Chapter

Microorganisms as Biocatalysts and Enzyme Sources

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Abstract

Microbial-catalyzed biotransformations have considerable potential for the generation of an enormous variety of structurally diversified organic compounds, especially natural products with complex structures like triterpenoids, flavonoids, steroids, steroidal saponins, and sesquiterpenoids. They offer efficient and economical ways to produce semisynthetic analogues and novel lead molecules. Microorganisms such as bacteria and fungi could catalyze *chemo*-, *regio*-, and *stereo*specific hydroxylations of diverse substrates that are extremely difficult to produce by chemical routes. During recent years, considerable research has been performed on the microbial transformation of bioactive compounds, in order to obtain biologically active molecules with diverse structural features. In green chemistry, biotransformations are an important chemical methodology toward more sustainable industrial processes.

Keywords: microorganisms, fungi, bacteria, microbial transformation, natural products, enzymes

1. 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 *regio*selectivity but is also enantiospecific, allowing the production of chiral products from racemic mixtures. The conditions for biotransformations are mild, and in the majority of cases, they do not require the protection of pre-existing functional groups. Furthermore, some reactions that do not occur when using chemical approaches are easily carried out by microbial transformation. Microbial factories show advantages, for instance, growing rapidly and ease of large-scale production [1–3].

The use of microorganisms may be a highly efficient method of production of these compounds. The reactions involved in biotransformation of organic compounds by whole cells of various microorganisms include oxidation, reduction, hydroxylation, esterification, methylation, demethylation, isomerization, hydrolysis, glycosylation, and hydrogenation [4, 5].

Biotransformation may be carried out with isolated enzyme systems or with intact organism. Although isolated enzyme systems may be more specific and

efficient for certain biotransformation, these reactions may involve isolating the enzyme system, and, for some classes of enzyme-catalyzed reaction, a recycling sequence may be required to regenerate the enzyme [6].

Fungi are playing a prominent role in the catalysis of organic compounds and in the production of commercially and industrially important compounds, because of their ability to catalyze novel reactions [7]. Fungi are commonly used in the industry for production of fermented beverages, foods, physiologically active substances, solvents, organic acids, polysaccharides, antibiotics, etc. Of the zygomycota, *Mucor* and *Rhizopus* are commonly used in the industry. *Rhizopus* strains are important in citric acid production. *Mucor* strains make a significant number of important lipases and catalyze the hydroxylation of a wide range of chemical compounds [2–4].

The use of the microbial model offers a number of advantages over the use of animals in metabolism studies, mainly: (1) simple, easy, and can be prepared at low cost; (2) screening for a large number of strains is a simple repetitive process; (3) the large number of metabolites formed allows easier detection, isolation, and structural identification; (4) newer metabolites can be isolated; (5) utilized for synthetic reactions involving many steps; (6) useful in cases where *regio-* and *stereo*specificity is required; (7) maintenance of stock cultures of microorganisms is simpler and cheaper than the maintenance of cell or tissue cultures or laboratory animals; (8) ease of setup and manipulation; and (9) more reliable and reproducible [8, 9].

The objective of this review is to highlight the importance of microorganisms or enzymes isolated from them in the biotransformation process of natural products or xenobiotic compounds, according to green chemistry or white biotechnology.

2. Microbiological transformations of some selected natural products with different microorganisms

2.1 Sesquiterpene lactone

Artemisinin (**1**), a sesquiterpene lactone endoperoxide and an antimalarial drug, is effective against chloroquine-resistant parasites; but its toxicities and low solubility in water hamper its therapeutical use. Studies on modification of **1** through biological and chemical methodologies have been reported to yield more effective and water-soluble derivatives. A wide array of microbial transformations of **1** involve oxidation, reduction, and degradation reactions by different microorganisms, such as *Aspergillus niger*, *A. flavus*, *A. adametzi* (ATCC 10407), *Cunninghamella echinulata, Caenorhabditis elegans, Mucor polymorphous, M. rammanianus, Streptomyces griseus, Penicillium simplicissimum, P. chrysogenum, P. purpuresceus*, *Pestalotiopsis guepini* (P-8), *Eurotium amstelodami, Trichoderma viride* (T-58)*, Saccharomyces cerevisiae*, *and Pichia pastoris.* Biotransformation of **1** usually includes the processes such as hydroxylation of methyl, methine and methylene groups, deoxidation reactions, hydration and acetylation reactions, epimerization, and breakdown of heterocyclic rings (**Table 1**).

2.2 Triterpene

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid, UA, **2**), a natural pentacyclic triterpene, is broadly used in food, cosmetics, and biomedical industries. As a ubiquitous constituent in the plant kingdom and the major component of many traditional medicine herbs, ursolic acid remarkably exhibits a lot of biological

Microorganism	Products	Action	Reference
A. niger	3β-hydroxy-4,12-epoxy-1-deoxyartemisinin Artemisinin G 3,13-epoxyartemisinin 4α-hydroxy-1-deoxyartemisinin	Epoxidation, hydroxylation C-3β site Endoperoxide function reduction Breakdown of heterocyclic rings Epoxidation C-3 and C-13 Hydroxylation C-4, endoperoxide function reduction	[10]
A. flavus (MTCC 9167)	14-hydroxyartemisinin Artemisinin G 4α-hydroxydeoxyartemisinin Deoxyartemisinin	Hydroxylation of C-14 site Breakdown of heterocyclic rings Hydroxylation of C-4α site Endoperoxide function reduction	[11, 12]
C. elegans (ATCC 9245)	7β-hydroxy-9α-artemisinin 4α-hydroxy-1-deoxoartemisinin 7β-hydroxyartemisinin 6β-hydroxyartemisinin 7α-hydroxyartemisinin 6β,7α-dihydroxyartemisinin	Hydroxylation C-7β site Epimerization C-9 Hydroxylation C-4α site Hydroxylation C-7β site Hydroxylation C-6β site Hydroxylation C-7α site Hydroxylation C-6β and C-7α sites	[13, 14]
P. simplicissimum	9β-acetoxyartimisinin 9α-hydroxyartemisinin	Acetylation of C-9β site Hydroxylation of C-9α site	[15]
R. stolonifer	Deoxyartemisinin 1α-hydroxyartemisinin 10β-hydroxyartemisinin	Endoperoxide function reduction Hydroxylation of C-1 site	[16]
S. griseus (ATCC 13273)	9-artemisitone 9α-hydroxyartemisinin 9β-hydroxyartemisinin 3α-hydroxyartemisinin	Oxidation of C-9 site Hydroxylation of C-9α site Hydroxylation of C-9β site Hydroxylation of C-3α site	[17]
N. corallina	Deoxyartemisinin	Endoperoxide function reduction	[11]

Table 1.
*Products obtained from the biotransformation of artemisinin (**1**) by different microorganisms.*

activities, such as antibacterial, anti-allergic, antioxidative, anti-inflammation, and antitumor activities [18].

Microbial transformation of ursolic acid (**2**) by *Bacillus megaterium* CGMCC 1.1741 yielded five metabolites identified as 3-oxo-urs-12-en-28-oic acid (**3**, 6.2%); 1β,11α-dihydroxy-3-oxo-urs-12-en-28-oic acid (**4**, 13.5%); 1β-hydroxy-3-oxo-urs-12-en-28,13-lactone (**5**, 5.0%); 1β,3β,11α-trihydroxy-urs-12-en-28-oic acid (**6**, 26.9%); and 1β,11α-dihydroxy-3-oxo-urs-12-en-28-O-β-D-glucopyranoside (**7**, 8.6%) [19]. The biotransformation studies of **2** by *Alternaria longipes* AS 3.2875 have led to the isolation of six products of hydroxylation or glycosylation. Their structures were identified as 3-carbonyl-ursolic acid-28-O-β-D-glucopyranosyl ester (**8**), ursolic acid-3-O-β-D-glucopyranoside (**9**), ursolic acid-28-O-β-D-glucopyranosyl ester (**10**), 2α,3β-dihydroxy-ursolic acid-28-O-β-D-glucopyranosyl ester (**11**), 3β,21β-dihydroxy-ursolic acid-28-O-β-D-glucopyranosyl ester (**12**), and 3-O-(β-D-glucopyranosyl)-ursolic acid-28-O-(β-D-glucopyranosyl) ester (**13**). Glycosylation reaction on pentacyclic triterpenoid fulfilled with difficulty in the process of chemical synthesis is facile by microbial transformation [20]. Biotransformation of **2** by *A. alternata* eight metabolites were found to be 2α,3β-dihydroxyurs-12-en-28-oic acid (corosolic acid, **14**), urs-12-en-2α,3β,28-triol (**15**), 3β,23-dihydroxyurs-12-en-28-oic acid (**16**), 2α,3β,23-trihydroxyurs-12-en-28-oic acid (**17**), 2α,3β,23,24-tetrahydroxyurs-12-en-28-oic acid (**18**), 3β,28-dihydroxy-12-ursene (**19**), urs-12-en-3β-ol (**20**), and urs-12-en-2α,3β-diol (**21**). The reduction of the C-28 carboxyl group and hydroxylation at C-2, 23, and 24 are steps in the metabolic pathway of **2** [21].

Biotransformation of UA by *S. racemosum* (3.2500) yielded five metabolites 3β,7β,21β-trihydroxy-urs-12-en-28-oic acid (**22**); 3β,21β-dihydroxy-urs-11-en-28-oic acid-13-lactone (**23**); 1β,3β,21β-trihydroxy-urs-12-en-28-oic acid (**24**); 3β,7β,21β-trihydroxy-urs-1-en-28-oic acid-13-lactone (**25**); and 1β,3β-dihydroxy-urs-12-en-21-oxo-28-oic acid (**26**) which were afforded [22]. Additionally, of the biotransformation of **2** with by *S. racemosum* compounds **27–30** and 11,26-epoxy-3β-21β-dihydroxy-urs-12-en-28-oic acid were obtained (**31**), (**Figure 1**) [23].

The endophytic fungi *Pestalotiopsis microspora* isolated from medical plant *Huperzia serrata* can transform **1** to afforded 3-oxo-15β,30-dihydroxy-urs-12-en-28-oic acid (**32**), 3β,15β-dihydroxy-urs-12-en-28-oic acid (**33**), 3β,15β,30-trihydroxy-urs-12-en-28-oic acid (**34**), and **30** [24].

Microbial transformation of ursolic acid by *Mucor spinosus* AS 3.3450 were isolated and their structures were identified as **9**, **22** and 3β,7β-dihydroxy-ursolic acid-28-ethanone (**35**) (**Figure 1**) [25].

The gum resin *Boswellia serrata* has been used for the treatment of inflammatory and arthritic diseases. Its major active constituents are ursane triterpenoids, which include 11-keto-β-boswellic acid (KBA, **36**), β-boswellic acid (BA), and acetyl-β-boswellic acid (ABA). Microbial transformation **36** by *Cunninghamella blakesleeana* (AS 3.970) yielded ten regioselective transformed products: 7β-hydroxy-11-keto-β-boswellic acid (**37**), 7β,15α-dihydroxy-11-keto-β-boswellic acid (**38**), 7β,16β-dihydroxy-11-keto-β-boswellic acid (**39**), 7β,16α-dihydroxy-11-keto-β-boswellic acid (**40**), 7β,22β-dihydroxy-11-keto-β-boswellic acid (**41**), 7β,21β-dihydroxy-11-keto-β-boswellic acid (**42**), 7β,20β-dihydroxy-11-keto-β-boswellic acid (**43**), 7β,30-dihydroxy-11-keto-β-boswellic acid (**44**), 3α,7β-dihydroxy-11-oxours-12-en,24,30-dioic acid (**45**), and 3α,7β-dihydroxy-30-(2-hydroxypropanoyloxy)-11-oxours-12-en, 24-oic acid (**46**). Bioconversion of **36** with *Bacillus megaterium* based on a recombinant cytochrome P450 system yielded *regio*- and *stereo*selective 15α-hydroxylation (**47**) of substrate (**Figure 2**) [26].

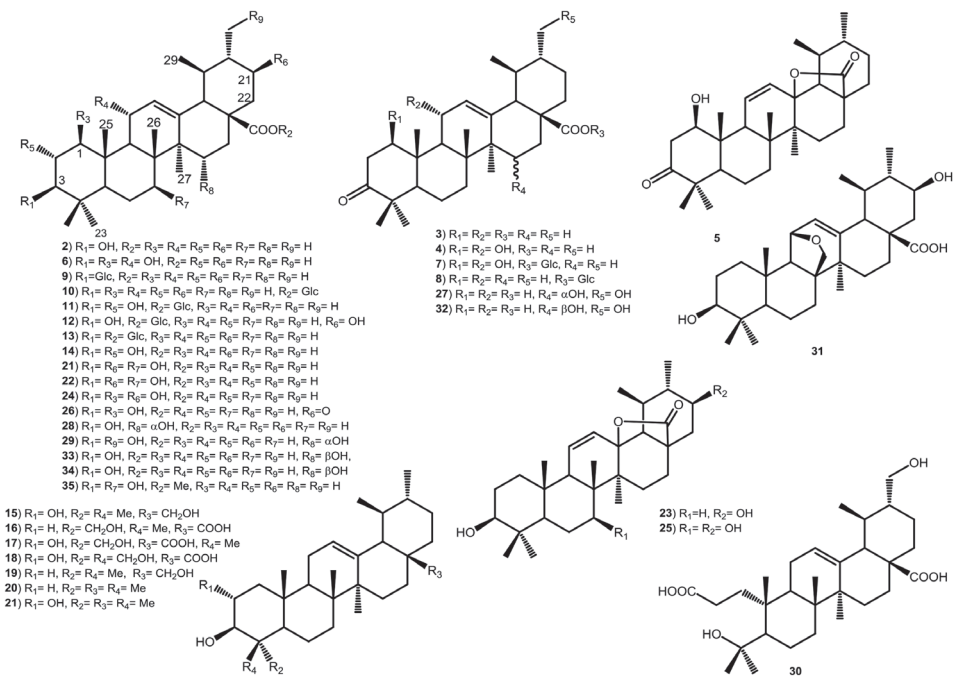
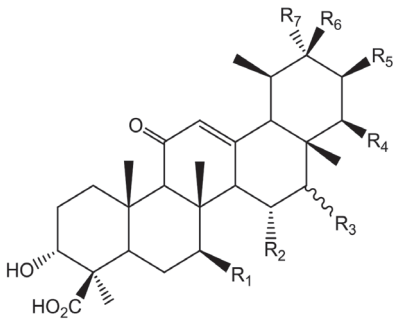


Figure 1.
Biotransformation products of ursolic acid (2).

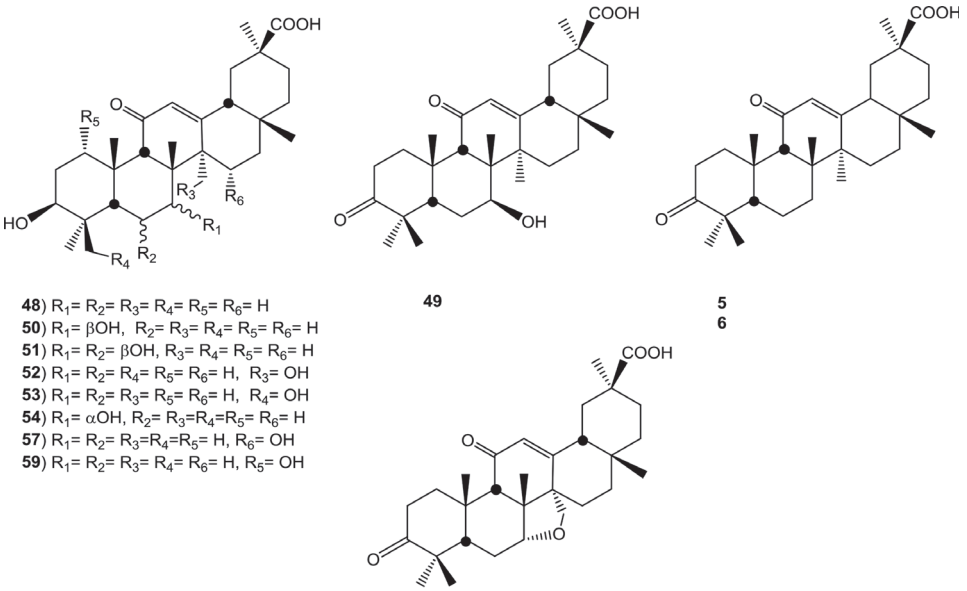


36) R_1= R_2= R_3= R_4= R_5= R_6= H, R_7= CH_3
37) R_1= OH, R_2= R_3= R_4= R_5= R_6= H, R_7= CH_3
38) R_1= R_2= OH, R_3= R_4= R_5= R_6= H, R_7= CH_3
39) R_1= OH, R_3= βOH, R_2= R_4= R_5= R_6= H, R_7= CH_3
40) R_1= OH, R_3= αOH, R_2= R_4= R_5= R_6= H, R_7=CH_3
41) R_1= R_4= OH, R_2= R_3= R_5= R_6= H, R_7= CH_3
42) R_1= R_5= OH, R_2= R_3= R_4= R_6= H, R_7= CH_3
43) R_1= R_6= OH, R_2= R_3= R_4= R_5= H, R_7= CH_3
44) R_1= OH, R_2= R_3= R_4= R_5= R_6= H, R_7= CH_2OH
45) R_1= OH, R_2= R_3= R_4= R_5= R_6= H, R_7= COOH
46) R_1= OH, R_2= R_3= R_4= R_5= R_6= H, R_7= $OCOCH(OH)CH_3$
47) R_1= R_3= R_4= R_5= R_6= H, R_2= OH, R_7= CH_3

Figure 2.
*Biotransformation products of 11-keto-b-boswellic acid (***36***).*

18β-glycyrrhetinic acid (**48**) is the active form of glycyrrhizin which is the major pentacyclic triterpene found in licorice (*Glycyrrhiza glabra* L.). Glycyrrhetinic acid has been shown to possess several pharmacological activities, such as antiulcerative, anti-inflammatory, immunomodulating, antitumor, antiviral, antihepatitis effects, and anticancer. Biotransformation **48** with a fungus *C. blakesleeana* (AS 3.970) yielded 3-oxo-7β-hydroxyglycyrrhetinic acid (**49**) and 7β-hydroxyglycyrrhetinic acid (**50**) [27], while of **48** using *Absidia pseudocylindrospora* (ATCC 24169), *Gliocladium viride* (ATCC 10097) and *Cunninghamella echinulata* (ATCC 8688a) afforded seven derivatives: **51**, **52**, 7β,15α-dihydroxy-18β-glycyrrhetinic acid (**53**), 15α-hydroxy-18β-glycyrrhetinic acid (**54**), 1α-hydroxy-18β-glycyrrhetinic acid (**55**) and 13β-hydroxy-7α,27-oxy-12-dihydro-18β-glycyrrhetinic acid (**56**), and the epimer of compound **53** on C-17 (**Figure 3**) [28].

Ginsenoside Rb1 (**61**) is the most predominant protopanaxadiol-type ginsenoside in *Panax* species (ginseng). Several microbial transformations of this substrate (Ginsenoside Rb1) have been accomplished with an ample and varied group of microorganisms, all of these having β-glucosidase activities.



48) R_1= R_2= R_3= R_4= R_5= R_6= H
50) R_1= βOH, R_2= R_3= R_4= R_5= R_6= H
51) R_1= R_2= βOH, R_3= R_4= R_5= R_6= H
52) R_1= R_2= R_4= R_5= R_6= H, R_3= OH
53) R_1= R_2= R_3= R_5= R_6= H, R_4= OH
54) R_1= αOH, R_2= R_3=R_4=R_5= R_6= H
57) R_1= R_2= R_3=R_4=R_5= H, R_6= OH
59) R_1= R_2= R_3= R_4= R_6= H, R_5= OH

49

5
6

60

Figure 3.
*Biotransformation products of 18β-glycyrrhetinic acid (***48***).*

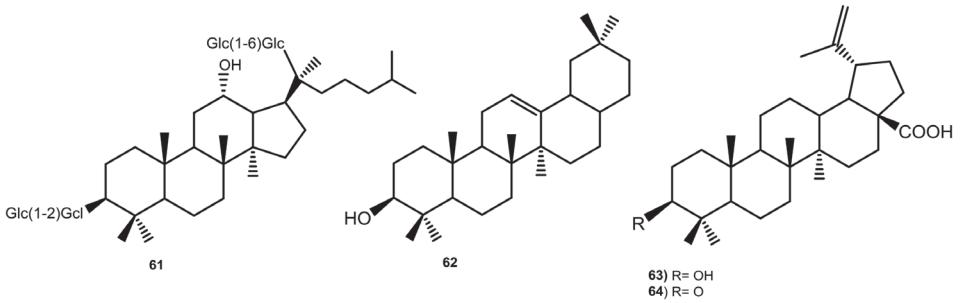


Figure 4.
*Triterpenic acid: ginsenoside Rb1 (**61**), oleanolic acid (**62**), betulinic (**63**) and betulonic acid (**64**).*

Deglycosylation appears to be the major transformation pathway, and the intermediate and the final hydrolysis products of **61** depended on the microorganisms used. The biotransformation of various triterpenes, such as **61–64**, has been described in the literature. For each triterpenoid, the transforming microorganism together with the type and site of the reaction catalyzed is given in **Table 2** (**Figure 4**) [29].

Biotransformation of oleanolic acid (**62**) with *Bacillus subtilis* (ATCC 6633) resulted in five more polar metabolites as 28-O-β-D-glucopyranosyl oleanic acid (**63**), 3β-O-β-D-glucopyranosyl oleanic acid (**64**), 3-O-(β-D-glucopyranosyl)-oleanic acid-28-O-β-D-glucopyranoside (**61**), 24-hydroxyl-oleanolic acid (**62**), and 3β-24-dihydroxy-olean-12-en-28-O-β-D-glucopyranosyl-oic acid (**63**), while echinocystic acid (**64**, 250 mg) was metabolized to three more polar metabolites as 28-O-β-D-glucopyranosyl echinocystic acid (**65**), 3-O-(β-D-glucopyranosyl)-echinocystic acid-28-O-β-D-glucopyranoside (**66**), and 24-hydroxyl-28-O-β-glucopyranosyl echinocytic acid (**67**), and then biotransformation of betulinic acid (**68**) contributed four metabolites as 28-O-β-D-glucopyranosyl betulinic acid (**69**), 3-O-(β-D-glucopyranosyl)-betulinic acid-28-O-β-D-glucopyranoside (**70**), 23-hydroxy-betulinic acid (**71**), and 23-hydroxy-28-O-D-β-glucopyranosyl betulinic acid (**72**). In this way there were two types of reactions in the biotransformation of triterpenic acids **58**, **64**, and **68**: hydroxylation and glycosylation [41]. Biotransformation of **58** by *C. muscae* yielded nine hydroxylated and glycosylated metabolites. The specific hydroxylation (7β, 15α, and 21β) was main reaction type. In addition, the selective glycosylation at C-28 was another main reaction type. It was also observed that the 3β-OH group was selectively dehydrogenated into carbonyl group [42].

A C-3 oxidized derivative of oleanolic acid **73** (3-oxoolean-12-en-28-oic acid) was transformed by the *Chaetomium longirostre* (RF-1095) into 4-hydroxy-3,4-seco-olean-12-ene-3,28-dioic acid (**74**) and the corresponding 21-hydroxylated derivative (**75**). Analogous ring-A cleavage oxidation reactions have been observed in the biotransformation of triterpenoid substrates with the fungi *Septomyxa affinis* ATCC 6737 and *Glomerella fusarioides* ATCC 9552. (**Figure 5**) [4, 43].

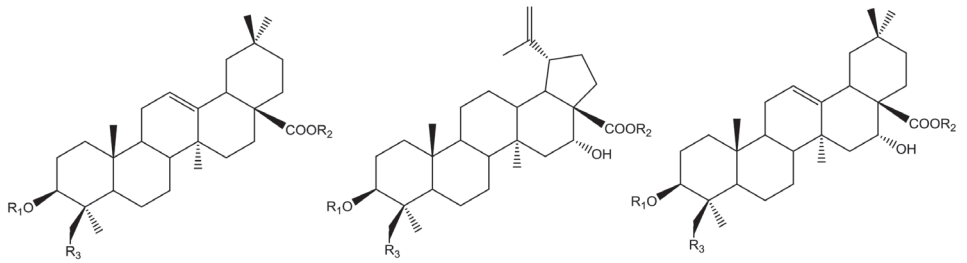
2.3 Steroidal saponins

Diosgenin [(25R)-spirost-5-en-3β-ol, **76**] is an important natural starting material in the pharmaceutical industry to produce steroid drugs and hormones since the last century. In recent years, a wide array of new biological activities of **76** has been disclosed. Diosgenin was subjected to several structural modification studies to secure new derivatives via microbial transformation. Several microorganisms have been found to be capable of degrading **76**, *Bacillus megaterium*, *Corynebacterium mediolanum*, *Mycobacterium fortuitum*, *M. phlei*, *Nocardia rhodochrous,* and *F. solani*.

Triterpenoid	Microorganism	Reaction	Reference
Ginsenoside Rb_1 (**61**)	*A. niger* (KTC 6909)	Deglycosylation at the C-3 and C-20 sites	[30]
	A. niger (AS 3.1858)	Deglycosylation at the C-3 and C-20 sites	[30]
	A. usamii (KTC 6956)	Deglycosylation at the C-3 and C-20 sites	[30]
	F. sacchari	Deglycosylation at the C-3 and C-20 sites	[31]
	P. oxalicum	Deglycosylation at the C-3 site	[32]
	C. lunata (AS 3.1109)	Deglycosylation at the C-20 site, hydration $\Delta^{24(25)}$ Formation of tertiary alcohol	[33]
	R. stolonifer (AS 3.822)	Deglycosylation at the C-3 and C-20 sites	[33]
Oleanolic acid (**62**)	*C. blakesleeana*	Diverse hydroxylation at the C-1β, C-7β, C-13β sites	[4]
	F. lini	Dehydrogenation C-13 and C-18. oleanderolide formation	[4]
	P. chrysogenum	Hydroxylation on C-21. Oxidation of the hydroxyl group in C-3	[4]
	C. phomoides	Hydroxylation in C-6β	[4]
	A. ochraceus (NG 1203)	Hydroxylation in C-11α	[4]
	Chaetomium longirostre	Oxidative ring A cleavage, hydroxylation at the C-21β sites	[34]
	Nocardia sp. (NRRL 5646)	Methyl esterification of the C-28 carboxyl group	[4]
	R. miehei (CECT 2749)	Hydroxylation of the C-7β, C-15α and C-30 sites Deshidrogenation $\Delta^{9(11)}$	[35]
Betulinic acid (**63**)	*B. megaterium* (ATCC 14581)	Dehydrogenation of the C-3 secondary alcohol group, hydroxylation at the C-6α and C-7β sites	[36]
	B. megaterium (ATCC 13368)	Dehydrogenation of the C-3 secondary alcohol group, hydroxylation at the C-7β and C-15α sites	[37]
	C. elegans (ATCC 9244)	Hydroxylation at the C-1β and C-7β sites	[4]
	Cunninghamella sp.	Introduction of a β-glucopyranosyl at the C-28 carboxylic acid group	[38]
Betulonic acid (**64**)	*B. megaterium* (ATCC 13368)	Ketone α-hydroxylation at the C-2 site	[37]
	Ch. longirostris	Oxidative ring A cleavage, hydroxylation, decarboxylation	[39]
	C. lunata (ATCC 13432)	Hydroxylations at the C-7β and (or) C-15β sites	[4, 40]

Table 2.
*Examples of biotransformed triterpenes (**61–64**) with different microorganisms.*

Three major products were accumulated, diosgenone (**77**), 1-dehydrodiosgenone (**78**), androst-4-en-3,17-dione (AD, **79**), and androsta-1,4-diene-3,17-dione (ADD, **80**) (**Table 3**) [44, 45]. In addition, two side-chain cleavage intermediates of **76** were produced by *C. elegans* and *Aspergillus nidulans*. Microbial transformation



62) R_1= R_2= R_3 = H
63) R_1= R_3= H, R_2 = Glc
64) R_1= Glc, R_2= R_3 = H
65) R_1= R_2= Glc, R_3 = H
66) R_1= R_2= H, R_3 = OH
67) R_1= H, R_2= Glc, R_3 = OH

63) R_1= R_2= R_3 = H
72) R_1= R_3= H, R_2 = Glc
73) R_1= R_2= Glc, R_3 = H
74) R_1= R_2= H, R_3 = OH
75) R_1= H, R_2= Glc, R_3 = OH

68) R_1= R_2= R_3 = H
69) R_1= R_3= H, R_2 = Glc
70) R_1= Glc, R_2= R_3 = H
71) R_1= H, R_2= Glc, R_3 = OH

Figure 5.
*Biotransformation products of oleanolic acid (**62**), echnocystic acid (**68**) and betulinic acid (**63**).*

Fungi	Diosgenona (77)	AD (79)	ADD (80)	Progesterone (102)	16-AD
A. nidulans	++	++	++	++	
C elegans	++			++	
F. solani	++				++
Rhizopus sp.	++	++	++		

AD androst-4-ene-3,17-dione; ADD androsta1,4-diene-3,17-dione; 16-AD androst-4-ene-3,16-dione.

Table 3.
*The ability of different fungi to transform diosgenin (**76**).*

of **76** using white-rot fungus *Coriolus versicolor* afforded eight polyhydroxylated steroids, 7β-hydroxydiosgenin (**81**), (25R)-spirost-5-en-3β,7β,21-triol (**82**), (25R)-spirost-5-en-3β,7β,12β-triol (**83**), (25R)-spirost-5-en-3β,7α,15α,21-tetraol (**84**), (25R)-spirost-5-en-3β,7β,12β,21-tetraol (**85**), (25R)-spirost-5-en-3β,7α,12β,21-tetraol (**86**), and (25R)-spirost-5-en-3β,7β,11α,21-tetraol (**87**). The 3β-hydroxyl group and double bond in the B-ring of 76 were found to be important structural determinants for their activity [46].

Microbial transformation of **76** using *Cunninghamella blakesleeana* AS 3.970 afforded polyhydroxylated derivatives, such as (25R)-spirost-5-en-3β,7α,12β-triol (**88**), (25R)-spirost-5-en-3β,7α,12β,15α,21-pentaol (**89**), (25R)-spirost-5-en-3β,7α,12β,18-tetraol (**90**), (25R)-spirost-5-en-3β,7α,12β,15α-tetraol (**91**), (25R)-spirost-5-en-3β,7α,11α,21-tetraol (**92**), (25R)-spirost-5-en-3β,7β,15α,21-tetraol (**93**), and (25R)-spirost-5-en-3β,7β,12β,18-tetraol (**94**) [47], specifically, the hydroxylation, ketonization, and methoxylation by *Cunninghamella blakesleeana*, *C. elegans, Helicostylum piriforme*, and *Streptomyces virginiae*, at C-7, C-9, C-11, C-12, and C-25 positions of **76**. Biotransformation of **76** by *Syncephalastrum racemosum* afforded (25R)-spirost-5-en-3β,7α,9α-triol (**95,** 1%), (25R)-spirost-5-en-3β,9α,12α-triol-7-one (**96**, 2%), (25R)-spirost-5-en-3β,9α-diol-7,12-dione (**97**, 1.5%), (25R)-spirost-4-en-9α,12β,14α-triol-3-one (**98**, 0.66%), and (25S)-spirost-4-en-9α,14α,25β-triol-3-one (**99**, 0.66%) [48]. *C. echinulata* (CGMCC3.2716) metabolized **76** to afford **81** (0.9%), **83** (7.7%), (25R)-spirost-5-en-3β,7β-diol-11-one (**100**, 7, 1.5%), and (25R)-spirost-5-en-3β,7β,11α-triol (**101**, 6.2%) (**Figure 6**) [49].

2.4 Steroids

Microorganisms are able to hydroxylate steroids in different positions C-1 to C-21. These represent the most widespread type of steroid bioconversion carried

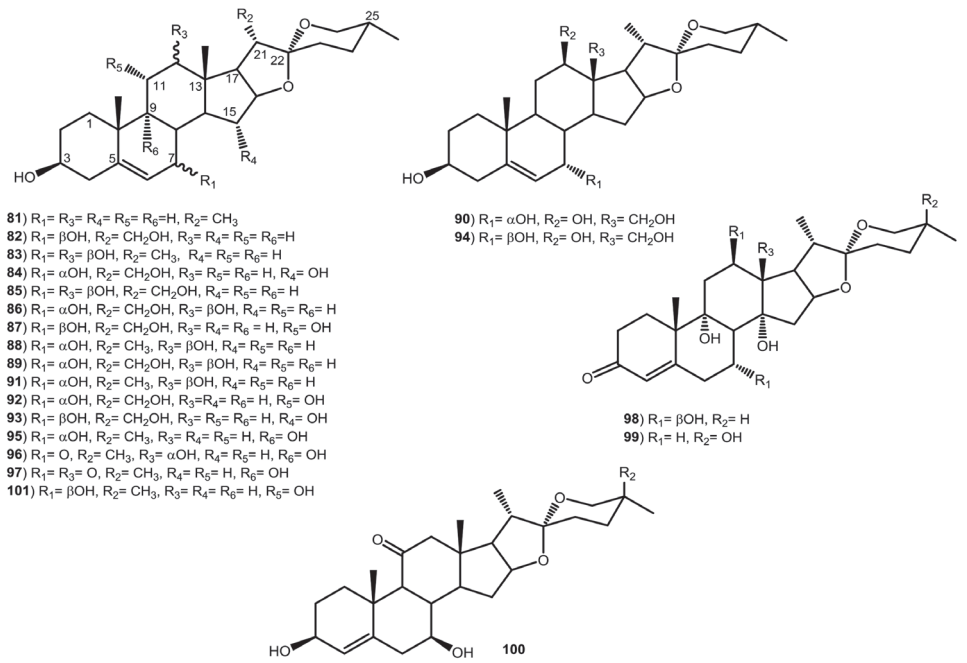


Figure 6.
*Biotransformation products of diosgenin (***76***).*

out by fungi. The commercialized microbial process in the steroid field was in the production of 11α-hydroxyprogesterone. This process was realized for the first time by Peterson and Murray (1952), which patented this process of 11α-hydroxylation of progesterone (**102**) by *Rhizopus* species [50]. Microbial hydroxylation of **102** by *A. griseola* produced two hydroxylated pregnane identified as 6β,14α-dihydroxyprogesterone (**103**) and 7α,14α-dihydroxyprogesterone (**104**). *R. pusillus* produced 6β,11α-dihydroxyprogesterone (**105**) with excellent yield (65.5%) and 7α,14α-dihydroxyprogesterone (**106**) (**Figure 7**) [51].

Industry, which is carried by different microorganisms, such as different species of *Curvularia* spp., *Cunninghamella* spp. and fungi *Trichoderma hamatum*, *Cochliobolus lunatus*. Structural transformation of steroidal compounds through microorganisms has emerged as an important application in the steroidal drug industry. Microbial conversions of steroids generally involve dehydrogenation, esterification, halogenation, isomerization, methoxylation, and side-chain modification of steroidal skeleton. Recently, *Mucor circinelloides lusitanicus* transformed 5-en-3β-ol steroids (**108** and **109**) into di- and trihydroxy products. The compound

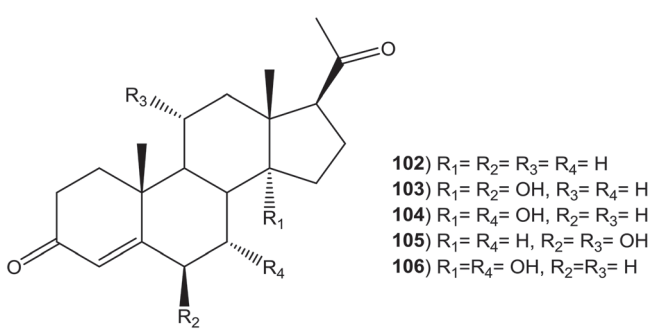
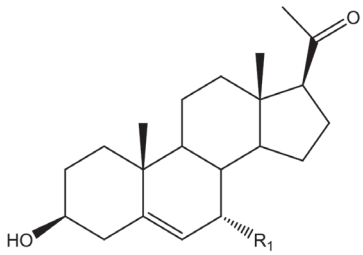
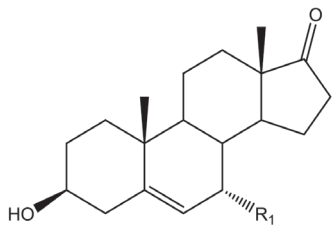


Figure 7.
*Biotransformation products of progesterone (***102***).*



108) pregna-5-en-20-one, R_1= H
110) 3β,7α,11α-trihydroxypregna-5-en-20-one, R_1= OH



109) androst-5-en-17-one, R_1= H
111) 3β,7α-dihydroxi-androst-5-en-17-one, R_1= OH

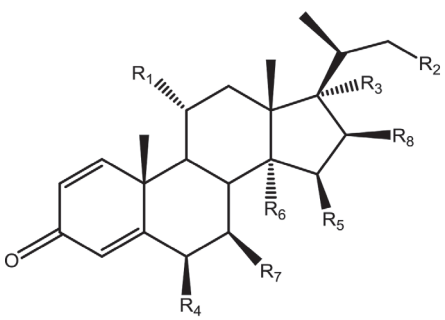
Figure 8.
Biotransformation products of 5-en-3β-ol steroids.

108 yielded 3β,7α,11α-trihydroxypregna-5-en-20-one (**110**, 46.4%), and **109** afforded **111** (3β,7α-dihydroxyandrost-5-en-17-one, 43.6%) (**Figure 8**) [52].

Microbial transformation of (20S)-20-hydroxymethylpregna-1,4-dien-3-one (**112**) is by four filamentous fungi, *Cunninghamella elegans* (**113–119**), *Macrophomina phaseolina* (**115**, **117**, **120–122**), *Rhizopus stolonifer* (**113**, **123**), and *Gibberella fujikuroi* (**115–117**, **123**). These metabolites were obtained as a result of biohydroxylation of **112** at C-6β, 7β, 11α, 14α, 15β, 16β, and 17α positions (**Figure 9**) [53].

The 11α-, 11β-, 15α, and 16α-hydroxylations are currently established processes in the steroid industry mainly for the production of adrenal cortex hormones and their analogues. 11α-, 11β-, and 16α-hydroxylations are usually performed using *Rhizopus* spp. or *Aspergillus* spp., *Curvularia* spp. or *Cunninghamella* spp. and *Streptomyces* spp., respectively (**Figure 10**) (**Table 4**) [54].

Boldenone (**124**) is an important steroid hormone drug which is the derivative of testosterone. Biotransformation of **124** by *Arthrobacter simplex* and recombinant *Pichia pastoris* with 17β-hydroxysteroid dehydrogenase from *Saccharomyces cerevisiae* produces BD (**124**) from androst-4-ene-3,17-dione (**79**, AD) efficiently [65]. Many microorganisms such as *Mucor racemosus*, *Nostoc muscorum*, and *Arthrobacter oxydans* can utilize androst-1,4-diene-3,17-dione (**80**, ADD) as substrate to produce testosterone through 17β-carbonyl reduction reactions (**Table 5**). The ability of



112) R_1= R_2= R_3= R_4= R_5= R_6= R_7= R_8= H
113) R_1= OH, R_2= OAc, R_3= R_4= R_5= R_6= R_7= R_8= H
114) R_1= R_4= R_5= R_6= R_7= R_8= H, R_2= R_3= OH
115) R_1= R_2= OH, R_3= R_4= R_5= R_6= R_7= R_8= H
116) R_1= R_2= R_4= OH, R_3= R_5= R_6= R_7= R_8= H
117) R_1= R_2= R_5= OH, R_3= R_4= R_6= R_7= R_8= H
118) R_1= R_2= R_3= OH, R_4= R_5= R_6= R_7= R_8= H
119) R_1= R_4= R_7= R_8= H, R2= R3= R_5= R_6= OH
120) R_1= R_3= R_4= R_5= R_6= R_8= H, R_2= R_7= OH
121) R_1= R_3= R_4= R_6= R_7= R_8= H, R_2= R_5= OH
122) R_1= R_2= R_3= R_6= R_8= H, R_2= R_5= R_7= OH
123) R_1= R_3= R_4= R_5= R_6= R_7= H, R_2= R_8= OH

Figure 9.
*Biotransformation products of (20S)-20-hydroxymethylpregna-1, 4-dien-3-one (**112**).*

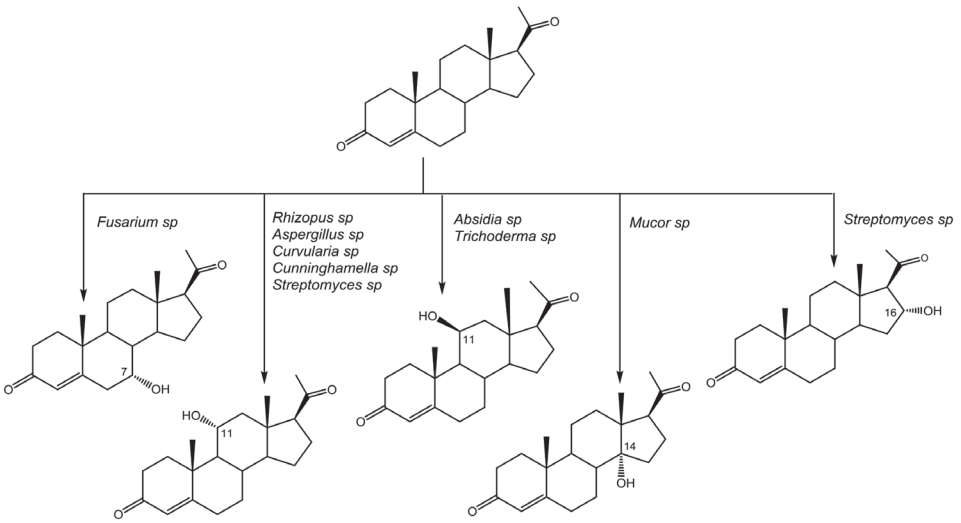
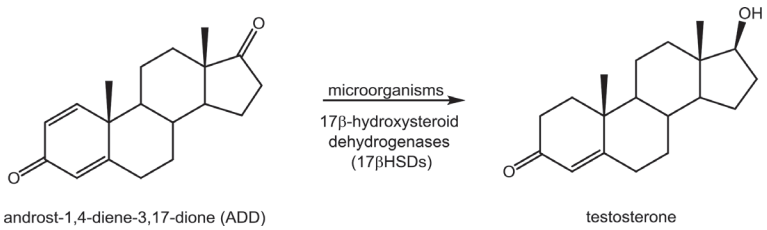


Figure 10.
*The ability of different microorganism to transform progesterone (***102***).*

Hydroxylation sites	Microorganisms	Applications	Reference
C-7α	*Fusarium* sp., *Gibberella* sp., *Nigrospora* sp., *Acremonium* sp., *Phycomyces* sp.	Production of bile acids and drugs for neuropsychiatry and immunology	[55, 56]
C-7β	*Mortierella* sp.	Obtaining drugs for prostate cancer	[56, 57]
11β	*Curvularia* sp., *Absidia* sp., *Cunninghamella* sp., *Trichoderma* sp., *Cochliobolus* sp.	Obtaining anti-inflammatory drugs, like hydrocortisone, prednisone acetate, dexamethasone	[56–60]
11α	*Aspergillus* sp., *Rhizopus* sp.	Obtaining of anti-inflammatory, immunosuppressive, anti-allergic drugs, and production of contraceptive drugs	[56, 61]
14α	*Mucor* sp.		[56]
15β	*Bacillus* sp.		[56, 62]
16α	*Streptomyces* sp.		[63, 64]

Table 4.
Some examples of steroid hydroxylation reactions promoted by microorganisms and their applications.

microorganisms to reduce 17-keto- to 17β-hydroxysteroids was evidenced for a wide variety of substrates and microorganisms of different taxonomy: bacteria, fungi, and yeast [54, 56, 57, 66].



Fungi		Yeast	Bacteria
Actinomucor elegans	*Fusarium culmorum*	*Candida albicans*	*B. stearothermophilus*
Agaricus silvaticus	*F. oxysporum* var. *cubense*	*C. pelliculosa*	*Bacteroides fragilis*
A. pantherina	*F. solani*	*C. pseudotropicalis*	*Brevibacterium sterolicum*
A. spissa	*Mucor piriformis*	*C. robusta*	*Clostridium paraputrificum*
Armillaria mellea	*M. spinosus*	*C. tropicalis*	*Comamonas testosteroni*
Corticium centrifugum	*P. chrysogenum*	*C. utilis*	*Lactobacillus bulgaricus*
Fusarium spp.	*P. crustosum*	*Cryptococcus albidus*	*Mycobacterium* spp.
Gibberella saubinetti	*P. blakesleeanus*	*C. laurentii*	*B. stearothermophilus*
Mucor spp.	*R. stolonifer*	*C. tsukubaensis*	*Bacteroides fragilis*
Penicillium spp.	*Septomyxa affinis*	*Debaryomyces hansenii*	*Brevibacterium sterolicum*
Aphanocladium album	*T. piriforme*	*D. kloeckeri*	*Clostridium paraputrificum*
Aspergillus chevalieri	*Trichoderma viride*	*D. nicotianae*	*Comamonas testosteroni (syn. Pseudomonas testosteroni)*
A. flavus	*Zygodesmus* sp.	*D. subglobosus*	*Lactobacillus bulgaricus*
A. oryzae		*D. vini*	*Pediococcus cerevisiae*
A. tamarii		*Hansenula anomala*	*Sarcina lutea*
B. obtusa		*H. califórnica*	*Staphylococcus aureus*
C. aphidicola		*H. schnegii*	*Streptomyces globisporus*
Ceratocystis paradoxa		*H. suaveolens*	*S. sphaeroides*
C. lunatus		*Kloeckera jensenii*	*S. viridochromogenes*
Colletotrichum musae		*Saccharomyces carlsbergensis*	*S. hydrogenans*
C. radicicola		*S. cerevisiae*	*S. lavendulae*
Exophiala jeanselmei var. *lecaniicorni*		*S. fragilis*	
		S. lactis	
		S. oviformis	
		S. turbidans	
		S. validus	
		Pichia farinosa	
		P. membranaefaciens	
		Torulopsis spp.	
		Hortaea werneckii	
		Phaeotheca triangularis	
		P. herbarum	
		P. ostreatus	
		Rhodotorula aurantiaca	
		R. mucilaginosa	

Table 5.
Reduction of the C-17 carbonyl group of steroids by (17βHSDs) different microorganisms.

The oxidation of 17β-hydroxyl group was observed along with hydroxylation of steroids at C_5 (*Penicillium crustosum, P. chrysogenum*), C_6 (*Bacillus stearothermophilus, B. obtusa, P. blakesleeanus*), C_7 (α/β) (*A. coerulea*, *Botrytis cinerea*, *B. obtusa*, *P. blakesleeanus*, *Rhizopus stolonifer*), C_{10} (*Absidia glauca*), C11 (α/β) (*A. coerulea*, *B. obtusa*, *Cephalosporium aphidicola*, *R. stolonifer*), C_{12} (*A. glauca*, *B. obtusa*), C_{14} (*Bacillus* sp.), and C15 (*A. glauca*, *Aspergillus fumigatus*, *B. obtusa*) [54, 56, 57, 67–69]. The biotransformation of **79** with different microorganisms is shown. Compound **79** is an endogenous weak androgen steroid hormone and intermediate in the biosynthesis of estrone and of testosterone from dehydroepiandrosterone (DHEA) [70]. DHEA is an endogenous steroid hormone. It functions as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids. Various microorganisms have had the ability to biotransform steroidal compounds such as AD (**79**) [54], DHEA (**125**)

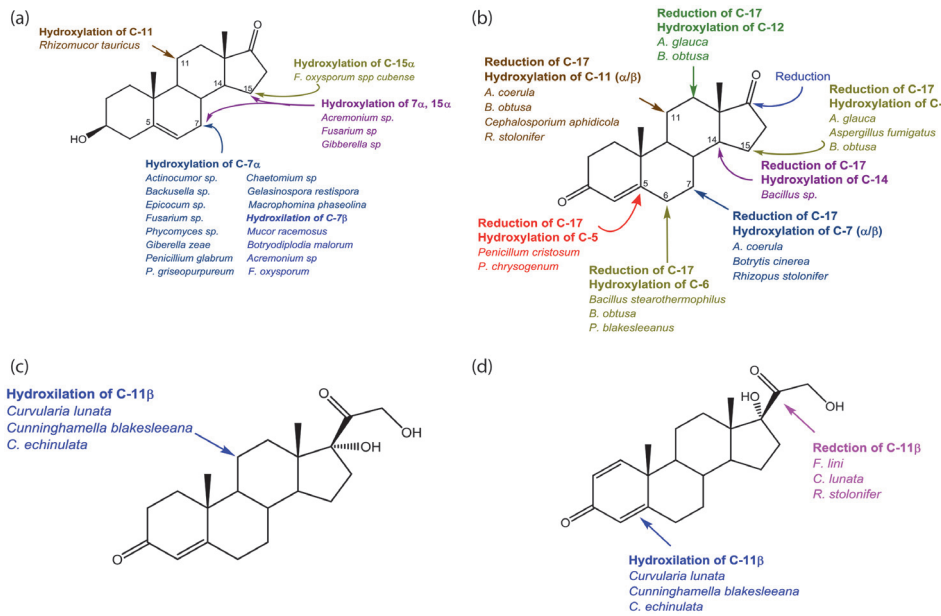


Figure 11.
*The ability of different fungi to transform DHEA (**125**), testosterone, cortexolone (**126**) and prednisone (**127**). (a) Hydroxilation of 3β-hydroxy-5-androsten-17-one (DHEA) by various microorganisms. (b) Reduction of C-17 and hydroxilation of testosterone by various microorganisms. (c) Hydroxylation of cortexolone (**123**) by various microorganisms. (d) Reduction and hydroxylation of prednisone (**126**) microorganisms.*

[54, 55, 70–73, 76, 77, 80, 81], testosterone [54, 55, 74–76, 81], cortexolone (**126**) [78, 79], and prednisone (**127**) (**Figure 11a–d**) [54, 55, 82].

2.5 Diterpene

Sclareolide (**128**) is a natural product isolated from several plant species which displays phytotoxic and cytotoxic activities against several human tumor cells lines. This compound has also been used as starting material for the synthesis of various bioactive products. Regarding the biotransformation of the **128** with different microorganisms, mono- (**130**, **131**, **135**, **140–142**) and dihydroxylation (**132–134**, **136**, **139**, **143**, **146**), oxidation (**129**, **144**), hydroxylation/oxidation (**145**), epimerization (**137**), and cyclization (**138**) products have been obtained [83]. The microbial transformation of **128** by *Curvularia lunata* yielded 3-ketoesclareolide (**129**), 1β-hydroxysclareolide (**130**), 3β-hydroxysclareolide (**131**), 1α,3β-dihydroxysclareolide (**133**), and 1β,3β-dihydroxysclareolide (**134**) [84]. The incubation of **128** with *Cunninghamella elegans* afforded **129**, **131**, **133,** and **135–137** [85]. *C blakesleeana* metabolized **128** to afford **129**, **135**, **134**, and **138–140**. Biotransformation of **128** with *C. echinulata* yielded 5-hydroxysclareolide (**141**) and 7β-hydroxysclareolide (**142**) [86]. Fermentation of **148** with *A. niger* using a nutrient-rich culture medium yielded **141** and **144–146** (**Figure 12**) [83].

2.6 Flavonoids

As most important phytochemicals in food, the dietary flavonoids exert a wide range of benefits for human health. Recent researches have explored diverse biological and pharmacological activities of natural flavonoids—antioxidant activity, anti-inflammatory activity, anti-Alzheimer's disease, antibacterial activity, antifungal activity, anti-HIV activity, anticoagulant activity, antileishmanial activity, and

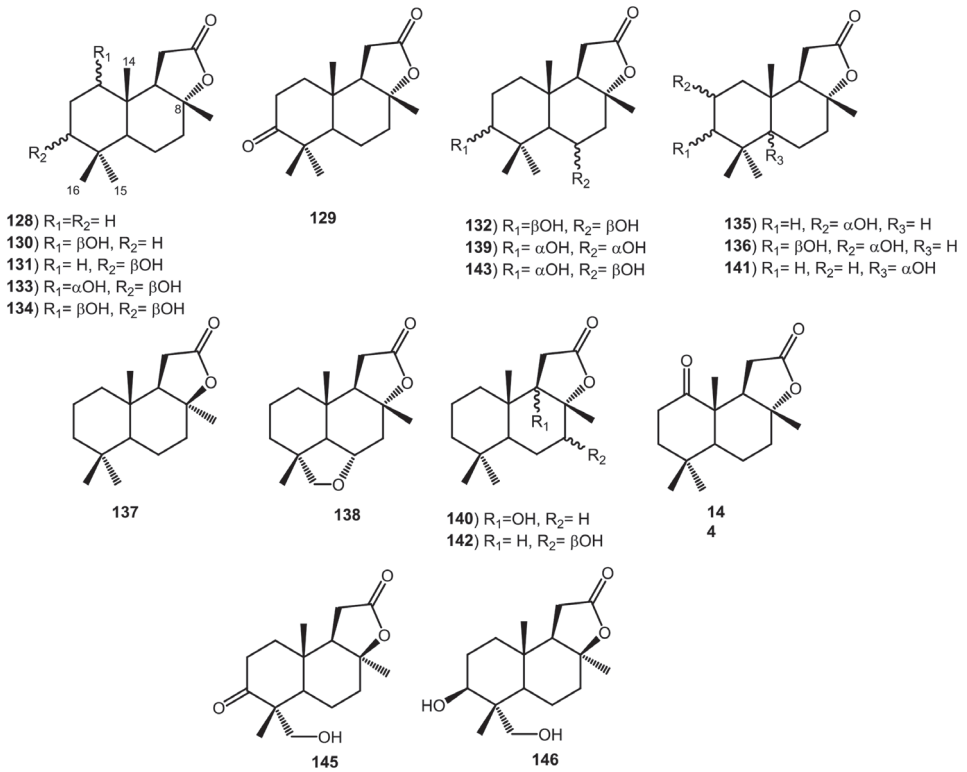


Figure 12.
*Biotransformation products of sclareolide (**128**).*

anti-obesity activity [87–91]. Microbial biotransformation strategies for production of flavonoids have attracted considerable interest because they allow yielding novel flavonoids, which do not exist in nature.

The main reactions during microbial biotransformation are hydroxylation, dehydroxylation, O-methylation, O-demethylation, glycosylation, deglycosylation, dehydrogenation, hydrogenation, C ring cleavage of the benzo-γ-pyrone system, cyclization, and carbonyl reduction. *Cunninghamella*, *Penicillium*, and *Aspergillus* strains are very popular to biotransform flavonoids, and they can perform almost all the reactions with excellent yields (**Figure 13**). Isoflavones are usually hydroxylated at the C-3′ position of the B ring by microorganisms. Chalcones **147-152** were regioselectively cyclized to flavanones (**Figure 14**). Hydrogenation of flavonoids was only reported on transformation of chalcones to dihydrochalcones (**Figure 14**) [92, 93].

Aspergillus niger is one of the most applied microorganisms in the flavonoids' biotransformation; for example, *A. niger* can transfer flavanone to flavan-4-ol, 2′-hydroxydihydrochalcone, flavone, 3-hydroxyflavone, 6-hydroxyflavanone, and 4′-hydroxyflavanone. The hydroxylation of flavones by microbes usually happens on the ortho position of the hydroxyl group on the A ring and C-4′ position of the B ring, and microbes commonly hydroxylate flavonols at the C-8 position. Natural flavonoids, such as naringenin (**166**), hesperetin (**167**), chrysin (**168**), apigenin (**169**), and luteolin (**170**) were subjected to microbiological transformations by *Rhodotorula glutinis* (KCh 735). Yeast was able to regioselectively C-8 hydroxylate **167**, **168, 169**, and **170** to generate **171** (17%), **172** (31%), **173** (12.9%), and **174** (25%), respectively. Naringenin (**166**) was transformed to carthamidin (**175**) and isocarthamidin (**176**) in a ratio of 1:19, respectively (**Figure 15**) [94].

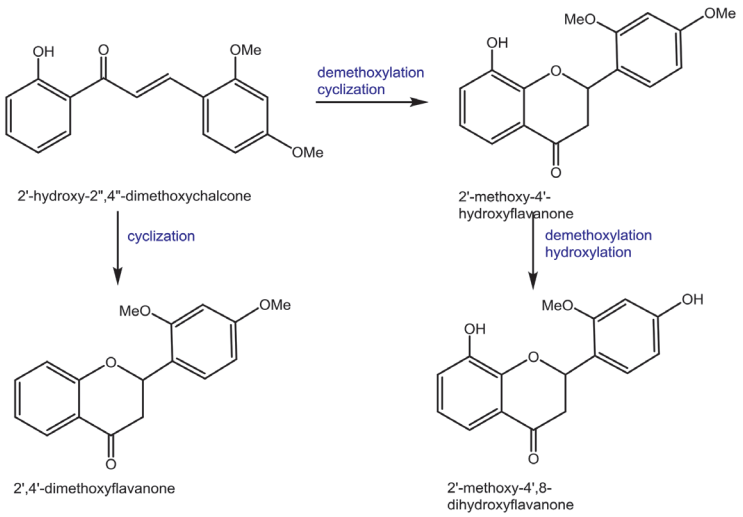
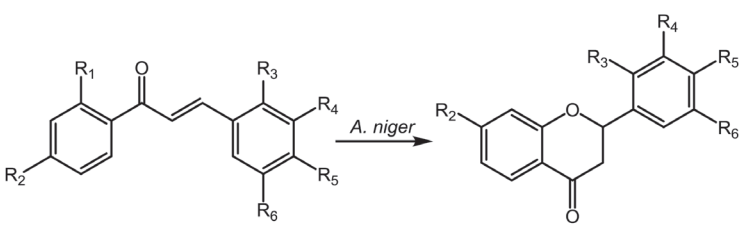


Figure 13.
The main reactions during biotransformation of chalcone whit microorganisms.

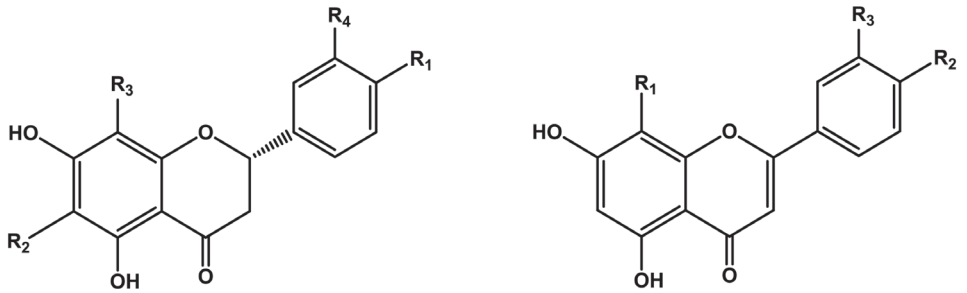
The microorganisms tend to hydroxylate flavanones at the C-5, 6, and 4′ positions; however, for prenylated flavanones, dihydroxylation often takes place on the $\Delta^{4(5)}$ double bond on the prenyl group (the side chain of A ring), although cyclization of the prenyl group to dihydrofurane derivatives is rather common biotransformation pathway of prenylated flavonoids. Prenylated flavanones are a unique class of naturally occurring flavonoids characterized by the presence of a prenylated side chain (prenyl, geranyl) in the flavonoid skeleton [95]. The prenyl chain generally refers to the 3,3-dimethylallyl substituent (3,3-DMA), geranyl and lavandulyl. It is proposed that the prenyl-moiety makes the backbone compound more lipophilic, which leads to its high affinity with cell membranes. The prenylation brings the flavonoids with enhancement of antibacterial, anti-inflammatory, antioxidant, cytotoxicity, larvicidal, as well as estrogenic activities. **Figure 16** demonstrated



147) R_1= OH, R_2= H, R_3= OMe, R_4= H, R_5= OMe, R_6= H
148) R_1= OH, R_2= H, R_3= OMe, R_4= OMe, R_5= H, R_6= H
149) R_1= OH, R_2= H, R_3= H, R_4= OMe, R_5= OMe, R_6= H
150) R_1= OH, R_2= H, R_3= H, R_4= OMe, R_5= H, R_6= OMe
151) R_1= OH, R_2= OMe, R_3= OMe, R_4= OMe, R_5= H, R_6= H
152) R_1= OH, R_2= OMe, R_3= OMe, R_4= H, R_5= OMe, R_6= H

153) R_1=H, R_2= H, R_3= OMe, R_4= H, R_5= OMe, R_6= H
154) R_1= H, R_2= H, R_3= OMe R_4=H, R_5= OH, R_6=H
155) R_1=O, R_2= H, R_3= OMe, R_4= H, R_5= OH, R_6= H
156) R_1= H, R_2= H, R_3= OMe R_4= OMe, R_5= H, R_6= H
157) R_1= H, R_2= H, R_3= OMe, R_4= OH, R_5= H, R_6= H
158) R_1= H, R_2= H, R_3= H, R_4= OMe, R_5= OMe, R_6= H
159) R_1= H, R_2=H, R_3=H, R_4= OH, R_5= OH, R_6= H
160) R_1= H, R_2= H, R_3= H, R_4= OMe, R_5= H, R_6= OMe
161) R_1=H, R_2= H, R_3= H, R_4= OH, R_5= H, R_6= OMe
162) R_1= H, R_2= OMe, R_3= OMe, R_4= OMe, R_5= H, R_6= H
163) R_1=H, R_2= OMe, R_3= OMe, R_4= OH, R_5= H, R_6= H
164) R_1=H, R_2= OMe, R_3= OMe, R_4= H, R_5= OMe, R_6= H
165) R_1=H, R_2= OMe, R_3= OH, R_4= H, R_5= OMe, R_6= H

Figure 14.
Biotransformation products obtained from biotransformation of chalcones **147-152** *with A. niger.*



166) R_1= OH, R_2= R_3= R_4 = H
167) R_1=R_2= OH, R_3= R_4= H
171) R_1= R_3= OH, R_2= R_4= H
175) R_1= OMe, R_2= R_3= H, R_4= OH
176) R_1= OMe, R_2= H, R_3= R_4= OH

168) R_1= R_2= R_3= H
169) R_1= OH, R_2= R_3= H
170) R_1= R_3= H, R_2= H
172) R_1= R_2= OH, R_3= H
173) R_1= H, R_2= R_3= OH
174) R_1= R_2= R_3= OH

Figure 15.
*Biotransformation products of flavanone (***166***,* **167***) and flavone (***168–170***).*

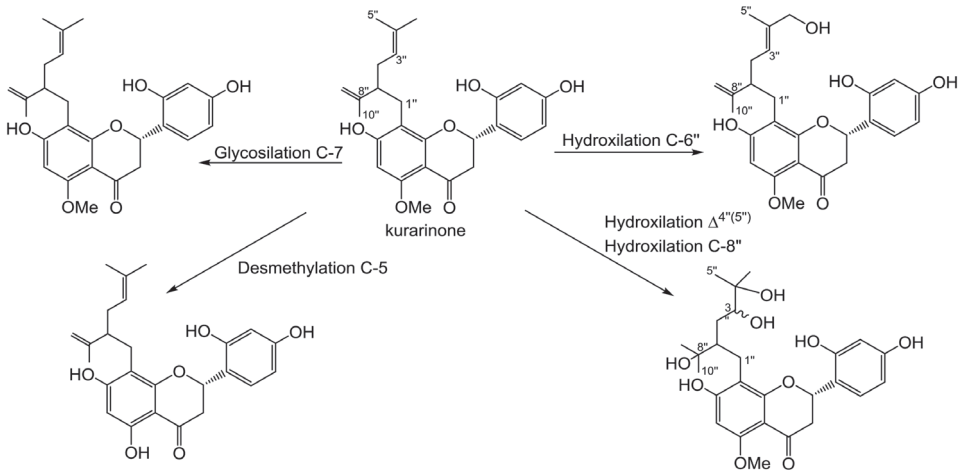


Figure 16.
*Microbial biotransformation of kurarinone (***177***) using C. echinulate and C. militaris.*

the microbial biotransformation of kurarinone (**177**) using *C. echinulata* and *C. militaris* [96, 97].

Incubation of *Absidia coerulea* (AM93) with prenylnaringenin (**178**) led to metabolite **179** (8-prenylnaringenin 7-O-β-D-glucopyranoside, 49.3%), while *B. bassiana* transformed **178** into **180** (8-prenylnaringenin 7-O-β-D-4″-O-methyl-glucopyranoside, 32.9%); the metabolites **179** and **180** originated in Sabouraud medium. In the absence of glucose in the culture of *A. coerulea*, the sulfation of substrate **178** (8-prenylnaringenin-7-sulfate, **181**, 31.1%) occurs, while *B. bassiana* into the same product (**180**). The capacity of some fungi—*Cunninghamella elegans*, *Streptomyces fulvissimus*, *Mucor ramannianus*, and *B. bassiana*—in the sulfation of certain phenolic compounds has been reported (**Figure 17**) [98].

Regioselective glycosylation of biologically active flavonoid aglycones catalyzed by microorganisms is an interesting and desired reaction, which significantly increases the water solubility of the compound and, therefore, may improve bio-availability of flavonoids. *Absidia glauca* AM177, *A. coerulea* AM93, *Rhizopus nigricans* UPF701, *Beauveria bassiana* AM278, and *B. bassiana* AM446 are able to conjugate

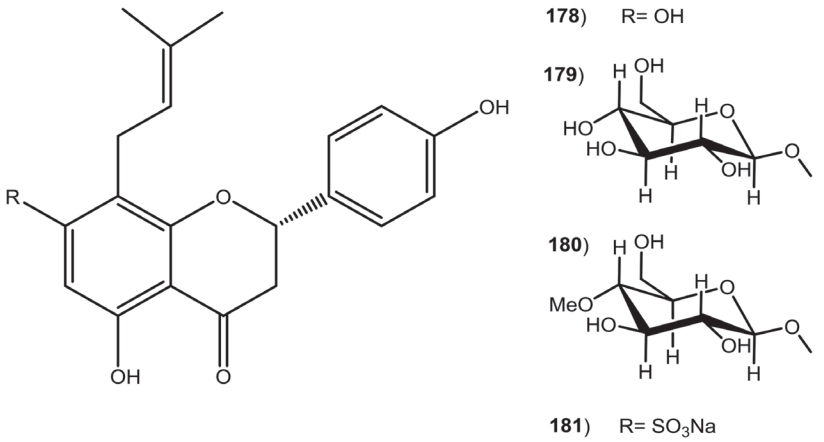


Figure 17.
*Biotransformation products of prenylnaringenin (**178**).*

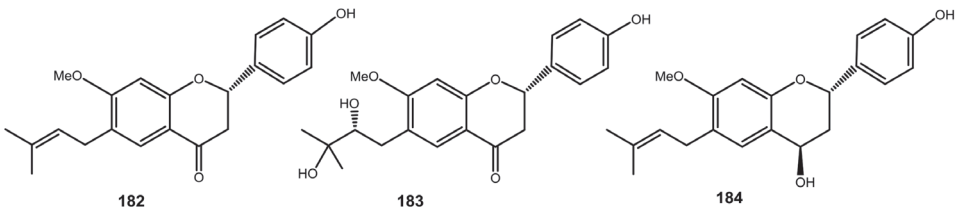


Figure 18.
*Biotransformation products of bavachinina (**182**).*

sugar moiety to chalcones, flavanones, and isoflavanones with high regioselectivity. Therefore, it is possible to use *Beauveria* and *Absidia* for the microbial transformation of simple or prenylated flavonoids by glycosidation reactions [97, 99].

Bavachinin (**182**) is one kind compound of flavanones and isolated from the aerial parts and dried fruits of *Psoralea corylifolia*, and bavachinin displays a broad range of biological activities, such as antioxidant, antibacterial, antifungal, anti-inflammatory, antitumor, anti-pyretic, and analgesic properties [100, 101]. Bavachinin (**182**) was subject to biotransformation by cultured cells of *A. flavus* (ATCC 30899); *C. elegans* (CICC 40250) afforded the same product **183** [(S)-6-((R)-2,3-dihydroxy-3-methylbutyl)-2-(4-hydroxyphenyl)-7-methoxychromen-4-one]. On the other hand, one major product **184** [(2S,4R)-2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)-chromen-4-ol] was obtained by *P. raistrickii* (ATCC 10490) by the reduction at the position of ketone group of the C-ring (**Figure 18**) [102].

The biotransformation of xanthohumol (**185**), a prenylated chalcone isolated from hops by selected fungi, *Absidia coerulea* (AM93), *Rhizopus nigricans* (UPF701), *Mortierella mutabilis* (AM404), and *Beauveria bassiana* (AM446), was investigated. The incubation of *A. coerulea* with **185** resulted in the isolation of xanthohumol 4′-O-β-D-glucopyranoside (**186**, 29%). This metabolite was also produced by *R. nigricans* (**186**, 14.2%). Biotransformation of **185** with *B. bassiana* and *M. mutabilis* yielded xanthohumol 7-O-β-D(4‴-O-methyl)-glucopyranoside (**187**, 23%) and isoxanthohumol 7-O-β-glucopyranoside (**188**, 49%), respectively (**Figure 19**) [103]. The compounds **188** (9.3%) and **186** (12%) were also observed as products of **185** transformation by *Cunninghamella elegans* [104]. Another way to obtain **188** is by the transformation of isoxanthohumol (**189**, 61.6%) with *Absidia glauca*; although

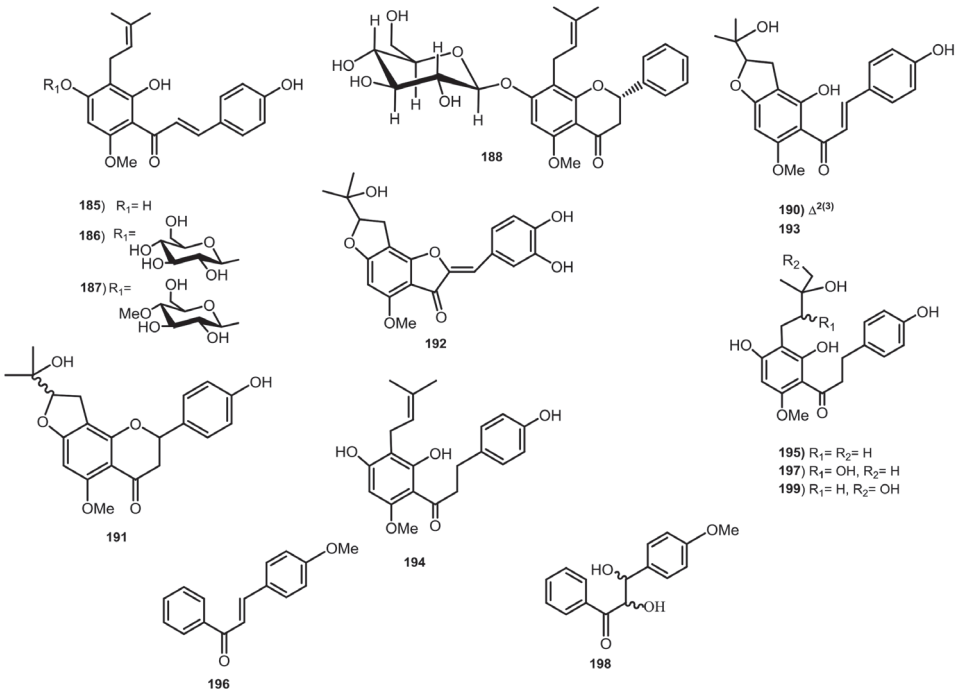


Figure 19.
*Biotransformation products of xanthohumol (***185***).*

the efficiency of this process was high (61.6% yield), it required the chemical isomerization of **185** to **189**, prior to biotransformation [105].

2″-(2″-hydroxyisopropyl)-dihydrofurano-[4″,5″:3′,4′]-4,2′-dihydroxy-6′-methoxychalcone (**190**), mixture of diastereoisomers of (2S, 2″S) and (2S, 2″R) 2″-(2″-hydroxyisopropyl)-dihydrofurano-[4″,5″:7,8]-4′-hydroxy-5-methoxyflavanone (**191**), and (Z)-2″-(2″-hydroxyisopropyl)-dihydrofurano-[4″,5″-6,7]-3′,4′-dihydroxy-4-methoxyaurone (**192**) were obtained by transformation of **185** in *Aspergillus ochraceus* (AM 465) culture (**Figure 19**) [106].

Incubation of xanthohumol (**185**) both with *Fusarium avenaceum* (AM11) and *F. oxysporum* (AM727) gave a single metabolite 2″-(2″-hydroxyisopropyl)-dihydrofurano-[4″,5″:3′,4′]-4′,2-dihydroxy-6′-methoxy-α,β-dihydrochalcone (**193**), which turned out to be the product of the prenyl group cyclization and α,β-double bond reduction. *F. tricinctum* reduced α,β-double bond of **185** to give 4,2′,4′-trihydroxy-6′-methoxy-3-prenyl-α,β-dihydrochalcone (**194**). *Penicillium albidum* (AM79) oxidized **185** at the double bond of prenyl group to xanthohumol H (**195**) [107]. The culture of the yeast, *Rhodotorula marina* (AM 77), converted **185** and 4-methoxychalcone (**196**) to α,β-dihydroxanthohumol (**197**) and 4-methoxydihydrochalcone (**198**) with the yields of 18% and 20%, respectively [108]. *Penicillium albidum* (AM79) dihydroxylated the $\Delta^{2''(3'')}$ double bond of xanthohumol to produce 3′-[3″-hydroxy-3″-methylbutyl]-4,2′,4′-trihydroxy-6′-methoxychalcone (199, 22.84%) (**Figure 19**).

B. bassiana AM278 and *Absidia glauca* AM177 converted isoxanthohumol (**189**) into glucoside derivatives (**200**, **201**), whereas *Fusarium equiseti* AM15 transformed it into (2R)-2-(2-hydroxyisopropyl)-dihydrofurano-[2,3:7,8]-4-hydroxy5-methoxyflavanone (**202**) (**Figure 20**) [95, 106].

C. echinulata (ATCC 9244) sulfated silybin (**203**) to silybin-7-sulfate (**204**) and 2,3-dehydrosylibin-7-sulfate (**205**). Sulfonation at the C-7 position of silybin

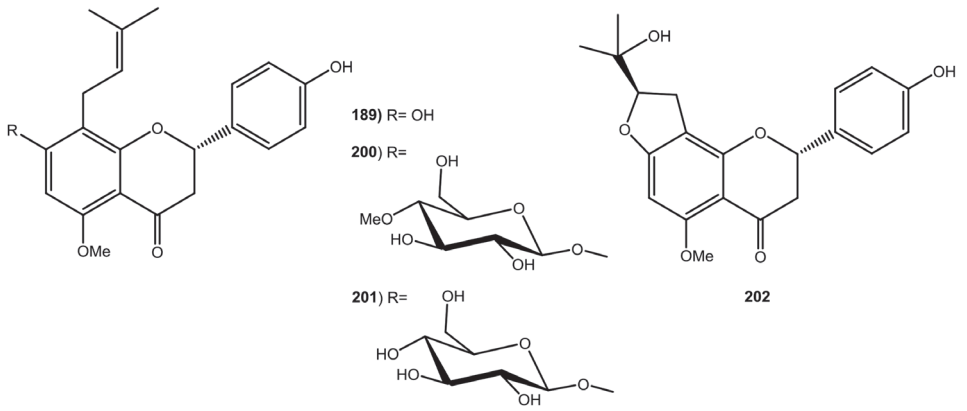


Figure 20.
*Biotransformation products of isoxanthohumol (**189**).*

significantly decreased the DPPH free radical scavenging potential; however, further dehydrogenation $\Delta^{2(3)}$ to 2,3-dehydrosilbyn-7-sulfate (**206**) drastically enhanced the DPPH free radical scavenging potential activity [109] (**Figure 21**).

2.7 Enzymes isolated from microorganisms and their application

Enzymes are the most proficient catalysts, offering much more competitive processes than chemical catalysts. A number of enzyme-based processes have been commercialized for producing several valuable products. During the 1980s and 1990s, engineering of enzymes based on structural information allowed extension of their substrate ranges, enabling the synthesis of unusual intermediates. Accordingly, the use of enzymes has been expanded to the manufacture of pharmaceutical intermediates and fine chemicals [110]. Microorganisms and enzymes (biocatalysts) are highly enantio-, chemo-, and regioselective in a wide range of reaction conditions. Selectivity is extremely desirable in the synthesis of different synthesis products, since it offers advantages such as minimizing the side reactions that do not require protection and deprotection steps, which allows for shorter synthesis. Biocatalysis provides a technology that is environmentally safer, and it effectively reduces the level of waste and even eliminates the waste generation rather than remediation and disposal of wastes at the end of the process. In addition

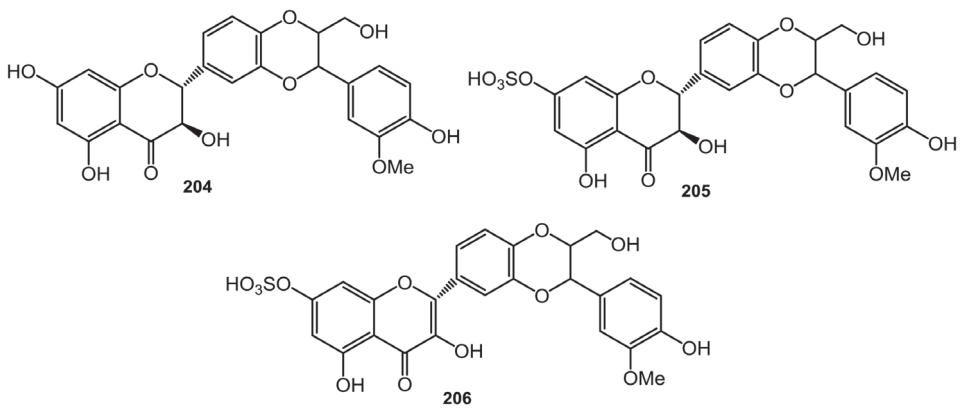


Figure 21.
*Biotransformation products of sylbin (**204**).*

to, biocatalysts have many attractive features in the context of green chemistry and sustainable development. Various enzymes used in different industrial processes have been described in the literature. **Table 6** indicates some enzymes, their source, and some applications [111–113].

2.8 Extremophiles

A very interesting research area in biology and biotechnology is the of extremophile microorganisms. Extremophiles can be divided into group according to (i) temperature tolerance, (ii) salt concentration, (iii) pH range, or (iv) pressure conditions. Enzymes from extremophilic microorganisms offer versatile tools for

Microbial enzymes	Microorganism	Application
α-Amylase	*Bacillus amyloliquefaciens* *B. stearothermophilus* *B. licheniformis*	Baking, brewing, starch liquefaction Clarification of fruit juice Textile industry Paper industry
Glucoamylase	*Aspergillus niger* *A. awamori* *Rhizopus oryzae*	Beer production High glucose and high fructose syrups
Proteases	*A. usami*	
Lactase (β-galactosidase)	*Kluyveromyces lactis* *K. fragilis*	Lactose intolerance reduction in people Prebiotic food ingredients
Lipase	*Candida antarctica* *C. cylindraceae Ay30* *Helvina lanuginosa* *Pseudomonas* sp. *Geotrichum candidum*	Cheese flavor development Textile indutry Medicinal applications Use in cosmetics Use as biosensors Use in biodegradation
Phospholipases	*Fusarium oxysporum*	Cheese flavor development
Esterases	*Bacillus licheniformis*	Enhancement of flavor and fragrance in fruit juice
Xylanases	*Streptomyces* sp. *Bacillus* sp. *Pseudomonas* sp.	Clarification of fruit juice Beer quality improvement
Glucose oxidase	*A. niger* *Penicillium glaucum* *P. adametzzi*	Food shelf life important Food flavor improvement
Laccase	*Funalia trogii* *Bacillus licheniformis* *Bacillus vallismortis*	Polyphenol removal from wine baking
Pectinases	*A. niger* *A. wentii* *Rhizopus* sp.	Clarification of fruit juice
Catalase	*A. niger* *Metarhizium anisopliae* *Psychrobacter piscatorri*	Food preservation Removal of H_2O_2 from milk prior to cheese production
Peroxidase	*Streptomyces viridosporus*	Development of flavor, color and nutritional quality of food

Table 6.
Enzymes, source, and some applications.

sustainable developments in a variety of industrial applications as they show important environmental benefits due to their biodegradability, specific stability under extreme conditions, improved use of raw materials, and decreased amount of waste products. Although major advances have been made in the last decade, our knowledge of the physiology, metabolism, enzymology, and genetics of this fascinating group of extremophilic microorganisms and their related enzymes is still limited [114–116].

The outstanding properties of thermozymes are suited to industries that employ elevated temperatures, such as the pulp and paper, food, brewing, and feed processing industries. Thermophiles are often highly resistant to harsh conditions such as chemical denaturing agents, wide pH ranges, and/or nonaqueous solvents. Examples of such enzymes are cellulases, xylanases, pectinases, chitinases, amylases, pullulanases, proteases, lipases, glucose isomerases, alcohol dehydrogenases, and esterases. Thermophilic enzymes have played important roles not only at the industrial level but also in pharmaceutical applications requiring use of specific aldolases for the synthesis of enantiopure compounds (**Table 7**) [118].

Source	Enzyme	Activity	Bioprocess/industry	Reference
Sulfolobus solfataricus S. acidocaldarius Thermoproteus texas Hyperthermus butylicus	Aldolase	Stereoselective C-C bond formation	Pharmaceutical industry	[117]
Pyrococcus furiosus	Hydrogenase	Final stage of glucose oxidation by oxidative pentose phosphate cycle	Enhanced production of biohydrogen	[119]
Geobacillus thermoleovorans	Carboxylesterase	Carboxyl ester hydrolysis	Agriculture, food, and pharmaceutical industries	[120]
Bacillus pumilus	Acidic thermostable lipase	Degradation of palm oil	Treatment of palm oil-containing wastewater	[121]
Geobacillus sp.	Lipase	Hydrolysis of diver's lipid substrates	Biofuel, cosmetics, or perfume production, leather and pulp industries	[122]
Microbial community from solid-state fermentation reactor	Protease	Degradation of hair waste from tannery	Leather industry	[123]
Sulfolobus tokodaii	Chitinase	Hydrolysis of β-(1, 4)-glycosidic bonds in chitin	Biomedical, pharmaceutical, food, and environmental	[124]
Acidothermus cellulolyticus	Endoxylanase	β-(1,4)-xylan cleavage	Biofuel production from lignocellulose	[125]
Thermotoga neapolitana	Pullulanase	Hydrolysis of α-(1, 6)-glucosidic linkages	Biofuel production	[126]

Table 7.
Extremophile microorganisms and some applications of their enzymes.

3. Conclusion

Due to microorganisms' abundant multienzyme systems, microbial transformation possesses advantages against chemosynthesis of environmental friendliness, mild reaction conditions, and high *stereo*-, *regio*, and *chemo*-selectivities as well as in improving conversion rates and reducing cost. Thus, microbial transformation technique is being increasingly used to structurally modify natural and synthetic compounds.

The hydrolytic and reductive capabilities of microorganisms have been known and are currently used in preparative and industrial reactions. Various classes of bioactive organic compounds have been subjected to enzymatic transformation to obtain more active and less toxic substances or to elucidate their metabolic pathways.

For example, biotransformation-derived steroids are used for a wide range of pharmacotherapeutic purposes, such as anti-inflammatory, immunosuppressive, progestational, diuretic, anabolic, as neurosteroids, and as contraceptive. Researchers continue to discover more useful steroid compounds and to isolate microorganisms that can perform the structural transformations desired. New technologies such as genomics, metanogenomics, gene shuffling, and DNA evolution provide valuable tools for improving or adapting enzyme properties to the desired requirements.

An alternative may be extremophilic microorganisms such as biocatalysts for countless future industrial applications that are more environmentally friendly.

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Conflict of interest

The authors report no conflicts of interest.

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