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Review

The biocatalytic reactions of *Beauveria* spp.

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Abstract

Fungi of the *Beauveria* spp., typified by *Beauveria bassiana* ATCC 7159, are among the most frequently used whole cell biocatalysts. They have been reported to catalyse many different reactions, including oxidative, reductive and hydrolytic transformations, of a wide range of substrates. This review covers the range and application of biocatalytic reactions of *Beauveria* spp., with emphasis on the scope and utility of *Beauveria*-catalysed reactions for preparative biotransformations.

Keywords: *Beauveria* spp.; Biocatalytic reactions; Preparative biotransformations

1. Introduction

Beauveria spp., belonging to the Moniliaceae family of fungi imperfecti, constitute a small group of closely related microorganisms. The 1997 catalogue of the American Type Culture Collection lists only thirty eight types, consisting of *Beauveria aranearia* (one example), *B. bassiana* (30 examples) *B. brongniartii* (five examples), *B. caledonica* (one example) and an unclassified *Beauveria* spp. (one example) [1]. It is remarkable that of this small selection of strains, *B. bassiana* ATCC 7159 is second only to *Aspergillus niger* as the most frequently used fungal biocatalyst, and is surpassed in applications only by the latter organism, *Pseudomonas putida*, and baker's yeast [2].

B. bassiana was originally isolated as a laboratory contaminant and classified as *Sporotrichum sulfurescens*. This classification was later changed to *B. sulfurescens*, and more recently to *B. bassiana*. However, the continuation of a single accession number such as ATCC 7159 or CBS 209.27 (both of which represent an identical strain in two different culture collections) has ensured the identity of the strain. To avoid possible confusion with the original literature, the following discussion will use the classification referred to in the original citation, and to assist reproducibility will provide strain accession numbers where these have been reported.

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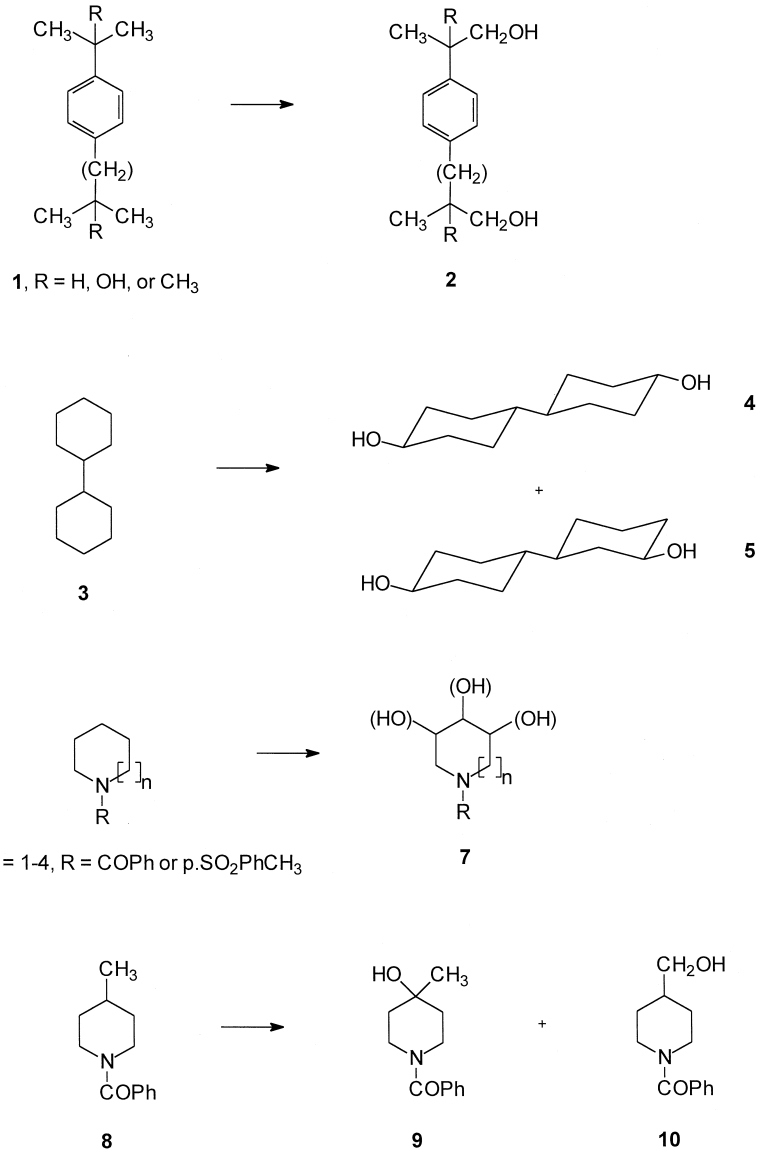
2. Hydroxylation reactions

2.1. Hydroxylation at saturated carbon

The inaugural paper in the field of *Beauveria*-catalysed hydroxylations begins with the sentence, ''The oxygenation of an unactivated methylene group is a reaction at which microorganisms are still more adept than organic chemists, is spite of many advances made in recent years by the macroorganisms'' [3]. The fact that this comment is as true today as it was when originally expressed over thirty years ago underlines the still undeveloped potential possessed by microbial biocatalysts for this reaction.

2.1.1. Hydroxylation of hydrocarbons and alcohols

The validity of the above comment is exemplified by the use of *S. sulfurescens* ATCC



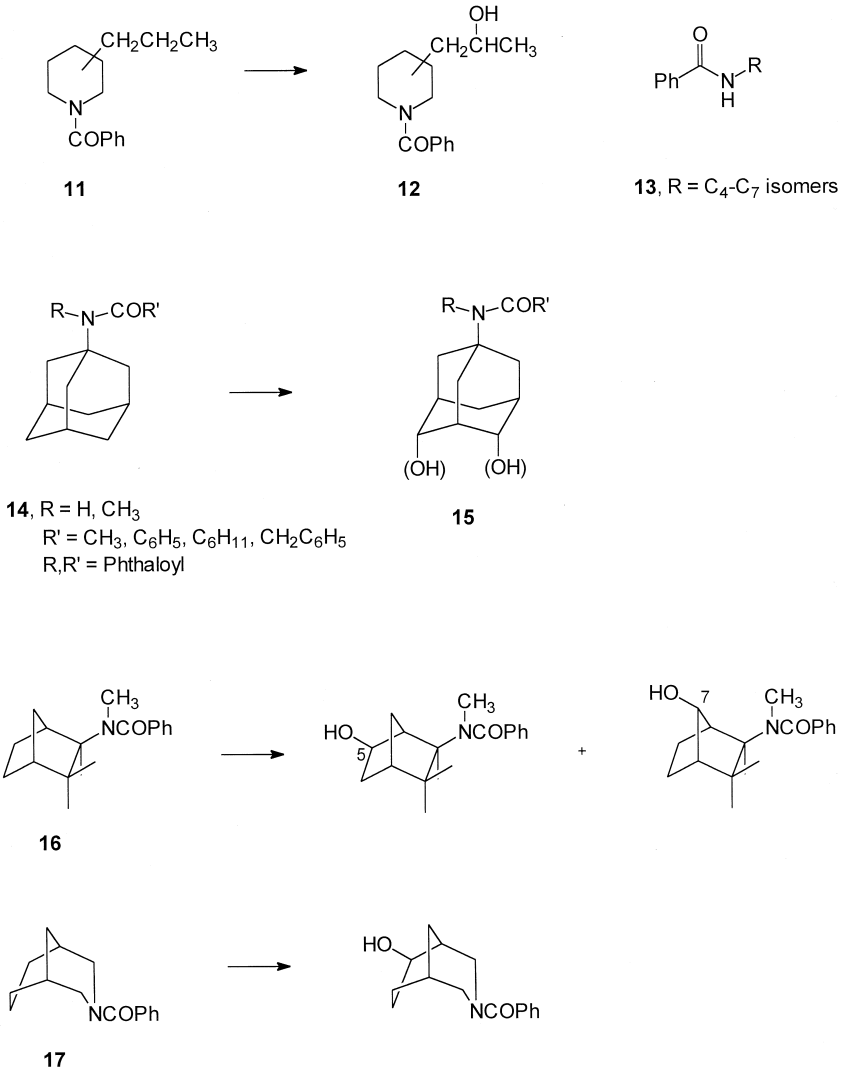
Scheme 1.

7159 to convert cyclododecanol into products oxygenated at C-5, C-6, and C-7 [3]. The product distribution from this biotransformation, together with the observation that cyclododecane was not a substrate, led to the proposal of the first ''active site'' model for *Beauveria*-catalysed hydroxylations, considered in more detail in Section 2.3.

In contrast to the failure of *S. sulfurescens* to hydroxylate cyclic saturated hydrocarbons, *meta*- and *para*-substituted dialkyl benzenes are hydroxylated at benzylic positions (when available) and/or benzylic methyl groups, summarised by the conversion of **1** to **2** [4]. Both hydrocarbon (R = H) and the corresponding mono-alcohol substrates (R = OH) gave the same products, but yields were higher from the latter. *B. bassiana* ATCC 7159 has also been reported to convert cyclohexylcyclohexane **3** to a 3:1 mixture of the 4,4′ and 3,4′ diequatorial diols **4** and **5** in low yield [5].

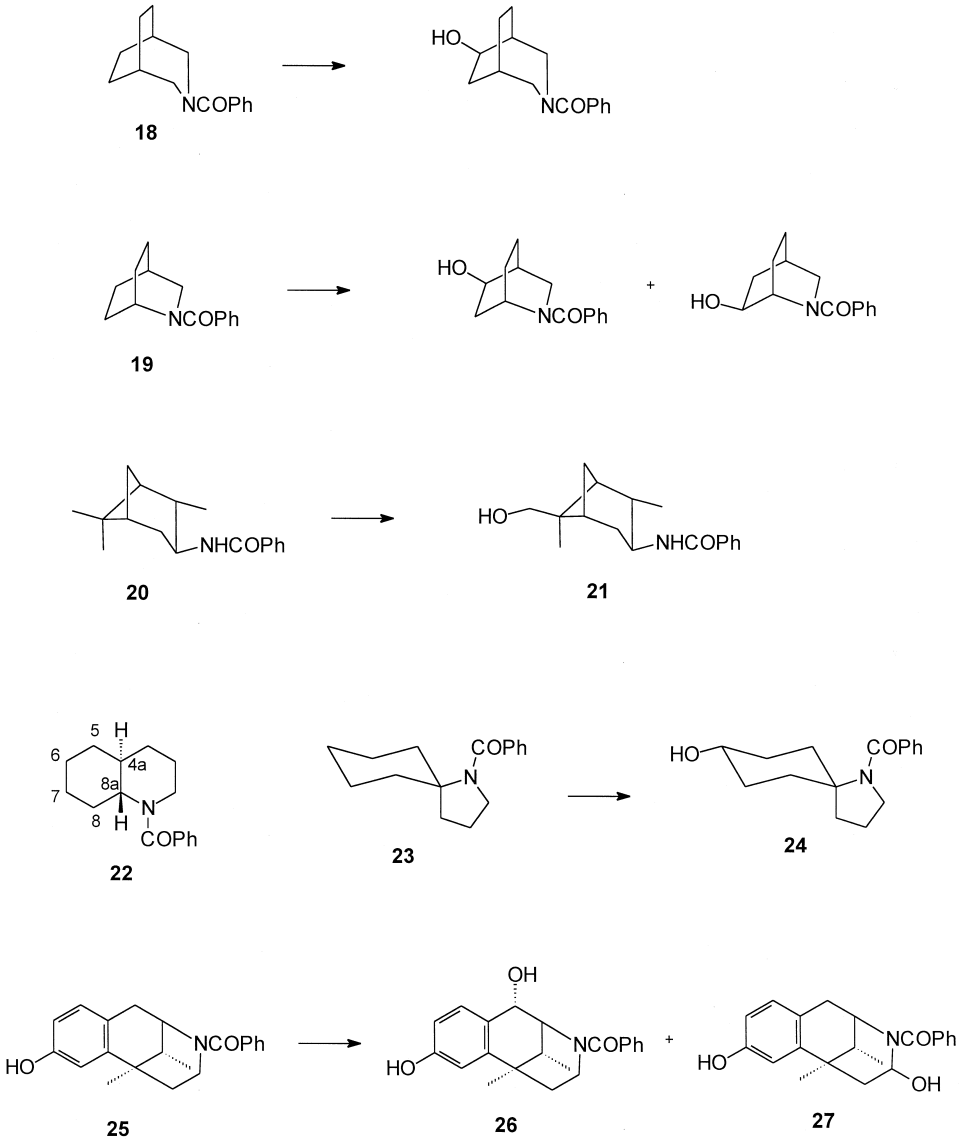
2.1.2. Hydroxylation of amides and related substrates

The ability of *B. bassiana* (originally as *S. sulfurescens* ATCC 7159) to hydroxylate a vari-



Scheme 2.

ety of amides, lactams, carbamates, azides, and sulfonamides has been extensively explored, beginning with work by the Upjohn group on the use as substrates of benzamide and *p*-toluenesulfonamide derivatives of piperidine and hexa-, hepta- and octamethyleneimines [6,7]. Yields of hydroxylated products were generally in the range 25%–60%, with attack occurring preferentially in a substrate-dependent manner at C-3, C-4 or C-5 as summarised by the conversion of **6**–**7**. The conversion of 2-, 3- and 4-methyl-substituted piperidines showed a similar regioselectivity, with hydroxylation occurring predominantly at C-4, illustrated by the conversion of **8**–**9** in 13% yield, but hydroxylation of the alkyl group was also observed for the latter substrate, giving rise to **10** in 23% yield [8]. The latter mode of reaction was also observed for 2-

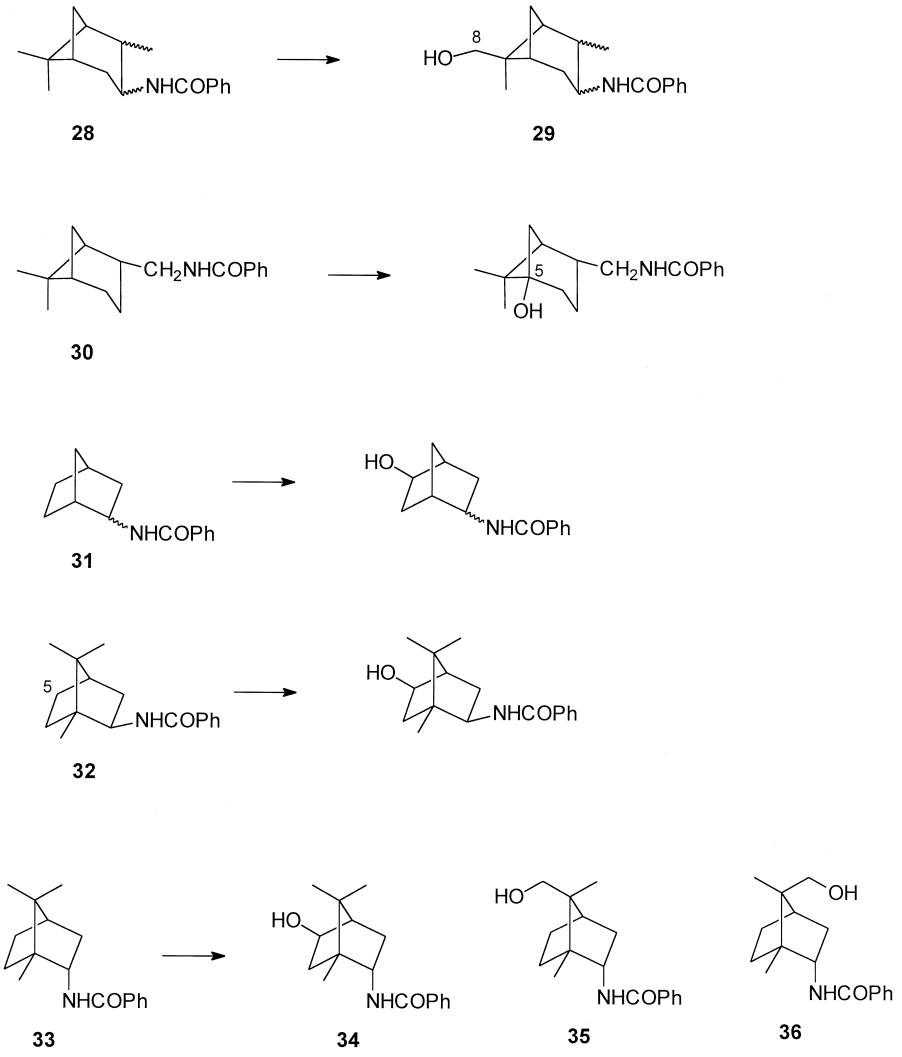


Scheme 3.

and 4-substituted *n*-propyl substrates **11**, which were converted to **12** in addition to the products of hydroxylation at C-3 and C-4 [8]. Acyclic benzamides **13** were converted to terminal or subterminal alcohols in substrate-dependent yields of 5%–70% [9]. The relative stereochemistry of hydroxylation products obtained from piperidine-based substrates has been examined, and a preference for equatorial introduction of the hydroxyl group clearly established [10].

Hydroxylation of a series of adamantyl amides **14** occurred mainly at a methylene site, giving mono- and diol products represented by **15**, with minor amounts of methine hydroxylation when R,R′ = methyl [11], and the *N*-benzoyl derivative of the ganglionic blocking agent mecamylamine, **16**, was hydroxylated at both C-5 (21% yield) and C-7 (29% yield), the latter product being isolated following oxidation to the ketone [12].

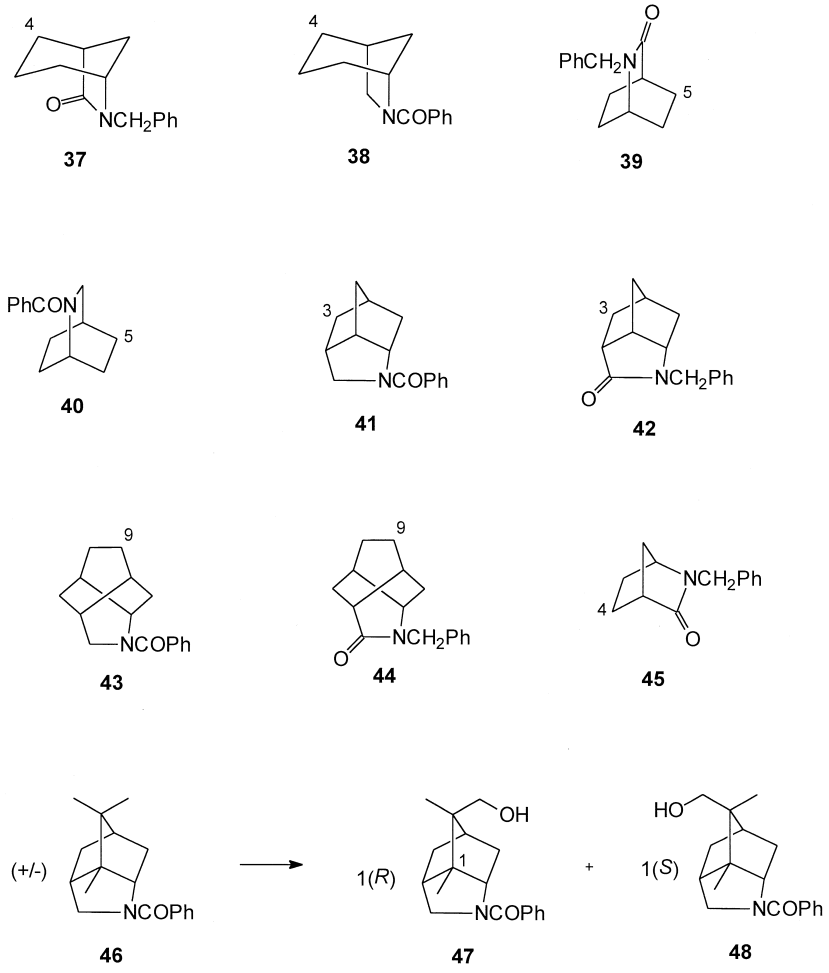
A series of azabicycloalkanes is also subject to hydroxylation, products resulting from attack at methylene groups within the substrate. The azabicyclo[3.3.1], azabicyclo[3.2.2], and azabi-



Scheme 4.

cyclo[2.2.2] substrates **17**–**19** were hydroxylated at analogous endo sites in yields of 45%–70% [13], while *N*-benzoylisopinocamphenylamine **20** was converted to the hydroxymethyl compound **21** in 62% yield from the (+)-isomer of substrate, but only 28% yield from the (−)-isomer [10]. 1-Benzoyl-*trans*-decahydroquinoline **22** gave a mixture of products hydroxylated at C-5, C-6, or C-7 in a total yield of 80%–90% [14]. The product of hydroxylation at C-6 was racemic, but hydroxylations at C-5 and C-7 were enantioselective for the 4a(*S*),8a(*R*) and 4a(*S*),8a(*S*) configuration of substrate, and specific for the formation of an equatorial alcohol product. Hydroxylations of both (+)- and (−)-**22** were also carried out and gave optically pure alcohol products [14]. The azaspirodecane **23** was also examined as part of this series of substrates, and gave the equatorial alcohol **24** in 54% yield [10]. *S. sulfurescens* ATCC 7159 was reported to be among a group of several microorganisms capable of converting the racemic azabicyclononane **25** to the benzylic alcohol **26**, but was unique in also forming the hemiaminal **27**, albeit in very low isolated yield (1%) [16].

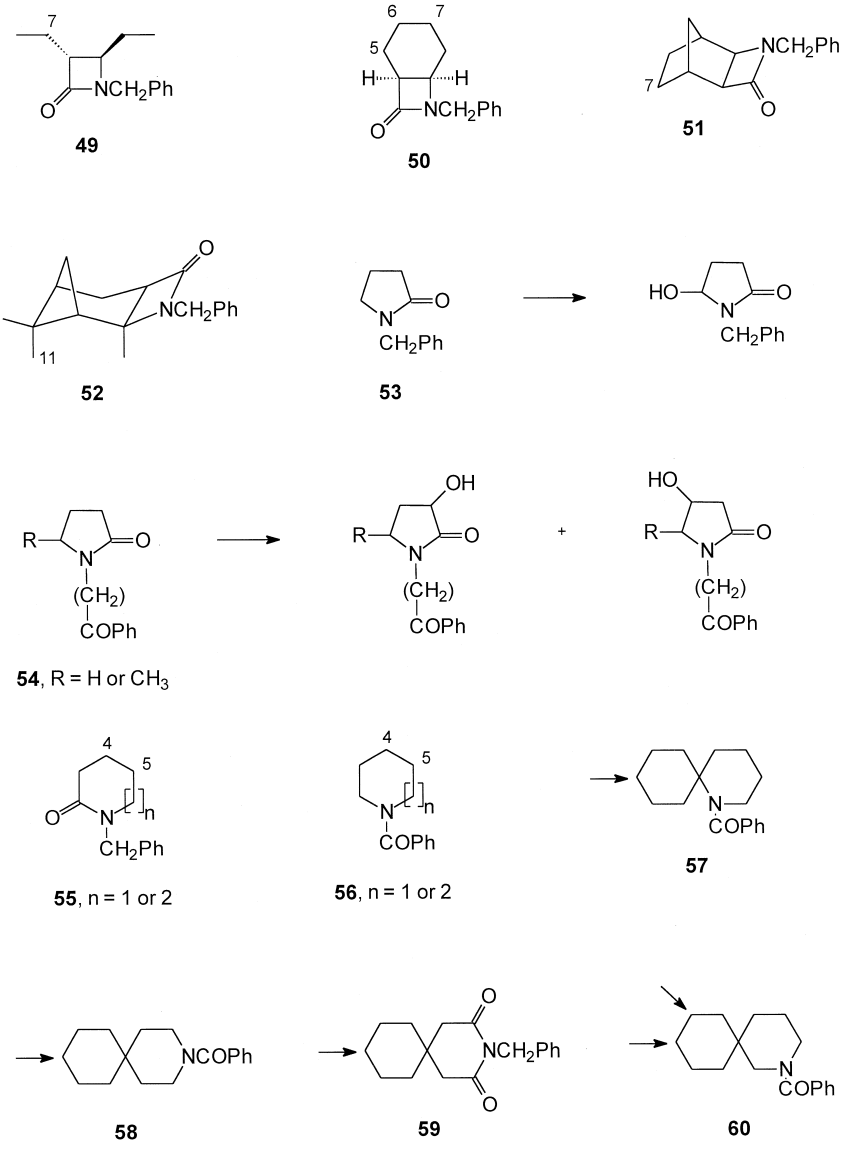
The examination of the hydroxylation of amide derivatives of simple monoterpenes and



Scheme 5.

other compounds related to **20** was expanded by Archelas et al. [17] to include a series of the four isomeric benzamides **28**, all of which were hydroxylated to give the C-8 hydroxymethyl derivatives **29** in 36%–60% yield (cf. the conversion of **20**–**21**). In contrast, the isomeric substrate **30** was hydroxylated exclusively at C-5, suggesting a role for the amide group in directing the regiochemistry of the reaction [17].

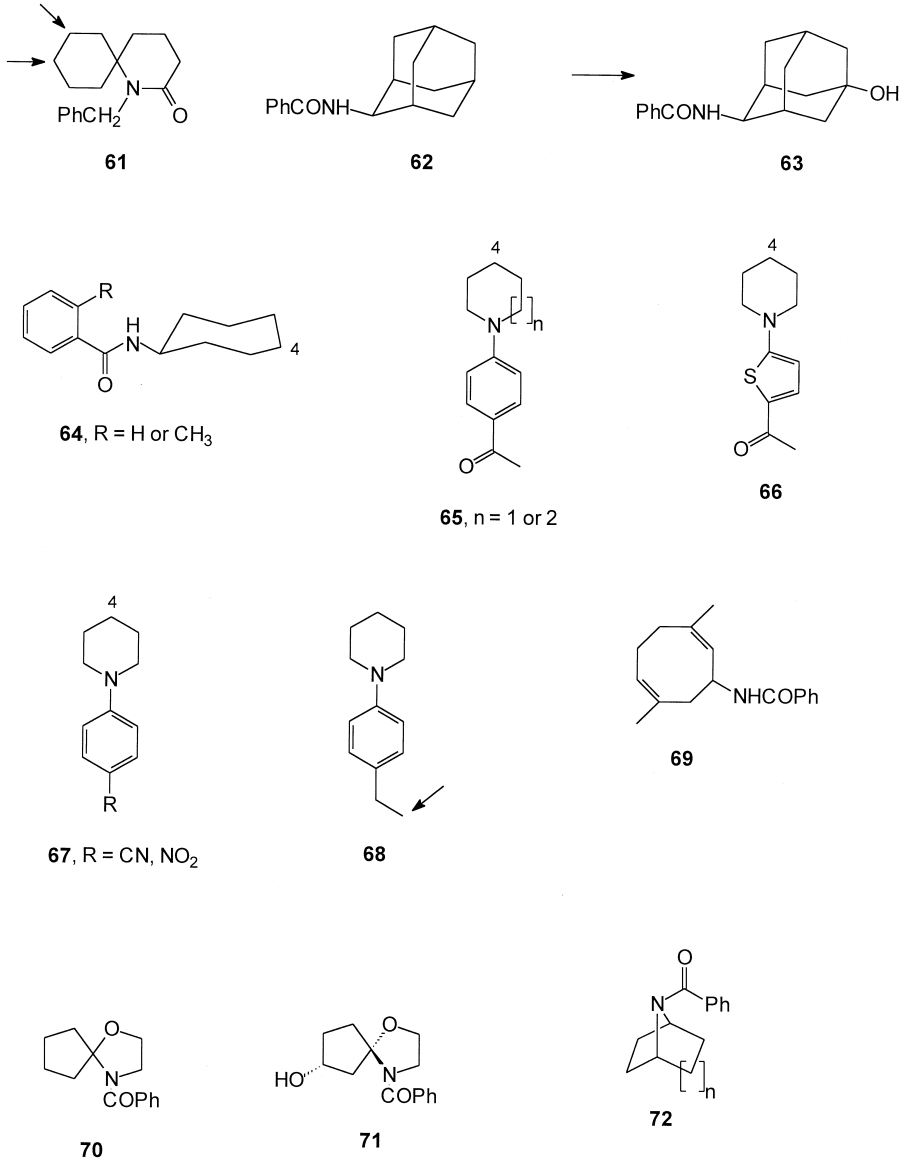
A similar analysis of the hydroxylation products obtained from isomeric bornylamides **31** and **32**/**33** supported this view, and established that the regioselectivity of hydroxylation at the *gem*. dimethyl group (leading to **35** or **36**) was dependent upon the absolute configuration of **33** [18]. Epimerisation of *endo* deuterium label at C-5 to the *exo* position during hydroxylation of **32** and **33** was observed, and attributed to the presence



Scheme 6.

of a radical intermediate in the hydroxylation process [19].

Expansion of the list of azabicyclic substrates for hydroxylation included the isomeric amides **37** and **38**, both of which were hydroxylated in good yield at the C-4_{axial} position [20]; **39** and **40**, which undergo C-5-endo hydroxylation [20]; **41** and **42**, both hydroxylated at C-3-*exo* [21,22]; and **43** and **44**, which give mixtures of products predominantly hydroxylated at C-9 [21,22]. The simple azabicyclo[2.2.1]heptane **45** was hydroxylated predominantly at C-4-*exo* in 30%–35% yield [23,24], and hydroxylation of the enantiomers of **46** was found to be regioselective in a manner analogous to that of hydroxylation of **33**, giving the 1(*R*) product **47** in 90% e.e. and the 1(*S*) product **48** in 85% e.e., each product being obtained in 30% yield [25].



Scheme 7.

In addition to the above examples, the range of amide substrates for hydroxylation by *Beauveria* also includes a series of monocyclic and polycyclic β-lactams **49**–**52**, hydroxylated at the numbered positions in 10%–65% yields [26], pyrrolidones **53** [27] and **54** [28], converted to ring-hydroxylated products in low (10%–20%) yields, and piperidone-derived and caprolactam-derived substrates **55** and **56**, hydroxylated at C-4 ($n = 1$) and C-3, C-4 and C-5 ($n = 2$) [27]. Azaspiroundecanes **57**–**61** were hydroxylated at the sites indicated (→) in 30%–80% yields [29], while the benzamide derivative of 2-aminoadamantane **62** gave the tertiary alcohol **63** in 78% yield [30]. The hydroxylation of *N*-cycloheptylbenzamides **64** gave isomeric alcohols at C-4 when R = methyl, but only the 4(*S*)-isomer when R = H [31]; the aryl amines **65**–**67** were hydroxylated at C-4 in 37%–66% yield; the C-4 methyl substituted analogue of **65** ($n = 1$) was also hydroxylated at C-4 in 55% yield, but the 4′-ethyl substrate **68** was hydroxylated only at the ethyl terminus [32]. Transformation of 1-benzoylamino-3,7-dimethylocta-2,6-diene **69** by *B. bassiana* BKMF-3111D has been reported, but no product details are available [33,34]. In contrast, biotransformation of the *N*-benzoyloxazole **70** has been fully characterised, giving the alcohol **71** in up to 80% conversion under optimised conditions [35].

The hydroxylation of bridgehead azabicycloalkanes **72** occurred to give endo alcohols **73**–**75** in 30%–56% yield, but **74**, a possible chiral precursor of the analgesic epibatidine, was formed in only 22% e.e. [36]. Azabicyclo[2.2.1]alkanes derivatised as carbamate esters **76** and as sulfonamides **77** are also hydroxylated in the same way, the former to give the 1(*S*) endo products **78** in low yield and e.e., but the azabicyclo[3.3.1]nonane **79** gave the *cis* alcohol **80** in 30% yield [37]. The sulfonamide **77** (R = Ph) gave a product analogous to **78** [36], but when R = *p*-CH_3Ph, **77** was hydroxylated preferentially at the benzylic position, giving the hydroxymethyl product **81** in 34%–54% yield [36,37].

The hydroxylation of substrates containing carbamate, sulfonamide, and other amino-protecting groups has been less extensively examined than that of amides, but nevertheless a substantial literature exists on hydroxylation of the former group of compounds by *Beauveria* spp.. The hydroxylation of a series of alkyl phenylcarbamates **82** has been examined [38,39]. When R′ = H, hydroxylation in the alkyl portion of the substrate was concomitant with hydroxylation in the *para* position of the aromatic ring, but when R′ = CH_3 the former mode of reaction was uniquely observed; hydroxylations in the alkyl portion of the substrates occurred at methylene or methyl sites remote from the ester oxygen. A recent study of *B. bassiana*-catalysed hydroxylations of the series of rigid and flexible carbamate derivatives of monocyclic and bicyclic alcohol substrates **83** reported similar findings [40,41]. The phenylcarbamate derivative of dihydroartemisinin **84** was converted to the hydroxy derivative **85** by *B. sulfurescens* ATCC 7159 in low yield (3.4%), but with retention of the reactive peroxide bridge [42,43]. In contrast, the ethyl ether **86** was hydroxylated at the methyl group and at C-9β, in addition to giving products arising from reduction of the peroxide bridge [43].

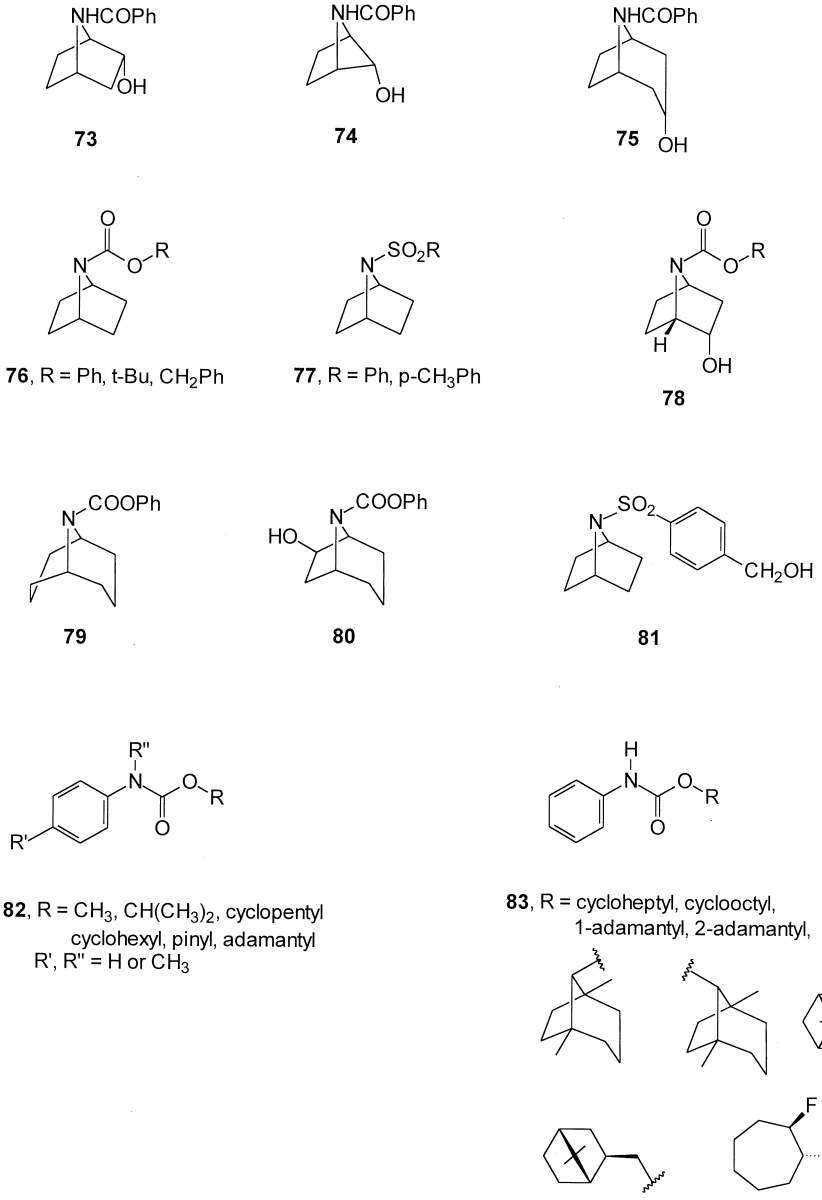
1-Azidoadamantane **87** was hydroxylated at the positions indicated (→) to give three mono-alcohol products in a total yield of 18%, but hydroxylation of *N*-benzoyl-3-noradamantanamine **88** occurred with higher regioselectivity to give a single product in 58% yield [44]. The hydroxylation of a series of unsubstituted and methyl-substituted cbz-protected piperidines **89** has also been examined, and found to proceed in close parallel with the hydroxylation of the corresponding *N*-benzoyl substrates, with reaction occurring principally at C-3 and C-4 [45].

2.1.3. Hydroxylation of miscellaneous substrates

In contrast to the wide use of amide and related substrates for hydroxylation by *Beauve-*

ria, only a limited range of other substrate types has been examined. The following discussion will focus on the use of *Beauveria* for the hydroxylation of drugs, steroids, terpenes, and benzylic carbons.

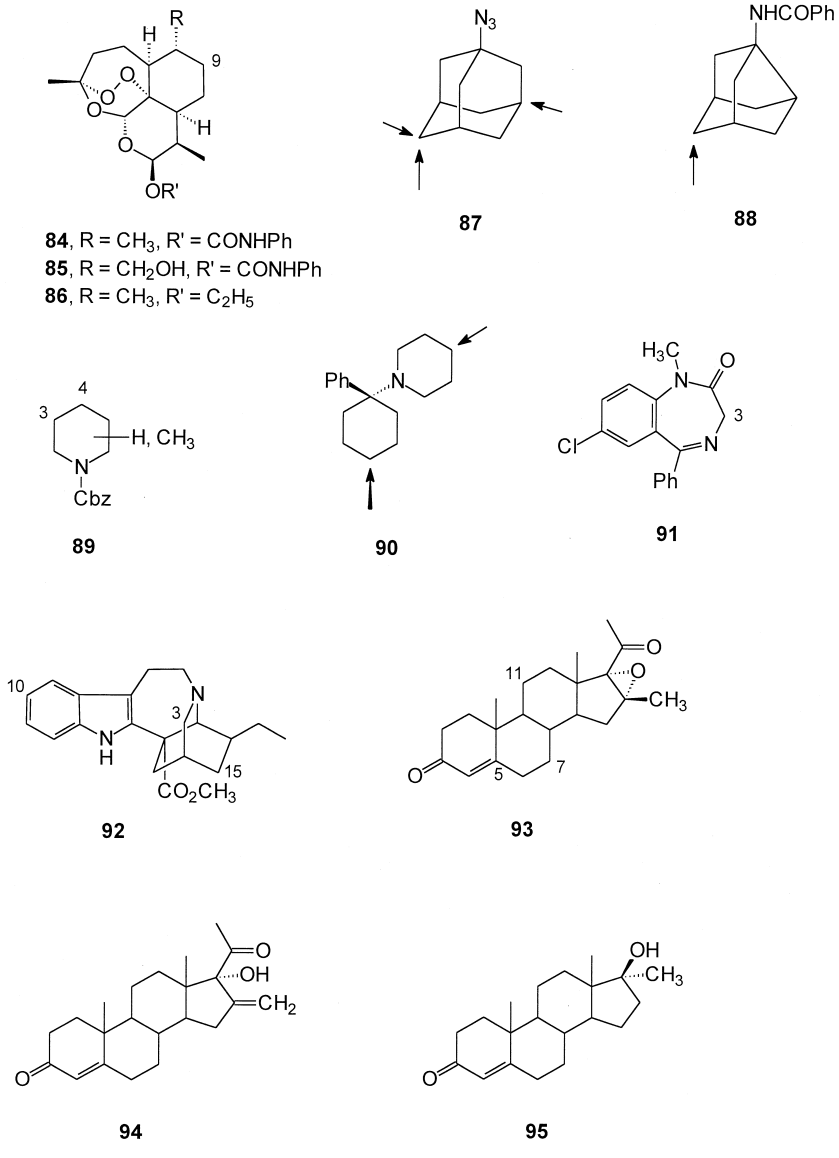
The piperidine derivative phencyclidine **90** was metabolised in low yield by *B. sulfurescens* ATCC 7159, by hydroxylation as indicated to give a mixture of two isomeric products, but interestingly a strain of *B. bassiana* (ATCC 13144) was reported to give no biotransformation products [46]. Diazepam **91** is reported to give derivatives hydroxylated at C-3 on biotransformation by *B. bassiana* IMI 12939 [47],



Scheme 8.

and the indole alkaloid coronaridine **92** gave products of hydroxylation at C-15-exo and C-3 on incubation with *S. sulfurescens* ATCC 7159, the latter being isolated as the amide [48].

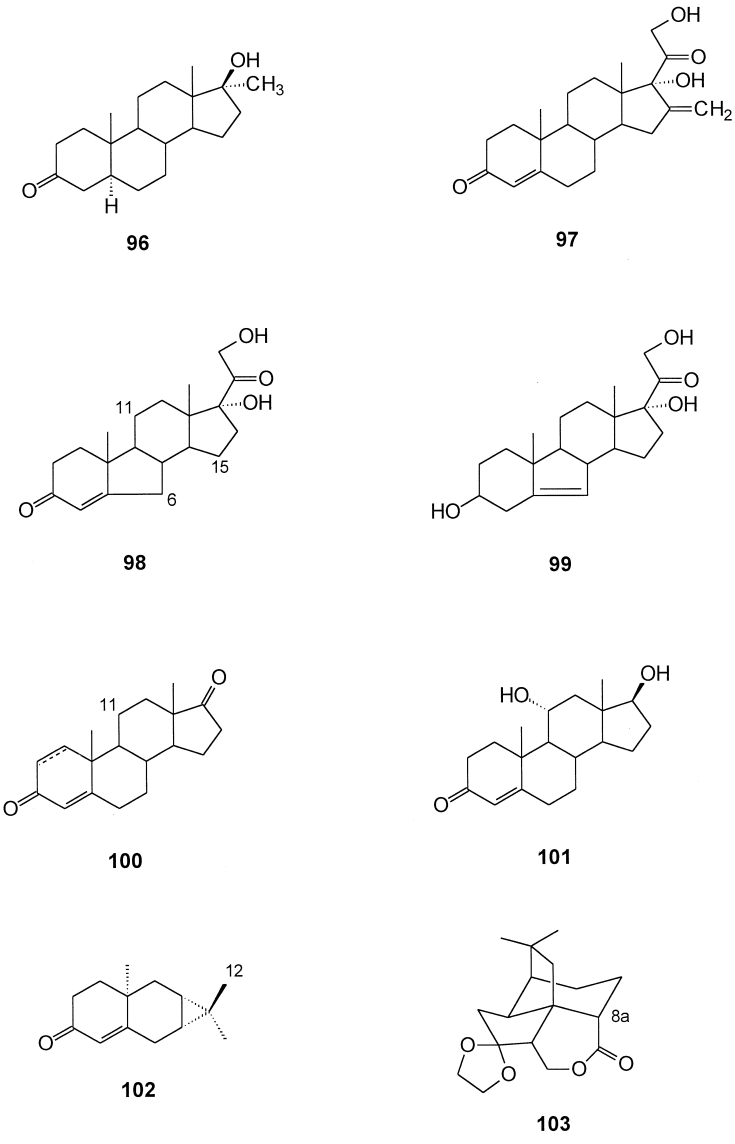
In contrast to many other fungi commonly used for biocatalysis, *Beauveria* spp. have not been extensively used for the transformation of steroid substrates. *B. globulifera* has been reported to convert steroids **93**–**96** by hydroxylation at C-11α and C-7β (in the case of **93** only), but concomitant with reduction of the 4,5 double bond to the 5β configuration [49]. *B. bassiana*, however, was able to hydroxylate **97** at C-11α without reduction at C-4(5) [50]. The B-norsteroid **98** was hydroxylated at C-6α, C-11α, C-11β, and C-15α by *B. bassiana*, but **99** was subject to hydroxylation at only the latter three positions [51]. Both androst-4-ene-3,17-di-



Scheme 9.

one and androsta-1,4-diene-3,17-dione, jointly represented by structure **100**, were hydroxylated at C-11α by an unidentified *Beauveria* spp. [52], while *B. bassiana* IMI 12939 converted testosterone to the 11α-hydroxy derivative **101** [47].

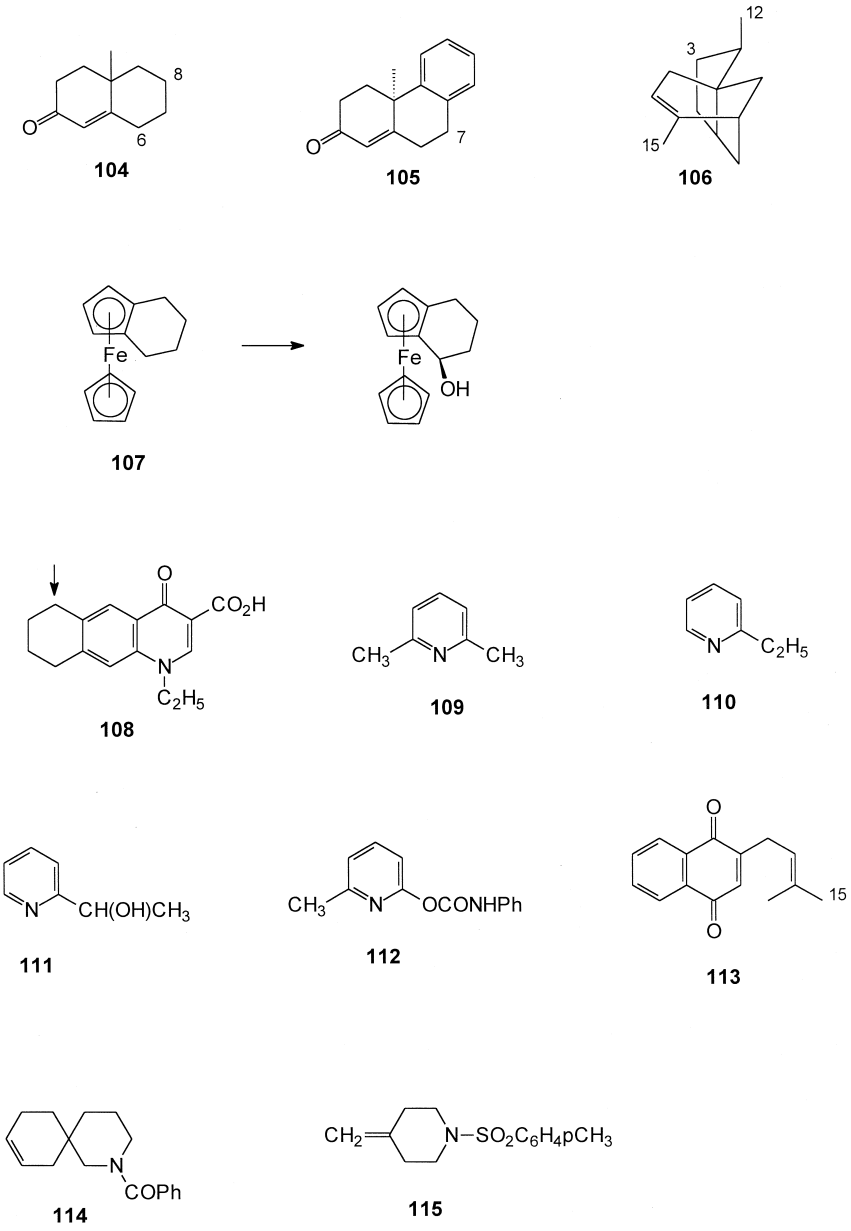
Biotransformation of terpenoids by *Beauveria* is similarly under exploited, but *B. bassiana* ATCC 7159 has been reported to hydroxylate the sesquiterpene **102** at C-12 in 80% yield [53], and to hydroxylate the ethylene ketal of quadrone **103** at C-8a [54]. The simple conjugated cyclic ketone **104** was hydroxylated at C-6 and C-8 by ATCC 7159 in a substrate enantioselective ratio: (*R*)-**104** gave mainly the latter product (36% vs. 6%), but (*S*)-**104** gave equal amounts (15%) of each product [55,56]. In contrast, ketone **105** gave only the product of



Scheme 10.

hydroxylation at the benzylic position, C-7, in 65% yield [56]. Incubation of the odiferous sesquiterpene cedrene **106** with *B. sulfurescens* gave three hydroxylation products in low yield (2%–5%); all contained an allylic hydroxyl at C-15 and two had additional hydroxyl groups at C-3 and C-12 [57]. Hydroxylation at C-3 was also observed for the related substrate cedrol [57].

Hydroxylation at a benzylic or allylic position is a common mode of oxidative biotransformation. *S. sulfurescens* ATCC 7159 converted the ferrocene derivative **107** to the corresponding exo alcohol in 13% yield [58], and



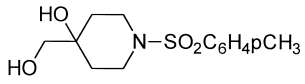
Scheme 11.

hydroxylated **108** in 39% yield at the benzylic position indicated [59]. 2,6-Dimethylpyridine **109** was converted to 2-methyl-6-pyridylmethanol by *B. bassiana* ATCC 7159 [60], and analogous products were also obtained from other dimethylpyridines [61–63]. 2-Ethylpyridine **110** gave the optically pure (−)-alcohol **111** in 60% yield [62,63], but only a low yield of benzylic alcohol was obtained when 4-ethylpyridine was used as substrate [62]. A 40% yield of benzylic alcohol was obtained from **112**, but more complex heterocyclic substrates were not hydroxylated by *B. bassiana* [63]. The allylic hydroxylation of lapachol **113** at C-15 by *B. sulfurescens* ATCC 7159 is reported to occur in 45% yield [64].

2.2. Hydroxylation at unsaturated carbon

2.2.1. Oxidation of alkenes

Whole-cell oxidative biotransformation of alkenes frequently results in the formation of



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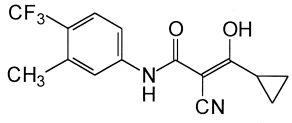

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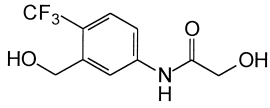

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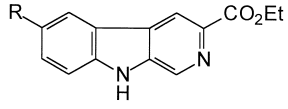
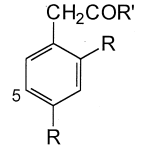
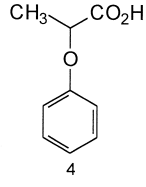


119, R = H



120, R = H, OH, OMe, F, Cl, Br
R' = OH, OMe, NH_2



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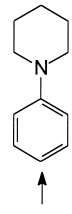

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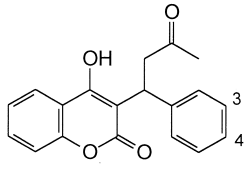

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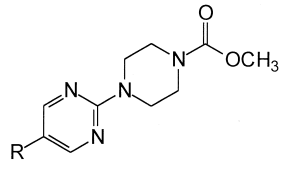


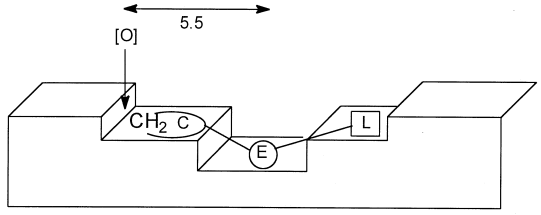
124, R = H
125, R = OH

Scheme 12.

1,2-diols by enzymic or nonenzymic hydrolysis of intermediate epoxides. *B. sulfurescens*-catalysed biotransformation of the olefin **114** resulted in isolation of the corresponding epoxide in 74% yield [29], but conversion of the *exo*-methylene substrate **115** gave diol **116** as the only isolatable product in 20% yield [30]. A more complex mode of oxidative biotransformation by *B. bassiana* ATCC 7159 was observed with substrate **117**, the immunomodulating agent HR325, which gave the diol **118** together with a glucosidated derivative [65].

2.2.2. Hydroxylation of arenes and heteroarenes

The ability of *S. sulfurescens* ATCC 7159 to convert arenes to phenols was established by



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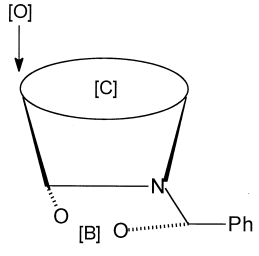
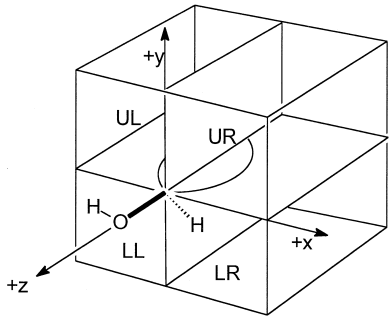
C = cyclic system
E = electron rich binding group
L = lipophilic substituent
[O] = site of hydroxylation



127

[C] = cyclic structure
[B] = binding site for amide oxygen
[O] = site of hydroxylation



128

Scheme 13.

Boyd et al. who reported the conversion of anisole to 2-methoxyphenol in moderate yield [66,67]. The more complex alkaloid substrate coronaridine **92** gave, in addition to products of oxidation at C-3 and C-15, a phenolic product derived from oxidation at C-10 [48], and a similar regiochemistry of oxidation was observed for the β-carboline **119**, converted by *S. sulfurescens* ATCC 7159 to the corresponding phenol (R = OH) in 62% yield together with an additional 15% of derived glycosides [68]. The phenylcarbamates **82** (R′ = H) [38,39,69] and **83** [41] were converted to the corresponding *para*-substituted phenols by *B. sulfurescens* ATCC 7159 in up to 40% yield, but hydroxylation in the aliphatic ring was the dominant mode of biotransformation for these substrates. Hydroxylation of the *N*-benzyl group of **45** also occurred in the *para* position to give a low yield of phenol [24].

An unspecified *B. bassiana* mutant was used to hydroxylate a series of substituted phenylacetic acid derivatives **120** at C-5 [70,71], and *B. bassiana* LU700 used for the hydroxylation of 2-phenoxypropionic acid **121** at C-4 [72]. *B. bassiana* ATCC 7159 was used for the *para*-hydroxylation of *N*-phenylpiperidine **122** [32], and of the rodenticide warfarin **123** at C-3 and C-4 [73]. *B. bassiana* IMI 12939 had previously been reported to hydroxylate warfarin only at the C-4 position [47,74]. *B. bassiana* ATCC 7159 has been used for the conversion of the pyrimidine heterocycle **124** to the phenol **125** in 11% yield, a further 8% of product being obtained as the corresponding glucoside conjugate [75].

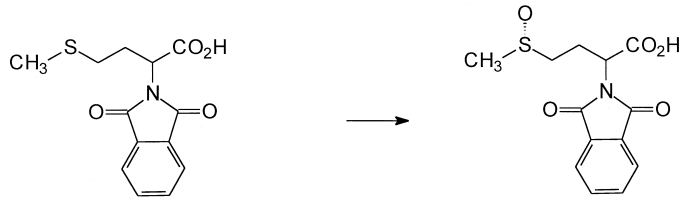
2.3. The selectivity of hydroxylation

As one of the fungi most frequently used for microbial hydroxylations, *Beauveria* has been successfully utilised for the hydroxylation of a range of natural products, synthetic cyclic amides, substituted aromatic compounds and hydrocarbon substrates, and its application for the hydroxylation of alcohols was the subject of the first systematic investigation of the parameters that may influence the site and stereochemistry of microbial hydroxylation [3]. The model **126** was initially proposed by the Upjohn group to account for the regioselectivity of the hydroxylation of cyclic alcohols by *S. sulfurescens* ATCC 7159, and was later extended to cover the hydroxylation of amides [15]. It proposes hydroxylation at a methylene group which is part of a ring system, C, to which is attached an electron-rich binding group E at an optimum distance of 5.5 Å from C. Group E may also carry a lipophilic substituent L, which may or may not be part of C. A refined version of this model, **127**, was proposed by Furstoss et al. to take into account the absolute stereochemistry of the hydroxylation reaction, and the experimental observation that hydroxylations could occur over a range of 4–7 Å from the binding centre [19,28,57,68,76].

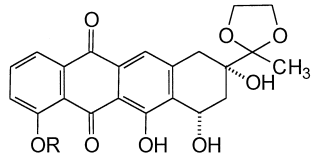
Hydroxylations of cyclic substrates by *B. sulfurescens* have also been analysed in terms of the octant system **128** for the definition of substrate-product relationships [15,22]. In this model, the newly introduced hydroxyl group is placed along the *z* coordinate of an *xyz* coordinate system, with the carbon undergoing hydroxylation located at the origin. The bulk of the substrate then preferentially occupies the rear quadrants labelled UL, UR, LL, and LR, in the order UR > UL ≫ LL and LR. A recent analysis of *B. bassiana*-catalysed hydroxylations by Pietz et al. [40,41] largely confirmed the validity of the Upjohn model when applied to both rigid and flexible carbamate derivatives of monocyclic and bicyclic alcohol substrates, and suggested that an ‘‘induced fit’’ of substrate into the enzyme’s binding site is followed by hydroxylation at a saturated carbon located at an optimum distance of 5.5 Å from the oxygen atom directly attached to the carbocyclic part of the substrate. This ‘‘induced fit’’ phenomenon was proposed in order to account for the combination of high regioselectivity yet low substrate specificity observed for the *B. bassiana*-cata-

lysed hydroxylation of a variety of substrates such as alcohols, amides, lactams, carbamates, azides, and sulfonamides, with the implication that a single enzyme of very wide substrate specificity was nevertheless able to catalyse hydroxylation in a highly regioselective manner. The existence of a binding region of the *B. bassiana* hydroxylase enzyme specific for an aromatic ring of the substrate has also been proposed to account for the parallel outcome of the hydroxylations of *N*-benzoyl and *N*-cbz protected piperidines [45].

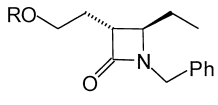
The models **127** and **128** were developed as an extension of the model **126** for alcohol substrates, specifically to explain the hydroxylation of cyclic amides and related substrates such as carbamates. None of these models satisfactorily account for other *B. sulfurescens*-catalysed hydroxylations such as the formation of phenols and hydroxylation of unactivated hydrocarbons. A reappraisal of the hydroxylations performed by *B. sulfurescens* suggests the possible existence in this microorganism of as many as four distinct types of hydroxylase enzymes, one specific for amides and related substrates whose stereo-and regiochemistry of action is subject to analysis by the above models, one specific for the hydroxylation of unactivated hydrocarbons without a directing influence by any existing substrate functionality, one for benzylic hydroxylations, and one for the conversion of arenes to phenols [77].



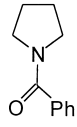
129 **130**



131, R = Me
132, R = H



133, R = Me
134, R = H



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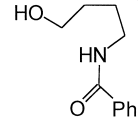

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Scheme 14.

3. Sulfoxidation reactions

A single report of the stereoselective oxidation of sulfides to sulfoxides by *Beauveria* spp. describes the use of *B. bassiana* ATCC 7159 for the conversion of *N*-phthaloyl D-methionines and L-methionines **129** to the corresponding (*S*) sulfoxides **130** in over 80% yield and high d.e. [78]. Both products could be purified to stereochemical homogeneity and were converted to the corresponding D-amino acid and L-amino acid (S_S) sulfoxides in high yield.

4. *O*- and *N*-dealkylation

It is not surprising to find that organisms of the genus *Beauveria* are able to catalyse other P450-dependent processes in addition to P450-dependent hydroxylations, such as heteroatom oxidation and dealkylation. Incubation of the 13-blocked anthracyclinone **131** with *B. bassiana* ATCC 7159 yielded 20% of the *O*-demethylated derivative **132** [79], and the β-lactam derivative **133** is demethylated by the same strain to yield alcohol **134** in 70% yield [26].

N-Dealkylations have been noted in drug metabolism studies using *Beauveria*. Diazepam **91** is transformed by *B. bassiana* IMI 12939 to four products including two metabolites, with and without hydroxylation at the 3-position, that exhibit demethylation at the nitrogen atom adjacent to the carbonyl [47]. In addition, biotransformation of the β-lactam **49** by *B. bassiana* ATCC 7159 yielded 20% of the debenzylated derivative in addition to hydroxylated product [26]. The same organism transformed *N*-benzoylpyrrolidine **135** to the ring-opened alcohol **136** in 7% yield [27].

5. Glucosidation

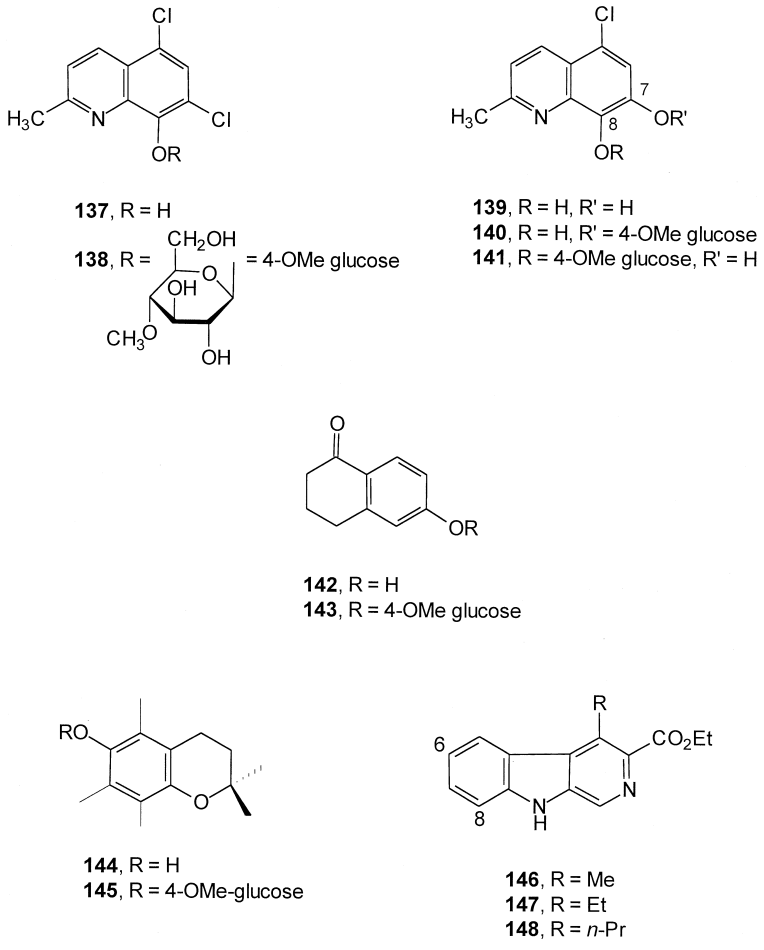
Many hydroxylation studies using *Beauveria* spp. have also reported the glucosidation of hydroxylated compounds and metabolites. The group of Kieslich observed that incubation of 5,7-dichloro-2-methyl-8-quinolinol **137** with *B. bassiana* ATCC 7159 resulted in a 30% yield of the phenolic glucoside **138** [80]. The dihydroxy derivative **139** yielded a mixture of glucosidated products at the 7 (**140**, 6%) and 8 (**141**, 1%) positions. In addition, 6-hydroxy-α-tetralone (**142**) was transformed by the fungus to the 4-*O*-methyl-β-glycoside (**143**) in 12% yield.

The hydroxylation of the herbicide Propham® [**82**, $R = CH(CH_3)_2$] by *B. bassiana* ATCC 7159 was followed by glucosidation to its phenolic 4-*O*-methylglucoside, isolated in 22% yield [69]. The adamantyl derivative **82** was transformed in a similar manner to its glucosidated metabolite [39]. The glucosidation of 2,2,5,7,8-pentamethyl-6-hydroxychroman **144** was observed to yield 10% of the conjugated aromatic phenol **145** at low concentrations of glucose in the fermentation medium, but at elevated concentrations of glucose (10 g l^{-1}), the yield was increased to 75% [81].

A series of ethyl β-carboline derivatives **119** and **146**–**148** was converted to their phenolic glucosides subsequent to aromatic hydroxylation at C-6 and/or C-8 by *B. bassiana* ATCC 7159 [68]. The selectivity of hydroxylation and hence glucosidation for the 8-position was improved on an increase in size of the alkyl group adjacent to the carboxyethyl moiety (Table 1). Studies of microbial models for mammalian drug metabolism have also shown that warfarin **123** was converted to 3′,4′-dihydroxywarfarin[4-methoxyglucoside] **149** by *B. bassiana* ATCC 7159 [73]. In addition, incubation of 240 mg of the synthetic immunomodulating agent

Table 1
Biotransformation of ethyl-β-carboline derivatives by *B. bassiana* ATCC 7159

Substrate	6-Hydroxylation (%)	6-Glucoside (%)	8-Glucoside (%)
119	62	8	7
146	–	20	18
147	–	–	70
148	–	–	68



Scheme 15.

HR325 **117** with the same strain resulted in 110 mg of its 4-*O*-methylglucoside **151** via oxidatively cleaved metabolite **150** [65]. A by-product of the hydroxylation of pyrimidine derivative **124** by *B. bassiana* ATCC 7159 was the 4-*O*-methyl glucoside of **125**, isolated in 8% yield [75]. The same strain transformed the fungicide Cyprodinil® **152** to glucosidated derivative **153** in 80% yield, with no reported detection of the presumed hydroxylated intermediate [82].

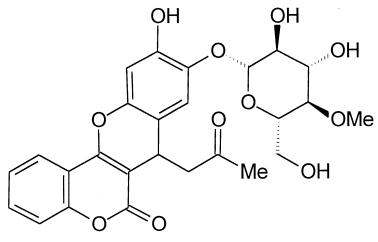
6. *N*-Acetylation

The sole report of *N*-acetylation by *B. bassiana* ATCC 7159 describes the transformation of *p*-cymene-related drug **154** to derivative **155** in 70% yield [83].

7. Epoxide hydrolysis

The presence of epoxide hydrolase activity in *Beauveria* spp. may be a further consequence of marked cyt-P450 activity in the genus, these enzymes being implicated in the degradation to vicinal diols of epoxides resulting from the oxidative action of cyt-P450 enzymes on alkenes in mammalian systems.

When *B. bassiana* ATCC 7159 was incubated with styrene oxide **156**, rapid hydrolysis resulted in residual (*R*)-epoxide isolated in 19%



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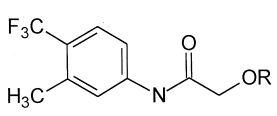
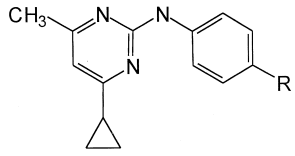
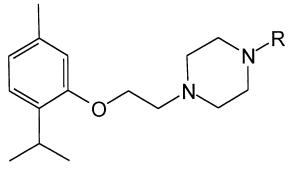


150, R = H
151, R = 4-OMe-glucose



152, R = H
153, R = 4-OMe-glucose



154, R = H
155, R = $COCH_3$

Scheme 16.

yield with 98% e.e. The diol **163** of (*R*)-configuration in 47% yield and 83% e.e. was obtained as a result of nucleophilic attack on the more hindered benzylic carbon atom, causing an inversion of configuration [84]. This hypothesis was confirmed by 99% incorporation of ^{18}O from labelled water into this position in the fast reacting enantiomer [85]. A general acid catalysed process for the hydrolysis of styrene oxide and six *para*-substituted derivatives **157**–**162** was proposed as the conserved regioselectivity and enantioselectivity of ring opening was congruent with the chemical acid catalysed ring opening of these epoxides [86]. Only *para*-nitrostyrene oxide **162** was resolved to the complementary (*S*)-enantiomer. The results showed a decrease in reaction rate with an increase in electron withdrawing power of the *para*-substituent, consistent with the presence of a carbonium ion intermediate in the hydrolytic mechanism (Table 2).

The synthetic utility of *Beauveria* epoxide hydrolase was expanded to include a series of substituted styrene oxide derivatives **170**–**175**

Table 2
Biohydrolysis of *para*-substitituted styrene oxides by *B. bassiana* ATCC 7159

Substrate	Reaction time (h)	Yield epoxide (%)	e.e. epoxide (%)	Product	Yield diol (%)	e.e. diol (%)
156	2	34	98 (*R*)	**163**	45	83 (*R*)
157	1	30	> 98 (*R*)	**164**	45	76 (*R*)
158	0.75	25	96 (*R*)	**165**	50	78 (*R*)
159	1	20	54 (*R*)	**166**	66	72 (*R*)
160	1	33	96 (*R*)	**167**	52	72 (*R*)
161	0.75	59	15 (*R*)	**168**	25	50 (*R*)
162	1	50	20 (*S*)	**169**	36	49 (*R*)

Table 3
Biohydrolysis of substituted styrene oxide derivatives by *B. bassiana* ATCC 7159

Substrate	Reaction time (h)	Yield epoxide (%)	e.e. epoxide (%)	Product	Yield diol (%)	e.e. diol (%)
170	5	10	53 (*S*)	**176**	80	10 (*R*)
171	4	42	20 (1*R*,2*S*)	**177**	42	99 (1*R*,2*R*)
172	3	30	98 (1*R*,2*R*)	**178**	38	90 (1*R*,2*S*)
173	–	no hydrolysis	–	–	–	–
174	1	20	98 (1*R*,2*S*)	**179**	48	69 (1*R*,2*R*)
175	0.5	38	98 (1*R*,2*S*)	**180**	49	77 (1*R*,2*R*)

[87]. *trans*-β-Methyl styrene oxide **172**, dihydronaphthalene oxide **174** and indene oxide **175** were all resolved to the epoxide with (*R*)-configuration at the benzylic position in accordance with the proposed mechanism, but tetrasubstituted epoxide **173** was not hydrolysed. The anomalous ring opening of *cis*-β-methylstyrene oxide **171** to diol of high purity but racemic residual epoxide prompted a second run to completion wherein an 85% preparative yield of the (1*R*,2*R*) diol with 98% e.e. was obtained. Hydrolysis of epoxide enantiomers with distinct and opposite regioselectivity was proposed to account for this enantioconvergence (Table 3).

Table 4
Biohydrolysis of alkyl substituted epoxides by *B. bassiana* ATCC 7159

Substrate	Residual enantiomer	Selectivity (*E*)
181	(*S*)	3.4
182	(*S*)	5.3
183	(1*S*,2*S*)	1.8
184	(1*R*,2*S*)	1.3

Comparatively poor selectivity was exhibited by the same strain of *Beauveria* for the hydrolysis of dialkyl substituted epoxides **181**–**184** (Table 4), with selectivity as determined by enantiomeric ratio not rising above 5.3 [88]. Residual epoxide was, in all but one case (and in contrast to the aromatic series), of the (*S*)-configuration.

An illustrative model of the active site of *B. bassiana* ATCC 7159 epoxide hydrolase was proposed (Fig. 1) in which a lipophilic pocket

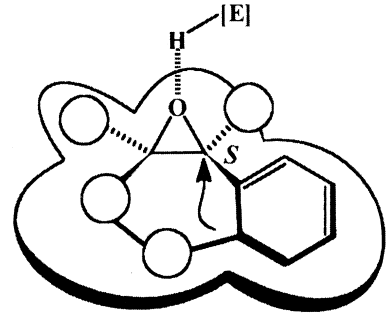
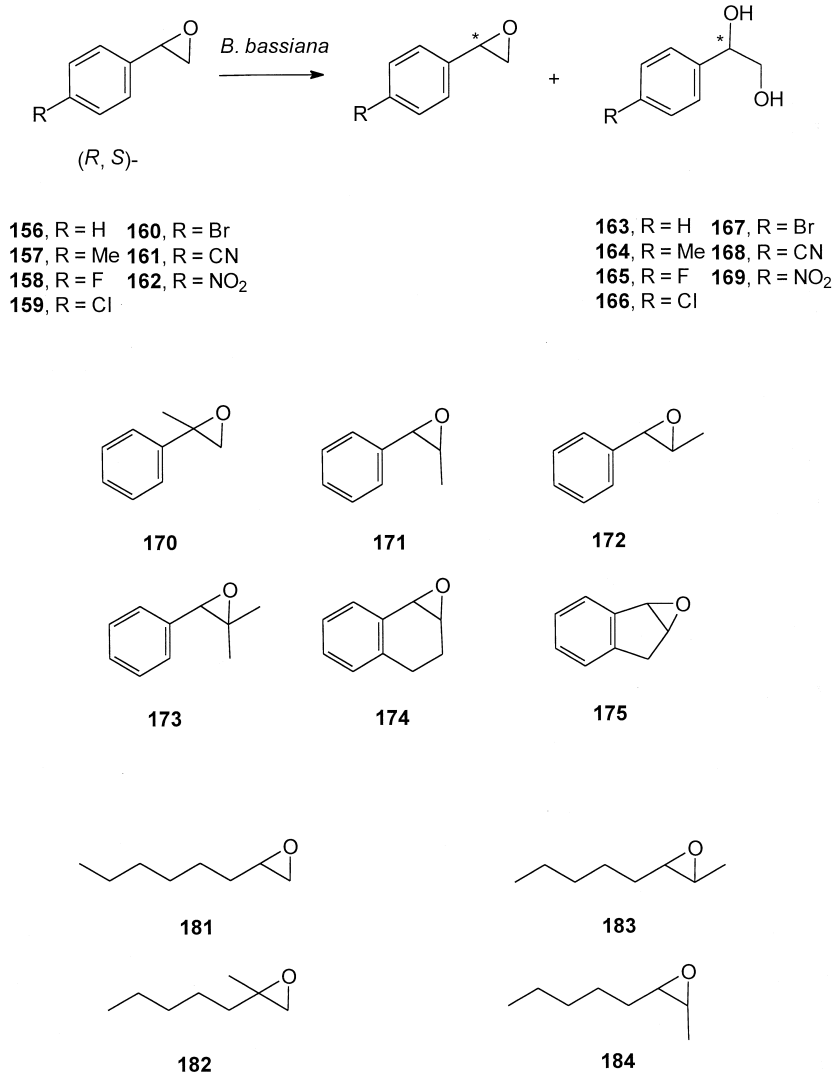


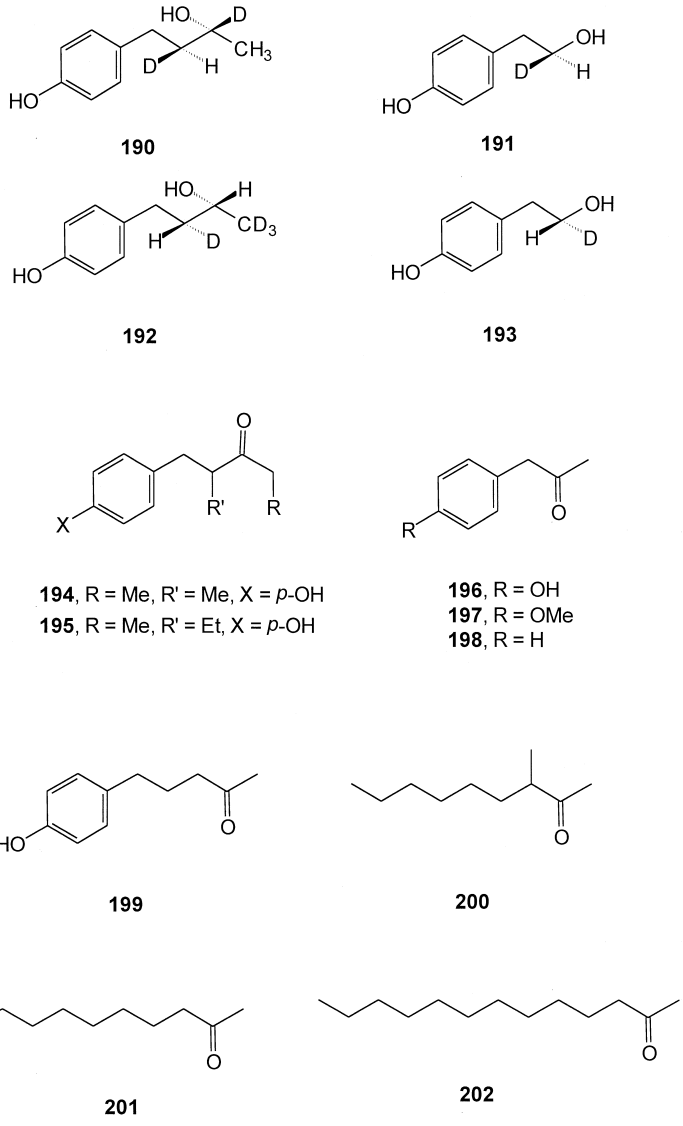
Fig. 1. Active site model proposed for epoxide hydrolase from *B. bassiana* ATCC 7159 [87].



Scheme 17.

exists at the right frontside to accommodate a phenyl moiety, with space available at the left frontside of the model to accommodate β-alkyl substituents and the active site nucleophilic residue beneath the benzylic carbon atom to allow for the observed selectivity [87]. Hydrolysis of α-methyl styrene oxide **170** was the only example not to fit the model. It was suggested that this exception was due to particular steric factors affecting binding in the active site.

Selectivity of the nucleophilic species for the benzylic carbon atom is conserved in *B. densa* CMC 3240. Styrene oxide is hydrolysed with similar selectivity, and restricted access to the benzylic carbon atom by substitution with chlorine at a position *ortho*- to the epoxide substituent results in no detectable hydrolysis [89]. Hydrolysis of *meta*-chlorostyrene oxide resulted in 60% yield of racemic epoxide, but a 10% yield of diol of 21% e.e., suggesting an enantio-



Scheme 18.

convergence of ring opening similar to that observed with *cis*-β-methyl styrene oxide hydrolysis by *B. bassiana* ATCC 7159.

8. Baeyer–Villiger oxidation

The ability of twelve *Beauveria* spp. to carry out Baeyer–Villiger type oxidations was implied by the transformation of progesterone to testosterone by various strains [90]. Although no detailed mechanistic studies were presented, this degradation of methyl ketone to secondary alcohol is strongly indicative of oxygen insertion in a Baeyer–Villiger-type process, followed by ester hydrolysis.

The group of Fuganti have studied the biogeneration and biodegradation of raspberry ketone **185** and related structures in growing cultures of *B. bassiana* ATCC 7159 (Fig. 2) [91]. In studies directed toward the biocatalytic preparation of this compound, incubation of 4-

(4′-hydroxyphenyl)-but-3-en-2-one (*p*-hydroxybenzylideneacetone, **186**) with the fungus resulted at short incubation times in raspberry ketone and 4-(4′-hydroxyphenyl)-butan-2-ol **187** via bioreduction. Further incubation resulted in quantitative transformation to tyrosol, **189**. A mechanism was proposed (Fig. 2) whereby raspberry ketone is converted via an enzymatic Baeyer–Villiger reaction to its acetate ester **188**, which is then hydrolysed to yield tyrosol. Incubation of the putative intermediate ester did indeed result in tyrosol, and deuterium labelling experiments showed that the product was unequivocally derived from raspberry ketone. Retention of configuration at the migrating carbon centre was demonstrated by transformation of chiral deuterated precursors **190** and **192** to **191** and **193** with the fungus. This phenomenon is consistent with a Baeyer–Villiger type reaction in this instance [92].

The structural requirements of this process were investigated with the incubation of raspberry ketone analogues **194**–**199** with *B. bassiana* ATCC 7159 [93,94]. All but **196** were transformed to mixtures of their Baeyer–Villiger products and the saturated carbinol, although the yield of Baeyer–Villiger product for compounds **195** was low, suggesting that when

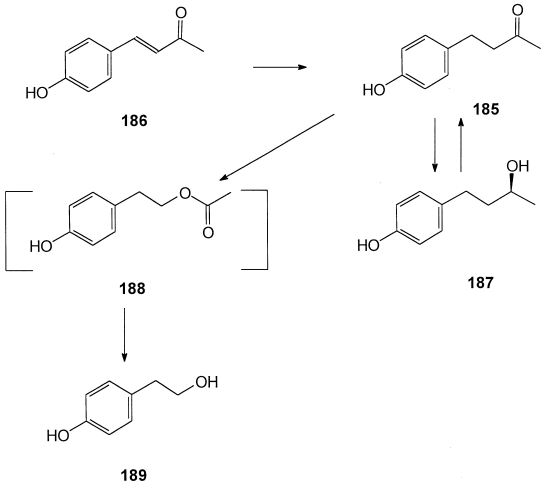


Fig. 2. Biotransformation of *p*-hydroxybenzylideneacetone **186** by *B. bassiana* ATCC 7159 [91].

Table 5
Biotransformation of raspberry ketone **185** and analogues by *B. bassiana* ATCC 7159

Substrate	Saturated carbinol at 72 h (%)	Baeyer–Villiger oxidation product at 72 h (%)
185	2	98
194	1	59
195	7	< 1
196	8	–
197	29	28
198	52	70
199	37	15
200	< 1	–
201	1	–
202	15	–

R = methyl and R′ = Et the substrate was not well tolerated by the oxidative enzyme. Derivatives with one carbon atom less in the chain were not transformed unless the *para*-substituent was methoxy, **197**, or H, **198**. Derivative **199**, with an extra carbon atom between phenyl ring and ketone was also transformed, but no Baeyer–Villiger products resulted from incubation of alkyl ketones **200**–**202** (Table 5).

9. Oxidoreductase activity. Reduction of conjugated double bonds and keto-alcohol conversions

The ability of *Beauveria* to perform the reductions of conjugated carbon–carbon double bonds was first noted by Protiva et al. In transformations of steroids **93**–**96** by *B. globulifera*, saturated analogues of products hydroxylated at the 11β position were also isolated [49]. Later, the group of Veschambre, while investigating the transformation of cyclopentanone derivatives **203** using *B. bassiana* ATCC 7159, noted instead their reduction to chiral cyclopentanones **204** [95]. Significant reduction was not observed when R_2 was an alkyl group or when R_1 was larger than methyl (Table 6). When an analogous series of cyclohexenones **205** was tested, a mixture of saturated ketone **206** and saturated

Table 6
Reduction of cyclopentanone derivatives **203** by *B. bassiana* ATCC 7159

R_1	R_2	Yield cyclopentanone **204**
H	H	90
Me	H	90
Et	H	5
H	Me	0
Me	Me	0

alcohol **207** was obtained, both with high optical purity. The same steric restrictions applied to the reduction of these derivatives as to the cyclopentanone series (Table 7).

An extension of this protocol allowed the synthesis of chiral 2- and 3-deuterated (*S*)-cyclopentanone and cyclohexanone via asymmetric reduction by *B. bassiana* ATCC 7159 [96]. 2-Deuterocyclohexanone also yielded an equal amount of the saturated alcohol. Addition of hydrogen to the double bond was determined to be *trans*-(anti-) with proton addition to the si-face at C-2 and the re-face at C-3. A later report expanded the utility of this transformation to the synthesis of optically active (2*R*,3*S*)-2,3-deuteriocyclohexanones and (2*R*,3*S*)-2-methyl-3-deuteriocyclohexanone via the same mechanism [97].

A series of acyclic analogues **208** was also reduced to varying mixtures of saturated ketone **209** and saturated alcohol **210**, where R_1 = H, Me, Et, R_2 = H, Me, *n*-Pr, and the *trans*-substituent on the β-carbon was not alkyl [95,98]. When R_2 was *n*-Bu or *n*-Pentyl there was no reaction (Table 8). Ketones of type **211** were also transformed, but exclusively to the optically pure saturated ketone **212**. Analogous unsaturated aldehydes **213** were also reduced to mixtures of saturated alcohols **214** and unsaturated alcohols **215** depending on the substitution patterns in the substrate. Only unsaturated alcohol was obtained when R_1 = H or Me; R_2 = Me or H and R_3 = H (Table 9). The saturated alcohols obtained in this way were optically active [99].

In general, the configuration of the carbon α to the carbonyl was (*S*) when R_1 = methyl in the acyclic series and (*R*) in the ethylketone and cycloalkanone series. This difference was rationalised by application of Prelog's rule where, if the carbon–carbon double bond is analogous to the carbonyl, attack of hydrogen occurs from behind or from the front of the molecule if the

Table 7
Reduction of cyclohexenone derivatives **205** by *B. bassiana* ATCC 7159

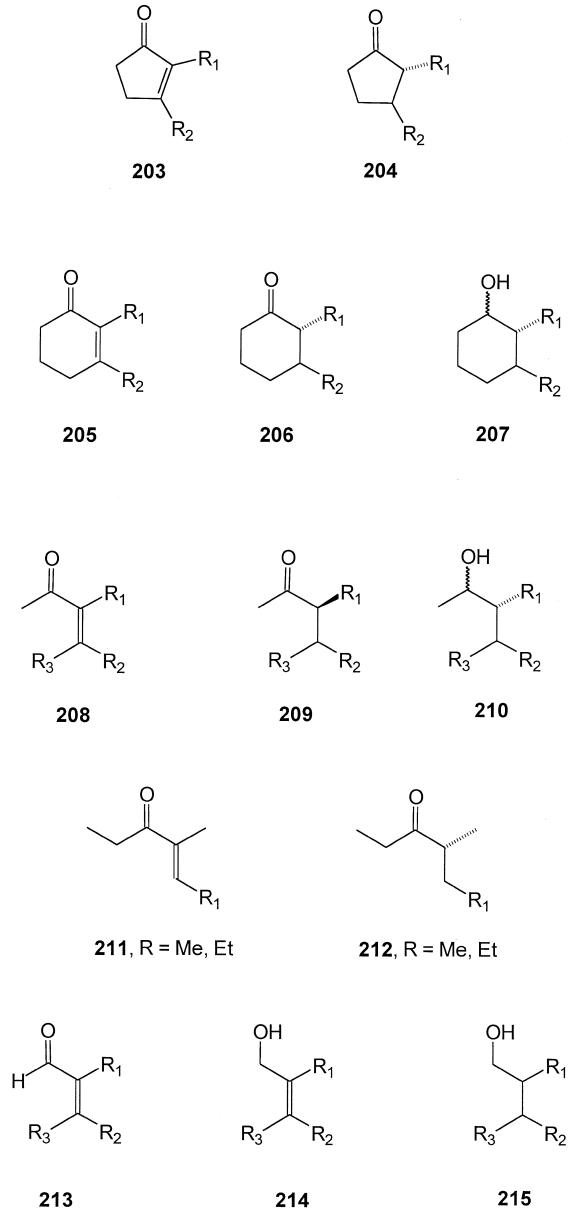
R_1	R_2	Yield saturated ketone **206**	Yield saturated alcohol **207**
H	H	40	45
Me	H	30	55
H	Me	0	0
Me	Me	0	0
Et	H	0	0

Table 8
Reduction of alicyclic unsaturated ketones **208** by *B. bassiana* ATCC 7159

R_1	R_2	R_3	Yield saturated ketone **209**	Yield saturated alcohol **210**
Me	H	H	70	20
H	Me	H	75	15
Me	Me	H	80	10
Et	Me	H	90	0
H	Me	Me	0	0
Me	Me	Me	0	0
n-Bu	Me	H	95	0
Me	Et	H	90	5
Me	*n*-Pr	H	92	3
Me	*n*-Bu	H	0	0
Me	*n*-Pentyl	H	0	0

Table 9
Reduction of unsaturated aldehydes **213** by *B. bassiana* ATCC 7159

R_1	R_2	R_3	Yield unsaturated alcohol **214**	Yield saturated alcohol **215**
H	Me	H	80	0
Me	H	H	68	0
Me	Et	H	31	69
H	*n*-Pr	H	37	63
Me	*n*-Pr	H	58	42
Et	*n*-Pr	H	0	0
H	*n*-Pentyl	H	25	25
H	Me	Me	0	0



Scheme 19.

larger substituent is on the left or the right, respectively (Fig. 3). For methylketones **208**, the methyl group is larger than the acetyl group. For ethylketones **211**, the methyl group is smaller than the propionyl group [98].

Experiments to determine the order of reduction of functionality in these substrates determined that, for acyclic substrates, carbonyl reduction preceded double bond reduction, but that for cyclohexenone substrates, the reverse case was true. For example, the reduction of 5- and 6-methylcyclohexenone showed a gradual increase in accumulation of saturated alcohol over time at the expense of initially generated saturated ketone [99]. These techniques were applied to the syntheses of (2*R*,5*R*)-(−)-2,5-dimethylcyclopentanone **219** and (5*S*)-(+)-1,5-dimethyl-2-cyclopentanone **218** by reduction of racemic 2,5-dimethyl-2-cyclopentanone **217** [100]. If the reaction were stopped at 5 d or 10 d, either optically pure saturated ketone or unsaturated ketone was obtained, respectively.

Reduction of the enantiomers of carvone **216** proceeded with different selectivity, the (+)-isomer yielding a large excess of the saturated alcohol (85%) over the saturated ketone compared to the (−)-isomer which yielded a 60:40 mixture of the respective products. All products were recovered with high optical purity [97].

The mechanism of microbiological reduction of α,β unsaturated alcohols and aldehydes was determined by incubation of 1,1-dideuterio-2-methyl-2-penten-1-ol **220** by *B. bassiana* ATCC 7159 [101]. Mass spectrometry clearly showed that product **223** (Fig. 4) was only monodeuterated and must have arisen via intermediates **221** and **222** and not as a result of direct transformation of the substrate.

It has proved possible to effect the transformation of compounds not previously recognised as substrates for the *B. bassiana* ATCC 7159

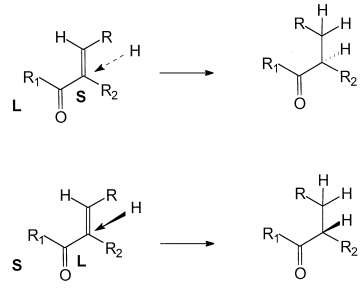


Fig. 3. Prelog's rule as applied to the reduction of methyl ketones (top) and ethyl ketones/cyclic ketones (bottom) by *B. bassiana* ATCC 7159 [98].

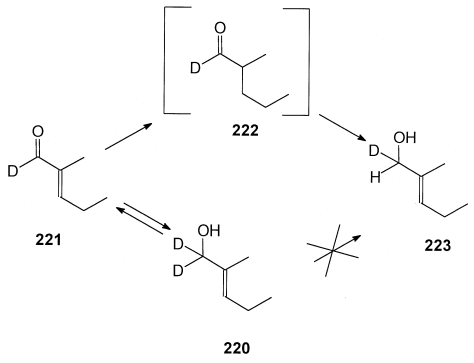


Fig. 4. Pathways for the reduction of 1,1-dideuterio-2-methyl-2-penten-1-ol **220** by *B. bassiana* ATCC 7159 [101].

enone reductase system by using washed cell preparations of the fungus that had been prepared from fungus grown with cyclohex-2-enone as inducer [102]. In this manner, substrates **224**–**226** were transformed, with racemic 2,6-dimethylcyclohex-2-enone **225** yielding resolved (6*S*)-(−)-substrate and optically pure saturated ketone **227** and saturated alcohol **228** as products (Fig. 5). An unspecified strain of *B. bassiana* was reported to reduce the conjugated exocyclic double bond of the sesquiterpene lactone parthenin **229** to metabolite **230** in 37% yield [103].

The ability of *B. bassiana* ATCC 7159 to reduce double bonds in conjugated systems leading to raspberry ketone **185** from 4-(4′-hydroxyphenyl)-but-3-en-2-one **186** has been addressed. This activity was observed to extend to analogues possessing *para*-fluoro, *para*-methoxy or an additional *meta*-methoxy substituent or the ethylketone derivative. In these

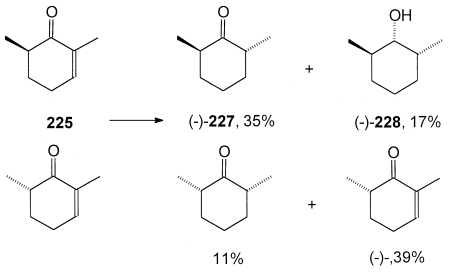
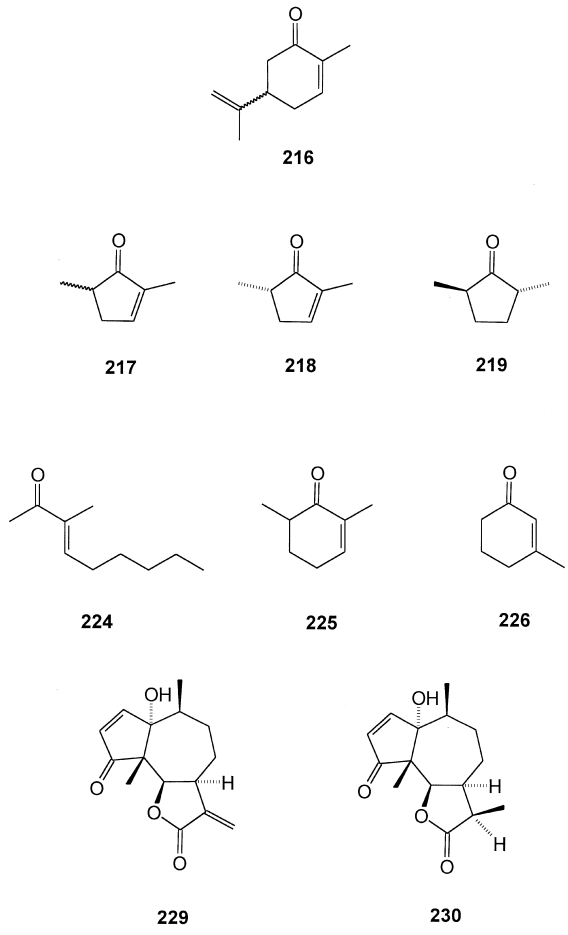


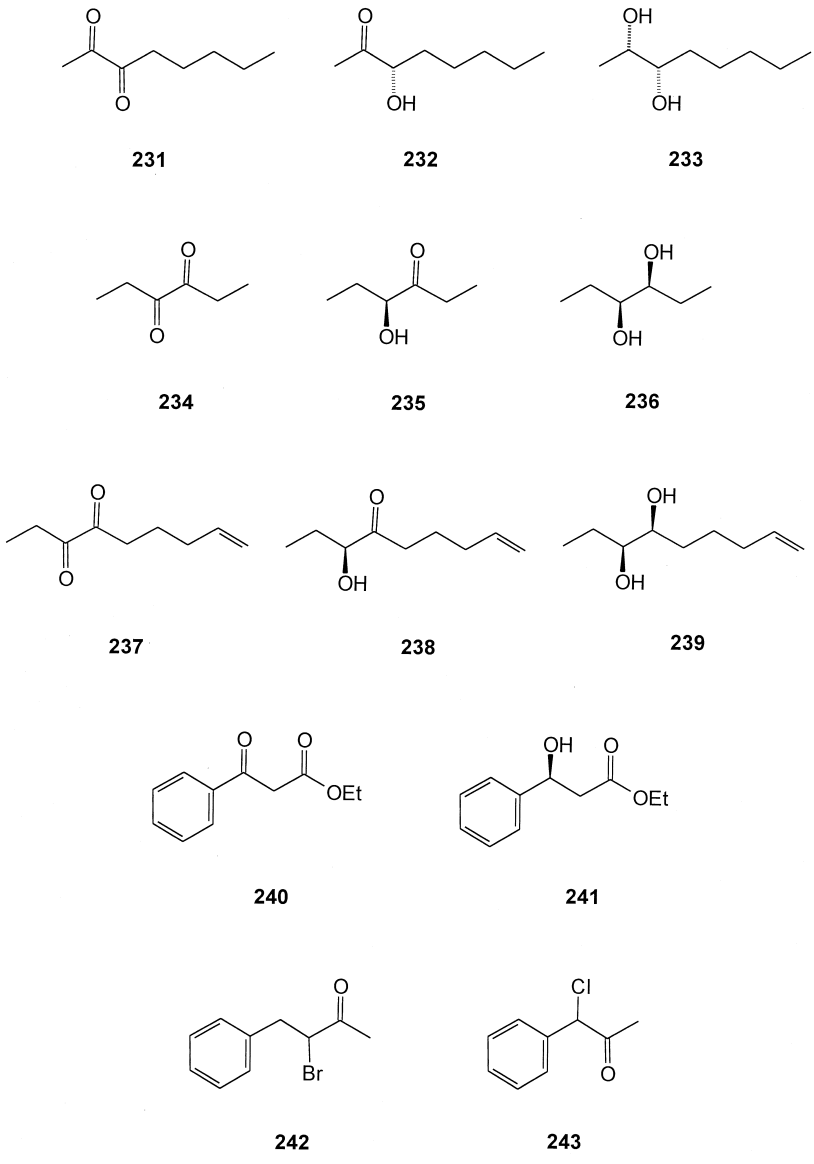
Fig. 5. Reduction of 2,6-dimethylcyclohex-2-enone (**225**) by *B. bassiana* ATCC 7159 [102].



Scheme 20.

cases, large amounts of reduction products, including the corresponding saturated carbinols, were observed after 24 and 48 h, prior to further degradation by the Baeyer–Villiger mechanism previously discussed [94].

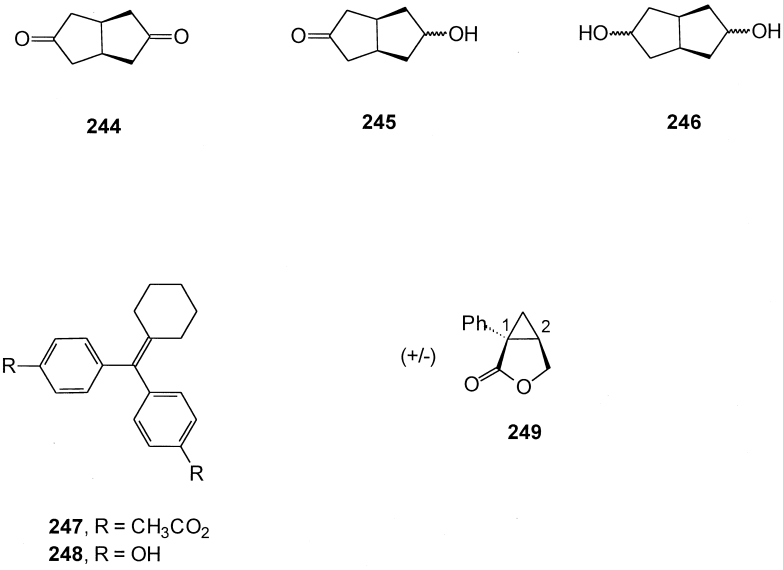
The interconversion of nonconjugated carbonyls and secondary alcohols by *B. bassiana* ATCC 7159 was first noted in studies of hydroxylation of *N*-benzamides such as **6** by the group of Johnson, in which keto-products were often isolated in addition to those such as **7** arising from simple hydroxylation [8]. Subsequently, this activity has found application in synthesis for the production of a pheromone component of *Xylotrechus pyrrhodeus* by reduction of octane-2,3-dione **231** by *B. bassiana*



Scheme 21.

ATCC 7159 via the mono-ol **232** to yield the (2*S*,3*S*)-diol **233** of 99% e.e. [104]. In addition the fungus was shown to transform hexane-3,4-dione **234** to the (*S*)-hydroxyketone **235** in 70% yield and 98% e.e. in 1 h. When left for 48 h, the (3*S*,4*S*)-diol **236** was formed in 20% yield and $>$98% e.e. [105]. The same report details the reduction of non-8-ene-3,4-dione **237**, initially to the (*S*)-hydroxyketone **238** in 75% yield, 48% e.e. in 3 h, which was subsequently transformed to the (*S*,*S*)-diol **239** in 70% yield with $>$98% e.e. This diol was then used in a synthesis of the pheromone *exo*-brevicomin.

Ethyl benzoacetoacetate **240** was reduced by *B. bassiana* ATCC 7159 to give (*S*)-3-hydroxy-3-phenyl propionate **241** in 72% yield with 96%



Scheme 22.

e.e. This chiral ester served as a starting point for a synthesis of enantiomers of both (*R*)-fluoxetine and (*S*)-fluoxetine [106].

The reductive ability of *B. bassiana* has recently been applied to the synthesis of chiral epoxides 4-phenyl-2,3-epoxybutane and 1-phenyl-1,2-epoxypropane from α-halohydrins obtained from reduction of the corresponding haloketones. 4-Phenyl-3-bromo-2-butanone **242** was reduced to a 50:50 mixture of (2*S*,3*S*)-syn-bromohy-drins and (2*S*,3*R*)-anti-bromohydrins with a combined yield of 75% in 95% e.e. and 94% e.e., respectively. Reduction yields of 1-phenyl-1-bromo-2-propanone and its chloro-analogue were poor, although the isomeric 1-phenyl-2-chloropropanone **243** was reduced to a 50:50 mixture of *syn*-halohydrins and *anti*-halohydrins in 43% yield in 85% e.e. and 8% e.e., respectively [107].

Further carbonyl reduction by an unspecified *Beauveria* strain has been observed in studies of the hydroxylation of androst-4-ene-3,17-dione **100** [52]. A recent publication also reports the reduction of *cis*-bicyclo[3.3.0]octane **244** by *B. bassiana* ATCC 7159 to the mono-ol **245** and diol **246** in yields of 8% and 28%, respectively [108].

10. Ester hydrolysis

The stability of ester functionality to hydrolysis by presumably, native lipase and/or esterase systems in *Beauveria* strains, seems to be very dependent on the structure of the ester concerned. It is apparent that the ester moieties present in coronaridine **92** [48] and ethyl benzoacetoacetate **240** [105] are stable to hydrolysis by *B. bassiana* ATCC 7159, but the diester bis-(4-acetoxyphenyl)methylenecyclohexane **247** is rapidly saponified to the diol **248** by the fungus [80].

The preparative enantioselective hydrolysis of the lactone (±)-**249** represents the only example of a synthetic application of any such hydrolytic activity. This lactone was hydrolysed to the hydroxyacid with high selectivity by *B. bassiana* ATCC 7159 (37% yield, 95% e.e. of residual (+)-(1*R*,2*S*) lactone) but unfortu-

nately, yielded the wrong enantiomer for synthesis of the antidepressant Milnacipran® [109].

11. Conclusions

The wide range of catalytic ability demonstrated by *Beauveria* spp. presents the chemist with a powerful synthetic tool, but at a price. It will be apparent from the above discussion that the biotransformation of many substrates by *Beauveria* involves more than one type of reaction, and that multiple products are frequently obtained. In addition, the predictability of substrate-product relationships with the high level of accuracy associated with chemical transformations is not always possible, even for those transformations such as hydroxylation, for which this aspect of the process has received considerable attention.

In spite of these shortcomings, however, biocatalysts of the *Beauveria* spp. possess considerable potential, and, with careful selection of substrate substitution pattern and functional group derivatives, can make significant contributions to synthetic chemistry.

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