# Meetings

## Peptides

The properties and synthesis of biologically active peptides and proteins were the subjects under discussion at the first American Peptide Symposium, which was held at Yale University on 12 to 15 August 1968. The conference opened with an outline of the current state of peptide synthesis by M. Bodanszky (Case Western Reserve University). The remainder of this session was devoted to a consideration of coupling agents and detailed schemes for the preparation of important peptides. The application of pentachlorophenyl-active esters to the joining of polypeptides from the COOH-terminal residue was discussed by A. Kapoor (St. John's University). For example, stepwise addition of phenylalanyl and glycyl pentachlorophenyl ester hydrochlorides to *N*-benzyloxycarbonylglycine yielded pure *N*-benzyloxycarbonylglycylphenylalanylglycine, which was converted into a sequence polymer. A new series of activated esters was described by D. S. Kemp (Massachusetts Institute of Technology). The 7-hydroxy-2-ethylbenzisoxazolium derivatives are very resistant to base-catalyzed racemization, yet undergo replacement reactions in aqueous or buffered solution. If coupled with tetramethylammonium or tetramethylguanidine salts of amino acids in dimethyl sulfoxide solution, then the esters afford excellent yields of peptides. Isotopic labeling experiments were used both to identify the products and determine the amount of racemization.

A summary of synthetic work on gramicidin-S and related analogs was presented by N. Izumiya (Kyushu University), who described the preparation of blocked pentapeptides followed by cyclization to the decapeptides. Biological activity is very sensitive to changes in ring size and steric factors. An account was provided for the related retrogramicidin and an open-chain derivative, similar to the natural *N*-formylethanolamide precursor of the antibiotic.

Charge-transfer complexes based on cyclic polypeptides with a 5 by 4 Å central cavity were considered by R. Roeske (Indiana University School of Medicine). The compounds are based on two separated *p*-aminobenzoic acid units with spacers in the form of amino acid residues. Combination of the tripeptide units glycylglycylglycyl and histidylseryllysyl with cyclohexyl moieties has allowed the preparation of several enzyme models. B. F. Gisin (Rockefeller University) developed a solid-phase route to valinomycin, a cyclodepsipeptide. After removal of the suitably blocked peptide from a resin, the intermediate was cyclized by use of benzenesulfonylchloride and dicylohexylcarbodiimide. A solid-phase synthesis of a 43-unit peptide sequence from staphylococal nuclease was studied by D. A. Ontjes (National Institutes of Health). The final product possessed some biological activity as compared to the native material. Detection, estimation, and purity checks on the compound were by fingerprinting techniques, rather than standard amino acid analysis. A novel point was the protection of the ε-amino function of lysine as the trifluoroacetyl derivative; the latter group was cleaved by treatment with aqueous piperidine. The tandem employment of a mass spectrometer for analysis of both synthetic and natural peptides in combination with a gas chromatograph was described by E. Bayer (University of Houston). Improvements in peptide synthesis may appear in the direction of better separation methods, the use of pellicular resins in solid-phase procedures, and the possible combination of both solid-phase and fragment-condensation routes.

The second session concentrated on the practical relation between structure and biological activity of peptides. G. C. Windridge (University of California Medical School, San Francisco) outlined the pertinent facts for angiotensin II. Aspartic acid analogs exert little or no change, while arginine is important in maintaining full biological properties. A single basic group located several residues from the valyl residue seems critical in the maintenance of activity. F. M. Bumpus (Cleveland Clinic Foundation) reviewed the many physiological factors necessary for the evaluation of angiotensin analogs. Of importance are the spatial arrangements between active groups in the peptide and a receptor site. Aldosterone release may be a significant factor in pressor activity and a clue to the evaluation of new and modified compounds. The application of circular dichroism and optical rotatory dispersion measurements to a conformational analysis of angiotensin was demonstrated by M. Goodman (Polytechnic Institute of Brooklyn). Nuclear magnetic resonance spectra gathered at 220 Mhz revealed the existence of different conformations favored by various substituted angiotensins. The existing data does not permit one to determine if these particular conformations have any specific relation to the receptor region. G. Marshall (Washington University) presented a movie made by a computer display of real-time graphics. Such projections, based on van der Waals radii energy considerations, allow one to visualize interactions or special conformations. The module concept in computer design was discussed in detail. Cyclic prolyl peptides were introduced by C. M. Deber (Harvard Medical School). Models for a dimer reveal that the carbonyl groups are on opposite sides, but the trimers have carbonyl groups facing outward together. Optical measurements were developed for these compounds and special attention was paid to the evidence for *cis* amide bonds. Although most peptides are tasteless, R. H. Mazur (G. D. Searle) reported that aspartylphenylalanine methyl ester is 100 to 200 times sweeter than sucrose. This observation was made during an effort to synthesize the COOH-terminal tetrapeptide portion of gastrin, for which the above dipeptide constitutes the initial segment. Many structural analogs similar to the compound have been prepared, but, so far, none are more active. The product may be marketed as a dietary substitute. Recent advances in the chemistry of gastrointestinal hormones were outlined by M. A. Ondetti (Squibb Institute for Medical Research). A β-aspartylsecretin, identified as a by-product impurity during a synthesis of secre-

tin, has only a fraction of the activity of the pure hormone. This fact suggests that the intact $NH_2$-terminal end of secretin is necessary for biological purposes. Later, the problems associated with the preparation of an octapeptide residue in cholecystokininpancreozymin were pointed out, especially in regard to residues containing *O*-tyrosyl sulfonate. Use of the fat cell to explore structure-activity relations was considered by D. Rudman (Emory University School of Medicine). The reduction in free fatty acid concentration may indicate the degree of activity of both hormones and proteins, and such information can be used to correlate different peptide sequences. It was postulated that the unit tyrosyl-$A_1$–$A_2$–glutamyl–$A_3$–$A_4$–arginyl is important to the activity of a peptide hormone; further synthetic efforts are needed to verify this assumption. J. T. Potts (National Institutes of Health) presented a way of improving the Edman degradation scheme, in which gas-liquid chromatography was utilized for the analysis of the phenylthiohydantoin derivatives. The modified technique, in combination with new enzymatic procedures for the total hydrolysis of proteins, was applied to the elucidation of the hormone thyrocalcitonin. To complete the story, J. Pless (Sandoz) considered various problems associated with a total synthesis of thyrocalcitonin by a fragmentation-condensation route, which made use of some novel protecting groups.

G. W. Anderson (American Cyanamid) reviewed methods for the detection of racemization. The mixed anhydride procedure was greatly improved by using *N*-methylmorpholine rather than triethylamine as the base in the reaction. Similarly, the coupling of peptides by a mixture of *N*-hydroxysuccinimide and dicyclohexylcarbodiimide have several advantages and, under certain conditions, are free of racemization. Various oxazolones, implicated as the intermediates involved in racemization, were prepared by C. Glazer (Brooklyn Polytechnic Institute). A study of the reaction kinetics revealed that the rate of racemization is faster than the rate of ring opening and is dependent on hydrogen bonding effects. This information is very useful in the designing of new coupling agents. Racemization in cysteine peptides was explored by J. Kovacs (St. John's University), who used sulfur labeling techniques. Benzyl mercaptan served as a convenient test system in this study. N. Izumiya, in a second paper, used an ion-exchange column to separate the L-L and L-D diasteroisomeric forms of leucylalanine and leucylvaline. An extension to various tripeptides furnished a sensitive scheme for the determination of racemization. For example, *N*-benzyloxycarbonylglycyl-L-alanine was coupled with L-leucine benzyl ester in the presence of some agent, then the resulting product was hydrogenolyzed to furnish glycyl-L-alanyl-L-leucine, plus any glycyl-D-alanyl-L-leucine. An ion-exchange analyzer provided an accurate quantative measure of the two compounds. The application of gas-liquid chromatography to the separation of asymmetric isomers was explained by J. Westley (Hoffman-LaRoche). *N*-Trifluoroacetylprolyl chloride reacts smoothly with racemic amino acid methyl esters to furnish volatile derivatives, which have different retention periods. Another compound, (−)-menthyl chloroformate, has given good results in the separation of depsipeptide hydrolyzates. Nuclear magnetic resonance shifts were utilized for both the detection and determination of racemization in a wide variety of peptides by B. Weinstein (University of Washington). Generally, any dipeptide or tripeptide containing the elements L(or D)-alanyl-L-aromatic amino acid or the reverse will produce two different sets of methyl doublet signals. The application of optically active solvents to the nuclear magnetic resonance resolution of enantiomers was considered to be a useful technique.

The last part of the program dealt with special problems in the analysis and synthesis of peptides. E. Gross (National Institutes of Health) elucidated the main features of nisin as judged by reaction with cyanogen bromide. Cleavage of a methionylysyl bond afforded two major fragments, consisting of cyclic peptides with internal disulfide links. Unusual features of the primary sequence were clarified, and a tentative structure was proposed for this antibiotic. Cystine residues are found in many proteins, but existing preparative procedures for these compounds are difficult and lack wide application. R. G. Hiskey (University of North Carolina) resorted to thiocyanogen in combination with thioethers to construct large disulfide rings. A feature of this work was a possible extension to a new synthesis of insulin.

J. Meienhofer (Children's Cancer Research Foundation) delineated routes to the preparation of actinomycin D.

The peptide portion of the molecule is essential for biological activity, but its precise role is unclear and needs elaboration. The capsular polypeptide derived from anthrax bacillus was studied by D. E. Nitecki (University of California Medical School, San Francisco). The material appears to be a pure form of poly-δ-D-glutamic acid. A detailed preparation was given for the individual oligomeric peptides, including the hexamer. The solid-phase procedure was generally used, but difficulties were encountered in achieving complete purity. The stereospecific preparation of dipeptides from alkyliminophenylacetyl amino acids was outlined by K. Harada (University of Miami). Hydrogenation led to partial optical activity, which is dependent on the bulkiness of the residues, the distance between the reaction center and the catalyst, as well as chelation factors. R. Walter (Mt. Sinai Medical School) described some selenium-containing oxytocins and compared their activity to deamino-oxytocin. L-Selenocysteine was also prepared and incorporated into various peptides. Effects of chemical modification on conformation can be estimated by comparison of optical rotatory dispersion spectra.

The antigenic properties of penicillin were discussed by N. Grant (Wyeth). Antigenicity arises from an opening of the β-lactam ring to yield a penicillenic acid, followed by polymerization or diketopiperazine formation. Present evidence favors the oligomeric formulation for the antigenic factor. S. W. Fox (University of Miami) revealed that thermal polymerization of common amino acids will produce a polymer having properties similar to histones. An equivalent reaction with amino acid adenylates formed high-molecular-weight materials which are characterized as modified proteinoids. These procedures offer a model for DNA-independent formation of biologically active high molecular weight compounds in prebiotic times.

Finally, the selective labeling of the COOH-terminal amino acid in proteins in terms of deuterium or tritium was developed into an analytical technique by H. Matsuo (University of California, Berkeley). The reaction scheme involves oxazolone formation with the aid of acetic anhydride, then incorporation of deuterium at the optical center by exposure to deuterium oxide-pyridine and ring opening to the starting peptide. Hydrolysis yields the labeled COOH-terminal amino acid. This method

should have wide application in the elucidation of peptide sequence.

The edited symposium papers are to be made available in a book (Marcel Dekker, New York), which should appear early in 1969.

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## Calendar of Events

**Nucleic Acid and Protein Interactions,** Spetsai, Greece, 6–19 July. This is the fourth Advanced Study Institute of Molecular Biology. Application forms and information may be obtained from the NATO Advanced Study Institute Secretary, M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge, England.

**Water Pollution Control,** Washington, D.C., 3–7 February. This course is designed for engineers, scientists, and researchers who are working in the field of control of water pollution. The scope of the course includes principles of hydrology, treats to water resources, sources of pollution, workshop on abatement technology, system analysis, economic analysis, legislation and legal aspects, and enforcement. (Jack E. Mansfield, Coordinator of Continuing Engineering Education, School of Engineering and Applied Science, George Washington University, Washington, D.C. 20006)

**Fundamentals of Dynamic Measurements as Applied to the Ocean Sciences,** San Diego, Calif., 11–14 Mar. The course is designed for practicing engineers, scientists, and technicians who use sophisticated electronic instrumentation in ocean research. The course outline includes units on recorders, signal generators, transducers, analog and digital conversion, waveforms, filters, amplifiers, voltage regulators, and the ocean research applications of these units. (Third Ocean Sciences Short Course, Instrument Society of America, Education & Research Services, 530 William Penn Place, Pittsburgh, Pa. 15219)

**Epidemiology,** Minneapolis, Minn., 15 June–3 July. Is designed primarily for teachers in medical schools, but post-doctoral fellows, graduate students, and residents in departments of preventive medicine and other medical school departments may qualify. In addition to the courses in the fundamentals of epidemiology and of biostatistics; epidemiology of cancer, cardiovascular diseases, and infectious diseases; genetics and epidemiology, new courses in epidemiology of mental disorders and of neurological diseases will be offered. Tuition: $120. Limited stipends are available. (Dr. Leonard M. Schuman, Director, Graduate Summer Session in Epidemiology, University of Minnesota School of Public Health, 1558 Mayo Building, Minneapolis 55455)