Tetrahedron Letters No.26, pp. 3083-3088, 1968. Pergamon Press. Printed in Great Britain.

RETROGRAMICIDIN S

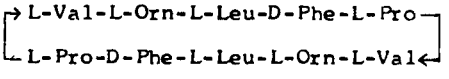
Michinori Waki and Nobuo Izumiya

Laboratory of Biochemistry, Faculty of Science

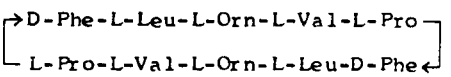
Kyushu University, Fukuoka, Japan

(Received in Japan 28 February 1968; received in UK for publication 1 April 1968)

In studies of the relationship between chemical structure and biological activity of gramicidin S (GS), various analogs wherein several amino acid residues are replaced by other amino acids have been synthesized in this laboratory. For example, we discovered that the synthetic [5,5'-glycine]-gramicidin S (1) possesses stronger antibacterial activity than GS. In order to determine the influence of the direction of the peptide bonds on the biological activity we have desinged the synthesis of a different type of synthetic analogs of GS, that of retroGS. RetroGS can be regarded as a cyclodiastereomer (2) of GS.



gramicidin S (GS)



retrogramicidin S (retroGS)

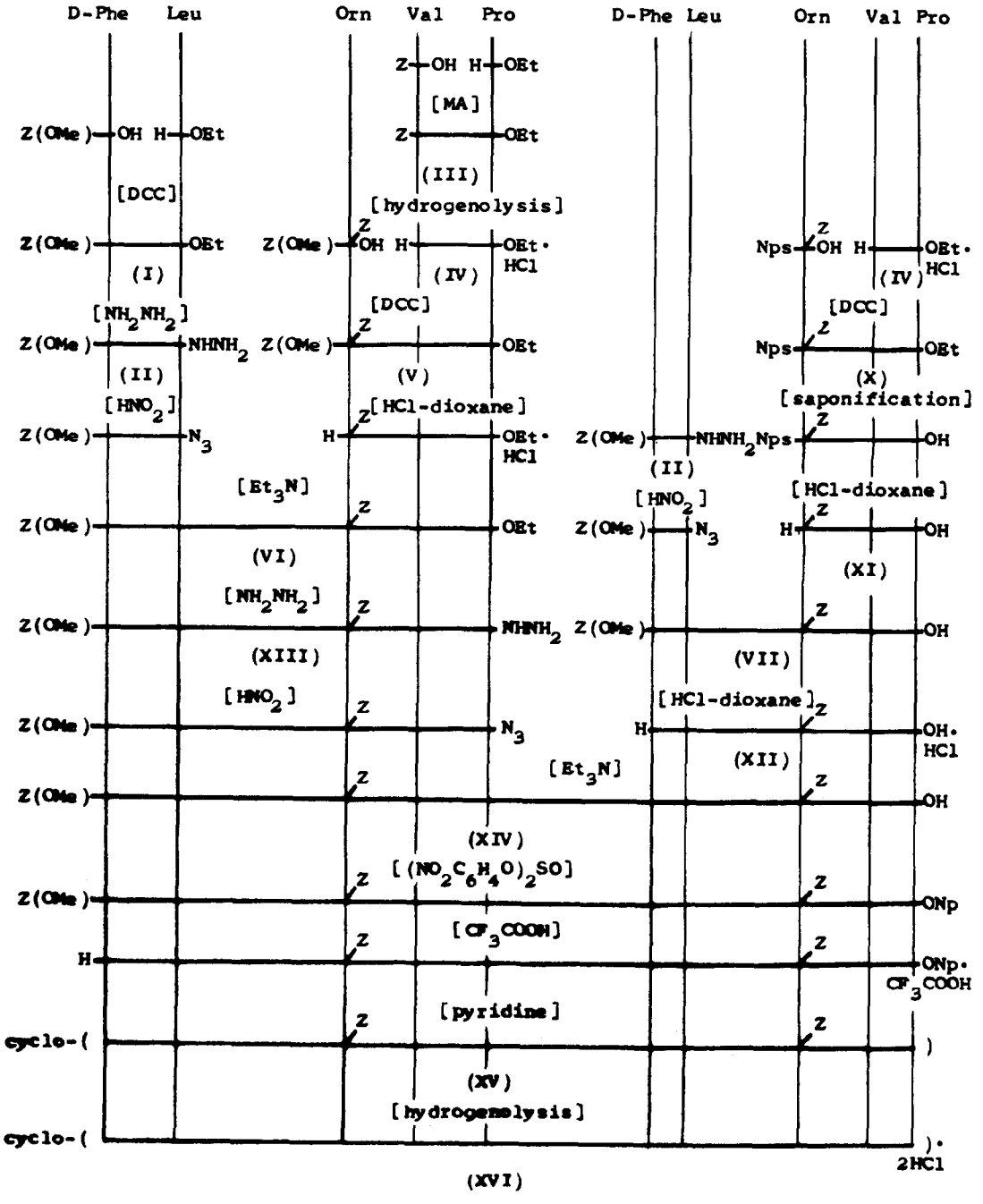
For the conformation of GS, several models have been proposed (3). A possible model is the intramolecular antiparallel β form with four hydrogen bonds which has been favored by the results of X-ray crystallographic investigation (4). In this model the hydrophilic ornithyl side chains are on

one side of the pleated plane, thus the model appears to be a cationic detergent (5). In the retroGS model which is constructed as the antiparallel β form with the basis of molecular model study, the hydrophobic proline residues are directed to the same side where the ornithyl side chains are located on. Thus, we anticipated that retroGS would show weaker activity than GS.

In studies of synthetic analogs of linear active peptides, a retro analog of bradykinin has been synthesized by two groups (6); they observed that retrobradykinin is completely inactive. As an analog of cyclic active peptides, Shemyakin et al. demonstrated that retroenatio-[5,5'-glycine]-GS possesses commensurative high activity with that of [5,5'-glycine]-GS (7).

In the synthesis of retro GS a sequence of steps was chosen specifically to preclude racemization as shown in the diagram. Z(OMe)-D-Phe-Leu-OEt (I) (8), mp 108-109°, $[\alpha]_D$ -0.6°, was prepared in 77% yield from Z(OMe)-D-Phe-OH (9) and H-Leu-OEt by the DCC method. The compound I was treated with hydrazine to afford Z(OMe)-D-Phe-Leu-$NHNH_2$ (II) in 95% yield, mp 176-177°, $[\alpha]_D$ -7.5°. Oily Z-Val-Pro-OEt (III), which was prepared in 69% yield, from Z-Val-OH and H-Pro-OEt by the mixed anhydride method, was converted to oily H-Val-Pro-OEt·HCl (IV) in 96% yield by hydrogenation. Condensation of Z(OMe)-Orn(δ-Z)-OH (10) with IV by the DCC method gave oily Z(OMe)-Orn(δ-Z)-Val-Pro-OEt (V) in 96% yield. Then, Z(OMe)-D-Phe-Leu-Orn(δ-Z)-Val-Pro-OEt (VI), 68%, mp 158-159°, $[\alpha]_D$ -31.0°, was prepared by condensation of the azide derived from II with oily H-Orn(δ-Z)-Val-Pro-OEt·HCl which was obtained from V in 98% yield by treatment of 2 N hydrogen chloride in dioxane. Saponification of VI with 2 equiv of sodium hydroxide gave a mixture of compounds. The mixture was separated into two pure components by the use of a Sephadex LH-20 column (solvent, dioxane): Z(OMe)-D-Phe-Leu-Orn(δ-Z)-Val-Pro-OH (VII), 28%, mp 136-137°, $[\alpha]_D$ -30.6°, and 2-isobutyl-5-[1-carboxy-2-(phenyl)-ethyl]-hydantoyl-Orn(δ-Z)-Val-Pro-OH (VIII) (11), 36%, mp 121-123°, $[\alpha]_D$ -23.2°.

The important intermediate VII was obtained more efficiently as follows. Dicyclohexylammonium salt (IX) of Nps-Orn(δ-Z)-OH, mp 167-168°, $[\alpha]_D$ -25.0°, was prepared in 71% yield from H-Orn(δ-Z)-OH and nitrophenylsulfenyl chloride


D-Phe
Leu
Orn
Val
Pro
Z(OMe)
OH H
OEt
[DCC]
(I)
[NH2NH2]
NHNH2
(II)
[HNO2]
N3
[Et3N]
(VI)
(XIII)
(XIV)
[(NO2C6H4O)2SO]
ONp
[CF3COOH]
ONp·CF3COOH
[pyridine]
(XV)
[hydrogenolysis]
cyclo-(
)
(XVI)
Z-OH H-OEt
[MA]
Z-OEt
(III)
[hydrogenolysis]
OEt·HCl
(IV)
[DCC]
OEt
(V)
[HCl-dioxane]
H
OEt·HCl
Nps
OH H
OEt·HCl
(IV)
[DCC]
Nps
OEt
(X)
[saponification]
Nps
OH
[HCl-dioxane]
H
OH
(XI)
OH
(VII)
[HCl-dioxane]
OH·HCl
(XII)
OH
)·2HCl

by the usual way (12). Condensation of IX with IV by the DCC method gave oily Nps-Orn(δ-Z)-Val-Pro-OEt (X) in 99% yield. Saponification of X afforded oily Nps-Orn(δ-Z)-Val-Pro-OH, 62%, which was converted to H-Orn(δ-Z)-Val-Pro-OH (XI), 68%, mp 174-176° dec, $[\alpha]_D$ -34.0° (AcOH), with treatment of hydrogen chloride in dioxane. Then, VII with mp 137-138° was obtained in 70% yield by condensation of the azide derived from II with XI. Treatment of VII with hydrogen chloride in dioxane yielded H-D-Phe-Leu-Orn(δ-Z)-Val-Pro-OH·HCl (XII), 90%, mp 201-203° dec, $[\alpha]_D$ -53.6°. Z(OMe)-D-Phe-Leu-Orn(δ-Z)-Val-Pro-$NHNH_2$ (XIII), mp 160-161°, $[\alpha]_D$ -29.4°, was prepared in 83% yield from VI with excess hydrazine in dimethylformamide. Condensation of the azide derived from XIII with XII gave Z(OMe)-D-Phe-Leu-Orn(δ-Z)-Val-Pro-D-Phe-Leu-Orn(δ-Z)-Val-Pro-OH (XIV), 75%, mp 174-175°, $[\alpha]_D$ -31.4°. Treatment of XIV with excess di-p-nitrophenyl sulfite gave an amorphous acyldecapeptide p-nitrophenyl ester which was then dissolved in trifluoroacetic acid to remove the methoxybenzyloxycarbonyl group. The decapeptide p-nitrophenyl ester trifluoroacetate thus obtained was treated with excess pyridine for the cyclization reaction. Purification of the crude product by passing its aqueous methanol solution through columns of Dowex 50 (H^+ form) and Dowex 1 (OH^- form) gave cyclo-[D-Phe-Leu-Orn(δ-Z)-Val-Pro-]$_2$ (XV), 49% from XIV, mp 250-251° dec, $[\alpha]_D$ -41.3° (AcOH) (Calcd: mol wt, 1410. Found: 1404 (13)). Hydrogenation of XV in the presence of 2 equiv of hydrogen chloride in methanol yielded crystalline retroGS·2HCl·$6H_2O$ (XVI), 92%, mp 273-276° dec, $[\alpha]_D$ -36.3° (c 0.1, EtOH) (Anal. Calcd for $C_{60}H_{106}O_{16}N_{12}Cl_2$: C, 54.49; H, 8.08; N, 12.71. Found: C, 54.64; H, 7.89; N, 13.51).

The synthesis of XV was also attempted by possible dimerization reaction of the pentapeptide p-nitrophenyl ester trifluoroacetate (XVII·CF_3COOH) derived from VII. The trifluoroacetate XVII was treated with pyridine (concentration of XVII, 3 x 10^{-3} M) (14); the throughout effluent from Dowex 50 and 1 columns contained two components. These were separated by the use of a Sephadex LH-20 column (solvent, methanol): XV, 22.6% from VII, mp 251-252° dec, and cyclo-[D-Phe-Leu-Orn(δ-Z)-Val-Pro-] (XVIII), 25.5% from VII (15), mp 230-232° dec, $[\alpha]_D$ -50.9° (AcOH) (Calcd: mol wt, 707. Found: 681

'13)). Hydrogenation of XVIII yielded retrocyclosemiGS·HCl·$3H_2O$ (XIX), 84%, mp 252-253° dec, $[\alpha]_D$ -60.0° (c 0.5, EtOH). Homogeneity of XVI and XIX was also ascertained by paper and thin-layer chromatographies, paper electrophoresis and carboxymethylcellulose column chromatography.

The antibacterial activity toward several microorganisms was examined (16). The compound XIX showed no activity for any of the microorganisms (17). GS and XVI showed also no activity for the Gram negative microorganisms (E. coli and others) at 100 μg/ml. On the other hand, minimum concentrations of growth-inhibition for the Gram positive microorganisms (Staph. aureus and B. subtilis) were found to be 5 μg/ml with GS and 50 μg/ml with XVI. These results correspond to out expectation.

REFERENCES AND FOOTNOTES

1. H. Aoyagi, T. Kato, M. Ohno, M. Kondo and N. Izumiya, J. Am. Chem. Soc. 86, 5700 (1964); Bull. Chem. Soc. Japan 38, 2139 (1965).
2. V. Prelog and H. Gerlach, Helv. Chim. Acta 47, 2288 (1964).
3. See for recent example; D. Balasubramanian, J. Am. Chem. Soc. 89, 5445 (1967).
4. D. C. Hodgkin and B. M. Oughton, Biochem. J. 65, 752 (1957).
5. E. P. Abraham, Comprehensive Biochemistry, Vol. 11, p. 181. Elsevier, Amsterdam (1963); Y. Miki, J. Osaka City Medical Center 9, 4005 (1960).
6. S. Lande, J. Org. Chem. 27, 4558 (1962); K. Vogler, P. Lanz and W. Lergier, Helv. Chim. Acta 45, 561 (1962).
7. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov and I. D. Ryabova, Experientia 23, 326 (1967). They have described also with footnote that retro-[5,5'-glycine]-GS is highly active as [5,5'-glycine]-GS.
8. Satisfactory elemental analyses and chromatographic data were obtained for all crystalline compounds described here. $[\alpha]_D$ refers to a solution in dimethylformamide at 20-25° otherwise noted. Z-, benzyloxycarbonyl; Z(OMe)-, p-methoxybenzyloxycarbonyl; Nps-, o-nitrophenylsulfenyl; ONp, p-nitrophenoxy. Amino acid symbols except D-Phe denote the L configuration.

9. T. Kato and N. Izumiya, Bull. Chem. Soc. Japan 39, 2242 (1966).

10. M. Kondo, H. Aoyagi, T. Kato and N. Izumiya, ibid. 39, 2234 (1966).

11. Saponification of Z-Arg(ω-NO_2)-Gly_4-OEt yielded 5-[1-carboxy-4-(3-nitroguanidino)-butyl]-hydantoyl-Gly_3-OH: M. Bodanszky, J. T. Sheehan, M. A. Ondetti and S. Lande, J. Am. Chem. Soc. 85, 99 (1963).

12. L. Zervas, D. Borvas and E. Gazis, ibid. 85, 3660 (1963).

13. With Hitachi Type 115 Osmometer (solvent, methanol).

14. M. Waki and N. Izumiya, J. Am. Chem. Soc. 89, 1278 (1967); Bull. Chem. Soc. Japan 40, 1687 (1967).

15. The data show that weight ratio of the monomer (XVIII) and the dimer (XV) in the crude product is 53:47. Whereas, weight ratio of the monomer and the dimer after the cyclization reaction of H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-ONp (XX) was found to be 32:68 (14). In spite of the profound difference of the amino acid sequences between XVII and XX, the formation of the protected cyclic decapeptide (XV or diZ-GS) in considerable amount after the cyclization reaction should suggest the stable conformation of XV or diZ-GS molecule.

16. The minimum amount of the compound necessary for the complete inhibition of growth was determined by a dilution method using a synthetic medium (We are indebted to Dr. M. Shibata of Takeda Chemical Industries, Ltd. for this assay).

17. We have observed that cyclic pentapeptides synthesized in this laboratory, cyclosemiGS (14) and its analogs, exhibit no activity.