

CIBA FOUNDATION SYMPOSIUM
ON THE
CHEMISTRY AND BIOLOGY
OF PURINES

Editors for the Ciba Foundation

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and

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*With 124 Illustrations
and structural formulae*



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PREFACE

At the Ciba Foundation's symposium on the Chemistry and Biology of Pteridines there was already talk of a similar conference on purines, and it was not long before Mr. G. M. Timmis put forward definite proposals to this end. Professor Adrien Albert proved as willing as usual to give us all possible help in its organization and the project was put in hand. For reasons of health the Director of the Foundation was not able to give the arrangements his personal attention, but his assistant, Miss Bland, admirably supported throughout by Professor Albert and Mr. Timmis, brought it successfully to completion.

Under the benign and knowledgeable Chairmanship of Professor Albert—whose move with his Department to Australia was, from our point of view, most happily delayed until after this symposium—members readily joined in the critical but co-operative discussions which, with the presented papers, are reproduced in this volume. On all such occasions at the Ciba Foundation the group is severely restricted in size, a rule which brings hard problems of selection but which has been found necessary if the conferences are to have a chance of being really useful. For those who could not be invited, it is hoped these proceedings will provide some sense of collaboration with the members present.

Although this will be the thirty-third book containing the papers and discussions of one of the Ciba Foundation's conferences, it may be helpful to add a few explanatory words about the Foundation and its other activities. It is an international centre, established as an educational and scientific charity under the laws of England. It owes its inception and support to its Founder, CIBA Ltd. of Switzerland, but is administered independently and exclusively by its distinguished British Trustees.

The Foundation provides accommodation for scientific workers who visit London from abroad, organizes and holds international conferences, conducts (in conjunction with the Institut National d'Hygiène) a postgraduate medical exchange scheme between England and France, arranges informal meetings for discussion, awards two annual lectureships, has initiated a scheme to encourage basic research relevant to the problems of ageing, assists international congresses and scientific societies, is building up a library service in special fields, and generally endeavours to give aid in all matters that may promote international co-operation in scientific research.

List of those participating in or attending the Symposium
on "The Chemistry and Biology of Purines",
8th-10th May, 1956

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OPENING REMARKS

G. M. TIMMIS

AFTER the success of the Ciba Foundation Symposium on the Chemistry and Biology of Pteridines, about two years ago, some of us who had been privileged to attend it felt that the purines, a field of work which is closely related both chemically and biologically, might well form a suitable subject for another symposium. We were able to persuade Dr. Wolstenholme that the time was about ripe for such a meeting and we were indeed most fortunate that Prof. Adrien Albert was prevailed upon to take on the vitally important office of chairman.

Since the early work of Emil Fischer on the chemistry of purines and, on the pharmacological side, the early investigations on the diuretic and stimulating properties of *N*-methylated purines, interest in the field somewhat declined and was only revived during and after the war by the work of Sir Alexander Todd and his school on nucleosides and nucleotides, in this country, and by American workers who were concerned mainly with simple purines and their analogues.

May I add here how sorry all of us are, who know Dr. George Hitchings and his work, that he is unable to be with us here.

Alongside the synthetic work and in a mutually interdependent way, remarkable advances have been made in the biochemistry of purines. Again, within the past few years the field has expanded in fresh and exciting directions with the discovery of vitamin B₁₂ and its purine-containing analogues, and of puromycin. In addition to the obvious potentialities of the B₁₂ analogues, the discovery of trypanocidal and anti-tumour activity in puromycin has furnished clues which may lead to new chemotherapeutic agents, depending for activity upon their purine structure.

Recently, some striking advances have been made in work on the biosynthesis of nucleic acid and the knowledge gained in elucidating this problem opens up the prospect of more fundamental investigations into chemotherapy, particularly perhaps of the virus diseases, and to be more optimistic but not, I feel, impossibly so, in the problem of cancer. Here, as so often happens, one or two drugs of some clinical value have been discovered as a by-product of a fundamental line of research.

Remarkable progress has also been made in the enzymology of purines and of their precursors and successors in the biosynthesis of nucleic acid and their complex derivatives. In all these fields the synthetic chemical work has played an invaluable part. In short, it seemed that the increasing number of significant discoveries in the chemistry, biochemistry and biology of purines made it very desirable for chemists and biologists to meet at a symposium like this and facilitate as far as possible joint planning of the work and, perhaps, to hasten the already impressive rate of progress.

SYNTHESIS AND PROPERTIES OF PURINES OF POTENTIAL BIOLOGICAL INTEREST*

AARON BENDICH, ALFREDO GINER-SOROLLA† and JACK J. FOX

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It is undoubtedly of considerable significance that purines occur in every living source which has been examined. The purines are integral parts not only of all the nucleic acids studied, whether of true cellular origin or in the less specialized structures, the viruses, but they are also essential constituents of coenzymes, ATP, etc. It is no surprise, therefore, that these compounds have been the object of intensive biological, biochemical and purely chemical investigations. Of particular significance has been the recent application of specific purines to the control of neoplastic disease (Rhoads, 1954), and this has provoked additional interest in this group of heterocycles.

Attention has been drawn (Bendich, Russell and Fox, 1954) to the types of structural features in purine derivatives which might be expected, from previous experience, to affect or interfere with biological systems. Whereas a limited success has resulted from this approach it would be idle to restrict new synthetic explorations to only certain structural types since the basis of such interference is largely unknown. In this paper, examples will be given of purines which have exhibited a hoped-for antagonism and of others which have been disappointingly inactive. The synthesis and certain properties of these are described.

* These investigations were supported in part by grants from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. C-471), The Atomic Energy Commission (Contract No. AT (30-1)-910) and the Ann Dickler Cancer League. The authors gratefully acknowledge the support and advice of Dr. G. B. Brown.

† Fellow of the International Institute of Education.

Synthesis

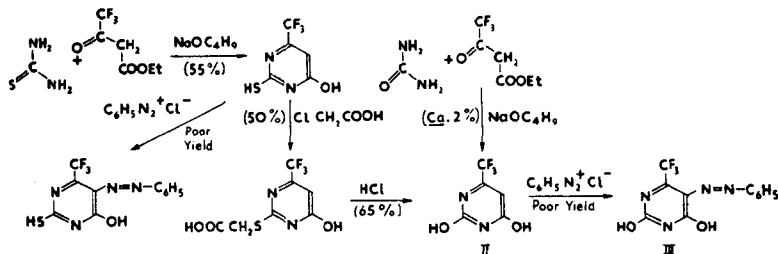
The various compounds synthesized were selected, in part, because they bore a structural resemblance to purines which had shown a particular biological effect. For example, inasmuch as 6-methylpurine (Gabriel and Colman, 1901) was found by Philips and his co-workers (1954) to be extremely toxic to mice and rats, it was decided to prepare 6-trifluoromethylpurine. Preparation of 6-*N*-hydroxylaminopurine and 6-azidopurine was prompted by the well-known inhibitory activities shown by analogues of adenine (Stock, 1954). The simplest member of the 1-*v*-triazolopyrimidine series was synthesized because of the inhibitory action displayed by its derivatives on tumours (Kidder *et al.*, 1949) and tobacco mosaic virus (Matthews and Smith, 1956). A discussion of some of the synthetic methods employed has appeared (Bendich, 1955).

However, some of the purines described in this paper are interesting purely from the standpoint of their chemistry. The reactions which some of them undergo are complicated and as yet poorly understood.

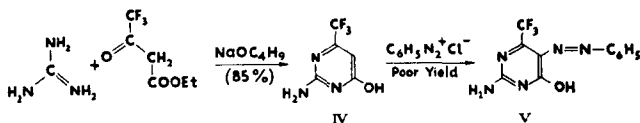
(I) Fluoro derivatives

Although the route to 6-methylpurine from 6-methyluracil (Gabriel and Colman, 1901) appeared applicable to the synthesis of 6-trifluoromethylpurine (I), it had to be abandoned because the analogous trifluoromethyluracil (II) was difficult to prepare in good yield, and a nitrogen function could not be introduced into the 5-position by nitrosation or nitration; the coupling reaction with benzenediazonium chloride gave poor yields of the 5-phenylazo derivative (III). The very sluggish behaviour of (II) in those reactions is undoubtedly due to electron depletion at C₍₅₎ resulting from the strong inductive effect of the trifluoro group. Some evidence for this effect on ring electron density is seen in the dissociation behaviour of 8-trifluoromethylpurine as contrasted with 8-methylpurine (*vide infra*). The condensation reaction of ethyl trifluoro-

acetoacetate with urea gave the pyrimidine (II) in poor yield in both boiling ethanol or *n*-butanol containing the corresponding sodium alcoholate; however, the reaction with



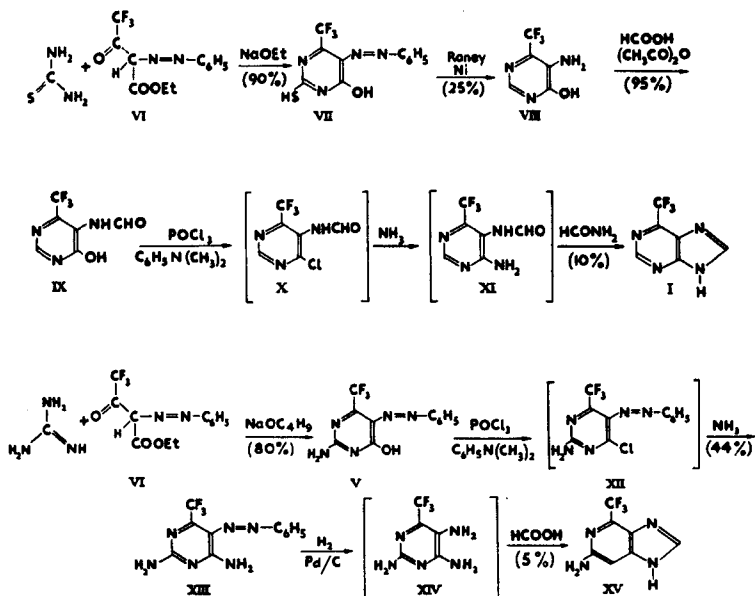
thiourea was much more successful (55 per cent yield). A yield of 85 per cent of 2-amino-4-hydroxy-6-trifluoromethylpyrimidine (IV) was obtained with guanidine. The pyrimidine (IV), however, failed to give encouraging yields of its 5-phenylazo derivative (V); nitration was ineffectual.



A more fruitful approach to I (cf. Baddiley, Lythgoe and Todd, 1943) proceeded via ethyl phenylazotrifluoroacetoacetate (VI) which condensed with thiourea to give the pyrimidine (VII) in excellent yields. Concomittant desulphurization and hydrogenolysis with Raney nickel afforded 5-amino-4-hydroxy-6-trifluoromethylpyrimidine (VIII).^{*} Conversion to the *N*-formyl derivative (IX) was necessary for replacement (X) of the 4-hydroxyl by the chlorine atom with $POCl_3$ and dimethylaniline, which, in turn, gave the amine (XI) upon treatment with cold ethanolic ammonia. The intermediates X and XI were not isolated in pure form; however their identities were established by conversion of XI by refluxing

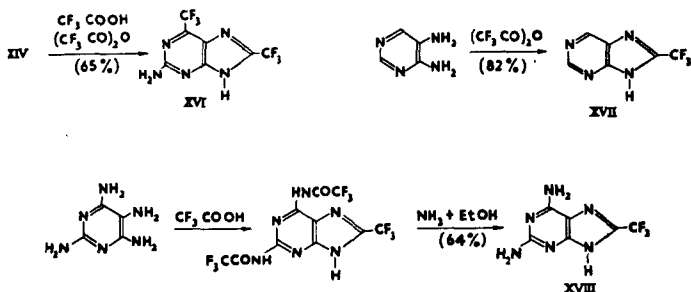
^{*} Alternatively, methylisothiourea was reacted with VI in ethanol/sodium ethylate and the 2-methyl derivative of VII was obtained in 83 per cent yield. The thioether, however, gave very unsatisfactory yields of VIII.

in anhydrous formamide to the desired purine (I), in 10 per cent overall yield. Guanidine and VI condensed smoothly to give 2-amino-4-hydroxy-5-phenylazo-6-trifluoromethylpyrimidine (V) directly. Replacement of the hydroxyl by the chlorine atom (XII) and subsequent amination resulted in the 4-amino derivative (XIII) in good yield. However, as 2 : 4 : 5-triamino-6-trifluoromethylpyrimidine (XIV) proved to be unusually unstable on exposure to air, its preparation from XIII with Raney nickel was discontinued in favour of the milder hydrogenolysis with palladium/charcoal at room temperature. 2-Amino-6-trifluoromethylpurine (XV) was obtained in low yield upon heating the triamine (XIV) in anhydrous formic acid.



When the triamine (XIV) was refluxed with a solution of trifluoroacetic anhydride in trifluoroacetic acid, 2-amino-6 : 8-bis (trifluoromethyl) purine (XVI) was obtained in 65 per cent yield. Two other purines in this series which have been pre-

pared are the 8-trifluoromethyl (XVII) and the 2:4-diamino-8-trifluoromethyl (XVIII) derivatives.



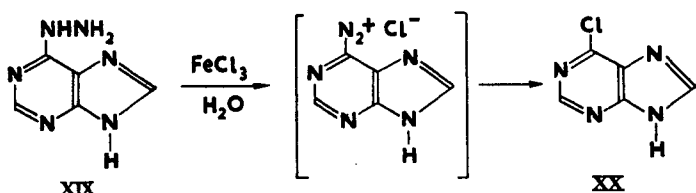
(II) Attempted synthesis of 6-fluoropurine

The antitumour activity of purine and 6-chloropurine (Bendich, Russell and Fox, 1954) made the synthesis of 6-fluoropurine desirable. Although all attempts have failed thus far, it is thought worthwhile to record some of the reactions which were tried.

Reaction of 6-chloropurine with aqueous solutions of silver nitrate failed to dislodge the chlorine atom, yet silver chloride was formed when silver fluoride was used instead. Although both reactions were complicated by the formation of the insoluble silver salt of the halopurine, and with silver fluoride a nearly quantitative yield of silver chloride was obtained, the desired fluoropurine was not formed. The chlorine-free product which formed appeared to be a polymer of a purine. When the imidazole portion of 6-chloropurine was blocked by acetylation, 6-chloropurine was recovered upon reaction with aqueous silver fluoride. Further synthetic attempts are under way.

Various diazotization reactions with adenine, including the Schiemann reaction (diazotization in presence of fluoroboric acid), failed to give fluoropurine. A fluorine-containing product could not be isolated following treatment of hypoxanthine with POF_3 in the presence or absence of proton acceptors.

An interesting lead came from the application of a little-used diazotization-type reaction (Seide, Scherlin and Bras, 1933); this is based on the conversion of phenylhydrazine to chlorobenzene upon warming with aqueous ferric chloride. When this reaction was applied to 6-hydrazinopurine (XIX), 6-chloropurine (XX) was obtained in 6 per cent yield. It also resulted upon reaction with a mixture of HCl and KClO_3 .



No evidence for the formation of 6-fluoropurine could be obtained from the reaction of 6-hydrazinopurine and ferric fluoride or with other oxidizing agents in presence of fluoride ion. It might be that the inability to prepare 6-fluoropurine may be due to its instability. Although the Schiemann reaction has been successfully applied to the synthesis of 2- and 3-fluoropyridine from the corresponding amino derivatives, 4-fluoropyridine appears to be too unstable to permit its isolation following the reaction (Roe and Hawkins, 1947).

(III) 6-Azidopurine

This compound (XXI) could be readily prepared from either 6-hydrazinopurine (XIX) by treatment with nitrous acid, or from 6-chloropurine (XX) and sodium azide.

