.3.

$C_{20}H_{12}O_3N_6 \cdot \frac{1}{2}H_2O$ requires, loss, 6.43; C, 61.1; H, 3.3%.

If the amino-alloxazine and amino-isoalloxazine are converted into their azo dyes in the same yields, it follows that this method of analysis gives:-

Alloxazine	37.2%
isoAlloxazine	66.6%.

3-Nitro-p-toluenesulphon-(β-diethylaminoethyl)-anilide.

Sodamide (1.47 g., 1.1 mol.) was added, over 15 minutes (c.f. Eisleb, 112) to a solution of 3-nitro-p-toluenesulphonanilide (10.0 g., 1 mol.) and β-chloroethyl-diethylamine (5.1 g., 1 mol.) in toluene (200 ml.) with vigorous stirring. The mixture was then heated to 100° (1 hour), kept at 100°(3 hours) and refluxed for 1 further hour. After filtering the toluene was extracted with hot dilute hydrochloric acid (200 ml. 5%), and on cooling the extract deposited the alkylated sulphonamide hydrochloride (10 g., 68%), which crystallised from water in colourless needles, m.p. 182°.

Found: C, 53.1; H, 6.0.

$C_{19}H_{25}O_4N_3S \cdot HCl$ requires, C, 53.3; H, 6.1 .

The picrate separated from aqueous ethanol in hexagonal yellow tablets, m.p. 156-7°

Found, after drying at 116° in vacuo:

C, 48.0; H, 4.5; S, 5.0.

$C_{19}H_{25}O_4N_3S.C_6H_3O_7N_3$ requires, C, 48.4; H, 4.5; S, 5.2%.

3-Nitro-β-diethylaminoethylaniline.

i) The above sulphonamide hydrochloride (6.0 g.) was dissolved in diluted sulphuric acid (55 ml. of 95%), and after 12 hours the mixture was poured onto ice and basified. The liberated nitroamine was collected with ether, and distilled as an orange-red oil, b.p. 125-30°(bath temperature)/0.03 mm. (2.5 g., 75%).

Found: C, 61.1; H, 8.0.

$C_{12}H_{19}O_2N_3$ requires, C, 60.7; H, 8.0%.

The picrate separated from aqueous ethanol in orange prisms, m.p. 151-2°,

Found: C, 46.5; H, 4.8.

$C_{12}H_{19}O_2N_3.C_6H_3O_7N_3$ requires, C, 46.4; H, 4.7%.

ii). 3-Nitro-p-toluenesulphonanilide (10.0 g.) and β-chloroethyldiethylamine (4.64 g.) were refluxed with a solution of sodium (0.79 g.) in ethanol (100 ml.) for 6 hours. The solvent was evaporated, and the residual material hydrolysed and worked up as in "i". 3-Nitro-β-diethylaminoethylaniline was obtained as a deep red oil,

b.p. 125-30°(bath temperature)/0.03 mm. (5.05 g., 62%), and characterised as the picrate, m.p. and mixed m.p. 151°.

A specimen of this nitro-amine (1.5 g.) was hydrogenated to the triamine (Raney nickel) in methanol (30 ml.), the filtered solution added to violuric acid (2.0 g.) in water (10 ml.) and the mixture heated on a steambath for 50 minutes. On cooling a red-brown solid (1.6 g. 52%), m.p. 290°(decomp.), was obtained, which dissolved in aqueous ethanol to give a yellow solution with a green fluorescence, very like that of the 3-amino-methylaniline-violuric acid condensate. It crystallised from water as a brick red powder with a green lustre, m.p. 300° decomp), and consisted of a violurate, probably of 7-amino-9-β-diethylaminoethyl-isoalloxazine and/or the isomeric alloxazine, but its constitution was not definitely ascertained.

Found, after drying at 120° in vacuo:

C, 49.6; 49.3; H, 5.2; 5.2; N, 25.6.

$C_{16}H_{20}O_2N_6.C_4H_3O_4N_3$ requires, C, 49.5;: H, 4.7; N, 26.0%.

6:7-Dimethoxyalloxazine.

i). 4:5-Dinitro-veratrole (1.08 g.) was hydrogenated (Raney nickel) to the diamine in methanol (20 ml.), filtered into concentrated hydrochloric acid (3 ml.) and the solution boiled with aqueous alloxan (0.9 g., in 10 ml.) for 15 minutes. The yellow alloxazine (0.65 g., 46%)

separated from the solution, after cooling was collected and washed with ethanol. It dissolved in warm 1% aqueous sodium hydroxide giving a yellow solution with a brilliant green fluorescence under u.v. light, and was precipitated by the addition of hot dilute hydrochloric acid. If the mixture is not kept boiling during the precipitation the alloxazine separates as a gel. The product, after 4 reprecipitations, separated as a yellow sesquihydrate, m.p. 345-55° (decomp., brown fumes evolved at c 270°),

Found: C, 48.2; H, 4.2; N, 18.4.

$C_{12}H_{10}O_4N_4.1\frac{1}{2}H_2O$ requires, C, 47.8; H, 4.3; N, 18.6%.

It lost water slowly at 150°C in vacuo,

Found: C, 51.7; H, 4.0.

$C_{12}H_{20}O_4N_4$. requires, C, 52.6; H, 3.7.

The intense red solution in concentrated sulphuric acid remained clear on heating, and the u.v. absorption spectrum is recorded on page 102 and 103.

ii). 4-Nitroveratrole (2.0 g.) was hydrogenated (Raney nickel) in dioxane (15 ml.), and the amine obtained by filtration and evaporation to dryness dissolved in acetic acid (15 ml.). Violuric acid (1.72 g.) was then added, and the mixture refluxed for 10 hours. The dark maroon precipitate (0.34 g.) was separated from the blue-black solution, and purified through alkali (using charcoal)

as in "i". The product was a yellow-brown powder, m.p. 333-5° (decomp. blackens c. 300°) dissolving in cold concentrated sulphuric acid to give an intense red solution, which charred slightly on heating.

Found, after drying at 150° in vacuo: C, 52.5; H, 4.1.

Found (undried): N, 18.4%.

The u.v. spectrum is recorded on p. 102 and 103.

iii). The amine and violuric acid (same quantities as in "ii") in aqueous solution (15 ml.) were heated on a steam bath for 7 hours. The solution went blue-black, and after cooling the precipitate (1.6 g.) was collected and purified through alkali (charcoal) giving a yellow powder (0.35 g.) which was redissolved and precipitated many times. The product, a yellow powder m.p. 340° (decomp.), did not analyse for the alloxazine.

Found: C, 60.5; H, 5.3; N, 8.8%.

It gave a faint red colour in cold concentrated sulphuric acid and charred badly on heating.

The u.v. spectra of the products of these three experiments were examined in N/10 alkali:-

Compound from "i"		"ii"		" iii"	
λ-max.	ε-max.	λ-max.	ε-max.	λ-max.	ε-max.
2100	20000	2700	26900	2300	27000
2600	26100	2800	26900	2810	12000
3500	7150	3300	4600	----	-----
4150	10400	4050	8700	4150	930

Compound from	"i"		"ii"	
	λ-min.	ε-min.	λ-min.	ε-min.
	3000	2090	3000	2500

* This is the value of ε at 3500Å: no extinction maximum was apparent in the curve at this wavelength.

These results confirm that the products from "i" and "ii" are identical, and show that if the main constituent of the product from "iii" has no absorption at 4100Å it contains about 9% of the alloxazine.

Part III. Benziminazoles

2-Benzylbenziminazole.

i). o-Phenylene diamine (0.54 g., 1 mol.) and phenylacetiminomethyl ether (0.75 g., 1.1 mol.) were refluxed in methanol (3 ml.) for one hour. No ammonia could be smelt, and no solid separated on standing overnight, even on seeding with benziminazole. Further refluxing (6 hours) after the addition of a crystal of iminoether hydrochloride caused the slow evolution of ammonia. Water was added, and the resulting precipitate crystallised from ethanol, 0.27 g (26%), m.p. 186° not depressed by addition of pure 2-benzylbenziminazole.

ii). The diamine (0.63 g., 1 mol.) and iminoether (0.87 g., 1.1 mol.) were heated together in an oil bath without a solvent. Ammonia began to come off at 115°, and rapidly at 135-40°. After 2½ hours at 150-60° the mixture was cooled and the black mass crystallised from aqueous ethanol (charcoal) giving the benziminazole as a dark brown solid, m.p. 183-4° (0.35 g., 35%, mixed m.p. 186-7°)

iii). Cold solutions of the diamine (2.16 g., 1 mol.) and phenylacetiminomethylether hydrochloride (3.71 g., 1 mol.) in methanol (8 ml. and 4 ml. respectively) boiled when mixed and ammonium chloride separated. After a few minutes the mixture was poured into water (100 ml.), and the precipitate of 2-benzylbenziminazole collected, m.p. 189°

(3.5 g., 84%). It crystallised from aqueous ethanol in colourless needles, m.p. 191^{0} (Found after drying at 100^{0}/vacuo:

C, 80.6; H, 5.8.

Calc. for $C_{14}H_{12}N_2$ C, 80.8; H, 5.8% .

iv). A mixture of the diamine (1.08 g., 1 mol.), hydrogen chloride (0.37 g., 1 mol.) and phenylacetiminomethylether hydrochloride (1.86 g., 1 mol.) in methanol (6 ml.) were refluxed for one hour, poured into water (50 ml.), neutralised, and the colourless 2-benzylbenziminazole collected, m.p. 190^{0} (1.65 g., 79%)

v). Suspensions of the diamine dihydrochloride (1.81 g., 1 mol.) and the iminoether hydrochloride (1.86 g., 1 mol.) in methanol (11 ml. and 3 ml. respectively) were mixed. No apparent heating effect occurred. After 20 hours the mixture was poured into water (50 ml.) and the benziminazole worked up as before, m.p. 185-6^{0} (0.85 g., 41%).

vi). A repetition of "v" using a total of 12 ml. of methanol, but refluxing the reaction mixture for one hour gave 1.1 g., (53%) of benziminazole, m.p. 188-9^{0}. Longer refluxing failed to improve the yield.

vii). Solutions of o-phenylene diamine (2.16 g., 1 mol.) and phenylacetiminothiobenzylether hydrochloride (5.56 g., 1 mol.) in methanol (8 ml. and 8 ml.) were mixed. A strong

smell of benzyl thiol rapidly developed, very little heat was evolved, and after 20 minutes the mixture, now containing a small precipitate, was poured into water (350 ml.) containing some concentrated hydrochloric acid (20 ml.). The mercaptan was removed with ether, the aqueous solution neutralised and the benzylbenziminazole collected, m.p. 187-8° (3.0 g., 72%).

viii). A mixture of the thioiminoether hydrochloride (3.78 g., 1 mol.) and o-phenylenediamine dihydrochloride (1.81 g., 1 mol.) in methanol (10 ml.) was left for 16 hours, poured into water, and the benzylbenziminazole worked up as in "vii", m.p. 187° (0.88 g., 42%).

1:2-Dimethylbenziminazole.

Acetiminomethylether hydrochloride (0.655 g., 1 mol.) was added to a solution of 2-amino-N-methylaniline (0.73 g., 1 mol.) in dry methanol (2 ml.). A vigorous reaction took place, the mixture boiled, and a yellow precipitate formed. After a few minutes the mixture was poured into water (30 ml.), the buff coloured precipitate (m.p. 66-9°) being collected and dried, m.p. 111-2° (0.52 g., 60%). (Phillips, 1950, gives m.p. 65° for the trihydrate, and m.p. 112° for the anhydrous compound.)

The picrate separated from aqueous ethanol in short

yellow needles, m.p. 243° (decomp.) Phillips, 120, gives m.p. 238°.

Found: C, 48.4; H, 3.4.

Calc. for $C_9H_{10}N_2.C_6H_3O_7N_3$, C, 48.0; H, 3.5%.

The yield of product was not decreased by using methanol containing 3% of water in the condensation.

2-Methylbenziminazole.

A mixture of o-phenylene diamine (1.08 g.) and acetiminomethylether hydrochloride (1.1 g.) in methanol (5ml.) were warmed on a steam bath for 10 minutes, diluted with water and the colourless precipitate of 2-methylbenziminazole collected (1.2 g., 91%), m.p. 175-6° (Phillips, 118, gives m.p. 176°).

It was characterised as the picrate, which separated from ethanol in long thin yellow prisms, m.p. 211-2°. (Phillips, 120, gives m.p. 248°)

Found: C, 46.6; H, 3.0.

Calc. for $C_8H_8N_2.C_6H_3O_7N_3$ C, 46.5; H, 3.0%.

1-Methyl-2-benzylbenziminazole.

i). This was similarly prepared from phenylacetiminomethylether hydrochloride (0.365 g., 1 mol.) and N-methyl-o-phenylenediamine (0.24 g., 1 mol.) in methanol (2 ml.). It separated as a pale yellow oil when the reaction mixture was poured into water. It was collected with ether and

characterised as the picrate, which separated from aqueous dioxane in yellow-brown dodecahedra, m.p. 249° (decomp.)

Found: C, 55.7; H, 3.7; N, 15.9.

$C_{15}H_{14}N_2.C_6H_3O_7N_3$ requires C, 55.9; H, 3.8; N, 15.5%.

ii). The diamine, from the catalytic hydrogenation (Raney nickel of 2-nitro-N-methylaniline (3.54 g., 1 mol.) in methanol (21 ml.) was filtered onto phenylacetiminothiobenzylether hydrochloride (6.46 g., 1 mol.). Benzyl mercaptan was instantly liberated, and after 2 hours the mixture was diluted with water (200 ml.), acidified strongly and the mercaptan removed with ether. The aqueous solution was neutralised with sodium carbonate and the liberated oil collected with ether and distilled. The benziminazole (3.4 g., 66%) distilled as a yellow oil, b.p. 244-8°/18 mm., which slowly solidified, m.p. 69-70°. After several recrystallisations it separated, from cyclohexane, in colourless prisms, m.p. 77-8°

Found: C, 80.8; H, 6.2.

$C_{13}H_{14}N_2$ requires C, 81.1; H, 6.3%.

The picrate had m.p. 245° (decomp.) and mixed m.p. (with that from "i") 247° (decomp.)

2-Methylbenzoxazole.

i). A mixture of o-aminophenol (2.5 g., 1 mol.) and acetiminomethylether hydrochloride (2.5 g., 1 mol.) in dry methanol (10 ml.) was heated on a boiling waterbath for

30 minutes. After the addition of water containing a little sodium carbonate to the semisolid sludge, the benzoxazole was collected with ether and distilled, b.p. 203-8° (2.38 g., 78%). It was obtained as a colourless oil, which rapidly turned red on standing, and was characterised as the picrate which separated from ethanol in yellow prisms, m.p. 117-7½°.

Found: C, 46.2*; H, 2.6*; N, 15.2.

$C_8H_7ON.C_6H_3O_7N_3$ requires C, 46.4; H, 2.8; N, 15.5%.

Wager, 48, gives m.p. 117-8° but no analysis.

ii). A small rise in temperature was noted when the iminoether hydrochloride (1.95 g., 1 mol.) and o-aminophenol (1.95 g., 1 mol.) were mixed in dry methanol (5 ml.). After two hours at room temperature the mixture was worked up as before, giving 0.7 g. (30%) of the benzoxazole, b.p. 200-210°.

iii). Acetiminothiobenzylether hydrochloride (2.15 g., 1 mol.) and o-aminophenol (1.15 g., 1 mol.) in ethanol (5 ml.) were heated on a boiling waterbath for 20 minutes, cooled, and filtered from the ammonium chloride precipitate. Picric acid (2.4 g., 1 mol.) was added to the heated filtrate and the benzoxazole picrate separated on cooling, m.p. and mixed m.p. 117-7½° (1.95 g., 51%).

1-(γ-Diethylaminopropyl)-2-methyl-5-chlorobenziminazole (XLIV, R=H, R'=Cl).

i). γ-Diethylaminopropyl-2-nitro-4-chloroaniline (2.02 g., 1 mol., prepared according to King and Acheson, 103), was hydrogenated to the triamine in methanol (15 ml., Raney nickel catalyst), filtered into concentrated hydrochloric acid (3 ml.) and evaporated to dryness in vacuo. The residual oil was refluxed with glacial acetic acid (0.75 ml., 1.9 mol.) in 4N hydrochloric acid (12.5 ml.) for 2 hours (c.f. Phillips, 118), the deep red solution basified and the benziminazole collected with ether. It was a thick yellow oil, b.p. 160-4° (bath temperature)/0.14 mm., solidifying in long needles, m.p. 53-4° (1.55 g., 79%)

Found: C, 64.1; H, 7.9; N, 15.3.

$C_{15}H_{22}N_3Cl$ requires C, 64.4; H, 7.9; N, 15.0%.

The monopicrate separated from ethanol in very small yellow needles, m.p. 165°. No good analyses were obtained for this compound owing to its tendency to disproportionate into the free base and the much less soluble dipicrate.

The dipicrate separated from aqueous ethanol in brown diamond shaped plates, with a green lustre, m.p. 239° (decomp.).

Found: C, 43.6: H, 4.1; N, 17.3: Cl, 4.2

$C_{15}H_{22}N_3Cl.2C_6H_3O_7N_3$ requires C, 43.9; H, 3.8; N, 17.1: Cl, 4.8%

ii). The nitroamine (1.27 g., 1 mol.) was catalytically

hydrogenated, as before, in methanol (10 ml.) and filtered onto acetiminomethylether hydrochloride (0.49 g., 1 mol.). The mixture darkened in colour, a precipitate soon formed, and after 4½ hours it was collected (0.84 g., m.p. 200°, 57%). (Identical results were obtained when the mixture was boiled vigorously for 90 minutes, and cooled). The filtrate was evaporated down, but no solid separated. It was then basified with aqueous alkali, the resulting dark oil being collected with ether and distilled, b.p. 165-70° /0.15 mm. (bath temperatures)(0.25 g.). The product, a pale yellow oil, did not solidify on seeding with benzimimazole, and analysis indicated a mixture (Found: C, 62.2; H, 6.2%). Treatment with one equivalent of alcoholic picric acid gave a red semisolid precipitate, a mixture of picrates, from which some benzimimazole dipicrate m.p., and mixed m.p. 237° (decomp.) was isolated by fractionation from aqueous alcohol. Treatment of a further portion of the oil with alcoholic picrolonic acid gave a precipitate, from which the benzimimazole dipicrolonate was obtained in yellow prisms by crystallisation from ethanol, m.p. 235-6° (decomp.)

Found: C, 57.1; H, 4.7; Cl, 4.6.

$C_{15}H_{22}N_3Cl.2C_{10}H_8O_5N_4$ requires C, 57.0; H, 4.7; Cl, 4.4%.

The solid compound, m.p. 200°, crystallised from methanol in colourless prisms, m.p. 204°, and analysed for the amidine hydrochloride (XLVI)

Found: C, 53.8; H, 7.9; Cl, 21.6; N, 15.0. 15.0.**

$C_{15}H_{25}N_4Cl.HCl$ requires C, 54.0; H, 7.8; Cl, 21.3; N, 16.8%.

This compound gave no ammonia with hot or cold alkali, and contained ionised halogen. The free base, obtained by basifying the aqueous hydrochloride and collecting with ether, was a pale yellow oil, b.p. 160°/0.2 mm. (bath temperature).

Found: N, 17.7.

$C_{15}H_{25}N_4Cl$ requires N, 18.9%.

The monopicrate, obtained by the action of aqueous sodium picrate on the aqueous hydrochloride, separated as the dihydrate from aqueous ethanol, in very deep red prisms, m.p. 113-4°.

Found: C, 45.2; H, 5.5; N, 16.8.

$C_{15}H_{25}N_4Cl.C_6H_3O_7N_3.2H_2O$ requires C, 44.9; H, 5.7; N, 17.4%.

After drying over phosphorus pentoxide in vacuo at room temperature the colour lightened somewhat and one molecule of water was lost, m.p. 115½°.

Found: C, 46.1; H, 5.4.

$C_{15}H_{25}N_4Cl.C_6H_3O_7N_3.H_2O$ requires C, 46.4; H, 5.5%.

Found, after drying at 100°/vacuo:

loss 1.88%; C, 46.9; H, 5.2.

$C_{15}H_{25}N_4Cl.C_6H_3O_7N_3.\frac{1}{2}H_2O$ requires loss 1.69%: C, 47.1: H, 5.2%.

The dipicrate separated from alcoholic picric acid in short yellow prisms, m.p. 156° (decomp.)

Found: Cl, 5.1.

$C_{15}H_{25}N_4Cl.2C_6H_3O_7N_3$ requires Cl, 4.7%.

iii). The nitro compound (2.07 g., 1 mol.) was hydrogenated in methanol (20 ml.) as before and filtered into ethanolic hydrogen chloride (containing 0.29 g., HCl, 1.1 mol.) before addition to the iminoether hydrochride (0.795 g., 1.0 mol.). A rise in temperature occurred on mixing the reactants and a precipitate formed. Next day the acetamidino derivative (0.65 g., m.p. 202°, 27%) was collected, and the filtrate evaporated almost to dryness. A solid, mainly ammonium chloride (0.2g., 52%), separated; the methanolic filtrate was basified with aqueous alkali and extracted with ether. Evaporation and distillation of the dried extract gave a yellow oil b.p. 165-70°(bath temperature)/0.2 mm., which failed to solidify when seeded with benziminazole. Analysis indicated a mixture, and alcoholic picric acid gave a picrate, from which a little benziminazole dipicrate was isolated, m.p. and mixed m.p. 237° (decomp.).

iv). "iii" was repeated, using more hydrogen chloride (0.53 g., 2 mol.), and no heating effect took place on mixing the reactants. Next day the dark solution was largely evaporated, some ammonium chloride separated, and the filtrate was worked up as before.

A pale yellow oil, b.p. 160°(bath temperature)/0.2 mm (1.47 g., 68%), was obtained, and analysed for the acetamidino derivative

Found: C, 60.9; H, 8.6; N, 17.5.

$C_{15}H_{25}N_4Cl$ requires C, 60.7; H, 8.4; N, 18.9%

It was further characterised by the formation of the mono- and dipicrates, and the hydrochloride, whose m.ps. and mixed m.ps. were 113-4°, 156° (decomp.), and 202-3° respectively.

v). Ringclosure of the amidine hydrochloride (0.3 g.) to the benziminazole was effected by refluxing with acetyl chloride (3.5 ml.) for 3 hours. The solid dissolved, and after reaction the mixture was cooled, diluted with water, basified and extracted with ether. Evaporation of the dried extract gave a yellow oil, which soon solidified in long needles, m.p. 52-4°, (0.24 g., 92%), not depressed by addition of pure benziminazole prepared via "i". The dipicrate had m.p. and mixed m.p. 238° (decomp.)

Found: C, 44.0; H, 3.9; Cl, 5.3%).

Attempted acetylation of 2-nitro-4-chloro-Y-diethylaminopropyl-aniline.

i). The aniline was recovered unchanged after heating to 200° for 6 hours with acetic anhydride and a trace of sulphuric acid in sealed tube.

11). The aniline (0.57 g.) in dry xylene (15 ml.) was refluxed with sodamide (0.11 g.) for a few minutes. Some ammonia was evolved and the solution went very dark. Acetyl chloride (0.2 ml.) in xylene (5 ml.) was added, after 15 minutes refluxing the solution was filtered and basic material isolated from the xylene with dilute hydrochloric acid. After basifying the extract a red oil (0.13 g.), which proved to be the original amine, was obtained.

1-(γ-Diethylaminopropyl)-2-methyl-6-chlorobenziminazole (XLIV, R=Cl, R'=H).

1). This was similarly prepared (p.110, "1") from 2-nitro-5-chloro-γ-diethylaminopropylaniline (prepared according to 121), by catalytic hydrogenation (over Raney nickel) in methanol, filtration into concentrated hydrochloric acid (5 ml.) and evaporation to dryness. The residual solid was refluxed with glacial acetic acid (1.1 ml., 1.1 mol.) in 4N hydrochloric acid (25 ml.) for 90 minutes, and the deep red solution worked up as before. The benziminazole was obtained as a thick yellow oil, b.p. 140°(bath temperature)/0.14 mm. (3.23 g., 66%).

The dipicrate separated from aqueous ethanol in yellow needles m.p. 217° (decomp.)

Found: C, 43.8; H, 4.1; N, 17.1.

$C_{15}H_{22}N_3Cl.2C_6H_3O_7N_3$ requires C, 43.9; H, 3.8; N, 17.1%.

The dihydrochloride was precipitated by passing hydrogen chloride into a solution of the free base in dry ether; it separated in colourless deliquescent prisms, m.p. 100-10° (decomp.)

Found: Cl, 26.9.

$C_{15}H_{22}N_3Cl.2HCl$ requires Cl, 30.2%.

ii). The same nitroamine (4.52 g., 1 mol.) was reduced as before in methanol (20 ml.) and filtered into a solution of acetiminothiobenzylether hydrochloride (3.40 g., 1.07 mol.) in methanol (5 ml.). Next day the methanol was evaporated and the residue treated with aqueous hydrochloric acid. The benzyl thiol was removed with ether, the aqueous solution basified, and the benziminazole collected with ether. It was a pale yellow oil, b.p. 140°(bath temperature)/0.14 mm. (2.1 g., 47%)

Found: C, 64.1; H, 8.0; N, 15.0.

$C_{15}H_{22}N_3Cl$ requires C, 64.4; H, 7.9; N, 15.0%

The dipicrolonate separated from aqueous ethanol in irregularly shaped yellow prisms, m.p. 236° (decomp.)

Found: C, 52.2; H, 5.0; N, 19.3; Cl. 4.4.

$C_{15}H_{22}N_3Cl.2C_{10}H_8O_5N_4$ requires C, 52.0; H, 4.7; N, 19.1; Cl, 4.4%

The dipicrate separated from aqueous ethanol in yellow needles and had m.p. and mixed m.p. with a sample

from "i", 217° (decomp.).

2-Nitro-4:5-dichloro-γ-diethylaminopropylaniline.

A mixture of 1:2-dinitro-4:5-dichlorobenzene (5.0 g.), freshly fused sodium acetate (6 g.) and γ-diethylaminopropylamine (5.2 ml.), after the vigorous initial reaction had subsided, was heated to 160° for 2 hours. The dark coloured reaction mixture was then diluted with a large volume of dilute hydrochloric acid, unreacted nitro compound removed with ether, the aqueous solution basified and the free amine collected with ether. Unless a large volume of solvent is used the amine hydrochloride separates. The amine was an orange red oil, b.p. 185-90°(bath temperature) /0.02 mm. (5.08 g., 75%.).

The hydrochloride separated from aqueous hydrochloric acid as a microcrystalline yellow powder, m.p. 216-7°

Found: C, 43.5; H, 5.7.

$C_{13}H_{19}O_2N_3Cl_2.HCl$ requires, C, 43.7; H, 5.6%.

The picrate separated from aqueous ethanol in fine yellow needles, m.p, 171-2°

Found, after drying at 116°/vacuo:

Found: C,41.6; H, 3.9; N, 15.5; Cl, 12.9.

$C_{13}H_{19}O_2N_3Cl_2.C_6H_3O_7N_3$ requires, C,41.5; H, 4.0; N, 15.3 Cl, 12.9%.

1-(γ-diethylaminopropyl)-2-methyl-5:6-dichlorobenzimidazole (XLIV, R=R'=Cl).

The above nitroamine (3.82 g., 1 mol.) was converted to the

benzimina<u>z</u>ole in the same way as the monochloro derivatives, using concentrated hydrochloric acid (3.6 ml.), glacial acetic acid (0.8 ml., 1.2 mol) and 4N hydrochloric acid (25 ml.). It was a pale yellow oil, b.p. 175-80°(bath temperature)/0.02 mm. (2.27 g., 61%)

Found: C, 57.1; H, 6.9; N, 13.4; Cl, 22.4.

$C_{15}H_{21}N_3Cl_2$ requires, C, 57.3; H, 6.7; N, 13.9; Cl, 22.6%.

The dipicrate separated from aqueous ethanol in long thin needles, m.p. 226-8° (decomp.)

Found: C, 42.3; H, 3.5; Cl, 8.7.

$C_{15}H_{21}N_3Cl_2.2C_6H_3O_7N_3$ requires, C, 41.9; H, 3.5; Cl, 9.2%.

Acetiminomethylether hydrochloride and 2-amino-4:5-dichloro-γ-diethylaminopropylaniline.

i). The amine, from the catalytic hydrogenation (Raney nickel) of the amine, liberated from 2-nitro-4:5-dichloro-γ-diethylaminopropylaniline hydrochloride (3.5 g., 1 mol.), in methanol, was filtered into acetiminomethylether hydrochloride (1.07 g., 1 mol.) in methanol (5 ml.). After boiling for 30 minutes, most of the solvent evaporated, the brown solution was basified and the precipitated amine collected with ether and distilled, giving a pale yellow oil, b.p. 145-55°(bath temperature)/0.04 mm. (2.07 g.,), which analysed for the acetamido or acetamidino derivative

Found: C, 54.6; H, 7.4.

$C_{15}H_{23}ON_3Cl_2$ requires C, 54.2; H, 6.9; N, 12.6.

$C_{15}H_{24}N_4Cl_2$ requires C, 54.4; H, 7.3; N, 16.1%.

Attempts to make a dipicrolonate failed, and picric acid gave an inhomogeneous product. Many recrystallisations of this material from aqueous ethanol gave a picrate, separating in yellow brown needles, m.p. 211-2° (decomp.), which in spite of its low decomposition point analysed for the benziminazole dipicrate

Found: C, 42.5; H, 3.9; Cl, 9.1%.

ii). The above was repeated, only filtering the methanolic amine (from 1.66 g. nitroamine base) into ethanolic hydrogen chloride (0.38 g., HCl, 2 mol.) before addition to the iminoether hydrochloride (0.57 g.). The mixture darkened, no precipitate separated, and was worked up after 4 hours. A pale yellow oil, b.p. 180-5° (bath temperature)/0.2 mm. (1.07 g.) which analysed for 2-(N-acetamidino)-5-chloro-γ-diethylaminopropylaniline was obtained

Found: C, 54.2; H, 7.5; N, 15.0%.

<u>1-(γ-Diethylaminopropyl)-2-methyl-5-methoxybenziminazole,</u>

(XLIV, R=H , R'=OMe).

2-Nitro-4-methoxy-γ-diethylaminopropylaniline (7.0 g., prepared from 2-nitro-4-methoxyaniline, according to 103), was hydrogenated to the triamine in methanol over Raney nickel,

the solution filtered and the filtrate and washings (54 ml.) divided into two parts.

i). Concentrated hydrochloric acid (4 ml.) was added to one half (37 ml.). The resulting solution was evaporated to dryness in vacuo, the residual gum refluxed with glacial acetic acid (1 ml.) and 4N hydrochloric acid (15 ml.), and the methoxy-benziminazole isolated in the usual way. It was a pale yellow oil (2.2 g., 64%) solidifying in long prisms, m.p. c. 40°, b.p. 186-8°/0.8 mm. (Simonov, 122, gives b.p. 184-5°/2 mm.).

Found:	C, 70.0;	H, 9.2;	N, 15.2.
Calc. for $C_{16}H_{25}ON_3$	C, 69.8;	H, 9.1;	N, 15.3%.

The dipicrate separated from aqueous n-propanol in clusters of short yellow prisms, m.p. 238-9° (decomp., after sintering c. 225°). Simonov gives up 236°.

Found:	C, 46.1;	H, 4.4;	N, 16.6.
Calc. for $C_{16}H_{25}ON_3 \cdot 2C_6H_3O_7N_3$	C, 45.8;	H, 4.2;	N, 17.2%.

ii). The rest of the triamine solution (37 ml.) was added to acetiminomethylether hydrochloride (1.4 g.) in methanol (3 ml.). The solution darkened, no precipitate formed, and after half an hour the solution was largely evaporated on a steam bath. On basifying ammonia was evolved, and the liberated base collected with ether and distilled, b.p. 178-188°/0.8 mm. (2.0 g.). It failed to solidify on

seeding, and analysis indicated a mixture. Treatment of a portion with alcoholic picric acid gave the benziminazole dipicrate (52% yield), m.p. 229° (decomp.) after one crystallisation from aqueous n-propanol.

Found: C, 46.2; H, 4.5%.

Part IV. Pteroylglutamic Acid and related compounds.

Chloroacet-2-nitroanilide.

Chloroacetyl chloride (16.8 ml., 1.1 mol.) was added to o-nitroaniline (28 g., 1 mol), and a vigorous reaction ensued. When it was over the mixture was heated till fused, cooled, and the brown solid ground up with water and washed with dilute acid. It crystallised from ethanol giving the chloroacetanilide in yellow needles (38.5 g., 88%), m.p. 86-7°. (145 gives m.p. 88°)

Chloracet-2-nitro-4-chloroanilide.(LI, R = Cl, R' = H).

This was similarly prepared from 2-nitro-4-chloroaniline in 78% yield, and crystallised from ethanol in pale yellow prisms, m.p. 141°.

Phenylglycine-2-nitroanilide. (LI, R = R' = H).

Chloracet-2-nitroanilide (2.15 g., 1 mol) and aniline (0.93 g., 2 mol.) were refluxed for 4 hours in n-propanol (5 ml.) and poured into water. The precipitate, crystallised twice from ethanol, gave the product (1.2g., 60%) in nearly cubical yellow prisms, m.p. 140°.

Found: C, 61.8; H, 5.0; N, 15.8.

$C_{14}H_{13}O_2N_2$ requires, C, 61.9; H, 4.9; N, 15.5%.

Unreacted chloracet-2-nitroanilide (0.6 g.,) was recovered by addition of water to the first ethanol mother liquors.

Phenylglycine-2-nitro-4-chloroanilide (LI, R = Cl, R' = H).
i). Chloracet-2-nitro-4-chloroanilide (2.3 g., 1 mol.) and aniline (1.8 g., 2.1 mol.) were refluxed in n-propanol (14 ml.) for 3 hours, and the product precipitated by water. It crystallised from ethanol (m.p. 132-3°, 2.0 g., 71%), and on recrystallisation separated in orange-yellow plates, m.p. 134-5°.

Found: C, 55.2; H, 4.0; Cl, 11.8.
$C_{14}H_{12}O_3N_3Cl$ requires, C, 55.0; H, 3.9; Cl, 11.6%.

ii). The chloroacetanilide (1.2 g., 1 mol.) and aniline (0.45 g., 1 mol.) were heated in pyridine (3 ml.). All dissolved, but after a few minutes a solid separated and violent bumping started. More pyridine was added, and refluxing was continued for ½ hour. Next day the solid was collected, and from the filtrate, on dilution with water, a small quantity of chloracet-2-nitro-4-chloroanilide (0.12 g.) was precipitated. The solid was soluble in water, contained ionised halogen, and separated from ethanol in very pale yellow needles, m.p. 232°(decomp.). It analysed for pyridiniumacet-2-nitro-4-chloroanilide chloride.

Found: C, 47.4; H, 3.3; N, 13.1.
$C_{13}H_{11}O_3N_3Cl_2$ Requires, C, 47.6; H, 3.4; N, 12.8%.

2-Phenylaminomethyl-5-chlorobenziminazole.

Phenylglycine-2-nitro-4-chloro-anilide was hydrogenated in methanol over Raney nickel to the corresponding amine, which separated from aqueous ethanol in colourless plates, m.p. 163°

Found: Cl, 12.5.

$C_{14}H_{14}ON_3Cl$ requires Cl 12.9%.

After refluxing this amine with aqueous methanolic hydrogen chloride for one hour, the solvent was evaporated. The residual benziminazole dihydrochloride, which easily lost hydrogen chloride, crystallised from ethanolic hydrogen chloride in colourless prisms, m.p. 250-1°

Found: C, 51.4; H, 4.8.

$C_{14}H_{12}N_3Cl.1.9HCl$ requires C, 51.4; H, 4.3%.

The picrate separated from aqueous ethanol in deep yellow prisms, m.p. 214-5°(decomp).

Found: C, 49.4; H, 3.2; N, 17.6.

$C_{14}H_{12}N_3Cl.C_6H_3O_7N_3$ requires C, 49.3; H, 3.1; N, 17.3%.

p-Carbethoxyphenylglycine-2-nitroanilide (LI, R = H, R' = COOEt).

Chloroacet-2-nitroanilide (4.3 g., 1 mol.), ethyl p-aminobenzoate (3.3 g., 1 mol.) and pyridine (1.6 ml, 1 mol.) were refluxed in n-propanol (10 ml.) for 30 hours, and poured into water. The precipitate, after one

crystallisation from ethanol, and one from n-propanol, gave the product (0.82 g., 12%), in irregularly shaped yellow plates, m.p. 186°

	Found:	C, 59.4;	H, 5.0;	N, 12.0.
$C_{17}H_{17}O_5N_3$	requires	C, 59.5;	H, 5.0;	N, 12.2%.

Lower yields were obtained in the absence of pyridine, or by refluxing for a shorter time.

2-(p-Carbethoxyphenylaminomethyl)-benziminazole (L, R = H, R' = OEt).

The above compound, on hydrogenation over Raney nickel in methanol, gave the corresponding amine, which separated from ethanol in irregular clusters of colourless needles, m.p. 169°

	Found:	C, 64.9;	H, 6.4;	N, 13.0.
$C_{17}H_{19}O_3N_3$	requires	C, 65.2;	H, 6.1;	N, 13.4%.

Ringclosure to the benziminazole was effected by two methods.

i). The amine (0.1 g.) was refluxed in ethanolic hydrogen chloride (2 ml.), containing one drop of water, for an hour and the solvent was removed in vacuo. The residual solid crystallised from ethanol giving the benziminazole dihydrochloride in irregularly shaped colourless prisms, ~~by addition of a sample made~~ m.p., and mixed m.p. with the same compound prepared by Spensley, 262°.

ii). The amine (0.1 g., 1 mol.) and picric acid (0.145 g., 2 mol.) were refluxed in ethanol (3 ml.) for 4 hours.

Next day the precipitated benziminazole picrate (0.15 g., 90%) was collected and crystallised from aqueous ethanol in yellow prisms, m.p. 216°(decomp.).

Found: C, 53.1; H, 3.9; N, 16.1.

$C_{17}H_{17}O_2N_3.C_6H_3O_7N_3$ requires C, 52.6; H, 3.8; N, 16.0%.

p-Carbethoxyphenylglycine-2-nitro-4-chloro-anilide (LI, R = Cl, R' = COOEt).

Chloroacet-2-nitro-4-chloroanilide (2.49 g., 1 mol.), ethyl p-aminobenzoate (1.65 g., 1 mol.) and pyridine (0.8 ml., 1 mol.) were refluxed in n-propanol (7 ml.) for 4 hours, and poured into water. The precipitate, after two crystallisations from ethanol, gave the product (c.0.4 g., LI, R = Cl, R' = COOEt) in fine yellow needles, m.p. 167 167-8°.

Found: C, 54.2; H, 4.2; Cl, 9.3.

$C_{17}H_{16}O_5N_3Cl$ requires C, 54.0; H, 4.2; Cl, 9.4%.

N-(2-quinoxaline)-4-aminobenzoic acid (LII, R = OH).

p-Aminobenzoic acid (0.69 g., 1 mol.) and 2-chloroquinoxaline (0.82 g., 1 mol.) were refluxed in n-propanol (6 ml.) for 2 hours, when the product slowly separated as a yellow powder (1.12 g., 85%), m.p. 340°(decomp.). It separated from nitrobenzene in yellow needles, and was further purified by dissolving in aqueous sodium carbonate and precipitating as a brown powder by dilute hydrochloric acid, m.p. 344-5°(decomp.).

Found, after drying at 150° in vacuo:

C, 66.3;) 66.6;) H, 4.4;) 4.5;) N, 15.5.

$C_{15}H_{11}O_2N_3.\frac{1}{2}H_2O$ requires, C, 66.6; H, 4.3; N, 15.6%.

Ethyl N-(2-quinoxaline)-4-aminobenzoyl-l-glutamate (LII, R = l-, NHCH(COOEt)CH_2CH_2COOEt).

2-Chloroquinoxaline (0.6 g., 1 mol.) and ethyl p-aminobenzoyl-l-glutamate (1.18 g., 1 mol.) were refluxed in ethanol (5 ml.) for 4 hours. A brown solid (1.3 g., 79%) separated, and after cooling it was collected and washed with a little ethanol. It crystallised from ethanol (charcoal) giving the quinoxaline derivative in yellow brown needles, m.p. 168°.

Found, after drying at 116°/0.5 mm.,

C, 64.0; H, 5.5; N, 12.2.

$C_{24}H_{26}O_5N_4$ requires, C, 64.0; H, 5.8; N, 12.4%.

N-(2-quinoxaline)-4-aminobenzoyl-l-glutamic acid (LII, R = l- NHCH(COOH)CH_2CH_2COOH).

A solution of the above ester (0.72 g.) in ethanol (12 ml.) was added to a solution of sodium hydroxide (0.24 g.) in water (2 ml.). A brown precipitate rapidly formed, and after 90 minutes it was collected and washed with ethanol. It dissolved in water, and the free acid (0.54 g., 86%) was precipitated by the addition of dilute hydrochloric acid. It was purified by dissolving in

aqueous sodium bicarbonate (100 ml.) and after treatment with charcoal was precipitated from the boiling solution by dilute hydrochloric acid. It was a yellow-brown powder, m.p. 252° (decomp.).

Found, after drying at 115° in vacuo:

C, 59.6; H, 4.5; N, 14.0.

$C_{20}H_{18}O_5N_4.H_2O$ requires, C, 59.6; H, 4.7; N, 13.9%.

2-Amino-β-hydroxyethyl-aniline.

o-Phenylenediamine (5.4 g., 1.0 mol.) and ethylene oxide (2.7 ml., 2.39 g., 1.14 mol.) were heated in a sealed tube for 20 hours at 120°. After cooling the residual crystalline sludge of 2-amino-β-hydroxyethylaniline was washed out with a little water and collected, m.p. 104-5° (3.1 g., 41%). It crystallised from water in colourless plates, m.p. 107° (Kremer, 148, gives m.p. 107°)

Found: C, 63.0; H, 7.7; N, 18.7.

Calc. for $C_8H_{12}ON_2$ C, 63.2; H, 7.9; N, 18.4%.

The monopicrate separated from water in orange-red prisms, m.p. 120° (decomp.)

Found: C, 44.0; H, 4.0; N, 18.9.

$C_8H_{12}ON_2.C_6H_3O_7N_3$ requires C, 44.1; H, 3.9; N, 18.4%.

The dipicrate crystallised from water in yellow prisms, m.p. 105-6°

Found: C, 38.9; H, 3.2.

$C_8H_{12}ON_2.2C_6H_3O_7N_3$ requires C, 39.3; H, 3.0%.

Di-p-toluenesulphonyl-o-phenylenediamine.

p-Toluenesulphonyl chloride (59 g., 2.2 mol.) was added to a solution of o-phenylenediamine (15 g., 1 mol.) in pyridine (75 ml.). After one hour on a boiling water-bath the solution was diluted with water (1l.) to precipitate the product, which crystallised from ethanol in colourless prisms, (46.2 g., 80%) m.p. 204° (Reverdin and Crepieux, 149, give m.p. 201-2°).

1:4-Di-p-toluenesulphonyl-2-hydroxymethyl-1:2:3:4-tetrahydroquinoxaline.

The above ditoluenesulphonyl derivative (88.2 g., 1.0 mol.), followed by 2:3-dibromopropanol (46.25 g., 1.0 mol.) in ethanol (200 ml.) was added to a solution of sodium (9.75 g., 1.0 mol.) in ethanol (1l.) After refluxing for 6 hours the ethanol was distilled off and the residual sticky yellow solid washed out with water and dried. This solid was refluxed with benzene (100 ml.) to remove gummy material, and after cooling the residue was collected and dissolved in boiling ethanol (1l.). On cooling the di-p-toluenesulphonylquinoxaline (44.5 g., 44%) separated in colourless prisms, m.p. 193°

Found: C, 58.1; H, 5.3; N, 5.7; S, 13.2.

$C_{23}H_{24}O_5N_2S_2$ requires C, 58.4; H, 5.1; N, 5.9; S, 13.5%.

2-Hydroxymethyl-1:2:3:4-tetrahydroquinoxaline.

i). The above ditoluenesulphonyl derivative (5.08 g.) was dissolved in diluted sulphuric acid (50 ml. d. 1.842 with 0.5 ml. water). After 2 days in a warm place the solution was poured onto ice, basified and extracted many times with chloroform. Evaporation of the chloroform gave the crude tetrahydroquinoxaline, which separated from ethanol in colourless prisms, m.p. 140-1°.

Found: C, 65.7; H, 7.3; N, 16.5.

$C_9H_{12}ON_2$ requires C, 65.8; H, 7.3; N, 17.1%.

The picrate separated from water, containing a trace of ethanol, in yellow prisms, m.p. 179-80° (decomp.)

Found: C, 45.3; H, 4.1.

$C_9H_{12}ON_2.C_6H_3O_7N_3$ requires C, 45.6; H, 3.8%.

The high yield recorded here was never reproduced in subsequent experiments. More dilute sulphuric acid failed to produce complete hydrolysis, and if the hydrolysis was carried out for a longer time very low yields of product were obtained, probably due to sulphonation.

ii). The di-p-toluenesulphonyl derivative (5.2 g.,) was heated with concentrated hydrochloric acid (20 ml.) to

170° for 9 hours. The resulting black mixture was filtered from unchanged starting material (0.5 g.), and the filtrate was basified and extracted with chloroform. Evaporation of the extract gave 2-hydroxymethyl-1:2:3:4-tetrahydroquinoxaline (0.7 g., 46%), m.p. and mixed m.p. 140° after one crystallisation.

Oxidation of 2-hydroxymethyl-1:2:3:4-tetrahydroquinoxaline.

Aqueous potassium ferricyanide (4.8 g., in 20 ml.) was added to a solution of the tetrahydroquinoxaline (0.56 g.) and sodium hydroxide (0.7 g.) in water (20 ml.). After half an hour the solution was filtered from inorganic material, and extracted seven times with chloroform. Distillation of the black tar (0.1 g.), obtained on evaporation of the extract, gave quinoxaline as a colourless oil, b.p. 130°/10 mm., which solidified on standing, m.p. 26° (Hinsberg, 150, gives m.p. 27°).

Found:	C, 73.4;	H, 4.8;	N, 21.4.
Calc. for $C_8H_6N_2$,	C, 73.8;	H, 4.6;	N, 21.5%.

2-Tribromomethylquinoxaline.

This was prepared in 79% yield (crude material, m.p. 91-2°) from freshly distilled 2-methylquinoxaline (152).

Methyl quinoxaline-2-orthocarboxylate.

2-Tribromomethylquinoxaline (5.6 g., 1 mol.) was added to a solution of sodium (1.2 g., 3.3 mol.) in methanol 30 ml.)

and the mixture refluxed for 4½ hours. The solvent was evaporated in vacuo. The residual orthoester solidified on treatment with water, and crystallised from methanol in colourless hair-like needles, m.p. 63-5°.

Found: C, 61.8; H, 6.0; N, 11.7.

$C_{12}H_{14}O_3N_2$ requires, C, 61.5; H, 6.0; N, 12.0%.

Methyl N-formyl-p-aminobenzoate

Methyl p-aminobenzoate (23 g.) was heated with aqueous formic acid (50 ml., of 50%) on a steam bath for 15 minutes, poured into water, and the precipitated formyl derivative crystallised from aqueous methanol. It separated in large colourless plates (20.0 g., 73%), m.p. 123½°.

Found: C, 59.9; H, 4.9.

$C_9H_9O_3N$ requires, C, 60.3; H, 5.0%.

2-Methylbenziminazole and 2-methyl-3-hydroxyquinoxaline.

1). A mixture of o-phenylenediamine (2.16 g., 1 mol.), ethyl isonitrosoacetoacetate (3.2 g., 1 mol.) and acetic acid (1.14 ml., 1 mol.) in ethanol (10 ml.) were refluxed for 5 hours. On cooling and scratching a pale yellow solid (0.25 g., 7.8%) separated, and was collected. The filtrate on treatment with aqueous ammonia gave an acid soluble oil, which did not react with 2:4-dinitrophenylhydrazine in 2N HCl. It was collected (with ether) and treated with alcoholic picric acid. 2-Methylbenziminazole picrate (1.8 g., 25%) precipitated, and after one

crystallisation from ethanol had m.p. and mixed m.p. with a sample described on p.107, 211-2°

Found: C, 46.8; H, 3.3.

Calc. for $C_8H_8N_2.C_6H_3O_7N_3$, C, 46.5; H, 3.0%.

The pale yellow solid, m.p. 227°, was soluble in aqueous sodium hydroxide, but insoluble in carbonate. Crystallisation from ethanol gave pale brown hairlike needles of 2-methyl-3-hydroxyquinoxaline, m.p. 245°, (154 gives m.p. 245°).

Found: C, 67.2; H, 4.8; N, 17.5.

Calc. for $C_9H_8ON_2$, C, 67.5; H, 5.0; N, 17.5%.

ii). o-Phenylenediamine dihydrochloride (1.81 g., 1 mol.) and ethyl isonitrosoacetoacetate (1.59 g., 1 mol.) were refluxed in ethanol (15 ml.) for 3 hours. Very little material precipitated on neutralising the solution, but 2-methylbenziminazole was isolated as the picrate (1.4 g., 39%), as before, m.p. and mixed m.p. 211-2°.

iii). o-Phenylenediamine (1.08 g., 1 mol.) and the isonitrosoester (1.59 g., 1 mol.) were refluxed in ethanol (7 ml.). 2-Methyl-3-hydroxyquinoxaline (0.5 g., 31%, m.p. and mixed m.p. 245°) slowly separated, and after 9 hours was collected from the cooled reaction mixture. 2-Methylbenziminazole was isolated from the filtrate as the picrate (1.2 g., 33%), m.p. and mixed m.p. 210°.

Ethyl 3-bromoacetoacetate (c.f. 155)

A solution of bromine (81 ml.) in chloroform 100 ml.) was added to ethyl acetoacetate (200 ml.) in chloroform (400 ml.) in 30 minutes, the temperature being kept below 7^0. After 24 hours standing at room temperature the chloroform was removed, and the residual liquid distilled in vacuo. After three fractionations 3-bromoacetoacetic ester was obtained as a colourless oil, b.p. $116\text{-}8^0/12$ mm., $n_D = 1.4818/17^0$ (70 g.). (156 gives $n_D = 1.4830/18^0$).

Ethyl N-(4-carbethoxyphenyl)-N-formyl-3-aminoacetoacetate.

1). A solution of ethyl N-formyl-4-aminobenzoate (4.0 g., 1 mol.) in xylene (75 ml.) was added to sodium (0.475 g., 1 mol.) powdered under xylene, and the mixture refluxed for 4 hours. 3-Bromoacetoacetic ester (4.33 g., 1 mol.) in xylene (10 ml.) was now added, and the mixture left for 4 hours, with occasional shaking. It was then refluxed for 2 hours, and filtered (hot) from inorganic material. The next day unchanged ethyl N-formyl-4-aminobenzoate (1.25 g.), which had separated from the dark brown solution, was collected and the filtrate evaporated down in vacuo. The residual almost black gum partially solidified on treatment with ether. This solid, after crystallisation from ether, then ethanol (charcoal), gave the alkylated amide as an orange yellow powder. It separated from ethanol in orange-yellow prisms, m.p. $113\text{-}4^0$ (Yield, 12-13 mg.).

Found: C, 59.2; H, 5.8; N, 3.2.

$C_{16}H_{19}O_6N$ requires, C, 59.8; H, 5.9; N, 4.4%.

There was not enough material for another nitrogen determination.

This compound, in ethanol solution, gave a mauve brown ferric chloride colour. It also gave a crystalline yellow precipitate when its solution (in ethanol) was treated with 2:4-dinitrophenylhydrazine in aqueous alcoholic 2N HCl.

ii). Ethyl N-formyl-4-aminobenzoate (5.02 g.) was added to a solution of sodium (0.598 g.) in ethanol (50 ml.) and the mixture was evaporated down three times with benzene in vacuo. The residual sodium salt was treated with 3-bromoacetoacetic ester (5.43 g.) in toluene (100 ml.) as in "i", but the only materials isolated from the reaction were the original formyl derivative, and succinylosuccinic ester (0.53 g.) crystallising from ethanol in pale pink prisms, m.p. 129° (157 gives 126-7°).

Found: C, 56.5; H, 6.3.

Calc. for, $C_{12}H_{16}O_6$, C, 56.3; H, 6.2%.

iii). An attempt to effect the condensation of the bromo-ester (5.18 g., 1.1 mol.) with the formyl derivative (4.35 g., 1 mol.) in the presence of sodamide (0.966 g., 1.1 mol.) in toluene (100 ml.) under the Eisleb conditions (112) failed. Only unchanged formyl derivative and succinylosuccinic ester (0.15 g.) m.p. 126°, were isolated from the reaction.

Quinoxaline-2-aldehyde.

This was prepared by the oxidation of 2-methylquinoxaline in xylene with selenium dioxide, as described by Borsche and Doller (151). It was not found possible to reproduce their yield (25%); a maximum of 12% was obtained, and the use of dioxane as a solvent (c.f. 158) caused a deterioration. The quinoxaline, which had a sickly smell, crystallised from light petroleum (60/80^0) in yellow platelets, m.p. 108^0.

N-(Quinoxaline-2-methylene)-4-aminobenzoic acid (LV, R = OH). Quinoxaline-2-aldehyde (0.46 g., 1 mol.) and p-aminobenzoic acid (0.4 g., 1 mol.) were heated to 100^0 in dioxane (5 ml.) for one hour. The reactants dissolved, and the product, a yellow solid, rapidly separated. It crystallised from dioxane in lemon yellow needles, m.p. 286-7^0 (decomp.) which reacted immediately with 2:4-dinitrophenylhydrazine in aqueous 2N HCl.

Found: C, 69.6; H, 4.2; N, 14.9;
$C_{16}H_{11}O_2N_3$ requires C, 69.3; H, 4.0; N, 15.2%.

All attempts to reduce this compound in alkaline solution to the secondary amine (LIII, R = OH) using sodium amalgam, or hydrogen over Raney nickel or palladised charcoal failed. Nothing homogeneous could be isolated from the reaction mixtures.

Ethyl N-(quinoxaline-2-methylene)-4-aminobenzoate (LV, R = OEt). Quinoxaline-2-aldehyde (1.9 g., 1 mol.) and ethyl p-aminobenzoate (2.0 g., 1 mol.) were refluxed in dioxane for 3½ hours, the solution was cooled and separation of the product (3.01 g., 82%) assisted by the addition of water. It crystallised from aqueous dioxane in very pale yellow needles, m.p. 139°.

	Found:	C, 71.0;	H, 4.8;	N, 13.6.
$C_{18}H_{15}O_2N_3$	requires	C, 70.8;	H, 4.9;	N, 13.8%.

Ethyl N-(quinoxaline-2-methyl)-4-aminobenzoate (LIII, R = OEt).

i). Ethyl N-(quinoxaline-2-methylene)-4-aminobenzoate in dioxane solution was not reduced by hydrogen in the presence of palladised charcoal; unchanged starting material was recovered.

ii). The same ester (0.527 g.) in dioxane (15 ml.) was hydrogenated over Adams catalyst (PtO_2) until the theoretical adsorption of hydrogen required to saturate one double bond had taken place. Further adsorption occurred if the reaction was left. The very dark brown solution was filtered, and evaporated to dryness in vacuo. Treatment of the residual black tar with ethanol gave the product as an almost colourless solid (0.188 g., 35%) which was washed with ethanol. It was almost insoluble in boiling ethanol, dioxane, water, and N aqueous hydrochloric acid, slightly

in aqueous β-ethoxyethanol, and easily in pyridine. It separated from the latter solvent, on cooling, in colourless prisms, m.p. 229-32° (slight decomp.).

Found: C, 70.0; H, 5.3; N, 13.3; Mol. Wt. 295.
$C_{18}H_{17}O_2N_3$ requires, C, 70.4; H, 5.5; N, 13.7%; Mol. Wt. 307.

The compound was soluble in warm aqueous hydrochloric acid (3-4N), and its solution gave no reaction with 2:4-dinitrophenylhydrazine in aqueous 2N HCl. It gave a deep blue solid with cold concentrated hydrochloric acid, and a deep purple solution in cold concentrated sulphuric acid, which gave a purple precipitate on dilution with water.

iii). The same ester (0.3 g.) in dioxane (5 ml.) was hydrogenated over Raney nickel. Adsorption ceased when 36% of the hydrogen required to saturate one double bond had been taken up, and after filtration the yellow brown solution was evaporated to dryness in vacuo. Treatment of the residual red oil with ethanol precipitated ethyl N-(quinoxaline-2-methyl)-4-aminobenzoate (0.1 g., 33%), m.p. 227-31° (decomp.). Nothing homogeneous could be isolated from the filtrate.

3-(4-Carbethoxyanilino)-2-bromoacrolein 4-carbethoxyanil (LVI, R = NC_6H_4COOEt).

Ethyl p-aminobenzoate (7.5 g., 2 mol.) and mucobromic acid (5.9 g., 1 mol.) in ethanol (40 ml.) were heated on a steam bath for 20 minutes. The orange-red precipitate (7.9 g., 62%) was collected next day, and on crystallisation from aqueous ethanol it gave the anil hydrobromide dihydrate in yellow needles, m.p. 249-50° (decomp.).

Found: C, 44.9; H, 4.5; N, 4.9.

$C_{21}H_{21}O_4N_2Br.HBr.2H_2O$ requires, C, 44.8; H, 4.6; N, 5.0%.

3-(4-Carbethoxyanilino)-2-bromoacrolein (LVI, R = O).

The above hydrobromide (9.7 g.) was boiled with water (1500 ml.) for 45 minutes. Next day the yellow solid was collected (5.1 g., 99%) and on crystallisation from ethanol it gave the aldehyde in almost colourless needles, m.p. 159-60°.

Found: C, 48.0; H, 3.8.

$C_{12}H_{12}O_3NBr$ requires, C, 48.3; H, 4.0%;

Attempts to condense this compound with o-phenylenediamine

i). in boiling ethanol,

ii) in boiling ethanol in the presence of sodium carbonate,

iii) in boiling aqueous ethanol with one or two equivalents of hydrochloric acid,

iv) in ethyleneglycol at 140°,

gave black solutions and small quantities of intractable black tars or solids.

Bromomethylglyoxal.

Bromine (8.4 ml., 1 mol.) in chloroform (50 ml.) was added to a solution of methylglyoxal (11.8 g., 1 mol.) in chloroform (100 ml.), the mixture was refluxed for one hour and large quantities of hydrogen bromide were evolved. The solvent was removed in vacuo and the residual liquid distilled with a green vapour, b.p. 116-140°/15 mm. It condensed to a yellow-green liquid which slowly thickened and became almost colourless.

Found: C, 24.2; H, 2.9;) Br., 51.7.)
C, 24.6; H, 2.8;) Br., 54.2.)

$C_3H_3O_2Br$ requires, C, 25.8; H, 2.0; Br, 53.0%.

Pteroylglutamic acid.

2:4:5-Triamino-6-hydroxypyrimidine bisulphite compound (0.95 g.) was boiled with N aqueous hydrochloric acid (a few ml.) till the evolution of sulphur dioxide ceased; the solution was filtered and added to p-aminobenzoyl-l-glutamic acid (1.2 g.) in water (150 ml.). Bromomethylglyoxal (0.68 g.) in ethanol (150 ml.) was added, over 30 minutes, with vigorous stirring at pH 3-4, and stirring was continued for a further 90 minutes. The brown precipitate (0.27 g.) was collected, washed with water and ethanol, and dried. Biological assay using S. lactis R. showed that it contained c. 5% of pteroylglutamic acid.

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