Lactone Formation in Yeast and Fungi

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Jolanta Krzyczkowska, Hanh Phan-Thi, and Yves Waché

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J. Krzyczkowska (✉)
Department of Chemistry, Faculty of Food Sciences, Warsaw University of Life Sciences – SGGW, Warsaw, Poland
e-mail: jolanta_krzyczkowska@sggw.pl

H. Phan-Thi • Y. Waché
Laboratory PAM, Joint Research Unit AgroSup Dijon-University of Bourgogne-Franche Comté, Dijon, France
e-mail: phanhanh_bq29@yahoo.com; ywache@u-bourgogne.fr

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Abstract

Lactones are important secondary metabolites for fungi. In this chapter are presented some lactones that are important in biotechnology such as flavoring lactones or fragrance macrocyclic musk compounds, whereas others are important for quorum sensing and health (mycotoxins). Different pathways or enzymes can give rise to lactones, and the pathways going through β-oxidation and ω-oxidation and the fungal polyketide pathway (relatively similar to the fatty acid synthesis pathway) are presented as well as the activity of Baeyer–Villiger monooxygenases and lactonases and their potential use in biotechnology.

Keywords

Lactones • Macrocyclic musk fragrances • Butyrolactone • Quorum sensing • Mycotoxins • Patulin • Aflatoxins • β-Oxidation • ω-Oxidation • Polyketide synthase • Baeyer–Villiger monooxygenase • Lactonase

List of Abbreviations

6MSA	6-Methylsalicylic acid
AHL	Acyl-homoserine lactone
AF	Aflatoxin
AFL	Aflatoxicol
ATP	Adenosine triphosphate
BVMO	Baeyer–Villiger monooxygenase
CDK	Cyclin-dependent kinase
DALs	Dihydroxyphenylacetic acid lactones
DNA	Deoxyribonucleic acid
ERK	Extracellular signal-regulated kinase
FAS	Synthesis of fatty acids
HSP	Heat shock protein 7
IC_{50}	Half maximal inhibitory concentration
MAP, MAPK, MAPKKK	Mitogen-activated protein kinase (MAP), kinase (K)
mRNA	Messenger ribonucleic acid
PKS	Polyketide synthase
QS	Quorum sensing
RALs	Resorcylic acid lactones
TAK	Transforming growth factor-activated kinase
ZAL	Zearalanol
ZAN	Zearalanone
ZEN	Zearalenone
ZEL	Zearalenol

1 Introduction

The lactone function is characterized by the presence of an ester in a cycle. A lactone is thus an oxygenated heterocycle resulting from the cyclization (or lactonization) of hydroxy acids. γ-Lactones and δ-lactones, also corresponding to 4- and 5-olides, represent the two structures most frequently identified in the composition of aroma compounds. They result from the cyclization of acids hydroxylated in 4 or 5. But there are several other groups of lactones exhibiting various important properties. Among them are volatile lactones exhibiting flavoring and perfuming properties as well as lactones involved in cell-to-cell communication, but lactones can exhibit other bioactivities such as antimicrobial, anti-inflammatory, and anticancer ones. Fungi are able to produce several types of lactones. Yeast is the reference organism for the biotechnological production of musk macrocyclic lactones and of the flavoring γ-decalactone. Besides, communication homoserine lactones, although having been more studied in bacteria, exist also in fungi. Less-known lactones exhibiting antimicrobial effects have also been identified in fungi. Eventually, fungal polyketide synthases are versatile tools to produce lactones.

This chapter aims to introduce pathways for lactone formation in yeast and other fungi, and, after giving a short introduction on the principles of lactone formation, it will present the pathways for the production of the various lactones cited above.

2 General Ways of Lactone Formation

Lactone can result from different enzymatic pathways, and three main biosynthetic pathways are presented in this part. Some of these ways (β- or ω-oxidation) can be related to the main metabolic pathways of oxidation of lipids in fungi (Fig. 1). Main pathways for catabolism of hydrophobic compounds give potentially rise to lactones, while another is specific for the synthesis of polyketides. Besides, some enzymes involved in various pathways (Baeyer–Villiger monooxygenases, lactonases) can also catalyze the synthesis or hydrolysis of lactones.

2.1 Lactone Resulting from Intra-esterification of Hydroxy Acids

This pathway is a very common way to get lactones [1]. When the hydroxyl group of a fatty alcohol can be in contact with the hydroxyl from the acid group, esterification can occur readily, especially in acidic conditions.

Different types of enzymes can be involved in this reaction. First, fatty acids have to be hydroxylated which is catalyzed by oxygenases, hydratases, or hydroxylases. Monooxygenases are available in the ω-oxidation pathway. They can oxidize alkanes at one end of the molecule or fatty acids in the ω-end of the molecule.

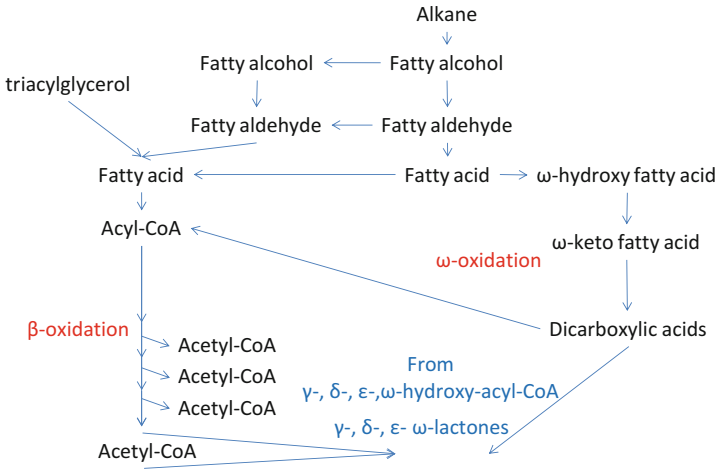


Fig. 1 Main pathways for catabolism of hydrophobic compounds giving potentially rise to lactones

Their role in metabolism and in biotechnology will be given in the part on macrocyclic lactones, and some fundamental or applied aspects have already been given in [2, 3]. Beside these α- and ω-regiospecific enzymes, there is a great deal of cytochrome P450 enzymes catalyzing oxidation of various substances [4]. However, in the case of fatty acid hydroxylation, most enzymes are not specific, giving rise to several different hydroxy acids with low yields. For instance, *Mortierella* sp. were used to transform caprylic acid into octalactone but gave rise to several different lactones [5].

If the hydroxyl group is not well located to react with the acid group, β-oxidation can occur first, resulting in a shortening of the distance between the two groups. This oxidation system involves a set of four reactions occurring in a cyclic way on energized fatty acids, acyl-CoA (Fig. 2). At each cycle, the length of the fatty acyl decreases of two carbons and an acetyl-CoA is created. Through this mechanism, a 10-hydroxylated fatty acid like ricinoleic acid **1** on Fig. 2 can be shortened of 6 or 8 carbons (3 or 4 β-oxidation cycles), giving rise to an ε- or γ-lactone, respectively. In the set of β-oxidation enzymes, there is an enoyl-CoA hydratase catalyzing the 3-hydroxylation of the acyl-CoA which is later dehydrogenated into its ketone. When lactonization occurs during this β-oxidation cycle, it can thus give rise to functionalized lactones. The catabolism of ricinoleic acid **1** on Fig. 2 giving rise to various lactones has been discussed in [6], and more information on biotechnological applications of β-oxidation will be given below, in the part on flavoring lactones.

In many cases, intra-esterification occurs readily, but some esterases have also been shown to exert a lactonase activity catalyzing cyclization or the opening of the lactone cycle. Actually, hydrolysis has been more investigated as it is involved in the catabolism of active compounds, whereas for esterification it is often difficult to state whether it has occurred readily or through an enzymatic catalysis. Readers interested in microbial lactonase activities can find information in a review on this subject [7].

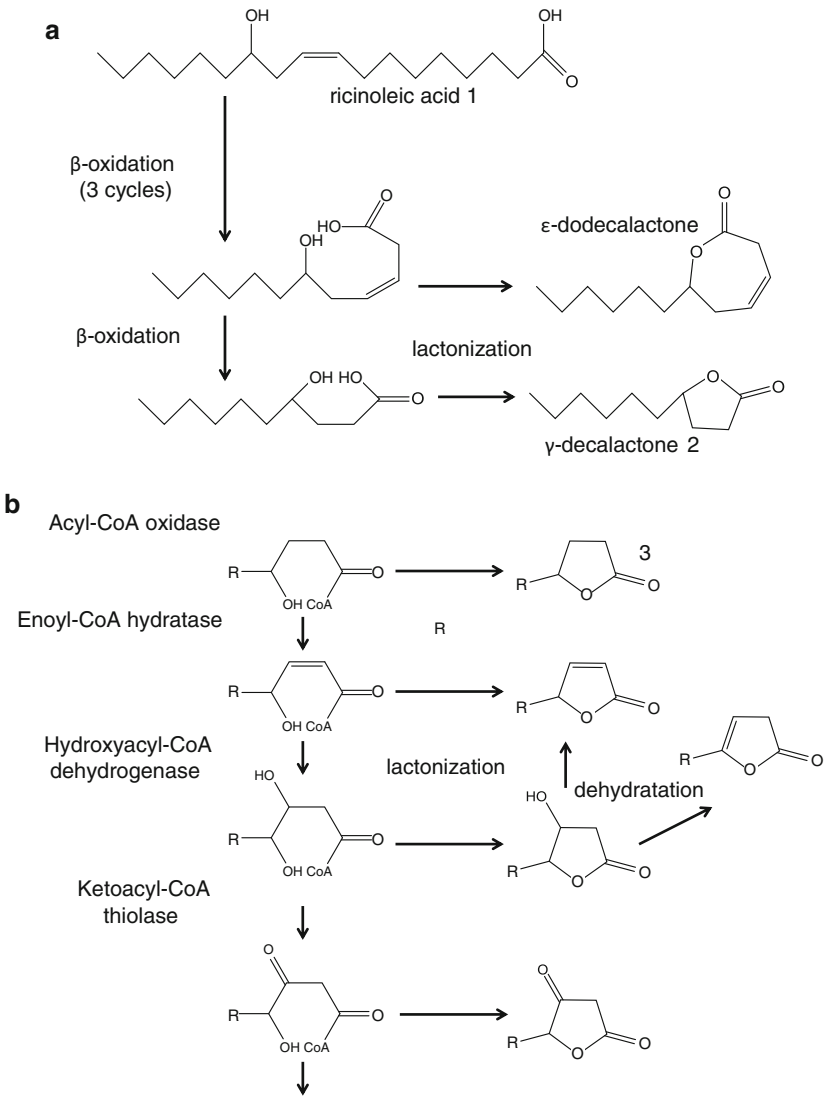


Fig. 2 β-Oxidation and synthesis of lactones (**a**) and synthesis of lactones from intermediates of β-oxidation cycle (**b**)

2.2 The Polyketone Pathway

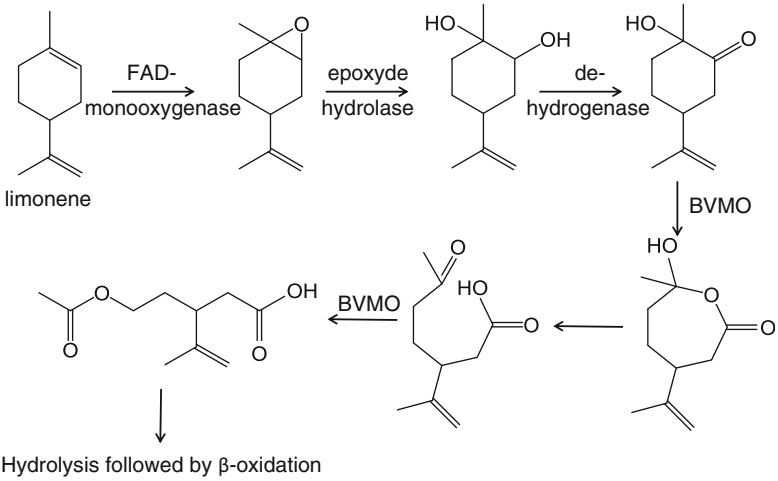
Beside the hydroxyl fatty acid pathway, a very common pathway in the synthesis of lactones is through the polyketide synthase pathway [8]. Polyketides are a family of complex secondary metabolites built from carboxylic acid building blocks. In microorganisms, they are produced by large, multifunctional proteins termed polyketide synthases (PKS). This pathway involves a set of basic reactions that are often

compared to the synthesis of fatty acids (FAS) as there is a start with a keto-synthase with an acyl group which condensates with acyltransferase-catalyzed loaded malonyl units onto an acyl carrier protein. Ketoreductase, dehydratase, and enoyl-reductase catalyze the processing of the compounds which is eventually terminated by a thioesterase. In fungi, this pathway has first been observed in the synthesis of patulin (see below) but is also at the origin of aflatoxins and many compounds. The better understanding of the PKS pathway in fungi enabled evolutionists to investigate the relationship between the various PKS and FAS systems [9]. This confirmed that the iterative fungal PKS-I system is directly related to the animal FAS-I system and far from the fungal FAS-I system.

2.3 Baeyer–Villiger Monooxygenases

Baeyer–Villiger (BV) oxidation consists in the transformation of a linear or cyclic ketone into its corresponding ester or lactone by insertion of an oxygen atom next to the carbonyl group (Scheme 1). It is catalyzed by Baeyer–Villiger monooxygenases (BVMOs, EC 1.14.13.x), which were first isolated in the 1960s, and their encoding genes, identified in the 1990s. There are different types of BVMO but the majority is sequence related (type I BMVOs), and they belong to the subclass B flavoprotein monooxygenases; the FAD cofactor is the prosthetic group, and they depend on NADPH as electron donor. An exhaustive review on the subject has been published some years ago [10].

The role of this enzyme is not fully elucidated, but it is remarkable that it has been only described in microorganisms where BMVO-specific protein sequence motifs have been found in each microbial genome investigated. However, some specific



Scheme 1 Degradation of limonene by the mountain pine beetle-associated pathogen *Grosmannia clavigera* (Adapted from Wang et al. [13])

filamentous fungi possess families of BMVO-encoding genes. These enzymes can fulfill a variety of functions, such as catabolic properties enabling microorganisms to grow on and degrade various ketones (shown for *Candida* sp. in [11]), cyclic alkanes, alcohol, or terpene into dicarboxylic acids (shown for bacteria in [12] and for *Grosmannia clavigera* in [13] (Scheme 1)), and, as shown below, they are involved in the synthesis of secondary metabolites.

3 Lactones as Flavors and Perfumes

3.1 Lactones as Flavors

3.1.1 Historic of Production

Short- and medium-sized length lactones resulting from the esterification of hydroxy fatty acids are an important family of aroma compounds. From butyrolactone (butyrolactone corresponds to two different molecules depending on the field, flavors, or quorum sensing; here it stands for molecule **3** in Fig. 2 with R = 0, while in the field of quorum sensing, butyrolactones correspond to lactones with a 4-carbon ring (or γ-lactones or 4-olides) like butyrolactone-I **10**) to C12 γ- or δ-lactones that can be functionalized or desaturated, there are several lactones possessing flavoring properties especially with fruity, fatty, and oily notes (Fig. 3). These lactones are present in many fruits where they are likely to result from the 1.1 pathway shown above. Yeasts are also able to synthesize these compounds that can be encountered in fermented food such as bread, beer, or whisky [14]. During fermentation, the pathway of synthesis involves first a hydroxylation step which can be fulfilled by lactobacilli before the β-oxidation step carried out by yeast [15]. With similar ways of synthesis from plants and

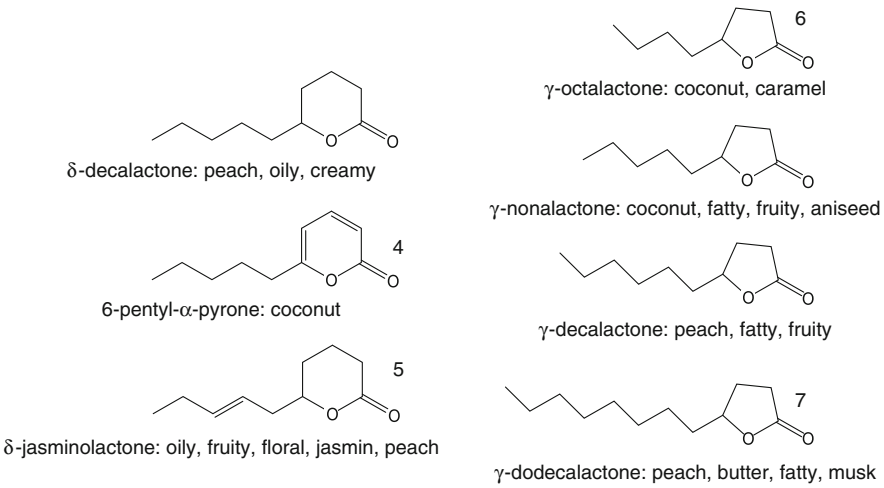


Fig. 3 Some lactones possessing flavoring properties and their characteristic notes

from yeasts, these latter catalysts have been employed to produce lactones with the natural label from the first observation of their capability to produce lactones which occurred from investigation of the metabolism of hydroxy fatty acids. When using *Candida* sp., γ-decalactone **2** accumulated during the catabolism of ricinoleic acid (hydroxylated in C10) [16]. In this study, ricinoleic acid **1** had been chosen because it was the only hydroxy acid available at low cost and in high amount. Indeed, due to a specific evolution in castor beans, castor oil possesses about 90 % ricinoleyl moieties. From ricinoleic acid, γ-decalactone will be produced. This lactone benefited from technology developments becoming the aroma compounds the most produced through biotechnology. Its cost followed the trend from a fine chemical price (around $12000/kg in 1986) to a low cost of natural aroma compounds ($500/kg in 1998) [17]. The development of research to produce lactones through biotechnology has also to be related to the consumers' trend toward natural compounds. There has been a first period of interest in the 1980s–1990s which followed the demand of European consumers (mainly in German-speaking countries in the beginning). During this period, most pathways of synthesis of aroma compounds had been investigated, and only some had been selected as potential sources of realistic price compounds [3]. Other lactones produced at this time were 6-pentyl-α-pyrone **4**, δ-jasminolactone **5**, γ-octalactone **6**, γ-dodecalactone **7**, δ-dodecalactone, etc. (Fig. 3). Finally, after a time of decrease in the interest, activities in lactone production began to rise again with the world interest for natural flavors in the 2000s. In the meantime, the price decreased again, making it more difficult for new companies aiming at beginning production. Moreover, despite some rare new biotechnological strategies or the identification of some efficient catalysts, most of the strategies published in the present period cannot address the real issues in the field which are described in the next part. Instead of this, most of the works published recently concern the optimization of production with a specific medium or the repetition of previous studies on the effect of aeration or on the performances of *POX*-mutants from *Yarrowia lipolytica*.

3.1.2 Limiting Steps of Lactone Production

Hydroxylation

As shown above, from fatty acids hydroxylated at various carbons, it would be possible to produce several lactones. However, production is limited to natural precursors that are already available at a low cost, such as ricinoleic acid **1**, and to precursors that can be specifically hydroxylated, such as unsaturated fatty acids that can be hydroxylated by some fungal activities. The ability to hydroxylate a fatty acid at a specific level and with good yields could have a high impact on lactone production (see [1] for review), but this step, which has been a real challenge in the first period of development of lactones, does not receive much attention now. Fungi able to hydroxylate before carrying out β-oxidation could be particularly interesting as the two steps of biotransformation could be carried out in one reactor. Some examples are shown in Fig. 4. Another strategy to overcome this problem is to

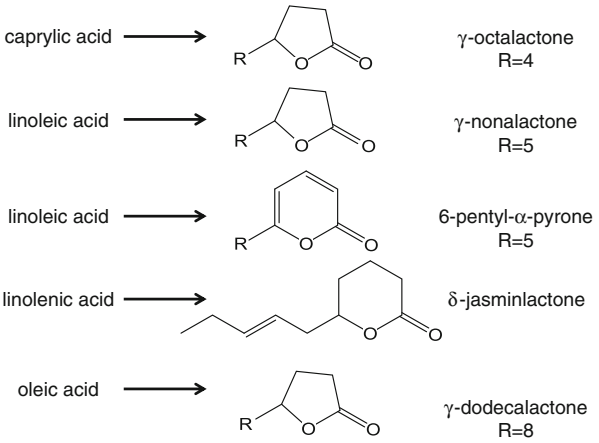


Fig. 4 Lactones produced from non-hydroxylated fatty acids (Adapted from Romero-Guido et al. [1])

modify plant substrates to make them produce hydroxylated fatty acids. Indeed, in the case of castor beans, it is remarkable that it is only a 4-amino acid evolution which changed the desaturase activity into a hydroxylase one [18]. From these facts, it seems promising to modify further enzymes of the fatty acid synthetic pathways to get a higher diversity of plant fatty acids.

β-Oxidation Control

The other crucial point is to control the flux of β-oxidation in yeast cells. Indeed, β-oxidation can go on after the stage of lactone formation, or the synthesized lactone can be further degraded by the producing cells. The fluxes of production have thus been investigated with several models for the production of γ-decalactone. Some interesting works have shown with *Sporobolomyces* sp. that several different models of channeling were present in the different strains tested [19]. In the meantime, works were carried out with mutant of the yeast *Yarrowia lipolytica*. This species is, for many producers, the γ-decalactone-producing species. It is also a reference species for the study of lipid metabolism as it possesses many multigene families, and it can be genetically manipulated [2]. The role of the various enzymes has thus been characterized [20–22], enabling to genetically engineer new strains [23] and to study fluxes in this species [24].

A physicochemical approach was also used to decrease both the lactone toxicity toward and the lactone degradation by the producing cells. Lactone was trapped using various materials exhibiting affinity, and a reactor was designed [25–27].

Finally, these studies show a diversity of yeast behavior with some strains exhibiting very efficient β-oxidation that requires non-favorable conditions to produce lactones [1, 3, 6, 28–30], whereas other strains exhibit a channeled production with an increased production in better conditions (Alchihab, personal communication).

Recently a strain of *Waltomyces lipofer* exhibiting no limitation of lactone production has been reported and patented [31, 32]. Depending on the hydroxylated

substrate, this strain was able to produce γ-dodecalactone, γ-decalactone, and γ-butyrolactone. This strain revolutionized the knowledge acquired on lactone production as (i) it was not sensitive to lactone toxicity and could produce more than 50 g/l of the highly toxic γ-dodecalactone; (ii) it could produce in a state of permeabilization with a 70 % ethanol treatment and other drastic treatment which did not seem to alter the β-oxidation pathway and all the cofactor regeneration required, if this pathway was also the one involved in this strain; and (iii) in some cases, accompanying the 100 % conversion of hydroxy acid into lactone, the cell produced de novo about 20 % of the same lactone from glucose, reaching yields about 1.16 to 1.22. This latter point suggests the ability to produce C10 hydroxylated fatty acids by this yeast.

3.2 Macrocyclic Musk Lactones

3.2.1 Macrocyclic Musk Fragrances

Musk-like fragrances have been used from the late antiquity and still have wide applications in the world of perfumes as bottom notes exhibiting warm, sweet, powdery, or animal notes that are long-lasting, tenacious, and substantive. They are particularly interesting in cosmetics where musk is among the most popular perfumes for shower gels and deodorants [33]. Three main categories of compounds exhibit these musk notes: aromatic nitro-musks, polycyclic musk compounds (e.g., galaxolide, which, with its low cost and strong and sweet floral musk smell, has been used in many perfumes), and macrocyclic musk compounds. The two first groups are used in the cosmetics and detergent industries, but their detection in human tissues and in the environment in addition to a suspicion of carcinogenic properties gave rise in the 1990s to a public debate on safety concerns resulting in their progressive replacement by compounds belonging to the 3rd group.

This latter group consists of macrocyclic ketones and lactones that are synthesized from fatty acids (Fig. 1). The compounds responsible for these sensorial notes were at the origin extracted from glands of animals such as the Asian musk deer (*Moschus moschiferus*, Moschidae) for macrocyclic ketones and from plant sources for macrocyclic lactones. From animal origin, the price was very high as about 30–50 animals had to be sacrificed to get one kg of musk grains (without the possibility of eating the meat due to the strong musk odor). Despite the high price, this source was common in many popular perfumes until the protection in 1979 of musk deer as an endangered species (CITES). In plants, macrocyclic lactones (or macrolides) were isolated from angelica root (e.g., 15-pentadecanolide), ambrette seed oil, galbanum resin, orchids, etc. Although most of these ketones and lactones were considerably more expensive than musk fragrances from the two other groups, interest to them increased with the process of replacement of synthetic compounds that begun in the 1990s. However, as the synthesis of precursors of macrocyclic ketones and lactones was possible through a fungal metabolic pathway, yeast cells were rapidly preferred to plant and animal extraction for production. A set of compounds produced through yeast biotechnology was soon available with ketones

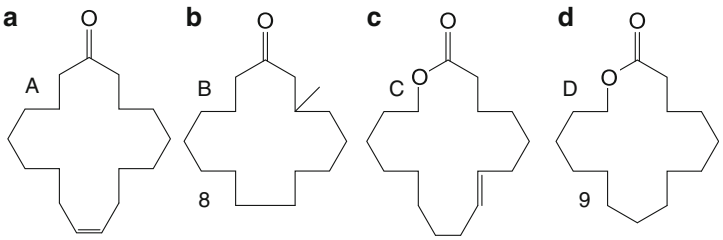
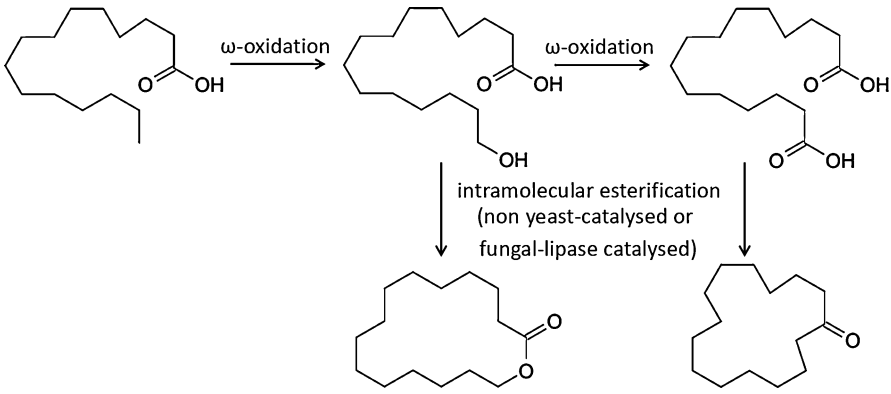


Fig. 5 Some macrocyclic musk produced commercially with their commercial names. (**a**) Civettone or civetone; (**b**) muscone; (**c**) ambrettolide; (**d**) exaltolide, muskalactone, pentalide, thibetolide (Adapted from Sommer [34])

such as muscone **8** and exaltone resulting from α,ω-dicarboxylic acids and macrocyclic lactones resulting from acid–alcohol. The pentadecanolide **9** isolated from angelica root was thus proposed by several fragrance producers under different commercial names (e.g., exaltolide, thibetolide, macrolide, pentalide, etc.) (Fig. 5). The fragrance of the compounds depends on their structure as well as on their chain length. With 14 carbons, a weak musk scent is exhibited, but the musk odor is strong and nice with 15- and 16-carbon compounds [34].

3.2.2 Pathway of Synthesis

The complete pathway of synthesis of macrocyclic molecules goes through the synthesis of a 15– 16-carbon-long fatty acid that is oxidized into ω-hydroxy fatty acids or α,ω-dicarboxylic acids and then cyclized into ketone or lactone macrocycles. The pathway of production is rather similar for the homologous cycles exhibiting animallike fragrances and for the heterologous cycles exhibiting plantlike fragrances, the first one going through the intra-esterification of ω-hydroxy fatty acids and the second one through the one of α,ω-diacids (Scheme 2). From Baeyer's strain theory and entropy studies, it has long been thought that such molecules with ring sizes over seven carbons were impossible to get [147]. However, they were not only present in nature but also possible to synthesize with the help of fungi catalysis. It must be stressed, however, that, contrary to the flavor lactones presented above, macrocyclic compounds result from a minor metabolic pathway which gives only rarely rise to macrocyclic ketones or lactones in the culture medium for wild-type yeasts. This pathway, through ω-oxidation (Fig. 1 and Scheme 2), enables the cell to oxidize alkanes and fatty alcohols into fatty acids that can enter the β-oxidation pathway. However, beside β-oxidation, alkanes and fatty acids can undergo the ω-oxidation pathway which is catalyzed by a cytochrome P450 oxygenase encoded by an *ALK* gene. The third step of the pathway, cyclization, is based on the esterification of the alcohol–acid or diacid molecule. This reaction occurs readily in many conditions, but the intra-esterification giving rise to macrocyclic compounds is competing with interesterification which results in polymers. The conditions and concentrations of precursors must be carefully chosen to favor the first reaction. It is also possible to favor lactonization with biocatalysis as several fungal lipases exhibit lactonase activity.



Scheme 2 Pathway for the synthesis of macrocyclic lactone and other α,ω-cyclic fragrances from a carboxylic acid

3.2.3 Biotechnological Developments

Due to the growing interest in natural and safe macrocyclic musk fragrances and to the limitation in the possibility of extraction from animal or plant tissues, biotechnologists have begun to imagine new ways to obtain these compounds in the beginning of the 1990s. The starting materials were natural C15 and C16 fatty acids available at a relatively low cost. The biocatalytic part began at this stage with yeast ω-oxidation. As mentioned earlier, this pathway is rather a minor one, and C15 or C16 fatty acids are likely to be oxidized in the β-oxidation loop in most yeast possessing an efficient ω-oxidation biocatalytic potential. As a result, the pioneering work by Picataggio [35, 36] consisted in engineering genetically a strain of *Candida tropicalis* to block the β-oxidation pathway and, in the meantime, to amplify the ω-oxidation monooxygenase enzyme. To block the β-oxidation pathway, the *POX* gene encoding the enzyme catalyzing the first reaction, the acyl-CoA oxidase (Aox) was deleted. As this strain possessed two copies of a family of two *POX* genes (*POX4 & POX5*), the authors had to delete 4 genes. This first step was required to avoid degradation of the macrocycle precursors in this pathway, but, to increase the ω-oxidation pathway, the monooxygenase encoding gene (*ALK1*) and the *CPR* genes coding for the NADPH–cytochrome P450 reductase had to be amplified. This strategy was a success which limited the access to yeast macrocyclic musk to competitors. A second group in Japan working with a related strain belonging to *Candida maltosa* investigated another strategy based on repeated mutagenesis and screening for higher dicarboxylic acid production. They selected strains overproducing dicarboxylic acids, and, through analysis of the resulting strains [37], it was shown that the overproducing strain of *C. maltosa* exhibited decreased level of β-oxidation proteins and an increased induction of synthesis of Alk proteins in the presence of alkanes.

4 Lactone for Quorum Sensing (QS) in Yeast and Fungi

4.1 Introduction to Lactones for Quorum Sensing

Quorum sensing (QS) is a phenomenon of the microbial communication whereby the accumulation of certain chemical compounds (signal molecules) enables a single cell to sense the population density. This phenomenon is widespread in microbial communities and mostly studied in bacteria. QS enables bacteria to coordinate gene expression according to the density of their local population and to coordinate certain of their behaviors such as biofilm formation, virulence, and antibiotic resistance. These responses include adaptation to availability of nutrients, defense against other microorganisms which may compete for the same nutrients, and the avoidance of toxic compounds potentially dangerous for bacteria. Quorum sensing is also prevalent in the unicellular (yeast) and filamentous fungi. It has been observed fifteen years ago with the discovery that farnesol controls filamentation in the pathogenic polymorphic fungus *Candida albicans* [38] and that phenylethanol and tryptophol stimulate morphogenesis and pseudohyphal growth formation in *Saccharomyces cerevisiae* [39]. Furthermore, quorum-sensing mechanisms are reported in various filamentous fungi including *Aspergillus nidulans*, *Aspergillus terreus*, *Penicillium chrysogenum*, and *Penicillium sclerotiorum* [40–42].

Lactone-containing compounds are widespread in nature and are involved in acting as signaling molecules in bacteria and fungi. A large group of QS signals including lactone-containing molecules such as acyl-homoserine lactones (AHLs), butyrolactone-I **10**, and γ-heptalactone **11** are found in several gram-positive and gram-negative bacteria and filamentous fungi (e.g., *A. nidulans*) [43]. AHLs, composed of a lactone ring and different-length and different-substituent acyl side chain, are the major class of QS molecules in bacteria and are produced by more than 50 different bacterial species. Each AHL is catalyzed by a specific AHL synthase enzyme belonging to the *LuxI* family and corresponds to a particular cytoplasmic DNA-binding regulator *LuxR*-type protein in bacteria such as *Pseudomonas aeruginosa* [44]. At high cell densities, the accumulated autoinducer AHLs bind to regulatory proteins *LuxR*, and then this complex recognizes and binds specifically to a QS-regulated promoter, thus activating the transcription of target genes (DNA sequences) and inducing a particular QS response [45].

Other lactone-containing compounds such as γ-butyrolactone are found as signaling molecules in filamentous bacteria *Streptomyces* sp. [46]. The high similarities between the filamentous bacteria and filamentous fungi triggered researchers to investigate the presence and role of γ-butyrolactone-containing molecules in filamentous fungi [43]. It was later reported that several γ-butyrolactone-containing molecules such as butyrolactone-I **10**, γ-heptalactone **11**, and multicolanic acid **12** act as putative QS molecules in filamentous fungi.

The study of quorum sensing belongs to microbial ecology and population biology. It is still very little known about the mechanisms of synthesis and

metabolism of signaling lactone-containing molecules involved in different bacterial and fungal species. The understanding of the above phenomena could have great potential for enhancing industrial production of commercially useful bacterial and fungal products. The genetic manipulations of the genes involved in the lactone QS and its metabolism processes could result in generation of engineered bacterial and fungal strains having implication in medicine, agriculture, and biotechnology with more specificity, i.e., antibiotic therapy, preventative therapy for plant disease, biosynthesis of antibiotics, and luminescent biosensors.

4.2 Lactone-Containing Molecules for Quorum Sensing in Fungi

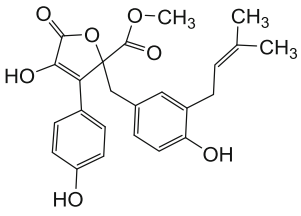
The lactone-containing molecules for QS phenomenon in filamentous fungi have only recently been observed; however, the criteria for existence of this system in fungi are based on proposals which are particularly verified in bacteria and yeast [47]. One of the fundamental characteristics of quorum-sensing signaling molecules is the increase in concentration as the microbial population grows and the subsequent autoinduction when the population density threshold has been reached, which ensures the correct timing of the physiological response [48]. For a molecule to qualify as a quorum-sensing entity, it should satisfy some critical characteristics: the molecule should be produced throughout the growth of the organism; however, the quorum-sensing response is only initiated at a certain stage of the growth [49]. It is at this stage of growth that the increase of the QS molecule reaching a specific concentration alters a coordinated response in the entire population's behavior, i.e., secondary metabolite production. In many fungi such as *Aspergillus nidulans*, *Aspergillus terreus*, *Penicillium chrysogenum*, and *Penicillium sclerotiorum*, oxylipins and lactone-containing molecules have been considered as signaling molecules and are reported to induce physiological responses including morphological changes, sporulation, and secondary metabolite production including mycotoxins and antibiotics [40–42]. Table 1 provides further evidences of various lactone-containing signaling molecules utilized by fungi and their biological functions.

Table 1 Filamentous fungi and lactone-containing QS with their corresponding target functions

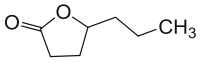
Filamentous fungi	QS lactone-containing molecules	Physiological response	References
Aspergillus terreus	Butyrolactone-I **10**	Hyphal branching, submerged sporulation, secondary metabolite production (lovastatin and sulochrin)	[40]
Aspergillus nidulans	γ-Heptalactone **11**	Increases penicillin production	[50]
Penicillium sclerotiorum	Multicolanic acid **12** and derivatives	Sclerotiorin production (antibiotic)	[51]

4.2.1 Butyrolactone-I

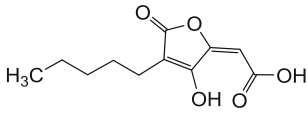
Butyrolactone-I **10** is produced as a secondary metabolite by *Aspergillus terreus*. Because small butyrolactone-containing molecules act as self-regulating factors in some bacteria, the effects of butyrolactone-I addition on the producing organism, specifically changes in morphology, sporulation, and secondary metabolism, were recently studied [40, 52]. Threefold or greater increases in hyphal branching, submerged sporulation, and secondary metabolism were observed when butyrolactone-I was added to cultures of *A. terreus*. Schimmel and co-workers observed that butyrolactone-I acts as a signaling molecule to enhance the production of the secondary metabolite lovastatin (threefold increase) and sulochrin (twofold increase) in similar growth conditions when compared to the control without butyrolactone-I addition [40]. It was also found to have an auto-stimulatory function as well as induction of lovastatin biosynthetic genes [51]. Lovastatin is known therapeutically important for the prevention of cardiovascular disease [53, 54], and sulochrin has weak antibacterial and antifungal properties [55]. Furthermore, these findings indicate that butyrolactone-I induces morphological and sporulation changes in *A. terreus* and enhances secondary metabolite production in a manner similar to the changes that were observed with small γ-butyrolactone-containing molecules in filamentous bacteria of the genus *Streptomyces*. This observation is in accordance with the idea that butyrolactone-I may function as a QS molecule in *A. terreus*. The practical application of these studies is the possibility that butyrolactone-I could be used to increase or promote the production of desired secondary metabolites in *A. terreus*, i.e., lovastatin and sulochrin production. Moreover, the mechanism by which butyrolactone-I is produced or diffused out of the fungal cells during the growth process is not known.



Butyrolactone-I **10**



γ-Heptalactone **11**



Multicolanic acid **12**

Besides, butyrolactone-I is known as an antitumor and anticancer molecule. Indeed, it is a potent and selective inhibitor of the cellular roles of cyclin-dependent kinase (CDK) enzymes, specifically inhibiting Cdk2 and Cdc2 kinase [56]. CDKs are protein kinases that control cell cycle progression in all eukaryotes and are regulated by phosphorylation and dephosphorylation of critical serine, threonine, or tyrosine residues. The inhibitory effect of butyrolactone-I due to competition with ATP binding at CDK is to block the phosphorylation of the transcription factor E2F-1. Therefore, it inhibited Cdc2 of unsynchronized cultured prostate cancer cells and interrupted the cell cycle progression toward cell division [57].

4.2.2 γ-Heptalactone

Another γ-butyrolactone-containing molecule, γ-heptalactone **11**, is an endogenously produced QS molecule regulating growth and secondary metabolite production by *Aspergillus nidulans* [50] that is a filamentous fungus well known for its ability to produce the secondary metabolite penicillin [58]. This fungus produces γ-heptalactone at a high cell density, and it can alter the organism's behavior at a low cell density, i.e., altering the organism's growth profile by shortening the lag phase. It also induces the production of the secondary metabolite penicillin. Indeed, the addition of this γ-butyrolactone-containing molecule to the wild-type *A. nidulans* strain led to a 31.9 % increase in penicillin production [50]. Because fungi coexist with bacteria in the environment, so they must rely on chemical defense mechanisms due to their lack of an active immune system. It can be suggested that *A. nidulans* has adapted a QS process and uses a range of regulatory circuits to adjust gene expression and coordinate cell-to-cell interactions.

The identification of γ-heptalactone as a QS molecule in *A. nidulans* can be further explored and hence exploited by the biotechnology industry to enhance yields of penicillin production. In flavor and fragrance industries as shown above, this lactone is widely used in peach, nut, maple, almond, caramel, and cream flavors, for a creamy finish in most vanilla, and in coconut and gardenia fragrances.

4.2.3 Multicolanic Acid 12 and Derivatives

Multicolanic, multicolosic, and multicolic acids were isolated by Gudgeon et al. [59] from *Penicillium sclerotiorum*. These compounds belong to a small group of chemicals called tetronic acid metabolites [60] which contain a γ-butyrolactone molecule. These γ-butyrolactone-containing compounds are synthesized by oxidative cleavage of an aromatic precursor 6-pentylresorcylate [61] and classified as hexaketides because of their polyketide origin [62].

In order to test whether γ-butyrolactone molecules produced by *P. sclerotiorum* exerted a physiological response in the cells, the effect of these potential quorum-sensing molecules on sclerotiorin production in these fungus was investigated [51]. This study suggests that addition of spent medium containing the putative quorum-sensing molecules has the ability to initiate production of sclerotiorin in a low-sclerotiorin-producing strain. The presence of γ-butyrolactone-containing molecules (multicolic acid, multicolosic acid, multicolanic acid, and related derivatives) in the spent medium increased sclerotiorin yield (6.4-fold). These data suggest that addition of γ-butyrolactone molecules had created an environment for the cells to respond similarly to the conditions where the threshold cell concentration was achieved, allowing for the expression of genes under quorum-sensing control [51]. However, the chemical structure of the molecule(s) responsible for the regulation of sclerotiorin was not precisely determined in this study.

The investigation in the effect of multicolanic acid and derivatives (i.e., dimethyl-*O*-methylmulticolosate, dimethyl dihydromulticolosate, and methyl-*O*-methylmulticolate acetate) as QS molecules in *P. sclerotiorum* open up a possible new way to enhance the ability of sclerotiorin production of this fungus. Thereon,

sclerotiorin is known as an aldose reductase inhibitor as well as a potent reversible lipoxygenase inhibitor [63, 64].

4.3 Perspectives

Few findings of lactone-containing compounds acting as quorum sensing on fungi have opened up a new front in further investigating this question with the potential for further basic and applied research. Once the importance of quorum sensing is established in pathogenic fungi and the mechanistic details are uncovered, the value of QS pathways as potential therapeutic targets can be assessed [65]. Hence, further elucidation of the mechanisms of QS in these pathogens and its effects on various metabolic pathways will lead to a better understanding of fungal pathogenesis facilitating the development novel antifungal approaches to combat human diseases. The mechanism of signal transduction in QS may be clarified by identification of the receptor proteins to which the γ-lactone binds to on the cell surface to enable signal perception.

As said above, the role QS lactones have in the organisms was more studied in bacteria than in fungi. For example, a mechanism involved in signal transduction from the detection of γ-lactone substrates/N-acyl-homoserine lactones (NAHSL) signals to the transcription of the *qsdA* operon in *Rhodococcus erythropolis*; an environmental gram-positive bacterium was illustrated in the review of Latour et al. [66]. A similar mechanism is presumed to control the qsdA operon with γ-lactone in the role of tetracycline [66] (Fig. 6). In the absence of a γ-lactone source, the QsdR (quorum-sensing signal degradation) regulator protein forms dimers that bind to the operator region, switching off the biosynthesis of catabolic enzymes. But the presence of γ-lactone binding to *qsdR*, a putative TetR family transcriptional regulator gene, changes the conformation and causes TetR detachment from the operator region and results in the expression of the gene encoding catabolic enzymes.

The industrial exploitation of QS lactone-containing molecules requires an optimization of their production from producing strains. The possible medium conditions were identified to maximize the production of butyrolactone-I from a butyrolactone-overproducing strain (Bty345) that had been derived by mutagenesis from *Aspergillus terreus* ATCC 20542 and selected for increased butyrolactone production which was available in the Merck (Elkton, VA) culture collection [67]. In this study, the yield of butyrolactone-I using optimized medium concerning the source and concentration of carbon and nitrogen represents a tenfold increase over the butyrolactone-I produced using the original, basic medium.

5 Lactone Mycotoxins and Other Bioactive Macrocyclic Lactones

The large group of lactones, apart from compounds that are flavoring components of food products (γ- and δ-lactones) [68, 69] or that reflect desirable aromas in the fragrance industry (coumarin, exaltolide) [70, 71] and that have been described

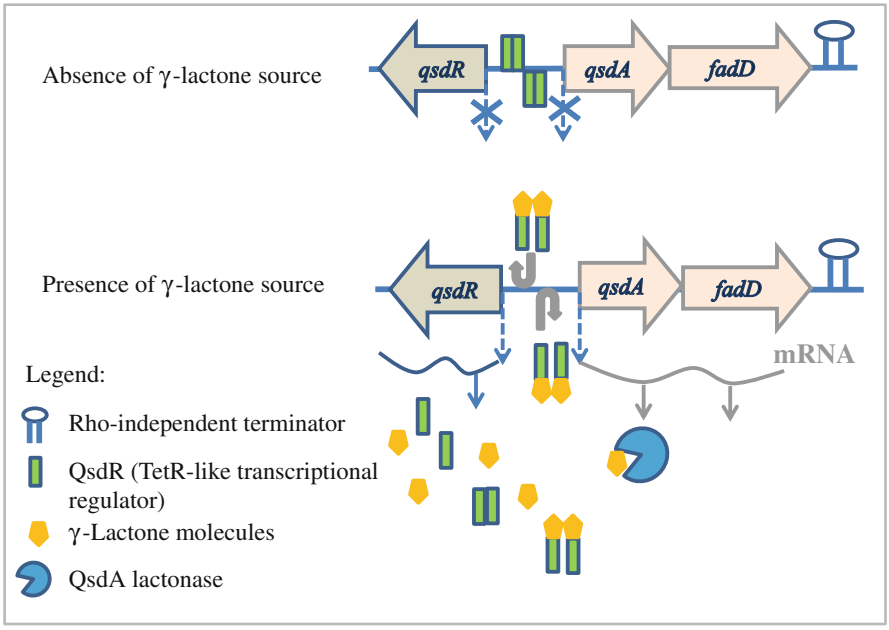


Fig. 6 The *qsdA* operon of *R. erythropolis* and its putative mechanism of regulation (Adapted from Latour et al. [66])

above, comprises lactones of diversified biological activity, including toxic (carcinogenic, teratogenic, mutagenic) and antitumor or anti-inflammatory effect.

Lactones having biological properties, isolated from natural sources, are currently a subject of study of many research centers. Research laboratories conduct ongoing works on the isolation and identification of active lactones, determine relationships between the structure of compounds and their biological properties, and in many cases synthesize analogs of these compounds characterized by a higher activity and stronger effect or try to conduct their inactivation.

5.1 Lactone Mycotoxins

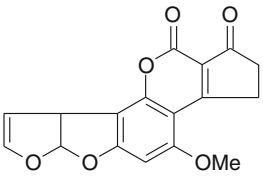
Some of naturally synthesized lactones exhibit strong toxic activity. They are mainly compounds which are low-molecular-weight ($M < 1.5$ kDa) secondary metabolites of filamentous fungi, or so-called mycotoxins, of different levels of toxicity both to humans and to animals, plants, and microorganisms. Toxic lactones can be stored as endotoxins in mycelium and conidia or can be excreted to the medium. These compounds cause contamination of raw materials and products of the food industry, fodders, and food of animal origin. The synthesis of lactones by molds is determined both genetically (metabolism of amino acids or fatty acids) and phenotypically (environmental factors).

In the group of mycotoxins, most of the studies were devoted to aflatoxins, comprising approximately 20 heterocyclic difuranocoumarin derivatives (coumarin is a lactone of *O*-hydroxycinnamic acid) produced by toxigenic strains of *Aspergillus* fungi, especially *A. flavus*, *A. parasiticus*, and *A. nominus* [72]. The pathway of biosynthesis of aflatoxins comprises at least 23 reactions catalyzed by enzymes. So far it was possible to identify 15 intermediates of these reactions. Genetic studies on the mechanism of the synthesis of aflatoxins by *A. flavus* and *A. parasiticus* allowed for cloning 29 genes responsible for the formation of enzymes necessary for this metabolic pathway [72, 73]. Aflatoxins are classified into two broad groups according to their chemical structure, and they include the difurocoumarocyclopentenone series (AFB_1, AFB_2, AFB_{2A}, AFM_1, AFM_2, AFM_{2A}, and aflatoxicol) and the difurocoumarolactone series (AFG_1, AFG_2, AFG_{2A}, $AFGM_1$, $AFGM_2$, $AFGM_{2A}$, and AFB_3) (Table 2) [74, 75]. These compounds have closely related structures (Scheme 4). Aflatoxin B_1 is formed by, among others, a lactone ring, which is adjacent to a benzene ring and forms the same system as in coumarin, and two furan rings, including the extreme one with double bond. In aflatoxin G_1 **15** the extreme ring with the ketone moiety is enriched with one atom of oxygen to form a lactone ring. Aflatoxins B_2 **14** and G_2 **16** are hydroxyl derivatives of aflatoxins B_1 **13**

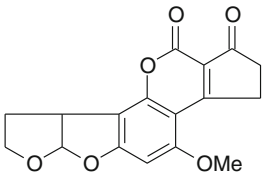
Table 2 The most important aflatoxin produced by the *Aspergillus* species [10, 12, 77]

Difuranocoumarins	Type of aflatoxin	*Aspergillus* species
Difurocoumarocyclopentenone series	Aflatoxin B_1 **13** (AFB_1)	*A. flavus, A. arachidicola, A. bombycis, A. minisclerotigenes, A. nomius, A. ochraceoroseus, A. parasiticus, A. pseudotamarii, A. rambellii*
	Aflatoxin B_2 **14** (AFB_2)	*A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, A. parasiticus*
	Aflatoxin B_{2a} (AFB_{2a})	*A. flavus*
	Aflatoxin M_1 **17** (AFM_1)	*A. flavus, A. parasiticus*
	Aflatoxin M_2 **18** (AFM_2)	Metabolite of aflatoxin B_2
	Aflatoxin M_{2A} (AFM_{2A})	Metabolite of AFM_2
	Aflatoxicol (AFL)	*A. flavus*, metabolite of AFB_1
Difurocoumarolactone series	Aflatoxin G_1 (AFG_1)	*A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, A. parasiticus*
	Aflatoxin G_2 (AFG_2)	*A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, A. parasiticus*
	Aflatoxin G_{2A} (AFG_{2A})	Metabolite of AFG_2
	Aflatoxin GM_1 ($AFGM_1$)	*A. flavus*
	Aflatoxin GM_2 ($AFGM_2$)	Metabolite of AFG_2
	$AFGM_{2A}$	Metabolite of $AFGM_2$
	Aflatoxin B_3 (AFB_3)	*Aspergillus* species not defined

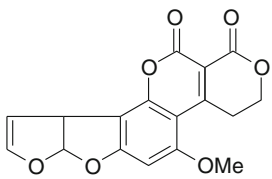
and G_1 **15**, respectively, while aflatoxins M_1 **17** and M_2 **18** are 4-hydroxyderivatives of aflatoxins B_1 and B_2 [72]. Among the aforementioned mycotoxins, aflatoxin B_1 is the most toxic. It is classified by the WHO as a group 1 carcinogen. Based on the toxicity, carcinogenicity, and mutagenicity of mycotoxic lactones of the aflatoxins group, they are classified in the following order: $AFB_1 > AFM_1 > AFG_1 > AFB_2 > AFG_2$ [74, 76, 77].



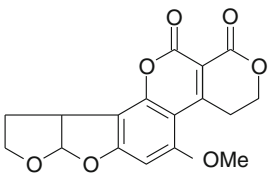
Aflatoxin B_1 13



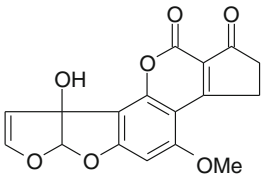
Aflatoxin B_2 14



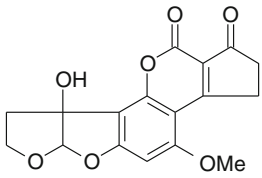
Aflatoxin G_1 15



Aflatoxin G_2 16



Aflatoxin M_1 17

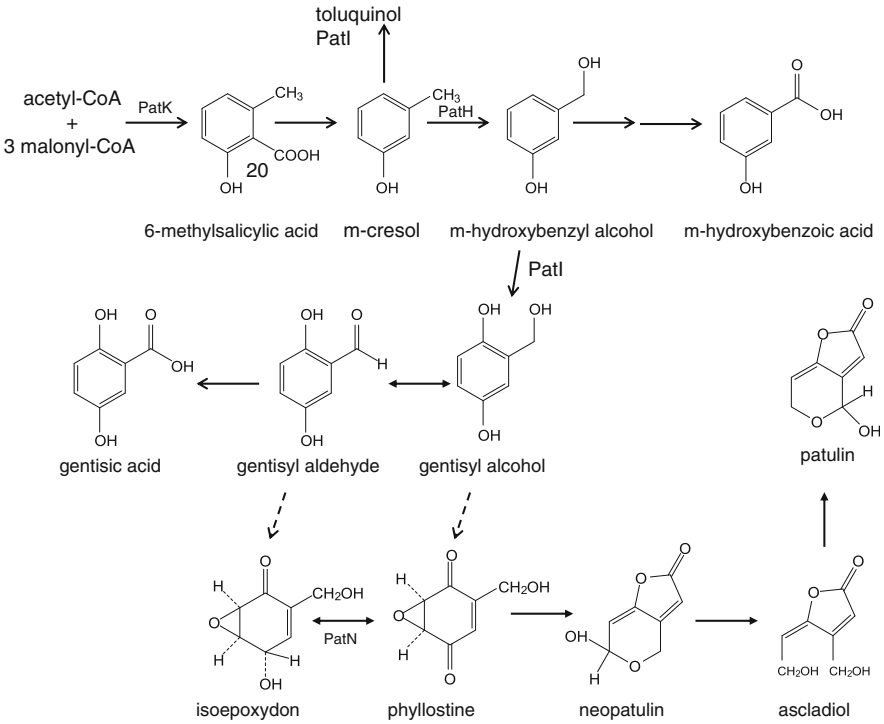


Aflatoxin M_2 18

The toxicity of these compounds is determined mainly by the lactone ring present in the coumarin moiety [78] and the double bond at position 8 and 9 of the furan ring. In the body, aflatoxins are transformed in the liver by cytochrome P450 enzymes into various metabolites and in case of AFB1 into particularly toxic AFB1-exo-8,9-epoxide (AFBO). These compounds interact with nucleic acids such as DNA or RNA and interfere with protein synthesis and glycolysis pathway. The formation of DNA adducts contributes to genetic mutations and cancer [77, 79].

The reduction of double bond in the extreme furan ring and the opening of the lactone ring and decarboxylation of the resulting –COOH group are substantially important to reduce the toxicity of aflatoxins. Inactivation of aflatoxins by ring opening can be conducted using, among others, acid or base hydrolysis. Additionally, the increase in temperature under these conditions to approx. 100 °C results in the removal of the methoxy group from the aromatic ring. Other chemical factors which cause a decomposition of aflatoxin structure are sodium hypochlorite, chlorine, and oxidizing agents such as hydrogen peroxide, ozone, and sodium metabisulfite [75].

The group of mycotoxic lactones comprises also patulin **19** produced by fungi of the *Penicillium* and *Byssochlamys* species. This compound was first isolated in 1940 from the culture of *Penicillium patulum*. In terms of chemical structure, patulin is a bicyclic lactone of the name 4-hydroxy-4H-furo[3,2c]pyran-2(6H)-one, soluble in water [80, 81]. This compound is a polyketide metabolite, the first for which the polyketide pathway has been characterized, synthesized in a



Scheme 3 Pathway of synthesis of patulin in *Penicillium* and *Aspergillus* sp. (Adapted from Puel et al. [82]). The Pat enzyme-encoding genes are organized in clusters in many fungi

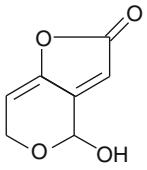
10-step pathway, starting from 6-methylsalicylic acid (6MSA compound **20** in Scheme 3) formed by the condensation of acetyl-CoA with three units of malonyl-CoA (Scheme 3). The reaction is catalyzed by a multifunctional enzyme, composed of four identical polypeptide chains of 176 kDa each, having the activity of acetyl- and malonyltransferase, ketoacyl synthase, ketoreductase, and dehydratase [82].

In the initial period of the study, patulin was tested for antibiotic properties, but because of its strong neurotoxic and teratogenic activity discovered in a later period, it was excluded from clinical use and in 1960 qualified as a mycotoxin [80]. In 1986, this compound was recognized by the IARC (International Agency for Research on Cancer) as a group 3 carcinogen. Patulin belongs to very reactive compounds that interact with nucleic acids and proteins. It exhibits strong affinity especially to thiol groups, which can result in severe damage to cells [82].

The process of detoxification of patulin employs chemical compounds based on oxidation and reduction of this lactone or the formation of less toxic thiol adducts. Detoxification of patulin using ammonia or potassium permanganate was performed with almost 100 % efficiency [83]. Sulfur dioxide was also an effective inhibitor of this toxin. At a concentration of 2000 ppm, a reaction of sulfur dioxide to the

hemiacetal ring of patulin, forming a carbonyl hydroxysulfonate and opening of the lactone ring structure at the double bond, was observed. Reduction of patulin toxicity was also possible thanks to the use of organic acids and vitamins, including ascorbic acid and vitamins of B group: thiamine hydrochloride, pyridoxine hydrochloride, and calcium-d-pantothenate [84].

A lactone with proven toxic properties, including carcinogenic properties, is penicillic acid **21** produced by fungi of the *Penicillium* and *Aspergillus* species. This compound was first isolated in 1913 from *Penicillium puberulum*. The carcinogenicity of this compound is determined by an α,β-unsaturated ring with a conjugated double bond at position 4 [81]. Penicillic acid, similarly to patulin, is a carcinogenic factor of group 3 (IARC 1998) [85]. It has been proven that this compound induces DNA strand breaks in HeLa cells [86].



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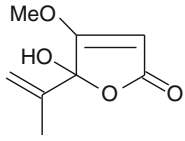
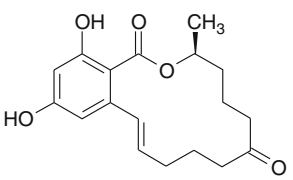
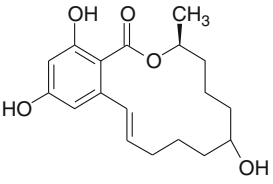


21

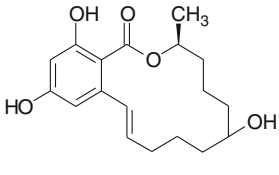
Mycotoxins produced by fungi of the *Fusarium graminearum* species (teleomorph *Gibberella zeae*) include zearalenone **22** (ZEN) – a lactone of resorcylic acid, chemically described as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone [80]. This compound is synthesized in the polyketide pathway involving polyketide synthases (PKSs), which catalyze sequential condensation reactions of acetate units to polyketide [87]. Zearalenone belongs to the compounds which disrupt a normal activity of the reproductive system. Because of its estrogenic properties, it is referred to as a nonsteroidal estrogen or mycoestrogen. The molecular structure of ZEN and its derivatives (α-zearalenol **24** [α-ZEL], β-zearalenol **25** [β-ZEL], α-zearalanol **26** [zeranol, α-ZAL], β-zearalanol **27** [teranol, β-ZAL], and zearalanone **23** [ZAN]) determines their ability to bind to estrogen receptors. ZEN is absorbed from the gastrointestinal tract and metabolized to ZEL or conjugated with glucuronic acid [88]. Estrogenic activity of ZEN depends on metabolic processes occurring in the body and on the immunologic status of the reproductive system of the contaminated organisms. It was demonstrated that ZEN affects the maturation and degree of degeneration of oocytes depending on the dose and time of exposure. In vivo and in vitro studies also show that ZEN reduces the activity of many enzymes, including those that are involved in the process of steroidogenesis in animals and belong to cytochrome P450scc and hydroxysteroid dehydrogenases of 3β- or 17β-type and their isomers, which are involved in conversion process of pregnenolone to progesterone or estrone to estradiol. In recent years, exposure to ZEN is associated with the occurrence of hormone-dependent cancers, including breast, cervical, and prostate cancer [89, 90].



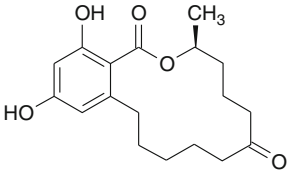
Zearalenone 22



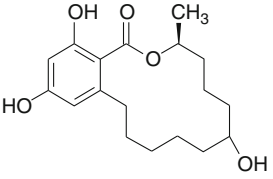
α-zearalenol 24



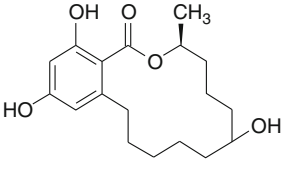
β-zearalenol 25



Zearalanone 23



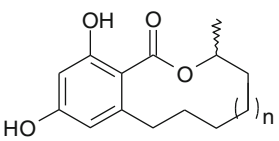
α-zearalanol 26



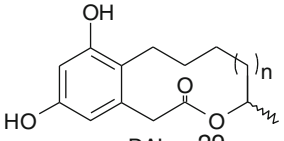
β-zearalanol 27

5.2 Other Bioactive Macrocyclic Lactones

The wide range of lactones includes a series of macrocyclic esters having a diversified biological activity, including antitumor, antimicrobial, antimalarial, or immunosuppressive activity [91]. They are a group of natural macrolides, synthesized in a pathway of polyketide synthase (PKS). Macrolides form a group of homologous compounds, which includes resorcylic acid lactones (RALs) (such as zearalenone and its derivatives) and dihydroxyphenylacetic acid lactones (DALs). Structurally RALs **28** and DALs **29** are formed by resorcinol fused to a lactone ring, at α- and β- or β- and γ-position, respectively.



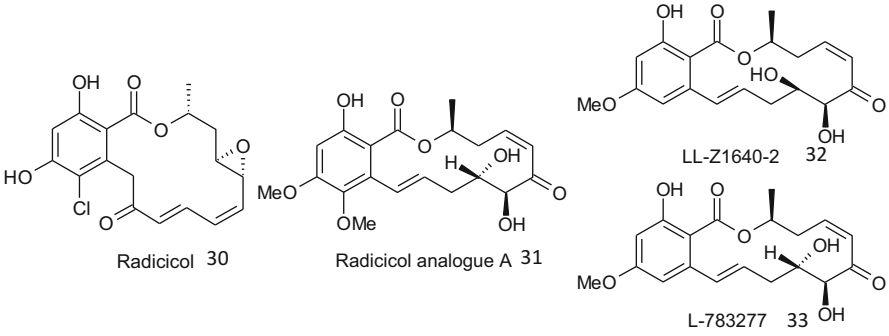
RALs 28



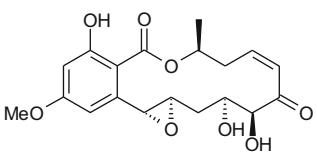
DALs 29

Resorcinol macrolides were discovered in 1953, when radicicol **30**, known initially as monorden, was first isolated [92]. Radicicol was initially identified as an antifungal antibiotic, and later studies assigned to it also several other biological activities, including a mild sedative effect [93]. In 1992, a group of scientists from Harvard University showed the inhibitory effect of radicicol in relation to the oncogenic Src kinase [94, 95]. Subsequently it was demonstrated that radicicol is a strong and selective inhibitor of heat shock protein HSP90, responsible for maturation and stability of many other oncogenic cellular proteins. It was shown that it contributes to the inhibition of tumor cell growth and their apoptosis by blocking HSP90 (radicicol blocks the ATP bond in N-terminal pocket of HSP90, thus preventing the conversion into a mature complex) [96].

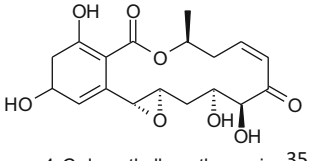
The family of resorcinol macrolides, which are conjugated *cis*-enones, comprises also other lactones of biological activity, including radicicol A **31**, LL-Z1640-2 **32**, and LL-783277 **33**. It was proved that these compounds inhibit irreversibly mitogen-activated protein kinases – MAP kinases, which are responsible for the regulation of many intracellular processes, including gene transcription, protein biosynthesis, cell division, cell differentiation, and survival or apoptosis [91]. Radicicol A inhibits the activity of cytokines IL-1β and accelerates the degradation of specific mRNA sequences containing adenylate-uridylate-rich elements [97]. LL-Z1640-2 exhibits the inhibitory effect in relation to TAK1 kinase (transforming growth factor-activated kinase 1) (IC_{50} = 8.1 nM) of the MAPK KK family [98] and ERK kinase (extracellular signal-regulated kinase) (IC_{50} = 8 nM) of the MAPK family [99], while L-783277 isolated from fungi of the *Phoma sp*. genus is characterized by a specific, strong inhibitory activity in relation to MEK1 kinase (4 nM) [100].



RALs which inhibit the activity of protein kinases include also hypothemycin **34**. This compound was first identified in 1980, after its isolation from the fungi *Hypomyces tricothecoides* [101]. Hypothemycin exhibits antifungal [102] and antimalarial activity, as well as cytotoxicity against various human cell lines [103]. According to a study of Fukazawa et al. [104], this compound contributes by binding cysteine, resulting to the inactivation of several protein kinases, including MEK1 (mitogen-activated protein kinase, whose activity is regulated by extracellular factors (IC50 15 nM)), ERK (extracellular signal-regulated kinase), and platelet-derived growth factor receptor. Solit et al. [101] demonstrated a strong activity of this lactone in the inhibition of protein BRAF mutation (BRAF V600E mutation is a point mutation, affecting the change in the protein activity, based on the replacement of valine 600 by glutamic acid). An analog of hypothemycin, 4-.-demethylhypothemycin **35**, isolated from *Hypomyces subiculosus* showed an equally strong cytotoxicity against a number of mutations of BRAF protein [105].

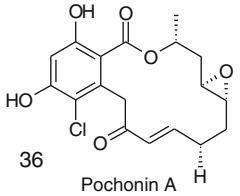


Hypothemycin 34

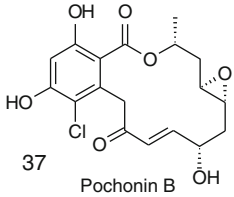


4-*O*-demethylhypothemycin 35

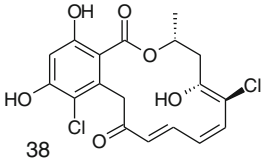
The group of less active lactones comprises also pochonins A–E **36–40**, isolated in 2003 from *Pochonia chlamydosporia* var. *catenulate* fungi. These compounds exhibit antiviral activity against, among others, herpes simplex virus 1 (the strongest activity is exhibited by pochonin C **38**) and are active against parasitic intestinal protozoa *Eimeria tenella* [106]. From 2009, the group of pochonins additionally comprises K–P analogs (K **41** L **42** N **43** O **44**) inhibiting expression of the WNT-5A protein and showing cytotoxicity against dermal papilla cells [107].



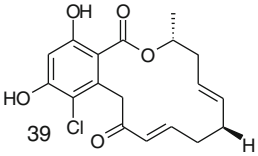
36 Pochonin A



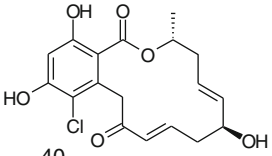
37 Pochonin B



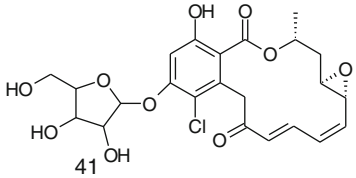
38 Pochonin C



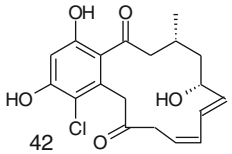
39 Pochonin D



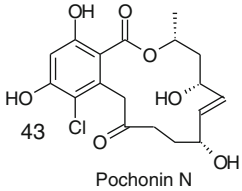
40 Pochonin E



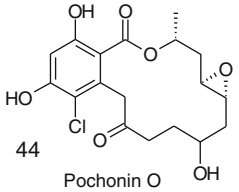
41 Pochonin K



42 Pochonin L

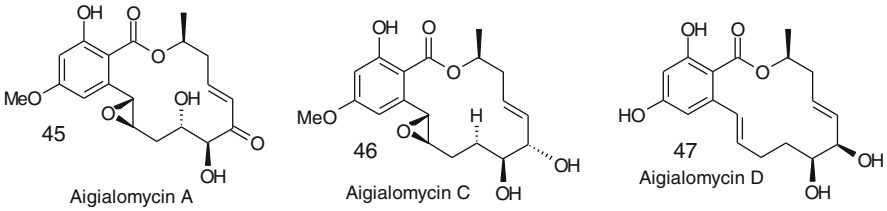


43 Pochonin N

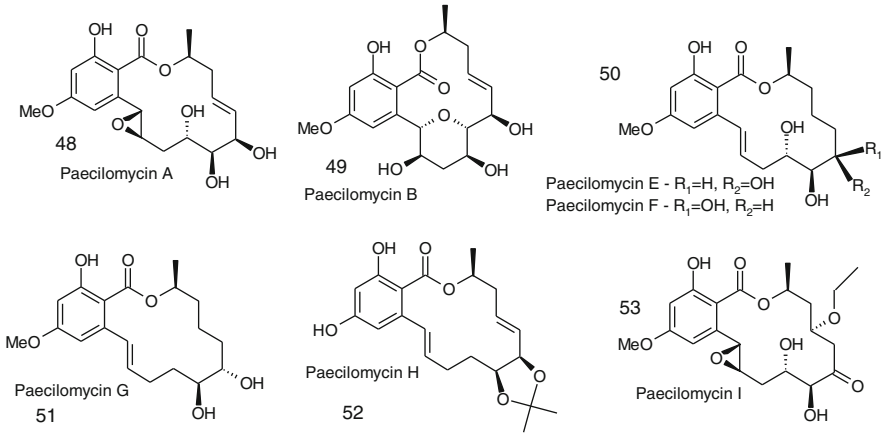


44 Pochonin O

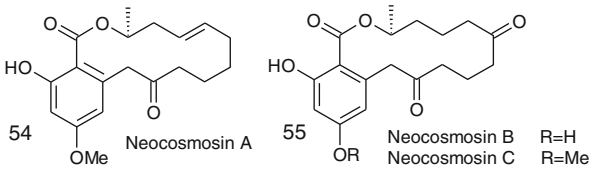
A diversified biological activity is also characteristic for aigialomycins A–E (A **45**, C **46**, D **47**), macrolides isolated in 2002 from a marine species of fungi – *Aigialus parvus*. Aigialomycin D has antimalarial activity (IC_{50} 6.6 μM) and exhibits cytotoxic effects against the cells of the KB type and BC-1 protein (which inhibits apoptosis) [108].



Another group of RALs comprises paecilomycins A **48**, B **49**, E **50**, F **50** [109], G-I **51–53** [110], and J–M [111] isolated from the solid medium of the fungus *Paecilomyces* sp. SC0924 in the years 2010–2013. These compounds exhibit inhibitory activity against a protozoan of the *Plasmodium* genus – *Plasmodium falciparum* – causing the most severe form of malaria in humans. Paecilomycin E is a strong inhibitor of the 3D7 strain of *Plasmodium falciparum* (IC_{50} 20 nM), while paecilomycin F inhibits the proliferation of the Dd2 strain.

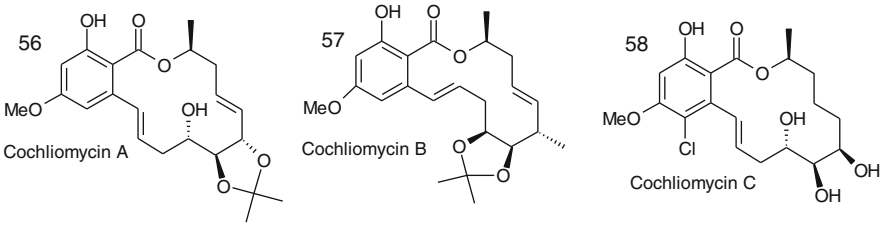


Neocosmosins A–C **54–55** belong to another recently identified group of resorcylic acid lactones synthesized by fungi of the *Neocosmospora* genus. These compounds, especially neocosmosin C, exhibit activity of agonists of opioid and cannabinoid receptors [112].

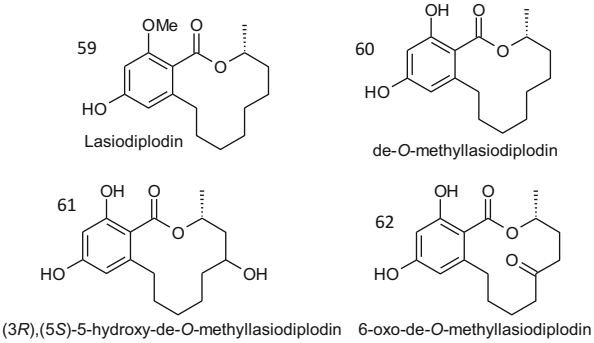


In 2011, the group of Shao [113] managed to determine the structure of three natural lactones – cochliomycins A–C **56–58** isolated from a broth culture of the *Cochliobolus lunatus* fungus originated from a gorgonian *Dichotella gemmacea* inhabiting the South China Sea. The studies involving cochliomycins showed,

among others, antibacterial activity of these compounds. It was demonstrated that these lactones exhibit inhibitory activity against bacteria *Staphylococcus aureus* and a lichen organism *Balanus amphitrite*.

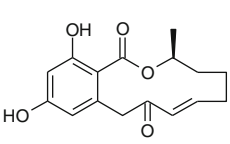


The family of lactones also comprises compounds which influence, among others, the regulation of plant growth. This activity was assigned to 12-membered RALs – lasiodiplodin **59** and de-*O*-methyllasiodiplodin **60**, first isolated in 1971 from the culture broth of fungi *Lasiodiplodia theobromae* [114, 115]. Later, both these lactones were also identified in plants. Based on several studies, it was demonstrated that lasiodiplodin exhibits an antileukemic activity, while de-*O*-methyllasiodiplodin was recognized, among others, to be an inhibitor of prostaglandin synthesis [116], a potential inhibitor of pancreatic lipase (IC 4.5 μM), and an antagonist of mineralocorticoid receptors, which may be effective in the treatment of hypertension and other cardiovascular disorders [117]. In 2011 also, a cytotoxic activity of de-*O*-methyllasiodiplodin against the KB (nasopharyngeal carcinoma cell line), BC1, and NCI-H187 (retinoblastoma cell line) cell lines was demonstrated [118]. According to the team of Buayairaks et al. [118], the group of lasiodiplodin derivatives comprises also 6-oxo-de-*O*-methyllasiodiplodin **62**, (3*R*),(5*R*)-5-hydroxy-de-*O*-methyllasiodiplodin, and (3*R*),(5*S*)-5-hydroxy-de-*O*-methyllasiodiplodin **61** isolated from fungi *Syncephalastrum racemosum*. To the latter lactone, a toxic activity against several tumors cells is attributed, especially against cholangiocarcinoma KKU-M139, KKU-M156, and KKU-M213.

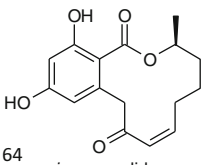


Further RALs are two isomers, *trans*- **63** and *cis*-resorcylide **64**, isolated from fungi of the *Penicillium spp*. [119], *Pyrenophora teres* [120], and *Acremonium zeae* [121] genera, which also exhibit a broad biological activity. These lactones are

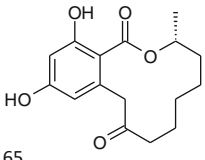
regarded as inhibitors of plant growth (*trans*-resorcylide isomer exhibits about tenfold stronger inhibitory activity than *cis*- isomer). Furthermore, *trans*-resorcylide is cytotoxic against a wide range of cancer cell lines, is considered to be an inhibitor of 15-hydroxyprostaglandin dehydrogenase (a key enzyme in the catabolism of prostaglandins), and is characterized by antimicrobial activity against *Pyricularia oryzae* [122]. *Cis*-isomer exhibits inhibitory activity against the coagulation factor XIIIa, responsible for the stabilization of fibrin [123].



63 *trans*-resorcylide



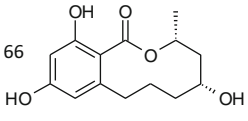
64 *cis*-resorcylide



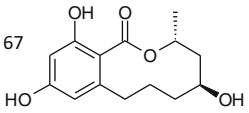
65 Dihydroresorcylide

Dihydroresorcylide **65** is a saturated analog of *cis*-resorcylide. It was identified by the team of Polling et al. [121] in an endophyte *Acremonium zeae*. Previous studies of this macrolide demonstrated its antifungal activity [124].

Lactones which are interesting in terms of their structure and biological activity are (*3R,5R*)-sonnerlactone **66** and its diastereoisomer (*3R,5S*)-sonnerlactone **67** colonizing a plant *Sonneratia apetala* [125]. Sonnerlactones exhibit antiproliferative activity against oral cavity cancer cell lines, resistant to numerous drugs.



(*3R,5R*)-sonnerlactone

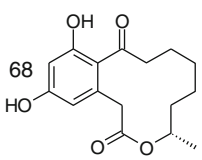


(*3R,5S*)-sonnerlactone

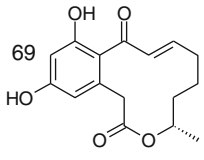
The group of DAL macrolides which are derivatives of dihydroxyphenylacetic acid lactones comprises a number of compounds of various biological activities, including curvularin **68**, 10,11-dehydrocurvularin **69**, and two epimers 11-α-methoxycurvularin **70** and 11-β- methoxycurvularin **71**. These lactones were identified in several fungal species, including *Penicillium* sp. such as *Penicillium citreoviride* [126–128], *Curvularia* sp. [129], *Chrysosporium lobatum* [130], *Eupenicillium sp*. [131], and *Nectria galligena* [132]. Curvularin is characterized by an antibiotic activity against numerous fungal species. It is an inhibitor of nitric oxide synthase [96] and an effective anti-inflammatory compound, inhibiting Janus kinases, which allows for its use in the development of drugs against chronic rheumatoid conditions [127]. Both curvularin and 10,11-dehydrocurvularin exhibit similar levels of cytotoxicity against several cancer cell lines including breast (MDA-MB-231 and MCF-7), cervical (HeLa), and lung (A549) cancer cell lines. In addition, 10,11-dehydrocurvularin is active against colon cancer cell line COLO 205 [130]. The other two abovementioned compounds of the curvularin group are also characterized by cytotoxic activity against, among others, lung NCI-H460, breast MCF-7, and pancreatic MIA Pa Ca-2 cancer cell lines [96, 130, 131].

A lactone of the DAL group, obtained in the polyketide synthase (PKS) pathway, is also citreofuran **72**. This compound is a metabolite of a hybrid strain *Penicillium citreoviride* ME 0005, isolated by Nakada and Yamamura [133]. However, its biological activity has not been reported yet.

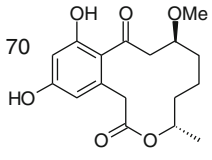
Sporostatin **73** is another example of a mycotoxic lactone, isolated from the fungus *Sporormiella* M5032. This compound exhibits strong inhibitory activity against tyrosine kinase of epidermal growth factor receptor. In addition, it is an inhibitor of a phosphodiesterase specific for cyclic adenosine-3′,5′-monophosphate [134].



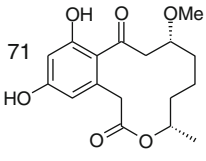
Curvularin



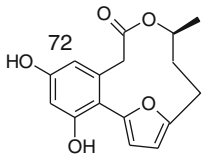
10,11-dehydrocurvularin



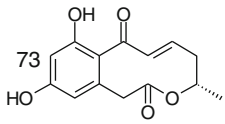
11-alfa-methoxycurvularin



11-beta-methoxycurvularin

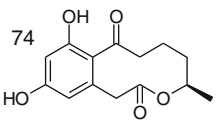


Citreofuran

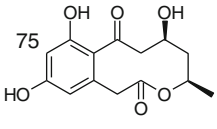


Sporostatin

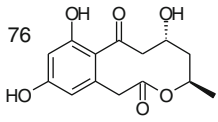
The DAL family also comprises xestodecalactones A–C **74–76**, isolated from the fungus *Penicillium cf. montanense* originated from marine sponges *Xestospongia exigua* [135], and D–F **77–79**, identified in fungi *Corynespora cassiicola* [136]. These compounds exhibit antifungal activity, and xestodecalactone B inhibits the growth of, among others, fungi *Candida albicans* [135].



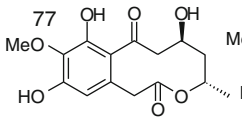
Xestodecalactone A



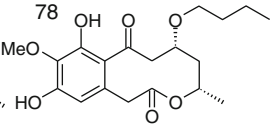
Xestodecalactone B



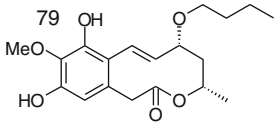
Xestodecalactone C



Xestodecalactone D



Xestodecalactone E



Xestodecalactone F

This review of macrocyclic lactones indicates a diversified structure and broad spectrum of biological activity. Valuable biological properties of lactones isolated from natural sources are the inspiration for the research works related both to the isolation of consecutive natural lactones occurring in nature and to the synthesis of new compounds containing lactone moiety in their molecules. Given the increasing

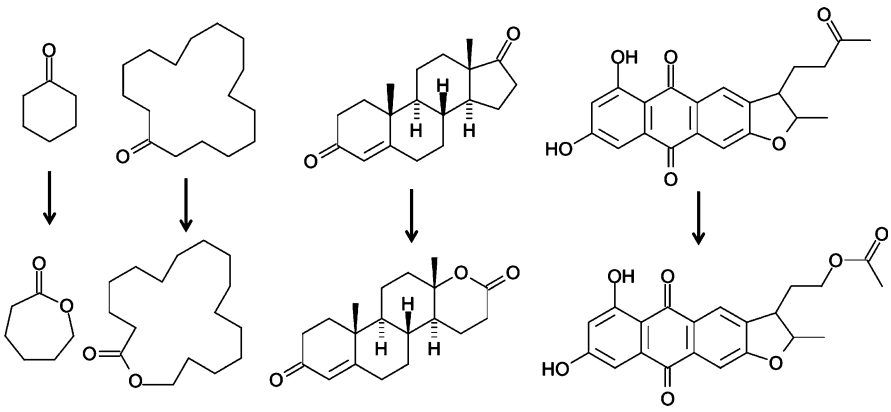
number of people suffering from cancer and continuous mutations of pathogenic microorganisms, it is understandable that many research centers began the search for natural and synthetic biologically active compounds, which in the future may become approved drugs.

6 Fungal Biocatalysts

Some of the fungal systems described above are used out of fungal metabolism for biotechnological applications. It is the case for Baeyer–Villiger monooxygenases, lactonases, and the polyketide synthase pathways, although more applications have been carried out from bacterial systems.

6.1 Baeyer–Villiger Monooxygenases

Baeyer–Villiger (BV) oxidation which consists in the transformation of a linear or cyclic ketone into its corresponding ester or lactone by insertion of an oxygen atom next to the carbonyl group is a precious reaction for oxidation of carbon chains or cycles (Scheme 4). The chemical reaction has been first described in 1899 by Baeyer and Villiger. In its traditional chemical catalysis, this reaction is not enantioselective, and catalysts are thus required that can result in enantiopure lactones. This property is exhibited by enzymes that are called Baeyer–Villiger monooxygenases (BVMOs – EC 1.14.13.x). First described in 1953 after studies on the degradation of steroids [137, 138], most of the known enzymes are bacterial, and the amount of studies resulted in a characterization of the enzyme



Scheme 4 Some lactone-related reactions catalyzed by Baeyer–Villiger monooxygenases. From left to right: the much studied (in *Acinetobacter* sp.) cyclohexanone oxygenation and the oxygenation of cyclopentadecanone, a macrocyclic ketone, in a way different from what described in (2), of androstenedione (steroid), and of hydroxyversicolorone (aflatoxin precursor) (Inspired from Torres Pazmiño et al. [144]

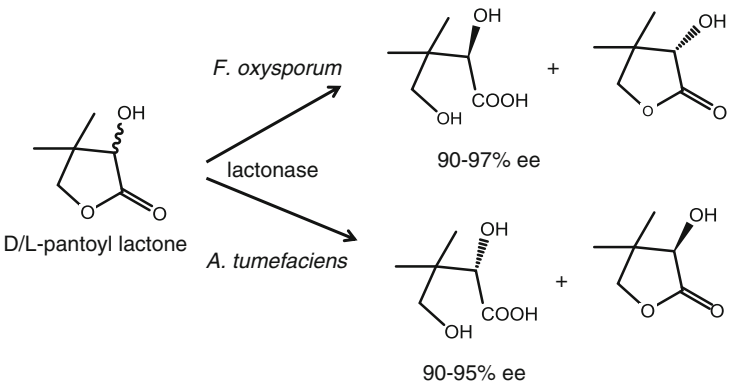
with the identification of a sequence motif [139] and a better knowledge of the role of BVMOs in metabolic pathways. All this aimed at developing biotechnological applications especially toward the enzymatic properties of regioselectivity and stereopecificity. Many important reactions can be catalyzed by BMVOs (Scheme 4). Recent work aimed at using directed evolution techniques to modify BVMOs and find new activities. It was thus found that a modification of only one amino acid could turn the BVMO of *Thermobifida fusca* into a NADPH oxidase [140], but some works have also been carried out to find new activities through chemical screening, genome mining, or evolution studies [141]. Several reviews on chemical and biotechnological applications of BVMO have been published [10, 12, 142–144], but most of them concerned bacterial catalysis. Although fungal BVMOs have been discovered in early research on the subject, the number of characterized fungal BVMOs is still low, although new attention has been devoted to these organisms with the possibility of genome mining investigation [145].

6.2 Lactonases

Due to the important place that lactones have in microbial metabolism, enzymes exhibiting the capability to catalyze their degradation through the opening of the cycle are important. The opening of the ring takes place usually through the hydrolysis of the ester bond, and enzymes able to open this belong to the esterase family and are called also lactonases. Different lactone rings can be hydrolyzed with lactonase catalysis, and an example is given Fig. 3 on the degradation of limonene which is first oxidized into a lactone with a BMVO catalysis and then hydrolyzed by a lactonase [13]. Like other esterases, lactonases may be highly enantiospecific which results in biotechnological applications in the resolution of racemics. As an example, lactonases are used for the resolution of racemics of pantoyl lactone (Scheme 5) (this example is related in the review on lactonases by [7]). Lactonases of *Fusarium oxysporum* or from *Agrobacterium tumefaciens* can be used, reaching the different enantiomers with enantiomeric excess (ee) at about 90–95 %. However, for the industrial reaction, it was easier to work with *Fusarium* lactonase and to immobilize it to keep activity.

6.3 The Polyketide Synthase Pathway

This system has been developed as a modular enzyme system enabling technologists to select the interesting activities to synthesize molecules. In terms of biocatalysis, this system is probably one of the most complex system developed. Several reviews have reported the advances in the field [9, 146, 148]. However, this part will not be developed in the present review as those megasynthases concern the bacterial system and not the fungal one.



Scheme 5 Resolution of D/L-pantoyl lactone racemic with *Fusarium oxysporum* and *Agrobacterium tumefaciens* lactonases

7 Conclusion

Lactones are important bioactive compounds for fungi. They play a significant role in fungal ecology as communication and antimicrobial molecules, but they have also a great impact on our lives through mycotoxins and can also be involved in positive aspects of human health as some compounds are active against cancer cells and other diseases. Beside health, many lactones are active on human senses such as flavor and fragrance lactones that can be produced by fungi in a natural way. In addition, pathways of production of lactones, β- and ω-oxidation, polyketide synthases, Baeyer–Villiger monooxygenases, and lactonases have been studied to understand and control the synthesis of fungal lactones, but they can also be used for industrial synthesis of building blocks or fine chemicals as they exhibit interesting properties for region-specific and region-selective oxidation.

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