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PART I

"EXPERIMENTS IN THE COLCHICINE SERIES"

PART II

"STUDIES IN THE SYNTHESIS OF DIAZACARBAZOLES"

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UNIVERSITY OF GLASGOW

THESIS

submitted for

DEGREE OF Ph. D.

by

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September, 1948.

ACKNOWLEDGEMENTS

The author would like to express his sincere thanks to Professor J. W. Cook and to Dr. T. S. Stevens for their constant help and advice during many years.

Financial support for this work was provided through the generosity of the Carnegie Trust (Junior Research Fellowship: one year) and of the Medical Research Council (Research Studentship: two years).

Thanks is also due to Mr. J. M. L. Cameron, who carried out the micro-analyses in these experiments; to the Research Department of I. C. I. (Dyestuffs and Pharmaceuticals) Ltd., who very kindly supplied some information and reagents; and to Mr. and Mrs. Gordon Evans for their invaluable assistance in typing and proofing the manuscripts.

NOTES

Nomenclature The naming of organic compounds in this thesis follows the principles set forth in American Chemical Abstracts, 1945, 39, 5867, with two general exceptions:

(a) groups attached to aromatic nuclei are named in order round the rings, starting from position 1, and not in alphabetical order.

(b) principal functions which are expressed terminally are written as (for example) phenanthrene-9-carboxylic acid, and not as 9-phenanthrenecarboxylic acid.

A few other exceptions are mentioned specifically in the text.

Indexing After each part of the thesis there is an index of the preparations carried out and reactions investigated. More general headings are given in the table of contents which precedes each introduction.

References References which are not given in full in the text are indexed under the authors' names at the end of each part. In all cases the abbreviations of the names of journals are those used by American Chemical Abstracts (1946, 40, supplement).

Formulae In the illustrations, the double bonds in benzenoid rings are omitted. Customary abbreviations are used (e. g. Me = CH_3, Et = C_2H_5).

PART I

"EXPERIMENTS IN THE COLORATURA SERIES"

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SUMMARY

The synthesis of a substance related in structure to colchicine has been completed. The new compound proved to be different from a degradation product of the alkaloid, with which it was isomeric. This observation has a bearing on the structure of colchicine.

A preliminary report shows that the substance synthesised inhibits cell-mitosis: a re-evaluation of the structural factors essential for biological activity in this type of substance is consequently regarded as necessary.

INTRODUCTION

The particular importance of the alkaloid colchicine arises from an unusual and specific biological activity which it possesses:

If colchicine is introduced in low concentration into plant or animal tissues, the division of reproducing cells is inhibited, mitosis being suspended at a particular stage of the process (at the metaphase). Tissue sections prepared at intervals show an increasing number of arrested mitotic figures.

In the affected cells the initial chromosome duplication has occurred as usual, but the processes leading to the systematic division of the cell as a whole have been disturbed. Later, a single nuclear membrane may form around all the chromosomes to give a single cell containing double the normal diploid chromosome number--a "tetraploid" cell. It is possible that this resumption of function occurs only after the effect of the colchicine poisoning has worn off.

The inhibition of mitosis appears to be quite a general effect, having been observed in a wide range of both animal and vegetable tissues. The phenomenon is naturally of the greatest interest in the study of the mechanism of cell-division--one of the most fundamental of life-processes.

Colchicine in Agriculture

In addition to theoretical aspects, practical applications of the colchicine effect in plant breeding experiments may prove to be of universal agricultural importance:

In plants (at least), the tetraploid cells which may arise on treatment with colchicine appear to be functioning units. Once the influence of drug has been removed, they are capable of reproduction in the normal manner, giving rise to further similar tetraploid cells. It has proved possible to develop not only tissues composed of tetraploid cells, but complete tetraploid individuals. As such plants are usually fertile and true-breeding, essentially new species may be said to have been originated.

The agricultural significance of the colchicine effect is due to the fact that tetraploid plants possess new and improved qualities: they are larger in size and produce improved yields of seed-crops and other products.

In connection with hybridisation and the genetical aspects of plant breeding artificial polyploidy may prove to be of even greater practical importance. The selective breeding of new hybrid species in which some of the better qualities of both ancestors are retained is often frustrated by sterility of the hybrid forms.

In such cases, where it has proved possible to induce the development of an offspring whose cells contain all the chromosomes of both types, fertility has been maintained. It should be pointed out that this type of hybrid (an allopolyploid) differs from the normal type in the nature of its genetic constitution. It might be said that there is a summation of characteristics rather than a random selection.

Of this form of hybridisation, Dobzhansky (1937) remarks

"It is no exaggeration to say that the production of allopolyploids is the most powerful tool yet available to a geneticist for moulding living matter into new shapes."

Owing to the long-term nature of plant-breeding experiments, and partly to the war-time suspension of research, it will probably be many years before the true importance of colchicine in the breeding of new plant species can be evaluated.

Other influences are known which may induce the formation of tetraploid cells, particularly in plants (in some cases spontaneous polyploidy has been observed); but colchicine is unique not only in its potency, but also in the specificity of its action, and in its almost universal application.

Colchicine in Cancer Therapy

Another aspect of the biological activity of colchicine has caused a great deal of interest: namely, its action on the development of malignant tumours. In early experiments, injection of the drug appeared to cause haemorrhage and regression of tumours in mice (Amoroso, 1935). However, subsequent work has not fulfilled early hopes for the use of colchicine in the treatment of cancer.

The question has recently been reviewed by Ludford (1945). In this paper evidence is given which shows that regression of tumours subsequent to colchicine treatment is principally the result of the haemorrhage which follows the disruption of newly-formed capillaries. Colchicine has been described by many workers as a "capillary poison," the symptoms of poisoning being due to dilation and haemorrhage of capillary systems. It is thus probable that the drug has no true specificity for malignant cells. No direct connection has so far been established between the toxic action of higher concentrations of colchicine and its effect on cell-division.

In the treatment of tumours in mice the doses required to produce permanent regression were near the sub-lethal maximum; in human subjects, where combined X-ray and colchicine therapy gave regression of the tumours, there was invariably a subsequent relapse,

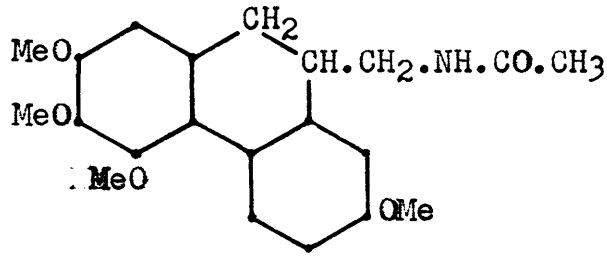
followed by the death of the patient.

In his review, Ludford sums up by saying,

"Extension of our knowledge of the chemical constitution of colchicine and related substances may reasonably be expected to lead to the discovery of new physiologically active compounds. Further progress is dependent upon finding among them some proclivity to a specific inhibition of malignant growth."

In addition to its importance on account of the above biological properties, the study of colchicine is of interest in general alkaloid chemistry.

The present work was part of an investigation of the structure and properties of the alkaloid and its derivatives, which was in progress in this laboratory. The substance formulated below is related in structure to colchicine, and was possibly identical with one of its degradation products. Its synthesis was undertaken both in connection with the elucidation of the structure, and for purposes of biological testing.



OCCURRENCE AND GENERAL PROPERTIES OF COLCHICINE

The plants in which colchicine occurs are members of Liliaceous species. It was originally isolated by Pelletier and Caventou (1820) from the seeds and corms of the autumn crocus (Colchicum Autumnale, Linn.), which is still the chief source of the alkaloid. In parts of this plant it is present to the extent of about 0.5%, and it has been found in a number of other species, notably Gloriosa Superba. The autumn crocus (also called the meadow saffron) is indigenous to the Old World and is found in meadows throughout Europe, the name "colchicum" being derived from the ancient province of Colchis on the shores of the Black Sea, where the plant was well known.

Since early times preparations containing colchicine have been used medicinally, particularly in the treatment of gout and rheumatism, and it is still prescribed, usually in the form of the salicylate, for these ailments. However, Henry (1939) states: "Up-to-date pharmacology has been unable to find any adequate reason for its use." Other authors report that in clinical practice colchicine alleviates the symptoms in acute attacks of gout, although in chronic cases it is of little help.

Commercially, the alkaloid is available as a yellow powder, m. p. 143-147°. When purified by crystallisation

from ethyl acetate it forms soft yellow needles, m. p. 155-157°, while from water crystals of the trihydrate can be obtained. Its anomalous solubility properties may have some bearing on the structure (see p. 21). According to Henry "it is miscible in all proportions with water, aqueous alconol or chloroform, but is less soluble in warm water (12% at 82°) or dry alcohol."

Colchicine is the methyl ester of an acidic compound, colchiceine, hydrolysis being very readily effected. The latter substance was isolated along with colchicine from the tissues of plants of the above-mentioned species, but Zeisel considered that it arose by hydrolysis during the extraction, and that the free acid does not occur naturally.

The parent alkaloid is a very toxic substance, causing, in man, as the principal symptoms, vomiting, haemorrhagic gastro-enteritis and diarrhoea, and in the later stages, ascending paralysis of the central nervous system.

Reference has already been made to the nature of the toxic effect; its relation, if any, to the inhibition of mitosis induced by high dilutions of colchicine is further discussed on pp.30-31.

A more specific biological activity was remarked by Dixon (1906), but the first critical observations of the action of colchicine on cell-division were not made until

the investigations of Lits (1934), Dustin (1934), and Ludford (1936). These experiments were made with animals, and only the inhibitory effects of colchicine on cell-division were observed. The development of tetraploid animal tissues, either in vivo, or in tissue culture, by the reproduction of tetraploid cells, was not reported. It is true however that in sections of treated tissue, cells were observed whose nuclei contained increased numbers of chromosomes, suggesting that, as in plants, the tetraploid cells are viable units. A number of studies with insects have now shown that it is possible for polyploid individuals to exist (cf. Rapoport, 1946); but the genetic complications introduced by sexual differentiation in animals cause the sex-determining mechanism to break down, and a normally propagating race cannot be achieved.

The applications of colchicine in plant breeding are discussed in an original paper by Blakeslee and Avery (1936).

STRUCTURE OF COLCHICINE

For many years it was assumed that the elucidation of the structure of colchicine was essentially complete. The derivation of the accepted formula (I) was given in 1924 by Windaus, in a paper in which the available evidence is summarised. However, in 1940, Cohen, Cook and Roe drew attention to several aspects of the chemistry of colchicine which made the proposed structure seem rather unlikely.

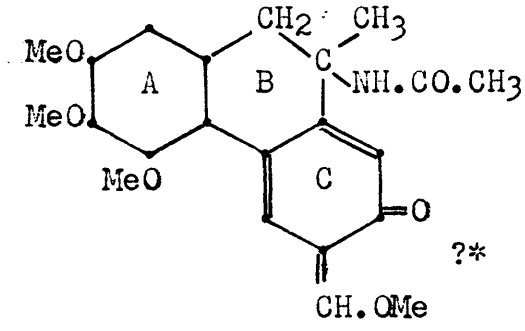
The evidence upon which Windaus based his hypothesis is first presented below, together with the criticisms which have been made. There follows a discussion of the significance of more recent observations, including the results obtained in the present work.

THE WORK OF ZEISEL AND WINDAUS

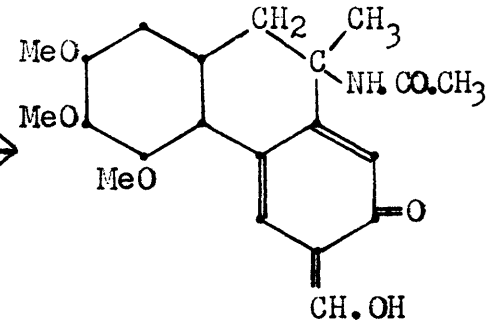
From preliminary investigations on the properties of colchicine, made by Zeisel (1883, etc.) and others, it was concluded that the colchicine molecule contains one labile methoxyl group, three more stable methoxyl groups, and an acetyl group attached to a basic nitrogen atom.

In his paper of 1924, Windaus proposed an essentially complete formulation for colchicine and its derivatives; the structures of colchicine and its classical degradation products, as represented in his hypothesis,

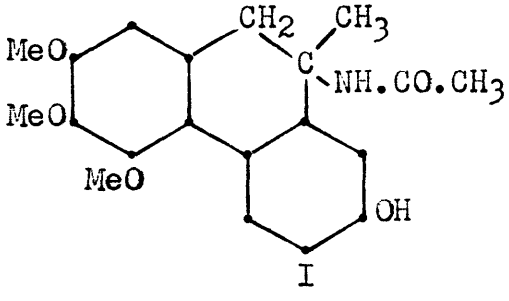
Windaus formulations



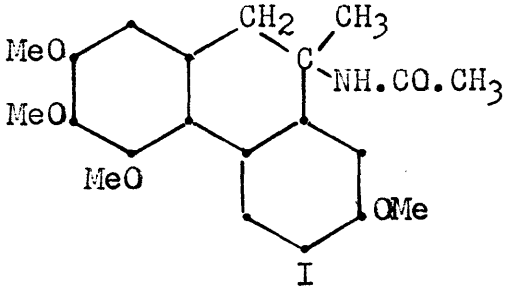
Colchicine (I)



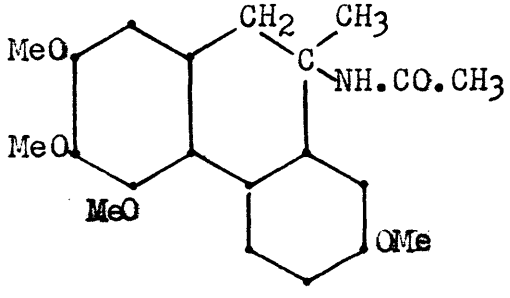
Colchiceine



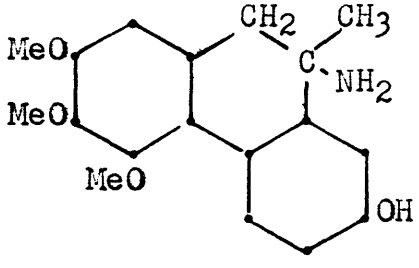
N-Acetyliodocolchinol



N-Acetyliodocolchinol methyl ether



N-Acetylcolchinol methyl ether



(Colchinol)

* Windaus was not able to establish the position of these groups. Subsequent work has established that, if this formulation is correct, then the orientation will be as shown (see p. 17).

are shown on the opposite page.

The preliminary steps in this classical degradation were brought about as follows: Hydrolysis of the labile methoxyl group was effected by the action of very dilute hydrochloric acid, and the resulting compound, colchiceine, possessed the properties of a weak acid.

On treatment of this acidic substance with iodine in aqueous potash, the elements of a formyl grouping were replaced by an iodine atom to give N-acetyliodocolchinol which behaved as an iodophenol.

Reduction of the methyl ether of N-acetyliodocolchinol with zinc dust removed the iodine atom to give N-acetylcolchinol methyl ether.

Elucidation of the Ring Structure

The evidence from which Windaus deduced the principal features of the ring structure of colchicine was derived mainly by oxidative 'degradation' of the above compounds. It may be summarised briefly as follows (see also illustrations, opposite):

A. Oxidation of colchicine, colchiceine, or deacetylcolchiceine*, gave, among other oxidation products, 3:4:5-trimethoxyphthalic acid.

B. If, before oxidation, ammonia was split off from

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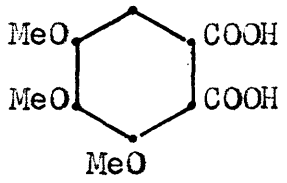
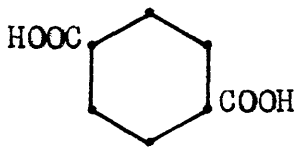
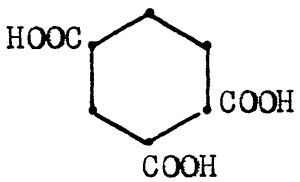
* also called trimethylcolchicinic acid.

A. 3:4:5-Trimethoxyphthalic acid

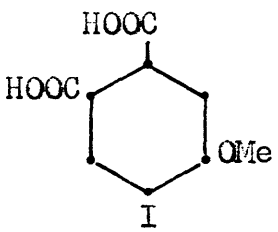


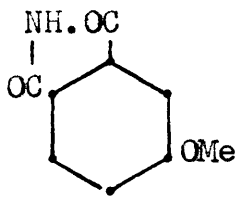
B. Terephthalic and trimellitic acids

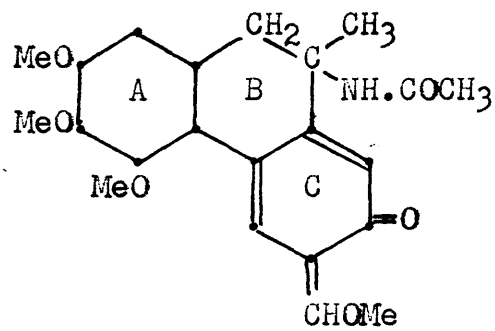




C. 4-Iodo-5-methoxyphthalic acid;
4-methoxyphthalimide







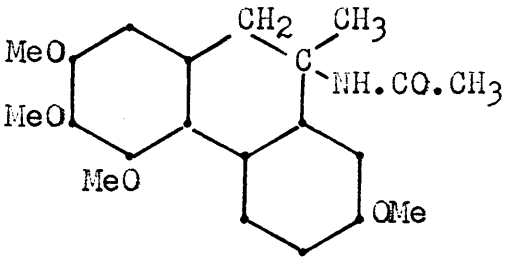
deacetylcolchiceine, then a number of benzene polycarboxylic acids, including notably terephthalic and trimellitic acids, could be detected among the oxidation products. This suggested that the elimination of ammonia caused a stabilisation (aromatisation?) of a second six-membered ring.

C. Oxidation of N-acetyliodocolchinol methyl ether with hot aqueous potassium permanganate yielded an iodomethoxyphthalic acid (later identified by Grewe (1938) as 4-iodo-5-methoxyphthalic acid), and oxidation of N-acetylcolchinol methyl ether gave rise to 4-methoxyphthalimide. From this last result a deduction could be made concerning the position of the nitrogen atom in the molecule.

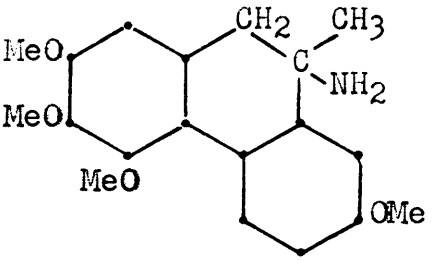
(If all the methoxy groups were first hydrolysed, then oxidation caused a complete breakdown of the molecule, and only succinic and oxalic acids were isolated. The production of the former is not particularly to be expected if the Windaus formulation is correct.)

From the above results Windaus deduced that the colchicine molecule contains three different six-membered rings. If the existence of a free methylene group is taken into account (see later), the number of hydrogen atoms present indicates that the rings will be doubly fused; i.e. that there is an anthracene or a phenanthrene nucleus.

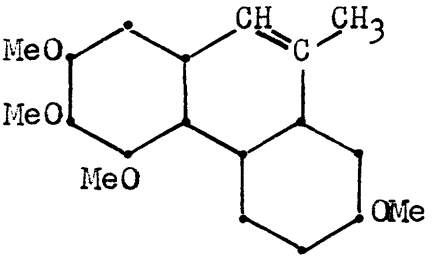
Windaus formulations



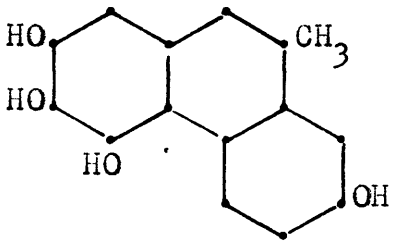
N-Acetylcolchinol methyl ether

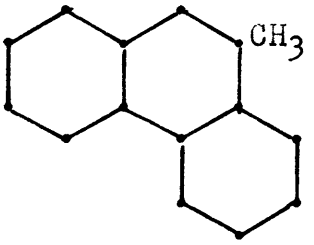


Colchinol methyl ether



Deaminocolchinol methyl ether





9-Methylphenanthrene

Windaus found that by the further degradation of N-acetylcolchinol methyl ether he was able to isolate ultimately 9-methylphenanthrene, which seemed to confirm the phenanthrene type nucleus in colchicine. This degradation is illustrated, using the Windaus formulae, on the left-hand page. The following steps are involved:

After deacetylation of N-acetylcolchinol methyl ether with hydrochloric acid the resulting colchinol methyl ether was submitted to a Hofmann degradation. Deaminocolchinol methyl ether was then treated with hydriodic acid to hydrolyse the methoxy groups, and the product was reduced by distillation with zinc dust. From the distillate a small quantity of 9-methylphenanthrene was isolated.

Ring A substituents:

Windaus correctly deduced the orientation of the three vicinal methoxy groups in ring A from the results of further oxidative experiments. His conclusion has since been confirmed by Barton, Cook, and Loudon (1945).

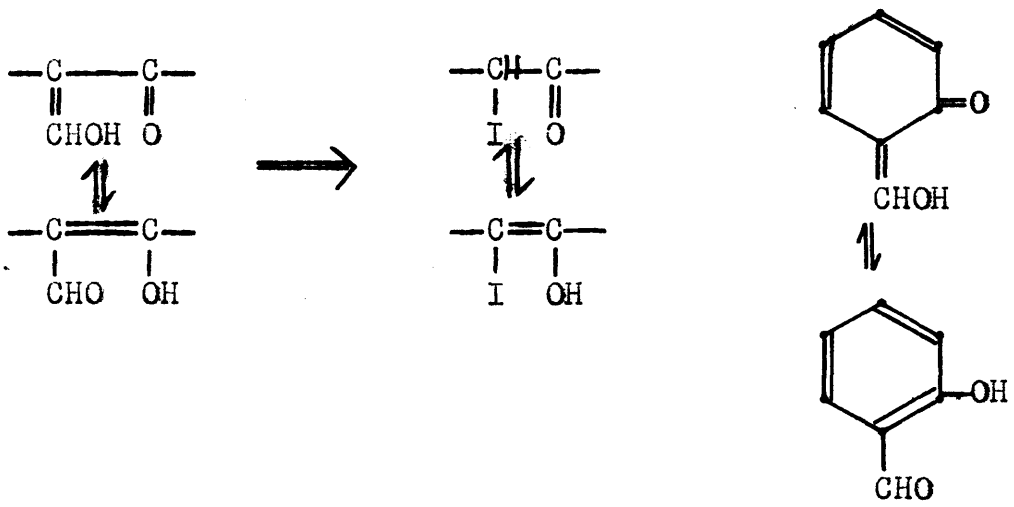
Ring B substituents:

The production of 4-methoxyphthalimide in the oxidation of N-acetylcolchinol methyl ether was taken as evidence that the basic group was attached to an α-carbon atom with respect to ring C. In another experiment it had been shown that N-acetylcolchinol methyl ether

contained a reactive methylene group. One of the two methylene groups of the 9:10-dihydrophenanthrene nucleus must therefore be unsubstituted, and the unplaced methyl group which is apparent in 9-methylphenanthrene must be attached also to the α-carbon atom with respect to ring C.

Ring C substituents:

The replacement of a formyl grouping by an iodine atom to give an iodo-hydroxy compound, by treatment with iodine in alkali, is a reaction which is given by both α-keto-hydroxymethylene compounds and o-hydroxy aromatic aldehydes. (In the tautomeric form the latter has an α-keto-hydroxymethylene structure.)



In addition, colchiceine exhibits a number of other properties characteristic of compounds of the above types. The existence of two isomeric forms of a dibenzoyl

derivative of deacetylcolchiceine (which has been demonstrated) could be accounted for by cis-trans isomerism about a methylene double bond. The structure finally suggested by Windaus as the one which accounted best for the observed properties was the tautomeric hydroxy-methylene form of an o-hydroxyaryl aldehyde.

In several respects this formulation is not satisfactory: the question is further discussed in a separate note on p. 21 .

If, however, the suggested formulation is assumed to be correct, then the work of Barton, Cook, and Loudon (1945) indicates that the orientation will be as shown in the accompanying illustrations.

Proof of a free methylene group

Careful oxidation of colchicine with chromic anhydride gave a substance, oxycolchicine, in which two hydrogen atoms in colchicine had been replaced by an oxygen atom. This product possessed the properties of a ketone. The oxidation of a reactive methylene group would account for this observation.

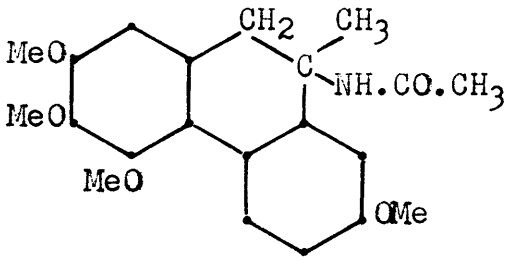
CRITICISMS OF THE WINDAUS HYPOTHESIS

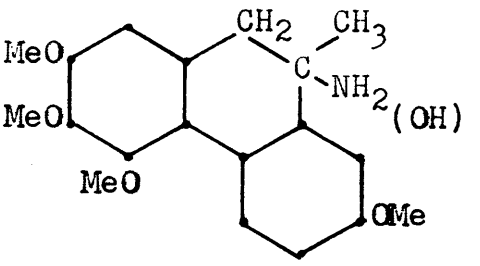
The present work is concerned mainly with the structure of ring B, which, as was observed by Cohen, Cook, and Roe

N-Acetylcolchinol methyl ether

Colchinol methyl ether (and corresponding carbinol)

1. (Windaus)





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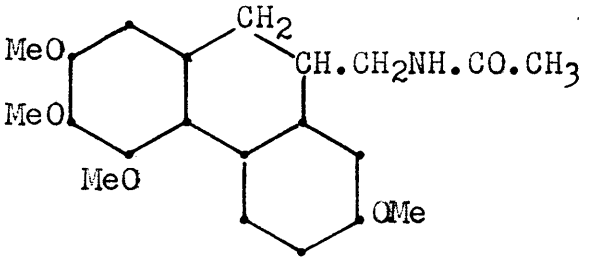

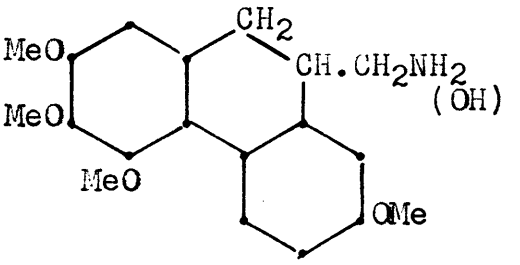

III

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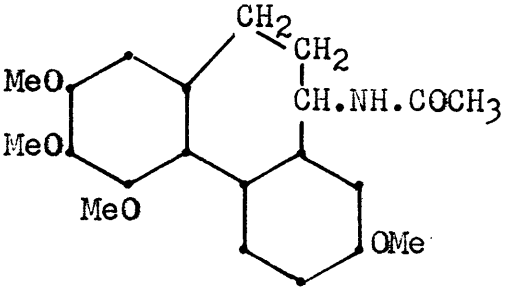
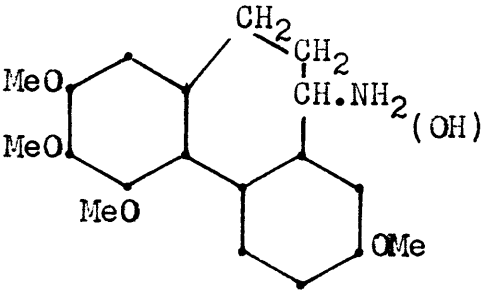




(1940), possesses properties which are not well accounted for by the above proposals:

For example, the amine, colchinol methyl ether, which is obtained by hydrolysing the acetyl group from N-acetylcolchinol methyl ether, does not readily lose ammonia to yield a phenanthrene as might be expected from the proposed structure. Cohen, Cook, and Roe, by treatment with nitrous acid, converted this amine to the corresponding carbinol. This carbinol likewise does not readily lose water to give a phenanthrene derivative.

ALTERNATIVE HYPOTHESES

Cohen, Cook, and Roe suggested two other possible formulations which might account for the observed properties. The formulae of N-acetylcolchinol methyl ether, colchinol methyl ether (and the corresponding carbinol), as represented by the three hypotheses are shown on the opposite page.

Against the second of these propositions it has been pointed out that N-acetylcolchinol methyl ether cannot readily be dehydrogenated to a phenanthrene derivative: it is recovered unchanged after treatment with platinum black at 280°. With known dihydrophenanthrenes this change takes place readily.

The fact that 9-methylphenanthrene was isolated by further degradation of colchinol methyl ether did not

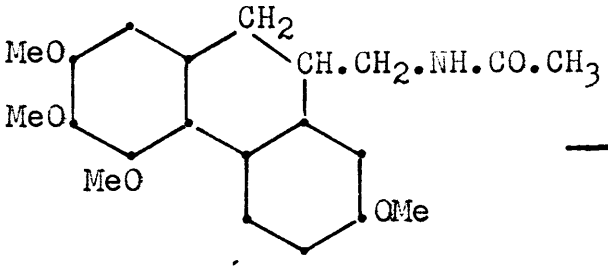
preclude the possibility of a seven-membered ring B. Rearrangement might well have occurred under the vigorous conditions of a zinc dust distillation.

THE PRESENT SYNTHESIS

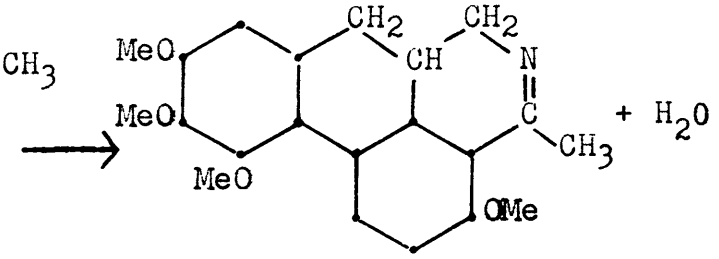
It seemed probable that the synthesis of 2:3:4:7-tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene (III) might be more easily achieved than that of the other compounds proposed as N-acetylcolchinol methyl ether. As this substance was also of interest for purposes of biological testing, its preparation was commenced.

While this work was in progress, further evidence became available (Barton, Cook, and Loudon (1945)) concerning the structure of colchicine degradation products. It was established that deaminocolchinol methyl ether was not, as Windaus suggested, a tetramethoxy methyl phenanthrene, but contained in fact a seven-membered ring (V). It would not be expected that the conditions used to convert N-acetylcolchinol methyl ether to deaminocolchinol methyl ether would bring about a rearrangement: it is therefore probable that the seven-membered ring is present also in the former substance (as in IV).

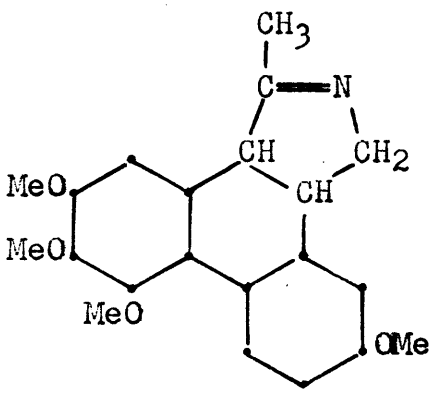
The properties of the new synthetic product (III) are not inconsistent with this view. It differs in melting point from N-acetylcolchinol methyl ether, and on heating

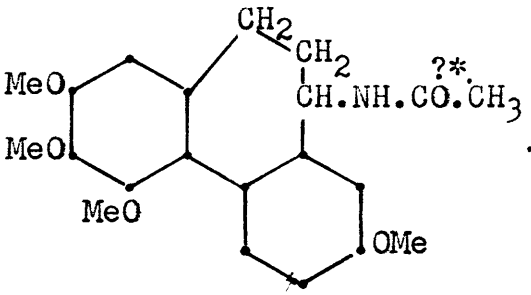


III



or?





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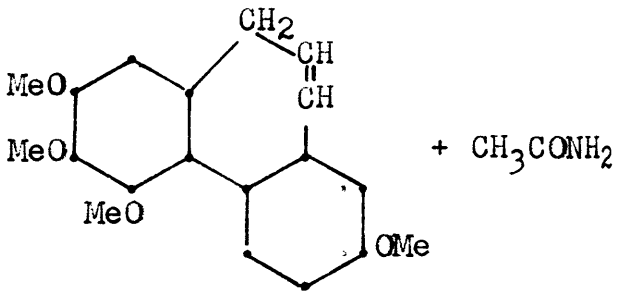
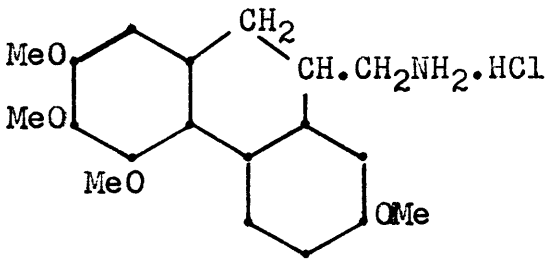


V



VI

* If the seven-membered ring does exist in colchinol derivatives there is still some doubt as to the position of attachment of the basic side chain.

with phosphoric oxide in xylene it loses the elements of water. N-Acetylcolchinol methyl ether, on similar treatment, eliminates acetamide and gives rise to the deaminocolchinol methyl ether (V) of Barton, Cook, and Loudon.

Similarly the corresponding amine hydrochloride (VI), which was also prepared, is different in its properties from the isomeric substance, colchinol methyl ether hydrochloride.

In view of these facts the hypothesis 2. for the structure of N-acetylcolchinol methyl ether is finally ruled out.

The only remaining alternative to a seven-membered ring is the original Windaus formulation, and this is still subject to the earlier criticisms, namely, that the amine, colchinol methyl ether, and the carbinol obtained from it by the action of nitrous acid, are both resistant to aromatisation. This stability is not consistent with the structures proposed by Windaus. Further evidence arising from the reactions of the "carbinol" with phthalic anhydride was given by Cohen, Cook, and Roe (1940). The interaction which occurred was "more characteristic of a secondary alcohol than of a primary or tertiary alcohol, but did not exclude a primary alcohol." The formulation of the carbinol involving a seven-membered ring contains a secondary alcoholic grouping.

There is thus a considerable body of evidence that

the seven-membered ring is present in colchinol derivatives as well as in deaminocolchinol in which its existence has been proved. Such an arrangement may well be present also in colchicine itself.

The position of attachment of the basic side chain in ring B remains to be confirmed.

(The identity of the methoxylated nucleus present in the substance synthesised in the present work (III) was confirmed by conversion of an intermediate to the known methyl ester of 2:3:4:7-tetramethoxyphenanthrene-9-carboxylic acid which had been prepared by a different route.)

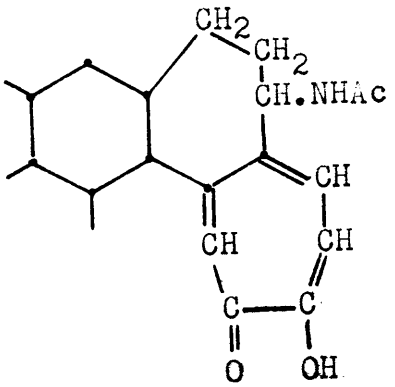
NOTE ON THE STRUCTURE OF RING "C" IN COLCHICINE

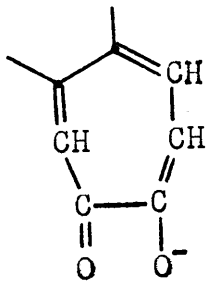
The evidence for a keto-hydroxymethylene grouping in colchiceine arises mainly from analogies with some rather unusual reactions common to α-hydroxymethylene ketones and o-hydroxy aromatic aldehydes such as salicylaldehyde. The form finally suggested by Windaus is the enolic state of an o-hydroxy aldehyde. In several respects this is unsatisfactory:

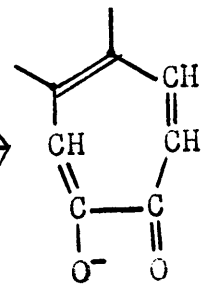
1. No explanation is given as to why the enol should exist to the exclusion of a benzene ring resonance. This does not occur in other phenolic aldehydes.

2. Colchiceine behaves as a stronger acid than would be expected from an iso-salicylaldehyde structure.

Colchicéine







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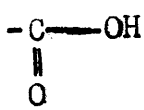
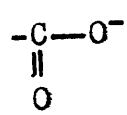
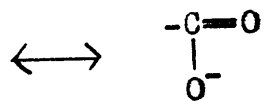
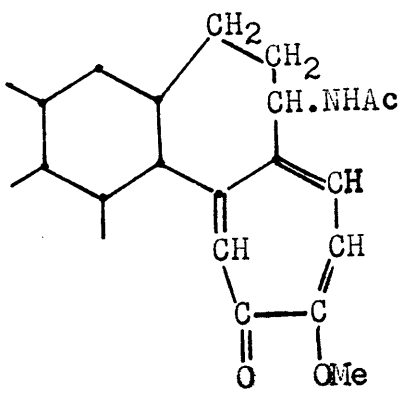
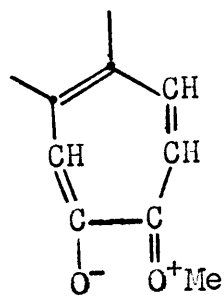


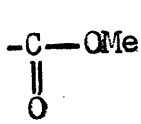


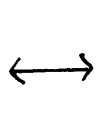


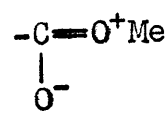
Colchicine

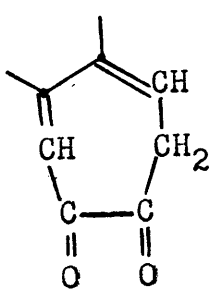


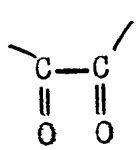


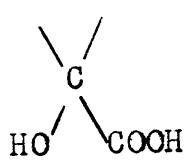












3. Neither colchicine nor colchiceine exhibits normal aldehyde or ketone reactions.

Dewar's Hypothesis

An interesting suggestion has been made by Dewar (1945, (1)), who proposes a seven-membered ring C. In this formulation for colchiceine a hydroxy group is conjugated around the seven-membered ring with a keto group.

As a carbonyl group and a hydroxy group separated thus by a conjugated double bond system would be expected to possess essentially the properties of the same groups as present in a carboxylic acid, this proposal would appear to be consistent with most of the observed properties of colchicine and colchiceine.

(In such a conjugated system the resonance alternatives possible are analogous to those which impart acidity and loss of ketonic properties in a carboxylic acid; this is illustrated on the opposite page.)

Dewar points out that rearrangement of such a seven-membered ring does not seem unreasonable. A diketo tautomer of the structure would be comparable to benzil, which readily undergoes the benzilic acid rearrangement.

It is difficult to account for the high water solubility of colchicine (it is more soluble than colchiceine). Dewar suggests that the ester-type zwitterion resonance is responsible; but a greater zwitterion contribution than

occurs in normal esters would be necessary to account for the observed solubility.

Hydrogen bond formation in colchiceine might help to account for its low solubility relative to colchicine.

A preliminary claim by Dewar (1945, (2)) to have confirmed a seven-membered ring structure by a lead-tetracetate oxidation has not so far been substantiated in a formal publication.

(In a recent paper Santavy (1946) claims that evidence from polarographic and spectrographic work shows colchicine to have the same characteristics as an ortho- or para-methoxy aromatic aldehyde. Further, colchiceine, deacetylcolchiceine, and colchicinic acid give results which would be expected from ortho- or para-hydroxy-aldehydes. Independent work by Brdicka gave rise to the same conclusions. It was also suggested by Santavy that the fact that an attempted Cannizzaro reaction on colchicine gave a high-melting product which no longer had the polarographic and spectrographic properties of a methoxy aldehyde was supporting evidence.

No experimental details are given for this work.)

STRUCTURE AND ACTIVITY IN COLCHICINE AND RELATED COMPOUNDS

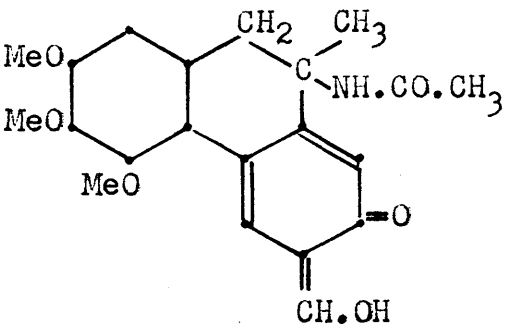
By the study of the chemical structure and functional properties of colchicine and other mitotic inhibitors, it may be possible to derive information concerning the biochemical processes which are involved in the reproduction of living cells. In such studies, perhaps the most obvious approach is the investigation of the effect of changes in chemical constitution on the biological activity.

In the case of colchicine the preparation and testing of related compounds is of additional value because of the possibility of finding new therapeutic substances for the treatment of cancer.

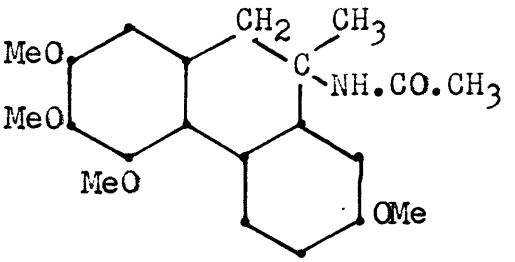
In a series of investigations, Lettré (1943, etc.) attempted to identify the structural components in colchicine which impart anti-mitotic activity. From his results he deduced that only compounds which contained a stilbylamine (α,β-diphenylethylamine) grouping are capable of causing the colchicine effect. However the fact that the substance prepared in the present work does not contain the required group, and yet is active, makes even this generalisation untenable.

A more detailed discussion of the work of Lettré follows:

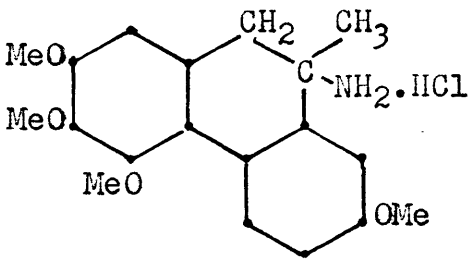
Windaus formulations



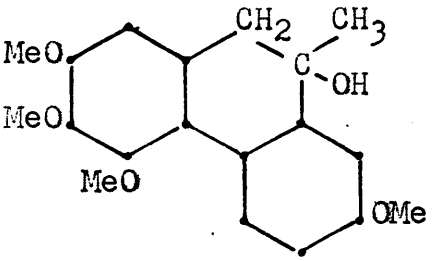
Colchiceine



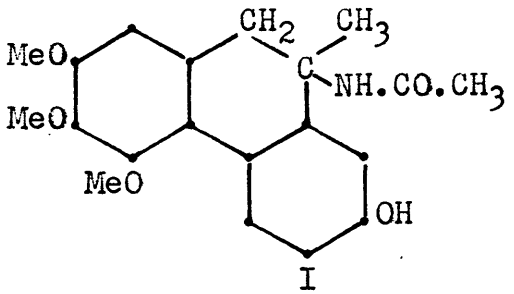
N-Acetylcolchinol methyl ether



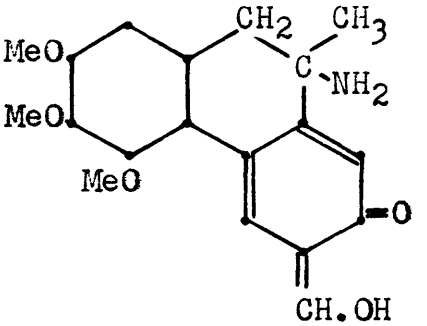
Colchinol methyl ether hydrochloride



Corresponding carbinol



N-Acetyliodocolchinol



Trimethylcolchicinic acid (Deacetylcolchiceine)

DERIVATIVES OF COLCHICINE

The first studies on the relationship between structure and activity were made by Brues and Cohen (1936).[1]

The classical degradation products of colchicine were studied, with the results summarised in the following table:

	Minimum effective dose (mg./100 g.)	Average lethal dose (mg./100 g.)
Colchicine	0.02	0.5
Colchiceine	0.8	3.0
Octahydrocolchicine	3.0	10.0
N-Acetylcolchinol	0.9	20.0
N-Acetylcolchinol methyl ether	8.0	12.0
Colchinol methyl ether hydrochloride	6.0	20.0
Corresponding carbinol	7.5	>20
N-Acetyliodocolchinol	10.0	20
Dimethylcolchicinic acid	--	10
Trimethylcolchicinic acid	--	20

1. As test subject Brues and Cohen made use of the rapidly regenerating liver tissue of partially hepatectomised rats, which is particularly suitable for observations on cell division. A suitable time after the administration of the substance under test the animal was killed, and an autopsy performed. The activity was expressed in terms of the minimum dose required to cause a reproducible inhibition of mitosis.

From these results it was concluded that certain structural factors are not clearly associated with the possession of activity: The N-acetyl group was not essential; the amino group could be replaced by a hydroxy group; the activity seemed relatively independent of the ring C substituents, although modification caused some loss of activity.

Brues and Cohen suggest that the unexpected inactivity of trimethylcolchicinic acid is related to the presence of a basic and an acidic grouping in the same molecule (with the attendant possibility of zwitterion formation).

In a further study of colchicine derivatives[1], Lettré and Fernholz (1943) found some activity in a number of other compounds in which ring C had been modified: these included colchicamide and azo-derivatives of N-acetylcolchinol; and a series of alkyl colchiceines prepared by the action of diazo-alkanes on colchiceine. In this last group of substances activity was found to fall off in the order R=methyl[2] > ethyl > propyl > butyl.

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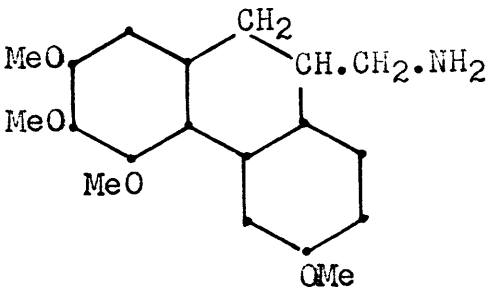
1. In all their studies Lettré and Fernholz detected anti-mitotic action in tissue cultures of chicken heart fibroblasts. The substances were also tested for an effect on the growth of Ascites tumour in mice, and it was found that the retardation in tumour growth roughly paralleled the inhibition of mitosis.

2. The methyl colchiceine obtained thus was not identical with colchicine. Lettré suggests that stereo-isomerism about the double bond of the methoxy-methylene system can account for this (but see p.).

Colchinol methyl ether



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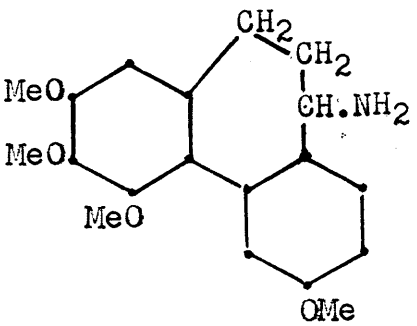

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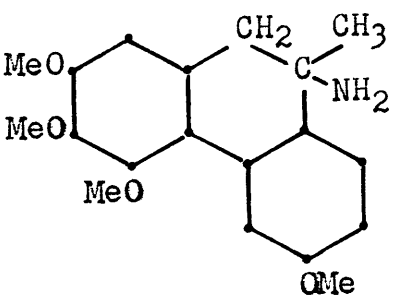
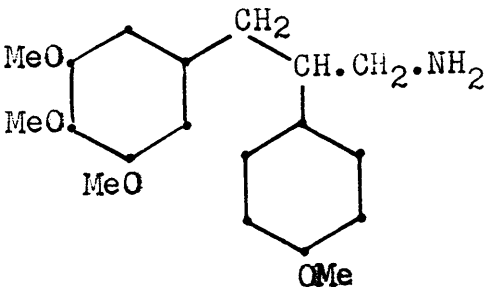
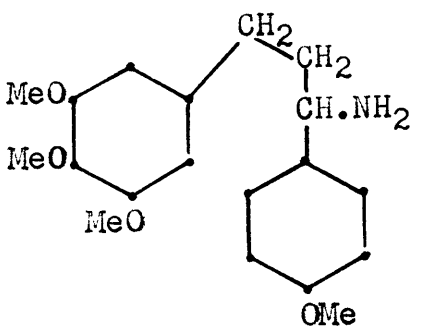


III

Simpler analogues



IV



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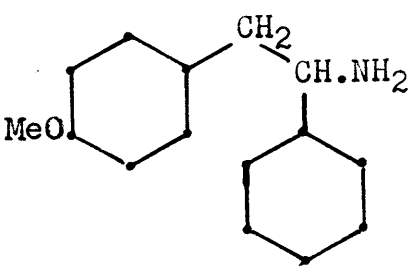


VI

SIMPLE SYNTHETIC ANALOGUES

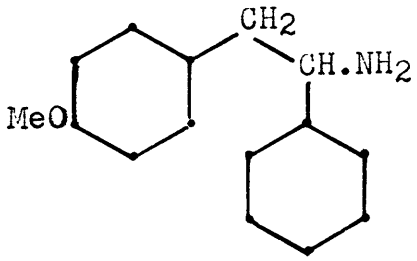
Lettré and Fernholz next studied a series of synthetic compounds of related structure in the hope of identifying the structural factors necessary for activity in some simpler derivative.

They related their results to the three possible formulae for colchicine which were discussed by Cohen, Cook, and Roe (1940). At this time the true methoxylation pattern had not been established, and a 2:3:4:6-distribution was wrongly assumed.

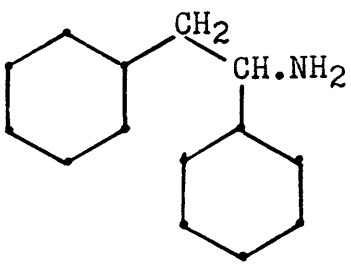
Cohen, Cook, and Roe have suggested the formulae I and II (opposite), as possible alternatives to the original Windaus hypothesis (III), for N-acetylcolchinol methyl ether. It was found by Lettré that the compounds IV and V, analogues of I and II respectively, possessed no specific anti-mitotic activity. In the case of V variations in the methoxylation pattern by removal of various groups did not promote an activity; the acetyl derivatives were likewise inactive.

However, a simpler analogue corresponding to the Windaus formulation, namely VI, was found to possess a very high activity.

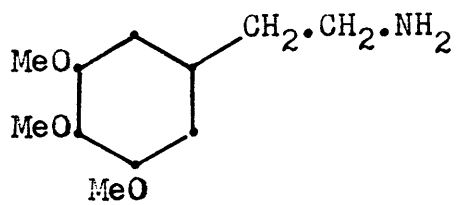
In his next study Lettré attempted to ascertain which features of this α-phenyl-β-(p-methoxyphenyl)ethylamine molecule were essential for activity. He therefore tested the compounds VII, VIII, and IX, in which various



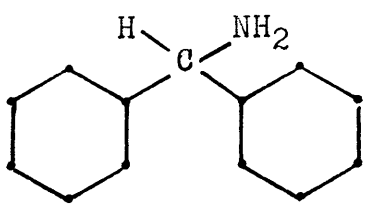
α-Phenyl-β-(p-methoxyphenyl)ethylamine (VI)



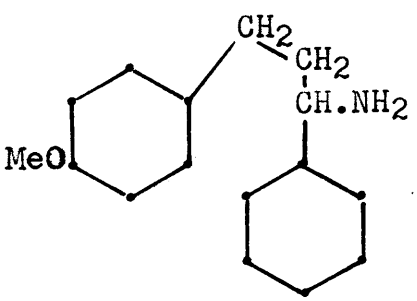
VII



VIII



IX



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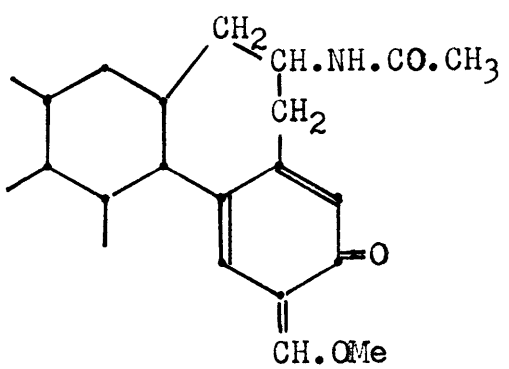


XI

groups in VI were lacking. In none of these substances, nor in a number of their derivatives, including their acetyl derivatives, was any activity found.

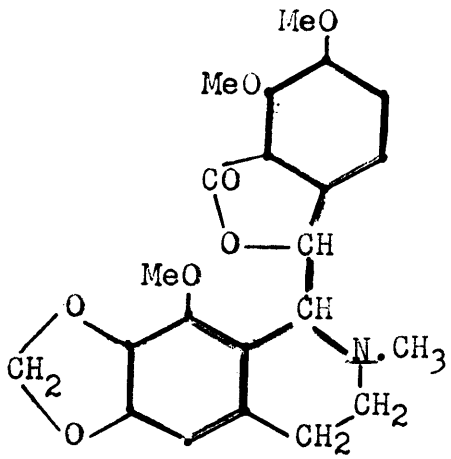
It therefore seemed that α-phenyl-β-(p-methoxyphenyl)-ethylamine represented the limit of simplification.

The fact that X also lacked activity suggested that exactly the grouping present in VI was necessary.

The hypothesis that a methoxylated stilbylamine grouping was characteristic of active substances received support from the results of further tests in which a number of substances containing the necessary grouping in their structure were examined:

A selection of alkaloids tested included representatives of all the classes which contained this grouping: the (colchicine), papaverine, berberine, and chelidonine alkaloids. Of the compounds tested, the two which showed anti-mitotic activity contained a stilbylamine structure in their molecules. These were narcotine and chelidonine (Lettré, 1943; Lettré and Albrecht, 1944).

A further study of stilbylamine derivatives (Lettré



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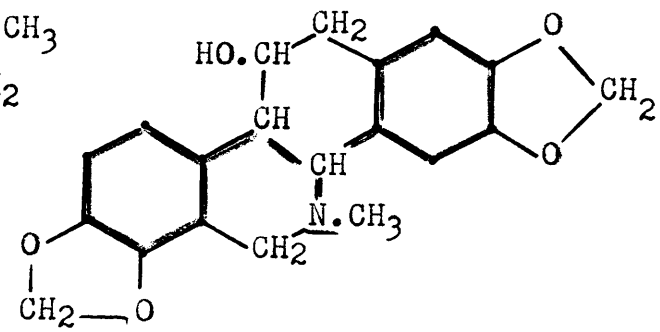

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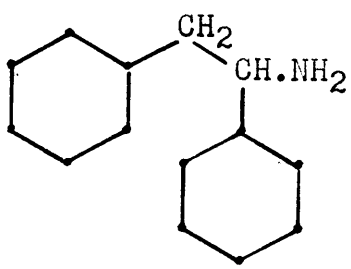

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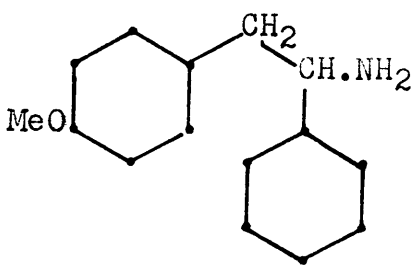

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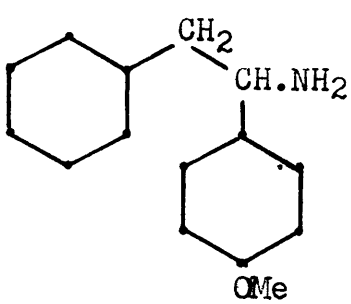

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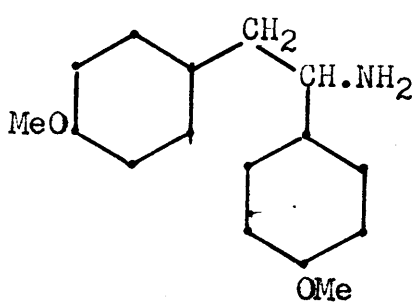

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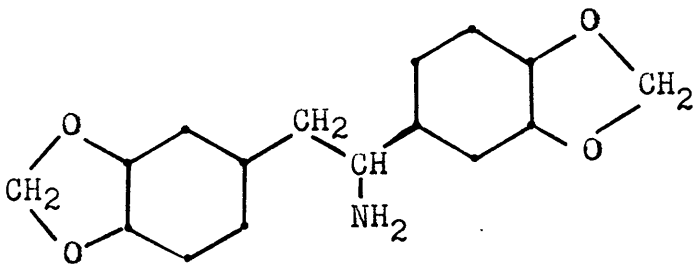

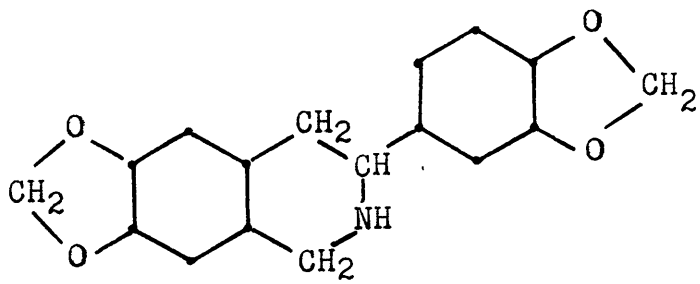

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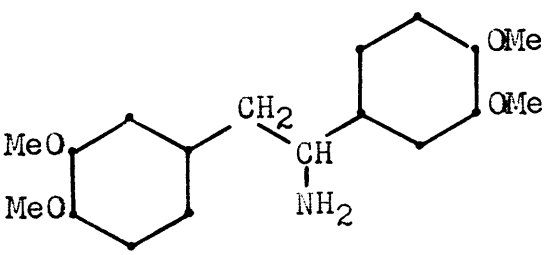
Active



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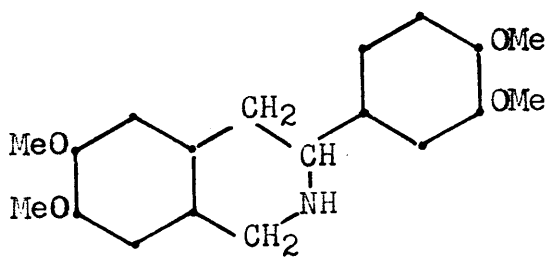

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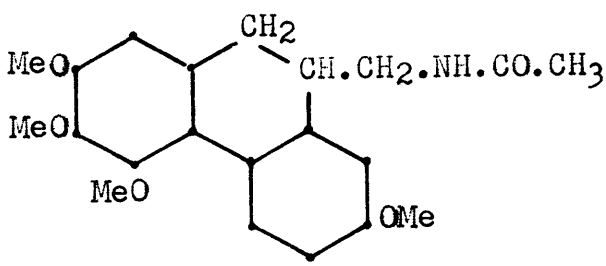


Active

(2:3:4:7-tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene, XII)

and Delitzsch, 1944) led to the general conclusion that activity was exhibited only by substances containing the stilbylamine grouping, and that this activity was further conditioned by the pattern of the substituent methoxy (or methylenedioxy) groups in the molecule.

The observations are illustrated by the formulae given on the left-hand pages.

ACTIVITY OF NEW SYNTHETIC PRODUCT

2:3:4:7-Tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene (XII) does not contain the grouping regarded as essential for activity.

A sample was sent to Dr. Lettré for testing, and a preliminary report indicates that the substance does however cause an inhibition of cell division. The above hypothesis of the structural requirements for biological activity will therefore require to be reconsidered.

In light of the newer knowledge of the structure of colchicine and its degradation products it is preferable that a new series of simple analogues should be tested before further deductions are made concerning the relationship between molecular grouping and anti-mitotic activity.

None of the above substances is active in as low a concentration as colchicine itself, either in the inhibition of mitosis or of tumour growth. The substance synthesised has not yet been tested for an action of the latter type.

THEORIES OF THE COLCHICINE EFFECT

The only conclusion which might be drawn from the results in the previous section is that, while activity is conditioned by the chemical structure, there is no functional group which is specifically responsible. This suggests that the inhibition of mitosis is effective via an oxidation (or other metabolic) product, derived from colchicine.

There is some evidence that the toxic effect of higher concentrations is due to the formation of oxidation products[1], but it has not been established that this toxic action is directly related to the anti-mitotic activity. The following evidence indicates a different mechanism:

(a) Much higher concentrations are required to cause the general symptoms of poisoning.

(b) Similar symptoms are induced by a variety of other substances which have no anti-mitotic action.

---

1. The evidence that the toxic action of colchicine is due to oxidation products is as follows:
   (a) Increase of temperature causes a rise in toxicity to frogs (Sanno, 1911).
   (b) Prior oxidation of the colchicine with ozone causes a similar rise. In mammals, oxidised colchicine seemed less toxic, however.
   (c) The latent period might correspond to the time necessary for oxidation to take place. (Brues and Cohen, 1936, have made the interesting observation that colchiceine, which is much less toxic than colchicine, has a shorter latent period).

(c) Similar symptoms are also produced by derivatives or analogues of colchicine, which show no anti-mitotic activity.

On the other hand:

(a) It is to be expected that the interruption of normal cell-division in body tissues would result in symptoms of some kind (for example, damage to capillary walls). The long latent period which follows the administration of colchicine is significant. (This latent period is not shortened by increasing the dose.)

(b) In a series of colchicine derivatives, Lettré found that anti-mitotic action roughly paralleled the inhibition of tumour growth. The latter effect is believed to be related to the toxic action of colchicine as a capillary poison (Ludford, 1945).

(c) In some recent experiments, Bucher (1946) has found that the presence of reducing agents depresses the anti-mitotic action of colchicine on tissue cultures.

No report on the anti-mitotic activity of colchicine oxidation products has been found.

It should be mentioned that free colchicine has been shown to interact with isolated proteins and nucleoproteins (Ehrenberg and Lofgren, 1945; Vlès, 1945).

A number of opinions have been expressed concerning the physical aspects of the colchicine effect, and these are discussed briefly below. By way of introduction a brief summary of the process by which normal division occurs might be of value. The descriptions which follow are somewhat generalised, for even in the case of a normal cell division the difficulty of making observations makes it hard to obtain a complete picture.

Normal Mitosis

In a normal cell division the sequence of events observed to occur is roughly as follows:

1. (Prophase) The chromosomes in the nucleus, which in the resting state form a tangled and seemingly confused network, become individualised. Reproduction of each then occurs and the two "daughter" chromosomes remain bound together along their longest axis. At this time the presence of "asters" or "poles" in the cytoplasm may be apparent. These poles are located on opposite sides of the nucleus, and rays which radiate from them may be observed.

2. (Metaphase) Following the break-down of the nuclear membrane a spindle-shaped volume is differentiated from the rest of the protoplasm. It stretches between the poles, and essentially constitutes the nuclear material. The still united chromosome pairs now move so as to place themselves on the equatorial plane of the "spindle"

(i.e. on the "metaphase plate").

3. (Anaphase) The pairs of new chromosomes then split apart, and the two members of each pair move off in opposite directions along the axis of the spindle and thus towards the poles.

4. (Telophase) When the new chromosomes have reached the neighborhood of the poles, a nuclear membrane is formed around each group. Division of the cell as a whole then separates the daughter nuclei and two new cells are formed.

The Colchicine Effect

A cell division under the influence of colchicine proceeds normally up to the point where the nuclear membrane breaks down. Spindle formation is then inhibited, and the chromosome doublets, instead of moving to the metaphase plate, remain at random in the cytoplasm. They may bunch together to a greater or lesser degree.

At approximately this point the process is suspended for a considerable period of time, and it is on account of this that the division appears to be "arrested at the metaphase." It is possible that the resumption of the mitotic process (~~in~~ the modified form) occurs only when the effect of the colchicine poisoning wears off.

Eventually the splitting of the chromosome pairs into constituent new chromosomes takes place, and a

nuclear membrane forms around the entire group. The final outcome is thus a single cell whose nucleus contains double the original diploid number of chromosomes--a tetraploid cell.

As has been previously mentioned, such a cell is capable of subsequent normal division with the formation of further tetraploids. Under the continued (or renewed) action of colchicine redoubling may occur to give viable polyploid forms, containing eight, sixteen, etc. times the normal number of chromosomes in the cell.

In actual experiment, particularly if the local concentration of colchicine is high, more profound abnormalities are seen, including necrosis of the cells and the formation of giant nuclei or scattered smaller nuclei, accompanied by general malformation of the cell.

OTHER MITOTIC INHIBITORS

In nature, spontaneous polyploidy appears to be a not infrequent occurrence. Indeed it is believed that this capacity has been a major factor in the course of evolution (of plants at least). An analysis of the chromosome numbers of plant species has given rise to the conclusion that many or most species with a large chromosome complement have originated by polyploid formation, with or without hybridisation, from species with lower chromosome number (cf. Dobzhansky, 1937). One important consequence

of this process may have been that in polyploid forms the extra genes which are not essential for the normal function of the plant may mutate to give new functions or qualities; and the resulting variations can undergo natural selection to give, in the course of evolutionary time, radically improved species.

A small number of plants are known in which both the normal diploid and a tetraploid form occur naturally; in other species tetraploid shoots or branches may arise spontaneously.

From this and from experimental work it is clear, firstly that polyploidy can be induced by natural causes, and secondly that certain species show a definite predisposition to polyploid formation.

Among the influences which have been found to induce, among other disturbances, a doubling of the chromosomes in the cells of plants are the following: tissue damage; the application of heat, cold, or pressure; dehydration; the injection of distilled water; bacterial infection; tumourous growth.

A wide range of foreign substances, mostly organic compounds, can induce similar disturbances provided that they are present in sufficient concentration.

It thus seems that ill-treatment of almost any kind may give rise to chromosome doubling; this suggests that the latter stages of cell division are more easily

disturbed than the initial chromosome reproduction.

Of the chemical substances other than colchicine which are capable of inducing tetraploid cell formation, only acenaphthene and certain of its derivatives have a specific action in low concentration. Sodium cacodylate also has a fairly specific action; but only if present in higher concentration. Derivatives of acenaphthene were studied by Shmuck (1938) and others. These compounds do not appear to be as widely effective as colchicine.

THEORIES OF ANTI-MITOTIC ACTION

Our ignorance concerning the nature of the mitotic process itself makes a discussion of the mechanism of colchicine action more or less intuitive (cf. Schrader, 1944, "Mitosis").

Koltzoff (1939) suggested that colchicine acts physico-chemically: "By penetrating the cell it changes the viscosity and hinders the arising of the spindle."

Similar views were expressed by Garrigues (1945) who found that by depriving a plant tissue of its full water supply (and, he presumes, thereby increasing the cellular viscosity), he induced colchicine-like anomalies in cell division. He suggested that the increased viscosity would hamper chromosome movement and thus inhibit the mitotic process.

Increased viscosity in cells treated with colchicine has actually been demonstrated by Kartashova (1945).

Östergren (1944) examined the more general anti-mitotic action exhibited by a large number of unrelated organic compounds, and discussed the mechanism of their action. He related activity in these substances to lipoid solubility and proposed a theory which links the colchicine effect with narcosis in general. His hypothesis is an extension of the Meyer-Overton lipoid theory of narcotic action (Meyer, 1899, etc.). It suggests that the narcotically active substances interact in particular with the lipophilic residues attached to the protein polypeptide chains, and so change the degree of folding of the latter. In narcotic action this would disturb the general cell metabolism; in the case of the colchicine effect, a contraction of the fibrous proteins presumed to constitute the spindle would result in an inhibition of mitosis. It is pointed out in support of this hypothesis that there is a contraction of the chromosomes themselves under the influence of colchicine.

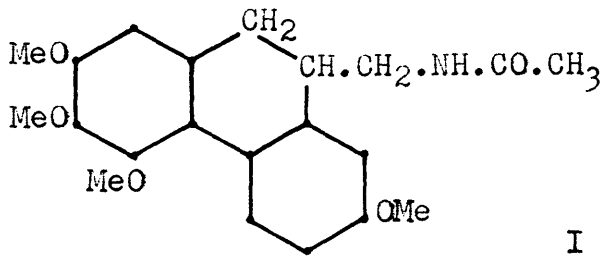
Alteration in the protein structure would presumably have some effect on viscosity.

However, colchicine, and to a lesser extent acenaphthene, differ from other agents in that they are active in exceedingly low concentration. Ludford (1936) claims to have detected activity with colchicine in a dilution

of 1 in 100,000,000.

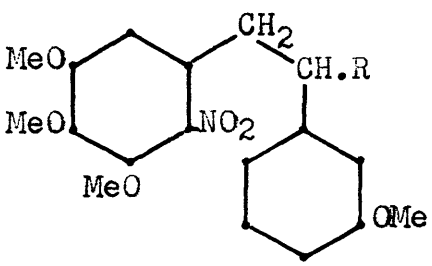
Östergren therefore suggested that colchicine has its effect on the degree of folding of the protein chains via some different and special mechanism.

Similar views are expressed by Gavaudin, Poussel, and Dody (1944), who relate anti-mitotic activity to thermodynamic activity and conclude that organic substances in general "act only through a physical mechanism like indifferent narcotics, whereas certain other substances, including colchicine, appear to act through some entirely different mechanism, probably purely chemical."

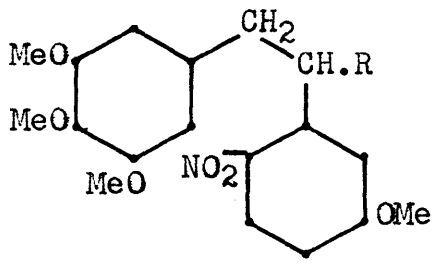


I

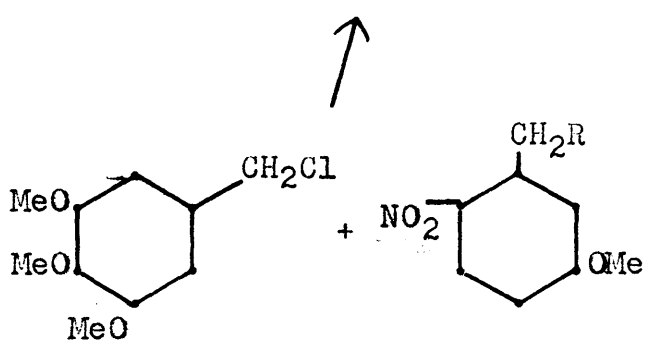
2:3:4:7-Tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene.



II



III



SYNTHETIC METHODS

In the synthesis of a substance of the type I, the Pschorr method could be applied in two senses: it is possible to start from a material containing a nitro group in the ortho position either in the trimethoxylated or in the monomethoxylated ring, as in II and III respectively.

As 2-nitro-3:4:5-trimethoxy compounds are difficult to obtain[1], it seemed of interest to explore the possibility of preparing a substance of type III with a view to attempting the Pschorr-type ring closure.

In the preparation of I, ring closure from the monomethoxylated on to the trimethoxylated ring has the additional advantage that a single product will be formed.

---

1. Nitration of polymethoxybenzenes. The products of attempted nitrations of methoxylated substances were studied by Harding (1911) who summed up his observations by saying that the groups, -H, -CHO, >CO, $-CH_3$, -COOH form a series, any member of which can be replaced by a nitro group. The directive effects of substituent methoxy groups apply to the replacement of the other groups of the above series just as to the replacement of hydrogen. An accumulation of methoxy groups will therefore cause an increased ease of displacement of another substituent by a nitro group, on nitration.
   Such nitrations may be further complicated by oxidative side-reactions.
   Methods for the preparation of 2-nitro-3:4:5-trimethoxybenzaldehyde, which is of particular interest in these studies, are discussed by Cook, Graham, et al. (1944). The procedure they finally recommend is that of Grosheintz and Fisher (1941).

Ring closure in the reverse sense would give rise to a mixture of two products in which the single methoxy group would be in two different positions. (This type of difficulty was overcome by Pschorr (1912) in one case, by blocking the relevant ortho-position by monobromination. The bromine atom was subsequently removed by reduction.)

Applications of the Pschorr method are discussed by Fieser (1936).

The most obvious synthesis of a structure of type III would depend on the condensation of 3:4:5-trimethoxybenzyl chloride with an α-substituted 2-nitro-5-methoxytoluene, as shown.

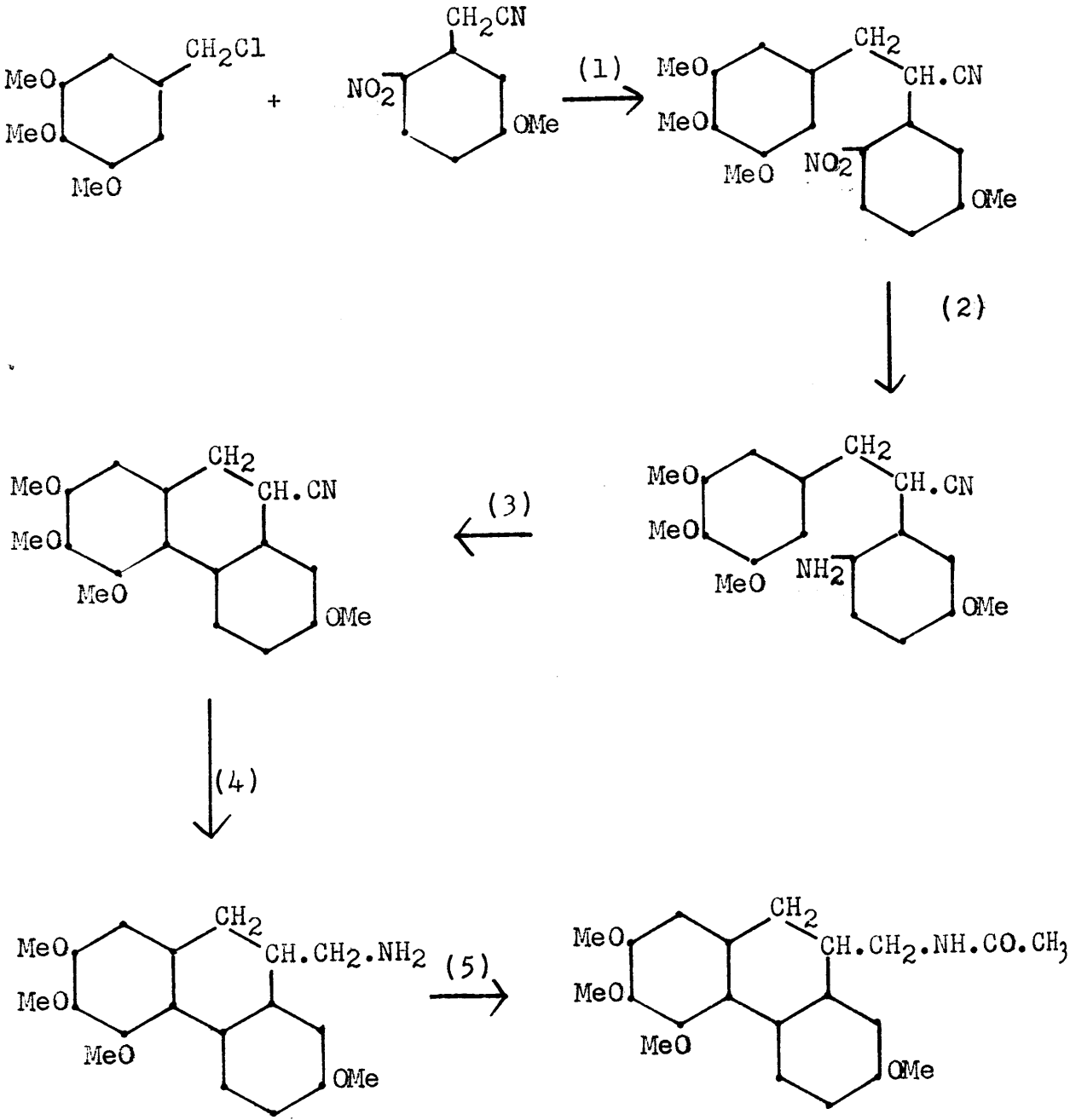
The nature of R in the nitrotoluene would be conditioned by two requirements:

1. that a suitable activation of the adjacent hydrogen atoms should be maintained.

2. that a method should be available for the conversion of R to the desired acetylaminomethyl side chain.

With R = -CN, both these requirements are fulfilled. By complete reduction and acetylation it is possible to change -CN to $-CH_2.NH.CO.CH_3$.

As reasonable methods were available for the preparation of the starting materials named above, the synthesis was commenced according to the scheme illustrated on the following page.


MeO
MeO
MeO
CH2Cl
+
CH2CN
NO2
OMe
(1)
CH2
CH.CN
NO2
(2)
NH2
(3)
(4)
CH.CH2.NH2
(5)
CH.CH2.NH.CO.CH3

DISCUSSION OF EXPERIMENTAL

The preparation of the starting materials is presented with the experimental on page 46. The steps in the synthesis, which are shown diagramatically on the opposite page, are discussed below:

(1) 2-Nitro-5-methoxy-α-(3:4:5-trimethoxybenzyl)-α-tolunitrile

An excellent yield (80% of theoretical) of pure nitrile was obtained by simple condensation at room temperature of molecular proportions of the two substances in alcohol in presence of 1.1 molecules of sodium ethoxide. The product separated on standing, and was recrystallised from alcohol, m. p. 164°.

(2) 2-Amino-5-methoxy-α-(3:4:5-trimethoxybenzyl)-α-tolunitrile

Hydrogenation of the nitro-compound in dioxan solution in presence of palladised strontium carbonate gave the required amine. Dioxan was the only solvent in which the nitro-compound was conveniently soluble.

The amine was at first obtained as a dark tar which showed no signs of solidifying. However the dark colour was later shown to be catalyst which had passed through the filters. It appears that the palladium in palladised strontium carbonate can form colloidal suspensions in dioxan. The difficulty was overcome by removing the dioxan by distillation in vacuo and extracting the residue with ether; the catalyst remained behind as an amorphous

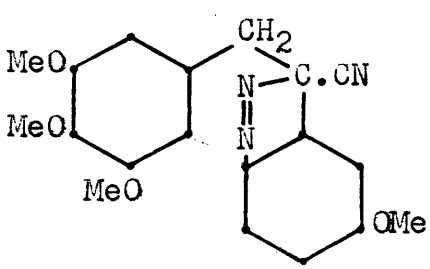
residue.

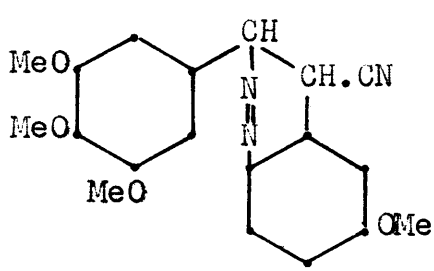
The amine, which was obtained on evaporation of the ether extract was now only very slightly coloured, but the tacky solid still could not be induced to crystallise, and was characterised as the acetyl derivative.

(3) 2:3:4:7-Tetramethoxy-9:10-dihydrophenanthrene-9-nitrile:

The ring closure of the amine by the application of the Pschorr method gave a satisfactory yield of the dihydrophenanthronitrile. Yield over stages (2) plus (3): 45-50% of theoretical.

It is interesting to note that, while the decomposition of the diazonium salt in acid solution with copper bronze gives elimination of nitrogen, decomposition at alkaline pH gives internal coupling. Two different isomeric products of the latter type were isolated (under different sets of conditions). Possible formulae for these substances are:





(4)(5) 2:3:4:7-Tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene:

The catalytic reduction of nitriles is complicated

by the fact that the amine formed tends to combine with the intermediate aldimine according to the following equations:

$$R.CN \xrightarrow{2H} R.CH{=}NH \xrightarrow{2H} R.CH_2NH_2$$

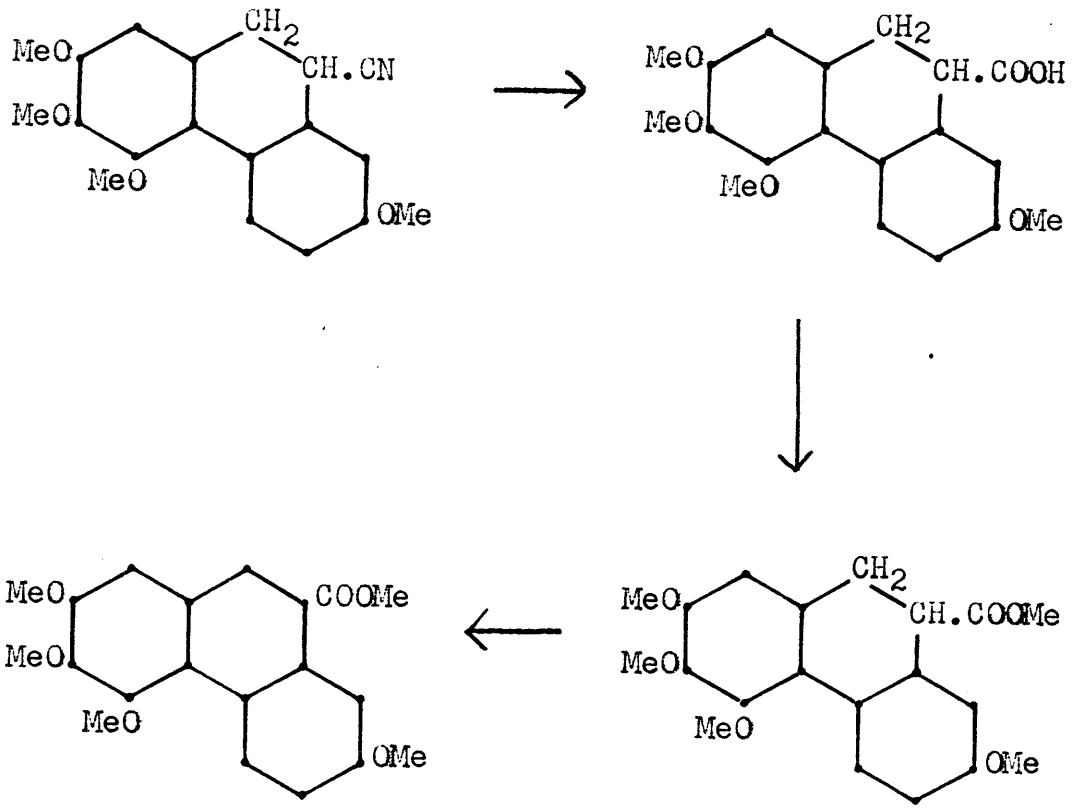
$$R.CH{=}NH + R.CH_2.NH_2 \longrightarrow R.CH_2.NH.CH(NH_2).R$$

$$\xrightarrow{2H} R.CH_2.NH.CH_2.R + NH_3$$

The final result is thus a mixture of primary and secondary amines and ammonia. However, if the primary amine is removed as it is formed, the reduction can be made to yield only the required product. This can be achieved by carrying out the hydrogenation in presence of acid, when the amine is fixed as the salt, or in acetic anhydride, where the acetylated amine is obtained in good yield.

The former method is used by Hartung (1928), who reduces benzonitrile in alcohol containing hydrogen chloride. Other procedures are discussed in Gilman's Organic Chemistry (2^{nd} Edition), p. 809.

The method found successful in this synthesis was that described by Lettré and Fernholz (1943) for the production of the simpler analogue, N-acetyl-β-(p-methoxyphenyl)-γ-(3:4:5-trimethoxyphenyl)-propylamine. Cook and Engel (1940), who attempted the same reduction in alcohol in absence of acid, obtained only poor yields


MeO
MeO
MeO
CH2
CH.CN
OMe
MeO
MeO
MeO
CH2
CH.COOH
OMe
MeO
MeO
MeO
CH2
CH.COOMe
OMe
MeO
MeO
MeO
COOMe
OMe

of the amine from a mixture of other products. Lettré and Fernholz used acetic acid containing a small proportion of sulphuric acid as solvent, and acetylated the base directly without isolating it.

In its application to the present synthesis this method gave the required amine in 60-70% yield, m. p. 149-150°.

2:3:4:7-Tetramethoxy-9-aminomethyl-9:10-dihydrophenanthrene (hydrochloride):

The reduction of the nitrile was also carried out using Hartung's method (above). Hydrogenation was effected in presence of a platinum black catalyst in a solution in methanolic hydrogen chloride, and the resulting amine was isolated as the hydrochloride, m. p. 226-228°.

Characterisation of 2:3:4:7-tetramethoxy-9:10-dihydrophenanthrene-9-nitrile:

The identity of the methoxylated nucleus present in the above products was confirmed by conversion of the nitrile to the known methyl ester of 2:3:4:7-tetramethoxy-9-phenanthrene carboxylic acid (prepared by Buchanan, Cook, and Loudon (1944)).

Prolonged treatment with methanolic potassium hydroxide hydrolysed the cyano group. The methyl ester of the resulting acid was dehydrogenated by heating with

palladium black in a slow stream of carbon dioxide. The identity of the product thus obtained was established by a mixed melting point with a sample of the ester which was very kindly supplied by Dr. J. Loudon.

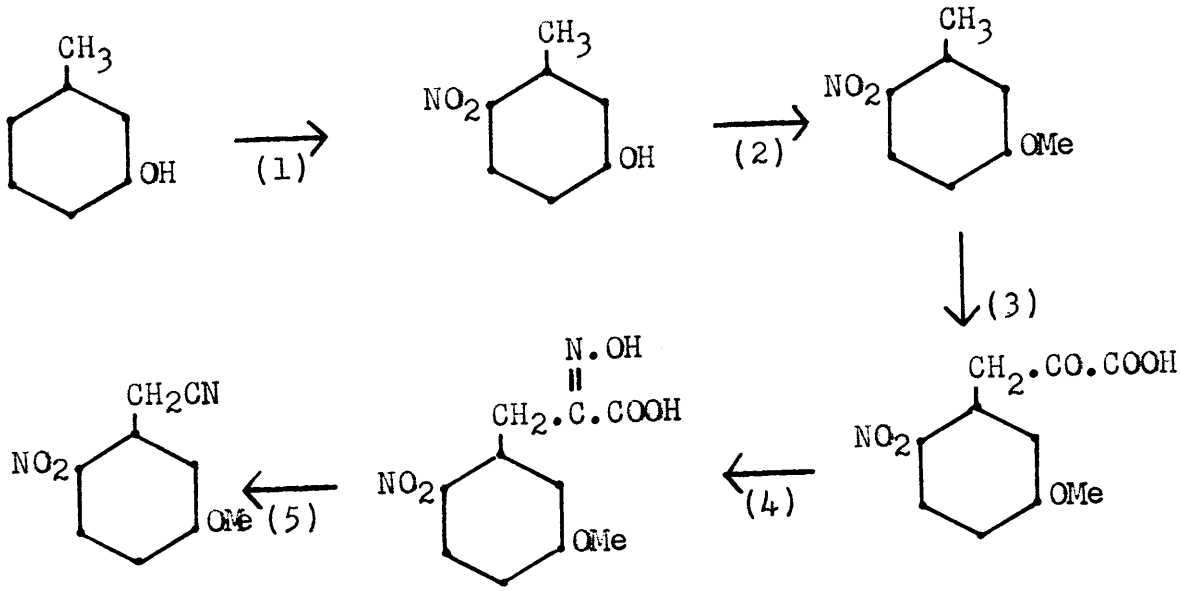
Properties of 2:3:4:7-tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene, (I), and N-acetylcolchinol methyl ester, (II):

The two substances are isomeric, possessing the molecular formula $C_{21}H_{25}O_5N$.

I has m. p. 150°, and hydrochloride m. p. 225-227°.

II has m. p. 199°, and hydrochloride m. p. 254°.

On treatment with phosphoric oxide in boiling xylene I loses the elements of water to give a yellow substance m. p. 156-157°. On similar treatment II eliminates acetamide to give a compound which crystallises in colourless plates, m. p. 110°.


CH3
OH
(1)
CH3
NO2
OH
(2)
CH3
NO2
OMe
(3)
CH2.CO.COOH
NO2
OMe
(4)
N.OH
CH2.C.COOH
NO2
OMe
(5)
CH2CN
NO2
OMe

EXPERIMENTAL

Melting points are uncorrected. Micro-analyses have been carried out on all new substances.

Starting Materials

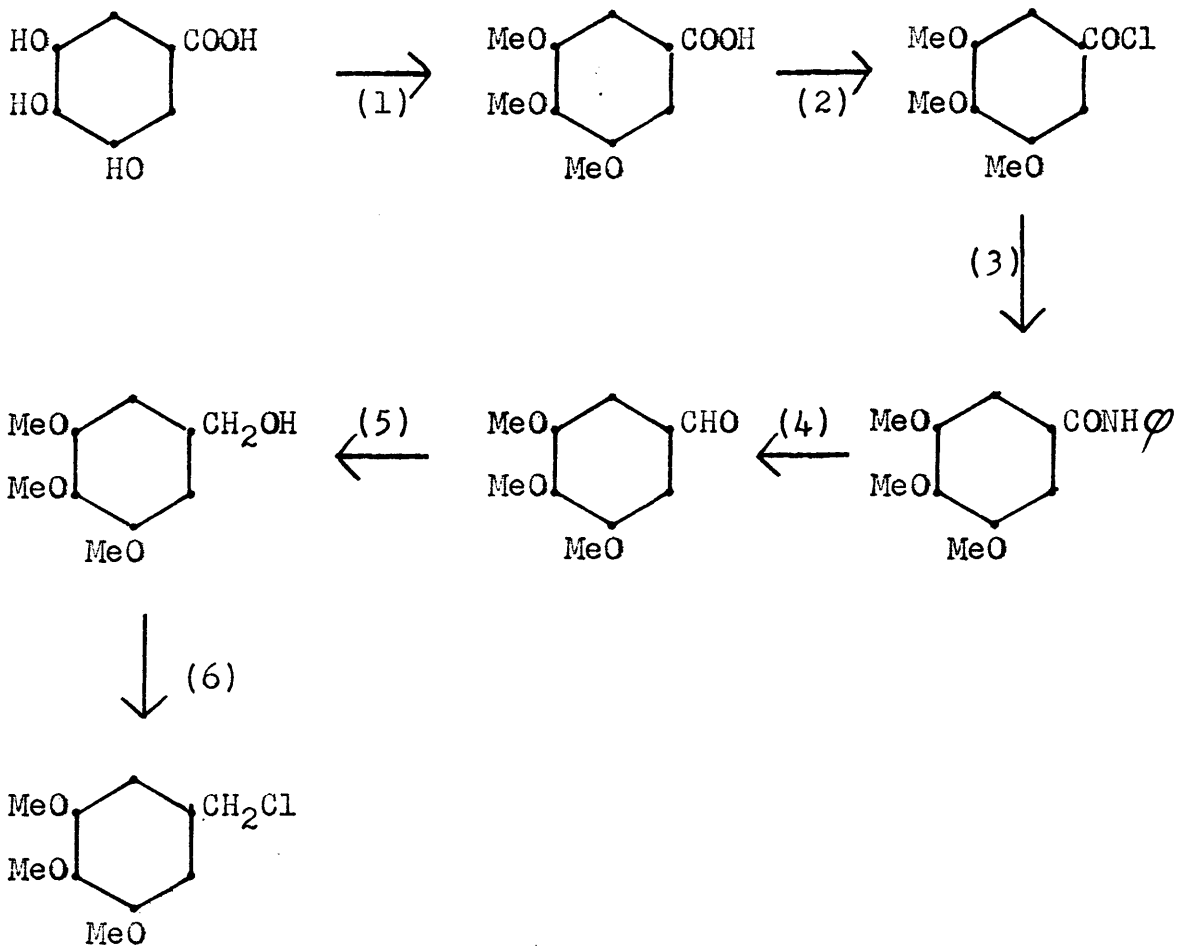
2-NITRO-5-METHOXY-α-TOLUNITRILE

The stages in the preparation are illustrated on the opposite page.

References:

(1)-(3) Blaikie and Perkin, J. Chem. Soc., 1924, 308. (see also Faltis, Wagner, and Adler, Ber., (1944) 77B, 686.)

(4),(5) Buchanan and Loudon (unpublished) converted 2-nitro-4-methoxyphenylpyruvic acid to the corresponding nitrile; the application of their method gave the corresponding 5-methoxy compound in good yield. The procedure is an extension of the general method of preparation of nitriles by the dehydration of oximes (cf. Organic Syntheses, Collective Vol. II, p. 622). In the case of the decomposition of a pyruvic acid oxime, decarboxylation accompanies the dehydration by acetic anhydride, and the nitrile is smoothly formed.


HO
HO
HO
COOH
(1)
MeO
MeO
MeO
COOH
(2)
MeO
MeO
MeO
COCl
(3)
MeO
MeO
MeO
CONHφ
(4)
MeO
MeO
MeO
CHO
(5)
MeO
MeO
MeO
CH2OH
(6)
MeO
MeO
MeO
CH2Cl

The oxime of 2-nitro-5-methoxyphenylpyruvic acid:

2-Nitro-5-methoxyphenylpyruvic acid (37 g.) in aqueous ethanolic sodium hydroxide (10 g. in 150 cc. of 1:1 mixture) was treated with hydroxylamine hydrochloride (17.5 g.) and refluxed for one hour. The solution was then diluted largely, and allowed to stand overnight. The light brown oil solidified to a dirty white solid which gave crystals from ether, m. p. 164-165° (Found: C, 47.87; H, 4.13. $C_{10}H_{10}O_6N_2$ requires C, 47.25; H, 3.94%).

2-Nitro-5-methoxy-α-tolunitrile:

The oxime did not crystallise readily, and the crude product (28 g.) was converted directly to the nitrile by boiling with acetic anhydride (500 cc.) for 4 hours. Most of the latter was then removed in vacuo, and the residual anhydride destroyed by heating with water. 2-Nitro-5-methoxy-α-tolunitrile, which solidified on cooling, crystallised from methanol (30 cc.; charcoaled) in brown crystals, m. p. 83-84° (Found: C, 56.21; H, 4.28. $C_9H_8O_3N_2$ requires C, 56.24; H, 4.17%).

The yield over the two stages was 50-60% of theoretical.

3:4:5-TRIMETHOXYBENZYL CHLORIDE

References:

(1)-(3) A. Cohen (unpublished).

(4)-(6) Cook, Graham, Cohen, et al, J. Chem. Soc., 1944, 322. (Cohen's procedure for steps (1)-(3) is quoted here in full.)

(1) 3:4:5-Trimethoxybenzoic acid (trimethyl gallic acid):

Gallic acid (112 g.) was suspended in water (300 cc.) and simultaneously a solution of sodium hydroxide (130 g.) in water (150 cc.) and methyl sulphate (340 g.) were slowly added with stirring, the mixture being kept warm and alkaline. It was finally warmed on the water-bath to remove excess methyl sulphate and hydrolyse a small amount of ester which had separated out. The cooled clear solution was acidified and the product collected and air dried. Yield 120 g.

(2) 3:4:5-Trimethoxybenzoyl chloride:

The above acid (80 g.) was refluxed with thionyl chloride (240 cc.) for two hours, the excess thionyl chloride removed under reduced pressure, and the residue distilled giving 71 g., b. p. 180°/13 mm. (Note: the product does not store well, even in a well-stoppered bottle. It should be used at once if possible.)

(3) 3:4:5-Trimethoxybenzanilide:

The acid chloride (71 g.) was dissolved in chloroform (300 cc.) and the solution heated with a chloroform solution of aniline (2 mols.) with shaking. The solution, after being left overnight, was shaken with dilute

hydrochloric acid and water, and dried, and then freed from solvent. The residue was recrystallised from aqueous methanol in large flat needles, m. p. 136-7°; yield 85 g.

(4) 3:4:5-Trimethoxybenzaldehyde:

The next step in Cohen's method is Sonn and Müller reduction of the above anilide. This source of 3:4:5-trimethoxybenzaldehyde has no certain advantages over other methods available. According to Buchanan, Cook and Loudon (J. Chem. Soc., 1944, 326), the best source of the aldehyde is the catalytic reduction of trimethoxybenzoyl chloride by the Rosenmund method (Ber., (1918) 51, 585), as modified by later workers (see Sharp, J. Chem. Soc., 1936, 1235).

In the Sonn and Müller method the anilide is treated with phosphorus pentachloride and the resulting iminochloride reduced to the anil of 3:4:5-trimethoxybenzaldehyde by means of stannous chloride. The anil is first obtained as a complex. It was found that the hydrolysis of this complex was troublesome, and the yields of the aldehyde uncertain.

(Note: A convenient method for the preparation of stannous chloride for use in the Sonn and Müller reduction is described by H. Stephen (J. Chem. Soc., 1930, 2786).)

(5) 3:4:5-Trimethoxybenzyl alcohol:

The time for the catalytic reduction of the aldehyde

can be reduced from 15 hours (found necessary by Cohen) to 20 minutes, by the addition of a trace of ferrous sulphate to the catalyst (platinum oxide) as promoter (cf. Carothers and Adams, J. Am. Chem. Soc. (1923) 45,1071; (1924) 46, 1680).

To the aldehyde (10 g.) in absolute ethanol (230 cc.) was added platinum black and a trace of ferrous sulphate as promoter (0.004 g. in 0.5 cc. water). The suspension was then shaken with hydrogen, and the theoretical quantity was absorbed in less than half an hour. After filtering off the catalyst, the solvent was removed, and the product distilled, b. p. 145-150°/0.4 mm.

SYNTHESIS OF 2:3:4:7-TETRAMETHOXY-9-ACETYLAMINOMETHYL-9:10-DIHYDROPHENANTHRENE

2-Nitro-5-methoxy-α-(3:4:5-trimethoxybenzyl)-α-tolunitrile:

2-Nitro-5-methoxy-α-tolunitrile (5 g.), dissolved in warm lime-dried ethanol (65 cc.), was treated with sodium (0.66 g.; 1.1 atoms) in ethanol (18 cc.), and to the resulting purple solution 3:4:5-trimethoxybenzyl chloride (5.56 g.; 1 mol.), also in warm ethanol (40 cc.), was added. The vessel, protected with a calcium chloride tube, was left at room temperature for 48 hours. The crystals which had separated out were then filtered off, washed with ethanol, and recrystallised from the same

solvent (450 cc.; charcoaled).

Yield 8 g. (80%); m. p. 163-164° (Found: C, 61.40; H, 5.46; N, 7.47. $C_{19}H_{20}O_6N_2$ requires C, 61.30; H, 5.38; N, 7.53%).

2-Amino-5-methoxy-α-(3:4:5-trimethoxybenzyl)-α-tolunitrile:

The above nitro compound (8 g.), dissolved (by gentle warming) in sodium-dried dioxan (300 cc.), was hydrogenated at ordinary pressure in presence of 2% palladised strontium carbonate (8 g.) (Thorpe: Dictionary of Organic Chemistry). Absorption was complete in 2 to 3 hours. The catalyst was filtered off and washed through with warm dioxan. As the palladium was found to form colloidal suspensions in dioxan, the latter was removed by distillation in vacuo and the residue extracted with warm ether; the catalyst remained behind as an amorphous solid. The ether extract, after treatment with a little decolourising charcoal, was reduced to dryness. A sticky buff-coloured residue remained, and this could not be induced to solidify or crystallise.

The product was therefore characterised as an acetyl derivative which was obtained by heating 0.3 g. of the crude amine on the water-bath for 20 minutes with acetic anhydride (5 cc.) containing a trace of sulphuric acid (0.01 cc.). The excess anhydride was destroyed by heating with water, and on standing at room temperature the product

solidified to a bright orange solid. This crystallised from ethanol with m. p. 171-172° (Found: C, 65.42; H, 6.40; N, 7.33; $C_{21}H_{24}O_5N_2$ requires C, 65.63; H, 6.23; N, 7.29%).

The free amine forms a sparingly soluble hydrochloride and sulphate. The nitrate and acetate are readily soluble.

2:3:4:7-Tetramethoxy-9:10-dihydrophenanthrene-9-nitrile:

The crude amine (above) was dissolved in dioxan (25 cc.) and the solution poured slowly into 3N hydrochloric acid (72 cc.), cooled in ice. The resulting suspension of the hydrochloride was treated at -8° with a solution of sodium nitrite (1.28 g. in 20 cc. water), added during 15 minutes. After stirring for 1 hour at -8°, copper bronze (8 g.) was added, and an effervescence resulted. The mixture was stirred at -8° for 15 minutes and at room temperature for 30 minutes. The product, which had begun to separate out, was extracted by shaking with a little ether, and the two-layered mixture was left at 0° overnight. The next day the copper was filtered off and washed with warm ether, and the combined ether extracts were washed with brine, dilute sodium hydroxide, and dried over anhydrous potassium carbonate. The residue after removal of the ether crystallised from methanol (40 cc.), m. p. 135-136° (Found: C, 70.34; H, 5.83; N, 4.54. $C_{19}H_{19}O_4N$ requires C, 70.15; H, 5.84; N, 4.31%).

The yield over the two stages was 50-60% of theoretical.

A slight blue fluorescence was observed in the ethereal solutions and the final product possessed a faint pink colour which was not readily removed with charcoal. It was insoluble in dilute hydrochloric acid, and readily soluble in organic solvents. There was no sign of decomposition at (or above) the melting point.

The identity of the nitrile was established by converting it to the known methyl ester of 2:3:4:7-tetramethoxy-9-phenanthrene carboxylic acid (see p. 55).

2:3:4:7-Tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene:

The nitrile (0.7 g.), dissolved in a mixture of acetic acid (30 cc.) and sulphuric acid (1.2 cc.),was shaken with hydrogen in presence of active Adams' catalyst (0.07 g.). Absorption was complete in several hours. The catalyst was filtered off and washed with a little acetic acid; the filtrate (plus washings) was diluted with ether (250 cc.), and then extracted with water (200 cc. in all). The cooled aqueous extract was neutralised with a moderate excess of ice-cold 15% sodium hydroxide solution, and the alkaline solution was shaken vigorously with acetic anhydride (10 cc. plus 5 cc.). On standing, a white solid separated and was filtered off*, dried, and purified by crystallisation from a super-saturated ether solution

(800 cc. reduced by distillation to 80 cc.).

(*An ether extract of the filtrate left a negligible residue on evaporation.)

The acetylamine was obtained as white micro-needles, yield 65-70%, m. p. 149-150° (Found: C, 68.18; H, 6.61; N, 3.94. $C_{21}H_{25}O_5N$ requires C, 67.92; H, 6.74; N, 3.77%).

2:3:4:7-Tetramethoxy-9-aminomethyl-9:10-dihydrophenanthrene hydrochloride:

This reduction of the nitrile was effected by hydrogenation (of 0.15 g.) in methanol (18 cc.) to which had been added 2 cc. methanol saturated with hydrogen chloride. In presence of Adams' catalyst (0.015 g.) absorption was complete in several hours. It was found preferable to warm the flask occasionally during the hydrogenation to prevent the separation of the unreduced nitrile. The filtrate on removing the catalyst was reduced in volume, and the addition of ether caused the precipitation of the hydrochloride of the amine. Purification by reprecipitation from methanol-ether gave a white product, m. p. 226-228° (Found: C, 61.87; H, 6.57. $C_{19}H_{24}O_4NCl$ requires C, 62.37; H, 6.57%).

ACTION OF PHOSPHORIC OXIDE ON 2:3:4:7-TETRAMETHOXY-9-ACETYLAMINOMETHYL-9:10-DIHYDROPHENANTHRENE

The acetylamine (0.12 g.) was refluxed with phosphoric oxide (0.3 g.) in xylene (5.5 cc.) for 30 minutes. The xylene was then decanted from the solid oxide, which had become yellow in colour, and this residue was washed (by decantation) with xylene and dry ether. From the xylene and washings some unchanged starting material was isolated, its identity being established by a mixed melting point.

The yellow residue, on treatment with water (6 cc.), gave a suspension of a yellow solid. On making alkaline with dilute sodium hydroxide the colour was lost, and the now white suspended material could be extracted with ether. The product obtained from this extract formed yellow crystals from ether, m. p. 156-157° (Found: C, 71.69; H, 6.48. $C_{21}H_{23}O_4N$ requires C, 71.39; H, 6.51%; this analysis corresponds to the loss of one molecule of water from the starting material).

IDENTIFICATION OF 2:3:4:7-TETRAMETHOXY-9:10-DIHYDROPHENANTHRENE-9-NITRILE

2:3:4:7-Tetramethoxy-9:10-dihydrophenanthrene-9-carboxylic acid:

The nitrile (0.3 g.) was heated with methanolic

potassium hydroxide (1 g. in 8 cc.) under reflux for 48 hours. Most of the methanol was then removed in vacuo and ice was added. A small insoluble residue was removed by extraction with ether, and the aqueous layer was neutralised with an excess of dilute hydrochloric acid. The white product which separated, after several recrystallisations from methanol, had m. p. 190-192° (Found: C, 65.94; H, 5.74. $C_{19}H_{20}O_6$ requires C, 66.27; H, 5.81%). A sodium fusion test for nitrogen was negative; the substance had acidic properties.

This acid (35 mg.) was converted to the methyl ester by the action of dry hydrogen chloride on a solution in methanol, and the reagents were removed by evaporation in vacuo over solid potassium hydroxide. An ethereal solution of the residue, after washing with a little sodium carbonate solution, and drying, was reduced to small bulk. On standing, crystals (25 mg.) were obtained, m. p. 121°.

Methyl ester of 2:3:4:7-Tetramethoxyphenanthrene-9-carboxylic acid:

The dehydrogenation was carried out by mixing the above dihydrophenanthrene ester (20 mg.) with palladium black (powdered; 10 mg.), and heating at 220-230° (oil bath temperature) in a slow stream of carbon dioxide for several hours. The apparatus was arranged so that starting material which distilled unchanged condensed on a

further supply of catalyst (10 mg.), and could then receive a second heat treatment. The product was distilled directly from the catalyst in vacuo (0.2 mm.; oil bath temperature, 245°), on to a cooled surface. The condensate, after two recrystallisations from methanol, formed yellow crystals, m. p. 101-102°.

Micro-melting points:

This specimen:	98-100.5°
Genuine ester:	101-102.5°
Mixed m. p.:	99-102°

The specimen of the known ester (Buchanan, Cook, and Loudon, J. Chem. Soc. 1944, 328) was very kindly supplied by Dr. J. Loudon.

ALKALINE DECOMPOSITION OF 5-METHOXY-α-(3:4:5-TRIMETHOXYBENZYL)-α-TOLUNITRILE-2-DIAZONIUM CHLORIDE

In connection with attempts to bring about a ring closure of this substance two other products were obtained in addition to the desired dihydrophenanthrene derivative. These two substances were isomeric, and their analyses suggested that intra-molecular coupling had occurred, with the elimination of hydrogen chloride from the diazonium salt (or of water from the hydroxide). Possible formulae for

these substances are given on p. 42 .

Substance A.

A solution of the amine (derived from 1 g. nitro compound) in dioxan (3 cc.) was poured into 3N hydrochloric acid. The resulting suspension of the hydrochloride was diazotised at -8° with the theoretical quantity of sodium nitrite, dissolved in a little water.

After a short time the mixture was rendered alkaline by the addition of a slight excess of dilute sodium carbonate, and warmed gradually on the water bath until the temperature reached 100°. On cooling and standing overnight, the substance which had separated solidified. It was filtered off, triturated with dilute hydrochloric acid, with water, and dried in a desiccator. The product was recrystallised several times from absolute ethanol, and finally from light petroleum (b. p. 60-80°) when almost colourless crystals were obtained, m. p. 122° (Found: C, 64.73; H, 5.55; N, 12.36. $C_{19}H_{19}O_4N_3$ requires C, 64.59; H, 5.38; N, 11.90%).

Substance B:

This compound was obtained (in small yield) when a similar solution of the diazotised amine was poured into warm (50°) sodium acetate solution (10%) containing suspended copper bronze. The solution was heated up to 70°,

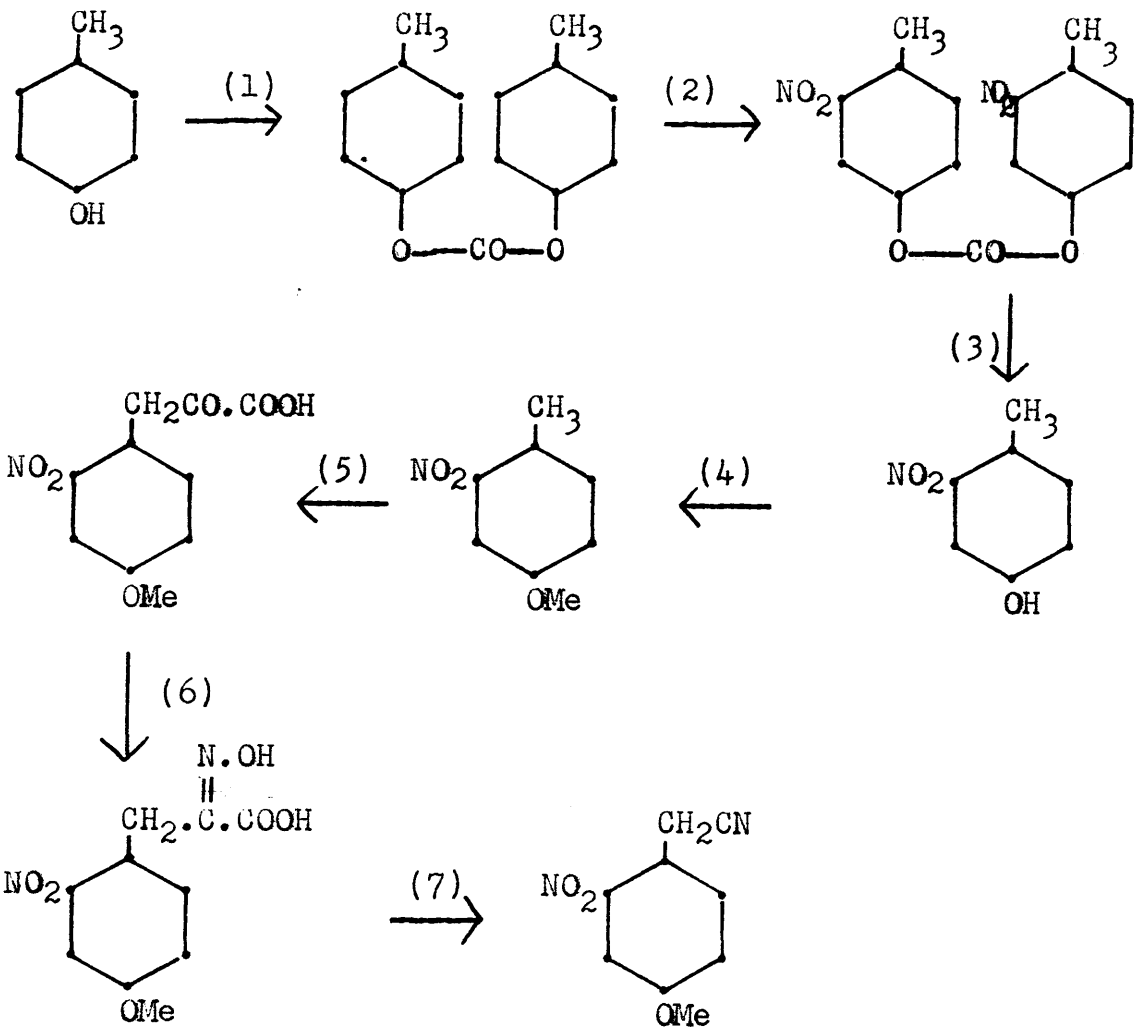
and then left at room temperature overnight. The next day the solid matter was filtered off and extracted with ether. The extract, after washing with dilute hydrochloric acid and water, was dried and reduced to dryness. The residue crystallised from absolute ethanol, after treatment with decolourising charcoal, to give a colourless product, m. p. 142° (Found: C, 64.46; H, 5.22; N, 12.03. $C_{19}H_{19}O_4N_3$ requires C, 64.59; H, 5.32; N, 11.90%).

A mixed melting point of A and B showed a marked depression.

2-NITRO-4-METHOXY-α-TOLUNITRILE

At the time when this investigation was commenced (April, 1944) the position of the methoxy group in ring C of the relevant colchicine degradation products had not been established. Work was initially commenced on the synthesis of 2:3:4:6-tetramethoxy-9-acetylaminomethyl-9:10-dihydrophenanthrene, but this synthesis was abandoned when the true methoxylation pattern became known (Barton, Cook and Loudon, J. Chem. Soc., 1945, 176).

The preparation of 2-nitro-4-methoxy-α-tolunitrile as one of the starting materials was begun according to the scheme shown opposite. No new substances were prepared in this work, but 2-nitro-p-tolyl carbonate, which had not previously been characterised, was purified by several


CH3
OH
(1)
CH3
CH3
O—CO—O
(2)
NO2
NO2
CH3
CH3
O—CO—O
(3)
CH3
NO2
OH
(4)
CH3
NO2
OMe
(5)
CH2CO.COOH
NO2
OMe
(6)
N.OH
CH2.C.COOH
NO2
OMe
(7)
CH2CN
NO2
OMe

recrystallisations from ethanol and submitted for analysis. (M. p. 148-149°) (Found: C, 54.81; H, 3.69; N, 8.50. $C_{16}H_{12}O_7N_2$ requires C, 54.21; H, 3.64; N, 8.44%).

The steps in the synthesis are described by the following workers:

(1),(2),(3): Copisarow, J. Chem. Soc., 1929, 25.

(4): Harvey and Robson, ibid., 1938, 99.

(5): Kermack, Perkin, and Robinson, ibid., 1921, 163.

(6),(7): Buchanan and Loudon, unpublished.

(1)-(5): Schlittler, Helv. Chem. Acta, (1932) 15, 394.

ATTEMPTED METALLATION OF 9:10-DIHYDROPHENANTHRENE

Gilman and Gibb (J. Am. Chem. Soc., (1939) 61, 109) describe the metallation of a variety of substances, in most cases by prolonged treatment with butyl lithium. Metallation in reactive nuclear positions is possible, and subsequent carbonation of the lithium compounds gives rise to carboxylic acids.

9:10-Dihydroanthracene, on such treatment, gives as the principal product, 9:10-dihydroanthracene-9-carboxylic acid. A small amount of the 9:10-dicarboxylic acid was also formed. The use of this reaction on a preparative scale is described by Burtner and Cusic (J. Am. Chem. Soc., (1943) 65, 1582).

It seemed possible that a similar method might be used to obtain 9:10-dihydrophenanthrene-9-carboxylic acid, and an attempt to carry out the metallation was made.

Experimental: The method applied was essentially that of Burtner and Cusic. Dihydrophenanthrene was refluxed in ether with butyl lithium, both solutions being prepared and maintained in an atmosphere of nitrogen. Mixing of solutions was effected by a syphon arrangement such that no air was admitted to the system.

Even with prolonged refluxing and a large excess of butyl lithium, no substantial reaction was detected, and the dihydrophenanthrene was recovered unchanged.

In a trial experiment made with dihydroanthracene in order to check the method, the 9-carboxylic acid was satisfactorily obtained.

It is possible that a more vigorous treatment of 9:10-dihydrophenanthrene with n-butyl lithium would give at least an interaction between the two substances.

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PART II

"STUDIES IN THE SYNTHESIS OF BENZOXAZOLES"

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SUMMARY

Certain derivatives of 1:3-diazacarbazole were of interest in connection with the search for new antimalarials. Attempts to synthesise compounds of this type were unsuccessful, as the new heterocyclic nucleus proved rather inaccessible. The reactions studied should form a useful basis for further work in this field, and it is considered probable that a continuation of the research would yield diaza-carbazoles.

INTRODUCTION

The protozoal infection, Malaria, afflicts the inhabitants of a large part of the world. It has been estimated that there are annually 300,000,000 cases, resulting directly in the death of some 3,000,000 people. The indirect effect of the general debility in those who survive is probably a more serious consequence of the disease.

The organisms responsible for malaria (there are four varieties) are species of Plasmodia (a genus of Protozoa). In the life cycle of the Plasmodium parasite two hosts are involved: the Anopheles mosquito[1] and man. In the former the malarial organism exists in sexual forms, but in man, it is modified to a single asexual type which undergoes a reproductive cycle ~~in man~~ by the process of schizogony (multiplication by repeated division). A useful brief description of the life-cycle of the malarial plasmodia is given by Goodman and Gilman, 1941.

The fever symptoms which characterise an attack of malaria are due to the fact that the asexual form in man is parasitic on the red blood cells. When reproduction by schizogony occurs, the host cell is disrupted. As a fairly regular reproductive cycle occurs, large numbers of red blood cells may be destroyed at the same time; the

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1. Only a fraction of the two hundred odd Anopheles species are malaria carriers.

foreign protein and other substances thus liberated cause the characteristic fever symptoms (chill, shivering, prostration, high temperature, nausea). In the various forms of the disease the reproductive cycle of the asexual form has a duration of from 36 to 72 hours, and the patient is subject to recurring bouts of acute fever at regular intervals which are similarly spaced.

If the victim is untreated, these attacks continue for several weeks, with decreasing intensity as immunity develops. This immunity is neither complete nor permanent.

Thus, although the infection is not usually fatal, the serious symptoms cause a general reduction of the vitality and resistance of the victims.

With the out-break of the war with Japan, the necessity for sending large numbers of troops into malaria-infested areas became apparent; and from a consideration of the available antimalarial therapies, it seemed that the degree of protection necessary for military efficiency could not be provided. The short-comings of the existing therapeutics were as follows:

Quinine, which was capable of suppressing acute symptoms[1], was not available in sufficient amount; for the

---

1. Quinine does not eradicate any form of the Plasmodia in man; however, by suppressing the multiplication of the asexual form in the blood, it prevents the large-scale disruption of red blood cells which gives rise to the acute fever.

prevention of the occurrence of acute fever, continuous regular prophylactic administration of the drug is necessary. Further, prolonged intake of therapeutic doses of quinine gives rise to undesirable symptoms of poisoning.

Of the synthetic substances which had been developed as antimalarials, only two were effective:

Mepacrine (hydrochloride) was similar to quinine in its action, but caused more immediate toxic reactions. Little was known of the effects of prolonged prophylactic administration; there seemed to be a possibility of serious cumulative effects.

Pamaquin was known to be dangerously toxic and could only be administered under careful medical supervision.

It thus seemed that the success of the war with Japan might depend on the discovery of a less toxic therapeutic agent which was at least capable of suppressing acute symptoms.

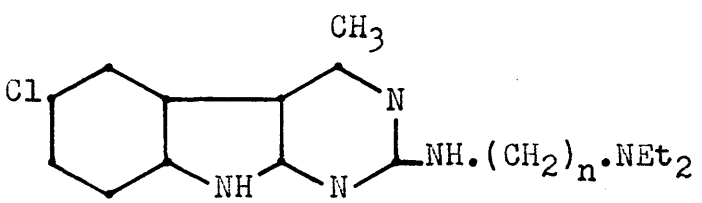
A large-scale search for new antimalarials was therefore initiated in various parts of the world including notably America and Britain.

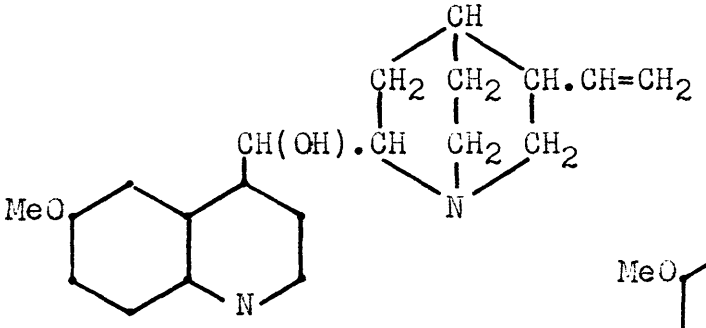
Fortunately for the progress of the war, however, the early fears concerning the use of mepacrine proved groundless, and its general use became possible. The initial toxic symptoms are due to an irritation of the stomach lining on oral administration of the drug; and a

degree of immunity ~~to this~~ develops in time. Its continued use causes an undesirable pigmentation of the skin, but the only signs of poisoning are very mild cerebral disturbance after prolonged administration.

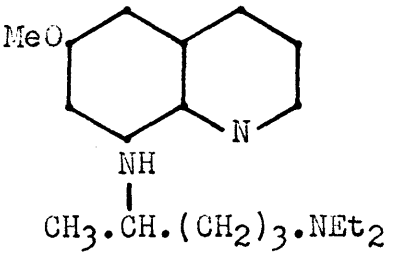
The present work was an extra-mural research associated with a war-time antimalarial project organised by the research department of Imperial Chemical Industries (Dyestuffs and Pharmaceuticals).

In the section which follows this introduction, evidence is given which suggests that antimalarial activity might be found in diazacarbazoles of the type shown below. The preparation of such diazacarbazoles was the object of this research.

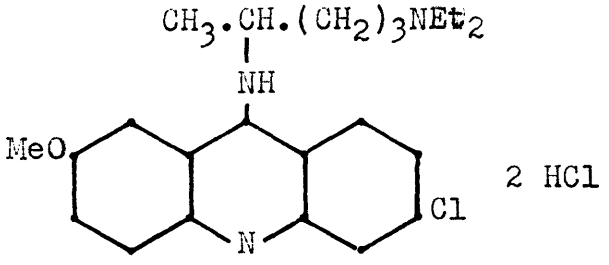




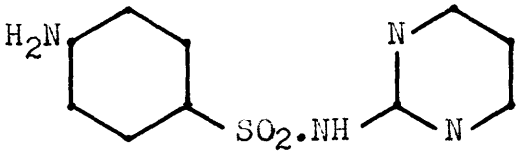
Quinine



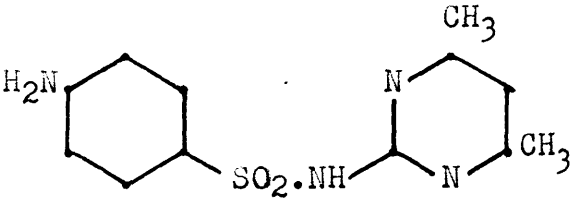
Pamaquin



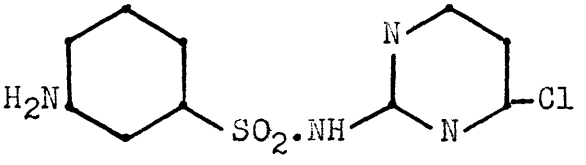
Mepacrine



Sulphadiazine



Sulphadimethyldiazine



Metachloridine

(which shows antimalarial activity)

ANTIMALARIAL ACTIVITY AND CHEMICAL STRUCTURE

In initiating a search for new less toxic antimalarial substances, the obvious starting point was the variation of the structure of known therapeutic compounds, in this case, quinine, pamaquin, and mepacrine (opposite). Fairly extensive investigations had already been carried out in this field, and a number of workers had attempted to correlate chemical structure with antimalarial activity. In particular, Magidson et al (1934, 1936) had suggested that the antimalarial activity was associated with the substituted quinoline or acridine nucleus, while the basic side chain served only a pharmacological purpose, being responsible for the facile absorption and distribution of the drug and finally its penetration to the tissues of the infecting Plasmodia.

Curd and Rose (1946a), who were about to embark on attempts to synthesise new antimalarials, took into account, in addition to the above hypothesis, a similar theory of the action of the sulphonamide drugs (some of which also have antimalarial activity).

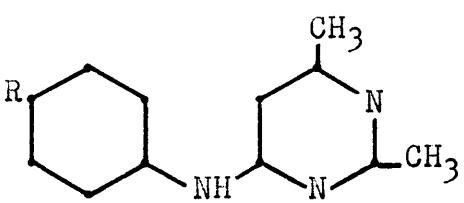
They suggested that here again the basic sulphonamide or substituted sulphonamide grouping served a pharmacological purpose, while the substituted phenyl residue was the bacteriocidal factor.

In the sulphonamides the basic groupings which seemed to be most efficient from the pharmacological standpoint were those present in sulphonamide derivatives of 2-amino-pyrimidine and 2-amino-4:6-dimethylpyrimidine.

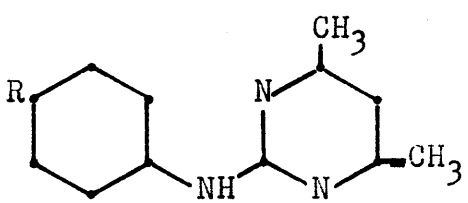
Curd and Rose conceived the idea of using pyrimidine rings as the pharmacological factors in synthetic anti-malarials. The fact that pyrimidine derivatives are common cell constituents made it seem possible that anti-malarials based on this structure might prove less toxic than other types. (It may well be that the easy penetration of pyrimidine derivatives into the tissues is derived from the same circumstance.)

The substances first investigated by Curd and Rose consisted of pyrimidine rings attached to aromatic nuclei, in which the substitution was in some way characteristic of that in known antimalarial compounds.

For example, the following compounds were tested:

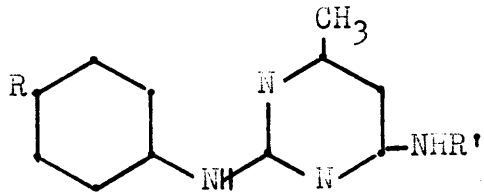


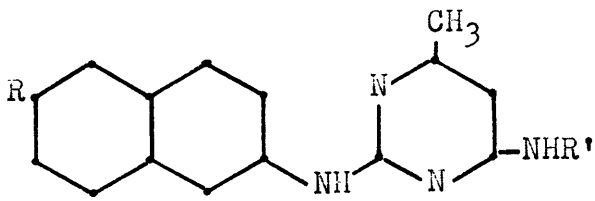
and

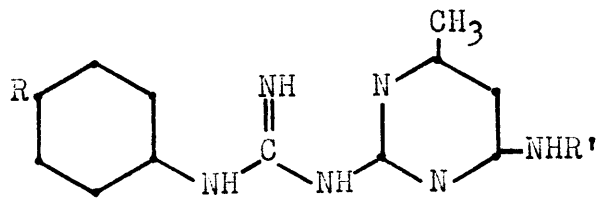


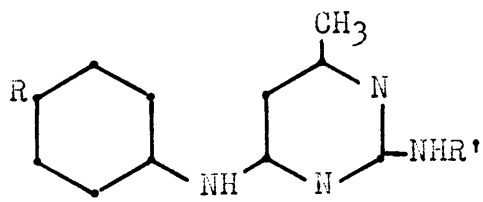
where R = -Cl or -OMe.

However no antimalarial activity was observed in these or a number of related compounds.

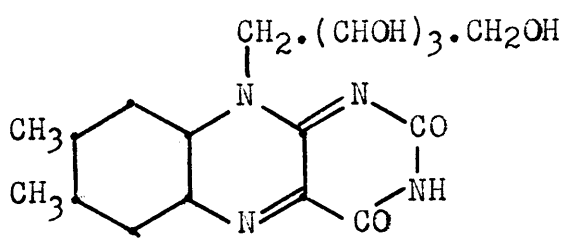






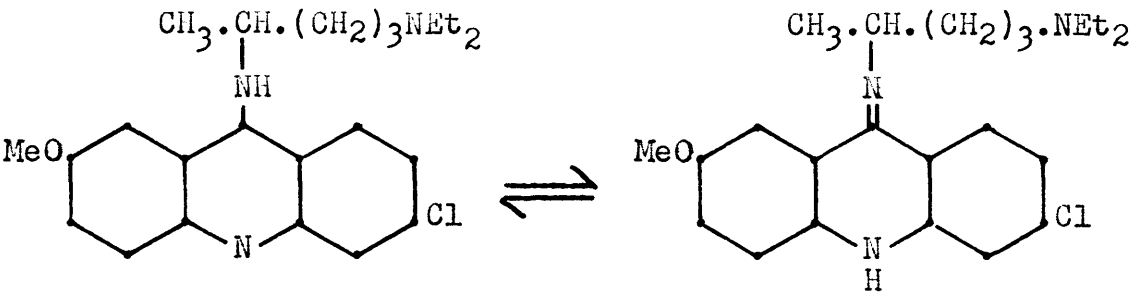


(R= Cl or MeO; R'= $-(CH_2)_n.NEt_2$)



Riboflavin

In view of the lack of activity, attention was turned to another hypothesis--that of Schönhöfer (1942), who suggested that activity was associated with the possibility of an equilibrium with a quinonoid form, thus:



In view of this hypothesis, Curd and Rose introduced into their pyrimidine derivatives the group -NHR, in positions such that such a tautomerism might be possible (involving the ring nitrogen atoms).

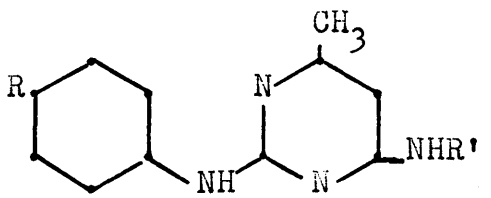
In compounds of this new type, a marked antimalarial activity was found.

A selection of the active substances is shown on the opposite page.

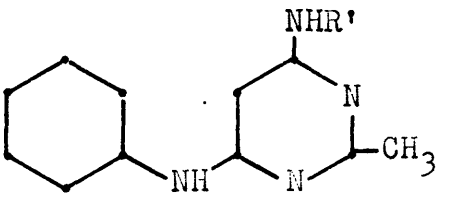
These new substances still maintained a vague structural resemblance to riboflavin, and it had been suggested on various occasions that the antimalarial effect of mepacrine derivatives may be due to a competitive effect in riboflavin metabolism. In the investigations which followed, modifications were made which enhanced the similarity of the structures to the riboflavin molecule (for

example, dimethyl groups were introduced into the benzene ring). However the results were rather inconclusive, and no further increase in activity was obtained.

An examination of the structures of a number of active antimalarials of the new type brought attention back to the consideration of the tautomer hypothesis of Schönhöfer. It was observed that, while I (below) showed a marked activity, II was quite inactive.



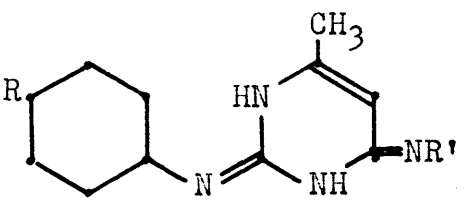
I



II

A significant difference exists between I and II, with respect to the possible tautomeric alternatives. In I, there are two linked -HN-C=N- systems which are capable of independent tautomerism. In II there are also two tautomeric systems, but these are not similarly independent.

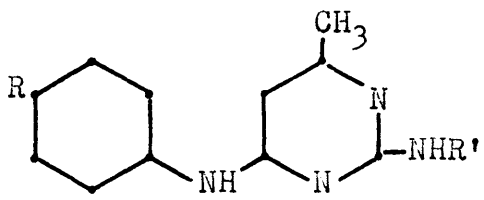
If both systems in I are in the changed form we have the structure III.



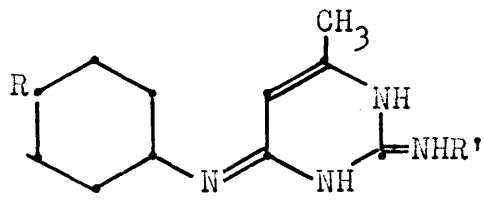
III

The substance II cannot give rise to a corresponding doubly tautomeric isomer (except in unlikely zwitterion forms).

It was therefore suggested that a capacity for simultaneous double tautomerism of linked -HN-C=N- systems might be connected with the possession of activity. This idea received support from the finding that IV (below) is also active. In IV, as in I, the doubly tautomeric form (V) can exist in addition to the two singly tautomeric forms.

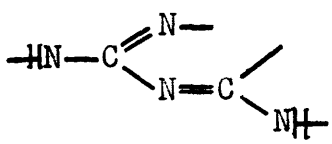


IV



V

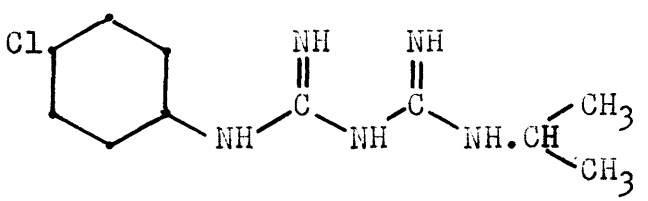
From these observations, Curd and Rose (1946b) made the generalisation that a structural requirement for activity in this type of substance is the existence of the arrangement:



Resonance Alternatives. It was pointed out that there are also limitations to the number of stable resonance

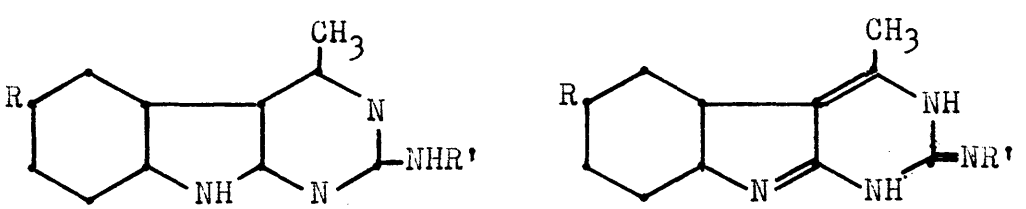
alternatives in type II which are not to be found in types I and IV. This may be the more important consideration.

Working on the above hypothesis, Curd and Rose considered the possibility that a pyrimidine ring per se might not be essential. The examination of substances in which this ring was open (incomplete) led to the discovery of Paludrine, which has proved to be the most effective and least toxic antimalarial so far obtained.



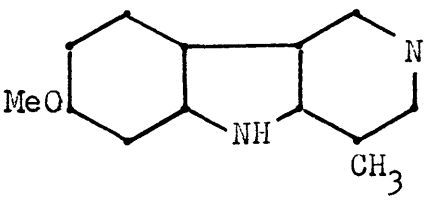
Paludrine

In the present work a different variation which still contained the required tautomeric arrangement was considered (VII):



VII

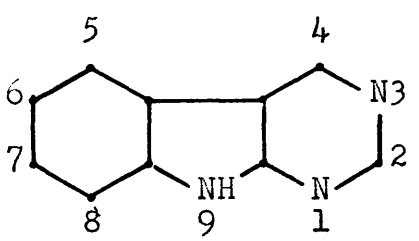
The fact that monoazacarbazoles are found naturally (in the Harmala alkaloids) is also of interest in relation to possible biological activity in substances of the **type** VII.



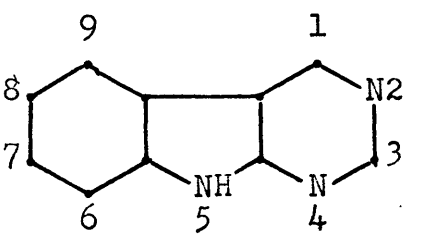
Harmine

Nomenclature of Diazacarbazoles

For reasons of convenience the term diazacarbazole has been used rather than the systematic name (below). The numbering of the heterocyclic ring system is shown, along with the systematic naming and numbering (Proposed International Rules for Numbering Ring Systems, Patterson, "The Ring Index," 1940, p. 599).



9-(1:3)-Diazacarbazole

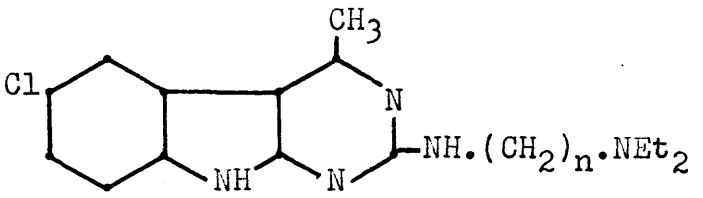


5-Pyrimido[4.5-b]indole

The "9" and the "5" in these names refer to the position of the "extra" hydrogen atom (cf. "The Ring Index").

Other heterocyclic nuclei and complex radicals are named systematically. Oxy-pyrimidines are classed as hydroxy-pyrimidines and not as pyrimidones.

SYNTHETIC METHODS



Before considering the condensations by which it might be possible to build up the heterocyclic nucleus in the above molecule, it was necessary to examine the requirements set by the existence of the substituent groups.

The Basic Side Chain

The fact that the basic side chain is attached at a reactive position made it possible to leave its introduction to a later stage, provided that some suitable replaceable radical was present. The following groups might reasonably be converted to the required diethylaminoalkylamino side chain:

(1) -Cl, by direct treatment with the appropriate diethylaminoalkylamine, $NH_2.(CH_2)_n.NEt_2$.

(2) -OH, via -Cl.

(3) -SMe, by direct treatment with the amine, or via -OH. A mercapto group can be hydrolysed by heating with chloracetic or hydrobromic acid.

(4) $-NH_2$, but not by direct action of the diethyl-

aminoalkyl halide ($Cl.(CH_2)_n.NEt_2$). In the reactive α and γ positions in nitrogenous six-membered rings, an amino group behaves anomalously and may even possess the tautomeric imino structure (=NH). Condensation with an alkyl halide is liable to give attachment to the ring-nitrogen.

In the present case it might be possible to hydrolyse the amino group, and convert the hydroxy pyrimidine via the chloro-compound to the required base.

The Methyl Group. Although a methyl group can be derived from other substituents, it was preferable to include it in the simpler molecules which were to be condensed to give the diazacarbazoles.

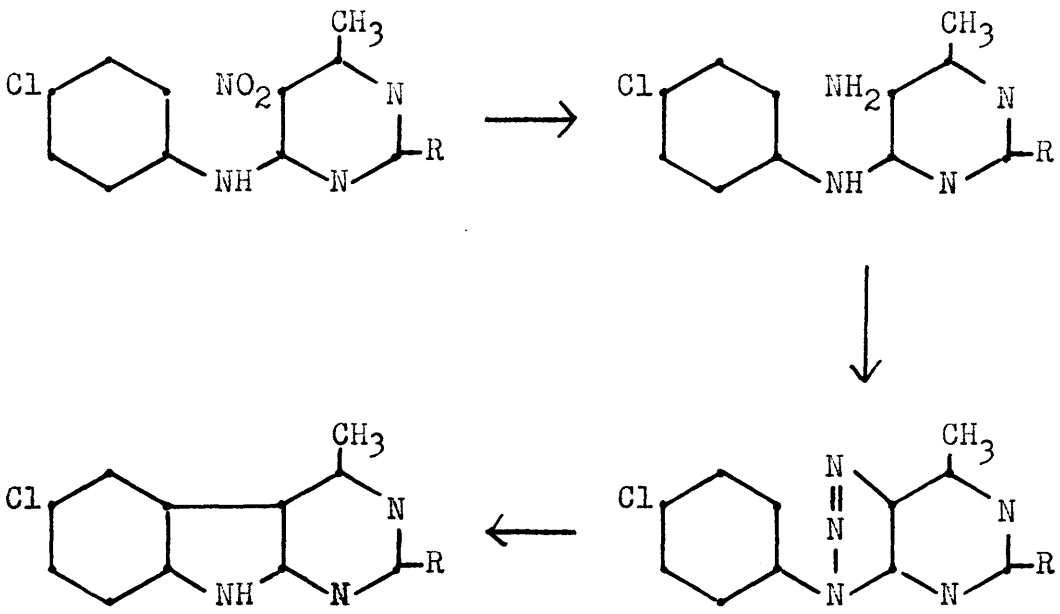
The Chloro Group. Direct chlorination of the 1:3-diazacarbazoles might be expected to give at least some of the 6-chloro derivative. However, ambiguity with respect to the placing could be avoided by its inclusion in the starting materials.

The Diazacarbazole Nucleus

A number of possible approaches to the preparation of this heterocyclic system suggested themselves; they are summarised individually below.

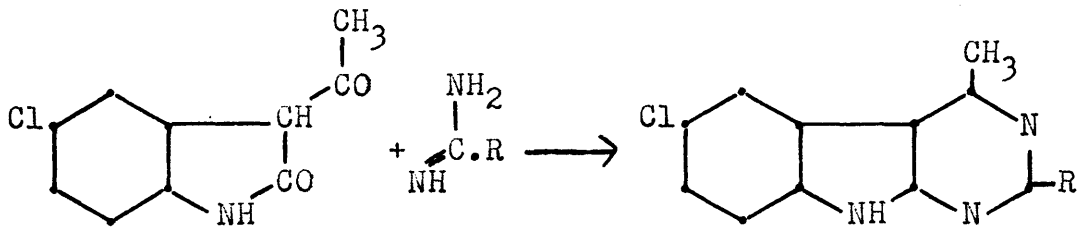
APPROACH A:

The classical Graebe-Ullmann carbazole synthesis, by the thermal decomposition of a triazole:



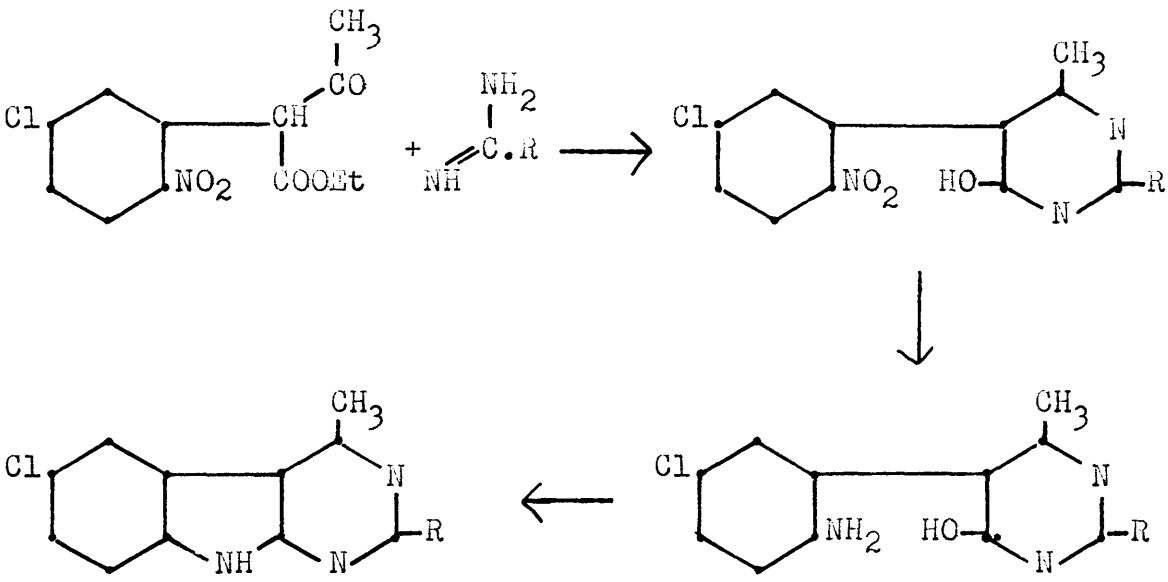
APPROACH B:

Addition of a pyrimidine ring to an indole derivative by condensation with an amidine-type substance (i.e. a guanyl derivative; $-C(=NH).NH_2$ ≡ guanyl).



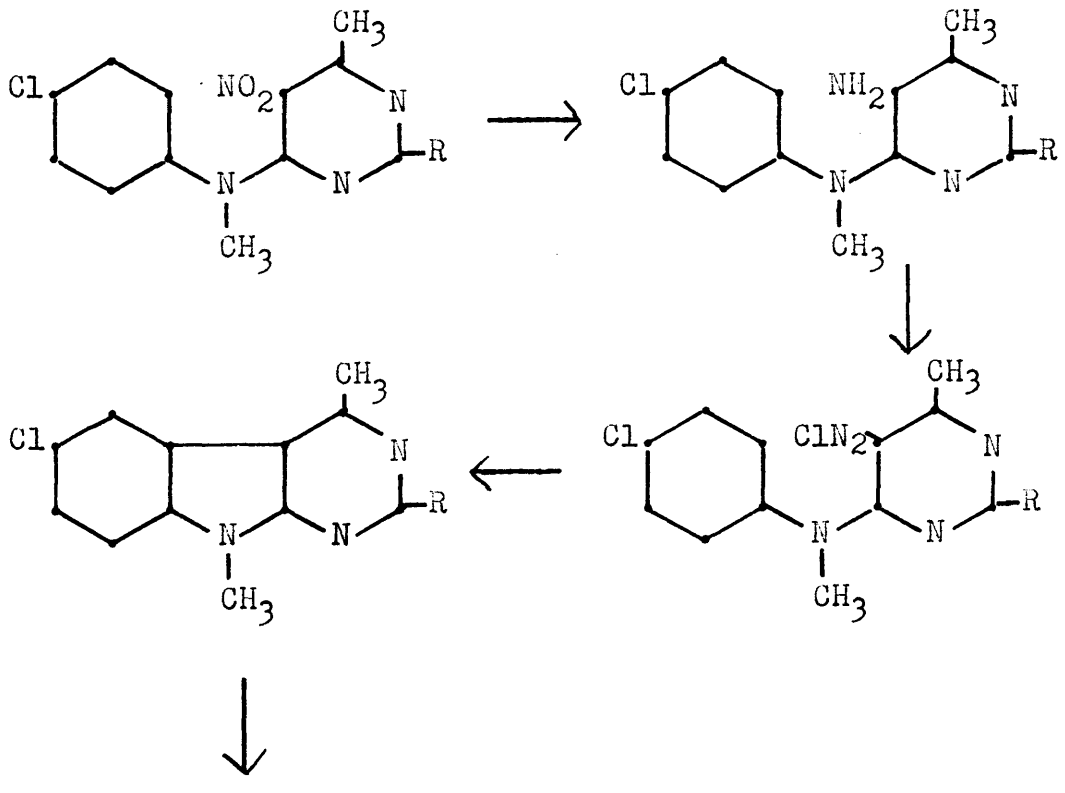
APPROACH C:

Condensation of an α-(o-nitrophenyl)acetoacetic ester with a guanyl derivative to obtain a pyrimidine ring, followed by a second ring-closure to complete the carbazole system:

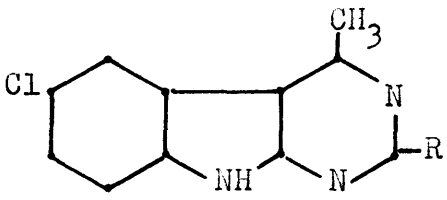


APPROACH D:

Blockage of triazole formation in approach A, and Pschorr decomposition of the diazotised amine to give a 9-methyl-(1:3)-diazacarbazole which could later be demethylated.



(over)



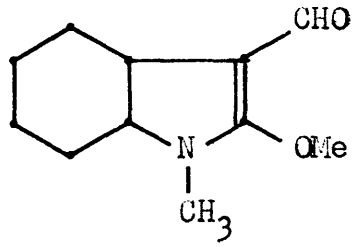
The above approaches were all investigated without the desired products being isolated.

The work was, however, suspended while modifications of approach D were still being studied.

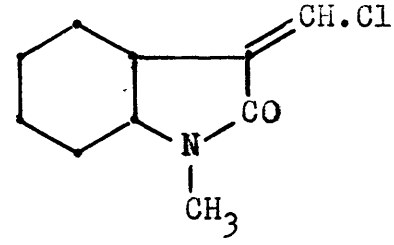
It is considered probable that further work would result in the isolation of diazacarbazoles.

Other approaches:

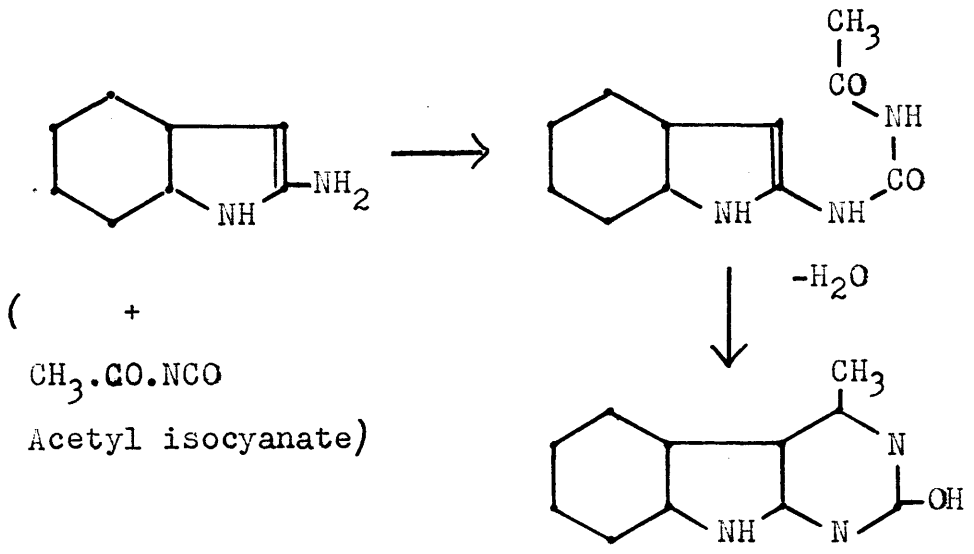
Some very preliminary experiments with compounds (below) related to those used in approach B suggested that at least the diazacarbazole nucleus might be obtained by their condensations with amidine-type substances.



and



Another approach which might be considered is the following:

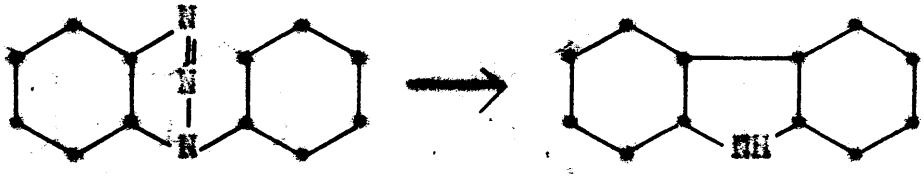


(+ $CH_3.CO.NCO$ Acetyl isocyanate)

DISCUSSION OF EXPERIMENTAL

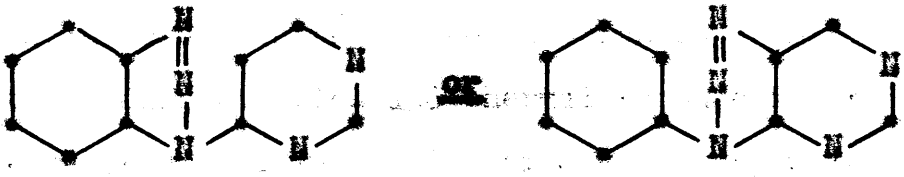
APPROACH A

In the classical carbazole synthesis (Graebe, Ullmann, Ann., 1896, 291, 16), ring-closure to form the central ring is effected by the thermal decomposition of a triazole, thus:



The triazole is usually heated in liquid paraffin or syrupy phosphoric acid until decomposition begins. In favorable cases high yields of the carbazole are obtained.

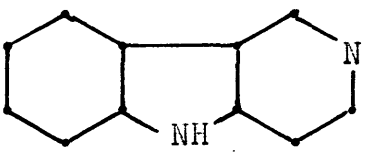
To form a diazacarbazole of the type desired, the carbazole synthesis might be applied in two ways: by pyrolysis of a benz-triazole or of a pyrimido-triazole, i.e. from



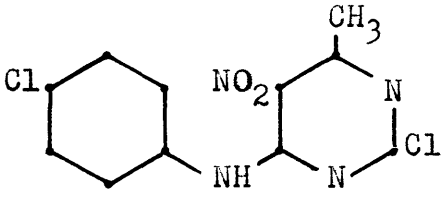
Little evidence is available to indicate which of these ring-closures is most likely to succeed. In one case where a monoazacarbazole (I, below) has been prepared in both ways, the better yield was reported by the

decomposition of the pyrido-triazole (Robinson and Thornley, J. Chem. Soc., 1924, 2169; Bremer, Ann., 1934, 514, 279).

In the present synthesis the pyrimido-triazole was selected for an attempted pyrolysis, mainly because its preparation seemed more straight-forward; a method for the synthesis of II (below) was known (Curd and Rose, private communication).



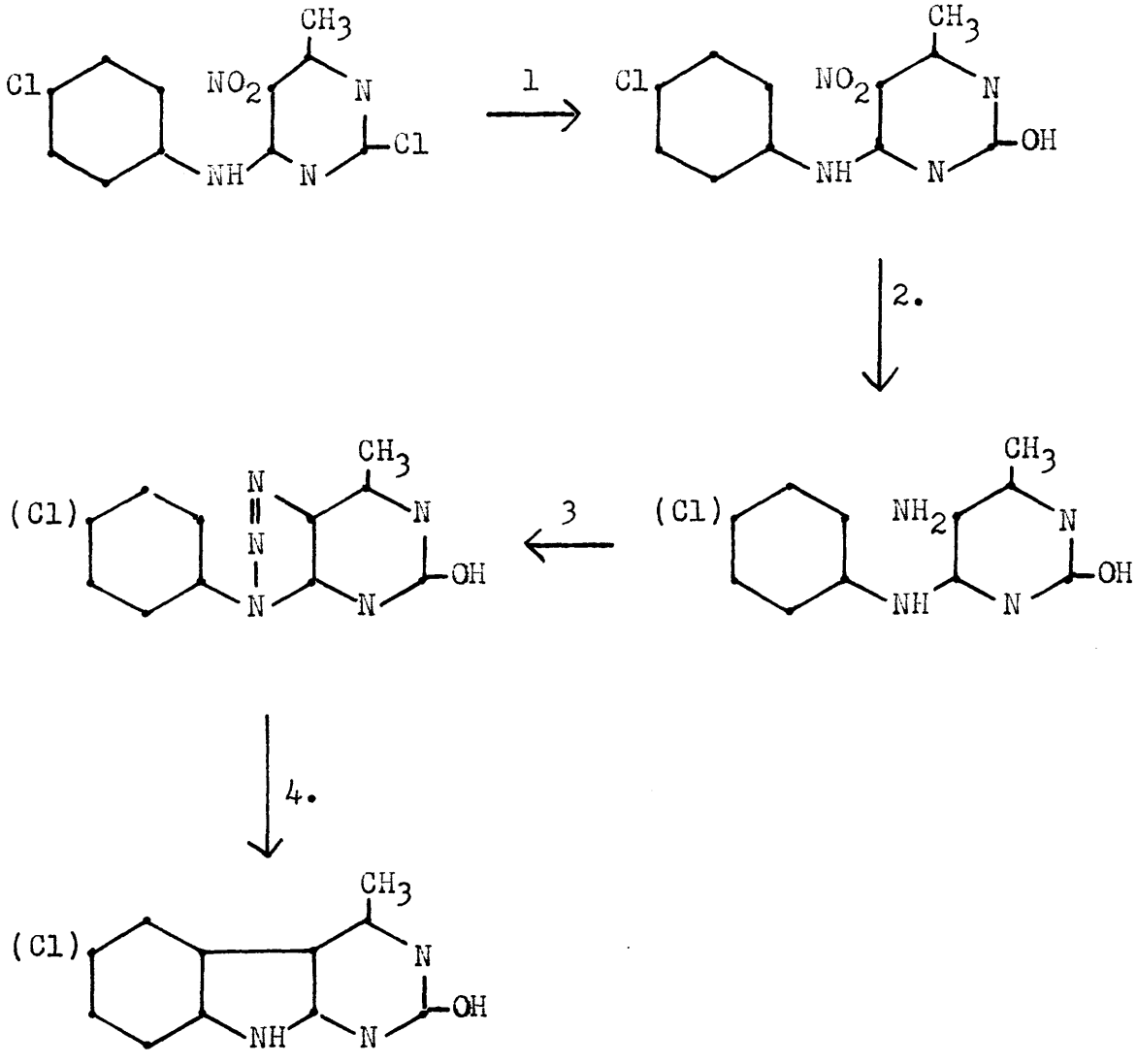
I



II

In this substance the benzene ring chlorine atom and the methyl group are in required positions (The compound had been prepared as an intermediate in a related antimalarial investigation).

The reactive chlorine atom in II would appear to offer a convenient means of introducing the basic side chain; however, in the compound which is submitted to reduction, diazotisation and thermal decomposition it is necessary to have in the 2-position a group which will be stable under the various conditions used. The very reactive chlorine atom present in II might be reduced along with the nitro group; it would certainly complicate the


CH3
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N
Cl
NH
N
1
CH3
Cl
NO2
N
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NH
N
2.
CH3
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NH2
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OH
NH
N
3
CH3
(Cl)
N
N
N
N
OH
N
4.
CH3
(Cl)
N
OH
NH
N

pyrolysis of the triazole. If the basic side chain were introduced at an early stage, it might likewise prove unstable under the violent conditions of a thermal decomposition. The expedient was adopted of hydrolysing the chloro group to a hydroxyl group. If the diazacarbazole were obtained, subsequent conversion to the basic radical might be effected via the chloro group thus:

$$-OH \xrightarrow{POCl_3} -Cl \xrightarrow{RNH_2} -NHR$$

The tentative procedure thus adopted is shown on the opposite page; the various stages are discussed below.

Experimental (p. *111*)

Stage 1. Hydrolysis was effected by heating with aqueous acetic acid.

Stage 2. The low solubility of the hydroxy-nitro compound in all ordinary solvents (in the cold) made it necessary to carry out the hydrogenation of an aqueous solution of the alkali salt. Under these conditions, the benzene ring chlorine atom was also reduced (the catalyst was palladised strontium carbonate). However, as triazole decomposition is liable to proceed more smoothly in absence of substituent groups, the synthesis was continued using the chlorine-free product.

Stage 3. On diazotisation of the 2-hydroxy-4-anilino-5-amino-6-methyl pyrimidine, the triazole was formed.

Stage 4. Attempted decomposition of the triazole by the methods usually applied gave in every case only tarry products.

Note on decomposition of triazoles

An examination of the literature made concurrently with the above experiments indicated that it was doubtful whether a successful decomposition of the triazole could be achieved. From described work on attempted preparations of monoazacarbazoles it was clear that

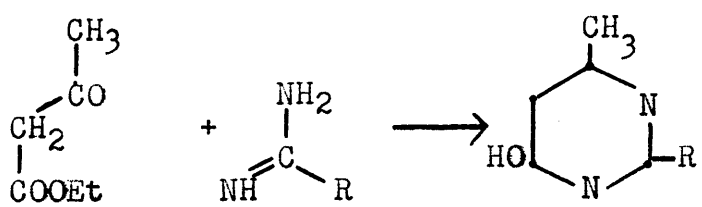
(a) azacarbazoles are obtained in poorer yields than the corresponding carbazoles,

(b) substituent groups interfere markedly with successful triazole decomposition, especially if they are reactive. The few attempts which have been made to prepare substituted monoazacarbazoles by the decomposition of triazoles failed (Bremer, Ann., 1934, 514, 279).

APPROACH B

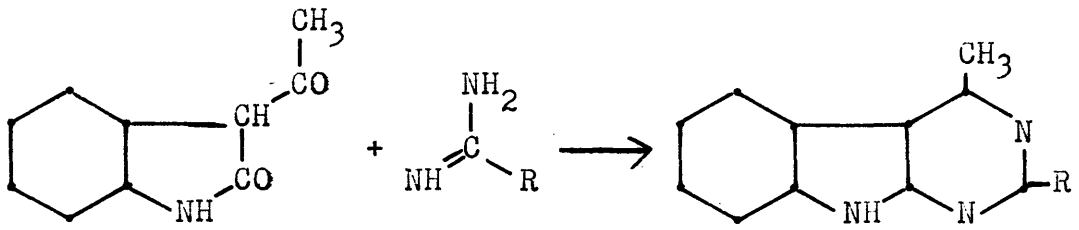
One of the classical methods of pyrimidine synthesis is the condensation of β-diketones (or keto-esters) with amidines and related compounds.

e.g.,



Two possible ways of applying this reaction to the present synthesis were apparent; the second of these is discussed in "approach C."

The application first considered involves a condensation of the type:



(R ≡ -OH, -SH, -SMe, or $-NH_2$)

It is to be expected that the presence of the indole -NH- group would have a depressive effect on the reactivity of the oxygen atoms in respect to condensation of this type. However the facility with which this pyrimidine synthesis normally occurs made it seem possible the above reaction might be brought about. (Condensations involving amide oxygen are known; cf. Pictet and Stehelin, Compt. Rend., 1916, 162, 876.)

In the condensations attempted, the amidines indicated were tried (i.e. urea, thiourea, S-methylisothiourea and guanidine). In each case the methods which have been successful for pyrimidine synthesis were employed. However no successful condensation was achieved. In general it seemed that hydrolysis of the acetyl group took place more

readily than the required condensation.

The substance 1-methyl-3-acetyloxindole was also prepared in the hope that the presence of the N-methyl group might aid condensation. Again it was found that deacetylation readily occurred, and that no products of the desired type could be isolated from the reactions.

Some interesting side-reactions occurred and are reported fully below.

In the above experiments the 5-chloro group was, for the moment, neglected. A method was worked out for the synthesis of 5-chloro-3-acetyloxindole.

Experimental (p. 115)

3-Acetyloxindole

The acetylation of oxindole by the method of Horner (Ann., 1941, 548, 117) gives only 24% yield. By modification of his procedure the yield was increased to 75%.

Attempted condensations of 3-acetyloxindole

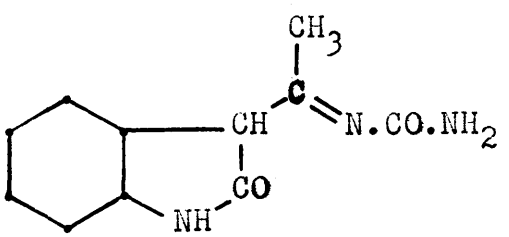
In the classical pyrimidine preparations a variety of conditions are used in the condensations of β-diketones and other compounds with amidines. In the following table some typical cases are summarised.

	Conditions	Amidine-type compound
1.	Concentrated aqueous KOH/days at room temperature	S-methylisothiourea
2.	In ethanol at room temperature in presence of conc. HCl or H_2SO_4	Urea, thiourea
3.	Refluxing with sodium ethoxide in ethanol	Thiourea, guanidine
4.	Simple heating (in ethanol)	Guanidine carbonate
5.	Heating with a small amount of acetic acid	Guanidine acetate

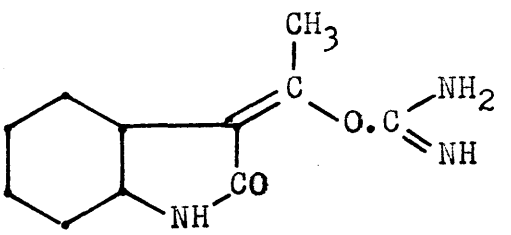
(The preparation of pyrimidines is discussed in Gilman, "Organic Chemistry," 1st Edition (1938), p. 948 and in Chem. Revs., 1933, 13, 209.)

Under similar conditions 3-acetyloxindole did not give the corresponding condensations.

In one experiment where this compound was heated alone with guanidine carbonate, a small quantity of a basic product was isolated, whose analysis suggested that the elements of ammonia had been eliminated. Possible structures for this compound are:



and

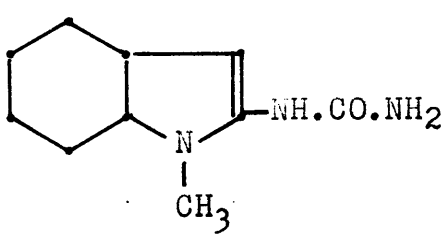


As the preparation gave only very low yields of the compound, and was not reproducible, the product was not further investigated. Numerous attempts to improve the procedure so that workable quantities could be obtained were unsuccessful.

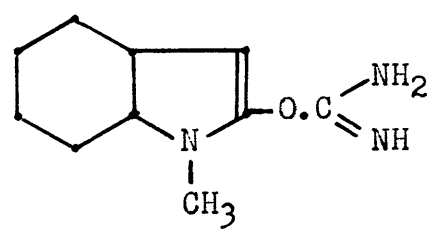
Condensations of 1-methyl-3-acetyloxindole

As with 3-acetyloxindole, attempts to bring about condensation with amidine-type substances were unsuccessful. Under alkaline conditions it was again found that the first ~~in~~reaction to occur was a deacetylation of the starting material.

When 1-methyl-3-acetyloxindole was heated with guanidine carbonate an interesting secondary reaction occurred. 1-Methyloxindole formed by deacetylation condensed with guanidine with the elimination of the elements of ammonia. The base formed may have one of two structures:



or



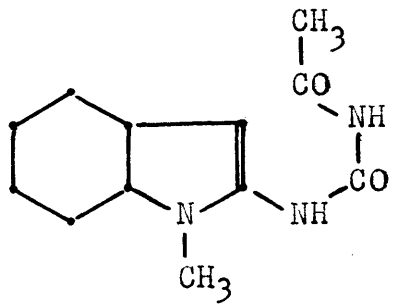
1-Methyl-2-ureidoindole

1-Methyl-2-isoureidoindole
(1-Methyl-O-guanyloxindole)

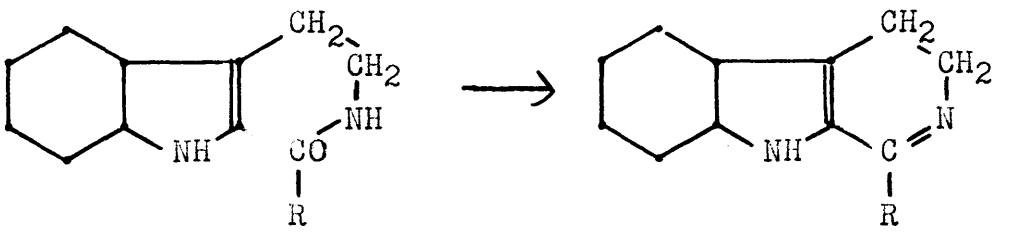
It was characterised as a picrate, a monoacetyl and a triacetyl derivative.

The same base was formed in 80% yield by direct condensation of 1-methyloxindole with guanidine carbonate under the same conditions.

One possible constitution of the monoacetyl derivative is I (below). A few experiments were made to see if it would undergo dehydration: (cf. Späth and Lederer, Ber., 1930, 63, 2102; II ⟶ III, below).



I



II III

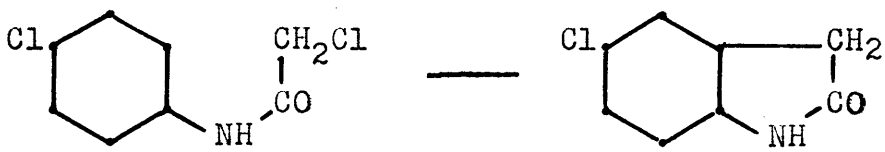
However it was unchanged by prolonged refluxing with phosphoric anhydride in xylene.

Cold concentrated sulphuric acid (cf. Knorr, Ann., 1886, 236, 83) caused deacetylation and mono-sulphonation of the free base. The same monosulphonic acid could be obtained by the action of sulphuric acid on the

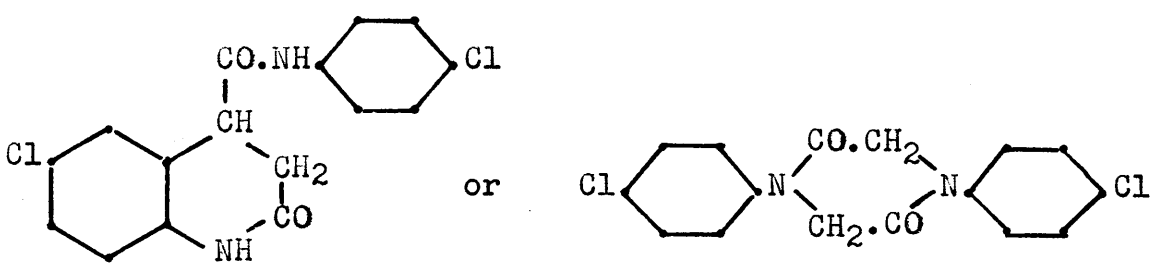
original 1-methyl-2-ureido (or isoureido?) indole.

The sulphonic acid had salt-like properties which suggested a zwitterion structure.

5-Chloroxindole is obtained in very poor yield (5%) by the method of Stollé (for oxindole):



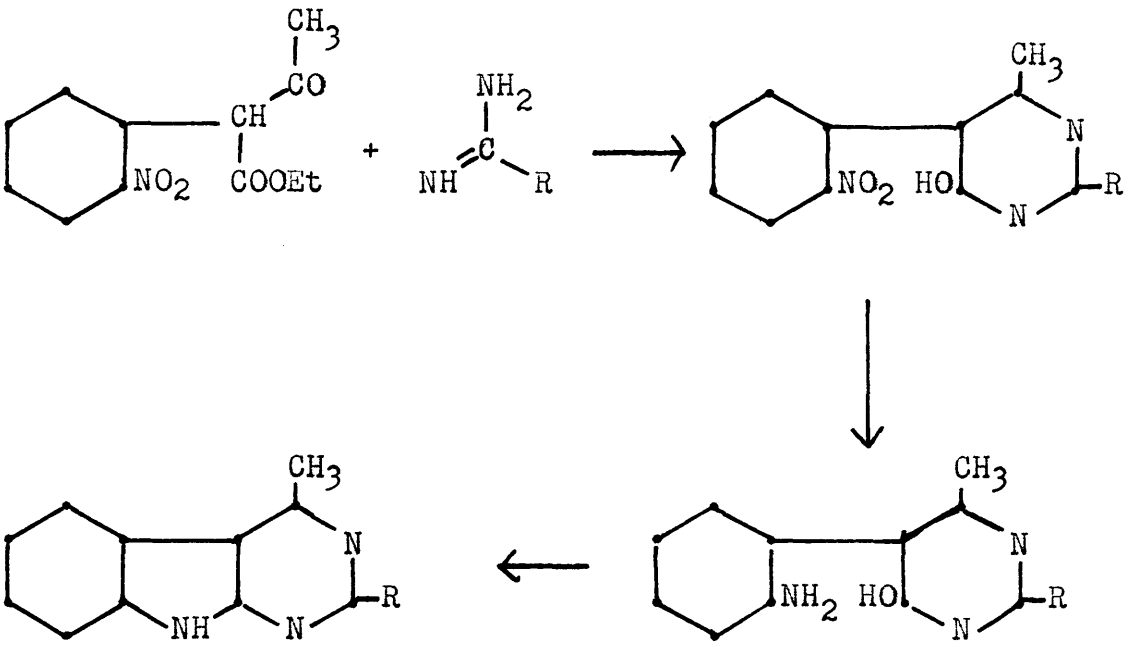
From such a Friedel-Crafts reaction another substance was isolated which gave the same analysis as 5-chloroxindole. This might be accounted for by the formation of an intermolecular condensation product such as



Better yields of 5-chloroxindole (30% of theoretical) were obtained by direct chlorination of oxindole, followed by fractional crystallisation of the products.

APPROACH C

A second application of the traditional pyrimidine syntheses might be made in the following way:



Preliminary attempts to prepare α-(o-nitrophenyl)-acetoacetic ester, which had not previously been described, were unsuccessful. Before spending more time on the preparation of starting materials, the more readily available 2:4-dinitrophenylacetoacetic and 2:4-dinitrophenylmalonic esters were examined to see if they would undergo the required condensations. Neither of these substances yields pyrimidines under the conditions previously described (Approach B, p. 93).

As these results did not hold out much promise of success, this approach was laid aside in favour of approach D.

Experimental (approach C) (p. 132)

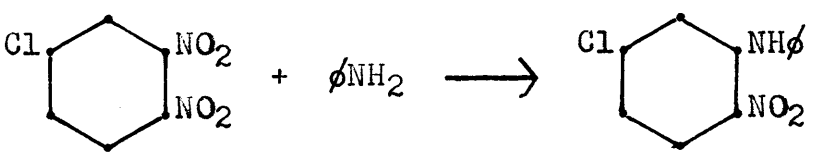
o-Nitrophenylacetoacetic ester

The chloro-group of o-nitrochlorobenzene is not suf-

ficiently reactive to react with sodioacetoacetic ester. (The reactions of o-nitrohalobenzenes and related substances with sodioacetoacetic and malonic esters are discussed by Borsche, Ber., 1916, 49, 2222.) Attempts were therefore made to introduce an acetyl group into o-nitrophenylacetic ester. Condensations using acetyl chloride or ethyl acetate in presence of sodium ethoxide gave only tarry products from which no crystalline benzoyl derivatives could be isolated. (The benzoylation of 2:4-dinitrophenylacetoacetic ester is described by Borsche, Ber., 1909, 42, 601.)

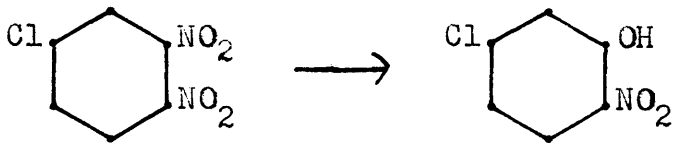
2-Nitro-5-chlorophenylacetoacetic ester

The substance 3:4-dinitrochlorobenzene possesses unusual properties (cf. Laubenheimer, Ber., 1876, 9, 760). The nitro group in the 3-position is labile, behaving in a manner similar to a reactive chlorine, as in 2:4-dinitrochlorobenzene. For example, 3:4-dinitrochlorobenzene reacts with amines to give 2-nitro-5-chlorophenylamines, the elements of nitrous acid being eliminated.

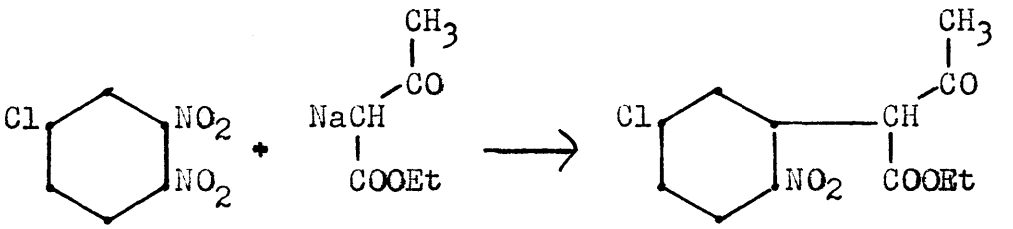


Similar condensations occur with hydrazine and phenylhydrazine.

If it is refluxed with sodium hydroxide solution 2-nitro-5-chlorophenol is produced:

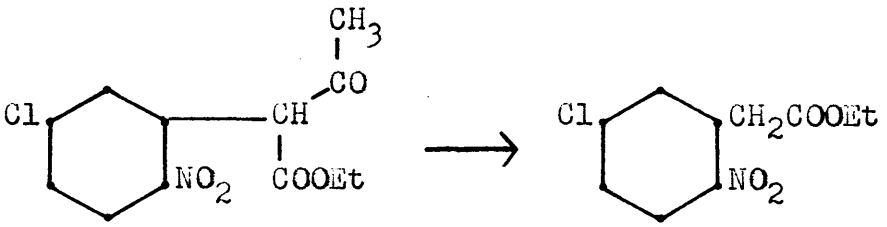


If 3:4-dinitrochlorobenzene could be induced to condense with sodioacetoacetic ester, then the substituents in the benzene ring would be suitably placed for the purposes of the present synthesis:



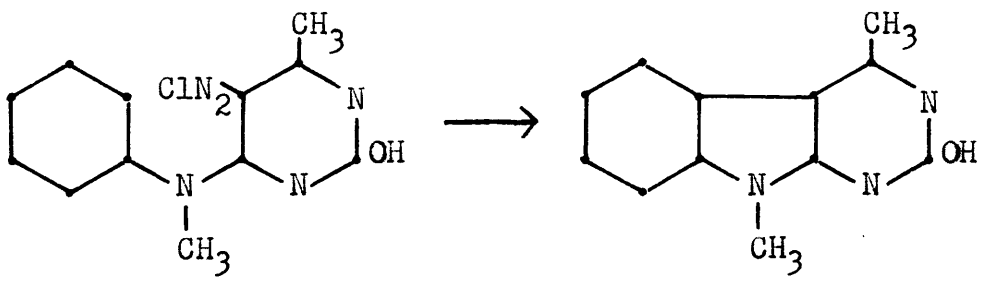
In an attempt to bring about the above condensation, ~~again~~ no solid benzoyl derivative could be obtained on benzoylation of the tarry reaction products. (In the working up, the precaution was taken of adding urea before acidification, to destroy any nitrous acid formed.)

However, an alkaline hydrolysis of the crude products of attempted condensation gave rise to a crystalline acid which analysed correctly for 2-nitro-5-chlorophenylacetic acid. This suggested that the required acetoacetic ester derivative was formed in the initial reaction:

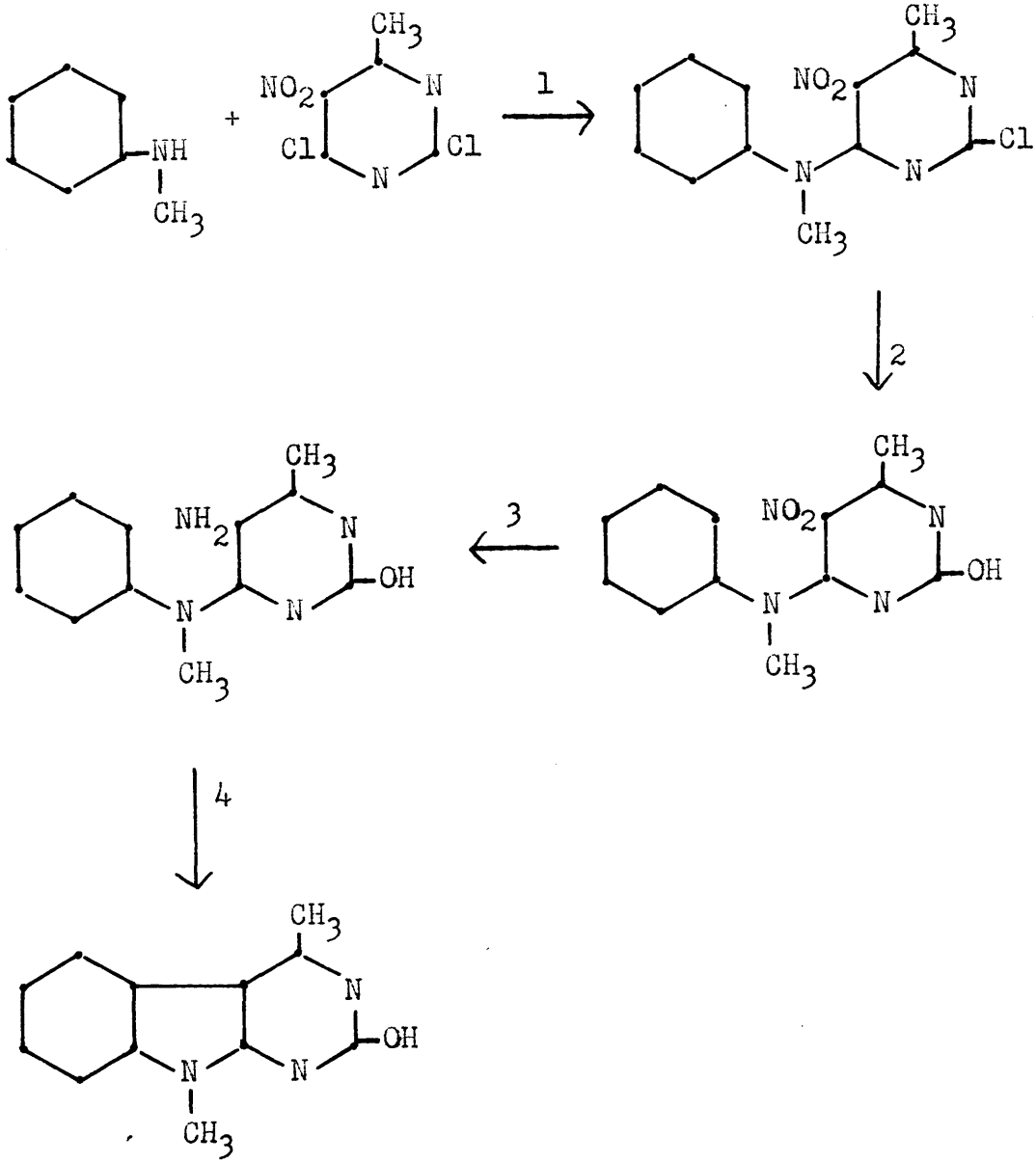


APPROACH D

In view of the above failures, a modification of the Graebe-Ullmann method was investigated, in which the hydrogen atom on the carbazole nitrogen (to be) was replaced by a methyl group. Triazole formation was thus inhibited and the diazonium salt was decomposed by the Pschorr method in the hope of bringing about the ring-closure shown.



It was found that the decomposition of the diazonium salts in these experiments was complicated by side-reactions (such as internal coupling with the pyrimidine ring methyl group, and in a synthesis where this was absent, by reduction of the products by the free copper bronze during the decomposition). Owing to shortage of time a full


CH3
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CH3
NO2
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OH
N
N
CH3
3
CH3
NH2
N
OH
N
N
CH3
4
CH3
N
OH
N
N
CH3

investigation of the reaction products was not completed. It is possible that the required diazacarbazole structure may yet be obtained from this type of approach.

Experimental (p. 135)

The steps in the first synthesis investigated are illustrated on the left-hand page.

Stage 1. The use of the method of Curd and Rose (p. 111) gave at best a 50% yield of the product. In this method one molecule of p-chloroaniline is used per molecule of dichloronitropyrimidine. The use of two molecules of the aniline is presumably avoided on account of the existence of a second reactive chlorine which is liable to condense also giving a di-(p-chloroanilino)pyrimidine derivative. When one molecule is used, the basic groups of product are capable of "accepting" the hydrogen chloride formed. However, part of the residual p-chloroaniline will be fixed (though in equilibrium) as the hydrochloride, and the reaction will be slowed in its latter stages to a greater or lesser degree.

It was felt that nearer to theoretical yields might be obtained if excess p-chloroaniline could be used, and experiments were made with 2 molecules present. (The relative reactivities of the chloro groups were not known; it was possible that the secondary reaction might not occur.) In order to minimise the risk of the further condensation,

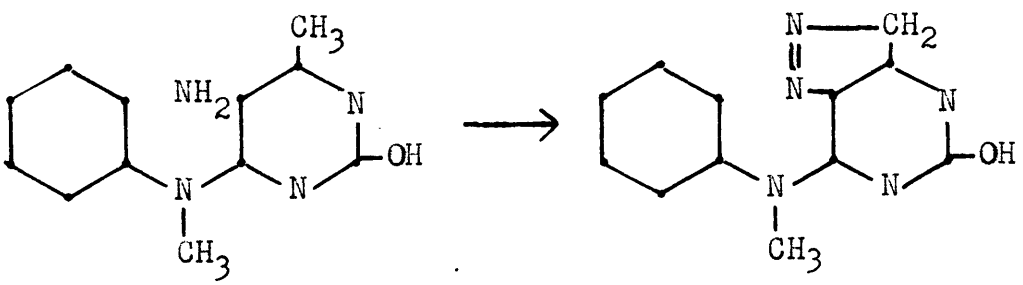
the amine (in ethanol) was added very slowly to a well-stirred solution of the dichloro-compound, which was cooled in a freezing mixture as low temperature would also inhibit the secondary reaction. Under these conditions the product crystallised from the solution more or less as it was formed, and was thus further protected.

Using this procedure 2-chloro-4-(p-chloroanilino)-5-nitro-6-methylpyrimidine was obtained in 85% yield in the pure state.

Stage 2. Hydrolysis was effected by warming with aqueous acetic acid.

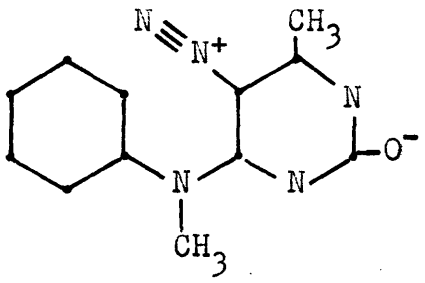
Stage 3. Reduction was effected by shaking with hydrogen in glacial acetic acid, and in presence of palladised strontium carbonate. The theoretical quantity was absorbed in a few hours.

Stage 4. Decomposition of the diazonium salt of the amine in warm sodium carbonate gave, instead of the desired Pschorr-type ring closure, an internal coupling:



The high melting point of the product and the easy

formation of a monoacetyl derivative showed that the product was not a stable diazo-oxide:

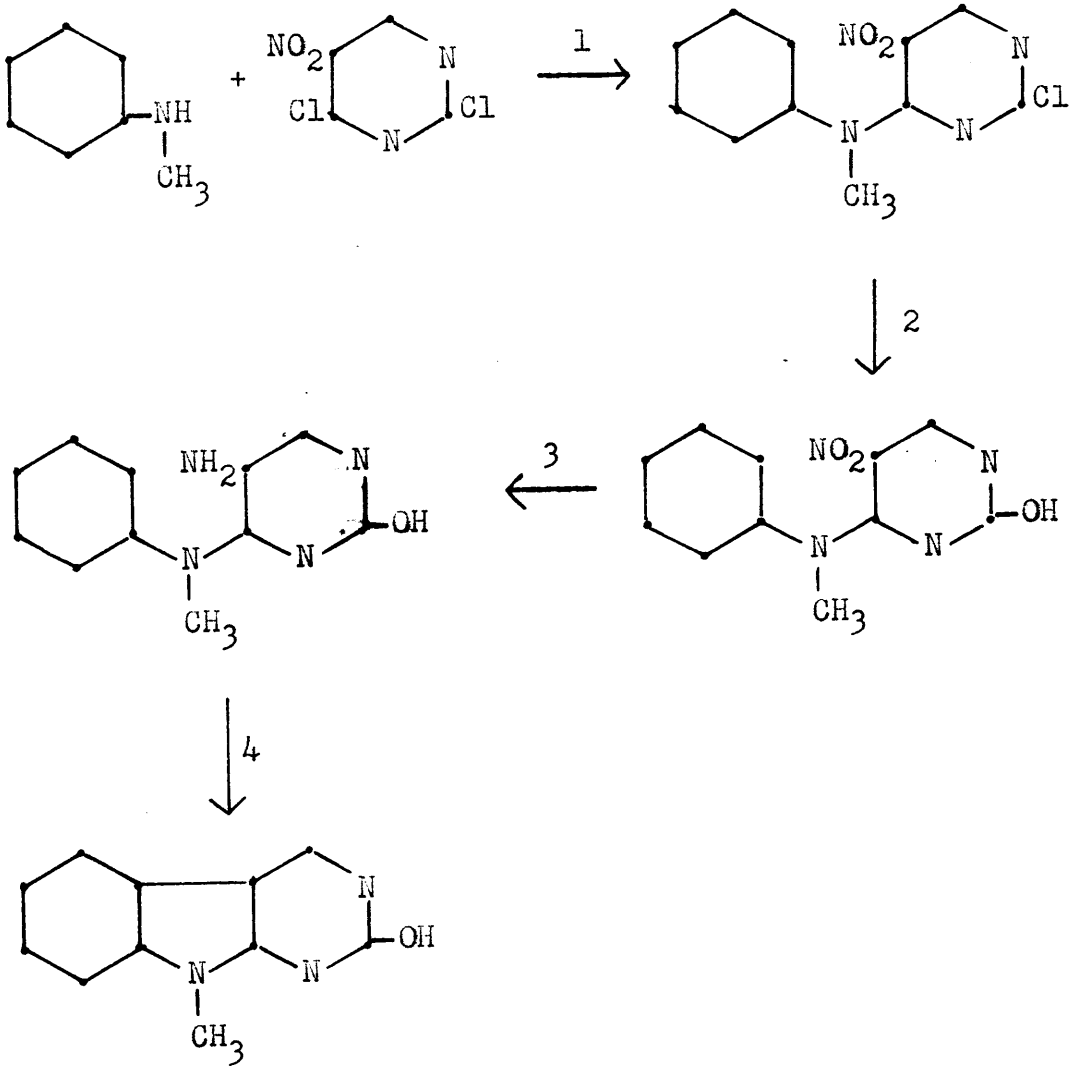


(plus other resonance forms)

It is possible that the use of copper bronze in acid solution may cause the required decomposition of the diazonium salt. At the time these experiments were made, the importance of pH in determining the type of reaction was not realised, and the synthesis in which the methyl group was absent was carried out in an attempt to obtain at least the desired diazacarbazole nucleus.

From the literature, it is clear that where internal coupling cannot occur the Pschorr synthesis may be achieved by alkaline decomposition of the diazonium salt; but where there is the possibility of coupling with a reactive hydrogen, the possibility of phenanthrene (or carbazole) formation under alkaline conditions is excluded.

(This became clear also in part I, where a similar observation was made. This stage of part I was carried out at a later date than the above experiments; part I and part II are not in strict chronological order.)


NO2
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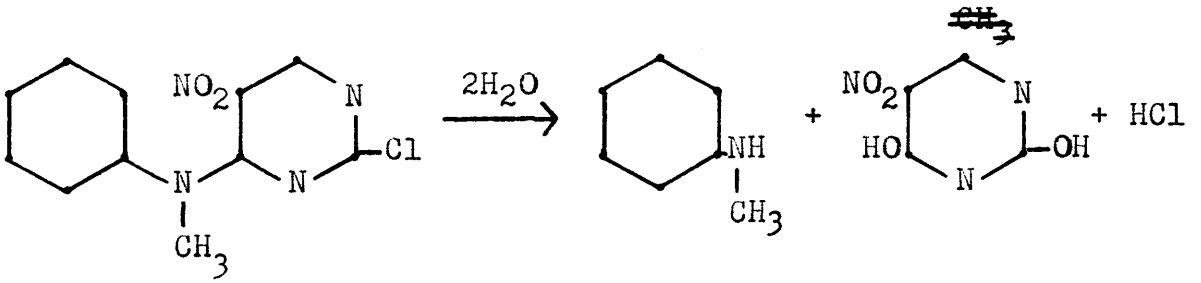
4
NH2
OH

The second synthesis, in which the reactive methyl group is absent, was carried out by the scheme which is shown on the opposite page.

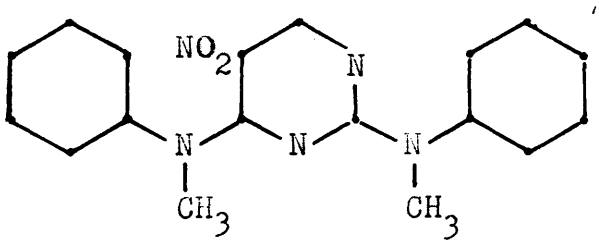
Some modifications were made of the methods (described in the literature) for the nitration of uracil and the preparation of dichloronitrouracil. (p. 140)

Stage 1. Application of the method worked out for the preparation of the 6-methyl analogue (p. 135) gave a 75% yield of the product.

Stage 2. An attempt to bring about hydrolysis of the chloro group with aqueous acetic acid gave unexpected results. The methylanilino group was also hydrolysed and the N-methylaniline liberated reacted with unchanged starting material thus:



(over)



+ HCl

The three products were all isolated and characterised (N-methylaniline as the acetyl derivative).

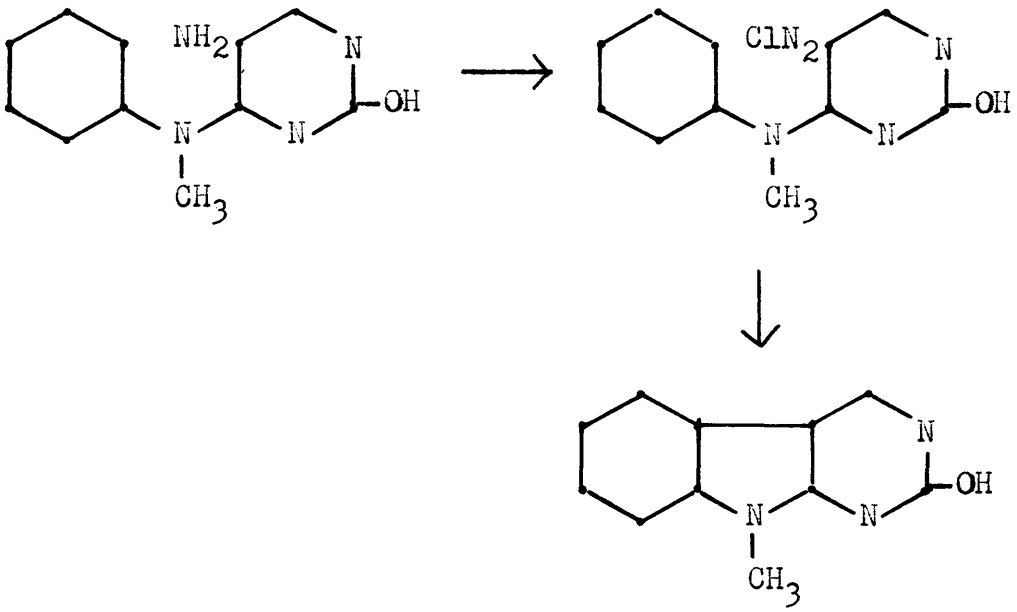
The identity of the di-(methylanilino)-5-nitropyrimidine was established by its formation in a direct condensation of the original chloro compound with N-methylaniline.

The high reactivity of the chloro group suggested that hydrolysis might be effected under very mild conditions. It was found that prolonged heating in aqueous acetone in presence of powdered calcium carbonate gave complete hydrolysis. The same effect could be obtained by a shorter treatment with sodium carbonate in aqueous acetone. However a purer product seemed to be formed by warming the chloro-compound with anhydrous sodium acetate in glacial acetic acid, and this was the procedure adopted.

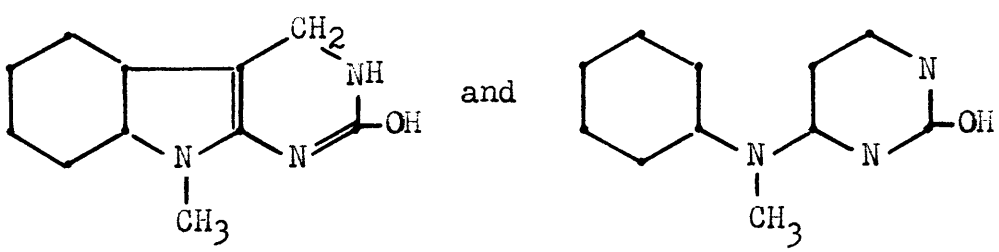
Stage 3. Hydrogenation of an alkaline solution of the crude 2-hydroxy-4-methylanilino-5-nitropyrimidine was complete in a few hours, and the amine was isolated in a 60-65% yield (over the two stages).

Stage 4.

Pschorr-type synthesis.



Decomposition of the diazotised amine with copper bronze was accompanied by the solution of copper, and an organic copper salt or complex separated. The analysis of the products obtained after the removal of copper (with hydrogen sulphide) suggested that partial reduction accompanied the decomposition of the diazonium salt. Two types of reduction product could be formed:

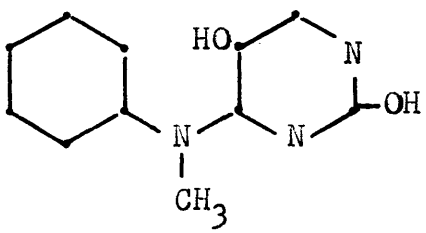


The fact that N-methylaniline was not isolated in an acid degradation of the product suggested that the product might be a dihydro-diazacarbazole, but this conclusion would require confirmation.

Other methods of decomposing the diazonium salt were considered, but time did not permit a full investigation of these reactions:

Decomposition in sodium carbonate solution

A low-melting colourless product was obtained by this method. It gave a yellow solution in sodium hydroxide, and possessed reducing properties. Its constitution was not established before the present work was suspended. One possible formulation for this product is:



Decomposition in glacial acetic acid

By cooling the acid solution of the diazotised amine in a freezing mixture it was possible to obtain the free diazonium salt (as a monohydrate), in the form of yellow crystals (in good yield).

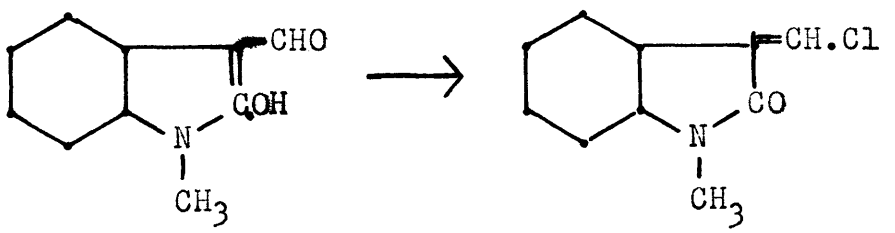
If this solid, after drying in a desiccator, was warmed in suspension in glacial acetic acid, an effervescence occurred at about 80°, and the solid dissolved. On working up, high-melting crystalline basic products were obtained; their identification was not completed.

OTHER APPROACHES

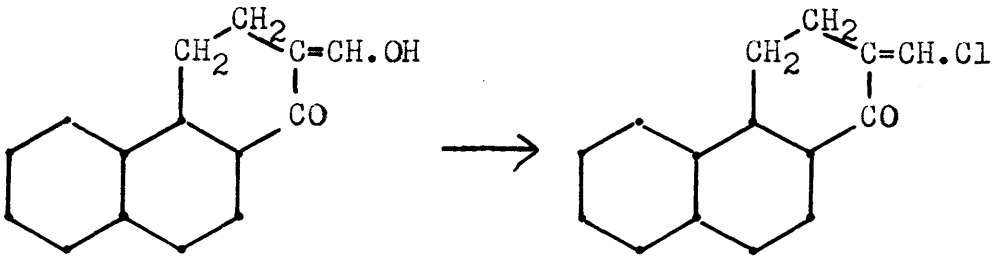
Two other substances were considered as possible starting points in these syntheses: namely, 1-methyl-3-chloromethyleneoxindole and 1-methyl-2-methoxy-3-formylindole. Some preliminary observations are described below:

Experimental (p. 150)

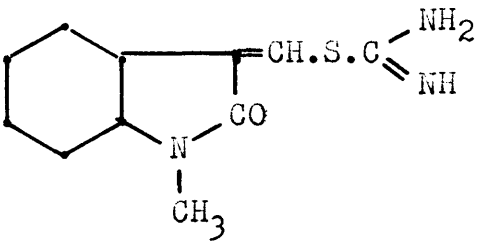
The preparation of 1-methyl-3-formyloxindole has been described in the literature; its conversion to 1-methyl-3-chloromethyleneoxindole was effected by treatment with thionyl chloride



(cf. W. H. S. Thompson, private communication:

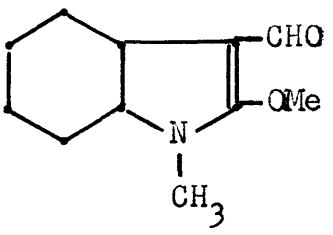


Thompson also described the conversion of the chloromethylene compound to a pyrimidine derivative by condensation with an amidine). The chloromethyleneoxindole was further characterised by a condensation with 2:4-dinitrophenylhydrazine. (It also gave a condensation product with thiourea, which may be



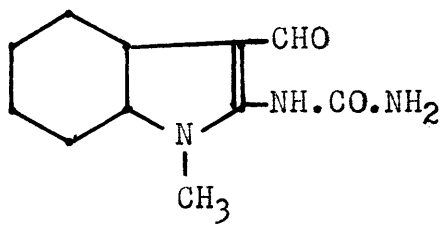
Condensations of thiourea with halogen compounds usually give isothiourea derivatives.)

1-Methyl-2-methoxy-3-formylindole.



This substance gave immediate precipitation of coloured

condensation products with both urea and guanidine in ethanol, in presence of sodium ethoxide. The guanidine condensation product was not identified, but an analysis of the orange substance indicated an elimination of methanol; the product might thus be



Continuation of this work should yield interesting results.

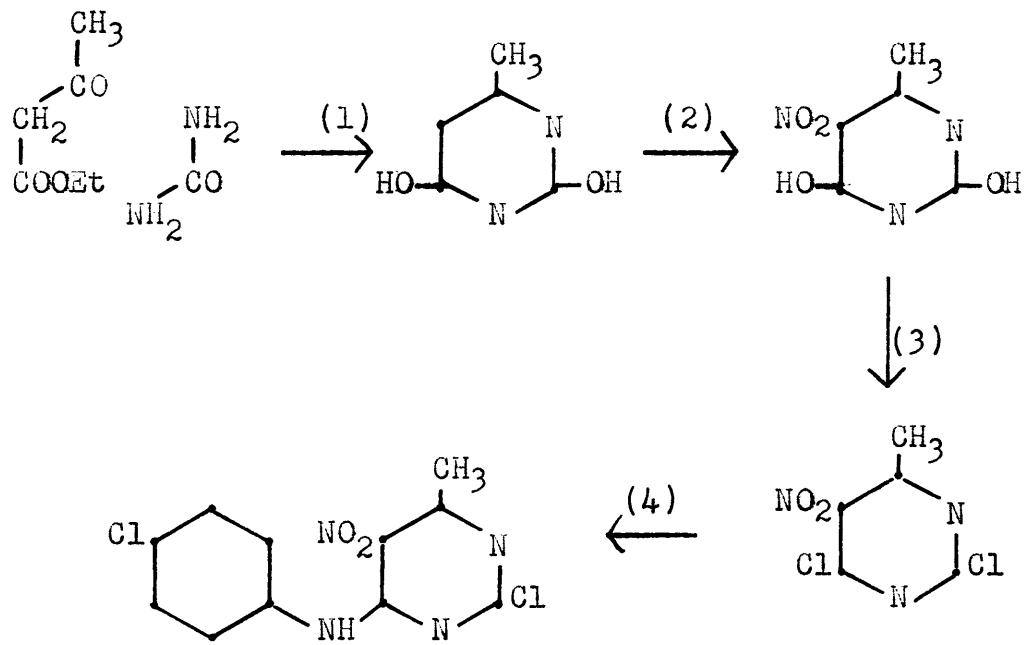
EXPERIMENTAL

Melting points are uncorrected. Microanalyses have been carried out on all new compounds.

APPROACH A

2-Chloro-4-(p-chloroanilino)-5-nitro-6-methylpyrimidine

This starting material was obtained thus:



References:

(1) 6-Methyluracil Behrend, Ann., 1885, 229, 9.

(2) 5-Nitro-6-methyluracil

Gabriel and Coleman, Ber., 1901, 34, 1234.

(3),(4) Curd and Rose (private communication).

Their procedures are described in full:

(3) 2:4-Dichloro-5-nitro-6-methylpyrimidine

"5-Nitro-6-methyluracil (34.2 g.) was refluxed with freshly distilled phosphorus oxychloride (171 cc.) and dimethylaniline (30 cc.) for 4 hours. The solution was cooled and poured on to crushed ice. After standing for 2 hours the solid product was filtered off, washed with water and dried in vacuo. Yield 34.15 g.

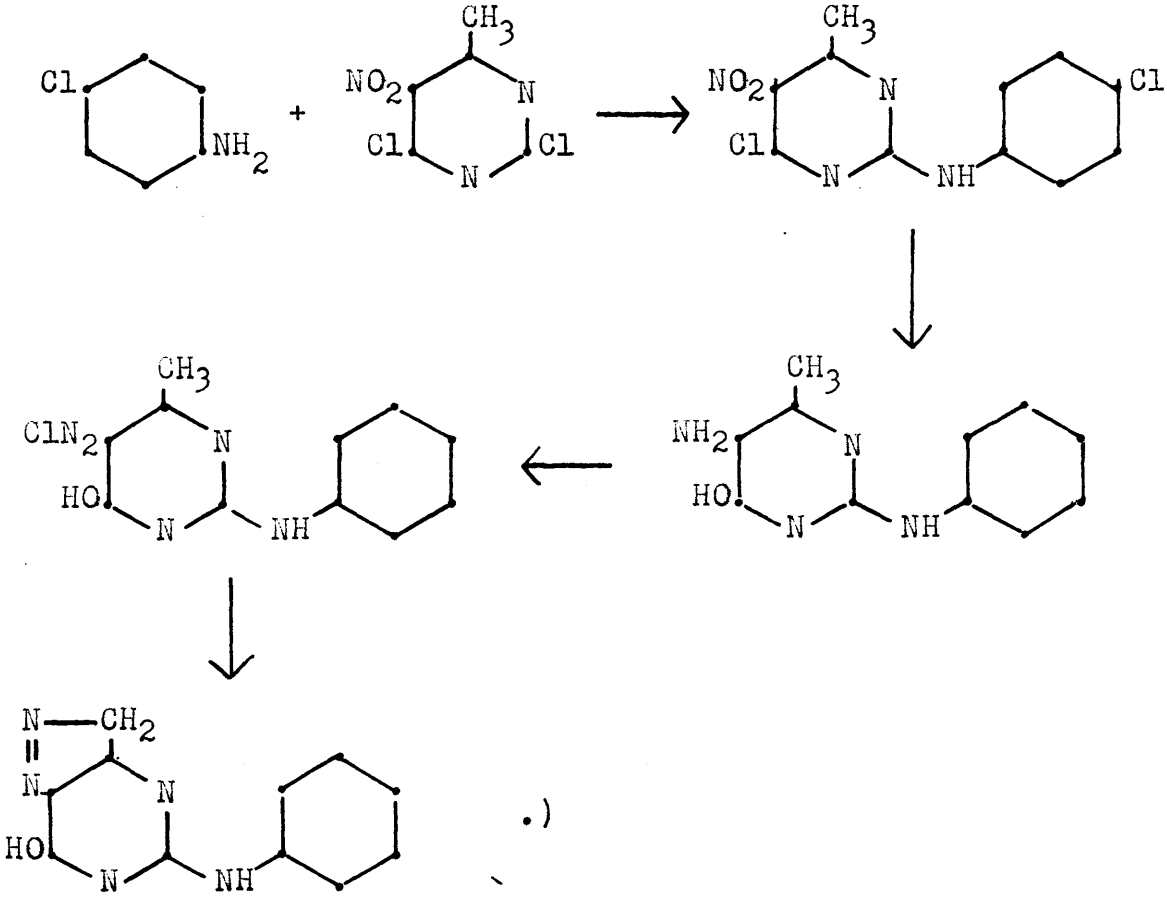
"The crude dichloro compound was extracted with hot petroleum ether (b. p. 60-80°), treated with decolourising charcoal and filtered. The solvent was then removed by distillation and the residue (32.4 g.) distilled at ordinary pressure, b. p. 240-242°. Yield 27.2 g.

(4) 2-Chloro-4-p-chloroanilino-5-nitro-6-methylpyrimidine

"2:4-Dichloro-5-nitro-6-methylpyrimidine (6.9 g.) was dissolved in absolute ethanol (40 cc.), and to this solution cooled in an ice-bath a solution of p-chloroaniline (4.25 g.) in ethanol (20 cc.) was added drop-wise with stirring. A precipitate of the product was formed. After diluting the mixture with ethanol (50 cc.) the mixture was stirred for 2 hours ice cold to complete the reaction. The precipitate was filtered off, washed with ethanol and dried at 60-65°. Yield 5.7 g. The product was purified by crystallisation from β-ethoxyethanol, m. p. 162-164°."

In this preparation Curd and Rose assume that it is the 4-chloro group in 2:4-dichloro-5-nitro-6-methylpyrimidine

which condenses with p-chloroaniline, on the grounds that a chloro group ortho to a nitro group is more reactive. That this assumption was correct is established by the smooth formation of the triazole. (The alternative possibility illustrated below is unlikely as internal coupling of this type would not occur in acid solution:



.)

2-Hydroxy-4-(p-chloroanilino)-5-nitro-6-methylpyrimidine.

The 2-chloro compound (10 g.) was refluxed for two hours in aqueous acetic acid (45 cc. glacial acid plus 5 cc. water), and the hot solution was diluted with a little water

(6 cc.). On standing the hydroxypyrimidine separated out (8.5 g.), m. p. 265° (with decomposition). The product was further purified by recrystallisation from 80% acetic acid, to give m. p. 265-267° (Found: C, 47.13; H, 3.44; N, 19.64. $C_{11}H_9O_3N_4Cl$ requires C, 46.95; H, 3.20; N, 19.92%).

The substance was sparingly soluble in acetone and dioxan, and dissolved in acetic acid only on heating.

2-Hydroxy-4-anilino-5-amino-6-methylpyrimidine.

Under the conditions described below a reduction both of the nitro group and of the benzene ring chlorine atom took place.

2-Hydroxy-4-(p-chloroanilino)-5-nitro-6-methylpyrimidine (1 g.), dissolved by warming in dilute potassium hydroxide solution (0.3 g. in 40 cc. water), was shaken with hydrogen in presence of 2% palladised strontium carbonate (4 g.). The reaction vessel was electrically warmed during the hydrogenation to diminish separation of the potassium salt of the starting material. Absorption was complete in about 2 hours. The catalyst was filtered from the hot solution and then further extracted with boiling water. The combined filtrates, on cooling, deposited colourless crystals. After recrystallisation from hot water (180 cc. per g.) the amine was obtained in needles, m. p. 294° (Found: C, 60.97; H, 5.45; N, 25.58.

$C_{11}H_{12}ON_4$ requires C, 61.10; H, 5.56; N, 25.92%).

3-Phenyl-5-hydroxy-7-methyl-3-v-triazolo-[d]-pyrimidine

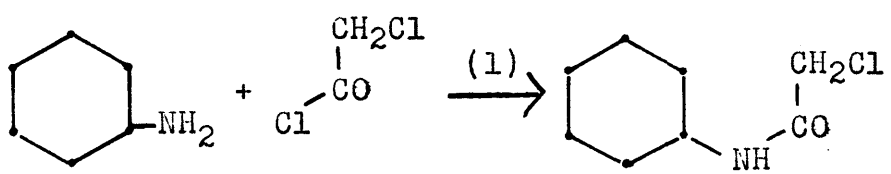
The above amine (1 g.), dissolved in dilute hydrochloric acid (30 cc.; 3N) was treated with sodium nitrite solution (0.33 g. in 10 cc. water; theoretical), all solutions being kept at 0°. A precipitate formed almost immediately. After standing for a short time, the pale yellow solid was filtered off, washed with water, and dried in air at 100°. This product was sparingly soluble in hot ethanol or dioxan but could be recrystallised from acetic acid as orange-yellow crystals (0.7 g.), m. p.: slow decomposition at 250-350° (Found: C, 58.16; H, 3.92; N, 30.55. $C_{11}H_9ON_5$ requires C, 58.16; H, 3.96; N, 30.84%).

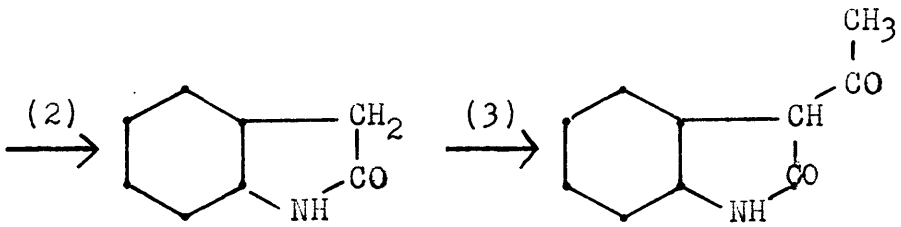
Decomposition of the triazole:

From various attempts to decompose the above triazole (by heating with phosphoric acid, liquid paraffin, or zinc chloride) only tarry products were obtained.

APPROACH B

3-Acetyloxindole





References:

Chloracetyl chloride

Barnett, Chem. News, 1921, 122, 220.

(1) Chloracetanilide

Holmberg, J. prakt. Chem., 1910, 82, 441.

It was found preferable to use more aniline than the amount recommended by Holmberg. Using 2 mols. of aniline per mol. of freshly distilled chloracetyl chloride, a 95% yield of chloracetanilide was obtained (compared with 80% using less aniline).

(2) Oxindole:

Stollé , J. prakt. Chem., 1930, 128, 1, as modified by McGuigan, unpublished. McGuigan's method is described below in full:

"Chloracetanilide (10 g.), mixed with pure anhydrous aluminium chloride (16 g.) was heated on the oil-bath. At 130° the mixture had become a brown liquid, and above this temperature hydrogen chloride began to be evolved. The temperature was raised to 223-225° for 1 hour, much hydrogen chloride being evolved at first.

"The melt was then cooled somewhat, poured on to ice, allowed to stand 1 hour, and the solid filtered off, washed, dried, and extracted with petroleum ether (b. p. 100-120°). The extract, on cooling, yielded crystals of oxindole which could be further purified if necessary by recrystallisation from petroleum ether or water. Yield 70-75%; m. p. 127°."

In the present work it was found that the first evolution of hydrogen chloride may be violent; caution should be exercised during the initial heating. A large volume of light petroleum is necessary to ensure complete extraction of the product.

(3) 3-Acetyloxindole:

(cf. Horner, Ann., 1941, 548, 117.)

To a solution of sodium (19.2 g.) in dry ethanol (600 cc.) were added oxindole (32 g.) and ethyl acetate (160 g.), and the mixture was heated under reflux for 6 hours. Dilution of the cooled mixture with water (1000 cc.) gave a clear solution. On acidifying with a slight excess of 6N hydrochloric acid a precipitate was formed. Later, the slightly pink solid was filtered off, washed with water, and dried in air at 45°. Weight 37 g.; m. p. 202-205°.

On recrystallisation from ethanol (550 cc.) the acetyloxindole (31.5 g.) was obtained as crystals, m. p. 205-206°.

ATTEMPTED CONDENSATIONS OF 3-ACETYLOXINDOLE

With S-methylisothiourea:

(1) Concentrated aqueous potassium hydroxide (cf. Wheeler and Merriam, Am. Chem. J., 1903, 29, 482).

S-methylisothiourea sulphate (0.16 g.) was dissolved in aqueous potassium hydroxide (0.15 g. in 1 cc. water), and 3-acetyloxindole (0.1 g.) was added. The resulting pasty suspension was left to stand in a stoppered flask for 1 week at room temperature. Water (8 cc.) was then added, and the solution carefully acidified with 6N hydrochloric acid. The solid matter which precipitated was filtered off, dried, and recrystallised from ethanol. M. p. 205-206°; mixed-m. p. with 3-acetyloxindole, 205-206°. Almost all the starting material was recovered unchanged.

Similar attempts using more concentrated potassium hydroxide solutions still gave back unchanged 3-acetyloxindole.

(2) Ethanolic potassium hydroxide

A mixture of 3-acetyloxindole (0.1 g.), pulverised S-methylisothiourea (0.16 g.) and potassium hydroxide (0.15 g.) in ethanol (6.5 cc.) was left to stand at room temperature for three weeks. On dilution with water (25 cc.) and acidification with excess concentrated hydrochloric acid (6 cc.) most of the 3-acetyloxindole precipitated

unchanged. After the removal of this solid by extraction with ether, the aqueous layer was brought to neutrality by adding sodium bicarbonate. No precipitation resulted. On repeated extraction of the solution with ether, and evaporation of the extract, no appreciable residue was left.

With Urea:

(1) Methanolic hydrogen chloride

(cf. Evans, J. prakt. Chem., 1893, 48, 492.)

3-Acetyloxindole (0.1 g.) was dissolved in warm methanol (5 cc.) and urea (0.8 g.) was added. Dry hydrogen chloride was passed into the solution for some time, and the vessel was stoppered and left at room temperature for 1 week. The contents were then diluted with water (14 cc.), and after standing for a few hours, the precipitate was filtered off, and the filtrate washed by extraction with ether. Neutralisation of this filtrate with sodium bicarbonate gave no precipitate and no product could be obtained from the neutral solution by repeated ether extraction. The solid initially filtered off represented almost all the unchanged starting material.

(2) Methanolic sulphuric acid

(cf. Hale and Vibrans, J. Am. Chem. Soc., 1918, 40, 1062.)

A similar experiment to (1), in which the hydrogen chloride was replaced by 5 drops of concentrated sulphuric acid, gave identical results.

With guanidine

(1) Sodium ethoxide in ethanol

(cf. Traube, Ber., 1900, 33, 1375.)

3-Acetyloxindole (0.2 g.), sodium (0.16 g.) in ethanol (6 cc.) and powdered guanidine acetate (0.1 g.) were well mixed and heated under reflux for 12 hours. The mixture was diluted with water (17 cc.) and the solution was worked up as in the preceding experiments. Again only unchanged starting material was isolated.

(2) Concentrated aqueous potassium hydroxide

3-Acetyloxindole (0.2 g.) and guanidine acetate (0.1 g.) were mixed with a solution of potassium hydroxide (0.32 g.) in water (0.6 cc.) and the mixture was heated at 45° for 24 hours. During this time most of the solid matter went into solution and the colour became deep yellow. An odour of ammonia was observed. On dilution with water (10 cc.), and working up, only unchanged 3-acetyloxindole and a quantity of free oxindole were obtained. Both were characterised by mixed melting points.

(3) Fusion at high temperature

Guanidine carbonate (0.25 g.), acetic acid (0.17 cc.) and 3-acetyloxindole (0.5 g.) were heated together at an oil-bath temperature of 170-180° for 2 hours. The resulting dark red tar was dissolved by warming with glacial acetic acid (4 cc.), and the cooled acetic acid solution was poured into hydrochloric acid (3N; 35 cc.). A reddish

precipitate was formed. This was dissolved by shaking with ether, and the ether layer was dried and evaporated. Several recrystallisations of the residue from ethanol gave a small quantity of a colourless product, m. p. 279-281° (Found: C, 60.88; H, 5.02; N, 19.45. $C_{11}H_{11}O_2N_3$ requires C, 60.82; H, 5.07; N, 19.33, corresponding to the elimination of H_2O and NH_3).

This substance was not easily soluble in dilute hydrochloric acid or sodium hydroxide solution. It was obtained in extremely low and variable yield. Repeated experiments in which the time of heating and the temperature were varied over a wide range gave no improvement. When milder conditions were used only unchanged 3-acetyloxindole could be isolated. More vigorous treatment gave tars from which no crystalline products were obtained.

Similar fusions in which anhydrous sodium acetate was included in the mixture gave no better results. Heating with guanidine carbonate alone and with phosphoric oxide was also tried without success.

N-Methylchloracetanilide:

(cf. preparation of chloracetanilide, J. prakt. Chem., 1910, 82, 441.)

Methylaniline (70 g.) in benzene (200 cc.), well stirred and cooled in ice, was treated with chloracetyl

chloride (37 g.) which was added slowly (during 1 hour). The next day the mixture, which had been left at room temperature, was filtered, and the filtrate was reduced in volume by distillation to 60 cc. On cooling and standing, a mass of crystals separated. This product was filtered off and washed with a little benzene. Recrystallisation from a small amount of ethanol gave a pure product (44 g.), m. p. 70-71°.

1-Methyloxindole:

(Stollé, Chem. Centr., 1921, II, 1065.)

Stollé does not give a description of this preparation (cf. preparation of oxindole, p. 116).

A mixture of N-methylchloracetanilide (5 g.) and crushed aluminium chloride (8 g.) was heated at an oil-bath temperature of 180-190° for 1 hour.

Initially, a vigorous evolution of hydrogen chloride took place. After cooling to about 100° the mixture was poured on to crushed ice (100 g.). The solid product was filtered, dried, and extracted with light petroleum (b. p. 60-80°). The product separated on cooling the extract (2.6 g.; yield 80% of theoretical; m. p. 89-90°).

1-Methyl-3-acetyloxindole:

(cf. Julian, Pikl and Wantz, J. Am. Chem. Soc., 1935, 57, 2026.)

Experimental details are not given in this paper.

1-Methyloxindole (15 g.) in lime-dried ethanol (60 cc.) was treated with sodium (4.8 g.) in ethanol (90 cc.), and ethyl acetate (19.6 cc.) was added. After heating for 2 hours under reflux the products were diluted with water (600 cc.) and the aqueous solution acidified with 6N hydrochloric acid. Later, the precipitate was filtered off, dried in air, and recrystallised from ethanol (40 cc.) to give crystals of 1-methyl-3-acetyloxindole (17.4 g.; yield 90% of theoretical; m. p. 110-111.5°).

CONDENSATIONS OF 1-METHYL-3-ACETYLOXINDOLE

With guanidine carbonate

N-Methyl-3-acetyloxindole (10.45 g.) and guanidine carbonate (10.45 g.) were heated at an oil-bath temperature of 170°, in presence of a small quantity of mesitylene (8 cc.) for 75 minutes. The reaction mixture was cooled and then triturated with dilute hydrochloric acid; the filtered acid solution was extracted with a little ether, and almost neutralised with solid sodium bicarbonate. Precipitation of the product was finally brought about by sodium carbonate solution. The base obtained (4 to 6 g.) had m. p. 240-250°.

No solvent found seemed suitable for the recrystallisation of this substance. An analysis specimen was

prepared by recrystallisation from a large amount of ether (0.15 g. from 1000 cc.). A silky white microcrystalline product was obtained, m. p. 252-253° (Found: C, 63.60; H, 6.39; N, 21.07. 1-Methyl-2-ureido(isoureido?)-indole, $C_{10}H_{11}ON_3$, requires C, 63.49; H, 5.82; N, 22.22%).

As this analysis was not conclusive the picrate was prepared and analysed (in duplicate); the analyses obtained were in excellent agreement with the suggested formulation.

The base (3.35 g.), in ethanol (130 cc.), was heated for a short time with picric acid (3.35 g.), also in ethanol (200 cc.). On cooling and standing, the picrate separated (5.5 g.), m. p. 201-202° (with decomposition). A second crystallisation gave m. p. 203-204° (Found: C, 46.03, 45.90; H, 3.26, 3.36; N, 20.49, 21.45. $C_{10}H_{11}ON_3 . C_6H_3O_7N_3$ requires C, 45.93; H, 3.35; N, 20.09%).

This picrate was decomposed with acid in the hope of obtaining a purified sample of the free base; the melting point of the recovered product did not however indicate a high degree of purity.

In an experiment in which 1-methyl-3-acetyloxindole was heated with guanidine carbonate at a lower temperature (160°; 20 minutes), both unchanged starting material and some free 1-methyloxindole were isolated from the reaction mixture, in addition to the above basic product.

This suggested that deacetylation precedes a condensation

of 1-methyloxindole with guanidine carbonate. The last-named substances were therefore heated together in the hope of obtaining direct condensation:

Condensation of 1-methyloxindole and guanidine carbonate:

The two substances (20 g. of each) were ground up together, and the intimate mixture was heated at an oil-bath temperature of 200° for 90 minutes. During this time the mixture fused, water and ammonia were evolved, and finally resolidification occurred. The solid products were ground up and thoroughly triturated with dilute hydrochloric acid (3N; 200 cc.). The filtered extract was washed by extraction with a little ether, diluted with water (100 cc.) and almost neutralised with solid sodium bicarbonate. Final precipitation of the product was effected by addition of sodium bicarbonate solution. The white basic solid had m. p. 240-250°.

The melting point of a sample of the picrate of this substance was not depressed on mixing with picrate of the base obtained (above) on heating 1-methyl-3-acetyloxindole with guanidine carbonate.

The base (1-methyl-2-ureido(isoureido?)-indole) forms a monoacetyl derivative:

5 g. was heated with acetic anhydride (15 cc.) at 100° for 10 minutes. After cooling and standing the yellow crystals which had separated out were filtered off, washed

with a little ether, and recrystallised from benzene (or ethanol), m. p. 221-222° (Found: C, 62.47; H, 5.51; N, 18.29. $C_{12}H_{13}O_2N_3$ requires C, 62.34; H, 5.63; N, 18.18%).

More vigorous acetylation (refluxing with acetic anhydride plus a trace of sulphuric acid) gave a triacetyl derivative which recrystallised from ethanol, m. p. 167-168° (Found: C, 60.89; H, 5.22; N, 13.14. $C_{16}H_{17}O_4N_3$ requires C, 60.95; H, 5.43; N, 13.33%).

On hydrolysis of the recrystallised monoacetyl derivative the base was obtained in a purer state, thus:

The acetyl derivative (1 g.) was heated at 100° with dilute sodium hydroxide solution (30 cc.) until the yellow crystals were replaced by a bulky precipitate (10-15 minutes). This precipitate was filtered from the cooled mixture, washed with water, redissolved in dilute hydrochloric acid, and reprecipitated with sodium bicarbonate. The well-washed product, after drying, had m. p. 252-253°.

In the condensation of 1-methyl-3-acetyloxindole a small quantity of another product, m. p. 266-267°, was formed. It was sparingly soluble in dilute hydrochloric acid or dilute sodium hydroxide solution, or in hot ethanol; it could be recrystallised from glacial acetic acid, and separated very slowly from solutions in this solvent. (Found: C, 72.38, 72.86; H, 5.12, 5.09; N, 9.79, 9.96. $C_{17}H_{14}O_2N_2$ requires C, 73.38; H, 5.04; N, 10.07%. No

probable product seemed to have this constitution.) The substance was not further investigated.

Attempted condensations of 1-methyl-3-acetyloxindole under other conditions:

(1) Guanidine acetate plus acetic acid:

1-Methyl-3-acetyloxindole (1 g.), guanidine (0.48 g.) and acetic acid (0.61 cc.) were heated at an oil-bath temperature of 170° for 40 minutes. After partial cooling the tarry mixture was dissolved in a further 2 cc. of hot glacial acetic acid and the resulting solution was cooled and poured into ice-cold 3N hydrochloric acid (17 cc.). From the material which could be extracted in ether from this acid mixture, a quantity of 1-methyloxindole was obtained.

No basic products precipitated or could be extracted on neutralisation of the acid mother liquor.

(2) Sodium ethoxide in ethanol

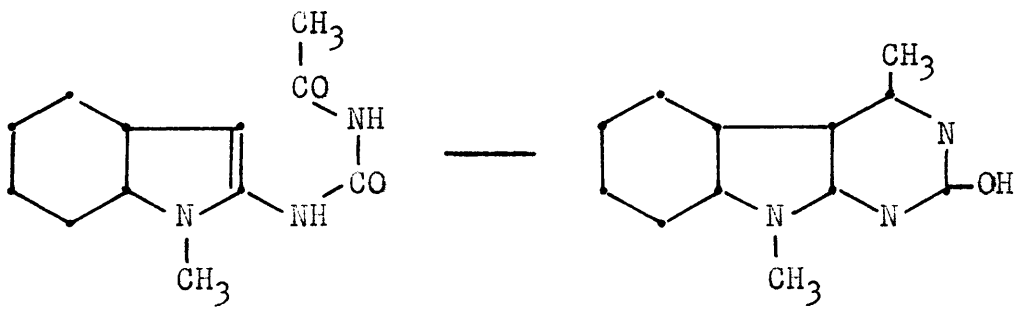
In presence of these reagents no interaction occurred between 1-methyl-3-acetyloxindole and guanidine:

Guanidine acetate (0.5 g.) in ethanol (2 cc.) was treated with the theoretical quantity of sodium ethoxide in ethanol (3 cc.). 1-Methyl-3-acetyloxindole (1 g.) was then added, and the mixture was heated at reflux (water-bath) for 5 hours. On working up as in previous experiments (above), almost all the 1-methyl-3-acetyloxindole was

recovered unchanged.

ATTEMPTED DEHYDRATION OF MONOACETYLUREIDO(isoUREIDO?)-METHYLINDOLE

As this substance possibly possessed the formula shown, the effect of dehydrating agents was investigated, in the hope of bringing about an intra-molecular ring-closure to a diazacarbazole:



Action of phosphoric oxide:

(cf. Späth and Lederer, Ber. 1930, 63, 2102.)

On heating the above acetyl derivative (m. p. 221-222°) for long periods (48 hours) with excess phosphoric oxide at the b. p. of benzene or xylene, only the unchanged starting material was recovered.

Action of sulphuric acid:

Treatment with cold concentrated sulphuric acid caused deacetylation and monosulphonation, the final product having the properties of an "inner salt."

The acetylated base (0.5 g.) was treated with concentrated sulphuric acid (3.5 cc.), a slight rise in temperature being observed during the mixing. The solution obtained was allowed to stand at room temperature for several days, and was then poured on to ice. The aqueous solution was neutralised with sodium bicarbonate. The resulting precipitate gave colourless crystals from water, m. p. above 320° (Found: C, 44.61, 44.59; H, 4.24, 4.36; N, 15.40. $C_{10}H_{11}O_4N_3S$ requires C, 44.61; H, 4.09; N, 15.61%).

The product was soluble in hot water (50 cc. per g.), cold dilute sodium hydroxide, and insoluble in cold dilute or concentrated hydrochloric or sulphuric acids, hot ethanol, benzene, or acetic acid. A sodium fusion test showed sulphur to be present; the aqueous solution gave no precipitate with barium chloride.

The sodium salt appeared to be sparingly soluble in cold water.

A substance with identical properties was obtained by similar treatment of the unacetylated base (i.e. of 1-methyl-2-ureido(isoureido?)indole).

Action of sodium ethoxide in ethanol

Refluxing for a short time caused quantitative deacetylation of the acetyl compound.

p-Chloro-chloracetanilide:

(cf. Holmberg, J. prakt. Chem., 1910, 82, 441; for chloracetanilide.)

A solution of p-chloroaniline (135 g.) in benzene (800 cc.) was cooled in a freezing mixture, and chloracetyl chloride (57 g.) was slowly added (during 45 minutes), with efficient stirring. Cooling and stirring were continued for several hours, and then the solid matter was filtered off, triturated with water and dilute hydrochloric acid (1N), dried in air, and recrystallised from ethanol (600 cc.). The product had m. p. 170-171°, and the yield was 80% of theory.

5-Chloroxindole:

McGuigan (unpublished) found that the application of Stollé's method (for oxindole) did not give satisfactory yields of the 5-chloro derivative. In his investigation, McGuigan chromatographed the products of the aluminium chloride fusion and obtained a low yield of 5-chloroxindole along with other products.

In the present work, p-chloro-chloracetanilide (5 g.) was heated with pure anhydrous aluminium chloride (10 g.) at 230-240° for 1 hour, cooled to 100°, and poured on to ice. Next day the solid was filtered off and dried (3.8 g.). Fractional crystallisation from benzene gave 5-chloroxindole (10% yield), m. p. 195-196° (Found: C, 57.13; H, 3.84;

N, 8.30. 5-Chloroxindole, C_8H_6ONCl requires C, 57.32; H, 3.58; N, 8.36%).

From the mother liquors of this fractional crystallisation a second substance was isolated, m. p. 194-195°, mixed-m. p. with 5-chloroxindole obtained otherwise 150-160° (Found: C, 56.66; H, 3.62. C_8H_6ONCl requires C, 57.32; H, 3.58%). Inter-molecular condensation could give a product with this empirical formula.

Chlorination of oxindole:

(cf. Reed, J. Chem. Soc., 1907, 1553, chlorination of acetanilide.)

Oxindole (3 g.) and anhydrous sodium acetate (4 g.) were dissolved in glacial acetic acid (40 cc.), and a crystal of iodine was added. Pure dry chlorine was passed into the stirred solution until the weight had increased by 0.8 g. After stirring for a further 30 minutes the mixture was poured on to ice; the solid matter was filtered off and dried. It appeared to consist of a mixture of products. Fractional crystallisation from ethanol gave a 30% yield of 5-chloroxindole, m. p. 195-196°.

3-Acetyl-5-chloroxindole

The method applied was that used in the similar preparation of 3-acetyloxindole from oxindole (above).

5-Chloroxindole (0.5 g.) was treated with ethyl acetate

(1.58 g.) in dry ethanol (14 cc.) in presence of sodium ethoxide (0.275 g. sodium). Refluxing was continued for 5 hours, and then the cooled mixture was diluted with water (50 cc.), and acidified with dilute hydrochloric acid. The resulting precipitate was filtered off, dried, and recrystallised from ethanol (19 cc.). Almost colourless crystals were obtained, m. p. 245-248° (Found: C, 57.09; H, 4.33. $C_{10}H_8ONCl$ requires C, 57.28; H, 3.82%).

APPROACH C

o-Nitrophenylacetic acid (ethyl ester):

Dippy and Page (J. Soc. Chem. Ind., 1936, 55, 190T) claim that the most convenient preparation of this substance is by direct nitration of phenylacetic ester. Their method, which involves the fractional crystallisation of a mixture of nitration products, was not found satisfactory. The procedure adopted was that of Reissert (Ber., 1897, 30, 1036), as modified by Pschorr and Hoppe (Ber., 1910, 43, 2547). The esterification of the free acid is described by Reissert and Scherk (Ber., 1898, 31, 395).

Attempted preparation of o-nitrophenylacetoacetic ester

In a number of attempts to condense ethyl acetate or acetyl chloride with o-nitrophenylacetic ester, in

presence of sodium ethoxide, only unchanged starting material or tarry products were obtained. In experiments where the sodium salt of o-nitrophenylacetic ester was treated with acetyl chloride, the purple colour of the former vanished immediately. Benzoylation of the tarry products did not yield any crystalline material (cf. benzoylation of 2:4-dinitrophenylacetoacetic ester, Borsche, Ber., 1909, 42, 601).

3:4-Dinitrochlorobenzene:

(Manguino and Deliddo, Gazz. Chim. Ital., 1933, 63, 612).

In this preparation a purer product is obtained if refluxing of the nitration mixture is stopped after 40 minutes, instead of the 90 minutes prescribed.

2-Nitro-5-chlorophenylacetoacetic ester (?):

Sodio-acetoacetic ester (from 2.49 cc. acetoacetic ester), in dry ether, was treated with 3:4-dinitrochlorobenzene (2 g.). The mixture, which became dark brown in colour, was left to stand overnight, and next day it was shaken with a mixture of ice, dilute (1N) hydrochloric acid, and urea (to destroy any nitrous acid formed). The ether layer was separated off, extracted with water, dried and evaporated, when a thick red tar remained, from which crystalline products could not be obtained. Again, no benzoyl derivatives could be isolated.

However, if the tar first produced was heated with dilute sodium hydroxide solution (to give alkaline hydrolysis of acetoacetic ester derivatives), then, on working up for acid products, a crystalline substance was obtained, m. p. 174° (from ethanol). (Found: C, 44.35; H, 3.22; N, 6.81. 2-Nitro-5-chlorophenylacetic acid, $C_8H_6O_4NCl$, requires C, 44.55; H, 2.81; N, 6.50%.)

This suggested that some of the desired 2-nitro-5-chlorophenylacetoacetic ester is formed in the above attempted condensations.

A few unsuccessful attempts were made to react the crude tar with amidine-type compounds in the hope of isolating some crystalline condensation products which might be of use in the main synthesis (in addition to giving a characterisation of the substituted acetoacetic ester).

2:4-Dinitrophenylacetoacetic ester:

(Borsche, Ber., 1909, 42, 601.)

2:4-Dinitrophenylmalonic ester:

(Borsche, Ber., 1888, 21, 2473.)

Condensations of 2:4-dinitrophenylacetoacetic ester and 2:4-dinitrophenylmalonic ester:

(1) (cf. Jaeger, Ann., 1891, 262, 365; Majima, Ber., 1908, 41, 176.) Heating with guanidine carbonate alone or in

ethanol gave high-melting insoluble products from which no crystalline material could be isolated. It is possible that complex poly-condensation took place.

(2) On refluxing with guanidine in ethanol in presence of sodium ethoxide, no reaction occurred.

(3) Similar treatment of a mixture with thiourea again gave no condensation.

APPROACH D

2-Chloro-4-methylanilino-5-nitro-6-methylpyrimidine:

(cf. preparation p-chloroanilino analogue, Curd and Rose; see discussion p. 101 .)

N-Methylaniline (28.4 cc.) in absolute ethanol (55 cc.) was added slowly (during 4 hours) to an ice-cold solution of 2:4-dichloro-5-nitro-6-methylpyrimidine (26.8 g.) in ethanol (160 cc.). The mixture was well stirred and the temperature was maintained at 0° during the addition by means of a freezing mixture. After stirring for a further hour, with continuous cooling (at -5° C.), the yellow crystalline precipitate was filtered off, washed with a little ethanol, and dried in air, m. p. 112-114°, yield 85% of theoretical. The substance was already almost pure; recrystallisation from light petroleum (b. p. 80-100°) (3 cc. per g.) gave m. p. 113-114° (Found: C, 52.06; H, 3.78; N, 20.03. $C_{12}H_{11}O_2N_4Cl$ requires C, 51.71; H, 3.95;

N, 20.11%).

The compound is sensitive to light, turning from yellow to orange on exposed surfaces; it should be stored in a well-stoppered bottle.

2-Hydroxy-4-methylanilino-5-nitro-6-methylpyrimidine:

The above chloro compound (6 g.) was dissolved in a mixture of glacial acetic acid (5.4 cc.) and water (0.6 cc.), and heated at 100° for 2 hours. The dark yellow liquid was diluted at boiling point with boiling water (12.5 cc.). Crystalline hydroxy compound separated from this supersaturated solution, and after standing at 0° overnight, the product was filtered off and dried (weight 4.2 g., yield 75% of theoretical; m. p. 253-254°, with decomposition). (Found: C, 55.24; H, 4.45; N, 21.54. $C_{12}H_{12}O_3N_4$ requires C, 55.39; H, 4.62; N, 21.54%.)

The compound was sparingly soluble in hot water, and dissolved slowly in sodium carbonate or sodium hydroxide to give yellow solutions. It also dissolved slowly in dilute hydrochloric acid, and appeared to be fairly soluble in acetic acid. Like the chloro compound from which it was derived, the substance was light-sensitive, rapidly turning from pale yellow to orange on exposure to strong light (not necessarily direct sunlight). It should therefore also be stored in the dark.

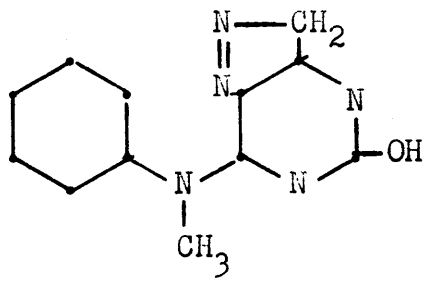
2-Hydroxy-4-methylanilino-5-amino-6-methylpyrimidine:

2-Hydroxy-4-methylanilino-5-nitro-6-methylpyrimidine (16.15 g.) was dissolved in acetic acid (160 cc.), and shaken with hydrogen in presence of 2% palladised strontium carbonate (16 g.). The theoretical quantity was absorbed in about 5 hours. The catalyst was filtered off, and washed through with more (hot) glacial acetic acid. The combined filtrates were reduced by distillation in vacuo to small bulk, and the residue dissolved directly by pouring into boiling water (500 cc.). On standing, a silvery crystalline solid separated, m. p. 251-253°. A further recrystallisation from water (36 cc. per g.) gave m. p. 254-255° (Found: C, 58.31; H, 6.21; N, 22.71. $C_{12}H_{14}ON_4.H_2O$ requires C, 58.06; H, 6.60; N, 22.58%).

The amine was sparingly soluble in cold water, cold ethanol, warm benzene; it was soluble in hot water, cold dilute sodium hydroxide (giving a yellow solution), and cold dilute hydrochloric acid. It was further characterised as the picrate, thus:

The base (0.33 g.) in ethanol (7 cc.) was mixed with picric acid (0.33 g.), also in ethanol (2 cc.). On cooling and standing a yellow solid crystallised out (0.55 g.). Recrystallisation from ethanol (20 cc.) gave the pure picrate, m. p. 177-178° (Found: C, 47.31; H, 3.65; N, 21.61. $C_{12}H_{14}ON_4.C_6H_3O_7N_3$ requires C, 47.07; H, 3.70; N, 21.35%).

5-Hydroxy-7-methylanilino-3-[4,3-d]diazolopyrimidine



A solution of 2-hydroxy-4-methylanilino-5-amino-6-methylpyrimidine (1 g.) in 1.5N hydrochloric acid (13 cc.) was cooled to 0°, and aqueous sodium nitrite (0.33 g. in 5 cc. water), also at 0°, was added; the acid solution immediately lost its slightly greenish colour. The now yellow liquid was added slowly to 3N sodium carbonate solution (60 cc.), which was maintained at 40°. When addition was complete, the temperature was slowly raised, and heating was continued at 80° for 15 minutes. After cooling and standing for some time the buff-coloured solid was filtered off and dried in air. The product was purified by crystallisation from glacial acetic acid, from which it tended to separate only very slowly. If decolourising charcoal was used, a colourless product could be obtained. The pure substance, on heating to high temperatures, did not melt; above 340° it decomposed slowly. (Found: C, 59.96; H, 4.88; N, 29.00. $C_{12}H_{11}ON_5$ requires C, 59.75; H, 4.56; N, 29.05%.)

The compound was soluble in dilute hydrochloric acid,

dilute sodium hydroxide solution, boiling acetic acid; and was sparingly soluble or insoluble in hot water or sodium carbonate solution, boiling ethanol, chloroform, pyridine, nitrobenzene or tetralin.

It was further characterised as the picrate, and as a monoacetyl derivative.

Picrate

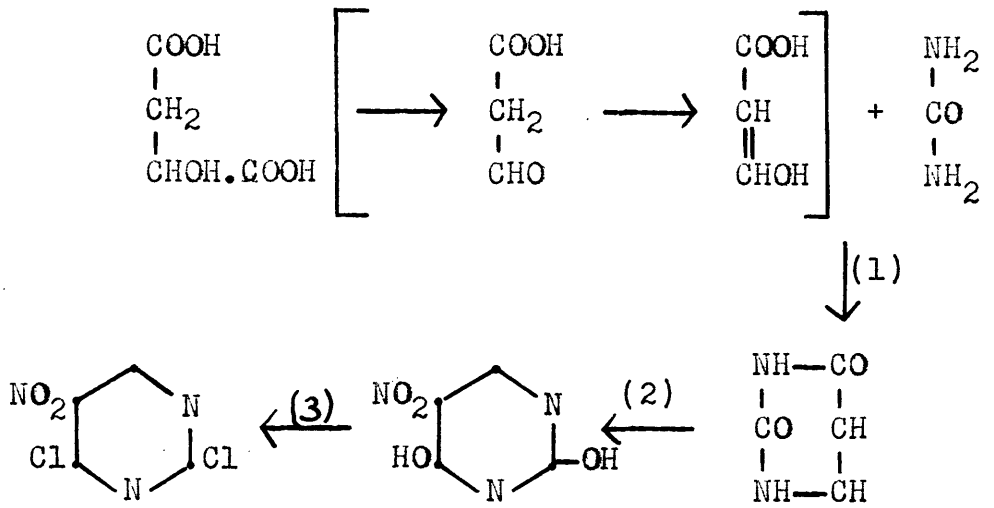
0.15 g. in glacial acetic acid (5 cc.) was treated with picric acid (0.15 g.) in the same solvent (1 cc.). After boiling with decolourising charcoal, the filtered yellow solution was cooled, and the yellow cushions of micro-needles which separated were recrystallised from more acetic acid for analysis, m. p. 237° (Found: C, 46.46; H, 3.27. $C_{12}H_{11}ON_5.C_6H_3O_7N_3$ requires C, 45.96; H, 2.98%).

Acetyl derivative

0.5 g. was refluxed with acetic anhydride (4 cc.) containing a trace (0.04 cc.) of concentrated sulphuric acid, for 1 hour. Most of the solid passed into solution. On hydrolysis of the excess acetic anhydride by heating with water (15 cc.), and cooling, a solid product was formed. This material was filtered off, triturated with dilute sodium hydroxide solution and with water, and finally recrystallised from ethanol, m. p. 235-240° (with decomposition) (Found: C, 59.22; H, 4.46; N, 24.78.

$C_{14}H_{13}O_2N_5$ requires C, 59.36; H, 4.59; N, 24.74%).

Preparation of 2:4-dichloro-5-nitropyrimidine



(1) Uracil

(Davidson and Baudisch, J. Am. Chem. Soc.,1926, 48, 2382.)

(2) 5-Nitrouracil

(Johnson and Matsuo, J. Am. Chem. Soc., 1919, 41, 783.)

In this preparation, unless the quality of the nitric acid is assured, it is preferable to ensure complete nitration by heating the nitric acid solution at very gentle reflux (on a gauze over a small flame) for 1 hour. Evaporation of the acid, as described, then leaves a residue of 5-nitrouracil which is washed with water; yield 95%.

The product can be recrystallised from water (20 cc. per g.).

The method of Johnson and Matsuo was found to be preferable to that of Wheeler and Bristol (J. Am. Chem. Soc., 1911, 33, 441) or of Blitz and Heyn (Ann., 1917, 413, 110).

(3) 2:4-Dichloro-5-nitropyrimidine

The methods described in the literature for this preparation are troublesome, involving heating under pressure in a sealed tube (Gabriel and Colman, Ber., 1901, 34, 1246; Isay, Ber., 1906, 39, 252). A satisfactory procedure was developed using phosphorus oxychloride in presence of dimethylaniline (cf. Curd and Rose, see p. 111):

Recrystallised 5-nitrouracil (47 g.) was refluxed with freshly distilled phosphorus oxychloride (235 cc.) and dry dimethylaniline (50 cc.), which was also freshly distilled. After 5 hours refluxing, most of the phosphorus oxychloride was removed by distillation in vacuo, and the residue was poured on to clean crushed ice. The tar which separated and the supernatant liquid were thoroughly extracted with ether; the ether extract was washed by shaking with very dilute ammonia solution and with water, and reduced to small bulk. Vacuum distillation of the residue gave dichloronitropyrimidine, b. p. 135-145°/14 mm. in 60-65% yield. The yellow oil could be solidified to a solid, m. p. 28-30°.

Care should be exercised during the final distillation as the residual fractions are liable to undergo spontaneous

decomposition.

The product does not keep well, even in a ground-glass stoppered bottle. It is preferable to prepare it as required.

2-Chloro-4-methylanilino-5-nitropyrimidine:

2:4-Dichloro-5-nitropyrimidine (5.5 g.) in dry ethanol (150 cc.) was treated drop-wise with methyl aniline (40 cc.) in ethanol (100 cc.) during 3 hours, the reaction vessel being cooled in ice. After stirring at 0° for a further hour, the crude product was filtered off; weight 35 g., yield 75% of theoretical. After recrystallisation from light petroleum (b. p. 100-120°), the m. p. was 131-132°. The compound was deposited in two different crystalline modifications: a pale yellow form, m. p. 132.5°, and deeper yellow crystals which melted one degree lower. The two forms usually appeared at once from solutions of the compound, and were deposited in varying proportions (Found: C, 50.46; H, 3.52; N, 20.53. $C_{11}H_9O_2N_4Cl$ requires C, 49.91; H, 3.40; N, 21.17%).

Action of aqueous acetic acid on 2-chloro-4-methylanilino-5-nitropyrimidine

An attempt to hydrolyse the chloro group by the method used in earlier similar hydrolyses (pp.113,136) was complicated by further reactions. Aqueous acetic acid brought

about hydrolysis of the methylanilino group in addition to the chlorine. A secondary interaction between the resulting free methylaniline resulted in the formation of 2:4-di(methylanilino)-5-nitropyrimidine. This substance, and also free uracil and N-methylaniline, were isolated from the hydrolysis and characterised:

The chloro compound (1 g.) was heated on the water bath for 4 hours in a mixture of acetic acid (2 cc.) and water (0.7 cc.). Colourless crystals (0.35 g.) which separated were filtered off (*), and were washed and recrystallised from acetic acid, m. p. 290° (with decomposition). (Found: C, 31.63; H, 2.06. 5-Nitrouracil, $C_4H_3O_4N_3$, requires C, 31.57; H, 1.91%.)

(*) This filtrate was diluted with water (2.4 cc.) and warmed. On cooling again and standing, crystals, m. p. 142-144°, separated (**). This product was purified by recrystallisation from ethanol, m. p. 144° (Found: C, 64.60; H, 4.84; N, 21.21. 2:4-Di-(methylanilino)-5-nitropyrimidine, $C_{18}H_{17}O_2N_5$, requires C, 64.49; H, 5.08; N, 20.89%). The substance contained no chlorine. Its identity was confirmed by its preparation by heating a mixture of 2-chloro-4-methylanilino-5-nitropyrimidine and methylaniline. Excess of the latter was used, and the heating at its boiling point was continued for 5 minutes. On cooling the dark solution, and diluting with a few ccs. of ethanol and cooling, golden yellow crystals

separated, m. p. 144°, mixed m. p. with the substance obtained above, 144°.

(**) The acid aqueous filtrate was extracted with ether, and then made alkaline with excess sodium hydroxide. An ethereal extract of the alkaline solution contained a small quantity of N-methylaniline, which was isolated and identified as the acetyl derivative (by a mixed-m. p.).

2-Hydroxy-4-methylanilino-5-nitropyrimidine:

(1) Aqueous acetone in presence of calcium carbonate

4 hours refluxing under these conditions gave back mostly unchanged starting material. However more prolonged treatment brought about the required hydrolysis:

2-Chloro-4-(p-chloroanilino)-5-nitropyrimidine (0.5 g.) and "Analar" calcium carbonate (3 g.) were added to aqueous acetone (1:1, 50 cc.). The suspension was refluxed for 36 hours, in a large stout round bottomed flask ("bumping" was rather violent). The acetone was distilled from the filtered solution, and the aqueous residue was reduced somewhat in vacuo. Addition of excess sodium carbonate solution precipitated the calcium as carbonate, which was filtered off. The filtrate was made just acid to litmus with hydrochloric acid, and, on standing, pale yellow needles, m. p. 270-280° (with decomposition), separated from the solution.

A small sample was recrystallised for analysis from much dioxan (400 cc. per g.) (Found: C, 54.02; H, 3.83; $C_{11}H_{10}O_3N_4$ requires C, 53.67; H, 4.06%).

The hydroxy-nitro compound was amphoteric, and it was always found to be difficult to bring about complete precipitation by neutralisation of its aqueous solution, although the pure substance seemed to be sparingly soluble even in hot water. It dissolved slowly in sodium bicarbonate solution, dilute hydrochloric acid, or boiling dioxan or ethanol.

(2) Sodium carbonate in aqueous acetone

The chloro compound (0.75 g.) was refluxed gently in a mixture of acetone (20 cc.) and 15% sodium carbonate solution (20 cc.) for 2 hours. Some discolouration of the solution occurred. The acetone was distilled out on the water-bath and the mother liquor extracted with ether. This extract contained little solid matter. The aqueous layer from the extraction was made just acid to litmus with dilute hydrochloric acid. On standing, a yellow solid precipitated which had properties similar to those of the compound obtained in (1).

(3) Sodium acetate in glacial acetic acid

These reagents also effected the hydrolysis, apparently without complicating side-reactions. The detailed procedure is described along with the next stage of the synthesis.

2-Hydroxy-4-methylanilino-5-aminopyrimidine

Owing to the difficulty in obtaining complete precipitation of the hydroxynitropyrimidine from its aqueous solutions, it was found preferable not to attempt its isolation. The crude material obtained directly by a sodium acetate-acetic acid hydrolysis of the chloro compound was hydrogenated directly; the combined procedure is described:

2-Chloro-4-methylanilino-5-nitropyrimidine (14 g.) was heated with sodium acetate (11.2 g.) in acetic acid (70 cc.) for 3 hours on the water bath. Most of the acetic acid was then removed by evaporation in a vacuum desiccator over solid potassium hydroxide, during several days. The residue (48 g.) was dissolved in ice-cold potassium hydroxide solution (37 g. in 320 cc. water), the vessel being cooled in a freezing mixture during the addition. 2% palladised strontium carbonate (14 g.) was added to the yellow solution and the suspension was shaken with hydrogen. The theoretical quantity was absorbed in several hours. After filtering off the catalyst, the cooled solution was carefully neutralised (litmus) with ice-cold 60% acetic acid, to give, on standing overnight, 10.1 g. crystalline precipitate. On recrystallisation from boiling water (2000 cc.) (and treatment with decolourising charcoal), the hydroxy-amine separated in light brownish plates, m. p. 260-280° (with decomposition). Yield over two stages:

64% of theoretical. (Found: C, 60.74; H, 5.25; N, 25.64. $C_{11}H_{12}ON_4$ requires C, 61.10; H, 5.56; N, 25.92%.)

ATTEMPTED PSCHORR-TYPE RING CLOSURES ON 2-HYDROXY-4-METHYLANILINO-5-AMINOPYRIMIDINE

(1) With copper bronze

The amine (1 g.) was dissolved in 3N hydrochloric acid (20 cc.), and the solution was cooled to -5°. An aqueous solution of sodium nitrite (0.32 g. in 3 cc. water) was then added slowly. Stirring was continued for a further 15 minutes, and during this time the diazonium salt tended to separate as a yellow crystalline solid. On the addition of copper bronze (1 g.), an effervescence took place, and the yellow crystals were replaced by a light brownish solid. This was made to dissolve by adding more 3N acid (10 cc.) and warming. The hot solution was filtered free from copper, and, on cooling, the pale green filtrate deposited fine colourless needles. An examination of the properties of this product led to the conclusion that it was a copper salt or complex.

In subsequent experiments, after filtration of the copper bronze from the hot acid solution, the filtrate was treated with hydrogen sulphide. The copper sulphide which precipitated was removed, and the now colourless solution was boiled to remove excess hydrogen sulphide. Neutralisation

of the hot solution gave, after cooling and standing, a precipitation of a colourless crystalline solid which could be recrystallised from water or ethanol, m. p. 312° (without obvious decomposition). (Found: C, 65.27; H, 5.28; N, 20.71. Diazacarbazole, $C_{11}H_9ON_3$, requires C, 66.33; H, 4.52; N, 21.10. Dihydro-compound, $C_{11}H_{11}ON_3$, requires C, 65.67; H, 5.47; N, 20.89%.)

This product was further characterised as a picrate, prepared in the usual manner in ethanol, m. p. (from ethanol) 240-243°. Two crystalline modifications of this picrate were observed. (Found: C, 46.97; H, 2.91; N, 19.35. Diazacarbazole picrate, $C_{17}H_{12}O_8N_6$, requires C, 47.66; H, 2.80; N, 19.63. Dihydro-compound picrate, $C_{17}H_{14}O_8N_6$, requires C, 47.43; H, 3.26; H, 19.54%.)

The evidence from these analyses is not conclusive of the dihydro formulation; however, the fact that copper has gone into solution suggests that a reduction has occurred.

An attempt to prepare an acetyl derivative was unsuccessful.

The colourless crystalline solid (m. p. 312°) was fairly soluble in hot water (220 cc. per g.) or ethanol or cold dilute hydrochloric acid or sodium hydroxide.

The products initially obtained in this and other similar experiments seemed to be of variable melting point.

The above substance was only obtained with a relatively sharp melting point above 300° after several recrystallisations. In one experiment a small crop of crystals was obtained from some mother liquors in which two crystal types were present with melting points 300-314° and 308-311°. A mixed melting point showed a marked depression.

It is possible that a mixture of reduced and unreduced products is obtained in the above decomposition.

A preliminary investigation of other methods of decomposing the diazonium salt of the above amine has been made:

(2) With sodium carbonate

The principal product on decomposition of the diazotised amine by warming in 15% sodium carbonate appeared to be a low-melting substance, which crystallised from hot water in colourless plates, m. p. 131-133°. It tended to separate rather as oil, even when fairly pure. Other solvents did not seem to be suitable for its crystallisation.

The compound gave an immediate yellow solution in sodium hydroxide, but dissolved only slowly in dilute hydrochloric acid. It was very soluble in ethanol, benzene, or chloroform. It appeared to possess reducing properties; neutral permanganate was decolourised on warming; warm alkaline copper sulphate turned black.

Investigation of this substance was not completed.

(3) With glacial acetic acid

It was found possible to isolate the diazonium salt of the amine in reasonable yield, by cooling the acid solution in a freezing mixture for some time. The filtered and dried product seemed quite stable, m. p. 178-180° (with decomposition) (Found: C, 46.52; H, 4.51. Monohydrate $C_{11}H_{10}ON_5Cl \cdot H_2O$ requires C, 46.78; H, 4.26%).

If this salt was suspended in glacial acetic acid and warmed, decomposition set in at about 80°. A preliminary investigation showed that crystalline products could be obtained from the reaction. These have not yet been investigated.

OTHER APPROACHES

1-Methyl-3-formyloxindole:

(Julian, Pikl, and Boggess, J. Am. Chem. Soc., 1934, 56, 1797.)

Attempted condensation of 1-methyl-3-formyloxindole:

Refluxing with thiourea for 7 hours in presence of sodium ethoxide in ethanol caused no condensation.

1-Methyl-3-chloromethyleneoxindole:

1-Methyl-3-formyloxindole (30 g.) was treated with

thionyl chloride (35 cc.), and the mixture was heated gently on the water bath so as to maintain a moderate rate of effervescence, and finally at reflux for 10 minutes. After cooling, the red liquor was poured into ice-cold dilute (1N) sodium hydroxide solution (2000 cc.). The red oil which separated soon solidified to a yellow solid; this solid was triturated with more sodium hydroxide, and with water, and dried in vacuo; weight 31.25 g. Recrystallisation from benzene gave yellow crystals in 70% yield, m. p. 85-86° (Found: C, 62.26; H, 4.19; N, 8.17; Cl, 18.14. $C_{10}H_8ONCl$ requires C, 62.04; H, 4.14; N, 7.24; Cl, 18.35%).

The product was further characterised by condensation with 2:4-dinitrophenylhydrazine. The derivative crystallised from glacial acetic acid in red plates with a golden lustre, m. p. 235-236°. It contained no chlorine. (Found: C, 53.94; H, 3.69; N, 19.52. $C_{16}H_{13}O_5N_5$ requires C, 54.07; H, 3.66; N, 19.72%, corresponding to the elimination of the elements of hydrogen chloride.)

The chloromethyleneoxindole also formed a yellow condensation product with thiourea on heating in dioxan.

If a mixture with powdered guanidine was heated on the oil bath, effervescence occurred at about 130°; this reaction was not further investigated.

1-Methyl-2-methoxy-3-formylindole:

(Julian, Pikl, Boggess, J. Am. Chem. Soc., 1934, 56, 1797.)

Condensations of 1-methyl-2-methoxy-3-formylindole (I):

(1) With guanidine

Addition of an ethanol solution of (I) to a mixture of guanidine hydrochloride and sodium methoxide in methanol gave an immediate scarlet precipitate.

This substance possessed unusual properties, some of which suggested that it was an unstable sodium salt:

Heating with water or treatment with acid caused a colour change to yellow (without solution). Addition of alkali brought back the red colour. A preliminary attempt to identify the product by recrystallisation from aqueous acetic acid, and analysis of the orangy precipitate were not successful.

(2) With urea

A mixture of (I) (0.3 g.) and urea (0.1 g.) in methanol (1 cc.) was treated with sodium in methanol (0.2 g. in 1 cc.). On mixing the warm solutions, immediate precipitation of an orange solid occurred. The precipitate was filtered off, washed with methanol, and dried. From aqueous acetic acid it recrystallised as an orange floc, m. p. 260-270° (Found: C, 61.16; H, 4.67; N, 20.32. $C_{11}H_{11}O_2N_3$ requires C, 60.82; H, 5.07; N, 19.35%, corresponding to the elimination of methanol).

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