

BIOCHEMICAL STUDIES ON NEW ANTIBIOTICS

THESIS

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ABBREVIATIONS USED*

AS-AZ-27	Ain Shams-isolate No. 27.
ATP	Adenosine triphosphate.
b.p.	Boiling point.
<u>B. subtilis</u>	<u>Bacillus subtilis</u> .
C	Degree centigrade.
^{14}C	Carbon 14.
Ci	Curie.
Cm	Centimetre.
CPM	Count per minute.
DNA	Deoxyribonucleic acid.
<u>E. coli</u>	<u>Escherichia coli</u> .
e.g.	For example.
Fig.	Figure.
g	Gramme.
^3H	Tritium.
IMP	Inosine monophosphate.
I R	Infrared.
kg	Kilogramme.
LD_{50}	Lethal dose killing 50% of the number of test animals.
log.	Logarithmic.
M	Molar.
mCi	Milli Curie.
M I C	Minimum inhibitory concentration.
mmole	Millimole.
mm	Millimetre.
mg	Milligramme.
m-RNA	Messenger ribonucleic acid.

min.	Minute.
N	Normality.
nm	Millimicron.
No.	Number.
p.	Page
O.D.	Optical density.
<u>P. chrysoqenum</u>	<u>Penicillium chrysoqenum.</u>
P-P	Pyrophosphate.
R _f	Retardation factor.
RNA	Ribonucleic acid.
r.p.m.	Round per minute.
<u>S.</u>	<u>Streptomyces.</u>
sp.	Species.
s-RNA	Soluble ribonucleic acid.
<u>St. aureus</u>	<u>Staphylococcus aureus.</u>
<u>S. typhosa</u>	<u>Salmonella typhosa.</u>
TCA	Trichloroacetic acid.
TLC	Thin layer chromatography.
t-RNA	Transfer ribonucleic acid.
uCi	Micro Curie.
UDP	Uridine diphosphate.
ug	Microgramme.
ul	Microlitre.
UTP	Uridine triphosphate.
UV	Ultraviolet.
V	Volume.
v/v	Volume per volume.

* :- Official abbreviations cited in "Current therapy" (1979).
 Edited by Howard H.; Cohn W.B.Saunders Company/Philadelphia/
 London/ Toronto. page. 914.

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PREFACE

A research project was started many years ago, to isolate actinomycetes from the soil of the Middle East. The isolated organisms were then subjected to a screening program to appraise their antagonistic properties against microbial growth. Subsequently, trials to isolate the antimicrobial agents and determine their physical, chemical and biological properties were conducted. The following new antibiotics were isolated in our laboratory : polychlorosubtilin, gluconimycin, ferramidochloromycin (FACM), polyketoacidomycin (PKAM), negabillin, AS-K-753, hodydamycin , yemenimycin, kuwaitimycin, nilemycin, cairomycin B and staphylococmycin (SCM).

Among the various workers engaged in this project, the author isolated nearly 500 pure Streptomyces cultures from soil samples collected from the ARAB REPUBLIC OF EGYPT. The potentialities of these isolates as antibiotic producers were examined. Out of this collection 100 isolates which possessed antagonistic properties were selected and were given serial numbers from 1 - 100. The isolate number 27 was selected for further studies due to its

highest antimicrobial activity mainly against Gram-positive bacteria and given the name Streptomyces AS-AZ-27.

Morphological, culture, biochemical and physiological characteristics for the isolate No. 27 were studied, and accordingly S. AS-AZ-27 could be classified as a new strain belonging to the series erythrochromogenes.

The antibiotic produced by the isolate was then separated and purified. On chemical analysis, the antibiotic AS-AZ-27 was found to contain C, H, N, O and Fe. Analysis of the acid hydrolytic product revealed the presence of six amino acids namely:- aspartic acid, alanine, lysine, valine, leucine and phenylalanine. Therefore, it could be classified as a new member of the iron containing polypeptide antibiotics.

Finally, the mechanism by which the antibiotic AS-AZ-27 inhibited the growth of B. subtilis was studied by both colourimetric techniques and by measuring the rate of incorporation of appropriate radioactive precursors into cellular macromolecules.

I- INTRODUCTION

I N T R O D U C T I O N

Nowadays, antimicrobial drugs play a large part in the control of infection. The use of these drugs is almost entirely a development of this Century.

Antibiotics are anti-infective agents of biological origin which interfere with the growth or other metabolic activities of organisms, have been known for many years. But it was the discovery of penicillin by Fleming in 1928 and its eventually successful use and large scale commercial production in 1941-1945 that provided the impetus to the modern intensive study of antibiotics. In common parlance the term has become restricted to substances interfering with biological activities of pathogenic microorganisms.

The term antibiosis was firstly used by Vuillemin⁽¹⁾ in 1889 to describe a type of association in which one living creature was destroying another in order to sustain its own life.

The term antibiotic was introduced by Waksman et al., 1942⁽²⁾ to designate a chemical substance of microbial origin which has the

property to inhibit the growth of microorganisms, bacteriostatic. Waksman⁽³⁾ in 1947 added that an antibiotic might also destroy bacteria and other microorganism, i.e. bactericidal.

Umezawa⁽⁴⁾ introduced the following definition: "Antibiotics are chemical substances which are produced by microorganisms and have the capacity in dilute solutions to inhibit the growth of the other microorganisms and in some situations to destroy them".

Waksman et al.⁽²⁾ in 1942 reported that antibiotic producing actinomycetes are widely distributed in nature especially in soils and composts. The antagonistic forms were found to belong to the genus Streptomyces.

Many investigators⁽⁵⁻⁸⁾ examining thousands of soil samples had found that some microorganisms which produce certain antibiotics were extremely common. Actinomycetes elaborating streptothricin, chloramphenicol, actinomycin and xanthomycin like antibiotics have a world wide distribution.

The differential susceptibility of various species of microorganisms to a given antibiotic permits the construction of a bacterial "spectrum" for the antibiotic, in which the relative effectiveness is determined for a standardized series of test organisms.

Such a spectrum may be useful in comparing two supposedly; identical or dissimilar antibiotics and in comparing synthetic compounds or derivatives with the naturally occurring antibiotics.

Classification of the antibiotics would be very useful for integrating descriptions of new antibiotics with the chemical classes or groups to which they belong. Classification of antibiotics is a difficult problem due to the incomplete information about newly isolated members. In practice, antibiotics were characterized by their chemical and physicochemical properties, their biological activities, toxicity and their mode of action.

Waksman and Lechevalier⁽⁹⁾ were the first to suggest a key for the classification of the antibiotics produced by actinomycetes. Their suggestion was based on the elementary composition of the antibiotic, its light absorption in U.V. and I.R. spectra, as well as its microbial and antitumour activities.

Kerzybiski et al.^(10,11) classified antibiotics according to their antimicrobial and antitumour activities as well as their origin.

Umezawa in 1964⁽¹²⁾ gave a detailed description of the physicochemical characteristics, biogenesis and mode of action of the isolated antibiotics.

In 1971, Sensi and Coronelli⁽¹³⁾ stated that a chemical system of classification of antibiotics is rather impossible to formulate at present due to lack of information about the chemical structures of numerous antibiotics.

Berdy⁽¹⁴⁾ in 1972 introduced a new classification of antibiotics based on their chemical structure, which raises a number of special problems. Berdy⁽¹⁴⁾ classified antibiotic families according to the following nine principal constituents:-

I- CARBOHYDRATE ANTIBIOTICS

A- Pure saccharides:-

1- Mono- and disaccharides:- among which streptozotocin^(15,16) produced by S. achromogenes strongly inhibited both Gram positive and Gram negative bacteria.

2- Polysaccharides:- among the antibiotics belonging to this series, statolon⁽¹⁷⁾, an antiviral substance, produced by Penicillium stoloniferum and mannan produced by Saccharomyces cerevisiae⁽¹⁸⁾.

B- Aminoglycoside antibiotics:-

1- Streptamine derivatives:- e.g. streptomycin and actinospectacin produced by S. griseus and S. spectabilis⁽¹⁹⁾ respectively, both antibiotics inhibit the growth of Gram positive and Gram negative bacteria.