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A New Paradigm for Biohydroxylation by *Beauveria bassiana* ATCC 7159

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Abstract: The biohydroxylation of a series of amides and related amino, keto and hydrocarbon substrates by the fungal biocatalyst *Beauveria bassiana* ATCC 7159 has been examined. The product distributions, together with data obtained from selective inhibition experiments using the cyt.P-450 inhibitors isosafrole, 1-aminobenzotriazole and phenylacetylene, suggest that *B. bassiana* contains a range of hydroxylase enzymes with different substrate specificities. A paradigm is presented for the interpretation of the results of microbial hydroxylation and for the application of existing active site models for *B. bassiana*.

Keywords: Amides, Enzymes and enzyme reactions, Fungi, Hydroxylation

INTRODUCTION

The fungus *Beauveria bassiana* ATCC 7159 is one of the most frequently used and versatile whole-cell biocatalysts. Over 100 papers in the open literature deal with the use of this fungus for the biotransformation of over 300 different substrates, involving a wide range of reactions such as hydroxylations of saturated[1] and aromatic[2] carbon, keto-alcohol redox reactions,[3] alkene reduction[4] and oxidation,[5] sulfide oxidation,[6] Baeyer-Villiger oxidations,[7] conjugation reactions,[8] the hydrolysis of epoxides[9] and esters,[10] heteroatom dealkylation,[11] and the reduction of peroxides to alcohols.[11]

The most widely reported biocatalytic reaction of *B. bassiana* is hydroxylation at saturated carbon; this was also the first of these processes to be systematically investigated in a now-classic series of papers by the Upjohn research group, starting in 1967[12] and continuing though to 1997.[13] This group proposed an "active site model" (Figure 1) to account for the substrate/product relationships observed for hydroxylations by *B. bassiana* (then known as *Sporotrichum sulfurescens*) in which hydroxylation occurred preferentially at a distance of 5.5Å from an electron-rich binding site of the substrate.[12] The model was initially proposed to account for the

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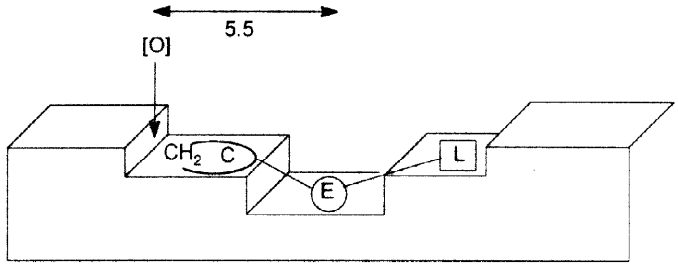
regiochemistry of hydroxylation of cyclododecanol and other monocyclic alcohols, but was later used to rationalise the regiochemistry of the hydroxylation of amides.[14]

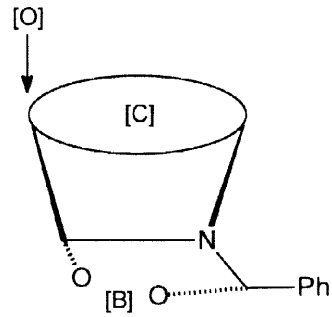
Figure 1. Original model for hydroxylation of monocyclic alcohols and amides by *Beauveria bassiana*.[12]



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

In the latter mode, the model was extended by Furstoss and co-workers (Figure 2) to include a representation of the absolute stereochemistry of the hydroxylation of cyclic amide substrates,[15] and modified to allow for variations in the orientation of substrate binding such that hydroxylation can occur at distances varying from 3.3 to 6.2Å from a substrate binding site.[16,17]

Figure 2. Modified model for hydroxylation of cyclic amides by *Beauveria bassiana*.[15]



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

The regio- and stereochemistry of the hydroxylations of amides by *B. bassiana* ATCC 7159 have also been subjected to analysis using an octant system to define forbidden and allowed regions for the orientation of substrate and product molecules,[14,15] with results that support the models presented in Figures 1 and 2.

A recent analysis of *B. bassiana*-catalysed hydroxylations by Haufe *et al.*[18] largely confirmed the validity of the original model when applied to both rigid and flexible carbamate derivatives of mono- 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 5.5Å 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*-catalysed 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.[1]

A necessary assumption for the accurate application of any "active site model" for prediction of the outcome of microbial hydroxylation of any hitherto untried substrate is that the reaction is catalysed either by a single enzyme or by a series of closely related isozymes. However, in spite of over three decades of work aimed at predicting the substrate-product relationships inherent in *B. bassiana*-catalysed hydroxylations, this aspect of the problem has never been specifically addressed. The implicit assumption that *B. bassiana* contains a single hydroxylase, responsible only for the hydroxylation of saturated carbon atoms of amide and related substrates, is clearly invalid. This biocatalyst is known to be capable of hydroxylation at saturated carbon atoms of substrates with no amide-analogous binding group, such as hydrocarbons,[19,20] steroids,[21] terpenes,[11,22-24] amines,[25] and alkaloids;[26] of hydroxylation at benzylic carbon atoms of alkylbenzenes,[19,27] and alkylpyridines;[28] and of hydroxylation at allylic positions of conjugated ketones.[29,30] It is also able to convert a wide range of aromatic substrates such as anisole,[31] warfarin,[32] propham,[17] and pyrimidine heterocycles[33] to phenolic products by hydroxylation at aryl carbon atoms.

In view of the wide range of hydroxylase activity exhibited by *B. bassiana*, the application of any model for the prediction of the outcome of hydroxylation of a new substrate by this biocatalyst is fraught with uncertainty. It is unclear, for example, whether the enzyme(s) responsible for the hydroxylation of amides are the same as that (those) responsible for the hydroxylation of hydrocarbons and/or arenes, and to what extent different enzyme activities, if present, may overlap. The model discussed above was initially proposed only for the hydroxylation of cyclic alcohols, but is now routinely applied to amides and related substrates, and its applicability to the hydroxylation of dialkyl benzenes has been tentatively suggested.[19]

In order to systematically examine the possible role of substrate functionality in determining the outcome of hydroxylation by *B. bassiana*, and in an attempt to delineate those substrates for which a predictive application of the models of Figures 1 and 2 might be appropriate, we have undertaken a study of the biotransformations by *B. bassiana* ATCC 7159 of four series of related substrates (Figures 3 - 6). The substrates were chosen so that,

within each series, a comparison could be made (within the limits of reasonable synthetic feasibility) of the effects on the outcome of the biotransformation of a systematic variation of the substrates by isosteric replacement of nitrogen by carbon, and of removal of carbonyl oxygen.

We have also examined the possibility of multiple hydroxylase activity in *B. bassiana* by a study of the biotransformation of one substrate (**IV-6**, Figure 6) that gave a series of products apparently arising from multiple enzyme activities, using a range of inhibitors known to be effective against cytochrome P-450-dependent hydroxylases. The P-450 inhibitor 1-aminobenzotriazole has been reported to prevent hydroxylation of a substituted pyrimidine ring by *B. bassiana* at high concentration,[33] but no selective inhibition studies of the hydroxylase activity of *B. bassiana* have been reported. Although mammalian cyt.P-450 isozymes are well documented, no data are currently available on the existence or activity of cyt. P-450 isozymes from filamentous fungi such as *B. bassiana*.[34]

RESULTS

The biotransformations outlined in Figures 3 - 6 were conducted by the standard protocol for hydroxylations using *B. bassiana*.[35] The reported yields refer to isolated, purified material; in the case of low yield biotransformations, mass balance was accounted for by the isolation of unreacted substrate. Product structures were determined by spectral analysis, ^{13}C NMR being particularly diagnostic of the site and relative stereochemistry of hydroxylation. The relative stereochemistry of the substituted cyclohexyl products **I-3a/I-3b, I-5a, II-1a, II-4a, III-3b**, and **IV-2a/IV-2b** was apparent from analysis of ^{13}C NMR chemical shifts of the cyclohexyl carbons, using published chemical shift parameters for the introduction of axial or equatorial hydroxyl into a substituted cyclohexane ring.[36] This is illustrated for the product **IV-2a** in Table 1, which presents the calculated and observed chemical shifts for both *cis* and *trans* isomers of **IV-2a**; the product is clearly the former.

Table 1. Calculated and observed ^{13}C NMR chemical shifts for the cyclohexyl carbons of **IV-2a**

carbon	calculated for *trans*-**IV-2a**	calculated for *cis*-**IV2a**	found
C-1	70.1	65.3	70.7
C-2	35.3	32.4	36.1
C-3	31.7	27.0	32.5
C-4	43.6	44.5	43.9

The absolute stereochemistry of the carbinol products **III-1a/III-3a**, **III-2a/III-4a**, and **III-3b** was determined

by a combination of optical rotation data and ^{1}H NMR analysis in the presence of *tris*-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium (III). The latter shift reagent gives configurationally-dependent chemical shift separations which, for a wide range of substituted 1-phenylethanols, result in the signal from the CHOH methine hydrogen of the (*R*) enantiomer appearing at lower field, and the signal from the *ortho*-aromatic hydrogens appearing at higher field, than the corresponding signals from the (*S*) enantiomer.[37,38] The absolute stereochemistry of 4-biphenylethanol **IV-8a** was determined by comparison of the above data with those from an authentic sample of the (*R*) isomer.[37]

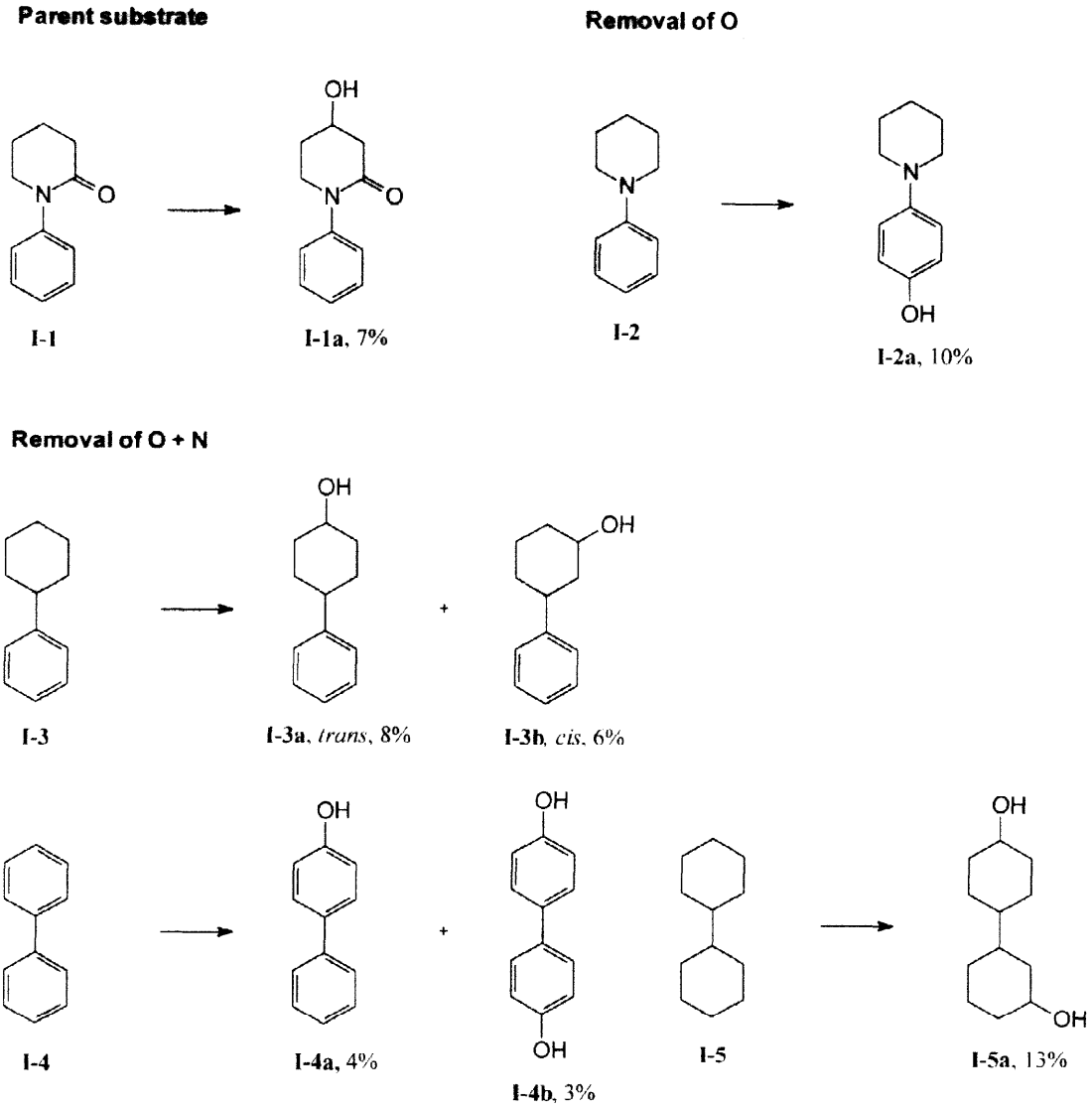
The distribution of products arising from the biotransformation of substrate **IV-6**, Figure 6, was monitored by HPLC analysis of products **IV-2**, **IV-6c** and **IV-6d** from biotransformations of **IV-6** conducted in the presence of varying concentrations of cytochrome P-450-dependant hydroxylase inhibitors.[39] These data are presented in Table 2. As shown in Figure 6, product **IV-6a** was converted to **IV-6c** by *B. bassiana*, and is clearly a precursor of product **IV-6c** in the biotransformation of **IV-6**. Similarly (but not shown), when diol **IV-6d** was used as a substrate it was converted in low isolated yield (23%) to ketone **IV-2**. The concentration of **IV-6a** in extracts from the biotransformation of **IV-6** in the presence of the inhibitors listed in Table 2 was very low, (<5% of the total product), and for these reasons **IV-6a** is not included in the product ratios listed in Table 2.

Table 2. Product distribution from the biotransformation of **IV-6** by *B. bassiana*

Inhibitor	Concentration	% Total Inhibition	Product Ratios*		
			IV-2	**IV-6c**	**IV-6d**
none		zero	27	32	41
carbon monoxide	15%	85	33	30	37
isosafrole	100μM	zero	25	30	45
	200μM	30	24	20	56
	500μM	40	16	16	68
	750μM	60	14	17	68
	1mM	75	12	16	72
1-aminobenzotriazole	100μM	zero	30	32	38
	200μM	43	25	40	35
	750μM	55	22	50	28
	1mM	60	17	57	26
phenylacetylene	100μM	zero	28	33	40
	1mM	10	38	23	39
	10mM	34	36	18	46
phenelzine	100μM	zero	30	35	35
	1mM	32	27	30	43
metyrapone	100μM	zero	30	34	36
	1mM	zero	28	32	40
	10mM	53	27	30	43

* molar percentage, ±4%

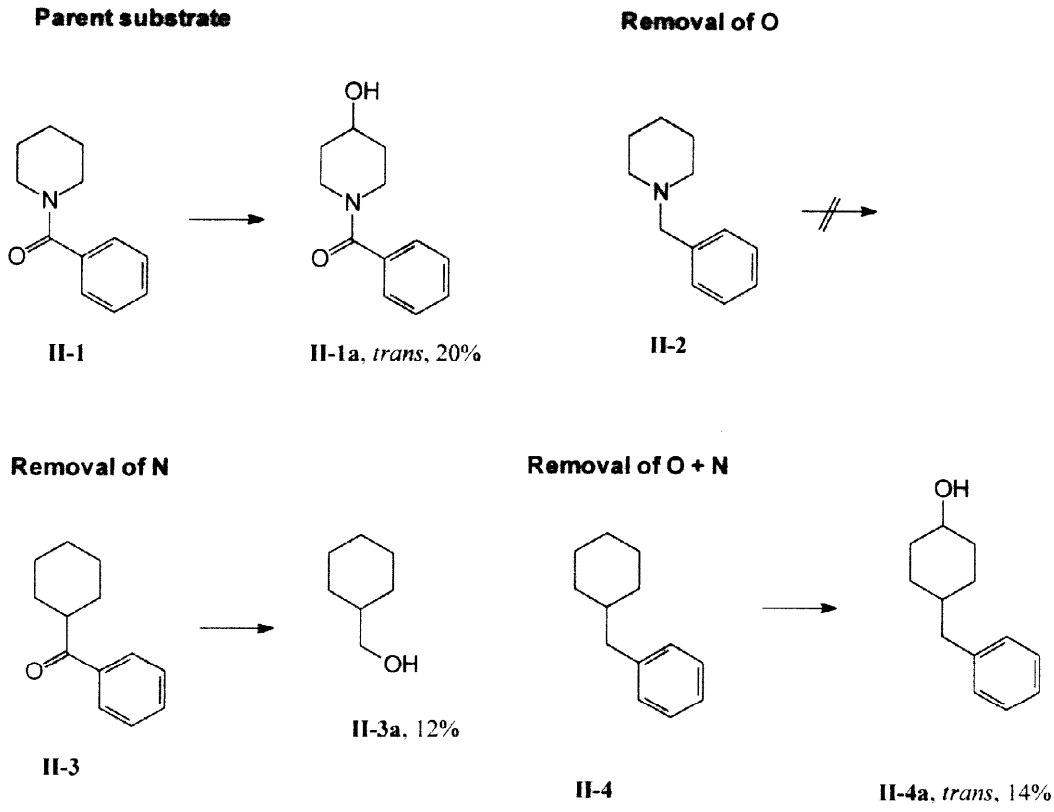
Figure 3. Substrates and Biotransformation Products from Series I



DISCUSSION

The biotransformations of Series I, presented in Figure 3, suggest that hydroxylation at C-4 of the amide **I-1** is not dependant on the presence of the amide group: substrates **I-3**, **I-4**, and **I-5** are hydroxylated with similar yields in similar regions of the molecule. The hydroxylation of **I-2** at the *para*-aromatic carbon to give **I-2a** can be accounted for by the activation of this position towards electrophilic attack: a survey of the literature of *B. bassiana*-catalysed hydroxylations of aromatic substrates suggests that similarly activated *para* aromatic carbons are frequently hydroxylated during biotransformation.[40] The Series II substrates of Figure 4 present a similar picture: removal of both oxygen and nitrogen atoms of the amide **II-1**, represented by substrate **II-4**, had no influence on the outcome of the hydroxylation reaction. The amino substrate **II-2** was not subject to

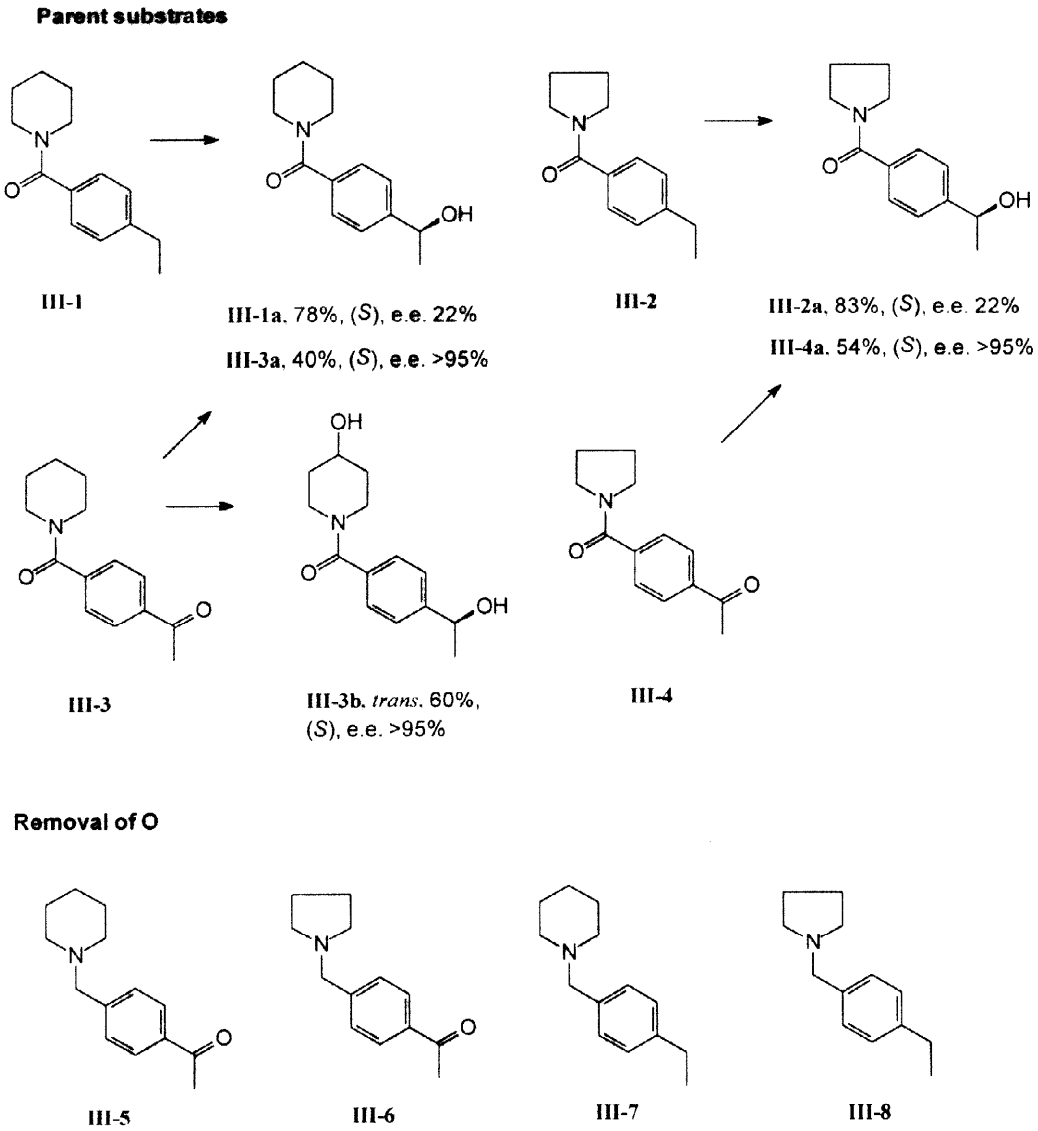
Figure 4. Substrates and Biotransformation Products from Series II



biotransformation, while the ketone **II-3** gave cyclohexylmethanol as the only detectable product, presumably *via* Baeyer-Villiger oxidation, ester hydroylsis and reduction of the resulting carboxylic acid, all of which are known enzymic activities of *B. bassiana*.[40] The substrates of Series III (Figure 5) present a comparison between the parent amides **III-1** - **III-4**, and corresponding substrates without carbonyl groups. The amines **III-5** to **III-8** were recovered unchanged from incubation with *B. bassiana*, but amide substrates **III-1** and **III-3** underwent different modes of hydroxylation, the former being converted only to the benzylic alcohol **III-1a**, while the latter was hydroxylated only at C-4 of the piperidine ring, concomitant with carbonyl reduction, giving **III-b**. Substrate **III-3** was also converted to the alcohol **III-3a** by simple reduction of the keto group. While reduction represented the only mode of biotransformation for the pyrrolidine derivative **III-4**, amide **III-2** with an available benzylic methylene position was converted to the benzylic alcohol **III-2a** in high yield.

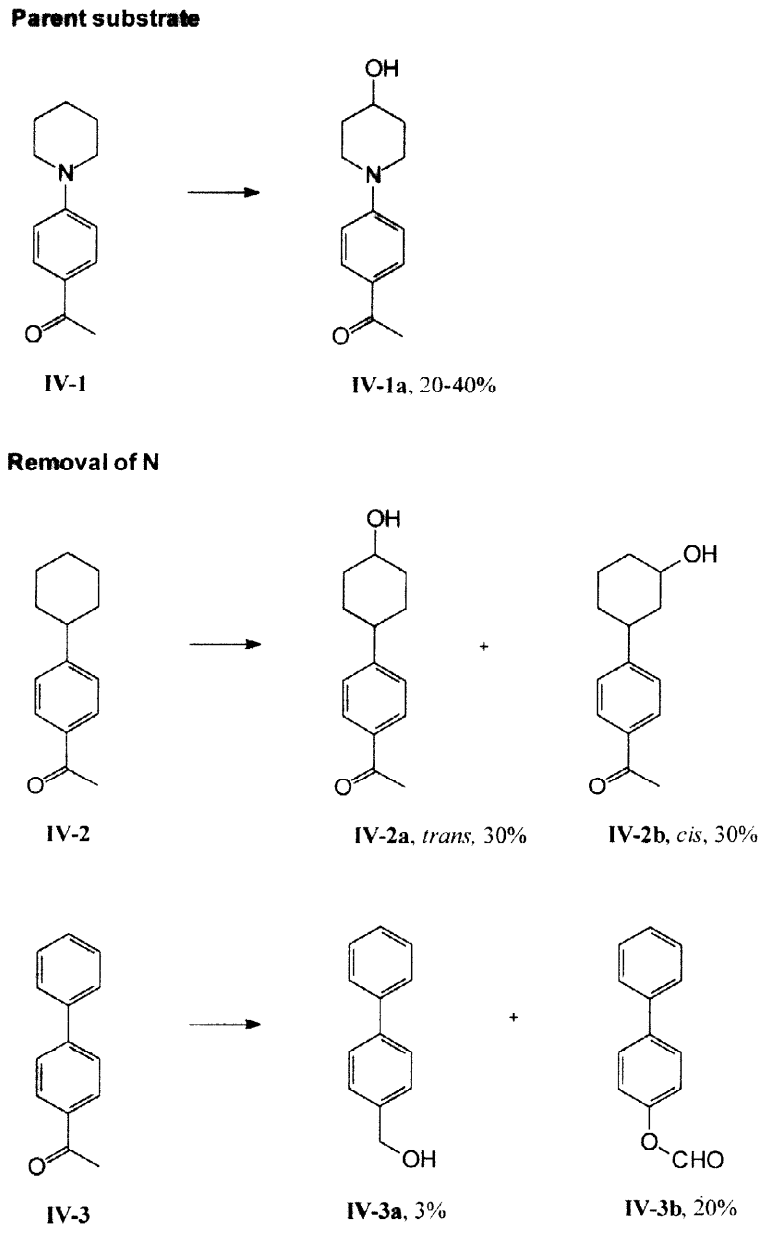
The substrates of Series IV, Figure 6, presented a complex pattern of biotransformation products resulting from various enzyme activities. The parent vinylogous amide, **IV-1**, is one of the classic substrates for *B. bassiana*-catalysed hydroxylation, giving the alcohol **IV-1a** as the sole biotransformation product.[14,35,41] The analogous

Figure 5. Substrates and Biotransformation Products from Series III



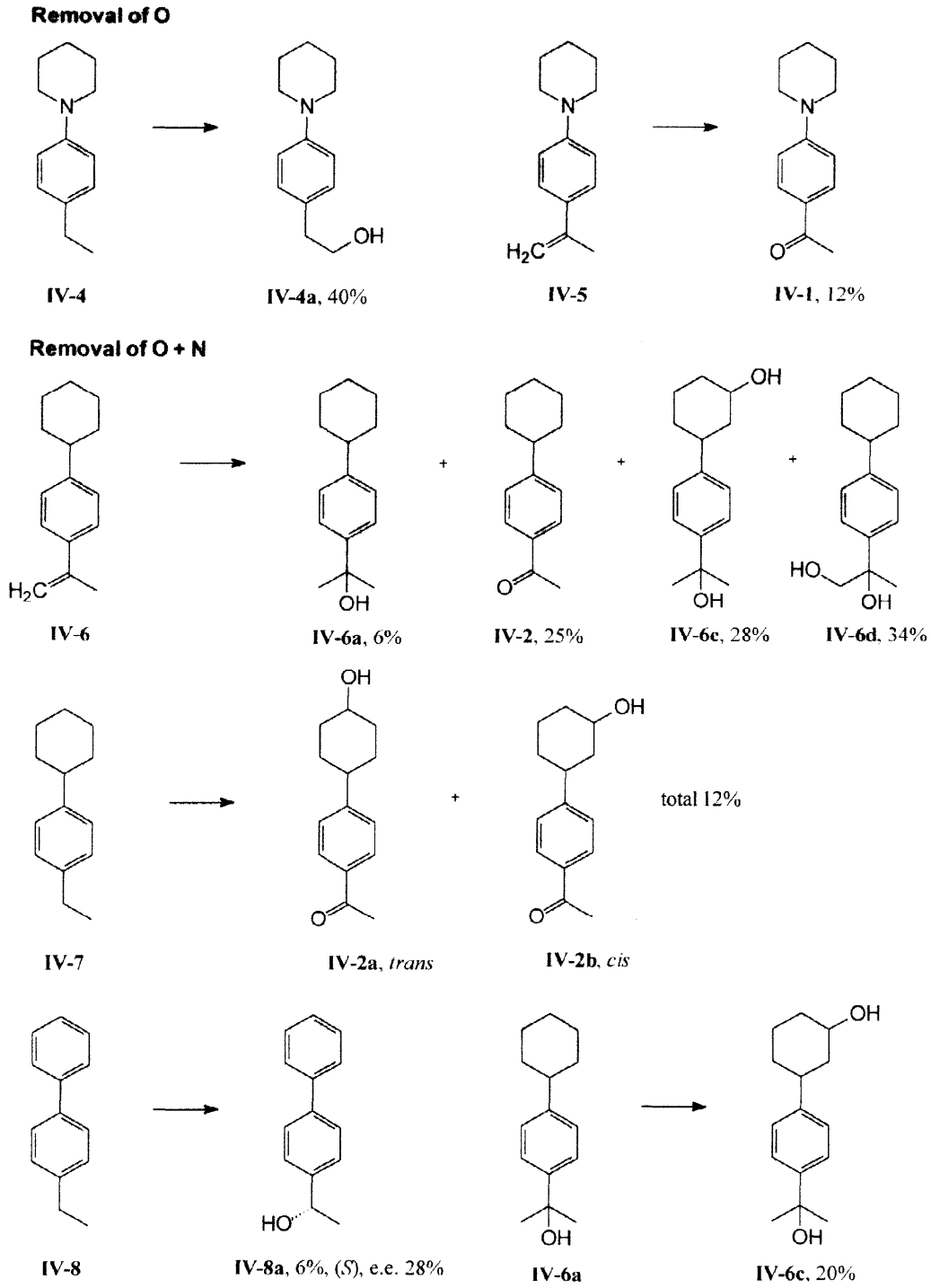
ketone, **IV-2**, was hydroxylated in a parallel manner to give **IV-2a**, but with reduced regiospecificity, as isomer **IV-2b** was also obtained. The corresponding biphenylketone **IV-3** was subjected to Baeyer-Villiger oxidation, giving the alcohol **IV-3a** and its formate, **IV-3b**, as the sole biotransformation products. Biotransformations of the amines **IV-4** and **IV-5** gave the alcohol **IV-4a** and the vinylogous amide **IV-1**, respectively. The formation of the primary alcohol **IV-4a** was unexpected: no hydroxylation of this substrate at the benzylic position was observed. The conversion of **IV-5** to **IV-1** was presumably the result of oxidation at the alkene followed by hydrolysis of the resulting epoxide, both known activities of *B. bassiana*,[5,9] followed by oxidation degradation of the resulting vicinal diol. A similar mode of biotransformation was observed for the alkene **IV-6**, which also gave tertiary alcohol **IV-6a** in low yield. The latter may arise from reduction opening of an epoxide, or by

Figure 6. Substrates and Biotransformation Products from Series IV



direct hydration of **IV-6**: both represent little-known pathways for biotransformation by *B. bassiana.* The formation of the diol **IV-6c** by hydroxylation of alcohol **IV-6a** parallels that of **IV-2b** from **IV-2**. It is noteworthy that substrate **IV-7** is only hydroxylated to similar products concomitant with hydroxylation and oxidation at the benzylic position; the latter position of the biphenyl **IV-8** is also susceptible to hydroxylation, giving the alcohol **IV-8a** in low yield.

Figure 6 (continued). Substrates and Biotransformation Products from Series IV



The range of products obtained from **IV-6**, and the disparate oxidative enzyme activities from which they may arise, led us to study of the biotransformation of this substrate in the presence of a series of cytochrome P-450-dependent hydroxylase inhibitors (Table 2). The classic inhibitor carbon monoxide was effective in reducing

the amount of oxidised product, but was not instrumental in changing the product ratio. However, both isosafrole and 1-aminobenzotriazole produced a concentration-dependent shift in product ratio, the former inhibitor favouring the relative accumulation of product **IV-6d** at the expense of both **IV-2** and **IV-6c**, while the latter led to an increase in the relative amount of **IV-6c** at the expense of **IV-2**. Other inhibitors were effective only at higher concentrations, and of these only phenylacetylene produced a significant change in product ratio, suppressing the formation of the diol **IV-6c**. The formation of **IV-6c** involves hydroxylation of **IV-6a**, and **IV-2** is produced from **IV-6d** by oxidative C-C bond cleavage. Both these processes are known activities of cytochrome P-450 dependent oxygenase enzymes.[42]

As a consequence of the intractable nature of membrane-bound hydroxylase enzymes,[39] a change in ratio of two or more hydroxylation products in response to a cyt.P-450 inhibitor in the biotransformation of a single substrate has been used as indirect evidence for the existence of two or more hydroxylase enzymes in a biocatalyst, that operate with different regio- or stereoselectivites.[43,44] The changes in product ratios discussed above are consistent with a scenario involving hydroxylase enzymes specific for (a) the oxidation of benzylic positions, the primary mode of attack of substrate **IV-6** and other substrates with an available benzylic site; and (b) hydroxylation of non-activated methylene positions, producing **IV-6c** and analogous products.

As a condition for the application of the models presented in Figures 1 and 2, the regiochemistry of hydroxylation must be directed by an existing substituent of the substrate. This mode of reaction is apparently observed for the conversions **I-5** → **I-5a** (for the introduction of the second OH group), **II-1** → **II-1a**, **III-3** → **III-3b**, **IV-1** → **IV-1a**, **IV-2** → **IV-2a** and **IV-2b**, **IV-4** →**IV-4a**, and **IV-6a** → **IV-6c**. However, a directing group is not a necessary requirement for methylene hydroxylation by *B. bassiana*: the hydroxylations of the unactivated substrates **I-3**, **I-4**, **I-5** (for the initial hydroxylation), and **II-4**, with product regiochemistries analogous to those observed for substrates that may possess a directing influence on the site of hydroxylation, illustrate that this influence is not a condition for the reaction to proceed in a regiochemically defined manner.

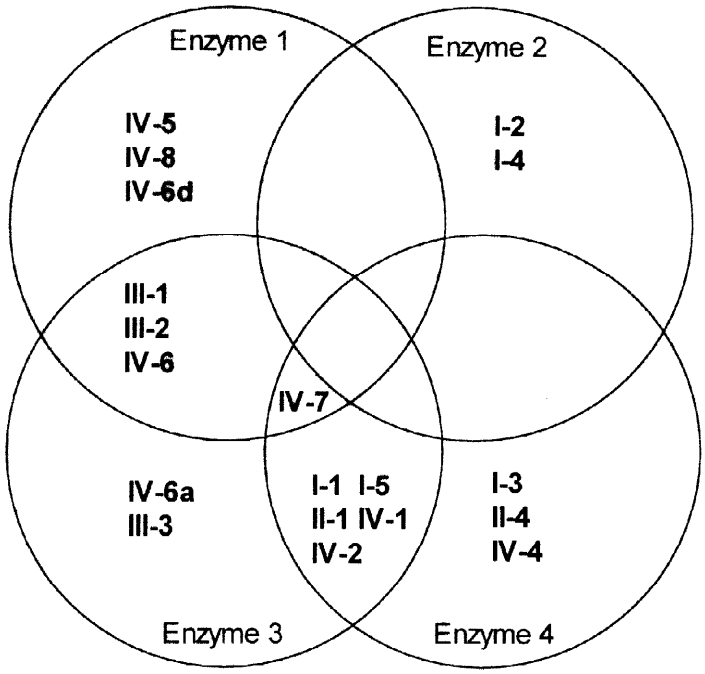
These observations, together with the selective inhibition experiments discussed above, lead us to suggest that *B. bassiana* may exhibit up to four distinct hydroxylase enzyme activities, defined as:

1. A hydroxylase with selectivity for hydroxylation or epoxidation at benzylic positions
2. An arene hydroxylase with selectivity for the *para* position of activated aromatic rings
3. A hydroxylase whose regioselectivity is susceptible to direction by existing functionality in the substrate
4. A hydroxylase with activity for unactivated hydrocarbon substrates

This group of enzymes may act in a competitive manner, or products may result from the activity of a single enzyme. This choice is dependent on substrate structure and enzyme specificity, and can be analysed by an approach analogous to the Venn diagram of symbolic logic illustrated in Figure 7 for the substrates of this

study, in which each enzyme activity is represented by a circle, and regions of overlap represent overlapping substrate specificity.

Figure 7. Analysis of the hydroxylase enzyme activities of *B. bassiana*



According to this proposal only biotransformations of those substrates that fall within the realm of action of enzyme 3 are susceptible to analysis by the active site models of Figures 1 and 2, and only then for the products that arise uniquely from the action of this enzyme. Biotransformations of several of the substrates used in this study cannot be unambiguously assigned solely to the action of a directed hydroxylase, enzyme 3, but give some products (e.g. **IV-6c** from **IV-6**) which may arise in this way. This analysis is therefore valuable in defining those substrates to which the previously published active site models for hydroxylation by *B. bassiana* should be applied, but does not attempt to replace those models. It allows for the existence other, distinct, hydroxylase enzyme activities in *B. bassiana* (benzylic hydroxylase, arene hydroxylase, and non-activated hydrocarbon hydroxylase) for which the exisiting models may be inappropriate, and considers the possibility of substrates being processed by more than one of the different hydroxylases present in *B. bassiana*.

Beauveria bassiana is a powerful and useful biocatalyst. Its application for the hydroxylation of a wide range of substrates presents the organic chemist with a powerful tool, under-utilised because of a lack of comprehensive understanding of its limitations and possibilities. The present study goes some way towards addressing these problems, but still leaves much work to be done before a complete understanding of the hydroxylation capabilities of *B. bassiana* can be achieved.

EXPERIMENTAL

Materials and Methods: Melting points were determined on a Kofler hot stage and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance series 300 spectrometer in $CDCl_3$ using residual $CHCl_3$ as the internal standard, chemical shifts are reported in p.p.m (δ) and the signals quoted as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The ^{13}C NMR spectra were recorded at 75 mHz in $CDCl_3$ solution on the above spectrometer. Mass spectra were obtained in EI mode (unless otherwise stated) using a Kratos 1S spectrometer. IR spectra were obtained using a Mattson Research Series FTIR spectrometer. Optical rotations were recorded at ambient temperature in the stated solvent using a Rudolph Autopol 3 polarimeter. Enantiomeric excess (e.e.) was determined by ^{1}H NMR analysis in the presence of *tris*-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium (III). TLC was performed on Merck silica gel F_{254} plates, 0.2 mm, and column chromatography used Merck silica gel, 230-400 mesh. HPLC analysis was performed on a Perkin Elmer Series 3 instrument using a μ-Bondapak C_{18} column, 3.9 x 300 mm, and acetonitrile:water, 1:1, 1.0 mL min^{-1}, as the mobile phase, with UV detection at 254 nm. *Beauveria bassiana* ATCC 7159 was maintained on Sabouraud dextrose agar slopes, grown at 26°C and stored at 4°C.

Preparation of Substrates: Compounds **I-2 - I-5**, **II-1**, **II-3**, **II-4**, **IV-1 - IV-3**, and **IV-8** were commercial samples. 1-Phenyl-2-piperidone (**I-1**) was prepared as described.[45]

1-Benzylpiperidine (**II-2**). Benzyl chloride (7.3 g, 0.06 mole) was added to a solution of piperidine (5 g, 0.06 mole) in acetonitrile (50 mL). Dry potassium carbonate (16 g, 0.12 mole) was then added, and the mixture stirred and refluxed for 1 h. The reaction was cooled, the precipitate removed by filtration and washed with acetonitrile, and the filtrate and washings evaporated to give 9.5 g (93%) of pale oil, sufficiently pure for use without further purification. ^{1}H NMR δ 1.4-1.6 (2H, m), 1.6-1.7 (4H, m), 2.3-2.5 (4H, m), 3.5 (2H, s), and 7.3-7.5 (5H, m) ppm; ^{13}C NMR δ 22.7, 24.3, 52.8, 62.2, 125.1, 126.3, 127.5, and 137.0 ppm; MS m/z(%) 175(80), 174(90), 98(68), 91(100), 84(47), M$^+$ 175.13605, calcd. ($C_{12}H_{17}N$) 175.13610; IR υ_{max} 2750-3000, 1500 cm^{-1}.

1-(p-Ethylbenzoyl)piperidine (**III-1**). Piperidine (1.7 mL, 0.017 mole) was mixed with aqueous sodium hydroxide (29 mL, 10%). The mixture was vigorously stirred and 4-ethylbenzoyl chloride (2.8 g, 0.02 mole) added in small portions. Stirring was continued for an additional 5 min, and the mixture then extracted with ether (3 x 30 mL), and the extract washed (5% HCl and water), dried and evaporated to give 3.5 g (96%) of a clear oil, sufficiently pure for use without further purification. ^{1}H NMR δ 1.3 (3H, t), 1.4-1.8 (6H, m), 2.7(2H, q), 3.2-3.5 (2H, t), 3.5-3.9 (2H, t), 7.2 (2H, d), and 7.3 (2H, d) ppm; ^{13}C NMR δ 15.7, 24.9, 26.5, 29.0, 43.6, 49.0, 127.3, 128.1, 134.1, 145.9, and 170.8 ppm; MS m/z(%) 217(44), 216(100), 134(10), 133(87), 77(9), M$^+$ 217.14608, calcd. ($C_{14}H_{19}NO$) 217.14666; IR υ_{max} 2800-3000, 1650 cm^{-1}.

1-(p-Ethylbenzoyl)pyrrolidine (**III-2**). Prepared as described above from pyrrolidine (1.4 mL, 0.017 mole) and 4-ethylbenzoyl chloride (2.8 g, 0.02 mole) to give 2.8 g (80%) of product, m.p. 85-86°C; ^{1}H NMR δ 1.3 (3H, t), 1.9-2.1 (4H, m), 2.7 (2H, q), 3.5 (2H, t), 3.7 (2H, t), 7.2 (2H, d) and 7.5 (2H, d) ppm; ^{13}C NMR δ 15.4, 24.5, 26.4, 28.7, 46.1, 49.6, 127.3, 127.6, 134.6, 146.2, and 169.8 ppm; MS m/z(%) 203(47), 202(20), 146(12), 134(11), 133(100), M$^+$ 203.13095, calcd. ($C_{13}H_{17}NO$) 203.13101; IR υ_{max} 2800-3000, 1630 cm^{-1}.

1-(p-Acetylbenzoyl)piperidine (**III-3**). A solution of p-acetylbenzoyl chloride (4.7 g, 0.026 mole) in dry THF (20 mL) was added slowly under an argon atmosphere to a stirred solution of piperidine (8.85 g, 0.104 mole) in dry THF (50 mL). The reaction mixture was then heated to reflux for 1 h, the evaporated and the residue dissolved in ether and the ethereal solution washed (5% HCl, 10% NaOH, water), dried and evaporated. The residue was chromatographed, eluting with benzene:ethyl acetate 1:1 to give 3.6 g (69%) of material; oil; ^{1}H NMR δ 1.4-1.8 (6H, m), 2.6 (3H, s), 3.3 (2H, t), 3.7 (2H, t), 7.5 (2H, d), and 8.0 (2H, d) ppm; ^{13}C NMR δ 24.9, 26.8, 43.5, 49.0, 127.3, 128.7, 130.4, 137.9, 169.5, and 197.0 ppm; MS m/z(%) 231(72), 230(100), 147(53), M$^+$ 231.12587, calcd. ($C_{14}H_{17}NO_2$) 231.12593; IR υ_{max} 2800-3000, 1650, 1600 cm^{-1}.

1-(p-Acetylbenzoyl)pyrrolidine (**III-4**). This was prepared as described above, starting with pyrrolidine (1.4 g, 0.02 mole). The reaction mixture was stirred at room temperature overnight and worked up as before to give, following chromatography, 0.45 g (41%) of product; oil; ^{1}H NMR δ 1.9-2.1 (4H, m), 3.6 (3H, s), 3.4 (2H, t), 3.7 (2H, t), 7.6 (2H, d) and 8.0 (2H, d) ppm; ^{13}C NMR δ 24.7, 26.6, 27.0, 46.5, 49.7, 127.6, 128.6, 138.1, 141.7, 168.8 and 197.8 ppm; MS m/z(%) 217(100), 216(25), 147(100), M$^+$ 217.11001, calcd. ($C_{13}H_{15}NO_2$) 217.11028; IR υ_{max} 2800-3000, 1650, 1570 cm^{-1}.

1-(p-Acetylbenzyl)piperidine (**III-5**). Lithium aluminium hydride (2.0 g, 0.05 mole) was added to a cooled solution of 1-(p-acetylbenzoyl)piperidine (**III-3**) (4.0 g, 0.017 mole) in dry THF (100 mL), and the resulting mixture heated at reflux for 48 h. After the usual work up the product (3.4 g, 94%) was, without further purification, dissolved in acetone and treated with Jones' reagent to give the title product in 74% overall yield; oil; ^{1}H NMR δ 1.4-1.7 (6H, m), 2.3-2.5 (4H, t), 2.6 (3H, s), 3.5 (2H, s), 7.4 (2H, d) and 7.9 (2H, d) ppm; ^{13}C NMR δ 22.9, 23.9, 25.2, 53.2, 62.0, 126.9, 127.8, 134.6, 143.1, and 196.6 ppm; MS m/z(%) 217(91), 216(100), 133(44), 98(75), 84(52), M$^+$ 217.14674, calcd. ($C_{14}H_{19}NO$) 217.14666; IR υ_{max} 1700, 1600 cm^{-1}.

1-(p-Acetylbenzyl)pyrrolidine (**III-6**). Prepared as described above from 1-(p-acetylbenzoyl)pyrrolidine in 45% overall yield; oil; ^{1}H NMR δ 1.7-1.9 (4H, m), 2.4-2.6 (4H, m), 2.6 (3H, s), 3.6 (2H, s), 7.5 (2H, d), and 7.9 (2H, d) ppm; ^{13}C NMR δ 23.9, 27.0, 54.6, 60.7, 128.8, 129.3, 136.3, 145.4, and 198.3 ppm; MS m/z(%) 203(92), 202(100), 133(42), 84(96), M$^+$ 203.13098, calcd. ($C_{13}H_{17}NO$) 203.13101.; IR υ_{max} 1700, 1600 cm^{-1}.

1-(p-Ethylbenzyl)piperidine (**III-7**). Prepared in 84% yield from 1-(p-ethylbenzoyl)piperidine (**III-1**) by reduction using lithium aluminium hydride as described above for the preparation of **III-5**; oil; ^{1}H NMR δ 1.2 (3H, t), 1.4-1.5 (2H, m), 1.5-1.7 (4H, m), 2.3-2.4(4H, t), 2.6 (2H, q), 3.5 (2H, s), 7.2 (2H, d), and 7.3 (2H, d)

ppm; ^{13}C NMR δ 16.3, 25.0, 26.5, 29.0, 54.9, 64.1, 128.0, 139.7, 136.2, and 143.1 ppm; MS m/z(%) 203(92), 202(100), 119(99), 98(68), 84(89), M^+ 203.16771, calcd. ($C_{14}H_{21}N$) 203.16740; IR υ_{max} 2800-3000, 1550 cm^{-1}.

1-(p-Ethylbenzyl)pyrrolidine (**III-8**). Prepared in 85% yield from 1-(p-ethylbenzoyl)pyrrolidine (**III-2**) by reduction with lithium aluminium hydride as described for the preparation of **III-5**; oil; ^{1}H NMR δ 1.3 (3H, t), 1.7-2.0 (4H, m), 2.4-2.6 (4H, t), 2.7 (2H, q), 3.6 (2H, s), 7.2 (2H, d) and 7.3 (2H, d) ppm; ^{13}C NMR δ 16.1, 23.9, 29.0, 54.6, 60.9, 128.1, 129.3, 137.0, and 143.1 ppm; MS m/z(%) 189(83), 188(98), 119(100), 84(62), 70(57), M^+ 189.15174, calcd. ($C_{13}H_{19}N$) 189.15175; IR υ_{max} 2800-3000, 1550 cm^{-1}.

1-(p-Ethylphenyl)piperidine (**IV-4**). Prepared in 34% yield from **IV-1** using the Huang-Minlon modification of the Wolff-Kishner reduction and purified by chromatography, eluting with hexane:ethyl acetate gradient; oil; ^{1}H NMR δ 1.3 (3H, t), 1.6-1.7 (2H, m), 1.7-1.9 (4H, m), 2.7 (2H, q), 3.1-3.3 (4H, m), 7.0 (2H, d) and 7.2 (2H, d) ppm; ^{13}C NMR δ 16.2, 24.8, 26.4, 28.4, 51.7, 117.3, 128.7, 135.6, and 150.9 ppm; MS m/z(%) 189(100), 188(79), 174(76), 118(15), M^+ 189.15147, calcd. ($C_{13}H_{19}N$) 189.15175; IR υ_{max} 2900, 1600 cm^{-1}.

1-(p-isopropenylphenyl)piperidine (**IV-5**). A solution of methylmagnesium bromide (4.8 mL, 3M in ether) was added to a cooled solution of p-piperidinoacetophenone (**IV-1**) (3.0 g, 0.015 mole) in dry ether (50 mL), and the resulting mixture stirred at room temperature overnight. The mixture was heated to reflux for 1h, cooled, and worked up following addition of satd. ammonium chloride to give 2.8 g (86%) of product, m.p. 60-62°C, which was dissolved in a mixture of acetone (23 mL) and water (1.35 mL). Conc. HCl (0.9 mL) was added, and the mixture heated to reflux for 1h, then cooled, diluted with satd. $NaHCO_3$ (23 mL), and extracted with ether. The extract was dried and evaporated to yield 2.1 g (70%) of yellow solid, m.p. 36-38°C; ^{1}H NMR δ 1.5-1.8 (6H, m), 2.1 (3H, s), 3.1-3.3 (4H, m), 5.0 (1H, d), 5.3 (1H, d), 6.9 (2H, d), and 7.4 (2H, d) ppm; ^{13}C NMR δ 22.1, 24.7, 25.7, 50.7, 109.9, 116.1, 126.5, 132.0, 143.0, and 151.8 ppm; MS m/z(%) 201(100), 200(80), 199(11), 145(15), M^+ 201.15148, calcd. ($C_{14}H_{19}N$) 201.15175; IR υ_{max} 2750-3000, 1650, 1600 cm^{-1}.

p-Isopropenylphenylcyclohexane (**IV-6**). Prepared in 85% overall yield from p-cyclohexylacetophenone (**IV-2**) using the method described above for the preparation of **IV-5**. Oil; ^{1}H NMR δ 1.3-1.6 (5H, m), 1.7-2.0 (5H, m), 2.2 (3H, s), 2.5-2.6 (1H, m), 5.1 (1H, d), 5.4 (1H, d), 7.2 (2H, d), and 7.5 (2H, d) ppm; ^{13}C NMR δ 22.2, 26.6, 27.3, 32.1, 34.7, 44.5, 72.7, 112.0, 124.8, 125.8, 127.0, 143.5, and 147.8 ppm; MS m/z(%) 200(100), 157(27), 144(20), 131(29), M^+ 200.15638, calcd. ($C_{15}H_{20}$) 200.15650; IR υ_{max} 2800-3000, 1650, 1600 cm^{-1}.

p-Ethylphenylcyclohexane (**IV-7**). Prepared in 46% yield from p-cyclohexylacetophenone using the method described for the preparation of **IV-4**. Oil; ^{1}H NMR δ 1.3 (3H, t), 1.5-1.7 (5H, m), 1.8-2.0 (5H, m), 2.5-2.6 (1H, m), 2.7 (2H, q), and 7.2 (4H, m) ppm; ^{13}C NMR δ 16.0, 26.7, 27.4, 28.8, 35.0, 44.6, 127.1, 127.9, 141.9, and 145.8 ppm; MS m/z(%) 188(100), 159(36), 145(69), 119(36), 117(45), M^+ 188.15687, calcd. ($C_{14}H_{20}$) 188.15650; IR υ_{max} 2950, 1500 cm^{-1}.

Biotransformation procedures: A growth medium composed of glucose (10 g) and corn steep liquor (20 g) per L of distilled water, adjusted to pH 4.85 with 1M NaOH (3 L) was distributed in 15 1L Erlenmeyer flasks which were stoppered with foam plugs and sterilised by autoclaving at 121°C for 20 min. The flasks were allowed to cool and inoculated under sterile conditions with *B. bassiana* taken from a 3-day old agar slope. The flasks were allowed to stand overnight at 27°C, then placed on a rotary shaker (1" orbit) at 180 rpm, 27°C. After three days, a solution of the appropriate substrate (0.75 g) in 95% ethanol (30 mL) was added, and growth allowed to continue for a further 3 days. The fungal mass was removed by filtration, and the filtrate continuously extracted with dichloromethane for 3 d. Filtrates from biotransformations of basic substrates were adjusted to pH 9 by the addition of 1M NaOH prior to extraction. The extract was then evaporated to give a residue that was purified by flash column chromatography using a benzene-ethyl acetate 10% stepwise gradient, followed by an ethyl acetate-methanol 5% stepwise gradient. The yields and e.e. values quoted refer to purified, homogeneous material and arise from the combination of (only) homogeneous column fractions without further purification (e.g. crystallisation) that could lead to changes in stereochemical enrichment values. Biotransformations in the presence of CO were conducted in closed flasks in an atmosphere of CO (15%) and air (85%). Concurrent control biotransformations were conducted in analogous closed flasks in 100% air.
Studies with cyt.P-450 inhibitors were performed following harvest of the fungal growth after the initial three-day period on the rotary shaker by filtration. The fungal material was then re-suspended in 1L flasks in a volume of distilled water equal to that of the initial growth medium containing an appropriate concentration of inhibitor. Duplicate flasks containing fungal biomass suspended in this medium were placed on the rotary shaker for a pre-incubation period of 2 h prior to the addition of substrate. Products were extracted as described above, followed by HPLC analysis of the extract. Data presented in Table 2 are the mean of duplicate analyses. For all assays, concurrent control biotransformations were performed in the absence of inhibitor.

Characterisation of products: Spectral data are listed below. Isolated yields are presented in the Figures.

4-Hydroxy-1-phenyl-2-piperidone (**I-1a**). Oil; ^{1}H NMR δ 1.8-2.1 (2H, m), 2.5/2.8 (2H, ABXq), 3.5 (1H, m), 3.8 (1H, m), 4.2 (1H, m), and 7.2-7.4 (5H, m) ppm; ^{13}C NMR δ 36.1, 49.8, 52.4, 68.9, 131.4, 131.7, 134.1, 148.4, and 173.9 ppm; MS m/z(%) 191(100), 172(11), 106(76), M$^+$ 191.09491, calcd. ($C_{11}H_{13}NO_2$) 191.09463; IR υ_{max} 3300, 1660 cm^{-1}.

1-(p-Hydroxyphenyl)piperidine (**I-2a**). M.p. 158-160°C (Lit. m.p. 160-161°C);[46] ^{1}H NMR δ 1.4-1.6 (2H, m), 1.6-1.8 (4H, m), 2.9-3.1 (4H, t), 6.6-6.9 (4H, m); ^{13}C NMR δ 24.5, 26.4, 53.1, 116.2, 119.6, 129.4, 151.1; MS m/z(%) 177(76), 176(89), 121(27), 64(84), 66(100).

trans-*4-Phenylcyclohexanol* (**I-3a**). M.p. 118-120°C (Lit. m.p. 120-121°C);[47] ^{1}H NMR δ 1.3-1.7 (4H, m), 1.8-2.0 (2H, m), 2.0-2.3 (2H, m), 2.5-2.7 (1H, m), 3.8 (1H, m), and 7.2-7.5 (5H, m) ppm; ^{13}C NMR δ 32.7, 36.2, 43.8, 70.9, 126.5, 127.4, 128.8, and 147.0 ppm; MS m/z(%) 176(53), 158(70), 117(47), 104(80), 91(100).

cis-*3-Phenylcyclohexanol* (**I-3b**). M.p. 93-95°C (Lit. oil);[48] ^{1}H NMR δ 1.4-1.9 (6H, m), 2.0-2.3 (2H, m), 2.6 (1H, m), 3.2 (1H, m), and 7.2-7.5 (5H, m) ppm; ^{13}C NMR δ 24.9, 33.9, 35.6, 43.2, 43.5, 71.3, 126.5, 127.4, 128.8, and 146.7 ppm; MS m/z(%) 176(50), 158 (90), 117(40), 104(80), 91(100), M$^+$ 176.12053, calcd. ($C_{12}H_{16}O$) 176.12011; IR υ_{max} 3100-3400, 2800-3000 cm^{-1}.

4-Phenylphenol (**I-4a**). M.p. 163-165°C; spectra identical with authentic material (Aldrich).

4,4'-Dihydroxybiphenyl (**I-4b**). M.p. >260°C; spectra identical with authentic material (Aldrich).

3,4'-Dihydroxybicyclohexyl (**I-5a**). M.p. 115-117°C; ^{1}H NMR δ 0.8-1.3 (10H, m) and 3.4-3.6 (2H, m); ^{13}C NMR δ 22.8, 26.6, 26.8, 26.9, 27.7, 34.4, 34.5, 38.3, 39.9, 40.5, 69.7, and 69.9; MS m/z(%) 198(1), 162(58), 97(40), 81(100), M$^+$ 198.16171, calcd. ($C_{12}H_{22}O_2$) 198.16198; IR υ_{max} 3100-3400, 2800-3000 cm^{-1}.

trans-*1-Benzoyl-4-hydroxypiperidine* (**II-1a**). Oil; ^{1}H NMR δ 1.2-1.5 (2H, m), 1.6-1.9 (2H, m), 3.0-3.3 (2H, m), 3.4-3.7 (1H, m), 3.8-4.1 (2H, m), and 7.1-7.4 (5H, m) ppm; ^{13}C NMR δ 34.0, 34.7, 40.0, 45.3, 66.9, 127.0, 128.8, 129.7, 136.3, and 170.8 ppm; MS m/z(%) 205(32), 204(67), 105(100), M$^+$ 205.10978, calcd. ($C_{12}H_{15}NO_2$) 205.11028; IR υ_{max} 3300, 1650 cm^{-1}.

Cyclohexylmethanol (**II-3a**). Oil; identified by spectral comparisons with authentic material (Aldrich).

Trans-*4-Benzylcyclohexanol* (**II-4a**). Oil (Lit. m.p. (undefined stereochemistry) 35-40°C);[49] ^{1}H NMR δ 1.3-2.2 (9H, m), 2.4 (2H, d), 3.7 (1H, m), and 7.2-7.4 (5H, m) ppm; ^{13}C NMR δ 29.1, 34.9, 40.5, 44.6, 69.7, 124.8, 126.8, 128.4, 142.1 ppm; MS m/z(%) 190(35), 172(50), 91(100).

(S)-1-(1-Hydroxyethylbenzoyl)piperidine (**III-1a** and **III-3a**). Oil; ^{1}H NMR δ 1.4 (3H, d), 1.6-1.8 (6H, m), 3.4 (2H, t), 3.7 (2H, t), 5.0 (1H, q), and 7.3-7.5 (4H, m) ppm; ^{13}C NMR δ 26.6, 27.3, 28.6, 45.2, 50.8, 72.1, 127.4, 129.1, 137.5, 149.2, and 172.3 ppm; MS m/z(%) 233(54), 232(93), 149(61), 127(96), 55(100), M$^+$ 233.14098, calcd. ($C_{14}H_{19}NO_2$) 233.14158; IR υ_{max} 3150-3500, 1650 cm^{-1}; $[\alpha]_D$, **III-1a** −6.9 (c 1.28, $CHCl_3$), e.e. 22%, **III-3a** −29.8 (c 1.71, $CHCl_3$), e.e. >95%.

(S)-1-(1-Hydroxyethylbenzoyl)pyrrolidine (**III-2a** and **III-4a**). Oil; ^{1}H NMR δ 1.4 (3H, d), 1.7-2.0 (4H, m), 3.3 (2H, t), 3.5 (2H, t), 4.8 (1H, q), and 7.3-7.5 (4H, m) ppm; ^{13}C NMR δ 24.7, 25.6, 26.7, 46.6, 50.0, 69.7, 125.6, 127.5, 135.8, 148.7, and 170.2 ppm; MS m/z(%) 219 (42), 149(46), 126(73), 85(56), 43(100), M$^+$ 219.12612, calcd. ($C_{13}H_{17}NO_2$) 219.12593; IR υ_{max} 3100-3500, 1650, 1600 cm^{-1}; $[\alpha]_D$, **III-2a** −5.9 (c 2.28, $CHCl_3$), e.e. 22%, **III-4a** −32.5 (c 1.1, $CHCl_3$), e.e. >95%.

trans-*(S)-1-(1-Hydroxyethylbenzoyl)-4-hydroxypiperidine* (**III-3b**). Semi-solid; ^{1}H NMR δ 1.5 (3H, d), 1.5-1.8 (5H, m), 3.4 (2H, t), 3.7 (2H, t), 4.1 (1H, d), 4.9 (1H, q), and 7.2-7.4 (4H, m) ppm; ^{13}C NMR δ 24.6, 24.9, 45.6, 49.2, 74.4, 75.5, 127.3, 127.6, 135.2, 144.2, and 170.6 ppm; MS m/z(%) 249(18), 248(32), 149(39), 128(12), 84(100), M$^+$ 249.13646, calcd. ($C_{14}H_{19}NO_3$) 249.13649; IR υ_{max} 3150-3700, 1650 cm^{-1}; $[\alpha]_D$, −61.9 (c 1.06, $CHCl_3$), e.e. >95%.

trans-*1-(p-Acetylphenyl)-4-hydroxypiperidine* (**IV-1a**). M.p. 123-125°C (Lit. m.p. 124-126°C);[41] spectral data as reported.[41]

trans-*4-(p-Acetylphenyl)cyclohexanol* (**IV-2a**). M.p. 128-130°C; ^{1}H NMR δ 1.3-2.2 (8H, m), 2.5-2.7 (1H, m), 2.6 (3H, s), 3.7 (1H, m),, and 7.3/7.9 (4H, ABq) ppm; ^{13}C NMR δ 26.9, 32.5, 36.1, 43.9, 70.7, 127.4, 129.1, 135.6, 152.7, and 198.3 ppm; MS m/z(%) 218(30), 203(100), 200(93), 185(91), 131(87), M$^+$ 218.13128, calcd. ($C_{14}H_{18}O_2$) 218.13068; IR υ_{max} 3200-3500, 1700 cm^{-1}.

cis-*3-(p-Acetylphenyl)cyclohexanol* (**IV-2b**). M.p. 118-120°C; ^{1}H NMR δ 1.3-2.2 (8H, m), 2.6 (3H, s), 2.7 (1H, m), 3.6 (1H, m), and 7.3/7.9 (4H, ABq) ppm; ^{13}C NMR δ 24.8, 27.8, 33.3, 35.6, 43.1, 43.2, 71.1, 127.4, 129.1, 135.6, 152.3, and 198.3 ppm; MS m/z(%) 218(33), 203(95), 200(100), 185(80), 131(77), M$^+$ 218.13116, calcd. $C_{14}H_{18}O_2$) 218.13068; IR υ_{max} 3200-3500, 1700 cm^{-1}.

4-Biphenylmethanol (**IV-3a**). M.p. 98-100°C; spectra identical with authentic material (Aldrich).

4-Biphenylformate (**IV-3b**). M.p. 216-218°C; ^{1}H NMR δ 6.7 (2H, d), 7.2-7.4 (7H, m) and 8.84 (1H, s) ppm; ^{13}C NMR (CD_3OD) δ 115.6, 126.2, 126.8, 127.9, 132.5, 140.3, 140.7, 157.2 ppm; MS m/z(%) 198(24), 183(33), 154(38), 143(42), 126(100), M$^+$ 198.06700, calcd. ($C_{13}H_{10}O_2$) 198.06808; IR υ_{max} 1700 cm^{-1}.

1-(p-2-Hydroxyethylphenyl)piperidine (**IV-4a**). Oil, ^{1}H NMR δ 1.5-1.6 (2H, m), 1.7-1.9 (4H, m), 2.8 (2H, t), 3.2 (4H, t), 3.4 (1H, s), 3.8 (2H, t), and 6.9/7.1 (4H, ABq) ppm; ^{13}C NMR δ 24.6, 26.2, 38.6, 51.5, 64.2, 117.4, 129.6, 129.9, and 151.2 ppm; MS m/z(%) 205(30), 174(100), 118(8), M$^+$ 205.14712, calcd. ($C_{13}H_{19}NO$) 205.14666; IR υ_{max} 3100-3600, 1600 cm^{-1}.

p-(2-Hydroxy-2-propylphenyl)cyclohexane (**IV-6a**). Oil; ^{1}H NMR δ 1.2-1.5 (5H, m), 1.6 (6H, s), 1.7-1.9 (5H, m), 2.4-2.6 (1H, m), and 7.2/7.4 (4H, ABq) ppm; ^{13}C NMR δ 24.5, 25.2, 30.0, 32.8, 42.4, 70.7, 122.6, 125.3, and 144.7 ppm; MS m/z(%) 218(5), 202(20), 201(100), M$^+$ 218.16752, calcd. ($C_{15}H_{22}O$) 218.16706; IR υ_{max} 3100-3600 cm^{-1}.

3-(p-(2-Hydroxy-2-propylphenyl))cyclohexanol (**IV-6c**). Oil; ^{1}H NMR δ 1.3-1.5 (4H, m), 1.6 (6H, s), 1.7-2.2 (4H, m), 2.5 (1H, m), 3.7 (1H, m), and 7.2/7.4 (4H, ABq) ppm; ^{13}C NMR δ 24.8, 31.3, 32.8, 36.1, 42.7, 43.3, 70.9, 72.8, 124.8, 126.9, 145.4 and 147.2 ppm; MS m/z(%) 234(6), 219(20), 217(100), M$^+$ 234.16166, calcd. ($C_{15}H_{22}O_2$) 234.16198; IR υ_{max} 3100-3600 cm^{-1}; $[\alpha]_D$ +5.3 (c 1.6, $CHCl_3$).

p-(1,2-Dihydroxy-2-propylphenyl)cyclohexane (**IV-6d**). Oil; ^{1}H NMR δ 1.2-1.4 (6H, m), 1.5 (3H, s), 1.7-2.0 (4H, m), 2.2-2.6 (1H + 2OH, m), 3.7 (2H, d), and 7.2/7.4 (4H, ABq) ppm; ^{13}C NMR δ 23.6, 26.4, 34.6, 42.7, 43.3, 71.3, 75.1, 125.6, 127.1, 143.2, and 145.4 ppm; MS m/z(%) 234(4), 219(100), 210(17), M$^+$ 234.16200, calcd. ($C_{15}H_{22}O_2$) 234.16198; IR υ_{max} 3100-3600 cm^{-1}; $[\alpha]_D$ –13.9 (c 1.35, $CHCl_3$).

(S)-1-(4-biphenyl)ethanol (**IV-8a**). M.p. 84-86°C (Lit. (racemate) m.p. 96.4-97.6°C);[50] ^{1}H NMR δ 1.54 (3H, d), 5.0 (1H, q), and 7.35-7.52 (9H, m) ppm; ^{13}C NMR δ 23.1, 70.6, 126.2, 127.5, 127.7, 129.2, 140.9, 141.2, 145.2 ppm; MS m/z(%) 198(62), 183(100), 155(90); IR υ_{max} 3300 cm^{-1}; $[\alpha]_D$ –8.5 (c 2, EtOH), e.e. 28%.

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