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THE AUSTRALIAN NATIONAL UNIVERSITY
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
DEPARTMENT OF MEDICAL CHEMISTRY

52/1964

ANNUAL REPORT - 1963

STAFF

Professor:

A.Albert, Ph.D., D.Sc., F.R.I.C., F.A.A.

Reader:

D.J.Brown, Ph.D., D.Sc.

Senior Fellows:

D.D.Perrin, M.Sc., Ph.D., F.R.I.C.; E.Spinner, M.Sc.Tech., Ph.D.

Fellow:

W.L.F.Armarego, Ph.D., F.R.I.C. (from October)

Microanalyst (Fellow):

Joyce E.Fildes, M.Sc., Ph.D.

Research Fellows:

G.B.Barlin, M.Sc., Ph.D.; J.Clark, Ph.D., F.R.I.C.;

R.F.Evans, D.Phil.; N.W.Jacobsen, M.Sc., Ph.D.

Honorary Research Fellow:

R.E.Willette, Ph.D. (U.S.A.National Institutes of Health Postdoctoral Fellow) (until December).

Head Technician:

J.S.Harper, A.R.A.C.I.

STUDENTS AND TEACHING ACTIVITIES

Mr.C.J.Hawkins, B.Sc., was awarded the degree of Doctor of Philosophy for his thesis 'Stability Constants and Oxidation-Reduction Potentials of Copper Complexes.' Mr.Y.Inoue, M.Sc., handed in a thesis entitled 'Reversible Addition of Water to the Carbon-Nitrogen Double-Bond', and has successfully completed requirements for the degree of Ph.D. The following are proceeding to the degree of Doctor of Philosophy: Mr.T.Teitei, M.Sc. (commenced 9th April 1962; subject 'Studies of Tautomerism in the Pyrimidine and Related Series'); Mrs. J.M.Lyall, B.Sc. (commenced 26th March 1962; subject 'A Study of the Amination of chloro-pyrimidines and -pteridines'); Mr.I.H.Pitman, B.Sc. (commenced 26th November 1962; subject 'Kinetic study of the Dimroth rearrangement'); Mr.R.V.Foster, M.Sc. (commenced 19th August 1963;

subject 'Studies in Pyrimidines'); Mr.J.G.Catterall, B.Sc. (commenced 11th October 1963; subject 'Investigations of bi-heterocyclyls'); Mr.I.G.Sayce, B.A., B.Sc. (commenced 4th November 1963; subject 'Complex-formation between metals and sulphur-containing ligands'); and Mr.J.Smith, A.R.C.S.T.(commenced 26th November 1963; subject 'Studies in Quinazolines and related compounds').

RESEARCH PROGRAMME

During the fifteen years of its existence, this Department has become recognized as a centre for fundamental studies of the chemistry of heterocyclic nuclei of biological interest. In the year under review, these studies have been broadened as well as extended. Work on the binding of metals by cell constituents has also been kept to the fore.

Although Michael-type condensations have hitherto always been base-catalysed, several heterocyclic examples have now been found to be acid-catalysed with a maximum near to pH 2.5. For this work, quinazoline was used as a model receptor, and acetylacetone or 4-methylquinazoline as a model donor. A reaction mechanism is proposed (Albert).

In aqueous solution, the cations of 3- and 8-nitro-1,6-naphthyridine have been shown to add water covalently across a double-bond. These, and earlier results have been used to show that both electron deficiency and resonance stabilization are necessary for covalent hydration of nitrogenous heteroaromatic substances (Albert, Armarego).

The azaindoles have been shown to exert pharmacological effects (on smooth muscle, and the central nervous system) which either mimic or oppose the actions of indole, depending on the position of the doubly-bonded nitrogen atom in the molecule. Thus, 2-, 4-, 5-, and 6-azaindoles resemble indole in producing clonic -tonic convulsions. The 5- and 6-isomers show this effect to a heightened degree, and have 0.2 of the potency of strychnine (Adler, Albert).

The apparent migration of an alkyl-group from a ring nitrogen to an exocyclic nitrogen atom (known as the Dimroth Rearrangement) has

been further studied in the pyrimidine series, and the project has been extended into the pyridine series. The methylation of 3- and 5-nitro-2-aminopyridine to two nitro-2-methylaminopyridines, described by Tschitschibabin *et al*. many years ago, has now been shown to proceed *via* 1,2-dihydro-2-imino-1-methyl-3(5)-nitropyridines which undergo facile rearrangement to the described products. For cases in which no aromatic stability is gained on rearrangement, the equilibrium is strongly displaced in favour of the form bearing the greater group on the exocyclic nitrogen atom. Thus 1-benzyl-1,2-dihydro-2-isopropyl-iminopyrimidine yields the 2-benzylimino- isomer, but not *vice versa* (Brown, Harper).

The methylation of pteridines aminated in the pyrimidine ring, as described in earlier reports, is now being compared with methylation of pteridines aminated in the pyrazine ring. The products are difficult to obtain, and their structures can be proven only by lengthy unambiguous syntheses of the pyrazines formed on degradation. 4,6-, And 4,7-diaminopteridines each gives a single monomethyl derivative, but only the second has so far been identified (it is the 1-methyl- derivative). 7-Aminopteridine gives two isomeric methyl derivatives, but 6-aminopteridine can not yet be methylated (Brown, Jacobsen).

An attempt is being made to find a simple experimental method of predicting the best conditions of time and temperature for the preparation of aminopyrimidines from chloropyrimidines (the literature is in a state of confusion and contradiction). With a moderate excess of *n*-alkylamine, the reactions approximate to the first order, and temperature coefficients have been found to be constant. Optimum conditions can now be accurately predicted for any such *n*-alkylamination by a single brief experiment culminating in a simple titration (Brown, Lyall).

To find the predominant tautomers present in 4-hydroxy-6-mercaptopyrimidine, the ultraviolet spectra have been compared with those of methylated derivatives corresponding to all possible tautomeric forms. It was found to exist mainly as tetrahydro-4-oxo-6-thiopyrimidine, a form in which C-5 is involved in hydrogen-gain

as in barbituric acid. A similar study of 4,6-dihydroxypyrimidine has shown that it also exists in the tetrahydro-dioxo form, and here the existence of the 5-methylene grouping has been utilized chemically by causing it to react with benzaldehyde (Brown, Teitei).

To verify a hypothesis that chloramphenicol exerts its anti-bacterial action by competition with uracil derivatives, steps have been taken to prepare relevant pyrimidine analogues (Brown, Foster).

Stability constants of copper complexes with various N,N'-disubstituted ethylenediamines have been determined. They support the hypothesis that antitubercular activity in these amines is related to their ability to form partially hydrolyzed metal complexes (Perrin, Hawkins).

Quantitative measurements have been made of the equilibrium involved in the hydrolysis of nickel ion. A theoretical expression has been derived which enables the effect of temperature on pK values of organic bases to be predicted. An index to the ionization constants of inorganic acids and bases has been prepared (Perrin).

Equilibrium and kinetic studies of reversible water-addition to nitrogen heterocycles have been extended to include derivatives of 2- and 6-hydroxypteridine, methylpteridines and triazanaphthalenes. Time-dependent hydration equilibria in 2,6-dihydroxypteridine have also been elucidated (Perrin, Inoue).

An investigation has been commenced into the detailed mechanism of the Dimroth rearrangement. It is thought that reaction proceeds through a reversibly hydrated intermediate (Perrin, Pitman).

Although the metal-binding properties of nitrogen- and oxygen-containing substances have been extensively studied in the past, little is known of the quantitative aspects of complex formation where the bonding is through sulphur atoms. This topic is being examined because of its relevance to many biological problems (Perrin, Sayce).

Spectral studies of substituted 2-hydroxypyridines and their ions have been continued. Infrared and Raman spectra have also been determined for some five-membered N-heteroaromatic ring systems and their ions (Spinner).

The infrared spectra of mono-, di- and tri- azanaphthalenes have been determined (Armarego, Barlin, Spinner).

Substituted acetate ions, like the free acids, and like carbonyl compounds in general, are capable of rotational isomerism. In aqueous solution, the equilibrium between the two conformations has been found to vary with the substituent, and the position is roughly the same for each acetic acid and its anion (Spinner).

Vibration spectral studies are being extended to some deuterated compounds and to the measurement of the depolarization ratios of Raman bands. The latter determinations have been carried out for the deuteroformate ion (Spinner).

The phenomenon of covalent hydration (see above) has been established in 1,4,5,8-tetraazanaphthalene and its C-methyl- derivatives, and found absent from phthalazine, 1-methylphthalazine, and an aza- derivative of the latter, (8-methyl-1,6,7-triazanaphthalene The "2-acetylnicotinic acid 1-oxide p-nitrophenylhydrazone anhydride" of Bain and Saxton is actually 6-p-nitrophenyl-8-methyl-5-oxo-1,6,7-triazanaphthalene 1-oxide (Armarego).

A study of the infrared spectra of quinazolines has revealed interesting correlations (Armarego, Katritzky, Ridgewell).

Preliminary investigation of indolizines has revealed that the site and degree of protonation is greatly altered by the presence of a methyl- group in various positions (Armarego).

A new method for the ultramicro-determination of sulphur in organic and biological materials is being devised (Fildes, Kirsten).

2-Amino- and 2-acylamino- pyrimidines were found to give the 1,4,5,6-tetrahydro- derivatives on catalytic hydrogenation in acidic media. Under similar conditions, 4- and 5- aminopyrimidines underwent hydrogenolysis, accompanied by formation of ammonia. However, 5-acetamidopyrimidine was satisfactorily reduced to the tetrahydro- derivative. Interesting basic and spectral properties were found in these tetrahydropyrimidines which are now, for the first time, becoming known (Evans).

A study has been made of the connexion between structure and resonance possibilities (on the one hand) and the magnitude of ionization constants (on the other) in diaminopyridines (Barlin).

An investigation of the kinetics of solvolysis of the azetidinone ring has been begun in order to throw more light on the mode of action of penicillin (Barlin).

Improved methods have been found for preparing 6- and 7-chloropteridine, and 6,7-dichloropteridine is described for the first time. In aqueous solution, these substances are (reversibly) covalently hydrated at the 3,4-double bond. Rate constants (first order) for the hydration and dehydration reactions were determined. In acid solution, these chloropteridines were degraded to pyrazines such as 2,3-dichloro-5-formamidopyrazine-6-aldehyde (Clark).

In attempts to find new syntheses of azaindoles, the hydrazone formed from ethyl pyruvate and 3- (and 4-)hydrazinopyridine 1-oxide were heated with polyphosphoric acid; esters of the hitherto unknown 3-nitro-2-(and 4-)pyridylcyanoacetic acid were reduced; and nitropicolines were condensed with oxalyl chloride (Willette).

OTHER ACTIVITIES

Professor Albert gave the opening address to a Symposium on "Chemical Structure and Biological Activity" held at the Cawthron Institute in Nelson, New Zealand during May, under the auspices of the Chemical Society (London).

In August Dr.D.J.Brown gave, as the Chairman's Address to the Canberra Section of the Royal Australian Chemical Institute, a lecture entitled "The Dimroth Rearrangement".

Dr.E.Spinner was programme secretary for the Fourth Australian Spectroscopy Conference, held in Canberra in August. In October, Dr. Spinner left Canberra to spend twelve months study leave in Canada, the United States, and Great Britain.

Dr.D.D.Perrin read a paper "The oxidation-reduction potentials of copper-complex ions" at the First Australian Electrochemistry Conference held in Sydney in February. He has been appointed an Associate Member of the International Union of Pure and Applied

Chemistry's Commission on Electrochemical Data (Division of Analytical Chemistry). For this Commission, he has prepared a critical compilation of the ionization constants of 3,800 organic bases.

Dr.J.E.Fildes spent five months of study leave in the Mikroanalyslaboratoriet of the Medical Chemistry Institute of the University of Uppsala, Sweden, investigating ultramicro-methods of organic elemental analysis. In London, during May and July, she took part in two Discussion Meetings on current methods of organic microanalysis, organized by the microchemistry group of the Society of Analytical Chemistry. In late November, she attended the International Symposium on Automated Analytical Chemistry, in Frankfurt. Useful visits were made to the leading microchemical research laboratories of Russia, Czechoslovakia, Switzerland, Western Germany, Great Britain, and the United States. Dr.Fildes lectured on microchemical methods in Sweden, Czechoslovakia, and Pakistan.

Professor Warren Kumler, Chairman of the Department of Pharmaceutical Chemistry, University of California Medical Center, San Francisco, spent from 1st to 21st June in the Department and gave a School Lecture on "The Conformation of Imides and Related Compounds". Professor A.Haddow, F.R.S., and Dr.K.Schofield (University of Exeter) made shorter visits.

Dr.C.Hawkins was awarded an I.C.I.Travelling Fellowship to work on chelation phenomena for two years at the Oersted Institute of the University of Copenhagen.

Specimens of substances discovered in this Department have been sent, upon request, to Professor A.Haddow (the Chester Beatty Institute of Cancer Research, London); Dr. Chester Stock (the Sloan-Kettering Institute of Cancer Research, New York); Dr. W. Jacobson (Leukaemia Division, Children's Hospital, Boston); Professor E.Boyland (Chester Beatty Institute); Dr.R.Ballard (U.K.Atomic Energy Authority, Harwell); Professor J.H.Green (Dept. of Nuclear and Radiation Chemistry, University of New South Wales); Dr.Lerek Hanssen (University of Oslo); Dr.K.Heirwegh, (Rega Institute, Belgium); and Dr.G.Gollin (Department of Zoology, University of Texas).

Two large-scale preparations and twelve pressure hydrogenations and similar tasks were carried out for colleagues in other departments. In the last five years, 750 gallons of alcohol have been purified for this and other departments at a saving of £4,575, a figure which greatly exceeds the capital cost of the apparatus.

During 1963, 189 ionization constants of 137 organic substances were determined by the pK_a service, supervised by Dr.D.D. Perrin. Spectroscopic service work supervised by Dr.E.Spinner, included 247 ultraviolet and 62 infrared spectra, 28 of the latter being for members of other departments.

A total of 1,793 requests for elemental microanalyses, special micro-manipulations and weighings were carried out on the 817 specimens submitted to the microanalytical section by 31 research workers. Slightly over 25% of this work was done for members of other departments in the School. This output represents a 7.4% increase over that for 1962.

PUBLICATIONS

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ALBERT, A.-

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"Correlations between Microbiological Morphology and the Chemistry of Biocides." In Advances in Applied Microbiology, 5, 1. Academic Press, New York and London (ed. Umbreit, W.W.).

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"Naphthyridines. Part II. Covalent Hydration, Electron-deficiency and Resonance Stabilization in 1,6-naphthyridines." J. chem. Soc., 4237.

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"Triazanaphthalenes. Part II. Covalent Hydration in 1,4,6-triazanaphthalenes." J. chem. Soc., 5156.

"Triazanaphthalenes. Part IV. Covalent Hydration in 1,4,5-triazanaphthalenes." J. chem. Soc., 5737.

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"Pteridine Studies. Part XXIV. Competitive Covalent Hydration of 2,6-Dihydroxypteridine. Kinetics and Equilibria." J. chem. Soc., 5151.

ALBERT, A., SERJEANT, E.P.**-

"Resolution of a Racemic Pteridine during Paper Chromatography." Nature, 199, 1098.

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ARMAREGO, W.L.F.-

"Quinazolines." In Advances in Heterocyclic Chemistry, 1, 253. Academic Press, New York and London (ed. Katritzky, A.R.).

"Covalent Hydration in 1,4,5,8-Tetraazanaphthalenes." J. chem. Soc., 824.

"Triazanaphthalenes. Part V. A Search for Covalent Hydration in 8-methyl-1,6,7-triazanaphthalene." J. chem. Soc., 6073.

BROWN, D.J., HARPER, J.S.-

"The Dimroth Rearrangement. Part I. Some Alkylated 2-Iminopyrimidines." J. chem. Soc., 1276.

BROWN, D.J., TEITEI, T.-

"Pyrimidine Reactions. Part V. Nuclear Methylation of Some 6-Substituted 4-Aminopyrimidines." J. chem. Soc., 3535.

"Simple Pyrimidines. Part V. Tautomerism in 4-Hydroxy-6-Mercaptopyrimidine." J. chem. Soc., 4333.

CORNFORTH, R.,[ØØ] CORNFORTH, J.,[ØØ] POPJAK, G.*

"Preparation of R- and S-Mevalonolactones." Tetrahedron, 18, 1351, 1962 [omitted from 1962 Annual Report].

HAWKINS, C.J., PERRIN, D.D.-

"Polynuclear Complex Formation. I. Copper(II) and 2,7-Diaminosuberic Acid." J. inorg. Chem., 2, 839.

"Polynuclear Complex Formation. II. Copper(II) and Ligands Related to Cystine." J. inorg. Chem., 2, 843.

"Oxidation-Reduction Potentials of Metal Complexes in Water. Part II. Copper Complexes with 2,9-Dimethyl- and 2-Chloro-1,10-phenanthroline." J. chem. Soc., 2996.

INOUE, Y., PERRIN, D.D.-

"Pteridine Studies. Part XXI. Kinetics of the Reversible Addition of Water to, and Ring-Opening of, Pteridine and its Methyl Derivatives." J. chem. Soc.,2648.

"Pteridine Studies. Part XXII. Kinetics of the Reversible Hydration of Substituted 2-Hydroxypteridines." J. chem. Soc., 3936.

"Pteridine Studies. Part XXIII. Kinetics of the Reversible Hydration of 6-Hydroxypteridines and Some Derivatives." J. chem. Soc., 4803.

"Triazanaphthalenes. Part III. Kinetics of Reversible Water Addition to 1,3,8-Triazanaphthalene, 2-Hydroxy-1,3,8-triazanaphthalene and 3-Hydroxy-1,4,6-triazanaphthalene." J. chem. Soc., 5166.

MAGUIRE, D.A.-

"A Microanalytical Sampling Technique for Hygroscopic Liquids." Chem. and Ind., 1655.

PERRIN, D.D.-

"The Dimroth Rearrangement. Part II. Kinetic Studies." J. chem. Soc., 1284.

"Buffers of Low Ionic Strength for Spectrophotometric pK Determinations." Aust. J. Chem., 16, 572.

SPINNER, E.-

"The Effect of N-Methylation (Quaternization) on the Electronic Spectra of Some N-Heteroaromatic Cations." Aust. J. Chem., 16, 174.

"The Electronic Spectra of Some Monosubstituted Pyridines and Pyridinium Ions." J. chem. Soc., 3855.

"The Vibration Spectra of Some Monosubstituted Pyridines and Pyridinium Ions." J. chem. Soc., 3860.

"Vibration-spectral Band Assignments for the Pyridinium Ion: Pyridine Deuteriochloride- and 1-Methylpyridinium Chloride." J. chem. Soc., 3870.

* Not a member of this University

ØØ Visiting Research Worker

** Based on work done while a member of the Department