

IMIDAZOLES AND IMIDAZOLE METABOLISM

by

G. E. TROUT

Thesis submitted for the degree of Doctor
of Philosophy at the University of the
Witwatersrand, Johannesburg, South Africa.

DECLARATION.

This is to certify that the work presented in this thesis is my own, and has not been presented previously for examination.

Signed ... *J. E. Trout*

Date ... *21/6/1966*

PUBLICATIONS.

Some of the data presented in this dissertation has been published as cited below:-

Studies on Imidazoles, Part I: The action of phosphorus pentachloride on N-butyl-N'-ethyloxamide. Trout, G.E. and Levy, P.R. Rec. trav. chim. 84, 1257, (1965).

Studies on Imidazoles, Part II: The action of phosphorus pentachloride on some symmetrical N,N'-dialkyloxamides. Trout, G.E., and Levy, P.R. Rec. trav. chim. in press.

The following article has been submitted to the same journal for publication:-

Studies on Imidazole, Part III. The synthesis of some 7,8-dialkylpurines. Trout, G.E., and Levy, P.R.

ANALYSES.

The majority of the micro-analyses reported in the following study were performed by either Drs. G. Weiler and F.B. Strauss, England or by Janssen Pharmaceutica, Belgium. A few analyses were undertaken by the C.S.I.R., and the Department of Chemistry, University of the Witwatersrand.

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It was at Professor P. R. Levy's suggestion that I undertook the present investigation, and I should like to express my deepest appreciation to him, not only for suggesting the problem, but also for his interest and assistance generously given at all stages of the work.

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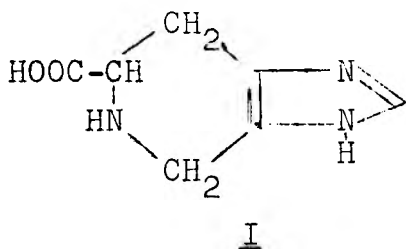
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INTRODUCTION.

Only a small number of compounds containing the imidazole ring are found in living systems, but most of these possess marked biological activity. The amino acid histidine and its decarboxylation product, histamine, are universally distributed. Histidine is not only a constituent of nearly all proteins, but in the dipeptide carnosine (β -alanyl-histidine) plays an important part in muscle biochemistry. Other derivatives of histidine include spinacin (I) which is found in shark liver (Ackermann and Skraup, 1949), and in crustaceans (Ackermann,



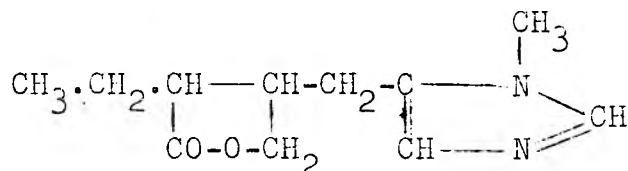
1962), and anserine (β -alanyl-3-methylhistidine) which is a constituent of striated muscle.

The imidazole ring is also an important constituent of several complex heterocyclic compounds ubiquitous in nature. In particular, it forms part of the purine nucleus, and consequently, is of fundamental importance in protein biosynthesis (nucleic acids) and in energy transfer (nucleotides). In primates the imidazole ring of the purines is resistant to rupture, being excreted as uric acid, though in other species rupture of the ring occurs to give allantoin (mammals) or allantoic acid (fishes). A number of purines have biological significance as

free nucleosides (Montgomery and Thomas, 1962). Examples of this are nebularin (9-~~β~~-D-ribofuranosyl-purine), spongosine (2'-methoxyadenosine), and crot-onoside (9-~~β~~-D-ribofuranosylisoguanine).

In the form of 5,6-dimethylbenzimidazole, the imidazole nucleus forms part of the vitamin B₁₂ molecule. Considerable modification can be made to this basic fragment, the activity of the vitamin still being retained (Lester-Smith, 1957 and 1960). Examples of such analogues are 5-aminobenzimidazole, 5,6-dichlorobenzimidazole, and 2,3-naphthamidazole. In addition, certain purines such as 2-hydroxypurine, 6-methylmercaptapurine, and 8-azadenine (Kon, 1957; Lester-Smith, 1960) can replace the benzimidazole and the resultant vitamin B₁₂ analogues still show activity in selected tests. While the essential biological function of vitamin B₁₂ is still unknown, the vitamin is universally distributed, and appears to have a fundamental role in the life process. It is known, for example, to be a co-enzyme for several enzymes including the important methylmalonyl-Co A-isomerase (Beck, 1962; Jaenicke, 1964).

The wide-spread natural occurrence of imidazole derivatives is illustrated further by such compounds as the pharmacologically-active alkaloids pilocarpine (II), caffeine, theobromine and theophylline, and the antibiotics 2-nitroimidazole (Nakamura, 1955; Beaman et al., 1965; Lancini and Lazzari, 1965), and puromycin (Porter et al., 1952; Waller et al., 1953), metabolites of the microorganism Streptomyces euroci-
dicus and Streptomyces alboniger respectively.



II

An interesting imidazole derivative is the synthetic plant hormone, kinetin (6-furfurylamino-purine). This compound is said to stimulate the growth of isolated leaves and leaf discs by an increase in cell size (Scott and Liverman, 1956), and to inhibit the yellowing of excised leaves. The mechanism of this effect is still obscure, but appears to involve an action on protein synthesis, possibly though the stimulation of nucleic acid synthesis (Mothes, 1963). While kinetin is of synthetic origin, the occurrence of natural substances with a kinetin-like action is considered highly probable (Goldacre and Bottomly, 1959).

Although a number of synthetic pathways to imidazoles (Hofmann, 1953), and purines (Lister, 1961 and 1963) are known, only in recent years have convenient methods become available for the preparation of substituted imidazoles for use as intermediates in the preparation of purines (eg. Shaw and Woolley, 1949). Thus, most of the methods for the synthesis of various purines have, hitherto, depended upon preformed pyrimidine intermediates (Lister, 1961).

Wallach, in 1877, first investigated the action of phosphorus pentachloride on N,N'-dialkylloxamides, the basic products of which were later shown to be 5-chloroimidazoles. The Wallach reaction has been employed from time to time (see page 6 of this thesis),

but has been restricted to dimethyl- and diethyl-oxamides. It appears to have been generally accepted that the reaction is limited to these two oxamides (for example, see Hofmann, 1953), and in a comprehensive review of the methods available for the synthesis of purines from imidazoles, Lister (1963) unequivocally states:

"No other homologues of N,N'-dialkyloxamides can be converted to 5-chloroimidazoles by this procedure."

The present author has found, however, that Lister's statement (above) is not correct.

This thesis deals with an investigation of the Wallach reaction as a general method for the preparation of 5(4)-chloro-1,2-dialkylimidazoles from a series of both symmetrical and nonsymmetrical N,N'-dialkyloxamides, and the use of these imidazoles as starting materials for the preparation of further derivatives, particularly the 7,8-dialkylpurines.

Part I of the thesis is devoted to the extension of the Wallach reaction to a wide range of N,N'-dialkyloxamides, the separation of isomeric products, and the orientation of the various substituent groups on the imidazole ring.

In Part II of the thesis, the synthesis of a series of 7,8-dialkylpurines are described using the various 5-chloro-1,2-dialkylimidazoles

as starting materials. In all, some 15 new purines are described, of which several were used to determine the influence of alkylation in the 7- and 8- positions on the metabolic activity of otherwise active purines. The test system employed depended upon the growth rate of purine-requiring bacteria. As all the compounds examined proved to be inactive, full data and discussion are present as an appendix (Appendix II).

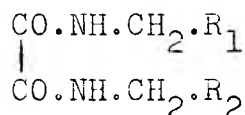
An interesting adjunct to this thesis was the demonstration of antifungal action by some of the Wallach bases and their nitro-derivatives. The present study, described in Part III, was restricted to a preliminary survey with eight pathogenic fungi. Nevertheless, the results are of interest as they provide a basis for further structural modification with greater potential anti-fungal activity. A more detailed study, embracing toxicity studies, was considered to be beyond the scope of the present investigation.

PART I.

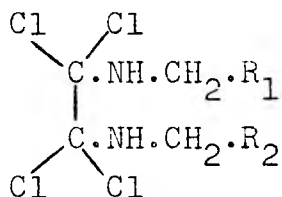
THE ORIENTATION OF THE 5(4)-CHLORO-1.2-DIALKYL-
IMIDAZOLES.

(a) The action of phosphorus pentachloride on
N,N'-dialkyloxamides.

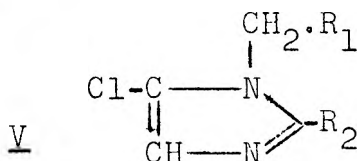
Ring closure of N,N'-dialkyloxamides (III) under the action of phosphorus pentachloride to give bases (later shown to be chloroimidazoles) was first described by Wallach (1877, 1881, 1882, and 1883); Wallach and Oppenheim (1877); Wallach and Schulze (1880 and 1881); and Wallach and Stricker, (1880). The reaction appears to take place in two stages (Wallach 1882, and 1883; Sarasin and Wegmann, 1924); first, the production of a diamidodichloride (IV), and secondly, loss of hydrogen chloride and cyclisation to form an imidazole derivative (V). A single chlorine atom remains attached to the imidazole ring either at the 4- or 5- position. Recently, it has been shown that in addition



III



IV.



V

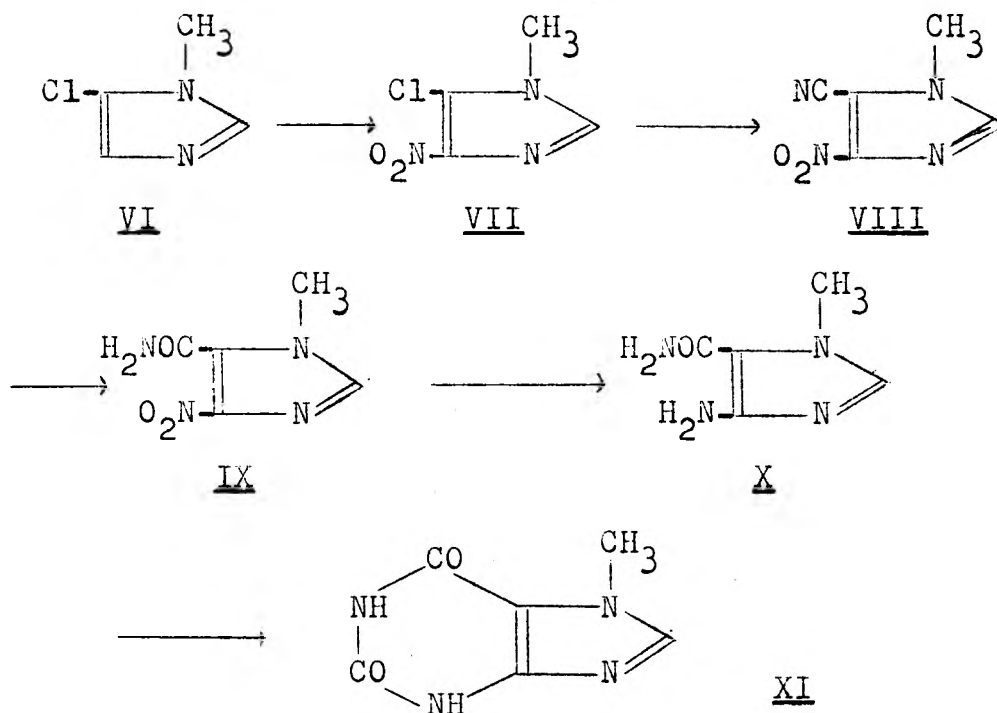
to 5-chloro-1-methylimidazole, the action of phosphorus pentachloride on dimethyloxamide produces significant quantities of 4,5-dichloro-1-methylimidazole and 2,4,5-trichloro-1-methylimidazole (Kochergin, 1964). It would appear, however, that these higher chloro derivatives are the result of chlorination of preformed 5-chloro-1-methylimidazole.

Several oxamides were investigated by Wallach and a series of bases prepared. The reaction of phosphorus pentachloride on di-propyloxamide, di-iso-amyloxamide and ethyl-phenyloxamide was reported to proceed with difficulty (Wallach and Schulze, 1880), and in fact, both Hofmann (1953) and Lister (1963) have stated that the above method is of practical value only for preparing the bases from dimethyloxamide and diethyloxamide.

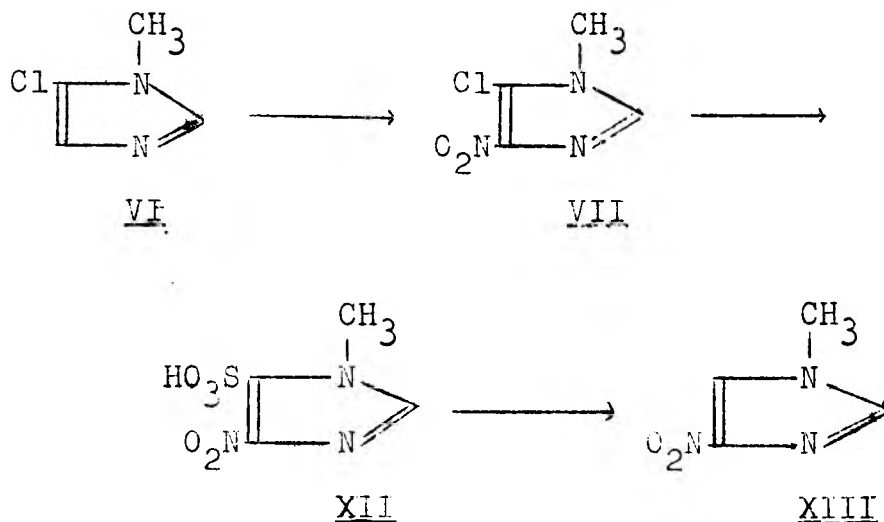
The present author has shown that, in contrast to the above statement, the reaction can be readily applied to a number of higher oxamides, both symmetrical and nonsymmetrical. Depending upon the oxamide used, a series of bases can be prepared in good yield which, for brevity, will be referred to as "Wallach bases". The reaction itself will be called the "Wallach reaction" (Gowan and Wheeler, 1960).

The constitution of the Wallach base from dimethyloxamide was determined by two groups of workers (Balaban and Pyman, 1924; Sarasin and Wegmann, 1924; Sarasin, 1923). Using different reaction sequences, both groups of workers succeeded in showing that the chlorine atom was attached to the 5-position of the imidazole ring, and the compound was therefore 5-chloro-1-methyl-imidazole. By the nature of the reaction, the methyl group can only be in the 1-position.

Sarasin and Wegmann (1924) determined the orientation of the chlorine atom in 5-chloro-1-methylimidazole (VI) by a five stage synthesis of 7-methylxanthine (XI) (hetero-xanthine). from the Wallach base and identified the product by comparison with authentic material prepared by a different route. It was clear from these reactions that the chlorine atom was attached to position 5- of the imidazole ring. Had the chlorine been in the 4- position, the isomeric 9-methylxanthine would have been formed. By analogy with dimethyloxamide, Sarasin inferred that the base from diethyl-oxamide also had the chlorine in the 5-position. The synthesis of several purines (Montequi, 1926 & 1927; Mann and Porter, 1945) from this same base confirmed that the chlorine atom is, in fact, in the 5-position.



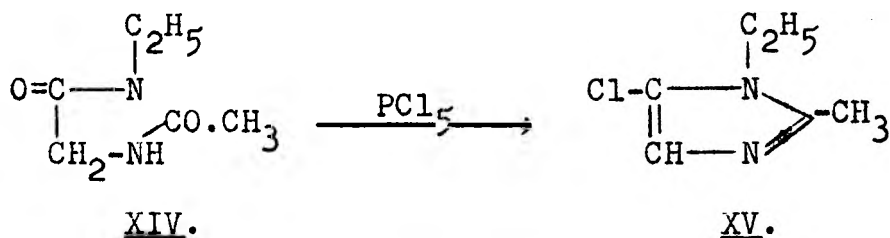
Balaban and Pyman (1924) exploited quite a different approach for the determination of the structure of 5-chloro-1-methylimidazole. Their method was essentially a dehalogenation of the nitrated base (VII) to form 1-methyl-4-nitroimidazole (XIII) already prepared independently (Hazeldine et al., 1924). Dehalogenation was accomplished by treatment of the nitro-chloroimidazole (VII) with 20% sodium sulphite to give the sulphonic acid (XII), which was then hydrolysed with 30% sulphuric acid. Since the product obtained was pure 1-methyl-4-nitroimidazole, the chlorine-containing base must have



been 5-chloro-1-methylimidazole*. The position of the chlorine atom in the Wallach bases from dimethyloxamide and diethyloxamide has therefore been unequivocally determined.

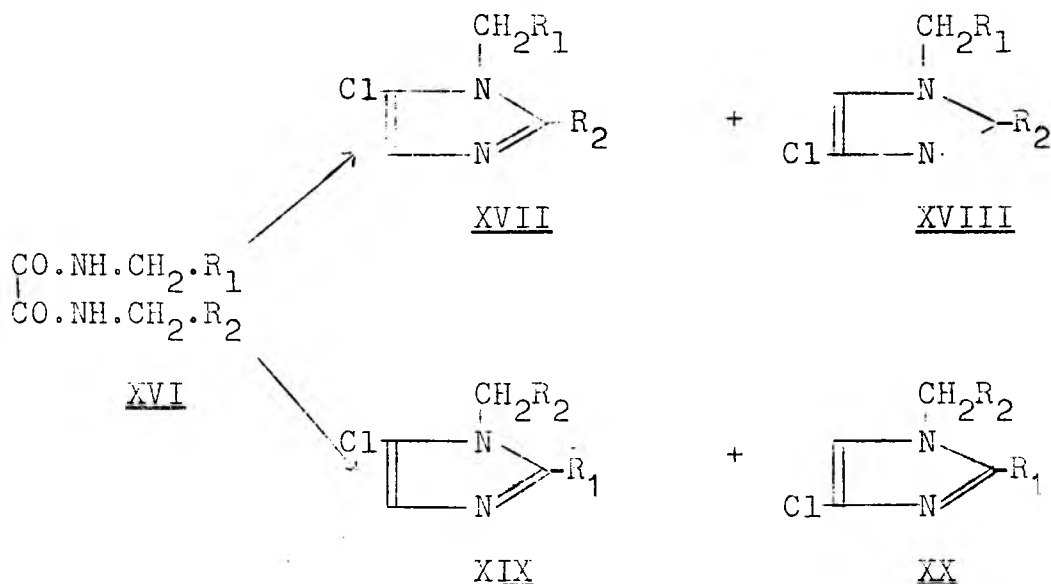
It is interesting to note that 5-chloro-1-ethyl-2-methyl-imidazole has been synthesised by an alternative route which leaves little doubt of its structure (Gränacher et al., 1925). Acetylglycine ethylamide was treated with phosphorus pentachloride in dry chloroform and 5-chloro-1-ethyl-2-methylimidazole was isolated according to the reaction:-

* The possibility of the base being 2-chloro-1-methylimidazole was not strictly excluded by these experiments. However, the general nature of the Wallach reaction was known to yield 5(4)-chloro-1,2-dialkylimidazole in which only the position of the chlorine was uncertain.



Very curiously, Gränacher (loc. cit.) makes no mention of Wallach's pioneering work, nor of Sarasin and Wegmann's studies on chloroimidazoles, and no attempt was made to collate findings from the several investigations. In comparing the properties of Gränacher's product with the Wallach base from diethyloxamide, the present author noted favourable agreement between the melting points of the picrates i.e. 154° (Gränacher) compared with $156-157^\circ$ in the present study.

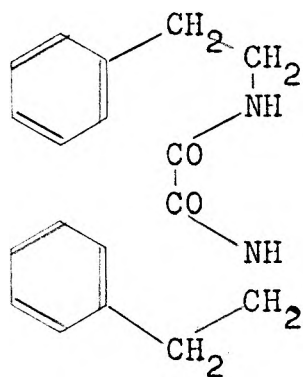
In the present investigation, the author has successfully extended the Wallach reaction to several dialkyloxamides not previously examined, and bases have been isolated in satisfactory yields. Although the products from dimethyloxamide and diethyloxamide have been shown to be the 5-chloro bases, it was felt that there was insufficient evidence to assume automatically that other imidazoles derived from similar reactions would also have chlorine atoms in the 5- position of the imidazole nucleus. Further, in the case of chloroimidazoles prepared from nonsymmetrical oxamides, the possibility of isomerism between the alkyl groups in the 1 and 2 positions would increase the number of products of the reaction as indicated in the following scheme:-



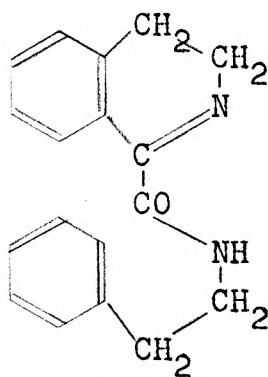
The postulated mechanism for the action of phosphorus pentachloride on N,N'-dialkyloxamides (page 6) permits the formation prima facie of 4- and 5-chloroimidazoles with equal probability. Even in the event of the reaction being biased in favour of one of the isomers, the presence of a significant proportion of the alternative isomer might reasonably be anticipated. However, a more detailed study of the products formed by the action of phosphorus pentachloride on dimethyloxamide (Kochergin, 1964) revealed only di- and trichloro derivatives as co-products of 5-chloro-1-methylimidazole. Nevertheless, there appears to be no reason why the 4-chloro isomer should not be produced in significant amounts when the reaction was extended to oxamides other than dimethyloxamide. For this reason, the bases prepared in the present investigation were carefully examined by gas chromatography and "reversed-phase" thin-layer chromatography in an attempt to detect the presence of isomers. No trace of the 4-isomer was found in any of the bases from the oxamides studied. The present author has shown that synthetic mixtures of 4-chloro (Sarasin, 1923) and 5-chloroimidazoles may readily be resolved by both the above techniques. (section d.)

The purpose of the present study was hence, first to investigate the applicability of the Wallach reaction to higher dialkylloxamides (both symmetrical and nonsymmetrical) and secondly, to determine the orientation of the alkyl groups and chlorine atom in the imidazole produced. Only from a larger number of cases than previously examined could a measure of certainty be placed on the postulate that the Wallach reaction yields 5-chloroimidazoles. A series of reactions leading to rupture of the imidazole ring was therefore investigated. From the nature of this degradation both the orientation of the chlorine atom and the alkyl groups was determined. A secondary aspect of the study was the confirmation of earlier findings (see above) that the base derived from diethyloxamide is 5-chloro-1-ethyl-2-methylimidazole.

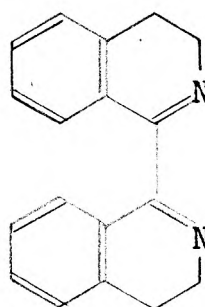
The conditions of reaction used in the present study for the preparation of chloroimidazoles did not differ markedly from those described by Decker et al., (1913) in a modification of the classical Bischler and Napieralsky reaction (1893) for the synthesis of 3,4-dihydro-iso-quinolines. Decker attempted to prepare, inter alia, 1,1-di-(3,4-dihydro-iso-quinoline) (XXIII) by treatment of di-(2-phenylethyl)-oxamide (XXI) in xylene solution with either phosphorus pentachloride or phosphoryl chloride.



XXI

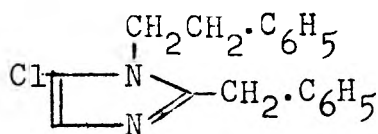


XXII



XXIII

He reported, however, that the di-iso-quinoline (XXIII) could not be prepared in this manner and that the product of the reaction was the intermediate compound (XXII). A picrate (m.p.167°) and a hydrochloride (m.p.193°) were described. Our attempts to repeat Decker's synthesis of this particular base were not successful, but we were finally able to isolate a very small quantity of base by treating (XXI) with phosphorous pentachloride directly. Our base gave a picrate (m.p.165°) and hydrochloride (m.p.228°). Because of the very small yield of material it was not possible to submit the compound to the degradation procedure described later for determining the structure of the Wallach bases. However, the distinct structural differences between the iso-quinoline derivative (XXII) and the imidazole base postulated in the present study (XXIV) allows the two structures to be readily distinguished by examination of the infrared spectra. In particular, the iso-quinoline derivative should show typical bands for

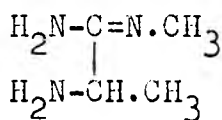


XXIV

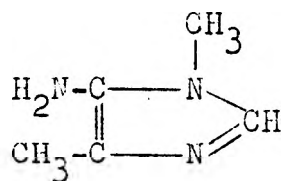
the amide group (1700 - 1670 cm^{-1} and 3460 - 3400 cm^{-1}) which are not present in the imidazole derivative. A number of infrared spectra of 5-chloro-1,2-dialkylimidazoles were examined for correlation purposes and the results are described in section (f). The use of infrared spectroscopy to elucidate the structure of the Wallach base from di-(2-phenylethyl)-oxamide is described in section (g).

(b). Reductive cleavage of the imidazole ring.

Attempts to reduce either 4(5)-nitroimidazole, 2-methyl-4(5)-nitroimidazole or 5(4)-methyl-4(5)-nitroimidazole with tin and hydrochloric acid in the cold (Fargher and Pyman, 1919; Fargher, 1920; Pyman, 1922) resulted in fission of the imidazole nucleus, two of the nitrogen atoms appearing as ammonia. Fargher originally suggested that the fission proceeded via an imidazolone derivative (Fargher and Pyman, 1919) but the subsequent isolation of alanine-N-methylamidine (XXV) and 5-amino-1,4-dimethylimidazole (XXVI) after reduction of 1,2-dimethyl-5-nitroimidazole with tin



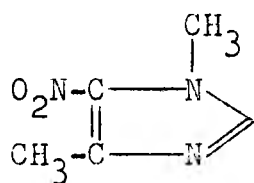
XXV.



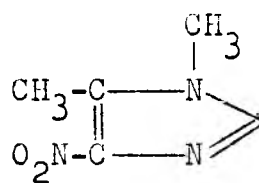
XXVI.

and hydrochloric acid (Pyman, 1922) suggested that the degradation arises from the elimination of the carbon atom in position 2- as (presumably) formic acid. In an investigation of the orientation of the groups in dimethylnitroimidazoles, Pyman (1922) postulated, and later confirmed, that if the reductive fission proceeded as suggested by the above intermediates, then the reduction of 1,4-dimethyl-5-nitroimidazole (XXVII) should lead ultimately to the formation of alanine, ammonia, methylamine and formic acid. On the other hand, 1,5-dimethyl-4-nitroimidazole (XXVIII) on reduction and subsequent fission, would yield N-methylalanine, ammonia (2 moles) and formic acid. The present author has applied these

observations to ascertain the orientation of the Wallach bases. If the chlorine atom is in the 5-position, the reaction indicated by Scheme I, leads



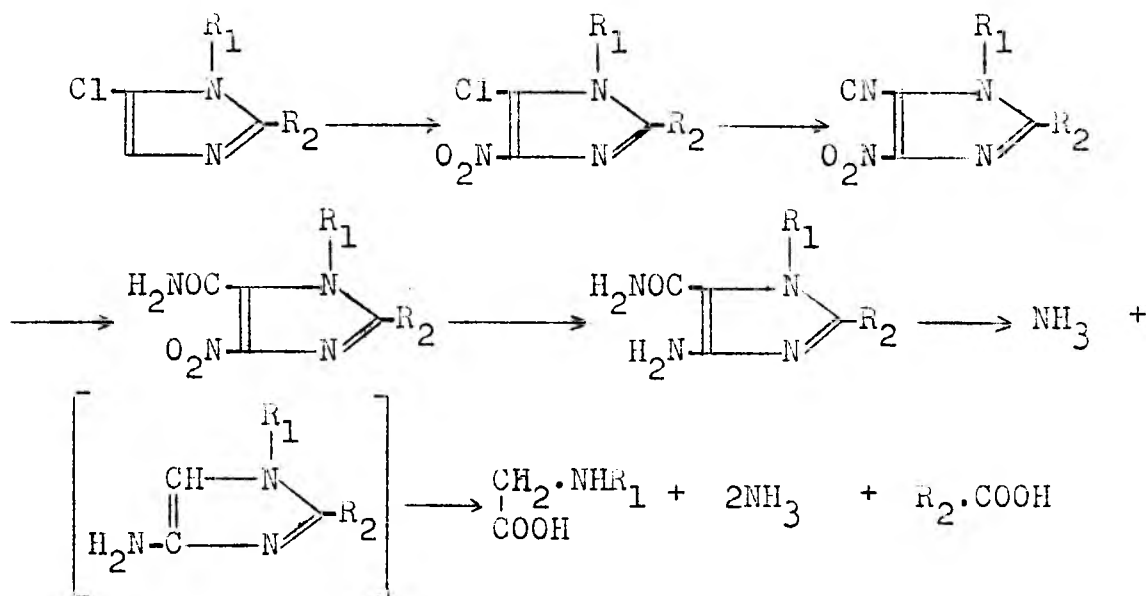
XXVII.



XXVIII.

ultimately to the formation of an N-alkylglycine, a carboxylic acid and 3 moles of ammonia per mole of original chloroimidazole. If, on the other hand,

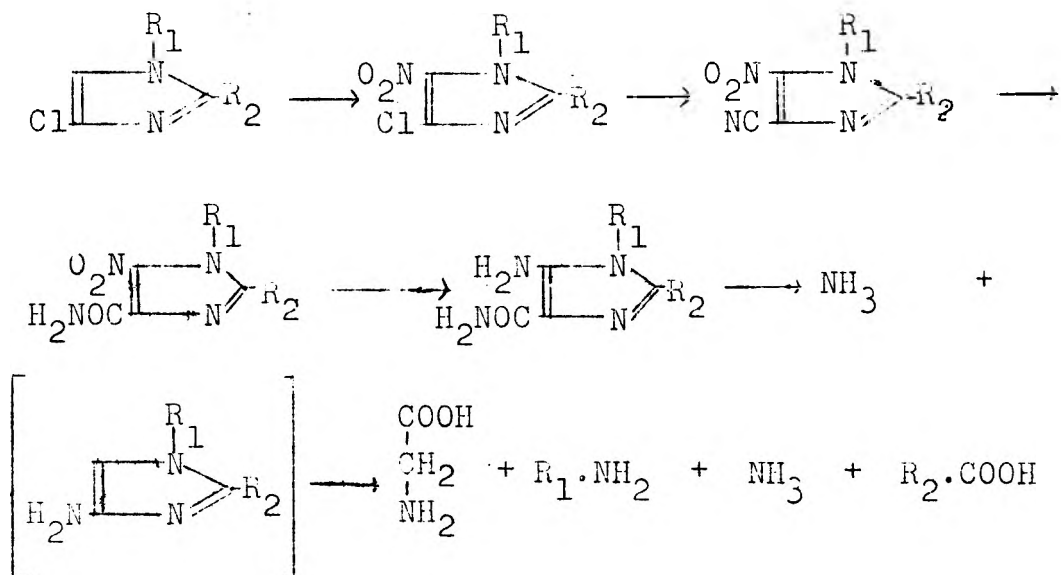
Scheme I: The degradation of 5-chloro-1,2-dialkyl-imidazoles.



the chlorine atom is in the 4-position of the imidazole ring, the same series of reactions will yield different fission products, namely:- glycine, an alkylamine, a carboxylic acid and 2 moles of ammonia per mole of original base.

Scheme II illustrates the suggested course of the reaction.

Scheme II:- The degradation of 4-chloro-1,2-dialkyl-imidazoles.



Hence, the isolation of an N-alkylglycine would indicate that the chlorine atom was situated in the 5-position in the original imidazole base. The isolation of glycine, by contrast, would show that the starting material was a 4-chloroimidazole. Further, the nature of the alkyl group in the N-alkylglycine indicates the orientation of the alkyl groups in the original compound. The identification of the carboxylic acid would complete the confirmation of the structure of the base.

In bases from symmetrical oxamides, no opportunity arises for isomerism in so far as the 1,2 alkyl groups are concerned since the C-2 atom of the imidazole ring arises from one of the original alkyl groups attached

to nitrogen and its substituent will therefore always be the immediate lower homologue. In the case of bases from nonsymmetrical oxamides a more complex situation arises, as two isomeric compounds are possible for each theoretical position of the chlorine atom (see page 12). However, the identification of the N-alkylglycine and of the carboxylic acid in the case of 5-chloro-1,2-dialkylimidazoles clearly determines the nature and position of the alkyl groups in the original compound. In the event of the original base being a 4-chloro-1,2-dialkylimidazole, the orientation and nature of the alkyl groups are indicated by the nature of the carboxylic acid and confirmation would depend on the identification of the alkylamine.

The possibility of a mixture of 4- and 5-chloroimidazole bases being formed cannot be discounted and would most easily be gauged by a quantitative examination of the products of degradation. Advantage might also be taken of the ease with which ammonia, produced in the degradation, may be estimated. Scheme II shows that a 4-chloro-1,2-dialkylimidazole yields 2 moles of ammonia per mole of base as compared with 3 moles of ammonia in the case of 5-chloro-1,2-dialkylimidazoles.

In the present investigation, small, accurately weighed amounts of each 4(5)-amino-1,2-dialkylimidazole-5(4)-carboxamide were treated with concentrated hydrochloric acid at 140° for 24 hours and the products quantitatively examined for ammonia by means of Nessler's reagent (see page 106).

(c). Synthetic techniques.

(i). Preparation of oxamides.

No difficulty was experienced in the preparation of the symmetrical oxamides (Dermer and Hutcheson, 1943; Rice et al., 1953). A low temperature is advisable as the reaction is exothermic and the amines used have low boiling points. The preparation of non-symmetrical oxamides, by contrast, provided considerable synthetic difficulties. The published preparation of ethylmethyloxamide (Wallach, 1877) consisted in adding one molar proportion of methylamine to diethyl oxalate, isolating the ethoxalylmethylamine, and then adding one molar proportion of ethylamine. The principal difficulty lies in obtaining pure products, particularly the intermediate ethoxalylmethylamine. The preparation of pure ethoxalylmethylamine was successfully undertaken by Tierie (1933) and depends upon fractional distillation of the crude product to remove unchanged diethyl oxalate, followed by freezing and gentle thawing of the distillate to just above the melting point when a precipitate of dimethyloxamide can be removed by filtration. This method has been extended successfully in the present study to the preparation of other ethoxalylalkylamines, particularly ethoxalylethylamine, ethoxalylbutylamine and ethoxalylbenzylamine from which a variety of non-symmetrical oxamides are readily prepared.

An alternative technique for the synthesis of ethylmethyloxamide has been examined. The synthesis depends upon the anticipated greater reactivity of an acid chloride group for a primary amine than that of an ester group (XXIX) - (XXXII).

TABLE I: Symmetrical oxamides and the corresponding 5-chloro-1,2-dialkyl-imidazoles.

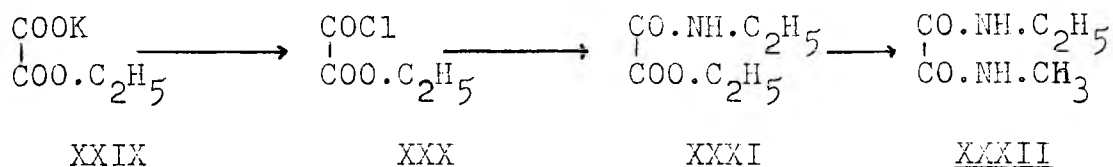
Oxamide.	M.p. °C.	5-chloro-1,2-dialkylimidazole.
Dimethyloxamide	217 - 217.5	5-chloro-1-methylimidazole ¹
Diethyloxamide	181	5-chloro-1-ethyl-2-methylimidazole
Dipropyloxamide	164.5-165.5	5-chloro-2-ethyl-1-propylimidazole
Di- <u>n</u> -butyloxamide	153.5-154.5	1-butyl-5-chloro-2-propylimidazole
Di- <u>iso</u> -butyloxamide	171.5-172.5	1- <u>iso</u> -butyl-5-chloro-2- <u>iso</u> -propyl-imidazole
Dibenzyloxamide	225.5-226.5	-
Di-(2-phenylethyl)-oxamide	188.5-189.5	2-benzyl-5-chloro-1-(2'-phenylethyl)-imidazole ² .

Footnotes:

1. Data taken from Wallach (1877).
2. Obtained in very small yield (3%) only.

TABLE II: Nonsymmetrical dialkyloxamides and the corresponding 5-chloro-1,2-dialkylimidazoles.

Oxamides.	M.p. °C.	1,2-dialkyl-5-chloroimidazole
Ethylmethyloxamide	153 - 154	5-chloro-1,2-dimethylimidazole
Benzylmethyloxamide	185.5	5-chloro-1-ethylimidazole
<u>n</u> -Butylethyloxamide	133.5 - 134.5	5-chloro-1-methyl-2-phenyl- imidazole.
<u>n</u> -Butylpropyloxamide	145 - 146	5-chloro-1-ethyl-2-propyl- imidazole.
<u>iso</u> -Butyl- <u>n</u> -butyl- oxamide	146 - 147	1-butyl-5-chloro-2-methylimidazole
		5-chloro-1,2-dipropylimidazole
		1-butyl-5-chloro-2-ethylimidazole
		1- <u>iso</u> -butyl-5-chloro-2- <u>n</u> -propyl- imidazole
		1- <u>n</u> -butyl-5-chloro-2- <u>iso</u> -propyl- imidazole



No particular advantage in respect of purity of the final product was noted and the relatively large number of stages involved is a disadvantage. It was therefore abandoned in favour of the Tirie method. Conversion of the ethoxalylkylamine to a nonsymmetrical oxamide is a simple process provided impurities are adequately removed at the intermediate ethoxalylalkylamine stage.

(ii). Preparation of 1,2-dialkyl-5-chloroimidazoles.

With the exception of di-(2-phenylethyl)oxamide all the oxamides studied undergo the Wallach reaction smoothly. Two methods of isolation of the base have been examined, selection of a particular technique depending on the properties of a particular base. The majority were isolated by extraction into chloroform followed by extraction into dilute hydrochloric acid. Regeneration of the base by treatment with sodium hydroxide and re-extraction with chloroform gave a reasonably pure product in a short time. The alternative technique, somewhat slower, though yielding a cleaner product, consists of a steam distillation of the crude alkaline material and extraction into chloroform after saturation of the distillate with potassium carbonate. The use of a short fractionating column in the final stage was found to improve the recovery of base from the chloroform extract.

TABLE III: Some 5-chloro-1,2-dialkyl-4-nitroimidazoles.

5-Chloro-1,2-dialkyl-4-nitroimidazole	M.p. °C.
5-Chloro-1-ethyl-2-methyl-4-nitroimidazole	88.3 - 89.1 .
5-Chloro-2-ethyl-4-nitro-1-propylimidazole	58 - 59
1-Butyl-5-chloro-4-nitro-2-propylimidazole	34.7 - 35.7
1- <u>iso</u> -Butyl-5-Chloro-4-nitro-2- <u>iso</u> -propyl- imidazole	109.5 - 110.5
5-Chloro-1-ethyl-4-nitro-2-propylimidazole	46 - 47
1-Butyl-5-chloro-2-methyl-nitroimidazole	-*
5-Chloro-1-methyl-4-nitro-2-(<u>p</u> -nitrophenyl) imidazole	156.0 - 157.5 .

* B.p. 180 - 181°/1 mm Hg.

(iii). Preparation of chloronitroimidazoles.

The chlorine atom in the imidazole ring is very unreactive and cannot be replaced, inter alia by cyanide, except after activation by nitration.

Nitration of 5-chloro-1-methylimidazole was originally achieved by the gentle evaporation to dryness of a mixture of base and excess dilute nitric acid, followed by treatment with hot concentrated sulphuric acid for several hours (Sarasin and Wegmann, 1924). The mechanism of the reaction is said to be the preliminary formation of a nitrate salt followed by re-arrangement of the nitro group to the 4 (or 5) position).

The present series of bases were successfully nitrated by a four to five hour treatment with a mixture of concentrated nitric and concentrated sulphuric acids (1:3) at the temperature of the boiling water bath. The violence of the immediate reaction was moderated by thorough cooling (-10°) of both acid mixture and base before, and during mixing. The above modification was considerably simpler and quicker than the Sarasin and Wegmann technique and give comparable yields.

(iv). Preparation of cyanonitroimidazoles.

Early attempts to replace the 5(4)-chlorine atom by the cyano group were based on the action of potassium cyanide and catalytic amounts of potassium iodide in absolute ethanol (Sarasin and Wegmann, 1924). Refluxing for many hours is essential. This method was used in some of the early experiments in this study, but suffers from two main disadvantages -

excessively long reaction time and uncontrollable "bumping". The action of cuprous cyanide in dry pyridine on the chloroimidazole (Neuman, 1937) was unsuccessful. The method of Friedman and Shechter (1960) was, however, readily adapted to imidazole derivatives. This latter method consists in heating the nitrochloroimidazole with sodium cyanide in partial solution in dimethyl sulphoxide. The present author found that it was imperative to reduce the temperature of the reaction from 130° to that of the boiling water bath (94°/620 mm) to minimise side reactions.

Isolation of the nitrile varies with the nature of the compound under study. In the case of oily nitriles, extraction into chloroform or ether proved satisfactory. Solid nitriles were removed by filtration and purified by recrystallisation from either ethanol or ethanol/water. It was observed that the purity of the nitrile was a critical factor in the successful conversion to the amide. For oily nitrile, thorough washing with water (at least four changes) of the chloroform or ether extract appeared to remove the impurity. Purification of liquid nitriles by distillation alone was not successful in removing the contaminants.

(v). Preparation of nitroimidazole-carboxamides.

Usually two hours heating on the water bath with excess sulphuric acid was sufficient to convert the nitrile into the amide. In a few cases up to four hours heating was shown to yield a purer product and this therefore became routine. The amide generally

TABLE IV: Some 5-cyano-1,2-dialkyl-4-nitroimidazoles.

5-Cyano-1,2-dialkyl-4-nitroimidazole	M.p. °C.	B.p. °C.
5-Cyano-1-ethyl-2-methyl-4-nitroimidazole	78-79	192-194/ 6 mm Hg
5-Cyano-2-ethyl-4-nitro-1-propylimidazole	62.5-63.5	-
1-Butyl-5-cyano-4-nitro-2-propylimidazole	36.5-37.5	194-198/ 1 mm Hg
5-Cyano-1-ethyl-4-nitro-2-propylimidazole	oil	-
1-Butyl-5-cyano-2-methyl-4-nitroimidazole	oil	-
5-Cyano-1-methyl-4-nitro-2-(p-nitrophenyl) imidazole	186.8-187.5	-
1- <u>iso</u> -Butyl-5-cyano-4-nitro-2- <u>iso</u> -propyl- imidazole	131.5	-

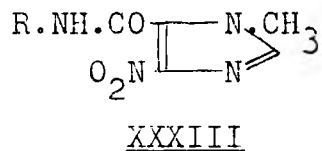
precipitates on pouring the acid solution into a large volume of water but occasionally several hours standing in the cold was required to effect crystallisation. The purity of the nitro-amide was critical in the successful reduction to aminoimidazole-carboxamide (see page 28). It was invariably necessary to recrystallise nitro-amides from acetic acid/water before submitting them to catalytic reduction.

(vi). Preparation of aminoimidazole-carboxamides.

It is known that an amino group in the 4- (or 5-) position in the imidazole ring behaves quite differently from the isomeric 2-amino compounds (Fargher and Pyman, 1919; Fargher, 1920). Thus the 2-aminoimidazole is a very stable, "non-aromatic" amino compound whereas the 4(5)-amino group is unstable (relatively) and can be diazotised and coupled with sodium β -naphthoxide. Alternatively the amine can be condensed with benzaldehyde - all properties of an aromatic nature (Fargher, 1920). The instability of some 4(5)-aminoimidazoles is such that they can only be kept as salts (Elderfield, 1957).

Original investigators (eg. Sarasin and Wegmann, 1924; Montequi, 1926) prepared the 4(5)-aminoimidazoles by reduction of the corresponding nitroimidazoles with tin and hydrochloric acid in the cold. Difficulty in obtaining satisfactory yields due to breakdown to smaller molecules were frequently recorded (Fargher, 1920; Pyman, 1922). Better yields have been reported using alternative reducing agents. Mann and Porter (1945), for example, noted that tin and hydrochloric

acid completely failed to reduce compounds of the type:-



due apparently to the presence of the substituted amide, but moderate yields were obtained with ferrous sulphate and ammonia or aluminium amalgam as reducing agents. When $\text{R} = \text{CH}_3$ in XXXIII, a 70% yield of amine was obtained after reduction with hydrogen and Raney nickel, but even this reagent failed when $\text{R} = \text{phenyl}$. A palladium catalyst, however, proved efficacious for these latter compounds provided one mole of hydrogen chloride was available for immediate neutralisation.

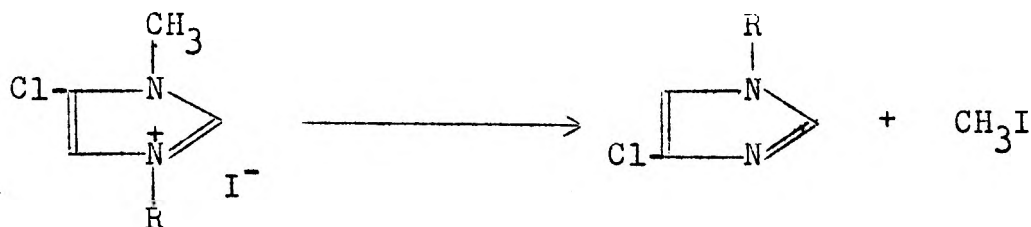
In the present study catalytic reduction of the nitro group was preferred, both Raney nickel and Adams' platinum oxide catalyst being satisfactory. Platinum appears to give a cleaner product and after a few initial experiments, was used exclusively. No reduction could be achieved in the present study with palladium as catalyst either as palladised charcoal or palladised barium sulphate.

The present author experienced some difficulty in isolating the amines, and two precautions were found to be essential for satisfactory results: very highly purified nitro-amides are essential as starting materials and since the reduction and subsequent evaporation of the ethanol solvent was found to be temperature sensitive, all operations

were conducted at a temperature not higher than 35°. Cooling the reaction to -5° did not improve yields. All the reductions in the present investigation were conducted in absolute ethanol, and the amino-amides isolated as the free bases. If the reduction is attempted in absolute ethanol containing dry hydrogen chloride, no reduction takes place. In absolute ethanol containing a small percentage of concentrated hydrochloric acid, on the other hand, reduction occurs rapidly and without the formation of dark coloured impurities, but great difficulty was experienced in isolating the amine hydrochloride due to its extreme solubility. Attempts to precipitate the amine hydrochloride from an absolute ethanol solution by the addition of dry ether produced only a "sticky" oil. Although solutions of the free amine rapidly darken, the product, once isolated in a relatively pure form, appears to be stable.

(vii). The synthesis of 4-chloro-1,2-dialkylimidazoles.

The distillation of an alkyl quaternary iodide of 5-chloro-1-methylimidazole has been shown (Sarasin, 1923) to result in decomposition of the salt, loss of methyl iodide, and the formation of a 1-alkyl-4-chloroimidazole according to the equation:-



In this manner Sarasin prepared a large number of

1-alkyl-4-chloroimidazoles.* The reaction was not, however, applied to other Wallach bases at this time. In the present study the author has extended the above reaction to several 5-chloro-1,2-dialkylimidazoles. The reaction has been used mainly to prepare the isomeric 4-chloro-imidazoles by using the appropriate alkyl iodide. The 4-chloroimidazoles were required (see page 39 & 43) for use as reference compounds in the chromatographic examination of the Wallach bases for the presence of 4-chloro and 5-chloro isomers.

(viii). Dechlorination of the 5-chloro-1,2-dialkylimidazoles.

The synthesis of 1,2-dialkylimidazoles from the relatively accessible 5-chloro-1,2-dialkylimidazoles provides a valuable route to these compounds. Wallach and Schulze (1881) reported the dechlorination of 5-chloro-1-methylimidazole, 5-chloro-1-ethyl-2-methylimidazole and 5-chloro-2-ethyl-1-propylimidazole by reduction with hydriodic acid and red phosphorus. The picrates were not described thus precluding comparison with the present series of dechlorinated imidazoles. Later Sarasin (1923) employed sodium in ethanol as an effective reducing agent and this reagent was employed in the present study. The method

*Sarasin incorrectly concluded the product formed by the action of phosphorus pentachloride on dimethyloxamide to be 4-chloro-1-methylimidazole and this fact must be considered when reading this publication. In 1924, Sarasin and Wegmann correctly determined the structure of the base as a 5-chloroimidazole.

is not particularly suitable for large scale use and was restricted to five gram amounts. In order to minimise complications due to the presence of unreacted starting material, a considerable excess of sodium was added. Because the volume of product was small and decomposition occurs on boiling at atmospheric pressure, boiling points for the dechlorinated bases were not determined. The melting points of the picrates of a series of bases are cited in Table V.

(d). The separation of isomeric Wallach bases.

The nature of the possible isomers produced by the action of phosphorus pentachloride on N,N'-dialkyloxamides has already been discussed (page 12). Briefly, in the case of symmetrical oxamides, isomerism in the position of the chlorine atom to give either 4-chloro-1,2-dialkylimidazole or 5-chloro-1,2-dialkylimidazole (or a mixture) is the only possibility. Nonsymmetrical oxamides, on the other hand, could in addition ring-close to give two isomeric 4-chloro- and 5-chloro-1,2-dialkylimidazoles depending on the nature of the alkyl groups attached to C-1 and C-2.

As indicated in the following section, attempts to demonstrate, by both gas chromatography and thin-layer chromatography, the presence of 4-chloroimidazoles as contaminants of the Wallach reaction failed. By contrast, most of the nonsymmetrical oxamides on treatment with phosphorus pentachloride were readily shown to yield an isomeric mixture of

TABLE V: The picrates of some 1,2-dialkylimidazoles and the corresponding 5-chloro-1,2-dialkylimidazoles.

5-chloro-1,2-dialkyl-imidazole		Picrate	1,2-dialkylimidazole		Picrate
R ₁	R ₂	M.p. °C.	R ₁	R ₂	M.p. °C.
butyl	propyl	96.5-97.5	butyl	propyl	108.7-109.4
<u>iso</u> -butyl	<u>iso</u> -propyl	173.5-174.5	<u>iso</u> -butyl	<u>iso</u> -propyl	139-140
propyl	ethyl	155.0-156.5	propyl	ethyl	139.0-140.3
ethyl	propyl	111.0-112.0	ethyl	propyl	124.5-125.2

5-chloro-1,2-dialkylimidazoles.

The five nonsymmetrical oxamides subjected to the Wallach reaction in the present investigation, and the possible 5-chloro isomers derived therefrom, are listed in Table II (page 21). The bases prepared from butylethyloxamide, butylpropyloxamide and n-butyl-iso-butyloxamide were shown to be mixtures, and their separation is discussed in detail below. The base resulting from the action of phosphorus pentachloride on benzylmethyloxamide was shown to be a single compound (5-chloro-1-methyl-2-phenylimidazole), the isomeric 1-benzyl-5-chloroimidazole not being detected either by thin-layer chromatography or by chemical means. The product obtained from ethylmethyloxamide was shown to yield a picrate of sharp melting point but as work on this base was discontinued early in the investigation, it is not possible to state whether the product was pure or a mixture of isomers with a congruent melting point.

The following section indicates the methods investigated to separate, either analytically or preparatively, the various isomeric bases listed in Table II, as well as the separation of 4-chloro and 5-chloro isomers by gas and thin-layer chromatography.

(i) Fractional distillation.

The basic product prepared by the action of phosphorus pentachloride on butylethyloxamide distilled at 232-236°/620 mm. and the picrate, despite repeated recrystallisations, melted over a wide range. The base was shown to be a mixture of two components by both thin-layer and gas chromatography. Separation into two isomers (later shown to be 5-chloro-1-ethyl-

TABLE VI: Summary of the methods used to separate isomeric 5(4)-chloro-1,2-dialkylimidazoles.

Dialkylloxamide	5-Chloro-1,2-dialkylimidazole.	G.L.C.	T.L.C.	Ion-exch. C.	Fract. dist.	Fract. cry.
Butylethyloxamide	5-Chloro-1-ethyl-2-propyl-imidazole 1-Butyl-5-chloro-2-methyl-imidazole	Yes	Yes	Yes	Yes	No
<u>n</u> -Butyl- <u>iso</u> -butyl-oxamide	1- <u>n</u> -Butyl-5-chloro-2- <u>iso</u> -propylimidazole 1- <u>iso</u> -Butyl-5-chloro-2- <u>n</u> -propylimidazole	Yes	No	No	No	Yes
Butylpropyloxamide	5-chloro-1,2-dipropylimidazole 1-Butyl-5-chloro-2-ethyl-imidazole	Yes	No	Yes	No	No
Dipropyloxamide	4-chloro-2-ethyl-1-propyl-imidazole 5-chloro-2-ethyl-1-propyl-imidazole	Yes	Yes	-	-	-
Dibutyloxamide	1-Butyl-4-chloro-2-propyl-imidazole 1-Butyl-5-chloro-2-propyl-imidazole..	-	Yes	-	-	-

Code: Yes: tried successfully; No: tried unsuccessfully; -: not attempted.

2-propylimidazole and 1-butyl-5-chloro-2-methylimidazole) was achieved by fractional distillation. Preliminary studies indicated that the boiling points of the isomers were close and a high efficiency fractionating column would be required to effect separation. The column finally used was 100 x 1.25 cm. in dimension and was filled with glass coils (4 x 4 mm). These packings were larger than recommended for a column of the size used, but smaller packings (Helipak 2.5 x 2 x 1 mm), though theoretically more effective, resulted in serious flooding of the column when the distillation was conducted under reduced pressure.

All the bases examined distilled satisfactorily at normal pressure but prolonged heating resulted in serious pyrolysis and rendered fractional distillation at normal pressure impractical. Experiment showed that a pressure of 56 mm. reduced the boiling point of the base by approximately 100° whence slow distillation became feasible but flooding of the column could be controlled. Even at this lower temperature, however, pyrolysis was considerable. A very slow "throughput" or vapour velocity was necessary to minimise flooding of the column and made careful heating of the distillation flask imperative. The use of a nitrogen "leak" to promote even boiling was impractical and was replaced by two pieces of a medical wooden throat spatula as suggested by Carney (1949).

Under such conditions a successful, if slow, fractional distillation was possible for the isomers already discussed.

A vacuum-jacketed vapour-dividing head and a reflux ratio valve operated by an electronic timer were also used. While on theoretical grounds a reflux ratio of 50:1 or larger would have provided the best separation, it was impractical to employ such large ratios in the present study owing to the slow "throughput". For the most part, it was convenient to collect the distillate for 2 or 3 seconds every 30 seconds - a ratio of 1:15 or 1:10. Heat losses were minimised during the distillation by vacuum jacketing the column. The remaining heat loss was compensated for by two independent heating coils with individual "variac" controls so that the distillation could be conducted, as far as possible, adiabatically.

Fractions of 2-5 ml. capacity were collected during the distillation in a collector which permitted removal of the sample without breaking the vacuum, and a few drops of each fraction converted to the picrate and the melting point examined. This was regarded as superior to following the boiling point as a measure of assessing the purity of the distillate.

The need for reduced pressure in the distillation was reflected in the closeness of the boiling points and was possibly responsible why other isomers examined could not be separated by distillation. Thus the present method failed to separate the isomers from butylpropyloxamide and n-butyl-iso-butyloxamide.

(ii). Fractional crystallisation.

The picrate of the basic material prepared by the action of phosphorus pentachloride on n-butyl-iso-butyloxamide melted in the region of 130-145°C. This wide range, not diminished by several recrystallisations from ethanol, was consistent with the presence of isomers as predicted by theory (page 12). Fractional distillation failed to separate the free bases (page 36) and attempts were then made to effect separation of the picrates by fractional crystallisation.

It was eventually shown that the slow crystallisation of a dilute solution ($\pm 10\%$) of the isomeric mixture of picrates from absolute ethanol resulted in the formation of a small quantity of material of sharp melting point. In some experiments, picrate crystals of melting point 149-152° (called Isomer A) were obtained, while in other seemingly identical experiments, crystals of a picrate melting between 159-163° (Isomer B) deposited. (A further crystallisation of these products improved the melting points to 150-152° and 163-165° respectively). In yet other recrystallisations of the isomeric picrates conducted under similar conditions, no improvement of the melting point occurred. Despite considerable investigation, the conditions controlling the deposition of a particular isomer or a mixture of picrates were not ascertained. "Seeding" the solution with crystals of the pure isomer failed to initiate selective crystallisation. Neither repeated crystallisation nor conventional fractional crystallisation over several stages produced an improvement in the melting point of

the product. Attempts to find alternative salts for recrystallisation, (3,5-dinitrobenzoates, styphnates or picrolonates), or better solvents (n-propanol, iso-propanol, acetone, dioxan, n-butanol or various mixtures) were all unsuccessful. Thus, while it proved possible to obtain small samples of the two isomeric picrates, the method was unreliable and was eventually abandoned.

Attempts to separate the isomers derived by the action of phosphorus pentachloride on other non-symmetrical oxamides were completely unsuccessful.

(iii). Gas chromatography.

An analytical study, by gas-liquid chromatography, of five Wallach bases prepared from both symmetrical and nonsymmetrical oxamides was undertaken to determine the presence of isomers in each sample. The high resolving power of the technique made it appear to be the one most likely to detect isomerism, if present, though the micro nature of the method precluded its use in any but analytical examination.

Two forms of isomerism were investigated. First, bases from both symmetrical and nonsymmetrical oxamides were examined for the presence of a mixture of the 4-chloro and 5-chloro- isomers (see page 12). As will be shown later, within the limits of the technique, no trace of the 4-chloro isomer in the samples studied could be detected. Secondly, in the case of the bases from butylpropyloxamide (other nonsymmetrical oxamides gave bases which, on chemical evidence alone, were clearly mixtures) the presence of a mixture of the two theoretically possible isomers, 5-chloro-1,2-

dipropylimidazole and 1-butyl-5-chloro-2-ethylimidazole, could not be conclusively determined on chemical evidence. Gas chromatography of the sample showed clearly that two compounds were present, presumably the suspected isomers.

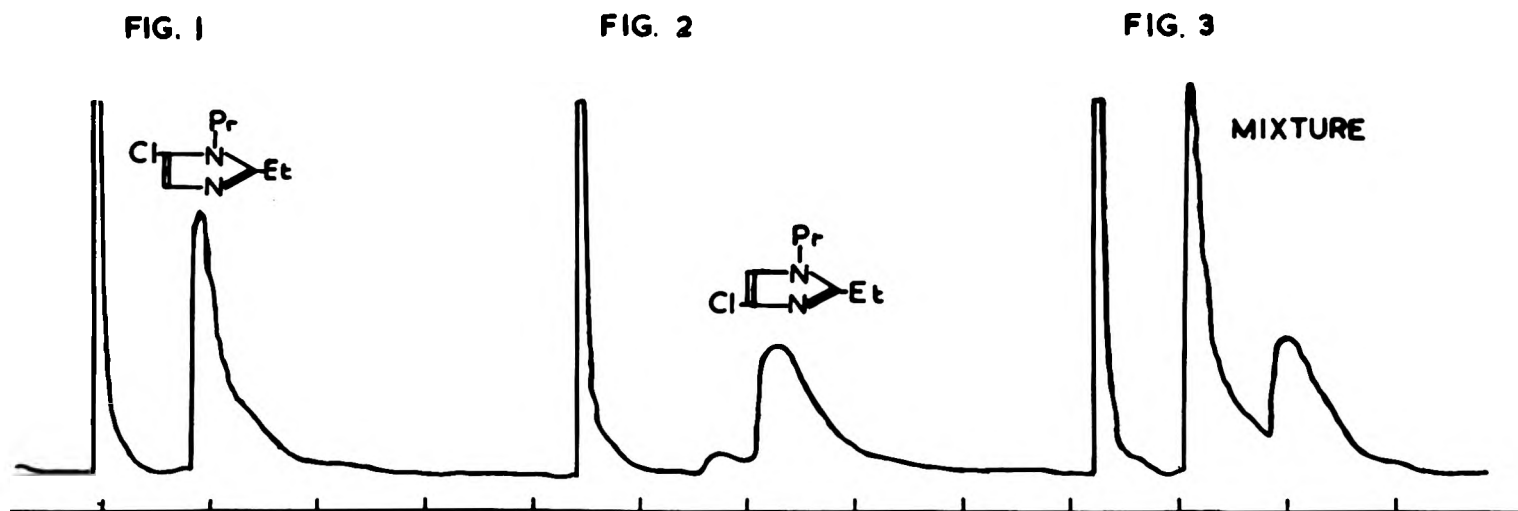
The resolving power of the column was tested for isomeric 4-chloro- and 5-chloroimidazoles with 5-chloro-2-ethyl-1-methylimidazole and 4-chloro-2-ethyl-1-propylimidazole [prepared from the 5-chloro-isomer as described by Sarasin (1923)]. Good resolution of the two isomers was obtained, the retention time for the 5-chloro isomer being less (5.4 mins.) than that for the 4-chloro isomer (10.2 mins.). The results are presented in Fig. III. A trace of the 5-chloro isomer was found in the sample of 4-chloro-2-ethyl-1-propylimidazole examined (Fig.2) but in view of the method of preparation, this was to be expected.

Bases from dipropyloxamide and di-n-butyloxamide were then examined in an attempt to detect the presence of 4- and 5-chloro isomers. In no case was more than one peak detected, an observation strongly indicative of the absence of isomers.

Examination of the bases from three nonsymmetrical oxamides showed in all cases, the presence of isomers, though resolution was not always good. Isomerism in these cases was due to variation in the alkyl groups.

Footnote: As the gas chromatography was conducted in the Organic Division of the C.S.I.R. by courtesy of the Director, it was not practical to extend this study to a variety of stationary phases as the present author would have wished.

GAS CHROMATOGRAPHIC SEPARATION OF 4-CHLORO-2-ETHYL-1-PROPYL-
IMIDAZOLE AND 5-CHLORO-2-ETHYL-1-PROPYLIMIDAZOLE

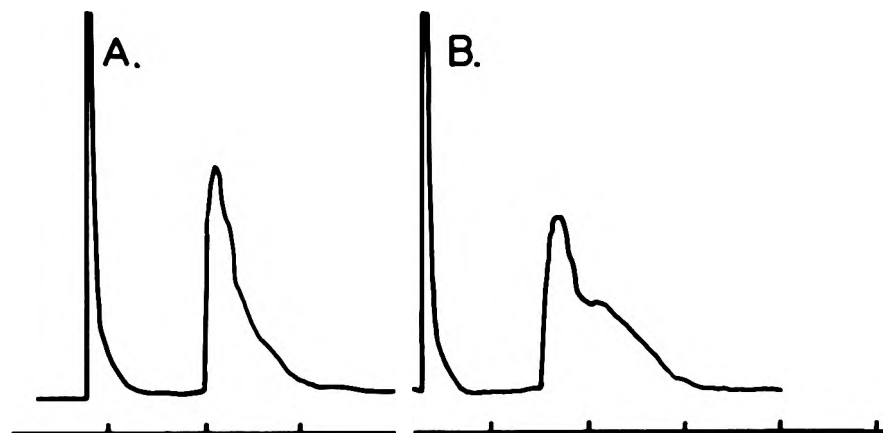


The product from the action of phosphorus pentachloride on butylpropyloxamide was shown to consist of two components. Chemical evidence in the case of this base was not conclusive in deciding whether or not the base was a single substance or a mixture of isomers. Attempts to purify the base by repeated crystallisation of the picrate gave a product of sharp melting point from which the free base was regenerated. Examination of the gas chromatogram showed the same two peak pattern described. No purification was therefore achieved. In order to check that the second peak in the above experiment is not a decomposition product formed by the action of heat on the base, 5-chloro-1,2-dipropylimidazole, was synthesised by an independent technique eliminating the possibility of isomerism (see page 110). The presence of a single peak only in the chromatogram indicates that the second peak in the original analysis was not, in fact, an artifact. The results are shown in Fig. IV.

(iv). Paper and Thin-layer chromatography.

The author examined a number of standard techniques for the separation of imidazole derivatives. Application of these methods to both 5-chloro-1,2-dialkylimidazoles and 5-chloro-1,2-dialkyl-4-nitroimidazoles was not successful. These techniques consisted of both paper chromatography and thin-layer chromatography on silica gel, alumina and cellulose. Invariably compounds moved either with the solvent front or with very high R_f values and poor or negligible resolution. It was concluded that normal adsorption or partition chromatography

FIG. IV. GAS CHROMATOGRAPHY OF THE WALLACH
BASES FROM BUTYLPROPYLOXAMIDE



- A. 5-CHLORO-1,2-DIPROPYLMIDAZOLE (GRÄNACHER'S METHOD).
- B. WALLACH BASES FROM BUTYLPROPYLOXAMIDE.

was unsuitable for the present compounds probably due to the poor solubility of the test materials in water, the stationary phase.

Attempts were subsequently made to separate the isomeric bases by "reversed-phase" chromatography by thin-layer chromatography on cellulose powder impregnated with formamide or dimethylformamide. Heptane, dekalin, cyclohexane, chloroform, benzene and various other organic solvents were examined for use as mobile phase without success. In all cases the bases ran close to the solvent front. The use of dimethyl-sulphoxide impregnated cellulose and benzene as mobile phase was likewise, unsuccessful. Somewhat better results were found with cellulose impregnated with diethyl tartrate developed with heptane. R_f values were small, however, and resolution inadequate.

Successful resolution of most of the bases examined was finally achieved by the use of propylene glycol impregnated cellulose as stationary phase and dekalin as mobile phase. A wide range of R_f values was observed for the Wallach bases examined, and the technique successfully resolved various known mixtures of 4-chloro and 5-chloro isomers. The isomeric bases from butylethyloxamide, namely 5-chloro-1-ethyl-2-propylimidazole and 1-butyl-5-chloro-2-methylimidazole were also successfully separated but the method failed to resolve the isomers from either butylpropyloxamide or n-butyl-iso-butyl-oxamide. The latter isomers formed and elongated

TABLE VII.

R_f values of chloroimidazoles on "reversed-phase" thin-layer chromatography.

Compound.	R _f
4-chloro-2-ethyl-1-propylimidazole	0.14
5-chloro-2-ethyl-1-propylimidazole	0.23
1-butyl-4-chloro-2-propylimidazole	0.32
1-butyl-5-chloro-2-propylimidazole	0.46
1- <u>iso</u> -butyl-5-chloro-2- <u>iso</u> -propyl- imidazole	0.50
2-benzyl-5-chloro-1-methylimidazole	0.20
5-chloro-1-ethyl-2-methylimidazole	0.09
5 chloro-1-ethyl-2-propylimidazole	0.21
1-butyl-5-chloro-2-methylimidazole	0.18

spot when examined by extended-run thin-layer techniques. Efforts to improve resolution by using a variety of solvents also failed.

Digol impregnated plates offered no advantage over propylene glycol. Spots were detected by the standard "Dragendorff" reagent for alkaloids. Table VII lists the R_f values for a number of bases. As the degree of impregnation of the cellulose varied, despite a standardised procedure, R_f values served only as a rough guide and standard materials were always run simultaneously with test materials.

Examination of crude Wallach bases for the presence of 4-chloro isomers, readily detected by the technique, showed them to be absent thus confirming the results of gas chromatography and the analysis for the ammonia produced on fission of the amino amide (see page 16 and 107).

(v). Ion-exchange chromatography.

The basic nature of the imidazoles under study suggested the application of ion-exchange chromatographic techniques, for provided the pK 's of the isomeric bases differ sufficiently, separation should readily be achieved. Advantage may also be taken of the high capacities of ion-exchange chromatography (as compared with) partition or adsorption chromatography) to separate isomeric bases on a preparative scale.

In order to establish suitable experimental conditions, a preliminary investigation was conducted with the bases prepared from butylethyl-

oxamide which had already been separated by fractional distillation (page 33). The strong cation exchange resin, Dowex 50 x 8% cross-linkage, was used in the H^+ form. The base in the eluent was detected by absorption in the ultra violet region of the spectrum. The U.V. absorption curve of a number of 5-chloro-1,2-dialkylimidazoles had shown that the maximum absorption usually arises in the region of 220 m μ and varies only slightly with pH. (see page 74). At this wavelength, interference from several solvents and inorganic ions becomes marked (Bladen, 1964). Ethanol, for example, has only 56% transmission at 220 m μ compared with that at 300 m μ . These features make impractical such techniques as eluting the free bases from a Dowex 50 column in the NH_4^+ form ^{with} ammonium chloride buffers in 50% ethanol (Parish, personal communication).

The technique finally adopted consisted in eluting the isomeric bases from a column of Dowex 50 in the H^+ form with N-hydrochloric acid and examination of the fractions for absorbance at 220 m μ . A large volume of acid was required and baseline values were usually elevated above that for unused eluent, presumably due to some measure of leaching of U.V. active material from the column packing.

Separation of the isomers produced by the action of PCl_5 on butylethyloxamide was achieved on a 22 cm. column as shown in Figure V. Experiments with the individual isomers showed that 5-chloro-1-ethyl-2-propylimidazole was eluted before 1-butyl-5-chloro-2-methylimidazole.

FIG. V. ION EXCHANGE CHROMATOGRAPHY OF THE ISOMERIC BASES FORMED BY THE ACTION OF PCl_5 ON BUTYLETHYLOXAMIDE.

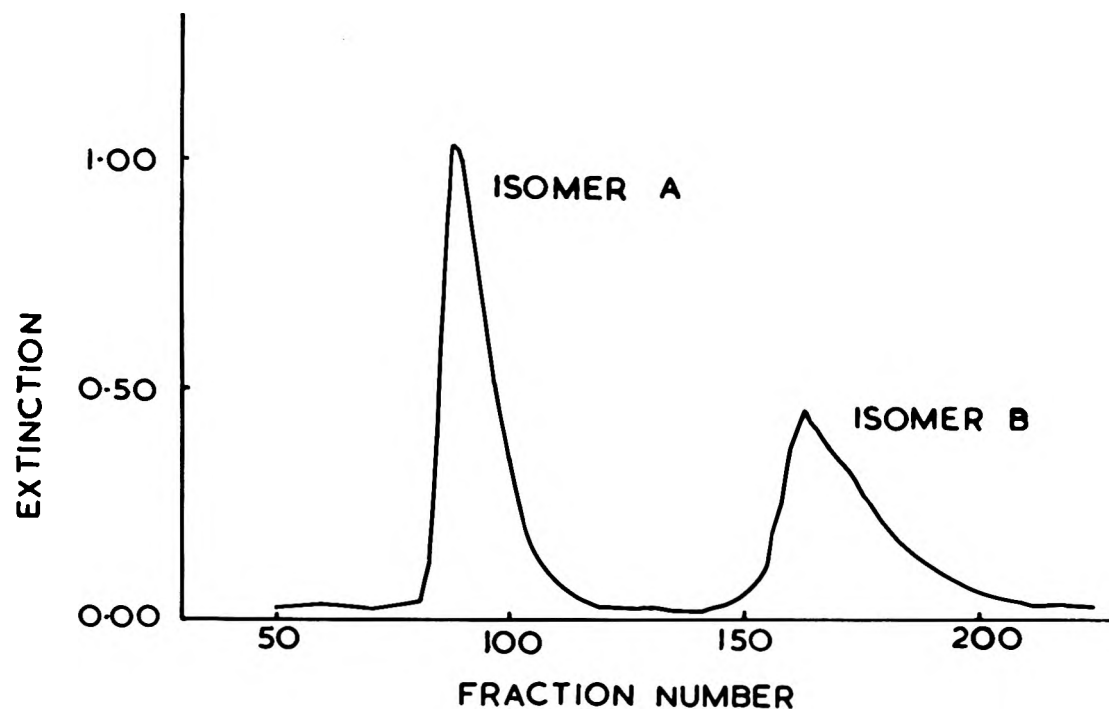


FIG. VI. ION EXCHANGE CHROMATOGRAPHY OF THE ISOMERIC BASES FORMED BY THE ACTION OF PCl_5 ON *n*-BUTYL-ISO-BUTYL-OXAMIDE.

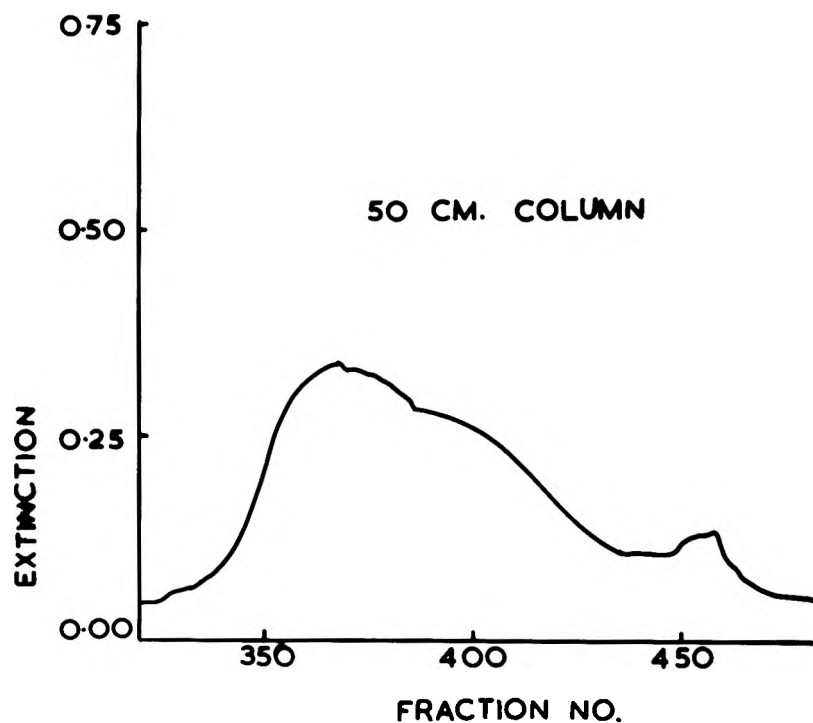
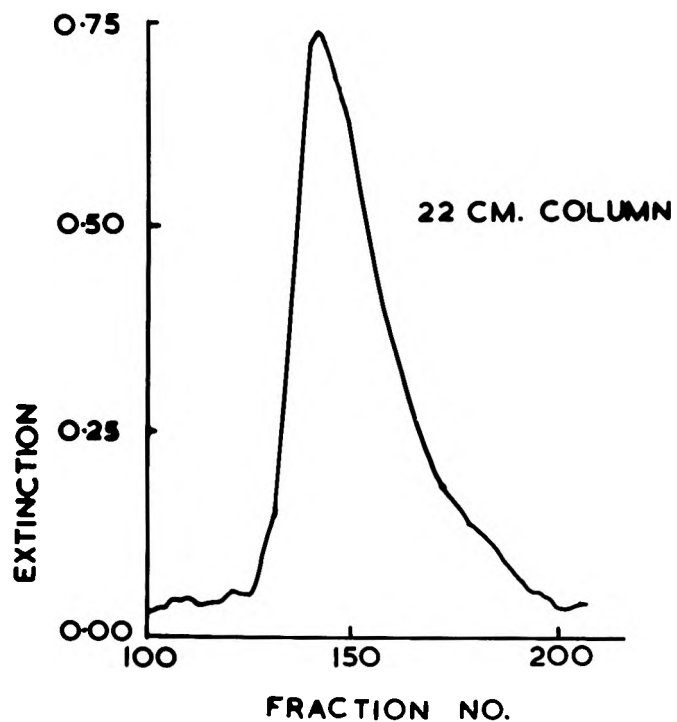
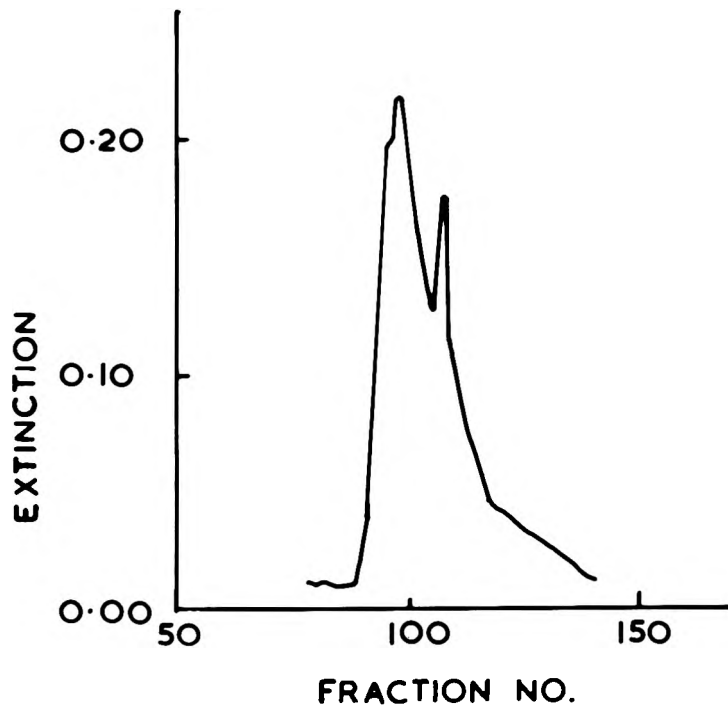


FIG. VII. ION-EXCHANGE CHROMATOGRAPHY OF THE ISOMERIC BASES FORMED BY THE ACTION OF PCl_5 ON BUTYLPROPYLOXAMIDE.



Extensions of the technique to the isomeric bases derived from n-butyl-iso-butyloxamide showed no separation but a single broad-based peak (Fig.VI). Increasing the length of the column from 22 cm. to 50 cm. also failed to effect separation of the isomers though the curve obtained (Fig.VI.) suggested the presence of two components. Because of the extended time required for elution of the isomers as well as the unsharp nature of the peaks, no further increase in column length was tested.

Attempts to separate the isomeric Wallach bases derived from butylethyloxamide were partially successful, though the peaks obtained were broad and low. An attempt to improve the separation by reducing the retention of the resin for the bases was made by increasing the cross-linkage of the resin, but with little success. Figure VII shows the separation of 18 mg of mixed isomers on a 50 cm. column of Dowex 50 x 12 in the hydrogen ion form. In this experiment the size of the fractions collected was 100 ml. Some 12 litres of 1-N hydrochloric acid were necessary for this separation.

(e). Interpretation of experimental findings.

Of the Wallach bases prepared in the course of this investigation, the following were submitted to the scheme of degradation outlined above (page 15):-

5-chloro-1-ethyl-2-methylimidazole
5-chloro-2-ethyl-1-propylimidazole
1-n-butyl-5-chloro-2-n-propylimidazole
1-iso-butyl-5-chloro-2-iso-propylimidazole
1-n-butyl-5-chloro-2-methylimidazole
5-chloro-1-ethyl-2-propylimidazole

With minor provisos, all the intermediates were isolated and characterised. The final stage, in which fission of the imidazole ring is produced by HCl at 140°, was shown in all cases to furnish an N-alkylglycine and not glycine. It was found to be of practical advantage to isolate the alkylglycine as the N-benzenesulphonyl derivative or, in the case of N-propylglycine, as the N-p-toluenesulphonyl derivative. The latter modification arises because N-benzenesulphonyl-N-propylglycine is difficult to isolate due to abnormally high solubility. In general, these derivatives possess sharp melting points and analyse well, two properties which ensure their suitability for identification purposes. Table VIII (page 53) tabulates the N-alkylglycine found, and the particular derivative employed for isolation and identification.

The carboxylic acid moiety, separated by steam distillation from the mixture of products produced by the HCl fission, was isolated and identified as the p-bromophenacyl esters. In the case of acetic acid, formed from bases containing a methyl group in position 2, several attempts to isolate the p-bromophenyl-acetate failed and successful isolation of this particular

acid was only achieved by precipitation of S-benzyl thiuronium acetate. In one case even this derivative could not be obtained (5-chloro-1-ethyl-2-methylimidazole).

Quantitative examination of the ammonia produced by acid degradation of the aminoamide of the 1,2-dialkylimidazole was undertaken on separate samples of a size more suitable for analysis by the Nessler procedure than in the main experiments. Within the limits of experimental error, all bases yield 3 moles of NH_3 per mole of original base, indicative of a 5-chloroimidazole and confirming, as outlined earlier, that the bases do not contain a significant proportion of the 4-chloroimidazole isomer. From these experiments it cannot be entirely discounted that a minor amount of 4-chloro-1,2-dialkylimidazole contaminating the major supply of 5-chloroimidazole could have been removed at one of the intermediate stages during (say) a recrystallisation. The symmetrical nature of the oxamide gives no basis to anticipate anything but a mixture of 4- and 5-chloro isomers. Nevertheless, taken in conjunction with other evidence, particularly that from chromatography which failed to show the presence of such isomers, the above results indicate that the 4-chloro isomers are, in all probability, absent.

It is concluded for the symmetrical oxamides investigated that diethyl oxamide gives 5-chloro-1-ethyl-2-methylimidazole; dipropyl oxamide gives 5-chloro-2-ethyl-1-propylimidazole; di-iso-butyl-oxamide gives 1-iso-butyl-5-chloro-2-iso-propyl-

TABLE VIII: Some Wallach bases and their degradation products.

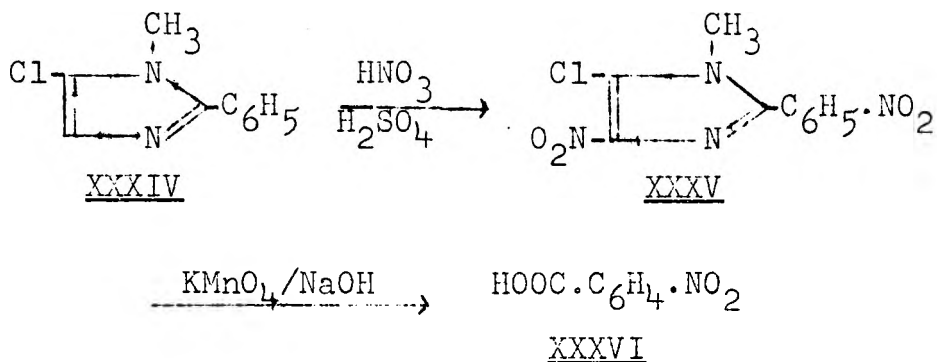
5-Chloro-1,2-dialkyl- imidazole	Degradation Products.			
	N-alkylglycine	Deriv.	Carboxylic acid.	Deriv.
5-Chloro-1-ethyl-2-methylimidazole	N-ethylglycine	A	acetic acid	E
5-Chloro-2-ethyl-1-propylimidazole	N-propylglycine	B	propionic acid	C
1-Butyl-5-chloro-2-propylimidazole	N-butylglycine	A	butyric acid	C
1- <u>iso</u> -butyl-5-chloro-2- <u>iso</u> -propyl- imidazole	N- <u>iso</u> -butyl- glycine	A	<u>iso</u> -butyric- acid	C
1-Butyl-5-chloro-2-methylimidazole	N-butylglycine	A	acetic acid	D
5-Chloro-1-ethyl-2-propylimidazole	N-ethylglycine	A	propionic acid	C

Code: A. N-benzenesulphonate.
 B. N-p-toluenesulphonate.
 C. p-Bromophenacyl ester.
 D. S-benzyl-thioronium salt.
 E. not isolated.

imidazole and di-n-butyloxamide gives 1-butyl-5-chloro-2-propylimidazole on treatment with phosphorus pentachloride. See Tables I and II.

The action of PCl_5 on benzylmethyloxamide can theoretically yield two bases - 5-chloro-1-methyl-2-phenylimidazole and 1-benzyl-5-chloroimidazole. Application of the established technique for the isolation of the Wallach bases produced a white crystalline solid of sharp melting point suggestive of a single compound. The possibility of a small amount of the isomeric base was not, however, discounted.

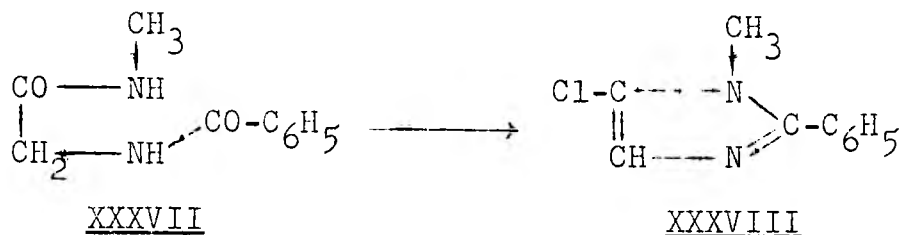
The structure of the base was ascertained by exploiting the ease with which the 2-phenylimidazoles are oxidised by alkaline potassium permanganate to give benzoic acid (Grant and Pyman, 1921) instead of the series of reactions employed hitherto. Hence, the nitrated Wallach base from benzylmethyloxamide was degraded to p-nitrobenzoic acid.



Had ring closure furnished the isomeric 1-benzyl-5(4)-chloroimidazole, the isolation of p-nitrobenzoic acid could only have arisen through the

somewhat unlikely oxidation of the N-benzyl group. These experiments show that the base is 5(4)-chloro-1-methyl-2-phenylimidazole. The position of the chlorine remains undetermined. By analogy, it is probable that the base is a 5-chloroimidazole.

This conclusion was amply confirmed by an independent synthesis of 5-chloro-1-methyl-2-phenylimidazoles by the method of Gränacher (1925). Hippuryl methylamide, synthesised by standard techniques, under the action of PCl_5 , ring closes to give the required base; it and its' picrate were identical with the base and its' picrate prepared by the Wallach reaction. The I.R. spectra



shown in Figure VIII (page 57) are superimposable.

Examination of the literature showed that 5-chloro-2-methyl-1-phenylimidazole has been synthesised (Gränacher, 1925) but the yield was so poor that only the picrate could be isolated. The latter had a melting point of 176° , very similar to that for the picrate of the Wallach base from benzylmethyl-oxamide (i.e. 177-178) and raises the possibility that the Wallach base is, in fact, 5-chloro-2-methyl-1-phenylimidazole; possibly by some rearrangement of the alkyl groups. Accordingly, the picrate of 5-chloro-2-methyl-1-phenylimidazole

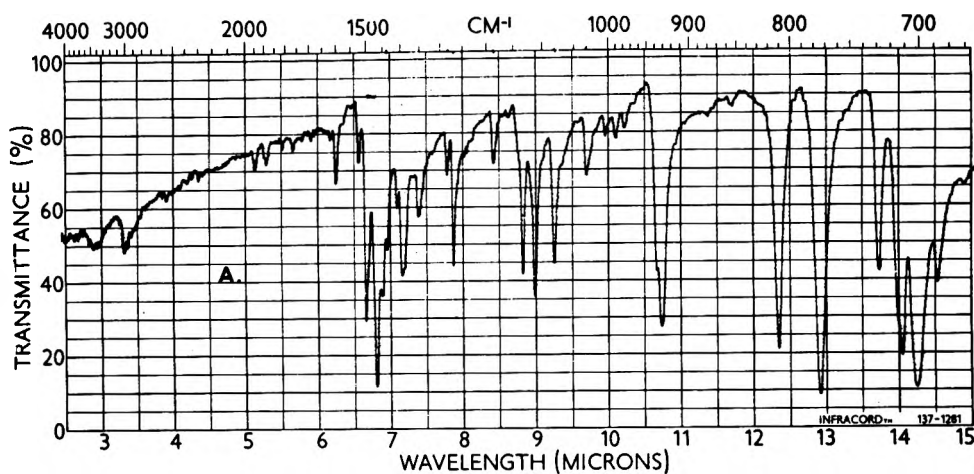
was prepared as described by Gränacher (loc.cit.) and compared with the picrate of the Wallach base. A mixed melting point determination showed clearly that the compounds were different. It may be stated therefore, that the product of the reaction of PCl_5 on benzylmethyloxamide is 5-chloro-1-methyl-2-phenylimidazole.

(f). The infrared spectra of the 5-chloro-1,2-dialkylimidazoles.

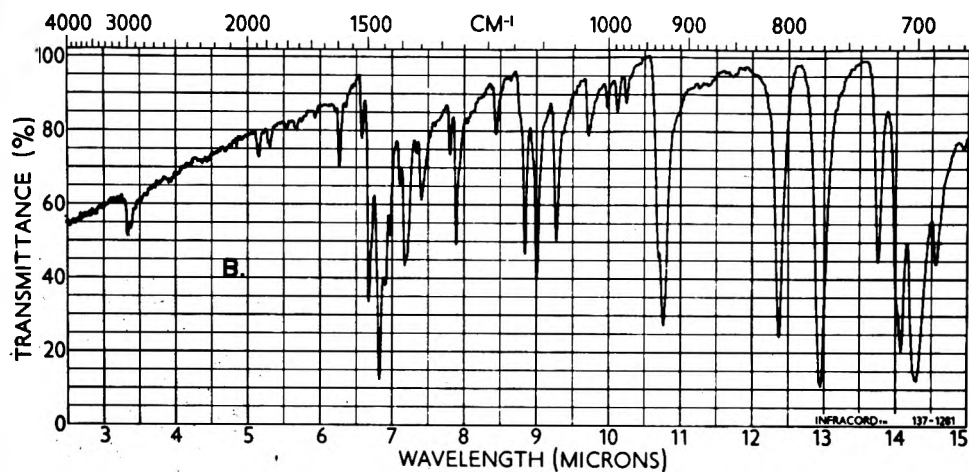
Relatively little data on the infrared spectra of substituted imidazoles is available in the literature. The spectrum of imidazole itself has been reported (Otting, 1956; Garfinkel and Edsall, 1958), and the latter workers also examined the spectrum of histidine. A detailed study of the infrared spectra of some arylimidazoles and arylisoimidazoles has recently been described (White and Sonnenberg, 1964), but these workers confined their data mainly to the 1625 cm^{-1} - 1475 cm^{-1} and 1200 cm^{-1} - 700 cm^{-1} ranges.

In the present study a number of 5-chloro-1,2-dialkylimidazoles were examined to establish the spectral pattern for this type of compound. The data were supplemented with spectra from a few other 1,2-dialkylimidazole derivatives (see Table IX) to assist in the interpretation of the peak produced by the aromatic type imidazole proton. The data were employed to confirm the structure of the base obtained by the action of phosphorous pentachloride on di(2-phenylethyl)-oxamide (see section g).

FIGURE VIII. INFRARED SPECTRA OF 5-CHLORO-1-METHYL-2-PHENYLIMIDAZOLE PREPARED BY TWO INDEPENDENT METHODS.



(A). By the Wallach reaction.



(B). By the Gräner reaction.

The data presented in the present study will be divided into two parts covering the ranges 3500 cm^{-1} - 2700 cm^{-1} and 1650 cm^{-1} - 1200 cm^{-1} . No relevant data appeared in the 2700 cm^{-1} - 1650 cm^{-1} range, and as the compounds were usually dissolved in carbon tetrachloride, solvent absorption limits the value of the results in the lower frequency range.

3500 cm^{-1} (2.86μ) - 2700 cm^{-1} (3.70μ) region.

Typical methyl stretching vibrations were observed at approximately 2970 cm^{-1} (3.37μ) and 2900 cm^{-1} (3.45μ) - the latter was frequently present as a shoulder - and methylene vibrations at 2930 cm^{-1} (3.41μ) and 2870 cm^{-1} (3.48μ). In the arylimidazole (8; Table X), the phenyl aromatic C-H vibrations were observed as a triplet at 3070 cm^{-1} (3.26μ), 3035 cm^{-1} (3.30μ) and 2990 cm^{-1} (3.34μ). An ill-shaped peak at about 3130 cm^{-1} (3.20μ), on scale expansion, became sharply defined and was ascribed to the aromatic-type C-H vibration at carbon atom number four in the imidazole ring. Evidence in support of this conclusion was available from two sources. First, nitration of the chloroimidazole eliminated the 3130 cm^{-1} (3.20μ) band as shown by two examples (2 and 4; Table IX). Secondly, dechlorination of the Wallach base (5) results in the formation of a doublet at 3134 cm^{-1} (3.19μ) and 3110 cm^{-1} (3.22μ) which appear to be due to the two ring aromatic-type C-H stretching vibrations. These two peaks are best observed on expansion of the scale. The

findings are summarised in Tables IX and X and the spectra presented in figures VIII to XVIII.

1650 cm^{-1} (6.06 μ) - 1200 cm^{-1} (8.33 μ) region.

Very little information is available on the absorption of imidazole in the 1650 cm^{-1} (6.06 μ) to 1200 cm^{-1} (8.33 μ) band and assignment of frequencies to particular groups or skeletal vibrations is largely speculative. Nevertheless, a relatively constant pattern of bands emerges from the present study. At about 1460 cm^{-1} (6.85 μ) C-C deformation bands from the alkyl groups were discernable, as were C-CH₃ symmetrical deformation bands at 1380 cm^{-1} (7.25 μ) to 1390 cm^{-1} (7.19 μ).

A series of four bands which could not be ascribed to particular groupings was observed consistently in the present series of compounds, namely at approximately 1520 cm^{-1} (6.58 μ), 1490 cm^{-1} (6.71 μ), 1410 cm^{-1} (7.09 μ) and 1260 cm^{-1} (7.94 μ) (except compound 4 where there is no band at 1260 cm^{-1} (7.94 μ) but bands at 1285 cm^{-1} (7.78 μ) and 1225 cm^{-1} (8.17 μ)). Five-membered heteroaromatic rings, in general, are said to have four bands in this range (Katritsky, 1959) corresponding to specific skeletal modes. Three of these appear consistently between 1600 cm^{-1} (6.25 μ) and 1350 cm^{-1} (7.41 μ) and the fourth somewhat more variable, but in thiophen, is at 1252 cm^{-1} (7.99 μ). In the case of imidazole typical bands are said to arise at 1550 cm^{-1} (6.45 μ), 1492 cm^{-1} (6.71 μ), and 1451 cm^{-1} (6.89 μ). In the present

TABLE IX: The C-H stretching frequencies of some 1,2-dialkylimidazole derivatives.

No.	Compound.	CH ₃	CH ₂	Ring C-H
1.	5-Chloro-1-ethyl-2-methylimidazole	2983 cm ⁻¹ 2900 cm ⁻¹	2938 cm ⁻¹ 2870 cm ⁻¹	3130 cm ⁻¹
2.	5-Chloro-1-ethyl-2-methyl-nitro- imidazole ^(a)	2990 cm ⁻¹ 2875 cm ⁻¹	2940 cm ⁻¹ 2815 cm ⁻¹	-
3.	5-Chloro-2-ethyl-1-propylimidazole	2972 cm ⁻¹	2935 cm ⁻¹ 2876 cm ⁻¹	3128 cm ⁻¹
4.	5-Chloro-2-ethyl-4-nitro-1-propyl- imidazole.	2975 cm ⁻¹	2940 cm ⁻¹ 2880 cm ⁻¹	-
5.	1-Butyl-2-propylimidazole	2962 cm ⁻¹ 2905 cm ⁻¹	2932 cm ⁻¹ 2874 cm ⁻¹	3134 cm ⁻¹ 3110 cm ⁻¹
6.	1-Butyl-5-chloro-2-propylimidazole	2960 cm ⁻¹ 2900 cm ⁻¹	2930 cm ⁻¹ 2872 cm ⁻¹	(b).
7.	1- <u>iso</u> -Butyl-5-chloro-2- <u>iso</u> -propyl- imidazole	2968 cm ⁻¹ 2910 cm ⁻¹	2930 cm ⁻¹ 2874 cm ⁻¹	(b).

Footnotes: (a) Potassium bromide disc. (b) Peak present but not measurable without expansion.

TABLE X.

C-H stretching frequencies in an aryl-
imidazole.

No.	Compound.	CH ₃	CH ₂	Aromatic C-H	Imidazole C-H
8.	5-Chloro-1-methyl-2-phenyl- imidazole	2955 cm ⁻¹ 2890 cm ⁻¹	2925 cm ⁻¹ 2850 cm ⁻¹	3070 cm ⁻¹ 3035 cm ⁻¹ 2990 cm ⁻¹	3130 cm ⁻¹

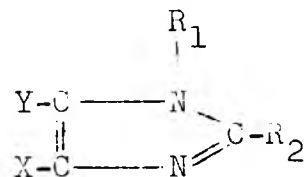
study the band at 1550 cm^{-1} (6.45μ) was resolved into two bands at 1540 cm^{-1} (6.49μ) and 1575 cm^{-1} (6.35μ) for imidazole in potassium bromide. It is tentatively suggested that the four bands found in the present series of compounds constitute a typical grouping for substituted imidazoles and possibly represents absorption frequencies for the specific skeletal modes discussed above.

In 5-chloro-1-methyl-2-phenylimidazole (8), the aromatic skeletal bands from the phenyl group appear at 1605 cm^{-1} (6.25μ), 1578 cm^{-1} (6.34μ), 1500 cm^{-1} (6.67μ), 1450 cm^{-1} (6.90μ). The 1578 cm^{-1} (6.34μ) band, more clearly defined on expansion of the scale, correlates well with the characteristic frequency for conjugated aromatic derivatives at 1580 cm^{-1} (6.33μ) (Randall, 1949).

(g). The infrared spectrum of the base formed by the action of phosphorus pentachloride on di-(2-phenylethyl)-oxamide.

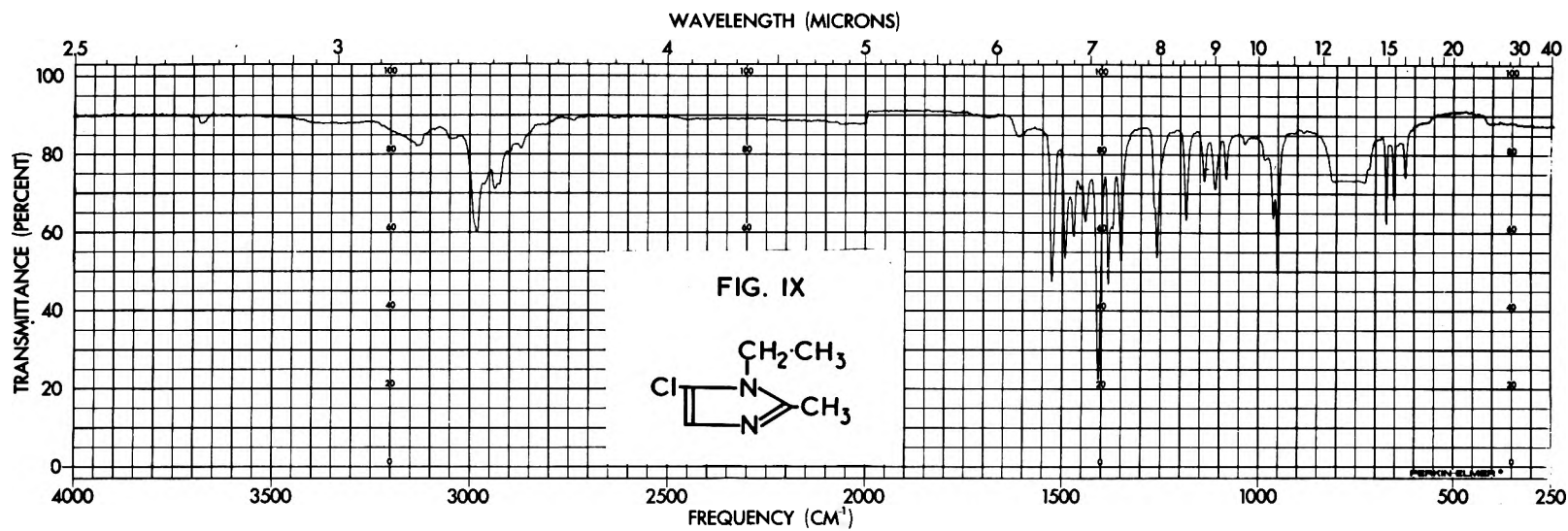
The spectrum is presented in Figures XIX and XX. Absorption at 3085 cm^{-1} (3.24μ), 3065 cm^{-1} (3.26μ), and 3029 cm^{-1} (3.30μ) is typical for aromatic C-H stretching in carbon tetrachloride solution, and indicates the presence of phenyl groups in the molecule as anticipated (see page 14). A small peak, clearly observed on expansion of the spectrum, at 3130 cm^{-1} (3.20μ) is consistent with the imidazole aromatic-type hydrogen already discussed (see page 58). Bands at 2925 cm^{-1} (3.42μ) and 2860 cm^{-1} (3.50μ) are indicative of methylene groups. A band at 2950 cm^{-1} (3.39μ) remains unexplained but is possibly an overtone of the band at 1480 cm^{-1} (6.76μ).

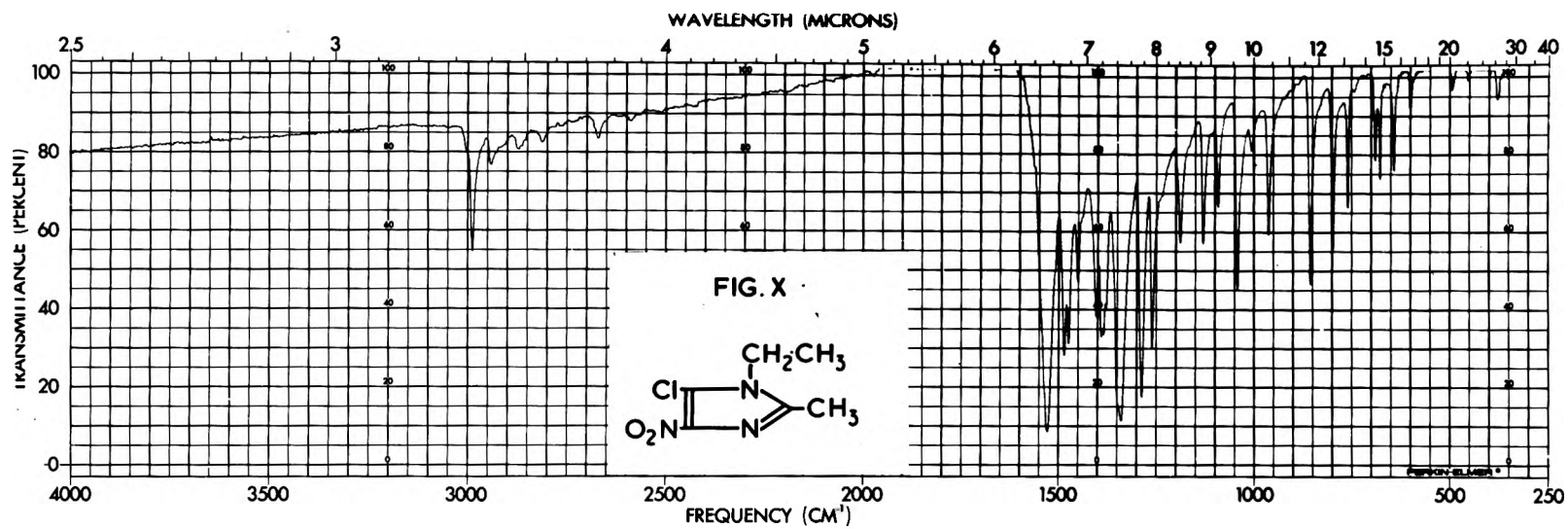
TABLE XI: Infrared Spectra of Imidazoles between 1600 cm⁻¹ and 1200 cm⁻¹.

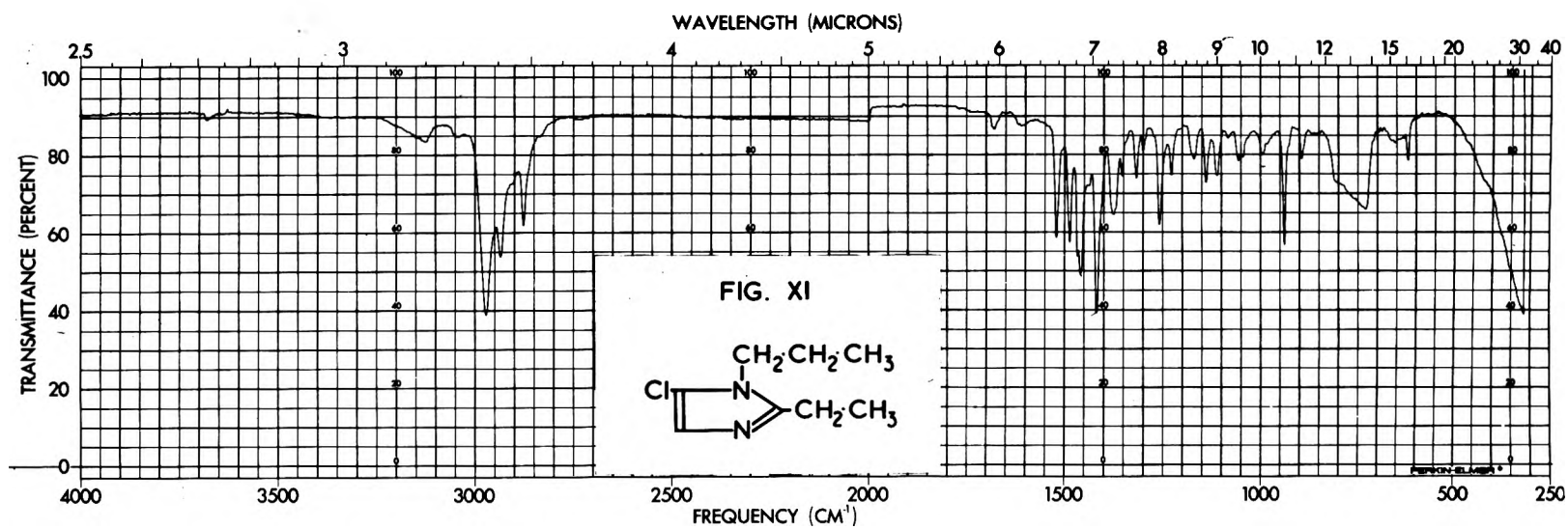


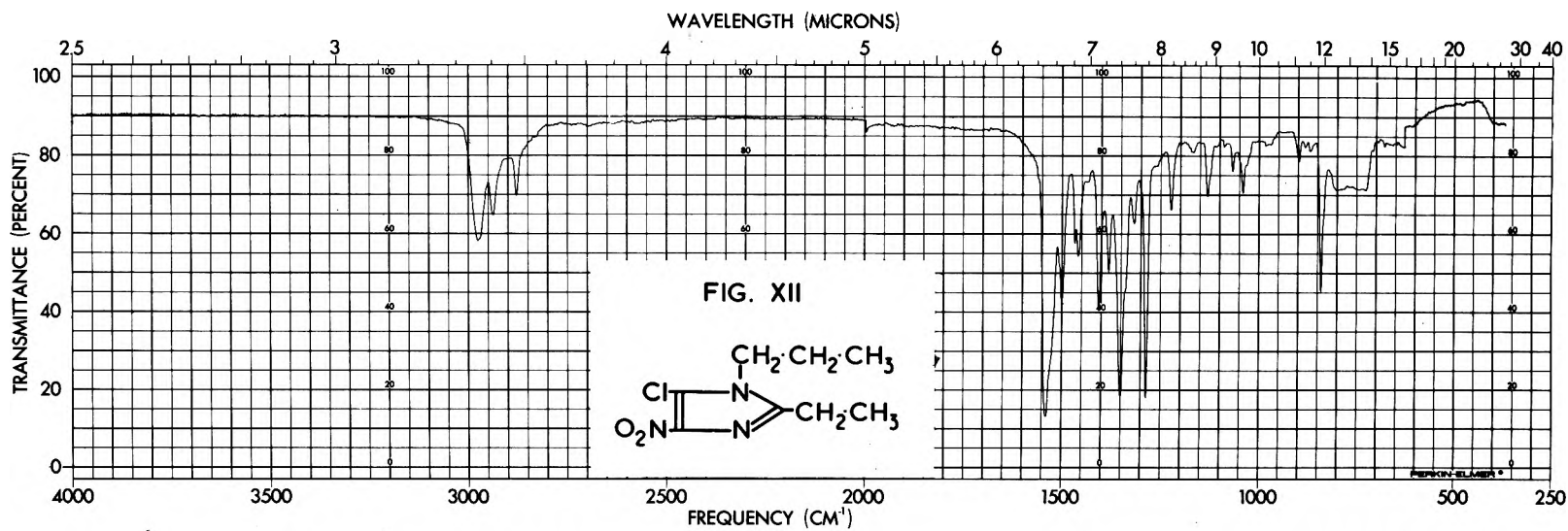
Cpd.	R ₁	R ₂	X	Y	1520 cm ⁻¹	1490 cm ⁻¹	1470 cm ⁻¹	1410 cm ⁻¹	1380 cm ⁻¹	1260 cm ⁻¹
1.	Et	Me	H	Cl	1527 (m)	1490 (m)	1470 (m)	1410 (m)	1380 (m)	1260 (m)
2*	Et	Me	NO ₂	Cl	1530 (s)	1485 (s)	1475 (s)	1404 (s)	1380 (s)	1260 (s)
3.	Pr	Et	H	Cl	1520 (m)	1487 (m)	1470 (m)	1420 (s)	1375 (m)	1260 (m)
							1460 (m)			
4.	Pr	Et	NO ₂	Cl	1540 (s)	1500 (m)	1470 (m)	1410 (m)	1380 (m)	1285 (s)
					1525 ^{sh} (m)		1460 (m)			1225 (w)
5.	Bu	Pr	H	H	1520 (w)	1490 (m)	1460 (m)	1428 (m)	1380 (w)	1260 (w)
6.	Bu	Pr	H	Cl	1520 (m)	1480 (m)	1460 (s)	1412 (s)		1260 (m)
7.	<u>iso</u> -Bu	<u>iso</u> -Pr	H	Cl	1520 (m)	1483 (m)	1465 (s)	1415 ^{sh} (w)	1390 (w)	1260 (m)
								1425 (m)		
8.	Me	Ph	H	Cl	1525 (w)	1490 ^{sh} (w)	1463 (s)	1402 (w)	1382 (m)	1265 (w)

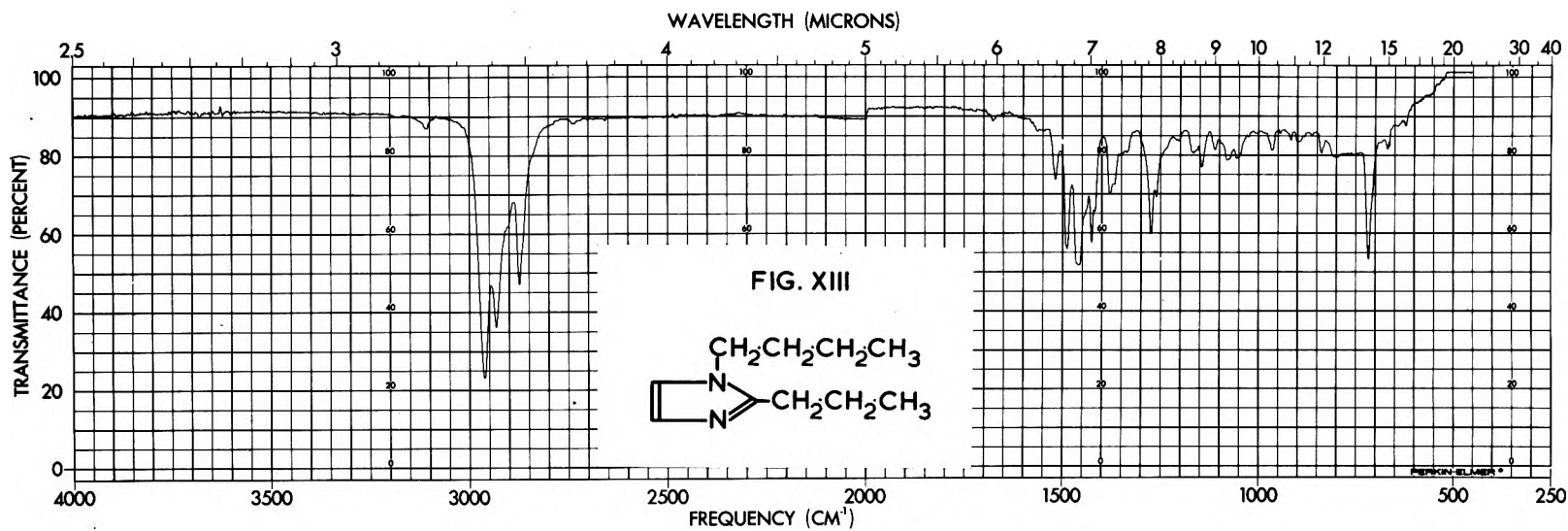
* in potassium bromide. sh=shoulder; s=strong; m=medium; w=weak

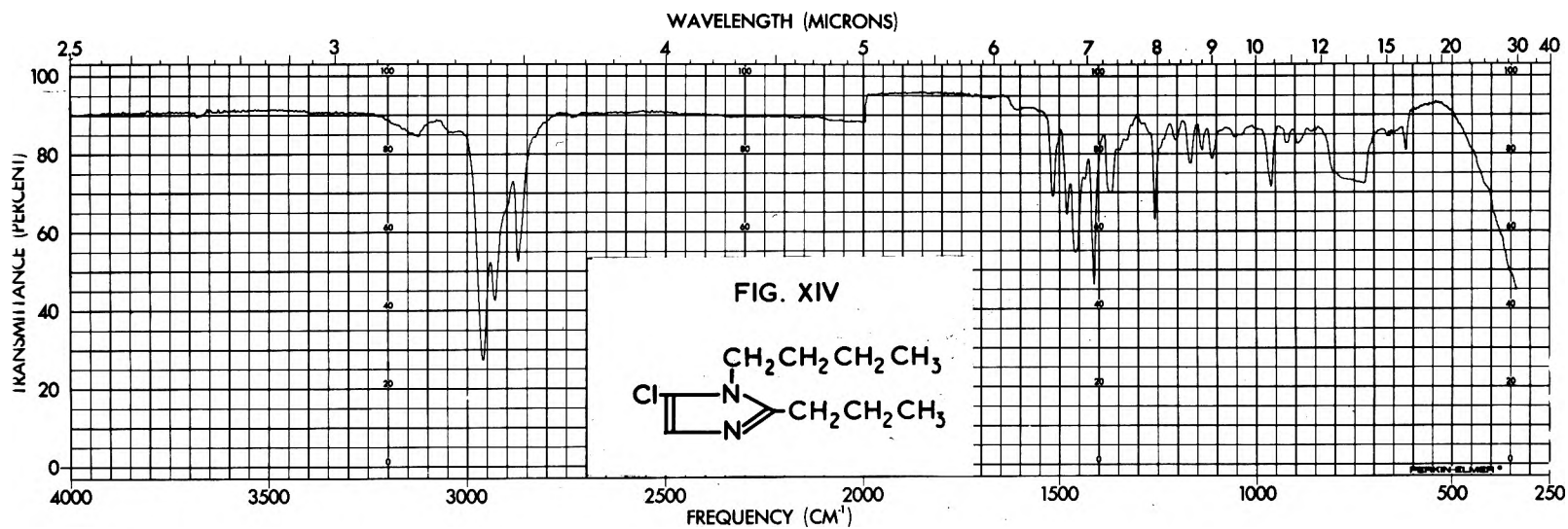


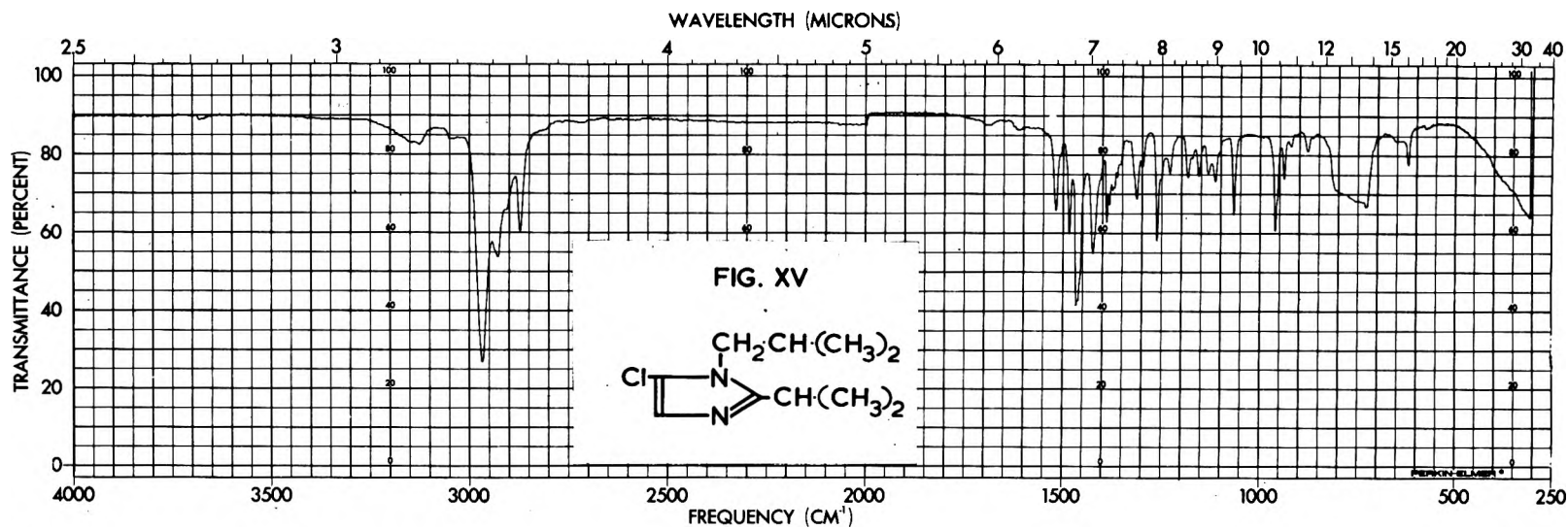












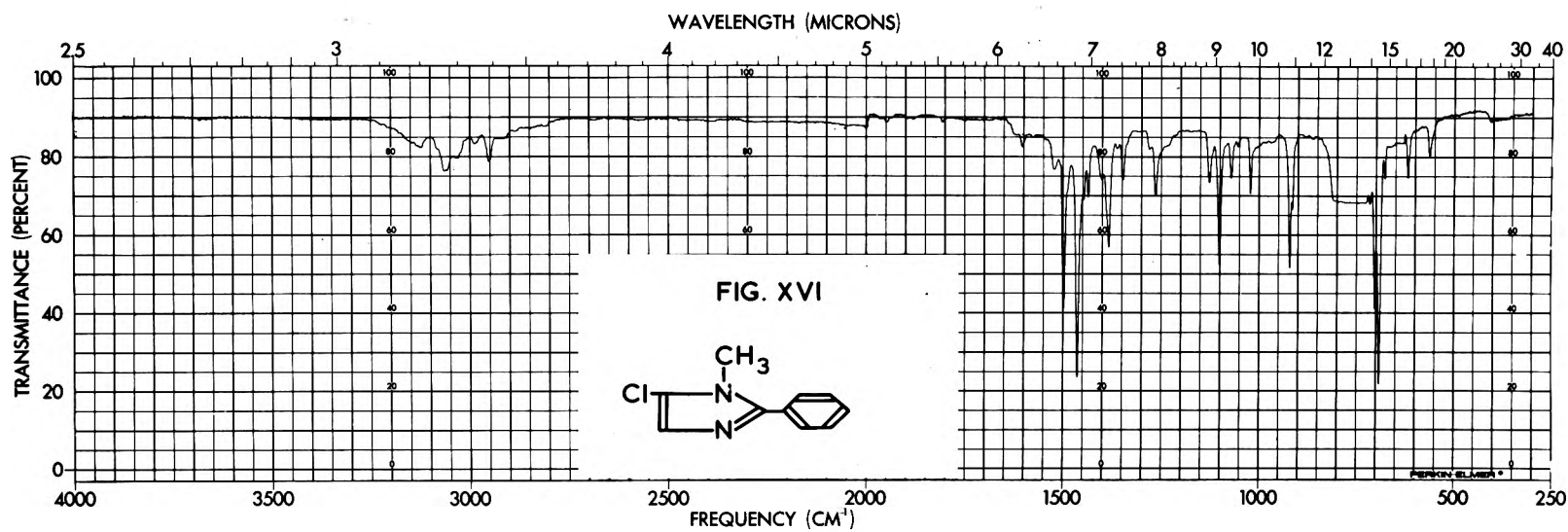


FIG. XVII. EXPANDED SCALE I.R. SPECTRA IN THE REGION 3300–3000 cm^{-1}
OF SOME SUBSTITUTED IMIDAZOLES.

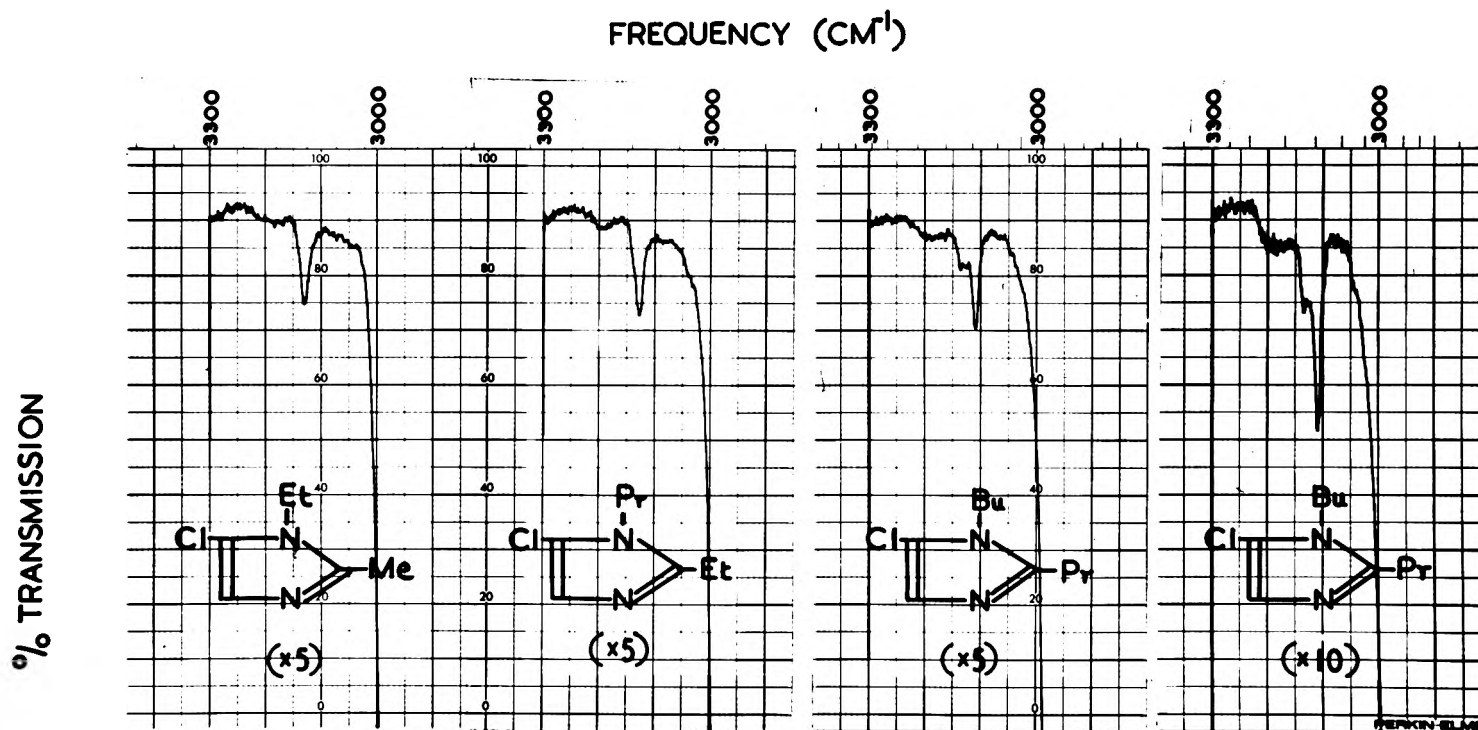
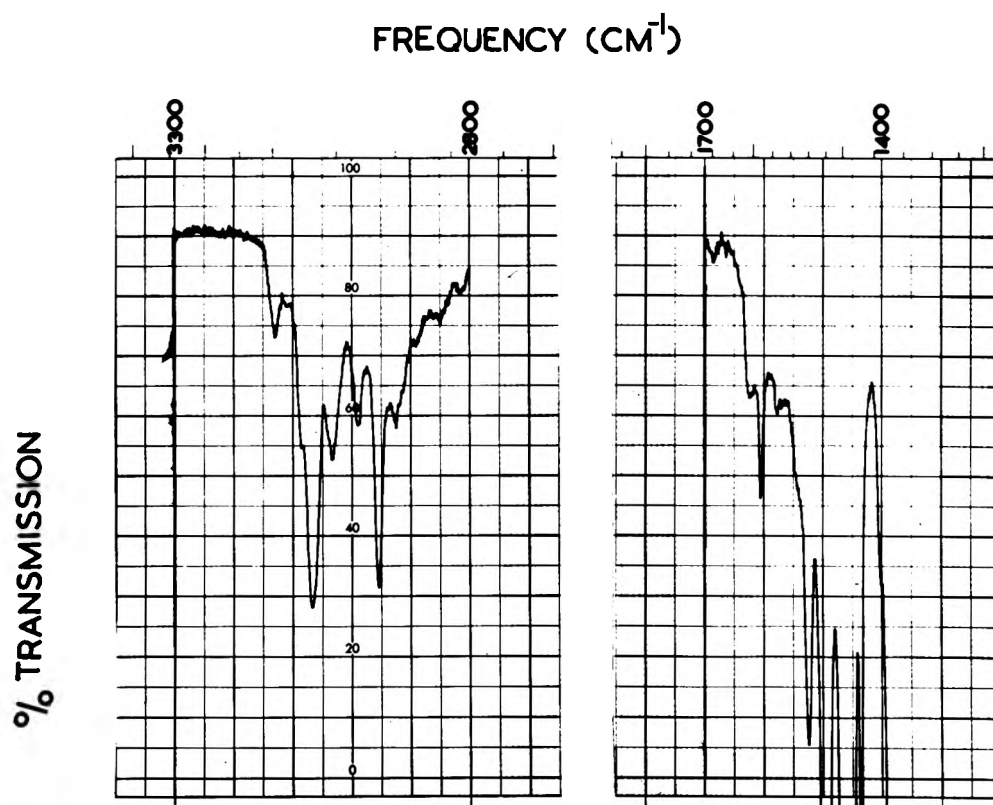


FIG. XVIII. EXPANDED SCALE (5X) I.R. SPECTRUM
IN THE REGIONS $3300\text{--}2800\text{ cm}^{-1}$ AND $1700\text{--}1400\text{ cm}^{-1}$
OF 5-CHLORO-1-METHYL-2-PHENYLMIDAZOLE.



of the spectra of a number of homologous bases. In view of the small effect alkyl groups were shown to have on characteristic absorption frequencies, a close correlation between the approximate value and that determined by experiment was anticipated.

The effect of pH on the absorption maximum of several 5-chloro-1,2-dialkylimidazole was determined by measuring the absorption in different buffer solutions and in normal hydrochloric acid. Table V summerises these results, and the various graphs are reproduced in Figure XXI.

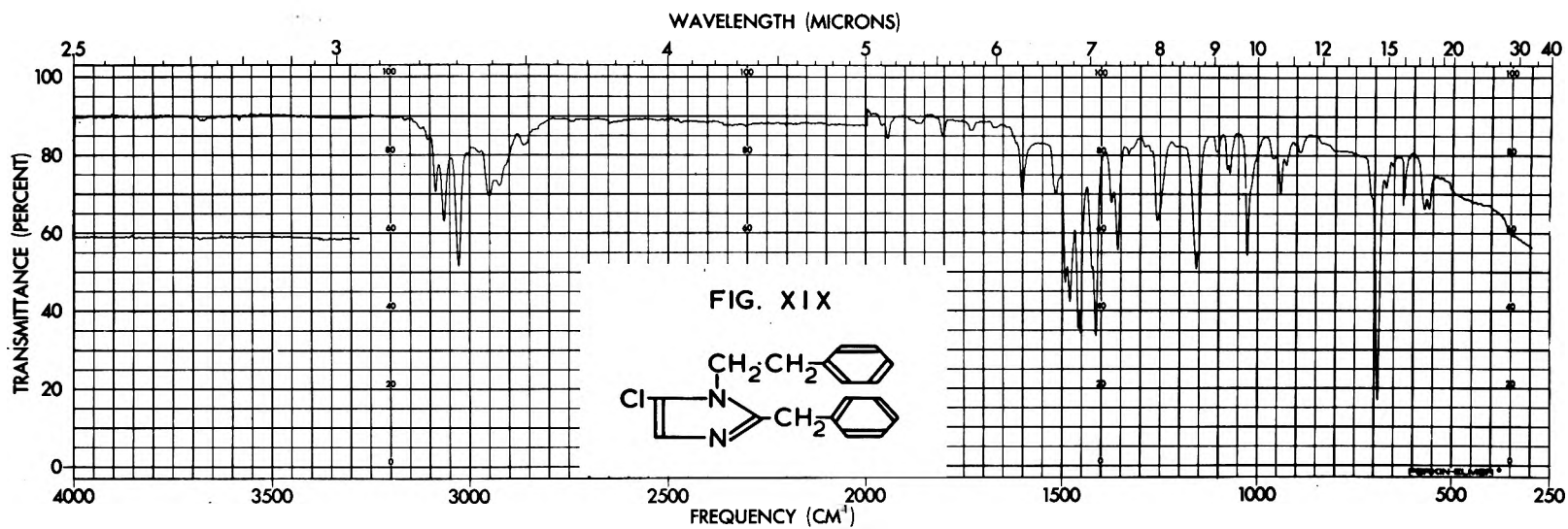


FIG. XX. EXPANDED SCALE (5X) I.R.
SPECTRUM IN THE REGION 3300 —
2800 cm^{-1} OF 2-BENZYL-5-CHLORO-1-
(2-PHENYLETHYL)-IMIDAZOLE.

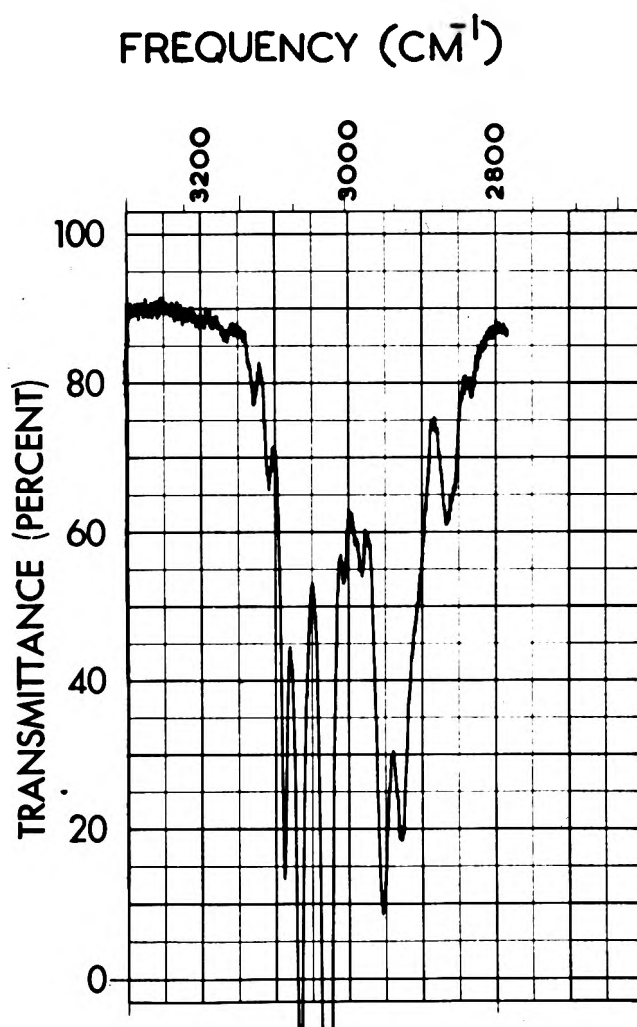


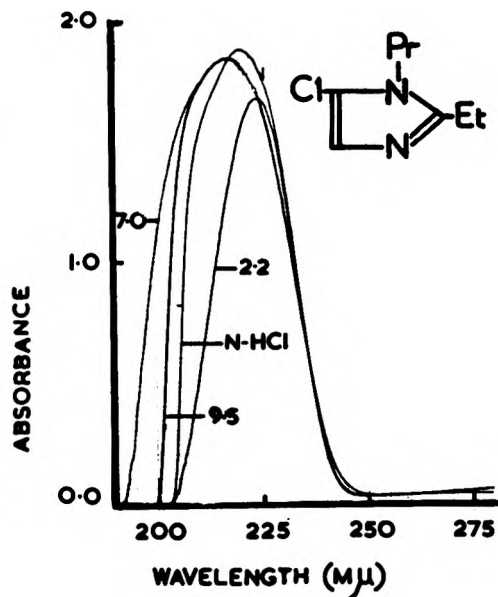
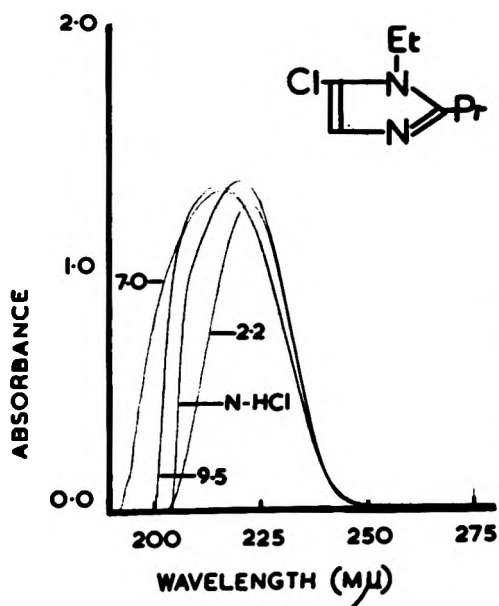
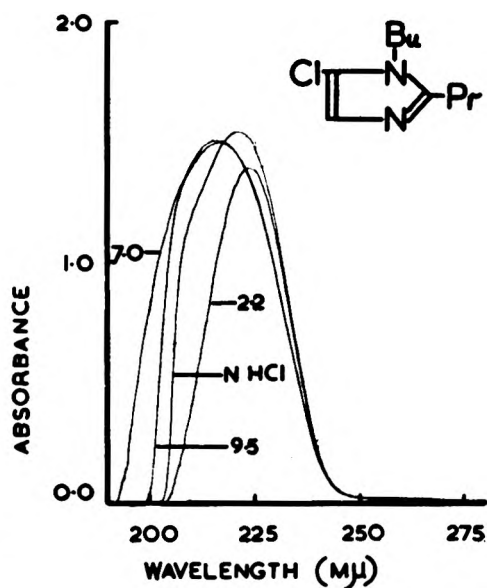
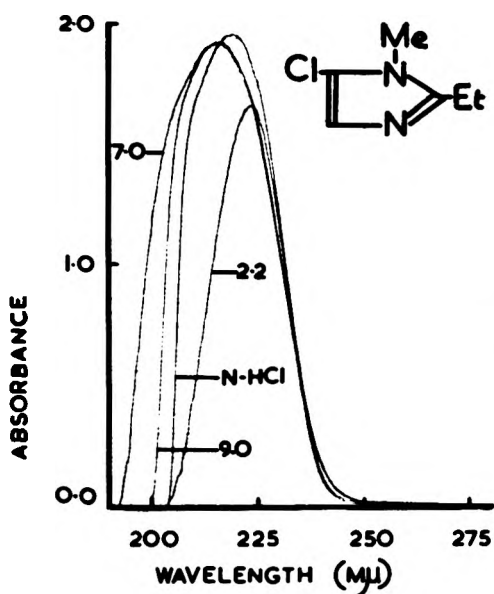
TABLE XII.

The ultraviolet absorption maxima for several 5-chloro-1,2-dialkyl-imidazoles at different pH values.

Compound.	Absorption maximum				
	pH 0	pH 2.2	pH 7	pH 9	pH 9.5
5-Chloro-1-ethyl-2-methylimidazole	219	228.5	215	216	-
1-Butyl-5-chloro-2-propylimidazole	221	223.5	217.5	216	-
5-Chloro-2-ethyl-1-propylimidazole	220	224	217	-	217
5-Chloro-1-ethyl-2-propylimidazole	220	222	215	-	215

FIG. XXI.

EFFECT OF pH ON THE ABSORPTION CURVE OF
SOME 5-CHLORO-1,2-DIALKYLIMIDAZOLES.



E X P E R I M E N T A L P A R T .

All melting points were recorded with Anschütz short-stemmed thermometers, and are corrected.

Preparation of symmetrical N,N'-dialkyloxamides.

(see Table I, page 20) Diethyloxamide. (Wallach, 1877). Ice-cold anhydrous ethylamine (2 moles, 90 g) was poured cautiously onto finely crushed ice (150 g) and diethyl oxalate (1 mole, 146 g) added dropwise with stirring and cooling (-5°). A heavy white precipitate rapidly formed. The mixture was warmed to room temperature and stirred continuously for 3 hours. It was then carefully boiled and additional water added until the oxamide was completely in solution. Traces of unreacted diethyl oxalate were removed by gently boiling the solution. On cooling, the diethyloxamide crystallised as long white needles which were dried at 50° . Yield= 66 g (46%) m.p. $180-181^{\circ}$.

Found	: C 50.1 %; H 8.5 %; N 19.1 %
Calc. for $C_6H_{12}N_2O_2$	: C 49.97%; " 8.43%; " 19.43%
(144.18)	

Di-n-butyloxamide. Diethyl oxalate (2 moles, 292 g) was dissolved in ethanol (1 l) and the mixture cooled to about -5° while a solution of n-butylamine (4 moles, 292 g) in 300 ml ethanol (95%) was added dropwise with stirring. The dibutyloxamide precipitated towards the end of the reaction. The mixture was left overnight and the solid product collected by filtration, washed with ethanol and dried at 55° . A higher temperature resulted in losses due to sublimation. A second

The characteristic aromatic bands at 1600 cm^{-1} (6.25μ), 1515 cm^{-1} (6.60μ), and 1450 cm^{-1} (6.90μ) are all present but the absence of the 1580 cm^{-1} (6.33μ) band indicates that the phenyl groups are not conjugated with the rest of the molecule. Absorption at 1460 cm^{-1} (6.85μ) is consistent with the $-\text{CH}_2-$ deformation mode. The four bands at 1520 cm^{-1} (6.58μ), 1490 cm^{-1} (6.71μ), 1412 cm^{-1} (7.08μ), and 1260 cm^{-1} (7.94μ) are all present although the 1520 cm^{-1} (6.58μ) band appears only as a weak shoulder. Nevertheless, these bands, together with the general pattern observed, suggest that there is a marked similarity between all the compounds examined, including the base derived from di-(2-phenylethyl)oxamide. Clearly the spectrum of this latter compound (Fig. XIX) contains no amide band at 1680 cm^{-1} - 1700 cm^{-1} (5.95μ - 5.89μ) as required for the iso-quinoline derivative (page 13). It was therefore concluded that the base obtained by the action of phosphorus pentachloride on di-(2-phenylethyl)-oxamide is 2-benzyl-5-chloro-1-(2'-phenylethyl)imidazole.

(h). The ultraviolet spectra of some 5-chloro-1,2-dialkylimidazoles.

An examination of the ultraviolet spectra of a series of 5-chloro-1,2-dialkylimidazoles was undertaken to provide data for detection of the bases in the eluent from column chromatography (see page 45). Several isomeric bases could not be examined directly as methods for their separation were not developed. Consequently, and approximate absorption maximum for these bases of $220\text{ m}\mu$ was estimated from a consideration

crop of oxamide could be obtained by concentrating the mother liquor. The material was sufficiently pure for immediate conversion to the chloroimidazole. A sample for analysis was recrystallised from ethanol. Yield=388g(97%) m.p.153.5-154.5°.

Found : C 60.0%; H 10.1%; N 14.0%

Calc.for:

$C_{10}H_{20}N_2O_2$ (200.28) : " 59.97%;" 10.07%;" 13.98%

Di-n-propyloxamide. Prepared by the above method.

Yield = 83%. m.p. 164.5 - 165.5°.

Found : C 56.4%; H 9.6%; N 16.5%

Calc.for:

$C_8H_{16}N_2O_2$ (172.23) : " 55.80%;" 9.36%;" 16.27%

Di-iso-butyloxamide. Prepared as above. Yield=71%
m.p. 171.5-172°.

Found : C 59.9 %; H 10.2 %; N 13.9 %

Calc.for:

$C_{10}H_{20}N_2O_2$ (200.28) : " 59.97%; " 10.07%; " 13.98%

Dibenzylloxamide. Obtained as a by-product in the preparation of ethoxalylbenzylamine (see page 85) of this dissertation). Recrystallised from glacial acetic acid. m.p. 225.5-226.5

Found : C 71.5%; H 6.2 %; N 10.5 %

Calc.for:

$C_{16}H_{16}N_2O_2$ (268.32) : " 71.62%;" 6.01%;" 10.44%

Di-(2-phenylethyl)oxamide. The above general method was modified by replacing the ethanol solvent by benzene as the product is too soluble in ethanol for ready isolation. The reaction was consequently conducted at 20°.

Yield = 97.5%. m.p. 188.5 - 189.5° (cf. 186°, Decker et al. (1913); 180°, Neubert, 1886).

Found : C 72.8 %; H 6.4 %; N 9.6 %

Calc. for:

$C_{18}H_{20}N_2O_2$ (296.37) : " 72.95%; " 6.80%; " 9.45%

Dimethyloxamide. A small sample of dimethyloxamide was prepared as a by-product in the synthesis of ethoxalylmethylaniline.

m.p. 217-217.5° (cf. Wallach, 1877).

Preparation of nonsymmetrical N,N'-dialkylloxamides.
Ethylmethyloxamide.

Method A: Potassium ethyloxalate was prepared according to the procedure of Crum-Brown and Walker (1893) and converted to ethoxalyl chloride as described by Southwick and Seivard (1949). Ethoxalyl ethylaniline was then prepared and converted to methylethyloxamide, in the following manner:-

Ethoxalyl chloride (55 g) was dissolved in sodium dried ether (200 ml) and treated slowly with anhydrous ethylaniline (40 g) in dry ether (200 ml). A vigorous reaction commenced and cooling to -5° was essential. A quantity of ethylaniline hydrochloride precipitated and was removed by filtration after the mixture had been allowed to stand overnight. The ether was removed by distillation and the oily residue cooled in the refrigerator. The

precipitate of diethyloxamide which formed was removed by filtration. Yield = 40 g (68.2%) Ethoxalylethylamine (0.36 mole, 40g) prepared above was dissolved in ethanol (40 ml) and cooled to -5° . Methylamine (35 ml of a 33% solution in ethanol) was then added dropwise with stirring when the product rapidly precipitated. After standing for 2 hours the product was collected by filtration. The crude oxamide was recrystallised from water or preferably, benzene. Yield = 17 g (47%) m.p. $153-154^{\circ}\text{C}$.
Found : C 46.0 %; H 7.5 %; N 21.5 %
Calc. for $\text{C}_5\text{H}_{10}\text{N}_2\text{O}_2$ (130.15) : " 46.14 %; " 7.74 %; " 21.52%

Method B: Ethoxalylmethylamine was prepared as described by Tierie, (1933) and converted to ethylmethyloxamide as described in method A only replacing the methylamine by an equimolecular proportion of ethylamine. Yield 44%. m.p. and mixed m.p. with a sample prepared by Method A $153-154^{\circ}$.

Ethoxalyl-n-butylamine. n-Butylamine (3 moles, 219 g) was added dropwise to a solution of diethyl oxalate (3 moles, 438 g) in 95% ethanol (2.1) at 0°C . The solution was left overnight at 5° and the precipitate of dibutyloxamide removed by filtration. The alcohol was removed by distillation under reduced pressure in a rotary evaporator, and the residue allowed to stand at 0° for 24 hours. A further deposit of dibutyloxamide was removed by filtration, and the oily filtrate carefully fractionated

at 75 mm. Hg through a 25 x 350 mm. column packed with glass coils. A small quantity of ethanol was removed at 32° followed by unchanged diethyl oxalate in the range 100 - 115° (proved by conversion to di-n-butyloxamide. m.p. and mixed m.p. 153-155°).

Ethoxalylbutylamine was collected at 188-190.5°.

Great care must be exercised to avoid contamination of the product from the residue of dibutyloxamide distilling above 191°. Yield = 372 g. (72%).

N-n-butyl-N'-ethyloxamide. A solution of anhydrous ethylamine (1 mole; 54 g) in ice-cold ethanol was added dropwise with stirring to an ice-cold solution of ethoxalylbutylamine (1 mole; 173 g) in ethanol (1.5 l.). After standing 3-4 hours at room temperature, the mixture was heated on the water bath until a clear solution formed. The crystalline material which deposited as fine white needles on standing overnight was collected by filtration, and washed with ethanol. Yield = 139 g. (81%). m.p. 133.5-134.5°.

Found : C 55.6 %; H 9.3 %; N 16.4 %

Calc. for
 $C_8H_{16}N_2O_2$ (172.23) : " 55.79%; " 9.36%; " 16.26%

N-n-butyl-N'-propyloxamide. Prepared from ethoxalylbutylamine by the addition of one molar proportion of propylamine as described above. Yield = 70%. m.p. 145-146°.

Found : C 58.3 %; H 9.6 %; N 15.1 %

Calc. for
 $C_9H_{18}N_2O_2$ (186.26) : " 58.04%; " 9.74%; " 15.04%

N-n-butyl-N'-iso-butyloxamide. Prepared as described above. Yield = 80%. m.p. 146-147°.

Found : C 60.2 %; H 10.3 %; N 14.1 %

Calc. for
 $C_{10}H_{20}N_2O_2$ (200.28) : " 59.97%; " 10.07%; " 13.89%

Ethoxalylbenzylamine. Prepared as described above for ethoxalylbutylamine, but replacing the butylamine by benzylamine. Dibenzylloxamide was formed in small quantity as a by-product. The product was collected in the range 218-220° under water pump vacuum (ca. 20 mm. Hg). On standing, the ethoxalylbenzylamine solidified. It was used directly for the preparation of benzylmethyloxamide. Yield = 223 g. (54%).

N-Benzyl-N'-methyloxamide. Prepared according to the method outlined for butylethyloxamide.

Yield = 76%. m.p. 185.5°.

Found : C 62.4 %; H 6.4 %; N 14.6 %

Calc. for
 $C_{10}H_{12}N_2O_2$ (192.22) : " 62.49%; " 6.29%; " 14.57%

Preparation of 5-chloro-1,2-dialkylimidazoles.

(A). Action of phosphorus pentachloride on N,N'-dialkyl oxamides.

The following synthesis of 1-butyl-5-chloro-2-propylimidazole serves as a general example of the method employed:-

Finely powdered, dry di-n-butyloxamide (1 mole; 200 g.) was shaken with finely crushed phosphorus pentachloride (2 moles; 416 g) in a round-bottomed flask fitted with a condenser and drying tube. After the initial vigorous reaction had subsided, the liquid mixture was heated on the steam bath for 6 hours. Phosphorus oxychloride was removed by distillation

under reduced pressure. The dark tarry residue was cooled to -10° and cautiously treated with ice-water (150 ml.) followed by 50% sodium hydroxide until strongly alkaline. The mixture was extracted with chloroform, and the chloroform in turn extracted with several portions of 4N-HCl. The acid extract was washed with fresh chloroform, and then made strongly alkaline with 50% sodium hydroxide. Basic material was re-extracted into chloroform. The chloroform extract was dried (K_2CO_3) and the solvent removed through a short fractionating column. The residue distilled almost completely between $252-256^{\circ}/620$ mm. Yield = 114 g (57%).

Found : C 60.0 %; H 8.7 %; N 14.0 %; Cl 17.5 %

Calc. for

$C_{10}H_{17}ClN_2$: " 59.84%; " 8.54%; " 13.95%; " 17.66%
(200.71)

The picrate crystallised as long yellow needles from ethanol. m.p. $100-101^{\circ}$.

Found : C 44.8 %; H 5.0 %; N 16.1 %; Cl 8.5 %

Calc. for

$C_{16}H_{20}ClN_5O_7$: " 44.71%; " 4.69%; " 16.29%; " 8.25%
(429.82)

(1). Chloroimidazoles from symmetrical oxamides.

1-iso-Butyl-5-chloro-2-iso-propylimidazole. Prep-
ared from di-iso-butyloxamide. The base was purified by steam distillation, and distilled. b.p. $234-236^{\circ}/620$ mm. Yield = 74%.

Found : C 59.8 %; H 8.7 %; N 13.7 %; Cl 17.5 %

Calc. for

$C_{10}H_{17}ClN_2$: " 59.84%; " 8.54%; " 13.95%; " 17.66%
(200.71)

Picrate (yellow needles from ethanol). m.p. 173.5-174.5°.

Found : C 44.8 %; H 4.8 %; N 16.5 %; Cl 8.2 %
 Calc. for $C_{16}H_{20}ClN_5O_7$: " 44.71%; " 4.69%; " 16.29%; " 8.25%
 (429.82)

5-Chloro-2-ethyl-1-propylimidazole. Prepared from dipropyloxamide. Yield = 77%. b.p. 228-232°/620 mm.

Found : C 55.6 %; H 7.6 %; N 16.5 %; Cl 20.3 %
 Calc. for $C_8H_{13}ClN_2$: " 55.65%; " 7.59%; " 16.22%; " 20.53%
 (172.66)

Picrate (from ethanol) m.p. 155-156.5°

Found : C 41.9 %; H 4.2 %; N 17.1 %; Cl 9.1 %
 Calc. for $C_{14}H_{16}ClN_5O_7$: " 41.85%; " 4.01%; " 17.43%; " 8.82%
 (401.77)

5-Chloro-1-ethyl-2-methylimidazole. Prepared from diethyloxamide. Yield = 53%. b.p. 228-232°/620 mm.

Found : C 49.8 %; H 6.5 %; N 19.6 %; Cl 24.4 %
 Calc. for $C_6H_9ClN_2$: " 49.83%; " 6.27%; " 19.37%; " 24.52%
 (144.60)

Picrate (from ethanol) m.p. 156-157°.

Found : C 38.7 %; H 3.2 %; N 19.0 %; Cl 9.8 %
 Calc. for $C_{12}H_{12}ClN_5O_7$: " 38.57%; " 3.24%; " 18.74%; " 9.49%
 (373.71)

2-Benzyl-5-chloro-1-(2'-phenylethyl)-imidazole.

Prepared from di-(2-phenylethyl)-oxamide.

Additional phosphorus oxychloride was added as solvent once the reaction had commenced.

Yield = 3%. m.p. 68-69°. (see section g, page 62).
The hydrochloride was precipitated from benzene by shaking with a little concentrated hydrochloric acid. m.p. 227.5-228.5°. Recrystallised from absolute ethanol.

Found . C 64.8 %; H 5.5 %; N 8.5 %; Cl 21.4 %
Calc. for
 $C_{13}H_{16}Cl_2N_2$. " 64.88%; " 5.44%; " 8.41%; " 21.28%
(333.26)

The picrate was prepared by precipitation with alcoholic picric acid from an aqueous solution of the hydrochloride. m.p. 165-166°.

(2). Bases from nonsymmetrical oxamides.
5-Chloro-1,2-dimethylimidazole or 5-chloro-1-ethyl-
imidazole.

The base was isolated by steam distillation. The crude product distilled at 204-206°/625 mm. Yield = 54%. The picrate crystallised as thick yellow needles from ethanol. m.p. 182-183° (uncor.) The sharpness of the melting point suggested that the compound was pure.

1-Benzyl-5-chloroimidazole or 5-chloro-1-methyl-
2-phenylimidazole.

Prepared from benzylmethyloxamide and phosphorus pentachloride by heating the reaction mixture for 8 hours at 150° (oil bath). The solid product was purified as follows.-

A chloroform solution of the crude base was extracted with 4 N-HCl. The acid solution (after charcoal treatment) was cooled and neutralised with 50% NaOH and left overnight at 5°. The crystalline product was removed by filtration, washed with

water until the washings were neutral to litmus, and dried in vacuum. Yield = 73%. Recrystallised from petroleum ether (b.p. 60-80°) and yielded white needles (charcoal). m.p. 104.5 - 105.5°.

Found : C 62.2 %; H 4.8 %; N 14.7 %; Cl 18.6 %
Calc. for $C_{10}H_9ClN_2$: " 62.34%; " 4.71%; " 14.51%; " 18.40%
(192.65)

5-Chloro-1-ethyl-2-propylimidazole and 1-butyl-5-chloro-2-methylimidazole.

A mixture of the above derivatives is formed by the action of phosphorus pentachloride on butyl-ethyloxamide. b.p. = 230-236°/619 mm. Yield = 76%.

1-Butyl-5-chloro-2-ethylimidazole and 5-chloro-1,2-dipropylimidazole.

Prepared by the action of phosphorous pentachloride on butylpropyloxamide. b.p. 238-245°/622 mm. Yield = 65%.

1-iso-Butyl-5-chloro-2-n-propylimidazole and 2-n-butyl-5-chloro-1-iso-propylimidazole.

A mixture of the above isomers is formed from n-butyl-iso-butyloxamide by application of the standard technique. b.p. = 243-250°/620 mm. Yield = 35.5%.

(B). Separation of isomeric bases arising from the action of phosphorus pentachloride on non-symmetrical oxamides.

5-chloro-1-ethyl-2-propylimidazole and 1-butyl-5-chloro-2-methylimidazole (from butylethyl-oxamide).

The mixture of isomers was separated by fractional distillation in a high efficiency column (for details see page 35). The distillation was conducted at a pressure of 56 mm. (manostat), at which pressure the boiling point of the bases was significantly reduced but flooding prevented. Two fractions were collected:-

A. b.p. = 147.5 - 149.5°/56 mm.

B. b.p. = 153.5 - 155.5°/56 mm.

Isomer A: (5-chloro-1-ethyl-2-propylimidazole).

Found : C 55.7 %; H 7.5 %; N 16.0 %; Cl 20.5 %

Calc. for

$C_8H_{13}N_2Cl$: " 55.66%; " 7.59%; " 16.24%; " 20.53%
(172.66)

The picrate from isomer A separated from ethanol as fine needles m.p. 111-112°.

Found : N 17.6 %; Cl 8.9 %

Calc. for

$C_{14}H_{16}N_5ClO_7$: " 17.43%; " 8.82%
(401.77)

Isomer B. (1-butyl-5-chloro-2-methylimidazole).

Found : C 54.8 %; H 7.8 %; N 16.5 %; Cl 20.4 %

Calc. for

$C_8H_{13}N_2Cl$: " 55.66%; " 7.59%; " 16.24%; " 20.53%
(172.66)

The picrate of isomer B separated from ethanol in yellow prisms. m.p. 151.5-152.5°.

Found : N 17.6 %; Cl 9.0 %
 Calc. for
 $C_{14}H_{16}N_5ClO_7$: " 17.43%; " 8.82%
 (401.77)

Nitration of 5-chloro-1,2-dialkylimidazoles.

Method A (cf. Sarasin and Wegmann, 1924) was employed for some of the earlier preparations but was later replaced by method B, described below for 5-chloro-2-ethyl-4-nitro-1-propylimidazole.

Method A. 5-chloro-1-ethyl-2-methyl-4-nitroimidazole.

5-Chloro-1-ethyl-2-methylimidazole (70g) was dissolved in 2.5 N nitric acid (700 ml) and evaporated to dryness on the water bath. The residue was treated with concentrated sulphuric acid (420 ml) and heated on the water bath for 3 hours. The mixture was cooled and poured onto crushed ice (500 g). The acidity was reduced by the cautious addition of $NaHCO_3$. A precipitate formed, which on standing overnight, was removed by filtration and recrystallised from water.

Yield = 59.5g (65%). m.p. 88-89°.

Found : C 37.9 %; H 4.5 %; N 22.3 %; Cl 18.7 %
 Calc. for
 $C_6H_8ClN_3O_2$: " 38.01%; " 4.25%; " 22.16%; " 18.70%
 (189.60)

Method B. 5-chloro-2-ethyl-4-nitro-1-propylimidazole.

5-Chloro-2-ethyl-1-propylimidazole (20g) was kept at -10° and treated with 80 ml. of a mixture of

nitric acid (sg = 1.4)/sulphuric acid (1/3) also cooled to -10° with shaking and cooling (ice bath). The acid mixture was added relatively rapidly so as to quench the initial heat of reaction. After the initial reaction was complete, the mixture was heated on the boiling water bath for 4-5 hours, then cooled and poured into water (4.1). The white oil which appeared solidified on standing overnight at 4° . After filtration, the crude nitro-chloroimidazole was washed thoroughly with water and allowed to dry in the air. The product was used without further purification. Yield = 22 g (88%). A sample for analysis was recrystallised from methyl cyclohexane. m.p. $58-59^{\circ}$.

Found : C 44.3 %; H 5.5 %; N 19.6 %; Cl 16.3 %
Calc. for
 $C_8H_{12}ClN_3O_2$: " 44.15%; " 5.56%; " 19.31%; " 16.29%
(217.66)

1-Butyl-5-chloro-4-nitro-2-propylimidazole.

Prepared as above. The product was a white oil which solidified on standing at 4° . It was purified by dissolving in chloroform, washing with water (three times), and drying over anhydrous sodium sulphate. After removal of the chloroform, the residue oil solidified on keeping at 4° . For analysis a sample was recrystallised from methyl-cyclohexane. m.p. $34.7-35.7^{\circ}$. Yield = 62%.

Found : C 48.9 %; H 6.4 %; N 17.3 %; Cl 14.2 %
Calc. for
 $C_{10}H_{16}ClN_3O_2$: " 48.88%; " 6.56%; " 17.10%; " 14.43%
(245.71)

1-iso-Butyl-5-chloro-4-nitro-2-iso-propylimidazole.

Prepared as described above, and recrystallised from 95% ethanol. Yield = 86%. m.p. 109.5-110.5°.

Found : C 48.7 %; H 6.3 %; N 17.2 %; Cl 14.8 %
Calc. for
 $C_{10}H_{16}ClN_3O_2$: " 48.88%; " 6.56%; " 17.10%; " 14.43%
(245.71)

1-Butyl-5-chloro-2-methyl-4-nitroimidazole.

A pale yellow oil was obtained by the standard method which failed to solidify on prolonged standing at 4°. Yield = 71%. A sample was purified for analysis by distillation under reduced pressure. b.p. 180-182°/1 mm.

Found : C 44.0 %; H 5.6 %; N 19.6 %; Cl 16.2 %
Calc. for
 $C_8H_{12}ClN_3O_2$: " 44.15%; " 5.56%; " 19.31%; " 16.29%
(217.66)

5-Chloro-1-methyl-4-nitro-2-(p-nitrophenyl)-imidazole.

The solid base was treated with nitric acid/sulphuric acid as in method B. On pouring the acid mixture onto ice a thick yellow paste separated which was filtered and thoroughly washed. In addition to the nitro group in the imidazole ring, it was shown that the above conditions resulted in the nitration of the benzene ring in the para position (see page 108 of this dissertation). Yield = 79%.

The product was recrystallised from benzene.
m.p. 156-157.5°.

Found : C 42.7 %; H 2.5 %; N 19.7 %
Calc. for
 $C_{10}H_7ClN_4O_4$: " 42.49%; " 2.50%; " 19.82%
(282.65)

Preparation of 5-cyano-1,2-dialkyl-4-nitroimidazoles.

The classical technique, method A (cf. Sarasin and Wegmann, 1924), was found to have several disadvantages and was replaced by the method of Friedman and Schechter (1960) - method B - after it was shown that the latter method could be adapted to chloronitroimidazoles (refer to page 24 of this dissertation). Both methods are illustrated below by detailed description of typical syntheses.

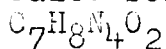
Method A: 5-Cyano-1-ethyl-2-methyl-4-nitroimidazole.

5-Chloro-1-ethyl-2-methyl-4-nitroimidazole (0.2 moles; 35.5 g) in absolute ethanol (800 ml) was refluxed for 10 hours with dry, pulverised potassium cyanide (0.4 moles; 26 g) and potassium iodide (0.02 moles; 3.32 g) as catalyst. A precipitate of potassium chloride gradually formed. The alcohol was removed on the water bath and the dark residue dissolved in ice water. The nitrile was extracted into chloroform, washed with water and dried over anhydrous potassium carbonate. The chloroform was removed and the product distilled in vacuo as a pale yellow oil.
b.p. 192-194°/6 mm. Yield = 26 g (72%).

On triturating with ethanol, the product solidified and was recrystallised from ethanol. m.p. 78-79°.

Found . C 47.0 %; H 4.5 %; N 31.4 %

Calc. for



(180.17)

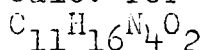
. " 46.66%; " 4.48%; " 31.10%

Method B. 1-iso-Butyl-5-cyano-4-nitro-2-iso-propyl-imidazole.

1-iso-Butyl-5-chloro-4-nitro-2-iso-propyl-imidazole (10 g) was dissolved on dimethyl sulphoxide (100 ml.) and dry, pulverised sodium cyanide (2.5 g) added. The mixture was heated on the boiling water bath for 2 hours with frequent shaking and finally poured into water (750 ml.) and allowed to stand overnight. The precipitate was separated by filtration and washed with water. Yield = 9 g. (94.5%). The compound was obtained as white needles from ethanol (charcoal). m.p. 131.5°.

Found . C 55.9 %; H 6.6 %; N 23.6 %

Calc. for



(236.28)

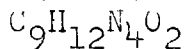
. " 55.92%; " 6.83%; " 23.71%

5-Cyano-2-ethyl-4-nitro-1-propylimidazole.

Prepared by method B. Recrystallised from 50% ethanol. Yield = 77%. m.p. 62.5-63.5°.

Found : C 51.8 %; H 5.8 %; N 27.2 %

Calc. for



(208.22)

. " 51.92%; " 5.81%; " 26.91%

1-Butyl-5-cyano-4-nitro-2-propylimidazole.

Prepared by method B. The crude nitrile was

extracted into ether from the aqueous reaction mixture and thoroughly washed with water (4x). After removal of the ether the product was obtained as a yellow oil which could be converted to the amide without further purification. Yield = 72%. A sample was recrystallised from ethanol for analysis. m.p. = 36.5-37.5°C. b.p. 194-198°/1 mm.

Found : C 55.7 %; H 6.7 %; N 23.6 %

Calc. for
 $C_{11}H_{16}N_4O_2$: " 55.92%; " 6.83%; " 23.71%
(236.28)

5-Cyano-1-methyl-4-nitro-2-(p-nitrophenyl)-imidazole.

Prepared as above; and the solid product recrystallised from aqueous cellosolve. Yield = 21%. m.p. 186.8-187.5°.

Found : C 48.5 %; H 2.6 %; N 25.8 %

Calc. for
 $C_{11}H_7N_5O_4$: " 48.36%; " 2.58%; " 25.62%
(273.20)

5-Cyano-1-ethyl-4-nitro-2-propylimidazole.

Prepared as above. The product was an oil which was used without further purification. Yield = 81%. The sample was not analysed.

1-Butyl-5-cyano-2-methyl-4-nitroimidazole.

A pale yellow oil prepared as described above but not purified. Yield = 71%.

Preparation of 1,2-dialkyl-4-nitroimidazole-5-carboxamides.

The following general method was used

throughout for the hydrolysis of the cyano group.

1-Butyl-4-nitro-2-propylimidazole-5-carboxamide.

1-Butyl-5-cyano-4-nitro-2-propylimidazole (15 g) was dissolved in concentrated sulphuric acid (100 ml) and heated on the boiling water bath for 2-4 hours. The cold acid mixture was poured onto ice (800 ml) and left overnight at 5°. The crude product was filtered and washed with water until acid free (litmus). Yield = 12 g (80%). Recrystallisation from aqueous acetic acid (charcoal) gave a product as white prisms, m.p. 184-186°. Several recrystallisations were frequently required to attain sufficient purity for successful reduction of the nitro group in later experiments.

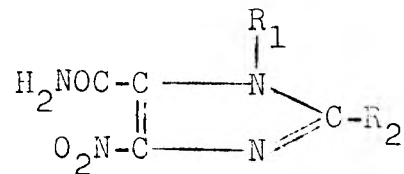
Found	: C 52.0 %; H 7.3 %; N 22.2 %
Calc. for $C_{11}H_{18}N_4O_3$ (254.29)	: " 51.96%; " 7.14%; " 22.03%

Physical data on all the nitroimidazole-carboxamides prepared are presented in Table XIII.

Preparation of 4-amino-1,2-dialkylimidazole-5-carboxamides.

Catalytic reduction of the nitro group was accomplished by the following general method described in detail for 4-amino-1-butyl-2-propylimidazole-5-carboxamide. Other nitroimidazoles were reduced similarly but required variation in the solvent for recrystallisation as indicated for each compound below.

TABLE XIII.

Physical data on some 1,2-dialkyl-4-nitroimidazole-5-carboxamides.

R ₁	R ₂	Formulae	Mol. Wt.	Yield	m.p. °C.	Analysis					
						Calculated			Found		
						C	H	N	C	H	N
Ethyl	Methyl	C ₇ H ₁₀ N ₄ O ₃	198.18	62%	278.5-279.5 (decomp)	42.42	5.09	28.27	42.4	5.0	28.5
Ethyl	Propyl	C ₉ H ₁₄ N ₄ O ₃	226.24	85%	210-210.5	47.78	6.24	24.76	47.8	6.5	24.2
Propyl	Ethyl	C ₉ H ₁₄ N ₄ O ₃	226.24	40%	221-221.5	47.78	6.24	24.76	47.8	6.2	25.0
Butyl	Methyl	C ₉ H ₁₄ N ₄ O ₃	226.24	65%	181	47.78	6.24	24.76	47.6	6.7	24.9
Butyl	Propyl	C ₁₁ H ₁₈ N ₄ O ₃	254.29	80%	184-186	51.96	7.14	22.03	52.0	7.3	22.2
<u>iso</u> -Bu	<u>iso</u> -Pr	C ₁₁ H ₁₈ N ₄ O ₃	254.29	79%	227-227.5	51.96	7.14	22.03	52.0	7.4	-

4-Amino-1-butyl-2-propylimidazole-5-carboxamide.

Highly purified 1-butyl-4-nitro-2-propylimidazole-5-carboxamide (2 g) was partially dissolved in absolute ethanol (100 ml) and Adams' platinum catalyst (50 mg) added. The theoretical volume of hydrogen was absorbed at atmospheric pressure and room temperature in about 3 hours. The catalyst was removed by filtration and the alcohol evaporated in vacuo at room temperature. The dry residue was recrystallised from hot water (45-50 ml) with charcoal treatment. The yield was 1.2 g (52%). m.p. 150-151°.

Found : C 58.9 %; H 9.4 %; N 24.9 %

Calc. for

$C_{11}H_{20}N_4O$: " 58.90%; " 8.99%; " 24.98%
(224.31)

4-Amino-1-iso-butyl-2-iso-propylimidazole-5-carboxamide.

The crude amine, after evaporation of the ethanol, was a pale yellow oil which solidified on standing overnight. It was recrystallised from benzene as white prisms. Yield = 52%. m.p. 156-156.5°.

Found : C 58.5%; H 9.5 %; N 25.1 %

Calc. for

$C_{11}H_{20}N_4O$: " 58.90%; " 8.99%; " 24.98%
(224.31)

A picrate was prepared (recrystallised from ethanol). m.p. 158-159°.

4-Amino-1-ethyl-2-methylimidazole-5-carboxamides.

Recrystallised from dioxan as pale brown needles. m.p. 167.5-168°.

Found : C 49.9 %; H 6.9 %; N 33.0 %

Calc. for
 $C_7H_{12}N_4O$ (168.20): C 49.99%; " 7.19%; " 33.31%

4-Amino-2-ethyl-1-propylimidazole-5-carboxamide.

Recrystallised from ethyl acetate. The compound was difficult to purify and was submitted for analysis as the picrolonate (recrystallised from ethanol). m.p. 200.5°-201.5° (decomp.)

Found : C 49.8 %; H 5.5 %; N 24.5 %

Calc. for
 $C_{19}H_{24}N_8O_6$ (460.46): C 49.56%; " 5.25%; " 24.33%

4-Amino-1-ethyl-2-propylimidazole-5-carboxamide.

Recrystallised from a relatively large volume of ethyl acetate. Yield = 52%. As purification of the free amine proved difficult, melting points and analyses were performed on the picrolonate after recrystallisation from ethanol, m.p. 206.5° (decomp).

Found : C 49.7 %; H 5.3 %; N 24.5 %

Calc. for
 $C_{19}H_{24}N_8O_6$ (460.46): C 49.56%; " 5.25%; " 24.33%

4-Amino-1-butyl-2-methylimidazole-5-carboxamide.

Recrystallised from ethyl acetate. Yield = 58%. m.p. 187-188°. Submitted for analysis as the picrolonate (recrystallised from ethanol). m.p. 219.5° (decomp).

Found :C 49.3 %; H 5.5 %; N 24.5 %

Calc. for

$C_{19}H_{24}N_8O_6$ (460.46): " 49.56%; " 5.25%; " 24.33%

Action of hydrochloric acid at 140° on 4-amino-1,2-dialkylimidazole-5-carboxamides.

The rupture of the imidazole ring and isolation and identification of the breakdown products was accomplished by a standard technique and is illustrated in detail by the following example:-

4-Amino-1-butyl-2-propylimidazole-5-carboxamide (2 g) was dissolved in concentrated hydrochloric acid (25 ml) and heated at 140° for 24 hours in a sealed pyrex glass tube (25 x 400 mm). When cold, the tube was cautiously opened (vigorous gas evolution) and the contents diluted with water (50 ml). Steam distillation removed the carboxylic acid fragment A (see below) for identification. The residue was evaporated to dryness on the water bath and then extracted for one-half hour with boiling absolute ethanol. The alcohol solution was evaporated to dryness and remaining traces of ammonium ions displaced by treatment with hot 10% sodium carbonate solution (15 ml). The mixture was again taken to dryness and extracted with boiling absolute ethanol. The ethanol was removed by evaporation and the residue dissolved in N-sodium hydroxide (20 ml) and filtered. The N-alkylglycine was isolated as the N-benzenesulphonyl derivative as described below (B).

(A). Identification of the carboxylic acid.

The butyric acid fragment was identified as the p-bromophenacyl ester as follows:-

The steam distillate (350-400 ml) was saturated with sodium chloride and extracted exhaustively with ether. After drying (Na_2SO_4) the ether was gently removed on the water bath. The acid residue was dissolved in water (3-5 ml) and N-NaOH added dropwise until the solution was just alkaline to phenolphthalein. Very dilute HCl was then added carefully until the solution had a pH of 5-6 (litmus). p-Bromophenacyl bromide (0.5 g) in ethanol (5 ml) was added and the mixture refluxed on the water bath for 1 hour. Additional ethanol was cautiously added until the precipitate which formed on addition of the p-bromophenacyl bromide just dissolved. On cooling, the p-bromophenacyl ester crystallised as feathery white plates which were purified by recrystallisation from aqueous ethanol. The p-bromophenacyl butyrate was identified by melting point and mixed melting point with an authentic specimen and confirmed by analysis. m.p. $62-63^\circ$.

Found : C 50.6 %; H 4.6 %

Calc. for
 $\text{C}_{12}\text{H}_{13}\text{BrO}_3$ (285.15) : " 50.54%; " 4.60%

In the case of acetic acid derived from 4-amino-1-butyl-2-methylimidazole-5-carboxamide it was found better to isolate the acid as the S-benzyl thiuronium salt by the following technique:-

The crude carboxylic acid neutralised to phenolphthalein was adjusted to about pH 5-6 (litmus) and a solution of S-benzyl thiuronium hydrochloride (0.5 g) in water (5 ml) added. A white crystalline material slowly appeared on standing for several hours at 0°. The salt was purified by recrystallisation from water. Identification was established as described for p-bromo-phenacyl esters.

(B). Identification of N-alkylglycine.

The alkaline solution of the crude N-butylglycine (20 ml) was shaken with benzenesulphonyl chloride (1 ml) until the odour of benzenesulphonyl chloride had disappeared (10-15 mins.). On addition of dilute hydrochloric acid the N-benzenesulphonyl-N-butylglycine separated as a white oil which solidified on standing at 5°. The product was purified by reprecipitation followed by recrystallisation from water. It was identified by melting point, mixed melting point with an authentic specimen, and by analysis. m.p. 101.5-103°.

Found : C 53.1 %; H 6.2 %

Calc. for

C₁₂H₁₇NO₄S (271.34) : " 53.12%; " 6.32%

In the case of N-n-propylglycine it was found better to isolate the N-p-toluenesulphonyl derivative as follows:-

The alkaline solution of N-propylglycine (20 ml) was shaken for 3 hours with an ethereal solution of p-toluenesulphonyl chloride (2 g in 50 ml). The ether solution was then separated and the N-p-toluenesulphonyl-N-propylglycine isolated by the dropwise addition of very dilute hydrochloric acid to the aqueous layer. Identification was by the criteria described above.

4-Amino-2-ethyl-1-propylimidazole-5-carboxamide.

Carboxylic acid: Identified as p-bromophenacyl propionate. m.p. 61.5-62.5°.

Found : C 48.8 %; H 4.2 %

Calc. for
C₁₁H₁₁BrO₃ (271.12) : " 48.73%; " 4.09%

Alkylglycine: Identified as N-p-toluenesulphonyl-N-propylglycine. m.p. 96.0-96.5°.

Found : C 53.3 %; H 6.4 %

Calc. for
C₁₂H₁₇NO₄S (271.34) : " 53.12%; " 6.32%

4-Amino-1-ethyl-2-propylimidazole-5-carboxamide.

Carboxylic acid: Identified as p-bromophenacyl-n-butyrate. m.p. 62-63°.

Found : C 50.4 %; H 4.8 %

Calc. for
C₁₂H₁₃BrO₃ (285.15) : " 50.54%; " 4.60%

Alkylglycine: Identified as N-benzenesulphonyl-N-ethylglycine. m.p. 115-116.5°.

Found : C 49.0 %; H 5.4 %

Calc. for

$C_{10}H_{13}NO_4S$ (243.29) : " 49.37%; " 5.38%

4-Amino-1-butyl-2-methylimidazole-5-carboxamide.

Carboxylic acid: Identified as S-benzyl-thiuronium acetate by melting point and mixed melting point with an authentic specimen only. Insufficient quantities of recrystallised material were available for satisfactory analysis.

Alkylglycine: Identified as N-benzenesulphonyl-N-n-butylglycine. m.p. 101.5-103°.

Found : C 53.2 %; H 6.4 %

Calc. for

$C_{12}H_{17}NO_4S$ (271.34) : " 53.12%; " 6.32%

4-Amino-1-iso-butyl-2-iso-propylimidazole-5-carboxamide.

Carboxylic acid: Identified as p-bromophenacyl-iso-butyrate. m.p. 76.5-77.5°.

Found : C 50.5 %; H 4.5 %

Calc. for

$C_{12}H_{13}BrO_3$ (285.15) : " 50.54%; " 4.60%

Alkylglycine: Identified as N-benzenesulphonyl-N-iso-butylglycine. m.p. 103-105° (uncorrected)

Found : C 53.2 %; H 6.3 %

Calc. for

$C_{12}H_{17}NO_4S$ (271.34) : " 53.12%; " 6.32%

4-Amino-¹-ethyl-2-methylimidazole-5-carboxamide.

Carboxylic acid: not isolated.

Alkylglycine: Identified as N-benzenesulphonyl-N-ethylglycine. m.p. 115-116.5°.

Found : C 49.4 %; H 5.6 %

Calc. for

C₁₀H₁₃NO₄S (243.29) : " 49.37%; " 5.38%

(C). Determination of the NH₃ component.

Ammonium chloride was detected in the ethanol insoluble residue after treatment with hydrochloric acid at 140° (see page 101). The quantitative estimation of this ammonia, however, was performed on small quantities of 4-amino-1,2-dialkylimidazole-5-carboxamides in separate experiments as follows:-

An accurately weighed amount of the relevant aminoimidazole derivative (20-30 mg) was heated in a sealed tube with 10 ml concentrated hydrochloric acid for 24 hours at 140°. After cooling, the tube was carefully opened and the contents quantitatively transferred to a 100 ml. volumetric flask and adjusted to volume with water. The ammonia content in duplicate 10 ml. aliquots was determined with Nessler's reagent. One ml of concentrated hydrochloric acid was added to blank and standard samples so that all tubes were of comparable acidity. The findings are shown in the Table XIV.

TABLE XIV.

Ammonia produced by treatment of 4-amino-1,2-
dialkylimidazole-5-carboxamides with hydro-
chloric acid at 140°.

1,2-dialkyl groups	Nitrogen Content		% Recovery
	Calc. N moles	Found N moles	
1-Ethyl-2-methyl	3.00	3.06	102
1-Ethyl-2-propyl	3.00	2.93	97.7
2-Ethyl-1-propyl	3.00	3.03	101
1-Butyl-2-methyl	3.00	2.90	96.7
1-Butyl-2-propyl	3.00	2.88	96.0
1- <u>iso</u> -Butyl-2- <u>iso</u> propyl	3.00	3.00	100

Permanganate oxidation of 5-chloro-1-methyl-4-nitro-2-(p-nitrophenyl)-imidazole.

A hot suspension of 5-chloro-1-methyl-4-nitro-2-(p-nitrophenyl)-imidazole (0.01 mole; 1.89 g) in 2N-potassium hydroxide solution (100 ml) was treated with potassium permanganate (5 g) in aqueous solution. The suspension which was initially orange in colour became purple, and after stirring for about one hour became bright green. After standing overnight excess permanganate was destroyed by boiling with a little ethanol. The solution was filtered hot and the pale yellow filtrate evaporated to small bulk, acidified with dilute hydrochloric acid and allowed to stand. A pale yellow crystalline precipitate formed which was recrystallised from hot water. The melting point, and mixed melting point with an authentic sample of p-nitrobenzoic acid, was 241-242°C.

Synthesis of 5-chloro-1-methyl-2-phenylimidazole from hippurymethylamide. (cf. Karrer and Gränacher, 1924; Gränacher et al., 1925)

(a). Hippurymethylamide. Hippuryl chloride was prepared from hippuric acid (Fischer, 1905) and converted to hippurymethylamide by heating under reflux with methylamine in ethanol (33% solution). The product **crystallised** as silvery-white plates on cooling and was recrystallised from water. The yield of pure material was 31% of the theoretical. m.p. 160.5-161.5°.

(b). 5-Chloro-1-methyl-2-phenylimidazole. Hippuryl-methylamide (2 g) was dissolved in chloroform (10 ml) and treated with small portions of phosphorus pentachloride until 4 grams had been added. A vigorous reaction ensued after each addition. The solution was refluxed for 4-5 hours with precautions against entry of moisture. The dark solution was cooled, and the hydrochloride of the base precipitated by the addition of ether. The supernatant liquid was decanted and the residue washed with ether. The tarry residue was finally dissolved in chloroform and extracted with 4N hydrochloric acid. The acid extract was treated with excess 50% sodium hydroxide and cooled. The base crystallised as long white needles. The product, 5-chloro-1-methyl-2-phenylimidazole, was used to confirm the identity of the Wallach base prepared by the action of phosphorus pentachloride on benzylmethyloxamide. (see pages 55 and 88). By means of the melting points and mixed melting points of the free bases (m.p. 104-105.5°) and the picrates (m.p. 177-178.6°), as well as the infrared spectra (page 57), the two imidazoles were shown to be identical.

Synthesis of 5-chloro-2-methyl-1-phenylimidazole.

Acetylglycine ester was prepared according to the method of Curtius (1916), and converted to acetylglycine anilide, m.p. 191°. The imidazole was prepared as described by Gränacher (1925), but the yield was very poor and only the picrate could be isolated. m.p. 177-178°.

Synthesis of 5-chloro-1,2-dipropylimidazole from butyrylglycine-propylamide.

(a). Butyrylglycine ethyl ester. (Curtius, loc. cit.)

A yellow liquid with a pungent "cheesy" odour, b.p. 168-172°/17 mm. The yield was 67%.

(b). Butyrylglycine-propylamide. Prepared as described for hippurylmethylamide (see above).

(c). 5-Chloro-1,2-dipropylimidazole. Butyrylglycine-propylamide (5 g) was suspended in chloroform (25 ml) and phosphorus pentachloride (12.5 g) was added as described above. The product of the reaction was isolated by the method already described.

Found : C 57.8 %; H 8.1 %

Calc. for
 $C_9H_{15}ClN_2$ (186.69) : " 57.90%; " 8.10%

Picrate: Yellow needles from ethanol. m.p. 111.5-112.5°.

Synthesis of 4-chloro-1,2-dialkylimidazoles (cf. Sarasin, 1923).

(a). Preparation of quaternary iodides of 5-chloro-1,2-dialkylimidazoles. Quaternary iodides were prepared by heating a benzene solution containing one molar proportion of the imidazole base with one molar proportion of the respective alkyl iodide under reflux for eight hours. In some cases the quaternary iodide precipitated as a white solid, but more frequently, would separate

as a heavy oil. This was separated and washed with benzene. Solification could usually be induced by "scratching" in the presence of ether. If prolonged attempts to induce crystallisation failed, the oil (thoroughly washed) was converted directly to the 4-chloroimidazole derivative (see below). Solid iodides were purified by solution in absolute ethanol and precipitation with ether until a sharp melting point was attained. It was used for the next stage in the reaction without analysis. Melting points are recorded in Table XV.

TABLE XV.

The melting points of some 5-chloro-1,2-dialkyl-imidazole-alkyl-quaternary iodides.

Compound	m.p. (°C)
5-Chloro-2-ethyl-1-propyl-imidazole propiodide	95-96
1-Butyl-5-chloro-2-methyl-imidazole butiodide	127.3-129.3
5-Chloro-1-ethyl-2-methyl-imidazole methiodide	208-209
1-Butyl-5-chloro-2-propyl-imidazole butiodide	oil
2-Benzyl-5-chloro-1-methyl-imidazole ethiodide	191.3-192.0

(b). Conversion of the alkyl quaternary iodides to 4-chloro-1,2-dialkylimidazoles. The quaternary iodide was distilled under vacuum and the high-boiling 4-chloroimidazole derivative condensed out.

The alkyl iodide fragment (see page 29) did not condense under the conditions used, but some contamination appeared to take place. Redistillation was essential to recover a purer product. A picrate was prepared from aqueous ethanol. The melting points of the picrates are listed in the accompanying table.

TABLE XVI.

The picrate of some 4-chloro-1,2-dialkyl-
imidazoles.

4-Chloro-1,2-dialkyl- imidazole.	Picrate m.p. (°C.)
4-Chloro-2-ethyl-1-propyl- imidazole	134.5-135.5
1-butyl-4-chloro-2-propyl- imidazole	123.3-124.3

Dehalogenation of 5-chloro-1,2-dialkylimidazoles.

Dechlorination of several Wallach bases was undertaken as outline^d by the following example:-
1-Ethyl-2-propylimidazole. A solution of 5-chloro-1-ethyl-2-propylimidazole (5 g) in absolute ethanol (100 ml.) was carefully treated with small pieces of sodium. A white precipitate of sodium chloride formed. After the reaction had appeared complete, the mixture was centrifuged to remove the very fine precipitate, and then a further quantity of absolute ethanol and sodium pieces added. This process was repeated until no further sodium chloride precipitated. The alcohol solution was evaporated to dryness, water (250 ml.) was added and the solution

TABLE XVII.

Some data on 1,2-dialkylimidazoles.

Compound	m.p. of Picrate(°C)	Analysis of Picrate					
		Calculated			Found		
		C	H	N	C	H	N
1-Ethyl-2-propylimidazole	124.5-125.5	45.78	4.67	19.07	46.2	4.9	19.5
2-Ethyl-1-propylimidazole	138.5-140.5	45.78	4.67	19.07	45.9	4.7	18.9
1-Butyl-2-propylimidazole	108.5-109.5	48.60	5.35	17.71	48.6	5.2	17.6
1- <u>iso</u> -Butyl-2- <u>iso</u> -propyl- imidazole	139-140	48.60	5.35	17.71	48.6	5.3	18.0

extracted three times with chloroform. The chloroform solution was washed with water and dried (K_2CO_3). After removal of the chloroform the oily residue was purified by distillation. The boiling point was not recorded. A picrate was prepared and characterised. m.p. $124.5-125.5^\circ$.

Found : C 46.2 %; H 4.9 %; N 19.5 %
Calc. for $C_8H_{14}N_2$ (138.22) : " 45.78%; " 4.67%; " 19.07%

Details of other dechlorinated bases are given in Table XVII.

Chromatography.

Gas-liquid chromatography: This was conducted on a non-commercial instrument equipped with a one meter column packed with 80-100 mesh Chromasorb containing 2% silicone polymer SE 30. The activity of the column was reduced by treatment with hexamethyldisilazane. Nitrogen gas (12 lbs./sq.in.) was the moving phase and the chromatography was conducted at temperatures between 80° and 150° , the most suitable being 120° . A convenient concentration of test material was 0.5 micro litres of a 1% solution in methanol. Detection of eluted components was by flame ionisation.

"Reversed-phase" thin-layer-chromatography:

Cellulose¹-coated plates were prepared in the

Footnote 1: supplied by Camag, Muttenz, Switzerland.

conventional manner (0.25 mm thick), and were immersed in an acetone solution of propylene glycol (30%). The acetone was removed by a current of cold air. The chromatogram, after "spotting" was developed with dekaline (B.D.H.) saturated with propylene glycol. Compounds were detected by spraying with Dragendorff reagent (Bloch et al., 1958). As the plates were not strictly reproducible, R_f values tended to vary between experiments. Standard materials run concurrently with the unknown materials were consequently of particular importance. Table VII contains some typical R_f values (Page 44).

Ion-exchange chromatography.

Dowex 50 x 8 (400 mesh) or Dowex 50 x 12 (400 mesh) was purified by the method of Moore and co-workers (1958), then washed exhaustively on the column with 4N hydrochloric acid, and finally with water until neutral. The columns (22 x 1.2 cm. or 52 x 1.2 cm.) were operated under slight pressure (nitrogen at ca. 2 lbs./sq. in.) to improve the flow rate to approximately 50 ml./hr. The isomeric bases (5-10 mg) dissolved in dilute hydrochloric acid (2 ml., ca. 0.1 N) were applied to the column and washed in with a little water. Elution from the column was effected with 1 N hydrochloric acid, collecting fractions of 19 ml each. Each fraction was subsequently examined for absorption at 220 μ . against a water blank.

Ultraviolet spectrophotometry.

The spectra of a series of 5-chloro-1,2-dialkylimidazoles at various pH values were obtained with a Unicam S.P. 800 recording spectrophotometer. The fractions obtained after ion-exchange chromatography of isomeric Wallach bases were analysed by means of a Beckman D.U. manual spectrophotometer.

Infrared spectrophotometry.

The infrared spectra reported were mostly obtained with a Perkin Elmer 521 I.R. spectrophotometer. A few spectra were obtained with a Perkin Elmer Infracord spectrophotometer, model 137.

P A R T II.

THE SYNTHESIS AND PROPERTIES OF SOME 7,8-DIALKYL-
PURINES.

(a). Purines occurring in biological material.

Relatively few purines occur in nature; the parent compound, purine itself, is a component of nebularine (9- β -D-ribofuranosylpurine) obtained from the fungus Agericus (Clitocybe) neblaris ((Löffgren and Luning, 1953; Brown and Weliky, 1953).

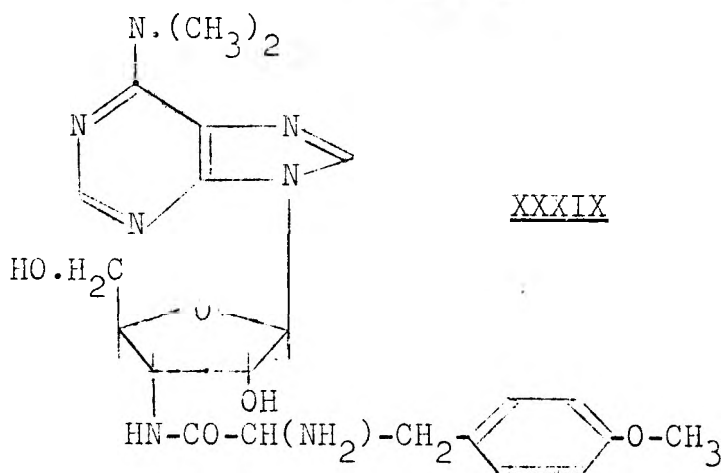
Adenine and guanine are the major purine constituents of the nucleic acids. Detailed study of RNA has shown, that in addition to adenine and guanine, 2-methyladenine, N⁶-methyladenine, N⁶,N⁶-dimethyladenine, 1-methylguanine, N²,N²-dimethylguanine and 6-methylguanine can all be isolated, though admittedly in trace amounts (Montgomery and Thomas, 1962).

The breakdown products of adenine and guanine, hypoxanthine, xanthine and uric acid are oxy-derivatives of purine, and both xanthine and uric acid are derived metabolically by the progressive oxidation of hypoxanthine under the action of the enzyme xanthine oxidase. Further unusual derivatives of adenine found in biological material include 2-methoxyadenine in the nucleoside spongosine (Bergman and Burke, 1956), and iso-guanine in crotonoside, a nucleoside

isolated from croton bean (Cherbuliez and Bernhard, 1932). Certain methylxanthines are found in plants; 3,7-dimethylxanthine (theobromine), 1,3-dimethylxanthine (theophylline) and 1,3,7-trimethylxanthine (caffeine) can be isolated in relatively large quantities. Less well known is paraxanthine (1,7-dimethylxanthine) which has been isolated in small quantities from whale liver and human urine. Paraxanthine has been shown to possess marked antithyroid action (Mann and Porter, 1945), "neutralising" the effect of ca 5,000 times its weight of thyroxine in selected tests. Investigation of the isomeric dimethylxanthines has shown that paraxanthine is unique in this group of compounds in its antithyroid action. On the other hand, however, some higher homologues of 1,7-dimethyl- and 1,7,8-trimethylxanthine also display marked antithyroid activity. Examples of such compounds are 1-methyl-7-ethylxanthine, 1,7-diethylxanthine, 1,7-diethyl-8-methylxanthine and 1-methyl-7-n-propylxanthine.

N⁶,N⁶-Dimethyladenine is a constituent of the nucleoside puromycin, an antibiotic produced by Streptomyces alboniger with

the following structure (Waller et al.; 1953):-



This antibiotic is especially valuable for its action against multicellular organisms and protozoa, particularly certain trypanosoma. It also appears to exhibit carcinostasis in mammary adenocarcinoma in C_3H mice, but the dosage required causes general toxicity (quoted by Hutchings, 1957). Studies have been made on analogues of the natural material to determine the effect of changes in structure on biological activity and the results have been summarised by Hutchings (1957). Attention may be directed, in passing, to the presence of the 3-amino sugar which is essential for antibiotic activity and was discovered in natural products for the first time in puromycin.

(b). Synthetic purines of biological interest.

Much of present-day knowledge of synthetic purines which do not occur in nature has arisen through a study of chemotherapeutics and anti-

metabolites of natural purines. Interest in these compounds arose from the observation that in neoplastic tissue there is a significant increase in purine metabolism as opposed to normal healthy tissue - an observation which equally well applies to other aspects of metabolism but assumes an added significance when viewed in the light of current knowledge of the importance of the purines in the nucleic acids, protein synthesis and the transfer of genetic information. In fact, certain antimetabolites of adenine and guanine are known to have a distinct cytostatic action (see Appendix II of this thesis), particularly 6-mercaptopurine, 2,6-diaminopurine, 8-azapurine and 6-chloropurine. Some of these are in use therapeutically.

Despite the enormous number of purine derivatives already synthesised, relatively few of these contain alkyl and/or aryl substituents in the 7- or the 8-positions. A number of naturally occurring purines (eg. caffeine and paraxanthine) have methyl groups in the 7-position, and several investigators have examined similar types of compounds (Mann and Porter, 1945); Bendich et. al., 1954; Prasad and Robins, 1957, Robins and Hu Lin, 1957 and Bergmann and Tamari, 1961). A few 8-substituted phenyl and benzyl purines have been described (Cook and Thomas, 1950). Adams and Whitmore (1945) commented at that time on the paucity of work on new purines and described

the synthesis of some 8-hydroxy-2,6-dialkylpurines and 8-alkylamino-2,6-dihydroxypurines. Some 8,8-disubstituted purines have been described by Bühler and Pflederer, (1965). In the field of 7,8-disubstituted purines, Montequi (1926) prepared 7-ethyl-8-methylxanthine from 5-chloro-1-ethyl-2-methylimidazole and the method is similar to that employed for 7-methylxanthine (Sarasin and Wegmann, 1924). Preparation of 7-methyl-8-phenylxanthine and 8-benzyl-7-methylxanthine (Cook and Thomas, 1950) added two more compounds to the small list of 7,8-dialkylpurines but the method is restricted in that the 7-alkyl group is invariably methyl.

Despite the preparation of such purines as cited above, no systematic investigation into the synthesis of 7,8-dialkylpurines has been made. The present study is an attempt to remedy, at least in part, this deficiency in our knowledge of these compounds. To this end, the chlorimidazoles produced in the Wallach reaction on a variety of dialkyloxamides, have been converted into 7,8-dialkylpurines of known structure via the important intermediate, 4-amino-1,2-dialkylimidazole-5-carboxamide, using a series of established synthetic techniques.

(c). The synthesis of purines from imidazoles.

Since their recognition as a distinct chemical class of compounds by Emil Fischer, a large number of purines have been described. Most of the known

synthetic methods fall into two general groups depending upon whether the pyrimidine or the imidazole ring is formed first. Historically the older, and the more widely exploited, syntheses start from a preformed pyrimidine. A comprehensive review of these methods has recently been published (Lister, 1961). Of later origin is the alternative procedure in which the imidazole ring is completed first, and the purine then formed by completing the pyrimidine ring from suitable groups in the 4- and 5- positions of the imidazole nucleus. Only the latter technique is of relevance to the present study.

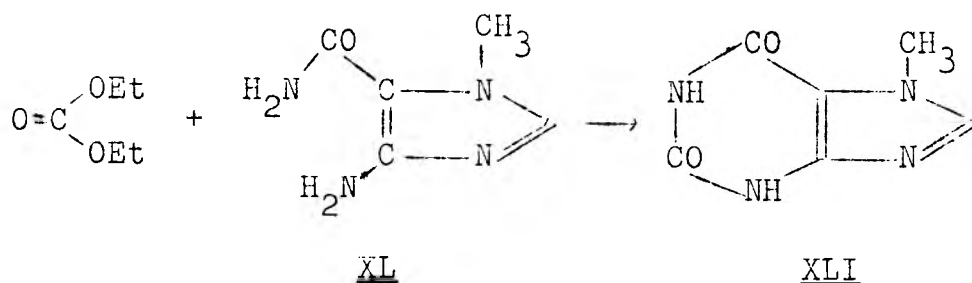
In spite of the success of early attempts to synthesis purines from imidazoles (eg. Sarasin and Wegmann, 1924) the method did not attract much attention and little development followed. Even as late as 1957, Taylor and co-workers were led to comment:-

" - several synthetic routes to purines have been described which utilise a preformed imidazole intermediate, but because of their inaccessibility, relatively few purines have been prepared in this manner. (Bendich, 1955; Cook and Thomas, 1950). -"

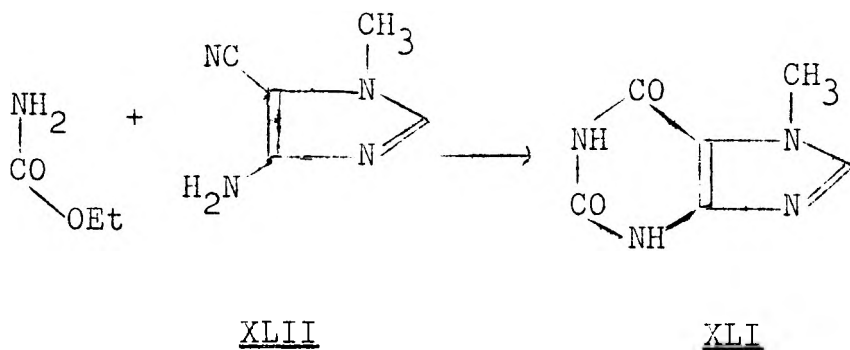
In recent years many more purine syntheses from imidazole derivatives have been published, possibly stimulated by the finding that much the same route is followed in the biological synthesis of nucleosides (for review see Buchanan, 1958-1959). No attempt will be made here to cover the entire field of these synthetic methods,

now adequately review by Lister (1963), and the present discussion will be confined to those syntheses of importance for the preparation of 7,8-dialkylpurines.

The first purine—7-methylxanthine (hetero-xanthine)—(XLI) to be synthesised from an imidazole intermediate was prepared by heating 4-amino-1-methylimidazole-5-carboxamide (XL) with diethyl carbonate under pressure (Sarasin and Wegmann, 1924). The following ring-closure took place:-

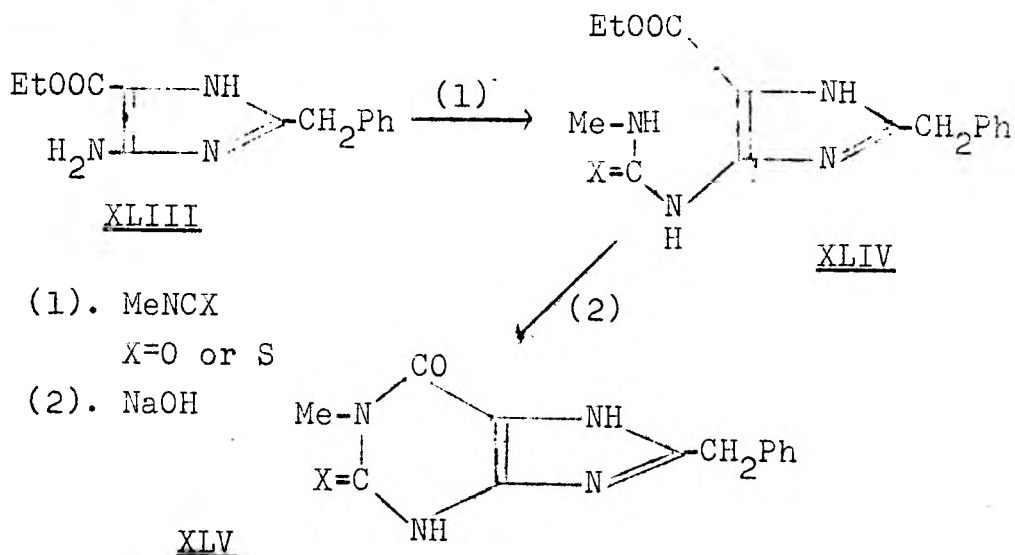


Somewhat later, the same purine was formed by the treatment of 4-amino-5-cyano-1-methylimidazole (XLII) with urethane (Montequi, 1926).



The synthesis of purines from imidazoles now lay dormant until revived, much later, for the synthesis of 1-alkyl-7-methylxanthines (Mann and Porter, 1945). Two variations of the method were studied by these workers of which only the first was successful. In this procedure 4-nitroimidazole-5-carboxamide was converted by the Bouveault reaction to the corresponding acid and then to the acid chloride. Treatment with an alkylamine gave a secondary amide. After reduction of the nitro group, ring-closure of the aminoamide was effected with diethyl carbonate. As the number of carbon atoms in the alkyl group of the secondary amide increased, it was noted that formation of the pyrimidine ring became increasingly more difficult to achieve. It was also shown that ethyl chloroformate was superior to diethyl carbonate in effecting ring-closure. In the second procedure a 4-nitroimidazole-5-carboxylate was reduced to the amino-ester. Cyclization with ethyl chloroformate could not be achieved, and the method was consequently abandoned. However, a closely related synthesis of purines from 2-substituted 4-aminoimidazole-5-carboxylates has been successfully accomplished (Cook *et al.*, 1949). In this reaction 4-amino-2-benzyl-5-carbethoxyimidazole was treated with either methyl iso-cyanate or methyl iso-thiocyanate to give a ureidoimidazole. Treatment of this derivative with sodium hydroxide solution resulted in the

formation of either a xanthine or a 2-thioxanthine respectively (XLIII - XLV). Further study by Cook and his co-workers (1950) showed that methyl iso-thiocyanate can, with advantage, be replaced by acetyl iso-thiocyanate.

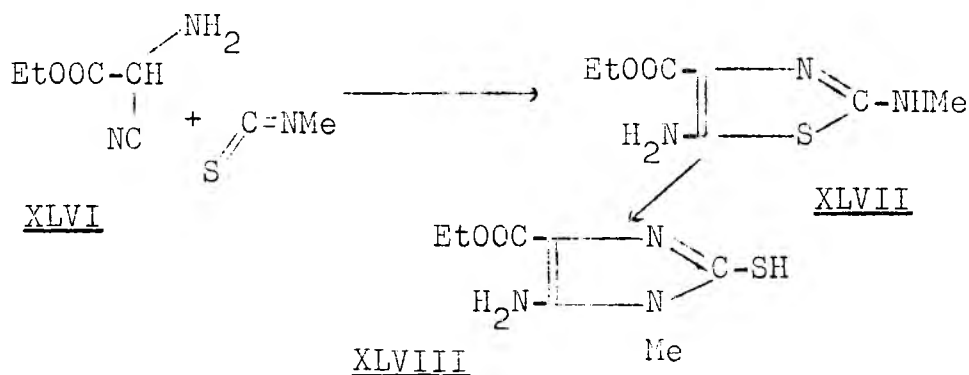


The above methods as well as several others of more recent origin (see Lister, 1963), have in common the use of a 4-amino-imidazole-5-carboxamide derivative or one in which the carboxamide group has been replaced by a related function such as a carboximidine, carboxylate or a cyano group. The general technique of purine synthesis from imidazoles may be divided into two stages:

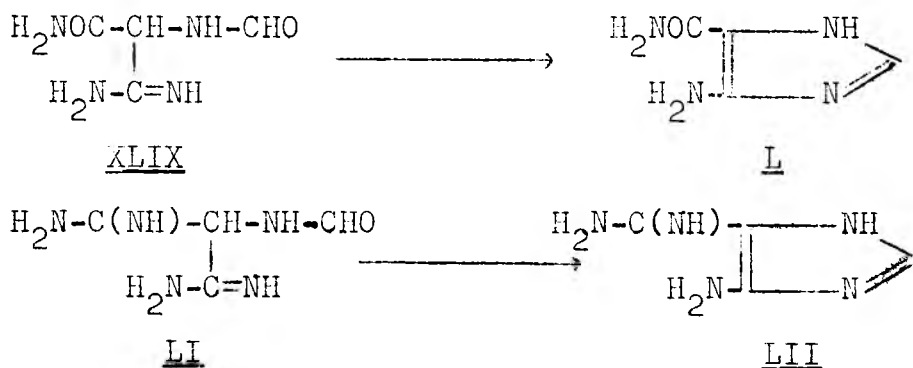
(a) the preparation of the 4-aminoimidazole-5-carboxamide from a suitable starting material, and (b) the ring-closure of this derivative to form the required purine.

Two routes are available for the preparation of 4-aminoimidazole-5-carboxamide (or related intermediates.)

The first depends upon an intermolecular re-arrangement of a thiazole, an example of which is the alkali conversion of a 2-methylaminothiazole (XLVII) to the imidazole (XLVIII) (Mongomery et al., 1959). The thiazole intermediate was prepared by the condensation of ethyl α -aminocynoacetate with methyl isc-thiocyanate (Shaw and Woolley, 1949).



The second route to 4-aminoimidazole-5-carboxamide depends upon the ring-closure of a linear intermediate. Into this group fall the important techniques of thermal cyclisation of formamidomalonamide hydrochloride (XLIX) (Shaw and Woolley, 1949) and formamidomalondiamidine (LI) (Shaw 1950).



Numerous alternative techniques for the preparation of suitable imidazole intermediates have also been described (Montgomery et al., 1959; Miller et al., 1950 and 1952; Shaw et al., 1959).

The preparation of 4-amino-1,2-dialkyl-imidazole-5-carboxamides [with the exception of 4-amino-1-methylimidazole-5-carboxamide (Sarasin and Wegmann, 1924) and 4-amino-1-ethyl-2-methylimidazole-5-carboxamide (Montequi, 1926; Mann and Porter, 1945)], have been little investigated. These latter imidazoles were prepared by a 6-stage synthesis from dimethyloxamide and diethyloxamide respectively. Numerous techniques are available for the completion of the pyrimidine ring to give the corresponding 7,8-dialkylpurine (see below). With an occasional exception (eg. Cook and Thomas, 1950), the preparation of higher 7,8-dialkylpurines appears to have received very little attention. Extension of the Sarasin and Wegmann technique (loc.cit.) to higher 5-chloro-1,2-dialkylimidazoles has been neglected, possibly as the Wallach reaction has been considered to be of practical value only for dimethyl- and diethyloxamide (Hofmann, 1953; Lister, 1963). However, an examination of the original literature (for reference see page 6) indicated to the present author that some higher 5-chloro-1,2-dialkylimidazoles have been prepared, but experimental details regarding yields are scanty.

Alternative syntheses for the preparation of

7,8-dialkylpurines from preformed pyrimidines have not been reported, but some 9-alkyl purines (Baker et al., 1954; Montgomery and Temple, 1957 and 1958; Schaeffer et al., 1959 and 1960) and a few 7-alkyl purines (Ruttink, 1946) have been prepared by this route. The synthesis of purines substituted with an alkyl or aryl group in the 8-position (starting from a pyrimidine precursor) has also been described (Elion et al., 1951a; Brederick et al., 1953; Acker and Castle, 1958; Prasad et al., 1959) though some difficulty has been reported in the case of higher alkyl groups (Montgomery and Temple, 1960). Thus, while purine syntheses from pyrimidines are suitable for the preparation of 9 (or 7)-alkylpurines and 8-alkylpurines, and could, presumably, be adapted to the preparation of 7,8-dialkylpurines, the methods appear to lack scope and convenience. In the present investigation, the successful exploitation of the Wallach reaction (See Section I) has provided a versatile procedure for the synthesis of suitable intermediates in the preparation of a variety of 7,8-dialkylpurines.

Completion of the pyrimidine ring of the purine may be accomplished by a variety of techniques. In the present study, 7,8-dialkylxanthines were most readily prepared by treatment of the respective 4-amino-1,2-dialkylimidazole-5-carboxamide with ethyl chloroformate in dioxan. Addition of potassium bicarbonate, as described for xanthine (Mann and Porter, 1945; Shaw, 1950) was found to be unnecessary.

On standing, the intermediate 4-carboethoxyamido-1,2-dialkylimidazole-5-carboxamide precipitated from the reaction mixture and was isolated. Ring-closure of this material to give the 7,8-dialkylxanthine was accomplished by heating the 4-carboethoxyamido intermediate in morpholine at 120° for $\frac{1}{2}$ hour. Alternatively, the same xanthine could be prepared by heating a mixture of the 4-amino-1,2-dialkylimidazole-5-carboxamide and urea for 2 hours at 175° (Shaw, 1950), but the yield was poor. Attempts to complete the pyrimidine ring by treatment of the aminoamide with diethyl carbonate (Sarasin and Wegmann, 1924) were not successful.

Conversion of the 4-amino-1,2-dialkylimidazole-5-carboxamide to a 7,8-dialkylhypoxanthine was best achieved in the present investigation by treatment with sodium formate in a mixture of formic acid and acetic anhydride (Shaw, 1950). Unlike the analogous procedure for hypoxanthine, the intermediate product - 1,2-dialkyl-4-formamidoimidazole-5-carboxamide - could not be isolated and the required hypoxanthine derivative was formed in a single-stage reaction, ring-closure being spontaneous. The preparation of hypoxanthine, by contrast, requires a separate stage to complete the pyrimidine ring by treatment of the intermediate formamidoimidazole with dilute alkali. A less satisfactory procedure for the synthesis of 7,8-dialkylhypoxanthines depends upon the action of ethyl orthoformate in acetic

anhydride on the corresponding 4-amino-1,2-dialkylimidazole-5-carboxamide (Richter and Taylor, 1955; Tayler and Loeffler, 1960). Higher orthocarboxylic acids can be used to prepare 2,7,8-trialkylhypoxanthines.

6-Chloro-7,8-dialkylpurines were readily prepared from the corresponding 7,8-dialkylhypoxanthines by treatment with phosphorus oxychloride (Prasad and Robins, 1957). It was not found necessary to conduct the reaction in the presence of a secondary or tertiary amine as required in the preparation of 6-chloropurine (Bendich et al., 1954). The chlorine atom in these purines is very reactive and can easily be replaced.

The importance of 6-mercaptopurine as an anti-cancer agent (see Appendix II) suggested that other mercaptopurines might be of value as purine anti-metabolites. Accordingly, a number of 7,8-dialkyl-6-mercaptopurines have been synthesised in the present study. Two general techniques for the preparation of these derivatives are available: first, by the action of thiourea on a 6-chloropurine, and secondly, by treatment of a hydroxypurine with phosphorus pentasulphide. In the present study the latter reaction only was explored to prepare 7-butyl-6-mercapto-8-propylpurine and 7-iso-butyl-6-mercapto-8-iso-propylpurine from the corresponding hypoxanthines. Application of this reaction to 7-butyl-8-propylxanthine was expected to give 7-butyl-2-hydroxy-6-mercapto-8-

TABLE XVIII.

7,8-Dialkylxanthines.

7,8-Dialkylxanthine	m.p. (°C)
7-Ethyl-8-propylxanthine	265.5-266.5
8-Ethyl-7-propylxanthine	224.5-225.5
7-Butyl-8-propylxanthine	212.5-213.5
7- <u>iso</u> -Butyl-8- <u>iso</u> -propyl-xanthine	246-247.5

TABLE XIX.

7,8-Dialkylhypoxanthines.

7,8-Dialkylhypoxanthine	m.p. (°C)
7-Ethyl-8-propylhypoxanthine	217.4-218.2
7-Butyl-8-propylhypoxanthine	202-202.5
7- <u>iso</u> -Butyl-8- <u>iso</u> -propyl-hypoxanthine	181.5-182.5

propylpurine, rather than 7-butyl-2,6-dimercapto-8-propylpurine. The structure of this compound was suggested by analogy with the formation of 2-hydroxy-6-mercapto-7-methylpurine prepared by the action of phosphorus pentasulphide on 7-methylxanthine (Prasad and Robins, 1957). However, satisfactory analysis could not be obtained.

E X P E R I M E N T A L P A R T.

7,8-Dialkylxanthines.

Several 7,8-dialkylxanthines were prepared by the following general methods, described in detail below for 7-butyl-8-propyl-xanthine.

7-Butyl-8-propylxanthine.

Method A: 4-Amino-1-butyl-2-propylimidazole-5-carboxamide (3 g) was dissolved in dioxan (100 ml) and ethyl chloroformate (6 g) added. The mixture was warmed on the water bath for $\frac{1}{2}$ hour, cooled, and the precipitate collected by filtration and washed with dioxan. The crude solid was dissolved in re-distilled morpholine (75 ml) and refluxed at 120° for $\frac{1}{2}$ hour. The morpholine was removed under vacuum. The oily residue solidified on treatment with a few millilitres of water. The crude product was recrystallised from aqueous ethanol.

Yield = 1.2 g (36%). m.p. $212.5-213.5^{\circ}$.

Found : C 57.8 %; H 7.7 %; N 22.4 %

Calc. for
 $C_{12}H_{18}N_4O_2$ (250.30) : " 57.58%; " 7.40%; " 22.40%

Method B. 4-Amino-1-butyl-2-propylimidazole-5-carboxamide (0.5 g) was mixed with urea (1.0 g) in a test tube, and heated to 180° for three hours. After cooling, the crystalline mass was dissolved in normal sodium hydroxide, boiled with a little charcoal, and filtered. The crude xanthine was precipitated by the dropwise addition of dilute

acetic acid and crystallised from aqueous ethanol (charcoal) as white plates. Yield = 0.1 g (18%) m.p. 212.5-213.5°, alone or when admixed with a sample prepared by Method A.

7-iso-Butyl-8-iso-propylxanthine. Prepared by method A. Yield = 1.5 g (45%) m.p. 246 - 247.5°.

Found : C 57.5 %; H 7.3 %; N 22.2 %

Calc. for $C_{12}H_{18}N_4O_2$ (250.30) : " 57.58%; " 7.40%; " 22.40%

7-Ethyl-8-propylxanthine. Prepared by method A.

Yield = 28% m.p. 265.5-266.5°.

Found : C 54.2 %; H 6.1 %; N 25.0 %

Calc. for $C_{10}H_{14}N_4O_2$ (222.25) : " 54.05%; " 6.31%; " 25.22%

8-Ethyl-7-propylxanthine. Prepared by method A.

Yield = 36%. m.p. 224.5-225.5°.

Found : C 54.2 %; H 6.5 %; N 25.0 %

Calc. for $C_{10}H_{14}N_4O_2$ (222.25) : " 54.05%; " 6.31%; " 25.22%

7,8-Dialkylhypoxanthines.

The following general methods, exemplified by the preparation of 7-iso-butyl-8-iso-propylhypoxanthine, were found satisfactory in the present investigation.

7-iso-Butyl-8-iso-propylhypoxanthine.

Method A: 4-Amino-1-iso-butyl-2-iso-propylimidazole-5-carboxamide (5 g) was mixed with anhydrous sodium formate (3.5 g) and dissolved in 98-100% formic

acid (20 ml). To this solution acetic anhydride (40 ml) was added and after a short while a moderately vigorous exothermic reaction commenced. When the reaction had moderated, the mixture was heated to 70° until no further evolution of gas occurred. At this stage the solvents were removed by distillation under reduced pressure, the product dissolved in dilute sodium hydroxide solution, and the hypoxanthine precipitated by the addition of dilute acetic acid. The crude product was purified by recrystallisation from water (charcoal).

Yield = 4.2 g (81%) m.p. 181.5-182.5°. (dried over P₂O₅/100°.)

Found : C 59.2 %; H 7.6 %; N 22.8 %

Calc. for
C₁₂H₁₈N₄O_{0.5}H₂O : " 59.24%; " 7.46%; " 23.03%
(243.31)

Method B: 4-Amino-1-iso-butyl-2-iso-propylimidazole-5-carboxamide (1 g) was refluxed for 2 hours with a mixture of ethyl orthoformate (10 ml) and acetic anhydride (10 ml). The solvents were then removed by distillation under vacuum and the sticky brown residue purified as in Method A. Yield = 0.32 g (31%). m.p. 181.5 - 182.5°.

7-Butyl-8-propylhypoxanthine. Prepared by both of the above methods. Yield = 88% (method A); 32% (method B). m.p. 202-202.5°.

Found : C 61.2 %; H 7.6 %; N 24.2 %

Calc. for
C₁₂H₁₈N₄O (234.30) : " 61.54%; " 7.69%; " 23.93%

7-Ethyl-8-propylhypoxanthine. Prepared by method A. Recrystallised from ethyl-methyl-ketone containing a small percentage of water.

Yield 81%. m.p. 217.4-218.2°.

Found : C 58.0 %; H 6.9 %; N 27.0 %

Calc. for
 $C_{10}H_{14}N_4O$ (206.15) : " 58.26%; " 6.80%; " 27.18%

7-iso-Butyl-6-chloro-8-iso-propylpurine. 7-iso-Butyl-8-iso-propylhypoxanthine (2.5 g) was refluxed for 6 hours with phosphorus oxychloride (75 ml). Excess phosphorus oxychloride was then removed by distillation under vacuum. Crushed ice (10 g) was added to the residue followed by sodium hydroxide solution (40%) dropwise until the pale yellow solid which formed was fully precipitated. The filtered product was recrystallised from dilute aqueous ethanol (charcoal). Yield = 2.3 g (88%).

Found : C 57.0 %; H 6.8 %; N 22.4 %; Cl 13.9 %

Calc. for
 $C_{12}H_{17}N_4Cl$ (252.75) : " 57.02%; " 6.73%; " 22.18%; " 14.06%

7-Butyl-6-chloro-8-propylpurine. Prepared as described above. The crude product was obtained as a yellow oil which was extracted into chloroform, washed with water, dried over anhydrous sodium sulphate and the chloroform removed by evaporation. The product was used without further purification for the synthesis of a number of N⁶-substituted adenines.

7,8-Dialkyl-N⁶-substituted-adenines.

Several 7,8-dialkyl-6-alkylaminopurines have been prepared by the action of the relevant primary

amine on the 6-chloro-purine. Individual details are described below.

7-Butyl-6-methylamino-8-propylpurine. Two grams of 7-butyl-6-chloro-8-propylpurine were dissolved in ethanol (50 ml) and 33% methylamine in ethanol (10 ml) was added. After refluxing (6 hours), the ethanol was removed by distillation under vacuum and the yellow oily residue solidified by scratching in the presence of water (10 ml). The crude product was filtered, washed with water, and then recrystallised from aqueous methyl cello-solve. Yield = 1.61 g (82%). m.p. 129.5-130.5°.

Found	:	C 63.1 %; H 8.4 %; N 28.5 %
Calc. for	:	
C ₁₃ H ₂₁ N ₅ (247.35)	:	" 63.12%; " 8.56%; " 28.31%

7-iso-Butyl-6-cyclohexylamine-8-iso-propylpurine.

A solution of 7-iso-butyl-6-chloro-8-iso-propyl-purine (1 g) in absolute ethanol (25 ml) was boiled under reflux with cylcohexylamine (2 g) for 8 hours. The ethanol was removed in vacuo and the residue dissolved in a little chloroform and washed thoroughly with water. The chloroform was then removed and the residue crystallised from dilute aqueous methyl cellosolve. Yield = 0.8 g (64%). m.p. 209-210.5°.

Found	:	C 68.6 %; H 9.0 %; N 22.3 %
Calc. for	:	
C ₁₈ H ₂₉ N ₅ (315.46)	:	" 68.53%; " 9.27%; " 22.20%

7-Butyl-6-butylamino-8-propylpurine. Prepared by the above general technique. The crude product was recrystallised from aqueous acetone. Yield = 65%. m.p. 82.0-82.7°.

Found : C 66.2 %; H 9.4 %; N 24.3 %

Calc. for
 $C_{16}H_{27}N_5$ (289.42) : " 66.43%; " 9.34%; " 24.22%

7-iso-Butyl-6-(2'-phenylethylamino)-8-iso-propylpurine. Prepared as described above. The product was isolated by adding first a little water to the residue and then ether. On standing a thick, white precipitate appeared at the interface, and was isolated by filtration. It was recrystallised from very dilute ethanol. Yield = 42%. m.p. 153-155°.

Found : C 70.9 %; H 8.0 %; N 20.8 %

Calc. for
 $C_{20}H_{27}N_5$ (337.47) : " 71.18%; " 8.01%; " 20.77%

7,8-Dialkyl-6-mercaptopurine.

The melting points of the following mercaptopurines were difficult to measure due to decomposition during the determination. Sharp values could, however, be obtained by preheating the oil to just below the melting point of the compound prior to immersing the sample. This technique necessitated a number of trial determinations at slightly different temperatures before a satisfactory result was obtained.

7-Butyl-6-mercanto-8-propylpurine. Dry, redistilled pyridine (150 ml) was carefully added to an intimate mixture of 7-butyl-8-propylhypoxanthine (2 g) and phosphorus pentasulphide (14 g). An exothermic reaction commenced.

The mixture was then refluxed for 4 hours. The pyridine was removed by distillation under reduced pressure, and cold water carefully added to the residue to a total volume of 250 ml. The mixture was heated on the boiling water bath for 3 hours, additional water being added to replace that lost by evaporation. Concentrated sodium hydroxide solution (40%) was added to dissolve the flocculant precipitate present at this stage, and the resulting dark brown solution filtered. The crude product was precipitated from this solution by the careful addition of 25% aqueous acetic acid, and then recrystallised repeatedly from aqueous methyl cellosolve. Yield = 1.4 g (68%). m.p. 262-264.5°.

Found : C 57.9 %; H 7.3 %; N 21.9 %; S 12.6%

Calc. for

$C_{12}H_{18}N_4S$: " 57.67%; " 7.25%; " 22.38%; " 12.81%
(250.37)

7-iso-Butyl-6-mercapto-8-iso-propylpurine. Prepared as described above. The crude product was recrystallised from dimethylsulphoxide containing a very small proportion of water. Yield = 68%. m.p. (immersion) 244.2-245.5°.

Found : C 57.6 %; H 7.2 %; N 22.2 %; S 12.8 %

Calc. for

$C_{12}H_{18}N_4S$: " 57.67%; " 7.25%; " 22.38%; " 12.81%
(250.37)

7-Butyl-2-hydroxy-6-mercapto-8-propylpurine. Attempts were made to prepare this compound by the above procedure but satisfactory analysis could not be obtained.

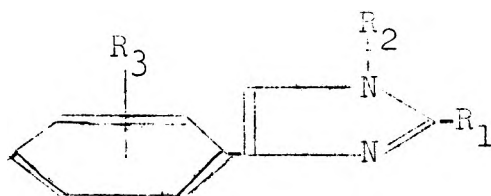
P A R T I I I .

THE ANTIFUNGAL ACTION OF SOME 5-CHLORO-1,2-DIALKYL-
IMIDAZOLES AND 5-CHLORO-1,2-DIALKYL-4-NITROIMIDAZOLES.

Relatively few imidazole derivatives have so far proved to be useful therapeutic agents against fungal or protozoal infections. The antibiotic azomycin (2-nitroimidazole) described by Nakamura (1955) has been shown to possess some anti-trichomonal activity, but is less effective than 1-(2'-hydroxyethyl)-2-methyl-5-nitroimidazole (Cosar and Julou, 1955), one of the few imidazoles used therapeutically. Some derivatives of 2-nitroimidazoles have been described (Lancini et al., 1963) as potential anti-trichomonal agents, but no report of the biological tests appears to have been published. The related thiazole derivative, 2-amino-5-nitrothiazole has been shown to possess marked anti-trichomonal activity (Cuckler et al., 1955).

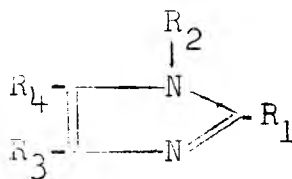
Two recent studies (Ellis et al., 1964a and b) of the antifungal and antiprotozoal activity of a series of substituted imidazoles have stimulated the present investigation into the anti-fungal action of a number of Wallach bases and their nitro derivatives. It was anticipated that the nitroimidazoles would show greater antifungal action than the parent bases. In this connection it is interesting to note that marked anti-trichomonal activity has been established for the heavily nitrated imidazole, 1-[(2,4-dinitropryrryl)-ethyl]-2-methyl-5-nitroimidazole (Brown et al., 1965).

The compounds studied by Ellis and co-workers, (1964a), for antifungal activity were of the general type:-



in which most of the chemical variation between compounds lay in the nature of the R^3 group. A large variety of mono or di-substituted phenyl derivatives, in which R^3 was halogen, nitro or other simple grouping, was tested. R^2 in these compounds varied to a lesser extent, being restricted to alkyl or 2-(pyrid-2-yl)ethyl-, and R^1 was a hydrogen atom except in one case when it was a methyl group.

A greater degree of complexity was introduced into the same basic imidazole molecule in studies of compounds with potential anti-protozoal activity (Ellis et al., 1964). These compounds were of the type:-



in which R^1 and R^2 were as previously described, R^3 was a substituted phenyl or styrene group (usually substituted with halogen or nitro groups), and R^4 a nitro group attached directly to the imidazole ring. Groups R^3 and R^4 were frequently interchanged.

While the Wallach bases and their nitro derivatives are less complex molecules than the compounds investigated by Ellis and co-workers, there remains a general similarity in structure. On the basis of this general similarity the present author undertook an examination of the potential anti-fungal action of a number of Wallach bases and nitro derivatives against a selection of eight pathogenic fungi. It was, in fact, demonstrated that some of these imidazoles possess antifungal activity against certain organisms, though the concentrations required were relatively large. It was possible, in addition, to assess in some measure the influence of an increase in the chain length of the alkyl group on anti-fungal activity. The results of this preliminary in vitro investigation are reported here. No attempt, however, has been made to evaluate any clinical importance of the results, as this lies outside the scope of the present work.

MATERIALS AND METHODS.

Organisms.

The following pathogenic fungi and yeast, isolated and identified during the course of routine mycological investigations on local patients, were employed as the test organisms.

1. *Aspergillus fumigatus*.
2. *Candida albicans*.
3. *Microsporum audouinii*.
4. *Microsporum canis*.
5. *Epidermophyton floccosum*.
6. *Trichophyton schoenleinii*.
7. *Trichophyton mentagrophytes*.
8. *Trichophyton rubrum*.

Methods.

All the organisms were cultured on Sabouraud's dextrose agar slants. The test materials were added to the media prior to sterilization (15 mins. at 15 lbs./sq.in.) in solution in Carbowax 300¹. The concentrations used were 50, 100, 200, 300, 400 and 500 μ /ml of medium. At no time did the total concentration of Carbowax 300 in the medium exceed 2% by volume, an amount shown to be without measurable effect on the growth rate of the organism. Slants were inoculated according to standard mycological practice. Stock sub-cultures of each organism were prepared at monthly intervals to minimise pleomorphic

1. Union Carbide Ltd.

changes.

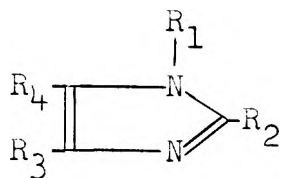
The inhibitory action of the imidazole derivatives was assessed by visual comparison of the size of the growth (i.e. surface area of medium covered by the organism) with the control slant. The concentration of fungistatic at which no growth was discernible after 7 days incubation at 29°C. was determined.

RESULTS.

Some preliminary results with several compounds on the yeast Candida albicans showed no inhibition even at high concentrations, and this organism was subsequently excluded from the survey. For the remaining 7 organisms the minimum concentration of active anti-fungal agent at which no growth of the organism could be detected on visual examination is listed in Table XXI. Most organisms required relatively large amounts of inhibitor to prevent growth, in many cases greater than 500 γ /ml of medium. Even the most active inhibitors were required at levels of about 100 γ /ml of medium. A large variation in the sensitivity of the various organisms to the drug was observed.

TABLE XX.

Compounds examined for anti-
fungal activity.



Cpd. No.	R ₁	R ₂	R ₃	R ₄
1.	Et	Me	H	Cl
2.	Pr	Et	H	Cl
3.	Bu	Pr	H	Cl
4.	<u>iso</u> -Bu	<u>iso</u> -Pr	H	Cl
5.	Me	Ph	H	Cl
6.	Et	Me	NO ₂	Cl
7.	Pr	Et	NO ₂	Cl
8.	Bu	Pr	NO ₂	Cl
9.	<u>iso</u> -Bu	<u>iso</u> -Pr	NO ₂	Cl
10.	Me	p-NO ₂ -Ph	NO ₂	Cl

TABLE XXI. Minimum concentration of test compound at which no growth of fungi was observed.

Cpd. No. table	Concentration of inhibitor(γ /ml.)						
	A.fumig.	M.audou.	M.canis	E.flocc.	T.schoen.	T.ment.	T.rubrum
1.	>500	>500	>500	>500	>500	>500	>500
2.	>500	>500	>500	>500	>500	>500	>500
3.	>500	200	200	300	300	500	500
4.	>500	>500	>500	>500	>500	>500	>500
5.	300	300	300	200	300	300	300
6.	>500	>500	200	200	300	400	400
7.	>500	>500	100	200	200	200	300
8.	>500	300	100	200	200	300	300
9.	>500	-	100	200	200	200	200
10.	300	200	200	200	200	400	100

DISCUSSION.

The 5-Chloro-1,2-dialkylimidazoles investigated above were relatively ineffective at concentrations up to 500 μ /ml. in inhibiting the growth of any of the organism studied. It was noted, however, that an increase in the length of the alkyl groups at C-1 and C-2 resulted in an increase in the anti-fungal activity as measured by a reduction in the area of agar covered by the organism. As the maximum concentration of inhibitor used in the study was less than that required for complete inhibition, the above conclusion is not illustrated by the data given in Table XXI. A further improvement in the anti-fungal activity was produced by replacing the alkyl group on the C-2 atom by a phenyl ring (Cpd.5).

The introduction of a nitro group in the 4-position of the imidazole ring (Cpds.6-10) produced a significant increase in inhibitory action against all the fungi tested. As with the non-nitrated Wallach bases, an increase in the number of C atoms in the alkyl groups was associated with an increase in anti-fungal activity. The greatest increase in inhibitory powers was noted in compound 10, (5-chloro-1-methyl-4-nitro-2-(p-nitrophenyl)-imidazole), and it is suggested that this increased activity is due, in part, to the presence of two nitro groups in the molecule. In this connection it is noteworthy that many established antifungal or antiprotozoal agents possess one or more nitro groups. The compounds 1-[(2,4-dinitrophenyl)-ethyl]-2-methyl-5-nitroimidazole and 1-(hydroxy-2'-ethyl)-2-methyl-5-nitroimidazole may be cited in support of this observation.

The present series of compounds were, in all cases, less active than those reported by Ellis and co-workers (1964a). The M.I.C. for the latter derivatives ranged for the most part between 5 and 25 μ /ml., and were thus some 4 to 20 times more active than the most active material examined by the present author. In addition the compounds tested by Ellis were frequently active against *Candida*, a feature absent from the present derivatives.

It is to be stressed that the present investigation was of a preliminary nature only. A number of aspects require further investigation. It was noted, for example, that in the investigation reported by Ellis and co-workers (1964) the minimum inhibitory concentration of five compounds for *Trichomonas* was between 20 and 250 times greater than for several species of *Trichophyton*. The possibility arises that the present compounds may have increased inhibitory activity against these protozoa. It is also to be remembered that compounds such as 1-(2'-hydroxyethyl)-2-methyl-5-nitroimidazole have in vivo activity as compared with the in vitro action described for the present compounds. The level of an inhibitory substance has to be assessed not only in terms of M.I.C. but also in terms of the toxicity of the compound and its ease of absorption. Such studies were considered beyond the scope of the present work but are essential to a more detailed investigation. Nevertheless, the finding recorded above may serve to stimulate further study of imidazole derivatives as useful drugs.

A P P E N D I X I.

THE PREPARATION OF N-ALKYLGLYCINE DERIVATIVES USED AS
REFERENCE COMPOUNDS.

The author examined a number of standard techniques and selected the following as suitable for the present study:-

N-benzenesulphonyl-N-ethylglycine (cf. Cocker, 1937).

To N-benzene-sulphonylglycine (12.5 g) (Cocker and Lapworth, 1931) dissolved in 3N sodium hydroxide (40 ml) was added ethyl iodide (16 g) and sufficient ethanol to form a homogeneous solution. The mixture was shaken for 3-4 hours and then filtered. Dilute hydrochloric acid was added dropwise to the solution and the product precipitated as an oil which solidified on standing at 5°. It was filtered and recrystallised from water. m.p. 115-116.5°.

Found : C 49.5 %; H 5.4 %

Calc. for
 $C_{10}H_{13}NO_4S$ (243.29) : " 49.37%; " 5.26%

N-benzenesulphonyl-N-n-butylglycine. Sodium (3.25-3.50 g) was dissolved in absolute ethanol (100 ml) and to this solution was added benzenesulphonylglycine (15 g) and the mixture boiled. Water (25-30 ml) was added carefully to effect a clear solution. The mixture was then refluxed for 22 hours with a slight excess of 1-iodobutane (15 g; 9.4 ml). After cooling, the mixture was acidified with dilute hydrochloride acid and then poured into a large volume of water (4 l). An oil separated which solidified on standing

for several days. The solid was filtered, dried and recrystallised from aqueous ethanol. m.p. 102-103°.

Found : C 53.2 %; H 6.4 %

Calc. for
 $C_{12}H_{17}NO_4S$ (271.34) : " 53.12%; " 6.32%

N-benzenesulphonyl-N-iso-butylglycine (Bilek et al., 1953). Glycine (5 g) was dissolved in 50% ethanol (200 ml) and iso-butyraldehyde (10 g) added. The mixture was reduced with hydrogen at atmospheric pressure and room temperature using palladium/charcoal (3 g) as catalyst. On completion of the reduction (ie. no further uptake of hydrogen) the solution was heated, one drop of acetic acid added, and the solution filtered. The solvents were removed on the water bath and the residue recrystallised from absolute ethanol. A second crop of product was obtained by addition of ether. m.p. 202-203°.

Found : N 10.4 %

Calc. for
 $C_6H_{13}NO_2$ (131.18) : " 10.68%

A solution of iso-butylglycine (0.5 g) was prepared in N-sodium hydroxide (25 ml) and shaken with benzenesulphonyl chloride (1 ml) until a clear solution was obtained (10-15 mins.). After filtration, dilute hydrochloric acid was added dropwise until a white oil formed. On standing at 5° overnight the oil solidified. It was filtered and recrystallised from water. m.p. 103-103.5°.

Found : C 53.3 %; H 6.4 %;

Calc. for
 $C_{12}H_{17}NO_4S$ (271.34) : " 53.12%; " 6.32%

N-p-toluenesulphonyl-N-propylglycine. N-propylglycine was prepared from glycine and propionaldehyde by the method described above for N-iso-butylglycine, and converted to N-p-toluenesulphonyl-N-propylglycine by standard techniques (Vogel, 1956). m.p. 96-96.5°.

Found : C 52.8 %; H 6.1 %

Calc. for
 $C_{12}H_{17}NO_4S$ (271.34) : " 53.12%; " 6.32%

A P P E N D I X II.

THE METABOLIC ACTIVITY OF SOME 7,8-DIALKYLPUINES.

Introduction.

Interference with nucleic acid biosynthesis and metabolism using various antimetabolic agents provides a valuable basis for cancer chemotherapy (Brown, 1955). The important therapeutic value of antitumour agents has stimulated a considerable amount of study into the synthesis and metabolic action of purine analogues. To assist in the preliminary selection of potentially-valuable antimetabolites, a number of biological assay systems have been developed depending upon the growth-rate of certain purine-requiring bacteria. Other, less widely used, methods have also been described (see later). In these experiments, anti-tumour and anti-metabolic activity are regarded as equivalent phenomena. Data obtained from the various screening tests may therefore be considered as complementary.

Elion and co-workers (1951) examined some one hundred purines for antimetabolic activity using the growth-rate of L.Casei in defined media as a test system (Hitchings et al., 1950), and reached a number of conclusions regarding structural change and consequent biological effects: replacement of the -OH group in either hypoxanthine or guanine by a -SH group produced compounds with strong inhibitory action; this inhibition could be reversed by a number of natural purines (see also Elion et al., 1953). The inhibitory action of these thiopurines has since been examined and found active in other micro-organisms (Collier

and Huskinson, 1957; Hutchinson, 1955), experimental tumours (eg. Skipper et al., 1959), and mouse leukaemia (Law, 1953). 6-Mercaptopurine was later given clinical trials and shown to possess therapeutic value (Burchenal et al., 1955).

Considerable data has now been collected collating chemical structure and biological activity, and several mechanisms have been proposed to account for the anti-tumour action. Despite the impressive weight of this and similar data, there is still no theoretical basis by which anti-metabolic activity may be predicted. At best, certain trends are occasionally discernible which may be used as guides in the development of more active compounds. Nevertheless, these trends are empirically determined and have as yet, no theoretical basis, and the presence of antimetabolic properties in a new compound can only be determined by experiment.

The large number of purine analogues examined in various test systems, and described elsewhere in this thesis, have already thrown considerable light on the influence of various structures on antimetabolic activity. Despite the interest shown in this topic, little attention has been directed towards the 7 and 8 positions, particularly with regards to substitution by alkyl groups. In view of the admitted empirical nature of the search for antimetabolites, it appeared worthwhile to consider the role of the 7 and 8 position in the purine ring in relation to the enhancement, or otherwise, of antimetabolite activity. The purpose of the present study, therefore, was to examine a number of 7,8-dialkylpurines previously synthesised (see part II) for either metabolic or antimetabolic

activity. If, in fact, these purines exhibit metabolic activity, it may be concluded that either the 7,8- positions are unimportant in the metabolism of purines, or that the organism is capable of dealkylating one, or both, of the 7,8-dialkyl groups. On the other hand, alkylation in the 7,8- positions may produce sufficient structural modification for the exhibition of antimetabolic activity.

E X P E R I M E N T A L
P A R T.

Purine-requiring organisms.

The following microorganisms were utilised in the present study.-

Lactobacillus Casei	ATCC 7469
Streptococcus Faecalis	ATCC 8043
Escherichia Coli	NCIB 8242

Culture conditions for various microorganisms.

Lactobacillus Casei. (Hitching et al., 1950)

The growth rate of this organism may be estimated either by the lactic acid production after 68-72 hours, or by measurment of the turbidity after 48 hours growth. In the present study, the lactic acid produced was measured by titration with 0.1 N sodium hydroxide using either thymol blue or titrating to pH 7.5 by means of a pH meter.

Preparation of Inoculum.

(A) Liquid culture medium.

(i). Micro inoculum broth.(Difco)

Yeast extract (Difco)	20 g
Proteose-peptone No. 3 (Difco)	5 g
D-Glucose	10 g
KH ₂ PO ₄	2 g
Tween 80	0.1 g
Water	1000 ml.

(ii). Tomato-enriched medium.

Peptonised milk	15 g
Yeast extract	5 g

D-Glucose	10 g
KH_2PO_4	2 g
Water	600 ml

Add 100 ml filtered tomato juice (a) and adjust the pH of the mixture to 6.5-6.8 with sodium hydroxide solution.

Then add:

Tween 80	10 ml soln (b)
Water	to make 1000 ml

(B). Agar culture medium.

Micro assay culture agar (Difco).

Yeast extract	20 g
Proteose-peptone No.3	5 g
D-Glucose	10 g
KH_2PO_4	2 g
Tween 80	0.1 g
Agar	10 g
Water	1000 ml.

Preparation of Test Media.

Basal Medium.

Vitamin-free Casamino acids	0.5 g
Sodium acetate. $3\text{H}_2\text{O}$	1.0 g
D-Glucose	1.5 g
Asparagine	25 mg
Tryptophan	10 mg
Cystine	10 mg
Alanine	20 mg
Glutamic acid	0.5 mg
Thiamin HCl	20 micro gram
Riboflavine	20 " "
Biotin (free acid)	0.5 " "
Calcium pantothenate	20 " "
Nicotinic acid	20 " "
Pyridoxine HCl	200 " "

Footnotes: (a). Pulp 1 lb. of ripe tomatoes and centrifuge to separate cellular material. Filter the clear liquid through a Whatman No 1 filter paper. (b). Dissolve 25 g Tween 80 in alcohol to make 250 ml.

p-Aminobenzoic acid	20 mg
Salt soln. A	0.5 ml
Salt soln. B	0.5 ml
Water	to 100 ml

Salt soln. A

KH_2PO_4	5.0 g
K_2HPO_4	5.0 g
Water	50 ml

Salt soln. B

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	10 g
NaCl	0.5 g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	2.0 g
$\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$	2.0 g
Water	250 ml.

Test Media.

To the basal medium O was added adenine sulphate (1 mg/100ml medium) and folic acid (4.6 μg folic acid/ 100 ml medium). Thus we have four media:-

Basal medium	O
Basal medium + purine	P
Basal medium + folic acid	OFA
Basal medium + purine + folic acid	PFA

Stock Culture.

The freeze-dried micro-organism was suspended in either of the liquid culture media and grown at 37° for 16-20 hours. Stab cultures (3) were then made in the "agar culture medium" and after incubation at 37° for 24 hours the cultures were stored at 5° . Fresh stab transfers were made weekly (see Snell and Strong, 1939).

Sodium chloride inoculum.

A sample from the stab culture was transferred on a sterile platinum loop to a tube containing either of the liquid culture media (10 ml) and incubated at 37° for 16-20 hours. The cell suspension was transferred aseptically to a centrifuge tube and centrifuged at 2000-2500 rpm for 10 minutes. The supernatant medium was decanted, the cells resuspended in 0.9% saline and re-centrifuged. This washing procedure was repeated at least four times to remove traces of trapped folic acid or other growth factors. The cells were finally suspended in 0.9% saline for use. It was advantageous to adjust the suspension so that approximately the same number of cell per ml of medium was used in each experiment. This was accomplished by diluting the cell suspension with saline to a percentage transmission of 40% at 660 mμ.

Assay method.

Tubes containing 10 ml of the various media (O, P, OFA, or PFA) were autoclaved (15 mins. at 15 lbs) and inoculated with one drop of the inoculum. The samples were incubated for 68 hours at 37° when the acid production was measured by titration with 0.1N sodium hydroxide. All the techniques used were standard microbiological procedures. A number of precautions and practical aspects of value in successful microbiological assay techniques were observed (Hunter et al., 1958).

Purification of casein hydrolysate (Flynn et al. 1951).

A solution of the casein hydrolysate (10 g/100 ml water) was adjusted to pH 3.0-3.5 and stirred at

80° for 1 hour with activated charcoal (1.0-1.5 g) previously heated at 100° for 2 hours.

Streptococcus Faecalis.

The organism S. Faecalis is a lactic acid producing bacterium and growth was estimated by titration of the lactic acid produced after 68-72 hours. The same procedure as described for L. Casei (page 155) was followed.

Preparation of inoculum.

Liquid culture medium.

Difco "Micro inoculum broth" (see page 155).

Agar culture medium.

Difco "Micro assay culture medium" (see page 156).

Preparation of test media. (Jukes, 1955)

Basal medium.

Acid-hydrolysed casein	5.0 g
L-Tryptophan	100 mg
Asparagine	300 mg
Cysteine	250 mg
D-Glucose	20 g
Sodium-citrate. 2H ₂ O	23.75 g
K ₂ HPO ₄	3.10 g
Glutathione	2.5 mg
Vitamin solution (a)	100 ml
Tween 80 solution (b)	10 ml
Salt solution (c)	5 ml
MnSO ₄ .H ₂ O	100 mg

Add the MnSO₄.H₂O to the medium after adjusting the pH to 6.8. Adjust volume to 500 ml with water.

Solutions.

(a). Dissolve 10 mg of p-aminobenzoic acid, 40 mg of pyridoxine hydrochloride, 4 mg of thiamine hydrochloride, 8 mg of calcium pantothenate, and 8 mg of nicotinic acid in 200 ml of water. Add enough biotin to furnish 200 μ g of biotin and 10 mg of riboflavin (dissolved in 200 ml of 0.02 M acetic acid). Then add 40 ml of sodium acetate buffer solution (pH 4.5; prepared by dissolving 18.75 g of acetic acid and 36.85 g of sodium acetate in water and diluting to 500 ml). Adjust volume to 2000 ml with water. Store in refrigerator.

(b). Dissolve 2.5 g of Tween 80 in warm water (45°) and dilute to 500 ml. Store in refrigerator.

(c). Dissolve 20 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g of NaCl, 1 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 750 mg. of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$. Add 1 ml of 37% HCl and dilute to 500 ml. Store in refrigerator.

Basal medium plus additives.

Test media containing various growth factors were prepared as described for the basal solution but included adenine, guanine and uracil (Soln. d), xanthine (Soln. e) and folic acid (Soln. f) as follows:-

Basal + purines/pyrimidine	5 ml soln(d), 10 ml soln.(e). per 100 ml medium
Basal + folic acid	4.6 μ g folic acid/ 100 ml medium

Basal + purines/pyrimidine	5 ml soln.(d)+ 10
+ folic acid	ml soln.(e)+4.6
	µg folic acid/
	100 ml medium.

Solutions.

(d). Dissolve 0.1 g each of adenine sulphate, guanine hydrochloride, and uracil with the aid of heat in 5 ml of 20% HCl, and add sufficient water to make 500 ml. Store in refrigerator.

(e). Suspend 0.1 g of xanthine in a small volume of water, add 4 ml of ammonium hydroxide, and heat until dissolved. Add water to make 500 ml and store in refrigerator.

(f). Stock folic acid: Dissolve 4.6 mg dry crystalline folic acid in 0.01N sodium hydroxide in 20% ethanol. Neutralise to pH 7-8 with HCl and dilute to 1 litre with 20% ethanol. (1 ml = 4.6 µg folic acid).

Standard folic acid solution: Dilute 1 ml stock solution to 1 litre with 20% ethanol. 1 ml = 4.6 µg folic acid.

Sodium chloride inoculum.

The organism was harvested and an inoculum in 0.9% sodium chloride prepared as described for L. Casei (page 158).

Escherichia Coli (purine-requiring strain NCIB 8242).

Two media have been investigated for use in the present experiments (Guthrie, 1949). Medium A, containing inorganic salts and glucose, and Medium B,

which in addition to the components of medium A, is supplemented with a number of amino acids and the B-group vitamins.

Medium A. (double strength).

NH ₄ Cl (a)	5.0 g
NH ₄ NO ₃	1.0 g
Na ₂ SO ₄	2.0 g
MgSO ₄ ·7H ₂ O	0.1 g
K ₂ HPO ₄	3.0 g
KH ₂ PO ₄	1.0 g
Glucose	5.0 g
Trace element solution (b)	1.0 ml
Water	500 ml
pH	adjust to 6.8-7.0

Medium B (double strength.)

NH ₄ Cl	5.0 g
NH ₄ NO ₃	1.0 g
Na ₂ SO ₄	2.0 g
MgSO ₄ ·7H ₂ O	0.1 g
K ₂ HPO ₄	3.0 g
KH ₂ PO ₄	1.0 g
Glucose	5.0 g
Trace element soln.	1.0 ml
"Vitamin-free" casamino acids	5.0 g
L-cystine	200 mg
DL-tryptophan	200 mg
Uracil	10 mg
Thymine	10 mg
Thiamin HCl	0.2 mg
Riboflavine	0.2 mg
Nicotinic acid	0.4 mg
Calcium pantothenate	0.4 mg

Footnotes: (a). all salts were reagent grade quality.
(b). B:0.01 mg/l; Mo:0.02 mg/l; Fe:0.2 mg/l; Cu:0.1 mg/l; Mn:0.02 mg/l; Zn:2.0 mg/l. (Horowitz and Beadle, 1943).

Folic acid	0.01 mg
Biotin	0.01 mg
p-Aminobenzoic acid	0.01 mg
Pyridoxine	0.2 mg
Inositol	0.4 mg
pH adjusted to	6.8-7.0
Water	500 ml

Preparation of inoculum.

(a). Liquid culture medium.

Heart infusion broth.

Beef heart, infusion from	500 g
Tryptose	10 g
NaCl	5 g
25 g solid medium is dissolved in 1000 ml water.	

(b). Agar culture medium.

Heart infusion agar.

Beef heart, infusion from	500 g
Tryptose	10 g
NaCl	5 g
Agar	15 g

Dissolve 40 g in 1000 ml water. Sterilise for 15 mins. at 15 lbs.

Stock cultures and inocula.

The freeze-dried specimen was suspended in heart infusion broth. From this sample, heart infusion agar slants were prepared and incubated at 37° for 16 hours. They were then stored at 5°.

Inocula were obtained by washing the bacteria off the agar slant with 0.9% saline, transferring the suspension to a centrifuge tube and isolating the cells (2000 rpm for 10 mins). The saline was

decanted and the cells washed by re-suspension in saline. After a further centrifugation the cells were again re-suspended in saline, the concentration being adjusted to give a predetermined value (40% transmission) at 660 mμ.

Quantitative studies on *E. Coli*.

Purine requirements

- (i) Adenine. A series of tubes containing medium B (5 ml) and a solution of adenine hydrochloride (5 ml) such that the final concentration of purine was between 10^{-5} and 10^{-3} M, were prepared in duplicate and sterilised (15 mins at 15 lbs./sq.in.). To each tube, one drop of inoculum (see page 161) was added, the tube shaken, and then incubated at 37° for 16 hours. The turbidity of each solution was measured in a colorimeter at 660 mμ against a water blank.
- (ii). Xanthine. Tubes were prepared as described above only replacing the adenine by xanthine.

Influence of 6-mercaptopurine on the growth curve.

6-Mercaptopurine (100 mg) was dissolved in 200 ml of medium B. Aliquots of this solution were then diluted with further quantities of medium B so as to give the following final concentrations of 6-MP:-250 μg/ml; 125 μg/ml; 62.5 μg/ml; 31.25 μg/ml. A blank solution was also prepared. Samples (5 ml) of each concentration were pipetted into test tubes (in duplicate) and the volume adjusted to 10 ml with

adenine hydrochloride solutions so that the final solutions were from 10^{-5} - 10^{-4} M with respect to adenine. Control samples containing only adenine in medium B were also prepared. After sterilization, the tubes were inoculated with one drop of E. Coli inoculum (page 161), shaken and incubated at 37° for 16 hours. The turbidity of the bacterial suspension was measured photoelectrically at 660 m μ .

Metabolic action of some 7,8-dialkylpurines on E.Coli.
Stimulation of the growth rate.

(a). In the absence of natural purines.

The following general method was used to determine the metabolic properties of a number of 7,8-dialkylpurines for E.Coli in a medium in which the 7,8-dialkylpurine was the sole source of purine:-

A series of tubes were prepared, in duplicate, containing 5 ml of double-strength medium B and varying amounts of the 7,8-dialkylpurine in the range 1×10^{-5} - 1×10^{-3} M. A blank and several control tubes containing either 2×10^{-5} or 5×10^{-5} M adenine were also prepared. Each tube was finally adjusted to 10 ml and sterilized (15 lbs/ sq.in./ 15 mins.). All samples were then inoculated with one drop of a saline suspension of the organism (see page 161), shaken, and incubated at 37° in an accurately controlled water bath for 16 hours. The growth of the organism was estimated by measuring the opacity of the suspension photoelectrically at 660 m μ against a water blank.

Details of particular purines.

7-Butyl-6-hydroxy-8-propylpurine. A standard aqueous solution was used as a source of the test purine.

7-Butyl-6-butylamino-8-propylpurine. The standard purine solution was prepared in very dilute aqueous acetone and a suitable control was run to ensure that the acetone was without effect at the concentration used.

(b). In the presence of sub-optimum quantities of natural purines.

The ability of certain 7,8-dialkylpurines to improve the degree of growth of E. Coli in medium B supplemented with a quantity of natural purine insufficient for maximum growth, was explored in a number of cases. The identical experimental arrangement also serves to detect any inhibitory action caused by the test purines (see later). The two following general techniques were employed:-

Procedure (1). Standard bacteriological tubes containing medium B (5 ml), a fixed amount of the test purine, and variable concentration of adenine or xanthine in the range 10^{-5} to 10^{-4} M. The volume of each tube was adjusted to 10 ml with water and then sterilised. The organism was inoculated and growth measured as described earlier.

Details of particular purines.

7-iso-Butyl-6-chloro-8-iso-propylpurine. The 7,8-dialkylpurine was dissolved directly in medium B to give four solutions of concentration 26.2 μ /ml, 12.6 μ /ml, 6.3 μ /ml, and 3.15 μ /ml in

addition to the blank medium B.

Procedure (2). Tubes containing medium B (5 ml), a fixed concentration of either adenine or xanthine, and a variable amount of the 7,8-dialkylpurine under examination were prepared and the total volume adjusted to 10 ml. Thereafter, the procedure was as described in the previous method.

Details of particular purines.

7-iso-Butyl-6-chloro-8-iso-propylpurine. As the concentration of the 7,8-dialkylpurine used was relatively high, the stock solution was prepared in dilute, aqueous carbowax 300 ($\pm 5\%$), a concentration well below that at which interference occurs. Various solutions of medium B were then prepared containing the test purine stock solution so that the final concentrations were in the range 30-500 μ /ml. The concentration of natural purine incorporated into each tube was 5×10^{-5} M. Adequate controls were prepared.

7-Butyl-2,6-dihydroxy-8-propylpurine. The purine was dissolved in dilute aqueous acetone ($\pm 5\%$) and added to the medium at a final concentration of 30-500 μ /ml. A standard concentration of 3×10^{-5} M adenine or 5×10^{-5} M xanthine was used throughout the experiment.

7-iso-Butyl-6-(2-phenylethylamino)-8-iso-propylpurine.

The details of the experiment were as described above with the exception that the test purine was dissolved in dimethyl sulphoxide, and added to the medium so that the final concentration of solvent was less than

4%. The concentration of purine in the medium was from 3.1 μ /ml to 250 μ /ml.

Inhibition of the growth rate.

The inhibitory action of 7,8-dialkylpurines was assessed by measuring their effect on growth of the organism, E. Coli. in medium B supplemented with either adenine or xanthine as described earlier (procedure (2) page 167). A decrease in the turbidity of the test solution after incubation at 37° for 16 hours would be evidence of growth inhibition.

Details of particular purines.

7-iso-Butyl-6-mercapto-8-iso-propylpurine. The purine was dissolved in 40% aqueous carbowax 300, and aliquots added to medium B supplemented with 5×10^{-5} M, adenine, so that the final concentrations were approximately 250 μ /ml, 125 μ /ml, 62.5 μ /ml, and 31.2 μ /ml. The total volume was adjusted to 10 ml. In the case of the higher concentrations of test purine, a turbidity due to precipitated purine was observed. A control for each concentration of test purine was prepared in which the effect of carbowax 300 only was determined.

<u>7-iso-Butyl-6-chloro-8-iso-propylpurine.</u>	)
<u>7-Butyl-2,6-dihydroxy-8-propylpurine.</u>	) as described
<u>7-iso-Butyl-6-(2'-phenylethylamino)-8-iso-propylpurine.</u>	) on page 167.

R E S U L T S.

Preliminary experiments for the selection of a suitable organism.

Lactobacillus Casei.

All attempts to prepare a suitable basal medium (ie. one which supported less than 20% of the growth obtained in the basal medium supplemented with adequate purines and folic acid) for L. Casei failed. It was subsequently shown that the "vitamin-free" casamino acids employed in the preparation of the medium contained significant amounts of folic acid, thus explaining the experimental difficulty described above. However, as a superior alternative bioassay system (viz. the E. Coli system to be discussed later) became available while studies with L. Casei were in progress, further experiments with the latter organism were discontinued.

Streptococcus Faecalis.

The influence of some physiological purines and folic acid on the growth of S. Faecalis is illustrated by the experimental findings presented in Table XXII.

Escherichia Coli (purine-requiring mutant).

The results presented in Table XXIII indicate the growth of E. Coli in both medium A and medium B supplemented with varying concentrations of adenine sulphate after incubation at 37° for 16 hours.

TABLE XXII.

Acid production by S. Faecalis in various media.

Code	Medium	Mean titration 0.1N NaOH
O	Basal	0.35 ml
A	Basal + adenine, guanine, uracil and xanthine	1.30 ml
OFA	O + folic acid	12.10 ml
AFA	A + folic acid	14.00 ml

TABLE XXIII.

Growth rate of E.Coli in various media.

Tube No.	Medium	%T @ 660 mμ
1	A	96.7
2	A + 0.2 mg adenine	95.9
3	A + 0.4 mg adenine	97.0
4	A + 1.0 mg adenine	96.5
5	B	96.8
6	B	96.6
7	B + 0.4 mg adenine	46.0
8	B + 0.4 mg adenine	46.0
9	B + 1.0 mg adenine	45.0
10	B + 1.0 mg adenine	46.7

Thus, in our hands, medium A supplemented with adenine appeared inadequate to support growth, whereas medium B in the presence of adenine, supported an intense growth of the organism. In view of the excellent results obtained with medium B, no further experiments were conducted with medium A.

Quantitative studies with *E. Coli* (NCIB 8242).

Both adenine and xanthine were shown independently to support growth of the purine-requiring strain of *E. Coli* in a medium containing inorganic salts, glucose, amino acids and the B-group vitamins (medium B). Adenine in the concentration 1×10^{-5} - 8×10^{-5} M progressively increased the growth response, but thereafter, no further growth took place (Fig. XXII). With xanthine as the only purine, growth increased rapidly over the range 1×10^{-5} - 4×10^{-5} M before reaching an equilibrium (Fig. XXIII). Concentrations of xanthine up to 2×10^{-3} M were examined but were found neither to stimulate nor to depress growth as compared with the level at 4×10^{-5} M.

Some quantitative studies with 6-mercaptopurine.

6-Mercaptopurine at all the concentrations tested (ie. 250 µg/ml - 31.25 µg/ml) completely inhibited growth of the organism in medium B supplemented with adenine. The results for the control specimen and the weakest concentration of 6-mercaptopurine (31.25 µg/ml) are shown in Fig. XXIV.

Action of some 7,8-dialkylpurines on the growth of *E. Coli*.

(i) Stimulation of the growth response in medium B in the absence of natural purines.

The addition of the adenine derivative, 6-butyl-

amino-7-butyl-8-propylpurine to medium B in concentrations from 10^{-5} - 10^{-3} M failed to promote the growth of E. Coli (Fig. XXV). This purine cannot therefore be metabolised in place of the physiological purines known to promote growth of this organism in medium B.

In similar vein, medium B supplemented with the hypoxanthine derivative 7-butyl-8-propylhypoxanthine also failed to support minimal growth of E. Coli. The effect of purine concentrations from 10^{-5} - 10^{-4} M are indicated in Fig. XXVI. Even concentrations of this 7,8-dialkylhypoxanthine up to 10^{-3} M (not shown in the graph) showed no metabolic value for the organism.

(ii) The stimulation/inhibition of growth of E. Coli on medium B and sub-optimum levels of natural purines.

The effect of a number of 7,8-dialkylpurines on bacterial growth was frequently determined by incorporating the test purine in the basal medium supplemented with sub-optimum levels of adenine or xanthine. In this manner, either growth response or growth inhibition induced by the test material could be determined in a single experiment. In none of the cases studied did the 7,8-dialkylpurines show any metabolic effect detectable by a change in the growth pattern. Individual findings are indicated in the tables or graphs below.

7-Butyl-2,6-dihydroxy-8-propylpurine.

A concentration of 7-butyl-8-propylxanthine of 31 to 500 μ /ml in the medium showed no effect on the growth of the organism. The results are presented in table XXIV.

FIG. XXII. E. COLI ON MEDIUM B + ADENINE.

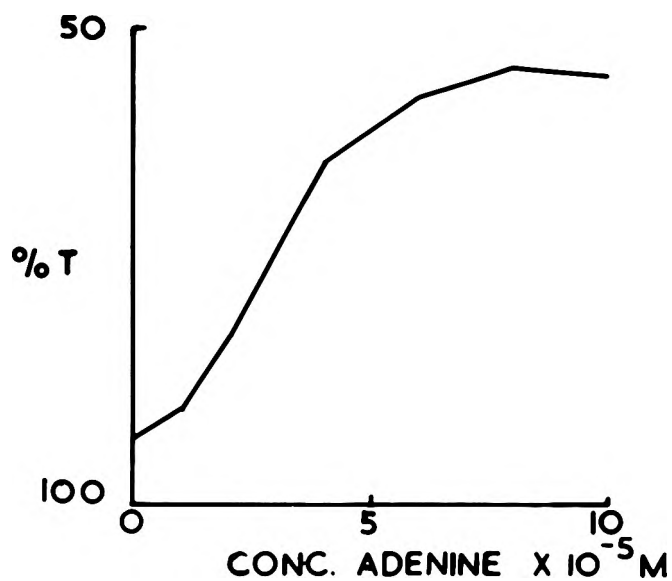


FIG. XXIII. E. COLI ON MEDIUM B + XANTHINE.

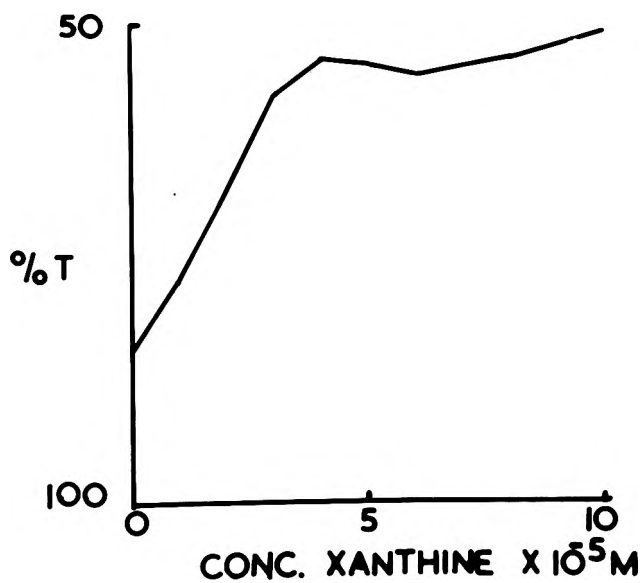


FIG. XXIV. INHIBITORY ACTION OF 6-MERCAPTO-
PURINE ON E. COLI.

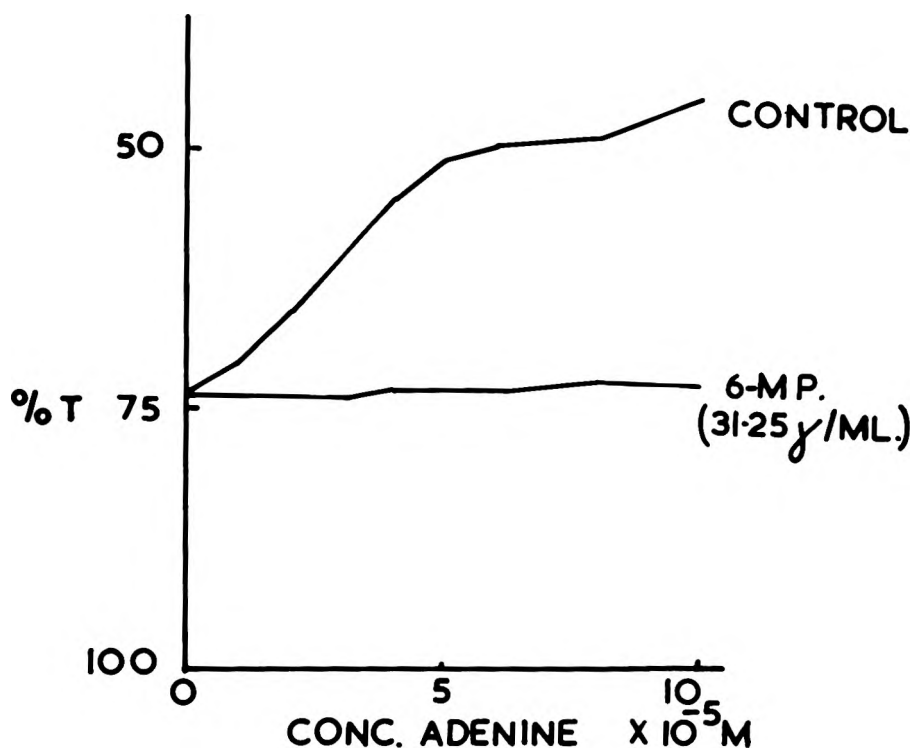


FIG. XXV. EFFECT OF 6-BUTYLAMINO-7-BUTYL-8-PROPYLPURINE ON E. COLI IN MEDIUM B.

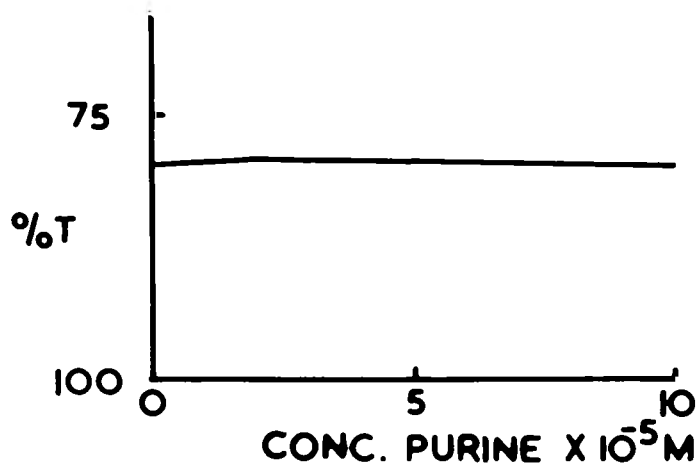


FIG. XXVI. EFFECT OF 7-BUTYL-8-PROPYLHYPO-XANTHINE ON E. COLI IN MEDIUM B.

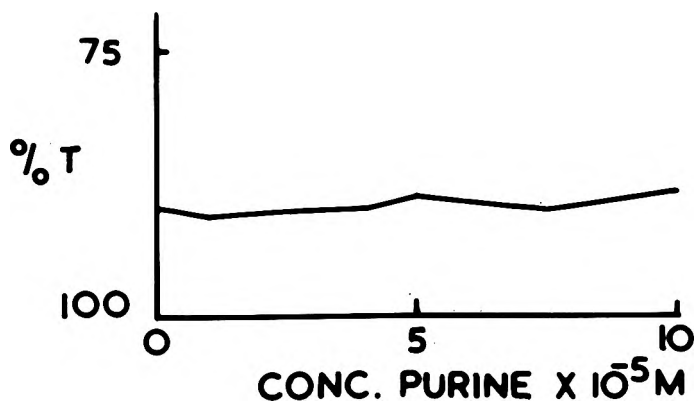


TABLE XXIV.

Influence of 7-Butyl-8-propylxanthine on the
growth rate of E.Coli

Purine γ /ml	Mean % T	
	$3 \times 10^{-5}M$ adenine	$5 \times 10^{-5}M$ xanthine
Blank	88.5	88.5
Control	65.3	64.4
31.2	64.0	64.0
62.5	64.9	66.0
125	66.5	64.3
250	67.0	64.3
500	67.5	63.5

7-iso-Butyl-6-chloro-8-iso-propylpurine.

Two experimental designs were employed with this purine.

(1) Fixed concentration of natural purine.

The results are tabulated below and illustrated graphically in Fig. XXVIII. Concentrations of 7,8-dialkylpurine for 7.8 to 500 γ /ml were without metabolic effect.

TABLE XXV.

Influence of 7-iso-butyl-6-chloro-8-iso-propyl-purine on the growth rate of *E.Coli*.

Purine γ /ml	Mean % T.	
	Purine Concentration	
	$5 \times 10^{-5} M$ adenine	$5 \times 10^{-5} M$ adenine
Blank	82.6	82.6
Control	48.0	62.2
7.8	50.0	61.2
15.6	48.9	63.1
31.2	48.6	61.4
62.5	48.8	61.3
125	47.8	61.5
250	48.8	61.1
500	48.0	63.9

(2) Fixed concentration of 7.8-dialkylpurine.

The normal growth-response curve of *E.Coli* on medium B supplemented with adenine over the range $0-10^{-5} M$ was shown to be independent of added 7-iso-butyl-6-chloro-8-iso-propylpurine when tested at

concentrations of 1.58 μ /ml, 3.16 μ /ml, 6.31 μ /ml, and 12.6 μ /ml. The full response-curves for the control and for the 1.58 μ /ml and 12.6 μ /ml concentrations are typical and are shown in Fig. XXVII.

7-iso-Butyl-6-(2'-phenylethylamino)-8-iso-propylpurine.

The above purine was without effect on the growth response of E. Coli as indicated by the data in Table XXVI. It was noted that a slight inhibition of growth (3%) appeared in the standard and was due to the presence of 4% dimethyl sulphoxide.

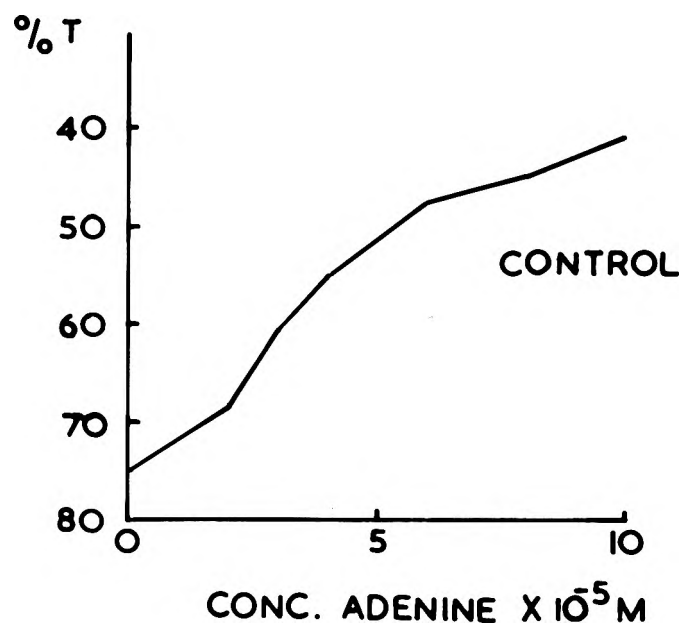
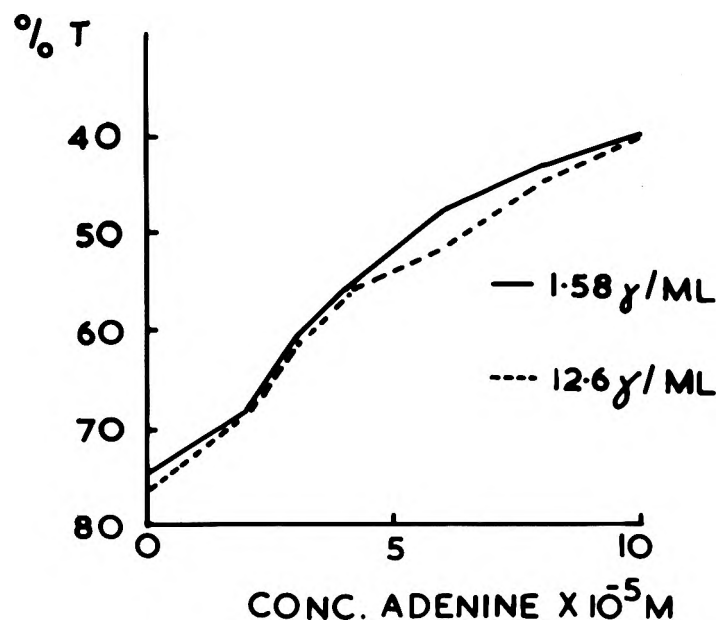
TABLE XXVI.

Influence of 7-iso-butyl-6-(2'-phenylethylamino)-8-iso-propylpurine on the growth rate of E. Coli.

Purine μ /ml	% T
	5×10^{-5} M adenine
Blank	80.0
Control	57.5
3.1	55.2
6.2	56.0
12.5	56.5
25.0	57.0
31.2	55.5
62.5	55.0
125.0	53.2*
250.0	48.0*

* A precipitate of 7,8-dialkylpurine formed, causing increased opacity.

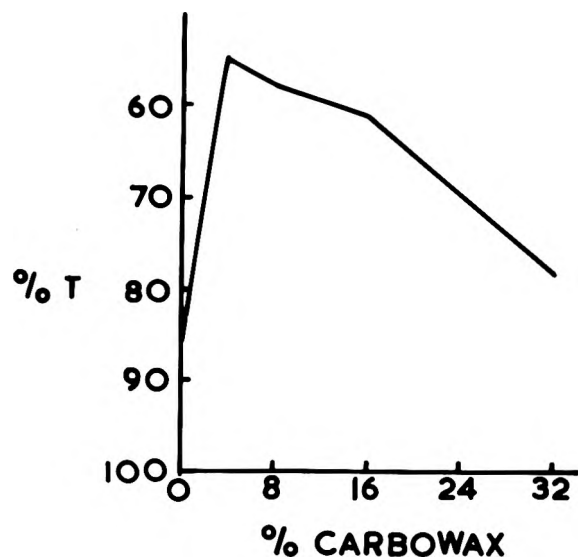
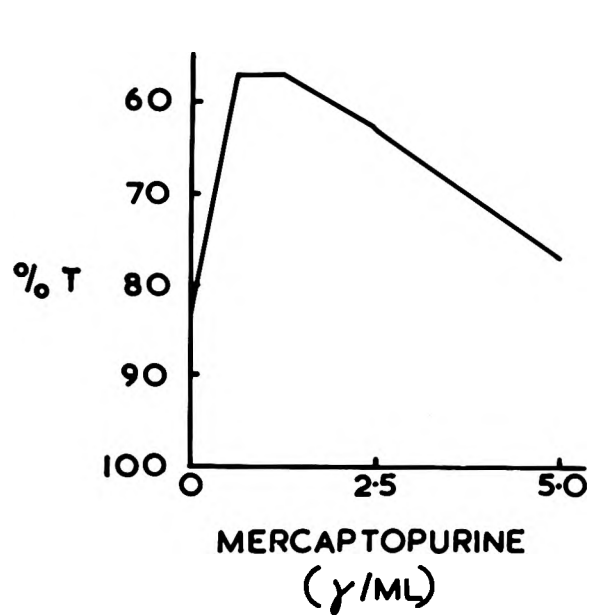
FIG. XXVII. EFFECT OF 7-ISO-BUTYL-6-CHLORO-8-ISO-PROPYLPURINE ON E. COLI
IN MEDIUM B.



7-iso-Butyl-6-mercapto-8-iso-propylpurine.

Growth inhibition of E. Coli was recorded in the test samples, but comparison with the control samples showed, within experimental error, that the same degree of inhibition was produced by the carbowax 300 alone. It was therefore concluded that the 6-mercapto-7,8-dialkylpurine was without measurable metabolic action. The results are presented in Fig. XXIX.

FIG. XXIX. EFFECT OF 7-ISO-BUTYL-6-MERCAPTO-8-ISO-PROPYLPURINE IN CARBOWAX 300
ON THE GROWTH OF E. COLI.



DISCUSSION.

(a) Experimental systems for the study of purine antimetabolites.

The growth rate of mutant strains of a relatively large variety of microorganisms has been shown to depend upon the presence in the medium of one or more purine derivatives (for a review of such findings up to 1945 see Peterson and Peterson, 1945). Such organisms include L. Casei (Snell and Mitchell, 1941), Escherichia Coli (Guthrie, 1949), Aerobacter Aerogenes (Balis et al., 1956), Acetobacter Melanogenium (Gray and Tatum, 1944), Group A Haemolytic Streptococcus (Pappenheimer and Hottle, 1940), Salmonella Typhimurium (Duggan et al., 1963), Neurospora Crassa (Mitchell and Houlahan, 1946), and the protozoan Tetrahymena Geleii (Kidder and Dewey, 1945 and 1948). In a number of cases, a linear relationship over a limited range between growth and the concentration of purine in the medium has been demonstrated, and the organism proposed for use as a purine bioassay system (eg. Hitchings, et al., 1950; Collier and Huskinson, 1957). It has also been shown by these latter investigators that purine-dependent organisms can be used to assay the antimetabolic activity of compounds, particularly "antipurine" and "anti-folic acid" activity (see page 171). Hence, purine-dependent bacterial systems have been employed as preliminary screening tests for compounds with potential anti-tumour activity. Such tests possess important advantages over alternative biological systems in that they are rapid and require relatively little material thus ensuring their importance in preliminary studies. It may be noted in passing however, that the antitumour activity of a compound,

shown to be an antimetabolite, need not be manifest via an antimetabolic action. 6-Mercaptopurine, for example, inhibits the growth of L. Casei and the effect can be reversed by addition of adenine to the medium. On the other hand, the antitumour effect of 6-mercaptopurine in animal experiments is not reversed by feeding additional amounts of adenine or similar natural purines (Sexton, 1963).

Relatively few of the organisms mentioned above have been developed for use as a screening test for antimetabolites. The most widely examined of these has been L. Casei (Hitchings et al., 1950) which is dependent upon both folic acid and the naturally occurring purines adenine, guanine, hypoxanthine and xanthine. In a medium lacking both factors relatively no growth is observed. It is probable that this marginal growth is a reflection of the difficulty of removing all traces of folic acid, to which the organism is particularly sensitive, from the medium. Both folic acid and purines independently support good growth, but in combination act synergically.

The organism S. Faecalis, a lactic acid producing bacterium, may also be employed in assaying antipurine activity (Balis et al., 1958), but has not been studied as closely as L. Casei. It has the advantage of being much less sensitive towards folic acid and consequently, precautions to exclude folic acid from the medium need not be

so rigorous. The dependency of these lactic acid-producing bacteria on a supply of folic acid appears to be due to the function of folic acid in the metabolism of one-carbon fragments, particularly in the biosynthesis of purines. Two carbon atoms in the purine molecule, viz. C-8 and C-2 (see, for example, Greenberg and Jaenicke, 1957) are known to arise from these fragments, and hence require catalytic quantities of folic acid.

L. Casei, because of the considerable study already undertaken on it, appeared to be the most suitable organism for the purpose of the present investigation. Accordingly, in a preliminary survey undertaken to select a suitable organism, a considerable amount of time was devoted to a study of the L. Casei model. No difficulty was experienced in growing the organism and preparing an inoculum, but attempts to prepare a suitable purine-free test medium failed. In a solution allegedly free of both purines and folic acid, almost as good a growth of the organism was constantly observed as was found in the control medium containing all the components designed to give maximum growth. As blank media should not support more than 20% of the maximum growth in a medium containing all necessary growth factors, such media were unusable. Several explanations of this unexpected growth were considered. The first was that the organism had undergone some mutation thereby ceasing to be either purine or folic acid dependent.

The chance that such a mutation had taken place was virtually eliminated by repeating the experiments with a culture of L. Casei obtained from an independent source. Identical findings made it very unlikely that a change in the metabolic requirements of the organism had taken place. The second, and more probable, explanation was contamination of the medium either by folic acid or purine (or possibly both). The extremely small requirement of the organism for folic acid (an optimum amount of 4.6 $\mu\text{g}/100\text{ ml}$) is indicative of the difficulty in eliminating this factor from the medium. Several components of the medium are known occasionally to contain elevated amounts of folic acid, particularly the casein hydrolysate and some samples of tryptophan, as well as some unexpected sources such as the cotton wool plugs frequently employed in bacteriological practice (Flynn et al., 1955). In the present study two commercially-available preparations of hydrolysed casein, certified to be vitamin-free, were used in preparing the media. Nevertheless, the blank medium continued to support good growth of the organism. Other components of the medium were replaced by alternative brands without success.

A more precise study of the possible folic acid contamination of the components of the medium was undertaken with the aid of a commercially-available medium for the microbiological assay of folic acid. Using L. Casei, the folic acid content of each component of the medium at approximately the concentration used was qualitatively assessed. The alleged "vitamin-free" casamino acid preparation used was found to be strongly contaminated with folic acid.

In similar tests using the less sensitive organism S. Faecalis, the same casein hydrolysate was judged to be free from this vitamin. It therefore appears that the level of residual folic acid in the casein hydrolysate is less than the detection limit for S. Faecalis but well above the minimum requirement of L. Casei which is said to be about six times more sensitive than the former organism. Attention was subsequently directed towards removing this trace of folic acid from the casein hydrolysate by adsorption onto activated charcoal at pH 3.0-3.5 (Flynn et al., 1955). Animal charcoal was successfully substituted for the "Darco 60" originally recommended. Casamino acid purified in this manner and assayed for folic acid with L. Casei in Difco "Folic acid casei medium" was shown to be virtually free of this vitamin.

As a direct result of the practical difficulties associated with the L. Casei system as described above, some preliminary experiments were conducted with S. Faecalis as an alternative bioassay organism. Because of the reduced sensitivity of the organism for folic acid (Jukes, 1955), no difficulty was experienced in preparing a medium in which the influence of folic acid and various purines on growth could be accurately observed. Nevertheless, the system was abandoned for the present study as relatively little differentiation was observed in the level of lactic acid produced by the organism in the absence and presence of the purine component of the medium. The relevant data is recorded in Table XXII (page 170).

An interesting alternative to the lactic acid bacteria is a purine-requiring mutant of E. Coli (NCIB 8242), already investigated in some detail (Guthrie, 1946; Collier and Huskinson, 1957), although the use of this organism in studies of anti-metabolites has been restricted to a few compounds such as 6-mercaptapurine. Two main advantages over the lactic acid bacteria are the independence of growth on folic acid and the rapid rate of growth. Thus only some 16 hours are required for an assay compared with 68 hours for L. Casei. With regard to the first point of the role of folic acid; while this strain of E. Coli requires folic acid in the medium, the limiting factor to growth is solely the level of purine in the medium. Any of the purines, adenine, guanine, hypoxanthine and xanthine are suitable; the organism apparently possessing the ability to permit interconversion. Graphs showing the influence of adenine and xanthine on growth are presented on page 173. A disadvantage of the E. Coli organism is the ease with which a culture can revert to a nonpurine requiring, wild-type organism when the culture is grown for too long a period. Two media have been described for this organism; Medium A, essentially a solution of inorganic salts and glucose, and Medium B, which is medium A supplemented with some pyrimidines and the B-group vitamins. Reversion to the nonpurine-dependent form occurs most readily in medium B, increasing rapidly after about 22 hours growth. With care, however, this characteristic does not impede the routine use of the organism in studies

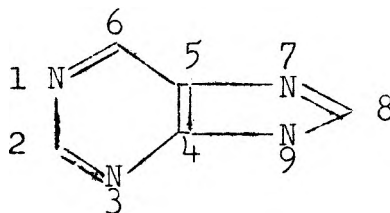
of purine metabolism. In the preliminary investigation Medium A was examined first, as not only was it simpler to prepare, but also gave less chance for undesirable mutation. Despite several attempts, however, it was not possible to obtain growth in this medium. As satisfactory growth was readily achieved in Medium B, no further attempts with the first medium were undertaken. In view of the satisfactory preliminary results with this strain of E. Coli, experiments with alternative organisms, (see above) were terminated.

Quantitative studies on E. Coli with the purines adenine and xanthine were successfully completed and growth found to increase over the range 1×10^{-5} - 8×10^{-5} M adenine, and with xanthine over the range 1×10^{-5} to 4×10^{-5} M (see Figs. XXII and XXIII). Experiments were performed with concentrations of purine up to 2×10^{-3} M in an attempt to see if growth was inhibited by high concentrations of the growth factor. Such a phenomenon has been described by Collier et al., (loc cit.) in Medium A but was not detected in the present study though admittedly, concentrations of purine were lower than described by Collier. As a test system the organism was grown in the presence of various concentrations of 6-mercaptopurine, of which the lowest concentration used (31.25 microgrammes/ml medium) is depicted in Fig. XXIV.. Complete inhibition of growth at all concentrations of adenine between 0 and 10^{-4} M was recorded. This compares favourably with data published for 6-mercaptopurine on E. Coli in medium A. (Collier et al., loc.cit.) The organism appeared therefore to be suitable for the present investigation.

The large number of purine-dependent bacteria, yeasts and protozoa mentioned earlier (page 183), while of potential value in studies of purine metabolism, do not seem to have been widely investigated or employed for assessing the activity of potential purine metabolites. They need not, therefore, be discussed further.

(b) The influence of chemical structures on biological activity in purines.

The purine nucleus has seven sites at which substitution can take place, namely the 2, 6 and 8 carbon atoms and the 1, 3, 7 and 9 nitrogen atoms.



Only in a few plant metabolites do the 1,3&7 positions seem to have an important biological function as for example, in such derivatives as caffeine (1,3,7-trimethylxanthine). The carbon atom at position 8, while very important in the excretory product uric acid, does not otherwise appear to be of biological significance. By contrast, the 2,6 and 9 positions are subject to biological (enzymic) modifications and are, in consequence, highly important metabolically. Substitution of the 2 and/or the 6 positions with hydroxyl or amino groups accounts

for all the common, biologically important, purines viz. guanine, adenine, hypoxanthine and xanthine. A few rare purines of natural occurrence have more complex groupings, usually in the 6 position. Thus kinetin, synthetic plant growth factor, is 6-furfurylaminopurine, and the antibiotic puromycin contains a 6-dimethylaminopurine as part of its structure. (Waller et al., 1953).

The proton at N-7 (or N-9) is readily substituted, particularly by carbohydrates (ribose and deoxyribose being the most common). In naturally occurring nucleosides (or nucleotides) however, it is usually the 9-position which is substituted (for example, see Montgomery and Thomas, 1962).

Since intensive study of purine analogues as antimetabolic and anticancer agents commenced, a large number of compounds have been examined (Elion et al., 1951; Skipper et al., 1959; Kelley et al., 1962; Schabel et al., 1961) and the effect of chemical change, both gross and subtle, in the purine molecule has been studied in several different biological systems. A wide variety of chemical groupings have been incorporated at different sites in the purine ring and the change in biological activity recorded. As example, the investigation of Skipper and co-workers (1959) may be cited in which some 125 purines analogues were tested against adenocarcinoma 755, an extremely sensitive in vivo assay system. As the object of the study was to gain fundamental knowledge of structure-activity relationships and not data of immediate clinical

importance, the sensitivity of the system was advantageous.

Presumably as a consequence of the importance of the 2-and the 6-positions of the purine ring in biosynthetic pathways, the most active antimetabolic agents so far prepared have their significant structural modification at these sites. A number of thiopurines were among the earliest of potent purine antimetabolites in the purine-requiring bacterium, L. Casei. Both 6-mercaptopurine and 6-thioguanine appeared worthy of further study and though the latter has proved too toxic for therapeutic use, 6-mercaptopurine is a valuable aid in the chemotherapy of cancer(Burchenal et al., 1955) and in control of the immune reaction (Schwartz and André, 1962). Other groups or substituents in the 6-position also show some antimetabolic activity but do not surpass that of the thiopurines discussed above. Such compounds include 6-chloropurine and some 6-alkylmercaptopurines. Attempts to increase the activity of 6-mercaptopurine by S-alkylation failed to improve the activity of the MTD* in Ad. 755 mice, but, as the length of the alkyl chain was increased from C₁ - C₈, the therapeutic index decreased from greater than 80 to 3. A further increase in the number of carbon atoms in the alkyl group to C₁₀, decreased the degree of antitumour activity and at C₁₂, activity was very small (Skipper et al., 1959). 6-Benzyl-thiopurine, on the other

* MTD - maximum tolerated dose

hand, is an active inhibitor of the Ad. 755 tumour and has a therapeutic index superior to that of 6MP.

It may be concluded, after consideration of all the various purine analogues examined by various investigators, that the 6-position of the purine ring is the most active site for introducing antimetabolic activity. The 2-position, though important, is much less so than the 6-position. Consideration of other positions in the purine ring, particularly the 7,8, and 9 positions, have been made (Elion et al., 1951) and the influence of substituent groups on biological activity assessed. However, no mention has been made of the influence of 7,8-dialkyl groups on activity and it was the purpose of the present investigation to determine what activity, if any, a number of such purines previously synthesised (see Part II) exhibited.

Some indication of the importance of the 7,8, and 9 positions particularly when substituted by alkyl groups, may be deduced from data in the literature. In imidazole the 1 and the 3 nitrogen atoms are equivalent but in the purine ring the corresponding 7 and 9 atoms are no longer identical, and replacement of the mobile proton by an alkyl group produces with the 7 and the 9 substituted derivative differing somewhat in properties. Alkylation of either the 7 or the 9 position of adenine, guanine, hypoxanthine or xanthine results in the loss of growth-promoting properties (for L.Casei) of the purine. In purine analogues with growth-inhibitory action, an alkyl group may reduce this inhibitory activity. Thus, 9-methyl-2,6-diamino--

purine is only slightly less active as a growth inhibitor than the parent compound - 2,6-diaminopurine. On the other hand, 7-methyl-2,6-diaminepurine was considerably less active. If the antimetabolite 6-mercaptapurine is converted to its 9-riboside, relatively no change in activity is found, but the therapeutic index* is considerably improved. 6-S-alkyl-mercaptapurine, 6-thioguanine, and 6-chloropurine are similarly influenced by conversion to the 9-ribonucleoside. The suggestion has been made (Brockman, 1960) that the metabolism of 6-mercaptapurine to the ribonucleotide constituting a lethal synthesis. If this is so, it is not surprising that there is little difference in activity between 6-mercaptapurine and its 9-ribonucleoside. It must be remembered, however, that a number of 9-alkyl-6-mercaptapurines have been shown to possess antitumour activity (Skipper et al., 1959); Hansen et al., 1964a). While it has been mooted that the antitumour activity arises from dealkylation of the base (Hansen et al., 1964b) it is currently believed that dealkylation is not an active part of the mechanism of action (Hansen et al., 1962; Hansen et al., 1964a).

The 8-position of the purine ring has been studied in some detail (Elion et al., 1951). The introduction of a-OH, -Cl or a methyl group into the 8-position diminished either the growth-promoting

* Therapeutic index = $\frac{MIC}{LD50} \times 100$

properties of the parent compound (as in 8-methyl-guanine and 8-methyladenine) or the inhibitory properties (as in 8-methyl-2,6-diaminopurine). Some evidence suggests that modification of the chemistry of the 8- position may increase the activity of known antitumour agents. Thus, the xanthine oxidase inhibitor 4-hydroxypyrazalo-(3,4-d)-pyrimidine, potentiates the antitumour action of 6-mercaptopurine. As the associated compound, 6-methyl-mercaptopurine, is known to be metabolised inter alia to 6-methyl-mercapto-2,8-dihydroxypurine by xanthine oxidase, it is possible that oxidation of the C-8 atom to non-active metabolites constitutes an important detoxification process. Inhibition of this C-8 oxidation by a suitable blocking agent in the 8- position may potentiate the action of 6-mercaptopurine. While the observation (see above) that an 8-methyl group results in reduced inhibitory or growth-promoting properties is in conflict with the above hypothesis, it is to be recalled that 8-arylpurines can be potent growth inhibitors of L. Casei. It is also interesting to note that the antitumour agent 8-azaguanine has a nitrogen atom in place of the position 8 carbon atom in guanine. The marked antitumour activity of this compound, produced entirely by a change at the 8-position, is a strong indication of the importance of this site in the purine ring.

In the present investigation several typical 7,8-dialkylpurines (viz 7-butyl-8-propylhypoxanthine; 6-butylamino-7-butyl-8-propylpurine; 7-iso-butyl-8-iso-propylxanthine; and 7-iso-butyl-6-(2'-phenylethylamino)-8-iso-propylpurine) previously

synthesised were examined for their ability to support the growth of a purine-requiring mutant of E. Coli. In all cases the test purine failed to support even marginal growth at the concentration tested (i.e. up to 10^{-4} M.). The maximum concentration used was in excess of that required of a physiological purine for maximum growth. It was concluded that the 7- and 8-positions of the purine ring in adenine, xanthine and hypoxanthine are critical positions and control metabolic activity.

The 7,8-dialkylation of purines with known antimetabolic activity was assessed by considering the growth rate of the E. Coli in a medium supplemented with adenine or xanthine and one of the experimental 7,8-dialkylpurines described in Section II. The low solubility of several of these purines precludes the use of concentrations in excess of about 10^{-3} M. At the concentrations employed none of the purines examined (viz. 7-iso-butyl-6-chloro-8-iso-propylpurine; 6-mercapto-7-iso-butyl-8-iso-propylpurine; 7-butyl-8-propylxanthine; and 7-iso-butyl-6-(2'-phenylethyl-amino)-8-iso-propylpurine) showed antimetabolic activity.

The relatively high antitumour activity of 9-ethyl-6-mercaptapurine and 9-butyl-6-mercaptapurine (Skipper et al., 1959; Hansen et al., 1962 and 1964b), indicates that the presence of an alkyl group in the 9-position does not eliminate antipurine activity. The presence of a 7-alkyl group in the present series of compounds probably modifies the activity of the parent compound in a similar way to the corresponding 9-alkylpurine, but is not responsible for the elimination of all activity. It appears more probable

that the absence of antipurine action is mainly the result of the 8-alkyl group.

In conclusion, it appears that the alkylation of the 7- and 8- positions of a physiologically-active purine produces such a significant change in the properties of the compound that metabolic activity becomes completely inhibited. In terms of the "lock and key" analogy of antimetabolic action in which an antimetabolite is likened to a key which is sufficiently similar to the correct key as to partially turn the lock before jamming, the 7,8-dialkylpurines are modified so much that they cannot turn the lock even a little. It appears reasonable to deduce, therefore, that either the 7- or the 8-positions or both, are vital for the normal function of ametabolically-active purine and cannot be alkylated without modification of this activity.

S U M M A R Y.

The Wallach reaction (the action of phosphorus pentachloride on an N,N'-dialkyloxamide) has been extended successfully to a series of symmetrical and nonsymmetrical oxamides yielding a group of 5(4)-chloro-1,2-dialkylimidazoles, to which the present author has given the collective name "Wallach bases".

A scheme was suggested for degrading the Wallach bases to identifiable fragments which permitted the orientation of both the chlorine atom and the alkyl groups to be determined. In all cases these bases were shown to be 5-chloroimidazoles. No trace of the isomeric 4-chloroimidazole was detected by any of the chemical or chromatographic methods employed.

The action of phosphorus pentachloride on nonsymmetrical oxamides was shown usually to yield two isomeric 5-chloro-1,2-dialkylimidazoles differing in the nature of the alkyl groups in the one and two positions. The orientation of these groups could be determined by the degradation described above.

An investigation was undertaken of techniques for the separation of isomeric imidazoles. The bases from butylethyloxamide were separated in large quantities by fractional distillation, and their structure determined by the above technique. A number of isomeric mixtures were separated on a small scale by various chromatographic and other techniques, but in insufficient quantity for further study.

Only one base was isolated in the reaction between phosphorus pentachloride and benzylmethyl-oxamide. This was shown to be 5(4)-chloro-1-methyl-

2-phenylimidazole by permanganate oxidation of the nitro derivative to inter alia p-nitrobenzoic acid. The position of the chlorine atom, and the final structure was confirmed by an independent synthesis which gave unequivocally the 5-chloro-1-methyl-2-phenylimidazole.

The infrared spectra for a number of 5-chloro-1,2-dialkylimidazoles and associated compounds were recorded. A tentative assignment of peaks and specific structural features has been made.

A very poor yield (3%) of basic material was obtained by the action of phosphorus pentachloride on di-(2-phenylethyl)oxamide and the product could not therefore be subjected to the degradation described. The general similarity of the infrared spectrum of this base with those of other Wallach bases strongly suggested that the product was 2-benzyl-5-chloro-1-(2'-phenylethyl)imidazole, and not an iso-quinoline as described by Decker et al., (1913).

The preparation of two 4-chloro-1,2-dialkylimidazoles, required for comparison purposes, is reported.

The dechlorination with sodium and alcohol of several Wallach bases has been described.

A number of 5-chloro-1,2-dialkylimidazoles and the corresponding nitro derivatives were shown to exhibit moderate anti-fungal activity when tested against a series of eight pathogenic fungi.

The metabolic and anti-metabolic action of several 7,8-dialkylpurines was examined by

measuring the growth rate of a purine-requiring mutant E. Coli. It was shown that none of the purines tested influenced, in any direction, the growth of this organism, and it was concluded that blockage of the 7 and 8 positions of the purine ring with alkyl groups eliminated all metabolic action.

B I B L I O G R A P H Y.

- ACKER, D.S., and CASTLE, J.E. A convenient laboratory synthesis of certain 6-hydroxypurines and 7-hydroxy-y-triazolo-[d]-pyrimidines. J. Org. Chem. 23, 2010, (1958).
- ACKERMANN, D., and SKRAUP, S. Endgültige Konstitutionsermittlung und Synthese des Spinacins. Zeit. physiol. Chem. 284, 129, (1949).
- ACKERMANN, D. "Über das Vorkommen von Spinacin in der Krabbe Crango Vulgaris. Zeit. physiol. Chem. 328, 275, (1962).
- ADAMS, R.R., and WHITMORE, F.C. Heterocyclic basic compounds. VI. Dialkylaminoalkylaminopurines. J. Am. Chem. Soc. 67, 1271, (1945).
- BAKER, B.R., JOSEPH, J.P., and WILLIAMS J.H. Puromycin. Synthetic studies. VI. Analogues of 6-dimethylaminopurine. J. Org. Chem. 19, 1793, (1954).
- BALABAN, I.E. and PYMAN, F.L. The bromo-derivatives of 1-methylglyoxaline and the constitution of "chloroxalmethylin". J. Chem. Soc. (1924), 1564.
- BALIS, M.E., BROOKE, M.S., BROWN, G.B., and MAGASANIK, B. The utilization of purines by purineless mutants of aerobacter aerogens. J. Biol. Chem. 219, 917, (1956).
- BALIS, M.E., HYLIN, V., COULTAS, M.K. and HUTCHISON, D.J. Metabolism of resistant mutants of S. Faecalis. II. Incorporation of exogenous purines. Cancer Res. 18, 220, (1958).
- BEAMAN, A.G., TAUTZ, W., GABRIEL, T., and DUSCHINSKY, R. The synthesis of azomycin. J. Am. Chem. Soc. 87, 389, (1965).
- BECK, W.S. The metabolic function of vitamin B₁₂. New. Eng. J. Med. 266, 708 & 814, (1962).¹²
- BENDICH, A., RUSSELL, P.J. Jn., and FOX, J.J. The synthesis and properties of 6-chloropurine and purine. J. Am. Chem. Soc. 76, 6073, (1954).
- BENDICH, A. Chemistry of Purines and Pyrimidines. In CHARGAFF, E., & DAVIDSON, J.N. ed. The Nucleic Acids. Vol. I. Academic Press Inc. N.York. (1955).
- BERGMAN, W., and BURKE, D.C. Contributions to the study of marine products XL. The nucleosides

- of sponges. IV. Spongosine. J. Org. Chem. 21, 226, (1956).
- BERGMANN, F., and TAMARI, M. The synthesis of 8-substituted purines. J. Chem. Soc. (1961), 4468.
- BILEK, L., DERKOSCH, J., MICHL, H., and WESSELY, F. Über der Zersetzung von α -amino-N-carbonsäureanhydriden mit Pyridin und Pyridinderivaten (Zur Frage der Bildung von höhermolekularen Cyclopeptiden). Monats. 84, 717, (1953).
- BISCHLER, A., and NAPIERALSKY, B. Zur Kenntniss einer neuer Isochinolinsynthese. Ber. 26, 1903, (1893).
- BLADON, P. Ultraviolet and Visible Spectroscopy. In SCHWARZ, J.C.P., ed. Physical Methods in Organic Chemistry. Oliver and Boyd, London. (1964).
- BLOCH, R.J., DURRUM, E.L., and ZWEIG, G. In A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press Inc. N. York. (1958).
- BREDERECK, H., HENNIG, I., PFLEIDERER, W., and WEBER, G. Synthesen in der Purinereihe, III. Mitteil Umsetzungen der Acetate des 4.5-Diaminouracils. Synthesen von Coffein, Theobromin und Theophyllin. Ber. 86, 333, (1953).
- BROCKMAN, R.W. A mechanism of resistance to 6-mercaptopurine: Metabolism of hypoxanthine and 6-mercaptopurine by sensitive and resistant neoplasms. Cancer Res. 20, 643, (1960).
- BROWN, G.B., and WELIKY, V.S. The synthesis of 9- β -D-ribofuranosylpurine and the identity of nebularine. J. Biol. Chem. 204, 1019, (1953).
- BROWN, G.B. The biosynthesis of nucleic acids as a basis for an approach to chemotherapy. Ann. N. Y. Acad. Sci. 60, 185, (1955).
- BROWN, G.W. Jn., WELLERSON, R. Jn., and KUPFERBERG, A.B. Antitrichomonal activity of 1-[(2,4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole and related compounds. J. Pharm. and Pharmacol. 17, 747, (1965).

- BUCHANAN, J.M. The enzymic synthesis of the purine nucleotides. Harvey Lectures. 54, 104, (1958-1959).
- BUHLER, E., and PFLEDERER, W. 8,8-Disubstituted xanthines: New types of purine derivatives. Angew. Chem. 77, 129, (1965).
- BURCHENAL, J.H., ELLISON, R.R., MURPHY, M.L., KARNOFSKY, D.A., SYKES, M.P., TAN, T.C., MERMANN, A.C., YUCEOGLU, M., MYERS, W.P.L., KRAKOFF, I., and ALBERSTADT, N. Clinical studies on 6-mercaptapurine. Ann. N. Y. Acad. Sci. 60, 359, (1955).
- CARNEY, T.P. Laboratory Fractional Distillation. Macmillan Co. New York. (1949).
- CHERBULIEZ, E., and BERNHARD, K. Recherches sur la graine de croton. I. Sur le crotonoside (2-oxy-6-amino-purine-d-riboside). Helv. Chim. Acta. 15, 464, (1932).
- COCKER, W. Preparation of the simpler α -alkyl-amino acids. Part I. J. Chem. Soc. (1937), 1693.
- COCKER, W., and LAPWORTH, A. A preparation of sarcosine. J. Chem. Soc. (1931), 1894.
- COLLIER, H.O.J., and HUSKINSON, P.L. The effects of potential antipurines on a purine-requiring strain of Escherichia Coli. Ciba Foundation Symposium on the Chemistry and Biology of Purines. (1957), 146.
- COOK, A.H., DAVIS, A.C., HEILBRON, I., and THOMAS, G.H. Studies in the azole series. Part XIV. A new synthesis of purines. J. Chem. Soc. (1949), 1071.
- COOK, A.H., and THOMAS, G.H. Studies in the azole series. Part XXIX. The preparation of some natural xanthines and related purines. J. Chem. Soc. (1950), 1884.
- COSAR, C., and JULOU, L. Activité de 1'(hydroxy-2'-éthyl)-1-méthyl-2-nitro-5-imidazole (8.823RP) Vis-a-vis des infections expérimentales a Trichomonas Vaginalis. Ann. Inst. Pasteur. 96, 238, (1959).
- CRUM-BROWN, A., and WALKER, J. Elektrolytische Synthese zweibascher Säuren. Ann. 274, 41, (1893).

- CUCKLER, A.C., KUPFERBERG, A.B., and MILLMAN, N.
Chemotherapeutic and tolerance studies on
amino-nitrothiazoles. Antibiotics and
Chemotherapy. 5, 540, (1955).
- CURTIUS, T. Gemischte Ketten aus Harnstoff,
Methylendiamin und Aminosäureresten.
J. Prak. Chem. 94, 116, (1916).
- DECKER, H., KROPP, W., HOYER, H., and BECKER, P.
II. Dihydroisochinolin-Synthesen.
Ann. 395, 299, (1913).
- DERMER, O.C., and HUTCHESON, J.W. Derivatives for
the identification of nitro-paraffins. Proc.
Oklahoma Acad. Sci. 23, 60, (1943). see
also C.A. 2006 (1944).
- DUGGAN, D.E., WEIGERT, M.G., GRIEB, W.E. and TITUS,
E.O. The metabolism of 6-methylaminopurine
in Salmonella Typhimurium. Biochem. Biophys.
Acta. 68, 519, (1963).
- ELDERFIELD, R.C. Heterocyclic Compounds. Vol. 5.
John Wiley and Son Inc. New York. (1957).
- ELION, G.B., BURGI, E., and HITCHINGS, G.H. Studies
on condensed pyrimidine systems. VII. Some
8-arylpurines. J. Am. Chem. Soc. 73, 5235,
(1951).
- ELION, G.B., HITCHINGS, G.H., and VANDERWERFF, H.
Antagonists of nucleic acid derivatives VI.
Purines. J. Biol. Chem. 192, 505, (1951).
- ELION, G.B., SINGER, S., and HITCHINGS, G.H. The
purine metabolism of a 6-mercaptapurine resist-
ant L. Casei. J. Biol. Chem. 204, 35, (1953).
- ELLIS, G.P., EPSTEIN, C., FITZMAURICE, C., GOLBERG,
L., and LORD, G.H. Antifungal activity of
some imidazole derivatives. J. Pharm. and
Pharmacol. 16, 400, (1964a).
- ELLIS, G.P., EPSTEIN, C., FITZMAURICE, C., GOLBERG,
L., and LORD, G.H. Synthesis and antiprotozoal
activity of some imidazole derivatives.
J. Pharm. and Pharmacol. 16, 801, (1964b).
- FARGHER, R.G. Orientation of the nitro- and aryl-
azo-glyoxalines. Fission of the glyoxalone
nucleus. J. Chem. Soc. 117, 668, (1920).

- FARGHER, R.G., and PYMAN, F.L. Nitro-, arylazo-, and amino-glyoxalines. J. Chem. Soc. 115, 217, (1919).
- FISCHER, E. Synthese von Polypeptiden, IX. Chloride der Aminosäuren und ihrer Acylderivate. Ber. 38, 605, (1905).
- FLYNN, L.M., WILLIAMS, V.B., O'DELL, B.L., and HOGAN, A.G. Medium for assay of vitamins with lactic acid bacteria. Anal. Chem. 23, 180, (1951).
- FRIEDMAN, L., and SHECHTER, H. Preparation of nitriles from halides and sodium cyanide. An advantageous nucleophilic displacement in dimethylsulphoxide. J. Org. Chem. 25, 877, (1960).
- GARFINKEL, D., and EDSALL, J.T. Raman spectra of amino acids and related compounds VIII. Raman and infrared spectra of imidazole, 4-methylimidazole and histidine. J. Am. Chem. Soc. 80, 3807, (1958).
- GOLDACRE, P.L., and BOTTOMLEY, W. A kinin in apple fruitlets. Nature. 184, 555, (1959).
- GOWAN, J.E., and WHEELER, T.S. Name Index Of Organic Reactions. Longmans. London. (1960).
- "
GRANACHER, Ch., SCHELLING, V., and SCHLATTER, E. Ueber Anhydride von Aminosäurederivaten. Helv. Chim. Acta. 8, 873, (1925).
- GRANT, R.L., and PYMAN, F.L. The nitro and amino derivatives of 4-phenylglyoxaline. J. Chem. Soc. 119, 1893, (1921).
- GRAY, C.H., and TATUM, E.L. X-ray induced growth factor requirements in bacteria. Proc. Nat. Acad. Sci. 30, 404, (1944).
- GREENBERG, G.R., and JAENICKE, L. On the activation of the one-carbon unit for the biosynthesis of purine nucleotides. Ciba Foundation Symposium on the Chemistry and Biology of Purines. (1957), 204.
- GUTHRIE, R. Studies of a purine-requiring mutant strain of Escherichia Coli. J. Bact. 57, 39, (1949).

- HANSEN, H.J., GILES, W.G., and NADLER, S.B. In vivo metabolism of 9-methyl-6-MP-S-35 and 9-ethyl-6-MP-S-35 by the rat. *Cancer Res.* 22, 761, (1962).
- HANSEN, H.J., VANDEVOORDE, J.P., BENNETT, K.J., GILES, W.G., and NADLER, S.B. Azaserine and thiopurines: I. Inhibition of S-180 mouse tumour and antibody synthesis. *J. Lab. Clin. Med.* 63, 801, (1964a).
- HANSEN, H.J., VANDERVOODE, J.P., GILES, W.G., and NADLER, S.B. Dealkylation of 9-E-6MP by the human. *Proc. Soc. Expt. Biol. Med.* 115, 713, (1964b).
- HAZELDINE, C.E., PYMAN, F.L., and WINCHESTER, J. The tautomerism of amidines. Part IV. The methylation of 4(or 5)-nitroglyoxaline and 4(or 5)-phenylglyoxaline. *J. Chem. Soc.* 125, 1431, (1924).
- HITCHINGS, G.H., ELION, G.B., FALCO, E.A., RUSSEL, P.B., SHERWOOD, M.B. and VANDERWERFF, H. Antagonists of nucleic acid derivatives. I. The Lactobacillus casei model. *J. Biol. Chem.* 183, 1, (1950).
- HOFMANN, K. Imidazole and its derivatives, Part I. Interscience Publishers, New York. (1953).
- HOROWITZ, N.H., and BEADLE, G.W. A microbiological method for the determination of choline by use of a mutant of Neurospora. *J. Biol. Chem.* 150, 325, (1943).
- HUNTER, S.H., CURY, A., and BAKER, H. Microbiological assays. *Anal. Chem.* 30, 849, (1958).
- HUTCHINGS, B.L. Puromycin. Ciba Foundation Symposium on the Chemistry and Biology of Purines. (1957), 177.
- HUTCHISON, D.J. Biological activities of 6-mercaptopurine. Effect on Streptococcus Faecalis. *Ann. N. Y. Acad. Sci.* 60, 212, (1955).
- JAENICKE, L. Vitamin and co-enzyme function: Vitamin B₁₂ and folic acid. *Ann. Rev. Biochem.* 33, 287, (1964).
- JUKES, T.H. Assay of Compounds with Folic Acid Activity. In GLICK, D., ed. *Methods of*

- Biochemical Analysis. Vol. 2. Interscience Publishers Inc., New York. (1955).
- KARRER, P., and GRÄNACHER, Ch. "Über Anhydride von Aminosäuren und Aminosäurederivaten. *Helv. Chim. Acta.* 7, 763. (1924).
- KATRITZKY, A.R. The infrared spectra of heteroaromatic compounds. *Quat. Rev. (London).* 13, 353, (1959).
- KELLEY, G.G., WHEELER, G.P., and MONTGOMERY, J.A. The effect of a series of 9-alkylpurines on the growth of sensitive and 6-mercaptopurine-resistant H. Ep. #2 cells. *Cancer Res.* 22, 329, (1962).
- KIDDER, G.W., and DEWEY, V.C. Studies on the biochemistry of *Tetrahymena* V. The chemical nature of factors I and III. *Arch. Biochem.* 8, 293, (1945).
- KIDDER, G.W., and DEWEY, V.C. Studies on the biochemistry of *Tetrahymena*. XIV. The activity of natural purines and pyrimidines. *Proc. Nat. Acad. Sci.* 34, 566, (1948).
- KOCHERGIN, P.M. Imidazoles Part 15. Product of the reaction of N,N'-dimethyloxamide with phosphorus pentahalide. *Zhur. obshchei Khim.* 34, 3402, (1964).
- KON, S.K. Biological and microbiological activity of purine analogues of vitamin B₁₂. Ciba Foundation Symposium on the Chemistry and Biology of Purines. (1957), 169.
- LANCINI, G.C., and LAZZARI, E. The synthesis of azomycin (2-nitroimidazole). *Experientia.* 21, 83, (1965).
- LAW, L.W. Resistance in leukemic cells to an adenine antagonist, 6-mercaptopurine. *Proc. Soc. Expt. Biol. Med.* 84, 409, (1953).
- LESTER SMITH, E. The chemistry of new purines in the B₁₂ series of vitamins. Ciba Foundation Symposium on the Chemistry and Biology of Purines. (1957), 160.
- LESTER SMITH, E. Vitamin B₁₂. Methuen and Co. London. (1960).

- LIGHT, L., and PYMAN, F.L. Bromo-derivatives of 2-methylglyoxaline. J. Chem. Soc. (1922), 2626.
- LISTER, J.H. Synthesis of purines from pyrimidines. Rev. of Pure and Applied Chem. 11, 178, (1961).
- LISTER, J.H. Synthesis of purines from imidazoles. Rev. of Pure and Applied Chem. 13, 30, (1963).
- LÖFGREN, N., and LÜNING, B. On the structure of nebularine. Acta Chem. Scand. 7, 225, (1953).
- MANN, F.G., and PORTER, J.W.G. The synthesis and properties of 1:7-dialkylxanthines. J. Chem. Soc. (1945), 751.
- MILLER, C.S., GURIN, S., and WILSON, D.W. C¹⁴ labeled 4(5)-amino-5(4)-imidazolecarboxamide in the biosynthesis of purine. Science. 112, 654, (1950).
- MILLER, C.S., GURIN, S., and WILSON, D.W. Substituted imidazoles as precursors of the purines. J. Am. Chem. Soc. 74, 2892, (1952).
- MITCHELL, H.K., and HOULAHAN, M.B. Adenine-requiring mutants of Neurospora Crassa. Fed. Proc. 5, 370, (1946).
- MONTEQUI, F. Sintesis de la 7-Etil-8-Metil-xanthine a partir de un Derivado del imidazol. Anales Real. Soc. Espan. Fis. Quim. (Madrid). 24, 731, (1926).
- MONTEQUI, F. Nueva sintesis de Purinas. Anales Real. Soc. Espan. Fis. Quim. (Madrid). 25, 182, (1927).
- MONTGOMERY, J.A., HEWSON, K., STRUCK, R.F., and SHEALEY, Y.F. A convenient synthesis of 4(5)-amino-5(4)-imidazole carboxamide hydrochloride. J. Org. Chem. 24, 256, (1959).
- MONTGOMERY, J.A., and TEMPLE, C., Jn. Synthesis of potential anticancer agents. IX. 9-Ethyl-6-substituted-purines. J. Am. Chem. Soc. 79, 5238, (1957).
- MONTGOMERY, J.A., and TEMPLE, C. Jn. Synthesis of potential anticancer agents. XII. 9-Alkyl-6-substituted-purines. J. Am. Chem. Soc. 80, (1958).

- MONTGOMERY, J.A., and TEMPLE, C. Synthesis of potential anticancer agents, XXII. Reaction of orthoesters with 4,5-diaminopyridines. J. Org. Chem. 25, 395, (1960).
- MONTGOMERY, J.A., and THOMAS, H.J. Purine nucleosides. Adv. in Carbohydrate Chem. 17, 301, (1962).
- MOTHES, K. Plant Hormones. Section d, Purines and other compounds. In FLORKIN, M., and STOTZ, E.H. ed. Comprehensive Biochemistry Vol. 11, Elsevier Publishing Co., Ltd. Amsterdam. (1963).
- NAKAMURA, S. Structure of azomycin, a new antibiotic. Pharm. Bull. (Japan). 3, 379, (1955).
- NEUBERT, A. Zur Kenntniss einiger Derivate des Phenethylamins. Ber. 19, 1822, (1886).
- NEUMAN, M.S. The preparation of 1-naphthonitrile. J. Am. Chem. Soc. 59, 2472, (1937).
- OTTING, W. Zur Struktur der heterocyclischen Fünfringe Pyrrol, Imidazol, 1,3,4-Triazol und Tetrazol. Ber. 89, 2887, (1956).
- PAPPENHEIMER, A.M. Jn., and HOTTLE, G.A. Effect of certain purines, and CO₂ on growth of strain of Group A Hemolytic Streptococcus. Proc. Soc. Expt. Biol. Med. 44, 645, (1940).
- PETERSON, W.H. and PETERSON, M.S. Relation of bacteria to vitamins and other growth factors. Bact. Rev. 9, 49, (1945).
- PORTER, J.N., HEWITT, R.I., HESSELTINE, C.W., KRUPKA, G., LOWERY, J.A., WALLACE, W.S. BOHONOS, N., and WILLIAMS, J.H. Achromycin: A new antibiotic having trypanocidal properties. Antibiotics and Chemotherapy. 2, 409, (1952).
- PRASAD, R.N., and ROBINS, R.K. Potential purine antagonists. VIII. The preparation of some 7-methylpurines. J. Am. Chem. Soc. 79, 6401, (1957).
- PRASAD, R.N., NOELL, C.W., and ROBINS, R.K. Potential purine antagonists. XVI. Preparation of some 2-, 6-, and 8-methylpurines. J. Am. Chem. Soc. 81, 193, (1959).

- PYMAN, F.L. Orientation of the 1,4 and 1,5- dimethylglyoxalines. Mode of fission of 5-aminoglyoxalines. J. Chem. Soc. (1922), 2616.
- RANDALL, H.M., FOWLER, R.G., FUSON, N., and DANGL, R. The infrared determination of organic structures. Van Nostrand. (1949).
- RICE, L.M., GROGAN, C.H., and REID, E.E. N,N'-Di-alkyloxamides. J. Am. Chem. Soc. 75, 242, (1953).
- RICHTER, E., LOEFFLER, P.K., and TAYLOR, E.C. Studies in purine chemistry. VIII, A convenient synthesis of hypoxanthines and adenines. J. Am. Chem. Soc. 82, 3144, (1960).
- RICHTER, E., and TAYLOR, E.C. Eine neue Synthese von Purinen. Angew. Chemie. 67, 303, (1955).
- ROBINS, R.K., and LIN, H.H. Potential purine antagonists. IV, Synthesis of some 9-methyl-6-substituted-purines. J. Am. Chem. Soc. 79, 490, (1957).
- RUTTINK, J. Investigations in the purine series, II. Synthesis of some purine derivatives. Rec. trav. chim. 65, 751, (1946).
- SARASIN, J. Sur les sels quaternaires des imidazols. Helv. Chim. Acta. 6, 370, (1923).
- SARASIN, J., and WEGMANN, E. Synthèse de l'hétéroxanthine à partir d'un dérivé de l'imidazol. Helv. Chim. Acta. 7, 713, (1924).
- SCHABEL, F.M., MONTGOMERY, J.A., SKIPPER, J.A., LASTER, W.R., and THOMSON, J.R. Experimental evaluation of potential anticancer agents, I. Quantitative therapeutic evaluation of certain purine analogues. Cancer Res. 21, 690, (1961).
- SCHAEFFER, H.J., and WEIMAR, R.D. Jn. Synthesis of potential anticancer agents. XVII. Preparation of 9-(substituted-cycloaliphatic)-purines. J. Am. Chem. Soc. 81, 197, (1959).
- SCHAEFFER, H.J., and WEIMAR, R.D. Jn. Synthesis of potential anticancer agents. XXV. Preparation of some cis- and trans-2-(6-substituted-9-purinyl)cyclopentanol. J. Org. Chem. 25, 774, (1960).

- SCHWARTZ, R., and ANDRÉ, J. The Chemical Suppression of Immunity. Second Intern. Symposium on Immunopath., Basel, (1962). 385.
- SCOTT, R.A., and LIVERMAN, J.L. Promotion of leaf expansion by kinetin and benzylaminopurine. Plant Physiol. 31, 321, (1956).
- SEXTON, W.A. Chemical Constitution and Biological Activity. (3rd. edition). E. and F.N.Spon Ltd. London. (1963).
- SHAW, E. A new synthesis of the purines adenine, hypoxanthine, xanthine, and isoguanine. J. Biol. Chem. 185, 439, (1950).
- SHAW, E., and WOOLLEY, D.W. A new and convenient synthesis of 4-amino-5-imidazolecarboxamide. J. Biol. Chem. 181, 89, (1949).
- SHAW, G., WARRENER, R.N., and (in part) BUTLER, D.N., and RALPH, R.K. Purines, pyrimidines, and glyoxalines. Part XIII. Some new unambiguous syntheses of 5-aminoglyoxalines and 5-amino-glyoxaline-4-carboxyamides, and a synthesis of 5-amino-1- β -D-ribofuranosylglyoxaline-4-carboxamide. J. Chem. Soc. (1959), 1648.
- SKIPPER, H.E., MONTGOMERY, J.A., THOMSON, J.R., and SCHABEL, F.M. Jn. Structure-activity relationships and cross resistance observed on evaluation of a series of purine analogues against experimental neoplasms. Cancer Res. 19, 425, (1959).
- SNELL, E.E., and MITCHELL, H.K. Purine and pyrimidine bases as growth substances for lactic acid bacteria. Proc. Nat. Acad. Sci. 27, 1, (1941).
- SNELL, E.E., and STRONG, F.H. A microbiological assay for riboflavin. Indust. and Eng. Chem. (Anal. Ed.). 11, 346, (1939).
- SOUTHWICK, P.L., and SEIVARD, L.L. The ring closure of N-alkoxalyl- β -anilinopropionic acids. J. Am. Chem. Soc. 71, 2532, (1949).
- TAYLOR, E.C., and LOEFFLER, P.K. Studies in purine chemistry, IX. A new pyrimidine synthesis from α -aminonitriles. J. Am. Chem. Soc. 82, 3147, (1960).

- TAYLOR, E.C., OSDENE, T.S., RICHTER, E., and VOGL, O. Some synthetic studies on purines and related heterocyclics. Ciba Foundation Symposium on the Chemistry and Biology of Purines. (1957). 20.
- TIERIE, G. Alkylsemioxamides. Rec. trav. chim. 52, 357, (1933).
- VOGEL, A.I. A Text-book of Practical Organic Chemistry. (3rd. Ed.). Longmans. London. (1956).
- WALLACH, O. Über die Einwirkung von Phosphorpentachlorid auf Säureamide. Ann. 184, 1. (1877).
- WALLACH, O. Zur Kenntniss der substituirten Oxamide, Formamide und der Diäthyloxaminsäure Ber. 14, 735, (1881).
- WALLACH, O. Über die Einwirkung von Phosphorpentachlorid auf Säureamide. Ann. 214, 257, (1882).
- WALLACH, O. Über Oxaline und Glyoxaline II. Ber. 16, 534, (1883).
- WALLACH, O., and OPPENHEIM, F. Zur Kenntniss der Basen $C_nH_{2n-3}ClN_2$. Ber. 10, 1193, (1877).
- WALLACH, O., and SCHULZE, E. Über Basen der Oxalsäurereihe. Ber. 13, 514, (1880).
- WALLACH, O., and SCHULZE, E. Über Basen der Oxalsäurereihe. Ber. 14, 420, (1881).
- WALLACH, O. and STRICKER, G. Über Oxaläthylin und Chloroxalallylin. Ber. 13, 511, (1880).
- WALLER, C.W., FRYTH, P.W., HUTCHINGS, B.L., and WILLIAMS, J.H. Achromycin. The structure of the antibiotic puromycin. J. Am. Chem. Soc. 75, 2025, (1953).
- WHITE, D.M., and SONNENBERG, J. Infrared spectra of arylimidazoles and arylisimidazoles. J. Org. Chem. 29, 1926, (1964).