volume 2

# CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

edited by
Boris Weinstein

# CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

A Survey of Recent Developments

◄ *Volume 2* ►

Edited by

**BORIS WEINSTEIN**

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

MARCEL DEKKER, INC., New York

MARCEL DEKKER, INC.

270 Madison Avenue, New York, New York 10016

LIBRARY OF CONBRESS CATALOG CARD NUMBER: 75-142896

ISBN: 0-8247-1769-4

Current printing (last digit):

10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

## ABOUT THE SERIES

The amide bond is one of the less reactive organic functional groups, yet it serves as the cornerstone for the building of the many peptides and proteins found in living systems. The evolving science of molecular biology has served to stress again that the chemistry and biochemistry of amino acids, peptides, and proteins is interwoven into a complex pattern, which on closer examination is found to be dependent on a host of secondary factors associated with individual compounds. There has been a need for a new review series in this area, especially if the interrelationships between the various disciplines are to be discussed in a detailed fashion. In an ideal sense, each volume should contain some chapters on recent developments and applications of established techniques, whereas others might describe the background and problems for topics still under investigation. Too, the subjects encompassed here do permit a variety of treatments without undue duplication or specialization.

One need not remind the reader of the many life processes that are dependent upon specific amino acid, hormone, and enzyme systems. Each functions in a very unique fashion, yet, in the end, they must involve the reactions of fundamental organic chemistry. Sometimes this point is overlooked, and it will be restated in greater detail through the series. To balance the scale, the brief comment is made here that new protecting, labeling, and coupling agents are always desirable, but these must be put to the test by the synthesis or degradation of actual compounds, for which practical use exists in Nature.

It is anticipated that these volumes can be useful both to the specialist and nonspecialist, and may provide a reference point to those who may do research in a broad region, or to the active worker in a small field. Most importantly, these volumes can serve the general purpose of presenting various points of view on the amide bond to interested observers, who, at present, are unknown to one another.

BORIS WEINSTEIN

*Seattle, Washington*
*December, 1970*

# PREFACE

This volume, the second in a continuing series, covers a variety of topics of much interest to active investigators in the interrelated areas devoted to the biochemistry and chemistry of peptides.

The first chapter by Nobuo Izumiya and Tetsuo Kato describes the field of gramicidin antibiotics and the effect of structure on biological activity. The second by Haruaki Yajima and Hiroki Kawatani is a survey of the related effort given to the connection between structure and effect for adrenocorticotropic hormone. The next by K. Jankowski discusses the reactions between amino acid and small-ring organic compounds. A review by Darrell J. Woodman appraises the isoxazolium salt coupling procedure from the viewpoint of both mechanism and application. The last by Kaoru Harada summarizes the data now available to explain the prebiotic synthesis of amino acids and peptides.

The initial review was received in the late Fall of 1971, while the last was obtained in the Summer of 1972. As a result, the literature is covered through 1970, and, in several cases, selected citations are given for 1971. The authors are gratefully thanked for their contributions, and any errors, omissions, and delays are the responsibility of the editor.

It is hoped that the good reception given to the first volume in 1971 will continue through this and succeeding ones. Any suggestions as to format and content will be welcomed by the editor.

BORIS WEINSTEIN

*Seattle, Washington*
*November, 1972*

## CONTRIBUTORS TO THIS VOLUME

Kaoru Harada, Institute for Molecular and Cellular Evolution, and Department of Chemistry, University of Miami, Coral Gables, Florida

Nobuo Izumiya, Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka, Japan

K. Jankowski, Université de Moncton, Moncton, New Brunswick, Canada

Tetsuo Kato, Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka, Japan

H. Kawatani, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Darrell J. Woodman, Department of Chemistry, University of Washington, Seattle, Washington

H. Yajima, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

## CONTENTS OF VOLUME I

## CONTENTS

# CHAPTER 1
# CHEMISTRY AND BIOCHEMISTRY OF GRAMICIDIN S AND RELATED COMPOUNDS

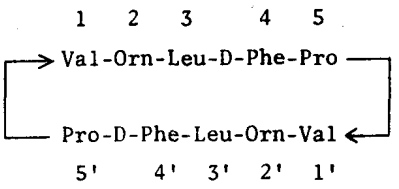
Tetsuo Kato and Nobuo Izumiya

Laboratory of Biochemistry
Faculty of Science
Kyushu University
Fukuoka, Japan

## I. INTRODUCTION

In 1939, Dubos isolated a crude antibiotic preparation, named tyrothricin, from cultures of Bacillus brevis [1]. It was recognized afterward that tyrothricin was composed of two crystalline components [2]. The first, a neutral fraction, was called gramicidin, and contained several linear polypeptide derivatives, while the second material consisted of a mixture of basic peptides. The latter, the tyrocidines, were separated by Battersby and Craig with the aid of countercurrent distribution techniques [3].

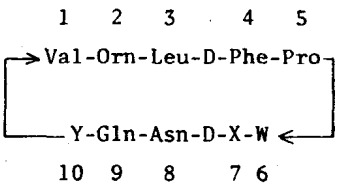
In search of a similar antibiotic from Russian soil, Gause and Brazhnikova carried out extensive microbiologic screening tests and discovered another peptide antibiotic from a strain of B. brevis [4]. This compound was named gramicidin S. After purification by crystallization, its homogeneity was established by classic analytical [5], diffusion [6], and countercurrent distribution methods [7]. Amino acid [5], sequence [8, 9] and 2,4-dinitrophenylamino acid determinations [10] indicated gramicidin S to be a cyclodecapeptide containing the pentapeptide sequence -Val-Orn-Leu-D-Phe-Pro-, which is repeated twice to form a 30-membered macrocyclic structure (1). The results of x-ray crystallographic studies [11, 12], diffusion measurements [6], and a molecular weight determination [13] provided further evidence for this structure.




(1)*

Pure gramicidin S dihydrochloride, m.p. 277-278°C, $[\alpha]_D$ = -289° ± 10° (c 0.43, 70% ethanol) [14], kills Staphylococci at a concentration of 3 μg/ml, and E. coli at 50 μg/ml in a nutritive medium [4].

Both the Craig and the Kurahashi groups [15-19] have determined the structures 2 to 6 of a number of tyrocidines. The results indicate that tyrocidines are also cyclic decapeptides and contain the same pentapeptide sequence as gramicidin S, but the other half of the molecule is built from different sequences. As a result, it may not be inappropriate to conclude that gramicidin S is just another particular variant of the tyrocidine family [20]. A typical example of the neutral gramicidins of Dubos, valine-gramicidin A, has the structure N-formylpentadecapeptide ethanolamide (7) [21].




(2)-(6)

*Abbreviations used are: Z, benzyloxycarbonyl; Trt, triphenylmethyl; Tos, p-toluenesulfonyl; Z($OCH_3$), p-methoxybenzyloxycarbonyl; Boc, tert-butyloxycarbonyl; OMe, methyl ester; ONp, p-nitrophenyl ester; TFA, trifluoroacetic acid; DCC, N,N'-dicyclohexylcarbodiimide. Amino acid symbols denote the L configuration unless otherwise indicated by D or DL.

| | | W | X | Y | References |
|---|---|---|---|---|---|
| Tyrocidine A | (2) | Phe | Phe | Tyr | 15 |
| B | (3) | Trp | Phe | Tyr | 16 |
| C | (4) | Trp | Trp | Tyr | 17 |
| D | (5) | Trp | Trp | Trp | 18 |
| E | (6) | Phe | Phe | Phe | 19 |

HCO-Val-Gly-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-
Trp-D-Leu-Trp-D-Leu-Trp-$NHCH_2CH_2OH$

(7)

## II. BIOSYNTHESIS OF GRAMICIDIN S

Ample evidence indicates that the biosynthesis of peptide antibiotics is independent of the ribosomal RNA-dependent process, such as protein synthesis; rather, it is dependent on a purely enzymatic process. Several oligopeptides that are possible intermediates in the biosynthesis of gramicidin S have been isolated in cell-free extracts of B. brevis; for example, H-D-Phe-Pro-OH [22], H-D-Phe-Pro-Val-OH [23], and H-D-Phe-Pro-Val-Orn-OH [24]. Yamada and Kurahashi [25] found an enzyme involved in the racemization of L-phenylalanine to the D- form. This observation explains the reason for the L-phenylalanine requirement by the bacteria.

Recently, Lipmann [26] discovered a multiple enzyme system composed of two active fractions, I and II, involved in the biosynthesis of gramicidin S. Fraction I is a complex capable of activating the four constituent L-amino acids of gramicidin S. Fraction II activates, racemizes, and incorporates phenylalanine into the product whenever this particular amino acid residue has the D- configuration. Further, II catalyzes a condensation

between the carboxyl group of D-phenylalanine, bound to an enzyme sulfhydryl group, and the free imino group of L-proline, which is one of the four L-amino acids all linked by their carboxyl functions to separate sulfhydryl groups within fraction I. Successive reactions within the enzyme system result in the generation of peptide-active thioester chains and ultimately in the formation of gramicidin S. However, there is no evidence for the existence of chains between six and nine residues long. A doubling cyclization reaction between two antiparallel pentapeptide units might then form gramicidin S in this biosynthetic procedure [26].

Within the past year, Laland [27] showed that the cyclization of two pentapeptides takes place on the same enzyme molecule in the form of a head-to-tail condensation and suggested a scheme for the cyclization reaction involving a 4'-phosphopantetheine arm. The phosphopantetheine appears to transfer each intermediate peptide to the corresponding enzyme in order to make a thioester linkage, as in the case of fatty acid biosynthesis [28].

On the other hand, a three-component enzyme system active in tyrocidine biosynthesis was prepared from B. brevis ATCC 8185. Each fraction activates phenylalanine, proline, and the remaining tyrocidine constituent amino acids [29]. Nine enzyme-bound intermediate peptides were isolated from cell-free systems [30]. All the peptides, including H-Phe-Pro-OH, H-Phe-Pro-Phe-OH, and others, up to the linear decapeptide H-Phe-Pro-Phe-Phe-Asn-Gln-Phe-Val-Orn-Leu-OH, were bound to the enzyme proteins by thioester linkages, as in the case of gramicidin S. An interesting difference between the gramicidin S and the tyrocidine [29] biosynthetic processes is that cyclization in the former seems to be a rather fast process, but in the latter case, cyclization probably is a rate-limiting process.

Pollard et al. [31] found another possible precursor of gramicidin S in cell-free extracts of B. brevis ATCC 9999. A linear decapeptide formula has been assigned to this product (8).

HCO-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-$NHCH_2CH_2OH$

(8)

Since the N-formylpentadecapeptide ethanolamide structure (7) of the linear gramicidins of Dubos resembles this possible intermediate (8) the two families of antibiotics found in Bacillus species, the linear gramicidins and the cyclic tyrocidines, appear to be more closely related to each other than their apparent structures would suggest. However, there is no direct evidence for compound (8) being a precursor of gramicidin S. This polyamide might be a mere artifact of the cell-free system in which the peptide is found free [31].

## III. CHEMICAL SYNTHESIS OF GRAMICIDIN S

Schwyzer and Sieber achieved the synthesis of gramicidin S in 1957 [14]. This was the first chemical preparation of a cyclopeptide antibiotic. The reactions are described in Fig. 1.

The protected pentapeptide methyl ester (9) derived from Z-Val-Orn(Tos)-$N_3$ and H-Leu-D-Phe-Pro-OMe was converted to the corresponding trityl derivative (11). After saponification, the protected pentapeptide acid (12) was coupled with the pentapeptide ester (10) through the use of dicyclohexylcarbodiimide to yield the decapeptide derivative (13). Saponification formed the protected decapeptide acid (14), which was converted to the corresponding p-nitrophenyl ester (15) by the action of di-p-nitrophenyl sulfite [32]. After cleavage of the trityl group, the decapeptide ester trifluoroacetate (16) was subjected to cyclization in a large volume of pyridine. Purification on the ion-exchange resins and crystallization gave ditosylcyclodecapeptide (17) in 28% yield. Removal of the tosyl groups by treatment with sodium in liquid ammonia afforded the desired cyclodecapeptide as a dihydrochloride salt (I·2HCl).

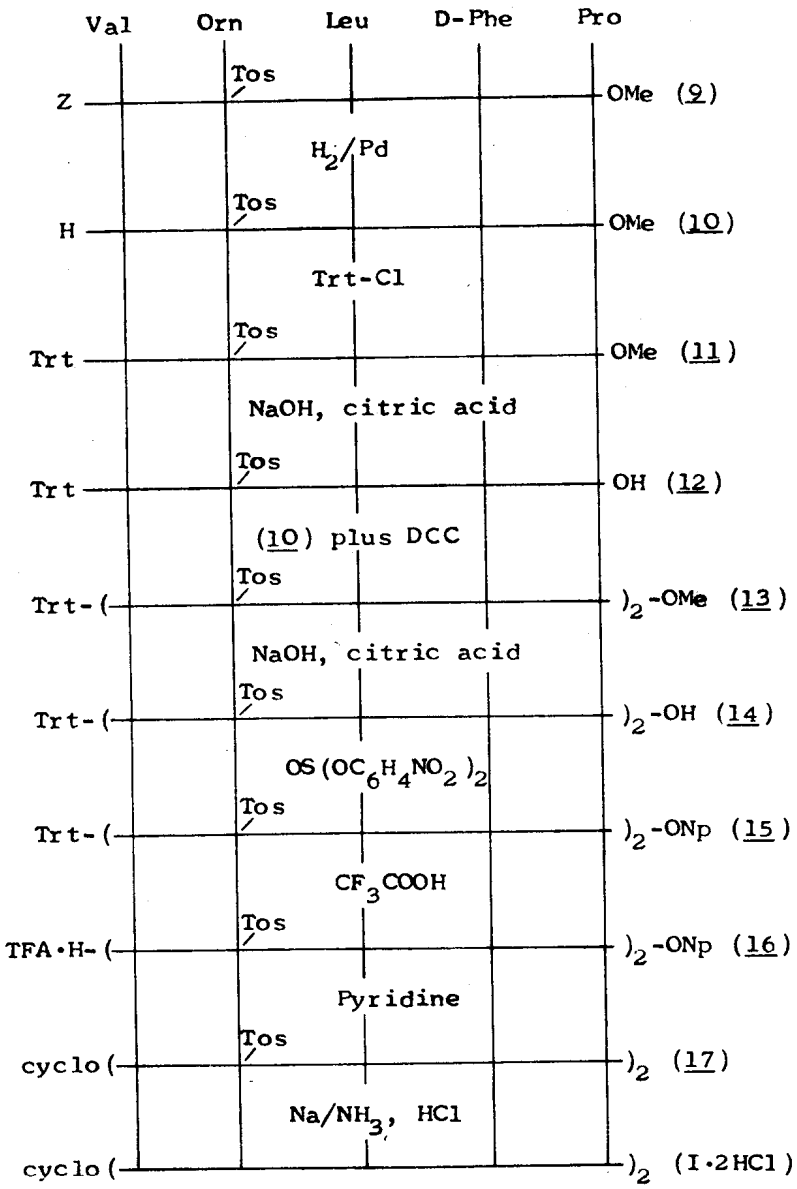



FIG. 1. Synthesis of gramicidin S.

The final cyclopeptide was found to be identical with natural gramicidin S on the basis of comparison of melting points, $R_f$ values on paper chromatograms in various solvent systems, infrared spectra, x-ray diffraction patterns, and microbiologic assays.

Schwyzer and Sieber synthesized gramicidin S not only from the corresponding decapeptide active ester, but also with the pentapeptide active ester H-Val-Orn(Tos)-Leu-D-Phe-Pro-ONp [33]. Two molecules of the latter combined to give ditosylgramicidin S through a doubling cyclization. Schwyzer explained the mode of this reaction as follows [34]: When the L-tripeptide sequence -Val-Orn-Leu- of the two molecules takes the antiparallel β-pleated sheet structure proposed by Pauling and Corey [35], the C-terminal active esters will be placed opposite the amino group of another molecule because the peptide backbone kinks at the position of D-phenylalanine and L-proline residues, as shown in Fig. 2. This orientation will lead to the doubling cyclization reaction and will afford the structure for gramicidin S depicted in Fig. 3.

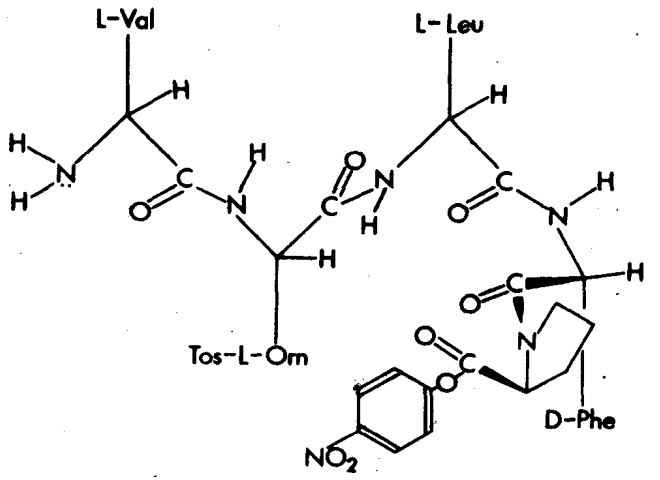



FIG. 2. A possible conformation of the pentapeptide active ester in a doubling cyclization reaction.