Volume 7

CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

edited dy

Boris Weinstein

CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

A Survey of Recent Developments

◄ *Volume 7* ►

Edited by

BORIS WEINSTEIN

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

MARCEL DEKKER, INC. New York and Basel

The Library of Congress has cataloged this serial publication as follows: 75-142896

MARCEL DEKKER, INC.

270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

ISBN: 0-8247-7027-7 (v.7)

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.

Seattle, Washington BORIS WEINSTEIN

PREFACE

The world of peptide chemistry continues to turn, and with the passage of time biochemical aspects of the subject become more and more important. Accordingly, it is a bit difficult to predict what may be of interest in a review book, as various subjects and techniques wax and wane over the years. This particular volume, therefore, attempts to tread a narrow, middle line, but the peptide connoisseur should find much of lasting interest here.

The first chapter by Richard K. Olsen concerns the interesting quinoxaline depsipeptide antibiotics, while Raniero Rocchi and Virgilio Giormani discuss in the second chapter the glycoproteins, with special emphasis on synthetic analogs. Jacques Tempé in the third chapter writes on open-chain imino acids, a subject of much interest to plant biologists, while the fourth chapter by Roger W. Roeske and Stephen J. Kennedy reviews the important area of ion-transporting peptides. Lastly, Arno F. Spatola in the fifth chapter covers reverse bond peptides, a recent development that has interesting possibilities. It is hoped that these selections shall be of value to a wide circle of readers and serve as permanent reference sources.

Seattle, Washington BORIS WEINSTEIN

ACKNOWLEDGMENT

We would like to thank Drs. Edward Orton and Loren Pickart for their assistance with the final editing of this volume during Dr. Weinstein's illness.

CONTRIBUTORS TO THIS VOLUME

VIRGILIO GIORMANI, Centro di Studi sui Biopolimeri del Consiglio Nazionale delle Ricerche, Università degli Studi di Padova, Padova, Italy

STEPHEN J. KENNEDY, Department of Physiology and Biophysics, University of Miami, School of Medicine, Miami, Florida

RICHARD K. OLSEN, Department of Chemistry and Biochemistry, Utah State University, Logan, Utah

RANIERO ROCCHI, Istituto di Chimica Organica, Università degli Studi di Padova, Padova, Italy

ROGER W. ROESKE, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Indiana

ARNO F. SPATOLA, Department of Chemistry, University of Louisville, Louisville, Kentucky

JACQUES TEMPÉ,* Crown Gall Research Laboratory, Genetics and Plant Breeding Station, National Center for Agricultural Research, Versailles, France

---

*Present address: Groupe de Recherche sur les Interactions Micro-organisms et Plantes, Institute de Microbiologie, Batiment 409, Universite de Paris-Sud, Orsay.

CONTENTS

CONTENTS OF OTHER VOLUMES

Volume 1

Volume 2

Volume 3

Volume 4

Volume 5

Volume 6

CHAPTER 1

THE QUINOXALINE DEPSIPEPTIDE ANTIBIOTICS

Richard K. Olsen

Department of Chemistry and Biochemistry
Utah State University
Logan, Utah

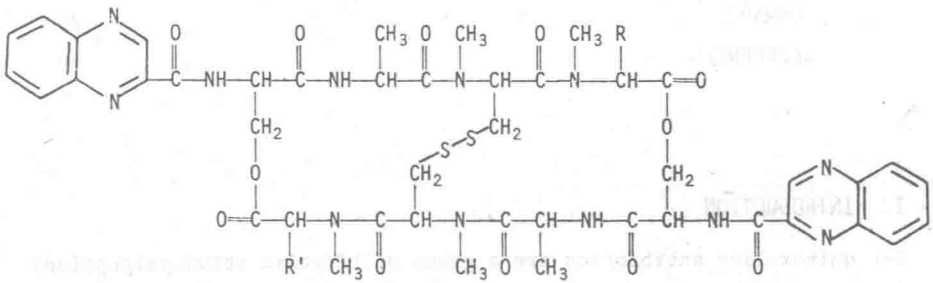
I. INTRODUCTION

The quinoxaline antibiotics are a group of bicyclic octadepsipeptides that are of considerable interest due to their potent biological properties and mode of action in binding to deoxyribonucleic acid (DNA). The antibiotics are produced by several species of streptomyces and the isolation of the first quinoxaline antibiotics was reported in 1954 [1,2].

Two families of the quinoxaline antibiotics, the triostins and quinomycins, are known. The structure of triostin A (1a) is composed of two units each of D-serine, L-alanine, N-methyl-L-cysteine, and N-methyl-L-valine. The two depsipeptide bonds occur between the respective hydroxyl groups of D-serine and carboxyl groups of N-methyl-L-valine, while a disulfide bridge exists between the N-methyl-L-cysteine units. A quinoxaline-2-carbonyl (Qxc) moiety is attached to the amino group of each D-serine. The molecule is symmetrical and possesses a twofold rotational axis passing through the disulfide bridge.

Echinomycin (2a), the most prominent of the quinomycin antibiotics, is identical to triostin A except for the novel unsymmetrical dithioacetal moiety present in place of the disulfide linkage common to the triostins. Other members of the triostins and quinomycins are known in which the position of the valine residues is modified by replacement by other aliphatic amino acids.

The quinoxaline antibiotics possess significant biological activity and currently are of considerable interest. The antibiotics show antimicrobial, antiviral, and cytotoxic activity. Of special significance, the quinoxalines have been shown to bind to DNA by a

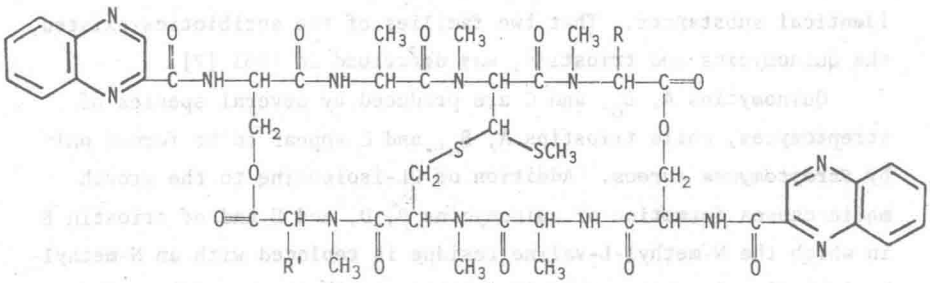


1a Triostin A R = R' = $CH(CH_3)_2$

1b Triostin B R = R' = $CH(CH_3)CH_2CH_3$

1c Triostin C R = R' = $CH(CH_3)CH(CH_3)_2$

1d Triostin B_o R or R' = $CH(CH_3)_2$ and $CH(CH_3)CH(CH_3)_2$



2a	Echinomycin (Quinomycin A)	R = R' = $CH(CH_3)_2$
2b	Quinomycin B	R = R' = $CH(CH_3)CH_2CH_3$
2c	Quinomycin C	R = R' = $CH(CH_3)CH(CH_3)_2$
2d	Quinomycin D	R or R' = $CH(CH_3)_2$ and $CH(CH_3)CH_2CH_3$
2e	Quinomycin E	R or R' = $CH(CH_3)CH_2CH_3$ and $CH(CH_3)CH(CH_3)_2$
2f	Quinomycin B_0	R or R' = $CH(CH_3)_2$ and $CH(CH_3)CH(CH_3)_2$

bifunctional intercalation mechanism involving both of the quinoxaline rings, resulting in the biological activity observed for these substances. The biological properties of the quinoxalines have been adequately discussed [3], while a similar summary [4] has appeared on the mode of action of the antibiotics in binding to DNA. This review, while surveying the above-mentioned subjects, will focus on recent structural and synthetic studies on the quinoxaline depsipeptide antibiotics. The literature on this subject has been surveyed through June, 1982.

II. ISOLATION AND DETERMINATION OF STRUCTURE

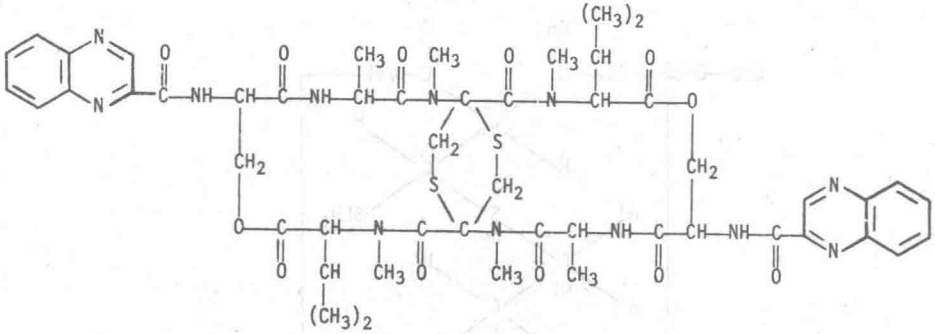
Since the quinoxaline antibiotics were independently isolated and reported in 1954 [1,2], other reports related to the isolation of additional quinoxaline antibiotics have appeared [5-7]. It was early recognized that certain of the antibiotics (i.e., levomycin, actinoleukin, antibiotic X-948, echinomycin, and quinomycin A) were

identical substances. That two families of the antibiotics existed, the quinomycins and triostins, was described in 1961 [7].

Quinomycins A, B_o, and C are produced by several species of streptomyces, while triostins A, B_o, and C appear to be formed only by *Streptomyces aureus*. Addition of DL-isoleucine to the growth media causes formation of quinomycins B, D, and E and of triostin B in which the N-methyl-L-valine residue is replaced with an N-methyl-L-alloisoleucine or an N, γ-dimethyl-L-alloisoleucine [8].

The structural elucidation of a quinoxaline antibiotic was first reported for echinomycin in 1959 [9]. Elemental analysis and molecular weight determination led to acceptance of a molecular formula of $C_{50}H_{60}N_{12}O_{12}S_2$. Analysis of hydrolysates established the presence of two residues each of D-serine, L-alanine, N-methyl-L-valine, and quinoxaline-2-carboxylic acid. A characteristic absorption spectrum was observed for the quinoxaline chromophore with maxima at 243 and 320 nm. The nature of the sulfur-containing amino acid component, however, was not readily apparent. Raney nickel desulfurization, followed by hydrolysis, produced N-methyl-L-alanine plus the three amino acids previously identified. Other studies provided evidence for the ester bond between serine and valine, while partial acid hydrolysis upon desthioechinomycin allowed deduction of the amino acid sequence as being D-serine, L-alanine, N-methyl-L-alanine, and N-methyl-L-valine. From consideration of the available data, a 1,4-dithiane ring was proposed for the sulfur moiety of echinomycin, with the complete structure of the antibiotic as shown in 3.

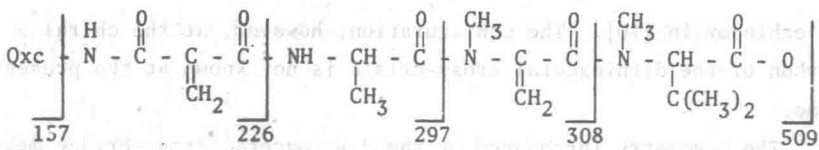
In 1975, two groups independently published nuclear magnetic resonance (nmr) and mass spectral data that led to a revised structure for echinomycin [10,11]. Most notably, the 100 MHz ^1H nmr spectrum of echinomycin showed the presence of an SCH_3 group as a singlet at δ 2.04, while lacking peaks assignable to the expected S-methylene groups of a dithiane ring. The existence of an -S-CHH'-CH-XY system and an S_2-CH-CH-X'Y' system was established by decoupling experiments. These data allowed assignment of an unsymmetrical dithioacetal bridge between the two peptide chains in



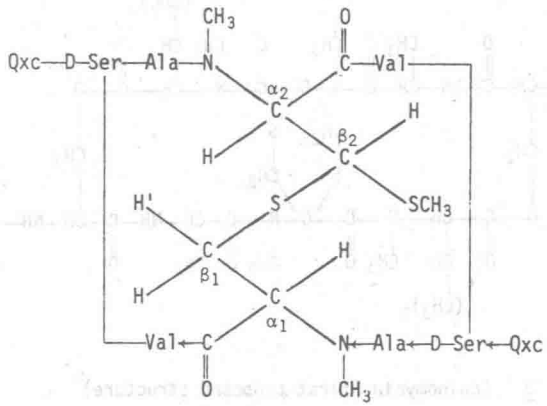
3 Echinomycin (first proposed structure)

echinomycin, as shown in Figure 1. The dithioacetal unit is equivalent to the occurrence of a β-methylthiolanthionine unit in echinomycin. The asymmetry introduced by the dithioacetal group was evident in that two singlets were observed at low field δ 9.36 and 9.41, for the H-3 protons of two nonequivalent quinoxaline rings.

^{13}C nmr data for echinomycin showed the presence of 51 carbon atoms, while off-resonance decoupling studies provided data consistent with the revised structure. A molecular ion at 1100 mass units consistent for a revised molecular formula of $C_{51}H_{64}N_{12}O_{12}S_2$, was observed using field desorption mass spectrometry [10,11]. The amino acid sequence in echinomycin was verified by the observed mass peaks resulting from fragmentation of the dehydroamino acid-containing tetrapeptide formed by scission of the cross-bridge and depsipeptide bonds of echinomycin, as shown below. Detailed mass spectral



studies on transformation products of echinomycin, formed by hydrolytic and reductive rupture of either the depsipeptide or dithioacetal functions, provided additional evidence for the revised structure



Protons(s)	Shift, δ*	Additional Data
SCH_3	2.04	Singlet
β_1CH H'	2.86	AB pattern, J_{AB} 15.0 Hz
β_1CH H'	3.40	JHα 9.0 Hz, JH'α < 4.0 Hz
α_2CH	4.96	Buried under multiplet at δ 4.5-5.0
α_1CH	6.10	Broad doublet, decoupled by irradiation at δ 2.86
β_2CH	6.44	Doublet (J = 9.0 Hz), decoupled by irradiation at δ 4.96

*Taken from reference 10.

FIG. 1. Revised structure of echinomycin and pertinent ^{1}H nmr data for dithioacetal function. (From Ref. 10.)

of echinomycin [10]. The configuration, however, at the chiral β carbon of the dithioacetal cross-bridge is not known at the present time.

The asymmetry introduced by the dithioacetal cross-bridge makes possible the existence of positional isomers in quinomycins D, E, and B_o due to the presence of two different alkyl side chains at the valine positions in each of these molecules (see 2d, 2e, and 2f).