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REVIEW

Biotransformation of Diterpenes

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Diterpenes are a versatile group of biologically active ingredients present in several phytoextracts. Structural modification of the diterpenes to enhance their pharmaceutical relevance can be efficiently carried out by the application of biotransformational processes using microorganisms or isolated enzymes. Over the past years, special attention has been paid to the biotransformation of diterpenes due to the fact that biocatalysts allows the production of enantiomerically pure compounds under mild and environmentally friendly processes. A wide range of microorganism have been assessed for these biotransformations and have produced encouraging results, as discussed in this review. This report reviews reactions mediated by fungi, published between 2000 and 2013.

1. Introduction and scope

The medicinal use of natural products precedes recorded human history probably by thousands of years. As such, they have proved invaluable in providing compounds, either directly or as leads, for therapeutic purposes, such as antibiotics or chemotherapeutic agents.[1]

Terpenoids are the largest and most widespread class of secondary metabolites; approximately 55,000 compounds have been identified to date with several new compounds being discovered every year.[2] Terpenoids are produced in plant cells via two distinctly localized routes. These pathways are named mevalonate pathway (MVA) and methylerythritol 4-phosphate pathway (MEP), respectively. The MEP pathway provides precursors mainly for the synthesis of mono- and diterpenes, isoprene, carotenoids, the phytohormones gibberellins and abscisic acid, phytol, the side chain of chlorophylls, tocopherols, phylloquinones, and plastoquinones while on the other hand, MVA pathway mainly provides isopentenyl diphosphate, essential for the synthesis of sesquiterpenes, sterols, brassinosteroids, polyprenols, and the moieties used for prenylated proteins.[3]

The diverse array of terpenoid structures and functions has ignited interest in their comercial use. Terpenoids exhibit several beneficial effects from a biological perspective, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihypercholesterolemic, antidiabetic, antiinflammatory, and immunomodulatory properties.[4] Based on these favorable biological activities, terpenoids have therefore received considerable phytochemical and biological attention.[5-7]

In particular, some diterpenoids with insect growth regulatory activity,[8] insect antifeedant,[9] or insecticidal activity,[10] have been

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isolated from higher plants. Ferruginol, an abietane diterpene, has shown antifungal and antibacterial activity.[11] Communic acids, diterpenes based on a labdane skeleton, has shown promising biological activities: antibacterial, antitumoral, hypolipidemic, and relaxing smooth muscle activities have been reported and reviewed recently.[12] They have been also used as building blocks for the semi-synthesis of other interesting bioactive compounds, such as quassinoids, antioxidant abietanes and ambrox, a compound with fixative properties particularly prized by perfumers.[13] Also, the clerodane has exhibited a wide range of biological activities. Of particular interest is asmarine A and B[14] which showed antiproliferative activity against several human cancer cell lines and, clerocidin,[15] a naturally occurring antibiotic which has also shown anticancer and antimicrobial activities. The tricyclic diterpene salvinorin A is a trans-clerodane diterpenoid isolated from the mexican plant Salvia divinorum.[16] It acts as a kappa opioid receptor agonist and it is the first non-alkaloid compound acting on this receptor.[17] Among this class of compounds of great relevance is taxol.[18] This compound has attracted growing attention because of its anticancer activity against several tumor cell lines non responsive to other treatments, such as ovarian and breast cancers, non-small-cell and small-cell lung cancer, and cancers to the head and neck.

According to several authors,[19] drugs derived from natural products can function not only as new drugs themselves, but also as lead compounds for chemical modifications that will furnish derivatives with increasedbetter activity and pharmacokinetic properties, new mechanisms of action, and fewer adverse side effects.[20] A multidisciplinary approach to drug discovery involving the generation of truly novel molecular diversity from natural product sources provides the best solution to increase the productivity in drug discovery and development. Recently, biocatalysis is gradually becoming an important tool for organic synthesis, especially for the production of derivatives, regio- and enantioselectivity, very difficult tasks to achieve by traditional chemical methods or too expensive to perform.[21]

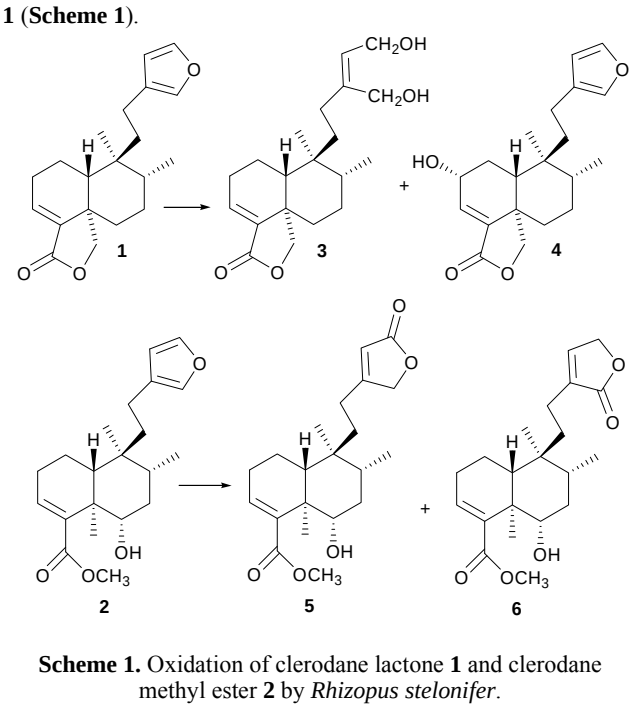
The term biotransformation can be applied to a specific modification or interconversion of chemical structures performed by enzymes contained in the cells or by an isolated enzyme. Biotransformation differs from fermentation in which the substrate is converted to a desirable product through a complex cell metabolic pathway.[22] There is an increasing body of information about use of biocatalysis for selective conversion of synthetic and natural products to intensify either their biological properties or to lead to new biological activities.[23] For instance, the ability of microorganisms to hydroxylate chemically inaccessible sites is a potentially powerful synthetic technique.[24] In particular, filamentous fungi contain numerous hydroxylating enzymes with broad specificities, able towhich catalyze regio- and stereoselective hydroxylation of nonactivated carbons on a variety of natural and synthetic organic compounds.[25] Special attention has been paid to the biotransformation of diterpenes because it allows the production of enantiomerically pure compounds, hemisynthesis intermediates, chiral auxiliaries, and chiral synthons of comercial interest under mild reaction conditions. Therefore, bearing in mind that in the last two decades microbial transformation of terpenes has gained increasing popularity and given the fact that there have been many new developments concerning the biomanipulation of diterpenoids we provide an overview of the most significant advances described in the recent literature. Thus, we have chosen to focus exclusively on diterpene biotransformation reactions from precursor molecules, although we excluded taxanes and gibberellins.

2. Clerodanes

Many of clerodane diterpenoids, especially those of a highly oxygenated nature, display potent insect antifeedant, antifungal,

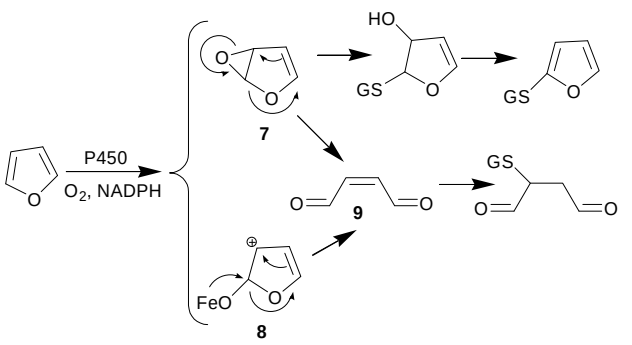
antibacterial, anticancer, and other desirable properties.[26] The biological activities and challenging structures of the clerodanes have stimulated much synthetic effort, including microbial transformation, that has culminated in many total syntheses.[27]

In this context, Atta-ur-Rahman and co-workers[28] describe the synthesis of oxidated derivatives of clerodane lactone **1** and clerodane methyl ester **2** by the plant pathogen *fungus Rhizopus stelonifer*. In both processes, the authors obtained the resultant products from a cytochrome P450-catalyzed furan ring oxidation, except in the case of **4** where the oxidation occurred not only in the furan ring but at the less active allylic position of clerodane lactone **1** (**Scheme 1**).



Scheme 1. Oxidation of clerodane lactone **1** and clerodane methyl ester **2** by *Rhizopus stelonifer*.

The structure of the reactive intermediate resulting from furan ring oxidation is somewhat ambiguous. Two structures have been proposed: an epoxide or a *cis*-enedione (**Scheme 2**). Furan oxidation by P450 enzymes is thought to proceed from one of two general mechanisms. The first one involves the direct formation of an epoxide, while the other involves the addition of the high valent iron(IV)-oxospecies to the π-system of the furan ring to produce a tetrahedral intermediate or cationic σ complex that can rearrange to yield either an epoxide **7**or a zwitterionic intermediate **8**. Either intermediate **7** or **8** can rearrange to form a *cis*-enedione **9**.[29]



Scheme 2. Proposed mechanism for enzymatic furan ring oxidation.

3. Pimaranes

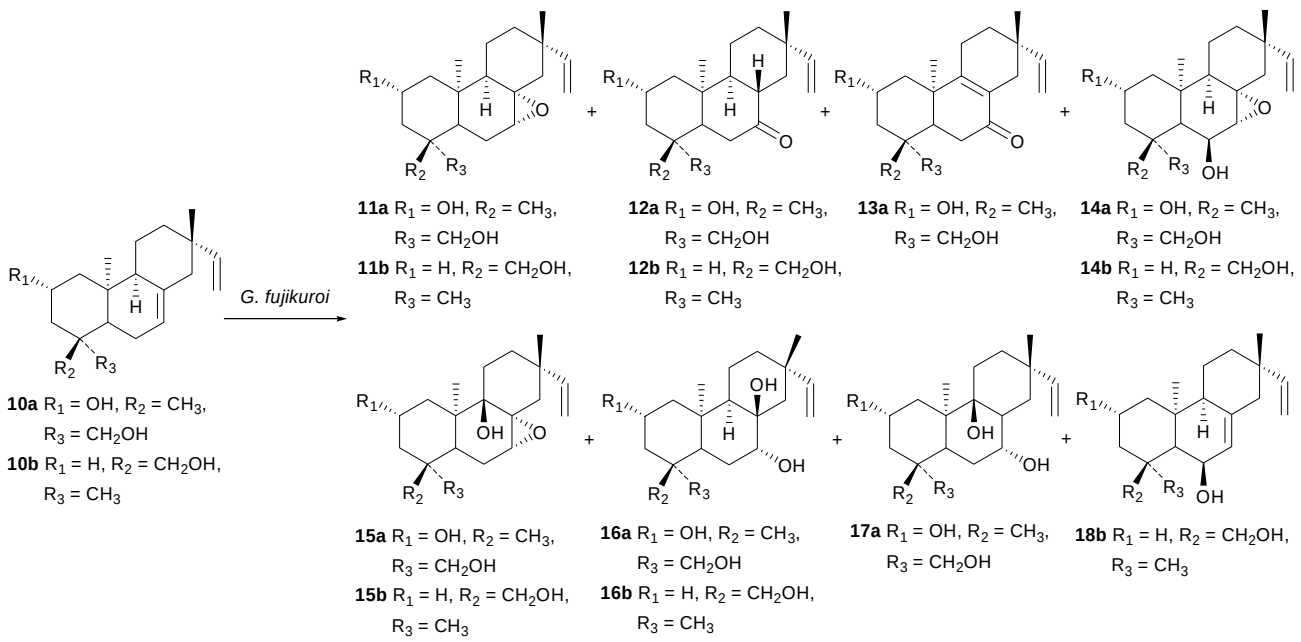
Gibberella fujikuroi was used in the microbiological transformation of *ent*-pimara-7,15-diene diterpenes.[30] In these biotransformations, C-19 oxidation did not occur, and the main reaction was the epoxidation of the 7,8-double bond, followed by rearrangement to afford 7-oxo derivatives (**Scheme 3**). These results were independient of the hydroxylation of C-2, and the angular methyls C-18 and C-19. This indicates a lack of specificity of the enzymes involved in these processes.

To change the double bond from C7-C8 to C9-C11, the reaction product was the epoxidation of the 9(11)-double bond, followed by rearrangement to afford allylic alcohols,[31] while in previous work, in the biotransformation of the *ent*-pimara-7,15-diene derivatives with this fungus,[30] the rearrangement of the 7,8-epoxide led to 7-oxo derivatives (**Scheme 4**). The results of the biotransformations are also influenced by the oxidative state of the methyl-19. Thus, in the incubation of the 13-epi-*ent*-pimara-9(11),15-diene-19-oic acid **19** (**Scheme 4**), a double oxidation at C-1 is produced, forming an oxo group, followed by a hydroxylation at C-2, either α or β, to give the corresponding α-hydroxy ketones **25** and **26**, or by a Baeyer-Villiger oxidation to afford the lactone **27**, while in the feeding of 19-hydroxy-13-epi-*ent*-pimara-9(11),15-diene **20**, two 7-oxo derivatives were obtained, **22** and **23**. The formation of a 1-oxo-2-hydroxy group in the biotransformation of **19** is very similar to that produced in the incubation of some 7-oxo-*ent*-kaur-16-ene derivatives to give the corresponding 7-oxo-6β-hydroxy derivatives.[32] The formation of the lactone **27**, represents the first time that a Baeyer-Villiger oxidation has been observed in a microbiological transformation with the fungus *G. fujikuroi*. It is also worth pointing out that during this enzymatic oxidation process, the "abnormal" lactone resulting from the migration of the less-substituted carbon atom is formed, while the same reaction using chemical reagents occurs at the most substituted position.

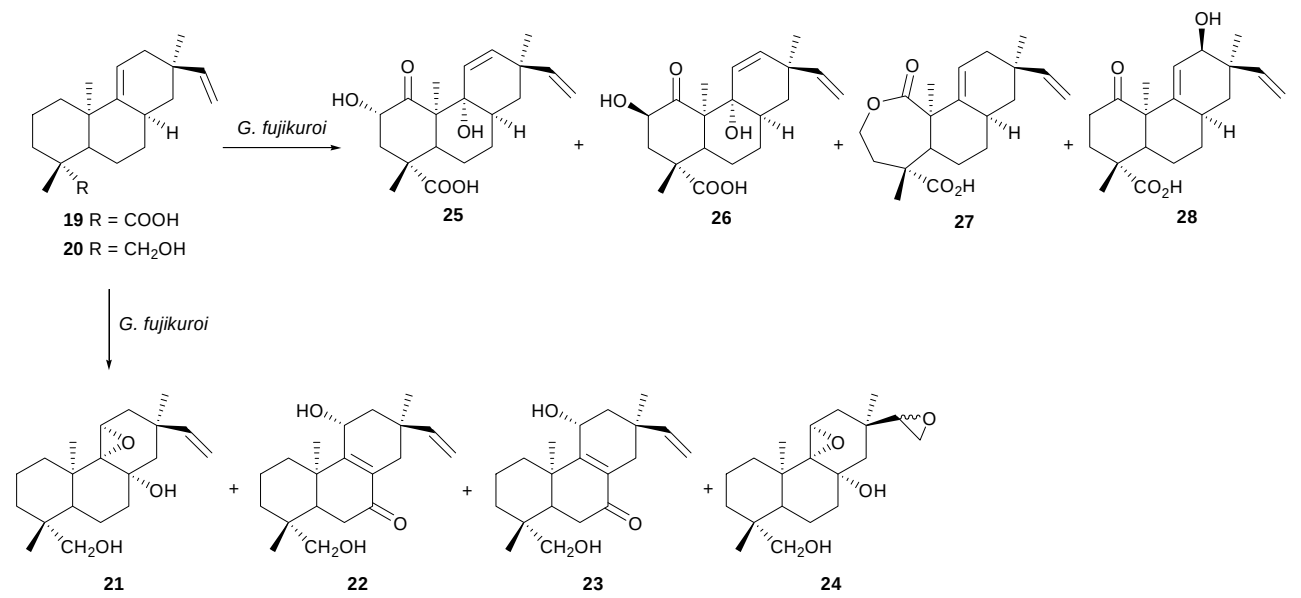
In the last few years, the development of enzymatic methodologies using Baeyer–Villiger monooxygenases (BVMOs) has allowed the preparation of several compounds that are of high interest in organic synthesis. These flavoproteins are oxidoreductases able to catalyse the Baeyer–Villiger oxidation as well as other oxidative processes which employ atmospheric oxygen as the natural oxidant.[33] The regioselectivity of the Baeyer–Villiger oxidation is established by steric, conformational and electronic effects leading to the migration of the higher substituted (the most nucleophilic) carbon centre. Nevertheless, in some rare cases the use of Baeyer–Villiger monooxygenases has led to the formation of unexpected lactones with high regioselectivities, formed by the migration of a less-substituted carbon atom,[34] increasing the synthetic potential of this class of enzymes.

Ambrosio and co-workers describe the biotransformation of two *ent*-pimaradienes metabolites isolated from the dichloromethane root extract of *Viguiera arenaria*:[35] *ent*-pimara-8(14),15-dien-19-oic acid **29** and *ent*-8(14),15-pimaradiene **30**. The incubation of **29** with *Glomerella cingulata*[36] afforded the bioreduction in the carboxylic acid moiety to an alcohol group as sole biotransformation product **31**, while its fermentation by *Mucor rouxii* yielded the isomerization of the endocyclic double bond **32** and oxidation at C-7 **33** as the main reaction products.[36] When *ent*-8(14),15-pimaradiene **30**, a substrate without the carboxylic

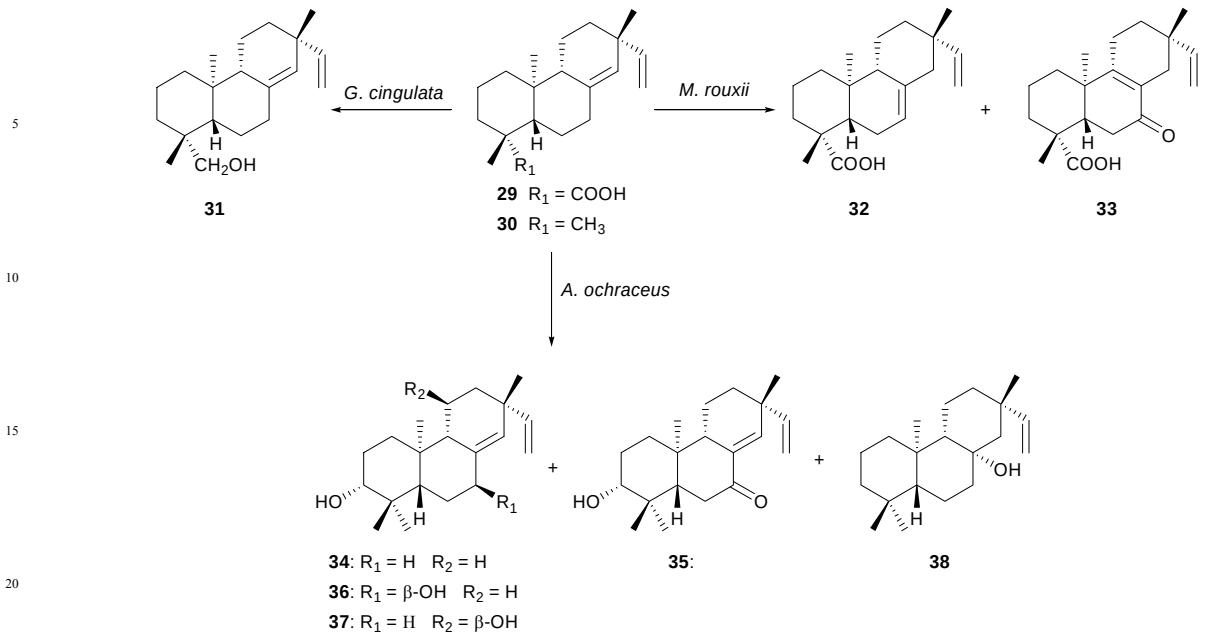
acid moiety at C-19, was transformed in the presence of *Aspergillus ochraceus*, five hydroxylated pimarane-type diterpenes were obtained.[37] The main transformations were the stereoselective hydroxylations at C-3, C-7 and C-11 in low yield. In this case, the orientation of hydroxyl group at C-3 was α in the four products (**Scheme 5**). Although the number of products in each biotransformation is smaller, these results are in agreement with the previously reported observation by Fraga and co-workers.[30-32]



Scheme 3. Microbiological transformation of 2α,19-dihydroxy-9-epi-*ent*-pimara-7,15-diene **10a** and 18-hydroxy-9-epi-*ent*-pimara-7,15-diene **10b** by *G. fujikuroi*.

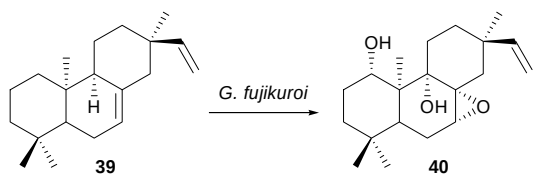


Scheme 4. Microbiological transformation of 13-epi-*ent*-pimara-9(11),15-diene-19-oic acid **19** and 19-hydroxy-13-epi-*ent*-pimara-9(11),15-diene **20** by *G. fujikuroi*.



Scheme 5. Biotransformation of *ent*-pimara-8(14),15-dien-19-oic acid **29** and *ent*-8(14),15-pimaradiene **30**.

The incubation of the hydrocarbon 9,13-epi-*ent*-pimara-7,15-diene diterpene **39**, obtained from its 18-hydroxy derivative by treatment with Ph_3P/CCl_4 and subsequent reduction with tri-*n*-butyltin hydride, with the fungus *G. fujikuroi* afforded 1α,9α-dihydroxy-7α,8α-epoxy-13-epi-*ent*-pimara-15-ene **40** as sole biotransformation product (**Scheme 6**).[38] The main difference between the microbiological transformations of the 18-alcohol (18-hydroxy-9,13-epi-*ent*-pimara-7,15-diene)[30] and the hydrocarbon **39**, lies in **39** the rearrangement of the 7α,8α-epoxygroup to give 7-oxo-derivatives was not observed. However, the sequence of reactions 7α,8α-epoxidation, hydroxylation at C-9(α) and subsequent C-1(α) hydroxylation occurred in both incubations.



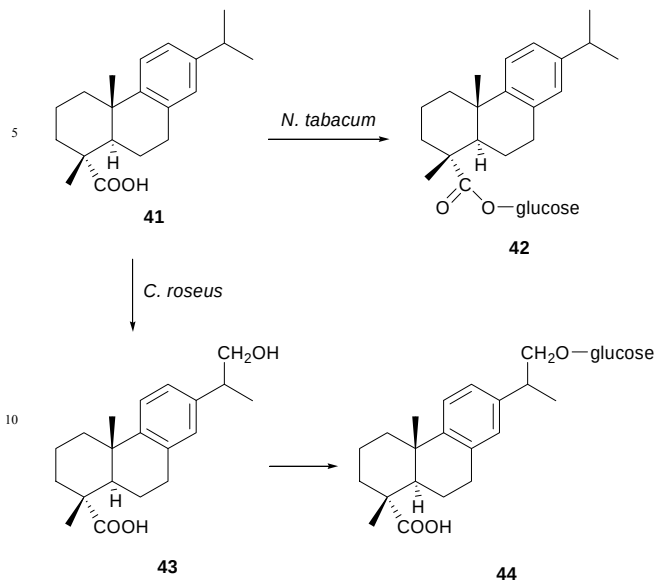
Scheme 6. Biotransformation of 9,13-epi-*ent*-pimara-7,15-diene **39** by *G. fujikuroi*.

4. Abietanes

4.1. Dehydroabietic acid derivatives

Diterpene resin acids are important defense compounds of conifers against potential herbivores and pathogens.[39] Dehydroabietic acid **41**, one of the major tricyclic diterpenoid constituents of pine resin, exhibits a broad spectrum of biological action. Several activities like antiulcer, antimicrobial, anxiolytic, antiviral, antitumor, anti-inflammatory and cytotoxic have been reported.[40] Recent studies have demonstrated that dehydroabietic acid **41** and some derivatives are chemical modulators, particularly openers, of large-conductance calcium-activated Kþ channels (BK channels).[41] This feature makes dehydroabietic acid a new scaffold in the treatment of acute stroke, epilepsy, asthma, hypertension, gastric hypermotility and psychoses. Also, dehydroabietic acid **41** was reported to have properties of enhancing the inhibitory activity of anticancer drugs in cervical carcinoma cells, hepatocellular carcinoma cells, or breast cancer cells. This broad spectrum of biological activities, indicate that the compound is a potentially useful starting material for the synthesis of industrial or pharmacologically important products. It is important to note that the microbial degradation or conversion of abietic acid has been scarcely studied due to its chemical lability, causing it to change readily into dehydroabietic acid.[42]

Häkkinen and co-workers assayed the bioconversion of dehydroabietic acid **41** in two plant species: *Nicotiana tabacum* and C*atharanthus roseus* cells,[43] where dehydroabietic acid **41** can be considered as a xenobiotic for these species, so both of the tested plant species were able to take up and modify this compound according to the typical detoxification pattern of each species. Such is one of the few examples about degradation or bioconversion of dehydroabietic acid **41** using plant cells. *Nicotiana tabacum* converted dehydroabietic acid **41** into the corresponding 18-*O*-glycoside **42** (**Scheme 7**). Madagascar periwinkle (*Catharanthus roseus*), which endogenously possesses the terpenoid biosynthesis machinery, converted the substrate into two bioconversion products. The first appearance was identified as a 17-hydroxy-dehydroabietic acid **43**, following the glycosylation by putative glycosyltransferase into dehydroabietic 17-*O*-glucoside **44**. Hydroxylation of dehydroabietic acid **41** follows a typical detoxification pattern of organic compounds.[44] Existing evidence indicates that hydroxylation is the dominant mechanism of resin acid degradation by fungi,[45] while several studies suggest that the main enzymes responsible for the oxidative reactions of xenobiotics in plants are cytochrome P450s.[46]



Scheme 7. Biotransformation of dehydroabietic acid **41** by *N. tabacum* and *C. roseus* cells.

Stereoselective hydroxylation at the C-1 position appeared to be the first degradation step of dehydroabietic acid **41** by two fungi: *Trametes versicolor* and *Phlebiopsis gigantean* (**Scheme 8**).[47] This hydroxylation at the C-1 position, has only been previously reported in cultures of two different *Fusarium* species[48] and *Aspergillus niger*.[49] The C-7 and/or C-16 hydroxylations produced in the incubation of dehydroabietic acid **41** with T. *versicolor* and *P. gigantea* have also been observed in the substrate biotransformation by the fungi *C. cochliodes*,[45b]*F. annosus*,[50]*A. niger*,[49] and *M. isabellina*.[51] Similarly, hydroxylation at C-7 has been reported in studies with the aerobic bacteria *Alcaligenes sp*., *Pseudomonas sp*.[52] and *Pseudomonas abietaniphila*.[46b, 53] Further oxidation of the hydroxyl group at C-7 to a carbonyl function, has equally been observed in studies of the conversion of dehydroabietic acid **41** by the aerobic bacteria *Flavobacterium resinovorum*[54] and *Moraxella sp*. (HR6).[46a] A possible pathways for the degradation of dehydroabietic acid **41** is shown in the **Scheme 8**.[47]

Nagasawa and co-workers have surveyed microorganisms to catalyze the hydroxylation of dehydroabietic acid **41**.[46a] Among 238 microorganisms from a soil sample, three bacteria [(HR1, M*oraxella sp*. (HR6), and *Pseudomonas sp*. (HR34)] and two molds [*Mucor circinelloides* (IT 25) and *Mortierella isabellina* (HR32)] exhibited dehydroabietic acid **41** derivative-converting activity. The substrate was converted *regio*- and *stereo*-selectively by *Mucor circinelloides* and *Mortierella isabellina* to give 2α-hydroxydehydroabietic acid **54** (**Scheme 9**).

Dehydroabietanol **56** and teideadiol **57** are two abietratriene diterpenes obtained from *Salvia pomifera*[55] and *Nepeta teydea*[56] respectively. The microbiological transformation of both products by *Mucor plumbeus*[57] led to 2α and 7β as the main hydroxylated products (**Scheme 10**). The core difference between both biotransformations is the 15-hydroxylation of dehydroabietanol **56**, which does not occur in teideadiol **57**. This could be due to the 1α-alcohol in the latter inhibits the C-15 functionalization. In the incubation of dehydroabietanol **56** with *Mucor plumbeus*, the 15- and 16-hydroxylations were produced, as in the biotransformation of dehydroabietic acid **41** by *Chaetomium cochliodes*, *Fomes annosus* and *Mortierella isabellina*,[47] thus implying a similar functionalization carried out by enzymes from four different genera of fungi.

4.2. Carnosic acid and carnosol

Carnosic acid **67**, an *O*-diphenolic abietane diterpene precursor of phenolic diterpenes featuring γ- and δ-lactone structures, and related metabolites, such as carnosol **68**, are the main compounds responsible for the distinctive antioxidant activity of the popular *Labiatae* herbs, rosemary and sage.[58] Many researchers suggested an oxidation and isomerization pathway for this transformation via an *O*-quinone intermediate.[59] Furthermore, it is suggested that these oxidation pathways were closely related to their antioxidant mechanism. This reactivity was used by San Andrés and coworkers to develop efficient transformations to obtain the minor biologically active abietatriene diterpenes in significative quantities from carnosol **68**.[60] In addition to their strong antioxidant character, carnosic acid **67** and carnosol **68** exert potent anti-inflammatory and anticarcinogenic properties.[61] These compounds inhibit cytochrome P450 activation of carcinogens in human cells in vitro[62] and enhance the activities of conjugating enzymes involved in carcinogen detoxification pathways *in vivo*.[63]

Rosazza and coworkers examined microbial transformations of carnosic acid **67** as a mean of obtaining novel derivatives.[64] They screened 49 microorganisms, and only *Nocardia sp*. (NRRL 5646) was capable of catalyzing the bioconversion of **67**, to produce three major metabolites (**Scheme 11**). In the mechanism proposed by the authors, the *Nocardia* carboxylic acid reductase reduces the carboxylic acid to an aldehyde group via a carbonyl-activated acyl-adenylate intermediate.[65] In whole cell *Nocardia* cultures, a separate NADPH-dependent alcohol oxidoreductase reduces aldehydes to the corresponding alcohols. The methoxyl group at position C-12 in **71** is likely introduced by means of an *S*-adenosylmethionine-dependent catechol-*O*-methyl transferase system.[66] The conversion of carnosic acid **67** to carnosol **68** likely involves enzymatic oxidation of **67** to a quinoid intermediate followed by an intramolecular Michael addition of the carboxylate anion at position C7.

4.3 Cryptotanshinone

Chemically, tanshinones are 20-norditerpenes with an abietane-type skeleton and a common *ortho*- or *para*-naphthoquinone chromophore in the C-ring, which represent the major chemical constituents present in the lipophilic extract of the rhizome of Chinese sage *Salvia miltiorrhiza Bunge*, a well-known chinese herb used in traditional medicine,[68] generally called Danshen.[67] Tanshinones share many clinical effects including inhibition of growth in lung cancer tumors,[69] atherosclerosis treatment[70] aldose reductase inhibitory activity,[71] neuroprotective effects,[72] apoptosis induction[73] and leishmanicidal and antiplasmodial activities.[74]

Cryptotanshinone **73** is one among more than 50 compounds of tanshinones and an active component of *S. miltiorrhiza Bunge*. Cryptotanshinone **73** was previously shown to possess the most powerful antibacterial activity among the tanshinones, and inhibits

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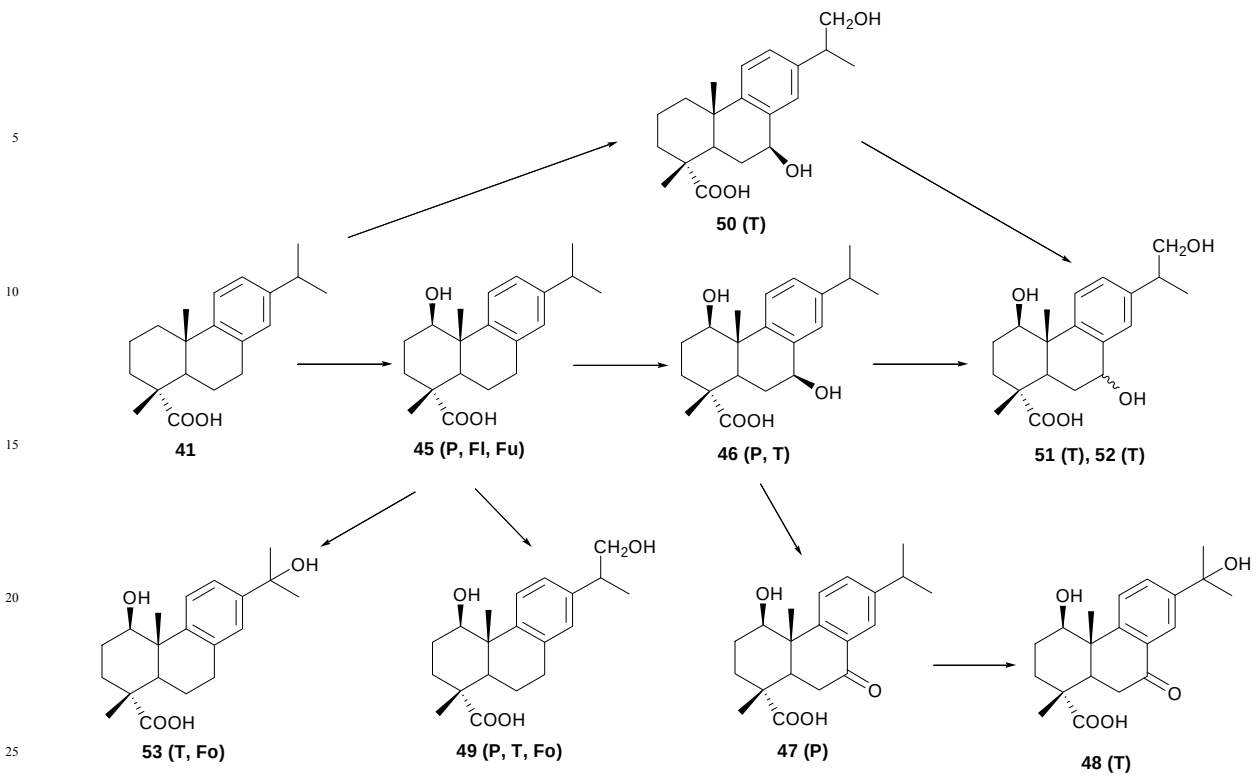
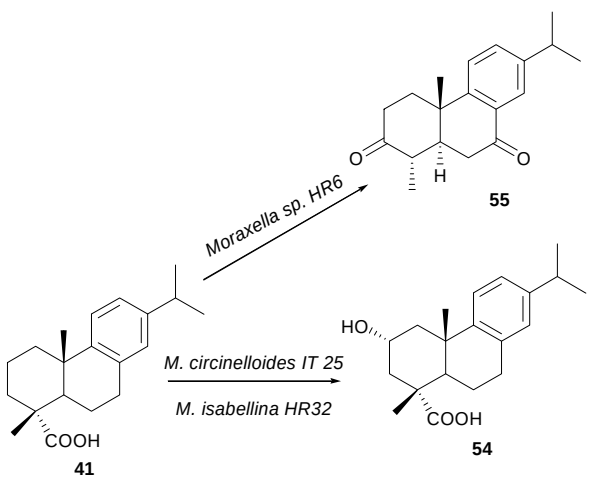


Scheme 8. Possible pathways for the degradation of dehydroabietic acid **41** by *Trametes versicolor* (T), *Phlebiopsis gigantea* (P), *Flavobacterium resinovorum* (Fl), *Fusarium oxyosporum* or *F. moniliforme* (Fu) and *Fomes annosum* (Fo).[47]



Scheme 9. Microbial conversion of dehydroabietic acid **41**.

the growth of the androgen-independent prostate cancer cell line in vitro and in mice.[75] It is an effective inhibitor of topoisomerase I[76] and exhibit significant cytotoxicity against a number of cultured human tumor cell lines.[77]

The biotransformation of cryptotanshinone **73** by *Cunninghamella elegans* yielded a pair of epimeric alcohols at C-3 and a third product where the unactivated methyl-18 group was oxidized (**Scheme 12**).[78] These biotransformed metabolites are identical to those formed *in vivo*, in rat bile sample after intravenous administration, which has demonstrated that this fungal biotransformation system could be used to predict and synthesize the mammalian drug metabolites.

The use of microorganisms for simulating the mammalian metabolism of many molecules of pharmacological importance is well documented. Smith and Rosazza postulated the concept of using microorganisms as models for mammalian metabolism of variety of xenobiotics in *regio*- and *stereo*-selective manners that are similar to those in mammalian enzyme systems, using both phase I (oxidative) and phase II (conjugative) biotransformation mechanisms.[79]

The use of microbial models surpass those of animals and offer a number of advantages mainly: (1) simple, easy, can be prepared at low cost. (2) Screening for a large number of strains is a simple repetitive process. (3) The large amount of metabolites formed allows easier detection, isolation, and structural identification. (4) Novel metabolites can be isolated. (5) Useful in cases where *regio*- and *stereo*-specificity is required. (6) Maintenance of stock

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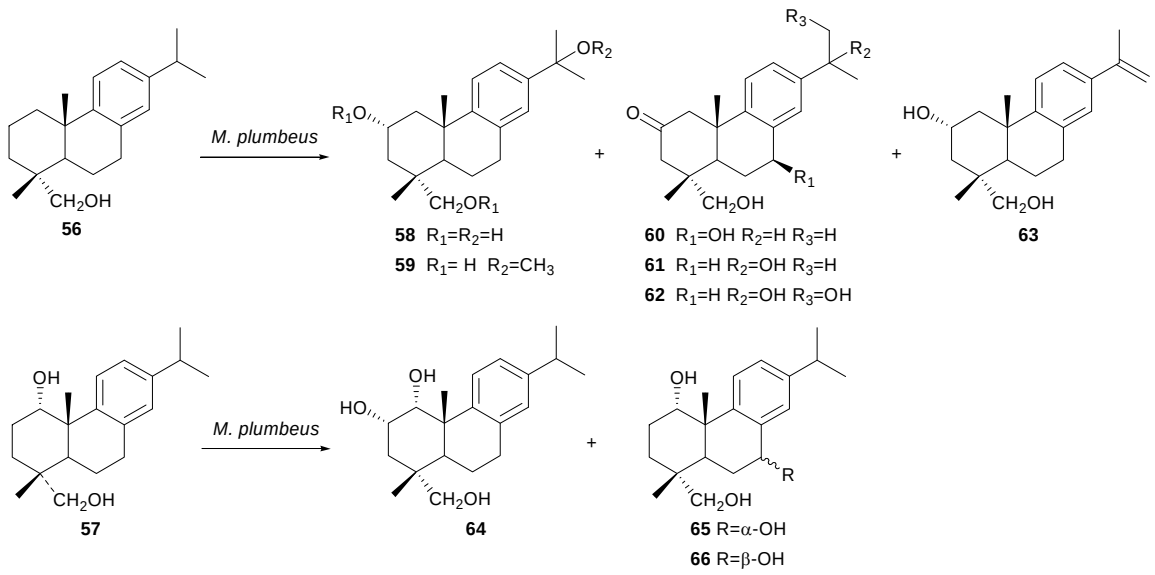
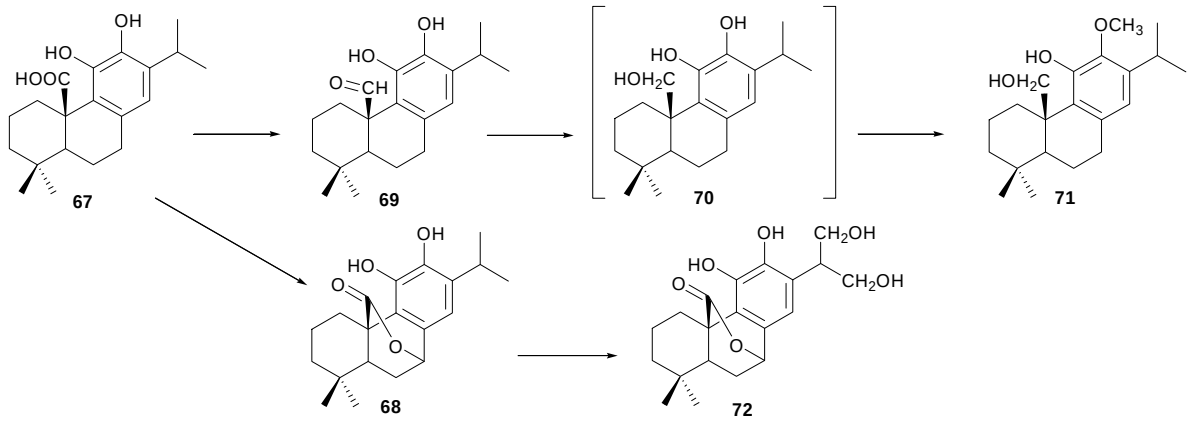
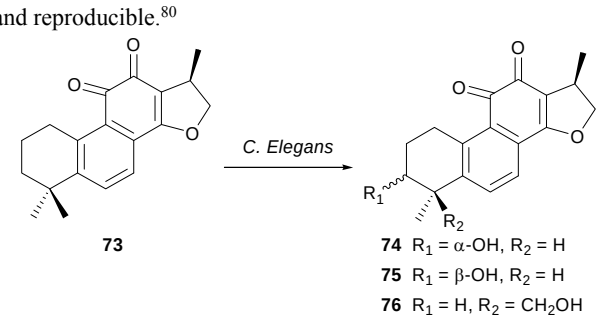


Scheme 10.Biotransformation of dehyroabietanol **56** and teideadiol **57** by*Mucor plumbeus*.



Scheme 11. Metabolites formed in the biotransformation of carnosic acid **67** by *Nocardia sp*.

cultures of microorganisms is easier and less expensive than those of cell or tissue cultures or laboratory animals. (7) More reliable and reproducible.[80]



Scheme 12. Biotransformation of cryptotanshinone **73** by *C. elegans*.

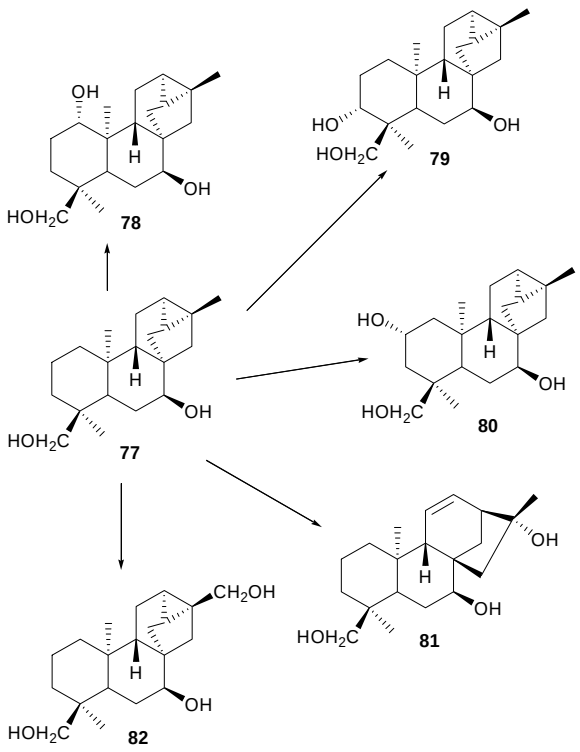
5. Trachylobane-type diterpenoids

Trachylobane-type diterpenoids are characterized by a pentacyclic carbon skeleton with a tricycle[3.2.1.0]octane system, closely related to the *ent*-kaurene series. It has been shown that *ent*-trachyloban compounds possess cytotoxic effects on HeLa and HL-60 cells, and was able to induce apoptosis in human promyelocytic leukemia cells, anti-microbial, anti-tumor, trypanocidal, antifeeding and anti-HIV.[81]

With the aim of obtaining some evidence about the transformation of *ent*-trachylobane skeleton into *ent*-kaur-11-ene derivatives, Fraga and co-workers described the microbiological transformation of trachinodiol **77** by the fungus *Mucor plumbeus*,[82] which led five polar compounds **78-82** (**Scheme 13**). The biotransformation of trachinodiol **77** to give sicanatriol **81** must occur via enzymatic abstraction of an H-11, this abstract is

favoured because such carbon is allylic to the cyclopropane ring, with concomitant cleavage of this ring, giving a carbenium ion at C-16, susequently neutralized with an OH anion, probably of water origin, to form the alcohol **81** (**Scheme 14**).

Litaudon and *co*-workers screened a range of oxidizing fungi (*Rhizopus arrhizus*, *Aspergillus terreus*, *Bauveria bassiana*, *Mucor plumbeus*, and *Cylindrocarpon radicicola*) for the biotransformation of *ent*-trachyloban-18-oic acid **83**.[83] In this case, biotransformation of **83** by *Rhizopus arrhizus* (this fungus was selected because it had the highest bioconversion yield and the highest diversity of metabolites) gave six oxidized compounds (**Scheme 15**), four of them rearranged derivatives by cleavage of the cyclopropane ring. Compounds **84** and **85** resulted from a direct enzymatic hydroxylation of compound **83** at positions C-17 and C-1, respectively, whereas a backbone rearrangement prior to the oxidation of *ent*-trachyloban-18-oic acid **83** occurred for compounds **87**-**89**. Finally, compound **86**, was a rearranged product.



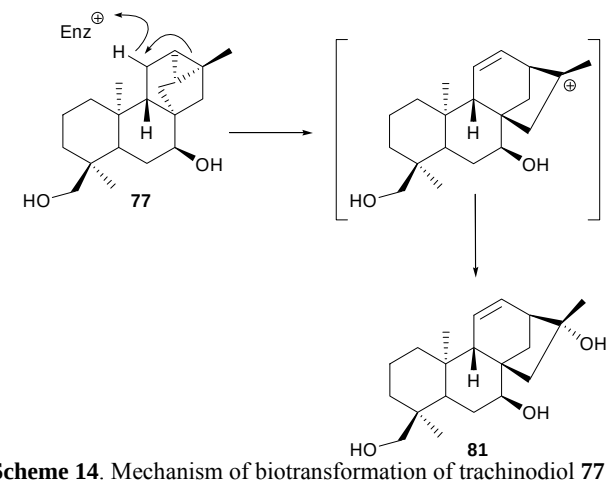
Scheme 13. Biotransformation of trachinodiol **77** by the fungus *M. plumbeus*.

The biotransformation experiment of trachyloban-19-oic acid **90**, epimeric compound of **83**, carried out with *R. Stolonifer*,[84] yielded two trachylobane type compounds (**Scheme 16**), the C-7β **91** and the C-17 **92** hydroxyl derivatives, and two rearranged *ent*-kaur-11-en-19-oic acids, the 16α **93** and the 9β,16α **94** hydroxylated compounds.

From a biosynthetic point of view, it is interesting to note that kaur-11-ene derivatives are scarce in nature and have only been found in plants where diterpenes with a trachylobane skeleton have also been isolated. Moreover, the rearrangement of a trachylobane diterpene lead *ent*-kaur-11-ene derivatives described below. All this facts support the biogenetic proposal based on the mechanism proposed by Fraga and *co*-workers, similar to that postulated by authors in plants of the *Sideritis* genus.[81d, 85]

6. Kaurenes

Kaurenes represent an important group of tetracyclic diterpenes. Their structures are constituted by a perhydrophenanthrene unit (A, B and C rings) fused with a cyclopentane unit (D ring) formed by a bridge of two carbons between C-8 and C-13. *ent*-Kaurenes and many natural derivatives of these diterpenes have significant anti-inflammatory, anti-hypertensive, and diuretic effects *in vivo*, in addition to antimicrobial, smooth muscle relaxant, and cytotoxic actions *in vitro*.[86]



Scheme 14. Mechanism of biotransformation of trachinodiol **77** into alcohol **81**.

Kauren-19-oic acid is one of the intermediate compounds involved in the biosynthesis of diverse kaurene diterpenes, including gibberellins, which represent an important group of growth phytohormones. Moreover, Kaurenoic acid is a bioactive compound with proven anticonvulsant, sedative, and hypotensive effects.[87] Microbiological transformations of diterpenoids having *ent*-kaurane skeletons have been widely investigated, leading to a large variety of functionalized compounds.

The microbiological transformation of candidiol (15α,18-dihydroxy-*ent*-kaur-16-ene) **95a** and 15α,19-dihydroxy-*ent*-kaur-16-ene **95b** by *Mucor plumbeus* yielded the same hydroxylations (at C-3α, C-6α or C-11β) and epoxidation of the exocyclic double bond reactions (**Scheme 17**).[88] This means that a change in the spatial orientation of the hydroxymethylene group at C-4, from equatorial in **95a** to axial in **95b**, does not affect the way in which these *ent*-kaurenes bind to the oxidative enzymes. Glucosides were formed in the feeding of **95b** (α-axial CH_2OH), but not in that of **95a** (β-equatorial CH_2OH). This was the first time that *ent*-kaurene derivatives of this type are formed in a biotransformation by a *Mucor* species. Other glucosyl derivatives had been obtained in the feeding of the mycotoxin zearalenone[89] and the steroid resibufogenin[90] with other species of *Mucor*.

In the case of candicandiol (7α,18-dihydroxy-*ent*-kaur-16-ene) **105** and epicandicandiol (7β,18-dihydroxy-*ent*-kaur-16-ene) **106**,[91] which differs both in the spatial change and in the orientation of the 7-hydroxyl group from equatorial in **105** to axial in the substrate **106**. The main difference in its fermentation with *Mucor plumbeus* was the formation of a 3α **107** and 9β **110**

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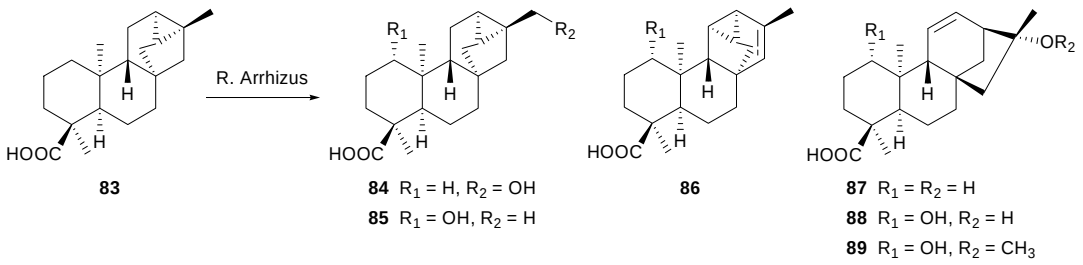
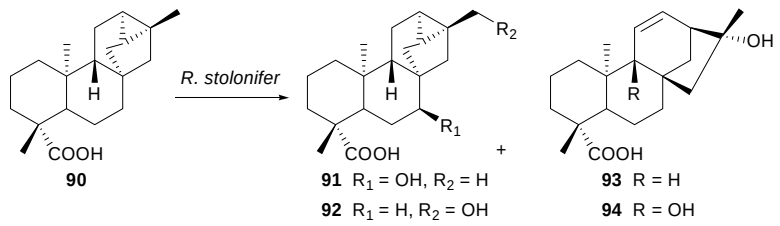


Scheme 15. Biotransformation of *ent*-trachyloban-18-oic acid **82** by the fungus *R. arrhizus*.



Scheme 16. Biotransformation of trachyloban-19-oic acid (**89**) by the fungus *R. stolonifer*.

hydroxylated derivative (**Scheme 18**), respectively, therefore a spatial change in the orientation of the hydroxyl group at C-7 affected the way in which these kaurenes bind to the oxidative enzymes affording a different hydroxylation pattern in the A and B rings. The formation of 16,17-dihydroxylated compounds, **109** and **112**, can be explained by enzymatic epoxidation of the exocyclic double bond to give the corresponding epoxides, followed by opening of these in the medium. For the formation of sideritriol **108** and of canditriol **111**, the authors proposed a mechanism where the enzymatic abstraction of a hydrogen at C-15 in with formation of a carbonium ion, followed by migration of the double bond to the 15,16-position and neutralization of the cation at C-17 by a hydroxyl group. Although these products could be biosynthesized by enzymatic opening of the 15,16-epoxide, this second mechanism is discarded, because analogously in the biotransformation of candicandiol **105**, the 7α-epimer of the diterpene sideritriol **108**, was not isolated.

To evaluate the importance of the presence of a 15β-alcohol and a 3-oxo group in the biotransformation of *ent*-kaurene diterpenes by the fungus *Gibberella fujikuroi*, Fraga and *co*-workers prepared substrates **113** and **114** from 3α-hydroxy-15β-angeloxy-*ent*-kaur-16-ene[92] previously isolated from *Elaseolinum tenuifolium*.[93] The biotransformations with the fungus *G. fujikuroi* (**Scheme 19**) in the presence of AMO 1618, a compound that inhibits the production of *ent*-kaur-16-ene without affecting the post-kaurene metabolism,[94] yielded the oxidation at C-19, **122-123**, so the existence of a 15β-hydroxyl group in the molecule does not inhibit this functionalization, in contrast to previous studies with the presence of 15α-alcohol,[95] and directs the hydroxylation at C-11β **115-121** and C-7α **116**, **119-121**, and **124-125**. The occurrence of 11β-hydroxylated *ent*-kaur-16-ene diterpenes with oxygen functions of the 15α-OH, 15β-OH, or 15-oxo type in liverworts[96] and higher plants[97] seems to indicate that the 11β-hydroxylation should be directed by the presence in the molecule of a 15-oxygenated substituent, as occurs in the microbiological transformations with the fungus *G. fujikuroi*. A 3-oxo group in the molecule inhibits oxidation at C-19 **115-119**, but the presence of a 2,3-double bond does not affect this oxidation **122-123**.

7. Beyeranes

Stevioside, the major constituent of *Stevia rebaudiana* leaves extract (family*Asteraceae*), consists of a glycoside possessing steviol, an *ent*-beyerane tetracyclic diterpene with a ketone on the D ring, as its aglycon.[98] Stevioside is approximately 300 times sweeter than sucrose and is widely used as a noncaloric sugar substitute in many kinds of foods and as a food supplement in many countries of Asia and South America.[99] In addition to their sweetening properties, stevioside and steviol have been used to treat metabolic syndromes, such as hypertension, hyperglycemia, dyslipidemia, and diabetes. Its analog, isosteviol, produces vasodilation of rat aorta.[100] Isosteviol lactone **169** has been used in biotransformations, because small modifications to the structure of a compound can modify its biological activities.[101]

Many microorganisms are capable of reproducibly converting isosteviol **127** into many metabolites. To measure the antihypertensive activity of hydroxylated isosteviol, Liu and *co*-workers studied the microbial transformations of **127**, obtained by hydrolysis of stevioside **126** with dilute hydrochloric acid, by *Cunninghamella bainieri*, *Actinoplanes sp*., *Mucor recurvatus*, and *Cunninghamella blakesleeana* (**Scheme 20**).[102] The specie of *C. bainieri* have the ability to functionalize isosteviol **127** at the C-7 position, this is a common occurrence for other microbes with kaurane and beyerane skeletons, [92,103] while hydroxylation at the

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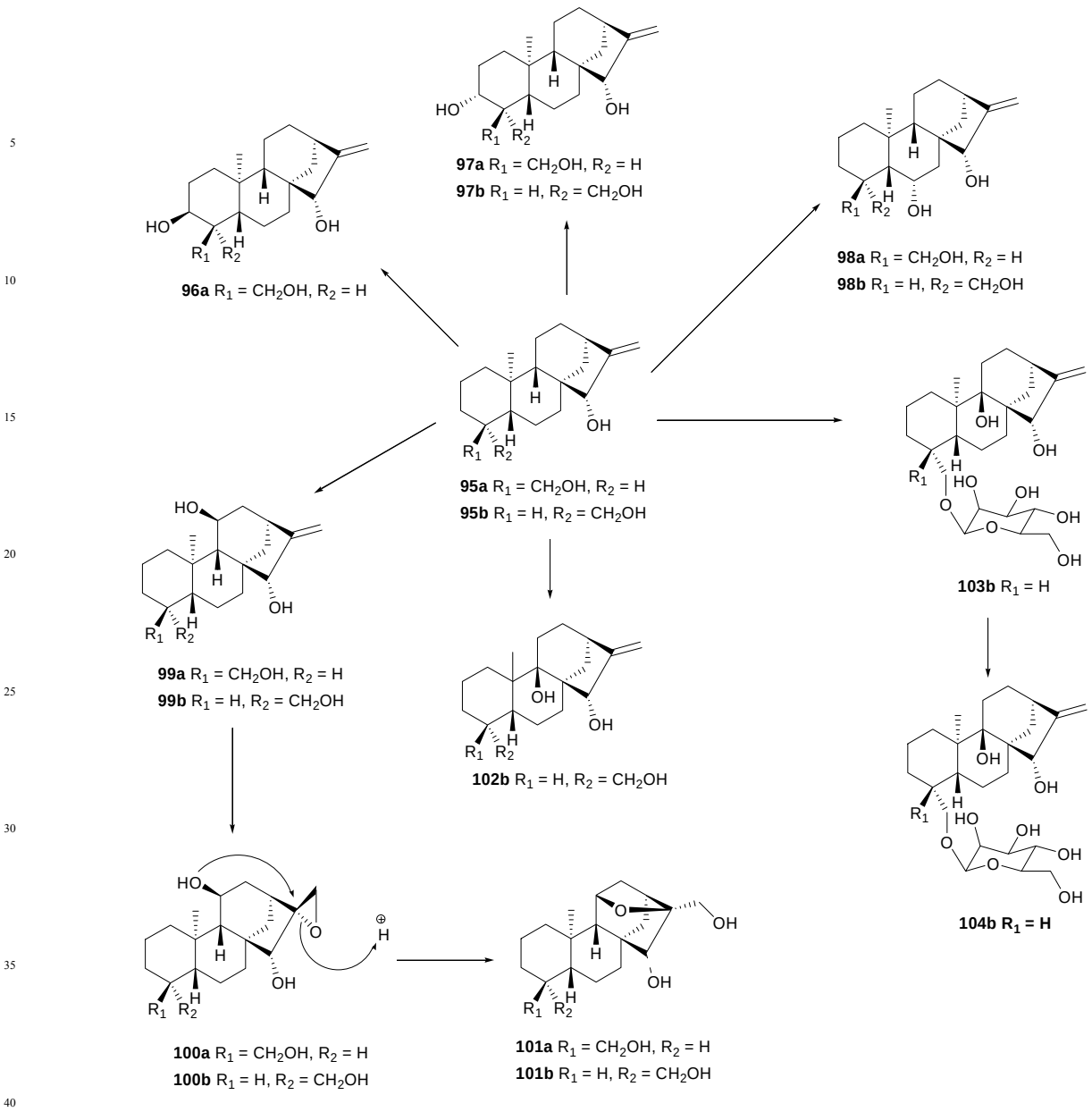
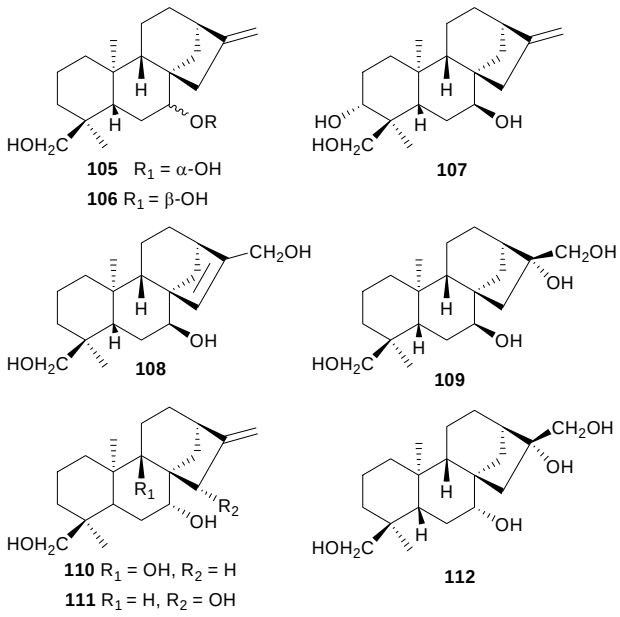


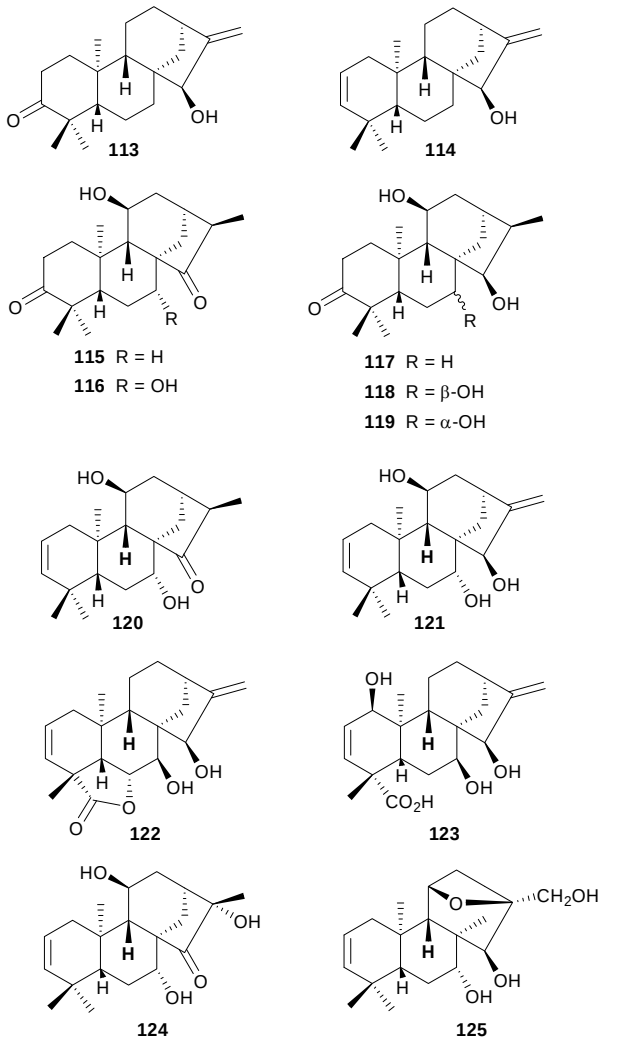
Scheme 17. Microbiological transformation of **89a** and **89b** by *Mucor plumbeus*.

9β-position is obtained for the first time on a beyerane skeleton. Previously, the dihydroxylation on isosteviol **127** was reported only at the 1α- and 7β- positions,[103b] but in this case, several hydroxylated isosteviol analogues were prepared by introduction of a hydroxyl group at: 11β,12β **132** and 11β,12β,17 **133** by *Actinoplanes sp*; 12β,15β **130** and 7β,15β **131** by *Mucor revurvatus*; 7β **128** and 7α **129** by *Cunninghamella bainieri*; 7β **128**, 9β **134** and 12β **135** by *Cunninghamella blakesleeana*.

In a posterior work, the same authors used three filamentous fungi, *Mucor recurvatus* (MR 36), *Absidia pseudocylindrospora* (ATCC 24169), and *Aspergillus niger* (BCRC 32720), for the biotransformation of **127** (**Table 1**).[104] The reactions involved *regio*- and *stereoselectively* introduction of hydroxyl groups at A, B, C, and D rings of isosteviol **127**. A comparison of the substrate specificity between these three selected fungi suggests that they possess different characteristics of reaction selectivity. *M. recuvatus* performs only mono and dihydroxylation on **127**; *A. pseudocylindrospora* is able to specifically hydroxylate **127** at C-17; while, *A. niger* has the ability to dihydroxylate **127** at the 1α,7β- and 7β,11β-positions followed by oxidation of the 1α- or 7β-OH, respectively, to yield the metabolites **151-153**. The dihydroxylation that occurred at the 1α,7β-positions of **127** by



Scheme 18. Microbiological transformation of **99** and **100**.



Scheme 19. Microbiological transformation of **107** and **108** by *G. fujikuroi*.

Asp. niger has previously been reported,[103b] although not the subsequent oxidation of an OH group to a ketone group.

Metabolites **142–145** were obtained previously from incubation of steviol-16α,17-epoxide **136**, prepared from isosteviol **127** by a reaction with *m*-chloroperbenzoic acid, with *Streptomyces griseus* and *Cunninghamella bainieri* (**Table 1**).[105] These two microorganisms have the abilities to produce not only *regio*- and *stereoselective* hydroxylation but also to rearrange the *ent*-kaurane into an *ent*-beyerane skeleton. While Akihisa and co-workers[106] obtained 7β-hydroxyisosteviol **137**, together with 11β-hydroxyisosteviol **155** and 12β-hydroxyisosteviol **156**, from microbial transformation of isosteviol **127** by the fungus *Aspergillus niger*; 17-hydroxyisosteviol **157** by the fungus *Glomerella cingulata*; and 7-oxoisosteviol **158** by the fungus *Mortierella elongate* (**Table 1**).

Yang, Lin and *co*-workers, studied the microbial biotransformation of isosteviol oxime (*ent*-16-*E*-hydroxyiminobeyeran-19-oic acid) **163**, prepared by reacting **127** with hydroxylamine hydrochloride, by *A. niger* and *A. pseudocylindrospora*,[107] which performed hydroxylation reactions **164–167**, Beckmann rearrangement **171**, and an abnormal Beckmann rearrangement **169-170** (**Scheme 21**). Although the isoteviol lactone **168** has been prepared previously by chemical methods, with acid catalysis (concentrated hydrochloric acid or 25% sulphuric acid) in an ampoule at 180°C,[108] this biotransformation yield this product in mild conditions, although in lower yields. The authors propose two possible mechanisms for the formation of isoteviol lactone **168**. According to the literature,[108] nitrile carbocation A (**Scheme 22**) is formed as in the Beckmann fragmentation reaction, however, it is further stabilised not by losing α-proton but by attaching a hydroxyl group and subsequent cyclization in imidate B, which is unstable and easily hydrolyses to lactone **168**. On the other hand, the isolation of ketone **168**, also suggests that substrate **163** might be hydrolyzed to isosteviol **127** first, and then converted to the **167** and **168** by hydroxylation and Baeyer–Villiger reaction, as reported in a previous works by Fraga and *co*-workers.[30-31]

Twenty-five selected microbial cultures were used to identify organisms capable of metabolizing isoteviol lactone **168**,[109] from these, *Cunninghamella bainieri* (ATCC 9244) and *Aspergillus niger* (BCRC 32720) were selected as biocatalysts for scaled-up biotransformations. Incubation of **168** with *C. Bainieri* (**Table 2**) afforded metabolites which involved isomerisation of lactone moiety **178-179**, hydroxylation **173** and **179**, and ring cleavage reactions followed by oxidation and selective *O*-methylation **182**. While *A. niger* possesses the abilities to not only isomerize the lactone ring to **178**, but to cause regio- and stereoselective mono-, di-, and trihydroxylation at the 1α- , 7β-, and 11β-positions.

The change of fungi to *Mucor recurvatus* (MR 36), *Aspergillus niger* (BCRC 31130) and *Absidia pseudocylindrospora* (ATCC 24169), the same authors obtained in the incubation of **168** thirteen diterpenoids (**Table 2**), where **183-194** were different to the previous work.[109] The reactions involved hydroxylation at the C-1, C-7, C-11, C-12 and C-17, and reduction to the ketone moiety **183-184**.

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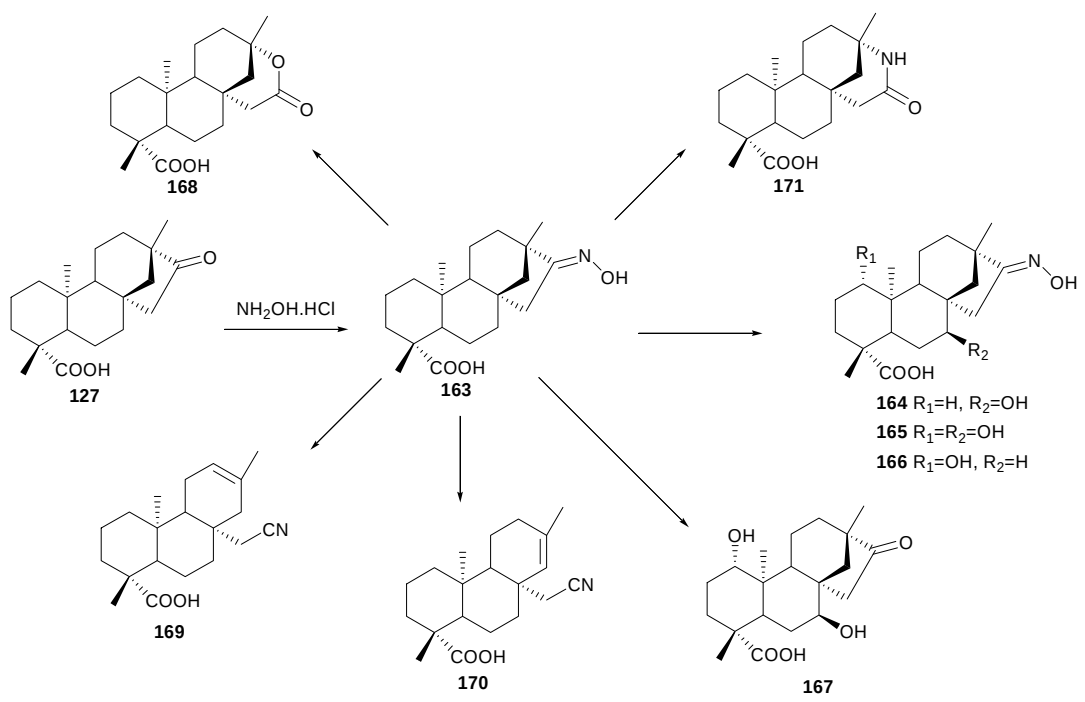
Scheme 20. Microbiological transformation of isosteviol **127**.



Scheme 21. Biotransformation of isosteviol 16-oxime **164** by *Aspergillus niger* and *Absidia Pseudocylindrospora.*

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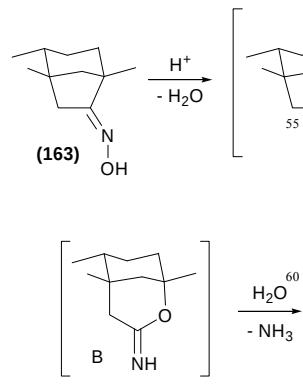
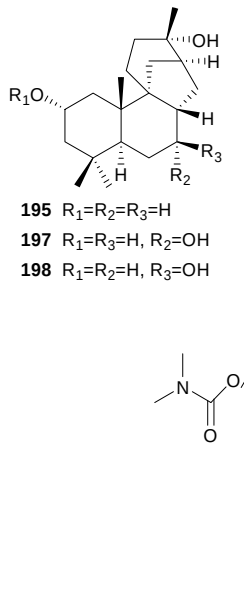


Scheme 22. Possible mechanism for isoteviol lactone **169** formation.



Scheme 23. Biotransformation of stemodin **196** and its carbamate derivative **197**.

8. Stemodanes

This type of compounds, isolated for the first time from *Stemodia maritime* L. (*Scrophulariaceae*) and used as a caribbean folk medicine for venereal diseases treatment,[111] have a unique tetracyclic framework: a *trans*-decalin (ring A/B) fused to a biciclo[3.2.1]octane (ring C/D). Stemodanes represent a synthetic challenge, and for this reason, structural modifications of these substrates and their derivatives via microbial transformation could be an alternative to obtain unique analogues with potential biological activity.

Reese and co-workers incubated stemodin **195** and their dimethylcarbamate derivative **196** by means of cultures of the fungi *Cunninghamella echinulata var. elegans* (ATCC 8688a) and *Phanerochaete chrysosporium* (ATCC 24725) (**Scheme 23**).[112]

The protection of alcohols as their carbamates promote stronger interactions between the substrate and the hydrophobic amino acids that are believed to constitute the enzyme's active site, for the nonpolar nature of the group. Furthermore, it is proposed that an electro-negative oxygen atom would help to anchor the substrate there. Carbamate derivatives of various substrates have been used successfully to improve the bioconversion yields as well as varying the sites where hydroxylation occurs.[23a]

Incubation of *C. echinulata* with stemodin **195** gave three trihydrolylated products **197-199** (**Scheme 23**), while the fermentation of 2α-(*N*,*N*-dimethylcarbamoxy)-13-hydroxystemodane **196** yielded only two monohydroxylated metabolites **201-202**. On the other hand, *P. chrysosporium* converted stemodin **196** into **198-200**. The dimethylcarbamate **196** was not transformed by this microorganism.

9. Conclusions

Biotransformation has gained significant importance in modifying the naturally occurring substance. In this review, we have highlighted the use of a series of diterpenes as starting materials for diverse biocatalytic strategies, in an effort to establish attractive methodologies for obtaining new pharmaceuticals, intermediates and analytical reagents Most biocatalytic reactions can be carried out under certain safety, health, environmental, and economical conditions.

The main structural modification observed is the regiospecific and stereospecific hydroxilation, and the position on the molecule where this occurs depends on both the substrate and the microorganism. Among the most frequently used microorganisms are Mucor plumbeus, Gibberella fujikuroi, and Aspergillus niger (**Figure 1**). The number of products resulting from biotransformation seem to vary with the microorganism, with few exceptions, microbial metabolism usually results in the generation of multiple products, which complicates downstream processing, in customarily low yields.

Figure 1. Comparative involvement of microorganisms utilized for biotransformation.

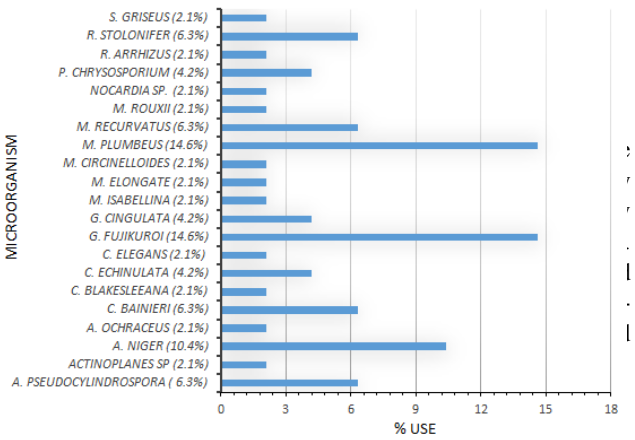


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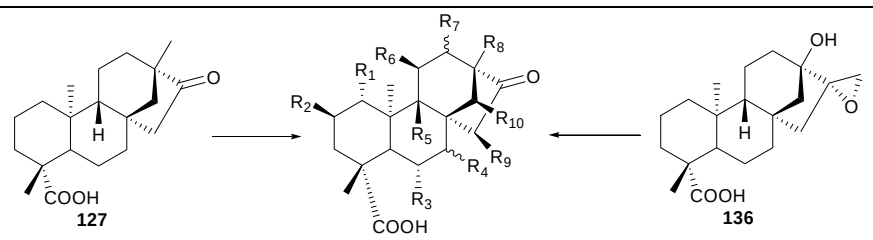
Table 1. Biotransformation of isosteviol **127** and steviol-16α,17-epoxide **136**.



127 **136**

SUBSTRATE	PRODUCT											ORGANISM	REF
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}		
Isosteviol **127**	**137**	H	H	H	β-OH	H	H	H	CH_3	H	H	*M. recurvatus*	104
												A. niger	106
Isosteviol **127**	**138**	H	H	H	α-OH	H	H	H	CH_3	H	H	*M. recurvatus*	104
Isosteviol **127**	**139**	H	H	H	H	H	H	H	CH_3	OH	H	*M. recurvatus*	104
Isosteviol **127**	**140**	H	H	H	H	H	H	β-OH	CH_3	OH	H	*M. recurvatus*	104
Isosteviol **127**	**141**	H	H	H	β-OH	H	H	H	CH_3	OH	H	*M. recurvatus*	104
Isosteviol **127**	**142**	H	H	H	H	OH	H	H	CH_2OH	H	H	*A. pseudocylindrospora*	104
Steviol-16α,17-epoxide **136**												*C. bainieri*	105
Isosteviol **127**	**143**	H	H	H	H	H	H	β-OH	CH_2OH	H	H	*A. pseudocylindrospora*	104
Steviol-16α,17-epoxide **136**												*S. griseus*	105
Isosteviol **127**	**144**	H	H	H	H	H	H	H	α-OH	CH_2OH	H	*A. pseudocylindrospora*	104
Steviol-16α,17-epoxide **136**												*S. griseus*	105
Isosteviol **127**	**145**	H	H	H	α-OH	H	H	H	CH_2OH	H	H	*A. pseudocylindrospora*	104
Steviol-16α,17-epoxide **136**												*C. bainieri*	105
Isosteviol **127**	**146**	H	H	H	α-OH	H	H	β-OH	CH_3	H	H	*A. pseudocylindrospora*	104
Isosteviol **127**	**147**	α-OH	H	H	H	H	H	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**148**	α-OH	H	H	β-OH	H	H	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**149**	H	H	H	=O	H	OH	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**150**	α–OH	H	H	=O	H	H	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**151**	=O	H	H	β-OH	H	H	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**152**	H	H	H	β-OH	H	OH	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**153**	α–OH	H	H	β-OH	H	H	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**154**	α–OH	H	OH	β-OH	H	H	H	CH_3	H	H	*A. niger*	104
Isosteviol **127**	**155**	H	H	H	H	H	OH	H	CH_3	H	H	*A. niger*	106
Isosteviol **127**	**156**	H	H	H	H	H	H	β-OH	CH_3	H	H	*A. niger*	106
Isosteviol **127**	**157**	H	H	H	H	H	H	H	CH_2OH	H	H	*G. cingulata*	106
Steviol-16α,17-epoxide **136**												*S. griseus*	105
Isosteviol **127**	**158**	H	H	H	=O	H	H	H	CH_3	H	H	*M. elongate*	106
Steviol-16α,17-epoxide **136**	**159**	H	OH	H	H	H	H	H	CH_2OH	H	H	*S. griseus*	105
Steviol-16α,17-epoxide **136**	**160**	H	H	H	H	H	H	H	CH_2OH	H	OH	*S. griseus*	105
Steviol-16α,17-epoxide **136**	**161**	OH	H	H	H	H	H	H	CH_2OH	H	H	*C. bainieri*	105
Steviol-16α,17-epoxide **136**	**162**	H	H	H	β-OH	H	H	H	CH_2OH	H	H	*C. bainieri*	105

Table 2. Biotransformation of lactone **169**

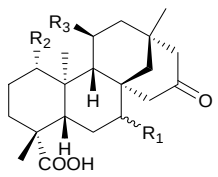
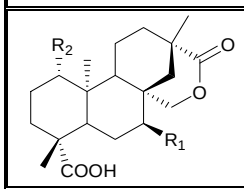
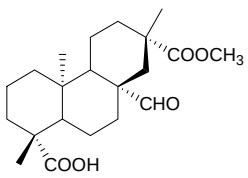
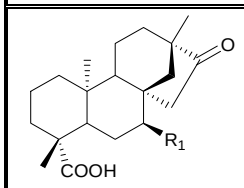
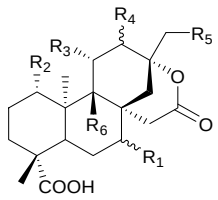
Structure	Substitution	Fungus				
		C. bainieri[109]	*A. niger*[109]	*M. Recur.*[110]	*A. niger*[110]	*A. pseudo.*[110]
	**172** R_1=R_2=R_3=H					
	173 R_1=β-OH, R_2=R_3=H					
	174 R_1=R_3=H, R_2=OH					
	175 R_1=β-OH, R_2=OH,R_3=H					
	176 R_1= =O, R_2=R_3=H					
	177 R_1=β–OH, R_2=OH,R_3=OH					
	**178** R_1=R_2=H					
	179 R_1=OH, R_2=H					
	180 R_1=H, R_2=OH					
	181 R_1=R_2=OH					
						
	182					
						
	183 R_1=H					
	184 R_1=OH					
	**185** R_1=R_2=R_3=R_4=R_5=R_6=H					
	186 R_1=β-OH, R_2=R_3=R_4=R_5=R_6=H					
	187 R_1=β-OH, R_2=R_3=OH,R_4=R_5=R_6=H					
	188 R_1=R_3=R_4=R_5=R_6=H,R_2=OH					
	189 R_1=R_2=R_3=R_5=R_6=H,R_4=β-OH					
	190 R_1=R_2=R_3=R_5=R_6=H,R_4=α-OH					
	191 R_1=α-OH, R_2=R_3=R_4=R_5=R_6=H					
	192 R_1=R_2=R_3=R_4=R_6=H,R_5=OH					
	193 R_1=R_2=R_3=R_4=H,R_5=R_6=OH					
	194 R_1=α-OH, R_5=OH, R_2=R_3=R_4=R_6=H					

Table 3. Summary of the microorganisms experimented for biotransformation and their relative percentage within the presented studies, the substrate, number of days used for the reaction to occur, the number of final Products, and the known modification they induced.

Microorganism	**Substrate**	**Time/ days**	**Number products**	**Modifications**
A. pseudocylindrospora				
ATCC 24169	Isosteviol	6	6	Hydroxylation
	Isosteviol 16-*E*-oxime	6	3	Hydroxylation; Hydrolysis oxime
	Isoteviol lactone	6	7	Hydroxylation
Actinoplanes sp				
	Isosteviol	6	2	Hydroxylation
A. niger				
BCRC 32720	Isosteviol	6	8	Hydroxylation
	Isosteviol	7	3	Hydroxylation
	Isosteviol 16-E-oxime	6	8	Hydroxylation; Hydrolysis oxime; Beckmann and abnormal Beckmann rearrangement
BCRC 32720	Isoteviol lactone	6	8	Isomerization;Hydroxylation
BCRC 31130	Isoteviol lactone	6	5	Hydroxylation;Reduction
A. ochraceus				
	Ent-8(14),15-pimaradiene	5	5	Hydroxylation
C. bainieri				
	Isosteviol	6	2	Hydroxylation (C-7)
	Steviol-16α,17-epoxide	6	6	Oxidation; Backbone rearrangement
	Isoteviol lactone	6	4	Isomerization;Hydroxylation; Ring cleavage reactions
C. blakesleeana				
	isosteviol	6	3	Hydroxylation
C. echinulata				
	Stemodin	10	3	Hydroxylation
	Stemodin dimethylcarbamate derivative	10	2	Hydroxylation
C. Elegans				
	Cryptotanshinone	5	3	Hydroxylation
G. fujikuroi				
	2a, 19-Dihydroxy-9-epi-ent-pimara-7,15-diene	6	7	Hydroxylation
	18-Hydroxy-9-epi-ent-pimara-7,15-diene	6	5	Hydroxylation
	19-Hydroxy-13-epi-ent-pimara-9(11),15-diene	6	4	Hydroxylation (C-2); Oxiadation (C1); Baeyer-Villiger reaction
	13-E*pi-ent*-pimara-9(11),15-diene-19-oic acid	6	4	Epoxidation; Hydroxylation (C-8); Oxidation (C-7)
	9,13-Epi-ent-pimara-7,15-diene diterpene	1	6	Hydroxylation, epoxidation
	15α-Hydroxy-3-oxo-ent-kaur-16-ene	6	5	Hydroxylation; oxidation (C-19)
	15α-Hydroxy-ent-kaur-2,16-diene	6	6	Hydroxylation; oxidation (C-19)
G. cingulata				
	Ent-pimara-8(14),15-dien-19-oic acid	7	1	Reduction of Acid moiety
	Isosteviol	7	1	Hydroxylation

Table 3(Continuation).

Microorganism	Substrate	Time/ days	Number products	Modifications
M. isabellina				
	Dehydroabietic acid	3	1	Hydroxylation (C-2α)
M. elongate				
	Isosteviol	7	2	Oxidation
M. circinelloides				
	Dehydroabietic acid	3	1	Hydroxylation (C-2α)
M. plumbeus				
	Dehydroabietanol	6	6	Hydroxylation
	Teideadiol	6	3	Hydroxylation
	Trachinodiol	6	5	Hydroxylation;Backbone rearrangement
	Candidiol	6	5	Hydroxylation
	15a,19-Dihydroxy-*ent*-kaur-16-ene	6	7	Hydroxylation
	Candicandiol	6	3	Hydroxylation
	Epicandicandiol	6	3	Hydroxylation
M. recurvatus				
	Isosteviol	6	2	Hydroxylation
(MR 36)	Isosteviol	6	5	Hydroxylation
	Isoteviol lactone	6	4	Hydroxylation reduction
M. rouxii				
	Ent-pimara-8(14),15-dien-19-oic acid	7	2	Isomerization doble bond; Oxidation (C-7)
Nocardia sp. NRRL 5646				
	Carnosic acid	3	3	Hydroxylation; Methylation
P. chrysosporium				
ATCC 24725	Stemodin	10	3	Hydroxylation
	Stemodin dimethylcarbamate derivative	10	0	
R. arrhizus				
	Ent-trachyloban-18-oic acid	14	6	Hydroxylation; Backbone rearrangement
R. stolonifer				
	Clerodane lactone	7	2	Oxidation furan; allylic hydroxilation
	Clerodane methyl ester	7	4	Oxidation furan
	Trachyloban-19-oic acid	20	4	Hydroxylation; Backbone rearrangement
S. griseus				
ATCC 10137	Steviol-16a,17-epoxide	6	7	Hydroxylation;Oxidation;Backbone rearrangement

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Notes

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