2-AMINOPHENAZINE CARBOXYLIC ACIDS

A Thesis

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for the Degree of

Doctor of Philosophy

by

D.J.H. BROCK, B.A. (Oxon)

Department of Chemistry,
University of Cape Town.

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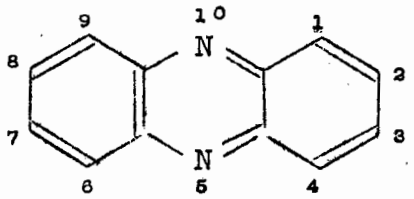
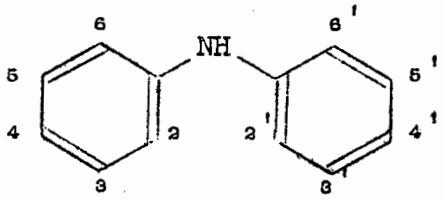
NUMBERING SYSTEMS

The numbering system for the phenazine nucleus used here is that of the Ring Index and Chemical Abstracts (but not of Beilstein).



Since the thesis is concerned largely with derivatives of 2-aminophenazine, compounds are named as such, even when this is not in accordance with I.U.P.A.C. numbering; e.g. 2-aminophenazine-9-carboxylic acid rather than 8-aminophenazine-1-carboxylic acid.

Diphenylamines are numbered as below. Since many reported here are

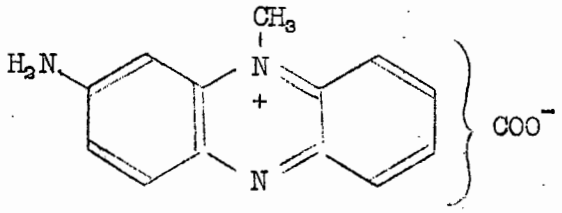


derivatives of 2,4 and 2,4'-dinitrodiphenylamine (or the diaminodiphenylamine) they are named as such to avoid confusion. This means that the compound (I)

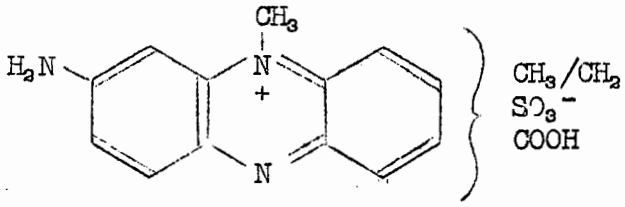
INTRODUCTION

The more common strains of the bacterium Pseudomonas aeruginosa produce a blue pigment, pyocyanine, the first compound containing the phenazine nucleus to be found in nature (1). Red strains of the organism have also been reported (2) but only superficially examined (3). When such a strain became available following a bacteriological survey in Groote Schuur Hospital (4) a programme of research was initiated, aimed at the isolation of the pigments producing the red colour and at the elucidation of their structures.

A long and involved sequence of purification allowed the eventual isolation of two crystalline red pigments (5), although there were indications that otherswere also present. These two pigments, named Aeruginosin A and B, were formed in such small quantity that information on their structures had to be gained almost entirely by physical methods. Infra-red and ultraviolet spectra, electrophoretic behaviour and elemental analysis, together with a micropyrolysis of A and examination of the products by paper chromatography, confirmed that the pigments were also based on the phenazine nucleus. Aeriginosin A appeared to be the N-methyl betaine of a 2-aminophenazine carboxylic acid (I) while B was further complicated by the presence of sulphonic and methyl or methylene groups (II). The evidence for these conclusions has been recently summarised (6).



(I)

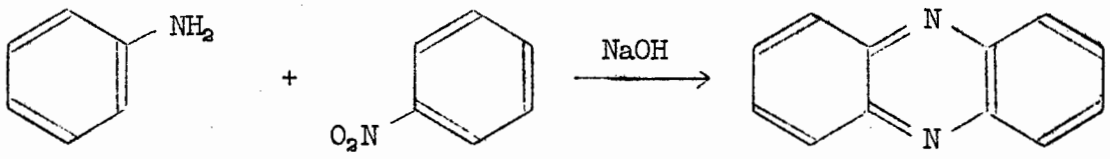


(II)

Further work was confined to A, which has the simpler structure and is more easily purified. The scant attention which has been paid to the infra-red spectra of substituted phenazines and the insolubility of the pigments in any suitable solvents, prevented use of characteristic substitution patterns for the location of the carboxyl-group. The small quantities of material available and the high stability of the symmetrical phenazine nucleus debarred a degradative investigation. Accordingly, the synthesis of the seven possible 2-aminophenazine carboxylic acids, none of which was known, was undertaken for the purpose of comparison with the alkaline-degradation product of Aeruginosin A.

In a project of this nature a general synthetic procedure which would apply to the whole series and which would give compounds of known orientation, is highly desirable. Although a large number of methods of preparing substituted phenazines have been reported (7), only three of these may be regarded as being in any way general. Each of the three is briefly discussed and reasons given for the lack of suitability for the work planned.

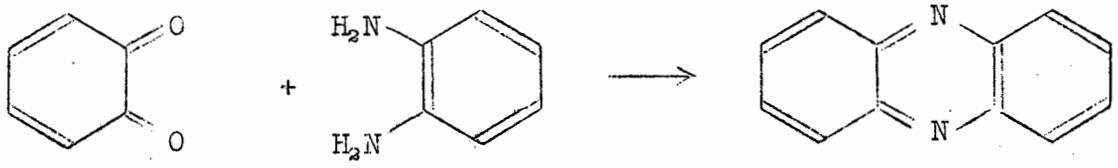
(1) In the past five years the most widely used method of phenazine synthesis has been by the Wohl-Aue reaction (8), where an aromatic amine and an aromatic nitro-compound are fused in the presence of powdered sodium hydroxide. Although modifications such as the use of inert solvents (9, 10)



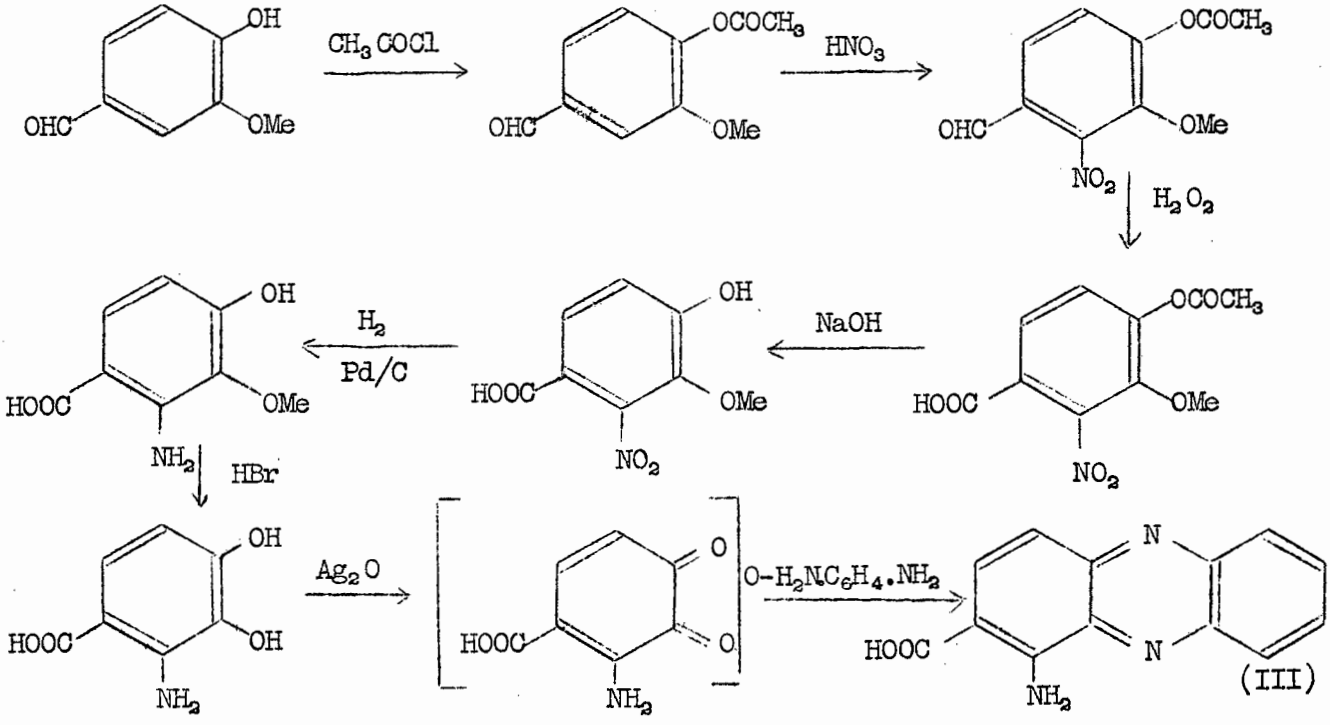
and the replacement of sodium hydroxide by sodamide (11) have refined the technique, yields are seldom greater than 10%, and column chromatography must frequently be used to purify the product (12, 13). The easy availability of starting materials is the sole advantage of the method. Only one aminophenazine has been made this way, and a modified procedure was necessary since nitroanilines are not stable to alkali (14). This, and the probable decarboxylations that would occur under the harsh, alkaline fusion-conditions made it an unsatisfactory method for the preparation of aminophenazine carboxylic acids. Furthermore, only in a limited number of cases would the orientation of the product be unambiguous.

(2) The condensation of an aromatic ortho-diamine with either a catechol (15), a cyclohexanedione (16) or an ortho-quinone (17) (with subsequent oxidation or dehydrogenation where necessary) affords a fairly direct route to the phenazine derivative (e.g. below). The chief

disadvantage of this method is that the orientation of the product can only

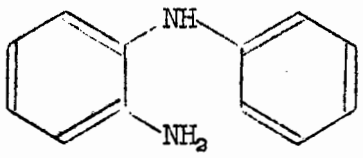


be guaranteed if both amino and carboxyl-groups are on the same ring. The difficulties of preparing a disubstituted o-quinone, catechol or cyclohexanedione are paralleled by the difficulties of preparing a disubstituted o-phenylenediamine. These are exemplified in the involved reaction sequence (see below) by which 1-aminophenazine-2-carboxylic acid (III) was made (18). This is the only aminophenazine carboxylic acid to have been

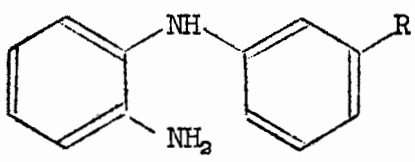


synthesised, apart from those reported in this thesis.

(3) In many respects the most promising method of phenazine synthesis is through the oxidative ring-closure of o-aminodiphenylamines (IV). It has the advantage that the orientation of the product is known (as long as only a single o-amino-group is present), except where cyclisation takes place on to a 3'-substituted ring (V). A large variety of oxidising



(IV)



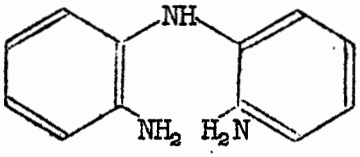
(V)

agents have been used, their success often depending on the nature of other substituents present.

With simple o-aminodiphenylamines, usually obtained without isolation from the corresponding o-nitrodiphenylamine (19), ring closure is difficult. It has been achieved by use of lead oxide (20), manganese dioxide in ammonia (21), and also by passing the vapours of the aminodiphenylamine over red hot lead oxide (22), but none of these methods is general, nor are they particularly suited to the phenazines here required. Waterman and Vivian have considerably simplified the procedure by treating o-nitrodiphenylamines with a reagent which abstracts oxygen without supplying hydrogen, thus shortening the usual reduction and oxidation to a single reduction (23). While their method is fairly general, it fails when

additional nitro-groups are present (24) and has not been used, probably due to the severity of the experimental conditions, to prepare phenazine carboxylic acids.

When an amino-group is present in the 2'-position of the o-aminodiphenylamine (VI), treatment with ferric chloride eliminates ammonia to give the phenazine (25). Although this oxidising agent does not appear to be successful except when both 2- and 2'-substituents are present, 2-amino-7-cyanophenazine and 2-amino-8-cyanophenazine have been obtained in quantitative yield in these laboratories by the ferric chloride oxidation of the appropriate diphenylamines (VII and VIII), neither of which



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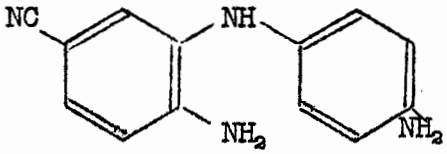

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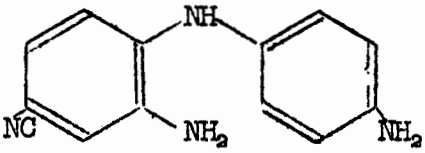
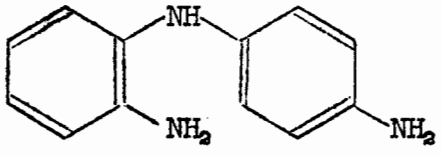


(VIII)

has a 2'-amino group. When applied to aminodiphenylamines bearing a carboxylic group, ring closure appeared to take place but the product was obtained in a unmanageable state. This is possibly due to complexing with ferric ions (26).

The complete absence of any satisfactory general method of synthesising a series of substituted phenazines stimulated a search for a new method. Attention was focussed on o-aminodiphenylamines which alone can give products of unambiguous orientation, and several different oxidising

agents were examined. Nitrobenzene as combined solvent and oxidant was an early success, and appeared to work well with both 2,4'-diaminodiphenylamines (IX) and 2-amino-4'-methoxydiphenylamines (X) (27, 28). However, the pattern of its behaviour was not at all clear, and a programme of work was initiated aimed at exploring the potentialities of the method and formulating a mechanism for the reaction (29).



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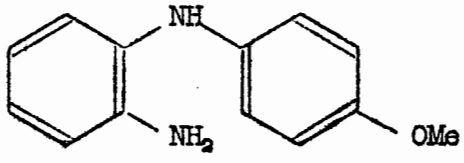
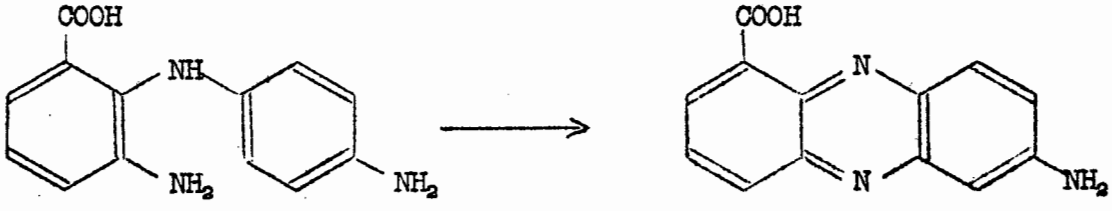
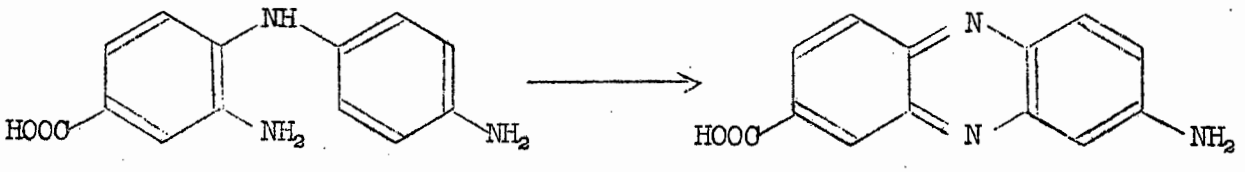


(X)

At this stage, the first two of the seven isomeric 2-aminophenazine carboxylic acids were synthesised, 2-aminophenazine-6-carboxylic acid (XII) from 2,4'-diaminodiphenylamine-6-carboxylic acid (XI), and 2-aminophenazine-8-carboxylic acid (XIV) from 2,4'-diaminodiphenylamine-4-carboxylic acid (XIII), both by the nitrobenzene oxidation method. By inspired guesswork



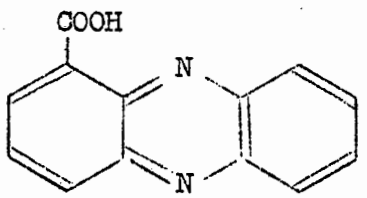
(XI) (XII)



(XIII) (XIV)

the correct acid was made first, and 2-aminophenazine-6-carboxylic acid proved identical to the alkaline degradation product of Aeruginosin A (6).

This did not, however, remove the incentive for completing the isomeric series. Although all ten phenazinediols have been prepared (in connection with the structure of another bacterial pigment, iodinin) (30) no complete series of disubstituted phenazines is known where the substituents are different. Such a series had every prospect of affording interesting correlations between the position of substitution and the properties of the compound. In this respect ultraviolet and infra-red data might well give information clarifying the position of substituents in Aeruginosin B. It also seemed likely that a chemical degradation of this pigment would lead to an aminophenazine carboxylic acid. Furthermore, in biogenetic studies on Aeruginosin A, an attempt was being made to establish that the final step in the biosynthetic sequence was the ammonolysis of phenazine-1-carboxylic acid (XV) or its N-methyl betaine (XVI) (31). If this were not the case, another aminophenazine acid might arise in this reaction, and the



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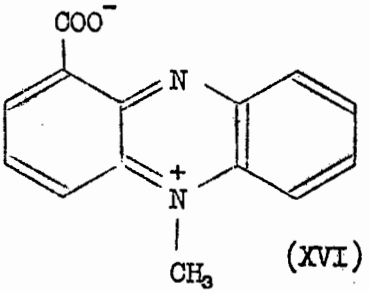
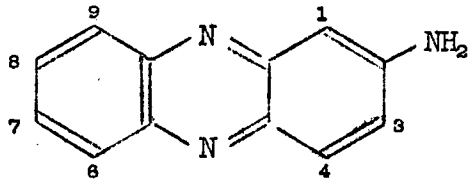


(XVI)

complete series would be of value in identifying this. And finally the preparation of a complete series of acids with the carboxyl-group in widely differing positions, all from ortho-aminodiphenylamines, would be a severe test of the newly developed nitrobenzene oxidation method.

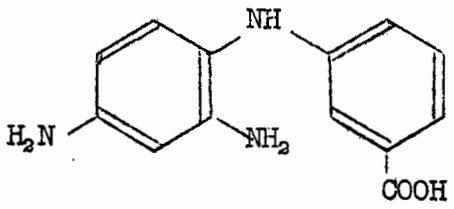
At the time that the work described in this thesis was begun the 2-aminophenazine-4,6 and 8-carboxylic acids (XVII) had been synthesised and characterised as their methyl esters. No attempt had yet been made



(XVII)

to prepare the 2,1 or 2,9-substituted acids, and experiments on the 2,3-acid had broken down at an early stage. Although 2-amino-7-cyanophenazine had been made the corresponding acid had not been directly prepared, nor had it been obtained by hydrolysis of the nitrile. Attention was therefore concentrated on the direct synthesis of these four acids.

Any disubstituted phenazine may be obtained theoretically from four different o-aminodiphenylamines. This is illustrated by the example of 2-aminophenazine-9-carboxylic acid, which may arise from any of the diphenylamines (XVIII to XXI). Because of the success of the nitrobenzene oxidation of 2,4'-diaminodiphenylamines in the preparation of



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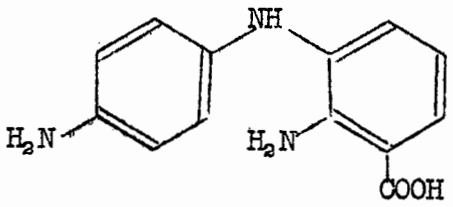

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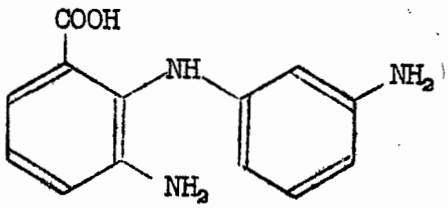

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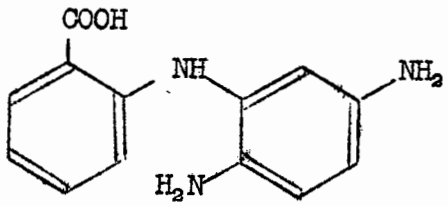


(XXI)

2-aminophenazines (28), attempts were made at first to synthesise the phenazine acids from this type of diphenylamine (e.g. XIX). However, severe difficulties were encountered in the preparation of 2,4'-dinitrodiphenylamines, only one of which had been previously made (32). Concurrent work on the oxidation method then established the suitability of 2,4-diaminodiphenylamines as intermediates (29), and since 2,4-dinitrodiphenylamines are easily prepared this line of approach was thereafter adopted (e.g. XVIII).

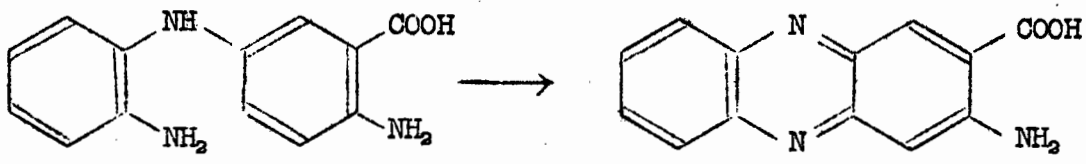
The discussion that follows is divided into two sections. Section I describes synthetic work completing the isomeric series of 2-aminophenazine carboxylic acids. It also includes a study on the relative rates of

formation of certain of the phenazines. Section II is an examination of selected physical and chemical properties of the series.

SECTION I PART I

THE SYNTHESIS OF 2-AMINOPHENAZINE-3-CARBOXYLIC ACID

For reasons given in the introduction, the most promising route to 2-aminophenazine-3-carboxylic acid (II) appeared in the earlier stages of this work to lie through the oxidation of 2,4'-diaminodiphenylamine-3'-carboxylic acid (I). This had the attraction of being theoretically capable of furnishing not only 2-aminophenazine-3-carboxylic acid (II), but also 2-aminophenazine-1-carboxylic acid (III), both of which were required to complete the series. The ratio of the two isomers formed would be an additional interesting feature of the reaction.



(I) (II)

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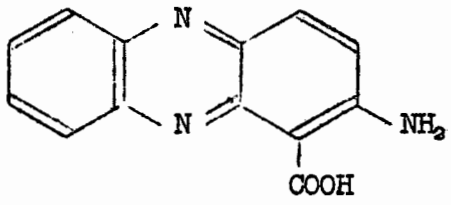
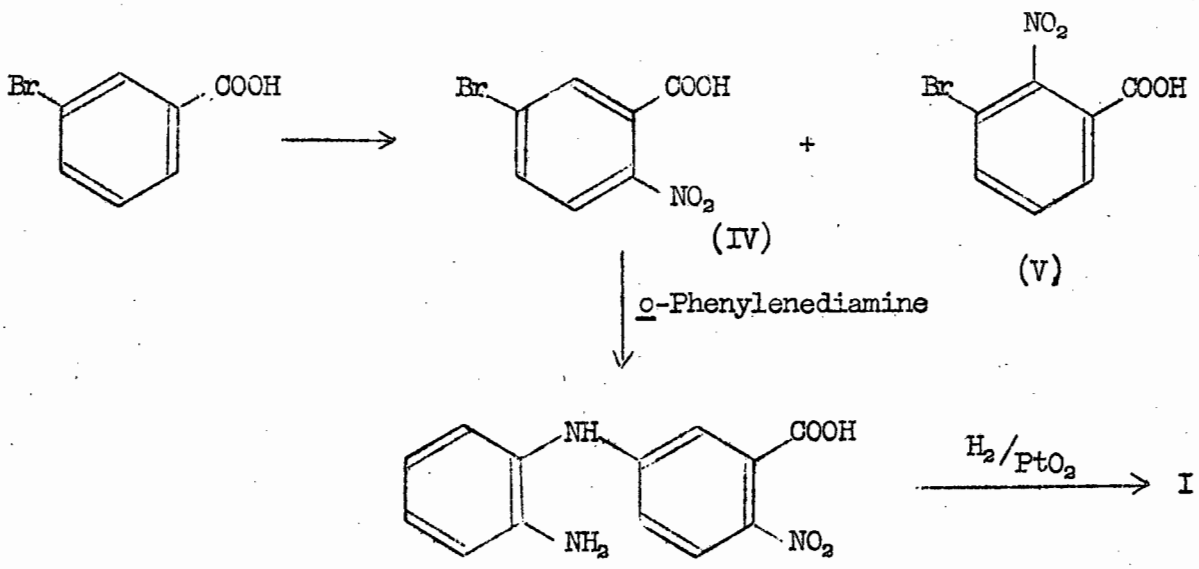


(III)

The proposed route to the diphenylamine is outlined below. Nitration of m-bromobenzoic acid under mild conditions leads to a mixture of 5-bromo-

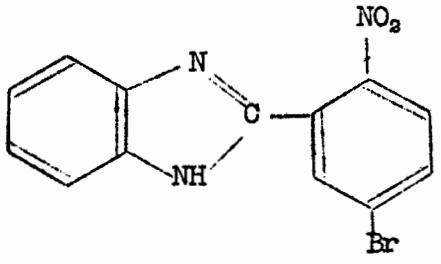


2-nitrobenzoic acid (IV) and 3-bromo-2-nitrobenzoic acid (V), with the former predominating in a ratio of 19 : 1 (33, 34). Separation of the isomers by fractional crystallisation from benzene (34) was found to be very much less satisfactory than an alternative method of Hollemann (35), using successively fractional crystallisation of the acids from aqueous alcohol and their sodium salts from water. The minor constituent (V), though isolated in very low yield, was required for a subsequent synthesis (Section I Part II).

It was hoped that the halogen atom of 5-bromo-2-nitrobenzoic acid (IV) would be rendered sufficiently labile by the para-substituted nitro-group to condense under mild conditions with o-phenylenediamine. Attempts to achieve this by an Ullmann reaction in a hydroxylic solvent led only to what appeared to be a molecular complex between the acid and the base. A recent review of molecular complexes (36) shows that a very large number

of these incorporate aromatic nitro-compounds as 'acceptors' and aromatic amines as 'donors', although there is no recorded case of o-phenylenediamine acting as a 'donor'. Where acids and bases are involved, a marked deepening in colour in the formation of the molecular complex has been used to distinguish this from a simple salt (37, 38, 39). The bright yellow colour of the addition product formed from the almost colourless constituents o-phenylenediamine and the bromonitro acid thus suggest that this is a true molecular complex.

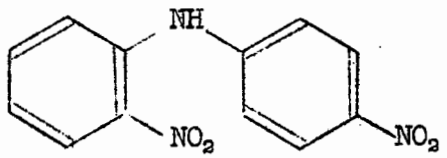
Since the complex was formed when the reaction was carried out in a hydroxylic solvent, it was felt that a direct fusion of the components might be successful in giving the diphenylamine. In this instance, however, fusion conditions could lead to the formation of a benziminazole (VI), these being prepared from o-diamines and carboxylic acids, usually in the presence of mineral acid (40) but often without (41). To prevent this, the bromonitro acid was esterified, and attempts made under a variety of



(VI)

conditions to condense the ester and the diamine. None of these were successful. Replacement of o-phenylenediamine by the very much weaker base o-nitroaniline was likewise unsuccessful, the product of reaction

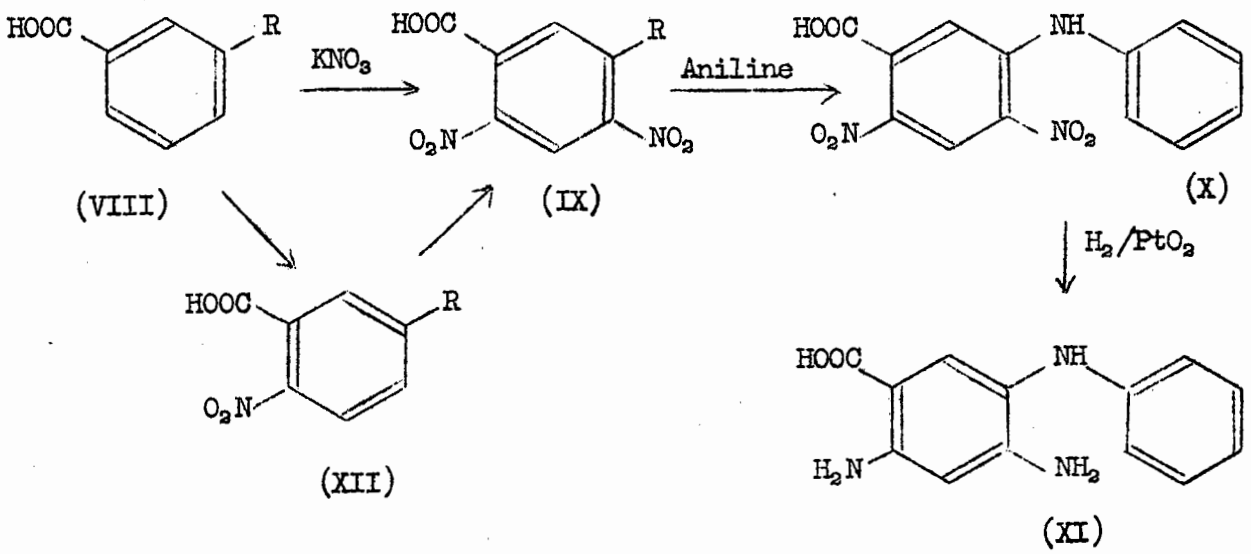
with the bromonitro acid (IV) being the decarboxylated material, 2,4-dinitrodiphenylamine (VII) and no reaction taking place with the bromonitro



(VII)

ester. A single attempt to condense the acid (IV) with o-aminoacetanilide also failed.

The difficulty of preparing 2,4'-diaminodiphenylamine-3'-carboxylic acid (I) directed attention to the alternative 2,4-diaminodiphenylamine (XI). The parent dinitro form of this (X) had already been made by Goldstein and Stamm (42) by the comparatively simple method outlined below (VIII - X). However, a preliminary investigation of this route



to the phenazine in these laboratories had been foiled by the inability to repeat the dinitration of m-chlorobenzoic acid, or to adapt it to m-bromobenzoic acid (43). Small quantities of the dinitro acid (IX, R = Cl) had been made by the nitration of 5-chloro-2-nitrobenzoic acid (XII, R = Cl) (the preparation of which in itself involves isomer separation) and the diphenylamine (X) made therefrom. A small scale reduction and cyclisation of this gave a compound, which on paper-chromatographic examination appeared to be the decarboxylation-product, 2-aminophenazine. The work, however, was not completed.

Since m-bromobenzoic acid was plentifully available* it was decided to concentrate on adapting the dinitration to this rather than to the chloro-compound. By using conditions based on those developed by Meisenheimer et al (44) for the dinitration of o-bromobenzoic acid this was achieved, though at all times a precarious balance was maintained between incomplete nitration and the reaction becoming violently and destructively exothermic. The product, 5-bromo-2,4-dinitrobenzoic acid (IX, R = Br) had the same melting point as that made by Goldstein and Stamm from the chloro-compound (IX, R = Cl) by ammonolysis, diazotisation and replacement of the diazo group by bromine. It furnished the same methyl ester and diphenylamine. It could also be made by the nitration of 5-bromo-2-nitrobenzoic acid (XII, R = Br).

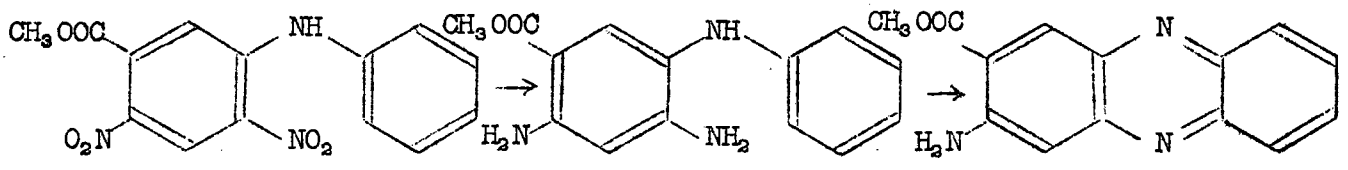
Reduction and cyclisation of the diphenylamine (X) obtained in this

---

* Student preparation.

way, confirmed that decarboxylation was taking place and that the product was indeed 2-aminophenazine. The probability that it was the diaminodiphenylamine (XI), a derivative of the easily decarboxylated 2,4-diaminobenzoic acid (45), rather than the aminophenazine (II) that was being decarboxylated, was confirmed in later studies. In subsequent runs the crude product from the nitration of m-bromobenzoic acid was esterified and then condensed with aniline. As only the halogen-group activated by both ortho and para nitro-groups (IX) was sufficiently labile to react under the conditions used, mononitro acids and unchanged m-bromobenzoic acid present as impurities in the reaction product from the nitration were effectively eliminated. In this way a pure sample of the diphenylamine ester was prepared in good yield.

Catalytic reduction of the dinitrodiphenylamine ester (XIII) gave methyl 2,4-diaminodiphenylamine-5-carboxylate (XIV) which was unusually stable, and could be isolated and purified. Refluxing this in nitrobenzene alone for 60 hours, or in nitrobenzene containing 5% palladium-charcoal for 30 hours gave methyl 2-aminophenazine-3-carboxylate (XV). (For a discussion of times of reflux see Section I Part IV). This was



(XIII) (XIV) (XV)

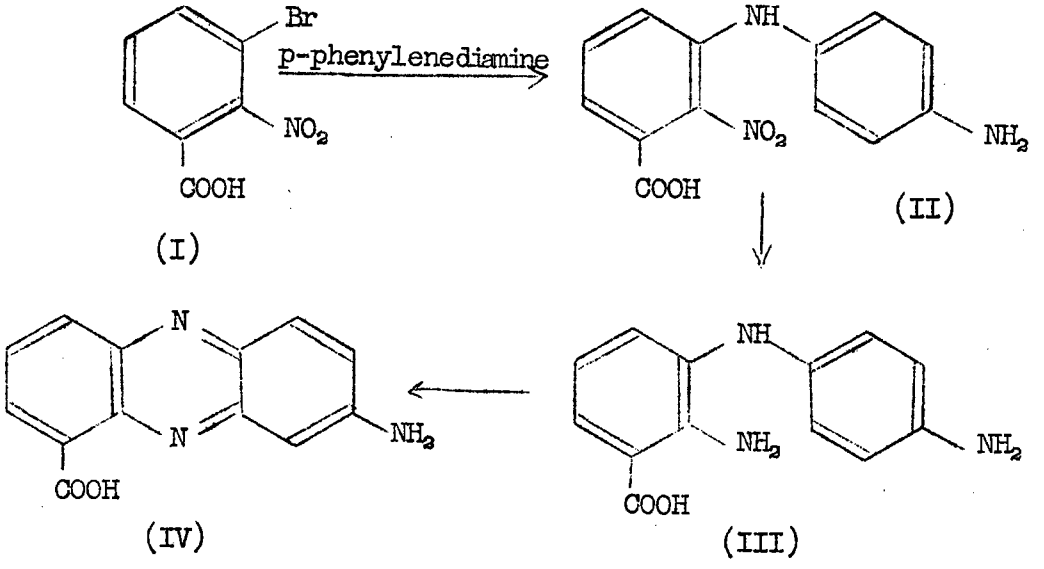
purified by adsorption chromatography on alumina. From the column trace quantities of 2-aminophenazine and larger quantities of 2-aminophenazine-3-carboxylic acid were also isolated. The latter arises partly from hydrolysis of the ester caused by the alkali of the alumina column. However, a careful estimate of the extent of this hydrolysis showed that a constant percentage of acid was produced for a given weight of ester chromatographed, and independent of the size and volume of the alumina column. The amount of acid thus produced was insufficient to account for that found in the reaction product. Furthermore, the presence of 2-aminophenazine indicates that some acid must be present in the refluxing nitrobenzene, since decarbomethoxylation is an unknown phenomenon. The nature of the hydrolysing agent remains puzzling, though superheated water formed during the oxidative ring closure and present in the refluxing medium could be responsible.

As is apparent from the preceding paragraph, the ester was easily hydrolysed under standard alkaline conditions to give 2-aminophenazine-3-carboxylic acid. This was obtained as an amorphous solid, resisting all attempts to render it crystalline.

SECTION I PART II

THE SYNTHESIS OF 2-AMINOPHENAZINE-9 AND 7-CARBOXYLIC ACIDS

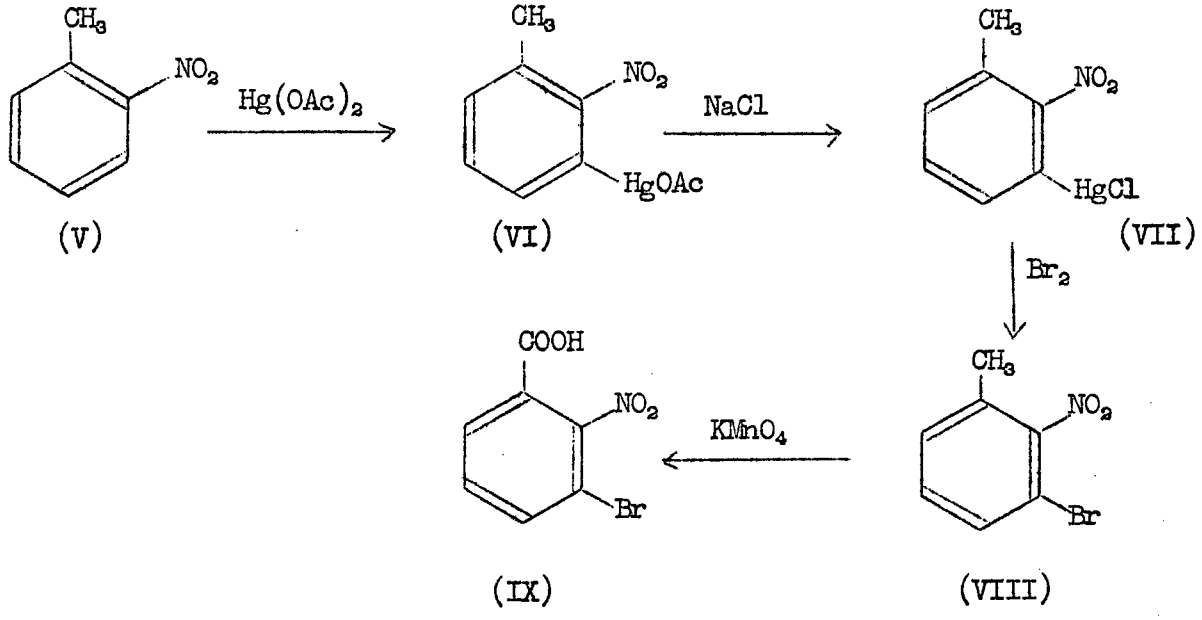
Once again, it was decided to make the initial approach to the synthesis of 2-aminophenazine-9-carboxylic acid (IV) through the intermediacy of a 2,4'-diaminodiphenylamine, i.e. (III). The proposed preparation of this involved the condensation of 3-bromo-2-nitrobenzoic acid (I) and p-phenylenediamine, with reduction of the ensuing aminonitrodiphenylamine (II).



Three different methods are reported in the literature for the preparation of the bromonitro acid (I). The first of these has already been mentioned; it is formed as a side-product in the mononitration of m-bromobenzoic acid (33, 34). When this was repeated, only 2.5 g. were

obtained for every 100 g. of m-bromobenzoic acid nitrated, and repeated recrystallisations were required to achieve purity. It was clearly not a suitable method for the production of the fairly large quantities needed for working out the right conditions for a diphenylamine condensation.

The second method employs the mercuration of o-nitrotoluene, replacement of the mercury group by bromine, and oxidation to the bromonitro acid (V → IX) (46). Some confusion exists, however, over the exact positions of

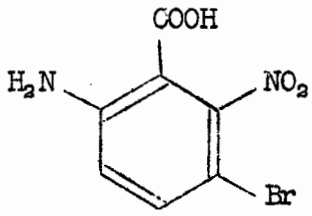


(V) (VI) (VII) (VIII) (IX)

entry of the acetoxymercuri group into o-nitrotoluene. Burton et al (46) report that the main product is the 4-substituted o-nitrotoluene, with the 3-isomer a side-product present in fair yield. Coffey (47), using identical experimental conditions, confirms that the 4-isomer is the major constituent, but claims that a relatively large amount of 6-acetoxymercuri-2-nitrotoluene was isolated, together with only small quantities of a third isomer. When

this work was repeated, oxidation of the crude mixture of bromonitrotoluenes and separation of the resulting acids, gave a very small yield of 3-bromo-2-nitrobenzoic acid. It did not seem worthwhile exploring the method further.

A third, more recent preparation of the bromonitro acid incorporates the deamination of 5-bromo-6-nitroanthranilic acid (X) (48). Although the preparation of the anthranilic acid (described in Section I Part III)



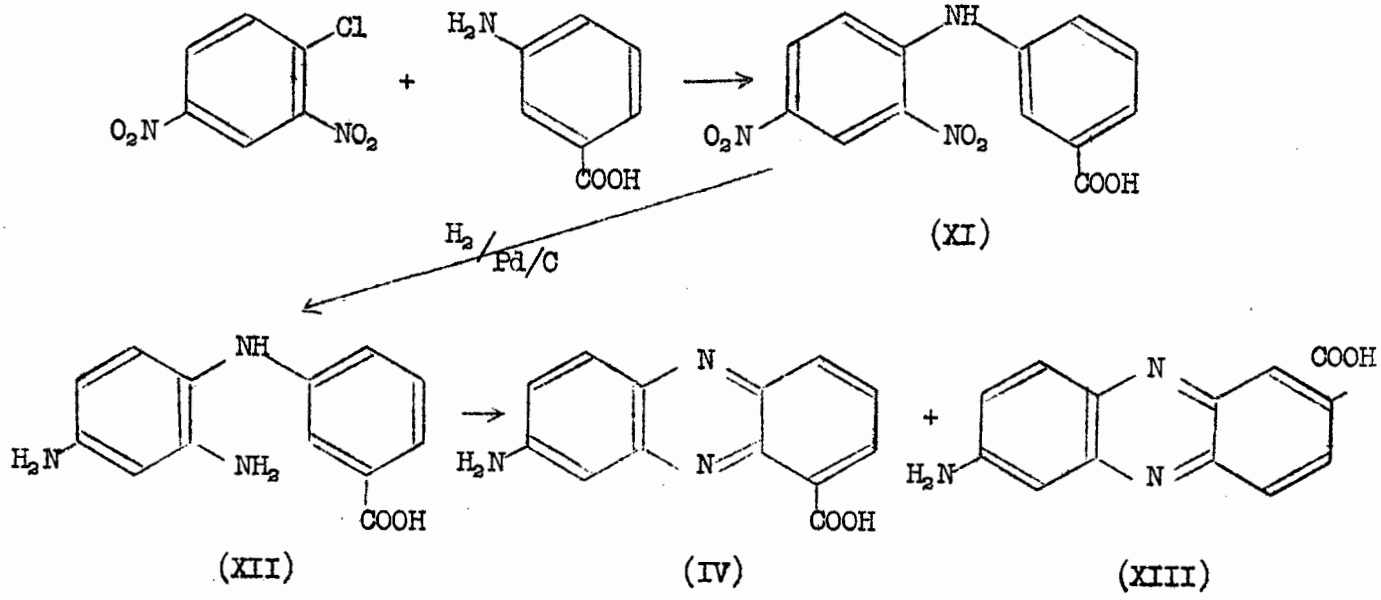
(X)

is a long one, this method of obtaining the bromonitro acid is attractive since it is the only one of the three to avoid isomer separation. However, numerous attempts to repeat the deamination in these laboratories had previously failed (49), and since the anthranilic acid was needed for another synthesis, the method was abandoned after a single unsuccessful endeavour.

Using the small quantities of 3-bromo-2-nitrobenzoic acid (I) obtained by the nitration of m-bromobenzoic acid, a preliminary investigation of the condensation with p-phenylenediamine and p-aminoacetanilide was carried out. The few attempts made were not successful, and in view of the difficulty of obtaining further batches of the acid, an alternative route to the

phenazine was sought.

Once again attention was directed to a suitable 2,4-diaminodiphenylamine. An attractive choice was 2,4-diaminodiphenylamine-3'-carboxylic acid (XII), which had already been made by condensation of commercially available 2,4-dinitrochlorobenzene and m-aminobenzoic acid, followed by reduction (50). Cyclisation of this could give rise to both the 2-aminophenazine-9-carboxylic acid (IV) and 2-aminophenazine-7-carboxylic acid (XIII), depending on the site of ring closure. Neither of these had been previously made.

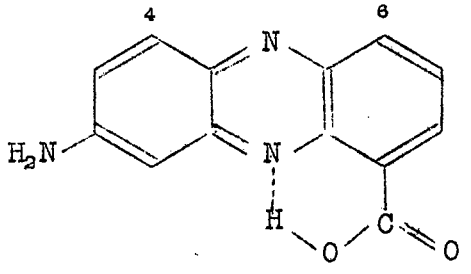


2,4-Dinitrodiphenylamine-3'-carboxylic acid (XI), made by a modification of the method of Linke (50), was hydrogenated over palladium-charcoal (the diamine being characterised as its diacetyl derivative) and refluxed in nitrobenzene. A paper-chromatographic examination of

the product indicated that both acids were present. Separation of the two, which have very similar physical properties and travel in paper-chromatographic solvents at almost identical rates, at first proved extremely difficult. Fractional crystallisation from water showed that one of the acids (later identified as 2,7-substituted) was more soluble than the other, but the sparing solubility of both in this solvent led to very incomplete separation. In nitrobenzene, the other acid (later identified as 2,9-substituted) was the more soluble, but again separation was imperfect. Partition chromatography on cellulose was totally unsuccessful, while adsorption chromatography on both normal and acid-washed, de-activated alumina allowed only small quantities of the more strongly adsorbed acid (the 2,7-substituted) to be collected in a pure state. Furthermore, the extremely low solubility of both acids in neutral organic solvents rendered chromatographic separation methods useless on all but a microscale.

Since organic acids do not lend themselves easily to adsorption chromatography (51), it was decided to esterify the acids and investigate the separation of their esters. Esterification in the presence of acid catalyst gave, however, a surprising result; 2-aminophenazine-7-carboxylic acid (XIII) was converted quantitatively to its methyl ester, while its isomer remained completely unchanged. This at once revealed a simple and elegant method of separating the two acids. It might be argued that the failure to esterify the 2,9-substituted acid (IV) is not completely unexpected, since it contains a carboxyl-group adjacent to one of the nitrogens of the ring, and capable of forming a hydrogen bond which could

impede the process of esterification. However, both the 2-aminophenazine-4 and 6-carboxylic acids contain similarly sited carboxyl-functions, and yet



(IV)

they may be esterified rapidly and quantitatively under identical conditions (49). A possible explanation for this phenomenon is discussed in Section II.

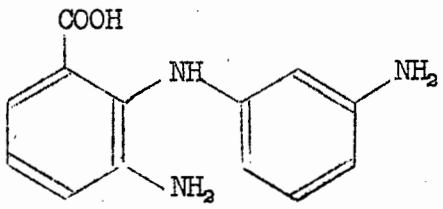
Separation and purification of the two acids was therefore carried out by refluxing the crude acid mixture in methanol/sulphuric acid, distilling the solution to small volume, making alkaline with ammonia and filtering. Slow evaporation of the ammoniacal filtrate released 2-aminophenazine-9-carboxylic acid as a finely crystalline material, representing 19% of the theoretical yield. Purification of the precipitated 2,7-substituted ester proved more difficult and was eventually achieved with some loss by chromatography on two successive alumina columns. In order to estimate the quantity of 2,7-substituted acid formed in the cyclisation, a weighed amount of crude ester was purified by paper chromatography and determined spectrophotometrically. This indicated that the 2,7-substituted acid had been formed in the ring-closing step in an amount representing 22% of the theoretical yield.

Hydrolysis of the ester under standard alkaline conditions proved easy,

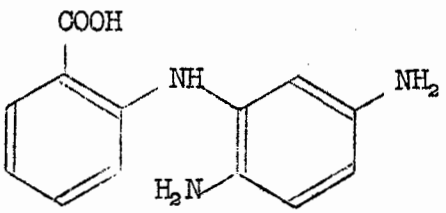
giving 2-aminophenazine-7-carboxylic acid as an amorphous solid, which like the 2,3-substituted acid, could not be rendered crystalline by any technique tried. Characterisation of 2-aminophenazine-9-carboxylic acid as its methyl ester was more difficult. Thionyl chloride could not be used to make the acid chloride, since it reacts with aromatic amines (52), and has also been reported to halogenate heterocyclic nuclei (53, 54). The almost complete insolubility of the acid in the normal organic solvents associated with the use of diazomethane hindered use of this versatile esterifying agent, though the method was eventually successful when dimethylformamide was employed as solvent.

At this juncture the orientation of the carboxyl-group in the two acids formed from 2,4-diaminodiphenylamine-3'-carboxylic acid (XII) had not been rigorously established. It seemed very probable that the acid which moved faster on paper and column chromatography, was less soluble in the polar solvents water and ethanol, was lower melting, and could not be esterified by the Fischer-Speier procedure was 2-aminophenazine-9-carboxylic acid, since these properties are all consistent with the existence of intramolecular hydrogen bonds (55, 56). In addition, the other acid could not be rendered crystalline by any technique tried, and such behaviour could be construed as following from the presence of intermolecularly hydrogen-bonded chains, preventing the packing of molecules into a crystal lattice. However, such pieces of evidence were purely circumstantial in nature, and what was needed was an absolute proof from the synthesis of one or both the acids through a different diphenylamine.

Of the remaining two o-aminodiphenylamines (XIV, XV) which had so far not been considered as intermediates in the synthesis of 2-aminophenazine-9-carboxylic acid, 6,3'-diaminodiphenylamine-2-carboxylic acid (XIV) was

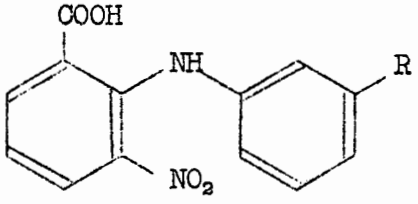


(XIV)

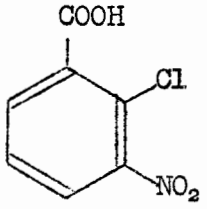


(XV)

the more attractive choice. Both the dinitro (XVI, R = NO_2) and aminonitro (XVI, R = NH_2) forms had already been made by the condensation of 2-chloro-3-nitrobenzoic acid (XVII) with the appropriately substituted aniline (57).

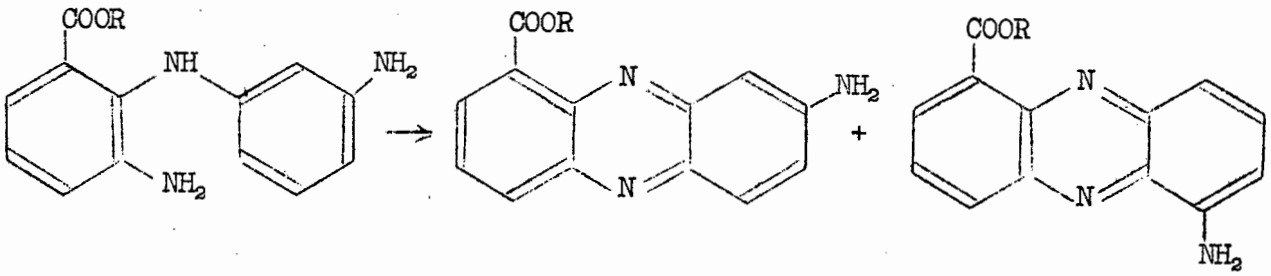


(XVI)



(XVII)

Oxidative cyclisation of the diamine (XVIII, R = H) could theoretically lead to both 2-aminophenazine-9-carboxylic acid (XIX, R = H) and 1-aminophenazine-6-carboxylic acid (XX, R = H). If both were formed, it was hoped to separate them by the differences in chromatographic adsorption



(XVIII) (XIX) (XX)

of 1 and 2-aminophenazines.

The preparation of the aminonitrodiphenylamine (XVI, R = NH_2) was achieved by the condensation of m-phenylenediamine and 2-bromo-3-nitrobenzoic acid, the bromo acid being more easily made than its chloro analog (XVII). Goldberg and Kelly (57) report that this diphenylamine melts over a wide range and cannot be purified, though it yields a pure acetyl derivative. The same behaviour was observed by the author, and though a pure crystalline hydrochloride could be made, liberation of the free base gave again a diffusely melting compound which could not be reduced catalytically. Preparation of the dinitrodiphenylamine (XVI, R = NO_2) furnished a seemingly-pure product which melted some 4° to 5° below the reported melting point, and which could not be purified by recrystallisation or column chromatography to the value given. Attempts to hydrogenate this over platinum oxide or palladium-charcoal led to the catalyst becoming poisoned and reduction remaining far from complete.

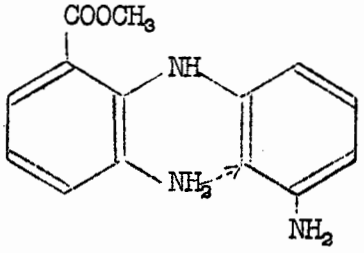
Difficulties in the preparation of the diphenylamine acid suggested use of the methyl ester, since if need be this could be purified by column

chromatography. The ester was made in two ways, by a direct condensation of methyl 2-bromo-3-nitrobenzoate and m-nitroaniline, and by esterification of the diphenylamine acid (XVI, R = NO_2) with diazomethane. The latter method furnished a product of greater purity, which on catalytic hydrogenation in absolute alcohol gave a colourless solution indicative of complete reduction. The unstable diamine was not isolated, but characterised as its diacetyl derivative.

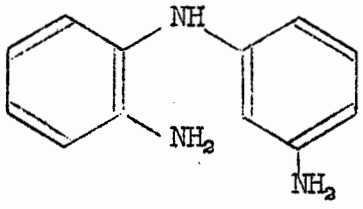
Refluxing the diaminodiphenylamine ester (XVIII, R = CH_3) in nitrobenzene for 30 hours was followed by chromatography of the product on alumina. From the column, both the expected products were recovered, methyl 2-aminophenazine-9-carboxylate (XIX, R = CH_3) as the major component in 28% yield, and methyl 1-aminophenazine-6-carboxylate (XX, R = CH_3) in only 2.5% yield. The 2-amino-9-substituted ester had identical melting-point and mixed melting point, infra-red spectrum and paper-chromatographic behaviour to the ester obtained from 2,4-diaminodiphenylamine-3'-carboxylic acid (XII), by cyclisation and subsequent esterification, and thus established the structure of this compound beyond any doubt. Since the other product was the first carboxyl-substituted 1-aminophenazine to be made, its structure was confirmed by hydrolysis of the ester, and decarboxylation of the ensuing acid to 1-aminophenazine. (A paper appeared subsequent to this work (18) describing the synthesis of another carboxyl-substituted 1-aminophenazine, 1-aminophenazine-2-carboxylic acid).

The preponderance of methyl 2-aminophenazine-9-carboxylate in this reaction is both interesting and somewhat unexpected. On purely steric

grounds it is the anticipated isomer, since the 3'-amino-group might be expected to impede to some extent the approach of the 2-amino-group to an ortho-position (see XXI) though not to a para-position. However, in

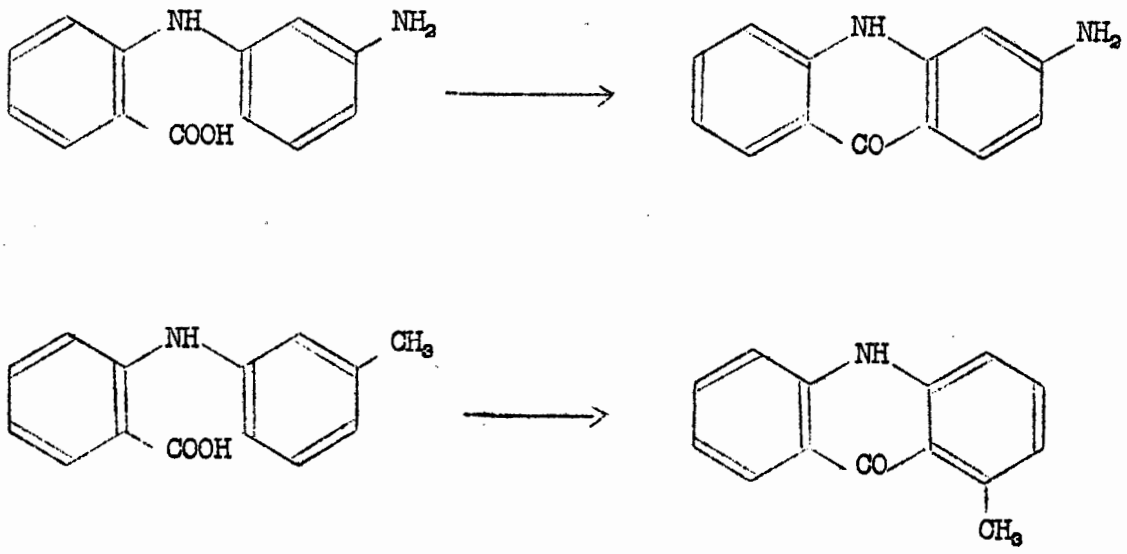


(XXI)



(XXII)

the closely analogous case of 2,3'-diaminodiphenylamine (XXII), Gaertner found that nitrobenzene cyclisation favoured ortho-ring-closure, and 1-aminophenazine outweighed 2-aminophenazine by a factor of 2 : 1 (58). How a carboxyl-group sited in the 6-position can so radically alter this ratio is difficult to understand. Suffice it to mention that ring closures on to 3'-substituted rings have been studied in other heterocyclic systems, and equally inexplicable results have been obtained. Thus, in the cyclisation of 3'-substituted diphenylamine-2-carboxylic acids to give acridones, predominantly para-ring-closure is obtained when the substituent is an amino-group (59) and ortho-ring-closure when it is a methyl-group (60, 61).

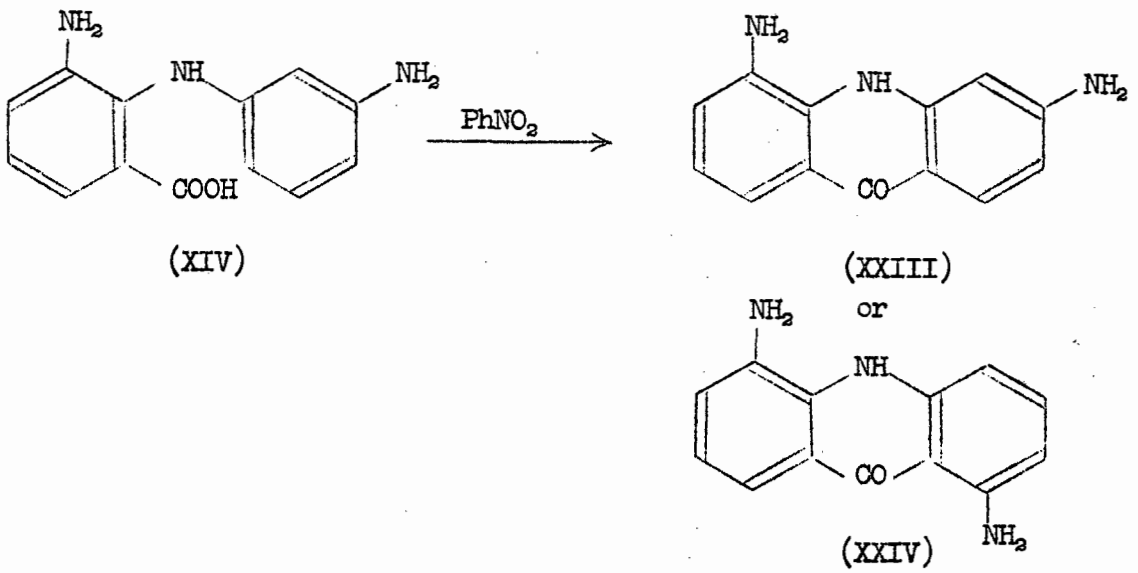


Both of these are electron-donating substituents. No explanation has been advanced to account for this.

Since methyl 6,3'-diaminodiphenylamine-2-carboxylate (XXI) gave a preponderance of the 2-aminophenazine, it was of some interest to discover whether cyclisation of the diphenylamine acid would give the same ratio of isomers. Unfortunately the ester could not be hydrolysed; refluxing in 5 N hydrochloric acid for several hours left it unchanged, dissolving in cold 100% sulphuric acid and pouring into water again left it unchanged, while refluxing in aqueous alkali gave a brown, resinified product under all conditions tried. It was decided therefore to work on a qualitative basis using the crude 6,3'-dinitrodiphenylamine-2-carboxylic acid (XVI, R = NO_2) made in the Ullmann condensation, and attempting to estimate the ratio of isomers by paper-chromatographic examination.

The crude dinitrodiphenylamine acid was hydrogenated as far as possible

over palladium-charcoal, and without isolation refluxed in nitrobenzene. Removal of the solvent and examination of the residue in different paper-chromatographic systems indicated the presence of two major components. One of these was the expected 2-aminophenazine-9-carboxylic acid. The other, a yellow material, remaining yellow in 5 N hydrochloric acid, was clearly not 1-aminophenazine-6-carboxylic acid, which has a deep blue-green hydrochloride. Its intense green fluorescence under the ultraviolet lamp suggested a substituted acridone, these being among the most fluorescent substances known (62). If the formation of acridones follows the same pattern in boiling nitrobenzene as it does in concentrated sulphuric acid, then the most likely compound was 1,8-diaminoacridone (XXIII) and not the isomeric 1,6-diaminoacridone (XXIV). Both of these were synthesised by the

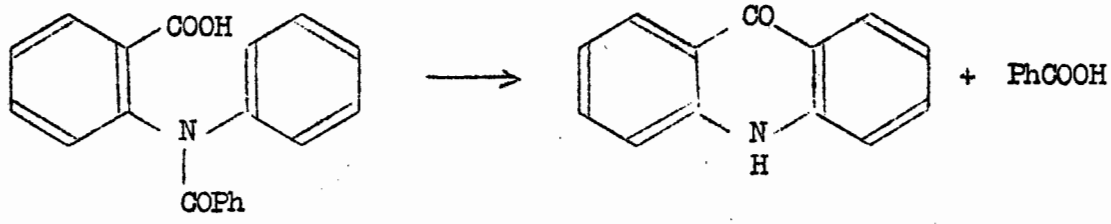


(XIV) (XXIII) or (XXIV)

method of Goldberg and Kelly (57), and the compound identified paper-chromatographically as the expected 1,8-diaminoacridone. This finding was effectively confirmed by a comparison of the infra-red spectra of the two

compounds (KCl disc). Difficulties in the reduction of the dinitrodiphenylamine and in the purification of the acridone and phenazine, did not allow an estimation of the relative proportions of the two products.

The formation of an acridone under the conditions used appears unprecedented. The cyclisation of diphenylamine-2-carboxylic acids to acridones is invariably acid-catalysed, and believed to proceed through a carbonium ion intermediate (63). However, a number of N-benzoyldiphenylamine-2-carboxylic acids have been converted to acridones with loss of benzoic acid purely by the action of heat (XXV → XXVI) (64 - 66). It is possible that had a purely thermal conversion of diphenylamine-2-carboxylic



(XXV) (XXVI)

acids to acridones been more extensively studied, further examples of this type of reaction would have occurred.

The rigorous proof of the structure of 2-aminophenazine-9-carboxylic acid described, established by difference that the other product in the cyclisation of 2,4-diaminodiphenylamine-3'-carboxylic acid (XII) was 2-aminophenazine-7-carboxylic acid. 2-Amino-7-cyanophenazine had also been synthesised in these laboratories as part of a scheme to make the corresponding acid, but attempts to hydrolyse it under various conditions

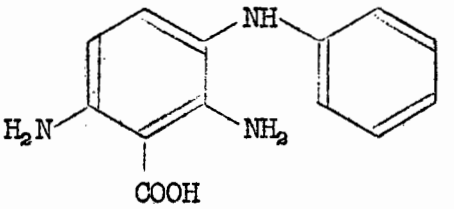
had not been successful (49). This was now achieved by an alkaline hydrolysis in the presence of sufficient alcohol to solubilize the water-insoluble nitrile. The acid thus produced was again a non-crystalline high-melting solid and had identical paper-chromatographic behaviour to the acid from oxidation of the diphenylamine. It was characterised as its methyl ester, and this compared and found identical to the ester from the diphenylamine route.

SECTION I PART III

THE SYNTHESIS OF 2-AMINOPHENAZINE-1-CARBOXYLIC ACID

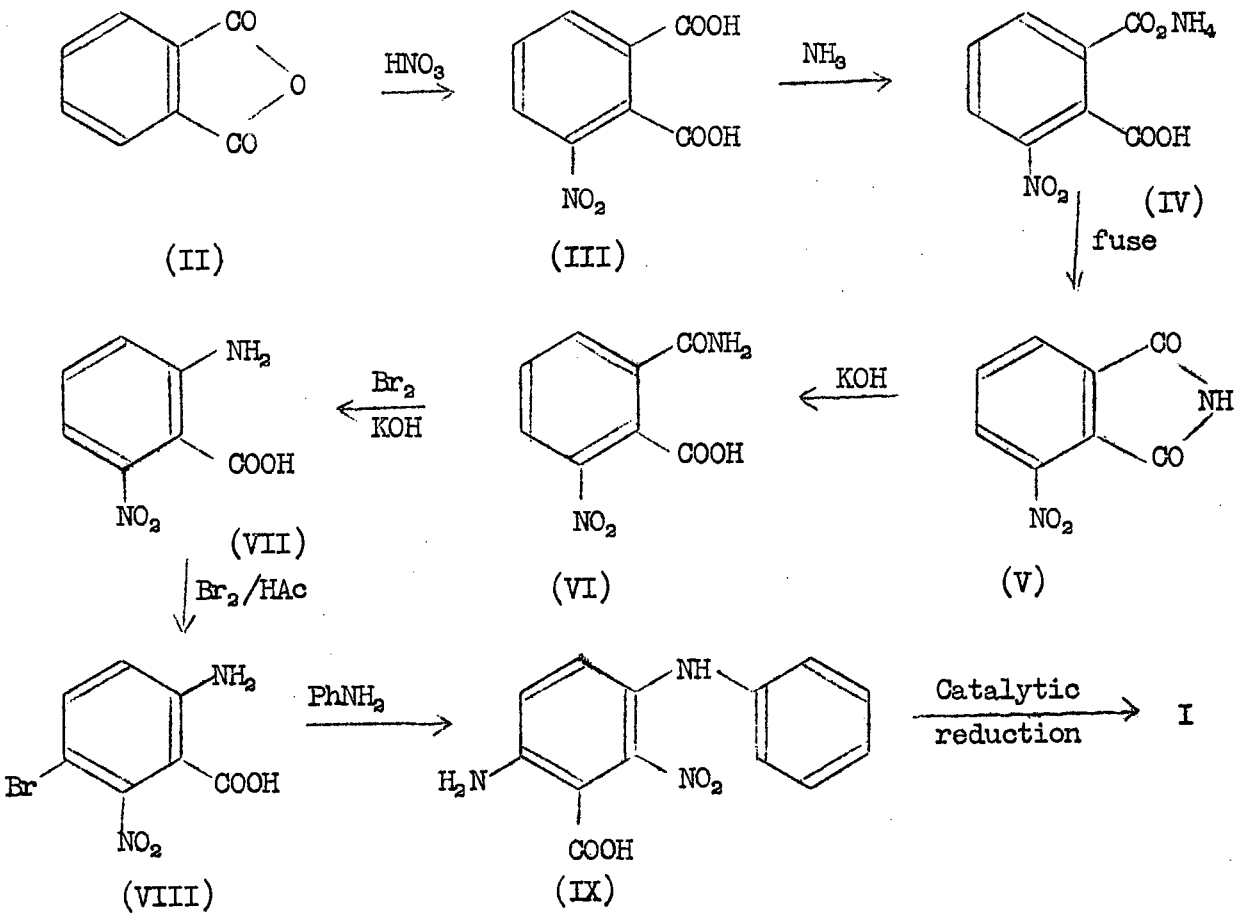
(1) From Methyl 2,4-diaminodiphenylamine-3-carboxylate.

Earlier, unsuccessful attempts to synthesise a substituted 2,4'-diaminodiphenylamine which would give 2-aminophenazine-1-carboxylic acid (together with the 2,3-substituted acid) have been described in Part I. This failure led to an investigation of the possibility of making the alternative 2,4-diaminodiphenylamine, i.e. 2,4-diaminodiphenylamine-3-carboxylic acid (I).



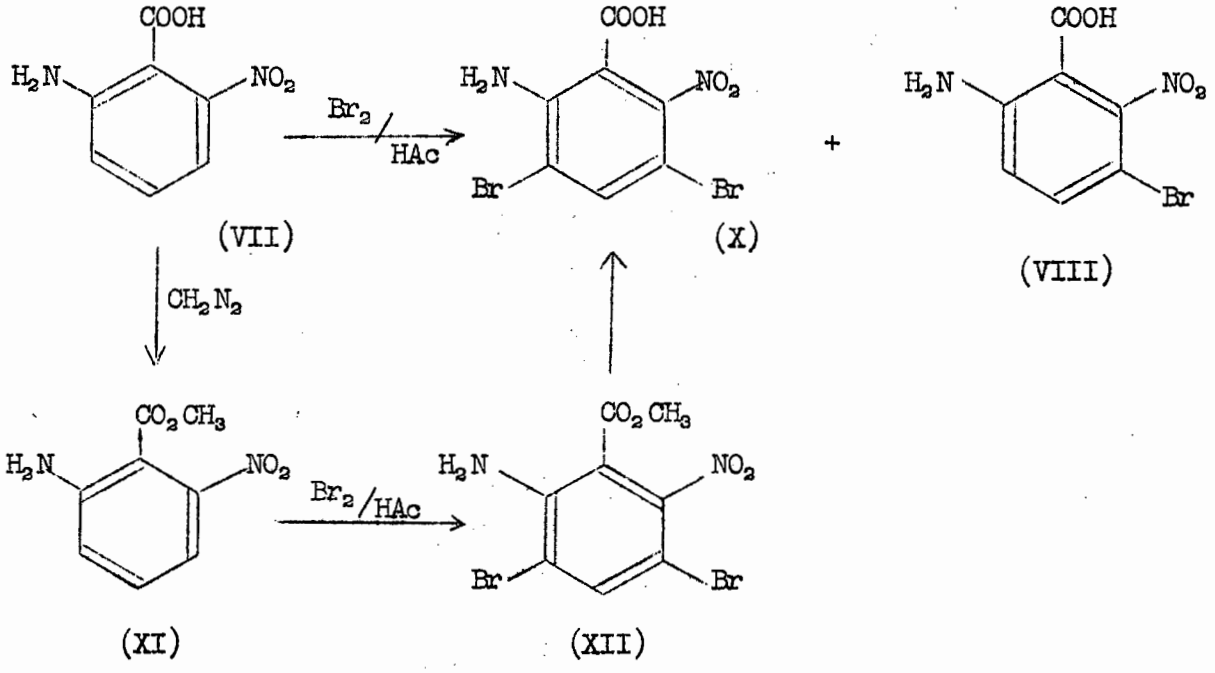
(I)

The preparation of a suitably substituted bromonitrobenzoic acid which could give the required diphenylamine has recently been described (48). 5-Bromo-6-nitroanthranilic acid (VIII) has been made by the reaction sequence shown (II → VIII), and if this could be induced to condense with aniline, could give on reduction the diphenylamine (I).



Repetition of this scheme went smoothly to the stage of 3-nitrophthalamic acid (VI). Conversion of this to 6-nitroanthranilic acid (VII) by a Hofmann reaction under the conditions described (67, 68) was only achieved with a few isolated batches of the phthalamic acid. The purity of the amide appeared to be critical, and since it reverts to the imide (V) on attempted recrystallisation, a method was sought which would apply to phthalamic acid of less rigorous purity. A modified technique suggested by Wallis and Lane for unreactive amides (69), in which an excess of both bromine and sodium hydroxide is used, was found to be successful.

Bromination of 6-nitroanthranilic acid (VII) is reported to give only 5-bromo-6-nitroanthranilic acid (VIII) (48). In the author's hands, significant quantities of a dibromo acid were also found in the reaction product, and had to be separated by fractional crystallisation of the sodium salts from water. Bromination of the methyl ester of the anthranilic acid (XI) gave only a dibrominated product together with unreacted ester, no monobromo derivative being discernible. This difference in behaviour of the acid and ester on bromination is probably due to the fact that the solubility of the ester allows it to be brominated in solution in acetic acid, while the acid must be treated in suspension. Hydrolysis of the dibromo ester to the acid showed that bromination had occurred in the same relative positions. Electronic considerations make it likely that this compound is 3,5-dibromo-6-nitroanthranilic acid (X).

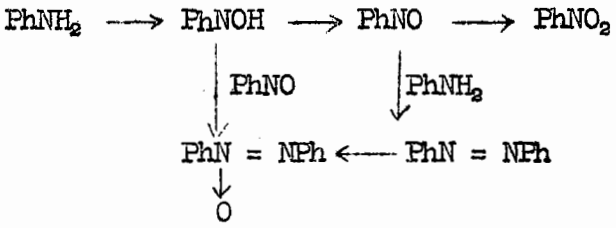


Attempts to condense 5-bromo-6-nitroanthranilic acid (VIII) with aniline under a wide variety of conditions met with scant success. The influence of the ortho-nitro-group on the halogen atom is offset by the para-amino-group, so that the susceptibility of the bromine atom to nucleophilic attack in this compound is probably not much different to that in bromobenzene. A very different state of affairs would follow if the amino-group could be converted to a nitro-group, to give a substituted 2,4-dinitrobromobenzene of enhanced reactivity.

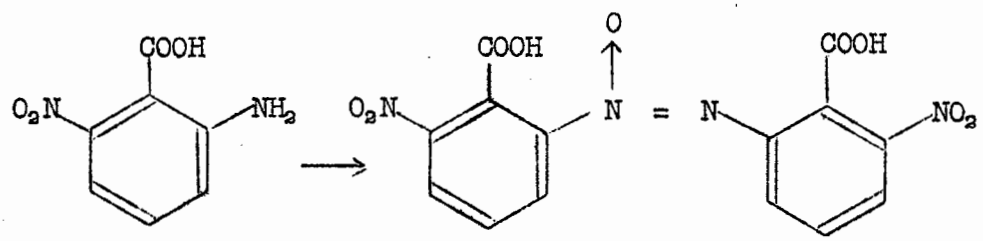
The oxidation of aromatic amines to nitro-compounds by organic peracids has been much investigated (70 - 74). With aqueous peracids yields are low, and substantial quantities of azoxy-compound are often formed (72, 74). Emmons has stepped up yields of the nitro-compound, but only by use of potentially hazardous anhydrous peroxyacetic acid (75). When negatively substituted amines are oxidised the even more dangerous peroxytrifluoroacetic acid must be used (76). These difficulties appeared to be avoided by Holmes and Bayer (77) who converted a series of amines to nitro-compounds simply by warming them with hydrogen peroxide and acetic acid in the presence of catalytic quantities of sulphuric acid. Several of the amines thus converted were negatively substituted. All were 2,6-dihalogenated. Since no reason was given for this curious choice of compound it was decided to test the method on a few amines closely related to 5-bromo-6-nitroanthranilic acid.

A possible reason for Holmes and Bayer's use of 2,6-disubstituted amines soon became apparent. In the oxidation of amines to nitro-compounds,

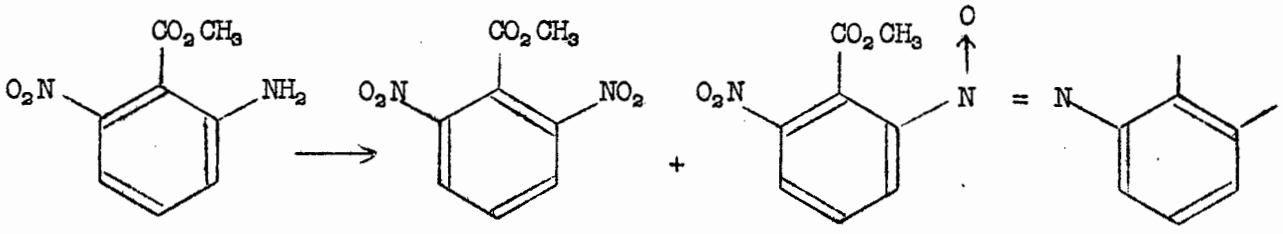
azo- and azoxy-compounds may arise from a competitive reaction between the intermediate nitroso-compound and unreacted amine, or nitroso-compound and phenylhydroxylamine (78) (see below). This intermolecular coupling would



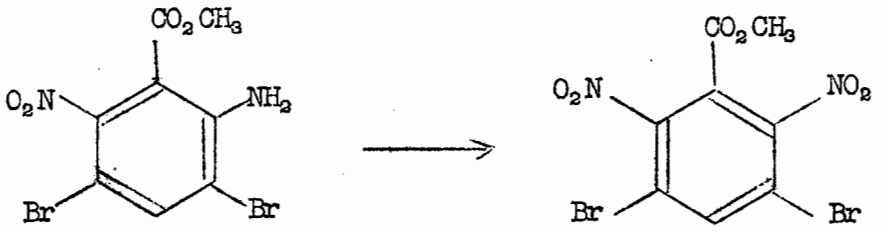
be retarded by the steric restrictions imposed by bulky ortho-substituents in the aromatic ring. Thus with 6-nitroanthranilic acid (VII) which has a single ortho-substituent, predominantly azoxy-compound was isolated. With methyl 6-nitroanthranilate (XI) which has a larger ortho-substituent both nitro- and azoxy-compounds were formed, with the former predominating. With the presumed methyl 3,5-dibromo-6-nitroanthranilate (XII), where two ortho-substituents are present, no azoxy-compound was formed, the product appearing to be the dinitro ester. This explanation accounts for the



(VII)



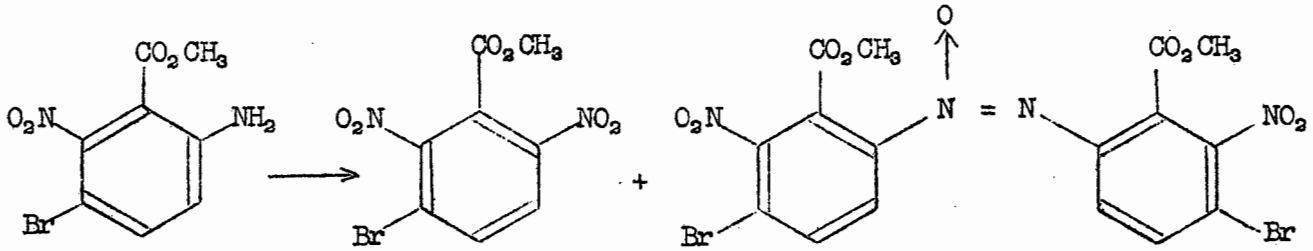
(XI)



(XII)

absence of azoxy-compounds in the products isolated by Holmes and Bayer.

In the light of these findings it seemed worthwhile attempting to convert the amino-group of methyl 5-bromo-6-nitroanthranilate (XIII) to a nitro-group by hydrogen peroxide, the ester being used instead of the acid for the reasons given. This was successful in that though both possible products were isolated, the dinitro ester (XIV) outweighed the azoxy ester (XV) by a factor of 5 : 1. By carrying out the reaction in a much larger

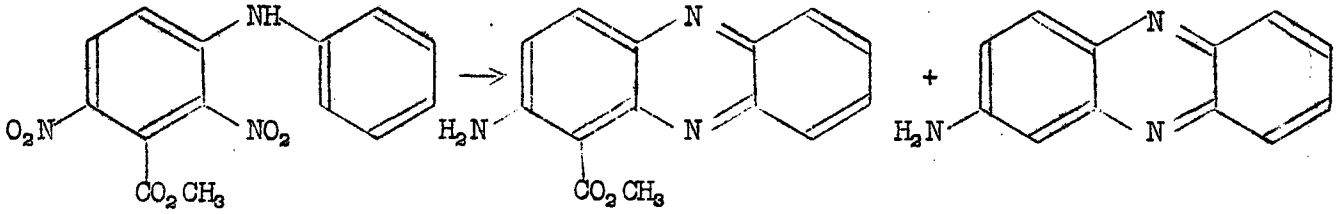


(XIII) (XIV) (XV)

volume of acetic acid, thus reducing the possibilities of intermolecular condensation, the ratio could be changed to 10 : 1.

Methyl 5-bromo-2,6-dinitrobenzoate (XIV) condensed easily and quantitatively with aniline to give the diphenylamine (XVI). This was

reduced catalytically in the normal way (the unstable diamine being isolated as its diacetyl derivative) and refluxed for 8 hours in nitrobenzene. Working up the product by chromatography on alumina revealed a whole spectrum of different bands, of which methyl-2-aminophenazine-1-carboxylate (XVII) and 2-aminophenazine (XVIII) were the most prominent. The presence of the latter in the reaction product made it difficult to



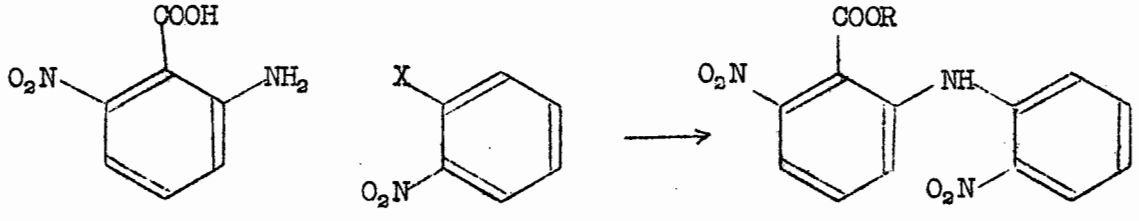
(XVI) (XVII) (XVIII)

purify the ester, since both were eluted from the column in the same fraction whatever eluting solvent was tried. Hydrolysis of the mixed fraction, however, allowed 2-aminophenazine-1-carboxylic acid to be separated from 2-aminophenazine and obtained in a pure state. Some of the phenazine acid was also isolated directly from the column, raising the yield to 15% of theoretical. In order to explain the presence of 2-aminophenazine it must be assumed that hydrolysis of the ester was being effected not only by the alkali of the alumina column, but also by the refluxing nitrobenzene medium. The considerably greater quantities of 2-aminophenazine found here than in the case of the 2,3-substituted ester (Part I) follow from the greater ease of decarboxylation of the 2,1-substituted acid.

The preparation of 2-aminophenazine-1-carboxylic acid just described involves twelve steps, several of which go in low yield, one of which requires isomer separation and the final one of which allows only a 15% recovery. The physical and chemical properties of this acid made it the most interesting of the series, and it was imperative to find a shorter and more convenient method of preparing it in reasonable quantity.

(2) From Methyl 3,2'-diaminodiphenylamine-2-carboxylate.

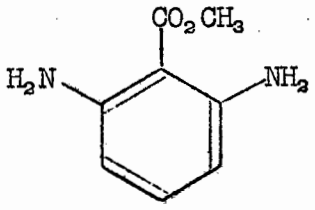
The separation and purification of the products of bromination of 6-nitroanthranilic acid (VII) was one of the more tedious and wasteful parts of the synthetic sequence just described. It was apparent that this and a subsequent step could be avoided if 6-nitroanthranilic acid could be forced to condense with an ortho-halonitrobenzene (XIX) giving 3,2'-dinitrodiphenylamine-2-carboxylic acid (XX, R = H). Reduction and cyclisation, particularly if the carboxyl-function were protected, should then give the 2,1-substituted phenazine acid. A previous synthesis (Section I, Part II) had already shown that cyclisation of 2,3'-diamino-diphenylamines could lead to the phenazine derivative.



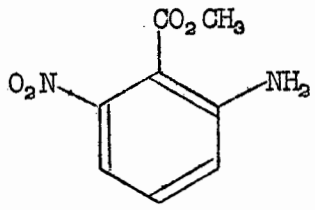
(VII) (XIX) (XX)

Owing to the electron-withdrawing effects of the nitro- and carboxy-groups, 6-nitroanthranilic acid is so weakly basic that it is insoluble in 5 N HCl. This and its proneness to decarboxylation did not make it a suitable compound for use in a Ullmann condensation. It was therefore esterified and the nitro-group reduced to give methyl 2,6-diaminobenzoate (XXI), which had the advantage of having two possible sites for condensation. Unfortunately the diamino ester, like its parent acid (79) is unstable under oxidising conditions or even in polar solvents in the cold, and all attempts to condense it with o-iodonitrobenzene led to black oily products.

This left methyl 6-nitroanthranilate (XI) yet to be investigated. Like the acid (VII) it is very weakly basic, and it was apparent that



(XXI)

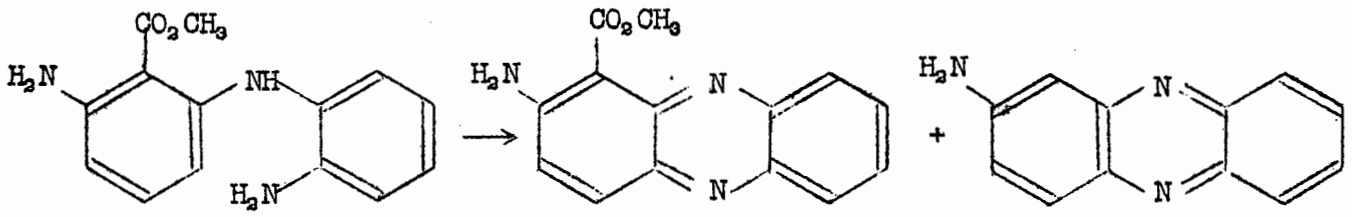


(XI)

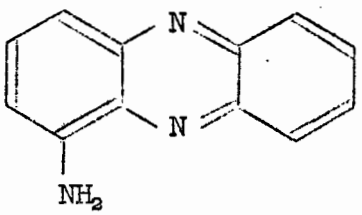
vigorous conditions would be needed to effect condensation. By fusing the ester, o-iodonitrobenzene and potassium carbonate at a high temperature for a very short time (a matter of minutes), and stopping the reaction as soon as the initial rapid carbon dioxide effervescence had slackened, the dinitrodiphenylamine (XX, R = CH_3) could be made. This synthetic procedure which has been much used in these laboratories (80) is a modification of the

conventional method in which fusions are carried cut for a number of hours at lower temperatures. It could, however, only be used for small scale preparations and in this case gave lowish yields.

The dinitrodiphenylamine was reduced catalytically (the diamine (XXI) being characterised as its diacetyl derivative) and refluxed in nitrobenzene for 16 hours with palladium-charcoal catalyst. Chromatography of the product again showed a large number of bands, three of which were isolated and identified. 1-Aminophenazine (XXII), which may here arise by elimination of the 2'-substituted grouping on cyclisation or from ring



(XXI) +



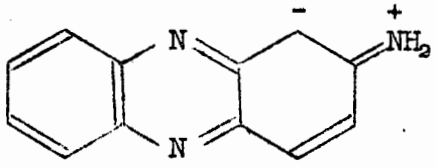
(XXII)

closure on to the hydrolysed and decarboxylated diphenylamine, was eluted first. A mixed band of the phenazine ester and 2-aminophenazine had to be separated by hydrolysis of the ester, the alkali-insoluble 2-aminophenazine being purified by rechromatography on alumina. The resulting acid was

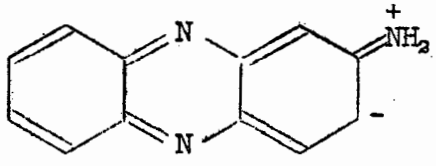
identical in all respects to 2-aminophenazine-1-carboxylic acid made by the previously described method. The total yield of the three products was extremely low, that of the acid being only 5% of theoretical. Shortening the synthetic sequence had thus not improved the ease of preparation of the phenazine acid.

(3) From 2-aminophenazine.

Failure to obtain 2-aminophenazine-1-carboxylic acid in reasonable yield from either of the suitable o-aminodiphenylamines suggested a quite different approach. This was based on the assumption that 2-aminophenazine, which is easily made from readily available starting materials (81, 82) is particularly susceptible to electrophilic attack in the 1-position. This follows from the possibility of localising a negative charge on C_1 in the activated state while leaving the adjacent aromatic quinoxaline system intact, e.g. XXIII. Localisation of the charge at any other carbon atom



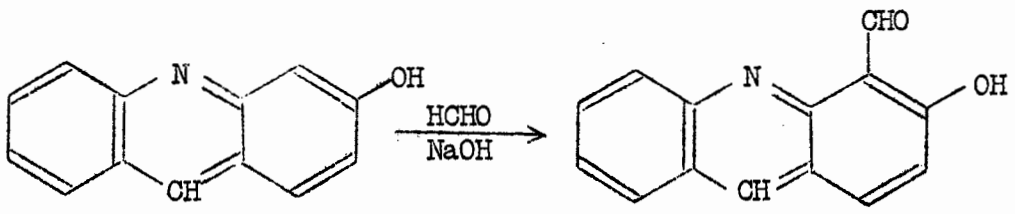
(XXIII)



(XXIV)

in the system must necessarily disrupt the aromatic nature of the molecule, e.g. XXIV. This type of argument has been used to explain why β-naphthol reacts in the 1-position (83) and to establish that the product of

formaldehyde and 2-hydroxyacridine (XXV) is 1-formyl-2-hydroxyacridine (XXVI) (84). In the phenazine series it has been used to account for the

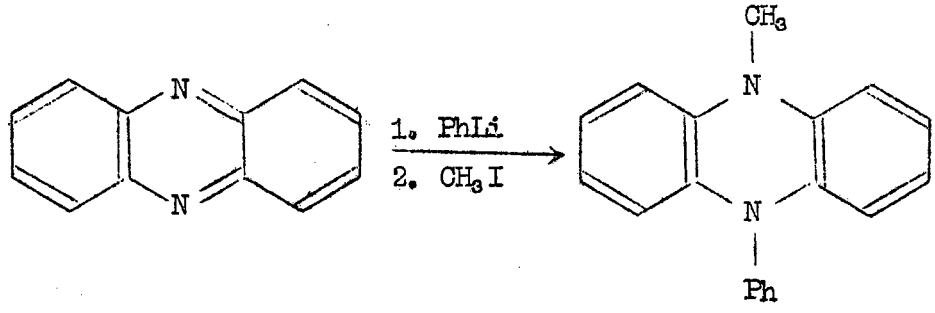


(XXV) (XXVI)

formation of 1-bromo-2-methoxyphenazine as the sole product of bromination of 2-methoxyphenazine (85).

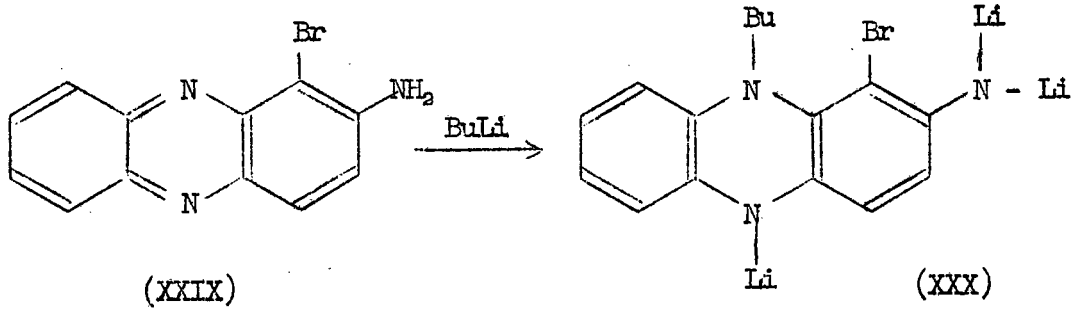
With the latter piece of work in mind, the bromination of 2-amino-phenazine was undertaken. A single product was obtained which was later established as 2-amino-1-bromophenazine. It was hoped that reaction of this with n-butyllithium would allow the halogen atom to be exchanged with lithium, to give a product which could be carbonated to the acid. However, the bromophenazine failed to react under the normal conditions for this type of exchange (86) or even under more vigorous conditions, and was recovered unchanged after each run. Since "the ease of halogen-metal interconversion is proportional to the degree of positive polarisation of the halogen atom" (87), the considerable localisation of negative charge on C_1 would be expected to retard the exchange. However, the analogous 1-bromo-2-methoxynaphthalene reacts with butyllithium in high yield to give the naphthoic acid (87), so that other factors, presumably steric, were also operating here. The postulate of steric hindrance is

supported by the observation that phenyllithium adds across the nitrogen atoms of phenazine (XXVII) (presumably reversibly) to give on treatment with methyliodide 9-methyl-10-phenyl-9,10-dihydrophenazine (XXVIII) (88). Thus in the reaction of 2-amino-1-bromophenazine with butyllithium, the first



(XXVII) (XXVIII)

product might be the lithiated derivative (XXX), which would present considerable resistance to halogen-metal exchange. The same factors would



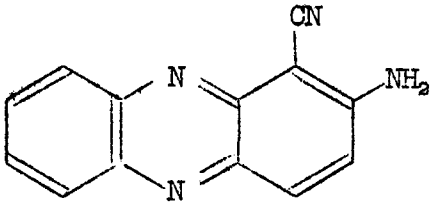
(XXIX) (XXX)

reduce the possibility of direct lithiation of 2-aminophenazine in the 1-position.

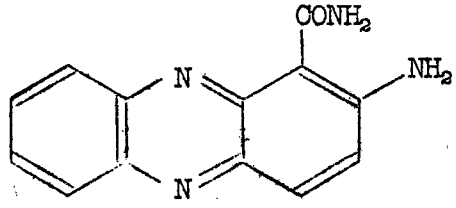
Conversion of 2-amino-1-bromophenazine to the corresponding 2-amino-1-cyanophenazine provided an alternative route to the acid. The nucleophilic attack by the cyanide ion is again favoured by electron-withdrawing ortho-substituents, and would be expected to be difficult in this case.

However, many heterocyclic halides, including one with an amino group para to the halogen (89) have reacted readily when fusion with anhydrous cuprous cyanide has been used. In this case the reaction was successful and yields of 70% could be obtained on small-scale runs in refluxing quinoline as solvent.

Hydrolysis of the ensuing 2-amino-1-cyanophenazine (XXXI) was complicated by several factors. Effectively, it is a di-ortho-substituted



(XXXI)



(XXXII)

aromatic nitrile and these are reported to be hydrolysed with difficulty if at all. This is particularly so when the substituents are electron-donating, since the ease of hydrolysis is proportional to the strength of the derived acid (90). Furthermore, in this case the resulting 2-amino-phenazine-1-carboxylic acid is decarboxylated with very great ease under both acid and alkaline conditions. Thus with alcoholic sodium hydroxide there was no hydrolysis of the nitrile even after 60 hours reflux, with 50% sulphuric/acetic acid mixture 2-aminophenazine was obtained and with alcoholic potassium hydroxide at 190° decarboxylation also occurred. Direct conversion of the nitrile to the ester, which might avoid the problem of decarboxylation, is also reported to be subject to the

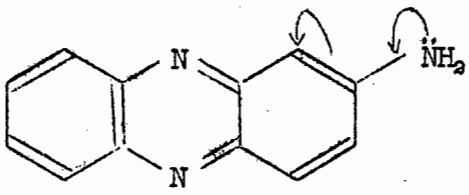
inhibiting influence of ortho-substituents (91) and was unsuccessful here.

The problem was partially solved by conversion of the nitrile (XXXI) to the corresponding amide (XXXII) with a large excess of 30% alkaline peroxide. However, this amide showed the same characteristics as the nitrile in its reluctance to yield the acid. Alkaline hydrolysis was ineffective, 100% phosphoric acid (92) gave 2-aminophenazine, while Bonveault's diazotisation method (93) hydrolysed the amide but also removed the amino-group. Fortunately, unlike the nitrile, the amide could be converted directly to the ester by a high pressure alcoholysis in the presence of sulphuric acid. The ester, as already reported, may be hydrolysed with ease to 2-aminophenazine-1-carboxylic acid.

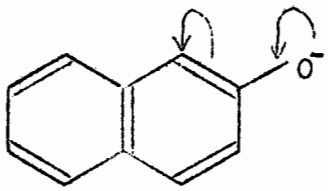
The acid thus obtained proved identical to that prepared by oxidation of the two different o-aminodiphenylamines (I and XXI). The position of bromination of 2-aminophenazine was therefore confirmed as being at C_1. Unfortunately this synthetic sequence suffers from a number of disadvantages. The failure of 2-amino-1-bromophenazine to react with butyllithium and the tortuous conversion of nitrile to acid through both amide and ester that must therefore be followed, complicated a seemingly simple route. Low yields in the nitrile → acid sequence are due to partial decarboxylation in each step, since 2-aminophenazine is a side-product in each of these reactions. Nevertheless this is certainly the preferred method of the three described for the preparation of the 2,1-substituted acid.

(4) Further attempts to prepare 2-aminophenazine-1-carboxylic acid.

Two further attempts were made to prepare the acid by single step reactions. The first of these derived from the expected similarity of 2-aminophenazine (XXXIII) and the β-naphthol anion (XXXIV) in their susceptibility to electrophilic attack in the 1-position. β-Naphthol in alkaline solution undergoes a Kolbe-Schmitt reaction to give 2-hydroxy-1-



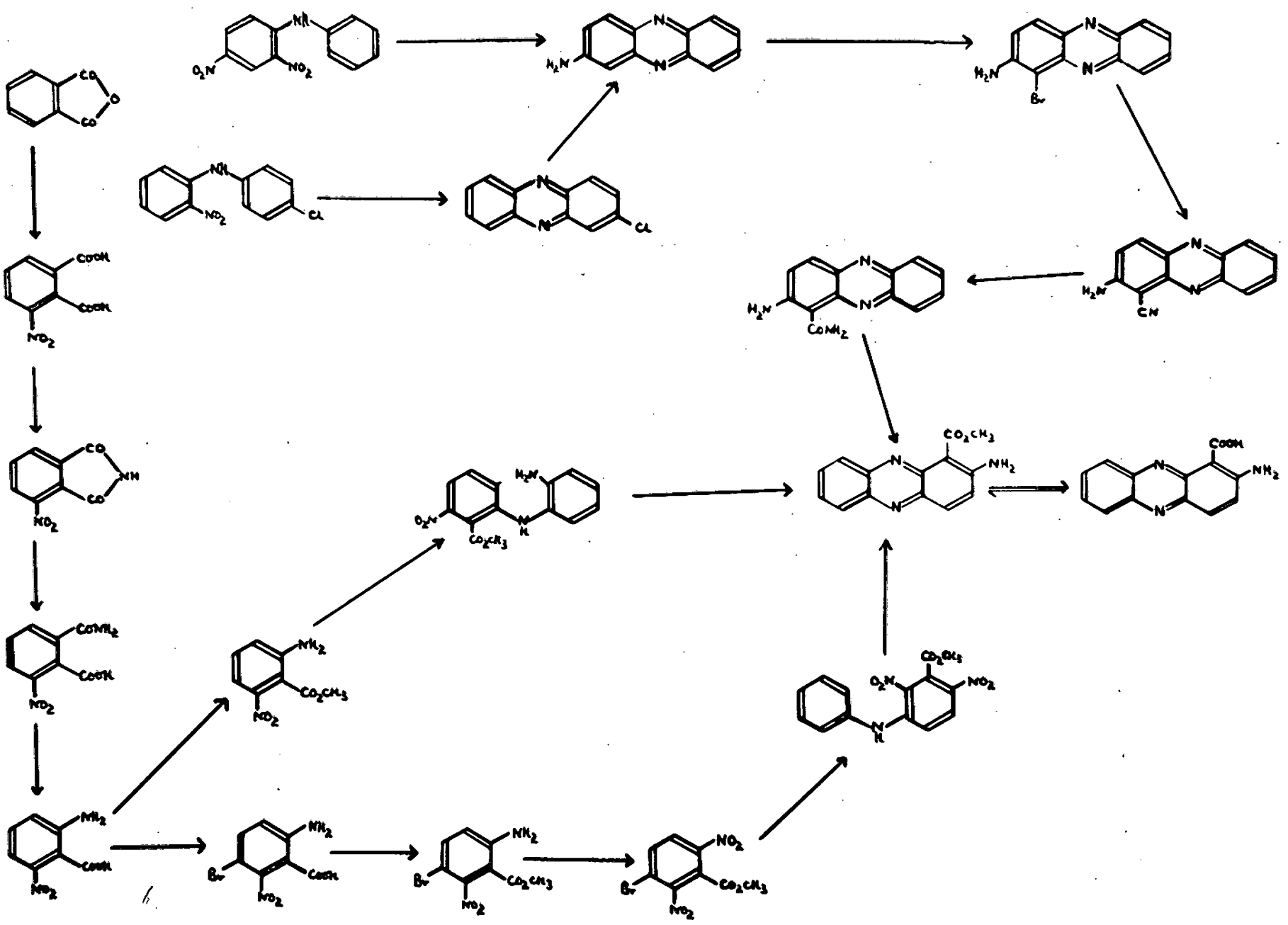
(XXXIII)



(XXXIV)

naphthoic acid (94). It was hoped that under the right conditions 2-aminophenazine might be induced to do the same. However, sufficient cognisance was not taken of the ease of hydrolysis of aminophenazines under high temperature and pressure conditions (95, 96), and the product of the reaction of 2-aminophenazine and carbon dioxide at 80 atmospheres and 150°C was 2-hydroxyphenazine. (Incidentally this is probably the cleanest and most quantitative way of converting 2-aminophenazine to the hydroxy-compound).

The second method employed a Wohl-Aue reaction between 6-nitro-anthranilic acid (VII) and aniline. A paper-chromatographic examination of the product failed to reveal any sign of the characteristically-



SECTION I, PART IV

COMPARATIVE RATES OF PHENAZINE FORMATION

Development of the nitrobenzene oxidation method revealed that the rate of phenazine formation from o-aminodiphenylamines varied widely with different substituents on the diphenylamine. This was the subject of a study by Gaertner (29) who examined comparative rates of phenazine formation and formulated a tentative mechanism for the reaction. In particular he investigated a number of 2,4 and 2,4'-diaminodiphenylamines with substituents in the 4' and 4-positions respectively.

In the synthetic work described in this thesis, phenazines have been prepared by the oxidation of 2,4-diaminodiphenylamines with carboxyl or carbomethoxyl-groups at different positions in the two rings. It seemed of interest to study the relative rates of phenazine formation from these, since it would complement Gaertner's work in which 2,4-diaminodiphenylamines with different groups at the same site (the 4'-position) were investigated. It was hoped that the results might throw further light on the mechanism proposed.

Rate studies were carried out by reducing a sample of dinitrodiphenylamine, and refluxing the diaminodiphenylamine in nitrobenzene without isolating it. Samples were withdrawn at intervals, the phenazine isolated by column chromatography and its concentration estimated spectrophotometrically. The use of alumina columns allowed the presence of coloured intermediates and by-products to be detected. The disappearance of diamino-

diphenylamine could also be followed, since these are brilliantly fluorescent under an ultra-violet lamp.

By this technique the yield of phenazine could be plotted against time. In each case the maximum yield coincided with the point at which diaminodiphenylamine could no longer be discerned on the column. Since maximum yields were in most cases far from quantitative and since side-products were invariably present, rate constants for the reactions could not be calculated. Instead a semi-quantitative comparison of reaction rates was made by collating maximum yields and times taken to reach them. These results are collected in Table I. It will be noticed that with one exception only the esters were examined, since the acids are not amenable to isolation by column chromatography. In the case of the acid recorded, isolation was achieved by removal of the nitrobenzene in steam, but this is not a particularly satisfactory method and was not repeated for the other acids. In all cases the results are little more than semi-quantitative and only gross trends are considered to have any significance.

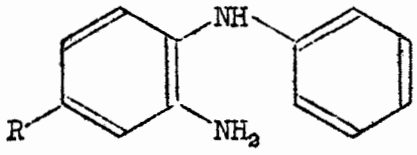
TABLE I

Substituted 2,4-diaminodiphenylamine	Substituted 2-aminophenazine	% Yield	Time, hrs.
1. No substituent *	No substituent	76	12
2. 3-Carbomethoxy	1-Carbomethoxy	70	8
3. 5-Carbomethoxy	3-Carbomethoxy	30	60
4. 6-Carbomethoxy	4-Carbomethoxy	55	40
5. 3'-Carbomethoxy	7 and 9-Carbomethoxy	80	10
6. 6-Carboxy	4-Carboxy	50	8

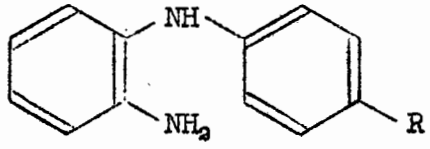
* Reported by Gaertner (29).

The experimental determination of rates was carried out by a technique identical to that used by Gaertner. For this reason the results recorded here are directly comparable with his. Since the majority of rate studies were done by Gaertner, his conclusions and proposed reaction mechanism are first reviewed, before any discussion of rates as recorded here is attempted.

For all ortho-aminodiphenylamines studied, Gaertner suggested that the initial step in the oxidation is the abstraction of a hydride ion from the -NH link of the diphenylamine. The postulate of hydride ion abstraction follows from a suggestion by Jackman that this is the normal mode of nitrobenzene oxidation (97). That it should be from the diphenylamine -NH rather than from the amino-group is indicated by the fact that a substituent has the same effect on the rate whether it is in the 4 or 4'position of the ortho-aminodiphenylamine (I and II). Similarly a 2,4-diaminodiphenylamine



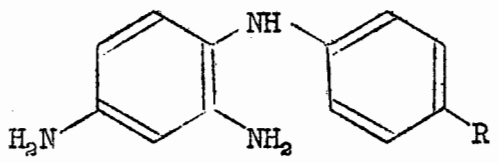
(I)



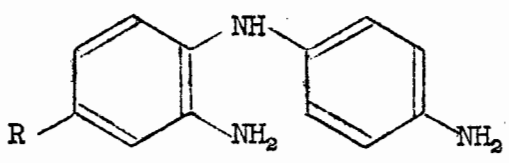
(II)

R = CH_3, OCH_3, Cl, CN.

with a substituent in the 4'-position (III) ring-closes at an identical rate to a 2,4'-diaminodiphenylamine with a substituent in the 4-position (IV).



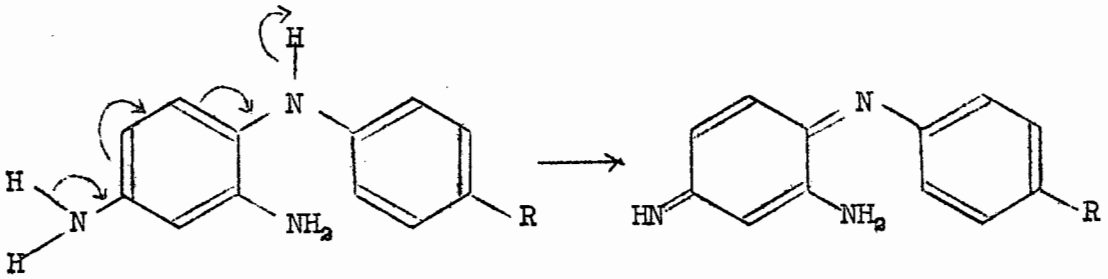
(III)



(IV)

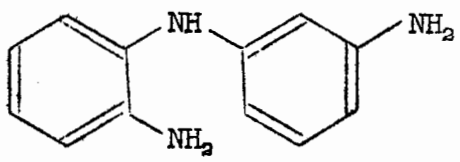
R = CH_3, OCH_3, Cl, CN.

In the case of 2,4-diamino and 2,4'-diaminodiphenylamines, Gaertner suggested that hydride ion abstraction is followed closely by removal of a proton from a 4 or 4'-amino-group to give as the first stable intermediate a para-quinone-imine type of structure (e.g. V). Evidence for this is that



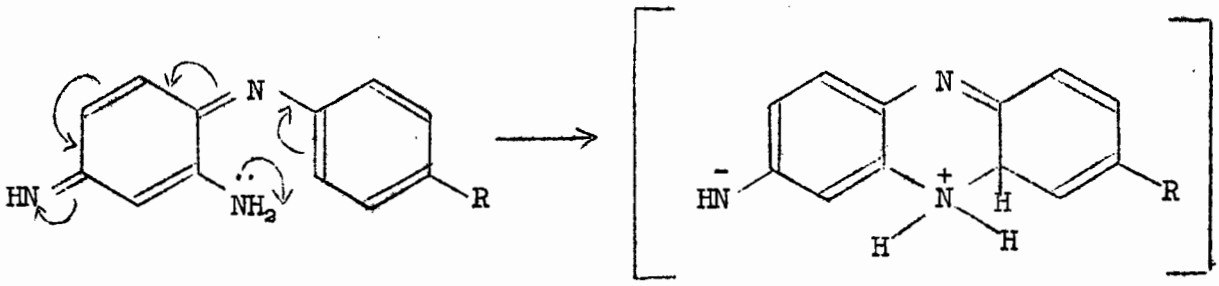
(III) (V)

quinone-imine intermediates, which are normally strongly coloured, have been isolated in the synthesis of more complicated phenazines (98, 99). In Gaertner's work deeply coloured bands appeared on the alumina columns during the course of the reaction, but disappeared once the maximum yield had been attained. Furthermore, these bands were absent in the oxidation of 2,3'-diaminodiphenylamine (VI), where no para-quinone-imine is possible.

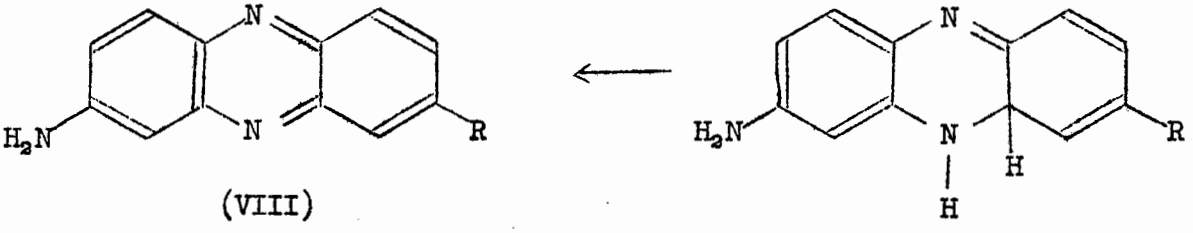


(VI)

In the proposed mechanism, quinone-imine formation is visualised as being followed by/or concerted with a nucleophilic attack by the amino-group on to the other ring to give a dihydrophenazine (VII). These are known to be oxidised to the phenazine (VIII) with great rapidity (100, 101).



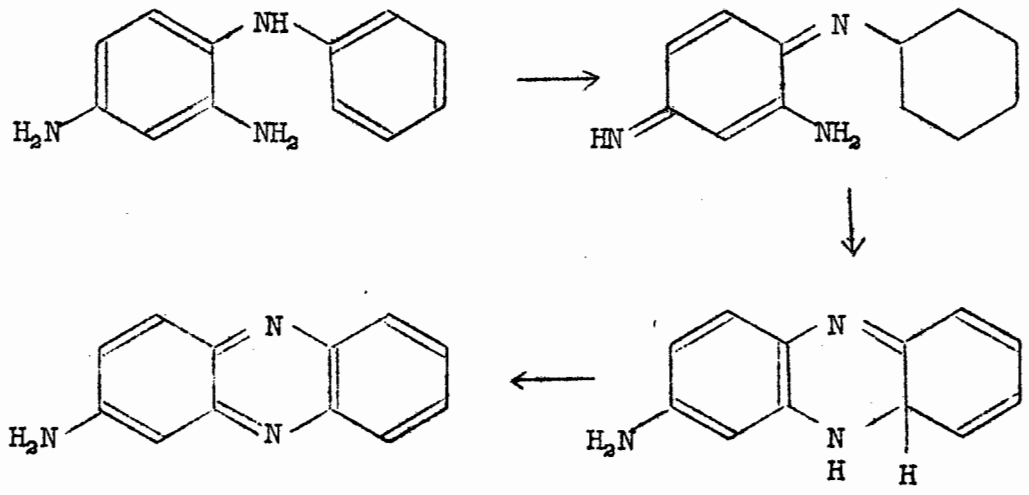
(V)



(VIII)

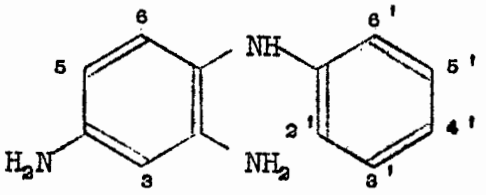
(VII)

Though space does not permit a summary of all the evidence presented by Gaertner in support of this mechanism, it seems fairly certain that the initial step in the reaction is hydride ion abstraction from the diphenylamine nitrogen. This leads either immediately or in a series of steps to a reaction intermediate of sufficient stability to be observed in favourable cases. Formulation of this as a quinone-imine seems probable, but awaits structural investigation of the coloured, transient bands. Cyclisation of the intermediate to the dihydrophenazine will then almost certainly involve a nucleophilic attack by the ortho-amino-group (or possibly an imino-group) on the second ring. Gaertner has summarised the mechanism as:



How far the results collected in Table I are consistent with this must now be examined.

When the carbomethoxy-group is in the 5 or 6-positions the rate of ring-closure and the maximum yield are both diminished relative to 2-amino-phenazine. An electron-withdrawing group in the 5-position (IX) will lower the basicity of the 2-amino-group and thus hinder its nucleophilic attack

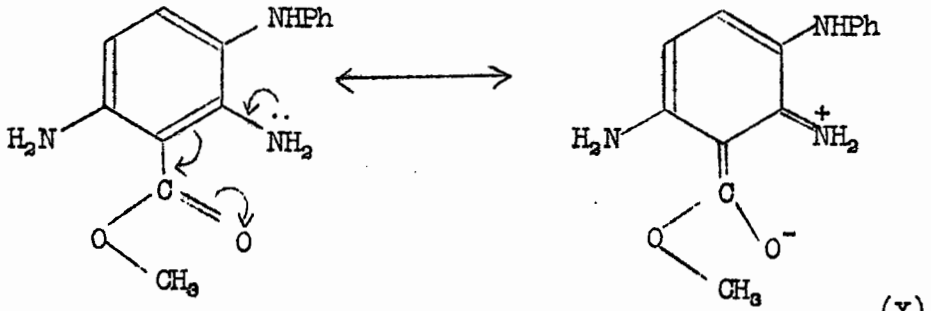


(IX)

on the second ring, while in the 6-position it will impede hydride ion abstraction from the diphenylamine N - H. In both cases the lower rates are entirely consistent with the proposed mechanism, though they have their origin in different parts of the reaction mechanism. In the 3'-position a carbomethoxy-group can have only a small influence on hydride ion abstraction, and that by a purely inductive effect. It will, however, probably assist the ring-closing step by lowering the electron-density at the 2'-position. In actual fact when allowance is made for the semi-quantitative nature of the results, the rate is found to be of very much the same order as in 2,4-diaminodiphenylamine itself.

A carbomethoxy-group in the 3-position would be expected to have much the same effect on the reaction rate as when it is in the 5-position. This, however, is found not to be the case, and phenazine formation takes place on a scale and at a rate similar to that in the unsubstituted diaminodi-

phenylamine. The most reasonable explanation for this phenomenon is that the carbomethoxyl-group is prevented from exerting its deactivating effect by steric inhibition of resonance. If it is to withdraw electrons from the ring and the ortho-amino-group by an electronic effect, contributions of the canonical form (X) are important. This, however, requires that both

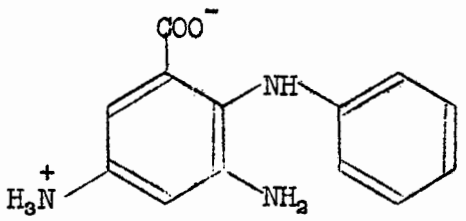


(X)

oxygens of the carbomethoxyl-group lie in the same plane as the ring, the nitrogen atoms of the amino-groups, and even the hydrogen atoms of one or both amino-groups. Sterically, this is not possible, and the electron-withdrawing power of the group by the electronic effect (though not the inductive effect) is thus prevented. Many examples of this type of phenomenon have been observed (102).

One further interesting result is obtained when the carbomethoxyl-group in the 6-position is replaced by a carboxyl-group. The retarding effect on the reaction appears to be removed and the diphenylamine now ring-closes at about the same rate, (though the yield is lower) as unsubstituted diaminodiphenylamine. The only possible explanation for this appears to be that the diaminodiphenylamine carboxylic acid exists as the zwitterion (XI) and that the carboxylate-group thus formed no longer

exerts anything like as strong an electron-withdrawing effect.



(XI)

So far nothing has been said about the presence of transient strongly coloured bands attributable to quinone-imine intermediates. Gaertner observed these in all cases, but in this study a deeply coloured transient band was found only with a carbomethoxyl-group in the 5-position. In itself this result is reasonable, since an electron-withdrawing group in the 5-position will have a retarding effect mainly on the ring-closing step, thus allowing the concentration of the quinone-imine to build up to an observable level. But taken in conjunction with Gaertner's results, where quinone-imines are claimed to be observed in every case, the result is less easily interpreted. A possible explanation for this may lie in the fact that the colour and ease of observation of individual quinone-imines may vary widely according to how they are substituted, and that in certain cases their presence may not be easily detectable. Thus electronic factors will control not only the production of the intermediate but also possibly the ease of observation of the intermediate. Before the reaction mechanism can be discussed in any greater detail, further work must be carried out on the proof of the existence of the quinone-imines.

Influence of catalysts: Various catalysts suitable for hydrogen transfer reactions were examined by Gaertner in the cyclisation of 2-amino and 2,4-diaminodiphenylamine. None were found to have any accelerating effect on the reaction. In this work, the influence of 5% palladium-charcoal on the oxidation of methyl 2,4-diaminodiphenylamine-5-carboxylate was examined. It was found that the maximum yield was attained in 30 hours, in contrast to the 60 hours needed without a catalyst, though the yield remained unchanged at 30%. Clearly this is an aspect of the problem worth further investigation, particularly for those diphenylamines which ring-close slowly, i.e. that need longer than 20 hours to reach completion.

SECTION II

PROPERTIES

In this section an investigation of selected physical and chemical properties of the series of acids and esters is reported. Such a study was considered of interest for two reasons: (i) because the properties of few substituted and even fewer disubstituted phenazines had previously been examined in any detail; (ii) because it was hoped that correlations might be found between the position of substitution and the properties of the particular aminophenazine. This could then give information in structural investigations on naturally occurring phenazines such as Aeruginosin B.

The properties chosen were the visible, ultra-violet and infra-red spectra of the acids and esters, and the ease of esterification of the acids. An obvious omission is an examination of acid and basic dissociation constants of the series (which have been extensively studied for the amino-acridines) (105), but certain obstacles, notably the low solubility of the compounds, meant that this would be too involved a study for the short time available.

At the end of Section II the physical characteristics of the acid and ester series are collected in table form.

ULTRA-VIOLET AND VISIBLE SPECTRA

Spectra were measured on a Beckmann Model D.U. Quartz Spectrophotometer and a Zeiss PMQ II Spectrophotometer. Both instruments were checked in the visible and ultra-violet against potassium chromate in 0.05 N potassium hydroxide solution (103). Absorption maxima are recorded in Table I, with logarithms of molecular extinction coefficients given in brackets. Selected absorption curves are reproduced in the appendix.

Solvents: The extremely low solubility of the aminophenazine carboxylic acids in water and organic solvents necessitated the use of dilute acid (N/10 HCl) or alkali (N/2 NaOH) as solution media. The esters were likewise measured in N/10 HCl, but were sufficiently soluble to be examined also in 96% ethanol.

From spectroscopic studies (104) it has been adduced that 2-aminophenazine (as well as the aminoacridines) (105) exists in dilute acid as the monocation, the proton being attached to the ring nitrogen meta to the amino-group. This very reasonable assumption is accepted in the work reported here. Since, however, free base and di-cation are probably also present in dilute acid solution, wherever comparisons are made they are made within a particular solvent system.

Discussion of results:

In general the absorption curves of the acids and esters are very similar to that of 2-aminophenazine itself. In all cases the absorption maxima may be classified into four groups, A, B, C and D, falling within fairly narrow spectral ranges. The molecular extinction coefficients of

TABLE I

Substituted 2-aminophenazine	Solvent	A	B	C	D
No substituent	HCl	231 (4.49)	281 (4.60)	383 (3.96)	520 (4.05
1-Carboxy	HCl	230 (4.32)	265 (4.61)	375 (4.07)	470 (4.09)
3-Carboxy	HCl	242 (4.42)	298 (4.47)	397 (3.96)	560 (3.95)
4-Carboxy	HCl	230 (4.49)	280 (4.62)	390 (3.91)	535 (4.08)
6-Carboxy	HCl	235 (4.64)	280 (4.84)	375 (4.01)	535 (4.07)
7-Carboxy	HCl	238 (4.31)	278 (4.50) 300 (4.58)	385 (3.89)	520 (4.17)
8-Carboxy	HCl	247 (4.44)	295 (4.53)	385 (4.00)	535 (4.04)
9-Carboxy	HCl	237 (4.46)	278 (4.61)	380 (3.97)	530 (4.16)
1-Carbomethoxy	HCl	225 (4.08)	266 (4.50) 305 (3.84)	381 (3.87)	465 (3.94)
3-Carbomethoxy	HCl	241 (4.45)	299 (4.49)	396 (4.01)	560 (3.98)
4-Carbomethoxy	HCl	230 (4.52)	280 (4.70)	388 (3.95)	530 (4.12)
6-Carbomethoxy	HCl	235 (4.54)	285 (4.66)	380 (4.02)	535 (4.11)
7-Carbomethoxy	HCl	238 (4.63)	278 (4.67) 300 (4.67)	383 (3.94)	520 (4.18)
8-Carbomethoxy	HCl	248 (4.51)	293 (4.57)	385 (4.06)	535 (4.10)
9-Carbomethoxy	HCl	237 (4.49)	280 (4.66)	372 (4.00)	525 (4.19)
1-Carboxy	NaOH	226 (4.34)	271 (4.64)	366 (3.83)	450 (3.80)
3-Carboxy	NaOH	240 (4.36)	276 (4.61)	379 (3.90)	472 (3.71)
4-Carboxy	NaOH	229 (4.31)	270 (4.70)	369 (3.85)	455 (3.85)
6-Carboxy	NaOH	231 (4.26)	271 (4.72)	372 (3.93)	452 (3.87)
7-Carboxy	NaOH	231 (4.06)	281 (4.60)	377 (3.76)	462 (3.89)
8-Carboxy	NaOH	239 (4.39)	277 (4.65)	373 (3.87)	463 (3.78)
9-Carboxy	NaOH	229 (4.14)	271 (4.66)	369 (3.82)	455 (3.84)
No substituent	EtOH	230 (4.43)	275 (4.71)	362 (3.82)	470 (3.88)
1-Carbomethoxy	EtOH	227 (4.14)	265 (4.58)	355 (3.66)	440 (3.78)
3-Carbomethoxy	EtOH	246 (4.41)	277 (4.67)	373 (3.83)	520 (3.74)
4-Carbomethoxy	EtOH	230 (4.44)	274 (4.76)	362 (3.76)	475 (3.96)
6-Carbomethoxy	EtOH	233 (4.50)	280 (4.75)	365 (3.87)	480 (3.93)
7-Carbomethoxy	EtOH	235 (4.26)	252 (4.34) 302 (4.66)	377 (3.49)	485 (4.07)
8-Carbomethoxy	EtOH	246 (4.53)	290 (4.70)	365 (3.78)	495 (3.87)
9-Carbomethoxy	EtOH	232 (4.39)	279 (4.71)	361 (3.75)	485 (3.95)
7-Cyano	HCl	240 (4.48)	278 (4.52) 302 (4.67)	380 (3.91)	520 (4.18)

the different bands in each compound are closely similar to equivalent bands in 2-aminophenazine and have approximately the same relative intensities.

A more detailed examination of the results shows that the seven isomers may be divided into two distinct groups. Where the substituent is in the 4, 6, 8 or 9-positions, the curves whether measured in acid, alkali or alcohol are virtually indistinguishable from one another or from 2-aminophenazine. The main difference is a small bathochromic shift of the long wave-length band D (relative to 2-aminophenazine) on the introduction of the carboxyl or carbomethoxyl function, but the magnitude of this is insufficient to make it of any diagnostic value. These four are therefore referred to in the subsequent text as the 'normal' acids and esters.

When the substituent is in the 1, 3 or 7-positions differences occur in the shape of the curve and in the position of band D, so that the identity of the isomer may be easily recognised from its absorption spectrum. The nature of these differences and possible reasons for them are discussed for the individual compounds.

(i) 7-Substituted 2-aminophenazines: When a carboxyl or carbomethoxyl-group is introduced into the 7-position of 2-aminophenazine, an additional absorption maximum occurs between 300 and 305 mμ. Though the intensity of this band is high (log ε 4.6 - 4.7), the positions and intensities of the remaining four bands are substantially unaltered. The band is also present in 2-amino-7-cyanophenazine, but disappears when the 7-substituted acid is examined in dilute NaOH. This would indicate the the extra absorption maximum is bound up with the presence of an electron-withdrawing group in

the 7-position. Unfortunately, no further 7-substituted 2-aminophenazines were available to verify this assumption.

In addition to furnishing an extra band in the ultra-violet, a -COOH, $-CO_2CH_3$ or -CN group in the 7-position causes a small hypsochromic shift in band D, when the spectra are examined in HCl. The band is then found at 520 mμ in contrast to the range 530 - 540 mμ for the normal members of the series. Though the shift is small, the effect on the visible colour is easily noticeable, 2-amino-7-substituted phenazines having the same rich red colour as 2-aminophenazine, while the normal isomers are a deep purple-red. This distinction disappears when the acids are examined in NaOH, or the esters in EtOH.

(ii) 3-Substituted 2-aminophenazines

The difference in colour between 2-aminophenazine-3-carboxylic acid (and its methyl ester) and other members of the isomeric series is striking, and is confirmed by measurement of ultra-violet and visible spectra. The 3-substituted acid and ester in HCl are a deep violet (560 mμ) in contrast to the red-purple of the normal members of the series (530 - 540 mμ). In ethanol the 3-ester is red (520 mμ) while the normal esters are orange (475 - 495 mμ); in NaOH the acid is orange (470 mμ), the normal acids in this solvent being yellow (450 - 460 mμ).

The pronounced bathochromic shifts described are accompanied by a broadening of band D and a lowerering of its intensity. The 3-substituted acid and ester are the only ones in the series in which band D has a lower intensity than band C, and the molecular extinction coefficients for band D

are considerably lower than the range of values for remaining members of the series. It is interesting to note that these trends persist even when the acid is dissolved in NaOH, in which its electronic effects must be considerably curtailed.

(iii) 1-Substituted 2-aminophenazines

Substituting 2-aminophenazine with an acid or ester grouping in the 1-position has an effect which is quite the reverse of 3-substitution. A pronounced hypsochromic shift in band D (λ_{max} in HCl is at 465 mμ in contrast to the value 530 - 540 mμ for the normal isomers) changes the colour from red to yellow. The shift persists in alcoholic solution but falls away when the solvent is sodium hydroxide. In addition a further band appears in the region 275 - 305 mμ. This, however, is quite different to the extra band observed in the 7-substituted isomers, in that it is of lower intensity (log ε 3.80 - 4.0) and sometimes present only as a shoulder. When the acid is examined in sodium hydroxide this shoulder disappears completely, in addition to the reversion of band D to the 'normal' value.

The spectra of three additional 1-substituted 2-aminophenazines, (the bromo, cyano and carboxamido compounds), which had been made as intermediates in the synthesis of the corresponding acid, were also examined. Absorption maxima and molar extinction coefficients are recorded in Table II.

Once again the most significant trends are observed in the position of band D. Whether the solvent is HCl or EtOH a hypsochromic shift is obtained compared to 2-aminophenazine which varies in magnitude in a

TABLE II

1-Substituted 2-aminophenazines	Solvent	A	B	C	D
1-Carboxy	HCl	230 (4.32)	265 (4.61) 275 - 300 (sh.)	375 (4.07)	470 (4.09)
1-Carbomethoxy	HCl	225 (4.08)	266 (4.50) 305 (3.84)	381 (3.87)	465 (3.94)
1-Cyano	HCl	225 (4.31)	265 (4.73) 280 - 300 (sh.)	370 (3.94)	465 (3.97)
1-Carboxamido	HCl	230 (4.35)	270 (4.59) 290 - 300 (sh.)	385 (3.98)	492 (4.00)
1-Bromo	HCl	234 (4.44)	288 (4.60) -	390 (4.08)	520 (4.05)
1-Carboxy	EtOH	226 (4.28)	265 (4.69) 298 (3.96)	360 (3.83)	450 (3.95)
1-Carbomethoxy	EtOH	227 (4.14)	265 (4.58) 290 - 315 (sh.)	355 (3.66)	440 (3.78)
1-Cyano	EtOH	225 (4.31)	265 (4.73) 280 - 300 (sh.)	370 (3.94)	440 (4.00)
1-Carboxamido	EtOH	227 (4.38)	267 (4.81) 280 - 310 (sh.)	355 (3.90)	450 (4.00)
1-Bromo	EtOH	230 (4.34)	280 (4.63) -	365 (3.84)	460 (3.85)

regular way. If the substituents are placed in order based on the size of the hypsochromic shift the following sequences are obtained:

Solvent HCl: $CO_2CH_3 \equiv CN > COOH > CONH_2 > Br$

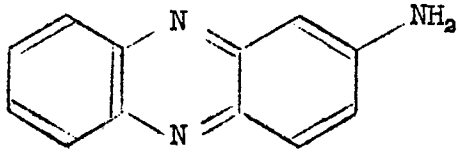
Solvent EtOH: $CO_2CH_3 \equiv CN > COOH \equiv CONH_2 > Br$,

with the bromo-compound being almost indistinguishable from 2-aminophenazine. It is significant that a hypsochromic shift occurs only when the substituent is capable of conjugating with the ring, and that the magnitude of the shift corresponds roughly to the electron-withdrawing power of the group. At the same time the shoulder or peak in the region 275 - 305 mμ is present in all cases where there is a hypsochromic shift in band D, but is absent in the bromo-compound.

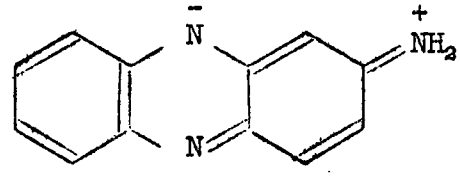
Possible origin of spectral shifts in 3 and 1-substituted 2-aminophenazines

Before reasons for the observed spectral shifts in 3 and 1-substituted 2-aminophenazines are suggested, the origin of colour in 2-aminophenazine itself must be briefly considered.

According to the valence bond resonance approach to light absorption the normal single classical structure of 2-aminophenazine must be replaced by a set of contributing structures, differing in the localisation of electrons. Two important contributors (representative of the whole spectrum of contributing structures) are the structures (I) and (II). The actual



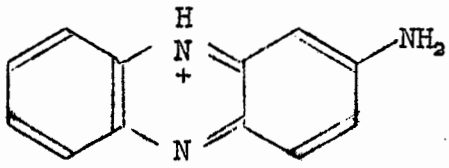
(I)



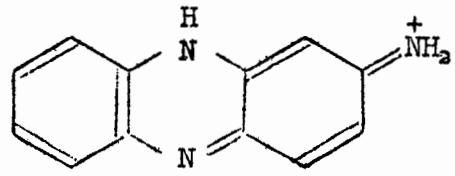
(II)

molecule is then a hybrid or superposition of these two structures and its wave function will be a combination of the wave-functions φ_1 and φ_2 of the individual structures. If φ_1 and φ_2 are closely similar or equal in energy, the splitting in energy between the ground state and first excited state of the molecule is small and the fundamental band is found at long wave-length. If on the other hand the wave-functions of the contributing structures differ appreciably in energy, a much greater splitting in energy between ground and first excited states is found, and the molecule absorbs at considerably shorter wave-length (106).

In the case of 2-aminophenazine, a considerable difference of energy between the structures (I) and (II) would be expected. Structure (II) will have higher energy: (i) because there is a loss of aromaticity in going from (I) to (II); (ii) because of the fundamental instability of a dipole (107, 108). For this reason the splitting between ground and first excited states is comparatively large and the molecule absorbs at relatively short wave-length (470 mμ, EtOH). If the solvent is N/10 HCl, where we are dealing with the monoprotonated form, the two main contributing structures are (III) and (IV). Since both are polar, the energy difference between the two must arise only from the loss in aromaticity in passing from (III) to (IV).



(III)

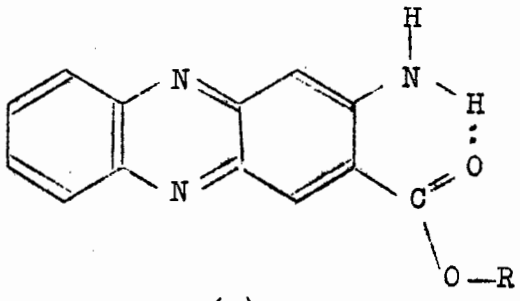


(IV)

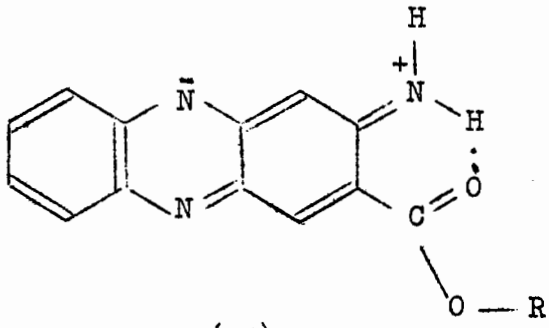
The difference is consequently smaller and the molecule shows a bathochromic shift (520 mμ, N/10 HCl).

From the principles enunciated it is apparent that any factor which will affect the energy of one of the contributing resonance structures and not the others will change the absorption characteristics of the system. In the case of the 3-substituted 2-aminophenazines described here, this state of affairs is possible. Here the carboxyl or carbomethoxyl-group is adjacent to the amino-group and thus capable of intramolecular hydrogen bonding (V

and VI). It is known that a hydrogen bond formed between a positively



(V)

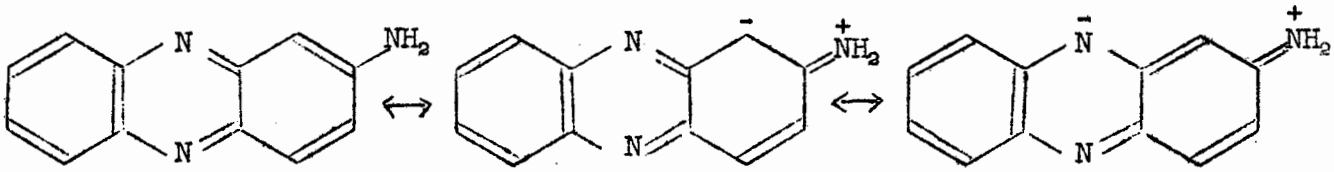


(VI)

charged nitrogen atom and a carbonyl oxygen is considerably stronger than a bond between an uncharged nitrogen and a carbonyl oxygen (109). It is therefore proposed that a possible explanation for the marked bathochromic shift in 3-substituted 2-aminophenazines has its origin in the stabilization or lowering of energy of the charge-separation structure (VI), due to hydrogen bonding, with respect to the Kekule structure (V). By thus rendering the energies of the contributing structures more equal, a smaller splitting between ground and first excited state is ensured, and a bathochromic shift results. The same argument applies to the protonatedspecies found in N/10 HCl. This postulate is supported by the fact that when the 3-acid is examined in sodium hydroxide solution where the electron-attracting power of the substituent must be severely curtailed, the bathochromic shift persists. Solution in sodium hydroxide will not restrict the hydrogen bonding abilities of the system. It therefore appears that the deepening in colour has its origin in a cause which is not electronic in nature. The suggestion that it is brought about by hydrogen bonding could be verified by replacement

of the carboxyl-function by an electron-withdrawing group incapable of hydrogen bonding, e.g. the linear cyano-group.

The consideration of extreme structures alone in drawing up the wave-function for a molecule has been criticised by Pauling (110). He points out that resonance interaction between extreme structures must take place through intermediate structures which are of much higher energy. This may be illustrated for 2-aminophenazine by the extreme structures (I and II) which interact through the high energy intermediate (VII). Normally the intermediate forms are not appreciably 'mixed in' with the extreme forms

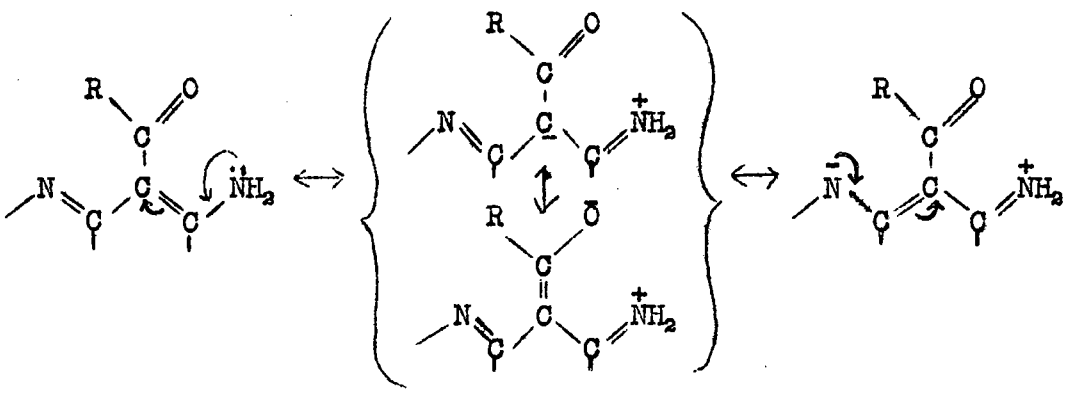


(I) (VII) (II)

in drawing up the wave-function for the molecule because their energies are too high. But if some additional factor lowers the energy of the intermediate structure to a value which is of the same order as the energy of the extreme structures (though it is still greater), appreciable resonance interaction takes place between all three structures, the splitting between ground and first excited state is increased, and the molecule absorbs at shorter wave-length (111).

A probable explanation for the hypsochromic shift in 1-substituted 2-aminophenazines now becomes apparent. If a carbonyl-function or a cyano-

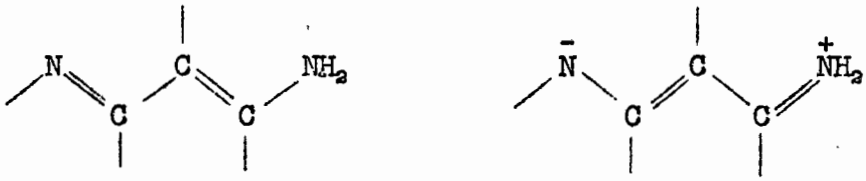
group is introduced at C_1 of 2-aminophenazine, the high energy intermediate structure has its energy lowered by the fact that it is itself resonance stabilized. Instead of a single structure, two structures may now be written for the intermediate, one having the negative charge localised on C_1, and the other having the charge localised on the oxygen atom of the carbonyl-group or the nitrogen atom of the cyano-group (e.g. below).



This leads to a fairly full interaction between all four structures described, a large splitting between ground and first excited state, and a hypsochromic shift in the fundamental band. The magnitude of the shift will be related to the ability of the conjugated atom in the substituent group to localise a negative charge upon itself.

The discussion so far has been based on the valence bond approach to quantum mechanics. A simpler procedure and one that gives a clearer physical picture is to use the quasi-classical model of Lewis and Calvin (112). They start with the idea that in the absorption of light the energy is taken up by electronic oscillations which are considered analogous to classical oscillations. This leads to the idea that 'when the colour of a substance is associated with an oscillation along a certain path in the

molecule and when it is due to an excess charge which may move towards one or other ends of this path, then the wave-length of absorption will be decreased by any influence that diminishes the amount of the charge'. In this particular case the excess charge is an electron pair and the path of oscillation is the vinylogous amidine moiety of 2-aminophenazine. Any electron-withdrawing group placed in the 1-position will diminish the



amount of charge oscillating in the system. The strong hypsochromic shifts observed when the substituent is -COOH, $-CO_2CH_3$ $-CONH_2$ or -CN, and the absence of a shift for -Br or $-COO^-$ are thus explained.

INFRA-RED SPECTRA

The low solubility of the aminophenazine carboxylic acids and their esters in organic solvents has already been alluded to. The acids were found to be almost totally insoluble in those organic solvents suitable for infra-red spectroscopy, and were therefore examined in the solid phase. The esters proved sufficiently soluble in chloroform to be measured as very dilute solutions, and for comparative purposes were also examined in the solid phase.

The interpretation of solid state spectra is confused by intermolecular interactions and anomalous dispersion effects (113). In particular the use of pressed halide discs has been criticised because of changes that may occur in the compound on grinding (114) and because interactions may take place with the alkali halide (115, 116). However, a comparison of the

spectra of several of the phenazine acids as Nujol mulls and as potassium chloride discs, showed that the major and identifiable peaks occurred at the same frequencies in both media. The spectrum was much more clearly defined for the discs - only the major bands being resolved in the mulls - and this dispersion medium was therefore persisted with.

One of the initial aims of the examination of infra-red spectra was that characteristic patterns might be discernible for differently substituted phenazines, such as have been recently described for other heterocyclic systems (117). This was foiled by the insolubility of the members of the series, and vibration patterns for halide disc spectra in the region 1225 - 950 $cm.^{-1}$ appeared to be unrelated to the position of substitution. For these purposes it seems imperative that spectra be examined in solution.

In the discussion that follows only those regions of the spectrum where bands can be assigned with reasonable certainty are considered. These are the 3500 - 3000 $cm.^{-1}$ region (NH stretch), the 1750 - 1650 $cm.^{-1}$ region (C = O vibration), and the 1650 - 1600 $cm.^{-1}$ region (NH deformation and C = C and C = N stretch). These are chosen also because considerable variations are observed among the individual compounds reported here, and in many cases these variations can be reconciled with molecular structures.

Frequencies of absorption bands are collected in Tables III to V. In Table III the spectra of the esters of the seven isomeric aminophenazine carboxylic acids are compared in chloroform solution, together with several other substituted phenazines which were soluble enough to be measured this way. 2-Aminophenazine-1-carboxylic acid, which has quite different

solubility properties to its isomers, is also included. Path lengths vary according to the solubility of the compound examined; in all cases the solutions are for spectroscopic purposes 'dilute'. In Table IV the spectra of the esters in potassium chloride discs are recorded, and in Table V the spectra of the acids in the same medium.

Measurements: Spectra were recorded on a Unicam S.P. 100 double-beam infra-red spectrometer equipped with an S.P. 130 sodium chloride prism-grating double monochromator operated under vacuum. Spectra of solutions were determined in matched cells. Potassium chloride discs were made from 250 mg. of halide and 0.7 mg. of compound

Discussion of results: Interpretation of absorption frequencies for spectra measured in chloroform solution is fairly straightforward. Potassium chloride disc spectra are complicated by the fact that three of the aminophenazine carboxylic acids (with the carboxyl-group in the 3,7 and 8-positions) were non-crystalline materials and gave poorly defined curves. Evidence for the non-crystallinity is largely adduced from the handling characteristics of the acids, but comparative X-ray powder photographs of the 9 and 3-isomers showed that whereas the former is found in a regular crystal lattice, the latter shows 'randomness in the third dimension' (117a). Possible reasons for this state are discussed in the ensuing text. The ester of 2-aminophenazine-1-carboxylic acid was not obtained in a state pure enough to give a satisfactory spectrum as a halide disc, and is recorded only in chloroform solution.

TABLE III

Chloroform Solution

Substituted Phenazine	Concn. w/v	Path-length	Frequency ($cm.^{-1}$)		
			NH stretch	C = O vib.	N - H deform. C = C) C = N) vib.
1. 1-amino	1.2%	0.2 mm.	3505 3395		1631 1610 1593
2. 2-amino	0.4%	0.5 mm.	3505 3410 3230 (w)		1642 1622 1608
3. 1-carbomethoxy	3.5%	0.2 mm.		1731	1627 (w) 1606 (w)
4. 2-amino-1-carbomethoxy	1%	0.2 mm.	3510 3415 (sh) 3355	1676	1637 1613 1590
5. 2-amino-3-carbomethoxy	1.2%	0.2 mm.	3510 3385	1714	1639 1612 1586
6. 2-amino-4-carbomethoxy	0.2%	1 mm.	3510 3415 3230 (w)	1735	1640 1620 1609
7. 2-amino-6-carbomethoxy	0.6%	0.5 mm.	3510 3415 3230 (w)	1730	1641 1620 1607
8. 2-amino-7-carbomethoxy	0.1%	1 mm.	3515 3420 3235 (w)	1724	1647 1624 1610
9. 2-amino-8-carbomethoxy	0.3%	0.5 mm.	3510 3415 3235 (w)	1726	1645 1625 1612
10. 2-amino-9-carbomethoxy	0.8%	0.2 mm.	3510 3415 3230 (w)	1730	1642 1623 1605
11. 1-carboxy	1%	0.2 mm.		1745 1725 (sh)	1627 (w) 1607 (w)
12. 2-amino-1-carboxy	1%	0.2 mm.	3500 3400 (sh) 3290	1684	1634 1617 1585
13. Unsubstituted	2.5%	0.2 mm.			*1632 (w) *1562 (w)

* Too weak to be definitely measured. sh = shoulder

TABLE IV

Potassium Chloride Discs

Substituted Phenazine	Frequency ($cm.^{-1}$)		
	NH stretch	C = O vib.	NH deform. C = C / C = N stretch
1. 2-amino 3-carbomethoxy	3450 3305 3180	1706	1633 1604
2. 2-amino 4-carbomethoxy	3385 3340 3215	1718	1657 1620 1600
3. 2-amino 6-carbomethoxy	3445 3330 3190	1721	1651 1630 1600
4. 2-amino 7-carbomethoxy	3425 3345 3200	1719	1661 1643 1602
5. 2-amino 8-carbomethoxy	3445 3330 3198	1717	1656 1633 1607
6. 2-amino 9-carbomethoxy	3430 3340 3215	1724 1710	1656 1635 1602
7. 1-carbomethoxy		1724	1625 (w) 1602 (w)
8. 1-amino 6-carbomethoxy	3445 3355	1707	1627 1608

TABLE V

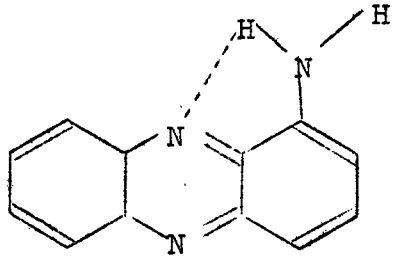
Potassium Chloride Discs

Substituted Phenazine	Frequency ($cm.^{-1}$)		
	NH stretch	C = O vib.	NH deform. C = C) C = N) stretch
1. 2-amino	3415 3330 3215		1654 1630 1604
2. 1-amino	3485 3345		1631 1612 1598 (sh)
3. 2-amino 1-carboxy	3370 3280 3200	1687	1640 1614
4. 2-amino 3-carboxy	3340 3250	1664	1644 1608
5. 2-amino 4-carboxy	3440 3355 3230	1727	1658 1636 1609
6. 2-amino 6-carboxy	3395 3330 3230	1728	1657 1630 1600
7. 2-amino 7-carboxy	3460 3345 3225	1710	1660 1610
8. 2-amino 8-carboxy	3360 3230	1707	1647 1613
9. 2-amino 9-carboxy	3435 3350 3240	1697	1640 1628 1596
10. 1-carboxy		1747	1624 1602
11. 2-carboxy		1715	1637 1607
12. 1-amino 6-carboxy	3480 3350	1719	1629 1609

The 3000 $cm.^{-1}$ region

Chloroform solution: In chloroform solution (Table III) all the aminophenazines examined show the normal two bands attributed to NH symmetric and asymmetric stretching modes, with the lower-frequency symmetric mode displaying the higher intensity. The Bellamy and Williams relationship (118), which requires that $\nu_{sym} = 345.53 + 0.876\ \nu_{asym}$, is obeyed in all cases where no intramolecular hydrogen bonding is possible. Where such bonding is possible the higher frequency band remains unchanged, while the lower frequency band is broadened and shifted to longer wave-length.

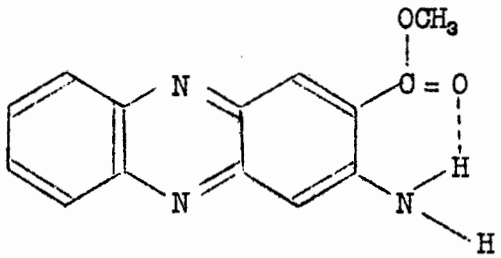
The normal separation between the two NH bands is 95 $cm.^{-1}$ in this series. Where hydrogen bonding is possible this separation increases and the magnitude of the increment correlates well with the expected strength of the hydrogen bond. Thus for 1-aminophenazine there is an increment of 15 $cm.^{-1}$ (a separation of 110 $cm.^{-1}$) which is consistent with the weak bonding which must exist in the strained five membered ring (VIII).



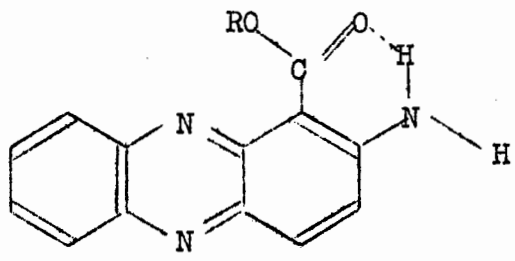
VIII

Similar bonding has been proposed for the closely analogous cases of 1-aminoacridine and 8-aminoquinoline (119). In 2-aminophenazine substituted by a carbomethoxyl-group in the 3-position, a six membered hydrogen bond ring is possible (IX) and a

larger increment is observed (30 $cm.^{-1}$). In 2-aminophenazine-1-carboxylic



IX



(X)

acid and its ester (X) the increments are very large indeed (115 $cm.^{-1}$ and 60 $cm.^{-1}$) and indubitably tied up with the phenomenon of conjugate chelation (to be discussed later). In these latter two cases a band attributable to the free symmetric stretching mode can be observed as a shoulder at the normal separation (95 $cm.^{-1}$) from the asymmetric frequency.

An additional very weak band is found at 3230 - 3235 $cm.^{-1}$ in all the aminophenazines where no intramolecular hydrogen bonding of the amino-group is possible (Nos. 2, 6, 7, 8, 9, 10, Table III). A similar band, but of greater intensity, has been reported by Angyal and Werner in their study of heterocyclic amines in chloroform solution (120). It seems probable that this is related to intermolecular interactions, but because the solutions were already so dilute, dilution studies could not be used to confirm this.

KCl disc spectra: The pattern of behaviour in the solid state (Tables IV, V) is not as clearly defined as in solution. In general there appear to be

three NH vibration frequencies, falling into the broad ranges 3450 - 3370 cm.$^{-1}$, 3350 - 3305 cm.$^{-1}$ and 3240 - 3180 cm.$^{-1}$. Although it has been stated that two NH bands in the solid state are usual (120), Bellamy has observed three absorption peaks for a number of amines and ascribed these to the simultaneous occurrence of free and intermolecularly bonded vibrations (121). If the non-crystalline 2-aminophenazine-3 and 8-carboxylic acids are ignored, it will be seen that the 2-aminophenazines show three NH bands, while the three 1-aminophenazines show only two such bands. This is of interest since Flett has observed a triplet of NH peaks for 2-aminoanthraquinone and β-napthylamine and only a doublet for 1-aminoanthraquinone and α-napthylamine when these compounds are measured in the solid state (122). No explanation for this point has so far been advanced, but it appears that it must be bound up with the steric influence on intermolecular association.

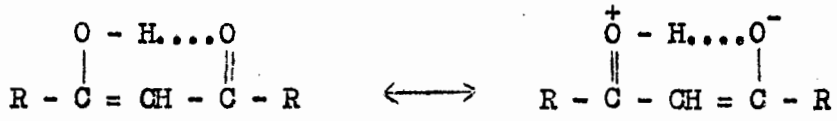
The carbonyl stretching region

In chloroform solution the carbonyl band for the esters falls in the range 1724 - 1735 cm.$^{-1}$, providing no intramolecular hydrogen bonding is possible (Table III, Nos. 3, 6, 7, 8, 9, 10). This accords well with the value of 1730 - 1717 cm.$^{-1}$ quoted by Bellamy, and 1723 - 1737 cm.$^{-1}$ quoted by Brooks et al for a series of substituted benzoates (123). The lowest values are found for the two esters where the carbomethoxyl-group is not adjacent to a ring nitrogen.

Where intramolecular hydrogen bonding with the amino-group is possible, the carbonyl band shifts to lower frequency. For methyl 2-aminophenazine-3-carboxylate (IX) the shift is a small one, the band being found at

1715 cm.$^{-1}$, but for the 1-substituted aminophenazine acid and ester (X) the carbonyl peak appears at the considerably lower values of 1684 cm.$^{-1}$ and 1676 cm.$^{-1}$ respectively. These shifts parallel the shifts in the symmetric NH vibration frequency already discussed. It must be noticed that although 2-aminophenazine-1-carboxylic acid has a higher C = O frequency (1684 cm.$^{-1}$) than its ester (1676 cm.$^{-1}$), the lowering of this frequency induced by hydrogen bonding is greater since the non-bonded phenazine-1-carboxylic acid has a higher C = O frequency (1745 cm.$^{-1}$) than its ester (1731 cm.$^{-1}$). This trend is parallelled in the NH frequency shifts.

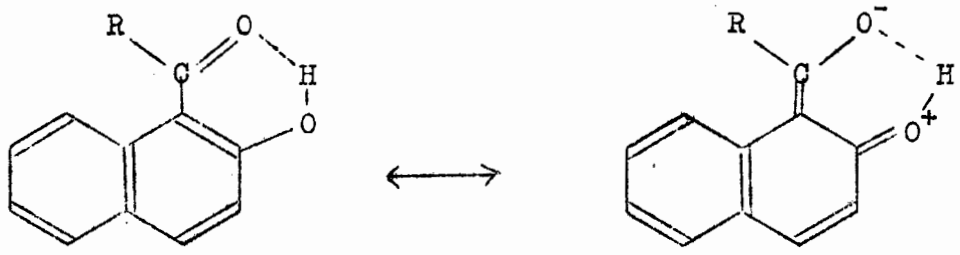
The considerable lowering of the carbonyl frequency in the 1-substituted 2-aminophenazines is certainly due to conjugate chelation. This phenomenon, which has been most extensively studied in the β-diketones (124), is quite different to normal hydrogen bonding in that the double-bond character of the carbonyl bond is reduced by a resonance effect and its vibration frequency accordingly lowered, e.g. XI and XII.



(XI) (XII)

Conjugate chelation has been described for hydroxy-esters (125), hydroxy-acids (126) and amino-esters and acids (125, 127), in all of which the carbonyl frequency is drastically modified by the reduction of its double-bond character. More important, this type of chelation has been used to measure the double-bond character of aromatic systems in which a certain amount of 'bond-fixation' is expected, i.e. napthalene (128) and

phenanthrene (129). The assumption here is that the greater the double-bond character of the ring bond separating the chelating groups, i.e. the 1,2-bond in napthalene (XIII), the greater will be the contribution of the charge-separation form (XIV) to the resonance hybrid and the greater the lowering



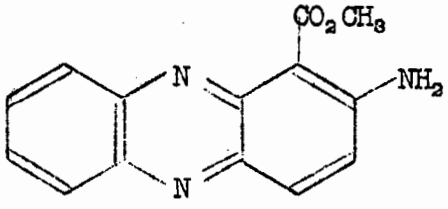
XIII (XIV)

of the C = O frequency. Percentage double-bond character thus calculated agrees well with the values assigned to these bonds by Pauling (130).

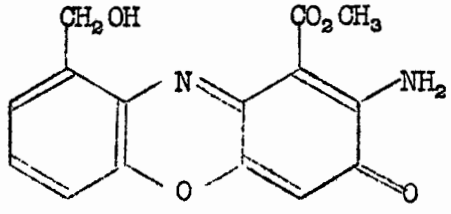
Quantitative calculations of double-bond character as carried out by Hunsberger for the napthalene-1,2 and 2,3-bonds and the phenanthrene-9,10-bond (128, 129) would be interesting for the 1,2 and 2,3-bonds of phenazine. Unfortunately the low solubility of many of the phenazine derivatives in chloroform restricts the amount of information available. It is interesting to note, however, that the lowering in the carbonyl band frequency when the carbomethoxyl and amino-groups straddle the 2,3-bond of phenazine is 16 $cm.^{-1}$ and when they straddle the 1,2-bond is 55 $cm.^{-1}$. These shifts are related to the position of the unchelated carbomethoxyl-group, and the assumption must be made that 2-carbomethoxyphenazine (which was not available) has its carbonyl peak at about the same frequency as 1-carbomethoxyphenazine. Similarly the shift in NH stretching frequency is 30 $cm.^{-1}$ for chelation

about the 2,3-bond and 60 cm.$^{-1}$ for the 1,2-bond. Since Hunsberger has shown that relative shifts are directly related to double-bond character, it may be deduced from these approximate figures that the 1,2-bond in phenazine has between two and three times the double-bond character of the 2,3-bond. This value agrees well with calculation of double-bond character by the Pauling method (130), and is also indicated by the experimental determination of bond-lengths in the molecule (131).

One further point of interest is that the carbonyl frequency in methyl 2-aminophenazine-1-carboxylate (XV) is identical to that in methyl cinnabarin (XVI), a compound of closely analogous structure (132).



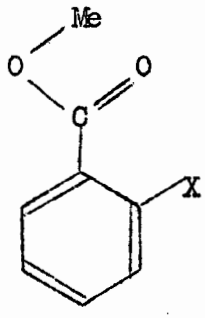
(XV)



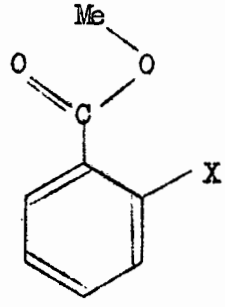
(XVI)

As potassium chloride discs the esters show the normal shift to lower frequencies associated with this change of phase and are found in the range 1718 - 1724 cm.$^{-1}$. The 2,3-substituted ester is somewhat lower at 1706 cm.$^{-1}$, presumably again due to intramolecular hydrogen bonding. The 1,6-substituted ester is likewise found at a low frequency (1707 cm.$^{-1}$), but here no hydrogen bonding is possible, and the reason for the low value is obscure. No other carboxyl-substituted 1-aminophenazines were available for comparison.

Methyl 2-aminophenazine-9-carboxylate is unique in the series in that it shows a double carbonyl band (1724 cm.$^{-1}$ and 1710 cm.$^{-1}$). A possible explanation is that this is a conformational effect of the type recently described for a number of ortho-substituted methyl benzoates (123). Splitting of the C = O band in these compounds is ascribed to conformational isomerism between the structures XVII and XVIII, and it requires no great innovation to extend this to the structures XIX and XX for the substituted

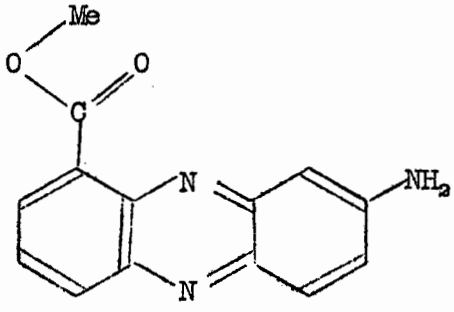


(XVII)

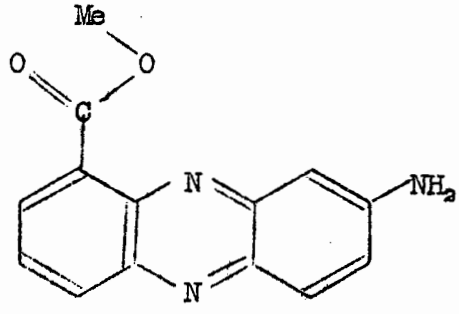


(XVIII)

phenazine. The absence of a carbonyl band splitting in the analogous 4



(XIX)

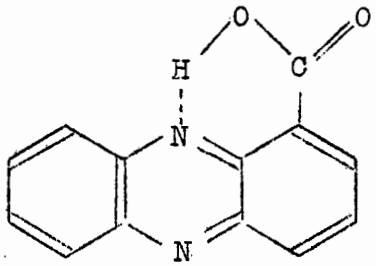


(XX)

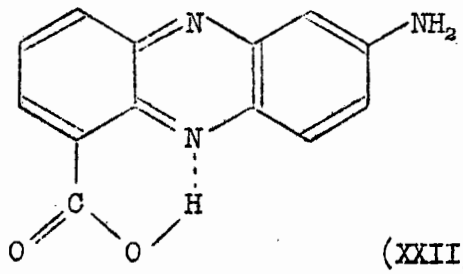
and 6-substituted esters may be related to the greater basicity of the nitrogen atom meta to the amino-group. In the ortho-substituted benzoates

the nature of the substituent is all-important in determining whether a carbonyl splitting occurs (123).

As potassium chloride discs the various phenazine acids examined show some diverse and interesting trends. When the carboxyl-group is adjacent to a ring nitrogen atom, but not adjacent to an amino-group, a considerably higher than normal value for the carbonyl frequency is found. Phenazine-1-carboxylic acid (XXI) absorbs at 1747 $cm.^{-1}$ and 2-aminophenazine-4 and 6-carboxylic acids at 1727 and 1728 $cm.^{-1}$ respectively. These are well outside the range 1680 - 1700 $cm.^{-1}$ quoted by Bellamy for aryl acids (133), 1710 - 1715 $cm.^{-1}$ for the pyridine carboxylic acids (134, 135) and ca. 1700 $cm.^{-1}$ for isonicotinic, quinaldinic and cinchoninic acids (136), all of which are believed to exist in the solid state as dimers. The high value indicates that the carboxyl-group in the three phenazines mentioned is not dimerised, and that it is the monomer frequency which is being recorded. Monomer frequencies for carboxylic acids have been reported to be in the range 1735 - 1750 $cm.^{-1}$ for dilute carbon tetrachloride solution (123). Normally, due to the powerful tendency to dimerisation the monomer frequency of a carboxyl-group can only be measured in very dilute solution and is then found together with the dimer frequency. But in these acids it is possible that dimerisation of the carboxyl-group is prevented by intramolecular hydrogen bonding of the hydroxyl-group of the carboxyl-function on to the nitrogen atom of the ring, e.g. XXI and XXII, and only the monomer frequency is thus observed.



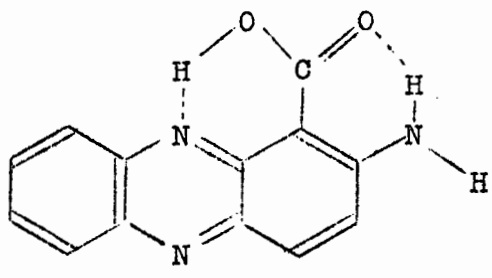
(XXI)



(XXII)

Although the prevention of dimerisation by a similar chelation has been reported for solution spectra, this has nothitherto been observed to persist in the solid phase (137). The lower values of the carbonyl frequency in the amino-acids compared to phenazine-1-carboxylic acid are in accordance with the well-established principle that the C = O frequency decreases with increasing electron-density of the ring-system to which it is attached (138, 139).

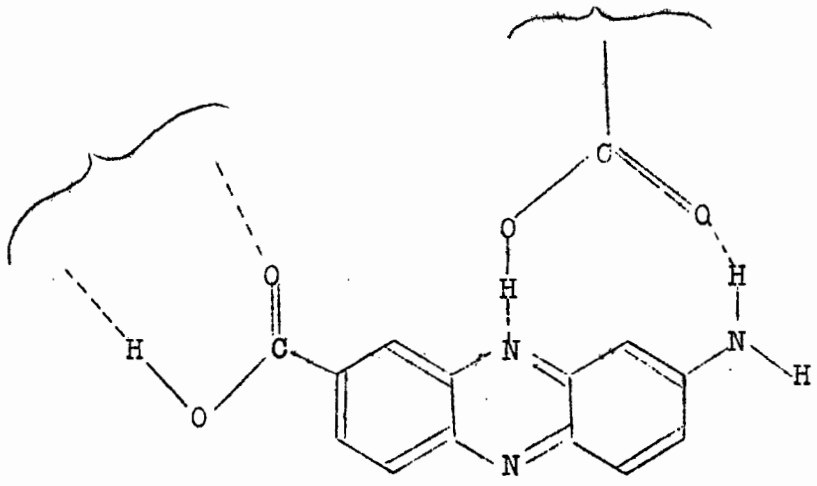
The carbonyl frequency of 1687 $cm.^{-1}$ for 2-aminophenazine-1-carboxylic acid (XXIII) is expected in view of the strong chelation which must exist in the molecule. But 2-aminophenazine-3-carboxylic acid, where the chelation is not expected to be as strong because of the reduced double-bond character of the 2,3-bond, has an even



(XXIII)

lower carbonyl band (1664 $cm.^{-1}$); and furthermore is obtained as a non-crystalline powder, whatever its mode of purification. Non-crystallinity is found also in the 7 and 8-carboxyl-substituted 2-aminophenazines and they too have depressed C = O frequencies at 1710 and 1707 $cm.^{-1}$ respectively.

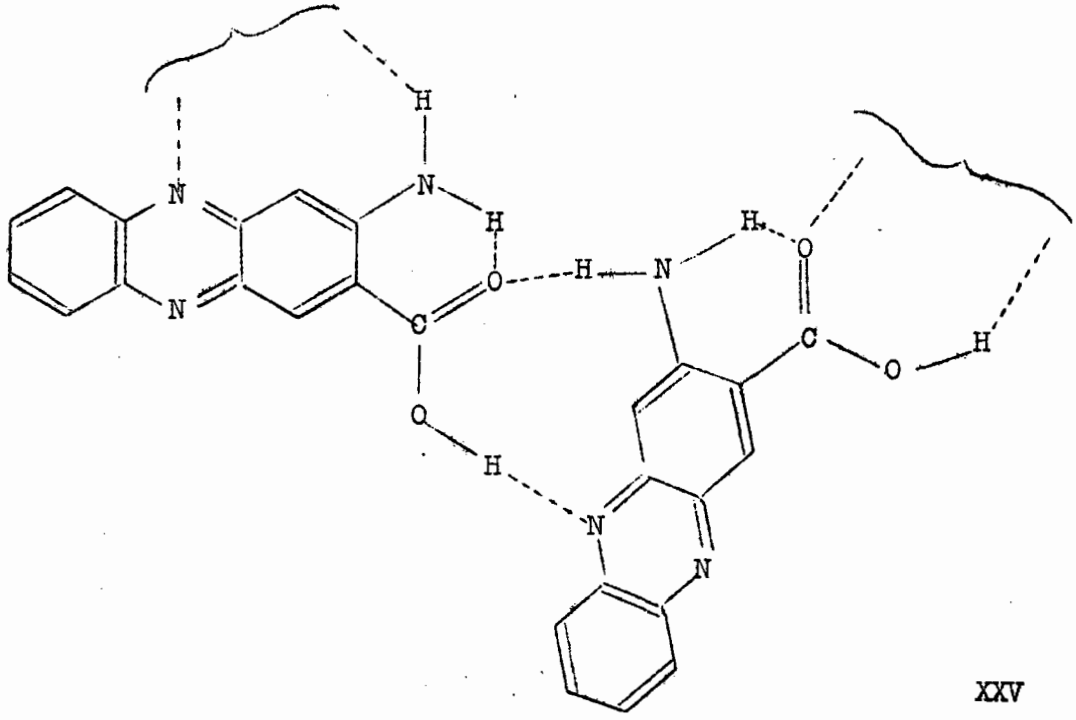
It seems unlikely that the lowered carbonyl frequency and non-crystallinity of these three acids is due to a simple dimerisation of the type normally ascribed to carboxylic acids in the solid state. Dimerisation is more probable for phenazine-2-carboxylic acid and this is crystalline, has a discrete melting point and a C = O band at 1715 $cm.^{-1}$. Rather it is felt that intermolecular association must be leading to hydrogen-bonded chains of molecules, which can then not conveniently pack into a crystal lattice. This association must involve the -OH group of the carboxyl-function (since it disappears on esterification), the C = O group of the carboxyl-function (because of the depression of the carbonyl frequency) and the amino-group (since it is absent in phenazine-2-carboxylic acid). A proposed structure consistent with these demands for 2-aminophenazine-8-carboxylic acid (or the 2,7-substituted isomer) is XXIV, in which the carboxyl-group of one molecule 'straddles' across the - N = C - C = C - NH_2 part of another molecule, to give indefinite chains of molecules. That such a 'straddle' is sterically possible has been confirmed by models and scale-drawings. Molecular chains of this type would be consistent with the observed properties of the acids,



XXIV

viz. no discrete melting points, insolubility in all but acidic or basic solvents, non-crystalline, but yielding a crystalline hydrochloride and sodium salt. It also explains why this behaviour is only found with the 3,7 and 8-isomers.

For 2-aminophenazine-3-carboxylic acid the representation of chains must be slightly different, for each carbonyl oxygen must simultaneously be engaged in both intermolecular and intramolecular hydrogen bonding, e.g. XXV. It has been demonstrated that a carbonyl-group can participate

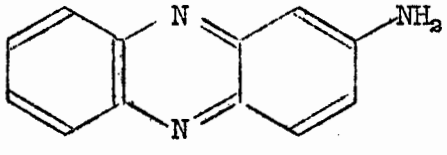


XXV

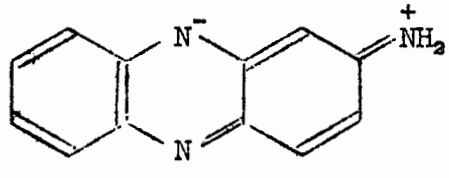
simultaneously in two different hydrogen bonds, either both intramolecular (122) or one intermolecular and one intramolecular (140). This leads to a lowering of the carbonyl frequency which is greater than the depression caused by a single hydrogen bond, and accounts well for the value of 1664 cm.$^{-1}$ found in the 2,3-acid.

The high carbonyl frequencies for 2-aminophenazine-4 and 6-carboxylic acids should be paralleled in the 9-substituted acid, where the carboxyl-group is likewise adjacent to a nitrogen of the ring. This is not the case, the band lying instead at 1697 cm.$^{-1}$. This puzzling value is not reproduced in the ester, for the C = O vibration returns to 1724 cm.$^{-1}$, though it has been split into a double peak. It appears that the decrease is therefore related to the hydroxyl-group of the carboxyl-function.

The following possible explanation is put forward somewhat tentatively. It has been pointed out in the ultra-violet section that the normal Kekule structure for 2-aminophenazine (I) does not adequately describe the true state of the molecule, and that a charge-separation structure (II) must contribute somewhat to the resonance hybrid. In other terms the ring

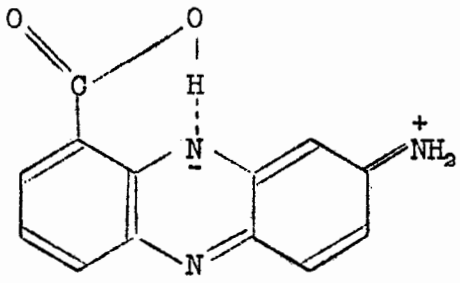


(I)



(II)

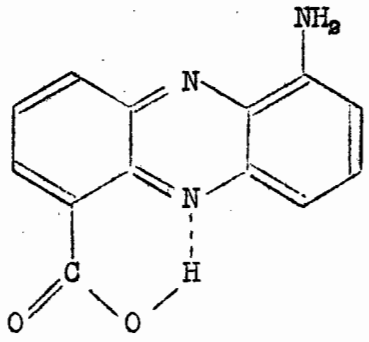
nitrogen meta to the amino-group has an enhanced basicity. This is obviously going to influence a carboxyl-group in the 9-position, by increasing its tendency to transfer a proton to the nitrogen atom. Complete transfer presumes a zwitterion structure and would leave a carboxylate-group absorbing around 1600 cm.$^{-1}$. This is not proposed. Instead it is suggested that the contribution of the dipolar structure (XXVI) to the resonance hybrid,



XXVI

increases the tendency of the hydroxyl oxygen to part with its proton to the nitrogen atom. This in turn increases the negative charge on that oxygen atom and thus reduces the double bond character of the carbonyl bond, in the same way that full ionisation of a carboxyl-function eliminates the discrete double-bond character of the carbonyl-group. Put slightly differently, the distance of hydrogen from oxygen in the O - H ..... N hydrogen bond is going to increase, and this will enhance the 'carboxylate' character of the carboxyl-group and thus decrease the C = O frequency.

The carbonyl band of 1-aminophenazine-6-carboxylic acid is also found at a somewhat depressed frequency (1719 cm.$^{-1}$). In this case, however, it is at a higher value than in the ester, and it seems likely that the structure (XXVII) describes the conformation of the molecule. The low



(XXVII)

values in both acid and ester are probably due to electronic effects characteristic of 1-aminophenazines. The absence of any other carboxyl-substituted 1-aminophenazines did not allow this point to be investigated.

The suggestion was made recently that quinoline and pyridine carboxylic acids exist in the solid state predominantly as zwitterions (135). Evidence for this was adduced from the lowered intensity of carbonyl bands in the 1700 $cm.^{-1}$ region relative to that of benzoic acid. However, in the spectra recorded no additional band appears in the region (1610 - 1550 $cm.^{-1}$) associated with the carboxylate ion absorption, and the intensity measurements must be suspect when the broadness and diffuseness of the carbonyl peaks are considered. Subsequent workers have disregarded this study and concluded that the presence of relatively sharp C = O bands in the 1700 $cm.^{-1}$ region presupposes a non-zwitterionic structure for heterocyclic carboxylic acids in the solid state (136, 134). The spectra of the phenazine carboxylic acids and aminophenazine carboxylic acids support this conclusion; not only because of sharp C = O bands in the 1700 $cm.^{-1}$ region, but also because the pattern of NH stretching vibrations is very similar in the aminoacids and the amino-esters. A zwitterionic structure would require a

NH^+ or NH_3^+ group, and this should produce absorption at lowered frequencies.

The 1650 - 1600 cm.$^{-1}$ region

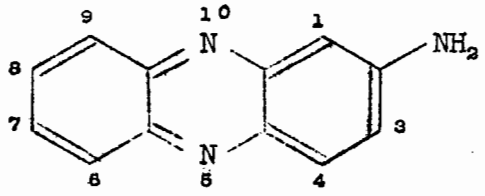
Though unambiguous assignment of bands in this region is not always possible, it seems fairly certain that the band near 1640 cm.$^{-1}$ in chloroform solution and near 1650 cm.$^{-1}$ in potassium chloride is due to the NH deformation mode. The general range 1650 - 1590 cm.$^{-1}$ has been quoted for this mode (141), and no band near 1650 cm.$^{-1}$ is found for phenazines not bearing an amino-substituent, i.e. 1-carboxy-, 1-carbomethoxy-, 2-carboxy- and phenazine itself. Furthermore in each case the frequency increases on passing from solution to the solid phase, a point characteristic of deformation modes, and one that has been described for primary amides (142). In the solid phase the frequency of this mode appears slightly lower for the 1-aminophenazines examined.

The remaining (usual) two bands in the 1650 - 1600 cm.$^{-1}$ region are very much less easy to assign. Absorptions arising from C = C and C = N vibrations have not yet been well positioned for different heterocyclic systems, particularly with more than one ring (117). Two points, however, are worthy of note. Firstly the introduction of an amino-group into the phenazine nucleus, not only gives rise to a third band in the region 1650 - 1600 cm. , but also greatly increases the intensity of the other two bands. Intensification of the C = C and C = N bands in heterocyclic systems by electron-donating substituents has been adequately described by Katritzky (143 - 146), and is a point in favour of assignment of these to the C = C and C = N vibration modes. Secondly the relative intensities of the three

bands in the 1650 - 1600 cm.$^{-1}$ region present an interesting pattern for the different 2-aminophenazines examined in chloroform solution. The same relative intensities are observed for the 2,1 and 2,3-substituted esters, and for the 2,4, 2,6 and 2,9-esters, while the 2,7 and 2,8-esters each have their own and different intensity patterns. Measurement of the spectra of further substituted phenazines would show whether these intensity patterns are of any diagnostic value for determining the position of substitution.

ESTERIFICATION

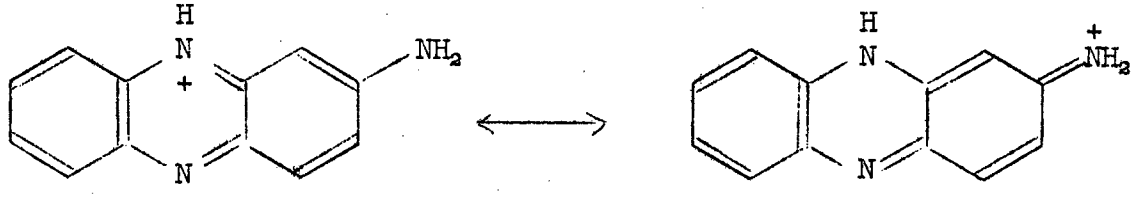
In Section I it was reported that 2-aminophenazine-9-carboxylic acid could not be converted to its methyl ester by refluxing in excess methanol containing about 2% by weight of sulphuric acid as catalyst. This was surprising in view of the easy esterification under identical conditions of 2-aminophenazine-4 and 6-carboxylic acids, both of which also contain carboxyl-groups adjacent to a nitrogen atom of the ring. The investigation was extended to the remaining members of the series and it was found that



the 7 and 8-substituted isomers were esterified rapidly, the 3-isomer extremely slowly and the 1-isomer not at all. In each case the same conditions were used and the progress of

the reaction followed semi-quantitatively by paper chromatography.

In order to explain these results the assumption must be made that under the conditions of the esterification, the aminophenazines exist predominantly in the monoprotonated form, and that the proton is attached to the nitrogen at position 10. Evidence for the latter assumption has been presented by Kehrmann from spectroscopic studies (147); the former condition also seems likely in view of the fact that distinctly different colours are observed for the mono-, di- and trications of 2-aminophenazine (148) and that the deep red colour under the conditions of esterification corresponds to that of the monocation. The resonating cation (III $\leftrightarrow$ IV) is therefore in all probability the species being dealt with.



(III) (IV)

In the acid-catalysed esterification of carboxylic-groups by primary alcohols, the first step is a rapid reversible protonation of the carboxyl-function (see below). The alcohol may then react with the conjugate acid

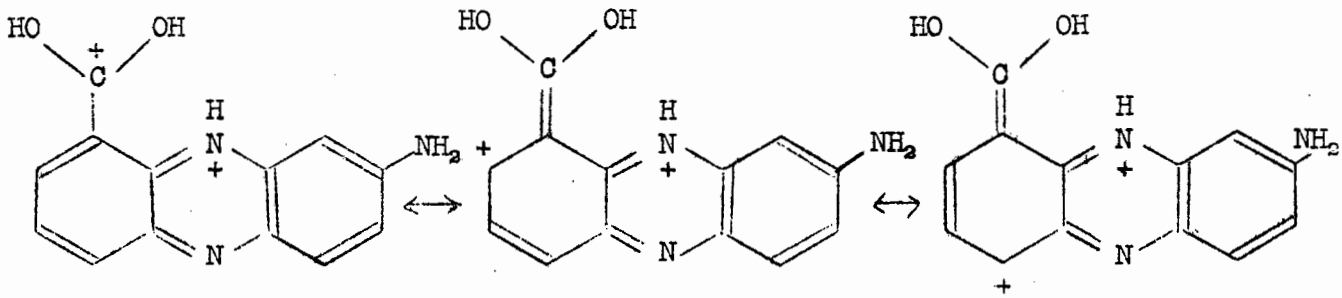
$$RCOOH + H^+ \rightleftharpoons [RCOOH_2]^+$$

either by bimolecular substitution or by acylium ion formation (149, 150). Though attack by the alcohol is normally the rate-controlling process, the ease of formation of the conjugate acid and its stability can also be

determining factors.

In the esterification of 2-aminophenazine-1,3 and 9-carboxylic acids, the carboxyl-group is in each case adjacent to a nitrogen atom bearing a partial positive charge. Protonation of the carboxyl-group will therefore be inhibited by electrostatic repulsion; this will be more strongly felt in the 1 and 9-isomers because of the greater contribution of structure (III) to the resonance hybrid, (see p. 70), than in the 3-isomer. As has been mentioned already, the 3-isomer can be esterified slowly, the 1 and 9-isomers not at all.

A further factor must be taken into account. If protonation of the carboxyl-group is possible, stabilisation of the conjugate acid formed is usually achieved by resonance among structures in which the positive charge is shared with the ring. For the 2,9-substituted isomer examples are XXVIII - XXX. But this presupposes that the oxygen atoms lie in the same



(XXVIII) (XXIX) (XXX)

plane as the ring, and the protonated nitrogen atom could make this difficult. An explanation of this type has been used to account for the failure of di-ortho-substituted benzoic acids to be esterified by the Fischer method (151).

TABLE VI

Physical Characteristics

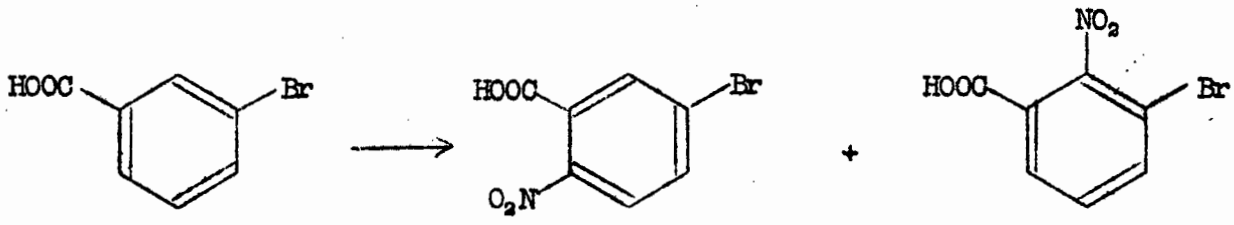
Substituted 2-aminophenazine	m.p. (uncorrected)	Appearance and recrystallising solvent	Colour in solution		Fluorescence in EtOH
			EtOH	N/10 HCl	
1. 1-Carboxy	274° - 276° (dec.)	Yellow-orange needles, H_2O/EtOH	Yellow	Yellow	Yellow-orange
2. 3-Carboxy	320° (dec.)	Purple powder, nitrobenzene	Red	Purple	Not fluorescent
3. 4-Carboxy	304° (dec.)	Magenta needles, methanol	Orange	Purple-red	Orange
4. 6-Carboxy	330° slow dec.	Magenta needles, nitrobenzene	Orange-red	Purple-red	Orange
5. 7-Carboxy	-	Red powder, nitrobenzene	Orange	Red	Orange
6. 8-Carboxy	-	Red powder, nitrobenzene	Orange-red	Purple-red	Orange
7. 9-Carboxy	346° (dec.)	Red-black needles, nitrobenzene	Orange-red	Purple-red	Orange
8. 1-Carbomethoxy	165°- 169°	Yellow microneedles, -	Yellow	Yellow	Yellow-green
9. 3-Carbomethoxy	235°	Red-purple needles, pet. ether	Red	Purple	Not fluorescent
10. 4-Carbomethoxy	230° - 235°	Red-orange needles, toluene	Yellow-orange	Purple-red	Yellow-orange
11. 6-Carbomethoxy	205° - 206°	Bright red needles, toluene	Yellow-orange	Purple-red	Orange
12. 7-Carbomethoxy	264°	Bright red needles, toluene	Orange	Red	Orange
13. 8-Carbomethoxy	256° - 257°	Bright red needles, toluene	Orange	Purple-red	Red
14. 9-Carbomethoxy	187 - 188	Red-orange needles toluene	Orange	Purple-red	Orange

EXPERIMENTAL SECTION

SECTION I PART I

THE SYNTHESIS OF 2-AMINOPHENAZINE-3-CARBOXYLIC ACID

Mono-nitration of m-bromobenzoic acid



The method of nitration used was that of Friedlander, Bruckner and Deutsch (34). The isomers were separated by the method of Hollemann (35). m-Bromobenzoic acid (100 g.) gave 5-bromo-2-nitrobenzoic acid (30.5 g.), m.p. 137° - 140° (given 140°), and 3-bromo-2-nitrobenzoic acid (2.35 g.), m.p. 246° - 250° (given 250°).

Reaction of 5-bromo-2-nitrobenzoic acid with o-phenylenediamine

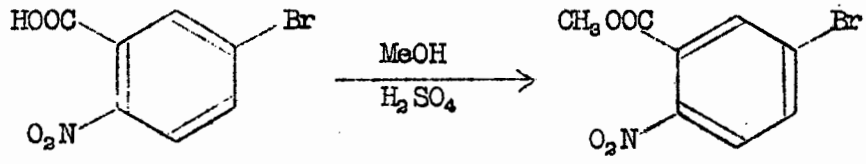
5-Bromo-2-nitrobenzoic acid (10 g.), o-phenylenediamine (6 g.) anhydrous potassium carbonate (7.4 g.) and copper powder (0.2 g.) were refluxed for 1 hour in amyl alcohol (100 cc.). The solvent was removed in steam and the residual solution acidified with concentrated hydrochloric acid. The precipitate of the molecular complex (11.8 g., 100%) was recrystallised from chloroform to give bright yellow needles, m.p. 154° - 156°.

Analysis:

Required for $C_{13}H_{12}N_3O_4$	Found
C 44.1	C 44.1
H 3.4	H 3.4
N 11.8	N 11.4

Ether extraction of an alkaline solution of the complex gave o-phenylenediamine, m.p. and mixed m.p. 102° - 103°, while extraction of an acid solution gave 5-bromo-2-nitrobenzoic acid, m.p. and mixed m.p. 138° - 140°. The compound could also be made by warming equivalent quantities of the acid and base for a few minutes in a minimum volume of water, and allowing to precipitate from the cooled solution.

Preparation of methyl 5-bromo-2-nitrobenzoate



5-Bromo-2-nitrobenzoic acid (15 g.) and concentrated sulphuric acid (15 cc.) were refluxed in absolute methanol (150 cc.) for 6 hrs. The methanol was distilled to small volume, and the residual solution poured into water. Washing the precipitate with a saturated $NaHCO_3$ solution gave the ester (16 g., 95%), m.p. 67° - 70°. It could be recrystallised from petroleum ether to give white needles, m.p. 68° - 70°.

Analysis:

$C_8H_6BrNO_4$ requires	Found
C 36.9%	C 37.0%
H 2.3%	H 2.25%
N 5.4%	N 5.2%
Br 30.7%	Br 30.5%

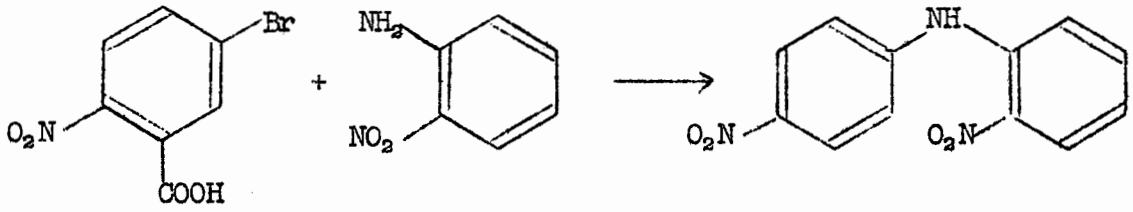
The entry in Heilbron (152) for this ester appears to be an error. The original paper (153) there cited refers to the preparation of methyl 3-bromo-5-nitrobenzoate.

Attempted preparation of methyl 2'-amino-4-nitrodiphenylamine-3-carboxylate

Attempts were made to condense methyl-5-bromo-2-nitrobenzoate and o-phenylenediamine in a molar ratio of 1 : 1.5 under a variety of conditions. Solvent, base, catalyst, reaction temperature and time, and product are tabulated below. The reaction mixture was worked up by removing the solvent (if any) in steam, extracting the residue repeatedly with boiling water and recrystallising from aqueous alcohol.

	Solvent	Base	Catalyst	Temp.	Time	Product
(1)	-	-	-	100°	2 hrs.	Unchanged ester
(2)	-	K_2CO_3		100°	4 hrs.	" "
(3)	EtOH	K_2CO_3	Cu	78°	4 hrs.	" "
(4)	AmOH	K_2CO_3	Cu	137°	1 hr.	" "
(5)	-	K_2CO_3	Cu	160°	3½ hrs.	Tar
(6)	-	K_2CO_3	Cu	100°	1½ hrs.	"
(7)	Cyclohexanol	-	Cu	161°	4 hrs.	"
(8)	Cyclohexanol	-	Cu	161°	2 hrs.	"

Reaction of 5-bromo-2-nitrobenzoic acid and o-nitroaniline



Compare Albert and Linnell, J.C.S., 1936, 1614.

The acid (4.9 g.), o-nitroaniline (4.2 g.), sodium carbonate (1.1 g.) and copper bronze (0.1 g.) were refluxed for half an hour in nitrobenzene (20 cc.). The nitrobenzene was removed in steam, and the dark red precipitate remaining dissolved in boiling ethanol, charcoaled, filtered and precipitated with water. It was insoluble in alkali and had m.p. 218° - 220°, undepressed on mixing with an authentic sample of 2,4'-dinitrodiphenylamine.

Attempted preparation of methyl 2',4-dinitrodiphenylamine-3-carboxylate

(a) Methyl 5-bromo-2-nitrobenzoate (2 g.), o-nitroaniline (1.5 g.) anhydrous potassium carbonate (1 g.) and copper powder (0.1 g.) were refluxed in nitrobenzene (10 cc.) for 2 hrs. Removal of the solvent and excess amine in steam left a brown gummy material which could not be purified.

(b) The above ester (1 g.), o-nitroaniline (0.7 g.) and copper powder (0.02 g.) were finely mixed and fused with slow stirring of the mobile melt at 200° for 2½ hours. Excess amine was removed in steam

to leave a black tar which could not be recrystallised.

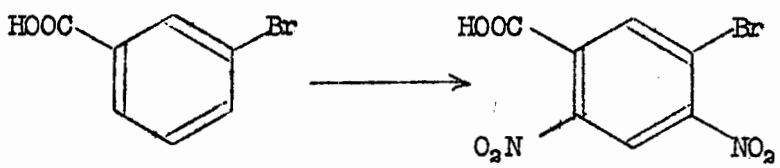
Attempted preparation of 2'-acetamido-4-nitrodiphenylamine-3-carboxylic acid

Compare Goldberg and Kelly, J.C.S., 1946, 102.

5-Bromo-2-nitrobenzoic acid (2 g.) and anhydrous potassium carbonate (1.5 g.) were refluxed with rapid stirring in amyl alcohol (20 cc.) and water (0.6 cc.), while about one tenth of the solvent was allowed to distil off. o-Aminoacetanilide (1.6 g.) and copper powder (0.1 g.) were added to the frothy potassium salt, and reflux continued with stirring for four hours. The solvent was steam-distilled, and the residual aqueous solution filtered hot and acidified with 5 N HCl. The precipitate was extracted with hot 5 N HCl, and the residue recrystallised from aqueous ethanol. The resulting pale brown crystals melted at 134° - 138°, the melting point being undepressed on mixing with starting acid.

Preparation of 5-bromo-2,4-dinitrobenzoic acid

(a) From m-bromobenzoic acid:



Compare Meisenheimer, Zimmermann and Kummer, Ann., 446, 213 (1926).

m-Bromobenzoic acid (10 g.) in concentrated sulphuric acid (100 cc.)

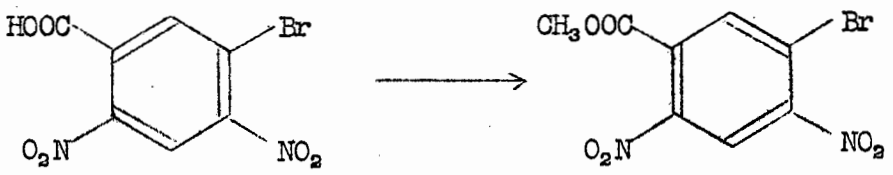
was warmed slowly to 50°C (internal temperature) with rapid stirring. Finely powdered potassium nitrate (5 g.) was added in portions at a rate consistent with keeping the temperature of the solution between 50° and 65°C. The solution was then heated slowly to 105°C and a further 20 g. of potassium nitrate added in such a way as to hold the temperature between 105° and 110°. (If the golden-yellow solution showed signs of darkening before 105° was reached, small portions of potassium nitrate were found to keep it clear and prevent the violent exothermic reaction which otherwise occurred). The solution was further heated to 125°C, and held there for half an hour, after which it was poured on to 500 g. of ice. The pale yellow precipitate of the dinitro acid weighed 9.4 g. (65%) and melted from 160° to 185°.

A portion recrystallised twice from aqueous alcohol gave pale, yellow needles, m.p. 190°. (Goldstein and Stamm (42) report 191°). The main bulk of the acid was used in subsequent steps without further purification.

(b) From 5-bromo-2-nitrobenzoic acid:

5-Bromo-2-nitrobenzoic acid (5 g.) was nitrated in a manner identical to that described for m-bromobenzoic acid. The crude dinitro acid (3.6 g., 59%) after recrystallisation from aqueous alcohol, gave pale yellow needles, m.p. 189° - 190°, undepressed on admixture with the dinitro acid obtained from m-bromobenzoic acid.

Preparation of methyl 5-bromo-2,4-dinitrobenzoate

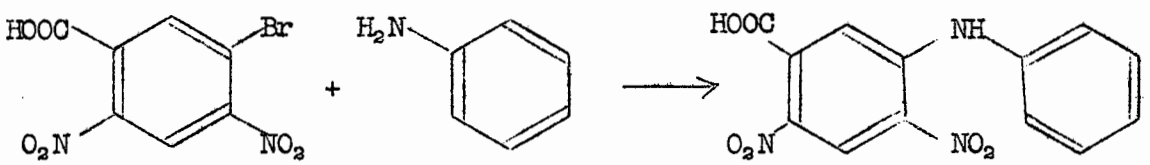


Goldstein and Stamm, Helv. Chim. Acta., 35, 1330 (1952).

Methyl-5-bromo-2,4-dinitrobenzoate, m.p. 96° - 103°, was prepared in 60% yield. Two recrystallisations from aqueous methanol raised the m.p. to 106° (given 107°).

The crude ester was used as such in the next step.

Preparation of 2,4-dinitrodiphenylamine-5-carboxylic acid



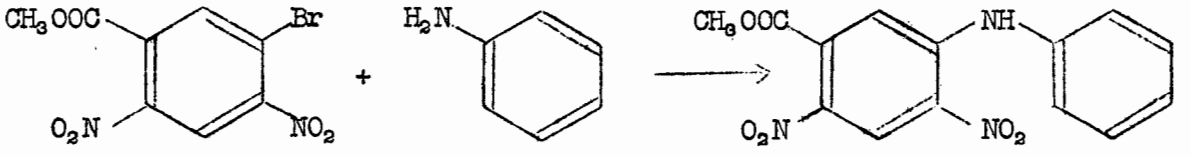
Goldstein and Stamm, Helv. Chim. Acta., 35, 1330 (1952).

The diphenylamine, m.p. 224° - 226°, was prepared in 50% yield. The authors cited quote m.p. 226° - 228°.

Reduction and cyclisation of 2,4-dinitrodiphenylamine-5-carboxylic acid

The above acid (0.200 g.) in absolute alcohol (30 cc.) was hydrogenated at 3 atmospheres over platinum oxide (0.020 g.). Nitrobenzene (25 cc.) was added to the filtered solution, the alcohol removed by distillation and reflux continued for 15 hours. The nitrobenzene was steam distilled, and the precipitated residue filtered. By mixed melting point (275° - 278°) and paper chromatography (BuOH : HCl : H_2O, BuOH : HOAc : H_2O), it was shown to be 2-aminophenazine.

Preparation of methyl 2,4-dinitrodiphenylamine-5-carboxylate



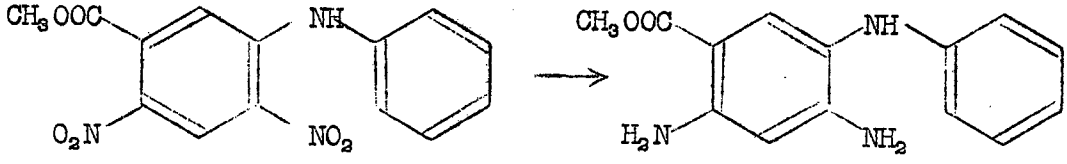
Crude 5-bromo-2,4-dinitrobenzoate (5 g.) and freshly distilled aniline (25 cc.) were heated on a boiling water-bath for 1 hour. The solution was cooled and poured with constant stirring and ice-cooling into 100 cc. of

5 N hydrochloric acid. The resulting precipitate was recrystallised from ethanol to give the diphenylamine ester (4 g., 77%) as yellow-orange plates, m.p. 142° - 146°. Further recrystallisation raised the melting point to 146° - 148°.

Analysis:

Required for $C_{14}H_{11}N_3O_6$	Found
C 53.0%	C 53.0%
H 3.5%	H 3.3%
N 13.2%	N 13.3%

Preparation of methyl 2,4-diaminodiphenylamine-5-carboxylate



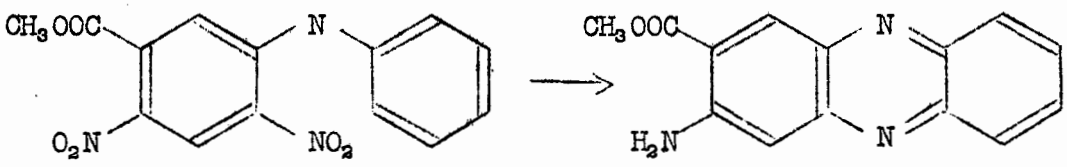
Methyl 2,4-dinitrodiphenylamine-5-carboxylate (0.5 g.) in absolute alcohol was hydrogenated at three atmospheres over platinum oxide. The solvent was removed under reduced pressure in a nitrogen atmosphere. The grey-white residue remaining (0.4 g.) was recrystallised from petroleum ether (charcoal) to give long silver needles of the diaminodiphenylamine, m.p. 163° - 166°.

Analysis:

Required for $C_{14}H_{15}N_3O_2$		Found	
C	65.0%	C	65.7%
H	5.8%	H	5.9%
N	16.3%	N	16.6%

The compound, stored in a stoppered bottle, showed no signs of decomposition after a year.

Preparation of methyl 2-aminophenazine-3-carboxylate



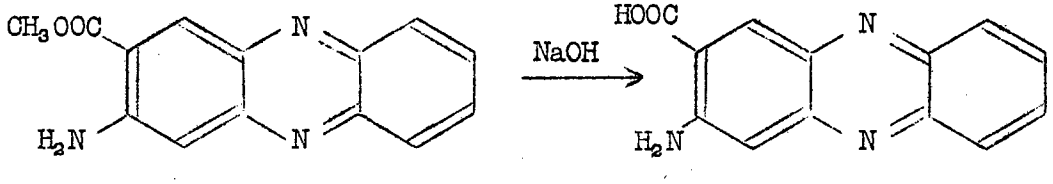
Methyl 2,4-dinitrodiphenylamine-5-carboxylate (1.23 g.) in absolute alcohol was hydrogenated over platinum oxide (0.2 g.) in the usual way. Nitrobenzene (150 cc.), and after distillation of the alcohol, 5% palladium-charcoal (1.2 g.) were added to the filtered solution, and reflux continued for 32 hours. The volume of the nitrobenzene was reduced in high vacuum to about 10 cc. and the oily solution adsorbed on to a Grade O alumina column (2 cm. x 30 cm.). Development of the column with benzene to remove the nitrobenzene, was followed by elution of the main red band with ether. Evaporation of the solvent and recrystallisation of the residue from a large volume of petroleum ether gave the phenazine ester (0.32 g., 32%) as deep red clusters of needles, m.p. 235°.

Analysis:

Required for $C_{14}H_{11}N_3O_2$	Found
C 66.4%	C 66.6%
H 4.3%	H 4.4%
N 16.6%	N 16.9%

Further elution of the column with ether removed an orange band which was identified paper-chromatographically as 2-aminophenazine. The eluting solvent was changed through acetone to water, and displaced a second deep red band. The aqueous eluate was concentrated, acidified with dilute hydrochloric acid, and the precipitate recrystallised from nitrobenzene. It was shown by paper chromatography (BuOH/HCl/H_2O; BuOH/HOAc/H_2O; BuOH/NH_3/H_2O) to be 2-aminophenazine-3-carboxylic acid (40 mg.), present on the column as the sodium salt.

Preparation of 2-aminophenazine-3-carboxylic acid



Methyl 2-aminophenazine-3-carboxylate (50 mg.) was warmed at 100° for 30 minutes in 3 N NaOH (5 cc.). The solution was diluted, filtered and cooled. With 5 N HCl the pH was adjusted to 8 and with acetic acid to 5. The dark purple amorphous precipitate of 2-aminophenazine-3-carboxylic acid (quantitative recovery) appeared pure on paper-chromato-

graphic examination (BuOH : HCl : H_2O; BuOH : NH_3 : H_2O). It reprecipitated from boiling nitrobenzene as an amorphous mass, m.p. 320° (dec.).

Analysis:

$C_{13}H_9N_3O_2$ requires		Found	
C	65.3%	C	65.2%
H	3.75%	H	4.0%
N	17.6%	N	17.4%

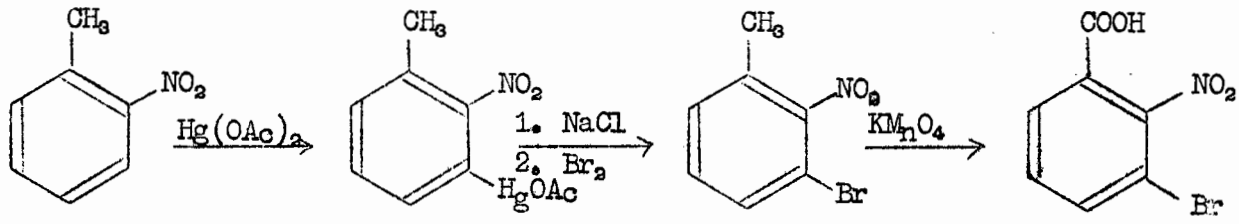
Attempts to render the compound crystalline by slow evaporation of an aqueous solution of its ammonium salt over concentrated H_2SO_4, or by reprecipitation from superheated water were unsuccessful.

SECTION I PART II

THE SYNTHESIS OF 2-AMINOPHENAZINE-9 AND 7-CARBOXYLIC ACIDS

Preparation of 3-bromo-2-nitrobenzoic acid

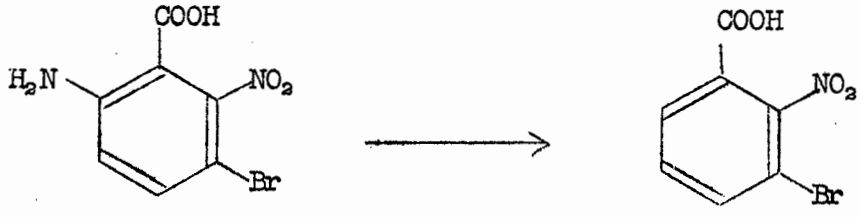
(a) From o-nitrotoluene



Burton, Hammond and Kenner, J.C.S., 1926, 1802.

A yield of 3-bromo-2-nitrobenzoic acid representing less than 1% of theoretical was obtained. The m.p. was 246° - 250° (given 250°). The authors cited do not quote a yield.

(b) From 5-bromo-6-nitroanthranilic acid



Erickson, Dechary and Pullig, J.A.C.S., 74, 5622 (1952).

The preparation described could not be repeated.

Attempted preparation of 2-nitro-4'-aminodiphenylamine-3-carboxylic acid

(1) 3-Bromo-2-nitrobenzoic acid (0.5 g.), p-phenylenediamine (0.4 g.), anhydrous potassium carbonate (0.5 g.) and a trace of copper powder were refluxed in amyl alcohol (5 cc.) for (a) 1 hour, (b) 6 hours.

(2) 3-Bromo-2-nitrobenzoic acid (0.4 g.) and potassium carbonate (0.4 g.) were refluxed in amyl alcohol (5 cc.) and water (3 drops) with rapid stirring, while a portion of the solvent was allowed to distil off. To the voluminous potassium salt remaining, p-phenylenediamine (0.3 g.) and a trace of copper were added and reflux continued for three hours. (cf. the general experimental technique of Goldberg and Kelly, J.C.S., 1946, 102).

Each of the above reaction mixtures was subjected to steam distillation, the solution remaining filtered and acidified with 5 N HCl. In each case the product was unreacted bromonitro acid.

Attempted preparation of 2-nitro-4'-acetamidodiphenylamine-3-carboxylic acid

3-Bromo-2-nitrobenzoic acid (0.5 g.), p-aminoacetanilide (0.45 g.) and anhydrous sodium acetate (0.5 g.) were fused

(1) at 130° for 15 minutes.

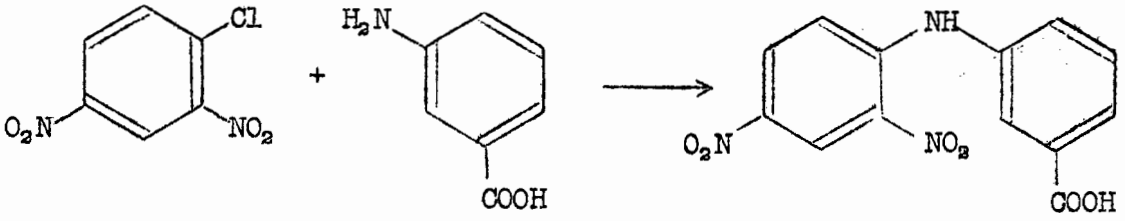
(2) with copper powder (25 mg.) at 150° for 1 hour.

(3) with copper powder (25 mg.) at 150° for 5 hours.

In each case the fused melt was extracted with hot sodium carbonate solution, filtered and acidified. In the first two experiments the

product was unreacted bromonitro acid, in the third a viscous black oil accompanied by a small amount of yellow precipitate melting over a wide range. The small amount of the latter recovered did not warrant its further investigation.

Preparation of 2,4-dinitrodiphenylamine-3'-carboxylic acid



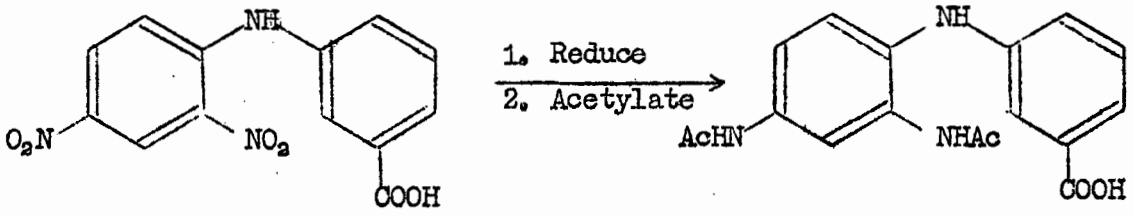
2,4-Dinitrochlorobenzene (36.5 g.), m-aminobenzoic acid (20 g.) and sodium carbonate (29 g.) were refluxed in 50% aqueous alcohol (200 cc.) for 6 hours. Acidification of the hot solution with acetic acid gave 40 g. (91%) of the diphenylamine, m.p. 254° - 255°. Recrystallisation from aqueous alcohol or aqueous acetic acid gave yellow micro needles, m.p. 256.5° - 258°.

Analysis:

Calc. for $C_{13}H_9N_3O_6$	Found
C 51.5%	C 51.8%
H 2.95%	H 3.25%
N 13.9%	N 14.1%

Linke (50) describes the acid as melting above 225°. A synthesis (154) reported subsequent to this work gives m.p. 262° (corr.).

Reduction of 2,4-dinitrodiphenylamine-3'-carboxylic acid and characterisation of the diamine

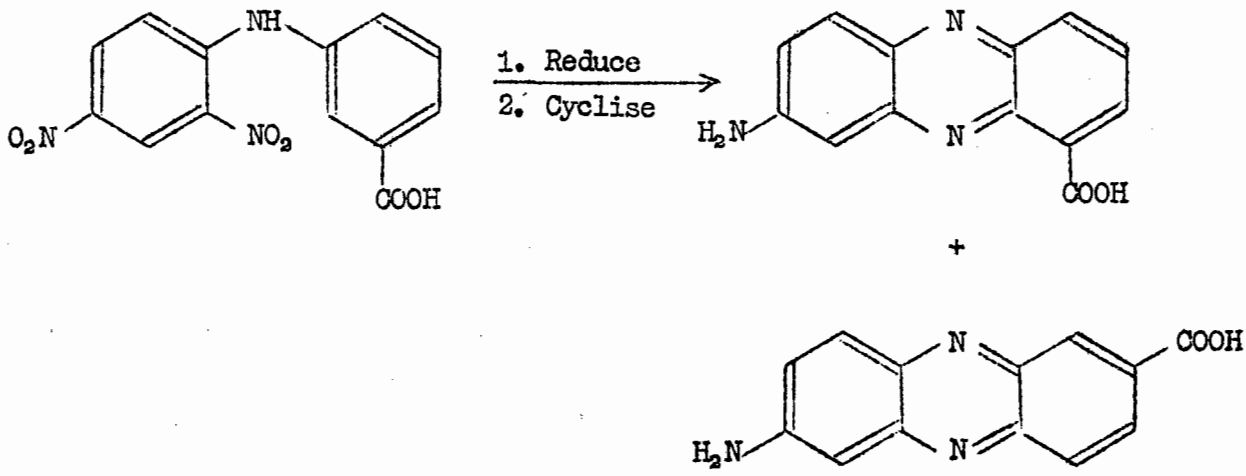


2,4-Dinitrodiphenylamine-3'-carboxylic acid (1 g.) in absolute alcohol was hydrogenated at 3 atmospheres over 5% palladium-charcoal (0.5 g.) to a clear solution. After filtering, the solvent was removed under reduced pressure in a nitrogen atmosphere. The white residue remaining darkened rapidly on exposure to air. Acetic anhydride (3 cc.) was added and the flask swirled until the solution set solid. A quantitative recovery of 2,4-diacetamidodiphenylamine-3'-carboxylic acid was obtained as white microneedles, m.p. 194° - 196° (dec.). No suitable solvent for recrystallisation could be found.

Analysis:

$C_{17}H_{17}N_3O_4$ requires:		Found:	
C	62.4%	C	61.7%
H	5.2%	H	5.3%
N	12.8%	N	12.5%

Reduction and cyclisation of 2,4-dinitrodiphenylamine-3'-carboxylic acid



The dinitrodiphenylamine (3 g.) was hydrogenated in absolute alcohol as described. The solution was filtered from catalyst, added to nitrobenzene (375 cc.), the alcohol distilled off, and reflux continued for 10 hours. The solution was filtered, the nitrobenzene stripped in vacuo, the residue dissolved in a minimum of hot dilute ammonia, filtered, and extracted with ether to remove remaining traces of nitrobenzene. The ammoniacal solution was acidified to pH 8 with 5 N HCl and to pH 5 with acetic acid. The colloidal precipitate of mixed phenazine acids, centrifuged and well-washed, weighed 1.65 - 1.75 g. (ca. 70%) and was a deep red-black in colour.

Separation of the components of the mixture. The above mixture (1.75 g.), absolute methanol (400 cc.) and concentrated sulphuric acid (4 cc.) were refluxed for 3 hours. The methanol was distilled to small volume, the

solution cooled, diluted to 100 cc. with water and made alkaline with ammonia. The precipitate of crude 2,7-substituted ester, after washing with dilute ammonia, weighed 1.1 g. The filtrate was put aside for working up of the 2,9-substituted acid.

Purification of methyl 2-aminophenazine-7-carboxylate: A weighed portion of the crude ester was paper-chromatographed in BuOH/HCl/H_2O, the spot corresponding to pure ester being cut from the paper, extracted and made up as a standard solution in N/10 HCl. Spectrophotometric estimation at 520 mμ indicated that the purity of the crude ester was of the order of 50%. (From this it could be calculated that the 2,7-substituted acid was formed in 22% yield in the cyclisation).

To isolate pure ester, the crude ester was dissolved in nitrobenzene, filtered from insoluble material, and adsorbed on to an acid-washed, methanol-deactivated, alumina column (2 x 35 cm.). After development of the column with benzene to remove nitrobenzene, the ester was eluted in ether, redissolved in nitrobenzene and passed through a second similar column. The bright red crystalline phenazine ester thus isolated weighed 0.300 g. (12% theoretical), had m.p. 264°, and could be recrystallised from toluene without raising the melting point.

Analysis:

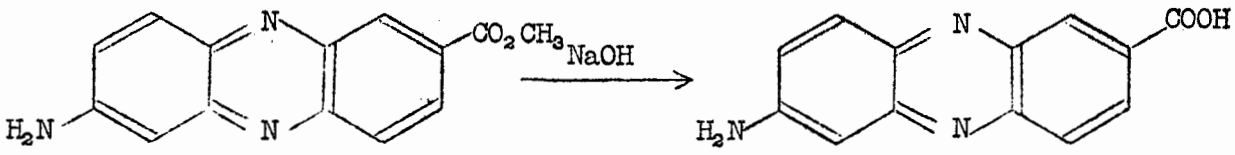
$C_{14}H_{11}N_3O_2$ requires:		Found:	
C	66.4%	C	66.6%
H	4.35%	H	4.65%
N	16.6%	N	16.4%

Purification of 2-aminophenazine-9-carboxylic acid. The ammoniacal filtrate from the esterification was evaporated slowly on a water-bath to small volume. 2-Aminophenazine-9-carboxylic acid separated as dark red gleaming needles with a greenish sheen, 0.450 g. (19%), m.p. 327° (dec.). Two recrystallisations from nitrobenzene raised the m.p. to 346° (dec.).

Analysis:

$C_{13}H_9N_3O_2$ requires:	Found:
C 65.3%	C 65.3%
H 3.75%	H 3.95%
N 17.5%	N 17.5%

Hydrolysis of methyl 2-aminophenazine-7-carboxylate



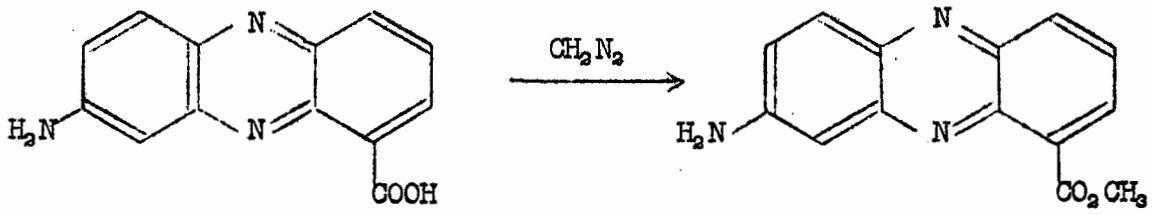
The above ester (0.170 g.) and 3 N NaOH (8.5 cc.) were refluxed for 2 hours. The solution was diluted, filtered hot, cooled and acidified with acetic acid. 2-Aminophenazine-7-carboxylic acid separated as a deep red amorphous precipitate, (0.140 g., 87%), melting above 360°. It reprecipitated from nitrobenzene, but could not be rendered crystalline by any of the methods tried for the 2,3-substituted acid. Paper-chromatographic examination (BuOH : HCl : H_2O, BuOH : NH_3 : H_2O) indicated

it to be pure.

Analysis:

$C_{13}H_9N_3O_2$ requires:	Found:
C 65.3%	C 65.3%
H 3.75%	H 3.9%
N 17.5%	N 17.5%

Esterification of 2-aminophenazine-9-carboxylic acid

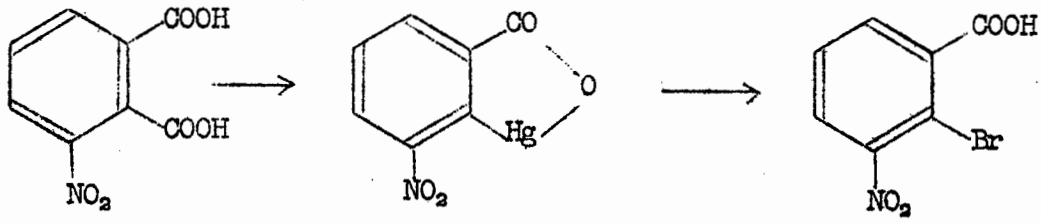


The acid (0.200 g.) in dimethylformamide (50 cc.) cooled to 0°C was treated with a slight excess of ethereal diazomethane with vigorous stirring. The deep red colour of the solution changed to a rich orange. Excess diazomethane was distilled off in ether at atmospheric pressure, and the remaining solvent removed in high vacuo. Recrystallisation of the residue from a benzene/petroleum ether mixture gave methyl 2-aminophenazine-9-carboxylate (0.170 g., 80%) as deep red needles, m.p. 186° - 188°.

Analysis:

$C_{14}H_{11}N_3O_2$ requires:	Found
C 66.4%	C 66.7%
H 4.35%	H 4.55%
N 16.6%	N 16.6%

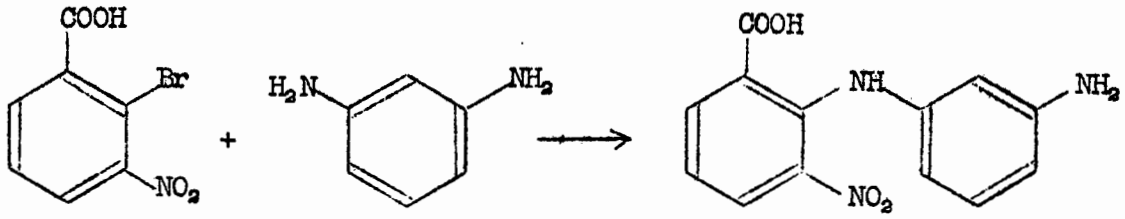
Preparation of 2-bromo-3-nitrobenzoic acid



Organic Syntheses, Coll. Vol. I, p. 48, 120.

2-Bromo-3-nitrobenzoic acid, m.p. 174° - 181°, was prepared in 56% yield. It was used without further purification in subsequent condensations.

Preparation of 6-nitro-3'-aminodiphenylamine-2-carboxylic acid



2-Bromo-3-nitrobenzoic acid (5 g.), m-phenylenediamine dihydrochloride (6.5 g.), anhydrous potassium carbonate (8.5 g.) and copper powder (0.15 g.) were refluxed for 90 mins. in amyl alcohol (25 cc.). The solvent was removed in steam and the aqueous solution acidified with 5 N HCl. The diphenylamine was obtained as a dark red brown material (2.4 g., 48%), melting over a wide range of temperature.

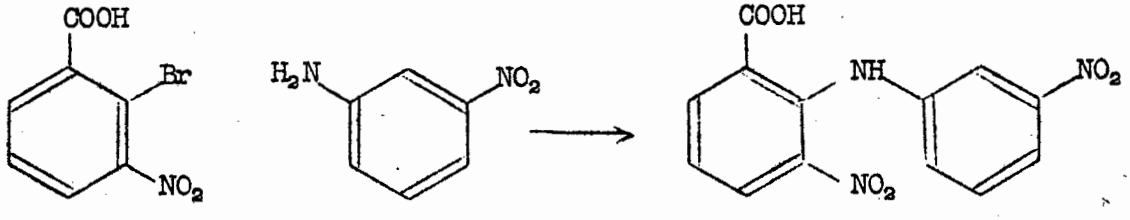
The aminonitrodiphenylamine (0.5 g.) in 12% sodium carbonate solution

(4 cc.) was treated with acetic anhydride (0.5 cc.) in the cold. After half an hour the solution was acidified, and the precipitate recrystallised from aqueous alcohol to give vermilion needles of the acetyl derivative, m.p. 223°.

Boiling a small portion of the aminonitrodiphenylamine and charcoal in slight excess of 5 N HCl gave on filtration and cooling, yellow needles of the hydrochloride, m.p. 172°. When this was suspended in warm water and treated with sodium acetate, the free base was regenerated as a gummy brown material, still melting over a wide range of temperature.

Goldberg and Kelly, (J.C.S., 1947, 595) prepared the diphenylamine from the chloronitro acid and m-phenylenediamine. They report a compound, m.p. 240° - 280° (slow decomposition), which could not be purified by recrystallisation. It yielded an acetyl derivative, m.p. 226° - 228°.

Preparation of 6,3'-dinitrodiphenylamine-2-carboxylic acid

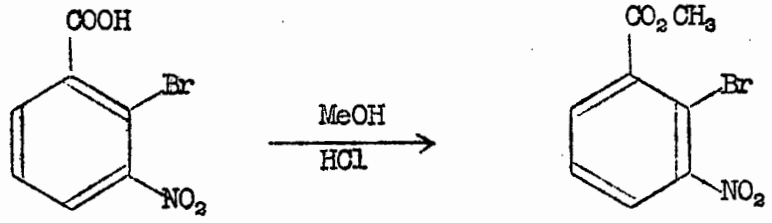


2-Bromo-3-nitrobenzoic acid (3.05 g.), m-nitroaniline (2.5 g.), anhydrous potassium carbonate (2.5 g.) and copper powder (0.1 g.) were intimately mixed and fused at 130° - 150° for 5 hours. The melt was extracted with boiling water (100 cc.), the extract filtered, cooled and

unchanged m-nitroaniline filtered off. The filtrate was then acidified with 5 N HCl at the boiling point, and the precipitate collected. The diphenylamine was obtained as a yellow powder (2.85 g., 70%), m.p. 188° - 193°. Repeated recrystallisations could only raise the m.p. to 193°. Chromatography on acid-washed alumina did not change this value.

Goldberg and Kelly, (J.C.S., 1947, 595) prepared this diphenylamine from the chloronitro acid and m-nitroaniline under more vigorous conditions. They record a m.p. 196° - 198°.

Preparation of methyl 2-bromo-3-nitrobenzoate

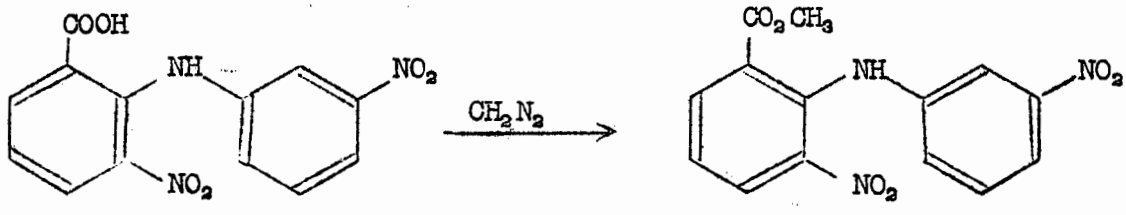


Stoughton and Adams, J.A.C.S., 54, 4426 (1932).

A 62% yield of the ester was obtained, m.p. 70° - 75°. It was used without further purification.

Preparation of methyl 6,3'-dinitrodiphenylamine-2-carboxylate

(a) By esterification of the diphenylamine acid



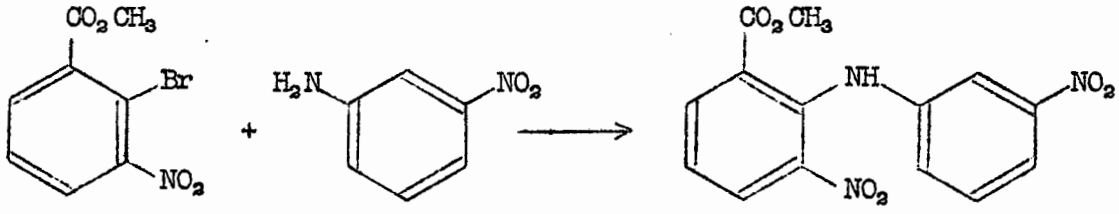
The diphenylamine acid (2.45 g.) in ether (120 cc.), cooled to 0°, was treated with slight excess of ethereal diazomethane. The ester slowly crystallised from solution and after several hours 1.8 g. (70%) of bright yellow needles, m.p. 143° - 146°, were filtered. Recrystallisation from aqueous ethanol or aqueous acetic acid did not raise the melting point. By evaporating the ether and recrystallising the residue from aqueous acetic acid, a further 0.35 g. of less pure ester (m.p. 138° - 141°) could be recovered. Overall yield 84%.

Analysis:

$C_{14}H_{11}N_3O_6$ requires:	Found:
C 53.0%	C 52.5%
H 3.45%	H 3.6%
N 13.2%	N 13.2%

The diphenylamine acid could not be esterified by the normal Fischer-Speier technique. When it was dissolved in methanol, treatment with even a large excess of diazomethane was only partially effective.

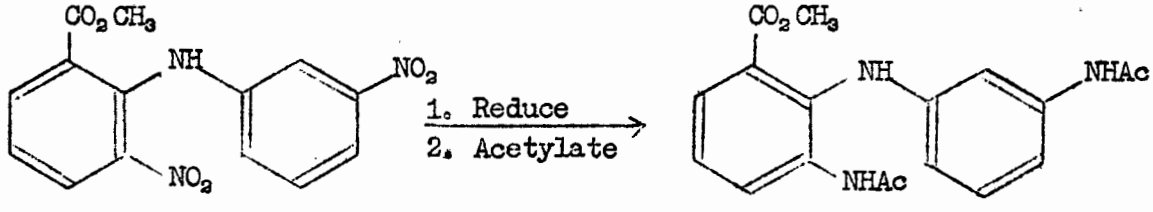
(b) By condensation of methyl 2-bromo-3-nitrobenzoate and m-nitroaniline



Methyl 2-bromo-3 -nitrobenzoate (1.55 g.), m-nitroaniline (1.25 g.), anhydrous potassium carbonate (1.25 g.) and copper powder (0.030 g.) were well mixed, and heated at 190° until the vigorous effervescence of carbon dioxide slackened (5 - 7 minutes). After being extracted with several portions of boiling water, the fused melt was recrystallised from aqueous acetic acid (charcoal) to give the diphenylamine ester (0.50 g., 26%) as dark yellow-brown needles, m.p. 139° - 142°. The dark colour could not be removed by recrystallisation, but by dissolving in benzene and running through an acid-washed, methanol-deactivated alumina column, followed by recrystallisation from dilute acetic acid, bright yellow needles, m.p. 143° - 145°, were obtained. These did not depress the melting point of the ester obtained directly from the diphenylamine acid.

The low initial yield of diphenylamine and the lengthy purification required, rendered this method of preparing the ester less satisfactory than the first method described.

Reduction of methyl 6,3'-dinitrodiphenylamine-2-carboxylate and characterisation of the diamine



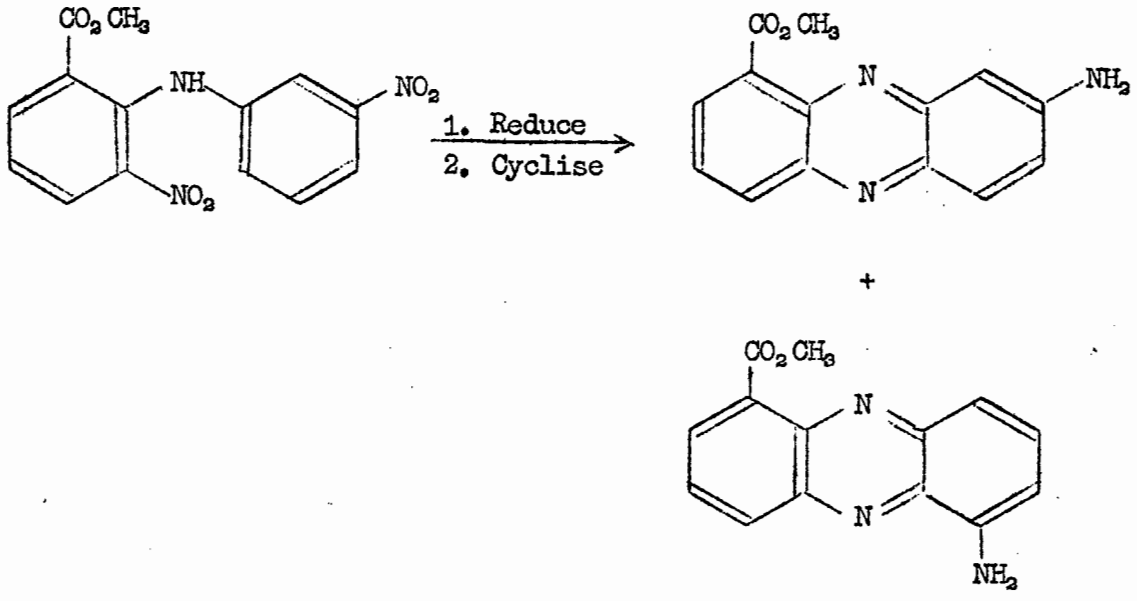
The dinitrodiphenylamine (0.5 g.) in absolute alcohol was hydrogenated at 3 atmospheres pressure over platinum oxide (0.1 g.) until gas absorption

ceased. The yellow-orange solution was filtered and the solvent removed under reduced pressure in a nitrogen atmosphere. The yellow diamine remaining darkened rapidly on exposure to air. Acetic anhydride (1.5 cc.) was added and the flask swirled until the solution set solid. A quantitative recovery of methyl 6,3'-diacetamidodiphenylamine-2-carboxylate was obtained, pale yellow plates from dilute acetic acid, m.p. 208° - 210°.

Analysis:

$C_{18}H_{19}N_3O_4$ requires:	Found:
C 63.4%	C 62.9%
H 5.6%	H 5.7%
N 12.3%	N 11.6%

Reduction and cyclisation of methyl 6,3'-dinitrodiphenylamine-2-carboxylate



The above diphenylamine (2 g.) was reduced over platinum oxide as described. After filtering, the yellow solution was added to nitrobenzene

(250 cc.), the alcohol removed and reflux continued for 30 hours. The solution was filtered and cooled, and the nitrobenzene stripped in high vacuo. The oily residue remaining was taken up in benzene and adsorbed on to an acid-washed, methanol-deactivated alumina column (30 x 2 cm.). Development of the column gave the following fractions.

(1) Elution with benzene removed a deep red band which was put aside for recovery of methyl 1-aminophenazine-6-carboxylate.

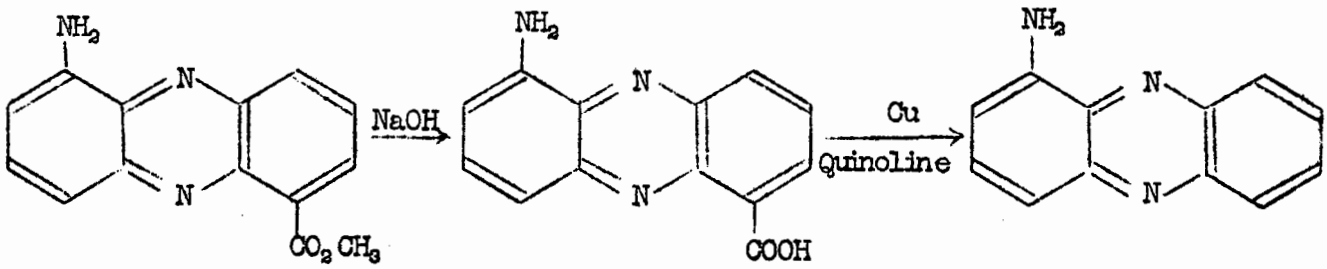
(2) Elution with ether stripped an orange band, which on evaporation gave deep red needles of methyl 2-aminophenazine-9-carboxylate (0.45 g., 28%), m.p. 185° - 187°. Recrystallisation from benzene/petroleum ether raised the melting point to 188°, not depressed on mixture with ester obtained by the alternative route (p. 119). The esters from the two different sources had identical infra-red spectra (KCl disc), and travelled at the same rate in three different paper-chromatographic solvents. (BuOH : HCl : H_2O; BuOH : NH_3 : H_2O; BuOH : HOAc : H_2O).

The first fraction (benzene) was evaporated to small bulk and run through a second similar alumina column. Evaporation of the solvent and two recrystallisations of the residue from aqueous alcohol gave methyl-1-aminophenazine-6-carboxylate, (40 mg., 2.5%) as bright red needles, m.p. 144°.

Analysis:

$C_{14}H_{11}N_3O_2$ requires:		Found:	
C	66.4%	C	66.5%
H	4.35%	H	4.4%
N	16.6%	N	16.5%

Hydrolysis of methyl 1-aminophenazine-6-carboxylate and decarboxylation of the ensuing acid



The ester (22 mg.) in N NaOH (3 cc.) was heated on a boiling water-bath for 30 minutes. The filtered solution was acidified with acetic acid, and the precipitate recrystallised from aqueous ethanol. 1-Aminophenazine-6-carboxylic acid separated as fluffy purple needles (16 mg., 77%), m.p. 305° - 307°.

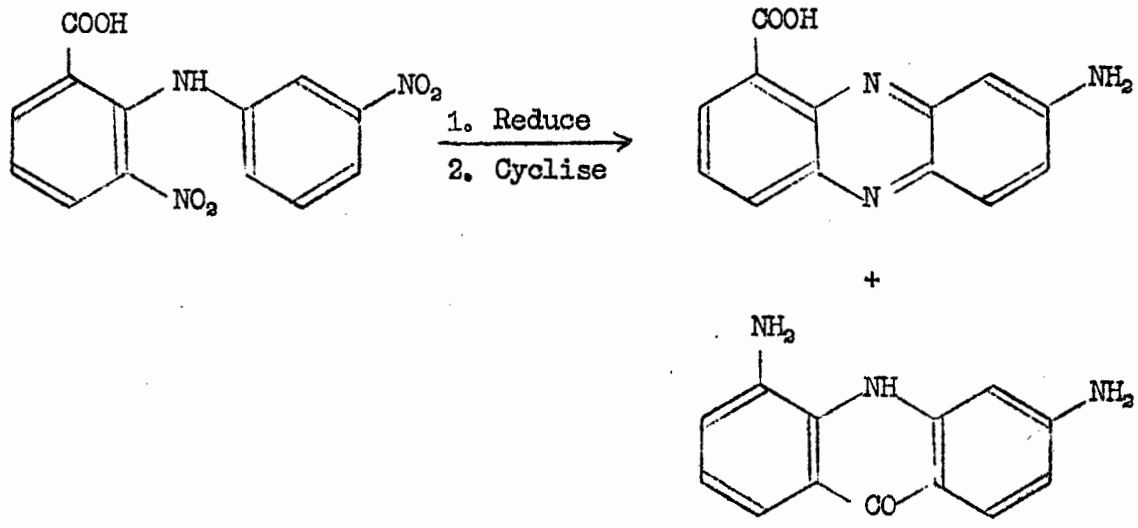
Analysis:

$C_{13}H_9N_3O_2$:	Found:
C 65.3%	C 64.8%
H 3.75%	H 3.7%
N 17.6%	N 17.3%

The phenazine acid (6 mg.) and copper powder (1 mg.) were refluxed for 10 minutes in quinoline (1 cc.), the course of the reaction being followed by a change in colour from deep purple to red. The solution was adsorbed in toto on to a Grade O alumina column (1 x 17 cm.), the quinoline being removed by development with benzene. Elution with ether and evaporation of the solvent gave deep red needles of 1-aminophenazine, m.p. 173° - 176°, mixed m.p. with an authentic specimen 174° - 176°. The nature of the product was confirmed by paper chromatography in BuOH : HCl : H_2O;

BuOH : NH_3 : H_2O; BuOH : HCOOH : H_2O.

Reduction and cyclisation of 6,3'-dinitrodiphenylamine-2-carboxylic acid



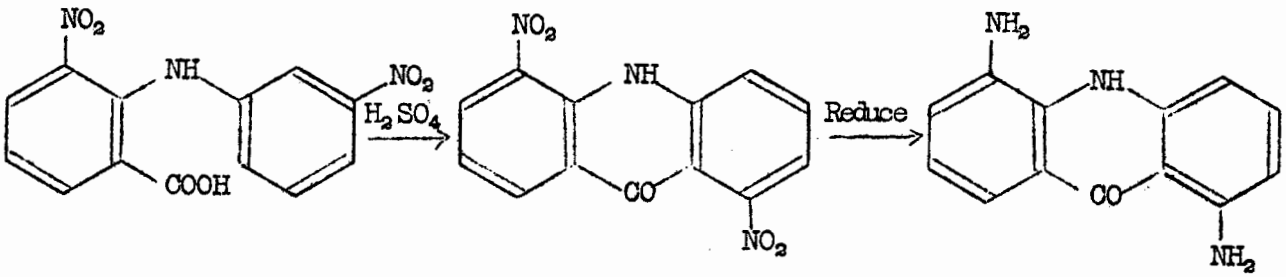
Crude 6,3'-dinitrodiphenylamine-2-carboxylic acid (1.45 g.) in absolute alcohol was hydrogenated over 5% palladium-charcoal (1 g.) as far as was possible. The yellow-red solution was filtered, added to nitrobenzene (112 cc.), the alcohol distilled off and reflux continued for 12 hrs. The bulk of the nitrobenzene was removed under reduced pressure, the remaining solution diluted with benzene and filtered. The precipitate (0.65 g.) was dissolved in 96% ethanol (2100 cc.) and adsorbed on to a Grade 0 alumina column (3.5 x 21 cm.).

Development of the column with a large volume of ethanol carried through a diffuse, widely-spread band, whose progress could be followed by the intense green fluorescence under untraviolet light. Evaporation of the solvent left a dark brown-yellow residue (0.200 g.), which could be

recrystallised from nitrobenzene to give brown-yellow needles, m.p. above 350°. Paper-chromatographic comparison (BuOH : HCl : H_2O; BuOH : HOAc : H_2O) and comparison of infra-red spectra (KCl disc) with 1,8-diaminoacridone, established the identity of this compound. (Melting points were too ill-defined to be of any use, though Goldberg and Kelly (57) quote 360° - 362°, while Albert and Linnell (155) record a melting point above 360°).

Elution of the column with methanol, followed by 5% acetic acid in ethanol removed a red band, established by paper chromatography as 2-aminophenazine-9-carboxylic acid. (BuOH : HCl : H_2O; BuOH : HAc : H_2O; BuOH : NH_3 : H_2O). Severe difficulties were encountered in purifying the phenazine, and no attempt was therefore made to estimate its relative quantity in the reaction product.

Preparation of 1,6-diaminoacridone

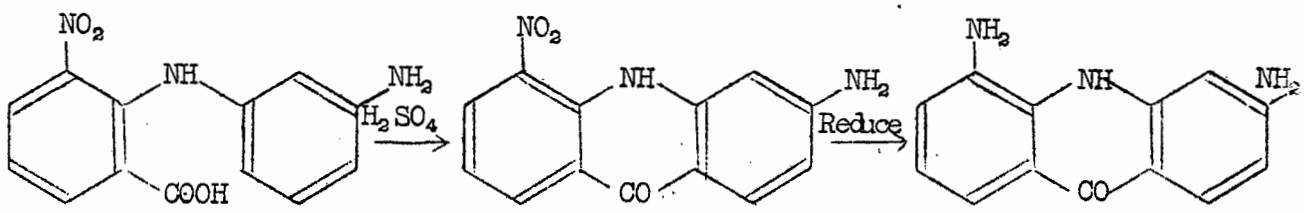


Goldberg and Kelly, J.C.S., 1947, 595.

The dinitroacridone was prepared as described by the authors. It had m.p. 332°, (given 332° - 334°). The diaminoacridone was made by catalytic reduction in absolute ethanol over platinum oxide, and not by the stannous

chloride method described. The melting point of the diamine was greater than 300°, but too poorly defined to be characteristic. However, the appearance of a brilliant green-yellow fluorescence, not shown by the dinitroacridone, indicated the success of the reduction.

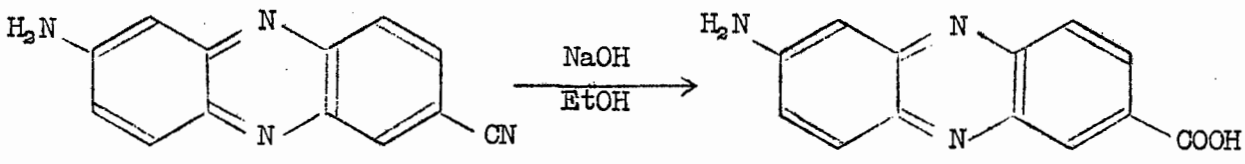
Preparation of 1,8-diaminoacridone



Goldberg and Kelly, J.C.S., 1947, 595.

The aminonitroacridone was prepared as described by the authors cited. It had m.p. 310° - 315°, (given 314° - 316°). Again, the diaminoacridone was made by catalytic reduction over platinum oxide, the course of the reaction being followed by the replacement of the red, non-fluorescent alcoholic solution, by a green-yellow highly fluorescent one. The melting point of this material was above 350°, but poorly defined.

Hydrolysis of 2-amino-7-cyanophenazine



2-Amino-7-cyanophenazine (0.25 g.) and sodium hydroxide (5 g.) were refluxed in 75% aqueous alcohol (25 cc.) for 1 hour. The solution was diluted, filtered hot and adjusted to pH 4 with 5 N HCl and acetic acid. 2-Aminophenazine-7-carboxylic acid (0.232 g., 82%) separated as a dark-red amorphous mass, which could be reprecipitated from nitrobenzene, but not rendered crystalline. Its melting point was greater than 360°.

Analysis:

Calc. for $C_{13}H_9N_3O_2$:	Found:
C 65.3%	C 65.2%
H 3.75%	H 4.05%
N 17.6%	N 17.5%

Comparison with 2-aminophenazine-7-carboxylic acid obtained by the cyclisation of 2,4-diaminodiphenylamine-3'-carboxylic acid could only be done by paper chromatography, (BuOH : HCl : H_2O; BuOH : NH_3 : H_2O; BuOH : HCOOH : H_2O; BuOH : CH_3COOH : H_2O). Rate of travel of the two acids in these solvents was identical.

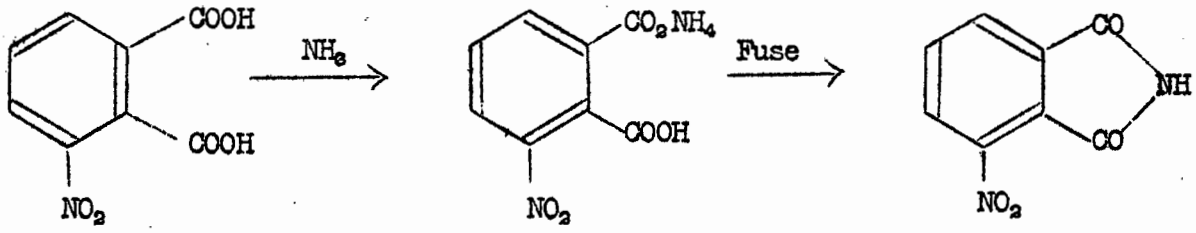
Characterisation of the acid as its methyl ester was carried out by the normal methanol/sulphuric acid technique. Methyl 2-aminophenazine-7-carboxylate thus prepared separated from toluene as red needles, m.p. 264°. It did not depress the melting point of ester obtained by the alternative method. The infra-red spectra of the two esters (KCl disc) were identical.

S E C T I O N I P A R T I I I

THE SYNTHESIS OF 2-AMINOPHENAZINE-1-CARBOXYLIC ACID

(1) From methyl 2,4-diaminodiphenylamine-3-carboxylic acid

Preparation of 3-nitrophthalimide

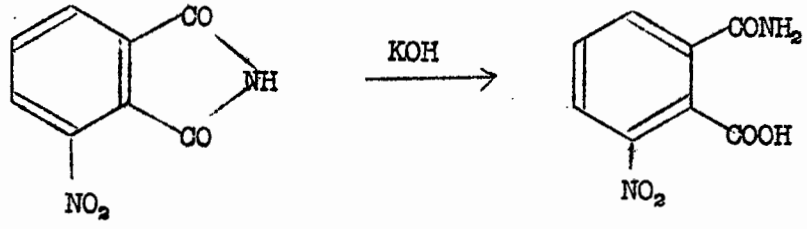


Bogert and Chambers, J.A.C.S., 27, 649 (1905).

Bogert and Boroschek, J.A.C.S., 23, 747 (1901).

3-Nitrophthalimide was prepared from 3-nitrophthalic acid (Org. Synth. Coll. Vol. I, p. 399) in 62% yield, m.p. 215° - 216°. The authors cited quote no yield, but m.p. 215° - 216°.

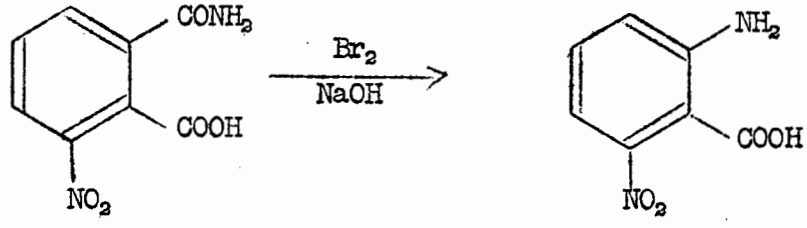
Preparation of 3-nitrophthalamic acid



Moser and Gompf, J. Org. Chem., 15, 585 (1950).

3-Nitrophthalamic acid, m.p. 152° - 153° with solidification and remelting 213° - 215°, was prepared in 81% yield. Moser and Gompf report an 80% yield of compound, m.p. 153° - 155°, 212° - 213°.

Preparation of 6-nitroanthranilic acid



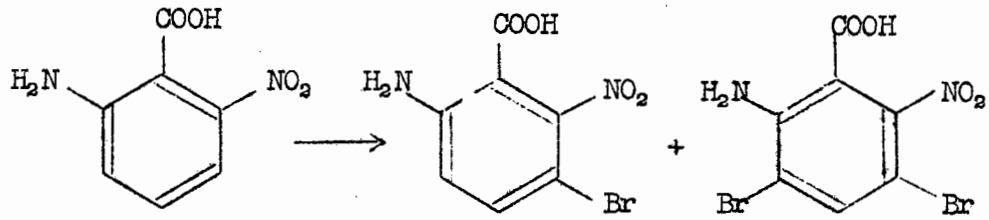
Kahn, Ber., 35, 3863 (1902).

Since the above method could not be repeated, a considerably modified technique was followed.

Finely powdered 3-nitrophthalamic acid (26 g.) was added in one portion to a solution of bromine (7.5 cc.) in 3 N sodium hydroxide (250 cc.) cooled to -6°C. Vigorous shaking dissolved the solid within 5 minutes, and the solution was immediately heated to the boil. The brick red colour of the sodium salt of the anthranilic acid developed before the boiling point, indicating the success of the reaction. The mixture was immediately cooled to 0°C in a freezing-mixture and acidified with cooling and stirring with 5 N HCl. 6-Nitroanthranilic acid separated as a yellow crystalline mass (16.8 g., 77%), m.p. 174° (dec.). Recrystallisation from water gave yellow needles, m.p. 176° - 178°. (Reported 180°).

The crude acid was used as such in subsequent reactions.

Bromination of 6-nitroanthranilic acid



Erickson, Dechary and Pullig, J.A.C.S., 74, 5622 (1952).

(1) By the method described 5-bromo-6-nitroanthranilic acid was obtained in 40% yield, m.p. 197° - 200°. The authors cited record 67% yield, m.p. 201° - 202.5°.

(2) To 6-nitroanthranilic acid (3.45 g.) suspended in glacial acetic acid (47 cc.) at 15°C, bromine (1 cc.) in acetic acid (8 cc.) was added with rapid mechanical stirring. The addition of sodium acetate trihydrate (2.7 g.) in water (4 cc.) gave a clear red solution which was poured with continuous stirring on to 40 g. of ice and 160 cc. of water. The yellow precipitate (0.85 g.) was collected and combined with the ethereal extract of the filtrate (2.9 g.).

The combined solids were dissolved in hot 0.5 N NaOH (125 cc.), cooled and filtered. Acidification of the filtrate with 2 N HCl and two recrystallisations of the product from aqueous alcohol (charcoal) gave 5-bromo-6-nitroanthranilic acid (2.15 g., 44%), m.p. 198° - 200°.

The precipitate was dissolved in boiling water, filtered and acidified. It was reconverted to its sodium salt and recrystallised from water.

To a rapidly stirred solution of methyl 6-nitroanthranilate (1.3 g., 0.007 m.) in glacial acetic acid (30 cc.), bromine (0.4 cc., 0.008 m.) in acetic acid (5 cc.) was added. A fine precipitate rapidly developed and was filtered, dissolved in water and treated with excess sodium acetate. Unchanged methyl 6-nitroanthranilate (0.3 g.), m.p. and mixed m.p. 105° - 108° was recovered.

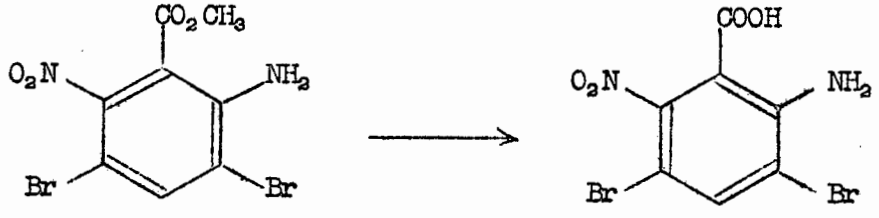
The filtrate was poured on ice, the resulting precipitate being recrystallised from aqueous methanol to give the presumed methyl 3,5-dibromo-6-nitroanthranilate (0.5 g., 36%) as fine yellow needles, m.p. 121° - 124°.

Analysis:

$C_8H_6Br_2N_2O_4$ requires:		Found:	
C	27.1%	C	27.2%
H	1.7%	H	2.2%
N	7.9%	N	7.8%
Br	45.2%	Br	45.6%

Attempts to brominate the ester as a suspension in a minimum volume of acetic acid (thus simulating bromination of the acid) gave a product which could not be purified.

Hydrolysis of methyl 3,5-dibromo-6-nitroanthranilic acid



The dibromo ester (0.100 g.) in 3 N NaOH (2 cc.) was warmed on a boiling water bath for 1 hour. The solution was cooled, the sodium salt of the acid collected, dissolved in boiling water (10 cc.) and acidified hot. Recrystallisation of the precipitate from aqueous ethanol gave long yellow needles of the acid, m.p. 228° - 230° (dec.), not depressed on mixing with the dibromo acid obtained by the bromination of 6-nitroanthranilic acid.

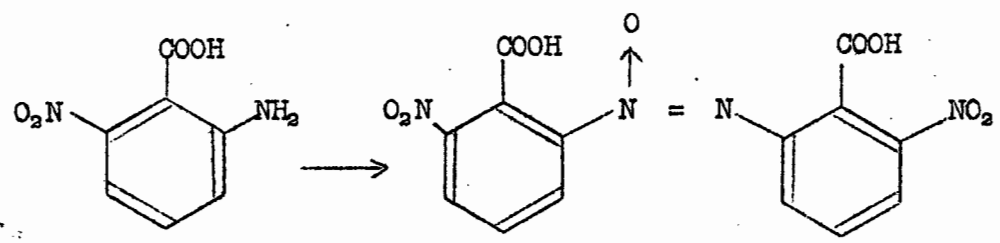
Attempted preparation of 4-amino-2-nitrodiphenylamine-3-carboxylic acid

Attempts were made to condense 5-bromo-6-nitroanthranilic acid and aniline under a variety of conditions. In each case the ratio of acid to aniline was 1 : 2. Solvent, base, catalyst, reaction temperature and time, and product are tabulated below. None of the attempts made were successful.

	Solvent	Base	Catalyst	Temperature	Time	Product
(1)	Ethanol	NaOAc	-	78°	16 hrs.	Starting material
(2)	-	NaOAc	-	170°	½ hr.	" "
(3)	-	K_2CO_3	Cu	180°	1 hr.	Tar
(4)	Ethanol	K_2CO_3	Cu	78°	7 hrs.	Starting material
(5)	Nitrobenzene	NaOAc	-	210°	16 hrs.	Tar
(6)	Nitrobenzene	K_2CO_3	Cu	210°	5 mins.	"
(7)	Amyl alcohol	K_2CO_3	Cu	160°	½ hr.	Starting material
(8)	-	K_2CO_3	Cu	170°	10 mins.	Tar

The reactions of various amines with hydrogen peroxide in acetic acid

(1) 6-Nitroanthranilic acid



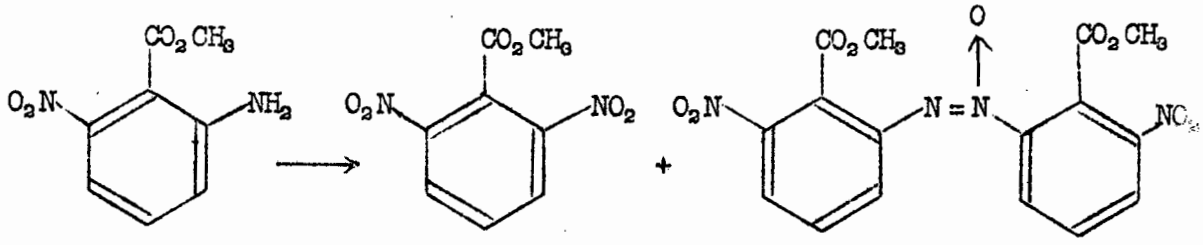
6-Nitroanthranilic acid (0.5 g.) in a solution of acetic acid (20 cc.), 30% hydrogen peroxide (2.5 cc.) and concentrated H_2SO_4 (15 drops) was heated slowly to 70°. The light yellow colour of the solution darkened through deep green (the nitroso stage) to a red-yellow. Heating was continued at 70° for 6 hours, further hydrogen peroxide (1.2 cc.) being added after 3 hours. Cooling the solution deposited a yellow-orange precipitate of the presumed 3,3'-dinitroazoxybenzene-2,2'-dicarboxylic acid (0.114 g., 22%), m.p. 268° - 271° (dec.). Recrystallisation (with loss) from a large volume of alcohol raised the melting point to 271° (dec.). The compound is sparingly soluble in organic solvents.

Analysis:

$C_{14}H_8N_4O_9$ requires:		Found:	
C	44.7%	C	43.4%
H	2.1%	H	2.6%
N	14.9%	N	13.9%

Though the analysis is not very satisfactory, it fits the azoxy-compound better than any of the other possibilities. This proposal is supported by infra-red spectral data (KCl disc). There are no bands in the 3500 - 3300 $cm.^{-1}$ or 1650 - 1580 $cm.^{-1}$ regions (N - H stretching and deformation). A double carbonyl peak at 1713 and 1707 $cm.^{-1}$ is as expected for an unsymmetrically substituted carboxyl-group. The high value of this (normal for aryl acids is 1700 - 1680 $cm.^{-1}$) is consistent with intramolecular hydrogen-bonding involving the hydroxyl hydrogen of the carboxyl-group, and thus preventing dimerisation. (See p. 87).

(2) Methyl 6-nitroanthranilate



Methyl 6-nitroanthranilate (0.200 g.) in a solution of acetic acid (4 cc.), 30% hydrogen peroxide (1 cc.) and conc. H_2SO_4 (6 drops) was heated slowly to 70°. Again, the colour change from light yellow through bottle-green to orange-yellow was observed. After 5 hours heating the solution was cooled and filtered, the filtrate being put aside for working up of the dinitro ester.

The precipitate of presumed dimethyl 3,3'-dinitroazoxybenzene-2,2'-dicarboxylate (0.040 g.) separated from acetic acid as yellow plates, m.p. 192° - 196°.

Analysis:

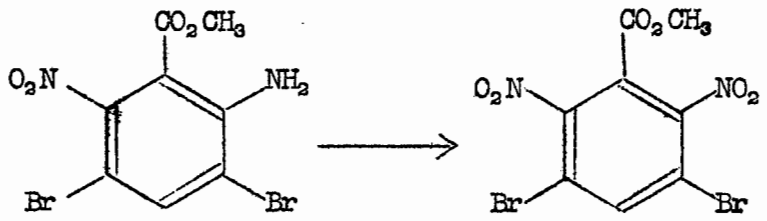
$C_{16}H_{12}N_4O_9$ required:	Found:
C 47.5%	C 47.5%
H 3.0%	H 3.5%
O 35.7%	O 36.3%

Infra-red spectral data (KCl disc) again support this formulation. The N - H bands present at 3495, 3390 and 1622 $cm.^{-1}$ in methyl 6-nitroanthranilate have disappeared, and the C = O band at 1718 $cm.^{-1}$ has been replaced in this product by a double peak at 1744 and 1734 $cm.^{-1}$.

The filtrate from the above reaction was diluted with 25 cc. of iced water and the precipitated methyl 2,6-dinitrobenzoate (0.116 g.) collected. Recrystallised from ethanol, it melted 139° - 146°. The melting point is given as 147°.

The infra-red spectrum (KCl disc) of the compound showed a single C = O peak at 1738 cm. . Bands in the 1550 - 1510 $cm.^{-1}$ and 1365 - 1335 $cm.^{-1}$ region, which may be assigned to aryl nitro-groups had doubled in intensity on passing from methyl 6-nitroanthranilate to this compound.

(3) Methyl 3,5-dibromo-6-nitroanthranilate



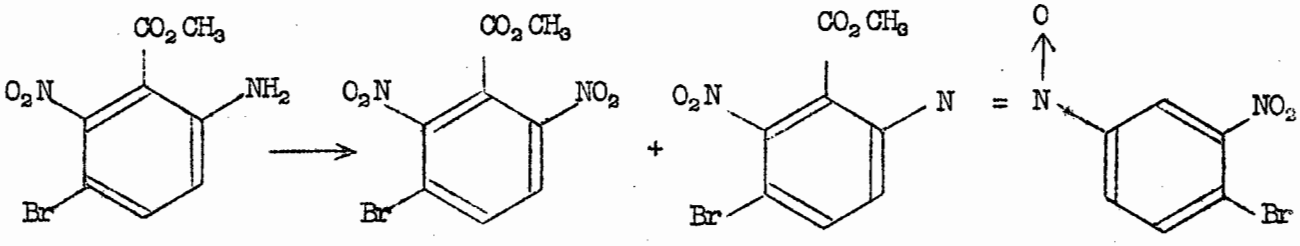
Methyl 3,5-dibromo-6-nitroanthranilate (0.200 g.) was heated slowly to 70° in acetic acid (2 cc.), 30% hydrogen peroxide (0.6 cc.) and conc. sulphuric acid (4 drops). Heating was continued for four hours, a further 0.66 cc. of peroxide being added after 2 hours. The solution was cooled and poured on ice, the resulting precipitate being recrystallised from methanol. The presumed methyl 3,5-dibromo-2,6-dinitrobenzoate separated as yellow plates, m.p. 162° - 166°.

Analysis:

$C_8H_4Br_2N_2O_6$ requires:	Found:
C 25.0%	C 26.7%
H 1.05%	H 3.9%
N 7.3%	N 8.2%
Br 41.7%	Br 38.5%

The analysis, though quite inadequate to establish the nature of the product, appears to rule out the possibility of an azoxybenzene having been formed. There was therefore no further point in attempting to purify the product.

The preparation of methyl 3-bromo-2,6-dinitrobenzoate



Methyl 5-bromo-6-nitroanthranilate (5.6 g.) in a mixture of glacial acetic acid (175 cc.), 30% hydrogen peroxide (40 cc.) and conc. H_2SO_4 (5 cc.) was heated at 70° for 5 hours. The usual colour changes were observed. The solution was cooled and filtered, the precipitate being put aside for working up of the azoxy compound.

The bright red filtrate was run under slight vacuum through a bed of charcoal, and poured on to 500 g. of ice and 500 cc. water. Recrystallisation of the precipitate from aqueous methanol (charcoal) gave methyl 3-bromo-2,6-dinitrobenzoate (3.8 g., 61%) as silky pale yellow needles, m.p. 118°.

Methyl 3-bromo-2,6-dinitrobenzoate (1.55 g.) and redistilled aniline (30 cc.) were heated at 100° for 3 hours. After being cooled to 0°C, the solution was poured with rapid stirring into 100 cc. of precooled 5 N HCl. Filtration gave the diphenylamine ester (1.45 g., 90%), m.p. 131° - 135°.

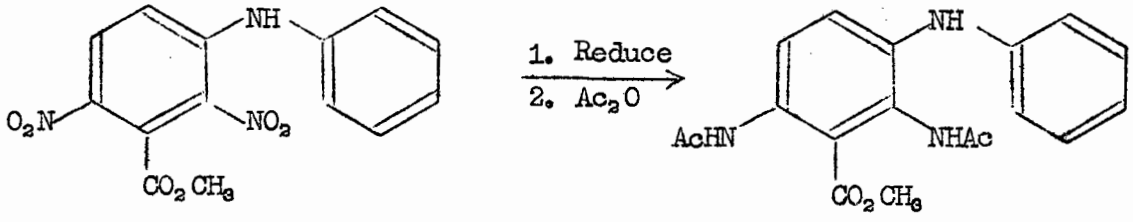
A portion (0.200 g.) of the crude ester, dissolved in benzene, was adsorbed on to a Grade O alumina column. Elution with ether gave bright orange needles, m.p. 136° - 139°. The ester could be recrystallised from aqueous alcohol, aqueous acetic acid or petroleum ether without raising the melting point.

Analysis:

$C_{14}H_{11}N_3O_6$ requires:	Found:
C 53.0%	C 53.6%
H 3.45%	H 3.55%
N 13.2%	N 13.9%

The crude ester (m.p. 131° - 135°) proved satisfactory for subsequent reduction and cyclisation.

Reduction of methyl 2,4-dinitrodiphenylamine-3-carboxylate and characterisation of the diamine



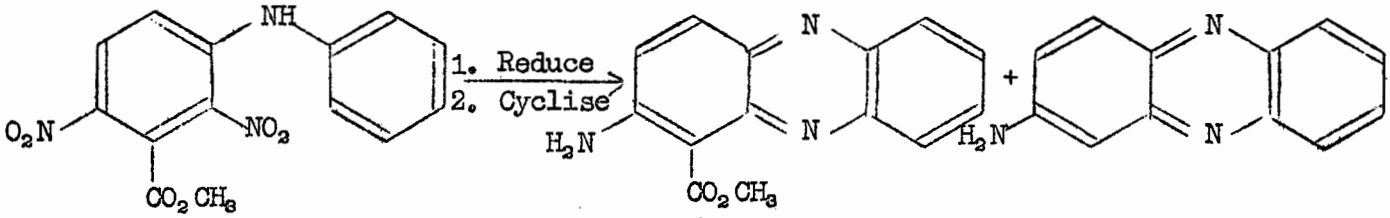
Methyl 2,4-dinitrodiphenylamine-3-carboxylate (0.200 g.) was

hydrogenated over platinum oxide catalyst in the normal way. The clear green-yellow solution was filtered and the solvent evaporated under reduced pressure in a nitrogen atmosphere. Acetic anhydride (1 c.c.) was added to the rapidly darkening residue and the flask swirled until the solution set solid. Recrystallisation from a chloroform/petroleum ether mixture gave grey-white micro needles of methyl 2,4-diacetamidodiphenylamine-3-carboxylate, m.p. 173° - 175°.

Analysis:

$C_{18}H_{19}N_3O_4$ requires:	Found:
C 63.4%	C 62.9%
H 5.55%	H 5.65%
N 12.3%	N 12.2%

Reduction and cyclisation of methyl 2,4-dinitrodiphenylamine-3-carboxylate



The dinitrodiphenylamine ester (1.21 g.) was hydrogenated over platinum oxide in the normal way, filtered into nitrobenzene (150 cc.), the alcohol distilled off and reflux continued for 8½ hours. The nitrobenzene was stripped in high vacuo to small volume, the residue taken up in benzene and adsorbed on to a Grade 0 Al_2O_3 column (2 cm. x 37 cm.).

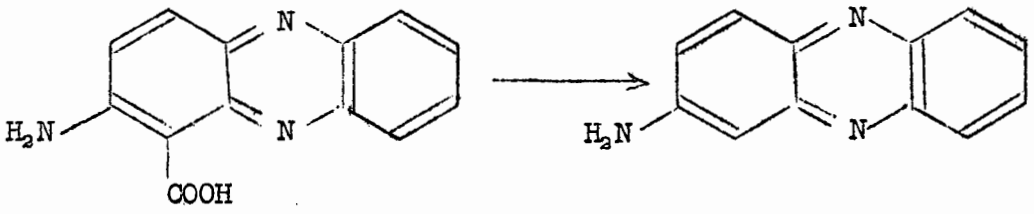
Elution of the column with ether revealed at least ten differend bands.

The main band, containing highly fluorescent yellow phenazine ester together with 2-aminophenazine was removed in ether, evaporated and the residue refluxed for 1 hour in 2 N NaOH (30 cc.). The solution was filtered hot from 2-aminophenazine, acidified carefully with HCl and acetic acid and the precipitate recrystallised from aqueous ethanol. 2-Aminophenazine-1-carboxylic acid (0.107 g., 11%) separated as yellow-orange needles, m.p. 274° - 276° (with decarboxylation). Changing the eluting solvent through acetone to water removed a second yellow band, which was concentrated, acidified and the precipitate recrystallised from aqueous ethanol. A further 36 mg. of the phenazine acid was thus obtained, m.p. 274° - 276°. This raised the overall yield to 15%.

Analysis:

$C_{13}H_9N_3O_2$ requires:	Found:
C 65.3%	C 64.7%
H 3.75%	H 3.55%
N 17.6%	N 17.6%

Decarboxylation of 2-aminophenazine-1-carboxylic acid

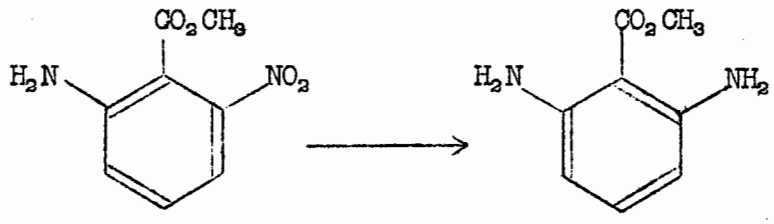


The acid (2 mg.) and copper powder (0.4 mg.) were refluxed in quinoline (0.2 cc.) for 10 minutes. The solution was diluted with benzene, adsorbed

on to an alumina column, and the orange band of 2-aminophenazine eluted in ether. It was identified by mixed melting point (278° - 280°) and paper-chromatographic (BuOH : HCl : H_2O) comparison with an authentic specimen.

(2) The Synthesis of 2-Aminophenazine-1-carboxylic Acid from Methyl 3,2'-Diaminodiphenylamine-2-carboxylate

The Preparation of methyl 2,6-diaminobenzoate



Methyl 6-nitroanthranilate (3 g.) in absolute alcohol (50 cc.) was hydrogenated at 3 atmospheres pressure over 5% palladium-charcoal (1.5 g.) until a colourless solution resulted. The solvent was removed in a nitrogen atmosphere and the residue recrystallised from water (charcoal). The diamino ester (1.45 g., 59%) separated as silver needles, m.p. 78° - 80°.

Analysis:

$C_8H_{10}N_2O_2$ requires:	Found:
C 57.8%	C 57.7%
H 6.0%	H 6.0%
N 16.9%	N 17.4%

The ester proved stable over a period of two years when kept in a dry,

stoppered container. Dissolved in polar solvents it rapidly darkened, and a black oil separated out. The free acid is spontaneously unstable (79).

Attempted preparation of methyl 3-amino-2'-nitrodiphenylamine-2-carboxylate

An intimate mixture of methyl 2,6-diaminobenzoate (0.200 g.) and o-iodonitrobenzene (0.300 g.) were

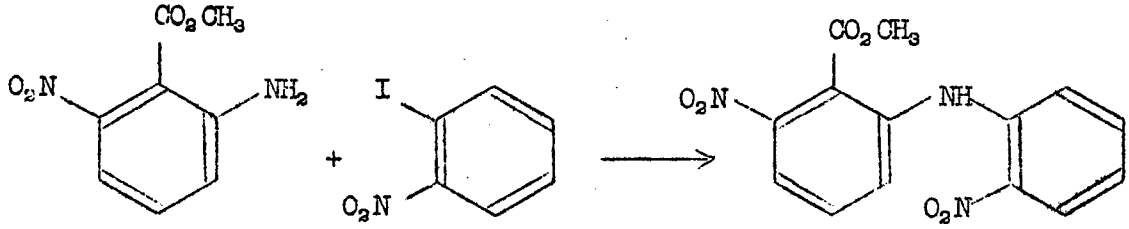
(1) fused at 140° for 4 hours with potassium carbonate (0.200 g.) and a trace of cuprous chloride;

(2) fused at 200° for 30 minutes with sodium acetate (0.150 g.), and a trace of copper, under a nitrogen atmosphere;

(3) refluxed for 1 hour in amyl alcohol (5 cc.) with potassium carbonate (0.200 g.) and a trace of copper, in a nitrogen atmosphere.

In each case the product was a dark viscous oil, which did not warrant further investigation.

Preparation of methyl 3,2'-dinitrodiphenylamine-2-carboxylate



Methyl 6-nitroanthranilate (1 g.), o-iodonitrobenzene (1.25 g.), potassium carbonate (1 g.) and copper powder (0.1 g.) were pulverized together. The mixture, contained in a boiling tube, was plunged into an

oil-bath at 200° and held there until the vigorous effervescence faded (5 - 6 minutes). The solidified melt was extracted twice with boiling water (10 cc.) and the residue recrystallised from glacial acetic acid (charcoal). The diphenylamine ester separated as glistening yellow-orange plates (0.55 g., 34%), m.p. 131° - 133°.

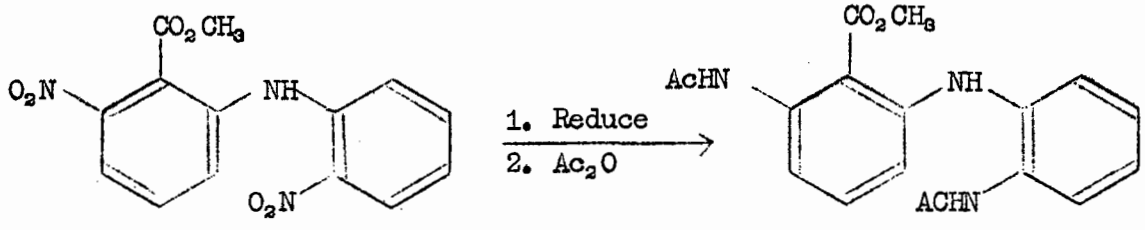
Attempts to prepare larger batches of diphenylamine led to considerably diminished yields and a darker product which was difficult to purify. If the temperature were allowed to exceed 210°, a violet exothermic reaction destroyed the product.

To ensure the purity of the compound, a portion (0.1 g.) in benzene was adsorbed on acid-washed, methanol-deactivated alumina and eluted in ether. Recrystallised from acetic acid, it separated as yellow-orange plates, m.p. 131° - 132°.

Analysis:

$C_{14}H_{11}N_3O_6$ requires:	Found:
C 53.0%	C 52.9%
H 3.45%	H 3.85%
N 13.2%	N 13.1%

Reduction of 3,2'-dinitrodiphenylamine-2-carboxylate and characterisation of the ensuing diamine

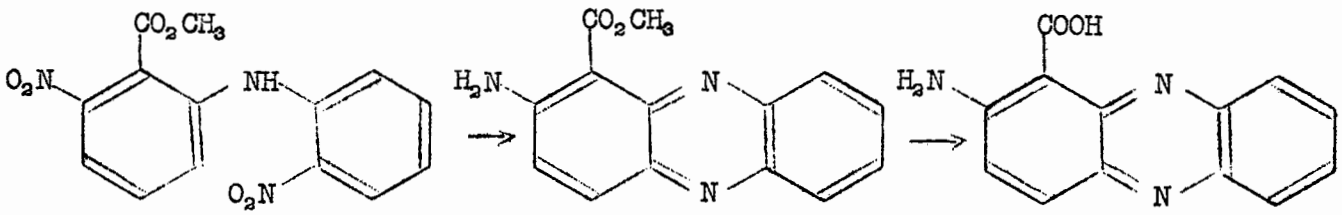


Methyl 3,2'-dinitrodiphenylamine-2-carboxylate (0.200 g.) was hydrogenated over platinum oxide in the usual way. The solution was filtered, and the solvent removed under reduced pressure with a nitrogen bleed. To the yellow oil remaining acetic anhydride (1 cc.) was added, and the mixture allowed to stand with intermittent shaking for 1 hour. Excess acetic anhydride was removed under reduced pressure and the residue recrystallised from a benzene/petroleum ether mixture. 3,2'-Diacetamidodiphenylamine-2-carboxylate separated as white spangles of needles, m.p. 168° - 169°.

Analysis:

$C_{18}H_{19}N_3O_4$ requires:	Found:
C 63.4%	C 63.1%
H 5.55%	H 5.8%
N 12.3%	N 12.3%

Reduction and cyclisation of methyl 3,2'-dinitrodiphenylamine-2-carboxylate



The dinitrodiphenylamine (0.600 g.) in alcohol was hydrogenated over platinum oxide in the normal manner. It was filtered into nitrobenzene (75 cc.), the alcohol distilled off, 5% palladium-charcoal (0.300 g.) added, and the solution refluxed for 16 hours. It was filtered hot and

the nitrobenzene removed under reduced pressure to small volume. The mixture was diluted with benzene and adsorbed on to an alumina column (2 cm. x 20 cm.). Development with ether revealed a large number of bands, three of which were isolated and identified.

(1) A deep red band travelling well ahead of the other main bands was eluted in ether. Evaporation of the solvent and recrystallisation of the residue from aqueous ethanol gave bright red needles of 1-aminophenazine (6 mg.), m.p. 175° - 178°, mixed m.p. with an authentic specimen 174° - 178°.

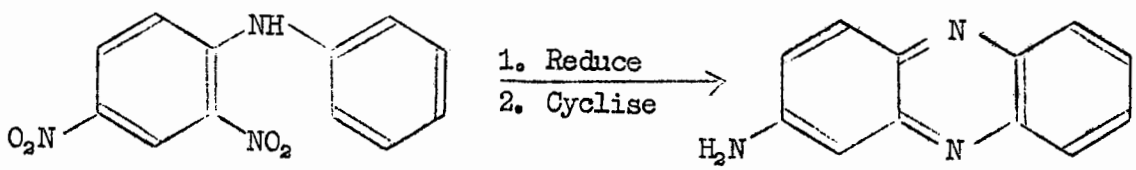
(2) A brilliantly-fluorescent yellow band tailing into an orange band was next collected. After evaporation of solvent, the residue was refluxed in 2 N NaOH for 1 hour. The solution was diluted, filtered, cooled and acidified with acetic acid. Recrystallisation of the precipitate from aqueous ethanol gave 2-aminophenazine-1-carboxylic acid (25 mg., 5%) as yellow-orange needles, m.p. 274° - 276° (dec.). Infra-red spectrum (KCl disc), mixed melting point and paper-chromatographic examination established it as identical to the acid obtained by the alternative route.

(3) The third orange fraction was refluxed for 1 hour in 2 N NaOH. The insoluble portion was dissolved in benzene and rechromatographed on alumina. 2-Aminophenazine (35 mg.), m.p. 274° - 275°, thus obtained did not depress the melting point of an authentic specimen and had identical paper-chromatographic behaviour.

(3) The Synthesis of 2-Aminophenazine-1-carboxylic Acid from 2-Aminophenazine

Preparation of 2-aminophenazine

(1) From 2,4-dinitrodiphenylamine



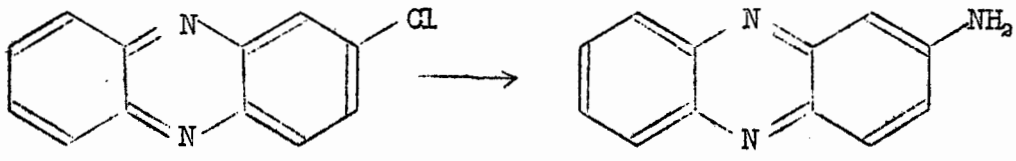
Gaertner, Thesis, p. 96.

2,4-Dinitrodiphenylamine (3 g.) (156) in alcohol was hydrogenated over 5% palladium-charcoal (0.5 g.) in the normal manner. It was filtered into nitrobenzene (375 cc.), the alcohol distilled off and the solution refluxed for 12 hours. The nitrobenzene was removed in high vacuo and the residue recrystallised from a minimum volume of nitrobenzene. 2-Aminophenazine (1.0 g., 42%) separated as shiny black needles, m.p. 275° - 280°.

Quoted values of the melting point of 2-aminophenazine range from 265° to 290° (157). Gaertner (loc. cit.) reports 270° - 274°.

The method recorded here is not suitable for the preparation of large quantities of 2-aminophenazine, since the volume of nitrobenzene needed soon becomes too great. A lower ratio of nitrobenzene to diphenylamine gave a black amorphous product which had to be purified by column chromatography.

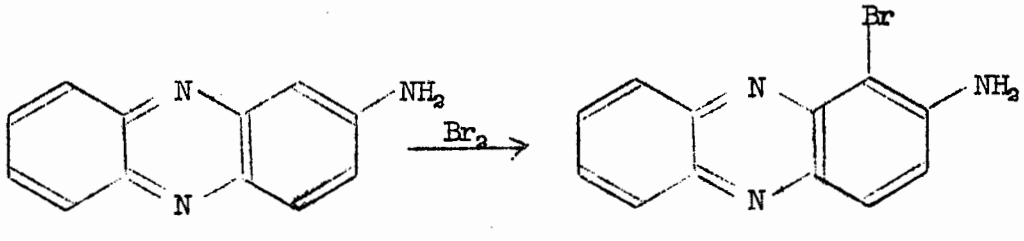
(2) From 2-chlorophenazine



Gray, Thesis, University of Cape Town, 1957.

2-Chlorophenazine (2.15 g.) (Vivian and Hartwell, J. Org. Chem., 18, 1068, 1953) cuprous chloride (0.43 g.), and a trace of copper bronze in an ammonia solution (100 cc. of sp. gr. 0.910) were heated for 18 hours at 200° in a high pressure hydrogenation apparatus fitted with a shaker. The cooled solution deposited 2-aminophenazine (2.0 g., 100%) as very dark black needles. Extraction of the precipitate with hot N HCl, filtration and basification gave the phenazine as a brick red precipitate (1.38 g., 70%), m.p. 273° - 275°.

The bromination of 2-aminophenazine



To 2-aminophenazine (1.48 g., 0.007 m.) in glacial acetic acid (30 cc.), a solution of bromine (1.28 g., 0.008 m.) in acetic acid (9 cc.) was added dropwise with vigorous shaking. The temperature of the solution was kept below 10°C during the addition. The precipitated hydrobromide was filtered, dissolved in boiling water, refiltered, and the filtrate made alkaline with 3 N NaOH. 2-Amino-1-bromophenazine (1.55 g., 75%) was obtained as an orange precipitate, m.p. 210° - 212°. Recrystallisation from aqueous alcohol gave orange needles, m.p. 211° - 212°.

Analysis:

$C_{12}H_8BrN_3$ requires:		Found:	
C	52.6%	C	52.7%
H	2.9%	H	3.0%
N	15.3%	N	15.2%
Br	29.2%	Br	28.8%

The reaction of 2-amino-1-bromophenazine with butyllithium

n-Butyllithium was prepared as described in Organic Reactions, Vol. VI, p. 353, and its activity determined by reaction with benzyl chloride.

(1) 2-Amino-1-bromophenazine (0.200 g., 0.00073 m.) dissolved in freshly distilled tetrahydrofuran (15 cc.) was cooled to -40°C. An ethereal solution (7 cc.) containing 0.0022 moles of n-butyllithium precooled to -40°C, was added in one portion with vigorous stirring. The solution was kept at -40°C for 15 minutes with intermittent shaking and then poured on to finely powdered dry ice. When it had warmed to room temperature, 2.5 N HCl (12 cc.) was added together with an equal

volume of ether. The solution was well shaken, the aqueous layer drawn off, freed from tetrahydrofuran by evaporation of the volatile solvent, and made alkaline. The filtered orange precipitate was shown by paper chromatography (BuOH : HCl : H_2O) to be unreacted starting material.

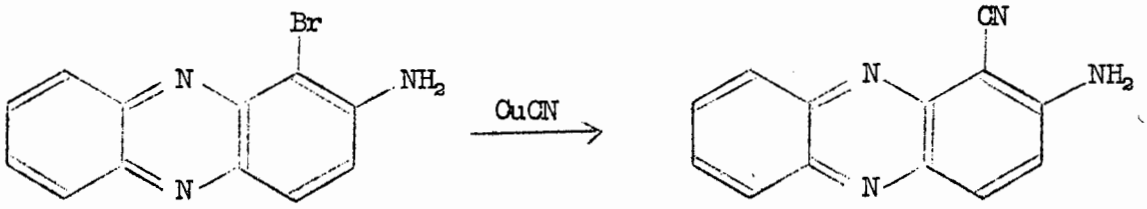
(2) This was carried out exactly as described above with the difference that 4 equivalents of n-butyllithium were added per equivalent of phenazine. The product was again unreacted starting material.

(3) 2-Amino-1-bromophenazine (0.050 g., 0.00018 m.), suspended in dry ether (10 cc.) and cooled to -35°C, was treated with an eight-fold excess of ethereal n-butyllithium. An intense violet colour developed which persisted for the 10 minutes during which the solution was kept at -35°C. Carbonation was followed by the normal working up, to give unreacted material.

(4) This was repeated as described above but the reaction was carried out at 0°C for 2 hours. No conversion was observed.

(5) 2-Amino-1-bromophenazine (0.050 g., 0.00018 m.) in tetrahydrofuran (5 cc.) and a three-fold excess of ethereal n-butyllithium were refluxed for 1 hour. The solution was carbonated and worked up as usual. No conversion was observed.

Preparation of 2-amino-1-cyanophenazine



2-Amino-1-bromophenazine (0.800 g.) and freshly-prepared anhydrous cuprous cyanide (0.500 g.) were refluxed for 30 minutes with vigorous stirring in quinoline (20 cc.). The solution was diluted with benzene, filtered and adsorbed on an alumina column (2 cm. x 30 cm.). Elution with ether removed the following bands:

(a) An orange band of unreacted bromophenazine.

(b) A pale orange band of 2-aminophenazine.

(c) A brilliantly fluorescent yellow band.

Evaporation of the solvent from the yellow band gave 2-amino-1-cyanophenazine (0.370 g., 58%), m.p. 280° - 285°. It separated from benzene as yellow needles, m.p. 284° - 285°.

Analysis:

$C_{13}H_8N_4$ requires:		Found:	
C	71.0%	C	71.1%
H	3.65%	H	4.1%
N	25.4%	N	24.6%

Runs using larger quantities of reactants gave considerably diminished yields, as did direct fusion or the use of nitrobenzene as solvent.

Attempted hydrolysis and alcoholysis of 2-amino-1-cyanophenazine

(a) Hydrolysis

(1) Crude 2-amino-1-cyanophenazine (15 mg.), sodium hydroxide (0.2 g.), ethanol (6 cc.) and water (6 cc.) were refluxed for 60 hours. Acidification of the solution and paper chromatographic examination showed only unchanged nitrile.

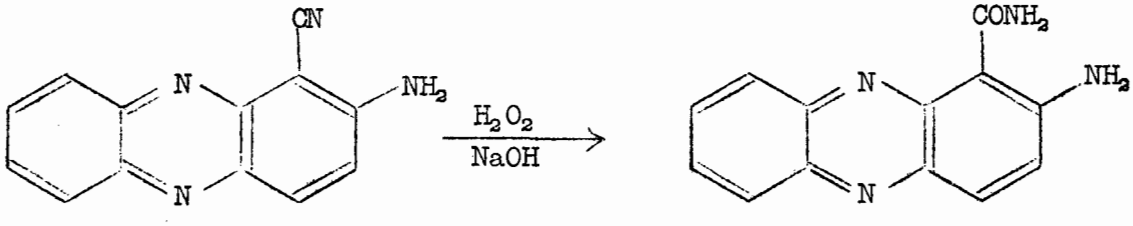
(2) Crude nitrile (15 mg.), conc. H_2SO_4 (2 cc.), acetic acid (2 cc.) and water (2 cc.) were refluxed for 1 hour. The solution was made just alkaline and the precipitate shown by paper chromatography (BuOH/HCl/H_2O) to be 2-aminophenazine.

(3) Crude nitrile (10 mg.), potassium hydroxide (0.2 g.) methanol (3 cc.) and a few drops of water were heated for 7 hours in a sealed tube at 190°. On cooling, dark red needles of 2-aminophenazine (m.p. and mixed m.p. 274 - 278°) separated.

(b) Alcoholysis

The nitrile (10 mg.), absolute methanol (3 cc.) and conc. H_2SO_4 (0.05 cc.) were heated in a sealed tube for 2 hours at 125°C. The solution was cooled, diluted with water (20 cc.) and made alkaline. The resulting precipitate was shown by paper chromatography (BuOH/HCl/H_2O; BuOH/HCOOH/H_2O; BuOH/NH_3/H_2O) to be unchanged nitrile.

Conversion of 2-aminophenazine-1-carbonitrile to 2-aminophenazine-1-carboxamide



To the nitrile (0.600 g.) in alcohol (140 cc.) and 25% sodium hydroxide (10 cc.), 30% hydrogen peroxide (20 cc.) was carefully added. The solution

was heated slowly to 70° and held there for 1 hour. A further portion of hydrogen peroxide (20 cc.) was cautiously added and heating continued for 2 hours. The alcohol was removed under reduced pressure, the residue well-washed with water and collected to give 2-aminophenazine-1-carboxamide (0.235 g., 36%), m.p. 265° - 270°. It recrystallised from aqueous ethanol as red-orange needles (becoming yellow on drying), m.p. 270°.

Acidification and ethereal extraction of the aqueous washings showed on paper-chromatographic examination traces of unchanged nitrile, 2-aminophenazine, 2-aminophenazine-1-carboxylic acid and an unidentified red spot, possibly an N-oxide.

Analysis:

$C_{13}H_{10}N_4O$ requires:		Found:	
C	65.5%	C	65.8%
H	4.2%	H	4.3%
N	23.5%	N	23.8%

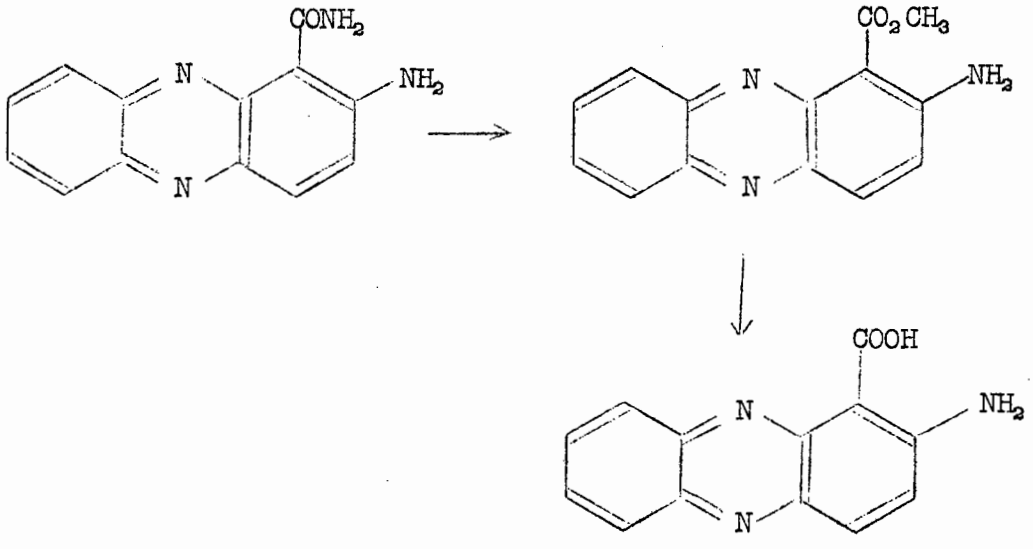
Attempted hydrolysis of 2-aminophenazine-1-carboxamide

(1) The amide (10 mg.) was refluxed for 4 hours in sodium hydroxide (2 cc.). At this point the aqueous solution was still quite colourless. The insoluble residue was collected and shown by paper chromatography ($BuOH/HCl/H_2O$; $BuOH/HCOOH/H_2O$; $BuOH/NH_3/H_2O$) to be unchanged amide.

(2) The amide (6 mg.) and 5 drops of 100% phosphoric acid (from 85% H_3PO_4 and P_2O_5) were heated at 140° - 150° for 1 hour. On dilution and basification, 2-aminophenazine separated and was identified by mixed melting point and paper-chromatography, (solvents as above).

(3) The amide (10 mg.) in conc. HCl (0.05 cc.) was cooled to 0°C and treated with 0.01 cc. of 27% sodium nitrite solution. The yellow suspension was heated over a period of 30 minutes to 80°C, the colour gradually changing to red. It was then heated for 3 minutes at 100°, the colour reverting suddenly to pale yellow. On basification no material was precipitated. Paper chromatographic examination in a basic solvent ($BuOH/NH_3/H_2O$) indicated that the product was an acid, though not identical to either 2-aminophenazine-1-carboxylic acid or phenazine-1-carboxylic acid. It was presumed therefore to be 2-hydroxyphenazine-1-carboxylic acid.

Esterification of 2-aminophenazine-1-carboxamide



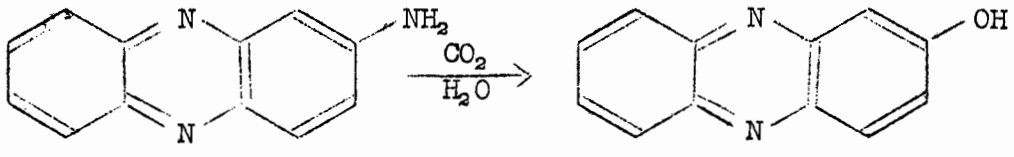
The amide (0.200 g.) in absolute methanol (30 cc.) and conc. H_2SO_4 (0.07 cc.) was heated in a sealed tube at 140°C for 5 hours. The cooled

solution was filtered from a small amount of dark material, the methanol evaporated and the residue taken up in 10 cc. of water. Adjustment of the pH to 8 with sodium hydroxide precipitated unchanged amide, while allowing the ester to remain in solution. No attempt was made to isolate the ester due to difficulties previously experienced in purifying it.

The amide was filtered and well-washed with water. The combined filtrate and washings were made strongly alkaline with sodium hydroxide and warmed on a boiling water-bath for 1 hour. (This had been previously established as the time giving the best compromise between hydrolysis of all the ester and decarboxylation of the resulting acid). The solution was filtered hot, cooled in ice and the pH adjusted to 5 with hydrochloric and acetic acids. The precipitate was redissolved in dilute ammonia, filtered, cooled and acidified as before. Recrystallisation of the precipitate gave 2-aminophenazine-1-carboxylic acid (0.043 g., 22%), m.p. 270° - 274°.

The acid thus produced was identical by mixed melting point, paper chromatography (BuOH : HCl : H_2O) and infra-red spectrum ($CHCl_3$ solution) to the acids obtained by oxidation of the different o-aminodiphenylamines.

(4) <u>The reaction of 2-aminophenazine and carbon dioxide</u>

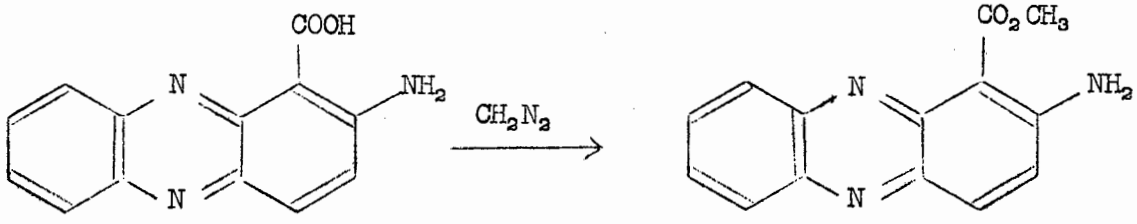


2-Aminophenazine (0.100 g.), water (100 cc.) and solid carbon dioxide (200 g.) were enclosed in a large hydrogenation apparatus fitted with a shaker and heated slowly to 150°C. The pressure rose to 90 atmospheres and was held there overnight. On cooling, red needles of 2-hydroxyphenazine (0.100 g., 100%) crystallised from solution, becoming yellow on drying. Paper-chromatographic examination (BuOH : HCl : H_2O) and comparison of infra-red spectra (KCl disc) with an authentic sample confirmed the nature of the product. The compound was best crystallised from nitrobenzene.

Attempted preparation of 2-aminophenazine-1-carboxylic acid by a Wohl-Aue reaction.

6-Nitroanthranilic acid (1 g.), aniline (1.6 cc.) and powdered potassium hydroxide (2.25 g.) were refluxed for 7 hours in benzene (10 cc.). The solvent was distilled and the black product remaining extracted with boiling water, filtered hot and carefully neutralized. The resulting precipitate was extracted with boiling 5 N HCl, filtered hot and concentrated. Paper-chromatographic examination of the concentrate showed no sign of the characteristic fluorescent orange spot of the phenazine acid.

Preparation of methyl 2-aminophenazine-1-carboxylate



A rapidly stirred solution of 2-aminophenazine-1-carboxylic acid (75 mg.) in absolute methanol (75 cc.) cooled to 0°C was treated with an excess of ethereal diazomethane. Stirring was continued for 2 hours after the addition. Excess diazomethane was distilled off at atmospheric pressure, and the solvent removed in high vacuo. The yellow partly-crystallised oil was extracted with cyclohexane, the solution filtered, and the cyclohexane removed in the cold. A yellow powder of the ester was left, m.p. 165° - 169°, which appeared pure by paper chromatography.

Analysis:

$C_{14}H_{11}N_3O_2$ requires:		Found:	
C	66.4%	C	70.0%
H	4.35%	H	6.2%
N	16.6%	N	12.6%

An infra-red spectrum of the ester contained bands assignable to cyclohexane. Inclusion of half a molecule of cyclohexane in the molecular formula improves agreement with the analytical figures. The cyclohexane could not be removed in high vacuo at room temperature, and the ester was not stable to heat.

Paper-chromatographic comparison of this ester with the esters obtained by cyclisation of the suitable diphenylamines and alcoholysis of 2-aminophenazine-1-carboxamide showed that all four were identical.

PART IV

COMPARATIVE RATES OF PHENAZINE FORMATION

General procedure:

The dinitrodiphenylamine (0.200 g.) in absolute ethanol was hydrogenated at 3 atmospheres pressure over platinum oxide (40 - 60 mg.). A colourless solution was taken as a criterion of completeness of reduction; in view of the strong colour of the dinitrodiphenylamines this is a reasonable assumption. The solution of diaminodiphenylamine was filtered into nitrobenzene (25 cc.), the alcohol and a few drops of nitrobenzene distilled off, and reflux commenced.

At suitable intervals reflux was interrupted, the solution cooled and a 1 cc. aliquot pipetted out. In the case of the esters this was adsorbed on to a column (1 x 25 cm.) of Peter Spence Grade 0 alumina prepared in benzene. The column was developed with benzene to remove the nitrobenzene and the phenazine ester eluted in ether. By examining the column under an ultra-violet lamp the presence of unchanged diaminodiphenylamine could be detected and its concentration roughly assessed. After removal of the solvent, the concentration of the phenazine was determined spectrophotometrically in N/10 HCl at a suitable wave-length by comparison with a standard curve. The identity of the compound was confirmed by paper-chromatographic comparison in BuOH/HCl/H_2O.

In two cases experiments were performed on solutions of phenazine ester in nitrobenzene of known concentration to determine the recovery from

an alumina column. In each case the recovery was about 90%, the loss being made up by hydrolysis of some of the ester to acid, which was retained on the column. Losses of this order mean that the maximum yields appear slightly lower than they should be, but there is no effect on the time taken to achieve this yield.

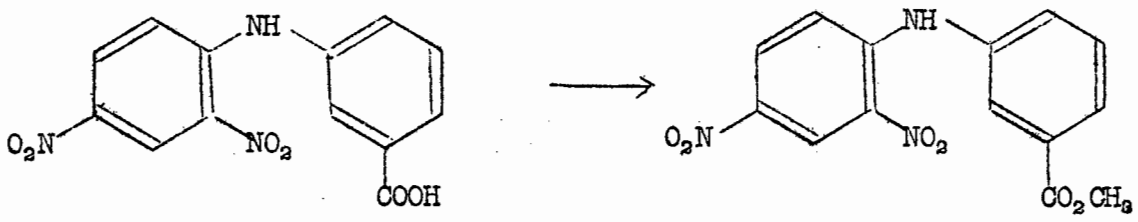
In the case of the acid, the nitrobenzene was removed from the 1 cc. aliquots by steam distillation. The residue was made up to a standard volume in N/10 HCl and determined in the normal way. Since the solution contained side-products and unchanged diaminodiphenylamine, the determination was made at 520 mμ where only deeply coloured materials would absorb. Paper-chromatographic examination showed that the phenazine was the only coloured material present in significant quantities.

At the conclusion of the rate study the final volume of nitrobenzene solution was measured. A mean of this and the starting volume was used in the calculation of yields.

The preparation of 2,4-diaminodiphenylamines with carbomethoxyl-groups in the 6 and 3'-positions, not mentioned in Parts I - III, is described here. 2-Aminophenazine-4-carboxylic acid and its methyl ester had already been prepared in these laboratories (26), and samples of these were used to identify the phenazines formed in the rate studies.

Preparative Work

Preparation of methyl 2,4-dinitrodiphenylamine-3'-carboxylate.



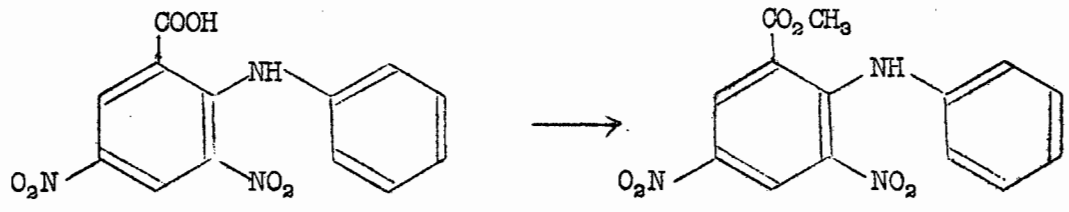
The dinitrodiphenylamine acid (2 g.) in absolute methanol (60 cc.) and concentrated sulphuric acid (2 cc.) was refluxed for 7 hours. On cooling, the ester crystallised from solution. A portion recrystallised from methanol gave shining yellow plates, m.p. 139° - 140°.

Analysis:

$C_{14}H_{11}N_3O_6$ requires:	Found:
C 53.0%	C 53.0%
H 3.45%	H 3.55%
N 13.2%	N 13.8%

Linke (50) reports that the ester crystallises from methanol as orange needles, m.p. 126°. No analysis appears to have been carried out.

Preparation of methyl 2,4-dinitrodiphenylamine-6-carboxylate



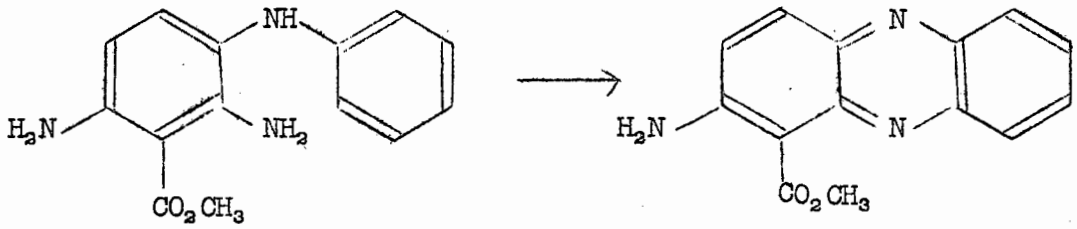
1.75 g. of the diphenylamine acid [Ullmann, Ann., 366, 83 (1909)] in ether (100 cc.) was treated dropwise and with mechanical stirring with excess diazomethane in ether. During the addition the ester began to crystallise from solution and after an hour 1.1 g. (60%) was collected, m.p. 145° - 146°. It could be recrystallised from methanol as brilliant yellow needles without raising the melting point.

Analysis:

$C_{14}H_{11}N_3O_6$ requires:	Found:
53.0%	52.7%
3.45%	3.15%
13.2%	13.1%

Rate Studies

Methyl 2-aminophenazine-1-carboxylate



Spectrophotometric determinations were carried out at 460 mμ.

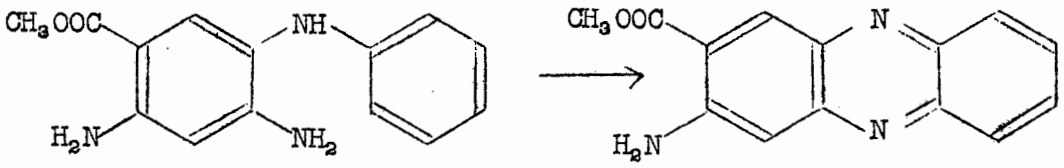
Time (hrs.)	2	4	5	10½	14
% Yield	37	64	67	58	43

Maximum yield 70% at 8 hours

Observations: In addition to the ester band several different coloured bands were present on the column. None of these were particularly intense and the author hesitates to ascribe any one of them to a transient intermediate. Under the ultra-violet lamp a green band became visible whose intensity diminished until it disappeared after 8 hours. This was removed and identified paper-chromatographically as the diaminodiphenylamine.

Recovery: 91%. A portion of the ester was hydrolysed to the acid, which could be eluted from the column in water, and identified by paper chromatography.

Methyl 2-aminophenazine-3-carboxylate



Spectrophotometric determinations were carried out at 560 mμ.

Time (hrs.)	12	18	24	36	48	60	72	96
% Yield	6.4	10.6	14.4	20.1	25.4	27.8	26.2	13.6

Maximum yield 28% at 60 hours.

In the presence of 0.200 g. of 5% palladium-charcoal.

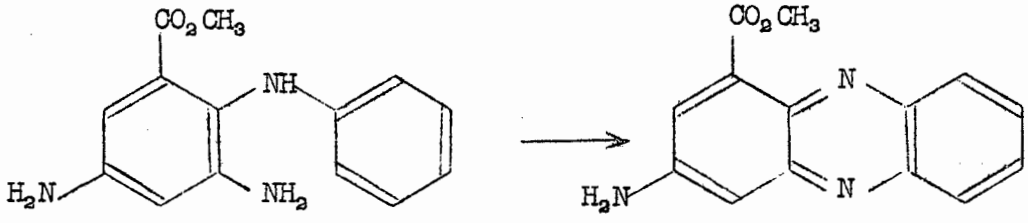
Time (hrs.)	15	20	24	30	36	42
% Yield	23	25.2	27.2	28.2	26.5	20.9

Maximum yield 28% at 30 hours

Observations: In each of the above a deep blue band and a band fluorescing a bright blue-green under the ultra-violet lamp were present up to the point of maximum yield. The fluorescent band was identified paper-chromatographically and by mixed melting point as the diaminodiphenylamine.

Recovery: 92%. A portion of ester was hydrolysed to the acid, which was eluted from the column and identified paper-chromatographically.

Methyl 2-aminophenazine-4-carboxylate



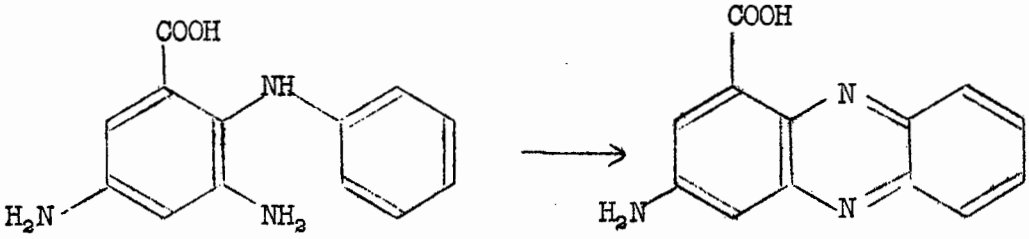
Spectrophotometric determinations were made at 520 mμ.

Time (hrs.)	1	6	12	24	32	47	72
% Yield	5.6	11.7	27.4	42	52	48.5	40.6

Maximum yield 55% at 40 hours

Observations: Diaminodiphenylamine, fluorescing a brilliant green-yellow under ultra-violet, and present to the point of maximum yield was identified paper-chromatographically. A pale orange band was also present throughout the reaction.

2-Aminophenazine-4-carboxylic acid

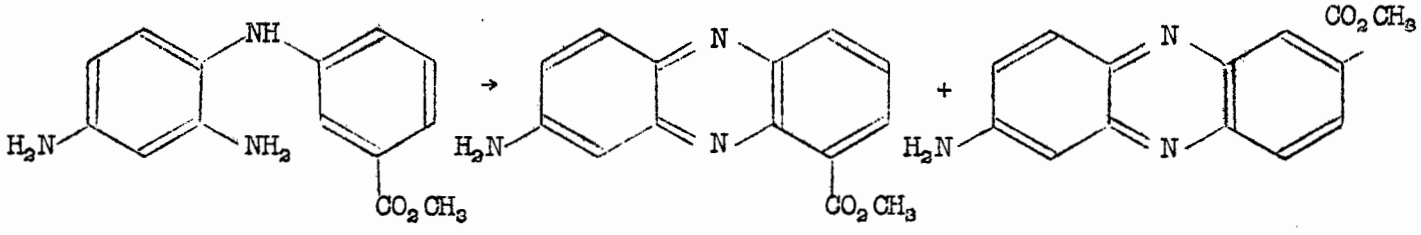


Spectrophotometric determinations were made at 510 mμ.

Time (hours)	2.5	4	6.5	8	10	13
% Yield	28.5	41	47	47	43	41

Maximum yield 47% at 8 hours

Methyl 2-aminophenazine-7 and 9-carboxylates



The standard curves at 520 mμ for methyl 2-aminophenazine-7-carboxylate and its 9-substituted isomer were almost exactly superimposable. The total concentration of the two phenazines was therefore determined without any attempt being made to separate them.

Time (hrs.)	2	3	5	7	8¼	11
% Yield	33	39	55	70	78	78

Maximum yield 80% at 10 hours

Observations: In addition to the mixed phenazine band, a band fluorescing green under ultra-violet light was present on the column to the point of maximum yield. This was identified as the diaminodiphenylamine by paper chromatography.

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Structures of compounds marked with an asterisk were not fully proved.

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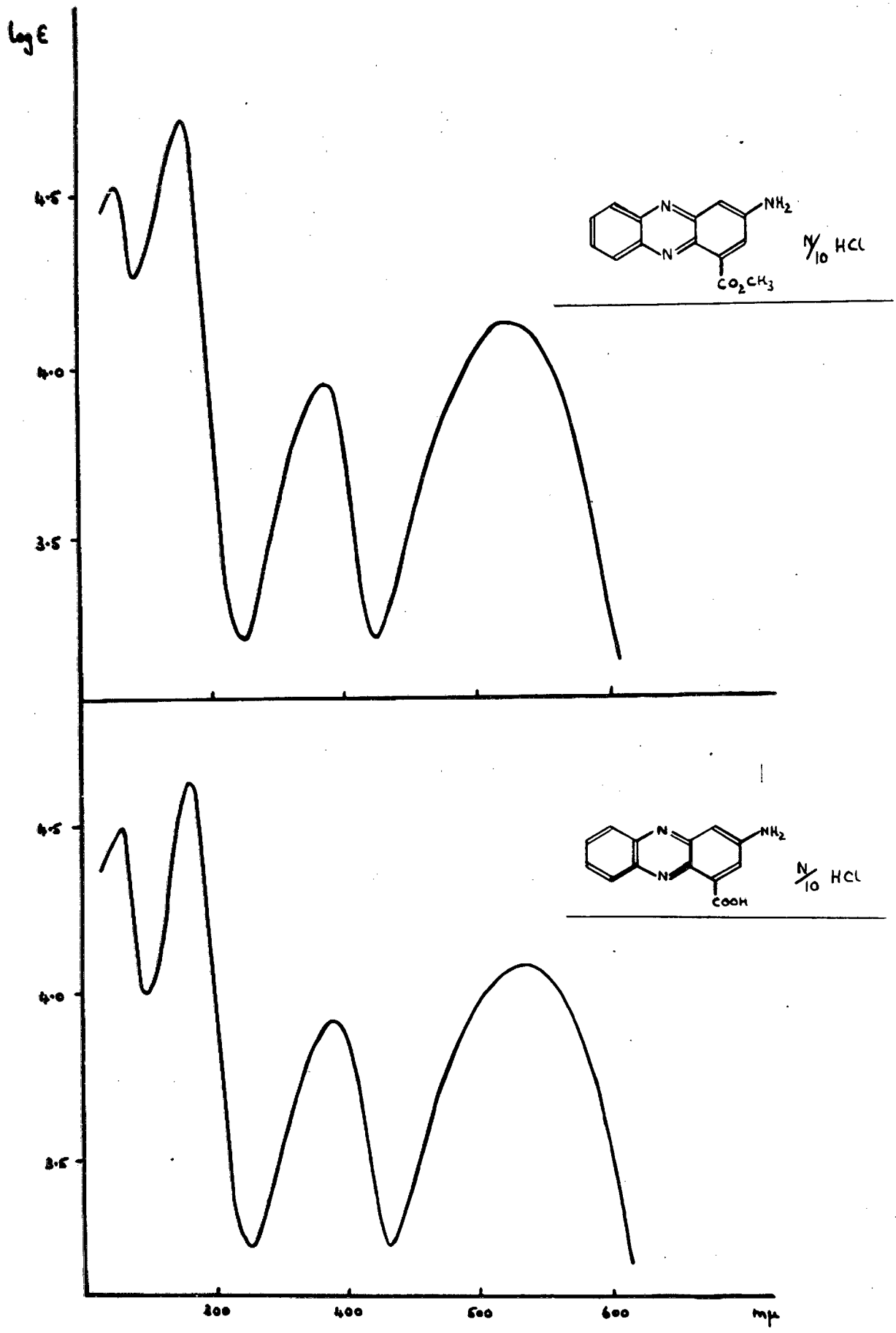
2-bromo-3-nitro-, methyl ester 122
3-bromo-2-nitro- 100, 112
5-bromo-2-nitro- 100
5-bromo-2-nitro-, methyl ester 101
2,6-diamino-, methyl ester 146
* 3,5-dibromo-2,6-dinitro-, methyl ester 140
2,6-dinitro-, methyl ester 139

Diphenylamine,
2,4-diacetamido-3-carboxy-, methyl ester 143
2,4-diacetamido-3'-carboxy- 115
3,2'-diacetamido-2-carboxy-, methyl ester 148
6,3'-diacetamido-2-carboxy-, methyl ester 124
2,4-diamino-5-carboxy-, methyl ester 108
2,4-dinitro-3-carboxy-, methyl ester 142
2,4-dinitro-5-carboxy- 106
2,4-dinitro-5-carboxy-, methyl ester 107
2,4-dinitro-6-carboxy-, methyl ester 164
2,4-dinitro-3'-carboxy- 114
2,4-dinitro-3'-carboxy-, methyl ester 164
2,4'-dinitro- 103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3·5
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N
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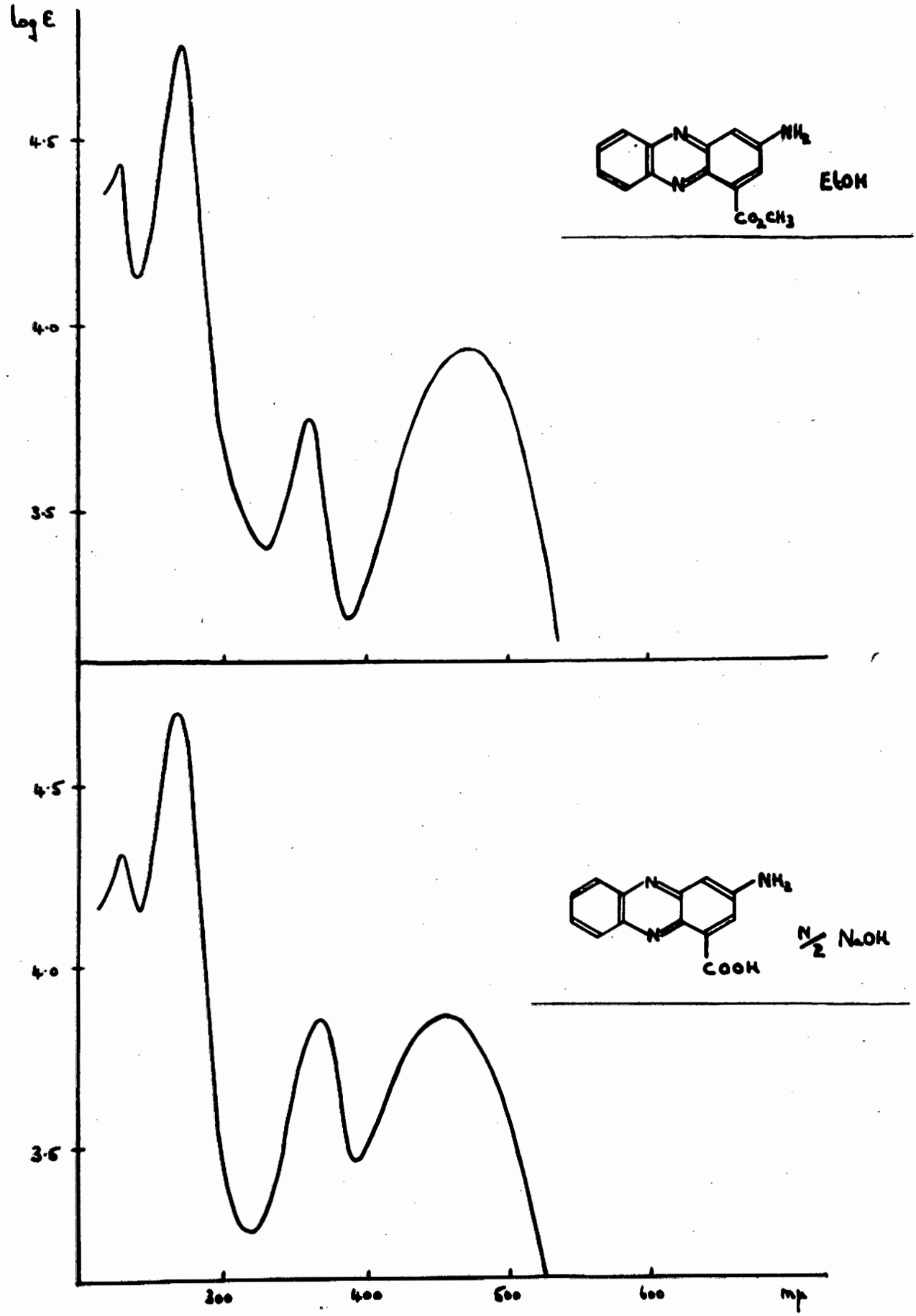
N/10 HCl
COOH
NH2
N
N
200
400
600
800
mμ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3·5
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N
N
CO₂CH₃
EtOH
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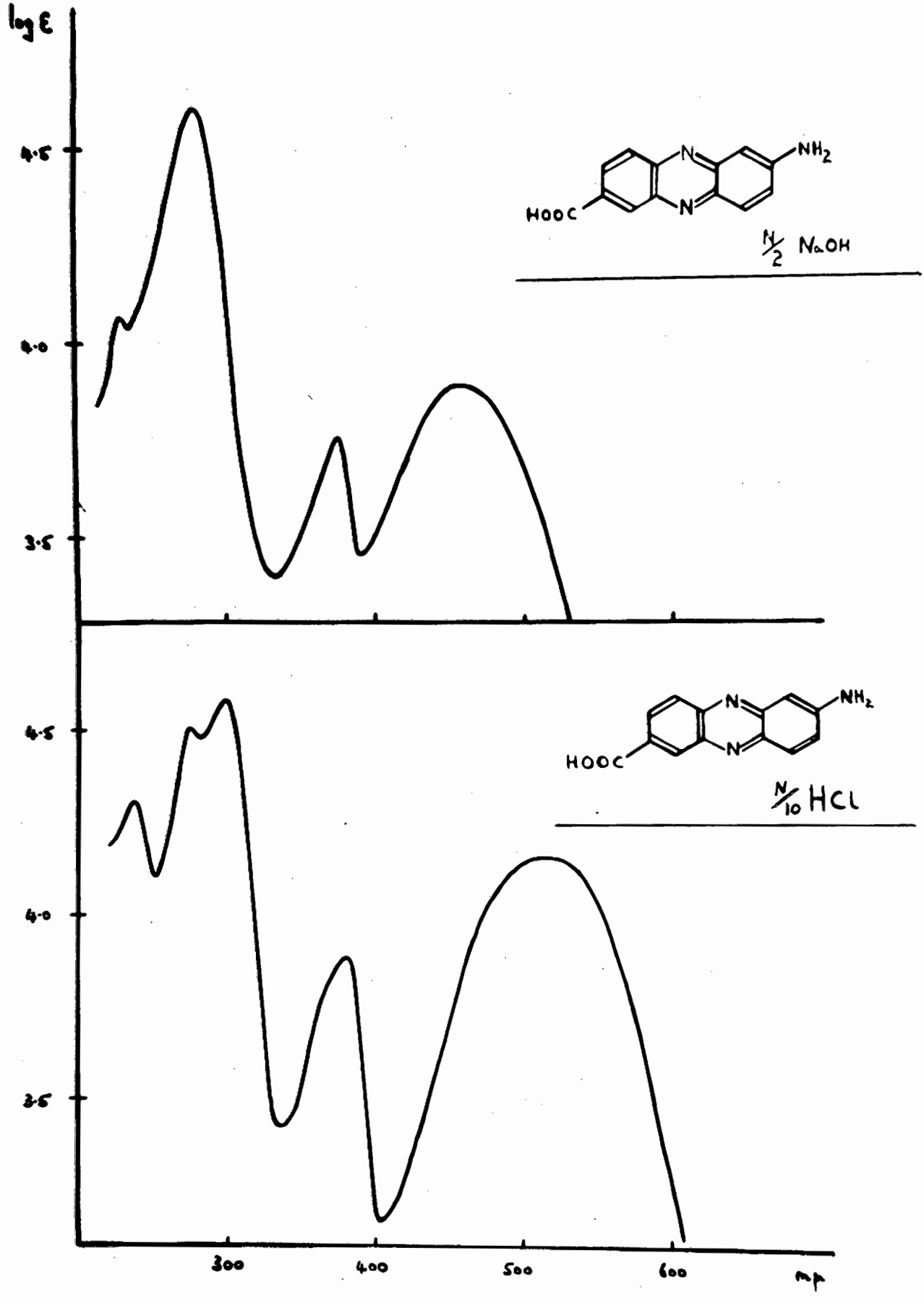
3·5
NH₂
N
N
COOH
N/2 NaOH
300
400
500
600
mμ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3·5
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N
N
½ NaOH
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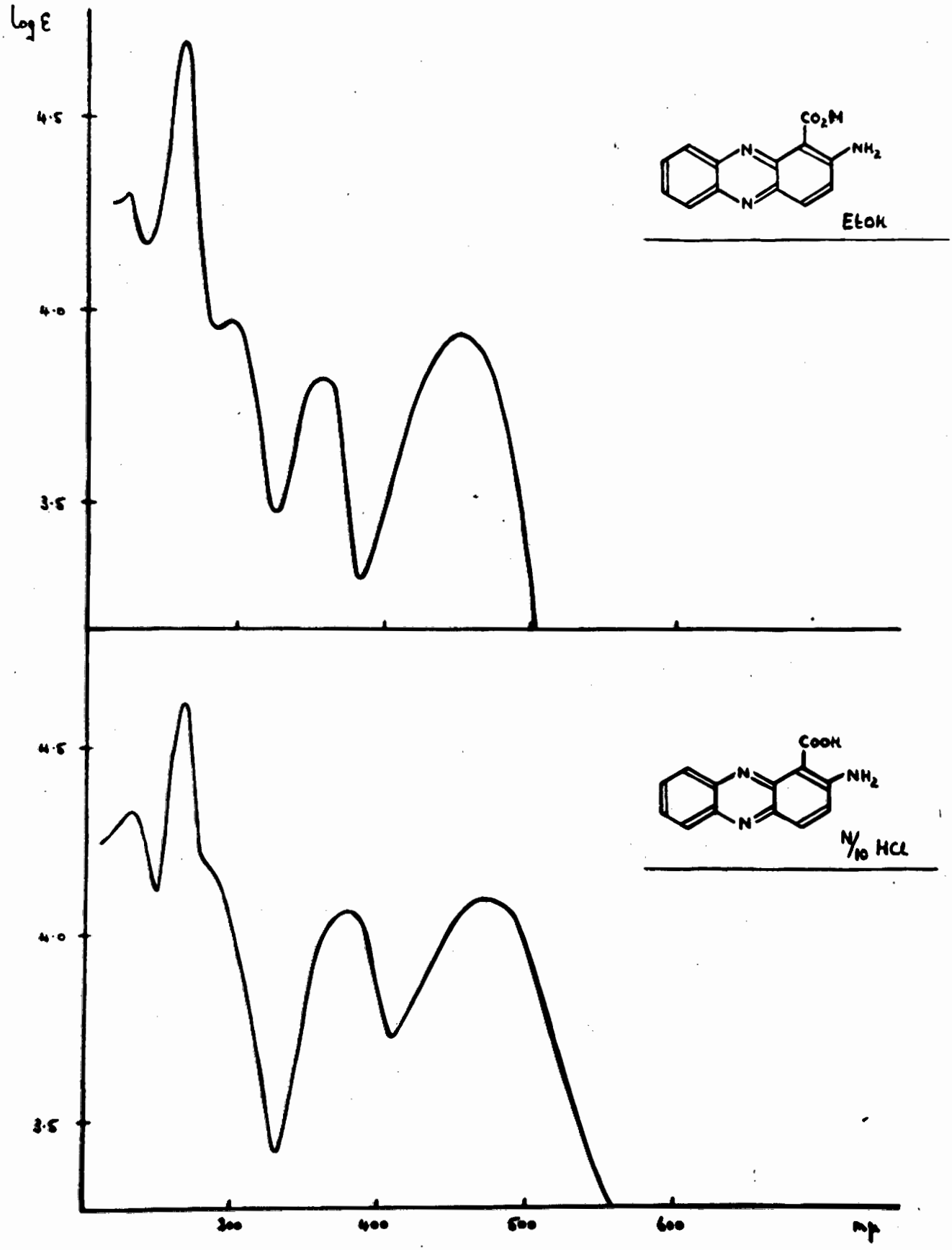
HOOC
NH2
N
N
N/10 HCl
300
400
500
600
mμ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3·5
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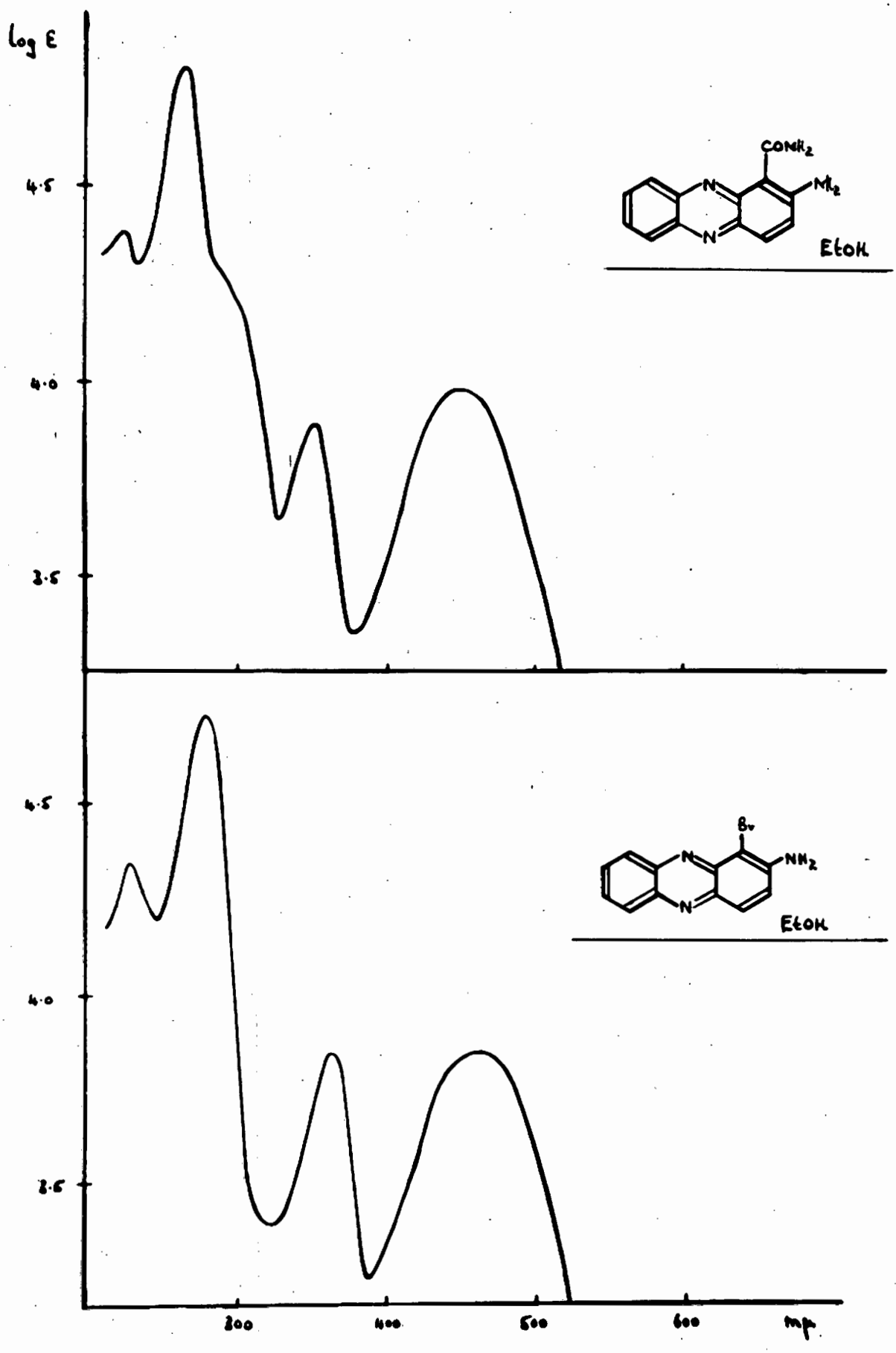
NH₂
N
N
EtOH
4·5
4·0
3·5
COOH
NH₂
N
N
N/10 HCl
200
400
600
800
mμ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4·0
3·5
CONH2
NH2
N
N
EtOH
4·5
4·0
3·6
Br
NH2
N
N
EtOH
300
400
500
600
mμ

A C K N O W L E D G E M E N T S

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