Biotransformation of Terpenoids: A Green Alternative for Producing Molecules with Pharmacological Activity

Y. Simeó[1] and J.V. Sinisterra[1,2]*

[1] *Organic and Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universidad Complutense de Madrid, Plaza de Ramón y Cajal s/n, 28040 Madrid, Spain*

[2] *Industrial Biotransformations Service, Science Park of Madrid, C/ Santiago Grisolía nº 2. 28760 Tres Cantos, Madrid, Spain*

Abstract: Terpenoids are natural products of great interest due to their broad application scope. They are employed as agrochemicals, drugs, fragrances, flavours and pigments. In the search of new derivatives with improved properties, the use of biocatalysts is being constantly increased, especially in redox processes. They can give rise to stereo- and regioselective products and/or compounds functionalized in remote positions difficult to reach by means of traditional organic chemistry. In this review, the application of whole cell catalyzed biotransformations of terpenoids to obtain new drug targets or to increase the pharmacological activity is presented.

Keywords: Terpenes, terpenoids, biotransformations, enzymes, redox process.

1. INTRODUCTION

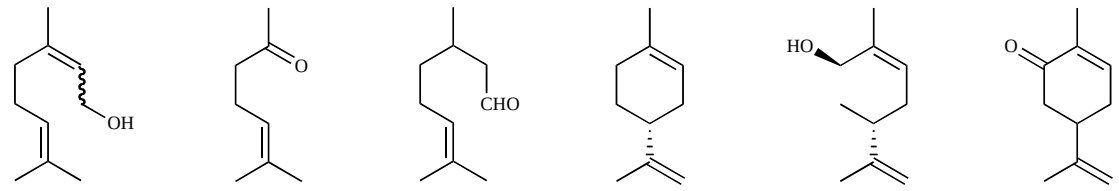
Due to the wide application scope of terpenes and terpenoids ranging from fragrances and flavors to their pharmacological activities such as antibacterial, antiviral, or cytotoxic properties, the interest of these compounds has lately been increased, driving the synthesis of new derivatives which can show improved properties or be a potential source of new building blocks for asymmetrical synthesis. In this sense, the bioconversion of terpenes plays an important role, since remote positions difficult to reach chemically can be biocatalytically functionalized with high regio- and stereoselectivity, as it occurs for instance in the hydroxylation of non functionalized methylene groups.

Biotransformations can be defined as the use of biological systems to produce chemical changes on synthetic or natural compounds. The employment of such processes is constantly growing due to several reasons: i) the reaction conditions are mild and frequently the protection of other reactive functional groups is not necessary; ii) high stereoselectivities and regioselectivities can be achieved, i.e. a single enantiomer is obtained; iii) the functionalization on remote non activated positions is possible; and iv) they are environmentally friendly and economically convenient.

2. MONOTERPENES

Monoterpenes are important fragrant molecules widely distributed in nature (more than 400 structures), which can be isolated from leaves, flowers and fruits of many plants. They are suitable starting materials for the biotechnological production of natural aroma chemicals useful in Food or Pharmaceutical industries.

Demyttenaere *et al.* [1] reported the biotransformation of pure geraniol and nerol (Fig. **1**), the mixture of both (*citrol*) and the mixture of the aldehydes (*citral*) to 6-methyl-5-hepten-2-one by sporulated surface cultures of *Penicilium digitatum*. Interestingly, it was proved that the spores retained their biotransformation capacity over a period of at least 6 weeks. Citral, an antitumoral terpene which imparts the characteristic lemon scent to plants like lemon grass, is a relatively inexpensive compound and represents an important ingredient in the perfumery industry employed for the synthesis of menthol enantiomers [2, 3] and of citronellal [4, 5]. Bacteria, yeasts and filamentous fungi were screened looking for enantiospecific reduction of the α,β-unsaturated carbon bond of this compound, remaining the other C=C bonds unaffected. The bacterial strains produced preferentially the (*S*)-enantiomer of citronellal with an enantiomeric excess (e.e.) of >99% for *Z. mobilis* and 75%



Z = Nerol
E = Geraniol — 6-Methyl-5-hepten-2-one — Citronellal — (*R*)-(+)-Limonene — *trans*-Carveol — Carvone

Fig. (1). Structure of some remarkable monoterpenes and their metabolites obtained by biocatalysed processes.

In this review we present several stereo- and/or regioselective redox biotransformations of terpenoids in order to obtain interesting molecules, which can be used as drugs due to their pharmaceutical activities or as key building blocks to prepare new synthetic or semisynthetic drugs.

for *Citrobacter freundii*. In contrast, the yeasts mainly produced (*R*)-citronellal, like *Candida rugosa*, with an e.e. value of more than 98%.

Another interesting aroma compound is (*R*)-(+)-limonene (Fig. **1**), which can be obtained from orange peel oil. Biotransformation of this monoterpene by the basidiomycete *Pletorus sapidus* led to *cis*/*trans*-carveol and carvone (Fig. **1**) as the main products [6]. (*S*)-Carvone (caraway-like flavor) and (*R*)-carvone (spearmint-like flavor) are important aroma compounds for foods and beverages. Both stereomers can be stereo- and chemoselectively reduced using fungi [7, 8].

*Address correspondence to this author at the Organic and Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universidad Complutense de Madrid, Plaza de Ramón y Cajal s/n, 28040 Madrid, Spain; Tel: +34-91-3941822; Fax: +34-91-3941820: E-mail: jvsgago@farm.ucm.es

1570-193X/09 $55.00+.00 © 2009 Bentham Science Publishers Ltd.

Furthermore, (+)-, (–)- and (±)-*trans*-carveol and *cis*-carveol were enantio- and diastereoselectively biotransformed by *Euglena gracilis Z* photoheterotrophically cultured [9].

3. SESQUITERPENES

Sesquiterpenoids are C_{15} compounds formed by the coupling of three isoprenoid units. They are widespread in nature, mainly distributed in higher plants. Considering the structure of these compounds, Ruzicka proposed the Biogenetic Isoprene Rule, which has been accepted as the base of terpenoid chemistry in general. This rule allowed establishing a kind of relationship within the numerous structures presented by sesquiterpenes, which exist as acyclic, monocyclic, bicyclic, tricyclic, and tetracyclic systems.

Farnesol and its derivatives are acyclic sesquiterpenes involved in terpenoid biosynthesis. Several microbial cultures were screened for their potential to oxifunctionalize α-farnesene (Fig. **2**) [10], since the introduction of oxygen in the terpene hydrocarbon (preferably at the allylic position) can yield flavour compounds. In fact, the major oxidation product in all cases, 3,7,11-trimethyldodeca-1,3(*E*),5(*E*)10-tetraen-7-ol, showed a pleasant citrus-like odour, which was measured by means of GC-olfactometry and a panel of five persons. In particular, an *Aspergillus niger* strain isolated from mango produced two terpene diastereomeric alcohols, menth-1-en-3-[2-methyl-1,3-butadienyl]-8-ol, which represents a new natural compound with an apricot-like odour.

Sesquiterpenoids also exist as monocycles with different ring sizes. Cyclonerodiol (Fig. **2**), isolated from marine-derived fungus *Myrothecium* sp., is an example of a sesquiterpene with a five carbon atoms ring. Preparative-scale fermentation of this compound with *Penicilium* sp. gave rise to a new glycosidic metabolite, 7-*O*-(β-D-mannopyranosyl)cyclonerodiol [11]. In turn, fermentation with a marine isolate of the actinomycete bacterium *Streptomyces* sp. afforded two oxidized geometrical isomers, 10(*Z*)- and 10(*E*)-cyclonerotriols (Fig. **3**). Interestingly, 10(*Z*)-cyclonerotriol has shown a preliminary decrease of 80% on the viability of HeLa cells, a cervical carcinoma cell line.

Limberger *et al.* [12] reported the efficient biotransformation of α-bisabolol (Fig. **2**) to bisabolol-oxide B (Fig. **3**) with the filamentous fungi *Bipolaris sorokiniana*. α-Bisabolol, a precursor of many natural products, is economically interesting due to its delicate floral odour and its anti-inflammatory and antiseptic activities. Therefore, it is being widely employed in the pharmaceutical industry. In the present methodology, the high regio- and stereoseletivity observed in the synthesis of one diastereomer of bisabolol oxide B along with the high yield (84 %) make this biotransformation very appealing. On the contrary, chemical oxidation of α-bisabolol with *m*-chloroperbenzoic acid is not selective, leading to a mixture of epoxidation products in poor yield.

Germacrane sesquiterpenes (Fig. **2**) are important intermediates in the biosynthesis of guaiane, eudesmane and other sesquiterpenes. The biotransformation of several germacrane epoxides by a suspension of fresh chicory root (*Cichorium intybus*) was investigated [13], leading to substituted guaianes and eudesmanes. Interestingly, a different sesquiterpenoid skeleton was formed depending on the position of the oxirane ring in the substrate. This way, (*E*,*E*)-1,5-germacrenes and germacrane-1,10-epoxides derived from (*E*,*E*)-1,5-germacrenes gave eudesmanes, whereas germacrane 4,5-epoxides derived from (*E*,*E*)-1,5-germacranes led to guaianes.

García-Granados' group has intensively investigated the biotrasformation of several kinds of sesquiterpenes, including germacranes [14] and eudesmanes [15-18], 4β-hydroxyeudesmane-1,6-dione among others (Figs. **2** and **3**). Eudesmane sesquiterpenes are extended in nature and they possess remarkable biological properties [19-22], such as antimicrobial, antimalarial, antifeedant, cell-growth-inhibiting and plant-growth-regulating activities. The majority of these biotransformations were carried out using filamentous fungi as *Gliocadium roseum*, which produced hydroxylation reactions in remote positions of the molecules [23-25]. Likewise, cadinane sesquiterpenes such as cadina-4,10(15)-dien-3-one (Fig. **2**) were also stereoselectively oxidized/reduced by *Beauveria bassiana* [26] and *Curvularia lunata* [27].

Other bicyclic sesquiterpenoids with different ring sizes (guaiane skeleton) such as (+)-γ-gurjunene (Fig. **2**) can also be biotransformed [28], producing regioselective hydroxylations in several positions of the molecule.

Tricyclic sesquiterpenoids such as (+)-1(10)-aristolene (Fig. **2**) from the crude drug *Nardostachys chinensis*, or plagiochilide (Fig. **2**) from the liverwort *Plagiochila fructicosa*, were biotransformed by *Chlorella fusca* var. *vacuolata*, *Mucor* sp. and *Aspergillus niger* [29]. Green algae *C. fusca* var. *vacuolata* and *Mucor* species oxidized the cyclohexane ring of aristolene. In turn, *A. niger* acted stereoselectively on one methyl of the *gem*-dimethyl group at C-11 of aristolanes and 2,3-secoaromadendrane, yielding a C-12 primary alcohol or a carboxylic acid.

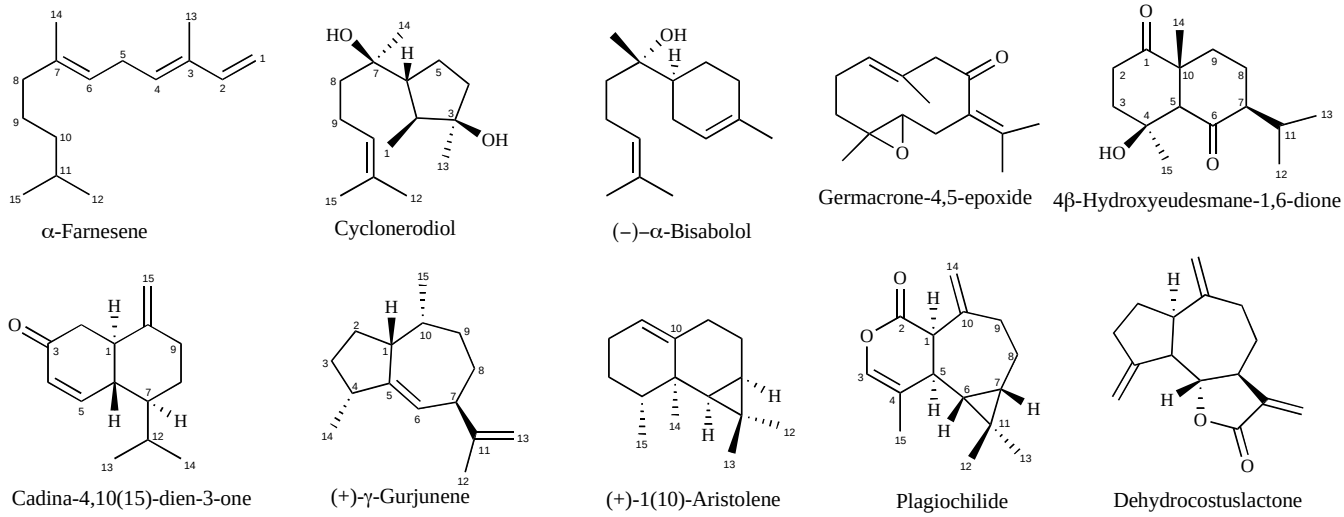
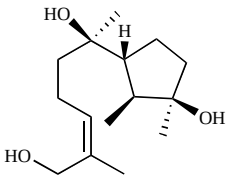
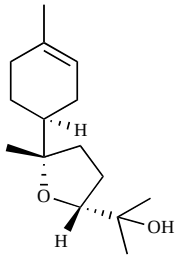


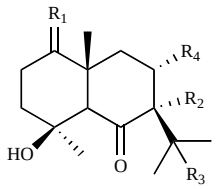
Fig. (2). Examples of most significant sesquiterpenoids.



10(*Z*)-Cyclonerotriol



Bisabolol oxide B



R_1 = O, R_2 = OH, R_3 = R_4 =H
R_1 = O, R_2 = R_4 =H, R_3 = OH
R_1 = O, R_2 = R_3 = OH, R_4 = H
R_1 = αOH, R_2 = R_4 =H, R_3 = OH
R_1 = αOH, R_2 = R_3 = H, R_4 = OH

Metabolites isolated fron the biotransformation of 4β-hydroxyeudesmane-1,6-dione by *Gliocadium roseum*

Fig. (3). Main sesquiterpene metabolites obtained by biotransformation.

Tricyclic lactones from cadinane skeleton, such as dehydrocostuslactone (Fig. **2**) and costunolide [30] or guaiane sesquiterpenes (arteannuin B) [31], have also been biocatalytically transformed. In the case of cadinane compounds, twenty strains of filamentous fungi (from nine genera) and four species of bacteria were screened. When *Mucor polymorphosporus* AS 3.3443 and *Aspergillus candidus* CICC 2360 were employed, stereo- and regiospecific hydrogenation reactions, epoxidation and epoxide hydrolysis occurred. Finally, other tricyclic sesquiterpenoids such as cedrol [32] or (-)-ambrox® [33] have been biocatalytically hydroxylated. Recently, a review about the regiospecificity in the bioconversion of sesquiterpenes by *Mucor plumbeus* has also been published [34].

4. DITERPENES

Diterpenoids form a large group of C-20 substances derived from geranylgeraniol pyrophosphate. They are mainly of fungal or plant origin and include the resin acids and the gibberellin plant growth hormones.

The research group of Reese has intensively investigated the biotransformation of terpenoids in general, paying much attention to sesquiterpenes and diterpenes in particular. Fungal transformation by *Beauveria bassiana* ATCC 7159 of *Stemodia maritima* L. terpenes stemodin, stemodinone and stemarin (Fig. **4**) was carried out with the intention of gaining some derivatives with enhanced antiviral activity than hydroxylated analogues previously produced [35]. Stemodin gave rise to 2α,13,18-trihydroxystemodane, stemodinone to 13,18-dihydroxystemodan-2-one and stemarin to two compounds, 1β,13,19-trihydroxystemarane and 13-hydroxystemarane-19-carboxylic acid. The synthesis and biotransformation of various derivatives of stemodin have also been studied.

The same research group also examined the influence of the C-2 oxygen function in the biotransformation of stemodin and analogues by *Rhizopus oryzae* ATCC 11145 [36] (Fig. **4**). In the case of stemodin, hydroxylation occurred at C-7, C-3 and/or C-16, yielding 2α,7β,13(*S*)-trihydroxystemodane and 2α,3β,13(*S*),16α-tetrahydroxystemodane. In turn, when stemodinone was subjected to *Rhizopus oryzae* cells, hydroxylation took place in C-6, rendering 6α,13(*S*)-dihydroxystemodan-2-one. These results provide useful information about the correlation between the functional groups of the substrates and the structure of the compounds isolated from the biotransformation. However, the yields of the metabolites obtained were rather low (1-10%). Microbial transformation of some rearranged stemodane derivatives has been studied by Reese *et al.*, rendering only hydroxylation reactions [37]. Stemodin and/or derivatives have been bioconverted by other microorganisms such as *Aspergillus niger* [38], *Mucor plumbeus* [39], *Whetzelinia sclerotiorum* [39], *Cunninghamella echinulata* [40] and *Phanerochaete Chrysosporium* [40].

The microbial transformation of terpenoids with fungi has also been one of the aims of Fraga *et al.*, particularly with *Gibberella fujikuroi*. This fungus produces the gibberellin plant hormones and the aim of these investigations was the preparation of new gibberellin analogues to obtain information about the substrate specificity of the enzymes involved in the biosynthesis of gibberellins. In this context, biotransformation of ribenone (Fig. **4**) by *Gibberella fujikuroi* produced several hydroxylated metabolites and 2,3-*seco*-acids [41]. It has been observed, that the compounds with a new oxygenated function may be more likely transformed by the fungus. Probably, the higher polarity with regard to ribenone facilitates its transport across membranes. Fraga *et al.* also investigated the biotransformation catalyzed by *Gibberella fujikuroi* of different skeletons such as *ent*-kauren-type diterpenes [42-43], *ent*-pimarene [44] and *ent*-manoyl oxide derivatives [45]. The latter substrates have been biotransformed with other microorganisms, such as *Curvularia lunata* [46], *Fusarium moniliformis* [47] and *Mucor plumbeus* [48].

Labdane-diterpenes are also substrates commonly biotransformed. When different strains were fed with isocupressic acid (15-hydroxylabda-8(17),13(*E*)-dien-19-oic acid) (Fig. **4**), several oxygenated metabolites were produced [49]. *Nocardia aurantia* ATCC 12674 catalyzed the cleavage of the 13,14-double bond to yield a new *nor*-labdane metabolite. In turn, *Cunninghamella elegans* NRRL 1393 hydroxylated the starting material in several positions, giving 7β-hydroxyisocupressic acid and labda-7,13(*E*)-diene-6β,15,17-triol-19-oic acid, in which a isomerization of one double bond took place. *Mucor mucedo* ATCC 20094 produced 2α-hydroxyisocupressic acid and labda-8(17),14-diene-2α,13-diol-19-oic acid. Likewise, cupressic acid (13-hydroxy-8(17),14-labdadien-19-oic acid), a diterpene obtained from *Araucarea angustifolia elegans*, was biotransformed by *Fusarium graminearum* producing four hydroxylated diterpene derivatives [50]. Sclareol, an antibacterial labdane-diterpene, has also been hydroxylated by several strains [51-53]. Furthermore, trachyloban-19-oic acid (Fig. **4**) was biotransformed by *Rhizopus stolonifer*, producing hydroxylation and rearrangement reactions [54].

The synthetic abietane diterpene triptophenolide (Fig. **4**) is metabolized by the fungus *Cunninghamella elegans* to produce triptoquinone (35%), 5α,14-dihydroxybutenolide (12%) and 14β-glucosyltriptophenolide (5%) (Fig. **5**). In order to increase the yield of triptoquinone (which shows anti-inflammatory activity) and to minimise the formation of the other metabolites, the influence of four factors (glucose, nutrient broth, and malt extract concentration,

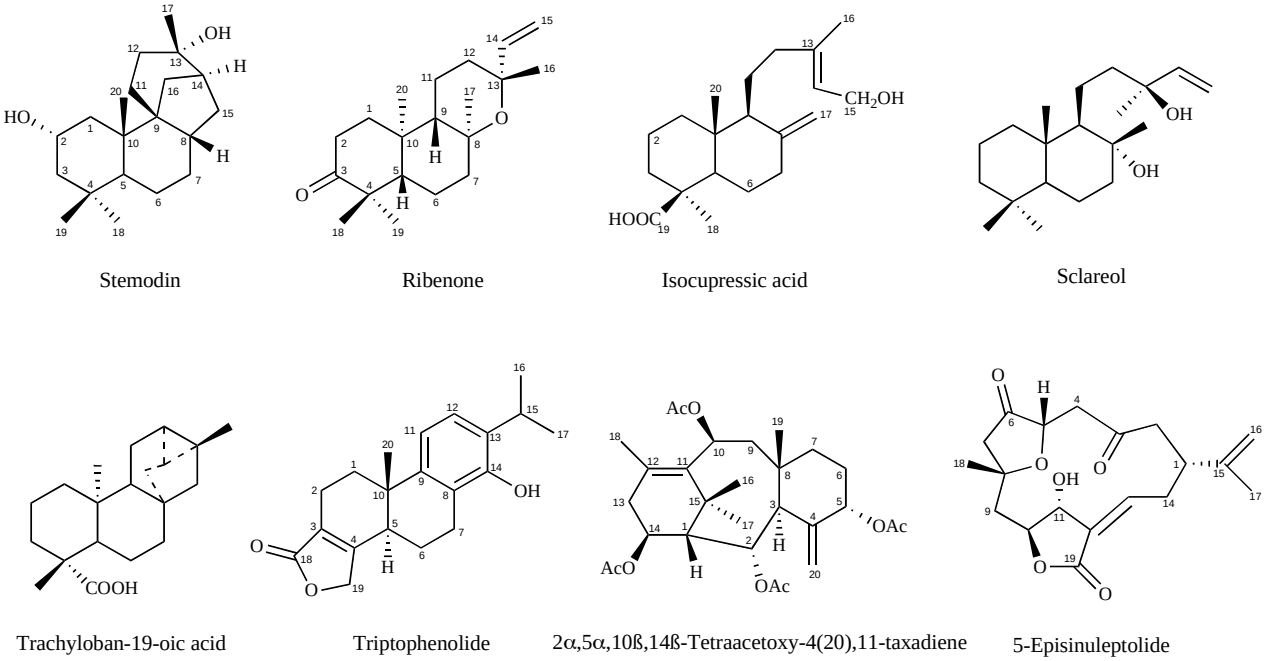


Fig. (4). Main diterpene structures.

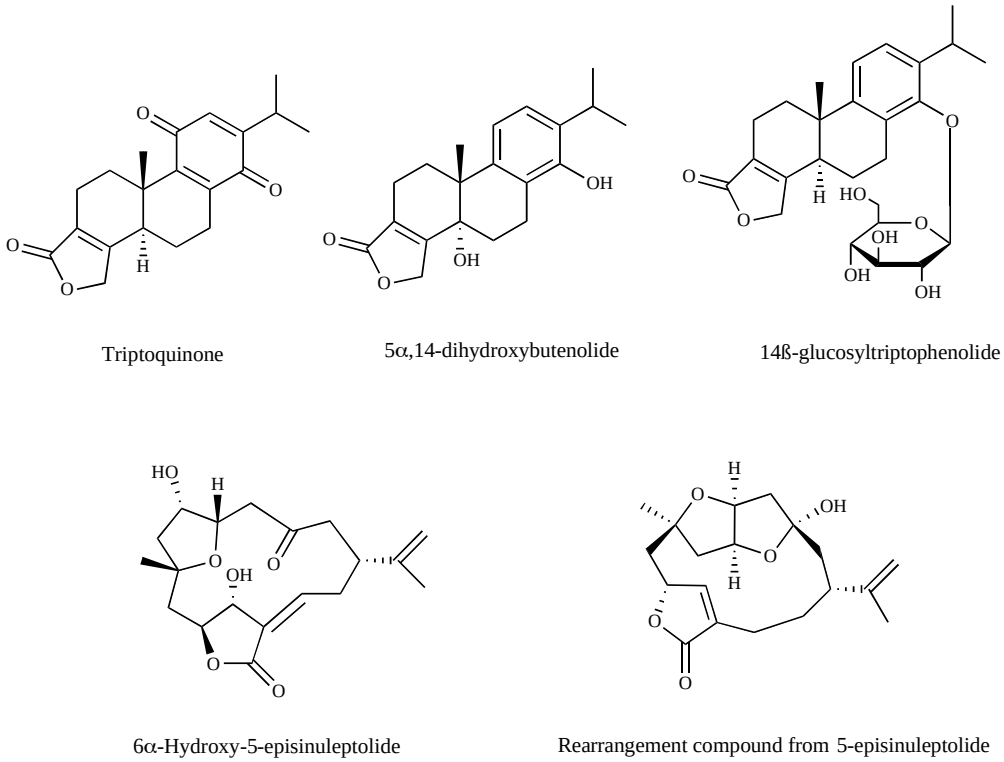
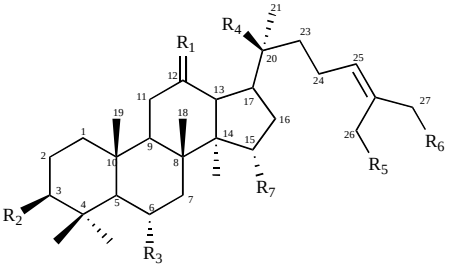


Fig. (5). Metabolites produced by bioconversion of several diterpenes.

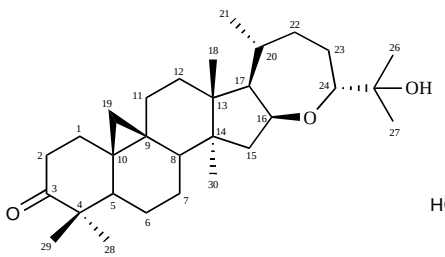
and biotransformation time) were examined [55], exhibiting that the biotransformation time was a critical reaction parameter.

As mentioned in the introduction, the biotransformation of biologically active molecules is of great interest due to the possibility of preparing products difficult to obtain by chemical means and with putative better properties. This is the case of taxol, which is a highly valued drug in cancer chemotherapy. Bioconversion of 2α,5α,10β,14β-tetraacetoxy-4(20),11-taxadiene by the fungi *Cunninghamella elegans* and *Cunninghamella echinulata* was examined [56]. Hydroxylation and deacetylation reactions in several positions took place. The same methodology has been pursued by Regueiro-Ren *et al*. to obtain hydroxylated derivatives of antifungal sordaricin [57]. Its antifungal activity was proved to be very sensitive to modifications in the steric hindrance and/or hydrophobicity of the diterpene skeleton.

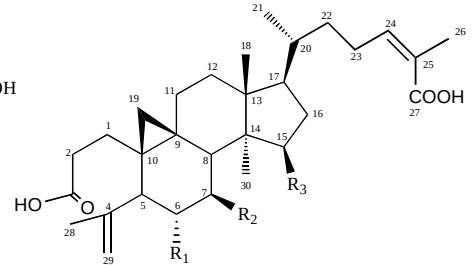
Finally, biocatalytic transformation studies with 32 bacteria and fungi of 5-episinuleptolide, isolated from several species of the soft coral genus *Sinularia*, were carried out. It is an abundant norcem-



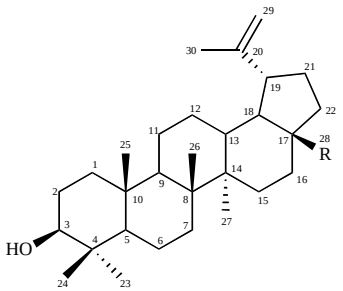
Ginsenoside Rb1, R_1 = βOH, R_2 = Glu(1-2)glu-, $R_3 = R_5 = R_6 = R_7$ = H, R_4 = Glu(1-6)glu-
Protopanaxatriol, R_1 = βOH, $R_2 = R_3 = R_4$ = OH, $R_5 = R_6 = R_7$ = H
12-oxo-26-hydroxy-20(S)-protopanaxatriol, R_1 = O, $R_2 = R_3 = R_4 = R_5$ = OH, $R_6 = R_7$ = H
12-oxo-15α-hydroxy-20(S)-protopanaxatriol, R_1 = O, $R_2 = R_3 = R_4 = R_7$ = OH, $R_5 = R_6$ = H
12-oxo-27-hydroxy-20(S)-protopanaxatriol, R_1 = O, $R_2 = R_3 = R_4 = R_6$ = OH, $R_5 = R_7$ = H
27-hydroxy-20(S)-protopanaxatriol, R_1 = βOH, $R_2 = R_3 = R_4 = R_6$ = OH, $R_5 = R_7$ = H



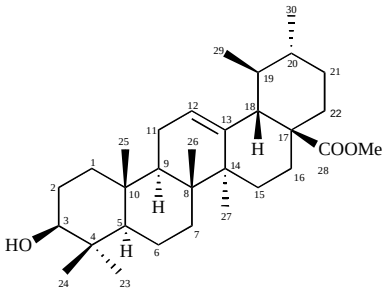
Argentatin B



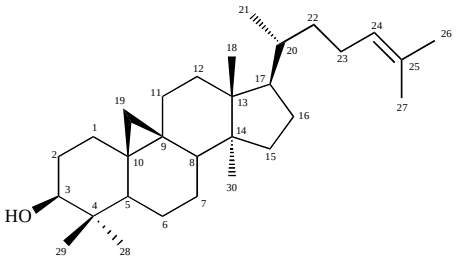
Nigranoic acid, $R_1 = R_2 = R_3$ = H
15β-hydroxynigranoic acid, $R_1 = R_2$ = H, R_3 = OH
6α,15β-dihydroxynigranoic acid, $R_1 = R_3$ = OH, R_2 = H
7β,15β-dihydroxynigranoic acid, R_1= H, $R_2 = R_3$ = OH



R = COOH, Betulinic acid
R = CH_2OH, Betulin



Methyl ursolate



Cycloartenol

Fig. (6). Significant triterpene compounds involved in biotransformation reactions.

branolide diterpene showing moderate cytotoxicity in assays against four human cancer cell lines. The most convenient strain, *Streptomyces lavendulae* ATCC 8664, was used for a preparative scale biotransformation of 5-episinuleptolide, rendering 6α-hydroxy-5-episinuleptolide and a compound with a 3,8-bicyclized cembranoid skeleton formed by rearrangement (Fig. **5**) [58]. Unfortunately, 6α-hydroxy-5-episinuleptolide was proven to be less active than starting material in cytotoxicity studies.

5. TRITERPENES

Triterpenoids constitute the largest group of terpenoids. They are widely distributed in nature, mainly in plants as free compounds or as esters or glycosides. However, some of them have also been found in the animal kingdom. There is a great structural variety of triterpenoids, but all of them originate biogenetically from squalene.

Ginsenosides, the major active component in *Panax ginseng* C. A. Meyer (Araliceae), have been reported to exhibit antitumor effects [59], particularly the inhibition of tumor-induced angiogenesis [60] and tumor invasion and metastasis [61, 62] and the control of phenotypic expression and differentiation of tumor cells [63, 64]. The most interesting aglycones of ginsenosides are the dammaranes 20(*S*)-protopanaxatriol and 20(*S*)-protopanaxadiol. The research group of Guo and colleagues tested the capability of 49 microbial strains to biotransform ginsenoside Rb_1 (Fig. **6**), a protopanaxadiol saponine, showing that the fungi *Rhizopus stolonifer* and *Curvularia lunata* produced four metabolites with different number of glucose units [65]. Similar metabolites were identified when the same substrate (ginsenoside Rb_1) was treated for 48 h with *Caulobacter leidyia* GP45, which was isolated from the soil of ginseng field [66].

Protopanaxatriol (Fig. **6**) has shown a strong cytotoxic activity against human leukemia cells (THP-1) by inducing DNA fragmentation and cell apoptosis [67]. These results, together with those of protopanaxadiol, suggest that the aglycones of ginsenosides are responsible for the antitumor effects. Searching for more active compounds derived from protopanaxatriol, four new metabolites were produced by the fungus *Mucor spinosus* (AS 3.3450) by means of oxidation reactions [68], 12-oxo-15α-hydroxy-20(*S*)-protopanaxatriol, 27-hydroxy-20(*S*)-protopanaxatriol, 12-oxo-26-hydroxy-20(*S*)-protopanaxatriol and 12-oxo-27-hydroxy-20(*S*)-protopanaxatriol. All the new metabolites as well as the substrate had significant cytotoxic effects on HL-60 cells (human leukemia cells).

Argentatin B is another naturally occurring tetracyclic triterpene (Fig. **6**). It presents a cycloartane-type structure and was isolated from *Parthenium argentatum* x *P. tormentosa*. The microbial transformation led to 16,24-epoxycycloartan-3α,25-diol (isoargentatin D) by *Nocardia corallina* var. *taoka* ATCC 31338, *Mycobacterium species* NRRL B3683 and *Septomyxa affinis* ATCC 6737, which also produced 16,24-epoxycycloartan-3β,25-diol (argentatin D) and 1,2-didehydroargentatin B (isoargentatin D) [69]. Cycloartane-type triterpenes are considered to be good sources to develop potent antitumor-promoters (cancer chemopreventive agents) [70]. Akihisa *et al.* investigated the fungal transformation of cycloartenol, 24-methylenecycloartanol and cycloartenone [71] using *Glomerella fusarioides*. Different reactions took place, such as hy-

droxylation, isomerization, oxidation, side chain fragmentation, methylation and demethylation. It is worthy to mention that the metabolite formed by side-chain degradation possessed a pregnane-type C_2 side-chain.

Nigranoic acid (Fig. **6**), an A-ring-secocycloartene triterpenoid showing activity against tumor cell lines and HIV, could also be biotransformed by the fungus *Gliocadium roseum* YMF1.00133. This microorganism hydroxylated the starting material in several remote positions, yielding 15β-hydroxynigranoic acid, 6α,15β-dihydroxynigranoic acid and 7β,15β-dihydroxynigranoic acid [72].

Lupane-type triterpenes constitute also an important class of biologically active compounds. Betulin, betulinic acid and their derivatives have been reported to exhibit a variety of biological properties, such as anti-inflammatory activity [73-75], inhibition of human immunodeficiency virus (HIV) replication in H9 lymphocyte cells [76], blockage of HIV type 1 entry into cells [77], inhibition of DNA polymerase β [78] and inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation [79]. The research group of Kouzi has paid much attention to different biotransformation reactions of these compounds. For instance, preparative biotransformation of betulinic acid (Fig. **6**) using resting-cells suspensions of *Cunninghamella* sp. afforded 28-*O*-β-D-glucopyranosyl-3β-hydroxy-lup-20(29)-en-28-oate, so that a selective biocatalytic glycosylation took place [80]. However, this metabolite was not active against the tested melanoma cell lines when compared to betulinic acid. These results suggest that the free carboxylic acid group at C-28 is essential for cytotoxic activity against melanoma. Incubation of betulinic acid with resting-cells suspensions of phenobarbital-induced *Bacillus megaterium* ATCC 14581, *Cunninghamella elegans*, *Mucor mucedo* and *Bacillus megaterium* ATCC 13368 resulted in the production of metabolites differently oxidized [81, 82]. The monooxygenase systems of these cultures have demonstrated a high degree of similarity with mammalian microsomal monooxygenases and the potential to serve as *in vitro* models of mammalian drug metabolism.

Although fungal transformations of ursane-type triterpenes are rare, methyl ursolate (Fig. **6**) was successfully biotransformed by *Mucor plumbeus* ATTC 4740, giving rise to a novel compound hydroxylated in C-7 and C-21 with poor yield (1.2%) [83].

CONCLUSIONS

The broad application of biocatalysis with whole cells to obtain new terpenoid derivatives has been shown. Oxidation/reduction reactions and hydroxylations are the most common transformations, although other processes are also possible. In several cases, molecules with improved pharmacological properties have been reported, enabling their potential application as drugs. These reactions must be performed using whole cells because coenzyme regeneration is necessary to scale up the process. Due to the advantages of biocatalysis compared to traditional organic synthesis, this field is gaining more and more interest, in particular with respect to industrial applications, so that a promising future of terpenoid biotransformations can be envisioned.

ACKNOWLEDGEMENTS

The authors thank the financial support by the SOLFSAVE project funded by the European Union and the project S-0505/PPQ/0344 by the Comunidad Autónoma de Madrid.

REFERENCES

[1] Demyttenaere, J.; De Kimpe, N. Biotransformation of terpenes by fungi: Study of the pathways involved. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 265-270.

[2] Trasarti, A.F.; Marchi, A.J.; Apesteguia, C.R. Highly selective synthesis of menthols from citral in a one-step process. *J. Catal.* **2004**, *224*, 484-488.

[3] Mäki-Arvela, P.; Kumar, N.; Kubicka, D.; Nasir, A.; Heikkilä, T.; Lehto, V.P.; Sjöholm, R.; Salmi, T.; Murzin, D.Y. One-pot citral transformation to menthol over bifunctional micro- and mesoporous metal modified catalysts: Effect of catalyst support and metal. *J. Mol. Catal. A: Chem.* **2005**, *240*, 72-81.

[4] Müller, A.; Hauer, B.; Rosche, B. Enzymatic reduction of the α, β-unsaturated carbon bond in citral. *J. Mol. Catal. B: Enzym.* **2006**, *38*, 126-130.

[5] Hall, M.; Hauer, B.; Stuermer, R.; Kroutil, W.; Faber, K. Asymmetric whole-cell bioreduction of an α,β-unsaturated aldehyde (citral): competing *prim*-alcohol dehydrogenase and C–C lyase activities. *Tetrahedron: Asymmetry* **2006**, *17*, 3058-3062.

[6] Onken, J.; Berger, R.G. Effects of *R*-(+)-limonene on submerged cultures of the terpene transforming basidiomycete *Pleurotus sapidus*. *J. Biotechnol.* **1999**, *69*, 163-168.

[7] Carballeira, J.D.; Álvarez, E.; Campillo, M.; Pardo, L.; Sinisterra, J.V. *Diplogelasinospora grovesii* IMI 171018, a new whole cell biocatalyst for the stereoselective reduction of ketones. *Tetrahedron Asymmetry* **2004**, *15*, 951-962.

[8] Carballeira, J.D.; Valmaseda, M.; Álvarez, E.; Sinisterra, J.V. *Gongronella butleri, Schizosaccharomyces octosporus* and *Diplogelasinospora grovesii*: novel microorganisms useful for the stereoselective reduction of ketones. *Enzyme Microb. Technol.* **2004**, *34*, 611-623.

[9] Noma, Y.; Asakawa, Y. Enantio- and diastereoselectivity in the biotransformation of carveols by *Euglena gracilis* Z. *Phytochemistry* **1992**, *31*, 2009-2011.

[10] Krings, U.; Hardebusch, B.; Albert, D.; Berger, R.G.; Maróstica, M.; Pastore, G.M. Odor-Active alcohols from the fungal transformation of α-farnesene. *J. Agric. Food Chem.* **2006**, *54*, 9079-9084.

[11] Li, X.; Kim, Y.H.; Jung, J.H.; Kang, J.S.; Kim, D.-K.; Choi, H.D.; Son, B.W. Microbial transformation of the bioactive sesquiterpene, cyclonerodiol, by the ascomycete *Penicillium* sp. and the actinomycete *Streptomyces* sp. *Enzyme Microb. Technol.* **2007**, *40*, 1188-1192.

[12] Limberger, R.P.; Ferreira, L.; Castilhos. T.; Aleixo, A.M.; Petersen, R.Z.; Germani, J.C.; Zuanazzi, J.A.; Fett-Neto, A.G.; Henriques, A.T. The ability of *Bipolaris sorokiniana* to modify geraniol and (-)-alpha-bisabolol as exogenous substrates. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 552-555.

[13] Piet, D.P.; Schrijvers, R.; Franssen M.C.R.; de Groot, A. Biotransformation of germacrane epoxides by *Cichorium intybus*. *Tetrahedron* **1995**, *51*, 6303-6314.

[14] García-Granados, A.; Gutiérrez, M.C.; Martínez, A.; Rivas, F.; Arias, J.M. Biotransformation of shiromool derivatives by *Rhizopus nigricans* cultures: Chemical-microbiological synthesis of michelenolide analogues. *Tetrahedron* **1998**, *54*, 3311-3320.

[15] García-Granados, A.; Gutiérrez, M.C.; Rivas, F.; Arias, J.M. Biotransformation of 4β-hydroxyeudesmane-1,6-dione by *Gliocladium roseum* and *Exserohilum halodes*. *Phytochemistry* **2001**, *58*, 891-895.

[16] García-Granados, A.; Gutiérrez, M.C.; Parra, A.; Rivas, F. Chemical-Microbiological Synthesis of cryptomeridiol derivatives by *Gliocladium roseum*: Semisynthesis of 11-hydroxyeudesmanolides. *J. Nat. Prod.* **2002**, *65*, 1011-1015.

[17] García-Granados, A.; Gutiérrez, M.C.; Rivas, F. Biotransformation of a 4α-hydroxylated eudesmane with *Exserohilum halodes*: Chemo-enzymatic synthesis of cryptomeridiol and 6-*epi*-colartin derivatives. *J. Mol. Cat. B: Enzym.* **2004**, *27*, 133-138.

[18] García-Granados, A.; Melguizo, E.; Parra, A.; Pérez, F.L.; Simeó, Y.; Viseras, B.; Arias, J.M. Chemical semisynthesis and biotransformation with *Rhizopus nigricans* of several sesquiterpenes: Obtention of new 1α- and 2α-hydroxyselinane derivatives. *Tetrahedron* **2000**, *56*, 6517-6526.

[19] Rodríguez, E.; Towers, G.H.N.; Mitchell J.C. Biological activities of sesquiterpene lactones. *Phytochemistry* **1976**, *15*, 1573-1580.

[20] Hooper, M.; Kirby, G.C.; Kulkarni, M.M.; Kulkarni, S.M.: Nagasampagi, B.A.; O'Neill, M.J.; Phillipson, J.D.; Rojatkar, S.R.; Warhurst, D.C. Antimalarial activity of parthenin and its derivatives. *Eur. J. Med. Chem.* **1990**, *25*, 717-723.

[21] Robles, M.; Aregullin, M.; West, J.; Rodríguez, E. Recent studies on the zoopharmacognosy, pharmacology and neurotoxicology of sesquiterpene lactones. *Planta Med.* **1995**, *61*, 199-203.

[22] Sattar, E.A.; Galal, A.M.; Mossa, G.S. Antitumor germacranolides from *Anvillea garcinii*. *J. Nat. Prod.* **1996**, *59*, 403-405.

[23] Miyazawa, M.; Honio, Y.; Kameoka, H. Biotransformation of the sesquiterpenoid β-selinene using the plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. *Phytochemistry* **1997**, *44*, 433-436.

[24] Alarcón, J.; Águila, S.; Cornejo. F.; Alderete, J. Biotransformation of 5α-hydroxy-14-eudesm-11-en-3-one by *Rhizopus nigricans*, *Cunninghamella elegans* and *Mucor plumbeus*. *J. Mol. Catal. B: Enzym.* **2007**, *48*, 23-27.

[25] Orabi, K.Y. Microbial transformation of the eudesmane sesquiterpene plectranthone. *J. Nat. Prod.* **2000**, *63*, 1709-1711.

[26] Buchanan, G.O.; Williams, L.A.D.; Reese, P.B. Biotransformation of cadinane sesquiterpenes by *Beauveria bassiana* ATCC 7159. *Phytochemistry* **2000**, *54*, 39-45.

[27] Collins, D.O.; Reese, P.B. Biotransformation of cadina-4,10(15)-dien-3-one and 3α-hydroxycadina-4,10(15)-diene by *Curvularia lunata* ATCC 12017. *Phytochemistry* **2002**, *59*, 489-492.

[28] Miyazawa, M.; Honjo, Y.; Kameoka, H. Biotransformation of the sesquiterpenoid (+)-γ-Gurjunene using a plant pathogenic fungus, glomerella cingulata, as a biocatalyst. *Phytochemistry* **1998**, *49*, 1283-1285.

[29] Furusawa, M.; Hashimoto, T.; Noma, Y.; Asakawa, Y. Biotransformation of aristolane- and 2,3-secoaromadendrane-Type sesquiterpenoids having a 1,1-

dimethylcyclopropane ring by *Chlorella fusca* var. *vacuolata*, *Mucor* species, and *Aspergillus niger*. *Chem. Pharm. Bull.* **2006**, *54*, 861-868.
[30] Ma, X.-C.; Zheng, J.; Guo, D.-A. Microbial transformation of dehydrocostuslactone and costunolide by *Mucor polymorphosporus* and *Aspergillus candidus*. *Enzyme Microb. Technol.* **2007**, *40*, 1013-1019.
[31] Elmarakby, S.Y.; El-Feraly, F.S.; Elsohly, H.N.; Croom, E.M.; Hufford, C.D. Microbial transformation studies on arteannuin B. *J. Nat. Prod.* **1987**, *50*, 903-909.
[32] Collins, D.O.; Reese, P.B. Biotransformation of cedrol by *Curvularia lunata* ATCC 12017. *Phytochemistry* **2001**, *56*, 417-421.
[33] Nasib, A.; Musharraf, S.G.; Hussain, S.; Khan, S.; Anjum, S.; Ali, S.; Atta-ur- Rahman; Choudhary, M.I. Biotransformation of (−)-ambrox by cell suspension cultures of *Actinidia deliciosa*. *J. Nat. Prod.* **2006**, *69*, 957-959.
[34] Arantes, S.F.; Hanson, J.R. The Biotransformation of sesquiterpenoids by *Mucor plumbeus*. *Curr. Org. Chem.* **2007**, *11* (7), 657-663
[35] Buchanan, G.O.; Reese, P.B. Biotransformation of diterpenes and diterpene derivatives by *Beauveria bassiana* ATCC 7159. *Phytochemistry* **2001**, *56*, 141-151.
[36] Martin, G.D.A.; Reynolds, W.F.; Reese, P.B. Investigation of the importance of the C-2 oxygen function in the transformation of stemodin analogues by *Rhizopus oryzae* ATCC 11145. *Phytochemistry* **2004**, *65*, 701-710.
[37] Martin, G.D.A.; Reynolds, W.F.; Reese, P.B. Stemodane skeletal rearrangement: chemistry and microbial transformation. *Phytochemistry* **2005**, *66*, 901-909.
[38] Chen, A.R.M.; Reese, P.B. Biotransformation of terpenes from *Stemodia maritima* by *Aspergillus niger* ATCC 9142. *Phytochemistry* **2002**, *59*, 57-62.
[39] Chen, A.R.M.; Ruddock, P.L.D.; Lamm, A.S.; Reynolds, W.F.; Reese, P.B. Stemodane and stemarane diterpenoid hydroxylation by *Mucor plumbeus* and *Whetzelinia sclerotiorum*. *Phytochemistry* **2005**, *66*, 1898-1902.
[40] Lamm, A.S.; Reynolds, W.F.; Reese, P.B. Bioconversion of *Stemodia maritima* diterpenes and derivatives by *Cunninghamella echinulata* var. *elegans* and *Phanerochaete chrysosporium*. *Phytochemistry* **2006**, *67*, 1088-1093.
[41] Fraga, B.M.; González, P.; Hernández, M.G.; Suárez, S. Formation of 2,3-*seco*-acids in the biotransformation of the diterpene ribenone by *Gibberella fujikuroi*. *Tetrahedron* **1999**, *55*, 1781-1792.
[42] Fraga, B.M.; Hernández, M.G.; García-Tellado, F.; González, P.; Perales, A. The biotransformation of two *ent*-15β,16β-epoxy-kaurane derivatives by *Gibberella fujikuroi*. *Phytochemistry* **1993**, *34*, 133-138; 1035-1040.
[43] Fraga, B.M.; Álvarez, L.; Suárez, S. Biotransformation of the diterpenes epicandicandiol and candicandiol by *Mucor plumbeus*. *J. Nat. Prod.* **2003**, *66*, 327-331.
[44] Fraga, B.M.; González, P.; Hernández, M.G.; Chamy, M.C.; Garbarino, J.A. The microbiological transformation of a 9-*epi*-*ent*-pimaradiene diterpene by *Gibberella fujikuroi*. *Phytochemistry* **1998**, *47*, 211-215.
[45] Fraga, B.M.; González, P.; Hernández, M.G.; Suárez, S. The biotransformation of the diterpene 2β-hydroxy-*ent*-13-*epi*-manoyl oxide by *Gibberella fujikuroi*. *Phytochemistry* **2003**, *62*, 67-70.
[46] García-Granados, A.; Jiménez, M.B.; Martínez, A.; Parra, A.; Rivas, F.; Arias, J.M. Chemical-microbiological synthesis of *ent*-13-*epi*-manoyl oxides with biological activities. *Phytochemistry* **1994**, *37*, 741-747.
[47] García-Granados, A.; Liñán, E.; Martínez, A.; Parra, A.; Rivas, F.; Arias, J.M. Preparation of polyoxygenated *ent*-13-*epi*-manoyl oxides by chemical-microbiological semisyntheses. *Phytochemistry* **1995**, *38*, 1237-1244.
[48] Fraga, B.M.; Hernández, M.G.; González, P.; López, M.; Suárez, S. Biotransformation of the diterpene ribenone by *Mucor plumbeus*. *Tetrahedron* **2001**, *57*, 761-770.
[49] Lin, S.-J.; Rosazza, J.P.N. Microbial transformations of isocupressic acid. *J. Nat. Prod.* **1998**, *61*, 922-926.
[50] Rodrigues-Filho, E.; Magnani, R.F.; Xie, W.; Mirocha, C.J.; Pathre, S.V. Hydroxylation of the labdane diterpene cupressic acid by *Fusarium graminearum*. *J. Braz. Chem. Soc.* **2002**, *13*, 266-269.
[51] Abraham, W.-R. Microbial hydroxylation of sclareol. *Phytochemistry* **1994**, *36*, 1421-1424.
[52] Díez, D.; Sánchez, J.M.; Rodilla, J.M.; Rocha, P.M.; Mendes, R.S.; Paulino, C.; Marcos, I.S.; Basabe, P.; Urones, J.G. Microbial hydroxylation of sclareol by *Rhizopus Stolonifer*. *Molecules* **2005**, *10*, 1005-1009.
[53] Choudhary, M.L.; Siddiqui, Z.A.; Hussain, S.; Atta-ur-Rahman. Structure elucidation and antibacterial activity of new fungal metabolites of sclareol. *Chem. Biodivers.* **2006**, *3*, 54-61.
[54] Silva, E.A; Takahashi, J.A.; Oliveira, A.B. An interesting backbone rearrangement and novel derivatives from the biotransformation of trachyloban-19-oic acid by *Rhizopus stolonifer*. *J. Braz. Chem. Soc.* **2002**, *13*, 101-105.
[55] Milanova, R.; Stoynov, N.; Moore, M. The optimization of triptoquinone production by *Cunninghamella elegans* using factorial design. *Enzyme Microb. Technol.* **1996**, *19*, 86-93.
[56] Hu, S.; Tian, X.; Zhu, W.; Fang, Q. Biotransformation of 2α,5α,10β,14β-tetraacetoxy-4(20),11-taxadiene by the fungi *Cunninghamella elegans* and *Cunninghamella echinulata*. *J. Nat. Prod.* **1996**, *59*, 1006-1009.
[57] Regueiro-Ren, A.; Carroll, T.M.; Chen, Y.; Matson, J.A.; Huang, S.; Mazzucco, C.E.; Stickle, T.M.; Vyas, D.V.; Balasubramanian, B.N. Core-modified sordaricin derivatives: synthesis and antifungal activity. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3403-3405.
[58] Kamel, H.N.; Fronczek, F.R.; Khalifa, S.I.; Slattery, M. Microbial transformation of 5-episinuleptolide. *Chem. Pharm. Bull.* **2007**, *55*, 537-540.
[59] Shibata, S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. *J. Korean Med. Sci.* **2001**, *16* (Suppl), S28-37.
[60] Sato, K.; Mochizuki, M.; Saiki, I.; Yoo, Y.C.; Samukawa, K.; Azuma, I. Inhibition of tumor angiogenesis and metastasis by a saponin of panax ginseng, ginsenoside-Rb2. *Biol. Pharm. Bull.* **1994**, *17*, 635-639.
[61] Mochizuki, M.; Yoo, Y.C.; Samukawa, K.; Azuma, I. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20 (*R*)-and 20 (*S*)-Ginsenoside-Rg3, of red ginseng. *Biol. Pharm. Bull.* **1995**, *18*, 1197-1202.
[62] Shinkai, K.; Akedo, H.; Mukai, M.; Imamura, F.; Isoai, A.; Kobayashi, M.; Kitagawa, I. Inhibition of *in vitro* tumor cell invasion by ginsenoside Rg_3. *Jpn. J. Cancer Res.* **1996**, *87*(4), 357-362.
[63] Odashima, S.; Ohta, T.; Kohno, H.; Matsuda, T.; Kitagawa, I.; Abe, H.; Arichi, S. Histochemical characterization of focal hepatic lesions induced by single diethylnitrosamine treatment in infant mice. *Cancer Res.* **1985**, *45*, 2781-2784.
[64] Ota, T.; Yamamoto, F.; Zong, Z.; Yamazaki, M.; Odashima, S.; Kitagawa, I.; Abe, H.; Arichi, S. Plant-glycoside modulation of cell surface related to control of differentiation in cultured B16 melanoma cells. *Cancer Res.* **1987**, *47*, 3863-3867.
[65] Dong, A.; Ye, M.; Guo, H.; Zheng, J.; Guo, D. Microbial transformation of ginsenoside Rb_1 by *Rhizopus stolonifer* and *Curvularia lunata*. *Biotechnol. Lett.* **2003**, *25*, 339-344.
[66] Cheng, L.-Q.; Kim, M.K.; Lee, J.-W.; Lee, Y.-J.; Yang, D.-C. Conversion of major ginsenoside Rb_1 to ginsenoside F_2 by *Caulobacter leidyia*. *Biotechnol. Lett.* **2006**, *28*, 1121-1127.
[67] Popovich, D.G.; Kitts, D.D. Structure–function relationship exists for ginsenosides in reducing cell proliferation and inducing apoptosis in the human leukemia (THP-1) cell line. *Arch. Biochem. Biophys.* **2002**, *406*, 1-8.
[68] Tian, Y.; Guo, H.; Han, J.; Guo, D. Microbial transformation of 20(*S*)-protopanaxatriol by *Mucor spinosus*. *J. Nat. Prod.* **2005**, *68*, 678-680.
[69] Maatooq, G.T. Z. Microbiological and chemical transformations of argentatin B. *Naturforsch.* **2003**, *58c*, 249-255.
[70] Akihisa, T.; Yasukawa, K. In *Studies in Natural Products Chemistry, Bioactive Natural Products (Part F)*; Atta-ur-Rahman, Ed.; Elsevier Science B.V.: Amsterdam, **2001**, Vol. *25*, pp. 43-87.
[71] Akihisa, T.; Watanabe, K.; Yoneima, R.; Suzuki, T.; Kimura, Y. Biotransformation of cycloartane-type triterpenes by the fungus *Glomerella fusarioides*. *J. Nat. Prod.* **2006**, *69*, 604-607.
[72] Dong, J.-Y.; Chen, Y.-G.; Song, H.-C.; Zhu, Y.-H.; Zhou, Y.-P.; Li, L.; He, Y.-P.; Cao, J.; Zhang, K.-Q. Hydroxylation of the triterpenoid nigranoic acid by the fungus *Gliocladium roseum* YMF1.00133. *Chem. Biodivers.* **2007**, *4*, 112-117.
[73] Yasukawa, K.; Yu, S.Y.; Yamanouchi, S.; Takido, M.; Akihisa, T.; Tamura, T. Some lupane-type triterpenes inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Phytomedicine* **1995**, *1*, 309-313.
[74] Manez, S.; Recio, M.C.; Giner, R.M.; Rios, J. L. Effect of selected triterpenoids on chronic dermal inflammation. *Eur. J. Pharmacol.* **1997**, *334*, 103-105.
[75] Safayhi, H.; Sailer, E.-R. Anti-Inflammatory actions of pentacyclic triterpenes. *Planta Med.* **1997**, *63*, 487-493.
[76] Evers, M.; Poujade, C.; Solers, F. Betulinic acid derivatives: A new class of human immunodeficiency virus type 1 specific inhibitors with a new mode of action. *J. Med. Chem.* **1996**, *39*, 1056-1068.
[77] Mayaux. J.F.; Bousseau, A.; Panwels, R.; De Clerq, E.; Pecq, J.B. Triterpene derivatives that block entry of human immunodeficiency virus type 1 into cells. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3564-3568.
[78] Ma, J.; Starck, S.R.; Hecht, S.M. DNA polymerase β inhibitors from *Tetracera boiviniana*. *J. Nat. Prod.* **1999**, *62*, 1660-1663.
[79] Konoshima, T.; Takasaki, M.; Kozuka, M.; Tokuda, H. Studies on inhibitors of skin-tumor promotion, I. Inhibitory effects of triterpenes from *Euptelea polyandra* on Epstein-Barr virus activation. *J. Nat. Prod.* **1987**, *50*, 1167-1170.
[80] Chatterjee, P.; Pezzuto, J.M.; Kouzi, S.A. Glucosidation of betulinic acid by *Cunninghamella* species. *J. Nat. Prod.* **1999**, *62*, 761-763.
[81] Kouzi, S.A.; Chatterjee, P.; Pezzuto, J.M.; Hamann, M.T. Microbial transformations of the antimelanoma agent betulinic acid. *J. Nat. Prod.* **2000**, *63*, 1653-1657.
[82] Chatterjee, P.; Kouzi, S.A.; Pezzuto, J.M.; Hamann, M.T. Biotransformation of the antimelanoma agent betulinic acid by *Bacillus megaterium* ATCC 13368. *Appl. Environ. Microbiol.* **2000**, *66*, 3850-3855.
[83] Collins, D.O.; Ruddock, P.L.D.; Chiverton de Grasse, J.; Reynolds, W.F. ; Reese, P.B. Microbial transformation of cadina-4,10(15)-dien-3-one, aromadendr-1(10)-en-9-one and methyl ursolate by *Mucor plumbeus* ATCC 4740. *Phytochemistry* **2002**, *59*, 479-488.

Received: March 14, 2008 **Revised: September 09, 2008** **Accepted: October 07, 2008**