In meiner etwas laienhaften Übersetzung etwa: «Bernhard von Tours pflegte zu sagen, wir seien wie Zwerge, die auf den Schultern von Riesen sässen; dass wir mehr und entlegeneres als diese sehen, ist offensichtlich nicht unserer besonderen Sehschärfe oder unserem hohen Wuchse zuzuschreiben, sondern der Tatsache, dass wir von ihrer gigantischen Grösse in die Höhe geführt und emporgehoben wurden». Einer dieser Riesen ist PAUL KARRER.

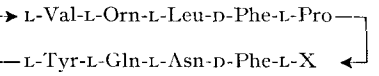
Summary. Polypeptides and proteins are compared with programmed machines capable of automatic functioning and of regulation. Among the regulatory processes, endocrine regulation is examined in more detail. Parallels are drawn to kybernetic devices, and concepts of organization and read out of information contained in polypeptide hormones are briefly dealt with. These views lead to recent work on a fluorescent derivative of ACTH, dansyllysine-21-ACTH-(1-24)-tetrakosipeptide, which is capable of binding to potential receptors. By means of fluorescence polarization it was discovered that this hormone derivative is specifically and strongly bound to glucose-6-phosphate dehydrogenase from cow adrenals.

Other work is described which pertains to the question of whether it is possible to write an original program in polypeptide language in order to obtain molecules with new (biological) activities. We were able to construct a bicyclic peptide, S,S'-*bis*-cycloglycyl-hemicystyl-glycyl-glycyl-prolyl, capable of specifically complexing potassium cations and transporting them through a lipophilic membrane. It is hoped to be able to achieve coupling of this transport to redox energy differences.

SPECIALIA

Synthesis of Tyrocidine B[1]

BATTERSBY and CRAIG[2] fractionated in 1952 a mixture of tyrocidine family into 3 components designated as tyrocidine A, B and C, using a technique of countercurrent distribution. In 1955 KING and CRAIG[3] isolated a crystalline hydrochloride of tyrocidine B (TB), and proposed the structure of TB to be a cyclic decapeptide shown as II in Figure[4]. However, they did not mention a quantitative feature of its antibacterial activity and data of some measurements such as melting point and specific rotation[3,4].



Structure of tyrocidine A (I) and B (II). X represents an amino acid residue such as Phe (I) and Trp (II).

We reported previously the synthesis of tyrocidine A[5] and E[6], and have been attempting to synthesize other tyrocidines. We wish to report here the synthesis of the cyclic decapeptide (II) designated as TB, and the chemical and biological properties of the synthetic product[7].

Z-Gln-Tyr-OEt[5] was hydrogenated in the presence of palladium black and an equivalent of hydrogen chloride in a mixture of ethanol and DMF to produce H-Gln-Tyr-OEt·HCl (III)[8], 94%, mp 157–158°, $[\alpha]_D$ +22.4°. Condensation of Z(OMe)-Asn-ONp[9] with III gave Z(OMe)-Asn-Gln-Tyr-OEt (IV), 63%, mp 213–214° dec, $[\alpha]_D$ −11.0° (DMF), which was treated with hydrazine in DMF to produce Z(OMe)-Asn-Gln-Tyr-$NHNH_2$ (V), 95%, mp 243° dec, $[\alpha]_D$ −11.0° (dimethyl sulfoxide). Condensation of the azide derived from V with H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH·HCl[5] gave Z(OMe)-Asn-Gln-Tyr-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (VI), 87%, mp 226–227°, $[\alpha]_D$ −22.2° (DMF). (Anal. calcd. for $C_{65}H_{85}O_{17}N_{11}\cdot 2H_2O$: C, 58.77; H, 6.75; N, 11.60. Found: C, 59.03; H, 6.88; N, 11.67.) Removal of the *p*-methoxybenzyloxycarbonyl group from VI by treatment with trifluoroacetic acid yielded amorphous H-Asn-Gln-Tyr-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH·CF_3COOH (VII) in quantitative yield.

[1] Presented at the 7th Symposium on Peptide Chemistry at Tokyo University, Tokyo (Japan), 22 November 1969.

[2] A. R. BATTERSBY and L. C. CRAIG, J. Am. chem. Soc. *74*, 4019 (1952).

[3] T. P. KING and L. C. CRAIG, J. Am. chem. Soc. *77*, 6624 (1955).

[4] T. P. KING and L. C. CRAIG, J. Am. chem. Soc. *77*, 6627 (1955).

[5] M. OHNO and N. IZUMIYA, J. Am. chem. Soc. *88*, 376 (1966). – M. OHNO, T. KATO, S. MAKISUMI and N. IZUMIYA, Bull. chem. Soc. Japan *39*, 1738 (1966).

[6] N. MITSUYASU, M. WAKI, S. MATSUURA, K. KUROMIZU and N. IZUMIYA, Abstract of 21st Annual Meeting of Chem. Soc. Japan, Tokyo (April 1968), vol. 3 p. 2242. – N. MITSUYASU and N. IZUMIYA, Experientia, submitted.

[7] The synthesis of Z-Val-Orn(δ-Tos)-Leu-Phe-Trp-Phe-OMe, has been reported; H. ZAHN and D. BRANDENBURG, Justus Liebigs Annln. Chem. *692*, 220 (1966).

[8] Satisfactory elemental analyses and chromatographic data were obtained for all crystalline compounds described here. $[\alpha]_D$ refers to a solution in methanol at 20° otherwise noted. Z-, benzyloxycarbonyl; Z(OMe)-, *p*-methoxybenzyloxycarbonyl; -ONp, *p*-nitrophenyl ester; DMF, dimethylformamide; HOSu, N-hydroxysuccinimide; DCC, dicyclohexylcarbodiimide. Amino acid symbols except D-Phe denote the L-configuration.

[9] E. SCHRÖDER and E. KLIEGER, Justus Liebigs Annln. Chem. *673*, 208 (1964).

Z(OMe)-Typ-OH dicyclohexylamine salt (VIII) was prepared in the usual manner[10], 71%, mp 151–152°, $[\alpha]_D$ +11.5°. The condensation reaction of VIII with H-D-Phe-OBzl by the DCC method gave Z(OMe)-Trp-D-Phe-OBzl (IX), 82%, mp 137–139°, $[\alpha]_D$ +3.6°, which was saponified to Z(OMe)-Trp-D-Phe-OH (XI), 80%, mp 84–86°, $[\alpha]_D$ −25.0°. XI (1.2 equiv.) in ethyl acetate was treated with HOSu (1.4 equiv.) and then DCC (1.1. equiv.) at 0 °C for 3 h. The filtrate from dicyclohexylurea was added to a solution of the octapeptide (VII) (1 equiv.) and triethylamine (1 equiv.) in DMF, the stirring being continued at 0° for 5 h. This treatment[11–14] gave Z(OMe)-Trp-D-Phe-Asn-Gln-Tyr-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (XII), 70%, mp 215–216° dec, $[\alpha]_D$ −33.1° (DMF). (Anal. calcd. for $C_{85}H_{104}O_{19}N_{14}\cdot 2H_2O$: C, 61.42; H, 6.55; N, 11.80. Found: C, 61.26; H, 6.42; N, 11.85.)

Treatment of XII with 10 equiv. of di-*p*-nitrophenyl sulfite gave amorphous acyldecapeptide *p*-nitrophenyl ester (XIII) and the *p*-nitrophenyl ester content of XIII was estimated to be 105% by means of the method described by SCHWYZER and SIEBER[15]. The decapeptide *p*-nitrophenyl ester trifluoroacetate, obtained from XIII by the action of trifluoroacetic acid, was treated with a large amount of hot pyridine (55–60°) for the cyclization reaction. Purification of the crude product by passing its aqueous dioxane-methanol solution through columns of Amberlite IRC-50 (H^+ form) and Amberlite IR-45 (OH^- form) gave cyclo-Trp-D-Phe-Asn-Gln-Tyr-Val-Orn(δ-Z)-Leu-D-Phe-Pro (XIV), 43% (from XII), mp 198–200°, $[\alpha]_D$ −86.9°. (Anal. calcd. for $C_{76}H_{94}O_{15}N_{14}\cdot 2H_2O$: C, 61.68; H, 6.68; N, 13.25; mol. wt., 1479. Found: C, 61.79; H, 6.58; N, 13.05; mol. wt., 1440.) Removal of the benzyloxycarbonyl group from XIV was achieved by catalytic hydrogenation in the presence of an equivalent of hydrogen chloride in methanol to provide the crystalline cyclo-Trp-D-Phe-Asn-Gln-Tyr-Val-Orn-Leu-D-Phe-Pro·HCl·$5H_2O$ (II·HCl·$5H_2O$) as a desiccator (with $CaCl_2$)-dried product; 62%, mp 236–237° dec, $[\alpha]_D$ −93.0° (c 0.5, MeOH). (Anal. calcd. for $C_{68}H_{88}O_{13}N_{14}\cdot HCl\cdot 5H_2O$: C, 56.87; H, 6.95; N, 13.66. Found: C, 56.71; H, 6.82; N, 13.62.) Its homogeneity was also ascertained by thin-layer and paper chromatographies, paper electrophoresis and carboxymethylcellulose column chromatography. Quantitative amino acid determination gave the following molar ratio: Trp 1.06, Phe 1.86, Asp 1.01, Glu 1.02, Tyr 1.07, Val 0.97, Leu 0.97, Pro 0.96, Orn 1.00, NH_3 2.20[16].

The antibacterial activity toward several microorganisms was examined[17]. It was found that levels of the activity of the synthetic peptide (II·HCl) was the same as that of the synthetic tyrocidine A[5] for the Gram positive microorganisms (*Staph. aureus* and *B. subtilis*) except a microorganism, *Candida albicans*; minimum concentrations of growth-inhibition for *Candida albicans* were found to be 12.5 μg/ml with the TA and 100 μg/ml with II. Work on syntheses of the cyclic decapeptide corresponding to the proposed structures for tyrocidine C[18] and D[19] is in progress in this laboratory.

Zusammenfassung. Die Synthese von Tyrocidin B durch Zyklisierung des aktiven Esters des linearen Dekapeptids wird beschrieben.

K. KUROMIZU and N. IZUMIYA

Laboratory of Biochemistry,
Faculty of Science, Kyushu University,
Fukuoka (Japan), 29 December 1969.

[10] S. SAKAKIBARA, I. HONDA, M. NARUSE and K. KANAOKA, Experientia *25*, 576 (1969).
[11] F. WEYGAND, D. HOFFMANN and E. WÜNSCH, Z. Naturforsch. *21b*, 426 (1966).
[12] J. E. ZIMMERMAN and G. W. ANDERSON, J. Am. chem. Soc. *89*, 7151 (1967).
[13] N. IZUMIYA and M. MURAOKA, J. Am. chem. Soc. *91*, 2391 (1969).
[14] The coupling method with HOSu-DCC was developed originally by WEYGAND et al.[11]. It was recognized that no racemization of L-Ala residue occurred absolutely by the use of a racemization test developed in this laboratory[13] when Z-Gly-L-Ala-OH and H-L-Leu-OBzl was coupled under the same conditions of this experiment.
[15] R. SCHWYZER and P. SIEBER, Helv. chim. Acta *40*, 624 (1957).
[16] We are indebted to Mr. K. NODA in this laboratory for the amino acid analysis. Molar ratios of Trp and Tyr were determined in conformity with the extinction at 280 nm and 293.4 nm in UV-spectra of II using 0.1 *N* sodium hydroxide-ethanol (1:1) solution as solvent; see, T. W. GOODWIN and R. A. MORTON, Biochem. J. *40*, 628 (1946).
[17] We are indebted to Meiji Seika Co. for the biological assay.
[18] M. A. RUTTENBERG, T. P. KING and L. C. CRAIG, Biochemistry *4*, 11 (1965).
[19] M. A. RUTTENBERG and B. MACH, Biochemistry *5*, 2864 (1966).

A Novel, Practical Synthesis of 18-Norsteroids

The syntheses of 18-norsteroids of potential medicinal importance have been reported by several groups[1]. The procedures are by either total synthesis or partial synthesis which involve multiple steps.

The author wishes to report a new, practical preparation of 18-norsteroids from an abundant steroid, hecogenin, by a simple sequence, which consists of the decarboxylation of a 12-keto-18-oic acid, a mechanism postulated to the biogenesis of fukujusonorone, the first naturally occuring 18-norsteroid isolated from *Adonis amurensis* by the author and co-workers[2].

3β-Acetoxy-20β-hydroxy-5α-pregnan-12-one (I), which is readily accessible from hecogenin[3], was treated with lead tetraacetate and iodine in cyclohexane expecting the hemiacetal (III), or iodine compound (IV)[4]. However, the main product directly obtained in 50% yield was the γ-lactone (II)[5], mp 209–211°, ν_{max}^{Nujol} 1760 (γ-lactone), 1730 (acetate), 1705 (12CO) cm^{-1}; τ (in $CDCl_3$): 8.93 (3H, s, 19-Me), 7.90 (3H, s, acetate), 5.54 (1H, q, J = 6.5 Hz, 20-H), 5.25 (1H, m, 3-H). The reason of this direct lactone formation may be attributed to activation of 18-methyl group by the 12-carbonyl function, though it is apart by one carbon unit. However, there are evidences of the formation of the hemiacetal (III) or the iodine derivative (IV), in small amounts. Thus, the solvolysis of the mother liquor in aqueous acetic acid followed by chromic acid oxidation gave an additional amount of the lactone (II) in 10% yield, and also treatment of the mother liquor with methanolic sodium hydroxide followed by chromic acid oxidation afforded a methoxyl compound (V), mp 209–210°, ν_{max}^{Nujol} 1710, 1700 cm^{-1}; τ (in $CDCl_3$): 8.89 (3H, s, 19-CH_3), 8.70 (3H, d, J = 7 Hz, 21-CH_3), 6.60 (3H, s, OCH_3), 5.89 (1H, q, J = 7 Hz, 20-H), 5.05 (1H, s, 18-H).