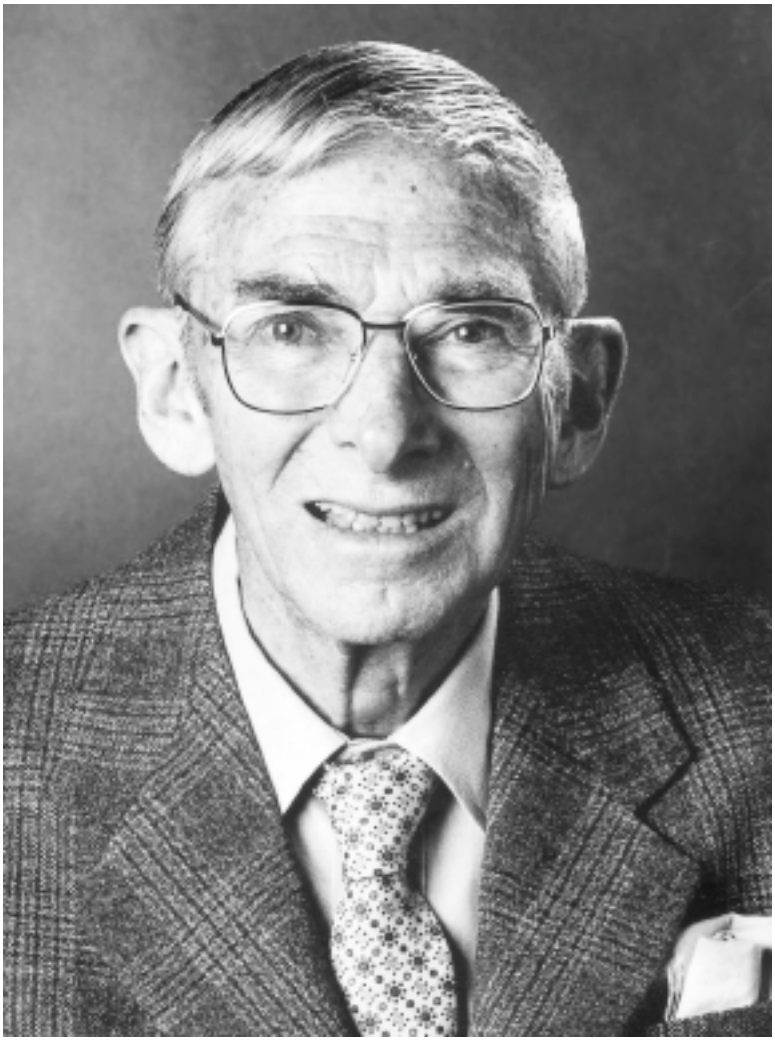
Liversidge Research Lecture

No. 15
1964

HETEROCYCLIC CHEMISTRY, AND SOME BIOLOGICAL OVERTONES

ADRIEN ALBERT

The Royal Society of New South Wales



Adrien Albert

This photograph is reproduced by permission of the Australian Academy of Science

Liversidge Research Lecturer No. 15, 1964

ADRIEN ALBERT 1907-1989

Adrien Albert was born in Sydney on 19 November 1907. After matriculation from Scots College (Sydney) in 1924 he undertook an apprenticeship in Pharmacy, attending the University of Sydney part-time. He became a registered pharmacist in 1928, but was drawn to pure science and returned to the University, graduating B.Sc (1st. class Hons.) with the University Medal in 1932. After working for short periods in the Pharmacy Department, University of Sydney, and then in a fabric dying firm, he went to London to work under W.H. Linnell at the College of the Pharmaceutical Society (London University) on aminoacridines, and graduated Ph.D. (Med) in 1937. He returned to Australia in 1938, and took a temporary teaching position in the Pharmacy Department, University of Sydney, and then a similar position in the Organic Chemistry Department of that Institution. He remained there during the Second World War, becoming involved with the war effort as part of a team supervised by Dr. Konrad Gibian of the Chemical firm Timbrol Ltd. During that time he prepared large scale amounts of both proflavine and the antimalarial "Atebrin"; in due course proflavine was replaced by 9-aminoacridine, a new compound first made by Albert as part of a research project that he had initiated. In the latter war years Albert's chemotherapy research was rewarded with funding by the newly formed National Health and Medical Research Council, and this support continued until 1947 when he went to London to work in the Wellcome Research Institution.

His original work on acridines resulted in the award of D.Sc (London) in 1947. He left the Wellcome Research Institution early in 1949 to take up the appointment as Foundation Professor and Head of the Medical Chemistry Department of the Australian National University that was temporarily set up in London pending construction of the building in Canberra. Soon afterwards he published his first monograph, '*The Acridines*' (1951), but his research was now in a new field, - that of the purines and pteridines, and in 1951 he published another monograph - '*Selective Toxicity*", which was to appear in another five editions over the next 35 years. It was seven years (1956) before Albert and his new Department moved to the new buildings in Canberra. Here, research continued on nitrogen heterocycles, including triazolopyrimidines ('8-azapurines'). He retired at the end of 1972, but became a Visiting Fellow at the Research School of Chemistry, and from 1981 held a similar position in the Department of Chemistry of the Australian National University. His last monograph 'Xenobiosis' earned him the Archibald Ollé prize in 1987; he died in 1989.

Adrien Albert was Visiting Professor in A.P. Grollman's Department of Pharmacological Sciences, the State University of Stony Brook on six occasions. During visits to the USA he also delivered the Patton, Blicke and Smissman Lectures at several campuses.

Honours and Awards

1946 The Liversidge Centenary Lecture, ANZAAS

1958 FAA

1960 The Inaugural Royal Society of Chemistry (Australian) Lectureship.

1964 Liversidge Research Lecture, Royal Society of New South Wales

1985 A biennial Adrien Albert Lectureship endowed in his honour by the Royal Australian Chemical Institute (RACI)

1987 Archibald D. Ollé prize, RACI

1989 Order of Australia (A.O.)

1990 D.Sc, *honoris causa*, University of Sydney (conferred posthumously).

Biographical Sources

(1) Brown, D.J., 'Adrien Albert 1907-1989', *Historical Records of Australian Science*, 1990, **8**, 63-75.

(2) Rae, I.D. and Spurling, T.H., 'Obituary', *Chemistry in Australia*, 1990, **57**, 116.

Scientific Publications by A. Albert

A list of 239 scientific publications by Adrien Albert, including several monographs, is given as an appendix to reference (1), Biographical Sources.

HETEROCYCLIC CHEMISTRY, AND SOME BIOLOGICAL OVERTONES*

ADRIEN ALBERT
Department of Medical Chemistry, John Curtin School of Medical Research, Australian National University, Canberra

Introduction

Mr. President, Ladies, and Gentlemen, I am indeed happy, and feel honoured, that the Royal Society of New South Wales chose me as this year's Liversidge research lecturer. I received the whole of my undergraduate training at the University of Sydney, first in Pharmacy and later in Science. Because I enrolled first in 1926, 1 was too late to come under Liversidge's *direct* influence, for in 1908 he had vacated the Chair of Chemistry after 35 years' tenure and he died in England in 1927. Yet his influence is felt by all scientists in Australia.

Archibald Liversidge, F.R.S., saw the whole of science as a single subject. At the age of 21 he worked in the Physiological Laboratory at Cambridge, whereas in Australia his chosen research field was mineralogical chemistry. As a constant background to this research, Liversidge was carrying out the organisational work for which Australian science will always be grateful. Edgeworth David wrote in 1931 that he thought Liversidge the greatest organizer of science that Australia has ever seen, and that he worked most unselfishly and with great singleness of purpose to advance the cause of science in Australia. "In 1879, after homeric battles with the powerful forces of Arts, he succeeded in winning for Science a Faculty of its own" (David, 1931). In 1885 he launched ANZAAS with the help of Australian and New Zealand colleagues, and this was, perhaps, his greatest work of all.

I had the good fortune to be chosen to give the Liversidge Centenary Lecture in 1946 at the ANZAAS meeting in Adelaide, and it is pleasing again to be doing honour to this great man.

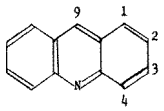
Because my deepest interest ever since school days, was to find out how drugs exerted their curative action, I studied biology alongside chemistry all through my undergraduate course. To-night I propose to describe some of the highlights of the research in which I became involved after completing my Ph.D. work in London and returning to Australia in 1937.

Monoaminoacridines

In my thesis work, under the supervision of W. H. Linnell, I had synthesized a number of new polyaminoacridines but the reason why only some of them were antibacterial quite eluded us. It was later, while I was working in the Department of Organic Chemistry in the University of Sydney, that I resolved to simplify the problem by examining the

---

*Liversidge Research Lecture, delivered before the Royal Society of New South Wales, June 9th, 1964. Reproduced by permission of the Royal Society of New South Wales from *J. Proc. Roy. Soc. N.S.W.*, 1964-5, **98**, 11-22.



Acridine (1)

*mono*aminoacridines. It can be seen from the formula of acridine (1) that five isomers are possible.

After preparing all five, I had the good fortune to interest my contemporary, S. D. Rubbo (now Professor of Bacteriology in the University of Melbourne), to test them on bacteria. The results were very clean cut: two isomers (the 3- and the 9-amino-derivatives) were highly active, and the other three isomers were almost inactive.

While I was wondering why this was so, an inner voice seemed to say, "It's the basic strength". Naturally I was tempted to disregard this inner prompting, because the suggestion did not follow logically from the evidence, and could only be tested by mastering a new technique. All of my undergraduate and postgraduate training had stressed that advances are made by logical analysis of the experimental evidence in the light of established principles. To teach a student how to proceed in this way, is one of the principal aims of formal tertiary education.

Fortunately there is another mental process available to the research worker. Whether this is referred to as "inspiration" or "intuition", it seems to depend on a portion of the brain that functions something like a computer, and tirelessly but unconsciously combines all sorts of odd strands of knowledge until finally it offers up to consciousness something that seems worth considering at that level. In giving credit to this process, which I am convinced is the research man's best friend, I do not want to undervalue formal education. It is only by the use of the formal universal language of science that ideas received in this way can be expressed in words, a process necessary both for their experimental testing and ultimate publication. But I must record also that in my mature scientific life I have met many bright young men, who, although they have absorbed an excellent formal training and can tackle any problem that one gives them, nevertheless can only proceed by small, logically-derived steps. Of course, we all have to do this when "inspiration" doesn't come, but possession of this ability alone does not seem to be enough qualification for undertaking full-time research, particularly in the difficult borderline subjects where so few principles are established. In other words, it seems fair to expect a proportion of discoveries to be surprising discontinuities, and not just inevitable extrapolations of known lines of work.

To return to my monoaminoacridines, I was shown how to determine their ionization constants by an honours student in Physical Chemistry. This was R. J. Goldacre who, by a steady broadening of interests, has become a Reader in Biology in the University of London.

The results of my collaboration with Rubbo and Goldacre are exemplified in Table 1. It is seen that two of the monoaminoacridines are much stronger bases than the others, that these two are far more highly ionized under the conditions of the test, and that these two isomers are by far the most highly antibacterial. This early success, led us to extend our approach to other acridines, about 106 of them in all, and to a wide range of pathogenic bacteria (aerobes, anaerobes, and Gram-positive and -negative species). As a result of this work, it became evident that the substituents did not influence antibacterial action except in so far as they influenced ionization. So long as the substance was substantially ionized as a cation at the

pH of the test it was highly antibacterial, and actively fell as ionization fell. Even the poorly antibacterial monoaminoacridines of Table 1 could, by lowering the pH to below the pKa values, be made to inhibit such organisms as *E. coli*, which grow well at low pH values (Albert *et al*., 1941, 1945).

TABLE 1
Examples of Dependence of Bacteriostasis on Ionization in the Acridine Series

Test organism: *Streptococcus pyogenes*, incubated for 48 hours at 37° and pH 7.3.
Medium: Meat broth, plus 10% serum.

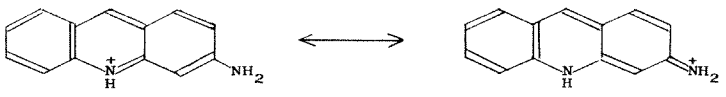
Acridine	pK_a in water at 20°	Per cent ionized (pH 7.3 and 37°)	Minimal bacteriostatic concentration
			1 in -
Unsubstituted	5.60	1	5,000
1-Amino	6.04	2	10,000
2-Amino	5.88	2	10,000
3-Amino	8.04	73	80,000
4-Amino	4.40	<1	5,000
9-Amino	9.99	100	160,000

A search of the literature revealed that this was the first correlation of ionization with a biological effect although there was one (unproven) suggestion of such a correlation (Stearn and Stearn, 1924), and one established correlation between antifungal action and *non*-ionization (Vermast, 1921).

Ionization Constants

Reverting to chemistry, I wanted to establish why these isomeric aminoacridines differed so much in basic strength. Inspection of the formulae suggested that possibilities for resonance, of the type (II) ⟷ (III) existed in the cations but not in the neutral species, of three of the isomers, namely the 1-, 3-, and 9-derivatives. Now, we had found that the 3- and 9-isomers had high basic strength (e.g. 20,000 times that of acridine for the 9-derivative), but the 1-isomer lacked it. We provisionally concluded that base-strengthening resonances of the type (II) ⟷ (III) were significant if paraquinonoid forms were involved, e.g. (III) and (IV), but not if neighbouring orthoquinonoid rings were created, as in (V) (Albert and Goldacre, 1943; *cf*. Gore and Phillips, 1949).

We then decided to transport to other series this concept of base-strengthening through the participation of a *p*-quinonoid form in the cation. At that time (1943), very few ionization constants of heterocyclic compounds were known (and hence few published ultraviolet spectra depicted pure ionic species). Sure enough, we found our base-strengthening principle upheld in the quinoline, pyridine, and many other nitrogenous heteroaromatic series (Albert *et al*., 1948). The later demonstration by Angyal and Angyal (1952) that the neutral species were primary amines, and not imines, rounded off this picture very well.



(II) (III)

(IV) (V)

By now, I had developed an interest in ionization constants that has always remained with me. This interest has proved its worth over and over again, whether for identification, for settling a point of constitution, for choosing the best pH to give a maximal yield in preparative chemistry, or for obtaining those *single species* ultraviolet spectra that alone are significant. It has led to writing a book on methods for determining ionizing constants (Albert and Serjeant, 1962) ; and also a review and compilation of heterocyclic pK_a values (Albert, 1963). One of the most interesting research projects of this kind, was the determination of the ionization constants of 87 hydroxy derivatives of various heteroaromatic nuclei and the estimation of the proportion of enol to amide forms present at equilibrium, e.g. (VI) and (VII) respectively.

(VI) (VII)

This proved often to be as little as 1 part in 100,000 (Albert and Phillips, 1956). Parallel studies of mercapto-heterocycles gave similarly interesting results (Albert and Barlin, 1959).

Significance of Flat Area for Antibacterial Properties

I should like now to return to the antibacterial work. In 1942, our highly potent 9-aminoacridine* was taken up by the Australian Army for use as a safe and effective dressing for badly infected war wounds. It was then adopted by the British Pharmacopoeia (in the latest edition of which it has displaced acriflavin) and the non-proprietary name Aminacrine was given it ("Monacrin" is one of the brand nantes). This was the first of the non-staining acridine antibacterials.

Although this clinically useful drug was culled in wartime from scientific studies that had been given a strong *applied* leaning because of the war, I do not think it desirable that University work during times of peace should be aimed at the discovery of new drugs. Rather should it be directed to discovering the principles responsible for drug action. Industry is much better equipped for the discovery and marketing of new remedies, but seldom has it the time or inclination to devote to the fundamental research for principles. If universities did not do this, who would do it?

---

*This was known, in those days, as 5-aminoacridine, before the advent of the I.U.P.A.C. numbering.

In 1946, S. D. Rubbo and I decided to investigate the antibacterial properties of cations other than those of acridines. We found that by removing a benzene ring from 9-aminoacridine to give 4-aminoquinoline (VIII) all antibacterial action was lost. The same thing happened if a benzene ring in 9-aminoacridine was reduced to give the 1,2,3,4-tetrahydro derivative. Yet all these substances were completely ionized under the test conditions. It occurred to me that a minimal flat area (about 38 sq. Å) was required before a molecule could have the antibacterial properties of an aminoacridine. (These properties can be defined as follows: active at high dilution, against a wide range of Gram-positive and -negative bacteria, even in the presence of protein). To test this idea we added, to the molecule of 4-aminoquinoline, a styryl-group to give 4-amino-2-styrylquinoline (IX), which proved to be a powerful acridine-type antibacterial. Encouraged by this and similar successes, we attached the highly ionizing guanidine group to the anthracene nucleus (which has the requisite flat area) and thus achieved a typical "aminoacridine" action in a non-heterocyclic series (Albert *et al.*, 1949).

TABLE 2

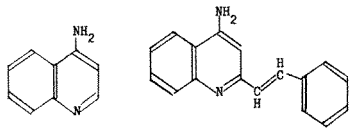
Decrease of bactericidal action as concentration of oxine is increased

Staph. aureus, in meat broth. Plated out after 3 hours at 20°

Oxine 1/M	200,000	100,000	50,000	25,000	12,800	6,400	3,200	1600	200
Growth	+++	-	-	-	+	+	+	+++	+++

Code: + + +, growth was prolific; +, up to 50 colonies; - , no growth.

Recent studies in the U.S.A. have shown that aminoacridines accumulate between the flat layers of purine and pyrimidine bases in deoxyribose nucleic acid (Lerman, 1963), and this may be their site of action.



(VIII)

(IX)

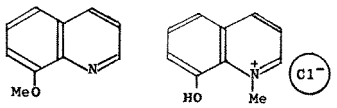
In the course of this acridine work some highly novel acridine syntheses and reactions were discovered, but these always seemed of secondary interest to the interdisciplinary studies in which the products were used.

Chelates

In a new collaboration with S. D. Rubbo, the mode of action of 8-hydroxyquinoline, a potent bactericide and fungicide, was investigated. This substance had long been used in analysis to segregate the ions of di- and tri-valent metals, and hence it seemed reasonable to suggest that its biological action was due to chelation (Albert, 1944). As has happened so often in the history of ideas, this suggestion was rejected by many as impossible, whereas to-day it is so well received that it now seems almost too obvious to mention! The biochemical grounds for rejection were that the human body contains many vital enzymes that have divalent metals as coenzymes, and hence a chelating agent would damage human and microbial cells equally. Many years later, it was possible to elaborate a quantitative basis for selectivity through, e.g. variations in stability constants, steric hindrance, and membrane permeability properties (which are related to partition coefficients). But in 1944 the first

practicable method for determining stability constants was still being worked out, by Bjerrum, in Denmark.

Briefly, what we did in 1944 was to prepare the six isomers of oxine (8-hydroxyquinoline) and show that they neither chelated nor were they antibacterial. Next we examined the two methyl-blocked derivatives of oxine, respectively (X) and (XI), and showed that these had neither chelating nor antibacterial properties. Thus it seemed certain that the biological action of oxine depended on chelation. It would have been facile to suppose that oxine acted by removing an essential divalent metal ion from the bacteria. But careful observation soon convinced us that exactly the opposite was occurring, namely we found that oxine was quite inactive unless it was supplied with a heavy metal from the medium.



(X) (XI)

The data which started this line of thought are shown in Table 2. It can be seen that oxine kills the test organism in a broth medium at a dilution of M/100,000, but progressively loses this property when the concentration of oxine is increased, so that a M/1600 solution has lost all bactericidal properties under the conditions of the test.

The explanation of this paradoxical "concentration quenching" came from the experiments performed in Table 3. The test organism, *Staphylococcus aureus*, was

TABLE 3

Effect of oxine and of iron on growth of bacteria Staph. aureus, 20°.
Plated out after 1 hour.

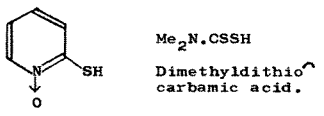
Oxine I /M	Fe^{2+} or Fe^{3+} 1/M	Growth	
		Distilled water	Meat broth
Nil	Nil	+ + +	+ + +
100,000	Nil	+ + +	-
Nil	100,000	+ + +	+ + +
100,000	100,000	-	-

+ + +, growth was prolific; - no growth.

incubated in distilled water with oxine for an hour, and all organisms found to be alive, whereas when the same experiment was performed in meat broth, all organisms were killed. Clearly the broth contained a co-factor without which the oxine was inactive, and this factor proved to be iron. When an iron salt, ferrous or ferric, was added to distilled water, oxine killed all the bacteria. Conversely when iron was removed from the broth (by prior shaking with a chloroform solution of oxine), oxine failed to kill. The paradoxical results of Table 2 could now be explained by postulating that the 1: 1 oxine-iron complex was the lethal agent, and that increasing the concentration of oxine without increasing that of the iron had produced the non-toxic 2 : 1 complex. We then showed that by maintaining a 1: 1 oxine-iron ratio, oxine remained active even in the highest concentrations (Albert *et al*., 1947, 1953). Cobalt was found to be a unique antagonist of this iron-oxine combination.

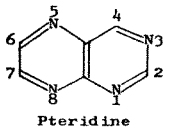
Later, working in London with different colleagues, I extended these studies to show that the activity of oxine derivatives was proportional to their lipophilic properties (as measured by oil/water partition coefficients) up to a plateau value. This suggested that penetration into the cytoplasmic membrane, or even into the interior of the cell, was a prerequisite for action (Albert *et al.*, 1954). By this time we had become quite skilled in determining stability constants, and brought these into the studies, to make sure that substances of similar metal-binding power were being compared.

Apart from straight derivatives of oxine, we found two other classes of antibacterial which were acting in the same way as oxine, in so far as they gave these same three tests: (i) less active (in broth) as the concentration is raised, (ii) inactive if iron is removed, (iii) inactive if both iron and cobalt are present (Albert *et al.*, 1956). These substances were 2-mercaptopyridine-*N*-oxide (XII) and dimethyldithiocarbamic acid (XIII) and their derivatives.



(XII)

(XIII)



(XIV)

Other workers extended our findings to other kinds of micro-organisms, namely: Williamson (1959) to the action of oxine on protozoa, Nordbring-Hertz (1955) to the action of oxine on yeasts, and both Anderson and Swaby (1951) and Block (1956) to the action of oxines on other fungi. Dimethyldithiocarbamate salts, which had long been dusted on crops as potent fungicides, were then shown also to act in the manner that we had worked out for oxine on bacteria (Sijpesteijn and Janssen, 1959). One small difference exists in the action of all these substances on bacteria and fungi: in the former iron is the co-toxicant, in the latter it is copper.

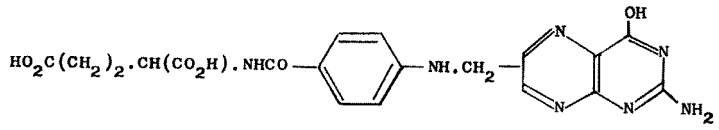
Our interest in chelation led us to discover and measure the metal-binding properties of the very successful antitubercular drug isoniazid (Albert, 1956), and of the tetracycline antibiotics (Albert and Rees, 1956).

Much of this work was done in London, where I went in 1947 as a Wellcome Research Fellow. In 1949, 1 was appointed to my present position by the Australian National University who asked me to stay in London until the John Curtin School of Medical Research was completed in Canberra. Meanwhile I was to hire laboratories, engage staff, buy apparatus and chemicals and commence research work. I was able to obtain suitable laboratories, mainly in the Wellcome Research Institution, but also others in University College, and Kings College. London provided a stimulating atmosphere in which to create a Research Department, but we were cramped for space, and expansion only became possible when we were able to move into the not quite finished John Curtin School in 1957.

Pteridines

Coincident with my joining the Australian National University, I took on a new research subject, the study of pteridine (XIV) and its derivatives. From their discovery by Gowland Hopkins in 1889, pteridines had been found only as constituents of insects, and their constitution was unknown before Purrmann's 1940 publications. The discovery in 1946 that the vitamin known as folic acid (XV) was a pteridine (Angier *et al.,* 1946), led to the discovery that a series of tetrahydrofolic acids were coenzymes in one of the most vital processes of the living cell, namely the synthesis of deoxyribose nucleic acid. These

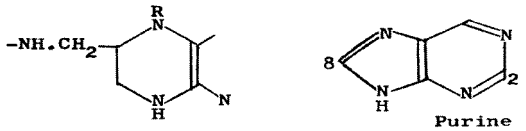
tetrahydrofolic acids, the pyrazine ring of which is shown in (XVI), have R = CH_3, or CH_2OH, or CHO, groups which are sometimes cross-linked to the nitrogen of the *p*-aminobenzoic acid portion. These coenzymes supply activated one-carbon fragments, from the R- group, for the biosynthesis of many cell constituents, notably the 2-, and the 8-carbons of all purines, and the 5-methyl-group of thymine.



Folic acid

(XV)

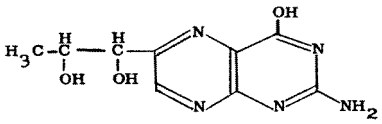
Fairly recently, another pteridine coenzyme has leapt into prominence. This is biopterin (XVIII) which enables molecular oxygen to participate in such important cellular activities as the oxidation of phenylalanine to tyrosine, of dihydroxyphenylalanine to the skin pigment (melanin), of the glyceryl ethers of the brain to glyceryl esters, and of steroids to 17-hydroxysteroids.



(XVI)

Purine

(XVII)



Biopterin

(XVIII)

The opportunity to commence a study of pteridine chemistry pleased me very much, because very little of it was then known. Our aim was to correlate structure with physical and chernical properties, and to proceed from simple to complex derivatives. It was an abrupt change to work in a series where few substances have a melting point; but paper chromatography proved a valuable substitute, and gave so much insight into reactions and their products that I have come to consider it essential even when dealing with substances of definite melting point.

I would now like to review some of the highlights of this pteridine work, which still goes on, and with which the name of my colleague D.J. Brown is closely coupled. First of all, I should mention that we devised some new syntheses, including one where a purine, set aside at room temperature with a two-carbon reagent, is converted to a pteridine by ring-expansion (Albert, 1957). This is now known to be the process by which pteridines (and hence riboflavine) are formed in Nature. But I do not dwell on syntheses in any part of this lecture because, interesting as they are, the more scientifically exciting thing is what one does with the products.

The high insolubility of the butterfly wing pteridines (which arise by degradation of the above coenzymes) had led early workers to think that these were substances of high molecular weight. These pteridines have three or more water attracting groups (such as -OH and $-NH_2$) per molecule, and their insolubility in water was considered puzzling. By preparing a whole series of pteridines, with an increasing number of such groups, we were able to show that

these so called water-attracting groups are the actual cause of the poor solubility in water (see Table 4).

Reference to Table 4 shows that pteridine itself is soluble in 7 parts of water (at 20°) and that each hydroxy-group added to this nucleus progressively decreases solubility (some smaller secondary effects are obviously imposed by positional isomerism). Our explanation was that these normally water-attracting groups were even more attracted to the nuclear nitrogen atoms of other molecules, thus producing a high crystal-lattice energy, and hence poor solubility. This was confirmed by showing that, when all bondable hydrogen atoms were blocked, either by *O*- or by *N*-methylation, the solubility in water actually increased although a hydrophobic group had been introduced, see Fig. 1 (Albert *et al.*, 1952).

TABLE 4
Solubility of pteridines in water at 20°

Pteridine	1 in	Pteridine	1 in
(Unsubstituted)	7		
2-hydroxy	600	2,4,6-trihydroxy	7,500
4- "	200	2,4,7- "	12,000
6- "	3,500	4,6,7- "	27,000
7- "	900		
4,6-dihydroxy	5,500	2,4,6,7-tetrahydroxy	58,000
4,7- "	4,000		
6,7- "	3,000		

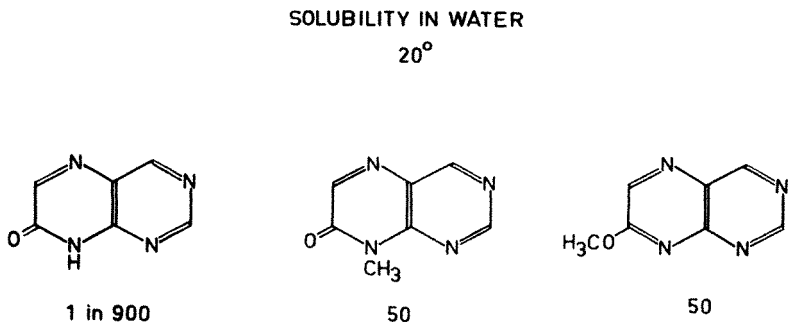


FIGURE 1
Solubility in water of 7-hydroxypteridine and its *N*- and *O*-methyl- derivatives

The most pleasant feature of these results is that we found the same insolubilizing effect of -OH and -NH_2 groups in all *N*-heteroaromatic series with six-membered rings, from pyridine to the most complex nuclei. The effect greatly decreases as the number of doubly-bound nitrogen atoms in the nucleus decreases (Albert, 1959).

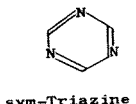
The naturally-occurring pteridines are noted for their resistance to hot, strong acid and alkali. However, inspection of the formula of the parent pteridine convinced me that it could have none of the chemical inertness of naphthalene or quinoline. The four doubly-bound nitrogen atoms, each equivalent to a nitro-group in inductive and mesomeric effects, seemed to be likely to attract the ten π-electrons and thereby cause such delocalization that aromatic stabilization would be severely reduced. The results, of treating a series of pteridines with acid and alkali, as in Table 5, gave substance to this idea. It can be seen that pteridine is

indeed very labile, and that even one electron-releasing group is insufficient to counteract this weakness. However two hydroxy-groups have the desired effect, and the presence of three such groups gives complete stability to acid (Albert *et al.*, 1952).

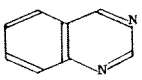
TABLE 5
Decomposition of pteridines by acid and alkali

Pteridine	Decomposition 1 hour at 110°	
	N - H_2SO_4	10N - NaOH
(Unsubstituted)	74%	57%
2-Hydroxy-	55	89
4-Hydroxy-	60	94
6-Hydroxy-	2	100
7-Hydroxy-	52	76
2,4-Dihydrox)-	6	4
6,7-Dihydrox)-	7	12
4,6,7-Trihydroxy-	0	4
2,4,6,7-Tetrahydroxy-	0	6

Here again, understanding of a phenomenon in the pteridine series provided a general explanation of the instability of various heterocyclic nuclei of graduated π-deficiency. Thus, whereas pyridine is stable to hot, concentrated acid and alkali, pyrimidine is degraded by boiling N-sodium hydroxide, and *sym*-triazine (XIX) is completely hydrolysed by cold water in one minute (formamide is the sole product). Yet, consistent with our pteridine studies, trihydroxytriazine (cyanuric acid) and triaminotriazine (melamine) are extraordinarily stable to hot acid and alkali.



sym-Triazine
(XIX)



Quinazoline
(XX)

Another interesting phenomenon in the pteridine series is photoreduction which occurs upon exposure to ultraviolet light for a few seconds. Thus 7-hydroxypteridine is converted to 5, 6-dihydro-7-hydroxypteridine, the hydrogen coming apparently from water (Albert, 1956).

Covalent Hydration

But the most interesting of all our discoveries has, so far, been the phenomenon of covalent hydration, interesting, too, because we extended the work to show that it occurs also in many other π-deficient heteroaromatic families. The abnormal titration curve of 6-hydroxypteridine, which forms a hysteresis loop (Fig. 2) was the first indication of this phenomenon. At first we suspected ring-opening and closing, but it was possible to exclude these in favour of the addition of water across a double-bond (Albert *et al.*, 1952; Albert, 1955). The location of the added water was determined by Brown and Mason (1956) who found that the hydroxyl-group went on to C-7, followed by the hydrogen on N-8. Their methods included comparison of the ultraviolet spectra and ionization constants with those of blocked derivatives, also infrared studies.

One of the simplest examples of this effect is furnished by quinazoline (XX). The ultraviolet spectrum of the neutral species of both quinazoline and 4-methylquinazoline are practically identical, but the spectrum of quinazoline is shifted to much shorter, and of

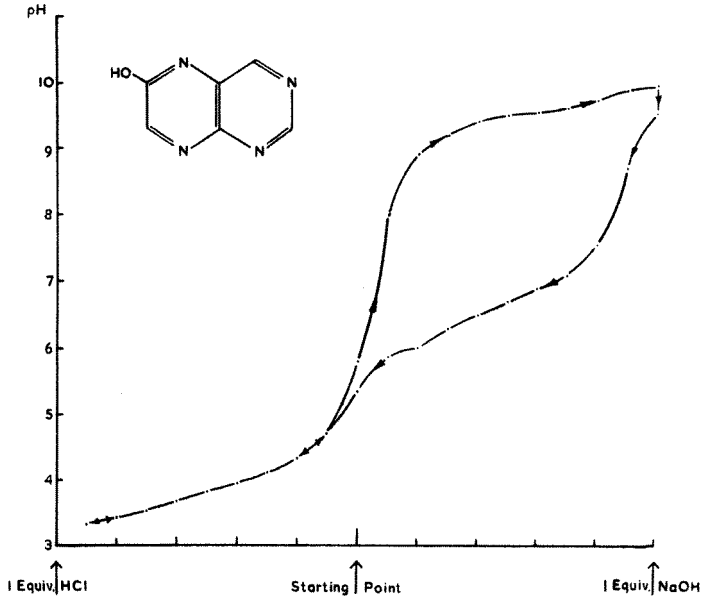


FIGURE 2
Titration curve of 6-hydroxypteridine (hsyteresis loop)

4-methylquinazoline to (the more normal) longer, wavelengths when each is converted to the cation (see Fig. 3). The correct explanation proved to be that each had an anhydrous neutral molecule, that quinazoline had a cation that was hydrated across the 3,4-position, and that the cation of 4-methylquinazoline was substantially anhydrous because of steric hindrance from the methyl-group (Albert *et al.*, 1961; Armarego, 1961).

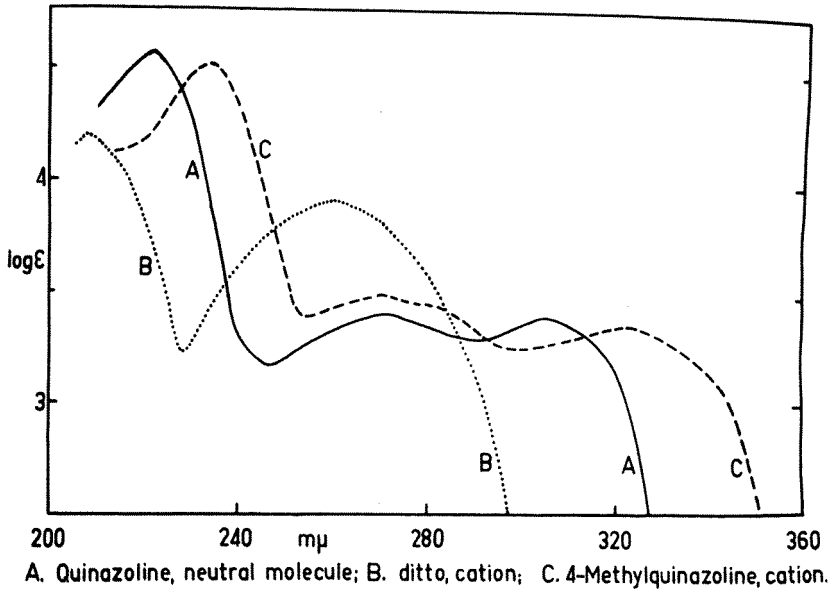


FIGURE 3
Ultraviolet spectra of quinazoline and 4-methylquinazoline

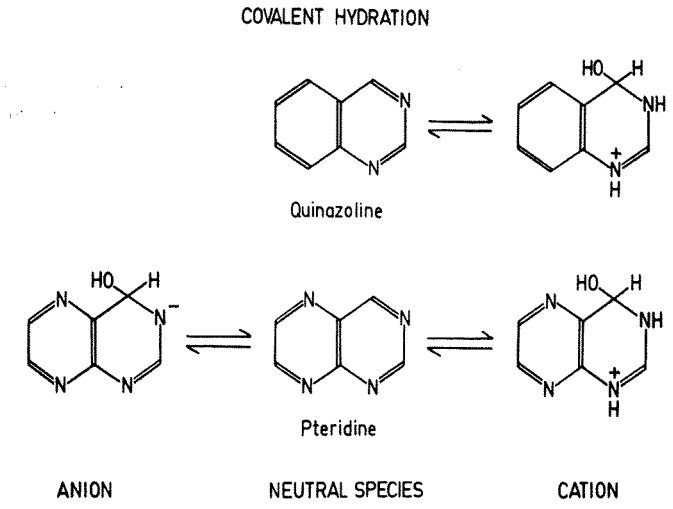


FIGURE 4
The most stable of the various ionic species of quinazoline and pteridine

Using the rapid reaction apparatus devised by Britton Chance, D.D. Perrin in my Department was able to obtain spectra of the two unstable species of quinazoline, *viz.* the hydrated neutral species and the anhydrous cation, and the true ionization constants, namely pK_a 7.77 for the two hydrated species and ~1.9 for the two anhydrous species. Thus the published pK_a of 3.51 represents only an equilibrium value for all four species, and is of relatively little interest. Similar studies with pteridine gave the corresponding values for these equilibria. Pteridine proved more complicated than quinazoline in two respects: the neutral species was itself hydrated (at equilibrium to the extent of about 20 %), and the hydrated form gives an *anion,* stabilized by resonance (see Fig. 4) (Perrin, 1962, 1963).

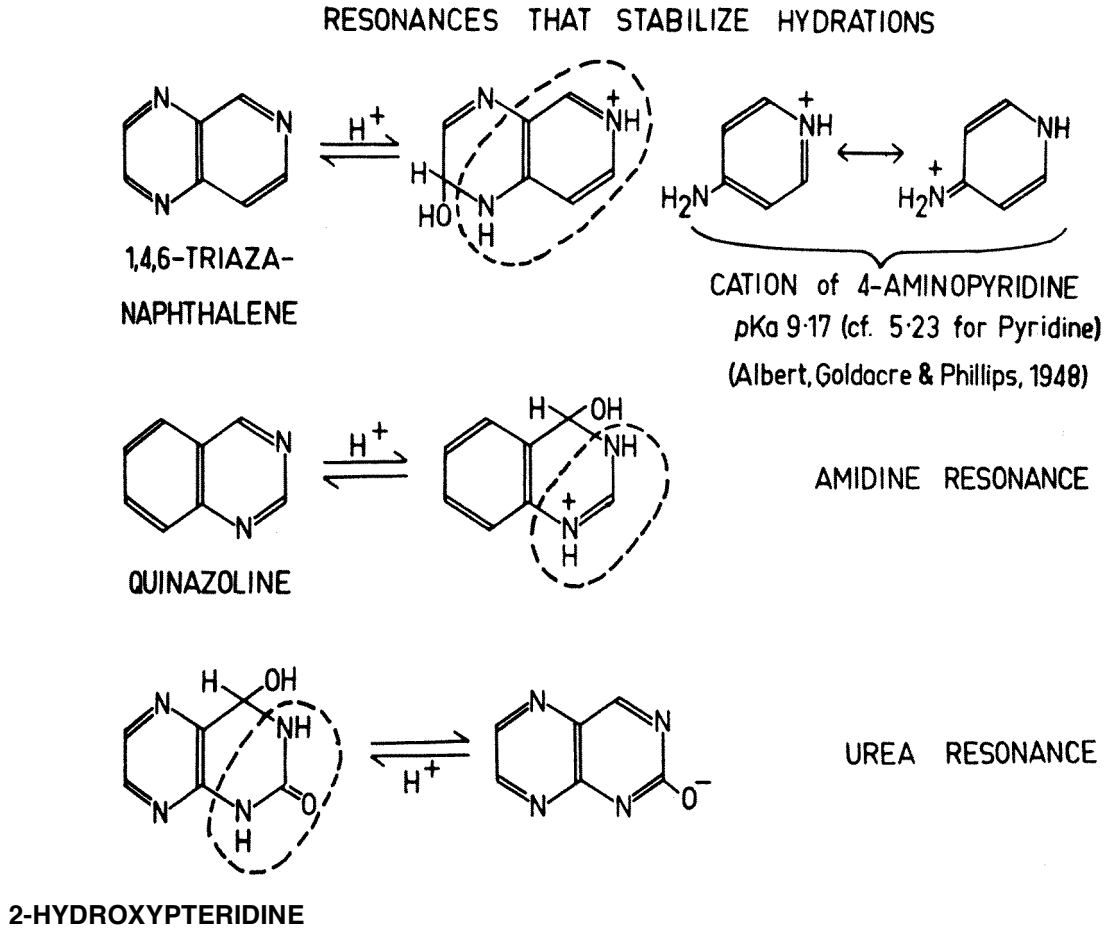


FIGURE 5
Resonances that stabilize covalent hydrations

Time will not permit an account of all the nuclei in which we have found covalent hydration, nor of the valuable kinetic work done by Dr. Perrin assisted by Mr. (now Dr.) Inoue. It must suffice to give our ideas on the genesis of the phenomenon. Covalent hydration tends to occur in all π-deficient N-heteroaromatic molecules in which the electrons of the π-layer are heavily delocalized on the nitrogen atoms. This leads to the creation of an independently polarized C=N bond, the carbon of which becomes attacked by the negatively charged end of a water molecule. The hydration, induced in this way, is quantitatively significant only if the hydrated molecule is stabilized by a new resonance (Albert and Armarego, 1963; Albert and Barlin, 1963). Some of these stabilizing resonances are shown in Fig. 5. Two reviews dealing with covalent hydration were published recently (Albert and Armarego, 1964; Perrin 1964).

Among the more interesting recent studies from my Department, an unusual note was struck by "Substance T", so called because paper chromatography produced twin spots (see A in Fig. 6). Each of these, when eluted from the paper and re-applied, was found to correspond to one of the two original spots (see B, and C), and to have opposite optical rotations. This resolution had been effected by the cellulose.

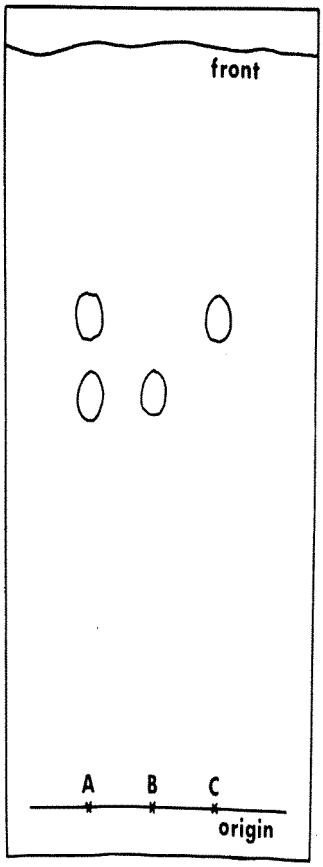


FIGURE 6
Behaviour of "Substance T" in paper chromatography (in water).
A. Original solution. B and C Eluates of the two spots from A

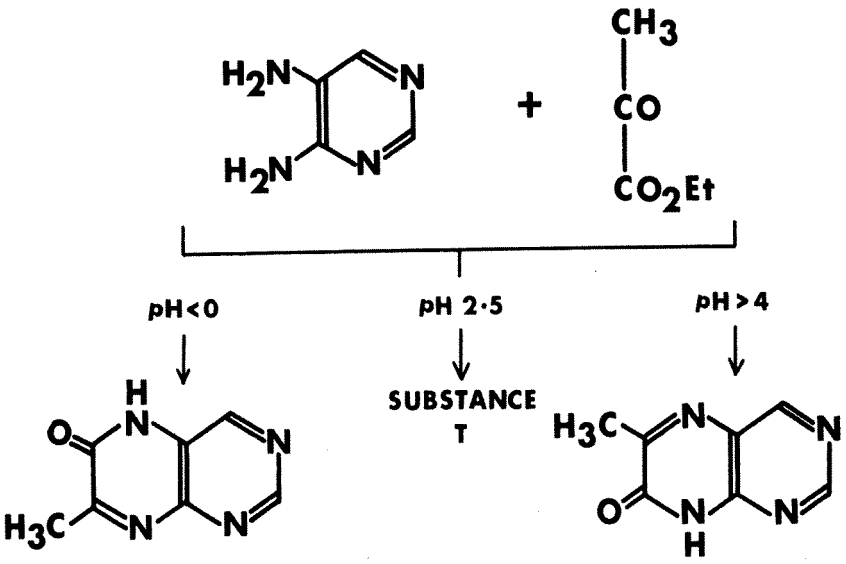
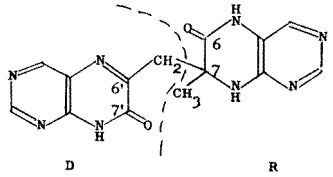


FIGURE 7
Chart showing effect of pH on the production of pteridines from 4,5-diaminopyrimidine and ethyl pyruvate

The generation of Substance T, from 4,5-diaminopyrimidine and ethyl pyruvate within a restricted pH range as shown in Fig. 7, led to its formulation as the bipteridyl-methane (XXI). This was formed from 7-hydroxy-6-methylpteridine (D) acting as a Michael donor, and 6-hydroxy-7-methylpteridine (R) acting as a Michael receptor. The structure was proved by

degradation. The underlying assumption, that Michael additions across a C=N bond can be acid catalysed, was verified by combining simple donors (such as acetylacetone) with simple receptors (such as quinazoline) (Albert and Serjeant, 1964).



Substance T

(XXI)

In conclusion, I shall say only that, although we have ranged widely within the limits set by the title of this lecture, there are more kinds of heterocyclic chemistry with biological overtones to explore than have yet been laid hands on. Who can say what surprises the near future may bring?

References

Albert, A., 1944. *Med. J. Aust.,* i, 245.

Albert, A., 1955. *J. Chem. Soc.,* 2690.

Albert, A., 1956. *Nature,* **177**, 525; **178**, 1672.

Albert ,A., 1957. *Biochem. J.,* **65**, 124.

Albert, A., 1959. "Heterocyclic Chemistry." London: Athlone Press.

Albert, A., 1963. "Ionization Constants ", in "Physical Methods in Heterocyclic Chemistry" ed. A. Katritzky. New York: Academic Press.

Albert, A., and Armarego, W.L.F., 1963. *J. Chem. Soc.,* 4237.

Albert, A., and Armarego, W.L.F., 1964. *Advances in Heterocyclic Chemistry* (ed. A.R. Katritzky), **4,** 1.

Albert, A., Armarego, W.L.F., and Spinner, E., 1961. *J. Chem. Soc.* pp. 2689, 5267.

Albert, A., and Barlin, G.B., 1959. *J. Chem. Soc.,* 2384 (cf. *ibid., idem.*, 1962, 3129).

Albert, A., and Barlin, G.B., 1963. J. *Chem. Soc.,* pp. 5156, 5737.

Albert , A., Brown, D.J., and Cheeseman, G.W.H., 1952. *J. Chem. Soc.,* pp. 1620, 4219.

Albert, A., Gibson M.I., and Rubbo, S.D., 1953. *Brit. J. exper. Path.,* **34**, 119.

Albert, A., and Goldacre, R.J., 1943. *J. Chem. Soc.,* 454.

Albert, A., Goldacre, R.J., and Phillips, J.N., 1948. *J. Chem. Soc.,* 2240.

Albert, A., Hampton A., Selbie, F.R.,*and* Simon, R.D., 1954. *Brit. J. exper. Path.,* **35**, 75.

Albert, A., and Phillips, J.N., 1956. *J. Chem. Soc.,* 1294.

Albert, A., and Rees C.W., 1956. *Nature,* **177**, 433.

Albert A., Rees, C.W., and Tomlinson, A.J.H., 1956. *Brit. J. exper. Path.,* **37**, 500.

Albert, A., Rubbo, S.D., and Burvill, M.I., 1949. *Brit. J. exper. Path.,* **30**, 159.

Albert, A., Rubbo, S.D., and Goldacre, R.J., 1941. *Nature,* **147**, 332.

Albert, A., Rubbo, S.D., Goldacre, R.J., and Balfour, B.G., 1947. *Brit. J. exper. Path.,* **28**, 69.

Albert, A., Rubbo, S.D., Goldacre, R.J., Davey. M.E., and Stone, J.D., 1945. *Brit. J. exper. Path.*, **26**, 160.

Albert, A., and Serjeant, E.P., 1962. " Ionization Constants of Acids and Bases.". London: Methuen.

Albert, A., and Serjeant, E.P., 1964. *J. Chem. Soc.*, 3357.

Anderson, B., and Swaby, R., 1951. *Austral. J. Sci. Research,* **B4**, 275.

Angier, R., Boothe, J., Hutchings, B., Mowat, J., Semb, J., Stokstad, E., Subbarow, Y., Waller, C., Cosulich, D., Fahrenbach, M., Hultquist, M., Kuh, E., Northey, E., Seeger, D., Sickels, J., and Smith, J., 1946. *Science,* **103**, 667.

Angyal, S.J., and Angyal, C.L., 1952. *J. Chem. Soc.,* 1461.
Armarego, W.L F., 1961. *J. Chem. Soc.,* 2697.
Block, S., 1956. *J. Agr. Food Chem.,* **4**, 1042.
Brown, D.J., and Mason, S.F., 1956. *J. Chem. Soc.,* 3443.
David, T.W.Edgeworth, 1931. *J. Chem. Soc.,* 1039.
Gore, P., and Phillips, J. N., 1949. *Nature,* **163**, 690.
Hopkins, F.G., 1895. *Phil. Trans. Roy. Soc.,* B, **186**, 661.
Lerman, L., 1963. *Proc. Nat. Acad. Sci., Wash.,* **49**, 94.
Nordbring-Hertz E., 1955. *Physiol. Plantarum,* **8**, 691.
Perrin, D.D., 1962. *J. Chem. Soc.,* 645.
Perrin, D.D., 1963. *J. Chem. Soc.,* 2648.
Perrin, D.D., 1964. *Advances in Heterocyclic Chemistry* (ed. A.R. Katritzky), **4**, 43.
Purrmann, R., 1940. *Liebigs Ann. Chem.,* **544**, 182 ; **546**, 98.
Sijpesteijn, A., and Janssen, M., 1959. *Antonie van Leeuwenhoek,* **25**, 422.
Stearn, A.E., and Stearn, E.W., 1924. *J. Bact.,* **9**, 491.
Vermast, P.G., 1921. *Biochem. Z.,* **125**, 106.
Williamson, J., 1959. *Brit. J. Pharmacol. Chemother.*, **14**, 443.