PEPTIDE CHEMISTRY

CHEMICAL SOCIETY SYMPOSIUM

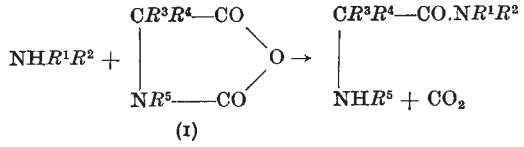
ON the occasion of the recent anniversary meetings in London of the Chemical Society, a symposium on peptide chemistry, arranged by Prof. D. H. Hey, was held on March 31 at the Royal Institution. The symposium consisted of five papers, two on physico-chemical aspects of the problem (Prof. H. D. Springall in the chair), and three on organic chemical aspects (Prof. Hey in the chair). A full account of the symposium will be issued as a special publication of the Chemical Society. The proceedings were opened by Prof. W. Wardlaw, president of the Chemical Society.

The first paper was by Prof. K. Linderstrøm-Lang (Carlsberg Laboratories, Copenhagen), who described some of his recent work on deuterium exchange between peptides and water. From earlier work it appeared likely that the rate of deuterium exchange in peptide systems would vary with the accessibility of the replaceable hydrogen atoms and the extent to which they are involved in hydrogen-bonding. In the present work, the quantitative study of rates of deuterium exchange has been applied to peptide systems of varying complexity and the results correlated with the structure of the peptides.

The experimental techniques were described. These depend on cryosublimation at −60° C., and density determinations, in the gradient tube. The results are as follows: Single-chain peptides, lacking intra-chain disulphide bridges, undergo immediate and complete exchange at 0° (alanylglycine, leucyltriglycine, oxidized insulin *A*-chain, oxidized ribonuclease). In the case of ribonuclease, this 128-residue single-chain peptide has four intra-chain disulphide bridges and, at *p*H 4·7, contains 245 exchangeable hydrogen atoms, 123 in side-chains and 122 in the backbone. About two hundred atoms (all those in the side-chains and about seventy-five in the backbone) undergo very rapid exchange. The rest require five hours at 40° for complete exchange, presumably due to the stabilizing of hydrogen-bonded structures by the disulphide bridges. In the case of insulin, the monomer has the two-chain (21 and 30 residues) structure with two inter-chain and one intra-chain disulphide bridges. There are 91 exchangeable hydrogen atoms, 43 in the side-chains and 48 in the backbone. 61 atoms (all those in the side-chains and 18 in the backbone) undergo very rapid exchange, 7 somewhat slower exchange, and 23 very slow exchange. It is suggested that the 23 very sluggish atoms are in the backbone sections between the inter-chain bridges.

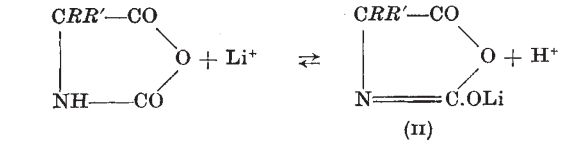
In the discussion of this paper, Dr. K. Schlögl (Vienna) asked if the exchange method could be used for studying a postulated enolization of peptide bonds in alkaline solution. Prof. Linderstrøm-Lang said that the exchange reaction, in some cases, is faster in alkaline solution, but he did not feel this to be due to enolization. Dr. D. Crowfoot Hodgkin (Oxford) pointed out that the 'loop' in insulin is stereochemically favourable for β-pleated sheet and α-helix peptide configurations and suggested the possibility of an exchange study of this. Dr. J. I. Harris (Copenhagen) directed attention to the fact that species variation of amino-acid composition of insulins is confined to the 'loop'. Dr. F. Sanger (Cambridge) confirmed this and asked if the difference between pig and sheep insulins (alanine for threonine and glycine for serine) could be detected by exchange methods; Prof. Linderstrøm-Lang thought this might be possible.

The second paper, by Dr. C. H. Bamford (Courtaulds, Maidenhead), dealt with recent work with Dr. D. G. H. Ballard on the mechanism of the N-carboxy-α-amino-acid anhydride synthesis of polypeptides. In the normal polymerization, the propagation reaction is of the type:



(I)

the product then reacting with more carboxy-anhydride. Recently, however, reactions of other types have been observed: notably those of (I; R^5 = H) with sodium and lithium ions in polar solvents to give cyclic polymers (VI) together with some of the 3-hydantoinylacetic acid (VII). It is suggested that these reactions proceed as follows:



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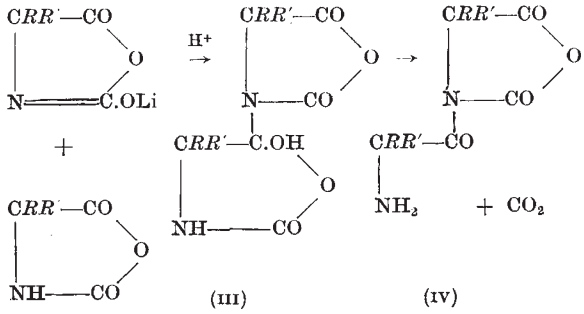

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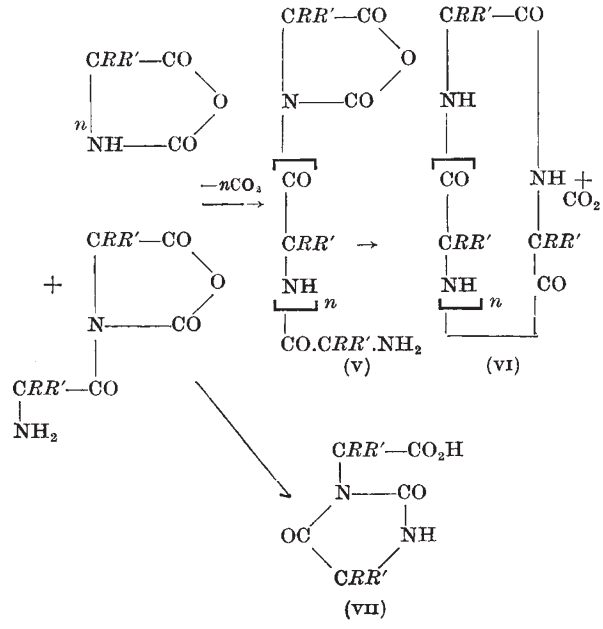


(V) (VI) (VII)

The grounds for these postulates are: (1) the nature of the products; (2) the essential nature of the NH group in the carboxy-anhydride; (3) the strong inhibiting effect of acids; and (4) the agreement between the kinetic data calculated from these equations and those actually observed.

The work described is a kinetic study (by rate of evolution of carbon dioxide) of the reactions of the anhydrides of N-carboxyl-DL-phenylalanine and γ-benzyl-N-carboxy-L-glutamate in nitrobenzene/N-methylformamide solution in the presence of sodium β-phenylpropionate. The kinetic studies and an analysis of the molecular weights of the reaction products support the view that "the essential feature of the salt-initiated polymerizations of the carboxy-anhydrides is the formation of a complex of type (II), which behaves as an activated form of the carboxy-anhydride. It initiates polymerization by reacting with the carboxy-anhydride to yield bifunctional intermediates, which couple and also enter into a growth reaction involving the complex. The initial rates of reaction are consequently very high, and the degrees of polymerization in general much greater than would be expected from the ratio [carboxy-anhydride] : [initiator]. In these respects the reactions differ fundamentally from the simple polymerizations catalysed by primary or secondary bases".

In the discussion on this paper, Dr. K. Schlögl criticized the suggested mechanism on several grounds, and Dr. Bamford gave a detailed reply to this criticism. Dr. J. A. Stock (London) asked if initiation by the sodium salts of amino-acids would yield cyclic polypeptides; Dr. Bamford felt that yields would be lower than with lithium chloride initiation.

In the third paper, Prof. V. du Vigneaud (Cornell University Medical College, New York) described his studies on the synthesis of cystine peptides, dealing especially with the reactions in liquid ammonia. These studies, extending over the past twenty-five years, have recently culminated in the brilliant synthesis of the peptide pituitary hormone, oxytocin. The following reactions, critically important for peptide work, have been achieved in liquid ammonia solution in the presence of sodium:

$$\text{Cy—S—S—Cy} \rightarrow 2\text{Cy—SH (as Cy—SNa)} \quad (1)$$

$$\text{Cy—SH} \xrightarrow{\varphi\text{CH}_2\text{Cl}} \text{Cy—S.CH}_2.\varphi + \text{NaCl} \quad (2)$$

$$\text{Cy—S.CH}_2\varphi \rightarrow \text{Cy—SH} + \text{CH}_3.\varphi \quad (3)$$

$$R\text{—O.CH}_2\varphi \rightarrow R\text{—OH} + \text{CH}_3.\varphi \quad (4)$$

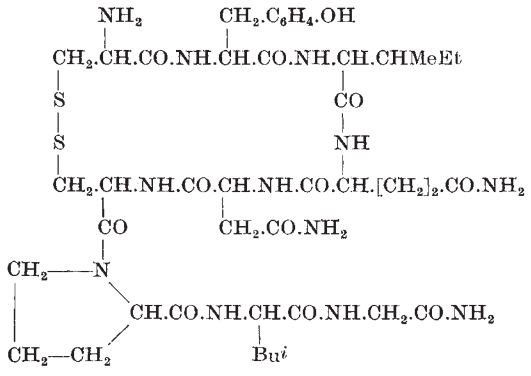
$$\text{Tos.NH—}R \rightarrow p\text{-CH}_3.\text{C}_6\text{H}_4.\text{SH} + \text{NH}_2\text{—}R \quad (5)$$

$$\varphi.\text{CH}_2.\text{O.CO.NH—}R \rightarrow \varphi.\text{CH}_3 + \text{CO}_2 + \text{NH}_2\text{—}R \quad (6)$$

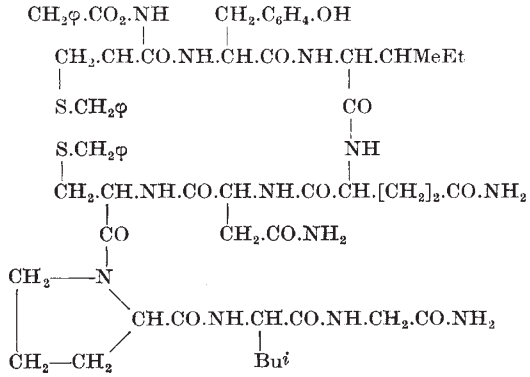
Moreover, favourable conditions have been devised for the reversal of reaction (1) by aeration in aqueous solution.

All these reactions, except (4), were used in the oxytocin synthesis. The first major application of these techniques was in the 1936 synthesis of glutathione, which had been synthesized in 1935 by Harington and Mead without resource to the liquid ammonia reactions.

The isolation of highly purified oxytocin and subsequent degradative studies, 1949–53, indicated the cystine bridge-cyclized nonapeptide triamide structure (I).



(I)

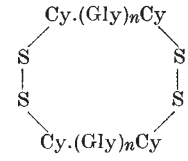


(II)

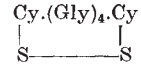
The compound (II) was successfully synthesized, the N- and S-protecting groups removed (Na/NH_3) and the disulphide bridge formed by air oxidation, yielding structure (I). The synthetic material was rigorously compared with natural oxytocin and found to be identical with it, in chemical, physical and physiological properties.

Sir Charles Harington opened the discussion on this paper with a tribute to Prof. du Vigneaud, and asked if a synthetic approach to insulin might be envisaged. Prof. du Vigneaud, remarking on the occurrence of the 20-membered loop in both insulin and oxytocin, said: "One has a right to dare to think that insulin could be synthesized—but it is of another order of magnitude of difficulty".

Prof. H. N. Rydon (Manchester) spoke briefly of his work on the air oxidation of the five peptides $HS.Cy.(Gly)_n.Cy.SH$ ($n = 0, 1, 2, 3, 4$). With $n = 0$ and 1, the main products are antiparallel cyclic dimers



and with $n = 4$, the cyclic monomer

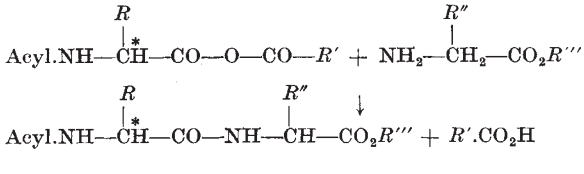


containing the 20-membered ring.

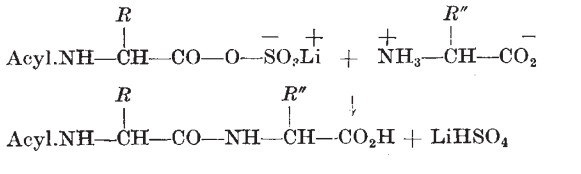
Dr. J. I. Harris (Carlsberg Laboratory, Copenhagen) read the fourth paper, describing his studies on C-terminal residue identification with carboxypeptidase. He outlined the experimental procedures and discussed the results obtained from insulin, somatotropin, α-corticotropin and tobacco mosaic virus. In general, cleavage is fastest with C-terminal aromatic amino-acids, very slow with an acidic or basic C-terminal residue and does not occur with C-terminal proline. Thus with the *B*-chain of insulin which has the terminal sequence . . . Pro.Lys.Ala., the alanine is released by carboxypeptidase, but reaction stops at that stage. With α-corticotropin, however, with the terminal sequence . . . Pro.Leu. Glu.Phe., the last three residues are split off in sequence. The summarized individual results per mole are: insulin gives one alanine and one asparagine, somatotropin, two phenylalanine; α-corticotropin, one phenylalanine, then one glutamic acid, then one leucine; tobacco mosaic virus, 2,900 + 100 threonine. The C-terminal sequences do not seem to be of critical importance for biological function. Dr. Harris, acknowledging the power of the enzymic methods, notably their very gentle experimental conditions, pointed out the need for a chemical method of study of C-terminal residues.

In the discussion, Prof. C. Fromageot (Paris) pointed out that ovomucoid is inert to carboxypeptidase but yields phenylalanine with lithium aluminium hydride. Dr. Harris felt that this must arise from some non-terminal fission. Dr. K. Schlögl advocated the Akabori hydrazine method for chemical study of C-terminal residues, to which Dr. Harris indicated the objections to a chemical method involving the complete degradation of the peptide chain.

Dr. G. W. Kenner (Cambridge) read the final paper, on mixed anhydride syntheses of peptides. He traced the development of the technique based on the reaction



from the work of Wieland, Vaughan and Boissonas ($R' = \varphi$ originally, then = Bu^i, then = OEt). There is grave danger of racemization at C* and there are difficulties in the removal of R'' (though these have been largely overcome using $R''' = CH_2\varphi$ and removing it by hydrogenolysis). To avoid these difficulties, Dr. Kenner has developed the use of mixed anhydrides of acylamino-acids and sulphuric acid, which will react, with very slight racemization, with free amino-acids and peptides in aqueous dimethylformamide solution



In the discussion, Dr. G. T. Young (Oxford), commenting on the importance of avoiding racemization, reported that, in the condensation of acetyl-L-leucine with glycine, ethyl ester configuration is retained using the Curtius azide method but lost using the Goldschmidt phosphorazo method. Prof. J. Baddiley (Newcastle upon Tyne) asked if the sulphuric anhydride method could be used to link an amino-acid to an amide NH_2 group, and to this Dr. Kenner replied that he felt it to be unlikely.

H. D. SPRINGALL

CAMBRIDGE OBSERVATORIES

REPORT FOR 1954

THE report of the Observatories Syndicate of the University of Cambridge for the year ending September 30, 1954*, is divided under the following headings: reconstruction and re-equipment; solar research; stellar photometry; other investigations; optics; buildings and grounds; lectures; and papers accepted for publication. The installation of the new 17–24-in. Schmidt telescope has been completed and tested extensively, and the original driving error—approximately a sine wave of total 6″—has been removed. The old Common 36-in. reflector has been dismantled and returned to the Science Museum, South Kensington, and the foundations for the new 36-in. reflector are ready for the telescope, which was expected to be delivered in December.

A great portion of the report dealing with solar research is devoted to the observations of the total solar eclipse of June 30, 1954, details of which have been already given in various publications. Plans were made, and preparations commenced, for observation of the total eclipse of June 20, 1955, when Dr. D. E. Blackwell will continue the work which he carried out at the 1954 eclipse—photographing the corona and zodiacal light, etc., to make accurate photometric measurements; a Sunderland flying-boat will be used, flying from the Fiji Islands over the Pacific Ocean (*Nature*, June 11, p. 1018). Prof. R. O. Redman, the director of the Observatories, and Dr. Z. Suemoto have completed the study of chromospheric line-widths, based on spectrograms obtained at the 1952 eclipse. Self-absorption and Stark effects, which are of greater importance than had been formerly supposed, having been taken into account, they have shown that the line-widths in the hydrogen Balmer series are consistent with a kinetic temperature not exceeding 10,000° K. and also that a model at 6,000° K. would represent the measures almost as well as at 10,000° K. In addition to other solar work, reference may be made to the critical examination in the 30-ft. spectrograph of two large, good-quality gratings, both with 600 lines per mm., one from Bausch and Lomb, of ruled area 20 cm. × 15 cm., and the other from the Mount Wilson Observatory, with ruled area 20 cm. × 13·7 cm.; the latter is on loan to the Observatories and "gives quite remarkable performance, theoretical resolving power being attained in the fifth order". It is to be embodied in a Babcock magnetograph for recording weak magnetic fields and Doppler effects on the sun's surface.

Under the heading of stellar photometry, it is very satisfactory to know that the Royal Astronomical Society has decided to publish in its "Memoirs" the results of the +15° Selected Areas programme of

* University of Cambridge. Report of the Observatories, for the year ending September 30, 1954. Pp. 4. (Cambridge: University Press, 1955.)