CIBA FOUNDATION SYMPOSIUM

ON

AMINO ACIDS AND PEPTIDES WITH ANTIMETABOLIC ACTIVITY

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, *O.B.E.*, M.A., M.B., B.Ch.

and

CECILIA M. O'CONNOR, B.Sc.

With 28 Illustrations

PREFACE

In 1956 Professor Sir Alexander Todd and Professor F. Bergel suggested to the Director of the Ciba Foundation that it would be useful and timely to hold one of the Foundation's small international symposia on the biochemistry of amino acids and peptides with antimetabolic and cytotoxic properties. They proposed the early part of 1958 as an appropriate date for the meeting. During the ensuing months the Deputy Director of the Foundation had a number of discussions with Professor Bergel, Sir Alexander Todd and Professor A. J. Birch and received a great deal of helpful advice. Professor Bergel also agreed to act as Chairman at the conference and although at one stage it seemed possible that a severe illness would prevent him from doing so, he happily recovered in time to take the Chair as originally planned. The meeting eventually took place in March 1958 and this book is a record of its proceedings.

Following the normal pattern of Ciba Foundation Symposia, the number of participants was small, only twenty-nine altogether. The size of the group thus allowed for very full discussion of each paper presented. It is hoped that these discussions which, with the papers presented, are fully set out in this volume, may be of interest to the many chemists, biochemists and pathologists who could not be invited to attend because the need for informality made strict limitation of numbers desirable.

For those readers who may not have previously come across the work of the Ciba Foundation, it should be explained that it is an educational and scientific charity administered by the distinguished Trustees and Members of Council whose names are set out on the opposite page. It is an entirely independent organization, although it was originally set up through the generosity of the Swiss firm CIBA Limited of Basle who

established it in London so that it would conveniently serve scientists from the old world and the new.

Here, it occupies a house nearly 200 years old and provides accommodation which is used each year by nearly 1,000 scientists from thirty to forty different countries. It arranges conferences such as the one reported here, as well as shorter meetings on scientific subjects relating to medicine and chemistry. Its further activities include annual lectureships, a medical postgraduate exchange scheme between Great Britain and France, and the support of basic research on ageing. Finally, by publishing the results of its work in volumes such as this, the Foundation attempts to provide some assistance for the individual research worker when distance and circumstances make it difficult for him to keep in touch with what his colleagues in the field may be doing in other countries.

List of those participating in or attending the Symposium on "Amino Acids and Peptides with Antimetabolic and Cytotoxic Properties" 18th–20th March, 1958.

E. P. Abraham . . .	Sir William Dunn School of Pathology, University of Oxford
K. Balenović . . .	University Chemical Laboratory, Zagreb
A. de Barbieri . . .	Istituto Sieroterapico Milanese, Milan
F. Bergel . . .	Chester Beatty Research Inst., London
A. J. Birch . . .	Dept. of Chemistry, University of Manchester
M. Brenner . . .	Dept. of Organic Chemistry, University of Basle
J. M. Buchanan . .	Division of Biochemistry, Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass.
L. C. Craig . . .	Rockefeller Inst. for Medical Research, New York
J. E. Dalby . . .	Radiobiology Dept., Christie Hospital & Holt Radium Inst., Withington, Manchester
S. Farber	Children's Cancer Research Foundation, Boston, Mass.
E. F. Gale . . .	Medical Research Council Unit for Chemical Microbiology, School of Biochemistry, University of Cambridge
J. Harley-Mason . .	University Chemical Laboratory, Cambridge
A. W. Johnson . . .	Dept. of Organic Chemistry, University of Nottingham
G. W. Kenner . .	Dept. of Organic Chemistry, University of Liverpool
L. J. Mullins . . .	Dept. of Biophysics, Purdue University, Lafayette
G. G. F. Newton . .	Sir William Dunn School of Pathology, University of Oxford
Natália I. Perevodchikova	Inst. of Experimental Pathology and Therapy of Cancer, Academy of Medical Sciences of the U.S.S.R., Moscow
H. Christine Reilly . .	Division of Experimental Chemotherapy, Sloan-Kettering Inst. for Cancer Research, New York
H. N. Rydon . . .	Dept. of Chemistry, University of Exeter
R. Schwyzer . . .	CIBA Limited, Basle

J. C. SHEEHAN . . .	Dept. of Chemistry, Massachusetts Inst. of Technology, Cambridge, Mass.
H. E. SKIPPER . . .	Kettering-Meyer Laboratory, Southern Research Inst., Birmingham, Alabama
J. A. STOCK . . .	Chester Beatty Research Inst., London
SIR ALEXANDER R. TODD .	University Chemical Laboratory, Cambridge
R. WADE	Chester Beatty Research Inst., London
TH. WIELAND . . .	Inst. of Organic Chemistry, University of Frankfurt
EVE WILTSHAW . . .	Chester Beatty Research Inst., London
T. S. WORK . . .	National Inst. for Medical Research, Mill Hill, London
A. WRETLIND . . .	Farmakologiska Institutionen, Karolinska Institutet, Stockholm
G. T. YOUNG . . .	The Dyson Perrins Laboratory, Oxford

CONTENTS

CHAIRMAN'S OPENING REMARKS

F. BERGEL

ONE of the privileges of the Chairman of such a conference as ours, apart from expressing his deep gratitude to the Director and Deputy Director and the Staff of the Ciba Foundation for organizing it, and voicing his pleasure at seeing so many distinguished participants from abroad and home, is to formulate roughly the philosophy behind the subject matter to be presented and discussed during the next three days.

You may consider this rather a continental attitude (I am not using this expression in a disparaging manner), but I hope you have no objections if I attempt the formulation of the motive which prompted the suggestion to Dr. Wolstenholme and Dr. Genese of the theme of the present symposium. Chemistry, biochemistry or biology of amino acid and peptide derivatives, in the widest sense, has so far only been dealt with between these pleasant four walls on the occasion of the Ciba Foundation Symposium on the Chemical Structure of Proteins in 1952. While purines, and in the background nucleic acids and pteridines, have been discussed here during the last two or three years, amino acids and peptides have, so far, been left aside. It was felt, therefore, that the time had come to bring these two groups of substances under review. In order to achieve a balance between the various disciplines of chemistry, biology and clinical science, the necessity arose to restrict the large subject matter to biologically active species. But even with such limitation the field is still too wide, as amino acids and peptides with hormonal (e.g. thyroxine, oxytocin), pharmacological (e.g. ergot), growth-promoting (e.g. strepogenin), plant-wilting (e.g. lycomarasmin) and other activities fall under the heading of such

subdivision. Consequently, it was proposed to cut from the large cake a slice which consisted of amino acids and peptides with antimetabolic and cytotoxic properties.

I hope that this rather arbitrary cutting down will not prevent us from touching on and discussing more general aspects, some of which, in form of a prediction, I shall mention in a few moments. I am here reminded of a remark which James Conant, the chemist-diplomat, used to quote when some of his experiments, demonstrated during his lectures, did not come off as promised by him beforehand to his audience. After one such *fiasco*, an elderly gentleman who had strayed as a visitor into his class gave him this advice, "Young man, it is always better to speak after the event as an historian than before the event as a prophet."

Let me, therefore, go first in the safer direction of recalling some early historical happenings inside the field of our subject, recently reviewed by Meister in his "Biochemistry of the Amino Acids" (1957). If one equates antimetabolic with antibiotic or antigrowth effects mainly on micro-organisms then the first clear examples of amino acid antagonists were produced after the pronouncement by Woods and Fildes in 1940 of their antimetabolite hypothesis (unless one quotes the toxicity of ethionine in rats as found by Dyer in 1938). It was McIlwain in 1941 who, following his success with pyridine-3-sulphonic acid as an antivitamin, tested α-amino-sulphonic acids on bacteria as growth inhibitors. A few years later, Fildes and Rydon (1947) demonstrated similar effects with methyl tryptophans and related compounds and American workers (Mitchell and Niemann, 1947; Ferger and du Vigneaud, 1949; Garst, Campaigne and Day, 1949) with *p*-halogeno phenylalanines and thienyl alanines, shifting the emphasis of antagonism to phenylalanine and tyrosine. The last type proved to be an inhibitor not only of microbial growth but also of growth in animals such as the rat, and with it we are among the as yet small group of potential antitumour amino acids, some of which will be mentioned during the coming sessions. The same applies to some antimetabolically active

amino acid derivatives of natural origin which were discovered during recent years. If we look for a beginning of the story of peptides possessing antimetabolic or cytotoxic properties, we could choose penicillin as filling this rôle, with its discovery in 1929 by Fleming and isolation in 1940–41 by Chain, Abraham and Florey and co-workers. But considering the rather special structural formula of penicillin, perhaps Dubos' discovery (Dubos, 1939; Dubos and Cattaneo, 1939; Dubos and Hotchkiss, 1941) of what were later known as gramicidin and tyrocidine should be given the same place from a purely historical point of view.

Since then, of course, a number of other peptides or peptide-like structures, and as I said before, amino acid derivatives with toxic, cytotoxic or antimetabolic activities have been added to those which opened up this interesting field.

Now, what can we expect from this symposium, apart from the description of chemical, biochemical, biological and clinical properties? We should hear something about methods of chemical preparation of such compounds, and pathways of biochemical synthesis of those which are found in Nature. If the chemical methods of preparation are novel they should be of interest also to anybody who studies the synthesis of amino acid and especially peptide derivatives outside our limited field. In this way, our biological and clinical friends will learn something about the endeavours carried on in the organic laboratory, just as *vice versa* the chemists will be informed about the biological characteristics and medical usefulness of some of their products.

Disregarding the warning in Conant's story, I venture to predict that some results will emerge which, despite the prudent reservations to be made, may directly or indirectly throw some light on structure/activity relationships, whereby structure stands for physical and chemical properties of whole molecules or of certain groups or groupings of such molecules, and activity should be defined in terms as rigid as biological experimentation will allow. The question arises immediately whether the amino acid derivatives with antagonist or

cytotoxic activities when built into peptide chains or even hooked on to isologous proteins would gain in efficacy and lose some of their toxic effects. With good reason I dare to predict that we might learn of more and novel facts concerning the mode of action of at least some of the substances. How far are they acting as true antagonists, with which part of cell metabolism do they interfere, what does the rather loose expression "cytotoxic" mean in terms of the biochemist and biologist, are there any connexions between some antibacterial and antitumour drugs, how many different kinds of action mechanisms are operating in the case of substances producing apparently similar or identical biological effects?

Many more questions could be asked. But if we obtain only a few answers it could be said that our meeting has contributed substantially to the solution of problems connected with synthetic chemistry, fundamental biochemistry and therapeutics.

REFERENCES

ABRAHAM, E. P. *et al.* (1941). *Lancet*, **ii**, 177.
CHAIN, E., FLOREY, H. W. *et al.* (1940). *Lancet*, **ii**, 226.
DUBOS, R. J. (1939). *J. exp. Med.*, **70**, 5.
DUBOS, R. J., and CATTANEO, C. (1939). *J. exp. Med.*, **70**, 249.
DUBOS, R. J., and HOTCHKISS, R. D. (1941). *J. exp. Med.*, **73**, 629.
DYER, H. M. (1938). *J. biol. Chem.*, **124**, 519.
FERGER, M. F., and DU VIGNEAUD, V. (1949). *J. biol. Chem.*, **179**, 61.
FILDES, P. (1940). *Lancet*, **i**, 955.
FILDES, P., and RYDON, H. N. (1947). *Brit. J. exp. Path.*, **28**, 211.
FLEMING, A. (1929). *Brit. J. exp. Path.*, **10**, 226.
GARST, R. G., CAMPAIGNE, E., and DAY, H. G. (1949). *J. biol. Chem.*, **180**, 1013.
MCILWAIN, H. (1941). *Brit. J. exp. Path.*, **22**, 148.
MEISTER, A. H. (1957). Biochemistry of the Amino Acids. New York: Academic Press.
MITCHELL, H. K., and NIEMANN, C. (1947). *J. Amer. chem. Soc.*, **69**, 1232.
WOODS, D. D. (1940). *Brit. J. exp. Path.*, **21**, 74.

THE STEREOCHEMISTRY OF NATURALLY OCCURRING β-AMINO ACIDS

K. BALENOVIĆ
University Chemical Laboratory, Zagreb, Jugoslavia

β-Amino acids and related compounds in Nature

β-Alanine has been described as a product of decarboxylation of aspartic acid by *Rhizobium leguminosarum* (Virtanen, Rintala and Laine, 1938), and as a well-known component of carnosine, anserine, coenzyme A, pantothenic acid, pantethein and pantethin.

Some other compounds related to β-alanine were also found in Nature, such as β-nitropropionic acid and β-aminopropionitrile.

β-(γ-L-Glutamyl)-aminopropionitrile is the toxic principle of *Lathyrus odoratus* seeds; this dipeptide can induce the skeletal abnormalities characteristic of lathyrism (McKay *et al.*, 1954; Schilling and Strong, 1954). β-Aminopropionitrile itself also has teratogenic properties.

The toxic β-nitropropionic acid (hyptagenic acid) was first isolated from *Hyptage benghalensis* (cf. Carter and McChesney, 1949). The same substance was produced by a strain of *Aspergillus flavus* (Busch, Touster and Brockman, 1951), and was also identified as the toxic constituent of *Indigofera endecaphilla* (Morris, Pagan and Warnke, 1954; Cooke, 1954).

(−)-α-Methyl-β-alanine has been isolated from human urine (Crumpler *et al.*, 1951). Approximately ten per cent of humans excrete about 200 mg. of this compound per day, whereas most humans excrete about one-tenth of this amount. Fink and his co-workers found that administration of dihydrothymine or thymine to rats resulted in urinary excretion of α-methyl-β-alanine (Fink, Henderson and Fink, 1951, 1952; Fink *et al.*, 1956). It has been found, furthermore, that on

incubation with rat liver slices dihydrothymine yields α-methyl-β-alanine.

β-Dimethylamino-β-phenylpropionic acid was obtained by hydrolysis of taxine (Winterstein and Guyer, 1923); Taxine I, the major alkaloid of the yew (*Taxus baccata* L.) is the ester of this acid (Baxter *et al.*, 1958). Mild hydrolysis of Graf's Taxine B (Graf, 1956; Graf and Boeddeker, 1956) gave the optically active (+)-β-dimethylamino-β-phenylpropionic acid, $[\alpha]_D + 9{\cdot}2°$.* Taxine I and Taxine B are probably identical compounds.

(+)-β-Lysine has been isolated from hydrolysates of a number of antibiotics, such as viomycin from *Streptomyces puniceus* and *Streptomyces floridae* (Haskell *et al.*, 1952), streptothricin from *Streptomyces lavendulae* (Carter *et al.*, 1952, 1954; van Tamelen *et al.*, 1956), streptolin from "*Streptomyces* No. 11" (Smissman *et al.*, 1953; van Tamelen and Smissman, 1953; van Tamelen *et al.*, 1956), roseothricin from *Streptomyces roseochromogenus* (Nakanishi, Ito and Hirata, 1954) and geomycin from *Streptomyces xanthophaeus* (Brockmann and Musso, 1955).

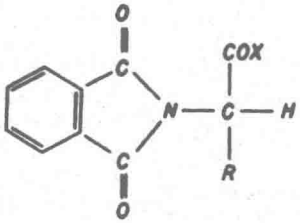
β-(2-Thiazole)-β-alanine has recently been isolated from degradation products of the antibiotic bottromycin obtained from *Streptomyces bottropensis, n. sp.* (Waisvisz, van der Hoeven, and te Nijenhuis, 1957). This β-amino acid was described as an optically inactive compound. By using milder conditions of hydrolysis it is probable that the optically active compound might be obtained, as in the hydrolysis of taxine.

Preparation of optically active β-amino acids

Fischer, Scheibler and Groh (1910) have described the resolution of β-amino-β-phenylpropionic acid into optical antipodes; Fischer and Scheibler (1911) also resolved β-aminobutyric acid. These resolutions were described as very tedious procedures.

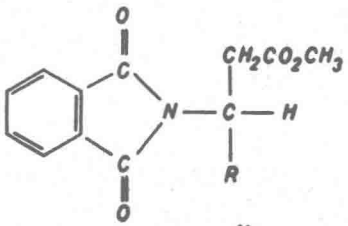
* This β-amino acid was recently proven to be of the L-configuration (Graf, 1958, personal communication).

A more convenient method for the preparation of optically active β-amino acids is the Arndt-Eistert homologization of α-amino acids (Balenović, 1947; Balenović *et al.*, 1951) through the reaction stages I–III.

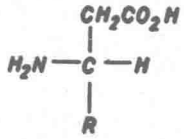


a, X = OH
b, X = Cl
c, X = CHN_2

I

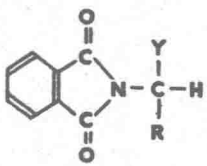


II



III

The Arndt-Eistert reaction was applied to the diazoketone I*c*, which was prepared from the optically active *N*-phthaloyl-



IV

a, Y = COOH
b, Y = COCl
c, Y = $COC(N_2)R_1$
d, Y = $CH(R_1)CONHPh$

α-amino acid I*a*. This method has been applied to a number of amino acids, as shown in Table I.