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Non-terpenoid biotransformations by *Mucor* species

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Abstract Biotransformation is an important tool for the structural modification of organic compounds, especially natural products with complex structures, which are difficult to achieve using ordinary methods. It is also useful as a model for mammalian metabolism due to similarities between mammalian and microbial enzyme systems. The development of novel biocatalytic methods is a continuously growing area of chemistry, microbiology, and genetic engineering, and novel microorganisms and/or their enzymes are being screened intensively. This review covers the transformation of non-terpenoid compounds such as steroids, coumarins, flavonoids, drugs, pesticides and others by *Mucor* spp. up to the end of 2012.

Keywords Biotransformation · *Mucor* sp. · Non-terpenoid

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Introduction

Microbial transformation is regarded as an enzymatic reaction by using the metabolic activities of microorganisms to modify the chemical structures of bioactive substrates for finding the new chemical derivatives with the potent bioactivities and physical–chemical characteristics. It has a number of advantages over chemical synthesis such as higher stereo- and regioselectivity, milder reaction conditions, lower cost and less pollution. Furthermore, some reactions that do not occur when using chemical approaches are easily carried out by microbial transformation (Chen et al. 2009).

Microorganisms can be used as a reliable and efficient alternative to in vivo studies or to synthetic chemistry to obtain sizable amounts of a number of drug derivatives in metabolism studies. Metabolism is the structural modification of drugs and chemicals by enzymatic systems which leads to the formation of relatively polar substances which are easily excreted from the organism. An important factor in the evaluation of the safety and effectiveness of any drug is knowledge of its metabolism (Asha and Vidyavathi 2009). The use of microorganisms to simulate the mammalian metabolism of many pharmacologically important molecules is well documented (Rosazza and Smith 1979; Smith and Rosazza 1975, 1983).

On the other hand, the use of the biotransformations to carry out useful chemistry processes may be complicated by inhibitory and toxic properties of

reactants and/or products, biocatalysts sensibility to environmental extremes of temperature and pH, and low quantity to derivatives production. However, there are biological and process solutions to many of these problems and methods to compare strategies and techniques for biotransformation operation are being developed (Lilly 1994).

From among the fungi used in biotransformation processes, *Mucor* species have the ability to metabolize a wide variety of compounds in manners that are similar to those in mammalian enzyme systems.

Microbial reactions have been used to achieve chemical transformations as a part of synthetic procedures integrating green chemistry principles into drug design. The aim of this review is to trace the biotransformation of non-terpenoid compounds by *Mucor* species up to the end of 2012.

Biotransformation of steroids

Steroids are widely distributed in the animal and plant kingdom. The basic skeleton consists of 17 carbon atoms arranged in the form of a perhydrocyclopentanophenanthrene. They vary widely in structure and contain vital compounds such as cholesterol, bile acids, sex hormones, vitamin D, corticoid hormones, cardiac aglycones, antibiotics, and insect molting hormones (Bhatti and Khera 2012).

Fungi have proved to be powerful biocatalysts in steroid biotransformations since their enzymatic and metabolic systems can be used to modify a wide range of this class of compounds.

A variety of steroids are widely used as anti-inflammatory, diuretic, anabolic, contraceptive, anti-androgenic, progestational and anticancer agents, as well as in other applications. Hydroxysteroids have been reported as having useful biological activities and several microorganisms, e.g. *Mucor* spp., are capable of bringing about these hydroxylations (Mahato and Mukherjee 1984; Mahato and Banerjee 1985; Mahato and Majumdar 1993; Mahato and Garai 1997; Al-Footy 2008a, b). Steroid hydroxylation is also an important enzymatic reaction in mammalian organisms due to the detoxification of exogenous steroid drugs (Lacroix et al. 1999).

The regio-chemistry of the microbiological hydroxylation of steroids has been rationalized in terms of the positions of existing hydroxyl or carbonyl groups on the steroid skeleton which act as directing groups. These groups facilitate the binding of the steroid to the hydroxylase, possibly by hydrogen bonding, and bring the centre which is to be hydroxylated within the sphere of the oxidative co-enzyme (Al-Fouti and Hanson 2002).

Fungi from the *Mucor* genus very effectively carry out hydroxylations of several C19 and C21 steroids at the C-7 and C-14 positions to produce important intermediates used in the synthesis of pharmacologically active steroid derivatives. The 14α-hydroxylase appears to have broad substrate specificity. However, steroids with a bulky substitution at the C-17 α-position or without the 4-en-3-one group are not accepted as substrates by the 14α-hydroxylase system (Madyastha 1994; Hu et al. 1995).

Several fungi have been shown to introduce a hydroxyl group at the 14α-position of progesterone (**1**) and some other steroids in reasonable yields. Thus, *Mucor parasiticus* ATCC 6476 and *Mucor griseocyanus* ATCC 1207 were found to oxygenate progesterone (**1**), deoxycorticosterone (**2**), 11-deoxycortisol (**3**) and testosterone (**4**) to the corresponding 14α-hydroxylated steroids (Fig. 1; Eppstein et al. 1958). The biotransformation of the hydroxyl-derivatives from **1**, **2** and **4** by *M. griseocyanus* ATCC 1207 also mainly produced 14α-hydroxy derivatives. However, in the case of 17α-methyl-substituted steroids, the main transformation products were the 7α-hydroxy derivatives (Singh et al. 1967).

Progesterone (**1**) was converted by *Mucor hiemalis* NRRL 2684 to 7α,14α-dihydroxyprogesterone, which inhibited sodium retention induced by deoxycorticosterone (**2**) and this bioprocess generated a patent (Dodson and Tweit 1960). *Mucor piriformis* was also used to study the mode of transformation of progesterone (**1**). The major metabolites isolated and identified were 14α-hydroxyprogesterone, 7β,14α-dihydroxyprogesterone, 7α,14α-dihydroxyprogesterone, and 6β,14α-dihydroxyprogesterone. Time-course studies of transformation product formation showed that hydroxylation at the 14α-position is the first step in the formation of dihydroxyprogesterones (Madyastha and Srivatsan 1987).

The first commercialized microbial process in the steroid field was in the production of 11α-hydroxyprogesterone, a compound with antiandrogenic and blood-pressure-regulating activity. In this case, *Mucor racemosus* NRRL 3639 and mutants from *M.*

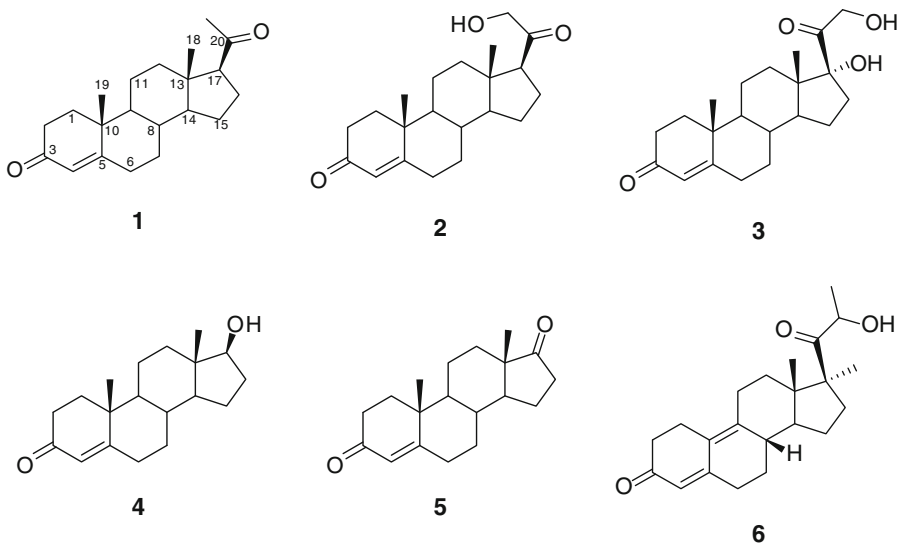


Fig. 1 Steroids used in biotransformations by *Mucor* spp

racemosus NRRL 3639 efficiently hydroxylated progesterone (**1**) to 11α-hydroxy progesterone (El Minofy et al. 2000; Hamdi et al. 2000; Khattab and Abd-El Salam 2012).

Screening of fungal cultures for their ability to monohydroxylate steroids at unusual sites showed that fungal strains were capable of dehydrogenating ring B of progesterone (**1**) and androstenedione (**5**) at positions C6–C7. Smith et al. (1989) described the use of *M. racemosus* to produce 11α-hydroxy-6-dehydroprogesterone from progesterone (**1**), which would be difficult and time-consuming to synthesize by conventional synthetic chemical methods.

17-Methyltestosterone, an important anabolic 17-methyl steroid derived from testosterone (**4**), has therapeutic uses such as weight gain after surgery, treatment of trauma, birth control, regulation of inflammation, and treatment of other diseases. The potential of *M. racemosus* in the biotransformation of methyltestosterone was investigated by Torshabi et al. The derivatives obtained were characterized as 7α-hydroxy, 15α-hydroxy and 12,15α-dihydroxy derivatives. This microorganism failed to carry out 14-hydroxylation on methyltestosterone which indicates that hydroxylation at C-14 may be inhibited by the presence of a 17-methyl group (Torshabi et al. 2011).

Therefore, it is stated that the α-hydroxylation catalyzed by *Mucor* species of steroids such as progesterone (**1**), and 17-methyltestosterone is an interesting tool to provide derivatives with pharmacological potential.

Microbial models produce satisfactory results when used to study mammalian steroid metabolism pathways. The ability of microbial species to produce different metabolites which are sometimes not achievable by chemical synthesis has made them indispensable to the pharmaceutical industry. Androst-4-ene-3,17-dione (**5**) is among the most important intermediates in the production of some valuable pharmaceutical steroid compounds. Several *Mucor* spp. have been shown to carry out different kinds of transformations on that molecule. Thus, 14α,17β-dihydroxyandrost-4-en-3-one monohydrate and 14α,17β-dihydroxyandrosta-1,4-dien-3-one monohydrate were identified in *M. piriformis* biotransformations from androst-4-ene-3,17-dione (**5**) (Krishnan et al. 1991). The ability of *M. racemosus* to produce different modifications of androst-4-ene-3,17-dione (**5**), including C-6β, C-7α, C-7β, C-11α, C-14α hydroxylations and 17-carbonyl reduction into the C-17β hydroxyl, was also demonstrated (Faramarzi et al. 2008). These studies showed that *M. racemosus* produced more modifications than *M. piriformis* in the structure of androst-4-ene-3,17-dione (**5**).

Also, Lacroix et al. reported the microbial transformation of RU27987 (Trimegestone®, **6**), a 3-keto-$\Delta^{4,9(10)}$-19-norsteroid drug developed by Hoechst–Marion–Roussel as a progestomimetic and calcium assimilation regulator for the treatment of osteoporosis.

The microbial transformation of this drug appeared to have potential for future investigation as a source of useful information concerning its metabolism in mammals. However, since it was extremely active, it had to be used at very low doses in experiments with animals and women and the amount of main metabolites recovered was insufficient for clear structural identification. The incubation of **6** with *Mucor circinelloides* CBS 108-16, *M. griseocyanus* ATCC 1207a, *M. hiemalis* BO, *Mucor plumbeus* CBS 110-16, *M. plumbeus* ATCC 4740, *M. racemosus* BO and *Mucor rouxii* CBS 416-77 afforded 11β, 11α and 15β-hydroxy derivatives. This study demonstrated the usefulness and versatility of microbial transformation systems as a tool for the early identification and easy production of potentially active mammalian and non-mammalian metabolites (Lacroix et al. 1999).

Microbial hydroxylation studies are important in gaining insight into cytochrome P450 enzyme mediated reactions. Furthermore, the exploitation of previously underutilized fungi is essential in the production of novel compounds. *M. plumbeus* has been employed in the hydroxylation of a range of steroids at the 1β, 5β, 6β, 7β, 9α, 11β, 12β, 15α and 16α positions, methods reported in three patents by Murray and Peterson (1952, 1956, 1957) and several articles (Eroshin 1962; Al-Fouti and Hanson 2002; Hanson et al. 2003). Lamm et al. performed bioconversion reactions by *M. plumbeus* ATCC 4740 using a series of androstanes (**7** and **4**), pregnanes (**1**, **10**, **13** and **14**), and an estrane (**15**) as substrates (Fig. 2). This group concluded that *M. plumbeus* ATCC 4740 appeared to possess a number of mono-oxygenases: one enzyme could be responsible for 7α/11β-hydroxylation, while another for functionalization at C-6β/14α. Furthermore, 17 and 20-carbonyl moieties were reduced in substrates **7**, **15** and **13** to give **8**, **12** and **17**, respectively. Steroid **8** was further hydroxylated at the C-7α position to yield **9**. Similarly, **10** was dihydroxylated at C-7α and C-11β, possibly by the same enzyme, yielding **18**. Compound **1** was difunctionalized at the C-6β and C-14 positions to give **16**. Transformation of **4** gave **11** and **16**, products of 6β and 14-hydroxylation, respectively. Compound **14** was not transformed (Lamm et al. 2007).

Phytosterols have received much attention due to their biological activities and health promoting effects. It was assumed that natural 7-hydroxysteroids could have a neuroprotective function, and in recent years 5-androstene-3β,7α,17β-triol (**19**) and 5-androstene-3β,7β,17β-triol (**20**) have attracted the attention of more and more scientists because of their significant bioactivity. In this connection, Li et al. (2008) reported obtaining these two important neuroactive steroids, 5-androstene-3β,7α,17β-triol (**19**) and 5-androstene-3β,7β,17β-triol (**20**), through microbial transformation of 5-androstene-3β,17β-diol (**8**) using *M. racemosus* ATCC 0401.

The pharmaceutical industry has great interest in steroid transformation for the production of steroid hormones. 7α-Hydroxylation of steroids, a common reaction in the biotransformation of these compounds, has potential for industrial exploitation. It is claimed that some 7α-hydroxylated derivatives may be useful in treating certain cancers and Alzheimer's disease by bolstering the immune response and as an anti-obesity agent, in addition to exhibiting anti-glucocorticoid action (Ge et al. 2008). Pregnenolone (**10**) is a major hormone mainly present in human nerve tissues and its therapeutic role in repairing neurons has been well documented. Recent studies indicate that the 7α-hydroxylated metabolite of **10** performs many important activities such as bolstering the immune response in mice; it has antiglucorticoid potential, improves spatial memory and acts as a neuronal activator to stimulate locomotor activity. Previous studies carried out by Shan et al. showed that *M. circinelloides* var. *lusitanicus* can transform various 5-en-3β-ol steroids into their 7α-hydroxy derivatives. Thus, pregnenolone (**10**) was biotransformed by *M. circinelloides* var. *lusitanicus* to 3β,7α,11α-trihydroxypregn-5-en-20-one (**21**), which could then be easily crystallized with high yield (Shan et al. 2009).

The introduction of aromatic groups into steroids has led to new physiological activities. Thus, compound **21** was reacted with benzaldehyde derivatives using the Claisen–Schimidt condensation reaction producing compound **22** (Fig. 3). The in vitro cytotoxicity of these compounds against the human esophageal cancer cell line, EC109, was evaluated. The results showed that both compounds **21** and **22** exhibited stronger inhibition activity than **10**. This indicates that the 7α- and 11α-hydroxyl groups and the benzylidene structure are essential for the cytotoxic activity being investigated (Shan et al. 2009).

The transformation of 3β-hydroxy-5-en-steroids with varying substituents at C-16 and/or C-17, i.e.: 16-dehydropregnenolone (**23**), 16α,17α-epoxy-

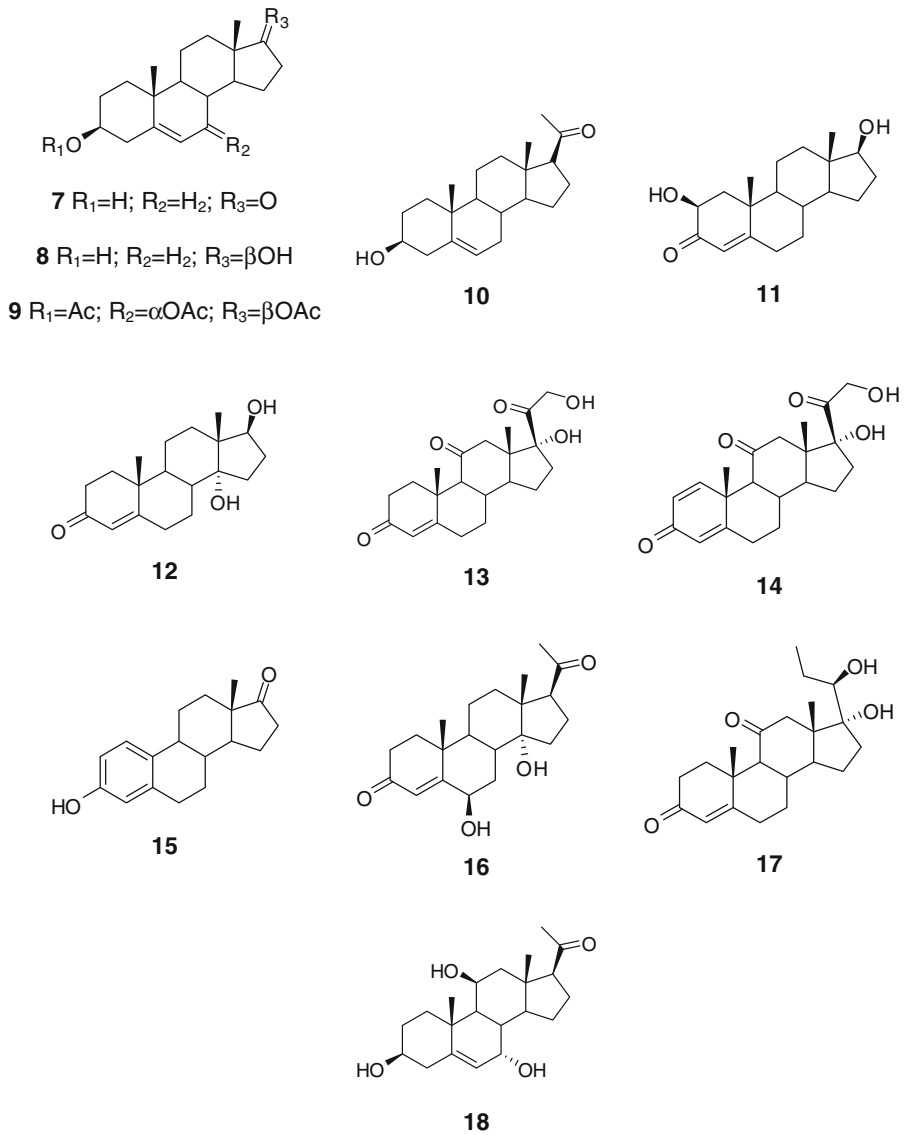


Fig. 2 Substrates and products from the biotransformation processes using *M. plumbeus* ATCC 4740

pregnenolone (**26**), and pregnenolone (**10**) using *M. racemosus* ACCC 0401 were investigated by Ge et al. to study the correlation between substrate structure and the course of hydroxylation (Fig. 4). In these transformations the substrates were mainly hydroxylated at the 7α position. The fermentation of **23** or **26** resulted in the formation of 16α-methyloxy-steroids, which seemed to be catalyzed by the sterol methyltransferase. In the transformation of **10**, besides the mainly 7α-hydroxyl-steroids, there was a little amount of 11α-hydroxyl-steroid. These results showed that a C5–C6 double bond had a significant impact on the position of hydroxylation. In conclusion, this strain of *M. racemosus* may have a very bright future in the pharmaceutical industry insofar as it can lead to the valuable 16α-methoxyl steroids and 11α-hydroxylated intermediates for steroidal drugs (Ge et al. 2008).

In recent years there has been increasing interest in immobilizing whole cells of microorganisms. Unfortunately, most of the work on cell entrapment has been performed using bacteria and yeasts given the relative simplicity of immobilizing their whole cells.

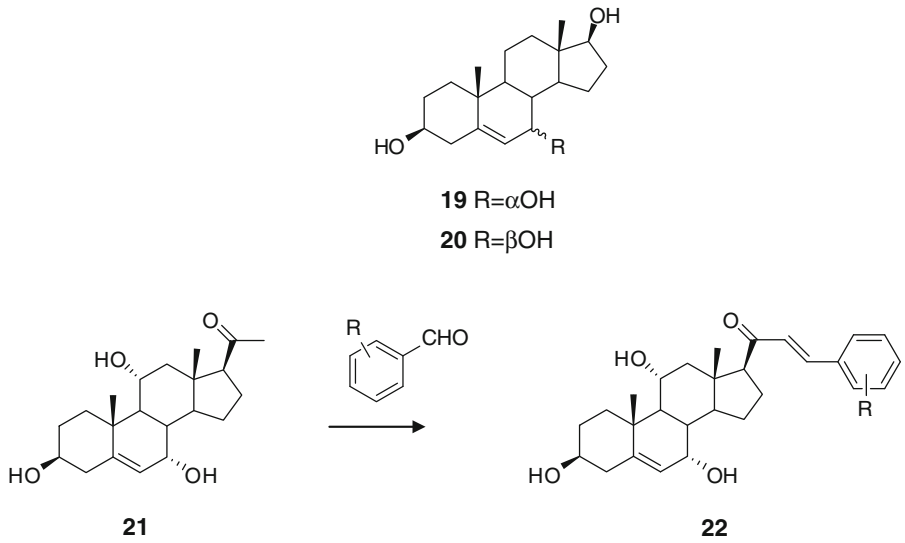


Fig. 3 Synthesis of compound **22**

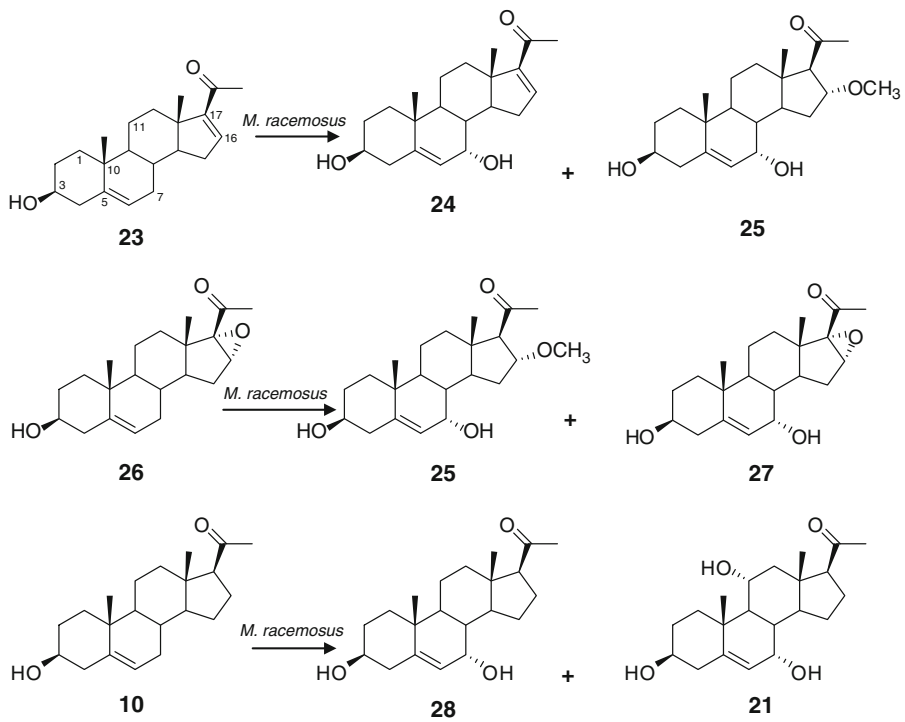


Fig. 4 Transformation of 3β-hydroxysteroids by *M. racemosus*

Nevertheless, there have been many modifications of the different methods to make them applicable to filamentous fungi. Thus, transformation reactions on 3β,17β-dihydroxyandrost-5-ene (**7**) using *M. plumbeus* ATCC 4740 in the free fungal cells were compared with those carried out by macerated mycelia, immobilized in calcium alginate beads. The results showed for the first time that encapsulated mycelial fragments essentially carried out the same bioconversions as those observed with growing cells. Two

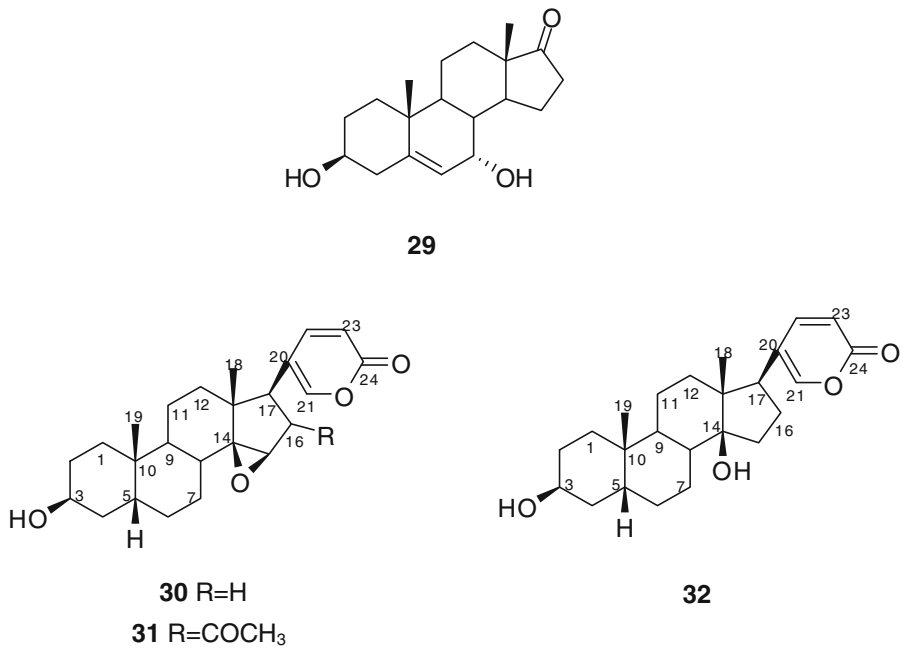


Fig. 5 Main bufadienolides isolated from Chansu

transformation products were obtained from these incubations, namely 3β,7α,17β-trihydroxyandrost-5-ene (**19**) and 3β,7α-dihydroxyandrost-5-ene-17-one (**29**). The advantages of this method over previous ones are its applicability in cases where the fungus does not produce spores (many endophytic fungi, for example) and easy purification of the transformed products since cells in the matrix do not produce secondary metabolites (Peart et al. 2012).

Bufadienolides are the bioactive components of the traditional Chinese medicine Chansu, a product from the skin secretions of local toads, which exhibit cardiotonic, respiratory, antiviral and antineoplastic biological activities (Zhan et al. 2003). These compounds have a steroidal A/B *cis* and C/D *cis* structure with a characteristic α-pyrone ring at the C-17 position. Resibufogenin (**30**), cinobufagin (**31**) and bufalin (**32**) are the major bufadienolides isolated from Chansu (Fig. 5; Ye et al. 2005a).

Zhan et al. (2003) reported the microbial hydroxylation of resibufogenin (**30**) by *Mucor subtilissimus* AS 3.2454 to 12β-hydroxyresibufogenin. Later, in 2006, these authors reported a series of reactions from resibufogenin (**30**) when catalyzed by enzymes existing in *M. subtilissimus* AS 3.2454 including hydroxylases, isomerase and glycosyltransferase. Hydroxylase played an important role in this process since most products came from hydroxylation. The products obtained from resibufogenin (**30**) were characterized as monohydroxylated derivatives at the 11β-, 16α-, 12α- and 7α-positions, dihydroxylated derivatives at the 1β,11β-, 12β,16α-, 12α,16α- and 1β,12β-positions, in addition to 12-*O*-β-*D*-glucoside and 12β-hydroxy-3-*epi* derivatives (Zhan et al. 2006).

In order to discover new bufadienolide derivatives with more potent bioactivities and improved physicochemical properties as drug candidates, Ye et al. reported the biotransformation of resibufogenin (**30**) by *Mucor polymorphosporus*. The transformation reactions involved hydroxylations at C-1β, C-5, C-7α, C-7β, C-12α, C-12β and C-16α, as well as the epimerization or dehydrogenation of 3-OH. Hydroxylations at C-12α, C-12β and C-16α were the major reactions. Some of the products exhibited diminished but still potent cytotoxicity. These results provided guidance for future directed synthesis of bufadienolides of pharmaceutical interest (Ye et al. 2005b).

Cinobufagin (**31**), a bufadienolide with a 14β,15β-epoxy ring, is one of the major active components of Chansu. Zhang et al. have focused on the structure optimization of some important active natural products using biological systems and, as part of their ongoing efforts, they carried out the biotransformation of **31**

using *Mucor spinosus*. The derivatives obtained from cinobufagin (**31**) were characterized as 1β-hydroxycinobufagin, 12β-hydroxycinobufagin and 1β,12β-dihydroxycinobufagin. Cytotoxic activity assays showed that the derivatives were less active than pattern structure **31** (Zhang et al. 2005). The 12β-hydroxylation is also the main reaction in the biotransformation of cinobufagin (**31**) by other *Mucor* sp. such as *M. polymorphosporus* or *M. subtilissimus* (Ye et al. 2004a).

He et al. isolated several microbial transformation products of cinobufagin (**31**) using *M. spinosus* AS 3.2450 and their antitumor activity was evaluated to obtain the structure–activity relationships and provide information about the metabolism of cinobufagin (**31**) in mammals. The compounds obtained were 1β-hydroxyl, 12β-hydroxyl, 1β,12β-dihydroxyl, desacetyl, 12β-hydroxyl desacetyl, 3-oxo-desacetyl, 3-oxo, 3-*epi*-12β-hydroxyl and 5β-hydroxyl derivatives. The cytotoxic activities were notably altered by hydroxylation of cinobufagin (**31**) at different positions: i.e. were increased significantly after mono-hydroxylation at C-1β, C-12β or when cinobufagin (**31**) was transformed into bufalin (**32**). However, activity decreased after losing the acetyl group, hydroxylation at C-5β and further oxygenation or epimerization of the hydroxyl group at C-3β (He et al. 2006).

The cytotoxicity of bufadienolides oxygenated at different sites, which are difficult to obtain by chemical means, remain unknown. However, biotransformation is an alternative tool to structurally modify complex natural products due to its capacity to catalyze novel reactions and its regio- and stereoselectivity. Thus, Ye et al. reported the production of new bufadienolides from bufalin (**32**) by *M. spinosus* AS 3.3450. The biotransformation products obtained in this study were mono- or dihydroxylated derivatives with novel oxyfunctionalities at the C-1β, C-7β, C-11β, C-12β and C-16α positions. The cytotoxicity of the products obtained, and that of several bufadienolides derived from cinobufagin (**31**), determined using four human cancer cell lines, suggested that 3-OH glucosylation or hydroxylation at C-1β or C-12β could be promising reactions to obtain more polar bufadienolides with enhanced cytotoxic activities. Bufalin (**32**) derivatives were more active than their corresponding cinobufagin (**31**) analogues. Thus, the 14β,15β-epoxy ring appears to reduce cytotoxicity (Ye et al. 2004b, 2005a).

At this moment, the literature shows that in the biotransformation of the major bufadienolides isolated from Chansu, resibufogenin (**30**), cinobufagin (**31**) and bufalin (**32**), were employed only three *Mucor* species: *M. subtilissimus*, *M. polymorphosporus and M. spinosus.* In front of the biological activities of these substrates and their metabolites, the investigation of the catalytic potential of other *Mucor* species could be an interesting approach to obtain new pharmacological active compounds.

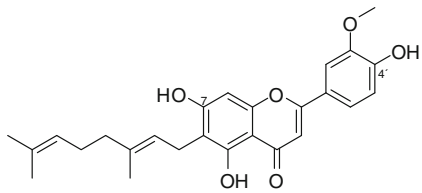
Biotransformation of flavonoids and coumarins

Flavonoids (Fig. 6) are important polyphenolic phytochemicals found in fruits, vegetables and beverages. They add color and flavor to many foods as well as make up an important part of the human diet. Flavonoids are in many dietary supplements and have been described as health-promoting and disease-preventing. They are associated with a wide range of biological activities, many beneficial, though some may exhibit mutagenic properties and interfere with essential biochemical pathways. The use of microorganisms is a valuable tool in the identification of possible mammalian metabolites and could shed some light on the metabolic pathways of flavonoids (Mikell and Khan 2012).

Cannabis sativa L., a plant originating in Central Asia, is cultivated worldwide as a source of fiber, energy, food and medicinal or narcotic preparations. Several flavonoids have been isolated from cannabis featuring seven chemical structures. Cannflavin A (**33**), a methylated isoprenoid flavone, was the first aglycone flavonoid uniquely isolated from cannabis, with strong antileishmanial activity. Cannflavin A (**33**) metabolites produced by *Mucor ramannianus* ATCC 9628 were reported by Ibrahim et al. The enzymatic reactions included hydroxylation, *O*-glycosylation and sulfate conjugation, as well as combinations of these transformations. The metabolites isolated showed *O*-glycosylation at C-4′, while sulfate conjugation occurred at the C-7 hydroxy (Ibrahim et al. 2010).

Microbial transformation using *M. ramannianus* ATCC 9628 of 7-hydroxyflavanone (**34**), a flavonoid associated with biological activities including antifungal effects, 20S proteasome inhibitory activity, and aromatase inhibitor properties, was reported by Mikell

Fig. 6 Flavonoids biotransformed by *Mucor* spp. and some metabolites



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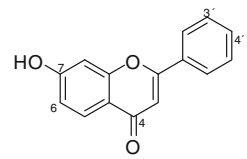

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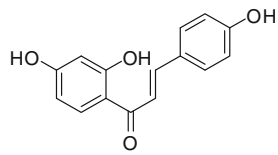

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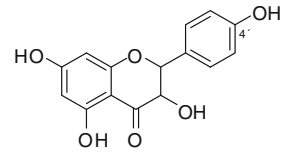

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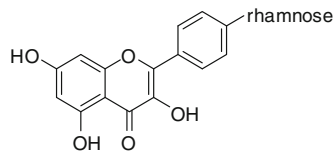

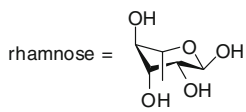

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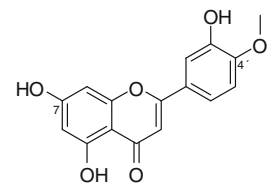

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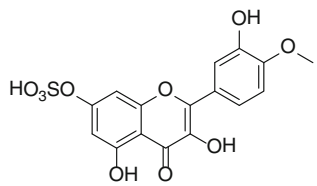

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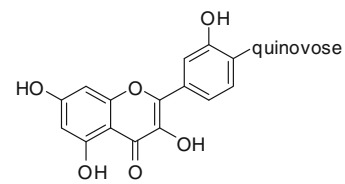

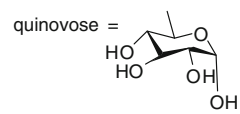

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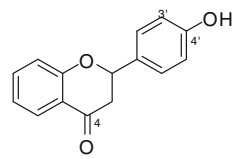

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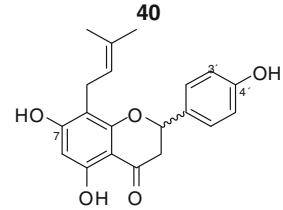

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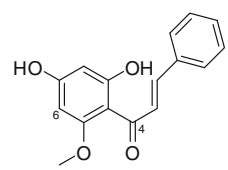

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and Khan as part of their ongoing examination of flavonoid metabolism. The array of reactions observed with **34** included hydroxylations at C-3′ and C-4′, methylation at C-6, sulfation and *O*-glycosylation at C-7. In addition, 7-hydroxyflavanone (**34**) was transformed by the opening of the C-ring in 4,2′,4′-trihydroxychalcone (**35**) (Mikell and Khan 2012).

Nerium oleander L. is a plant with cardiotonic, antibacterial, anticancer, and anti platelet aggregation activity and depresses the central nervous system. Kaempferol (**36**) is a flavonol isolated from *N. oleander* L. and from a wide range of medicinal herbs. This compound possesses a wide spectrum of bioactivity, is one of the most potent antioxidant drugs isolated from natural sources and also exhibited antiatherogenic, anti-inflammatory and antidepressant activity. When evaluating the safety and effectiveness of a drug, it is important to know how the drug is metabolized. Incubation of kaempferol (**36**) with *M. ramannianus* ATCC 9628 led to the isolation of one metabolite identified as kaempferol 4′-*O*-α-*L*-rhamnopyranoside (**37**) (Ibrahim et al. 2008).

Hesperetin (**38**) is the most commonly consumed flavonoid accounting for about 30 % of the total intake. Biological activities of **38** include antioxidant, bone-sparing and lipid-lowering effects. Its antioxidant properties together with the ability to penetrate the blood–brain barrier is suggested to help in neuroprotection against oxidative damage. Hesperetin (**38**) also plays a significant role in inflammation and cancer inhibition. Since microorganisms can be used as predictive models for mammalian drug metabolism, the microbial transformation of hesperetin (**38**) was investigated to isolate and characterize metabolites which may help to predict its effect on mammalian systems. Investigation addressing the biotransformation of **38** by *M. ramannianus* ATCC 20129 reported the isolation of hesperetin 7-sulfate (**39**) and eriodictyol 4′-*O*-α-qinovopyranoside (**40**) as the prominent metabolites, sulfation and glycosylation proving to be the metabolism pathway of hesperetin (**38**) in fungi (Herath and Khan 2011).

4′-Hydroxyflavanone (**41**), a synthetic analogue of flavanones, is associated with biological activity including endothelium-independent full vasorelaxing effectiveness on rat aortic rings and aromatase inhibitor properties. It also decreases collagen concentration in human dermal fibroblasts and exhibits moderate binding ability to the rat uterine estrogen receptor. Its metabolic transformation by rat liver microsomes resulted in the formation of 3′,4′-dihydroxyflavanone as the major metabolite, which was consistent with the observation that flavonoids with no hydroxyl groups in the B-ring, or those that had one at the 4́ position, are converted to the corresponding catechol by microsomal enzymes. Mikell et al. subjected 4′-hydroxyflavanone (**41**) to microbial transformation with *M. ramannianus* ATCC 9628 in view of low metabolite yields in previous experiments preventing complete structure elucidation and to obtain transformed products with enhanced biological properties. The formation of C-4 carbonyl group reduction compounds showed metabolic pathways similar to the ones observed in the in vitro biotransformations by mammalian microsomes (Mikell et al. 2011).

The female flowers of the hop plant (*Humulus lupulus* L.) have been used as a flavoring ingredient in brewing processes. Several biological activities and chemical compositions of prenylflavonoids in hops have received much attention in recent years. Of them, (±)-8-prenylnaringenin (**42**) was identified as the most potent phyto-estrogenic substance in hops, with an activity level comparable to or greater than that of other established plant estrogens. Subsequent in vitro and in vivo bioassays showed the detailed estrogenic activity of this compound and its possible use in treating menopausal symptoms. Investigation on the in vitro metabolism of (±)-8-prenylnaringenin (**42**) by human liver microsomes showed that most metabolites were produced by oxidation on two terminal methyl groups, a double bond on the prenyl group and C-3′ position on the B ring of the flavanone skeleton. In addition, its glucuronide and sulfate conjugates at C-7 and C-4′ were identified in the models of hepatic and intestinal metabolism in vitro using human hepatocytes. Kim et al. (2008) reported the biotransformation of (±)-8-prenylnaringenin (**42**) by *M. hiemalis* KCTC 6165 and the metabolites were identified as 8-prenylnaringenin 7-*O*-β-*D*-glucopyranosides.

The microbial transformation of 7,8-dimethoxyflavone by *M. ramannianus* produced metabolites hydroxylated at C-3′, C-4′ and C-7 (Herath et al. 2009). The study of microbial glycosylation of cardamonin (**43**) by *M. spinosus* CGMCC 3.3450 resulted in the isolation of two new products. Their structures were elucidated and 4-*O*-β-*D*-glucopyranosyl and

6-*O*-β-*D*-glucopyranosyl groups were assigned (Xu et al. 2011).

Osthole (**44**) is one of the major active components of *Cnidium monnieri* (L.) Cuss, with an isopentenoxy-coumarin structure. Pharmacological studies have demonstrated that it exhibits a wide variety of bioactivities such as anti-osteoporosis, antiallergic, antibacterial, anti-cancer, anticonvulsant and antidiabetic activities. However, its poor solubility in water and poor oral availability has limited its clinical use. The biotransformation of osthole (**44**) by *M. spinosus* AS 3.3450 was studied in search of some new chemical entities of **44** in the biotransformation process and to improve its water-solubility and activity. In addition, the anti-osteoporosis activities of all of the biotransformed metabolites were also evaluated. The hydroxylation, dehydrogenation, demethylation and glycosylation reactions of **44** by *M. spinosus* AS 3.3450 were reported in this study. The results suggested that the dehydrogenation, demethylation and glycosylation reactions could decrease bioactivity. Hydroxylation at C-4′ could enhances anti-osteoporosis activity and water-solubility. Interestingly, the *Z*-configuration of the double-bond at C-2′ and C-3′ provided better bioactivity than *E*-configuration (Lv et al. 2012).

Mucor sp. MNP801 was used in the biotransformation study of another coumarin, the 6,7-dimethoxy-coumarin (scoparone, **45**), producing different derivatives (Wang et al. 2010).

Biotransformation of miscellaneous compounds

The conversion of aromatic molecules to nonaromatic compounds is a very difficult reaction to perform due to the stability of the aromatic ring thus requiring severe conditions and the use of chemical reagents and/or toxic, metal-derived components. Biotransformations, on the other hand, can be performed under very mild conditions and offer an environmentally friendly alternative in tune with the current tendency towards clean chemical technologies (Quintana and Dalton 1999).

In this sense, derivatives of hexa-hydronaphthoic acid were obtained by *M. griseocyanus* (Capek et al. 1962) and 2-hydroxybiphenyl was hydroxylated by *Mucor* spp. (Herber et al. 1969). Phenolic and nonphenolic *p*-cymene-related compounds were biostransformed by *M. rouxii*, *M. hiemalis* and *Mucor janssenii.* Reactions such as hydrolysis, *N*-demethylation, desacetylation and the production of glycosylated metabolites were identified (Moussa et al. 1997). Biotransformation of vanillic acid to vanillyl alcohol by several strains of Zygomycetes, such as *Mucor bacilliformis* and *M. plumbeus*, showed the presence of extracellular phenoloxidases in their enzymatic system (Seigle-Murandi et al. 1992).

Nitrobenzene and its chlorinated derivatives are important chemical intermediates in the manufacture of dyes, agricultural chemicals, pharmaceuticals and industrial agents. Exposure to chloronitrobenzene occurs primarily in industrial settings, but environmental exposure can occur where chloronitrobenzenes are present in waste water and rivers and through accidental spills. In addition, chloronitrobenzenes can be formed in the environment during the biotransformation of chloroanilines. Chloronitrobenzenes are hematotoxic, splenotoxic, hepatotoxic and immunotoxic (Hong et al. 2002). Several chlorinated nitrobenzenes were biotransformed by *Mucor javanicus* and the main metabolites were the corresponding dichloroanilines (Hafsah et al. 1984, 1987a, b).

Aromatic hydrocarbons are commonly found in nature as components of fossil fuels. There is evidence that some fungal species metabolize aromatic hydrocarbons to metabolites identical to those formed by mammalian enzymes. Thus, Cerniglia et al. used naphthalene as a model substrate to survey a wide taxonomic and phylogenetic spectrum of fungi in order to determine whether the capacity to metabolize aromatic hydrocarbons is ubiquitous among fungi. In this work, all organisms tested from the Mucorales order oxidized naphthalene with *Mucor* species showing the greatest activity. The predominant metabolite formed by most organisms was naphth-1-ol. Other products identified were *trans*-hydroxylated derivatives (Cerniglia et al. 1978).

(*R*)-Phenylacetylcarbinol or (*R*)-1-hydroxy-1-phenylpropan-2-one is a chiral intermediate in the production of the pharmaceutical compounds ephedrine and pseudoephedrine and is currently produced industrially via a biotransformation of benzaldehyde by fermenting yeast cultures. In the literature, *M. circinelloides* was reported to carry out acyloin condensations with acyclic unsaturated aldehydes but its ability to catalyze benzaldehyde conversion was not investigated. Thus, strains from the *Mucor*

genera were included in a screening for (*R*)-phenylacetylcarbinol formation from benzaldehyde. The best initial productivities of (*R*)-phenylacetylcarbinol were achieved with extracts of *M. rouxii*, indicating the potential for a rapid biotransformation process (Rosche et al. 2001).

Mucor spp. is known to have enzyme systems with high proteolytic and lipolytic activity and is used in traditional fermentation of sufu and lobster sauce. Thus, Ma et al. used a strain of *Mucor* isolated from nature in biotransformations of cinnamaldehyde, cinnamic acid and acetophenone in order to study the characteristics of the reactions and develop novel applications for this fungus. Cinnamaldehyde and acetophenone could be reduced to their corresponding alcohols by *Mucor* sp. JX23, whereas cinnamic acid was biotransformed to acetophenone with α,β-oxidation in the form of degradation instead of being reduced to its corresponding alcohol. Previous reports showed that the reduction of non-activated carboxyl acids to alcohols is catalyzed by at least two enzymes. First, acids are reduced to aldehydes by reversible aldehyde oxidoreductases. Second, the reduction of aldehydes to alcohols is catalyzed by alcohol dehydrogenases. Thus, Ma et al. (2011) inferred that *Mucor* sp. JX23 contained alcohol dehydrogenases but did not contain aldehydes oxidoreductases.

The ability of *M. hiemalis* to oxidize biphenyl was studied and compared to mammalian systems. The monohydroxylation of biphenyl occurred at three positions to produce 2-hydroxy, 3-hydroxy and 4-hydroxy derivatives, the last being the main metabolite. 4-Hydroxybiphenyl was also the main metabolite formed by pig liver microsomes. 2-Hydroxybiphenyl used as substrate was further metabolized by *M. hiemalis* into 2,5-dihydroxybiphenyl, which is also present in rat urine after in vivo administration of 2-hydroxybiphenyl. Moreover the fungal cultures were also able to produce a phase II metabolite: biphenyl-*O*-glucoside. There was ample evidence to indicate that *M. hiemalis* was able to oxidize biphenyl in a similar pathway to that observed in mammals and by similar mechanisms (Decolin et al. 1985).

The biotransformation of anthraquinones was investigated to study their structural modification. *M. spinosus* was used in the biotransformation of three anthraquinones and glucosylated metabolites were obtained. When these anthraquinones were incubated with *M. spinosus* culture containing galactose as the carbon source instead of glucose, the corresponding product was a mixture of the metabolites with the glucopyranoside group rather than the expected galactose glycoside. Thus, glycosylation was influenced by the addition of different carbon sources to the medium (Zhang et al. 2003).

Thebaine (**46**) is an isoquinoline alkaloid which is very often used as a starting material to synthesize various morphine agonists as well as antagonists. One of the steps involved in their preparation is the *N*-dealkylation reaction and the microbial process could certainly offer an alternative approach. In this connection, northebaine (**47**) was identified as the major metabolite formed during the biotransformation of thebaine (**46**) by *M. piriformis* (Madyastha 1994; Madyastha et al. 2000).

Sorbic acid, an $\alpha\beta,\gamma\delta$-unsaturated carboxylic acid, has a strong antimicrobial effect and is widely used as a food preservative. This compound was used in the biotransformation experiment as a handy substrate to study the microbial metabolism of carboxylic acids containing conjugated double bonds. The sorbic acid was converted to *trans*-4-hexenol by *Mucor* sp. A-73. Investigation of the biological reduction capacity of this fungus showed that *Mucor* sp. A-73 performed two independent reductions: (1) transformation of the carboxyl group to alcohol and (2) of α,*β*-unsaturated alcohol to α,*β*-saturated alcohol. Furthermore, α,*β*-unsaturated alcohols were temporarily detected in the course of fungal reductions of some α,*β*-unsaturated acids. This suggests that the reduction of α,*β*-unsaturated acids to α,*β*-saturated alcohols was initiated by reaction (1) and followed by (2) (Kurogochi et al. 1974, 1975). Other experiments were carried out in the wake of these studies to clarify the metabolism of long-chain *trans*-2-alkenoic acids by *Mucor* sp. A-73 resulting in the isolation and identification of the neutral and methylated acid metabolites (Tahara et al. 1977).

Biotransformation of drugs

Biotransformation of antibiotics

The antimicrobial spectrum of 6-*O*-methylerythromycin A (**48**, clarithromycin), a macrolide antibiotic, is similar to that of erythromycin A. Furthermore, it exhibits notably higher activity in vivo than erythromycin A due to its improved acid stability and

pharmacokinetic characteristics. In phase I of the clinical trials with 6-*O*-methylerythromycin A (**48**), an active major metabolite was isolated and identified as its 14*R*-hydroxy derivative, which was found in human urine. Owing to the apparent difficulty involved in performing partial chemical synthesis to obtain this derivative by introduction of a hydroxyl group at C-14, Sasaki et al. experimented with the microbial transformation of 6-*O*-methylerythromycin A (**48**). The study described the microbial transformation of **48** and of the related compound 6-*O*-methylerythromycin B (**49**) by *M. circinelloides* f. *griseo-cyanus* IFO 4563 to their 14*R*-hydroxy derivatives, and also reported the antibacterial activity of these macrolide antibiotics and their transformation products. Analysis of the antibacterial activity assays suggested that hydroxylation of 6-*O*-methylerythromycins A (**48**) and B (**49**) at C-14 barely reduced their in vitro activity (Sasaki et al. 1988). Clarithromycin (**48**) also was used in the biotransformation studies using *M. circinelloides* whereby 14-, 15- and 16-hydroxyclarithromycins were obtained. It was interesting to note that the carbons near the lactone group were preferentially hydroxylated by this microorganism (Adachi et al. 1989).

In the studies with clarithromycin (**48**), there was reported that *M. circinelloides* generated the obtention of hydroxylated derivatives in three different positions, while the biotransformation of the same substrate by *M. circinelloides* f. *griseo-cyanus* IFO 4563 showed only 14*R*-hydroxy derivatives (Fig. 7).

Fluoroquinolones are synthetic antimicrobial agents which are active against a broad spectrum of pathogenic Gram-negative bacteria and some Gram-positive bacteria and mycoplasmas. These drugs work by inhibiting DNA gyrase and topoisomerase IV in bacteria. Fluoroquinolones in the environment often persist nearly intact for long periods of time, perhaps by binding to organic matter. However, a few studies have shown the capacity of microorganisms to transform some fluoroquinolones (Parshikov et al. 1999). Ciprofloxacin (**50**), enrofloxacin (**51**) and sarafloxacin (**52**) are fluoroquinolone-series drugs widely used as antimicrobial remedies for veterinary use (Fig. 8). The need for information about the environmental effects caused by the pervasive use of fluoroquinolones prompted Parshikov et al. to investigate the metabolism of ciprofloxacin (**50**), enrofloxacin (**51**) and sarafloxacin (**52**) using a common soil fungus, *M.*

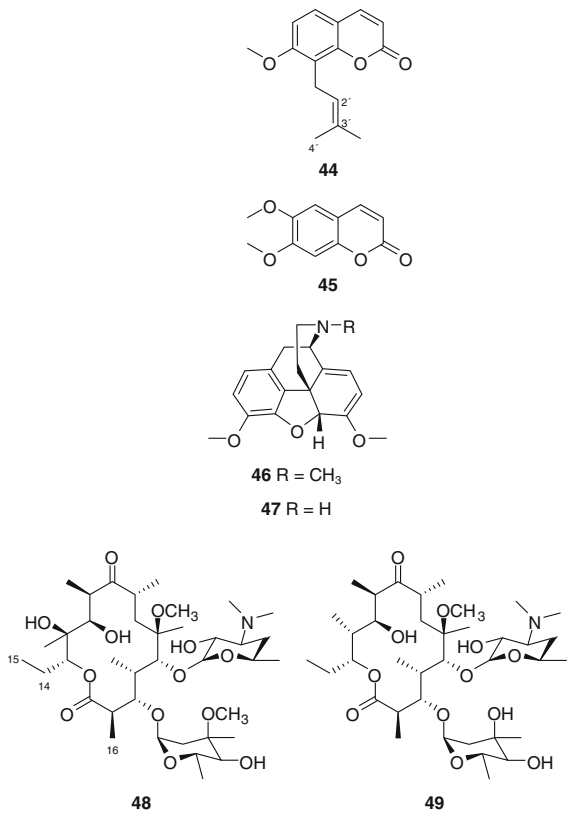

Fig. 7 Structures of 6-*O*-methylerythromycin A (**48**) and B (**49**)

ramannianus R-56. The results showed that *M. ramannianus* transformed **50** by means of a regiospecific pathway to a single metabolite, *N*-acetylciprofloxacin (Parshikov et al. 1999). Three metabolites were identified from the biotransformation of enrofloxacin (**51**): enrofloxacin *N*-oxide, *N*-acetylciprofloxacin and desethylene-enrofloxacin (Parshikov et al. 2000). Some reports showed that sarafloxacin (**52**) was metabolized to a glucuronide by mice, rabbits, and dogs; to *N*-acetylsarafloxacin by mice and rabbits; to 3′-oxosarafloxacin by rabbits and humans; and to an ethylene diamine-substituted quinolone and an aminoquinonlone by humans. Parshikov et al. (2001) showed that *M. ramannianus* R-56 was able to biotransform sarafloxacin (**52**) to *N*-acetylsarafloxacin and desethylene-*N*-acetylsarafloxacin.

Biotransformation of other drugs

Naftazone (**53**) is an orally active drug which protects the vascular system. It inhibits the activity of the

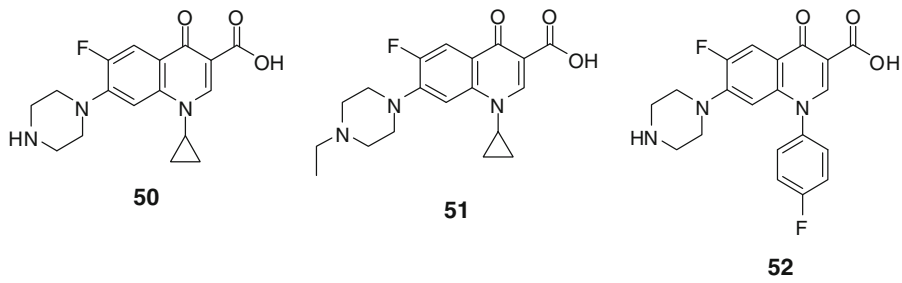

Fig. 8 Veterinary fluoroquinolones used in biotransformations by *M. ramannianus* R-56

component forms of nitric oxide synthases in rat endothelial cells. Nitric oxide (NO) synthases catalyze the formation of nitric oxide, an efficient endothelium-derivated relaxing factor. Nitric oxide is also involved in other physiological processes including neurotransmission, cytotoxicity and cell-mediated defense. Pharmacokinetic studies on naftazone (**53**) have shown that this compound is metabolized in rats and humans mainly by reduction and glycoconjugation reactions. A glucuronide derivative was identified in the blood and urine of rats treated with naftazone (**53**), and was also obtained by incubating **53** with rat and human liver microsomes in vitro. In 1995, Ouzzani et al. reported the biotransformation of naftazone (**53**) by *M. plumbeus* LCM which converted this substrate into compound **54** (Fig. 9). Additionally, the fungal metabolite of naphtazone (**53**) was assayed for the induction and activity of the inducible form of nitric oxide synthase in activated murine peritoneal macrophages. The authors concluded that compound **54** was able to inhibit both the induction and the activity of NO synthase in activated murine peritoneal macrophages (Ouazzani et al. 1995).

A family of non-peptide orally active angiotensin II receptor antagonists has been successfully developed for the treatment of hypertension. The human metabolism of this drug family mainly involves the *N*-glucuronidation of one of the tetrazole nitrogens and/or the *C*-hydroxylation of alkyl substituents. A systematic evaluation of these metabolic products has shown similar or increased antihypertensive activities. Irbesartan (**55**), a highly selective and potent drug of the same family, was metabolized in animals and humans to give several urinary metabolites. Exhaustive screening of fungal and microbial strains led to high yields of some of the animal metabolites of irbesartan (**55**) enabling researchers to

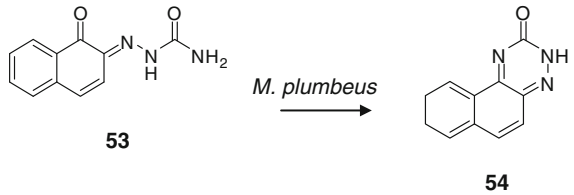

Fig. 9 Biotransformation of naftazone (**53**) by *M. plumbeus*

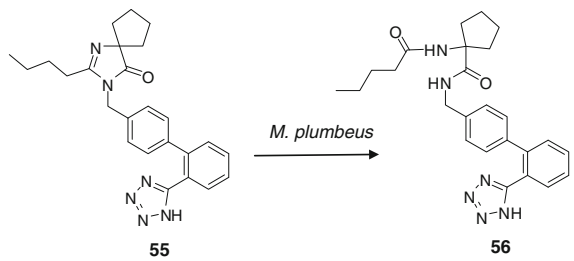

Fig. 10 Hydrolysis of irbesartan (**55**) by *M. plumbeus* CBS 110-16

elucidate their structural and stereochemical characteristics. In this screening, small amounts of metabolites were detected in fungal incubations. In the biotransformation of **55** by *M. plumbeus* CBS 110-16, a hydrolyzed metabolite (**56**) was detected (Fig. 10; Alexandre et al. 2004).

Daunomycin (**57**) is an antitumor agent which has been found to be remarkably effective in the treatment of leukemias and solid tumors. However, the search continues for improved drugs both in terms of their effectiveness and cardiotoxicity, the latter of which severely limits the utility of **57**. To this end, daunomycin (**57**) was metabolized by *M. spinosus* similar to the way it is metabolized by mammalian cells. The reaction found was the reduction of the ketonic carbonyl group at C-13 to a hydroxyl group (Marshall et al. 1978; Fig. 11).

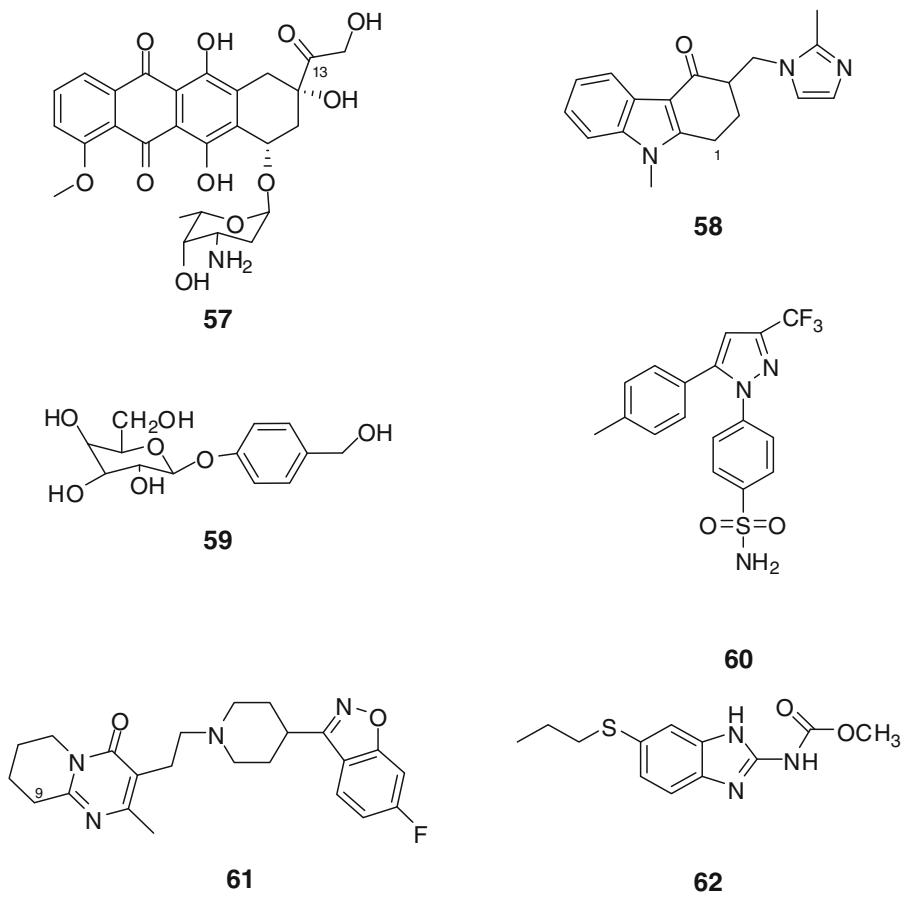

Fig. 11 Several drugs used in biotransformations by *Mucor* spp

Ondansetron (**58**) is a potent selective serotonin 5-hydroxytryptamine-type receptor antagonist which has a role in the prophylaxis of postoperative chemotherapy/radiotherapy induced emesis. Hydroxylation of the benzene ring followed by glucuronide or sulfate conjugation is a major route of metabolism, whereas *N*-demethylation is a minor route of metabolism in rats and humans. Study of the biotransformation of ondansetron (**58**) by *M. circinelloides* AS 3.3421, revealed that this fungus was able to hydroxylate ondansetron (**58**) at C-1 giving two diastereoisomers (Duan et al. 2006).

Gastrodia elata Blume belongs to the family of Orchidaceae and it can improve circulation and is usually prescribed for rheumatism, paralysis, hemiplegia, lumbago, headache, vertigo, tetanus and convulsant diseases. Gastrodin (**59**) has been detected as the major active ingredient in the herb *G. elata* Blume, and pharmacological tests show that it has a sedative, anaesthesic and neuroprotective effect, improves memory, prevents convulsions and acts as an antioxidant and free radical scavenger (Zhang et al. 2008). Microbial transformation of gastrodin (**59**) by *M. spinosus* 3.3450 resulted in a product identified as *p*-hydroxybenzyl alcohol which has a transformation rate of close to 100 % (Zhan et al. 2001).

Celecoxib (**60**), a non-steroidal anti-inflammatory drug, is the first specific cyclooxygenase-2 (COX-2) inhibitor approved by the US Food and Drug Administration (FDA) for the treatment of osteoarthritis, rheumatoid arthritis, and familial adenomatous polyposis. The selective inhibition of COX-2 by celecoxib (**60**) is believed to reduce discomfort in the upper gastrointestinal tract which is mediated by COX-1 through inhibition by conventional non-steroidal anti-inflammatory drugs. Celecoxib (**60**) is extensively metabolized in humans and rats to produce major metabolites by methyl hydroxylation and their

subsequent conversion to carboxylic acid. The metabolic pathway involves oxidation of the methyl group to produce a hydroxymethyl metabolite, which is then converted to carboxylic acid. Since the drug celecoxib (**60**) is lipophilic in nature, it should be eliminated mainly by metabolism, and thus the importance of studying metabolic pathways. In this regard, Srisailam and Veeresham studied the metabolites of celecoxib (**60**) formed by several microorganisms and compared them with those produced in mammals. In the biotransformation of celecoxib (**60**) by *M. rouxii* MTCC 386, a hydroxymethyl metabolite was detected where the hydroxylation took place on the methyl group of 5-(4-methyl) phenyl moiety (Srisailam and Veeresham 2010).

Risperidone (**61**) is a benzisoxazole derivative class of an atypical neuroleptic agent. Studies have shown that **61** has fewer side effects compared with traditional antipsychotic drugs. Risperidone (**61**) selectively antagonizes dopamine and serotonin receptors and has a lower propensity to induce extrapyramidal side effects at therapeutic dose levels. It is effective in treating schizophrenia and other psychiatric illnesses in adults and children, including pervasive developmental disorders, autism and attention deficit disorder. Risperidone (**61**) is metabolized mainly by the liver with an efficiency rate of >99 %. Once metabolized, risperidone (**61**) yields two metabolites, 7-hydroxyrisperidone and 9-hydroxyrisperidone. As it is known that microbial transformations of compounds play a vital role in the preparation of new derivatives with biological activities, a study was conducted on risperidone (**61**) biotransformation by *M. rouxii* NRRL 1894 which showed that this fungus was able to enantioselectively metabolize risperidone (**61**) into its chiral active metabolite, (−)-9-hydroxyrisperidone, with high enantiomeric excess (Jesus et al. 2011).

Chiral interaction is often a typical attribute of enzymatic reactions, messenger-receptor interactions and metabolic activities. There are several drugs currently being marketed as racemic mixtures, however the pharmacokinetic and pharmacodynamic profiles of the enantiomers have proved this wrong. Albendazole (**62**, ABZ) is a wide spectrum antihelminthic prochiral drug and its effectiveness is mostly due to its sulfoxide metabolite, albendazole sulfoxide (ABZSOX). **62** is oxidized to the active and chiral ABZSOX metabolite in the human liver by flavin monooxygenases, mostly by the cytochrome CYP3A4. ABZSOX is then oxidized to the inactive albendazole sulfone ($ABZSO_2$) metabolite. Today, ABZSOX is marketed as a racemic mixture under the generic name ricobendazole. It was in this context that Hilário et al. developed a chiral HPLC method using the polar organic mode to analyze albendazole (**62**) and its metabolites following stereoselective fungal biotransformation. Under the conditions studied, the fungus *M. roxii* NRRL 1894 was able to biotransform albendazole (**62**) to ABZSOX, however the formation of $ABZSO_2$ was not observed. Additionally, *M. roxii* NRRL 1894 may be an excellent alternative to obtain the (+)-ABZSOX enantiomer with good biotransformation efficiency (Hilário et al. 2012).

Biotransformation of pesticides

In the last few decades, a number of compounds used as pesticides or biocides have been released into the environment or inadequately disposed of and this has led to world-wide contamination of ecosystems. The use of microorganisms to transform these potentially toxic compounds into friendly ones, a process called bioremediation, has received much recent attention (Lièvremont et al. 1996).

DDD (*l*,*l*-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and DDE (*l*,*l*-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) are formed from DDT (*l*,*l*,*l*-trichloro-2,2-bis(*p*-chlorophenyl)ethane) in soil, water, plants, and animals. Although these compounds can be generated by photochemical reactions, microorganisms are apparently important in their formation in certain ecosystems. Anderson et al. (1970) observed that *Mucor alternans* converted DDT to water-soluble compounds and hexane-soluble products. Because the water-soluble compounds may represent new groups of DDT metabolites generated by degradation pathways not yet characterized, a study was initiated of the possible synthesis of water-soluble products from DDT by marine microorganisms. Several water-soluble compounds generated from DDT by *M. alternans* were partially characterized and were found to be different from known DDT metabolism products (Juengst and Alexander 1976).

Many kinds of chloronitrobenzene- or chloroaniline-based pesticides are in use and it is believed that most are degraded in soil as a result of microbial action. Tahara et al. investigated the metabolism of

2,4-dichloro-1-nitrobenzene by *M. javanicus* AHU 6010. In addition to the corresponding benzenamine derivative, two other metabolites were detected. It was suggested that two biological reactions, namely reduction of the nitro group and/or substitution of the *ortho*-chlorine atom by a methylthio group, were responsible for the formation of these metabolites (Tahara et al. 1981).

Nitroaromatic compounds such as pentachloronitrobenzene (PCNB) have been produced industrially on a massive scale. Most are highly resistant to degradation. They are mainly used as intermediates in the synthesis of a wide range of industrial chemicals including explosives, biocides and dyes. Lièvremont et al. conducted a study to determine whether *M. racemosus* could degrade PCNB at concentrations far greater than those typically encountered in the environment (100 mg L^{-1}), and to evaluate the ability of the fungus to eventually adsorb this chemical. Since biodegradation can lead to hazardous metabolites, sorption by fungal biomass may represent a potential and efficient removal mechanism. *M. racemosus* cultures demonstrated high PCNB-transforming ability (78–81 %) (Lièvremonr et al. 1996).

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methyl carbamate), a class of *N*-methyl carbamate pesticides, is highly toxic and an inhibitor of acetylcholinesterase, an enzyme that is vital for the operation of the central nervous system. As a consequence of extensive and diverse use and unintentional discharge into the environment, carbofuran has become one of the most frequently detected pesticides in water resources. Accumulation of carbofuran and its metabolites in the environment could potentially pose health hazards. This prompted Seo et al. to study the biodegradation of carbofuran and carbofuran phenol by *M. ramannianus*, a common soil fungus, to better understand the role of fungi in the degradation of these compounds. The molecular structures of the metabolites were not identified, although the results, which were the first reports of the metabolism of carbofuran phenol by fungus, will provide valid information for the environmental risk assessment related with pesticide carbofuran (Seo et al. 2007).

Pentachlorophenol (PCP) is a xenobiotic of great environmental concern. It was commonly applied for many years as a bactericide, fungicide, defoliant, herbicide, wood preservative and detergent supplement in soaps. This biocide is slightly soluble in water and very resistant to biotic and abiotic attack, which leads to a constant increase in its concentration in soil, water sediments and living organisms. Szewczyk et al. set out to identify PCP biodegradation metabolites formed in *Mucor ramosissimus* IM 6203 cultures and optimize medium composition to enhance PCP removal in the presence of engine oil acting as a carbon source. Pentachlorophenol (PCP) to tetrachlorohydroquinone (TCHQ) transformation was the most interesting transformation performed by the strain tested (Szewczyk and Dlugonski 2009). The co- and direct-metabolism of PCP by *M. plumbeus* was also studied. Surprisingly *M. plumbeus* was able to directly metabolize PCP, leading to its complete depletion from media (Carvalho et al. 2009).

Therefore, it is a fact that several *Mucor* species are employed in the bioremediation, being able to biotransform efficiently pesticides or biocides. There were cases in that *Mucor* sp. accomplished the complete depletion of the pesticide from media.

Conclusions

Mucor genus is widespread in nature and some species are used extensively in biotechnology for enzyme and useful compound production. Biotransformation can be used as a very convenient way of producing compounds, particularly when the structure is complex and they can neither be isolated as metabolites nor chemically synthesized.

Moreover, *Mucor* species have the ability to metabolize a wide variety of substrates in ways that mirror mammalian enzyme systems, making them useful as in vitro models for the simulation of mammalian drug metabolism. The microbial simulation of mammalian metabolism also sheds light on the mechanism of action, toxicity, and pharmacological activity of the drugs and thus aids in the discovery of new drug molecules.

It is safe to conclude that biotransformation by *Mucor* species is of great importance due to their wide-ranging use in the stereospecific production of compounds of commercial interest, and because they facilitate the study of the metabolism of such compounds in order to obtain novel agents with many interesting biological activities.

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