HANDBOOK of TOXICOLOGY

Volume II

Antibiotics

Editor: SPECTOR

HANDBOOK of TOXICOLOGY

Volume II: Antibiotics

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Prepared under the Direction of the Committee
on the Handbook of Biological Data
DIVISION OF BIOLOGY AND AGRICULTURE
THE NATIONAL ACADEMY OF SCIENCES
THE NATIONAL RESEARCH COUNCIL

W. B. SAUNDERS COMPANY
Philadelphia and London

Made in the United States of America

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 56-6976

Foreword

This compilation of data on antibiotics--their physical, chemical, biological and toxicological properties--comprises Volume II of the Handbook of Toxicology. The Handbook was prepared under Aero Medical Laboratory Contract No. AF 33(616)-2875 between The National Academy of Sciences and the Wright Air Development Center, United States Air Force. Under the same contract additional volumes are scheduled for publication in the coming year. The contract was administered under the direction of the Aero Medical Laboratory, Directorate of Research, Wright Air Development Center, Dr. George Kitzes acting as Project Director, Project No. 7159, "Health Hazards of Air Force Materials." The United States Army Chemical Center has also generously assisted in support of this contract.

Data for all volumes were contributed by experts in various areas of the fields represented. The material was reconciled, assembled, compiled, and edited by the Handbook Staff and reviewed and authenticated by specialists in the subjects covered. The work was carried out under the direction of the Committee on the Handbook of Biological Data, operating under the Division of Biology and Agriculture of The National Academy of Sciences-National Research Council.

Acknowledgment is made, on behalf of the Committee, to the Wright Air Development Center for the foresight and scientific judgment inherent in the commission to prepare this Handbook; to the Army Chemical Center for its generous support and cooperation; to Dr. John N. Porter and Mr. Gilbert C. de Mello of the Research Division, American Cyanamid Company, who compiled the data appearing in this volume and who served as guides and advisors in its preparation; to The National Research Council Committee on Toxicology, under the chairmanship of Dr. Harold C. Hodge, for encouragement and advice in planning the contents of the Handbook of Toxicology; to Mrs. Dorothy Dittmer for her tireless editorial efforts, without which this volume never would have reached the stage of publication; to Miss Florence Lochrie for her meticulous proofing of the manuscript; to Mr. John F. Sobrofski and Mr. John P. Henner for their excellent performance in the most tedious job of layout and drafting the entire volume; to the many biologists and toxicologists who served as contributors and reviewers for the Handbook; and finally to all the Handbook staff members who so willingly shared the multitude of tasks inherent in the preparation of the book.

May 28, 1957

W. S. Spector

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Contents

Volume II

Introduction

This volume presents data on the physical, chemical, biological, and toxicological properties of 340 antibiotics. The word "antibiotic" is used in the strictest sense, i.e., "a chemical substance produced by microorganisms which has the capacity, in dilute solutions, to inhibit the growth of, or to destroy, bacteria and other microorganisms." Other anti-infectives and chemotherapeutic agents have not been listed in this book.* The data herein are recorded as accurately as possible from the literature and are current as the book goes to press. Because the field of antibiotics is tremendously fast-moving, it is by no means beyond possibility that some of the biological and toxicological information may be out of date by the time the volume appears in print--or that some of the facts may be contradicted by more recent reports.

The guiding principles in selection of material have been that it be of basic importance and from reliable literature sources. Some data of value have had to be omitted either because they were not on hand for publication or because time has not permitted the necessary preparatory steps for printing. The fact that certain data have been compiled and are already in print, or available in other form, has not been regarded as a reason for excluding them from this Handbook. Every page of this volume has been examined for accuracy by the contributors.

In the compilation of this information, one of the chief objectives has been clarity of presentation. To attain this clarity, only the most fundamental data appear in the body of the text. In several instances footnotes have been used to supply additional facts and preserve the conciseness of the text. Other pertinent material prohibited by limitations of space is to be found in the literature, and for this reason the bibliography is presented in sets of references, with a set for each antibiotic listed. Chemical nomenclature has been kept exactly as contributed and is, in every case, identical with that commonly found in the literature. However, a cross index at the back of the book contains numerous synonyms and proprietary names to facilitate location of any compound listed.

In an attempt to increase the usefulness of the volume, two appendices have been added. The first of these is an antibiotic efficacy listing in which organisms are compiled alphabetically and each is followed by the code numbers of all antibiotics showing moderate to high activity against it. The second appendix is a source list of antibiotics, alphabetized by the source organism.

It must be emphasized that the data presented in these tables, particularly those values concerned with toxicology, are by no means absolute and should be interpreted only as a "yardstick" of activity for the compounds listed. Again, the literature reference, in most cases, will reveal the number of determinations, the number of animals in each determination, and conditions under which determinations were made. Some of the conditions which influence toxicity or biological effect of any given compound are as follows:

a. Dose: Generally, the larger the dose the more rapid the action.
b. Rate of absorption: The faster this rate, the quicker the action of the drug. With oral administration the lethal dose may be considerably influenced by the condition of the gastrointestinal tract, especially by the amount of food and fecal material in the stomach and intestine.
c. Route of administration: For the most part, toxicity or efficacy is greatest by the route that carries the active substance to the bloodstream most rapidly. In descending order of speed of action, routes for most drugs are: intravenous, inhalation, interperitoneal, intramuscular, subcutaneous, oral, and cutaneous. Food in the alimentary canal may delay or decrease activity; digestive enzymes may destroy or alter the compounds with resultant changes in the effectiveness thereof. Certain compounds are harmless if taken orally and lethal when introduced parenterally; in some cases the converse is true. The efficacy of the drug may also vary considerably with the form in which it is administered, i.e., solid, in suspension, or in solution. In the last instance the activity again may be influenced by the solvent and the concentration.
d. Site of injection: With subcutaneous injections, toxicity may be affected by the density of the subcutaneous tissue. With intravenous administration, whether the injection is made into the femoral or jugular vein may be of importance, but in any case the rate of injection, or the amount of toxic material injected per minute, will considerably influence the value of the toxic dose.
e. Other important influences: Idiosyncrasy, disease, environmental temperature, habit and tolerance, diet, season of the year (especially with hibernating animals) may all affect the activity or toxicity of an antibiotic. These properties will also vary with the species of animals used, and sometimes with different strains of the same species and within the same strain they may differ with age, weight, sex, and the general condition of the animals.

With all of the above variables exerting their individual or collective influences, it is important that the activity be delineated with reference to the time of death, the period of time for which fatalities are counted, or whatever other end-point is employed.

Unfortunately, only in rare instances are all these factors considered and specified in the literature on toxicity determinations. This renders the duplication of such data by different investigators extremely difficult if not impossible. At the present time, attempts are being made to put toxicity data on a quantitative basis. The older literature often refers simply to "lethal doses" (LD) or "minimal lethal doses" (MLD), meaning doses which will be

*Data on these compounds will be presented in a forthcoming volume entitled "Anti-infectives."

fatal or the smallest dose which will kill a limited number of animals. By using a larger number of animals of comparative weight and sex for each level tested, attempts are now being made to determine more precisely the dose which will kill 50 per cent (LD_{50}). These values can be further certified by the application of various statistical methods, by stating the degree of deviation of single values from the mean or the slope of the toxicity curve.

The data in this Handbook are, in the judgment of the contributors, as authentic as can be procured under the conditions as they exist. It is recognized, however, that all data, and particularly those in the fields of toxicology and antibiotics, are subject to continuing revision as investigators standardize techniques and make more measurements. The user of the volume is warned against attributing significance to small differences from species to species. He is invited to submit any data he feels should be given consideration.

TOXICITY CLASSES

The toxicological data presented in this Handbook are the result of extensive tests on laboratory animals. Frequently, toxicologists, industrial hygienists, industrial physicians, etc., are asked to translate these data into terminology that will readily describe the hazards associated with their use. Consequently, classes have been established to define the toxicity of a chemical material, in common terms, with reference to data obtained by specified animal tests. The following tabulation of toxicity classes is useful only for those data which are applicable.

COMBINED TABULATION OF TOXICITY CLASSES*

		Various Routes of Administration			
Toxicity Rating	Commonly Used Term	LD_{50} Single Oral** Dose Rats	Inhalation 4-hr Vapor Exposure Mortality 2/6-4/6 Rats	LD_{50} Skin Rabbits	Probable Lethal Dose for Man
1	Extremely toxic	1 mg or less/kg	<10 ppm	5 mg or less/kg	A taste, 1 grain
2	Highly toxic	1-50 mg	10-100	5-43 mg	1 teaspoon, 4 cc
3	Moderately toxic	50-500 mg	100-1000	44-340 mg	1 ounce, 30 g
4	Slightly toxic	0.5-5 g	1000-10,000	0.35-2.81 g/kg	1 pint, 250 g
5	Practically non-toxic	5-15 g	10,000-100,000	2.82-22.59 g/kg	1 quart
6	Relatively harmless	15 g and more	>100,000	22.6 or more g/kg	>1 quart

* Hodge, H. C., and Sterner, J. H., American Industrial Hygiene Association Quarterly, 10:4, 93, Dec. 1943.

** Standards for intravenous LD_{50} for rats and rabbits may be obtained approximately by dividing the oral toxicity standards for rats by 10.

1. ABIKOVIROMYCIN

SOURCE Streptomyces rubescens, n. sp, and S. abikoensum, n. sp [1] .

STABILITY Unstable to heat, acid, exposure in dry state [1] .

OTHER REACTIONS Red color on heating 5 minutes at 100°C in 1% phosphate buffer at pH 7; positive Molisch, Tollens, Ag mirror; sublimes during freeze drying. [1]

BIOLOGICAL ACTIVITY In vitro. Weak antibacterial and antifungal activity. Inhibits, at 62.5-250 μg/ml, Shigella dysenteriae, Salmonella paratyphi, S. typhimurium, Klebsiella pneumoniae, Proteus vulgaris, Bacillus anthracis, Mycobacterium phlei, and Candida albicans. Brucella melitensis, Pseudomonas aeruginosa, Bacillus subtilis and Micrococcus pyogenes var. aureus are not sensitive, requiring 500 μg/ml or more. Inhibits western and eastern equine encephalitis virus in dilution of 1:8,000,000. No activity against Venezuelan equine encephalitis and Japanese encephalitis viruses.[2] Poliomyelitis virus rendered non-infective to mice when mixed with crude filtrates. Partially purified product exhibited antiviral activity in dilution of 1:25,000.[3]

ACUTE TOXICITY LD_{50} in mice, approximately 6.6 mg/kg, i.v., and 66 mg/kg, s.c. [1] .

2. ACHROMOVIROMYCIN

SOURCE Streptomyces achromogenes, n. sp [1] .

REACTIONS Extractable with ethyl acetate at pH 2; related to, but not identical with, abikoviromycin and sarcidin [1] .

BIOLOGICAL ACTIVITY In vitro. Inhibits only Sarcina lutea, no other bacteria or fungi. Crude preparation inhibited Japanese B encephalitis virus in mice in dilution of 1:16,000 [1] .

ACUTE TOXICITY Mice tolerated s.c. doses of approximately 100 mg/kg [1] .

3. ACTIDUINS

SOURCE Streptomyces sp [1] .

COLOR Yellow to reddish substances [1] .

MELTING POINT (°C) None definite; darken at approximately 280, but no melting at 350.[1]

SOLUBILITY s. strong mineral acids; sl. s. butanol, ethanol, pyridine, acetic acid, cyclohexanone, dimethylformamide; i. water, ether[1].[1]

STABILITY Stable at acid and neutral pH; unstable at alkaline pH [1] .

OTHER REACTIONS Yellowish fluorescence in organic solvents, less so in aqueous solvents; activity may be obscured in beers by faster diffusing substances; contain C, H, N, S, and one contains Cl; precipitated by dilution after dissolving in strong acids; crystalline reineckates soluble in acetone. Infrared peaks: actiduin II, 6.04, 6.54, 6.86, 7.05, 7.27, 7.47, 7.67, 8.1, 8.3, 8.6, 8.73, 9-9.14, 9.45, 9.87, 10.91, 11.9, 12.2, 12.7, 13.33 μ; actiduin III, 6.04, 6.41, 6.86, 7.04, 7.27, 8.1, 8.3, 8.61, 8.71, 9-9.14, 9.45, 10.91, 11.9, 12.2, 12.7, 13.33, 13.91, 14.37 μ; actiduin VI, 6.04, 6.54, 6.86, 7.28, 7.48, 7.67, 8.05, 8.3, 8.58, 9-9.14, 9.4, 9.85, 10.1, 10.9, 12.67, 13.3, 13.9 μ. On hydrolysis they yield both a ninhydrin positive and a fluorescent, somewhat acidic material, the former separating into acidic and neutral fractions on paper ionophoresis.[1]

QUANTITATIVE DETERMINATION Microbiological: Micrococcus pyogenes var. aureus[2] [1] .

BIOLOGICAL ACTIVITY Actiduins II, III, IV, VI inhibit M. pyogenes var. aureus in dilution of 1:60,000,000-1:360,000,000. Also highly active against Streptococcus pyogenes, Diplococcus pneumoniae, Corynebacterium xerose. No activity against Gram-negative bacteria and Mycobacterium tuberculosis.[1]

4. ACTINOLEUKIN

SOURCE A strain of Streptomyces aureus [1] .

MOLECULAR FORMULA $(C_9H_{12}N_2O_3)_n$ [1] .

CRYSTAL FORM AND COLOR White platelets or needles [1] .

MELTING POINT (°C) 191-192 (d.)[3] [1] .

/1/ Different actiduins, however, are soluble in acetone, methanol, or chloroform, and can be separated on this basis. /2/ Preferably using serial tube dilution. /3/ After repeated crystallization.

4. ACTINOLEUKIN (Concluded)

UV ABSORPTION MAXIMA 243, 312 mμ [1] .

SOLUBILITY s. methanol, ethanol, butanol, acetone, ethyl acetate, butyl acetate, dioxane; sl. s. benzene, chloroform; i. ether, petroleum ether, water.[1]

OTHER REACTIONS Positive $FeCl_3$; negative ninhydrin, biuret, Tollens, Fehling; purple ring in Molisch test. Analysis: C, 55.53, 55.68; H, 6.05, 5.98; N, 14.05, 14.01.[1]

BIOLOGICAL ACTIVITY In vitro. Inhibits Micrococcus pyogenes var. aureus, Sarcina lutea, Bacillus anthracis, B. subtilis at 0.005-0.04 μg/ml. Gram-negative bacteria not susceptible.

In vivo. Slight inhibition of Ehrlich carcinoma in mice when 6 μg daily were injected, i.p., for 9 days.[1, 2]

ACUTE TOXICITY LD_{50} in mice, 1 mg/kg, i.v. [1] .

5. ACTINOMYCELINE

SOURCE Streptomyces sp related to S. antibioticus [1] .

NATURE Neutral, yellowish-green pigment [1] .

SOLUBILITY s. water, ethanol, methyl acetate; less s. acetone; sl. s. amyl acetate, chloroform; i. ether, benzene.[1]

STABILITY Thermolabile; stable at pH 7, less stable at pH 2.0; destroyed in 0.1 N NaOH in 24 hours.[1]

OTHER REACTIONS Intense fluorescence in ethanol [1] .

BIOLOGICAL ACTIVITY In vitro. Active against Gram-positive bacteria; no activity against fungi and mycobacteria.[1]

ACUTE TOXICITY Rats tolerated s.c. doses of 25 mg/kg [1] .

6. ACTINOMYCETIN

SOURCE Streptomyces albus [3] .

NATURE Probably polypeptide enzyme [1] .

SOLUBILITY s. water; i. ether, ethanol.[1]

STABILITY Thermo- and acid labile [1] . Inactivated by UV shorter than 300 mμ [2] .

OTHER REACTIONS Precipitated by acetone, ethanol, $(NH_4)_2SO_4$; repeated precipitation with $(NH_4)_2SO_4$ yields 10-fold increase in activity.[1, 4]

BIOLOGICAL ACTIVITY Contains a lytic substance, "actinozyme," which dissolves dead and, to a lesser extent, living bacteria, e.g., Micrococcus pyogenes var. aureus, Streptococcus pyogenes, Diplococcus pneumoniae, Bacillus megatherium [4] .

7. ACTINOMYCINS[1]

SOURCE Streptomyces antibioticus, S. chrysomallus [12, 14, 21] .

NATURE Weakly basic chromopeptides, quinonoid [4, 7, 12] .

MOLECULAR FORMULA AND WEIGHT

Actinomycin A: $C_{41}H_{58}N_8O_{11}$ [4] ; 1000 ± (Rast); 768-780, 813 (cryo.); 716 ± 15 (cryst.).[1, 2, 6]

B: $C_{61}H_{88}N_{12}O_{16}$ or $C_{65}H_{88}N_{12}O_{20}$; 1240 ± 20 (found); 1245, 1356 (calc.).[7, 9]

C: $C_{62}H_{89}N_{11}O_{17}$ or $C_{60}H_{83}O_{16}N_{11}$; 915 (Beckman); 876-944 (Barger-Rast); 1260.4, 1214 (calc.).[5]

C_2: 1296 ± 35 [26] .

C_3: $C_{64}H_{90}O_{16}N_{12}$ [32] ; 1307 ± 35. [26]

D: $C_{60}H_{76}O_{15}N_{12}\cdot 3H_2O$ (proposed); approximately 1200 (by analogy).[23]

J_1: 1305 ± 35.

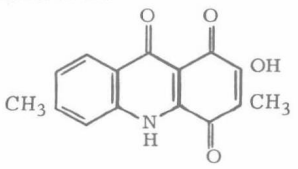
X_1: 1320 ± 35.

X_2: 1307 ± 35.

$X_{o\beta}$: 1393 ± 35.[26]

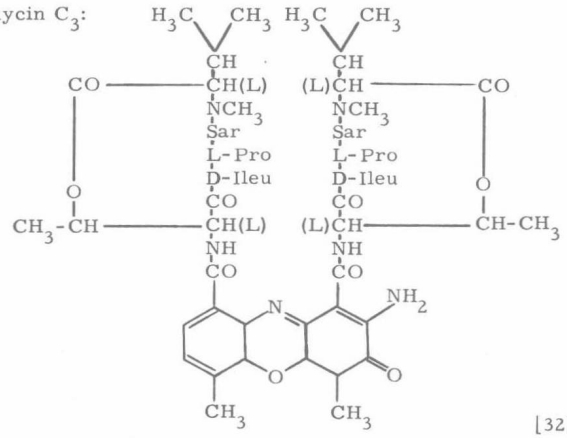
/1/ Actinomycin C also known as actinochrysin, Sanamycin.

STRUCTURE Actinomycins appear to have a common chromophore but differ in peptides. Peptide-free chromophore (actinomycinol) postulated to be:



[25]

Actinomycin C_3:



[32]

CRYSTAL FORM AND COLOR

Actinomycin A: red-vermilion platelets[1] [1, 2].
B: red plates[2], orange needles[3] [8].
$C_1, C_2, C_3, I_o, I_1, X_1$: hexagonal bipyramids, six-angled prisms or needles[4] [10, 11, 13].
D: bright red rhomboid prisms [23].
X_2: rhombic plates[5] [10, 11, 13].
$X_{o\beta}$: yellow needles [31].

MELTING POINT (°C)

Actinomycin A: 250, 252 (d.) [1-3].
B: 250-252 (d., corr.) [8].
C_1: 241-243 (d.)[6] [10, 13].
C_2: 237-239 (d.)[6] [10, 13].
C_3: 232-235 (d.)[6] [10, 13].
D: 241.5, 243 (d.) [23].
I_o: 242-243 (d.)[6] [10, 13].
I_1: 240.5-242 (d.)[6] [10, 13].
X_1: 240-242 (d.)[6] [10, 11, 13].
X_2: 244-246 (d.)[6] [10, 11, 13].
$X_{o\beta}$: 245-247 [31].

OPTICAL ACTIVITY

Actinomycin A: $[\alpha]_D^{25} = -320^o \pm 5^2$ [1].
B: $[\alpha]_D^{31} = -332^o, -340^o$ [3, 8].
C_1: $[\alpha]_D^{20} = -349^o \pm 10$ [10, 13].
C_2: $[\alpha]_D^{19} = -325^o \pm 10$ [10, 13].
C_3: $[\alpha]_D^{19} = -321^o \pm 10$ [10, 13].
D: $[\alpha]_D^{28} = -315^o \pm 10^7$ [23].
I_o: $[\alpha]_D^{20} = -314^o \pm 10$ [10, 13].
I_1: $[\alpha]_D^{20} = -353^o \pm 10$ [10, 13].
X_1: $[\alpha]_D^{20} = -309^o \pm 10$ [10, 13].
X_2: $[\alpha]_D^{19} = -341^o \pm 10$ [10, 13].
$X_{o\beta}$: $[\alpha]_D^{20} = -261^o \pm 10^8$ [31].

/1/ From ether-acetone or ethyl acetate. /2/ Ethanol. /3/ Butanol. /4/ Ethyl acetate, methanol, benzene. /5/ Ethyl acetate. /6/ Koffler-Block MP determinations. /7/ c, 0.25 methanol. /8/ c, 0.22 acetone.