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Biotransformations by *Colletotrichum* species

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Abstract—Biotransformations by *Colletotrichum* sp. are reviewed. Various substrates and the *Colletotrichum* species used for the transformations are included in this review of the literature for the period 1975–2002.

Contents

1. Introduction

Fungi and yeasts, like bacteria and other lower organisms, have been used for some time for the biological transformation of organic compounds. Recently, the use of both whole microorganisms or their enzymatic systems alone to carry out stereospecific and stereoselective reactions has taken on greater significance, as these reactions have demonstrated their usefulness in the asymmetric synthesis of molecules with important biological activities.[1–3]

The anamorphic genus *Colletotrichum* (teleomorph *Glomerella*) is a member of the subdivision Deuteromycotinia of the form order Melanconidiales.[4] Species of the genus *Colletotrichum* are implicated in plant diseases, generally referred to as anthracnoses, throughout the world. In fact, the various *Colletotrichum* species comprise the most destructive post-harvest pathogens of a wide range of plants, including cereals, legumes, fruits and vegetables. Strawberries are a particularly susceptible host, suffering a variety of *Colletotrichum*-triggered diseases, including wilts, rots, and anthracnose.[5,6]

Several species of the genus *Colletotrichum* have been used in biotransformations in order to study the characteristics of the reactions that these organisms can perform and their possible synthetic or industrial applications. The species which has received the most attention has been *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. (teleomorph *Glomerella cingulata*). Traditionally, the species of *Colletotrichum* have not been specifically used to produce biotransformations, indeed it is only during the last 10 years that an important numbers of papers have been published on this subject.

In the 1970s, some studies began to appear concerning the fungal modification of phytoalexins and the preparation of optically active C16-juvenile hormone.[7,8]

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However, in the last 10 years biotransformations by *Colletotrichum* have received considerable attention; in particular, the work of the Japanese group headed by Miyazawa stands out.

2. Detoxification of phytoalexins by *Colletotrichum* spp.

Phytoalexins are low molecular weight antibiotic compounds produced by plants in response to infection by microbes. These antimicrobial compounds are thought to provide resistance to microbial invasion and colonization. The pterocarpanes: (–)-maackiaine **1** and (–)-medicarpine **2** are compounds that perform the role of phytoalexins in the plant species *Medicago sativa* (alfalfa) and *Trifolium pratense* (red clover).[9] Not only are both compounds structurally similar, but they have also been observed to act as fungicides against several species of fungi. However, the phytopathogenic fungi that are successful in attacking *M. sativa* and *T. pratense*, are capable of detoxifying these compounds.

The detoxification assays have been performed with nine pathogenic fungi, including three species of *Colletotrichum*: *C. trifolii*, *C. dematium f. truncatum* and *C. destructivum*. It should be noted that while these latter two species do not seem to be involved in the primary infection, they have been isolated from the lesions caused by the disease.[9]

All three species hydroxylate compounds **1** and **2** at position C-6a to afford metabolites **3** and **4**. The species *C. trifolii* does this by breaking down the C ring of both substrates to produce the isoflavonols sophorol **5** and vestitone **6**. Furthermore, this species is able to stereoselectively hydroxylate position C-1a to produce compounds **7** and **8**[9] (see Fig. 1). As should be expected, all the resulting compounds from this biotransformation exhibit lower toxicity than do the starting materials. In the biotransformation media another compound with higher polarity than some of the isolated compounds was detected; apparently this material was the result of a successive biotransformation of compounds **3** and **4**. These results thus indicate that *C. trifolii* can follow various metabolic pathways to transform these compounds.

All three of the *Colletotrichum* species tested degraded medicarpine **2** by elimination of methoxyl group on C-8 to afford demethylmedicarpine **9**, which is less toxic than the starting compound. Other researchers have reported the production of **9** in the biotransformation of **2** using species of *Colletotrichum* different from those studied here.[7,10]

Biotransformation of the phytoalexin maackiaine **1** by the species *C. gloeosporioides* occurs via stereoselective hydroxylation of position C-6a on the B ring to yield compound **3**, as is the case with the other species of this genus that attack alfalfa.[11] The product of this hydroxylation, (–)-6α-hydroxymaackiaine **3**, was isolated together with another polar compound that was identified as the diol (–)-6,6α-dihydroxymaackiaine **10**. This diol was found to be present in a 4 to 1 ratio with its epimer **11** (Fig. 2).

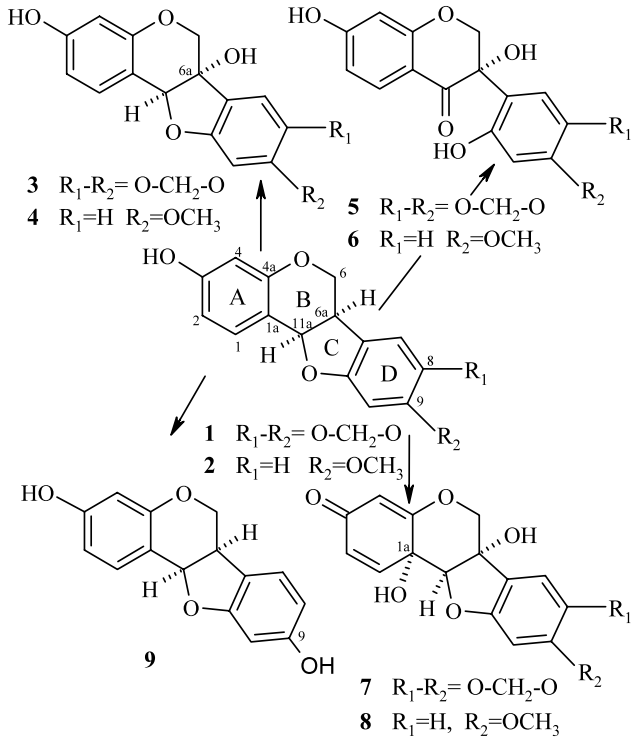

Figure 1. Biotransformation of the pterocarpans (–)-maackiaine **1** and (–)-medicarpine **2** by *Colletotrichum* spp.

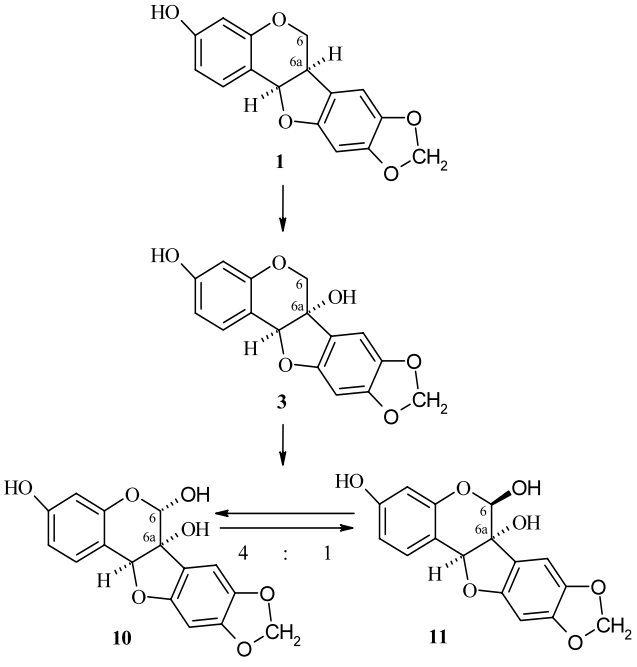

Figure 2. Biotransformation of the phytoalexin (–)-maackiaine by *C. gloeosporioides*.

3. Biotransformations of terpenoids

A good number of terpenes form part of the essential oils of many plants and are thus responsible for the typical aromas and flavors found in them. Because of their organoleptic properties, they have been used widely in industry to provide aroma and flavor; many have been synthesized or subjected to chemical transformations and biotransformation in order to enhance their natural characteristics.

The bibliographic information on biotransformations of terpenes has been organized according to the type of carbon chain that was subjected to biotransformation, starting with acyclic mono- and sesquiterpenoids, saturated and unsaturated components and continuing with cyclic terpenoids and related cyclic compounds. Finally, the biotransformation of steroids and miscellaneous compounds has been included.

4. Biotransformations of acyclic terpenoids

The reported results showed that in the biotransformation of acyclic terpenoids by *G. cingulata*,[12–22] unsaturated acyclic terpenoids were regioselectively oxidized at the double bond remote from the hydroxyl or carbonyl group as a main reaction, whereas saturated acyclic terpenoids were mainly hydroxylated at the tertiary carbon of the isopropyl moiety, which is most distant from the hydroxyl group.

4.1. Biotransformation of saturated acyclic terpenoids

The saturated, acyclic monoterpenes tetrahydrogeraniol **12** and tetrahydrolavandulol **13**[12] under biotransformation by *G. cingulata* were oxidized regioselectively at the isopropyl group. Thus, the principle compound isolated from the biotransformation of substrate **12** was 7-hydroxytetrahydrocitronellol **14**; likewise, the main compound isolated from **13** was the alcohol 5-hydroxytetrahydrolavandulol **15** (Fig. 3). The oxidation observed in both compounds represents an interesting general method for use in organic chemistry.

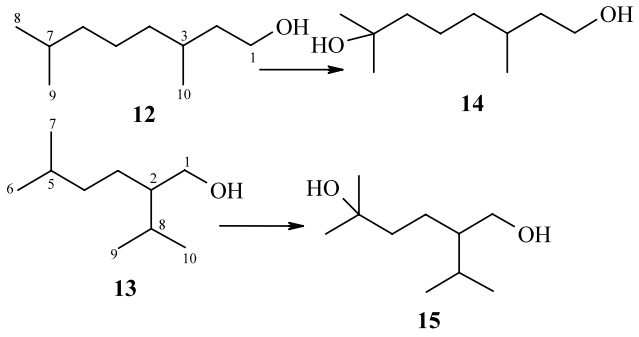

Figure 3. Biotransformation of the saturated, acyclic monoterpenes tetrahydrogeraniol **12** and tetrahydrolavandulol **13** by *G. cingulata*.

4.2. Biotransformation of unsaturated acyclic terpenoids

The compound (−)-dihydromyrcenyl acetate **16** underwent biotransformation by the fungus *G. cingulata*,[13] yielding dihydromyrcenol **17**, 3-hydroxydihydromyrcenyl acetate **18**, 3-hydroxydihydromyrcenol **19**, 3,7-dimethyloctane-1,2,7-triol-7-carboxylate **20** and 3,7-dimethyloctane-1,2,7-triol **21** (Fig. 4). The starting substrate, not found in nature, is obtained synthetically from α-pinene for its use in the perfume industry.

The starting material was consumed almost entirely during the 6 days of fermentation and metabolites **17**–**19** disappeared after 8–10 days. Compound **20**, on the other hand, began to increase in concentration on the third day, only to decrease rapidly from day 8 onwards. This coincided with the initial increase in concentration of metabolite **21**. The authors of this study thus propose two different pathways for the production of compound **21**:

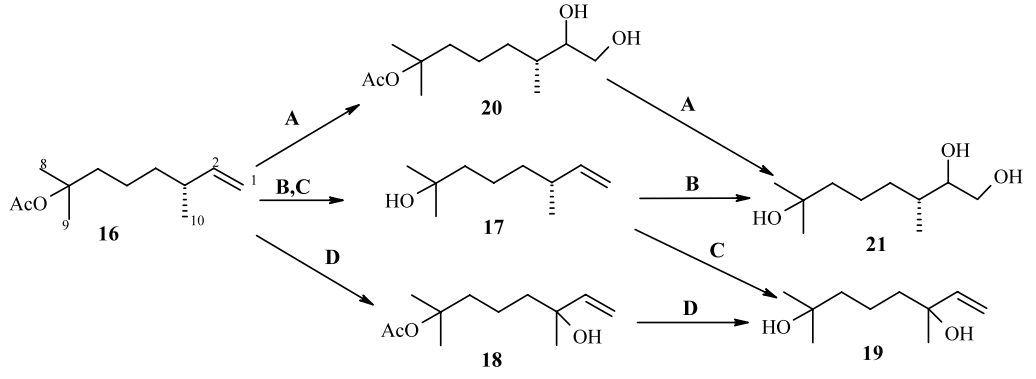

Figure 4. Biotransformation of the unsaturated, acyclic monoterpene dihydromyrcenyl acetate **16** by *G. cingulata*.

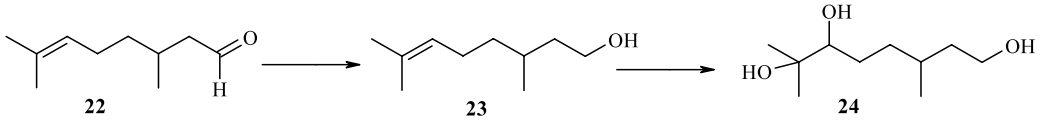

Figure 5. Biotransformation of the unsaturated, acyclic monoterpene citronellal **22** by *G. cingulata*.

from **20** via pathway **A** and from **17** via pathway **B**. Likewise, compound **19** arises from metabolite **17** via pathway **C** or from compound **18** via pathway **D** (Fig. 4).

The biotransformation of racemic (±)-citronellal **22** and (±)-citronellol **23** (see Fig. 5) yielded a mixture of diastereomers of 3,7-dimethyl-1,6,7-octanetriol **24**.[14,15] The authors hypothesized that the biotransformation of **22** to **24** occurs via the initial reduction of the aldehyde group to afford citronellol **23**, which is subsequently oxidized on the double bond between C-6–C-7 to yield the triol **24** directly. The reduction of the aldehyde group was not enantioselective and gave metabolite **23** as a racemic mixture. During this biotransformation, the presence of citronellic acid, habitually formed during the biotransformation of citronellol by *Pseudomonas* spp.[16] was not detected.

In 1976, Imai and Marumo published an interesting regio and stereoselective oxidation on the double bond between C-6–C-7 of methyl geranate **25** by *C. nicotianae*. In addition stereoselective conversions of (±)-methyl 6,7-epoxygeranate **26** and (±)-methyl 10,11-epoxyfarnesate **28** were carried out to apply the results to the synthesis of the juvenile hormone C_{16}-JH enantiomers,[8] (Fig. 6).

An interesting enantioselective cyclization was obtained on microbial transformation of the racemic monoterpene lavandulol **31**, using the fungus *G. cingulata* as a biocatalyst, to afford the enantiomerically pure (100% ee) **32**.[17] The authors proposed first the epoxidation of the substrate on the C-4–C-5 double bond furthest from the hydroxyl group, followed by cyclization to form the tetrahydropyran ring (Fig. 7).

In subsequent studies, the authors were also able to isolate and identify three other metabolites: the tetrahydrofurans **33** and **34**, along with 6-hydroxylavandulol **35**[18] (see Fig. 7). Compounds **32**, **33** and **34** were formed regioselectively from an initial epoxide intermediate formed on the C-4–C-5 double bond of the starting substrate while a regioselective hydroxylation of the methyl on position C-6 in **31** led to compound **35**. This hydroxylation of the methyl group had not been observed in the other biotransformations by *G. cingulata*.[18]

Furthermore, the regioselective oxidation at the isopropyl or isopropylidene moiety located in the remote

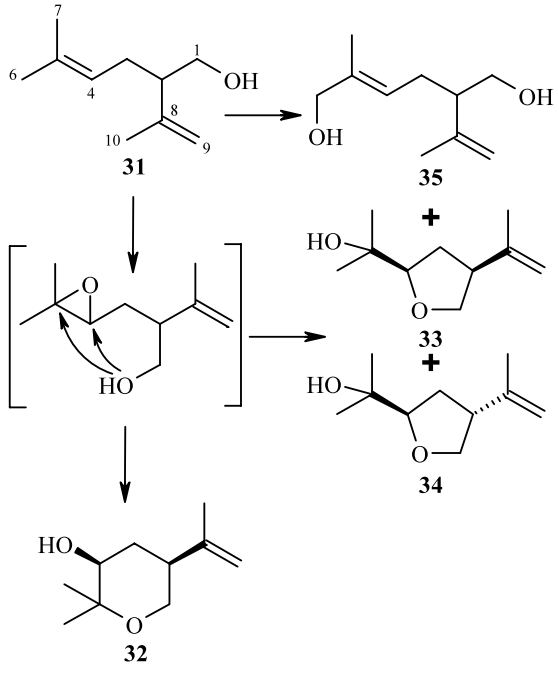

Figure 7. Biotransformation of lavandulol **31** by *G. cingulata*.

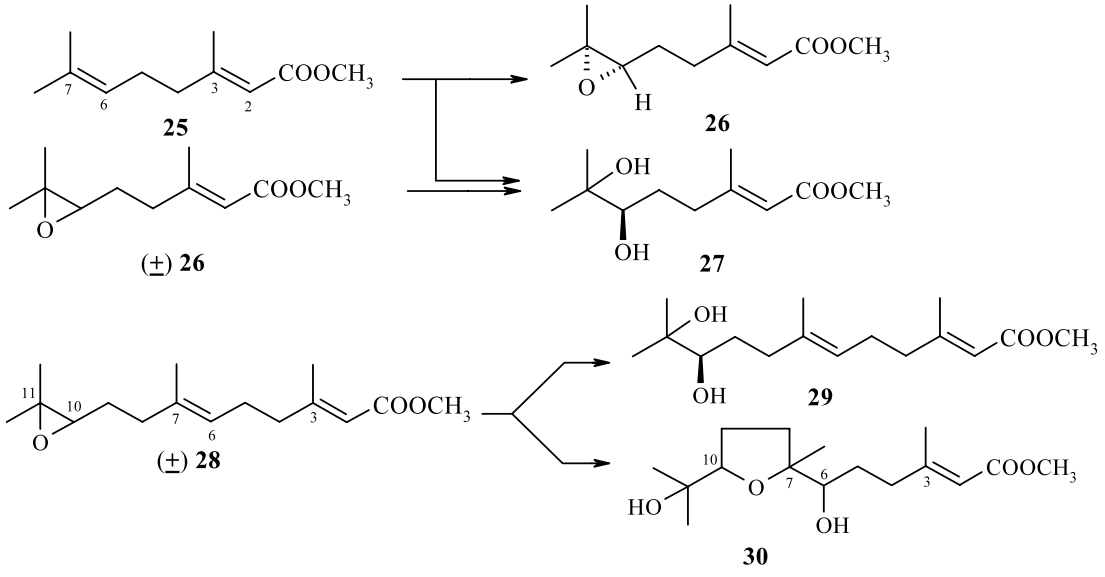

Figure 6. Biotransformation of methyl geranate **25**, methyl-6,7-epoxygeranate **26** and methyl-10,11-epoxyfarnesate **28** by *C. nicotianae*.

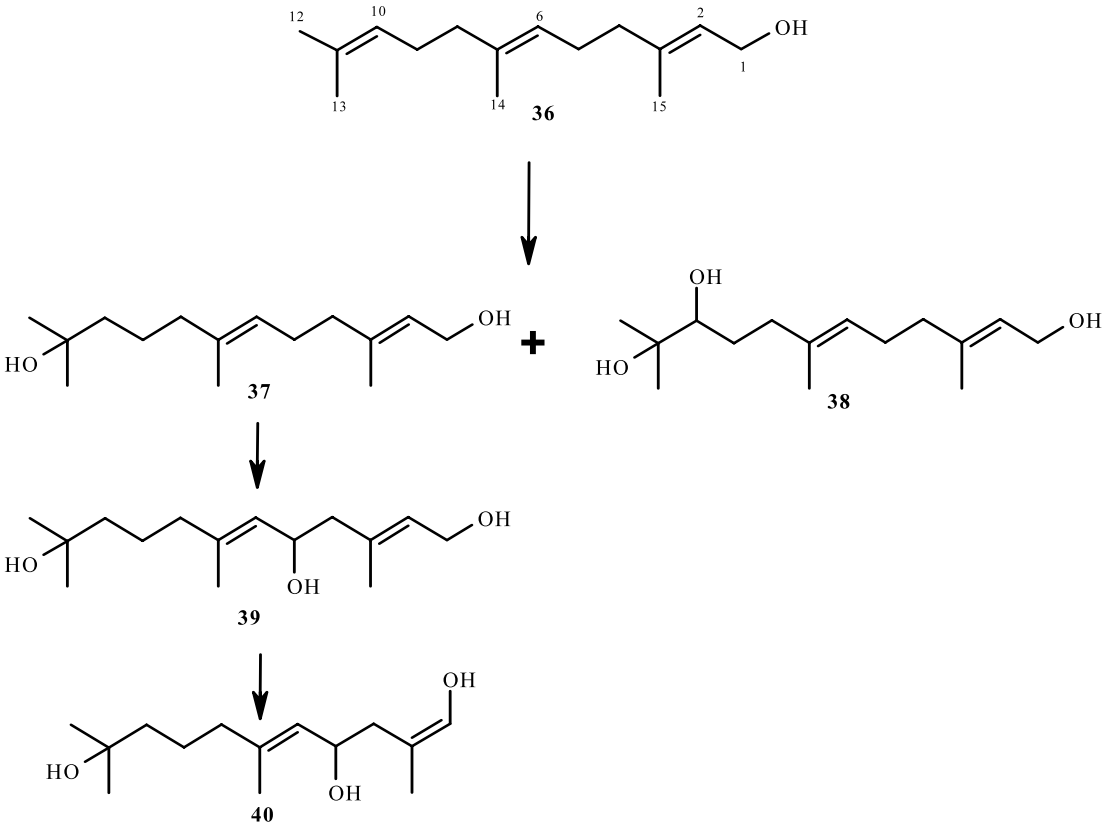

Figure 8. Biotransformation of (2*E*,6*E*)-farnesol **36** by *G. cingulata*.

position from the oxygenated functional group, in the terpenoid skeleton, has been observed and reported on acyclic terpenes geranylactone,[19] (±)-*trans*-nerolidol,[19] (±)-*cis*-nerolidol,[20] nerylacetone,[20] (2*E*,6*E*)-farnesol[21] and (2*Z*,6*Z*)-farnesol.[22]

The microbial transformation of (2*E*,6*E*)-farnesol **36** using the fungus *G. cingulata* afforded, in a first step, (2*E*,6*E*)-3,7,11-trimethyl-2,6-dodecadien-1,11-diol **37** and (2*E*,6*E*)-3,7,11-trimethyl-2,6-dodecadien-1,10,11-triol **38**. In a second step, compounds **39** and **40** were obtained from **37**[21] (Fig. 8). The results of this biotransformation were in good agreement with those observed by the same authors in the biotransformation of (2*Z*,6*Z*)-farnesol.[22]

5. Biotransformation of monoterpenoids and relative cyclic compounds

5.1. Biological reduction of cyclic ketone

The biotransformation of the racemic methylcyclohexanones: (±)-2-methylcyclohexanone **41**, (±)-3-methylcyclohexanone **44** and (±)-4-methylcyclohexanone **47** by the fungus *G. cingulata* led to both the diastereo- and enantioselective reduction of these compounds (Fig. 9).[23] Regioisomeric 2, 3- or 4-methylcyclohexanone gave the corresponding *cis*- and *trans*-methylcyclohexanols.

The major metabolites of (±)-**41** and (±)-**44** were *cis*-2 and *cis*-3-methylcyclohexanol. The reduction of **41** was stereospecific, with the (2*R*)-ketone being converted to the corresponding (+)-(1*S*-2*R*)-*cis*-2-methyl cyclohexanol **42a**, with an enantiomeric excess of 92%.

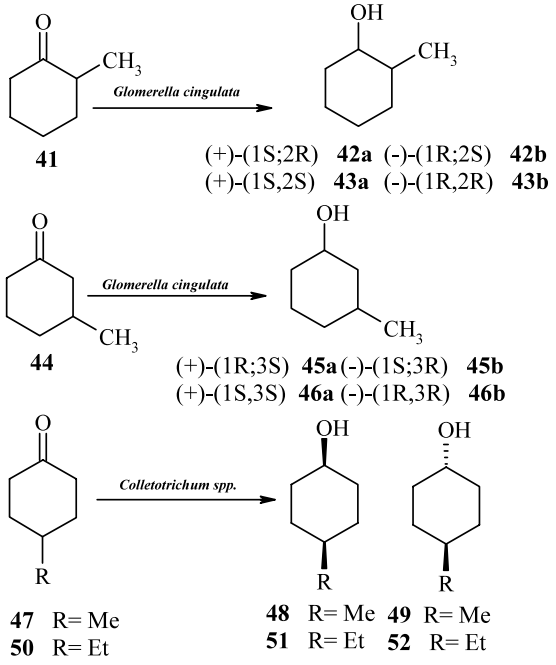

Figure 9. Biotransformation of the racemic mixtures of 2-methyl, 3-methyl, 4-methyl and 4-ethylcyclohexanones by the fungus *G. cingulata*.

During 8 days incubation with *G. cingulata*, the 90% of **44** was metabolized to give the corresponding metabolites **45** and **46**. The major product consisted of a mixture of the *cis*-enantiomers **45a** and **45b** in 75% yield. The predominant (−)-enantiomer was compound **45b** with an enantiomeric excess of 33%.

The biotransformation of racemic 4-methylcyclohexanone **47** and 4-ethylcyclohexanone **50** was carried out by the fungus *G. cingulata* as well as with the following *Colletotrichum* species: *C. fragarie*, *C. lindemuthianum* (three different strains), *C. lagenarium*, *C. atramentarium*, *C. trifolii*, *C. dematium* and *C. graminicola*.[24] Both starting substrates **47** and **50** were biotransformed in the *cis*- and *trans*-alcohols, respectively, and the composition of the final products varied depending on the species of fungus analyzed (Fig. 9). The species that afforded the highest degree of stereoselectivity in the reduction of the compound 4-methylcyclohexanone **47** were *C. lagenarium* and *C. lindemuthianum* (C-13). Both species metabolized the substrate stereoselectively to the *trans*-isomer. The *trans*- to *cis*-ratio of the products obtained was 81:1 with *C. lagenarium* and 18:1 with *C. lindemuthianum*.

The substrate 4-ethylcyclohexanone **50** exhibited similar behavior during its metabolic reductions. The highest degree of stereoselectivity was reached with the fungi *C. lagenarium*, which afforded the *trans*-isomer in 80% yield and with a *trans*- to *cis*-ratio of 10:1 and *C. dematium*, which gave the *trans*-isomer with a *trans*- to *cis*-ratio of 3.5:1. In contrast, the fungus *G. cingulata* and *C. graminicola* MAFF305460 performed the biotransformation stereoselectively to the *cis* isomer with a *cis*- to *trans*- ratio of 2:1 and 1.3:1, respectively.

The biotransformation of 4-propyl, 4-isopropyl, 4-*tert*-butyl and 4-*tert*-pentylcyclohexanone was examined using ten different anthracnose fungi (*C. lagenarium*, *C. dematium* MAFF410046, *C. trifolii* MAFF305389, *C. fragariae*, *C. atramentarium* MAFF712102, *C. lindemuthianun* (C-1), *C. lindemuthianum* (C-3), *C. lindemuthianum* (C-13), *C. graminicola* MAFF305460 and *G. cingulata*) as biocatalysts.[26–28] 4-Propylcyclohexanone and 4-isopropyl cyclohexanone were reduced to the corresponding *cis*- and *trans*-alcohols, transformed mainly to *trans*-4-alcohols by all ten fungi.[26] In particular, the ratio of *cis*- and *trans*-alcohol products was shown to be 1:13 (4-propylcyclohexanol) and 1:11 (4-isopropylcyclohexanol) with high stereoselectivity by *C. lagenarium* after a 7-day incubation period. The biotransformation of 4-*tert*-butylcyclohexanone[27] resulted mainly in its transformation to *trans*-4-alcohol, using *C. lagenarium*, *C. atramentarium* MAFF712102, *C. fragariae*, *C. graminicola* MAFF 305460, *C. lindemuthianum* (C-3) and *C. lindemuthianum* (C-13). The *cis*- and *trans*-alcohol products were obtained at a ratio of 1.0:6.0 with stereoselectivity by *C. lagenarium* after 7 days incubation. The *cis*-alcohol was stereoselectively formed by *G. cingulata*, *C. lindemuthianum* (C-1), *C. trifolii* MAFF305389 and *C. dematium* MAFF410046. 4-*tert*-Pentylcyclohexanone was transformed to mainly *trans*-4-*tert*-pentylcyclohexanol by all of the fungi.[28] In particular, the ratio of *cis*- and *trans*-alcohol products was shown 1:5.5 with stereoselectivity by *C. lindemuthianum* (C-3) after 7 days incubation period.

The microbial transformation of 2,6-dimethylcyclohexanone **53** and 3,5-dimethyl cyclohexanone using the fungus *G. cingulata* was reported (Fig. 10).[25] The results suggest that in the case of 2,6-dimethylcyclohexanone the *trans*-form only was metabolized, not the *cis*-form. (2*R*,6*R*)-Ketone **53b** was readily and stereoselectively converted to the corresponding (−)-(2*R*,6*R*)-2,6-dimethylcyclohexanol **54** with an enantiomeric excess of 70%. On the other hand, only the *cis*-meso form of 3,5-dimethylcyclohexanones **55a** was biotransformed to yield two metabolites: (1α,3α,5α)-3,5-dimethylcyclohexanol **56** (74%) and (1α,3β,5β)-3,5-dimethyl cyclohexanol **57** as the minor metabolite (26%). The *trans*-numeric form of 3,5-dimethylcyclohexanone **55b** showed no reaction for either enantiomer.

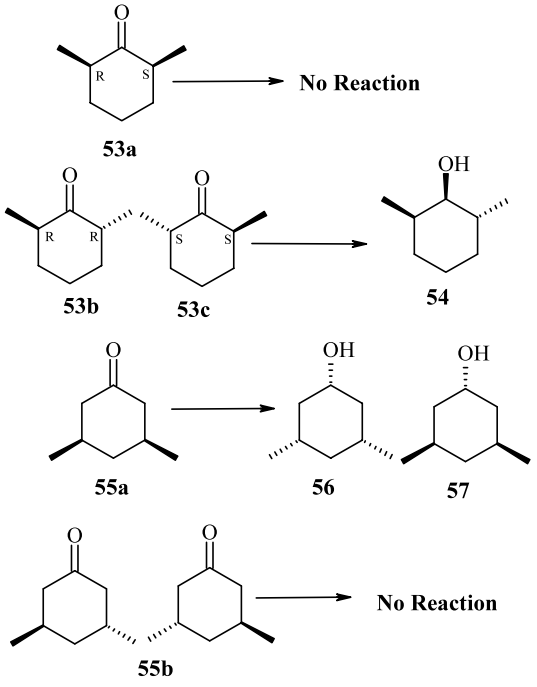

Figure 10. Biotransformation of 2,6- and 3,5-dimethylcyclohexanones by the fungus *G. cingulata*.

The microbial transformation of 3,3,5-trimethylcyclohexanone was investigated using the plant pathogenic fungus, *G. cingulata*. With this organism 3,3,5-trimethyl cyclohexanone gave the corresponding *cis*- and *trans*-3,3,5-trimethylcyclohexanols with the ratio of 20:1 forming the *cis*-isomer highly stereoselectively, upon 5 days incubation together with 3,3,5-trimethyl-2-cyclohexen-1-one (isophorone) as a minor product.[29]

Karahanoenol **59** was prepared by microbial asymmetric reduction of karahanaenone **58**[30] (Fig. 11). *G. cingulata* reduced compound **58** in a period of 24 h with excellent yield (98%) and enantioselectivity (100% ee).

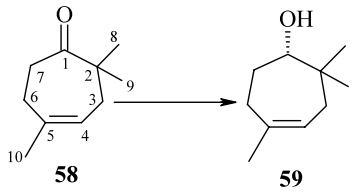

Figure 11. Biotransformation of the monoterpene karahanaenone by the fungus *G. cingulata*.

The biotransformation of bicyclic ketones (+)-camphorquinone **60**, (−)- camphorquinone **63** and (±)-bicyclo[3.3.1]nonane-2,6-dione **66** by various fungi has been reported.[31–33] The ketone (+)-camphorquinone **60** and (−)-camphorquinone **63** were readily reduced by various fungi (Fig. 12). The reduction, over a short time, of (−)-camphorquinone **63** by *Aspergillus niger* produced mainly (+)-(2*R*)-*endo*-hydroxyepicamphor **64b** with high stereoselectivity, whereas reduction of (+)-camphorquinone **60** by *G. cingulata* and *Mucor mucedo* afforded (−)-(3*S*)-*exo*-hydroxycamphor **61b** with stereoselectivity After 24 h, the reduction of (+)-camphorquinone **60** by *G. cingulata* produced (+)-2-*exo*-2,3-diol **62** with high stereoselectivity, whereas (−)-camphorequinone yielded (+)-2-*endo*-3-*exo*-2,3-diol **65** with high stereo- and enantioselectivity.[31,32]

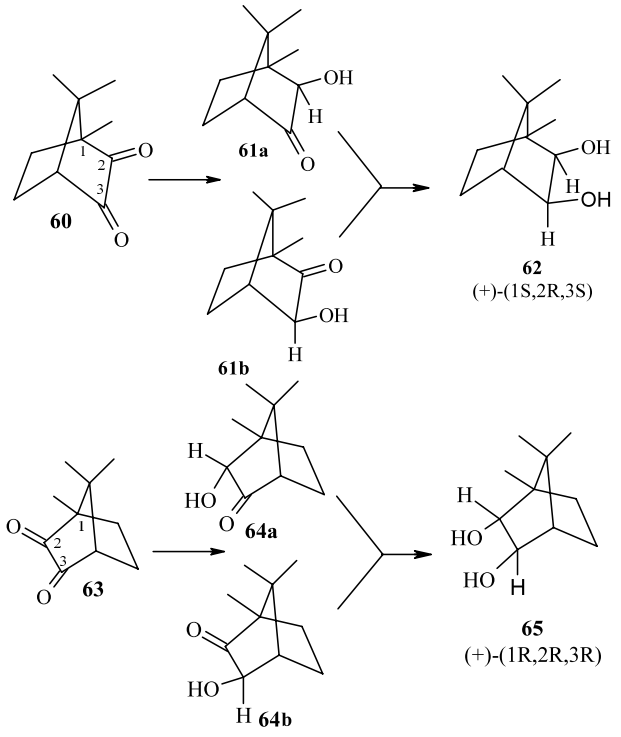

Figure 12. Biotransformation of the bicyclic, monoterpenic diketones (+)-camphorquinone **60** and (−)-camphorquinone **63**.

Stereoselective reduction of (±)-bicyclo[3.3.1]nonane-2,6-dione **66** by microorganisms has been reported[33] (see Fig. 13). Biotransformation with *A. niger* and *G. cingulata* gave excellent results for the reduction of **66** to obtain enantiomerically pure alcohols. Racemic ketone **66** was metabolized by *G. cingulata* to give the major metabolite ketoalcohols **67a** and **67b** (about 42 and 36% respectively).

These metabolites were transformed to diols **68**, being the major isomer **68a**, (17% ee at 7 days).

5.2. Biotransformation of cyclic monoterpenes

Some papers have been reported about biotransformation of monoterpenes by *G. cingulata*. The biotransformation of 1,8-cineole,[34] (−)-*cis*-myrtanol, (+)-*trans*-myrtanol,[35] (−)-nopol,[36] (±) bornyl acetate,[37] (+)-isopinocampheol, (−)-isopinocampheol[38] and lime oxide T[39] by *G. cingulata* led to the oxidation products.

The oxidations of the skeleton of (−)-nopol were confined mainly to the C-4 position to give (4*R*)-(−)-4-hydroxy- and 4-oxo-nopol. This enantioselective hydroxylation might be due to steric hindrance by the geminal dimethyl group at C-6 dictating that the hydroxyl group is incorporated from the opposite side of the molecule to the dimethyl group.[36] On the related skeleton of *cis*- and *trans*-myrtanol, both were oxidized at C-3, C-4 and C-5, whereas oxidation at C-9 and C-10 was found only with (+)-*trans*-myrtanol.[35] The biotransformation of (+)- and (−)-isopinocampheol by *G. cingulata* took place with enantioselectively. Both compounds were converted to three pinanediols, respectively. The main product of (−)-isopinocampheol was (1*R*,2*R*,3*S*,4*S*,5*R*)-3,4-pinanediol, and that of the (+)-enantiomer was (1*S*,2*S*,3*S*,5*R*,7*R*)-3,4-pinanediol.[38]

An interesting resolution of racemic *cis*- and *trans*-linalool oxide-pyranoid via esterification with malonic acid by *G. cingulata* has been reported[40] (Fig. 14). Two esters of malonic acid, (3*R*,6*R*)-*cis*-linalool oxide-pyra-

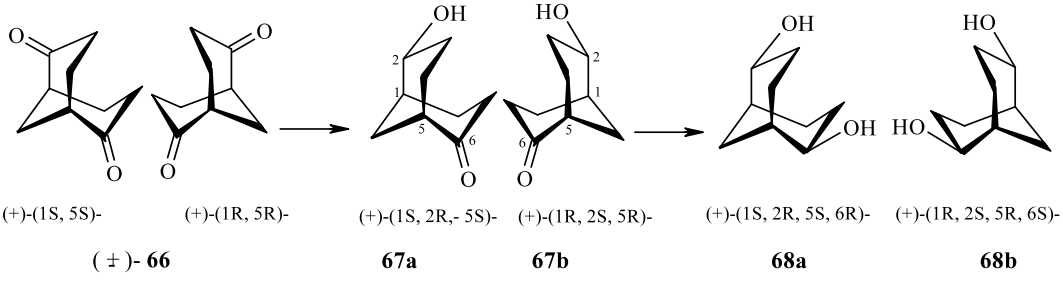

Figure 13. Biotransformation of bicyclo[3.3.1]nonane-2,6-dione **66** by the fungus *G. cingulata*.

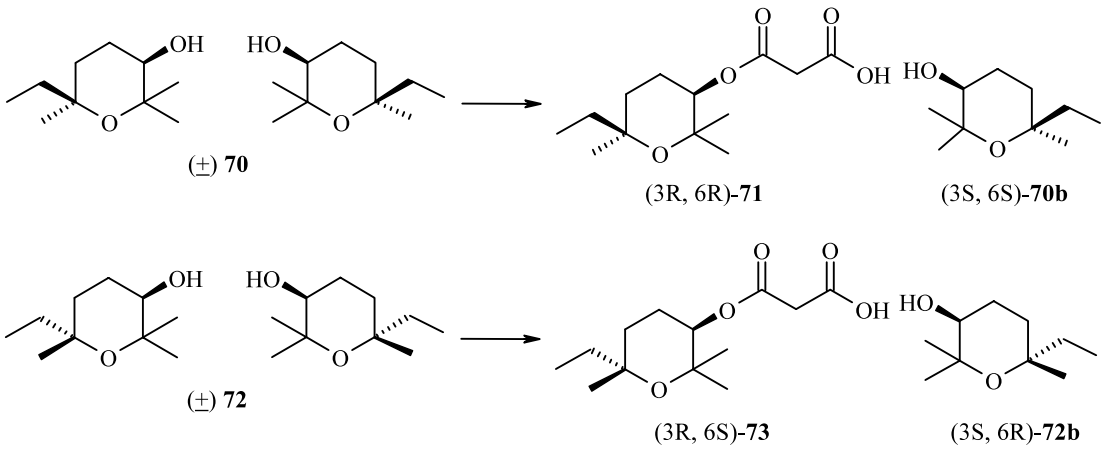

Figure 14. Resolution of the racemic *cis*- and *trans*-linalool oxide-pyranoid **70**, **72** by the fungus *G. cingulata*.

noid-3-yl malonate **71** and (3*R*,6*S*)-*trans*-linalool oxide-pyranoid-3-yl malonate **73**, as well as the pure (3*S*,6*S*)- and (3*S*,6*R*)-enantiomers **70b** and **72b** were obtained with 100% ee and in 50% yield after 24 h. The malonic esters were formed from malonic acid biosynthesized by the microorganism itself during the biotransformation.

5.3. Biotransformation of sesquiterpenes

Microbial transformation of the sesquiterpenes bisabolol,[41,42] selinene,[43] cedrol,[44,45] and the sesquiterpenes with a guaiane skeleton, gurjunene,[46] aromadendreno, alloaromadendrene,[47] globulol and ledol,[48] were investigated, in all cases the fungus led to hydroxylated compounds.

The biotransformation of the cyclic sesquiterpene (–)-α-bisabolol **74** carried out with the fungus *G. cingulata*[41,42] afforded the compounds (1*S*,3*R*,4*R*,7*S*)-3,4-dihydroxy-α-bisabolol **75**, bisabolol oxide B **76**, (1*S*,3*R*,4*R*,7*S*,10*S*)-3,4-dihydroxybisabolol oxide B **77** and (1*S*,3*S*,4*S*,7*S*,10*S*)-3,4-dihydroxybisabolol oxide B **78** (Fig. 15). Metabolites **75** and **76** underwent a subsequent biotransformation to give metabolites **77** and **78**. The metabolic pathways of the biotransformation of (–)-α-bisabolol by *G. cingulata* are also discussed.

In the biotransformation of the eudesmane sesquiterpene β-selinene **79**, carried out with the species *G. cingulata*,[43] the substrate was observed to undergo regioselective oxidation on the C-11 and C-13 positions of the double bond of the isopropenyl chain as well as on position C-1 to form a mixture of the diastereomeric triols (1*S*,6*S*,9*S*,10*R*,11*R*)- and (1*S*,6*S*,9*S*,10*R*,11*S*)-1,11,13-trihydroxy-β-selinene **80** and **81** (Fig. 16).

Interestingly, the biotransformation of the tricyclic sesquiterpene (+)-cedrol **82**[44,45] via stereoselective hydroxylation on C-3 and C-12, gave rise to compounds **83**, **84** and **85** (Fig. 17). Compound **83** was subsequently metabolized to 8-cedren-3α-ol **86** through dehydration across the bond C-8/C-9.

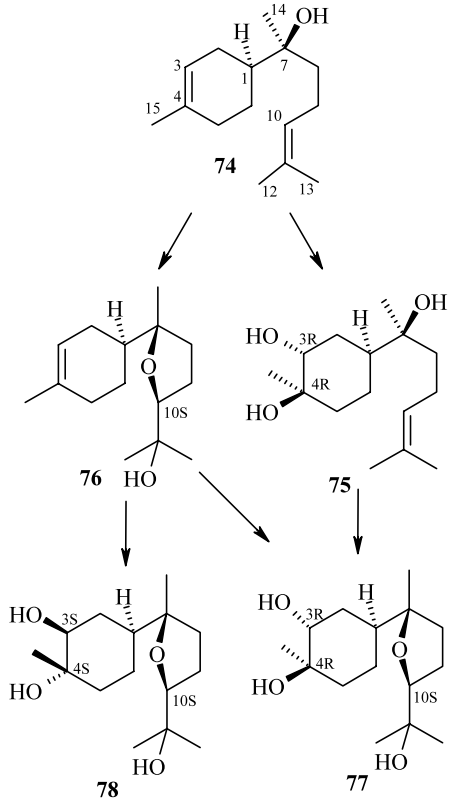

Figure 15. Biotransformation of the cyclic sesquiterpene (–)-α-bisabolol **74** by the fungus *G. cingulata*.

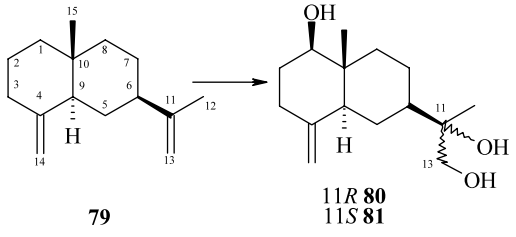

Figure 16. Biotransformation of the cyclic sesquiterpene (–)-β-selinene by the fungus *G. cingulata*.

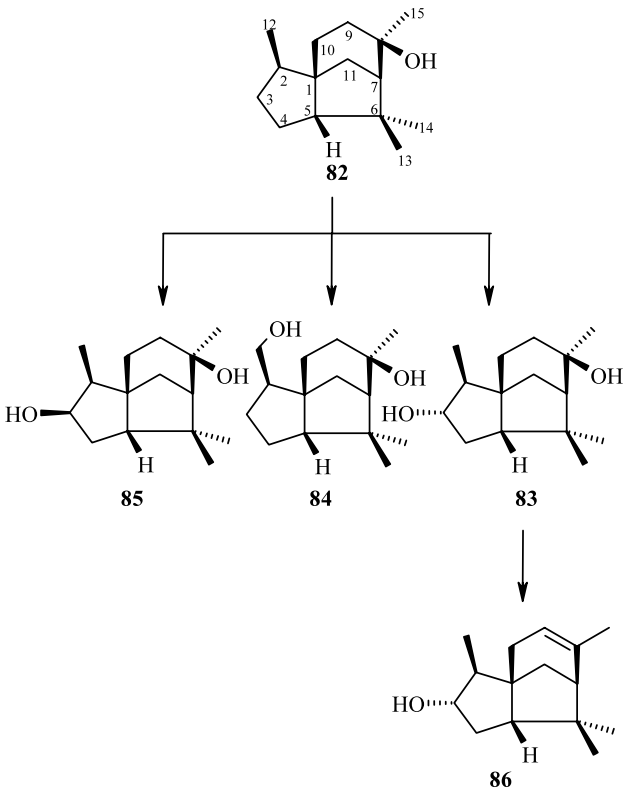

Figure 17. Biotransformation of the tricyclic compound (+)-cedrol **82** by the fungus *G. cingulata*.

An interesting similar behavior in the biotransformation of sesquiterpene compounds with a guaiane skeleton by *G. cingulata* was observed. So, in the biotransformation of compounds with a guaiane skeleton, the substrate was observed to undergo regioselective hydroxylation on the C-10, C-10/C-14 and C-13, C-11/C13.

In the biotransformation of the guaiane sesquiterpene (+)-γ-gurjumene **87** carried out by the species *G. cingulata*,[46] oxidation on the C-11 and C-13 positions on the double bond of the lateral isopropenyl chain was observed. This gave rise to the formation of the compound (1*S*,4*S*,7*R*,10*R*)-5-guaien-11,13-diol **88** (Fig. 18). This compound underwent a subsequent metabolic oxidation on C-10 to afford the triol (1*S*,4*S*,7*R*,10*S*)-5-guaien-10,11,13-triol **89**.

The sesquiterpenes (+)-(1*R*)-aromadendrene **90**, (−)-(1*S*)-alloaromadendrene **91**, (−)-globulol **92** and (+)-ledol **93** were oxidized by the fungus *G. cingulata* (see Fig. 19) and regioselectively hydroxylated on position C-13. In addition, compounds **90** and **91** were regioselectively hydroxylated on positions C-10 and C-14 of the double bond.[47,48]

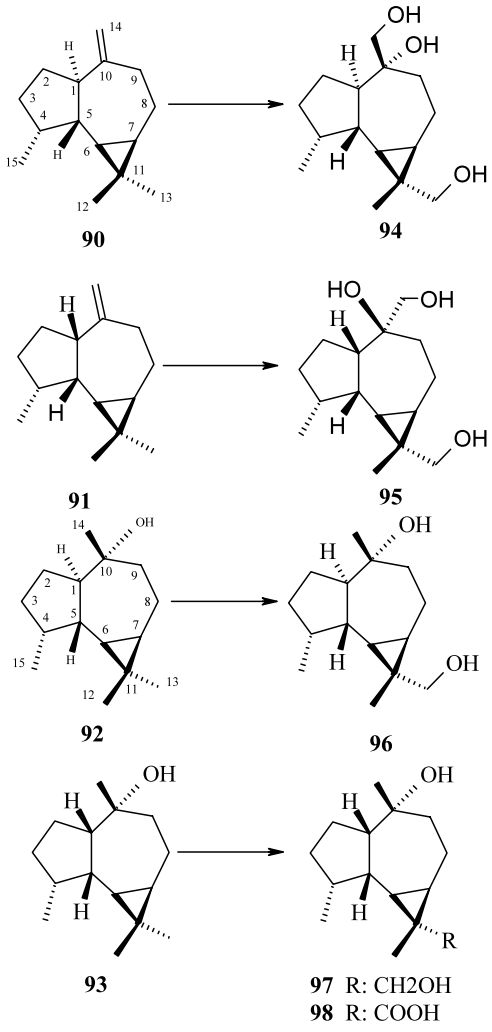

Figure 19. Biotransformation of the sesquiterpenes (+)-(1*R*)-aromadendrene **90**, (−)-(1*S*)-alloaromadendrene **91** and (−)-globulol **92** and (+)-ledol **93** by the fungus *G. cingulata*.

During the course of the fermentation, the formation of likely intermediate compounds such as epoxides, alcohols, or diols was not observed. Rather, both substrates **90** and **91** were oxidized rapidly to the corresponding

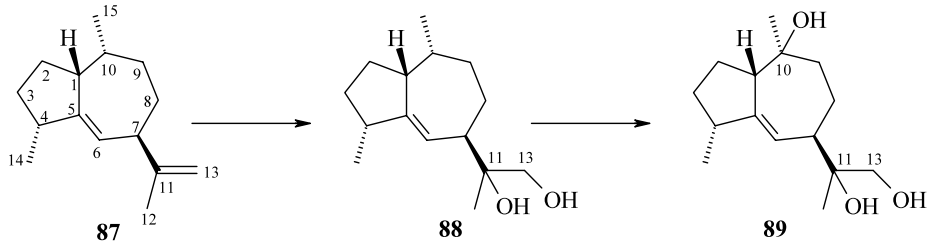

Figure 18. Biotransformation of the unsaturated, cyclic sesquiterpene (+)-γ-gurjumene **87** by the fungus *G. cingulata*.

triols: **94**, C-10 (*R*)-configuration, and **95**, with a C-10 (*S*)-configuration. The authors proposed that the difference in the orientations of the hydroxylations on C-10 for both metabolites is due to the configuration of position C-1 in each substrate.

Incubation of 6β,7β-epoxy-4β-hydroxyguaian-10-ene with *G. cingulata* yields exclusively 4β,13-dihydroxyguaian-6,10-diene. Antibacterial evaluations of this and other terpenoids have been recently published.[49]

5.4. Biotransformation of steroids

Colletotrichum musae and *G. cingulata* have been examined for their potential in the biotransformation of steroids.[50,51] The biotransformation with *G. cingulata* of 17,21-dihydroxypregn-4-ene-3,20-dione yielded the dehydrogenated derivative on C-1/C-2 position. The substrates **99**, **100** and **101** were metabolized by *C. musae*. Products isolated were those of oxidation and reduction, although α,β-unsaturated carbonyl functionalities in all substrates were left untouched. The formation of minute quantities of hydroxylated steroids was noted[51] (Fig. 20).

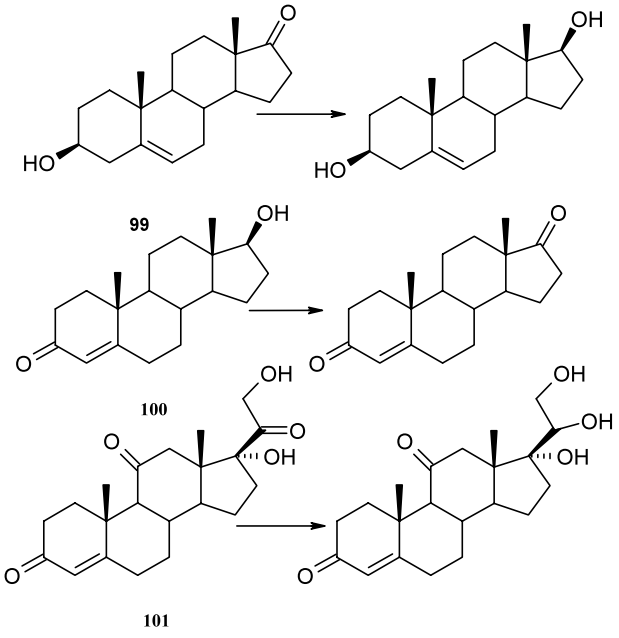

Figure 20. Biotransformation of steroids by *C. musae*.

5.5. Miscellaneous

The microbial reduction and resolution of the well-known herbicides (±)-2-(1-naphthoxy)propionic acid *N*,*N*-diethylamide and (±)-2-(2-naphthoxy)propionic acid anilide (commercial name Devrinol or Napropamide, and Naproanilide) has been carried out with *G. cingulata*. The enantiomeric excess of (*S*)-(+)-2-(1-naphthoxy)propanol reached 99% ee.[52]

A selection of 30 strains of micromycetes known as good degraders of polychlorinated aromatic compounds have been investigated to degrade fluorene. As a result of this study *C. dematium* degraded fluorene. The presence of 9-fluorenol and 9-fluorenone indicated that oxidation of the aliphatic ring occurs in detoxification of fluorene.[53]

On the other hand a direct access to 4-vinylphenol and 2-methoxy-4-vinylphenol has been described as result of biodegradation of cinnamic acids by *C. gloeosporioides*.[54]

6. Conclusions

In summary, it is clear that the *Colletotrichum* species have a considerable oxido-reductase activity. So, regioselective hydroxylations and diastereo- and enantioselective reductions on different substrates have been widely discussed above.

In the case of terpenoid compounds *Colletotrichum* displays a considerable hydrolase activity and strong reducing power. On the other hand, an interesting hydroxylase and oxidase activity is shown when these fungi metabolize phytoalexins or antifungal compounds as a detoxification mechanism.

The high stereospecificity of enzymatic reactions is challenging to organic chemists, because traditional organic chemical reactions often fail to reach the same degree of enantioselectivity. For this reason micro-organisms or their enzymes are used for stereoselective reactions. The biotransformations and results herein reviewed may provide useful tools for further in vivo and in vitro investigations on purified enzyme systems.

Finally, it can be concluded that biotransformations by *Colletotrichum* species have a significant importance due to their broad utility for the enantioselective production of compounds of commercial interest, as well as for permitting the study of the metabolism of such compounds in order to obtain novel antifungal agents with activity against these micro-organisms.

Acknowledgements

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