Tetrahedron: *Asymmetry* 20 (2009) 385–397

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Tetrahedron: *Asymmetry* Report Number 107

Stereoselective biotransformations using fungi as biocatalysts

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ARTICLE INFO

Article history:
Received 25 November 2008
Accepted 10 February 2009
Available online 16 March 2009

ABSTRACT

The development of novel biocatalytic methods is a continuously growing area of chemistry, microbiology, and genetic engineering due to the fact that biocatalysts are selective, easy-to-handle, and environmentally friendly. A wide range of reactions are catalyzed by microorganisms. Fungi can be considered as a promising source of new biocatalysts, mainly for chiral reactions. Chemo-, regio-, and stereoselective processes are very important in the synthesis of many chemical, pharmaceutical, and agrochemical intermediates; active pharmaceuticals; and food ingredients. This report reviews stereoselective reactions mediated by fungi, such as stereoselective hydroxylation, sulfoxidation, epoxidation, Baeyer–Villiger oxidation, deracemization, and stereo- and enantioselective reduction of ketones, published between 2000 and 2007.

Contents

1. Introduction

Biocatalysis has become an increasingly valuable tool for the synthetic chemists. 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 the subject of intensive screening. Frequently, biotransformation reactions are chemo-, regio-, and stereoselective, producing a wide variety of fine chemicals, that is, intermediaries and/or drugs,[1] food ingredients, and agrochemical intermediates[2]. A particular compound is modified by transforming functional groups with or without degradation of the carbon skeleton. These modifications result in the formation of novel and useful products that are difficult or impossible to be obtained through conventional chemical procedures.

Biotransformation is an alternative tool with great potential, especially for the development of sustainable technologies for the production of chemicals and drugs, that is, green chemistry. However, the number and diversity of applications are still modest considering the great availability of useful microorganisms and the broad scope of reactions which they can trigger. Some limitations

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doi:10.1016/j.tetasy.2009.02.009

such as enzyme availability, substrate scope, and operational stability could be overcome by recent scientific progress in genomics, directed enzyme evolution, and the exploitation of biodiversity.[3] Furthermore, exploration of the planet's biodiversity aided by bioinformatics and high-throughput screening facilitates the discovery, optimization, and availability of enzymes and/or active cells customized to suit required process conditions.[4,5]

The use of whole microorganisms and/or their enzymatic systems alone to carry out stereospecific and stereoselective reactions has taken on greater significance. These reactions have proven useful in the asymmetric synthesis of molecules with important biological activities. Additionally, biotransformation reaction technology is deemed economically and ecologically competitive in the search for new compounds of use to the pharmaceutical and chemical industries.

This review covers the major stereoselective reactions mediated by fungi, such as stereoselective hydroxylation, sulfoxidation, epoxidation, Baeyer–Villiger oxidation, deracemization, and stereo- and enantioselective reduction of ketones, published over the period 2000–2007. For further information concerning fungi-triggered biotransformation prior to the year 2000, see the literature.[6]

2. Choice of microorganisms

Active biocatalysts have been obtained by screening a broad variety of microorganisms. Microorganisms are widespread throughout Nature, and there are many habitats that can be exploited in the search for new microbial species. Bioprospection for novel microorganisms from all biotopes found on our planet, including those featuring extreme environmental conditions, such as geothermal ecosystems and hydrothermal vents, hypersaline and supercooled sea ice, could also lead to the discovery of new enzymes able to catalyze various types of reactions.

Fungi have traditionally been one of the most studied whole cell systems for microbial natural product isolation and also for biotransformation reactions. The isolation of fungi from the environment has sparked the interest of researchers because it is estimated that only very few existing fungal species are actually known. The incidence of fungi in plants occurs by natural infection in the environment favored by humid climates, and their isolation can be considered as a first step in understanding the emergence of secondary metabolites in plants and the activation of specific enzymes in fungi.

Pathogenic and endophytic fungi deserve attention among the fungi present in plants because they may be promising sources of biocatalysts with numerous applications. The term endophytic fungi has many definitions in the literature,[7–9] and has been employed to describe those fungi that can be detected at a particular moment within apparently healthy plant host tissue. They can inhabit the tissue of living plants for all or part of their life cycle. The colonization can be inter- or intracellular, localized, or systemic.[10] Endophytes invade the tissue of living plants causing unapparent and asymptomatic infections.[7] Pathogenic fungi can cause disease by colonizing parts of animals and plants internally or externally. Pathogenic fungi may also exhibit long latency periods, that is, symptom-free occupation of host tissue.[11] The differences between pathogenic and endophytic fungi are not very clear, and there are fungi which are pathogenic to one plant species, but can live as mutualistic endophytes in another host. The distinction between pathogenicity and endophytic behavior may be determined by a single gene.[12,13]

Endophytic fungi are an unexplored or at least under-explored source for microbial biotransformations. Endophytes were mentioned for the first time at the beginning of the 19th century, but it was DeBary (1866)[14] who first pointed out the difference between endophytes and phytopathogens. However, it was only in last century, at the end of the 1970s, that endophytic fungi began to acquire importance. It was found that they could protect plants against attack from insects, diseases, and mammalian herbivores. In addition, they may produce metabolites that are same as those produced by the host plant, and can also be used in chemical and drug biotransformation processes.[15–22] An important example of this is the fungus *Taxomyces andreanea* found inside the plant *Taxus brevifolia* which produces taxol, a complex anticancer diterpenoid of great interest to the pharmaceutical industry.[15,23]

3. Biotransformation reactions

3.1. Stereoselective hydroxylation

The process of hydroxylation involves the direct oxidation of a C–H bond to produce an alcohol.[24] These reactions may take place at various points on the molecule, especially hydroxylations of non-activated centers that are difficult to be achieved using classical chemical methods. Microbial hydroxylations are very well studied in terpenoids.[25–79]

Terpenes are a large and widespread class of bioactive secondary metabolites used in the fragrance and flavor industries, and these are also useful as chiral synthons for chemical synthesis.[25] They tend to be characterized by high structural complexity, meaning that chemical synthesis or structural modifications demand reactions with stereo- and enantioselectivity.[25] Microbial transformations have proven to be an efficient alternative to chemical methods in the regio- and stereoselective functionalizations of terpenes, frequently giving rise to more biologically active products.

L-Menthol **1**, utilized in the fragrance industry, was used as the substrate for biotransformation by 12 *Rhizoctonia solani* strains. Three strains were capable of producing products **2** (65.2%), **3** (32.4%), and **4** (18.4%) (Fig. 1).[25]

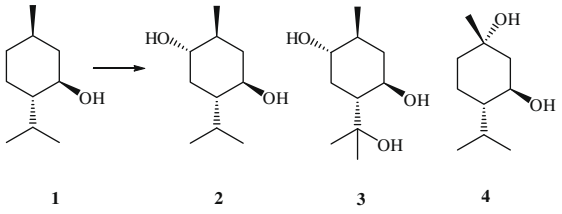


Figure 1. Biotransformation of L-menthol **1** by *Rhizoctonia solani* strains.

Other monoterpenes were biotransformed using fungi.[26–31] The monoterpene (−)-α-pinene **5** is the major constituent of many aromatic plants and is an important component of many essential oils. The biotransformation of **5** using *Botrytis cinerea* produced two compounds, **6** (10%) and **7** (16%) (Fig. 2).[26]

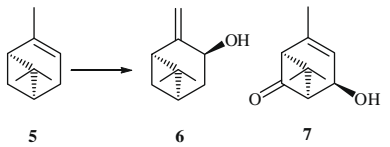


Figure 2. Biotransformation of (−)-α-pinene **5** by *Botrytis cinerea*.

Sesquiterpenes have been widely used as substrates in biotransformation; the products of these biotransformations are normally hydroxylated compounds.[32–57]

Squamulosone **8**, a sesquiterpene isolated in large quantities from the plant *Hyptis verticillata* Jacq., was incubated with *Curvu-*

laria lunata to yield stereoselectively hydroxylated analogues **9**–**11**. Furthermore, compound **12** was stereoselectively hydroxylated and epoxided. Compounds **8**–**11** were found to possess insecticidal activity, but, unfortunately, **12** was not isolated in quantities sufficient for bioassay (Fig. 3).[32]

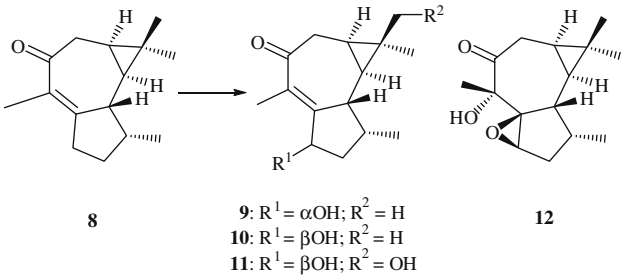


Figure 3. Biotransformation of squamulosone **8** by *Curvularia lunata*.

The biotransformation of nootkatone **13**, the grapefruit fragrance used in the cosmetic and fiber business, and valencene **18**, obtained inexpensively from Valencia oranges, was carried out using *Aspergillus niger*, *Fusarium culmorum*, and *Botryosphaeria dothidea*. Compound **13** was biotransformed by *A. niger* into stereomixtures of **14** and **15** (51.5% isolated yield), while *F. culmorum* biotransformed **13** to products **15** (47.2%) and **16** (14.9%). The fungus *B. dothidea* showed the best results for the stereoselective hydroxylation of **13,** yielding **14, 15** (54.2%) and **17** (20.9%).[33] The biotransformation of **18** by *A. niger* rendered compounds **14, 15** (13.5%), **19** (1.5%), and **20** (2.0%) (Fig. 4). These compounds exhibited no effective odor.[33]

The natural sesquiterpenoids patchoulol **21**, ginsenol **29**, and cedrol **34** exhibited antifungal activity against the phytopathogenic fungus *B. cinerea*. This fungus was able to biotransform **21** into **22**–**28** (Fig. 5), and **29** into **30**–**33** (Fig. 6) as a detoxification mechanism.[34] Compound **34** was also converted by *B. cinerea* into compounds **35**–**39**.[34] The incubation of **34** with *C. lunata* in PDB (potato dextrose broth) medium resulted in the production of three compounds **35**, **36** and **43**, and incubation in BEM (peptone, yeast extract, beef extract, and glucose in water) medium gave rise to a further five products **37**–**42** (Fig. 7).[35] None of the biotransformation products showed any significant antifungal activity. These results could contribute to the further development of selective antifungal compounds for the control of *B. cinerea*.

The biotransformation of four taxane diterpenoids by *Absidia coerula* caused highly regio- and stereoselective hydroxylation at the 1β and 9α positions.[58] Compound **44** was converted into 1β-hydroxylated metabolite **45** (58%) and 9α-hydroxylated derivative **46** (8%). Compounds **47**, **49**, and **51** were hydroxylated affording metabolites **48**, **50**, and **52**, respectively, but in low yields (Fig. 8). The 9α hydroxylation of taxoids had not been described previously, and these results indicate that the oxidases of *A. coerula* have high stereoselectivity. The most important member of this family is the diterpenoid paclitaxel, a drug used to treat cancer.[58]

Triptolide **53**, a diterpene triepoxide isolated from *Triptergium wilfordii*, has been shown to be effective in the treatment of autoimmune diseases and to have potent antileukemic and antitumor activities. It was submitted to biotransformation by the fungus

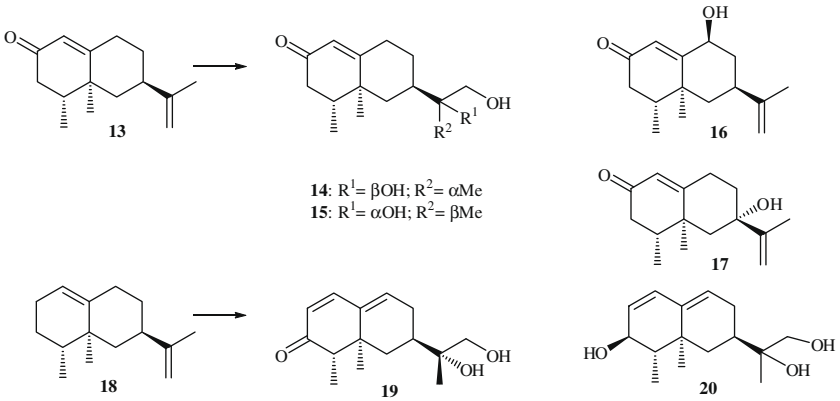


Figure 4. Biotransformation of nootkatone **13** and valencene **18** by *Aspergillus niger*, and biotransformation of nootkatone **13** by *Fusarium culmorum* and *Botryosphaeria dothidea*.

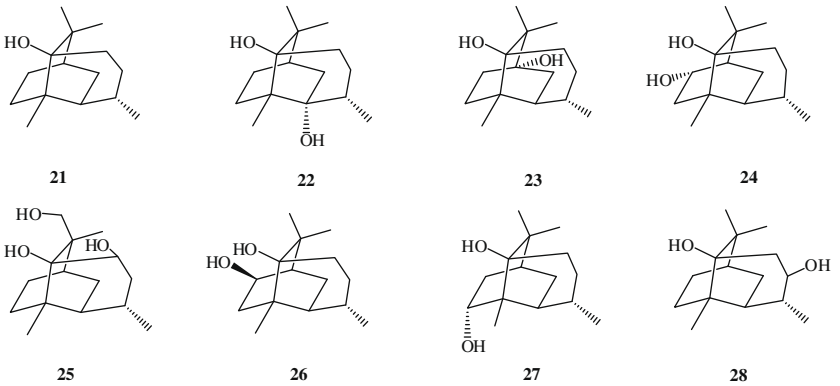


Figure 5. Biotransformation of patchoulol **21** by *Botrytis cinerea*.

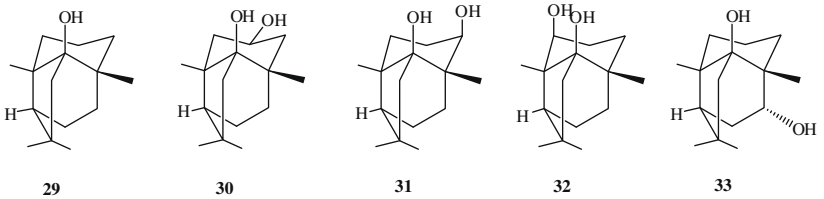


Figure 6. Biotransformation of ginsenol **29** by *Botrytis cinerea*.

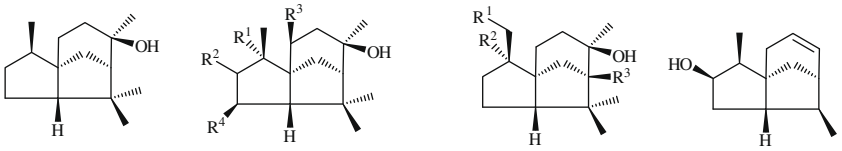


Figure 7. Biotransformation of cedrol **34** by *Botrytis cinerea* and *Curvularia lunata*.

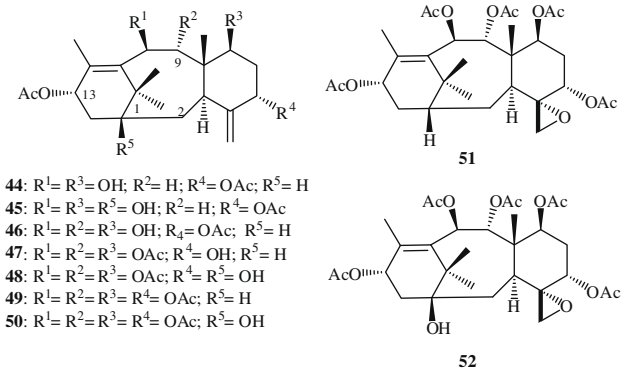


Figure 8. Biotransformation of four taxane skeleton products **44**, **47**, **49**, and **51** by *Absidia coerula*.

Cunninghamella blakesleana, yielding 5α-hydroxytriptolide **54**, 1β-hydroxytriptolide **55**, triptodiolide **56**, 19α-hydroxytriptolide **57**, and 19β-hydroxytriptolide **58** (Fig. 9). All the new biotransformed compounds exhibit potent in vitro cytotoxicity against human tumor cell lines KB, BGC_{823}, MCF-7, Hela, and HL-60.[59]

Stemodane diterpenoids are produced by plants from the *Stemodia* genus. These diterpenoids are attractive due to their structural similarity to aphidicolin, isolated from some fungal cultures and exhibiting antiviral and anticancer properties. The incubation of 13α,17-dihydroxy-stemodane **59** with *Mucor plumbeus* led to the isolation of eight hydroxylated metabolites **60**–**67**, while the incubation of 13α,14,dihydroxy-stemodane **68** rendered two hydroxylated products at positions 3β **69** and 2α **70**. Position C-3 is the one that is most frequently hydroxylated by this microorganism in diterpenoids, and it does not depend on the absolute configuration of the substrate (Fig. 10).[60] Stemodin **71**, stemodinone **75**, and stemarin **77** were biotransformed by *A. niger*.[61] Incubation of **71** with *A. niger* gave 2α,3β,13-trihydroxystemodane **72**, 2α, 7β,13-trihydroxystemodane **73**, and 2α,13,16β-trihydroxystemodane **74** (Fig. 11), while **75** was biotransformed to **76** (Fig. 12). The biotransformation of **77** gave rise to the new products 7β,18-dihydroxystemaran-19-oic acid **78**, 7α,18,19-trihydroxystemarane **79**, and 1β-hydroxystemaran-19-oic acid **80** (Fig. 13).[61]

The biotransformation of *ent*-manoyl oxides, labdane-type diterpenoids, led to hydroxylations at positions difficult to be achieved by other chemical means. The substrate *ent*-3β,12α-dihydroxy-13-*epi*-manoyl oxide **81** was incubated with *Fusarium moniliforme*, rendering the product *ent*-7β-hydroxylated **82** (35%) (Fig. 14). Chemical oxidation of **71** produced *ent*-3,12-dioxo-13-*epi*-manoyl oxide **83**, which was biotransformed stereoselectively by *Gliocladium roseum* to products **84** (19%) and **85** (7%). Incubation of **83** with *Rhizopus nigricans* gave rise to products **85** (4%), **86** (13%), and **87** (14%) (Fig. 15).[62]

Betulinic acid **88**, a triterpenoid found in many plants, and the closely related betulonic acid **89** have attracted attention because of their important pharmacological properties (anticancer and anti-HIV activities).[79] In order to obtain biologically active derivatives, both compounds **88** and **89** were biotransformed by the fungi *Colletotrichum* sp. and *Arthrobotrys* sp., respectively. *Colletotrichum* sp. (from corn leaves) biotransformed **89**–**92** (1.72%) and **93**

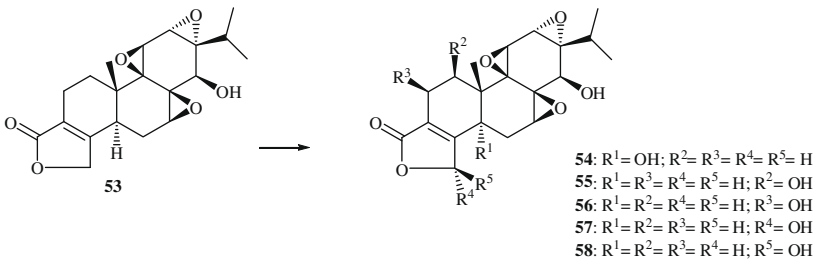
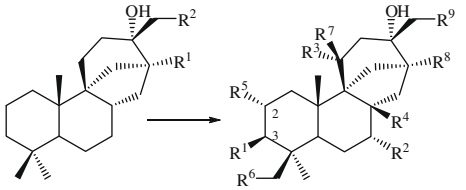


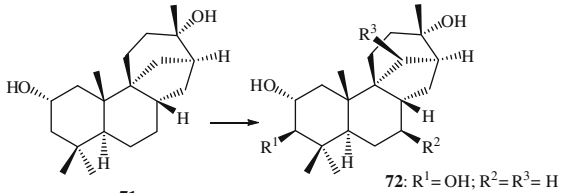
Figure 9. Biotransformation of triptolide **53** by *Cunninghamella blakesleana*.



59: R^1= H; R^2= OH
68: R^1= OH; R^2= H

60: R^1= R^9= OH; R^2= R^3= R^4= R^5= R^6= R^7= R^8= H
61: R^1= R^3= R^4= R^5= R^6= R^7= R^8= H; R^2= R^9= OH
62: R^1= R^3= R^9= OH; R^2= R^4= R^5= R^6= R^7= R^8= H
63: R^1= R^2= R^9= OH; R^3= R^4= R^5= R^6= R^7= R^8= H
64: R^1= R^4= R^9= OH; R^2= R^3= R^5= R^6= R^7= R^8= H
65: R^1= R^2= R^3= R^4= R^6= R^7= R^8= H; R^5= R^9= OH
66: R^1= R^2= R^3= R^4= R^7= R^8= H; R^5= R^6= R^9= OH
67: R^1= R^7= R^9= OH; R^2= R^3= R^4= R^5= R^6= R^8= H
69: R^1= R^8= OH; R^2= R^3= R^4= R^5= R^6= R^7= R^9= H
70: R^1= R^2= R^3= R^4= R^6= R^7= R^9= H; R^5= R^8= OH

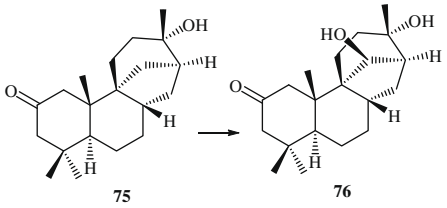
Figure 10. Biotransformation of 13α,17-dihydroxy-stemodane **59** and 13α,14,dihydroxy-stemodane **68** by *Mucor plumbeus*.



71

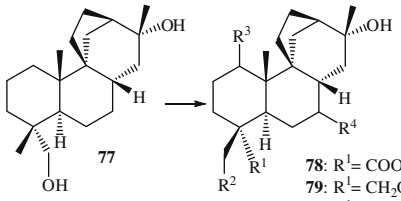
72: R^1= OH; R^2= R^3= H
73: R^1= R^3= H; R^2= OH
74: R^1= R^2= H; R^3= OH

Figure 11. Biotransformation of stemodin **71** by *Aspergillus niger*.



75 **76**

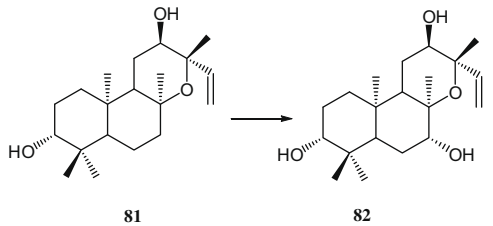
Figure 12. Biotransformation of stemodinone **75** by *Aspergillus niger*.



77

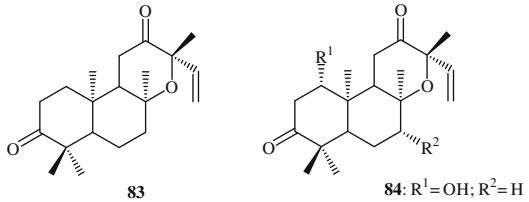
78: R^1= COOH; R^2= OH; R^3= H; R^4= βOH
79: R^1= CH_2OH, R^2= OH; R^3= H; R^4= αOH
80: R^1= COOH; R^2= R^4= H; R^3= βOH

Figure 13. Biotransformation of stemarin **77** by *Aspergillus niger*.

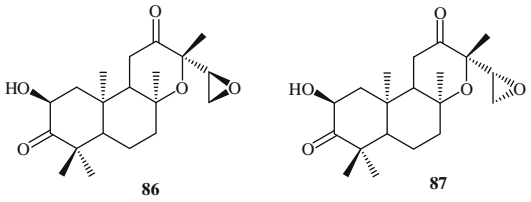


81 **82**

Figure 14. Biotransformation of *ent*-3β,12α-dihydroxy-13-*epi*-manoyl oxide **81** by *Fusarium moniliforme*.

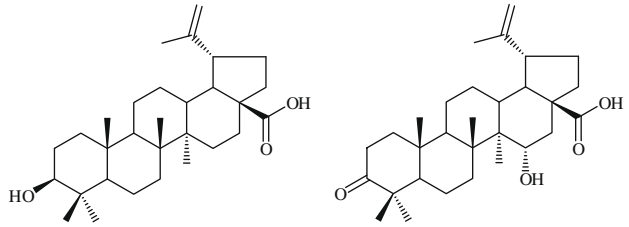


83 **84**: R^1= OH; R^2= H **85**: R^1= H; R^2= OH

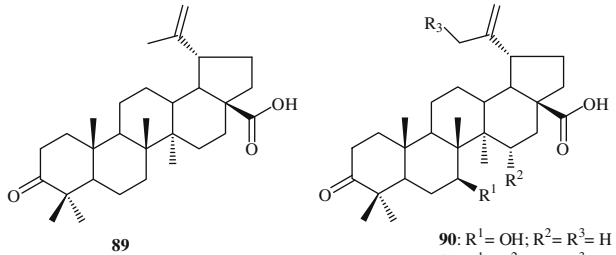


86 **87**

Figure 15. Biotransformation of ent-3,12-dioxo-13-*epi*-manoyl oxide **83** by *Gliocladium roseum* and *Rhizopus nigricans*.



88 **93**



89

90: R^1= OH; R^2= R^3= H
91: R^1= R^2= OH; R^3= H
92: R^1= R^3= OH, R^2= H

Figure 16. Biotransformation of betulonic acid **89** by *Arthrobotrys* sp., and biotransformation of betulinic acid **88** and betulonic acid **89** by *Colletotrichum* sp.

(2.97%), and converted **88**–**93** (2.34%). *Arthrobotrys* sp., isolated as an epiphytic fungus from *Platanus orientalis*, a plant producing betulinic acid derivatives, transformed **89** into 3-oxo-7β-hydroxylup-20(29)-en-28-oic acid (**90**, 1.64%), 3-oxo-7β-15α-dihydroxylup-20(29)-en-28-oic acid (**91**, 0.62%), and 3-oxo-7β,30-dihydroxylup-20(29)-en-28-oic acid (**92**, 1.33%). (Fig. 16).

Therefore, the biotransformation of plant-derived metabolites by microorganisms isolated from the plant hosts could increase the likelihood of obtaining novel natural product derivatives.[79]

Biotransformation studies have received more attention since the development of microbial hydroxylation of bioactive steroids or intermediary products for corticosteroid synthesis. The 11α-hydroxylation of progesterone in a single microbial step using *Rhizopus arrhizus* was described in 1952.[80] This reaction was very

important for the economic synthesis of adrenocortical hormones (corticosterone, cortisone, and hydrocortisone), and afforded interesting possibilities for the preparation of bioactive derivatives (prednisone, prednisolone, and triamcinolone).[81] The 11β-hydroxylation is also crucial for the biological activity of steroids. This reaction was first reported in 1953 using *Cunninghamella blakesleeana* and *C. lunata*. Cortexolone was hydroxylated at the 11β position producing hydrocortisone in a yield of 60–70% by *C. lunata*, higher yields being obtained when the substrates were acetylated at positions 17α and 21.[81]

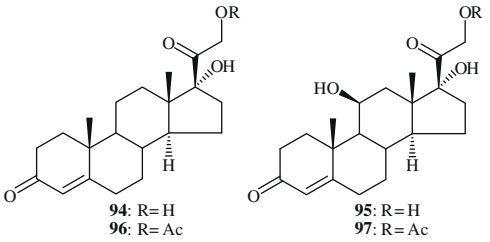


Figure 17. Biotransformation of cortexolone **94** to hydrocortisone **95** using four fungi (two isolates of *Cunninghamella blakesleana*, *C. echinulata*, and *Curvularia lunata*), and of cortexolone-21-acetate **96** to hydrocortisone acetate **97** using *Cunninghamella blakesleana*.

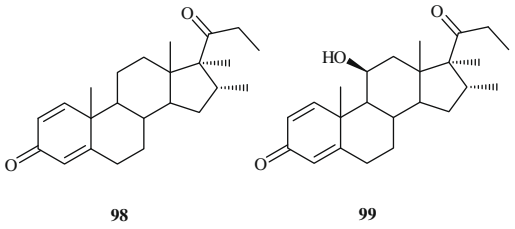


Figure 18. Biotransformation of 16α,17α-dimethyl-17-(1-oxopropyl)androsta-1,4-dien-3-one **98** by *Curvularia lunata*.

Other steroids have recently been biotransformed using fungi.[82–91] The 11β hydroxylation is a key structural factor for the bioactivity of steroidal drugs. This reaction has been achieved in the biotransformation of different steroidal substrates by some fungi. Cortexolone **94** was converted to hydrocortisone **95** by four fungal strains (two isolates of *C. blakesleana*, *C. echinulata*, and *C. lunata*).[82] Cortexolone-21-acetate **96** was also biotransformed to hydrocortisone acetate **97** (Fig. 17) using *C. blakesleana* ATCC 8688a[83], and 16α,17α-dimethyl-17-(1-oxopropyl)androsta-1,4-dien-3-one **98** was biotransformed to rimexolone **99** using *C. lunata* (Fig. 18).[84]

Resibufogenin **100**, a cytotoxic steroid, showed strong inhibitory activities against human hepatoma Bel-7402 cells, human gastric cancer BGC-823 cells, and human cervical carcinoma HeLa cells, with IC_{50} values of 0.13, 0.11, and 0.01 μmol/L, respectively. In an ongoing effort to obtain novel bufadienolide analogues with more potent cytotoxicity, **100** was biotransformed by *Mucor polymorphosporus* affording 20 products **101**–**120** (Fig. 19). All these products showed less cytotoxicity in comparison with **100**, but these results could contribute to structure–activity relationship studies for the design of novel bufadienolides of pharmaceutical interest.[85]

Several other substrates besides terpenes have been stereoselectively biotransformed by fungi.[92–105] (*R*)-(−)-Methyloctalone **121** and (*S*)-(+)-methyloctalone **122** were subjected to biotransformation by *Chaetomium* sp. and *Didymosphaeria igniaria*. *Chaetomi-*

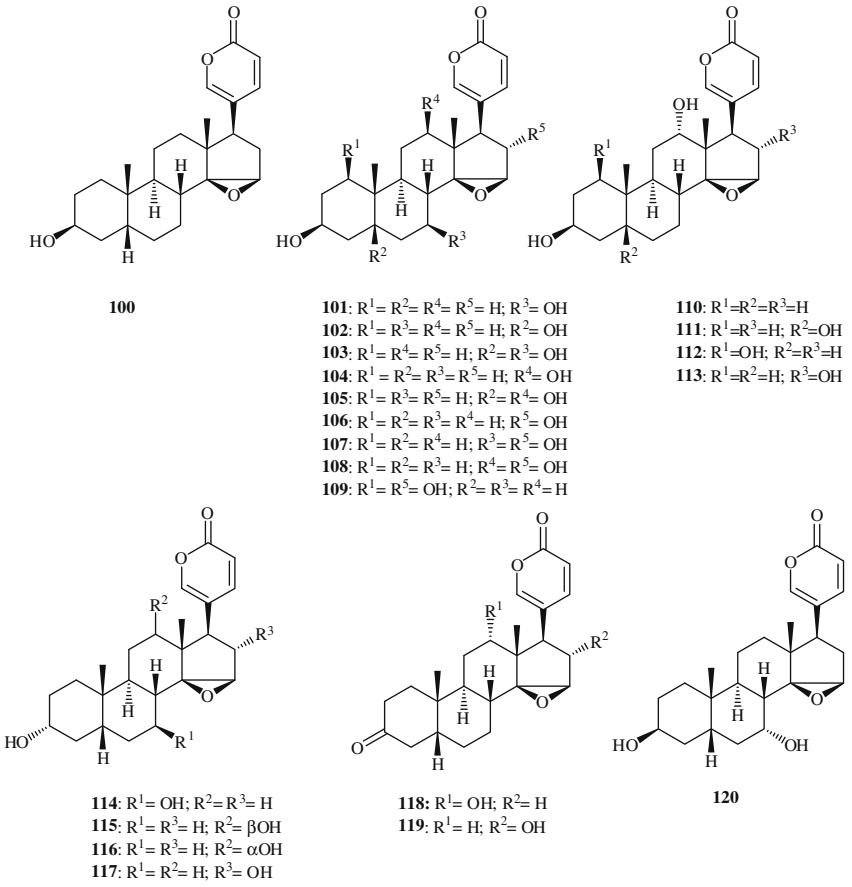


Figure 19. Biotransformation of resibufogenin **100** by *Mucor polymorphosporus*.

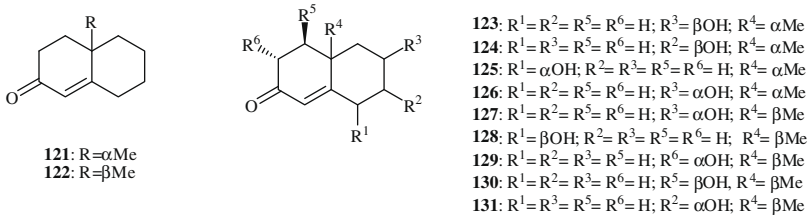


Figure 20. Biotransformation of (*R*)-(−)-methyloctalone **121** and (*S*)-(+)-methyloctalone (**122**) by *Chaetomium* sp. and *Didymosphaeria igniaria*.

um sp. converted **121** to products **123–125** (30%, 50%, and 6%, respectively). Furthermore, when using product **122** as a substrate, *Chaetomium* sp. produced compounds **127–130** (60%, 20%, <5%, <5%, respectively). *D. igniaria* biotransformed **121** to products **123–126** (7%, 20%, 128%, and 19%, respectively), and **122–128** (35%), **130** (14%), and **131** (11%) (Fig. 20).[92]

Botrytis species are fungi that affect many plant species, such as carrots, grapes, lettuce, strawberries, and tobacco, producing various leaf spot diseases and powdery grey mildews. Compound (±)-1-(4′-chlorophenyl) propan-1-ol exhibited high antifungal activity against *B. cinerea*. The (*R*)-enantiomer **132** was incubated with *B. cinerea* in order to gain a better understanding of the possible fungal detoxification mechanism. The hydroxylated products **133** and **134** were produced (Fig. 21). Antifungal assays have shown that the biotransformed products are less toxic to fungal growth than **132**. Therefore, *B. cinerea* has a mechanism to detoxify compound **132** by hydroxylating various positions of this molecule.[93]

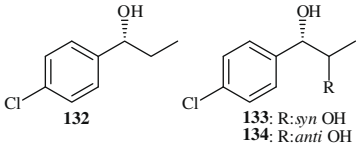


Figure 21. Biotransformation of (*R*)-(+)-1-(4′-chlorophenyl)propan-1-ol **132** by *Botrytis cinerea*.

Vinclozolin **135** is a fungicide used in Europe and the United States for the control of diseases caused by fungi in several plants. This compound was biotransformed by the fungus *Cunninghamella elegans* affording the (3*R*)- and (3*S*)- isomers of 3′,5′-dichloro-2,3,4-trihydroxy-2-methylbutyranilide **136** (33%), presumably formed by an epoxide hydrolase reaction, from epoxide derivative **137** (Fig. 22). This was the first study into the fungal metabolism of **135** and identification of its major metabolites.[94]

3.2. Sulfoxidation

In the last few years, chiral sulfoxides have become important building blocks for the synthesis of pharmaceuticals and biologically active compounds. An increasing number of applications are evident because they occur in a variety of functionalized amino acids possessing various biological activities. Several methods are available for the synthesis of these sulfoxides, however, there is still a significant need for enantioselective conversion methods. Microorganisms have been used for the production of chiral sulfoxides with high regio- and stereoselectivity.

The biotransformation of benzhydrylsulfanyl acetic acid **138** was tested using eight fungal strains. This compound can be used for the synthesis of (±)-modafinil, a psychostimulant agent. *Beauveria bassiana* (ATCC-7159) biotransformed benzhydrylsulfanyl acetic acid into (*S*)-sulfinyl carboxylic acid **139** in very good yield (89%) and in high enantioselectivity (99%) (Fig. 23). Other fungi exhibited poor enantioselectivity, but *Microsporum gypseum* (ATCC-11395) provided a good yield of the sulfinyl product (94%).[106]

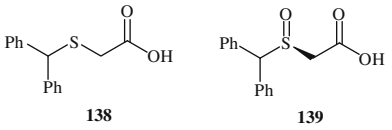


Figure 23. Biotransformation of benzhydrylsulfanyl acetic acid **138** by *Beauveria bassiana* (ATCC-7159).

The stereoselective kinetic biotransformation of thioridazine, a phenotizine neuroleptic drug, was investigated by using 12 endophytic fungi. Both enantiomers of thioridazine were efficiently biotransformed by four fungal strains (*Phomopsis, Glomerella cingulata, Diaporthe phaseolorum,* and *Aspergillus fumigatus*). The endophytes produced four diastereomers that were same as those produced by mammalian metabolism, but with different regio- and stereoselectivity.[21,22] These results corroborate that microbial systems could be used as an alternative for preliminary metabolism studies for drug candidates.

Other sulfides were biotransformed by fungi. A total of two organic sulfides were stereoselectively biotransformed by *B. cinerea*, *Eutypa lata*, and *Trichoderma viride* yielding high enantiomeric purity. The best results for the oxidation of thioanisole were obtained with *T. viridae*, which provided (*R*)-methyl phenyl sulfoxide in 70% enantiomeric excess (ee) on a static culture. The biotransformation of benzyl phenyl sulfide by *B. cinerea* afforded (*S*)-benzyl phenyl sulfoxide, but in low yield and enantiomeric excess. (*R*)-Benzyl phenyl sulfoxide was obtained with both *T. viride* and *E. lata*, with *T. viride* providing the best enantiomeric excess (>95% ee) and

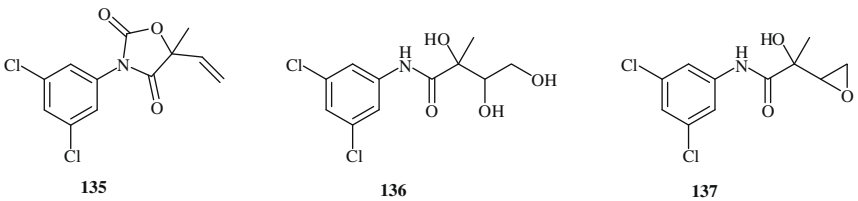


Figure 22. Biotransformation of vinclozolin **135** by *Cunninghamella elegans*.

good yield (60%).[107] A series of phenylthio-2-propanone and benzylthio-2-propanone were biotransformed using the fungi *Helminthosporium* sp (NRRL 4671)[28] and *Mortierella isabellina* (ATCC 42613) producing β-hydroxysulfoxides in good yields and enantiomeric purity (>95%).[108,109]

A comprehensive review regarding sulfoxidation using microorganisms has already been published. Further information is given in the literature.[110]

3.3. Epoxidation

Epoxides are formed during the biotransformation of several terpenoids[27,28,32,36,61,63–65] and other products.[111] More than 60 fungal strains were tested for their capacity to biotransform (*R*)-(+)- and (*S*)-(−)-limonene using solid-phase microextraction as the monitoring technique. *Penicillium* species biotransformed the (*R*)-(+)-limonene **140** to *trans*- and *cis*-limonene oxide **141**–**142** (Fig. 24).[27]

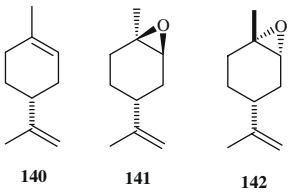


Figure 24. Biotransformation of (*R*)-(+)-limonene **140** by *Penicillium* species.

The sesquiterpene (4*E*,8*R*)-caryophyll-4-(5)-en-8-ol was biotransformed by *B. cinerea*. Epoxidation at the double bond yielded a product previously obtained in the biotransformation of caryophyllene oxide by *B. cinerea*.[36]

The incubation of the diterpene type 7-oxo-18-hydroxy-*ent*-kaur-16-ene **143** with *Gibberella fujikuroi* produced product 18-hydroxy-16α,17-epoxy-7-oxo-*ent*-kaurane **144** (Fig. 25). The α-stereochemistry was assigned considering that in these types of compounds epoxidation occurs at the α-face, this structure being confirmed by the chemical epoxidation of 7-oxo-*ent*-kaur-16-ene.[63]

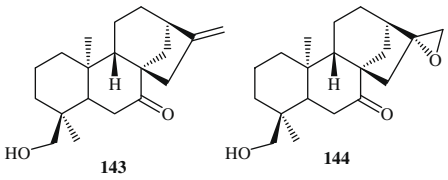


Figure 25. Biotransformation of 7-oxo-18-hydroxy-*ent*-kaur-16-ene **143** by *Gibberella fujikuroi*.

3.4. Baeyer–Villiger oxidation

The Baeyer–Villiger oxidation of linear and cyclic ketones into their corresponding esters or lactones is an important reaction in organic chemistry. Currently, to meet the growing demand for biologically active chiral molecules, it has become necessary to extend the available methods for asymmetric Baeyer–Villiger oxidation. Microorganisms are able to carry out this reaction with high regio- and enantioselectivity, and are therefore a good alternative in this respect.

A total of nine *Aspergillus* strains were used for the Baeyer–Villiger oxidation of two cyclic ketones. In some cases, the production of a chiral lactone was observed in up to 99% enantiomeric excess.[112]

Bicyclo[3.2.0]hept-2-en-6-one **145** is used for the synthesis of prostaglandins, and it is interesting as a precursor of some antibiotics. This compound was biotransformed by different *Fusarium* sp. affording (+)-(1*R*,5*S*)-lactone **146** in yields of 78% (73% ee) and 86% (70% ee). *Aspergillus terricola* and *A. amazonicus* produced (−)-(1*S*,5*R*)-lactone **147** from starting material **145** in reasonable enantiomeric excess (Fig. 26).[113]

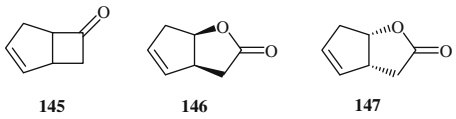


Figure 26. Biotransformation of bicyclo[3.2.0]hept-2-en-6-one **145** by isolates of *Fusarium* sp. and *F. solani*.

3.5. Deracemization

Deracemization by microbial stereoselective bioreduction or enantioselective hydrolysis is a very important reaction in biocatalysis, but it has been the subject of only a few papers.[114–119]

An important number of thermophilic filamentous fungi have been studied for enantiomerically and enantiotopically selective biotransformation.[114] Hydrolases from thermophilic fungi were studied using a stereoselective test reaction. The results indicated that these enzymes might be superior in synthetic biotransformation over the commercialized thermophilic fungal lipases with regard to the degree of enantiomer selectivity or direction/degree of enantiotopic selectivity.[114]

Deracemization by oxidation and by enantioselective bioreduction of acetophenone and its derivatives has been carried out by several fungi.[115–118] The hydrolytic kinetic resolution of 2-pyridyloxirane, using the overexpressed epoxide hydrolase, from the filamentous fungus *A. niger* has been reported.[119]

A number of papers have been published over the last several years on the deracemization of interesting intermediates in the synthesis of pharmaceuticals and agrochemicals or of structural elements in many syntheses of bioactive compounds.[102,120–123]

Simple chemoenzymatic access to enantiopure pharmacologically interesting (*R*)-2-hydroxypropiophenones from propiophenone has been reported.[99] Acetoxylation of propiophenone **148** with manganese (III) acetate followed by hydrolysis of the acetoxy derivative using *Rhizopus oryzae* as a biocatalyst yielded hydroxyacetone **149** in high enantiomeric excesses and in good yields (Fig. 27). The undesired acetoxy ketones were epimerized and recycled to give the (*R*)-enantiomer.

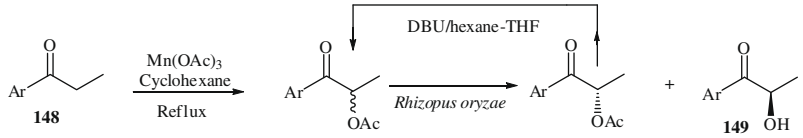


Figure 27. Biotransformation of propiophenone **148** by *Rhizopus oryzae*.

Deracemization of racemic compounds with interest for the fragrance and pharmaceutical industries has been reported using *Glomerella cingulata*,[121] *Trichosporon cutaneum*,[122] and *R. arrhizus*.[102]

3.6. Stereo- and enantioselective reduction of ketones

Several articles have reported on microbial reduction reactions for the stereo- and enantioselective reduction of ketones.[112,124–137]

The current interest in applying biocatalysis in organic synthesis is mainly related to the preparation of optically active compounds with high stereoselectivity under environmentally friendly conditions. Significant attention has been paid to the stereo-and enantioselective synthesis of enantiomerically pure compounds of chiral synthons needed under the increasing demand for the development of modern drugs and agrochemicals. From among the chiral compounds, enantiomerically pure alcohols are particularly useful as building blocks for the synthesis of pharmaceuticals and agrochemicals.

Biotransformation is a convenient method for preparing chiral alcohols. The use of whole microbial cells is particularly advantageous for carrying out the reduction of ketones since they do not require the addition of cofactors for their regeneration. Hence, several fungi and yeasts have been used for the stereo- and enantioselective reduction of prochiral ketones.

Experimental conditions using whole cells to select fungal strains for the specific bioreduction reaction of acetophenones and the formation of Baeyer–Villiger oxidation products were studied. Species of the *Trichothecium* genus were found to be effective biocatalysts for the enantioselective bioreduction of acetophenone and its analogous compounds to their corresponding (*R*)-alcohols in good enantiomeric excesses.[124]

(*S*) and (*R*)-Alcohols were prepared by the reduction of the corresponding ketones using different fungal strains. High acetophenone monooxygenase activity was observed with the fungus *Emericella nidulans* CCT 3119. The results proved useful for further investigations aimed at obtaining purified enzyme systems from this fungus.[125–127]

A comparative study has been reported using whole cells of the white-rot fungus *Merulius tremellosus* ono991 as a biocatalytic reduction system and ruthenium(II)-amino alcohol and iridium(I)-amino sulfide complexes as metal catalysts in an asymmetric transfer hydrogenation. It was concluded that the biocatalytic and transfer hydrogenation approaches appear to be complementary.[128]

Several organoseleno-acetophenones, 3,5-bis(trifluoromethyl)-acetophenones, and acetonaphthone derivatives[129–131] were reduced with whole fungal cells of the genera *Rhizopus*,[129,131] *Aspergillus*,[129,130] *Emicerella*,[129] *Lactobacillus*,[130] *Geotrichum*, *Candida*, and *Yarrowi*.[131]

Along these same lines, the potential of an important number of fungi in carrying out the biotransformation of cyclic ketones was investigated.[112,132–134] A set of 416 strains from public collections composed of 71 bacteria strains, 45 actinomycetes, 59 yeasts, 148 filamentous fungi, 33 marine fungi, and 60 basidiomycetes was used for a screening campaign searching for: (i) microorganisms that display reductase activity in the absence of oxidase activity,[133] and (ii) microbial biocatalysts for the stereoselective reduction of carbonilic compounds.[134] *Gongronella butleri*, *Diplogelasinospora grovesii*,[133] and *Schizosaccharomyces octosporus* were selected as the most interesting strains based on their productivity, their tolerance to high concentrations of ketones, and the absence of secondary products in the reduction of cycloalkanones.[134]

The stereo- and enantioselective microbial reduction of specific ketones derivatives has been studied,[135,136] and therefore the reduction of 3-methyl-4-phenyl-3-buten-2-one and its phenyl-substituted derivatives by microorganisms was investigated. *M. isabellina* DSM 1414 and *Geotrichum candidum* LOCK 105 strains reduced α,β-unsaturated ketones to the corresponding secondary alcohols in high enantiomeric excess (94–99%).[137]

Several oxo-sesquiterpene derivatives with different skeletons have been biotransformed to give interesting derivatives, some of which are difficult to be achieved by chemical means.[37,38,40,57,138,139]

The microbial transformation of 1-oxo and 6-oxoeudesmanes yielded other useful hydroxyselinane derivatives in high proportions as the result of a stereoselective reduction of the carbonyl groups at these positions by *R. nigricans* on the β-face.[37]

The biotransformation of sesquiterpene 4β-hydroxyeudesmane-1,6-dione by the filamentous fungi *Gliocadium roseum* and *Exserohilum halodes* was achieved.[39] While *G. roseum* yielded several hydroxylated metabolites, only one was obtained from *Exserohilum halodes* as a result of the regio- and stereoselective reduction of the keto group at C-1 which is difficult to be achieved by chemical methods.[39] Moreover, Garcia-Granados et al. increased biocatalysis rates from cyclic sulfite eudesmene derivatives, and considerable differences in the biotransformation of cyclic sulfites have been found. Promising 8α,11-dihydroxy derivatives isolated from the biotransformation of the (*S*)-diastereomer **150** (Fig. 28)

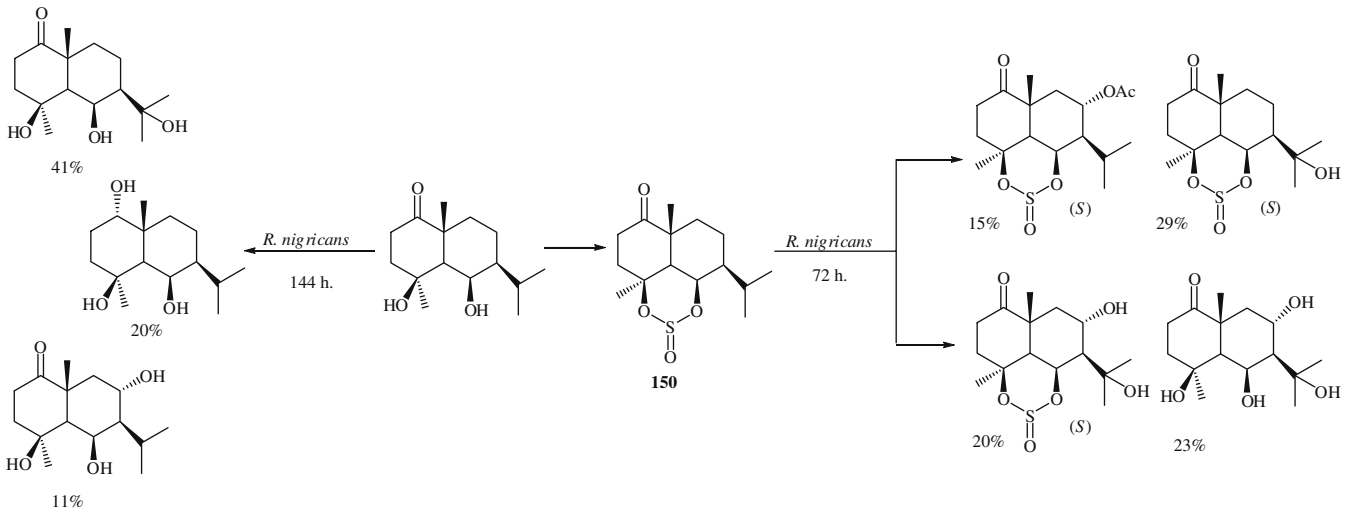


Figure 28. Biotransformation of 1-oxoeudesman-4β,6β-diyl-*S*(*S*)-cyclic sulfite **150** by *Rhizopus nigricans*.

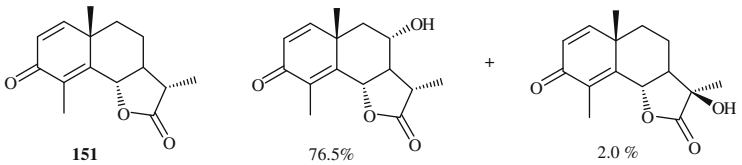


Figure 29. Biotransformation of α–santonine **151** by *Absidia coerulea*.

offer attractive new possibilities for the synthesis of natural product derivatives such as 8,12-eudesmanolides.[57]

In addition to the reduction of unsaturated ketones, interesting hydroxylations at C-8 and C-11 were obtained by the biotransformation of α–santonine **151** by the fungus *Absidia coerulea* (Fig. 29).[40] Also, the sesquiterpene lactone chinensolide B was specifically reduced (C-3 ketone to alcohol, and/or 11(13) methylene to methyl) by the same fungus *A. coerulea*, strain IFO 4011.[40]

Cadinane sesquiterpenes were biotransformed by *C. lunata* ATCC 12017 and *B. bassiana* ATCC 7159, several derivatives were obtained from reduction of the ketone. The insecticidal potential and phytotoxicity of the isolated metabolites have been evaluated.[38,138] Some other diterpene and steroid derivatives have been studied from the point of view of their bioconversion by different fungi species.[61,65,86]

The importance of optically active β-hydroxy acid derivatives as versatile building blocks in asymmetric synthesis is well established, and among the many existing methods to prepare them, microbial enantioselective reduction of β-keto esters has proven to be one of the most effective. 3-Hydroxybutanoic acid and its esters are prominent members of this category and have been used as synthetic building blocks and intermediates for the synthesis of several classes of natural products and several therapeutic agents. In particular, its alkyl ester has been exploited extensively for the synthesis of fine chemicals including pharmaceuticals, agrochemicals, flavors, and fragrances.

Alkyl oxo-ester derivatives[140–144] have been extensively studied from the point of view of their reduction using whole microbial cells, and an important number of microorganisms have been studied as potential biocatalysts (Table 1). Alkyl oxobutanoate derivatives were reduced enantioselectively (99% ee; 67% yield) to the corresponding (*S*)-alcohol by *Rhizopus* species.[140] Similar results were obtained with the fungus *Cylindrocarpon sclerotigenum*.[141] The dimorphic fungus *Mucor rouxii* showed good performance in whole cell biocatalysis in both aqueous and organic media. Both morphologies, mycelium and yeast, displayed interesting reductase activity. Interestingly, yeast-like cells and spores produced the best results in a non-polar medium using hexane as the solvent.[143]

New functionalized butyrolactone derivatives obtained from commercial (±)-α-acetyl-γ-butyrolactone and its corresponding (±)-*anti*- and (±)-*syn*-hydroxyl analogues are of interest as potential central nervous system (CNS) ligands (Fig. 30). *A. niger*,

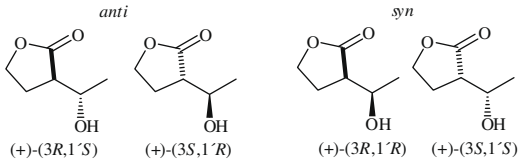
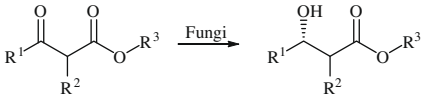


Figure 30. Obtained products of biotransformation from butyrolactones.

G. candidum, and *Kluyveromyces marxianus* strains produced (+)-(3*R*, 1′*S*)-α′-1′-hydroxyethyl-γ-butyrolactone in good to excellent conversions, diastereomeric and enantiomeric excesses. The corresponding enantiomer was obtained using *Hansenula* spp.[144]

C. lunata has been used for the stereoselective alkylation–reduction of β-keto nitriles. This fungus has proven its ability to α-alkylate and concomitantly reduce aromatic and heteroaromatic β-keto nitriles. After optimization of the conditions, the alkylation–reduction reaction led to the formation of a C–C bond

Table 1
Reduction of alkyl oxobutanoate using different microorganisms as potential biocatalyst



Substrate	R^1	R^2	R^3	Organism	ee %	Ref.
1	CH_3	H	$-CH_3$	*Rhizopus arrhizus*	70–71	140
2	CH_3	H	$-CH_2CH_3$	*Mucor rouxii*	60 (water)	143
				R. arrhizus	89–74	140
3	CH_3	H	$-CH(CH_3)_2$	*M. rouxii*	97 (hexane)	143
4	CH_3	H	$CH_2CH_2OCH_3$	*M. rouxii*	67	143
5	CH_3	CH_3	$-CH_2CH_3$	*M. rouxii*	99	143
6	CH_3	H	-Allyl	*R. arrhizus*	80–89	140
7	CH_3	H	-isoBut.	*R. arrhizus*	89–90	140
8	CH_3	H	-*t*-But	*R. arrhizus*	94	140
				Botrytis fabae	93	141
				B. alli	95	141
				Cylindrocarpon olidum	93	141
				C. sclerotigenum	>99	141
9	$ClCH_2$	H	$-CH_2CH_3$	*Penicillium purpurogenum*	83.4	141
				P. oxalicum	83	141
				Trichoderma polysporum	80	141
				T. longibrachatum	74.5	141

and two stereogenic centers in moderate yields of up to 69% and in high stereoselectivities of up to 98% ee and de in most cases.[145] The use of methanol as a cosolvent allows for the chemoselective reduction of aromatic β-keto nitriles, yielding the corresponding (*S*)-β-hydroxy nitriles in a highly enantioselective manner.[146]

Optically active β-hydroxysulfoxides and sulfones are of great utility in organic synthesis, and have been used in the preparation of many fine chemicals. One of the most useful strategies to access chiral β-hydroxy sulfones has been the Baker's yeast-mediated asymmetric reduction of β-keto sulfones.[147] However, a major factor in the enantioselectivity of these processes is the size of the substituents attached to the carbonyl group. The best results were obtained when the substituent was a methyl group.[148] β-Keto sulfones bearing bulky groups were reduced with high enantioselectivities to the corresponding optically active β-hydroxy sulfones by the fungus *C. lunata* CECT 2130, and the cells can be re-used without loss of their catalytic activity.[149]

The biotransformation of a series of substituted phenylthio-2-propanone, 1-(*p*-methoxyphenylthio)-2-propanone, and benzylthio-2-propanone was carried out using *Helminthosporium* sp.[108,109] and *M. isabellina* ATCC 42613, or *Rhodococccus erythropolys* IGTS8. Several fungal strains catalyze the oxidation of sulfide to sulfoxide and the reduction of carbonyl to secondary alcohol in different compounds producing β-hydroxysulfoxides in medium to high enantiomeric and diastereomeric purities. Fungal biotransformation using *Helmisthosporium* sp. and *M. isabellina* resulted in the opposite sulfoxide configurations of various β-hydroxysulfoxide products.[109]

Chiral 2-hydroxyalkanephosphonates have attracted attention due to their potential biological activity and versatility as substrates for the synthesis of a variety of organophosphorus derivatives. A series of 2-oxoalkanephosphonates have been screened for reduction with *G. candidum*. Only diethyl 2-oxo-propanephosphonate underwent asymmetric reduction to yield (+)-(*R*)-diethyl 2-hydroxypropane phosphonate in 98% ee. Under kinetic resolution conditions in the presence of various lipases, racemic 2-hydroxyalkanephosphonate was acetylated yielding the corresponding acetoxy-derivatives, and recovered alcohol in good yield and 93% ee.[150]

3.7. Miscellaneous

Extracts from 14 filamentous fungi were examined regarding their potential for the production of (*R*)-phenylacetylcarbinol ((*R*)-PAC) from benzaldehyde via pyruvate decarboxylase which is the chiral precursor in the manufacture of the pharmaceutical ephedrine and pseudoephedrine. (*R*)-PAC was obtained in 90–93% enantiomeric excess using *Rhizopus javanicus* and *Fusarium* sp. The study showed that (*R*)-PAC formation is not limited to the use of yeasts and the bacterium *Zymomonas mobilis*, but can be extended to filamentous fungi. Higher initial productivities and slightly higher final yields were obtained with *R. javanicus* than those obtained with extracts of the yeasts *Candida utilis* and *Saccharomyces cerevisiae*.[151]

Some terpenes have been biotransformed by fungi yielding different derivatives. The biotransformation of the enantiomers of the monoterpenes linalool and citronellol by *Aspergillus* sp. has been studied under different culture conditions using solid-phase microextraction as the analytical sampling technique.[152,153]

4. Conclusions and future trends

Biocatalysis is now becoming a key component in the chemical process for obtaining new pharmaceuticals, intermediates, and analytical reagents. Most biocatalytic reactions can be carried out under certain safety, health, environmental, and economical conditions. The ability of biocatalysis to reach its full potential in pharmaceutical synthesis will require cost-reduction techniques and complete integration with chemistry. In addition, microorganisms and their enzymes have been discovered by means of extensive screening, and these are now commonly used in industrial applications. This enzyme screening, in combination with current biotechnologies such as protein, metabolic, and genetic engineering, will pave the way to widespread industrial use of microbial enzymes. In this context, the synthesis of single enantiomers of drug intermediates and/or human metabolites is increasingly important in the pharmaceutical industry. Biocatalysis provides an enormous added opportunity to prepare pharmaceutically useful chiral compounds providing an environmentally viable alternative. The advantages of biocatalysis over chemical catalysis are that enzyme-catalyzed reactions are stereoselective and regioselective, and can be carried out at ambient temperature and atmospheric pressure. The different classes of enzymes can catalyze many types of chemical reactions affording a wide variety of chiral compounds. Over the course of the last decade, progress in biochemistry, protein chemistry, molecular cloning, random and site-directed mutagenesis, directed evolution of biocatalysts, and fermentation technology has opened up unlimited access to a variety of enzymes and microbial cultures which can be used as tools in organic synthesis. Therefore, the integration of biocatalysis and organic synthesis will spark the creation of new synthetic strategies and will open up new technological frontiers of both fundamental and practical interest.

Acknowledgments

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), sub-program BIOTA/FAPESP (Rede BIOprospecTA), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and for granting research fellowships. This work was supported by Grants of Junta Andalusia (Projects PAI-2005-FQM-00489 and P07-FQM-02689).

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