volume 4

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 4* ►

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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

Strong interest in amino acid chemistry and biochemistry continues on a wide scale, so it is with pleasure that volume four in the series is appearing at this time.

The first chapter by John S. Davies introduces the topic of D-amino acids, which are found in a variety of natural products, including cell walls and plants. The second by John H. Jones involves a discussion of sequential polypeptide synthesis, a topic of both practical and theoretical interest to both organic and polymer chemists. The third by James C. Powers concerns haloketone inhibitors of proteolytic enzymes, a broad and fascinating field that embraces both simple organic chemistry and various aspects of enzymology. The fourth by Anthony B. Mauger concerns the chemistry of cyclic α-imino acids, which includes a host of four, five, six, and seven membered compounds. The fifth, and last, by Charles N. C. Drey summarizes the chemistry and biochemistry of β-amino acids, a hitherto neglected area, but one that promises to be of greater interest in the near future.

The initial review was obtained in December, 1975, and the last in August, 1976. Where possible, the reviews were updated and, in general, the literature is covered through late 1976. The authors are due a well-earned acknowledgment, both for their writing duties and for the efforts put into the final production stages.

Work is underway on the next volume and any comments as to possible reviews would be welcomed.

Seattle, Washington BORIS WEINSTEIN

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CONTENTS OF OTHER VOLUMES

Volume 1

THE OPTICAL ANALYSIS OF AMINO ACID DERIVATIVES BY GAS CHROMATOGRAPHY, *John W. Westley*

THE CHEMISTRY OF CYCLOSERINE, *Charles H. Stammer*

THE USE OF HYDROGEN FLUORIDE IN PEPTIDE CHEMISTRY, *S. Sakakibara*

γ-GLUTAMYL PEPTIDES: CHEMISTRY AND IMMUNOCHEMISTRY, *D. E. Nitecki and J. W. Goodman*

PEPTIDE ALKALOIDS, *Mary Pais and Francois-Xavier Jarreau*

Volume 2

CHEMISTRY AND BIOCHEMISTRY OF GRAMICIDIN S AND RELATED COMPOUNDS, *Tetsuo Kato and Nobuo Izymiya*

SYNTHESIS OF ACTH-ACTIVE PEPTIDES AND ANALOGS, *H. Yajima and H. Kawatani*

REACTION OF SMALL HETEROCYCLIC COMPOUNDS WITH AMINO ACIDS, *K. Jankowski*

THE ISOXAZOLIUM SALT METHOD OF PEPTIDE SYNTHESIS, *Darrell J. Woodman*

SYNTHESIS OF AMINO ACIDS AND PEPTIDES UNDER POSSIBLE PREBIOTIC CONDITIONS, *Kaoru Harada*

Volume 3

CONFORMATIONS OF PEPTIDES IN SOLUTION AS DETERMINED BY NMR SPECTROSCOPY AND OTHER PHYSICAL METHODS, *Victor J. Hruby*

NATURALLY OCCURRING AMINO ACID AND OLIGOPEPTIDE ANTIMETABOLITES, *James P. Scannell and David L. Pruess*

THE CHEMISTRY OF DIOXYGENASES, *Dennis G. Brown*

CONTENTS

CHAPTER 1

OCCURRENCE AND BIOSYNTHESIS OF D-AMINO ACIDS

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I. INTRODUCTION

The biosynthesis of the amino acids has been intensively studied over the past three decades and two very comprehensive reviews [1] and a monograph [2] summarize the developments up to 1968. Interest in the subject has also justified periodic coverage in the Annual Review series [3], while a recent book [4] discusses in depth the shikimate pathway to the aromatic amino acids.

Techniques from the disciplines of organic chemistry, biochemistry, enzymology, genetics, and microbiology have been used to elucidate the details of the biosynthetic pathways and their regulatory control [5]. The stage has now been reached whereby precise stereochemical definition of specific steps in the pathways can be made. This coincides with the current interest in the use of stereochemically defined precursors [6,7]. The greater precision achieved by doubly labeled (^3H and ^{14}C) precursors and intermediates augment the detailed understanding of the pathways [8,9]. The use of prochirally labeled compounds--a technique exemplified by the use of prochiral valine, i.e., valine labeled with either ^{13}C or ^2H at the β-center [10]--points to current trends in this field.

The major emphasis, quite naturally, has been based on the 20 "protein" amino acids commonly found in proteins, all bearing the L-configuration. But contemporary to the developments in the biosynthesis of these protein amino acids, great progress has been made in the identification of large numbers of "nonprotein" amino acids with very diverse structures. Recent reviews estimate the total number of known amino acids to be of the order of 200-300. The majority of these amino acids have been found in plants [11] and microorganisms [12]. Many of the structures found represent only secondary modifications to those of the protein amino acids, and it can be presumed, therefore, that their biosynthesis in the main follow similar patterns to the primary pathways. However, at some stage a secondary modification has to be carried out, as for example in the formation of D-forms of amino acids. The aim of this chapter is to review the current evidence on the biosynthetic origins of D-amino acids. The review covers in detail papers published up to June 1975, but the literature was scanned to the end of 1976.

II. SOURCES OF D-AMINO ACIDS IN NATURE

Peptide antibiotics produced by a range of microorganisms appear to be the richest sources of D-amino acids [12], while an increasing number are being identified in plants. However, this rather limited distribution of D-amino acids inevitably reflects the screening methods. The peptides have been identified because of their antibiotic activity rather than as a means of assessing D-amino acid distribution. It is therefore quite conceivable that these amino acids have a much wider distribution. Proving that the amino acid has a D-configuration in its natural form has always been a problem, but since the majority of the peptide antibiotics have now been completely characterized, i.e., with the particular D-amino acid in position, there is good evidence for individual authentication of this configuration. Identifying a D-configuration in an isolated amino acid without the advantage of an attached group raises many more problems, since the possibility of racemization during isolation procedures, etc. cannot always be ruled out.

Table 1 updates earlier lists [12,13] of D-amino acids to include more recent characterizations, while Table 2 lists D-amino acids recently isolated from plants. Taking due note of the comments in the preceding paragraph about the possibility of the survey being restricted, it is still worth emphasizing that the amino acids which exist most widely in the D-form in microorganisms (Table 1) seem to have predominantly hydrophobic side chains, or side chain groups, capable of cross-linking to form cyclic structures. This observation might be taken as an indication of their function in nature as discussed in Sect. III.

TABLE 1

D-Amino Acids in Peptides Isolated from Microorganisms

D-Amino acid	Peptides (including those having nonpeptidic features)
Amino adipic acid	Cephalosporin C [14], penicillin N [14], peptides from *Cephalosporium* [15]
Alanine	Enduracidin [16], stendomycin [17], ostreogrycin B_1* [18], vernamycin B_γ* [19], pristinamycin I_C* [20], bacterial cell wall glycopeptides [21], mycosides from mycobacteria [22], glyco-amino acid esters [23], cycloheptamycin [24], prumycin [25]

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*Now known to be the same compounds.

TABLE 1 (continued)

D-Amino acid	Peptides (including those having nonpeptidic features)
αβ-Diaminobutyric acid	Amphomycin [26], aspartocin [27], polymyxins [28]
Aspartic acid	Mycobacillin [29], antibiotics A-128-OP and A-128-P [30], bacitracin [31]
erythro-Hydroxyaspartic acid	Phallocidin [32]
Glutamic acid (or as glutamine)	Mycobacillin [29], bacitracin [31], bacterial cell wall glycopeptides [21], viscosin [33], poly(γ-D-glutamyl) capsule in *B acillus lichenformis* [34]
allo-Hydroxyproline	Etamycin [35]
allo-Isoleucine	Actinomycins C_2 and C_3 [36], angolide [37], peptido-lipid from *Nocardia asteroides* [38], sporidesmolides [39], stendomycin [17]
Isoleucine	Monamycin [40], antibiotic S-520 [41]
Cysteine	Malformin [42]
Leucine	Malformin [42], circulin B [43], gramicidin A [44], polymyxins [28], surfactin [45], mycosides [22], etamycin [35], isaarin [46], sporidesmolides [39]
N-Methylleucine	Monamycin [40], griselimycin [47]
Lanthionine	Nisin [48], subtilin [48]
3-Methyl lanthionine	Nisin [48], subtilin [48]
Lysine	Peptidoglycan from *Butyribacterium rettgeri* [49]
Ornithine	Peptidoglycans from *B. rettgeri* [49], and *Corynebacterium betae* [50], enduracidins [16], bacitracin [31]
Penicillamine (β-mercaptovaline)	Penicillins [51]
Phenylalanine	Gramicidin S [52], polymyxins [28,53], fungisporin [54], gramicidin S precursor [55], bacitracin [31], tyrocidins A, B, C [56] and E [57], peptidolipid [58]
Pipecolic acid	Amphomycin [59], aspartocin [60], glumamycin [61]

TABLE 1 (continued)

D-Amino acid	Peptides (including those having nonpeptidic features)
Proline	Ostreogrycin G [62]
Serine	Enduracidins [16], polymyxin D [28], viscosin [33], quinoxaline antibiotics, e.g., triostin [63], antibiotics A-128-OP and A-128-P [30]
allo-Threonine	Stendomycin [17], viscosin [33], enduracidins [16], tripeptide from *Keratinophyton terreum* [64], antibiotics A-128-OP and A-128-P [30], antibiotic LL-A-0341 [65], peptidolipids from mycobacteria [22]
Threonine	Phallotoxins [32]
Tryptophan	Tyrocidins C [56] and D [66]
Tyrosine (OMe)	Cycloheptamycin [24], cyclotetrapeptide Cyl-2 [67]
Valine	Peptides from *Cephalosporium* [15], stendomycin [17], gramicidin A [44], actinomycins C_1 and C_2 [36], fungisporin [54], viscosin [33], monamycins [40], antibiotic S-520 [41], valinomycin [68], sporidesmolides [39]
Piperazic acid (and chloropiperazic acid)	Monamycins [40]
allo-Enduracididine	Enduracidins [16]
α-[4-Hydroxyphenyl]-glycine	Enduracidins [16]

TABLE 2

D-Amino Acids from Plant Sources

D-Amino acid	Source
1-Amino proline	As 1-[(N-γ-L-Glu)amino]-D-proline in flaxseed (*Linum usitatissimum*) [69]
Alanine	As D-alanyl-D-alanine in tobacco leaves [70] and pasture grass [71]; as γ-L-glutamyl and N-malonyl derivative from *Pisum sativum* [72]

TABLE 2 (continued)

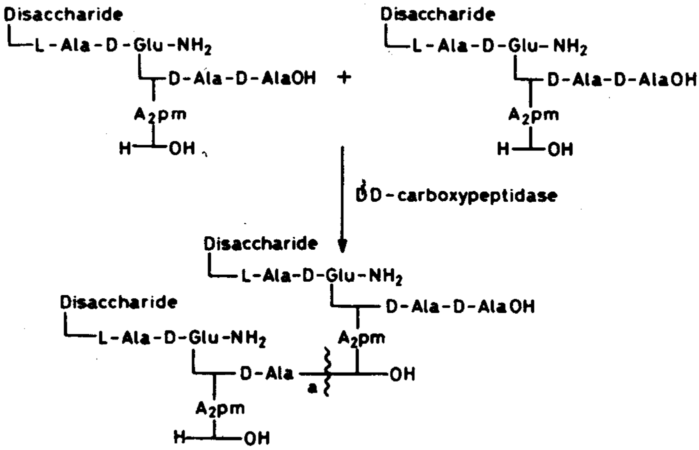
D-Amino acid	Source
4-Chlorotryptophan	As N-carboethoxyacetyl and N-carbomethoxyacetyl in *P. sativum* [73]
3-Carboxyphenylglycine (DL)	Bulbs of *Iris tingitana* and seeds of *Reseda luteola* [74]
3-Carboxy-4-hydroxyphenylglycine (DL) (enriched in D-isomer)	Seeds of *R. luteola* [74]
4-Hydroxymethylproline	Seeds of *Eriobotrya japonica* [75]
4-Methyleneproline (DL)	Seeds of *E. japonica* [76]
Methionine	As N-malonyl derivative in tobacco leaves [77]
Tryptophan	As N-malonyl derivative in *Caragana arborescens* seeds and *Malus pumila* fruits [78]

III. BIOLOGICAL ROLE OF THE D-AMINO ACIDS

No definite evidence seems to exist as to the exact function of D-form of amino acids. Therefore, this section can do no more than bring together cases where the D-form has been implicated as having a function. It is tempting, in broad terms, to dismiss their function to be a means of reducing the chances of degradation by L-specific enzymes, but it is obvious from the more recent work that their function may well be more subtle.

A. Bacterial Cell Walls

Studies on bacterial cell wall biosynthesis [79,80] have recently placed special significance on the role of the D-alanyl-D-alanyl sequence in a peptide precursor of the cell wall peptidoglycan. The final stages in the formation of peptidoglycan is envisaged as in Scheme 1 for a peptidoglycan in *Streptomyces*. Therefore the formation of the peptide bond ("a" in Scheme 1) corresponds to a



A_2pm = diaminopimelic acid

Scheme 1

transpeptidation at the D-Ala-D-Ala bond of the precursor with loss of a D-Ala residue. In vitro studies [80] confirm the necessity for the terminal D-Ala-D-Ala unit to be part of any enzyme substrate that will undergo transpeptidation. It is thus envisaged that the enzyme-substrate complex formed confers special reactivity on the D-Ala-D-Ala bond. Apart from the transpeptidation process, the enzyme is also capable of cleaving off the terminal D-Ala, thus preventing further cross-linking. For some time it was thought that the antibiotic activity of the penicillins involved stereochemical mimicking of the dialanyl residues at this late stage in cell wall biosynthesis, but the recent findings suggest that the penicillins bind to the enzyme at a different site to the natural substrate [81].

B. Peptide Antibiotics

The molecular structures of most of the peptide antibiotics listed in Table 1 can be classified as homodetic (with the amide bond as the only functional link in the ring) or heterodetic (where other atoms, e.g., sulfur or an ester bond are included) cyclic structures. The relatively small size of a number of the ring systems probably