Handbook of Toxic Fungal Metabolites

RICHARD J. COLE

RICHARD H. COX

Handbook of Toxic Fungal Metabolites

RICHARD J. COLE

United States Department of Agriculture
National Peanut Research Laboratory
Dawson, Georgia

RICHARD H. COX

National Institute of Environme
Health Sciences
Research Triangle Park, Nort

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London Toronto Sydney San Francisco

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Cole, Richard J.
Handbook of toxic fungal metabolites.

Includes bibliographies and index.
1. Mycotoxins--Handbooks, manuals, etc.
2. Mycotoxins--Spectra--Handbooks, manuals, etc.
3. Microbial metabolites--Toxicology--Handbooks, manuals, etc. 4. Food contamination--Handbooks, manuals, etc. 5. Feeds--Contamination--Handbooks, manuals, etc.
I. Cox, Richard H., Date. II. Title.
QP632.M9C65 615.9'5292 81-4082
ISBN 0-12-179760-0 AACR2

PRINTED IN THE UNITED STATES OF AMERICA

81 82 83 84 9 8 7 6 5 4 3 2 1

Preface

The science of mycotoxicology had its advent with the discovery of aflatoxin in the early 1960s in England. Since that time, interest and research on the aflatoxins and mycotoxins in general have steadily intensified. The result has been an ever-increasing awareness of the potential danger of these secondary metabolites to human and animal health.

The evaluation of the role and extent of mycotoxin contamination in food and feed requires the isolation of the toxic principle(s) from a suspect commodity or from cultures of fungi isolated from the suspected material. This handbook has been compiled with the aid of numerous individuals for the purpose of facilitating the identification of known or related mycotoxins. The book provides investigators in the field with a comprehensive accumulation of chemical, physical, spectral, and biological data on toxic fungal metabolites and related chemicals that would otherwise be widely scattered throughout the literature. Since mycotoxins represent a wide diversity of chemical species, the book will be particularly useful to other scientists interested in some aspect of a particular chemical species or related species other than its toxic nature.

The presentation, where possible, of actual copies of UV, IR, ^{1}H NMR, ^{13}C NMR, and mass spectra greatly facilitates the spectral identification of known mycotoxins or related metabolites by both chemists and researchers not knowledgeable in the interpretation of spectral data.

The handbook has been divided into twenty-one sections. Members were placed into sections on the basis of chemical relationships. The last four groups are the exception. These could not be placed into any group based on chemical considerations and, therefore, three groups were devised according to the genus most likely to produce them: *Aspergillus* toxins, *Penicillium* toxins, and *Fusarium* toxins. The final section contains metabolites that could not be classified under the aforementioned categories.

The handbook is oriented primarily toward fungal metabolites that elicit a toxic response in vertebrate animals; however, it does contain metabolites

that show little or no known acute toxicity. The latter are included because of their chemical or close biosynthetic relationship to a toxin or group of toxins.

Richard J. Cole
*Richard H. Cox**

*Present address: Philip Morris U. S. A. Research Center, P. O. Box 26583, Richmond, Virginia 23261

Acknowledgments

Special thanks are due to Mr. Joe W. Dorner, USDA, Mr. C. Pape, University of Georgia, and Mrs. Betty Shope for their valuable assistance in the production of this book. We also wish to thank the following investigators who provided editorial assistance, authentic samples, and/or spectra for this book.

Dr. Matazo Abe
Tokyo University of Education
Tokyo, Japan

Dr. W. Acklin
Laboratorium für Organische Chemie
Eidgenössische Technische Hochschule Zurich
Zurich, Switzerland

Dr. D. C. Aldridge
Imperial Chemical Industries Limited
Alderley Park, Macclesfield
Cheshire, England

Dr. Duilio Arigoni
Laboratory for Organic Chemistry
Zurich, Switzerland

Dr. J.-C. Bouhet
Service de Biochimie
Centre D'Études Nucléaires de Saclay
Gif-sur-Yvette, France

Dr. H. P. Broquist
Vanderbilt University
Nashville, Tennessee

Dr. George Büchi
Massachusetts Institute of Technology
Cambridge, Massachusetts

Dr. Tom Burka
Vanderbilt University
Nashville, Tennessee

Dr. H. Burkhardt
Western Regional Research Center
USDA, SEA, AR
Berkeley, California

Dr. Vincent J. Carroll
Pfizer Chemicals Division
Brooklyn, New York

Professor Roy W. Curtis
Purdue University
West Lafayette, Indiana

Dr. Horace G. Cutler
Richard B. Russell Research Center
USDA, SEA, AR
Athens, Georgia

Dr. Makoto Enomoto
Sagamihara Kyodo Hospital
Sagamihara, Japan

Dr. Pierre Fromageot
Service de Biochimie
Centre D'Études Nucléaires de Saclay
Gif-sur-Yvette, France

Dr. Rex Gallagher
Department of Science and Industrial Research
Applied Biochemistry Division
Palmerston North, New Zealand

Dr. A. O. Geiszler
Abbott Laboratory
North Chicago, Illinois

Dr. W. O. Godtfredsen
Leo Pharmaceutical Products
Ballerup, Denmark

Professor Thomas Harris
Vanderbilt University
Nashville, Tennessee

Dr. Donald Harvan
National Institute of Environmental Health Science
Research Triangle Park, North Carolina

Dr. A. Wallace Hayes
Director, Toxicology Research
Rome and Haas Company
Spring House, Pennsylvania

Dr. J. G. Heathcote
University of Salford
Salford, England

Dr. Robert A. Hill
University Chemical Laboratory
Lensfield Road
Cambridge, England

Dr. Dennis Hsieh
University of California
Davis, California

Dr. K. Ishii
Science University of Tokyo
Tokyo, Japan

Dr. Alexander Keyl
Richard B. Russell Research Center
Athens, Georgia

Dr. David G. I. Kingston
Virginia Polytechnic Institute and State University
Blacksburg, Virginia

Dr. Hiroshi Kurata
National Institute of Hygienic Sciences
Tokyo, Japan

Dr. Stephen H. Larsen
Lilly Research Laboratories
Indianapolis, Indiana

Dr. Louise S. Lee
Southern Regional Research Center
USDA, SEA, AR
New Orleans, Louisiana

Dr. E. B. Lillehoj
Southern Regional Research Center
USDA, SEA, AR
New Orleans, Louisiana

Dr. J. C. MacDonald
National Research Council of Canada
Saskatoon, Saskatchewan, Canada

Dr. A. G. McInnes
Atlantic Regional Laboratory
National Research Council of Canada
Halifax, Nova Scotia, Canada

Dr. Merle Sid Masri
Western Regional Research Center
USDA, SEA, AR
Berkeley, California

Professor C. J. Mirocha
University of Minnesota
St. Paul, Minnesota

Dr. Nobuichi Morooka
Kagawa University
Kagawa, Japan

Professor Dr. E. Mutschler
de Johann Wolfgang Goethe-Universität
Frankfurt, Germany

Dr. S. Natori
National Institute of Hygienic Sciences
Tokyo, Japan

Dr. Stanley Nesheim
Department of Health, Education, and Welfare
Food and Drug Administration
Washington, D.C.

Dr. S. V. Pathre
University of Minnesota
St. Paul, Minnesota

Dr. A. E. Pohland
Department of Health, Education, and Welfare
Food and Drug Administration
Washington, D.C.

Mme. J. Polonsky
Institut de Chimie des Substances Naturelles
Gif-sur-Yvette, France

Dr. John Richard
National Animal Disease Laboratory
Ames, Iowa

Dr. John W. Ronaldson
Ruakura Agriculture Research Center
Hamilton, New Zealand

Dr. Harry Schroeder
USDA, SEA, AR
Texas A&M University
College Station, Texas

Dr. Peter M. Scott
Health Protection Branch
Health and Welfare Canada
Ottawa, Ontario, Canada

Dr. William G. Sorenson
The University of Oklahoma
Norman, Oklahoma

Dr. Pieter S. Steyn
National Chemical Research Laboratory
CSIRO
Pretoria, South Africa

Dr. G. M. Strunz
Canadian Forestry Service
New Brunswick, Canada

Professor Dr. Ch. Tamm
Institut für Organische Chemie der Universität Basel
Basel, Switzerland

Dr. A. Taylor
Atlantic Regional Laboratory
National Research Council of Canada
Halifax, Nova Scotia, Canada

Dr. M. Tenabe
Stanford Research Institute
Menlo Park, California

Professor Y. Ueno
Science University of Tokyo
Tokyo, Japan

Dr. A. C. Waiss, Jr.
Western Regional Research Laboratory
USDA, SEA, AR
Albany, California

Dr. Steve Weinreb
Fordham University
New York, New York

Dr. John M. Wells
Southeastern Fruit & Tree Nut Research Laboratory
USDA, SEA, AR
Byron, Georgia

Dr. Benjamin J. Wilson
Vanderbilt University
Nashville, Tennessee

Dr. David Wilson
University of Georgia College of Agriculture
Coastal Plain Station
Tifton, Georgia

Professor Yuzuru Yamamoto
Kanazawa University
Kanazawa, Japan

Professor Dr. M. Yamazaki
Chiba University
Chiba, Japan

Spectral Format

Samples and/or copies of the various spectra were solicited from various individuals and we are grateful to those who responded to our request. Copies of spectra received from individuals were traced and photographed, otherwise spectra were photographed directly from an original black-ink recording on blank chart paper.

UV spectra of samples were taken with a Beckman model DB-G* recording spectrophotometer in methanol solution unless otherwise indicated. The recorder speed was 2.54 cm per minute. Spectra were calibrated with a holmium oxide standard.

IR spectra of samples were obtained with a Perkin-Elmer model 257 recording spectrophotometer equipped with a 3× beam condenser and baseline attenuator. Unless otherwise indicated, samples for analysis were prepared as a mull or as a thin film on a KBr window.

Mass spectra were obtained from individuals or institutions when possible. Mass spectra of collected samples were obtained on an A.E.I. MS-902 double focus instrument. Electron-impact spectra were run at 70 eV with an accelerating voltage of 8000 volts and a source temperature between 175° and 200°C. Chemical-ionization spectra were obtained on the same model instrument fitted with an S.R.I.C. chemical-ionization source. Positive ion spectra were recorded using isobutane as the ionizing reagent gas at ≃ 1 torr source pressure and source temperature at 175°–200°C.

Criteria for purity of samples were based on TLC analyses and correlation of the spectral data with those reported in the literature.

The appropriate reference scale with TMS at 0 ppm was added at the bottom of each NMR spectrum. For most of the ^1H spectra, a standard 10 ppm display

*Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or the National Institute of Environmental Health Sciences and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

is used. Many of the compounds contain peaks outside this range, and these peaks are recorded with an offset baseline above the normal 10 ppm spectrum at the left side of the chart. For spectra for which there is no scale for this portion of the spectrum, one can determine the offset by observing where the spectrum is located with respect to the 10 ppm scale. For example, a recording between 8 and 10 ppm corresponds to a sweep between 10 and 12 ppm and a recording between 7 and 10 ppm corresponds to a sweep between 10 and 13 ppm, etc.

An attempt has been made to assign as many of the ^1H spectra as possible using known trends and comparison among similar compounds. Assignments of specific protons are presented above each spectrum assigned.

The ^{13}C spectra presented are those obtained with proton noise decoupling using the Fourier transform technique. Chemical shifts are reported relative to internal TMS using the convention that downfield shifts are assigned positive ppm values. For D_2O soluble samples, dioxane was used as the internal reference, and the chemical shifts were converted to the TMS scale using the conversion factor of 67.4 ppm. In a few cases for which long-term accumulation was required, no reference was added and the chemical shifts were referenced to the solvent peaks and later converted to the TMS scale using 77.0 ppm for $CDCl_3$ and 40.4 ppm for DMSO. Sufficient numbers of pulses were accumulated to provide adequate signal-to-noise ratios.

The assignment of chemical shifts of each ^{13}C spectrum is presented above each spectrum. Assignments are based on single frequency off-resonance decoupling results, comparison with related compounds, and data reported in the literature. The results of the single frequency off-resonance decoupling spectra are reported along with the chemical shifts as s, d, t, q for quaternary, methine, methylene, or methyl carbons, respectively. In a few cases, no sample was available and spectra were traced; therefore, no single frequency off-resonance decoupling results are available. These spectra were not assigned unless a sufficient number of spectra of related compounds were available.

The ^1H spectra in this collection were obtained on either Varian HA-100 or XL-100-12 spectrometers or a JEOL PFT-100 spectrometer using 5 mm sample tubes. The XL-100-12 spectrometer was equipped with the Varian 620-L disk data system, and the PFT-100 spectrometer with the JEOL EC-100 disk data system. TMS was used as an internal lock on the HA-100 instrument, whereas the deuterium resonance from the deuterated solvent provided the lock signal on the XL-100-12 and PFT-100 instruments. For samples run in the Fourier transform mode, standard operating conditions were a 90° pulse with a repetition time of 4.0 seconds using 8K data points.

^{13}C spectra were obtained with PFT-100 and XL-100-12 instruments using 10, 5, and 1.7 mm sample tubes, depending on the amount of sample available. The deuterium resonance from the deuterated solvent provided the lock signal and TMS was used as an internal reference in most cases. All samples were

run in the Fourier transform mode and standard operating conditions were a pulse angle of 30° with a repetition of 3.0 seconds using 8K data points. An exponential weighting factor of −1 was applied to the free induction decay before Fourier transformation. Sweep widths of either 5000 Hz or 6250 Hz were used, depending on the type of carbonyl carbons present in the compound. Peak positions and calculated chemical shifts were obtained from computer printouts and the chemical shifts are accurate to ±0.1 ppm.

Most samples were run in $CDCl_3$ and/or DMSO. As a general rule, the solubility of the compound in question was first checked in $CDCl_3$. When the compound was not sufficiently soluble in $CDCl_3$ alone, a few drops of DMSO was added. At this point, if the compound was not sufficiently soluble, the $CDCl_3$ was removed and the compound was dissolved in DMSO. In a few cases, the solubility of the compound in either D_2O or acetone was known and spectra were obtained in these solvents. For most compounds, the ^{13}C spectrum was obtained first and the ^1H spectrum was obtained on the same sample. Despite our best efforts, many of the ^1H spectra obtained in DMSO show a peak for H_2O absorbed from the atmosphere during the interval between sample preparation and determination of the spectrum.

Contents

Preface / ix

Acknowledgments / xi

Spectral Format / xv

1 **The Aflatoxins** / 1

2 **Sterigmatocystins** / 67

3 **Versicolorin Group** / 94

4 **Ochratoxins** / 128

5 **The Trichothecenes** / 152

6 **The Cytochalasins** / 264

7 **Rubratoxins** / 344

8 **Tremorgen Group** / 355

9 **Toxic Lactones** / 510

10 **Roquefortines** / 527

11 **Epipolythiopiperazine-3,6-diones** / 569

12 **Alternaria Toxins** / 614

13 **Secalonic Acids** / 646

14 **The Malformins** / 670

15 ***Penicillium islandicum* Toxins** / 683

16 **Sweet Potato Toxins** / 716

17 **Viridiol Group** / 738

18 ***Aspergillus* Toxins** / 753

19 ***Penicillium* Toxins** / 823

20 ***Fusarium* Toxins** / 893

21 **Miscellaneous Toxins** / 911

Molecular Formula Index / 925

Molecular Weight Index / 927

Compound Index / 929

Microorganism/Plant Source Index / 933

1

The Aflatoxins

The aflatoxin group, notably aflatoxin B_1, has had the most profound impact on the development of the science of mycotoxicology. Their discovery in 1960 as the cause of "Turkey X" disease and subsequent implication in other mycotoxicoses has had the effect of stimulating an interest in and an awareness of other possible mycotoxin problems.

The four naturally occurring aflatoxins, B_1, B_2, G_1, and G_2, are acutely toxic and carcinogenic metabolites produced exclusively by *Aspergillus flavus* and the closely related fungus *A. parasiticus*. Other members of the group are derived from these four as metabolic products of microbial or animal systems (such as M_1, M_2, P_1, Q_1, and aflatoxicol) or produced spontaneously in response to the chemical environment (such as B_{2a}, G_{2a}, and D_1).

The aflatoxins are highly fluorescent, highly oxygenated, heterocyclic compounds characterized by dihydrodifurano or tetrahydrodifurano moieties fused to a substituted coumarin moiety. Aflatoxin B_1 is the most prevalent naturally occurring, acutely toxic, and carcinogenic member of the group.

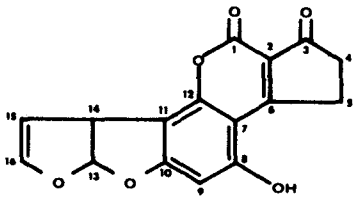
Although the toxicology of the aflatoxins varies considerably among species and with regard to age, sex, and nutrition, the primary organ affected is the liver. Gross clinical signs are growth retardation and weight loss due to reduced feed intake and efficiency, followed by severe tenesmus a few days before death. Postmortem examination may show fatty infiltration of the liver, liver fibrosis, ascites, visceral edema, bile duct proliferation, and hepatic carcinoma, depending on species involved. The ability of aflatoxin B_1 to induce liver carcinoma varies considerably with species. Trout and ducklings are very susceptible, but most animal species appear less prone to aflatoxin-induced hepatic carcinoma.

More subtle effects of aflatoxin ingestion are breakdown in the immune response and synergism or antagonism with various vitamins.

Several excellent reviews and at least one book which discuss various aspects of the aflatoxins are available.[2,10,11,24,50]

The importance of the aflatoxins in mycotoxicoses of animals and man cannot be overemphasized or underestimated.

Aflatoxin group	Molecular weight	Molecular formula
Aflatoxin P_1	298.0477	$C_{16}H_{10}O_6$
Aflatoxin D_1	286.0841	$C_{16}H_{14}O_5$
Parasiticol (aflatoxin B_3)	302.0790	$C_{16}H_{14}O_6$
Aflatoxin B_1	312.0633	$C_{17}H_{12}O_6$
Aflatoxin Q_1	328.0582	$C_{17}H_{12}O_7$
Aflatoxin G_1	328.0582	$C_{17}H_{12}O_7$
Aflatoxin M_1	328.0582	$C_{17}H_{12}O_7$
Aflatoxin B_2	314.0790	$C_{17}H_{14}O_6$
Aflatoxicol A	314.0790	$C_{17}H_{14}O_6$
Aflatoxicol B	314.0790	$C_{17}H_{14}O_6$
Aflatoxin G_2	330.0739	$C_{17}H_{14}O_7$
Aflatoxin M_2	330.0739	$C_{17}H_{14}O_7$
Aflatoxin B_{2a}	330.0739	$C_{17}H_{14}O_7$
Aflatoxin G_{2a}	346.0688	$C_{17}H_{14}O_8$
Aflatoxicol *O*-ethyl ether A	342.1103	$C_{19}H_{18}O_6$
Aflatoxicol *O*-ethyl ether B	342.1103	$C_{19}H_{18}O_6$



Common name[20]	Aflatoxin P_1
Molecular weight	298.0477
Molecular formula	$C_{16}H_{10}O_6$
General characteristics[6,20]	Pale yellow needles from methanol–benzene–hexane, mp >320°C $[\alpha]_D^{20}$ −574° (C = 0.08 in methanol)
UV data[6]	λ_{max}^{EtOH} nm (ϵ): 226(20,400), 267(11,200), 342(14,900), 362(15,400), and 425(2,500)
Source[6]	Aflatoxin P_1 is the principal urinary metabolite of aflatoxin B_1 in rhesus monkeys. It occurred in the urine in unconjugated (3%), sulfate (10%), and glucuronide (50%) forms.
Toxicity data[6]	In a mouse bioassay using IP injection, aflatoxin P_1 showed considerably less toxicity than aflatoxin B_1. At a dosage of 100 mg/kg, no mortalities were observed; at 150 mg/kg there were 2 mortalities in 15 animals; and at 200 mg/kg, no mortalities occurred. Aflatoxin B_1 in the same assay had an LD_{50} of 9.5 mg/kg.