Antifungal Chemotherapy

Edited by

D. C. E. Speller

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Preface

The writing of this book at this particular time reflects the growing interest of the medical profession in antifungal chemotherapy. The incidence of systemic fungal infections is increasing with modern medical and surgical treatment, and clinicians everywhere are becoming more aware of the problem. It has been appreciated that with prompt diagnosis and apt treatment the prognosis of these infections, even in the very impaired host, is far from hopeless. There is now a small, but increasing, range of effective drugs for systemic use in fungal infection. Amphotericin B has established its value, the role of flucytosine can now be more clearly assessed, and newer introductions, such as miconazole and other imidazoles, present a challenge to established treatment.

Systemic fungal infections are far less common than bacterial, but may occur sporadically in any hospital or practice. Although superficial fungal infections are among the most common in the population, their limited range and comparatively benign nature give them little prominence in medical education. Many clinicians and laboratory workers are diffident about managing fungal infections, feeling that they are intrinsically different from bacterial diseases. One of the main aims of this book is to display the particular characteristics of fungi and fungal infections, but to show that the principles of good treatment, and the laboratory techniques, devised for other infections may be applied to them also.

The first section of the book reviews the antifungals used in systemic treatment, with separate chapters devoted to important drugs and groups of drugs: the polyenes, flucytosine, the imidazoles, and griseofulvin. It is hoped that these, written by specialists with a particular interest in the drug concerned, will give a general survey and also contain the basic data required in practical and theoretical work. The minor drugs for systemic use—of specialized use, historical interest or recent introduction—are dealt with briefly. Throughout the book, references (with full titles) have been provided for important or controversial points.

In the second section the subject is approached from the clinical aspect, with advice on the treatment of fungal infections, setting these firmly in the background of clinical diagnosis and management, and not restricting treatment to the administration of the drugs. Superficial fungal infections are reviewed, and here the main preparations for topical use only are described. The deep mycoses are considered by the body site where they occur, and also more generally in the setting of the compromised host. In the case of the often exotic infections

caused by the so-called 'pathogenic fungi', more clinical, epidemiological, and diagnostic information is given to support the data about the treatment of these conditions. Inevitably, there is some duplication of material in these chapters and some diversity of opinion about details of treatment, but it is intended that the various approaches to the subject should have different practical usefulness in clinical medicine. Efforts have been made, by cross-referencing and the extensive index, to link material in the various chapters.

The final section deals with laboratory aspects, and it is hoped that the practical and well tried methods set forth will encourage many laboratories to assess and monitor antifungal drugs themselves when the need arises.

In general, only drugs effective against true fungi, rather than actinomycetes (such as *Actinomyces* and *Nocardia* spp.), have been considered extensively, but infection by *Nocardia* spp. is dealt with in Chapter 9, and mycetoma caused by actinomycetes in Chapter 7.

A book with many authors depends on the assistance of numerous people, and all contributors would acknowledge their secretarial assistance, the advice of colleagues, and the help of the medical and scientific advisers to the pharmaceutical industry, who have a most important role in the dissemination of information about antifungals. I must acknowledge with gratitude the cheerful and professional co-operation of all the contributors themselves, and particularly Professor D. W. R. Mackenzie's continuing advice, and Dr R. J. Hay's welcome assistance in the late stages of compilation. Many contributors to meetings of the British Society for Mycopathology have kept alive an interest in antifungals in Britain, given perspective to the subject, and suggested the form of this book.

Bristol, December 1978 D. C. E. SPELLER

NOMENCLATURE

Wherever possible the approved names of fungal diseases and the fungi causing them from the Medical Research Council Memorandum No. 23 (1977) have been used. Approved names of antifungal agents (British Pharmacopoeia Commission, 1977) have been used throughout. Extended chemical nomenclature is given in the various chapters in Part 1 of the book where the more commonly encountered proprietary names are also included.

British Pharmacopoeia Commission (1977) *Approved Names*, Her Majesty's Stationery Office, London.

Medical Research Council Memorandum No. 23 (1977) *Nomenclature of Fungi Pathogenic to Man and Animals* (4th edn), Her Majesty's Stationery Office, London.

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CHAPTER 1

The Polyenes

G. MEDOFF AND G. A. KOBAYASHI

INTRODUCTION

Since the first report of the discovery of nystatin (then called fungicidin) by Hazen and Brown in 1951, many other polyenes have been described. Fracidin

was discovered in the same year, followed by the isolation of rimocidin and endomycin in 1951 and ascosin, trichomycin, and antimycoin in 1952. Amphotericin B was first isolated and described by Gold *et al.* in 1956 and its antifungal properties and relatively low toxicity for humans were quickly exploited to treat systemic fungal infections in patients. The discovery rate of the polyenes has continued up to the present time and the list has now reached approximately 87 compounds, most of which have reasonable chemical delineation. Because it appears that the polyenes are the most commonly occurring single group of all the antibiotics produced by the genus *Streptomyces*, there is good reason to think that there will be many more polyene antibiotics discovered.

Among the large list of polyenes that have been isolated and described are some of the most effective antifungal agents known. Problems associated with solubility, stability, absorption, and toxicity, however, have made only a few of these agents therapeutically useful. Table 1.1 lists those polyene antibiotics that have been clinically and biologically useful and which will be covered in this review.

Table 1.1. Polyene antibiotics that have been useful experimentally and clinically

Name	Producing organism	Chemical composition	Molecular weight
Amphotericin B	*Streptomyces nodosus*	$C_{47}H_{73}NO_{17}$	924
Filipin complex	*S. filipensis*	$C_{35}H_{58}NO_{11}$	655
Nystatin	*S. albidus* or *S. noursei*	$C_{47}H_{75}NO_{17}$	926
Natamycin (pimaricin)	*S. natalensis*	$C_{33}H_{47}NO_{14}$	666
Trichomycin	*S. hachijoensis* and *S. abikoensis*	$C_{61}H_{86}N_2O_{21}.2H_2O$	1,230
Candidin	*S. viridoflavus*	$C_{47}H_{71}NO_{17}$	922
Candicidin	*S. griseus*	$C_{63}H_{85}N_2O_{19}$	1,200
Etruscomycin (lucensomycin)	*S. lucensis*	$C_{36}H_{57}O_{14}N$	739
Hamycin	*S. pimprina*	$C_{57}H_{89}N_2O_{14}$	1,147

CHEMICAL PROPERTIES OF POLYENE ANTIBIOTICS

All of the polyene antibiotics analysed to date have certain common structural features in addition to their conjugated double bond system. They are all characterized by a macrolide ring of carbon atoms closed by the formation of an internal ester or lactone. The presence of the lactone confers a highly characteristic peak on the infra-red spectra of these compounds. The ring sizes of the various polyenes vary from 12 to 14 up to 35 to 37 carbon atoms. The conjugated double bond system is contained exclusively within the cyclic lactone. The analysis of the characteristic ultra-violet spectra of the polyene antibiotics and also the X-ray crystallographic analysis of an *N*-iodoacetyl derivative of

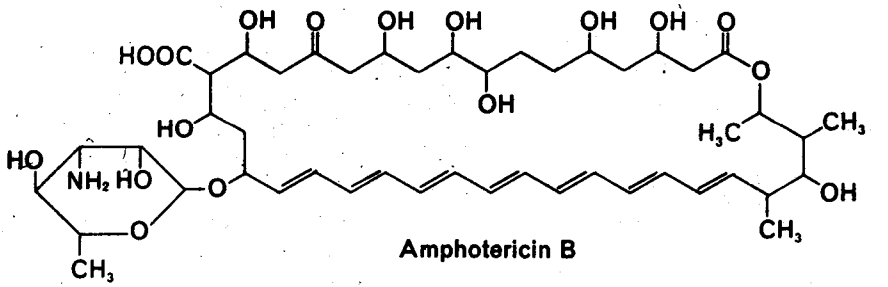
amphotericin B (Ganis *et al.*, 1971) indicate that all the double bonds are in the *trans* conformation. Certain theoretical considerations have been advanced suggesting that there should be $(4n+4)$ carbons in the macrolide ring, where n is the number of double bonds in the conjugated system (Oroshnik and Mebane, 1963). The possibility also exists that the polyene antibiotics may, under certain circumstances, undergo conformational changes in which some or all of the *trans* double bonds convert to *cis* isomers.

The second highly characteristic feature of the polyene antibiotics is the large number of hydroxyls present on the molecule. They are usually distributed along the macrolide ring on alternate carbon atoms. The number of hydroxyls varies from 6 to 14. The presence of such a large number of hydroxyl groups has posed challenging problems to the chemist who is concerned with their precise location. Also, the presence of the polar hydroxyl groups and multiple hydrophobic double bonds confer upon the polyene antibiotics the additional characteristic chemical property of being amphipathic. It is likely that this amphipathic feature plays an important role in the mode of action of these substances as they interact in various biological systems. Inspection of the structures given in Figures 1.1 and 1.2 reveals that these hydrophilic and hydrophobic components reside on opposite sides of the macrolide ring.

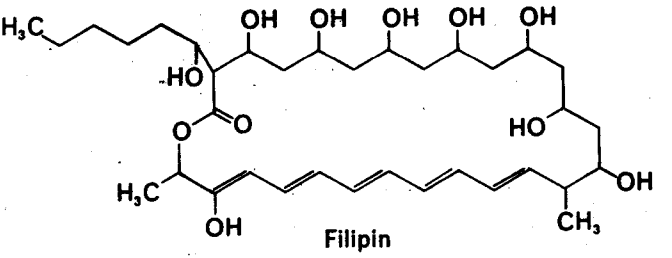
Elemental analyses of the polyene antibiotics indicate that they contain carbon, hydrogen, oxygen, and, in some instances, nitrogen. Many have a net charge conferred upon them by the presence of a single amino group, or a single carboxyl group, or both. In the latter instance they have a zwitterionic character.

The amino group present in some of the polyene antibiotics is associated with an amino sugar that is connected to the macrolide ring through a glycoside bond. In all instances the carbohydrate moiety has been found to be mycosamine. Its elemental analysis identifies it as $C_6H_{13}O_4N$, and its structure as 3-amino-3,6-dideoxymannose (Walters *et al.*, 1957). This is a 3-amino analogue of *D*-rhamnose or 6-deoxy-*D*-mannose. The structure was unequivocally established in 1961 by its chemical synthesis (Von Saltza *et al.*, 1961). Mycosamine has been found in the polyene antibiotics pimaricin, etruscomycin, trichomycin, candicidin, candidin, and amphotericin B. The α-pyranose ring form of mycosamine is more stable than the α-furanose ring structure.

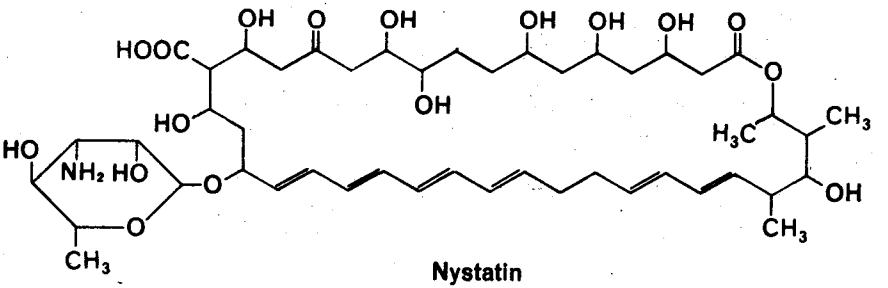
The most characteristic physical property of the polyene antibiotics is the ultra-violet absorption spectrum. The ultra-violet spectra of all the polyenes have a regular series of sharp peaks of absorption, which are separated by sharp troughs, all in the range of approximately 400–280 nm. Oroshnik and Mebane (1963) have given an extensive tabulation of the exact absorption maxima for many of the polyene antibiotics and have identified the ultra-violet absorbing system as being due to conjugated double bonds. Careful analysis of the ultra-violet spectra indicates that there are several distinct classes of chromophores: tetraenes, pentaenes, hexaenes, and heptaenes. The molar extinction



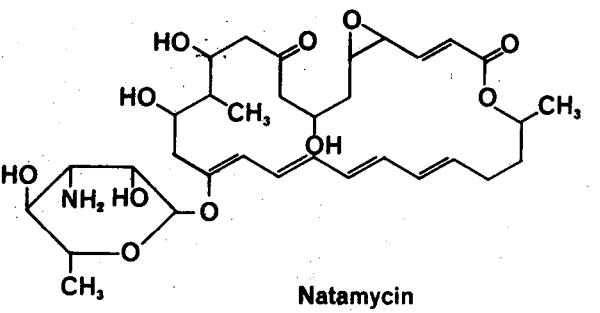
Amphotericin B



Filipin



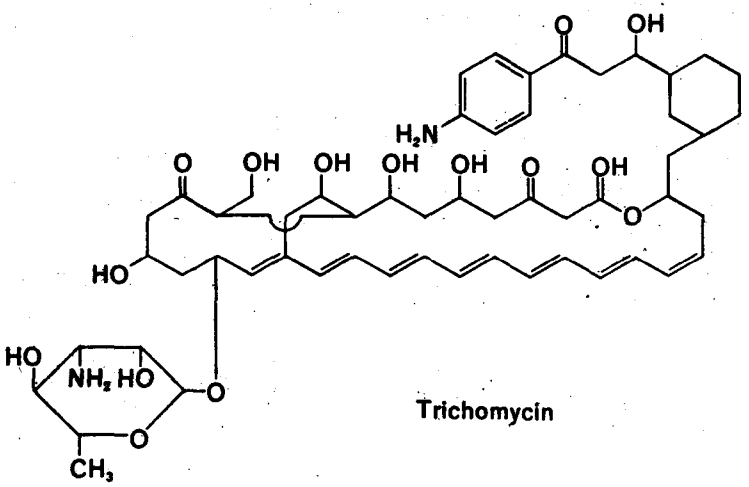
Nystatin



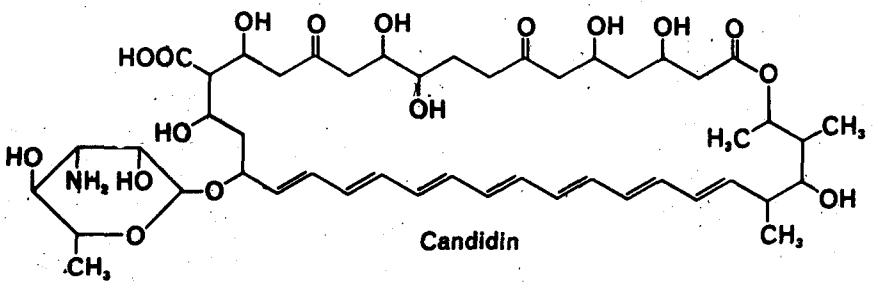
Natamycin

Figure 1.1.

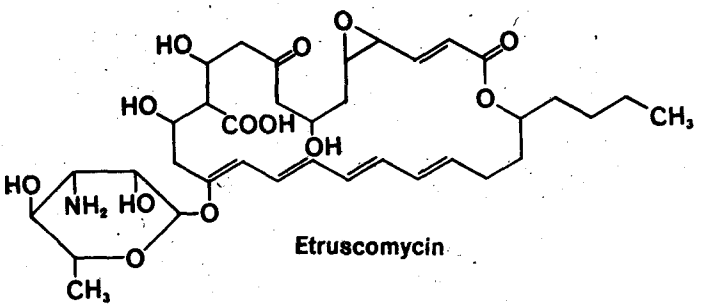
coefficients of the various absorption maxima range from 2 to 8×10^4, but it is not a simple matter to measure accurately the absorption of aqueous solutions of polyene antibiotics. For example, for filipin there is a difference in the molar extinction coefficients of the various ultra-violet maxima, depending on whether



Trichomycin



Candidin



Etruscomycin

Figure 1.2.

the antibiotic is dissolved in an organic solvent or an aqueous solvent. This phenomenon was first reported by Lampen *et al.* (1960). Norman *et al.* (1972b) have found that the exact diminution of the spectrum of equivalent concentrations of filipin in water as compared to methanol is determined in a complex manner by a number of variables which include: (1) the concentration of filipin; (2) the manner of introduction of filipin dissolved in dimethylformamide into the water; (3) the length of time of vigorous mixing of the aqueous solution; (4) the temperature; and (5) the age of stock solution of filipin dissolved in dimethylformamide. It is suspected that a portion of this reduction of ultra-violet absorbance of filipin in water is due to either micelle or aggregate formation. It was noted by these workers that filipin in organic solvents followed the Beer–Lambert relationship over the range $1\text{–}400 \times 10^{-6}$ M, but that in aqueous solutions filipin did not follow the Beer–Lambert law over the same range of concentration. In spite of these technical difficulties, Norman *et al.* (1972a) have been able to utilize the ultra-violet absorbing properties of the polyene antibiotics to carry out studies on their biological and biochemical mode of action.

Although there are at least 87 different polyene antibiotics classified according to the number of double bonds, we shall cover in this review only those of the greatest biological interest. The following is a brief description of the prominent structural features of these selected polyene antibiotics which have been chemically characterized. Figures 1.1 and 1.2 present their structure.

1. Amphotericin B

Amphotericin B, isolated from a strain of *Streptomyces nodosus*, is the only polyene antibiotic with known chemical structure and absolute configuration. Previous work has indicated that one molecule of amphotericin B contains a mycosamine and a free carboxyl group. The macrolide ring structure contains 37 carbon atoms and there are 7 double bonds and 7 free hydroxyls on the ring structure. One very interesting feature, appreciated for the first time as a consequence of X-ray analysis, is the presence of a 6-membered ketal ring which is included as an appendage to the macrolactone ring. Amphotericin B also contains 14 asymmetrical centres, all of which are present in the macrolide lactone ring. One additional unique feature that was determined from the X-ray analysis is that the mycosamine is bonded by an α-glycosidic linkage to the hydroxyl group at carbon 19. The 6-membered ketalic ring as well as the mycosamine pyranose ring are both in the 'chair' conformation. The hydroxyls at carbons 8, 15, and 35 are equational, whereas those at carbons 3, 5, 9, and 11 are in the axial configuration.

There are several interesting features of the crystal structure that became evident from the X-ray analysis. The molecule was found to be amphipathic, with all the hydroxyl groups and the carbohydrate moiety on one side of the lactone ring opposite the chromophoric double bond groups, located on the opposite