I. *Epilogue*

Lack of time and space put an end to this exploratory excursion into the border areas of organic chemistry. Many a promising trail has been uncovered. Often the temptation was great to follow these new paths and run the risk of getting overcommitted to applications. Once a generally useful principle or method is found by the chemical pathfinder, follow-up and application should be up to the experts in the neighboring disciplines. By living like the artist on several planes simultaneously, the scientist should avoid the ominous trend toward overspecialization.

My modest role as a chronicler and narrator ends here, but my indebtedness to my younger collaborators and associates continues.

Zusammenfassung. Im Rückblick und Ausblick wird anhand einiger ausgewählter Arbeiten aus dem Laboratorium des Verfassers gezeigt, wie die Ziele und Methodologie der modernen Naturstoff-Forschung sich im Laufe der letzten 20 Jahre geändert haben. Noch immer kommen die unerwartetsten Anregungen für ungewöhnliche Strukturen aus der Natur, so zum Beispiel die neuen Tier-Alkaloide *Histrionicotoxin* und seine Begleiter, die an einem Spiro-cyclohexyl-piperidin-Skelett, Azetylen- und Allen-Seitenketten enthalten. Mehr noch als die Struktur interessiert beim *Batrachotoxin* die selektive Wirkung auf den passiven Transport von Natrium-Ionen durch Membranen elektrogener Gewebe, ein Effekt, der durch *Tetrodotoxin* reversibel aufgehoben werden kann. Heute zählt man auch die Eiweiss-Stoffe zu den wichtigen Naturprodukten. Ihre Erforschung wurde durch die Einführung selektiver Spaltungsmethoden ermöglicht und erleichtert, was am Beispiel des *Immunoglobulins* und des *Kollagens* gezeigt wird. Diese Spaltungsmethoden beruhen auf der Ausnutzung benachbarter Gruppen-Effekte, die weiterhin auch als Basis zum Ausbau von Enzym-Modellen dienen. Die Beschäftigung mit der Chemie der Überträgersubstanzen der Nervenfortleitung, wie Dopamin und Noradrenalin, führte zur Auffindung von *6-Hydroxydopamin*, das zum ersten Mal selektiv «chemische Sympathektomie» erlaubt. In der physiologischen Inaktivierung dieser Nervenhormone spielt die *Brenzkatechin O-Methyltransferase* eine grosse Rolle im Stoffwechsel, der man durch Reindarstellung und Beschreibung des Enzyms näherzukommen versucht. Im Stoffwechsel von aromatischen Substraten bedeutet die erste *Isolierung eines Arenoxyds* einen wichtigen Fortschritt. Ein derart labiles Stoffwechselprodukt spielt in der langfristigen Toxikologie aromatischer Arzneimittel und in der Ätiologie von Krebs durch cancerogene Kohlenwasserstoffe eine Rolle. Zum Studium von Oxydationsmechanismen mikrosomaler oder Modell-Hydroxylierungen wird als Kriterium mehr und mehr die sogenannte *NIH-Verschiebung* herangezogen.

SPECIALIA

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Synthesis of a Structure Proposed for Scotophobin

Scotophobin[1], a behavioral 'memory code word', has been formulated as the pentadecapeptide[2] Ac-ser-asp-asn-asn-glu-gln-gly-lys-ser-ala-glu-gln-gly-gly-tyr-NH_2. A synthesis of this compound is now reported[3,4], as well as full biological data.

N-Benzyloxycarbonyl-O-*t*-butyl-tyrosine (I) was converted into a mixed anhydride (N-methyl morpholine and isobutyl chloroformate) and treatment with ammonia produced the amide (II). In turn, hydrogenation formed the amine (III); a coupling to N-benzyloxycarbonyl-glycine (IV) by the mixed anhydride procedure gave the dipeptide (V). Hydrogenation then afforded the S_{14-15} amine glycyl-O-*t*-butyl-tyrosinamide (VI).

The sequence S_{10-13} was prepared beginning with methyl glycinate (VII) and N-benzyloxycarbonyl-glutamine (VIII), which on joining by the mixed anhydride procedure, yielded methyl N-benzyloxycarbonyl-glutaminyl-glycinate (IX). Hydrogenation furnished the amine (X); a combination with N-benzyloxycarbonyl-γ-*t*-butyl- glutamic acid (XI) by the mixed anhydride method produced the tripeptide (XII). Hydrogenation formed the amine

[1] G. UNGAR, *Molecular Mechanisms in Memory and Learning* (Plenum Press, New York 1970), p. 149.

[2] G. UNGAR, personal communication, February 24, 1970

[3] A stepwise preparation of the scotophobin sequence was discussed in detail by B. WEINSTEIN at The Second American Peptide Symposium, Cleveland, Ohio, August 1970; the biological results for the tridecapeptide S_{3-15} were mentioned, too. Additional information on this synthesis was presented at the 160th National Meeting of the American Chemical Society, Chicago, Illinois, September 1970, Abstracts ORGN 008.

[4] The original route met with two major hurdles: The blocked peptides beginning with the subunit S_5-S_{15} are very insoluble and thus slow to react, while some fragments containing the S_{3-4} asn-asn sequence undergo facile hydrolytic cleavage.

(XIII) and a coupling to N-benzyloxycarbonyl-alanine (XIV) by a mixed anhydride step gave the tetrapeptide (XV). Hydrolysis by dilute alkali afforded N-benzyloxycarbonyl-alanyl-γ-*t*-butyl-glutaminyl-glycine (XVI). No evidence for glutaminyl hydrolysis was seen in this reaction.

The acid XVI was combined with the amine VI through a water-soluble carbodiimide to yield the S_{10-15} hexapeptide (XVII). Hydrogenation then furnished the amine (XVIII). A mixed anhydride coupling of N-benzyloxycarbonyl-ε-*t*-butyloxycarbonyl-lysine (XIX) with methyl O-*t*-butyl-serinate (XX) produced the dipeptide (XXI). Hydrolysis formed the corresponding acid (XXII), which was joined to the amine XVIII by a water-soluble carbodiimide in the presence of N-hydroxysuccinimide to give the octapeptide (XXIII). Hydrogenolysis then afforded the S_{7-15} fragment N-benzyloxycarbonyl-ε-*t*-butyloxycarbonyl-lysyl-O-*t*-butyl-seryl-alanyl-γ-*t*-butyl-glutamyl-glutaminyl-glycyl-glycyl-O-*t*-butyl-tyrosinamide (XXIV).

The preparation of the S_{3-7} portion was achieved in a different fashion. N-*t*-Butyloxycarbonyl-glycine (XXV) was joined to N-benzyloxycarbonyl hydrazine (XXVI) by the mixed anhydride reagent to give the protected hydrazide (XXVII). Cleavage with trifluoroacetic acid furnished the amine salt (XXVIII), which was combined with N-*t*-butyloxycarbonyl-glutamine (XXIX) by the mixed anhydride method to produce the dipeptide (XXX). Removal of the *t*-butyl group with trifluoroacetic acid formed the amine salt (XXXI). A mixed anhydride coupling with N-*t*-butyloxycarbonyl-γ-benzyl-glutamic acid (XXXII) gave the tripeptide (XXXIII). Treatment with trifluoroacetic acid afforded the amine salt (XXXIV), which on the addition of N-*t*-butyloxycarbonyl-asparagine *p*-nitrophenyl ester (XXXV) yielded the tetrapeptide (XXXVI). Removal of the *t*-butyl group in the usual manner furnished the amine salt (XXXVII), which on coupling to XXXV produced the pentapeptide (XXXVIII). Addition of trifluoroacetic acid then formed asparaginyl-asparaginyl-γ-benzyl-glutamyl-glutaminyl-glycyl-N^2-benzyloxycarbonyl-hydrazide trifluoroacetate (XXXIX).

The S_{1-2} unit was obtained in a simple fashion. A mixed anhydride coupling between N-benzyloxycarbonyl-O-*t*-butyl-serine (XL) and methyl β-*t*-butyl-aspartate (XLI) gave the dipeptide (XLII). Hydrogenation in the presence of one equivalent of pyridinium hydrochloride afforded the amine hydrochloride (XLIII) and the addition of acetic anhydride yielded the N-acetyl-dipeptide (XLIV). Hydrolysis with dilute base furnished N-acetyl-O-*t*-butyl-seryl-β-*t*-butyl-aspartic acid (XLV).

The acid XLV was combined by the mixed anhydride method with the amine salt XXXIX to produce the S_{1-7} heptapeptide (XLVI). Hydrogenation then formed N-acetyl-O-*t*-butyl-seryl-β-*t*-butyl-aspartyl-asparaginyl-asparaginyl-glutamyl-glutaminyl-glycyl hydrazide (XLVII). An organic azide coupling between XLVII and the amine XXIV gave the S_{1-15} pentadecapeptide (XLVIII). Treatment with trifluoroacetic acid afforded the deblocked trifluoroacetate salt (XLIX) and passage through an acetate ion-exchange resin yielded scotophobin acetate (L)[5].

Both scotophobin trifluoroacetate and scotophobin acetate were tested for the induction of a dark memory effect in untrained mice[6]. The former was lethal, but the latter possessed 2.5% of the activity of natural scotophobin at the 10.0 µg dosage level.

The lower biological behavior observed here, as well as the difference in R_f values (0.15 vs. 0.57), means that the postulated structure is in error, either in terms of sequence or in amino acid functionality. Work on several molecular variants is currently underway in order to clarify this situation[6].

Zusammenfassung. Es wurde ein biologisch interessantes Polypeptid mit der für Scotophobin vorgeschlagenen Sequenz synthetisiert. Da dieses nur eine geringe biologische Aktivität besass, wird angenommen, dass die für das natürliche Produkt vorgeschlagene Strukturformel nicht korrekt ist.

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Department of Chemistry, University of Washington, Seattle (Washington 98195, USA), 22 April 1971.

[5] All optically active amino acids are L; satisfactory microanalytical data were obtained for the new intermediates, while quantitative ammonia and amino acid assays were routinely performed on both blocked and deblocked products; thin-layer chromatography employed silica gel H as the support, chloroform-methanol (9:1) or acetic acid-*n*-butanol-water (1:4:1) for development, and iodine or ninhydrin for detection. Further experimental details will be given in the full paper.

[6] The pharmacology assays were furnished by Prof. G. Ungar, Baylor College of Medicine, Texas Medical Center, Houston (Texas, USA).

[7] We thank the National Institutes of Health (MH 19320) for the support of this work.

Periodic Acid as a New Oxidant for the Degradation of Bile Pigments. Isolation of a Biliverdine Type of Reaction Intermediate on Oxidation of Bilirubin with Periodic Acid[1]

As part of a continued interest in new applications of periodic acid in organic chemistry[2], the study has now been extended to oxidation of bile pigments[3], particularly to samples of bilirubin that we are studying for the purpose of developing a standard reference material for clinical analysis. The bile pigments are usually analyzed by using degradation by chromic acid, either in 50% sulfuric acid (Willstätter[4] degradation) or at conrolled pH, the procedure of Rüdiger[5]. It has been found in this laboratory[6] that an aqueous solution of paraperiodic acid (H_5IO_6) may be used to degrade such porphyrins as hemin, hematoporphyrin, protoporphyrin, or chlorophyll, as well as such bile pigments as bilirubin (I), biliverdine (II, without iodine), urobilin, and other rubins, verdines, and violines. The co-solvents used in these degradations include dimethyl sulfoxide (Me_2SO, which shows superior solvating power for bile pigments and porphyrins), acetic acid, tetrahydrofuran, N,N-dimethylformamide, or acetone.

The final degradation products from bile pigments or porphyrins, usually mono-pyrrole derivatives, can be separated by extraction with ether or ethyl acetate into an acidic fraction (hematinic acid[7]) and a neutral fraction (2-ethyl-3-methyl-maleimide[7], methylvinylmaleimide[8] or, in certain instances, pyrroledialdehydes[5]). Identification