

CHAPTER 1

Fungi and the Development of Microbiological Chemistry

1.1 Introduction

Fungi are widespread, non-photosynthetic microorganisms that play a vital role in the environment, particularly in the biodegradation of organic material. The study of their metabolites and metabolism has made many contributions to the overall development of chemistry. Although the biosynthetic pathways that fungi utilize to construct their metabolites have general features in common with those found in bacteria, plants and mammals, they differ in detail and the structures of the resultant natural products are often different. This book is restricted to fungal metabolites but the reader should not lose sight of other natural products produced elsewhere in the living world.

Since fungi do not contain chlorophyll and are not photosynthetic organisms, they gain their energy and many of the nutrients to supply their biosynthetic pathways through the degradation of plant and other matter. Their environmental role is that of recycling. Their widespread provenance is often illustrated in one of the first practical exercises of many microbiology courses. A Petri dish containing a nutrient agar is exposed to the atmosphere for a few minutes. It is then incubated to reveal the range of organisms, both bacteria and fungi, whose spores are present in the atmosphere and which fell onto the plate in a relatively short time. It was a chance contaminant of an agar plate that led to the isolation of penicillin and changed the face of medicinal chemistry.

Fungi are eukaryotic organisms with a distinct nucleus, unlike bacteria which are prokaryotes. This also distinguishes them from another wide family of soil microorganisms, the Actinomycetes (*e.g.* Streptomycetes), which are often considered along with the bacteria. Yeasts, however, are regarded as a unicellular form of a fungus. Some fungi grow in a symbiotic relationship with photosynthetic algae or cyanobacteria in the form of lichens.

Fungi do not grow in isolation. Some attack plants, insects and mammals as pathogens whilst others are saprophytic and grow on dead material. Some live in a positive symbiotic relationship with a host organism. Thus, there are mycorrhizal fungi that are associated with the roots of plants and facilitate the uptake of nutrients by the plant. Others are endophytic organisms that grow within the vascular system of the plant. Throughout the natural world there is a chemical language between the fungus and its host which determines the nature of this relationship. We are beginning to understand the role of fungal and plant metabolites in this ecological communication.

The chemistry of fungi impinges on many aspects of our daily life whether it be in the role of yeasts in the production of bread and wine, the edible mushrooms or the manufacture of antibiotics such as the penicillins. The fungal diseases of crops, ornamental plants and trees and the spoilage of stored foodstuffs are serious economic problems. The control of the phytopathogenic organisms and the detection of their toxic metabolites in the food chain provide further chemical problems.

The microbiological chemist is interested in the structure, chemistry and biological activity of fungal metabolites. The biosynthesis of these metabolites, the sequences, stereochemistry and mechanism of the individual steps, together with the structure and regulation of the enzymes involved, is a major area of enquiry. The ecological chemistry of fungal interactions with plants and insects has provided another area of chemical investigation. An understanding of the chemical basis of fungal bio-control agents may have useful agrochemical applications.

As biodegradative organisms, fungi can carry out microbiological transformations of extraneous chemical substances. They can behave as self-replicating, environmentally friendly, chiral reagents. Their ability to carry out transformations that are chemically difficult, *e.g.* hydroxylations at sites that are remote from other reactive centres, has been exploited commercially. The scope of these biotransformations and the development of predictive models so that the use of an organism can be built into a synthetic strategy is yet another area of investigation. The use of the biodegradative ability of fungi in the bio-remediation of contaminated land is a further application of chemical interest.

There are various estimates of the number of species of fungi. These range from 100 000 to 250 000. What is clear is that only a relatively small number, of the order of a few thousand, have been thoroughly investigated by microbiological chemists. Furthermore, there are often different strains of the same species. Whilst these may be morphologically similar, their metabolites can be quite diverse. Some metabolites may be produced consistently by all the strains of a particular species whilst other metabolites may be variable. The chemistry of an organism can also vary with the conditions under which it is grown. Unsurprisingly, therefore, some species of economic importance, *e.g.* *Penicillium chrysogenum*, have generated immense chemical interest.

1.2 Structure of Fungi

At first sight the structures of fungi appear quite diverse. The fruiting body of the common edible mushroom, *Agaricus bisporus*, is very different from the

green *Penicillium* species growing on the surface of some cheese. However, there are some common features. The basic structural units of most fungi are the filaments known as the hyphae. Collectively, hyphae can aggregate to form a felt known as the mycelium. In some of the higher fungi, the hyphae can aggregate to form long strands and even differentiate to create a structure almost like a boot-lace, which is known as a rhizomorph. Another name for the honey-fungus, *Armillaria mellea*, which does considerable damage to trees, is the 'boot-lace fungus', which aptly describes the rhizomorphs by which it spreads underground.

The higher fungi, the mushrooms and toadstools, develop complex and readily observable structures known as fruiting bodies. These sprout from their mycelium, particularly in the autumn, and produce spores. At the other extreme some unicellular micro-fungi, such as the yeasts, produce small globular or ellipsoid cells that are only visible under the microscope.

The hyphae may be long single multi-nucleate aseptate (undivided) cells through which the cellular cytoplasmic fluids may flow. Other hyphae are septate and have distinct divisions. In these much of the chemical activity takes place at the growing tip. The lower micro-fungi only become septate as the culture ages whilst the higher macro-fungi become septate at an early stage and, as rhizomorphs are formed, their function may differentiate.

The form a fungus takes can depend on the culture conditions. Some fungi will have a yeast-like form under one set of conditions and a filamentous form under others. Under inhospitable conditions, often exploited in the storage of cultures, an organism can develop a 'resting' stage. In the wild this can allow spores to over-winter in the soil. In the laboratory, fungal cultures are often stored at low temperatures on agar under oil or in sealed vials on sand.

When a fungus is grown in suspension in a nutrient medium contained within a conical flask, the mycelium will sometimes clump together whilst at other times a well-dispersed mycelial suspension or even a mycelial mat is formed. The aeration and hence the metabolic capabilities of these forms can differ. The aeration can be quite poor within tightly formed clumps and this can affect the metabolism of the fungus. It is often difficult to get higher fungi to produce fruiting bodies in laboratory culture and again this can affect their metabolite production. Some rapidly growing fungi such as *Rhizopus* species produce fine long hyphae that spread rapidly across the agar in a Petri dish. They may produce a covering of aerial mycelium with the appearance of household dust. Indeed, quite a lot of household dust is fungal mycelium.

Fungi usually reproduce by spores although they can also develop vegetatively from mycelial fragments. The spores may be pigmented and some may have a gelatinous polysaccharide coating to facilitate their dissemination by a carrier and their attachment to a host. They are often borne on a specific thallus or germ tube. Hyphae that carry these are known as conidiophores. A culture such as that of *Botrytis cinerea* may appear light grey as the mycelium spreads across a Petri dish and then it develops a ring of green-black sclerotial mycelium bearing spores.

1.3 Classification of Fungi

In the general taxonomic classification, fungi are grouped in terms of the following ranks: division, class, order, family, tribe, genus, section, and species. In the binomial description of a fungus, the first name is that of the genus and the second name is the species. The name (not italicized) that follows this may be that of the author who first described the species. There are often varieties and strains of particular species. The accession number in a culture collection can be important in defining the organism used to isolate a particular metabolite. Although some metabolites may be specific to particular strains, others may be more common and are found in a section of a genus. The structure of the reproductive organs and the mechanisms of reproduction form the basis of the classification of fungi. These organisms may be broadly grouped in the following way. There are the Phycomycetes or lower fungi, which have a simple thallus bearing the spores. They possess unicellular aseptate hyphae. In some classifications this class name is treated as a trivial name for the Mastigomycotina and Zygomycotina. Typical examples are the Peronosporales, which include plant pathogens such as *Pythium* and *Phytophthora* species and the Mucorales, which include the common *Mucor*, *Rhizopus* and *Phycomyces* species. The ‘damping-off’ fungus *Pythium ultimum*, found growing across over-zealously watered germinating seeds, is an example.

A second group are the higher fungi which have septate hyphae, and these can be divided into the Ascomycetes and the Basidiomycetes. In the Ascomycetes the spores are borne in a sac-like structure known as an ascus. This type of fruiting body or ascocarp is found in *Monascus* species. The fungus *M. ruber*, which produces the red colour on Chinese red rice, is an example of these. The genera *Penicillium* and *Aspergillus* belong to the class of Ascomycetes known as the Plectomycetes. The spores are held in a pear-shaped perithecium in another class known as the Pyrenomycetes. The saprophytic plant parasites of the Hypocreales are also members of this group. Some of the best known of the higher fungi are Basidiomycetes. Here the spores are borne in special distinctive fruiting bodies. The edible part of the common mushroom, *Agaricus bisporus*, is a typical example.

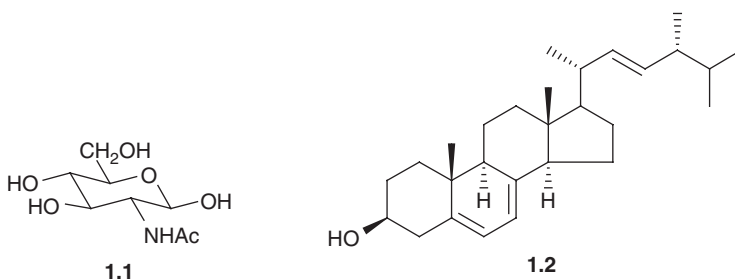
The final large group are the Fungi Imperfecti or Deuteromycetes. In these organisms, the perfect stage of reproduction is rare or unknown and for the most part they are cultured vegetatively. The *Fusaria* are the best known of these. This classification can be confusing because some fungi originally classified within the Fungi Imperfecti do have both an asexual imperfect stage and a perfect stage. Thus the fungus that produces the gibberellin plant hormones, *Gibberella fujikuroi*, is the perfect stage of *Fusarium moniliforme*.

The naming of fungi has undergone many changes over the years and this can be a source of confusion. For example, *Ophiobolus graminis* was the name given to a serious pathogen of wheat. This name was incorporated into that given to a family of terpenoid metabolites, the ophiobolanes, which were isolated from the fungus. However, the fungus is now known as *Gaeumannomyces graminis*. Ophiobolanes are also produced by a rice pathogen that was at one time known as *Helminthosporium oryzae* or *Drechslera oryzae* and is now described as *Bipolaris oryzae*. Many of the *Polyporus* species, which gave their

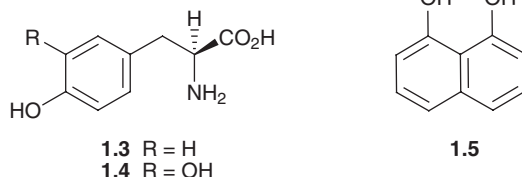
names to the triterpenoid polyporenic acids, have also been renamed as *Piptopteris*. When attempting to re-isolate a fungal metabolite, particularly from a culture that has been deposited in one of the culture collections, it is helpful to trace the provenance and naming of a particular isolate. When describing the isolation of a fungal metabolite, it is important to record the accession number of the culture in one of the major culture collections. If the strain of the organism is a new isolate it should be deposited in an accessible culture collection. Much valuable time has been wasted in unsuccessful attempts to re-isolate a fungal metabolite when the original culture has been lost.

1.4 The Fungal Cell Wall

The chemistry of the fungal cell wall contains some useful taxonomic markers. The cell wall is also a very important target for anti-fungal agents. The fungal cell wall differs in its structural components both from the bacterial cell wall and mammalian cell membranes. The fungal cell wall is a complex of chitin [a polymer of *N*-acetylglucosamine (**1.1**)], various mannoproteins together with α - and β -linked 1,3-D-glucans. Electron microscopy of the cell walls of the yeast *Candida albicans* shows that they are in layers attached to a plasma membrane. The major sterol in these is ergosterol (**1.2**) rather than cholesterol which is found in mammalian systems. Inhibitors of the biosynthesis of these components can, therefore, be selectively fungicidal.



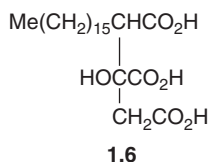
The development of novel anti-fungal agents is a continuing area of research. Furthermore, opportunistic fungal infections, particularly caused by *Candida* and *Aspergillus* species, are emerging as a source of morbidity and mortality amongst immunocompromised patients. Polyene antibiotics such as nystatin and amphotericin B bind to ergosterol much more avidly than to cholesterol and hence disrupt the fungal cell membrane. Ergosterol biosynthesis inhibitors such as the azole fungicides target a key stage in the biosynthesis of ergosterol, the C-14 α demethylation of lanosterol. Melanins are dark brown or black pigments that are present in fungal cell walls and are formed by the oxidation of phenolic precursors such as tyrosine (**1.3**), 3,4-dihydroxyphenylalanine (**1.4**) and 1,8-dihydroxynaphthalene (**1.5**). Some anti-fungal agents such as tricyclazole produce a weakening of cell walls by inhibiting melanin biosynthesis. More recently, several compounds that target the β -(1,3)-D-glucan and chitin synthases have been developed.



Part of the antagonistic interaction between fungi, such as that between *Trichoderma* and other organisms, includes the production of a chitinase. This allows the *Trichoderma* to attack the cell wall of its target organism. The hyphae of the *Trichoderma* can then penetrate the target fungus and sequester its nutrients.

1.5 History of Fungal Metabolites

The chemical activities of fungi have a long history. Many fungi, because of the competitive environment in which they live, produce antibiotics of varying efficiency. The Greek physician, Dioscorides described the use of an infusion that he called Agaricium, which was obtained from the larch polypore, *Fomitopsis (Polyporus) officinalis*, and was used for the treatment of consumption (tuberculosis). This biological activity has been attributed to the presence of agaric or laricic acid [α -cetylcitric acid (**1.6**)]. The ‘ice-man’, whose 5300 year old body was discovered some years ago in the ice in the Alps between Italy and Austria, had the birch polypore, *Piptoporus betulinus*, with him. This fungus is active against wound bacteria such as *Staphylococcus aureus*. There are records of the use of other fungi, particularly *Ganoderma lucidum*, in ancient Chinese medicine. The identification of moulds growing on cloth by their pigmentation and their treatment is described in the Old Testament of the Bible in Leviticus Chapter 13, Verse 47.



The hallucinogenic properties of fungi such as *Amanita muscari* were known to several peoples. It is possibly the Soma which was used in parts of Asia and Scandinavia. There are records from travellers in the 18th century of its use.

The toxicity of ergot was apparently known to the Syrians in 600 BC. The metabolites of the ergot fungus, *Claviceps purpurea* which grows on rye, contaminated rye bread and brought about the disease known in the Middle Ages as St Anthony’s Fire. Ergotism involved damage to the nervous system and vascular constriction, leading to death of the affected parts of the body. Subsequently, medicinal uses of ergot were developed. In the early nineteenth century ergot was used to induce childbirth and to prevent post-natal haemorrhage.

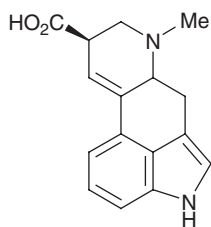
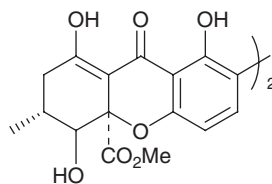
1.5.1 Fungal Metabolites in the Nineteenth Century

The fungus *Penicillium glaucum* was used by Pasteur in 1860 to degrade one enantiomer of tartaric acid and allow the resolution of a racemate in experiments that laid the foundations for the study of chirality. Pasteur was also one of the first to recognize the antagonism between microorganisms, which led in 1889 to the use of the term ‘antibioté’ by a French biologist Vuillemin to describe the substances involved. The term antibiotic was redefined much later by Waksman in 1941 to describe a natural product formed by a microorganism that inhibited the growth or killed another microorganism.

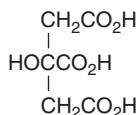
Fungi were recognized as the cause of several serious plant diseases in the middle of the 19th century. The need to control fungal diseases of plants, such as *Plasmopara viticola* infections of vines, led to the development by Millardet in 1883 of Bordeaux mixture (copper sulfate and lime).

The scale on which many natural product degradations were carried out in the late 19th century and the early 20th century precluded much structural work on fungal metabolites. With a few exceptions, fungal material was not available on a scale that would permit the isolation of the gram quantities of natural product that were used for structural studies in the days preceding spectroscopic methods.

One of the exceptions was ergot from *Claviceps purpurea*. The therapeutic as well as the toxic properties of ergot provided the stimulus for studies. Although an impure alkaloidal fraction of ergot had been described by Wenzell in 1866, a crystalline alkaloid, ergotinine, was isolated by Tanret in 1875. However, the structures of these alkaloids derived from lysergic acid (**1.7**) were not elucidated until 1935 when the work of Jacobs and Craig in New York and by Smith and Timons at the Wellcome Laboratories came to fruition. A crystalline yellow pigment, sclererythrin, was first isolated from ergot by Dragendorff in 1877. Subsequent studies led to the isolation of ergoflavin by Freeborn in 1912 and to ergochrysin in 1931 by Barger and Bergmann. The structures of these pigments (e.g. **1.8**), which differ from those of the alkaloids, were not established until the late 1950s and early 1960s. The ubiquitous fungal sterol ergosterol was first isolated by Tanret in 1879. The structure of ergosterol (**1.2**) was eventually established in 1933 by Chuang through an inter-relationship of the parent hydrocarbon ergostane with the cholic acids and cholesterol.

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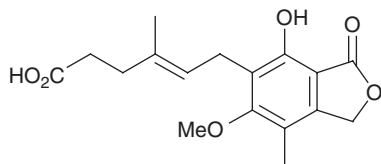
Several simple acids, such as oxalic and citric, were isolated from *Aspergillus niger* in 1891–1893. Commercial methods for the microbiological production of citric acid (**1.9**) were developed in the early 1920s.



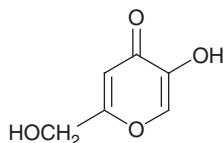
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1.5.2 Fungal Metabolites 1900–1940

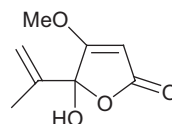
Mycophenolic acid, which is now used as an immunosuppressant to prevent organ rejection after transplant operations, was first isolated by Goscic from a *Penicillium* species in 1896. It was subsequently isolated from *P. stoloniferum* by Alsberg and Black in 1913 and from *P. brevicompactum* in 1932 by Raistrick. However, its structure (**1.10**) was not finally established until 1952. The much simpler fumaric acid was isolated by Ehrlich in 1911 from *Mucor stolonifer*. The fungus *Aspergillus oryzae* is used in Japan to produce the koji fermentation of rice to make saké. Kojic acid, 5-hydroxy-2-hydroxymethyl-4-pyrone (**1.11**), was first isolated from this organism in 1907 and its structure was established by Yabuta in 1924. The anti-bacterial agent penicillic acid was isolated from *Penicillium puberulum* by Alsberg and Black in 1910. Its structure (**1.12**) was established in 1936 by Raistrick, who had obtained it from *P. cyclopium*.



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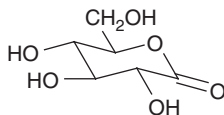


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1.12

The First World War saw the development of the bacterial fermentation of *Clostridium acetobutylicum* for the production of acetone and butanol. This provided a stimulus to microbiological chemistry. During the 1920s there were several studies on the effect of the composition of the medium on metabolite production, e.g. by *Aspergillus niger*, leading to the isolation of D-gluconic acid lactone (**1.13**) by Malliard in 1923. This compound had previously been obtained by Herrick and May from *Penicillium luteum* in 1912. Commercial fermentation methods using a chiral acetoin condensation mediated by the yeast, *Saccharomyces cerevisiae*, were developed in 1930 for the synthesis of L(-)-ephedrine from benzaldehyde.

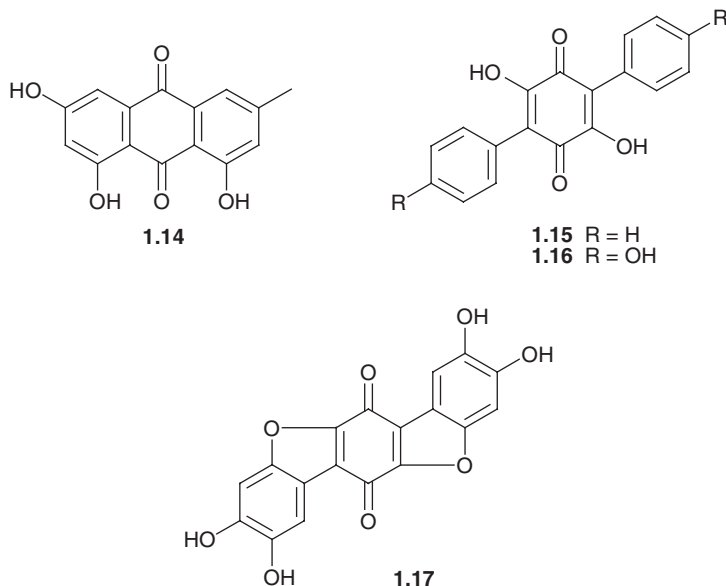


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During the 19th and early 20th centuries there had been several reports of poisoning arising from mouldy wall paper. In a study of microbiological

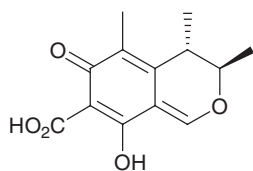
methylation, the formation of the poisonous and volatile trimethylarsine through the metabolism of arsenites present as pigments in wall paper by *Scopulariopsis brevicaulis* was established by Challenger in 1933.

Several quinonoid pigments of the higher fungi were identified in the 1920s by Kogl. These included frangulaemodin (**1.14**) from the blood-red agaric *Dermocybe sanguinea* in 1925, polyporic acid (**1.15**) from *Polyporus nidulans* in 1926 and atromentin (**1.16**) from *Paxillus atromentosus* in 1928. The terphenyl telephoric acid (**1.17**) is a widespread pigment of the Basidiomycetes.

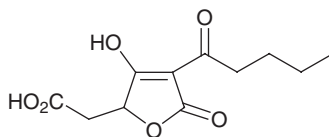


Raistrick studied the consumption of glucose from their growth medium by fungi and concluded that this was a useful guide to the production of fungal metabolites. By monitoring this to establish the period of a fermentation he was able to isolate and identify a series of mainly anthraquinone pigments from various *Penicillium*, *Aspergillus* and *Helminthosporium* species. Much of this work, which started in the Nobel Division of ICI at Ardeer in Ayrshire and was continued at the London School of Hygiene and Tropical Medicine, was published in the 1930s and 1940s.

The constituents of various *Penicillium* species growing as spoilage organisms on maize were investigated at this time in the context of a possible link with pellagra. Although this disease turned out to have a completely different origin, nevertheless the studies led to the identification and elucidation of the structure of several metabolites, including citrinin (**1.18**) from *Penicillium citrinum*, penicillic acid (**1.12**) from *P. cyclopium* in 1936 and a series of tetrone acids such as carlosic acid (**1.19**) from *P. charlesii* in 1934. Another metabolite, puberulic acid, isolated from *P. puberulum* in 1932 and a relative stipitatic acid isolated in 1942 from *P. stipitatum* were assigned theoretically interesting pseudoaromatic tropolone structures by Dewar in 1945.



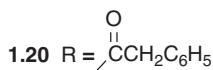
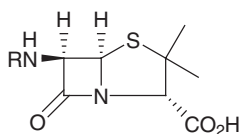
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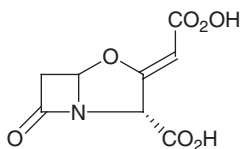
1.5.3 Fungi in the Antibiotic Era, 1940–1960

The discovery of penicillin by Fleming in the autumn of 1928, and which he reported in 1929, revolutionized medicinal chemistry after a lengthy gestation period. Fleming who was Professor of Bacteriology at St Mary's Hospital in Paddington, had an interest in the control of the bacterial infection of wounds by various agents, including lysozyme. During August 1928 he left a Petri dish containing a bacterial culture of *Staphylococcus aureus* for washing up. This plate became contaminated by fungal spores of a *Penicillium* species. Whereas *Penicillium* species grow quite well at 18–25 °C, most bacteria grow better at a higher temperature of 30–38 °C. At first, the weather that August was cool, allowing the *Penicillium* to develop. Subsequently, there was a hot period and the bacteria began to develop. However, there were clear zones of inhibition around the fungal contaminant, indicating the presence of an antibiotic. Fleming was able to isolate the fungus and demonstrate that it produced a powerful antibiotic. He originally identified the fungus as *Penicillium rubrum* but this was subsequently corrected by Raistrick and Thom to *P. notatum*. Fleming approached Raistrick for help in isolating the metabolite. However, the instability of penicillin precluded its isolation at that time. The isolation and structural work was taken up by Florey, Chain, Abraham and Heatley in 1938 in Oxford. The outbreak of the Second World War increased the urgency with which the research was undertaken. By 1940 the yield of penicillin had been increased and sufficient material was available for the first human trial to take place in February 1941. Collaboration with Oxford chemists (Robinson, Wilson Baker and Cornforth) and with the Northern Regional Research Laboratories in Peoria in the USA began in 1942/3. The β -lactam structure of penicillin (**1.20**) was eventually established in 1945 as a result of the chemical work and X-ray crystallographic studies by Dorothy Hodgkin. Further details of the work on penicillin are discussed later in the chapter on fungal metabolites derived from amino acids.

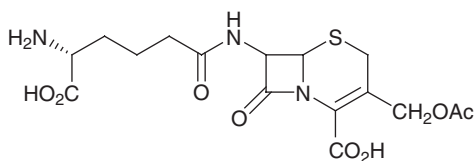


There are, however, several milestones in the development of the penicillins that can be recorded here. The core of the penicillin structure, 6-aminopenicillanic

acid (**1.21**), was isolated in 1959, paving the way for the preparation of semi-synthetic penicillins with enhanced biological activity. In 1960 the tripeptide, δ -(L- α -aminoadipoyl)-L-cysteinyl-D-valine (LLD-ACV) was isolated by Arnstein. This peptide has played a central role in biosynthetic studies, particularly by Abraham and Baldwin in Oxford, which have culminated in studies on the enzyme system isopenicillin N synthase and the demonstration of the role of this non-haem iron oxidase in the formation of the penicillin ring system. These studies have continued to the present time. The problem of the resistance of bacteria to the penicillins was already known in the 1940s. The discovery in 1976 of the β -lactamase inhibitor clavulanic acid (**1.22**), which is a metabolite of *Streptomyces clavuligenus*, made a significant contribution to reducing but not eliminating this problem. Methicillin resistant strains of *Staphylococcus aureus* (MRSA) pose a serious medical problem today.

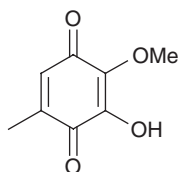
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The anti-bacterial activity of the penicillins stimulated a major search for other antibiotics. Many compounds were isolated from amongst the metabolites of *Streptomyces* obtained from soil. These included therapeutically useful antibiotics such as chloramphenicol, the tetracyclines, erythromycin and streptomycin. Several fungi yielded useful compounds. One of these was *Cephalosporium acremonium*. A biologically-active strain was obtained by Brotzu from a sewage outfall near Cagliari in Sardinia in 1945 and described in 1946. The β -lactam cephalosporin C (**1.23**) was isolated from this organism in 1954 and its structure determined in 1961 by Abraham and Newton.

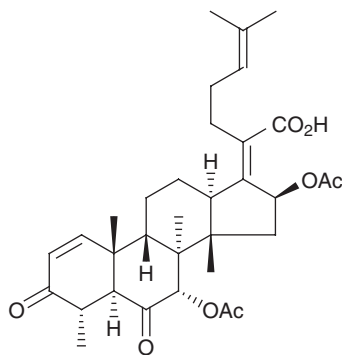
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Several investigations of the fungus *Aspergillus fumigatus* between 1938 and 1945 by Raistrick and by Chain and Florey led to the isolation of compounds with high anti-bacterial activity and diverse structure. These included a quinone, fumigatin (**1.24**), a steroidal antibiotic, helvolic acid (**1.25**), and a diketo-piperazine disulfide, gliotoxin (**1.26**). The latter had been isolated previously by Weindling in 1936 from *Gliocladium fimbriatum*. Griseofulvin was first isolated in 1939 by Raistrick from *Penicillium griseofulvum*. Studies of the antagonism

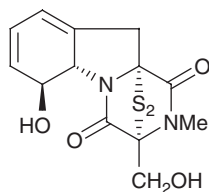
between fungi led to the isolation in 1946 of griseofulvin from *P. janczewskii* as an anti-fungal metabolite with a particular ‘curling’ effect on the hyphae of the plant pathogen, *Botrytis allii*. The structure of griseofulvin (**1.27**), which has been used in medicine to treat fungal infections of man, was established in 1952. It is discussed in Chapter 4.



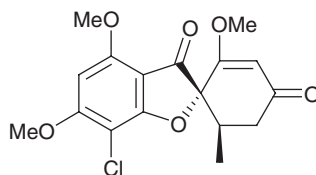
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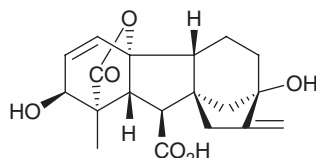
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1.5.4 Study of Fungal Plant Diseases 1940–1965

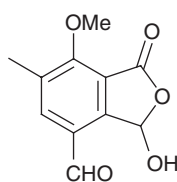
The association of fungi with plant diseases has provided the stimulus for many studies. One particular study was that of the ‘bakanae’ stem elongation disease of rice. Examination of the causative organism, *Gibberella fujikuroi*, led to the isolation of a crude gibberellin in 1938. Careful microbiological work at ICI afforded a strain whose major metabolite was gibberellic acid. This was described in 1954 by Cross. The full structure and stereochemistry of gibberellic acid (**1.28**) was established in 1962 through a combination of chemical and spectroscopic studies by Cross and Grove and X-ray crystallographic studies by Scott. Work on the biosynthesis of gibberellic acid, which is discussed later, led to the isolation of many other gibberellins and compounds possessing an ent-kaurene skeleton. In 1958 gibberellins were identified as plant growth hormones in higher plants by MacMillan. The enzymology and genetics of gibberellin biosynthesis in *Gibberella fujikuroi* are aspects of continuing interest. The metabolites of some other phytopathogenic organisms, such as *Alternaria* species, were identified at this time (see Chapter 8).



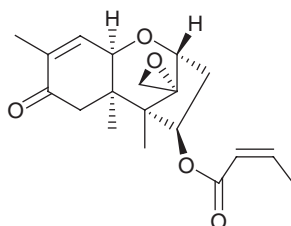
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1.5.5 Impact of Spectroscopic Methods on Structure Elucidation

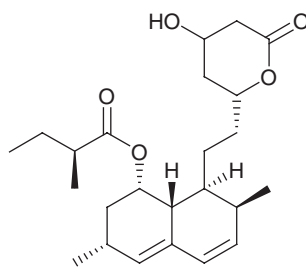
The development of modern spectroscopic methods from the 1950s onwards had an immense impact on the determination of the structures of fungal metabolites. The milligram scale on which these methods work meant that the amounts of material produced by different organisms in laboratory scale cultures, and that could be separated by chromatographic methods, could be studied. For example, correlations between the frequency of absorption and the structural environment of carbonyl groups played an important role in the determination of the structure of gladiolic acid (**1.29**) from *Penicillium gladiolii* in 1951 by Grove. The correlation tables published then have been used in many subsequent studies. The characteristic ultraviolet absorption of polyacetylenes was invaluable in their detection in the culture broths of many higher fungi in studies reported separately by Bohlmann and Jones between the 1950s and the 1970s. However, NMR spectroscopy has made the most important contributions. Some of the earliest applications of NMR spectroscopy to organic structure determination involved fungal metabolites such as gibberellic acid (**1.28**) (1959), cephalosporin C (**1.23**) (1961) and in correcting the structure of the trichothecenes (**1.30**) (1962). The structures of many mycotoxins and phytotoxins could not have been determined without the aid of ^1H and ^{13}C NMR spectroscopy. Advances in X-ray crystallography and in particular in the use of non-heavy atom methods have led to the solution of many natural product structures. The structure of a fungal metabolite, rosein III, was one of the first to be determined by direct methods in 1976.



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1.30

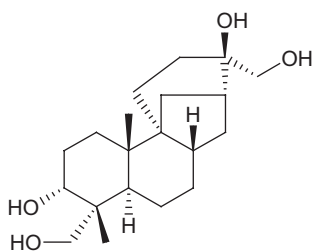


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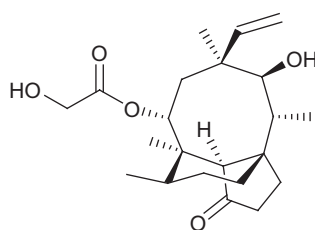
1.5.6 Fungal Metabolites 1965–2005

Over the last 40 years, many novel fungal metabolites have been isolated with quite diverse carbon skeleta. There have been two main driving forces behind this

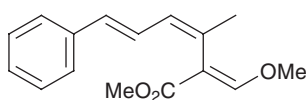
work. One has been the search for fungal metabolites with useful pharmaceutical activity; this has led to several compounds that have achieved commercial importance. These include the statins such as lovastatin (mevinolin) (**1.31**) from *Aspergillus terreus* (1974) which is used for the inhibition of cholesterol biosynthesis. The cyclosporins were discovered in 1970 as metabolites of the fungi *Cylindrocarpon lucidum* and *Tolypocladium inflation* and their undecapeptide structure was reported in 1976. They are widely used as immunosuppressant agents. The structure of aphidicolin (**1.32**) from *Cephalosporium aphidicola* was reported in 1972. This metabolite is a potent inhibitor of DNA polymerase α and has been examined as a potential tumour inhibitor and anti-viral agent. Pleuro-mutilin (**1.33**) was first described in 1951 from *Pleurotus mutilus* and its structure was established in 1962. It is the core for the antibiotic thiamulin[®]. The oudemansins (**1.34**) and strobilurins are a family of anti-fungal β -methoxyacrylates that were first isolated in 1977. They have subsequently been isolated from many Basidiomycetes, such as *Strobilurus tenacellus*. The β -methoxyacrylate moiety formed the lead structure for a novel range of commercial synthetic fungicides such as azoxystrobin[®].



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1.33

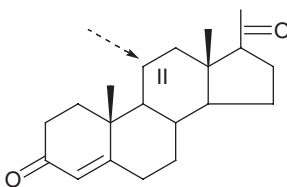


1.34

The second major area of investigation has been into those fungi responsible for plant diseases. Particular attention has been paid to Dutch elm disease (*Ceratocystis ulmi*), to *Botrytis cinerea*, which is a pathogen on many commercial crops, and to those fungi that are pathogens on the Gramineae such as wheat, barley and rice. The phytotoxic metabolites of the honey-fungus, *Armillaria mellea*, have been thoroughly examined by several groups. The constituents of other Basidiomycetes, particularly those growing wild in Western Europe, and their biological activity has attracted attention especially through the work of Steglich. Many of these metabolites are sesquiterpenoids.

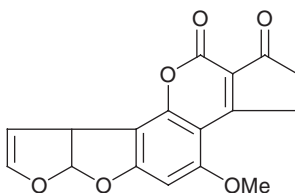
The microbiological hydroxylation of the steroid progesterone (**1.35**) at C-11 by *Rhizopus arrhizus* was reported in 1952. This biotransformation, which is now carried out commercially on a substantial scale, provided the facile means of

introducing functionality at a site that was chemically difficult to access. It meant that many analogues of the cortical steroid hormones became available for the treatment of rheumatoid arthritis and other conditions. This observation has stimulated a great deal of work in the use of fungi for biotransformations, not just using steroidal substrates.



1.35

The association of fungi with mammalian diseases came to the fore in 1960 with the discovery of the aflatoxins (**1.36**). The death of turkeys from liver damage having been fed on groundnuts contaminated with *Aspergillus flavus* led to the isolation of the highly carcinogenic aflatoxins. These developments are discussed in Chapter 9. It led to the awareness of the potential human health hazards from microbial metabolites and the implications of the presence of other mycotoxins in foodstuffs such as patulin in apple juice and the trichothecenes on corn. The development of analytical methods for the detection of mycotoxins has become an important aspect of food science.



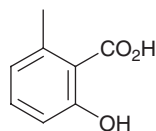
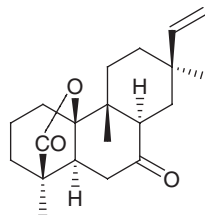
1.36

On a more positive note a mycoprotein known as Quorn[®], which was obtained from *Fusarium venenatum* (originally described as a strain of *F. graminearum*), was approved for human consumption in the United Kingdom in 1984 and marketed as a meat substitute in the 1990s. The original fungal isolate was discovered in a soil sample from Marlow in the 1960s.

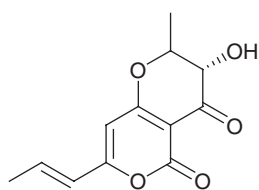
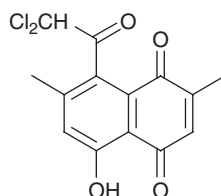
1.5.7 History of Biosynthetic Studies with Fungi

Microbial metabolites have proved to be more amenable to biosynthetic studies than the natural products obtained from higher plants partly because there are not the seasonal problems of production and also because the incorporation of labelled precursors is higher. In 1907 Collie had proposed that natural polyphenols might be biosynthesized from polycarbonyl compounds (polyketides)

derived from linking acetate units together. This idea was taken up by Birch in 1953. The incorporation of carbon-14 labelled acetate by *Penicillium* species into 6-methylsalicylic acid (**1.37**) and griseofulvin (**1.27**) was described in 1955 and 1958, respectively. The discovery in 1958 that mevalonic acid was the precursor of the isoprene units of cholesterol in mammalian systems was followed by biosynthetic experiments on fungal metabolites, including gibberellic acid (**1.28**) (1958), rosenonolactone (**1.38**) (1958) and trichothecin (**1.30**) (1960). These metabolites were subsequently studied in greater detail in the 1960s and 1970s.

**1.37****1.38**

The radiolabelling experiments required careful degradation to establish the specificity of the labelling. Stable isotope studies using ^{13}C NMR and more recently ^2H NMR have overcome these problems and provided additional information. The first experiments using carbon-13 in the biosynthesis of fungal metabolites were described by Tanabe in 1966 on the biosynthesis of griseofulvin. The sites of the carbon-13 labels were located by measuring the ^{13}C - ^1H satellites in the ^1H NMR spectrum. The first direct measurements of biosynthetic enrichments in the ^{13}C NMR spectrum were made by Tanabe in 1970 in studies with radicinin (**1.39**) produced by *Stemphyllium radicinum*. The identification of ^{13}C - ^{13}C coupling patterns reported by Tanabe in 1973 in connection with a study of mollisin (**1.40**) biosynthesis provided an important method for establishing the integrity of units. The measurement of carbon-13 enrichments, coupling patterns and the use of carbon-13 as a 'reporter' nucleus for deuterium and oxygen-18 labelling studies, particularly in the work of Simpson, Staunton and Cane, have shed a great deal of light on biosynthetic pathways in fungi. Many of these studies are discussed in detail in later chapters.

**1.39****1.40**

Microorganisms, particularly yeasts, had long been a source of enzyme preparations. Consequently, during the 1980s many enzyme systems associated

with secondary metabolite biosynthesis were isolated. Once the acetate labelling patterns of polyketide metabolites were established in the 1970s the emphasis of biosynthetic studies moved towards the study of the polyketide synthases. The ideas built upon knowledge gained from the fatty acid synthases. Although studies on 6-methylsalicylic acid synthase and lovastatin polyketide synthase have been reported, the main thrust of the work since the late 1980s has been with erythromycin polyketide synthase obtained from a Streptomycete, *Saccharopolyspora erythraceae*, rather than with a fungus. The results of this work are discussed in the Chapter 4 on polyketides. An interesting facet of this work is that the genes that determine the structure of these enzymes are clustered together. The significance of this is discussed later. The chemical enzymology of some fungal terpene synthases, including some of the sesquiterpene and gibberellin systems, has also been examined with results that are discussed in the relevant chapters.