CIBA FOUNDATION SYMPOSIUM

ON THE

CHEMISTRY AND BIOLOGY OF PURINES

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, *O.B.E.*, M.A., M.B., B.Ch.

and

CECILIA M. O'CONNOR, B.Sc.

With 124 Illustrations
and structural formulae

LITTLE, BROWN AND COMPANY
BOSTON

THE CHEMISTRY AND BIOLOGY OF PURINES

Ciba Foundation Symposia

General Volumes:

Visceral Circulation – – – – –

Mammalian Germ Cells – – – – – –

Preservation and Transplantation of Normal Tissues – – – – – – –

Hypertension: Humoral and Neurogenic Factors – – – – – – –

Leukæmia Research – – – – – –

Chemistry and Biology of Pteridines – –

Experimental Tuberculosis – – – –

Porphyrin Biosynthesis and Metabolism –

Histamine – – – – – – – –

Extrasensory Perception – – – –

Bone Structure and Metabolism – – –

Paper Electrophoresis – – – – –

Ionizing Radiations and Cell Metabolism –

The Nature of Viruses – – – – –

A leaflet giving fuller details of these volumes, also of the Ciba Foundation Colloquia on Endocrinology and Colloquia on Ageing, is available from the Publishers.

CIBA FOUNDATION SYMPOSIUM

ON THE

CHEMISTRY AND BIOLOGY OF PURINES

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, *O.B.E.*, M.A., M.B., B.Ch.

and

CECILIA M. O'CONNOR, B.Sc.

With 124 Illustrations
and structural formulae

LITTLE, BROWN AND COMPANY
BOSTON

Published in London by
J. & A. Churchill Ltd.
104 *Gloucester Place, W.*1

First published 1957

Printed in Great Britain

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.

CONTENTS

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

A. ALBERT	The John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T.
J. BADDILEY	Dept. of Chemistry, University of Durham
B. R. BAKER	Biochemistry Division, Southern Research Inst., Birmingham, Alabama
A. BENDICH	Sloan-Kettering Inst. for Cancer Research, New York
F. BERGEL	The Chester Beatty Research Inst., London
H. BREDERECK	Inst. für Organische Chemie, Technische Hochschule, Stuttgart
D. J. BROWN	The John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T.
D. M. BROWN	University Chemical Laboratory, Cambridge
G. B. BROWN	Sloan-Kettering Inst. for Cancer Research, New York
J. M. BUCHANAN	Division of Biochemistry, Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass.
H. O. J. COLLIER	Research Division, Allen & Hanburys Ltd., Ware, Herts.
J. DAVOLL	Parke, Davis & Co., Ltd., Hounslow, Middlesex
GERTRUDE B. ELION	The Wellcome Research Laboratories, Tuckahoe, New York
D. A. G. GALTON	The Chester Beatty Research Inst., London
R. GREENBERG	Dept. of Chemistry, University of Durham
A. HADDOW	The Chester Beatty Research Inst., London
B. L. HUTCHINGS	Research Division, American Cyanamid Co., Pearl River, New York
G. W. KENNER	University Chemical Laboratory, Cambridge
S. K. KON	The National Inst. for Research in Dairying, Shinfield, Reading
I. LEWIN	The Chester Beatty Research Inst., London
S. F. MASON	Dept. of Chemistry, University of Exeter
R. E. F. MATTHEWS	Dept. of Scientific and Industrial Research, Plant Diseases Division, Private Bag, Auckland

W. PFLEIDERER	Inst. für Organische Chemie, Technische Hochschule, Stuttgart
P. SCHMIDT	CIBA Ltd., Basle
E. LESTER SMITH	Glaxo Laboratories Ltd., Greenford, Middlesex
E. C. TAYLOR	Dept. of Chemistry, Princeton University, New Jersey
G. M. TIMMIS	The Chester Beatty Research Inst., London
Sir ALEXANDER TODD	University Chemical Laboratory, Cambridge
S. VARADARAJAN	University Chemical Laboratory, Cambridge; and Dept. of Chemistry, University of Delhi
D. D. WOODS	Microbiology Unit, Dept. of Biochemistry, University of Oxford

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 B_{12} and its purine-containing analogues, and of puromycin. In addition to the obvious potentialities of the B_{12} analogues, the discovery of trypanocidal and antitumour 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

The Sloan-Kettering Institute for Cancer Research, New York

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

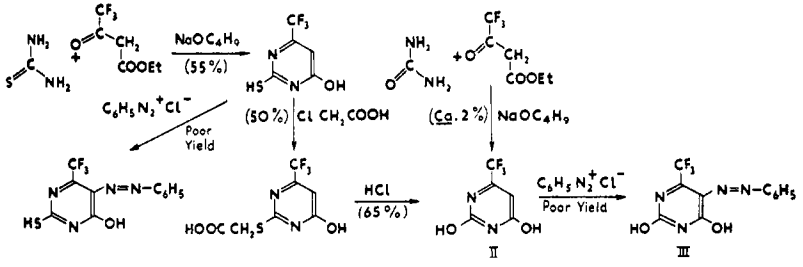
The various compounds synthesized were selected, in part, because they bore a structural resemblance to purines which had shown a particular biological effect. For example, inasmuch as 6-methylpurine (Gabriel and Colman, 1901) was found by Philips and his co-workers (1954) to be extremely toxic to mice 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 l-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.

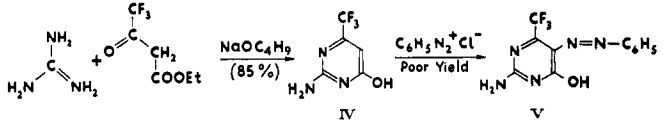
(I) Fluoro derivatives

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



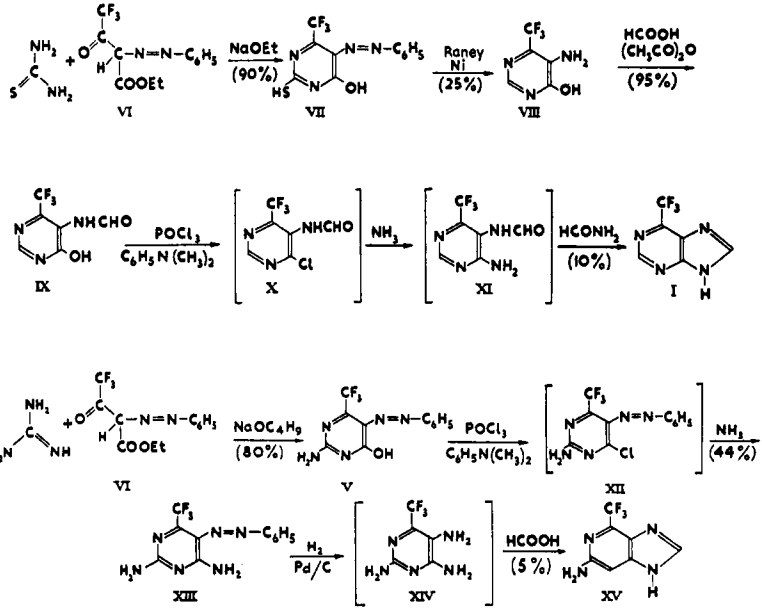
thiourea was much more successful (55 per cent yield). A yield of 85 per cent of 2-amino-4-hydroxy-6-trifluoromethylpyrimidine (IV) was obtained with guanidine. The pyrimidine (IV), however, failed to give encouraging yields of its 5-phenylazo derivative (V); nitration was ineffectual.



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

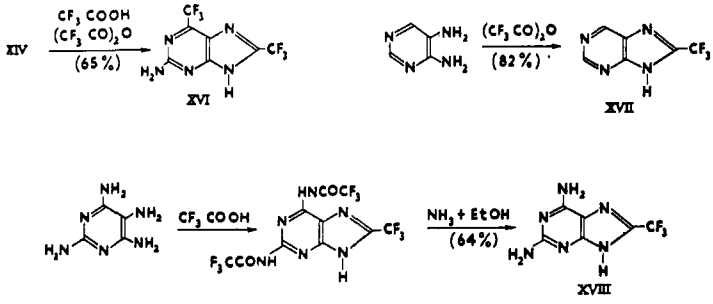
* Alternatively, methyl*iso*thiourea was reacted with VI in ethanol/sodium ethylate and the 2-methyl derivative of VII was obtained in 33 per cent yield. The thioether, however, gave very unsatisfactory yields of VIII.

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.



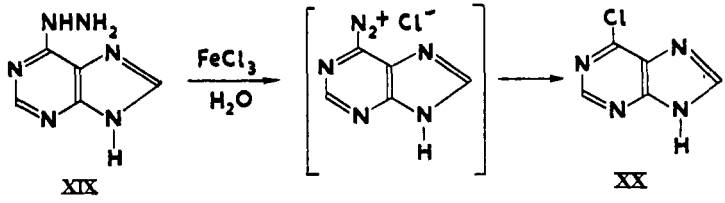
(II) Attempted synthesis of 6-fluoropurine

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 $KClO_3$.

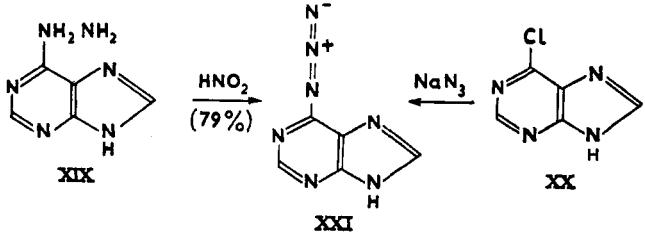


XIX XX

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

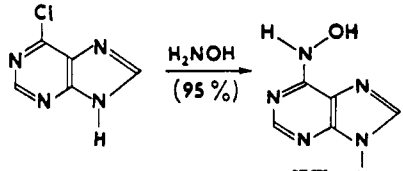
This compound (XXI) could be readily prepared from either 6-hydrazinopurine (XIX) by treatment with nitrous acid, or from 6-chloropurine (XX) and sodium azide.



XIX XXI XX

(IV) 6-*N*-hydroxylaminopurine

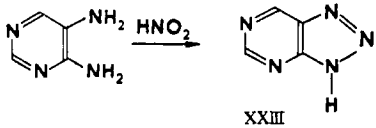
This compound (XXII), which is isomeric with guanine and which may be considered as an oxidation product of adenine, forms readily from 6-chloropurine (XX) and hydroxylamine.



XX XXII

(V) 8-Azapurine, or 1-v-triazolo(d)-pyrimidine

This compound (XXIII) has been prepared independently (Felton, 1955), but details of the method were not presented. We had attempted its synthesis, without success, by desulphurization of 8-aza-2-mercaptopurine. However, XXIII is obtained from 4 : 5-diaminopyrimidine by reaction with nitrous acid.



XXIII

Chemical Transformations

(I) 6-*N*-hydroxylaminopurine

Although it is quite stable at room temperature in N-HCl, 6-*N*-hydroxylaminopurine is rather unstable at high pH values and it was impossible to determine the ultraviolet absorption spectrum reproducibly in 0·1 N-NaOH. This behaviour in alkaline solution is reminiscent of the closely related 6-hydrazinopurine (Elion, Burgi and Hitchings, 1952) which is largely transformed into hypoxanthine by such treatment. With 1·0 N-NaOH, the hydroxylaminopurine is rapidly and irreversibly converted into a deeply red crystalline compound the elementary analysis of which is consistent with a disodium salt of 6 : 6′-azoxypurine monohydrate. The

structure of this product is under investigation; it changes to an amorphous yellow substance of unknown constitution upon neutralization. The hydroxylaminopurine reduces alkaline phosphomolybdate and ammoniacal silver nitrate. It is thought that in alkaline solution (as with other aromatic hydroxylamines), 6-*N*-hydroxylaminopurine is oxidized by oxygen of the air probably to 6-nitrosopurine which condenses rapidly with unreacted compound to give the azoxy derivative (cf. Sidgwick, 1945).

Adenine is obtained upon reduction of the 6-*N*-hydroxylamine with hydrogen (1 atmosphere, room temperature) in the presence of a palladium/charcoal catalyst.

(II) 6-Azidopurine

When treated with sodium nitrite and hydrochloric acid at 0° C, 6-hydrazinopurine is converted in high yield to 6-azidopurine. However, when fluoroboric acid is used in place of the hydrochloric acid, a colourless crystalline compound (containing fluorine and boron) is obtained which gives 6-azidopurine when treated with sodium acetate solution (pH 5). However, if the former compound (presumably the purinediazonium fluoroborate) is warmed to 40–50° C, it is transformed into a bright cherry-red compound (explodes at 240° C) which appears to be an azo derivative. The presumed azo derivative has been reduced with alkaline dithionite to a colourless hydrazo-like crystalline derivative which is distinctly different from the hydrazopurine obtained by condensing 6-chloropurine and 6-hydrazinopurine.

6-Azidopurine is unaffected, in either neutral or acidic solution, by hydrogen (1 atmosphere, room temperature) in the presence of a palladium/charcoal catalyst, but is reduced quantitatively to adenine if a Raney nickel catalyst is used instead. This reduction is of some interest since the volume of nitrogen produced is identical with the hydrogen consumed and hence there is a misleading constancy in the eudiometer reading:

$$C_5H_3N_7 + H_2 \xrightarrow{Pd/C} C_5H_5N_5 + N_2$$

A brisk evolution of nitrogen occurs when solutions of the azidopurine in N-HCl are heated to boiling; adenine was the only product visible on paper chromatograms in ultraviolet light. The compound is also unstable at room temperature in N-NaOH, but the identity of the reaction product was not established.

Physicochemical Properties

A few of the physical properties of the new purines are given in Table I which also includes adenine, guanine, and unsubstituted purine for comparison. The decrease in both

Table I

PHYSICAL PROPERTIES OF SOME PURINES

Purine Derivatives	*Melting Point* °C	*Solubility in water* 20° (± 2°) 1 *part in*	*pKa (in water)*	*Ref.*
Unsubstituted	216	2*a*	8·92 (±0·02) 2·52 (±0·05)	*b*
8-Aza	173–173·5 (d)	14	4·87 (±0·03) 2·12 (±0·05)	*c*
6-Methyl	235–236	5*a*	9·02 (±0·02) 2·6	*a*
6-Trifluoromethyl	254–255		7·35 (±0·05) <0	*d*
8-Methyl	271–273*a*	18*a*	9·87 (±0·05) 2·85 (±0·06)	*a*
8-Trifluoromethyl	192		5·12 (±0·05) 1·0 (±0·1)	*d*
6-Amino (adenine)	360 (d)	1,100*e*	9·80 4·15	*f*
6-Azido	185	244	7·60 (±0·03)	*g*
6-*N*-hydroxylamino	254 (d)	1,660	>12 9·83 (±0·05) 3·80 (±0·05)	*h*
2-Amino-6-hydroxy (guanine)	——	200,000*a*	12·3 9·2 3·3	*f*

a Albert and Brown (1954).
b Bendich, Russell and Fox (1954).
c Determined by titration in 0·1 M solution; Felton (1955) reported values of 4·9 and 2·1 obtained spectroscopically.
d Determined spectroscopically.
e Kossel (1886); at 40° C.
f Taylor (1948); by titration, conc. from 0·0012 to 0·005 M.
g Determined spectroscopically; no new dissociation was observable from pH 9·6 to 12. Instability at pH 1 or lower prevented determination of basic dissociation.
h Determined spectroscopically and by titration; instability at high values of pH prevented accurate estimation of pK>12.

the acidic and basic pKa's in 8-azapurine when contrasted with purine has been discussed previously (Felton, 1955). The pH of a 0·1 M solution of 8-azapurine is 3·47, in excellent agreement with 3·50 calculated from its pKa values of 4·87 and 2·12.

Similarly, 8-trifluoromethylpurine is a much stronger acid and a weaker base than 8-methylpurine, and this may be attributed to the strong inductive or electron-withdrawing effect of the trifluoromethyl group. The strong base-weakening effect caused by fluorine substitution in the pyridine system has been discussed (cf. Brown and McDaniel, 1955).

However, the trifluoromethyl group at position 6 does not have the same influence on the ionization behaviour of the purine moiety as it does at position 8. Of the two, the 8-trifluoromethyl group exerts the greater acid-strengthening effect (decrease of about 3·8 pK units from the acid dissociation of purine). One possible explanation of these differential effects can be suggested.

Since there can be little doubt that anion formation in alkaline media is the result of proton removal from the imidazole portion of unsubstituted purine (cf. Albert and Brown, 1954; Bendich, Russell and Fox, 1954) it should be expected that a trifluoromethyl group at position 8 would facilitate proton removal from adjacent imidazole nitrogen more than it would at the more remote position 6.

In regard to cation formation in acid solutions, however, the site of proton capture in the molecule is not known (*loc. cit.*). It would appear that addition of the proton in acidic solutions might be to the pyrimidine, rather than the imidazole part, as it can be expected that the greatest base-weakening effect would be shown by the group which is situated closest to that ring which accepts the proton. Since the base-weakening effect of the trifluoromethyl group at $C_{(6)}$ is greater (more than 2·5 units of pK lower than the basic dissociation of purine), it may be inferred that proton capture leading to the cation takes place in the pyrimidine moiety of these trifluoromethylpurines, at least.

An analogous effect of position of substitution on base strength is seen in the fluoropyridines. The values of pKa for pyridine, 2- and 3-fluoropyridine are, respectively, 5·17, — 0·44 and 2·97 (Brown and McDaniel, 1955).

Replacement of the amino group of adenine by the hydroxylamino function is accompanied by a large decrease in melting point, but little change in solubility in water. The resulting 6-*N*-hydroxylaminopurine exhibits an additional acidic dissociation, and thus resembles guanine in its ionization behaviour (Table I).

6-Azidopurine is more acidic than adenine and is much more soluble in water. The decrease in the acidic pKa of about 2·2 units may be taken to mean that the azido group behaves, in this system, more as an electron sink than as a donor. Unfortunately, the instability of the compound in acid

Table II

ULTRAVIOLET SPECTRAL PROPERTIES OF SOME PURINES

Purine	*pH*	*Species charge*	λ *max.* (*mμ*)	ε × 10^{-3}	*Ref.*
Unsubstituted	0·23	+	260	6·26	*a*
	5·94	0	262·5	8·16	
	11·90	—	270	7·98	
6-Amino (adenine)	2·10	+	262	13·2	*b*
	7·03	0	260	13·5	
	12·01	—	267	12·0	
6-*N*-Hydroxylamino	1·23	+	271	13·3	*c*
	6·73	0	268	11·8	
6-Azido	4·99	0	250, 258, 286	4·58, 5·05, 7·38	*d*
	10·25	—	232·5, 305	10·7, 7·31	
6-Trifluoromethyl	3N-HCl	mainly +	267·5	8·40	
	3·23	0	270	8·11	
	10·25	—	275	7·48	
8-Trifluoromethyl	3N-HCl	mainly +	262·5	7·04	
	3·0	0	264	7·72	
	7·98	—	270·5	8·80	
8-Aza	0	+	248	8·15	*e*
	3·77	mainly 0	262·5	7·02	
	7·22	—	268	7·59	

a Bendich, Russell and Fox (1954).
b Mason (1954).
c Instability at values of pH above pH 9 prevented accurate determination of spectra.
d Unstable in more strongly acidic solutions.
e Compare Felton (1955).

solutions prevented determination of the basic dissociation which is probably much weaker than that of purine.

The ultraviolet spectral properties of certain of these purines are listed in Table II. The determination of accurate spectra for 6-*N*-hydroxylaminopurine in alkaline media and for 6-azidopurine in strongly acidic media was rendered difficult because of the instability noted above.

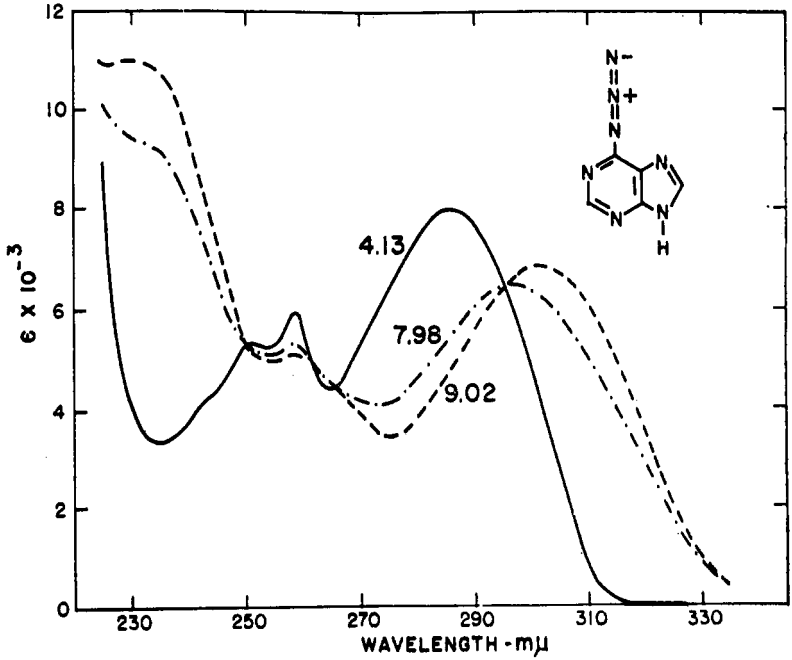


FIG. 1. Ultraviolet absorption spectra of 6-azidopurine at pH values indicated (pKa = 7·60 ± 0·03).

The spectra for 6-azidopurine at three values of pH are given in Fig. 1. Since the two dissociations (pKa of 2·12 and 4·87) of 8-azapurine (Table I) overlap to some extent, the determination of the spectrum of the neutral species involves some uncertainty. At pH = 3·5 (i.e., midway between 2·12 and 4·87), the ultraviolet absorption curve is mainly repre-

sentative of the neutral species with small, but significant contributions from the cationic and anionic species. The spectral data recorded in Table II for 8-azapurine (mainly the neutral species) are for pH = 3·77. An interesting analysis of the ultraviolet spectra of a compound (4 : 4′-diaminobenzophenone) which possesses severely overlapping dissociation constants (pKa's of 1·37 and 2·92) has been presented (Sager and Siewers, 1952).

Biological Activity

The compounds described here have been tested in the Division of Experimental Chemotherapy on mice bearing sarcoma 180, and, in a few instances, on normal mouse and sarcoma 180 cells in tissue culture.* The screening tests have been described (Stock, 1950, 1954). Of the new purines which have been synthesized in this study, only one showed some evidence of antitumour activity.

Although it was without apparent effect in these mice at a dose of 250 mg./kg., 6-*N*-hydroxylaminopurine was found to be differentially toxic to cells of mouse sarcoma 180 in tissue culture as seen in mitotic inhibition and induction of nuclear degeneration when compared with normal embryo skin fibroblasts. At a concentration of 0·05 μmole/ml., 6-methylpurine permits no mitosis in both sarcoma 180 and normal skin cells in tissue culture, but no toxicity is seen with 6-trifluoromethylpurine at 500 times this concentration.

REFERENCES

ALBERT, A., and BROWN, D. J. (1954). *J. chem. Soc.*, 2060.
BADDILEY, J., LYTHGOE, B., and TODD, A. R. (1943). *J. chem. Soc.*, 386.
BENDICH, A. (1955). *In* The Nucleic Acids, Vol. 1, p. 81, ed. E. Chargaff and J. N. Davidson. New York: Academic Press.
BENDICH, A., RUSSELL, P. J., Jr., and FOX, J. J. (1954). *J. Amer. chem. Soc.*, 76, 6073.
BROWN, H. C., and MCDANIEL, D. H. (1955). *J. Amer. chem. Soc.*, 77, 3752.

* These tests were carried out through the courtesy of Dr. J. J. Biesele, Dr. D. A. Clarke and Dr. C. C. Stock. The authors gratefully acknowledge the many helpful discussions with them.

ELION, G. B., BURGI, E., and HITCHINGS, G. H. (1952). *J. Amer. chem. Soc.*, **74**, 411.
FELTON, D. G. I. (1955). Discussion, Special Publication No. 3, p. 134. London: The Chemical Society.
GABRIEL, S., and COLMAN, J. (1901). *Ber. dtsch. chem. Ges.*, **34**, 1234.
KIDDER, G. W., DEWEY, V. C., PARKS, R. E., Jr., and WOODSIDE, G. L. (1949). *Science*, **109**, 511.
KOSSEL, A. (1886). *Z. physiol. Chem.*, **10**, 254.
MASON, S. F. (1954). *J. chem. Soc.*, 2071.
MATTHEWS, R. E. F., and SMITH, J. D. (1956). *Advanc. Virus Res.*, **3**, 49.
PHILIPS, F. S., STERNBERG, S. S., HAMILTON, L., and CLARKE, D. A. (1954). *Ann. N.Y. Acad. Sci.*, **60**, art. 2, 283.
RHOADS, C. P. (1954), consulting editor. Conference on 6-Mercaptopurine, *Ann. N.Y. Acad. Sci.*, **60**, art. 2, 185.
ROE, A., and HAWKINS, G. F. (1947). *J. Amer. chem. Soc.*, **69**, 2443.
SAGER, E. E., and SIEWERS, I. J. (1952). *J. Res. nat. Bur. Stand.*, **49**, 7.
SEIDE, O. A., SCHERLIN, S. M., and BRAS, G. V. (1933). *J. prakt. Chem.*, **138**, 55.
SIDGWICK, N. V. (1945). The Organic Chemistry of Nitrogen (revised and rewritten by Taylor and Baker), p. 163. Oxford: University Press.
STOCK, C. C. (1950). *Amer. J. Med.*, **8**, 658.
STOCK, C. C. (1954). *Advanc. Cancer Res.*, **2**, 425.
TAYLOR, H. F. W. (1948). *J. chem. Soc.*, 765.

DISCUSSION

Albert: You mentioned 6-chloropurine as being one of your key compounds. Although these gamma-chloro compounds are often very unstable, I think this one is rather stable?

Bendich: The key problem here, I believe, is the stability in acid solution, and chloropurine is rather unstable below a pH of 3. Now it seems to me that 6-fluoropurine should be a rather acidic compound; the pK_a of pyridine, for example, is 5·2, whereas 2-fluoropyridine has a pK_a of $-0{\cdot}4$ and 3-fluoropyridine has a pK_a of about 3·0. The corresponding chloropyridines are weaker acids. I should imagine then that 6-fluoropurine should be a stronger acid than 6-chloropurine, and as it would be isolated from acid solution it might be expected to be rather more unstable than 6-chloropurine, which is itself unstable in acid solution. (For reference to pK_a values of these pyridine compounds, see Brown, H. C., and McDaniel, D. H. (1955), *J. Amer. chem. Soc.*, **77**, 3752.)

Timmis: With the possibility of reduction *in vivo* in mind, have you examined, in the first case, the action of the azidopurine on purine-requiring bacteria with a view to ascertaining whether reduction to adenine had occurred? You might be able to get evidence by antagonism studies for its conversion to adenine if this were the case.

Bendich: We have not done that, but we do hope many people will examine these things; we have given some to Dr. Matthews. It should be possible to find out whether the azidopurine is converted to adenine in bacteria.