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Selectivity of the Biooxygenation of *N*-Phenylcarbamates by the Fungus *Beauveria bassiana*

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Abstract: The biotransformation with *Beauveria bassiana* of *syn*-1,5-dimethylbicyclo[3.2.1]oct-8-yl-*N*-phenylcarbamate (**11**), its *anti*-isomer **13** and cyclooctyl-*N*-phenylcarbamate **31** is described. Based on the observed hydroxylation positions and on earlier results of oxygenations using this fungus, a modified distance model for hydroxylations is proposed. The mode of binding of the substrate to the enzyme's active site depends on the intrinsic structure and electronic properties of its electron-rich group. The regio- and stereochemistry of hydroxylation, however, is determined mainly by the structure of the hydrocarbon moiety, namely by a specific gap from the anchoring group. This is represented by a distance of about 5.5 Å between the oxygen directly attached to the carbocycle and hydrogens which potentially can be substituted. © 1997 Elsevier Science Ltd.

INTRODUCTION

Since the pioneering work by Fonken et al.[1] in the sixties, biooxygenations of non-activated hydrocarbon positions by microorganisms[2] continue to attract the attention of organic chemists.[3] Particularly the fungus *Beauveria bassiana* ATCC 7159[4] achieves regio- and diastereoselective,[1,5] with proper substrates even enantioselective[5a,6] hydroxylations of non-activated hydrocarbon positions. At present no chemical method[7] is available which can compete with the selectivity and yield of biohydroxylations involving monooxygenases[8] in whole-cell systems.[1c,2] However, the prediction of the regio- and stereoselectivity of these types of reactions is still a subject of discussion. Fonken et al.[1] proposed an enzyme-substrate model in 1967 to account for the preferential hydroxylation by that micro-organism of certain positions in cycloalkanols and alicyclic carboxamides. This model suggests that an electron rich center, such as carbonyl oxygen atom, e.g. of the amide function,[1,9] becomes attached to the enzyme's active site in a first step and that hydroxylation occurs selectively at a carbon atom approximately 5.5 Å distant from the carbonyl oxygen. This optimum distance has since been enlarged to 4.5-6.2 Å[10] and became the first predictive tool for the regioselectivity of biohydroxylation of hydrocarbon positions using *B. bassiana*.

In the eighties, Furstoss et al. used this micro-organism for biohydroxylations of various amides,[5a,6b,11] lactams,[6b,12] and carbamates.[13] Indeed, they found that for these substrates, the distance between the carbonyl oxygen and the hydroxylated carbon atom varied from 3.3 to 6.2 Å, making this criterion unusable as a general predictive tool.[14] Because of the large substrate variety yet high regio- and stereoselectivity of hydroxylations by this fungus, they elaborated a concept that accounts for a relatively flexible oxygen activating site of the monooxygenase.[5a,14] Their trajectory model of the hydroxylating locus reveals that different hydroxylation positions can exist for the same hydrocarbon depending on the nature of the anchoring group and the place of attachment to tne carbon skeleton in several carboxamides and lactams. On the other

hand, studies of a broad variety of substrates bearing anchoring amide or carbamoyl groups by Johnson,[6c-6e] Furstoss,[5a,6b] Roberts[15] and ourselves[16] support the concept that a specific distance exists between the anchoring region and the catalytically active site of the oxygenating enzyme of *B. bassiana*. The position of the hydroxylation and the ratio of alternative products is at least partly governed by the distance from the electron rich anchoring function and the intrinsic nature of this group. This feature also requires that the selection between different oxygenation positions is made after the substrate's binding and makes it improbable that the monooxygenase binds one specific substrate in a variety of orientations. Further investigations revealed that this fungus prefers *trans*-hydroxylation in the 4-position relative to the anchoring amide[5c,9a] and carbamate[13b,c] substituents of many substrates.

Within the scope of our work, which focused on the selective hydroxylation of non-activated hydrocarbon positions, we investigated transformations with *B. bassiana* of *N*-phenylcarbamates.[16] We present here the results with some rigid bicyclic and flexible monocyclic alcohols, namely *syn*-1,5-dimethyl-bicyclo[3.2.1]oct-8-yl-*N*-phenylcarbamate (**11**), its *anti*-isomer **13** and cyclooctyl-*N*-phenylcarbamate (**31**). As a result and considering earlier results by other research groups [1a,13b,c] we present herein a modified distance model for the prediction of hydroxylation position(s) based on the two earlier models.[1a,5a,10,14]

RESULTS AND DISCUSSION

The results of oxygenations mediated by *B. bassiana* of different *N*-phenylcarbamtes of cyclic alcohols[1a,13b,c,16.] are summarized in the Table. Moreover, distances from the oxygen directly bound to the carbocycle to hydrogens which potentially can be substituted are calculated for stable conformers (AM1[17]) and given in the Table.

The first urethanes which have been transformed by the fungus were cyclohexyl systems such as **1**, **5** and **7** (Table). The biotransformation of the conformationally flexible cyclohexyl-*N*-phenylcarbamate (**1**) was shown to be highly regio- and diastereoselective (76% de) giving mainly the *trans*-4-alcohol **2**, the consecutive *p*-hydroxylated compound **3** and a minor amount of *cis*-alcohol **4**.[13b] The conformationally very rigid adamantane carbamates **5** and **7** gave only *trans*-4-hydroxylated products.

The 100% regioselectivity of these transformations can be interpreted exactly in terms of the distance model by Fonken et al.[1] The diastereoselectivity, however, cannot be explained by this model. On the other hand, the trajectory model by Furstoss et al.[5a,14] must define a geometry of the enzyme's active site, since the structure of the enzyme is not yet known. A substrate can be oriented to the geometry of this model to explain the hydroxylation position found in the product(s). However, a prediction of hydroxylation positions of a compound which is not already known to be a substrate seems to be difficult without knowledge of the structure of the enzyme's active site.

Proceeding on the assumption that the biotransformation runs out as a two-step process,[1a,14] a specific substrate will be attached to the enzyme in the first step by induced fit of the electron rich anchoring group like the phenyl carbamoyl group for the examples presented here. This seems to be possible for a broad variety of substrates, almost independently from the hydrocarbon part of the molecule and for several functional derivatives like alcohols, benzamides, lactams, carbamates, azides or sulfonamides, explaining the very low substrate specificity of the fungus.[1,5,6,9-15] After initial binding to the enzyme, positioning of the hydrocarbon moiety to the hydroxylating site is important for the hydroxylation position. However, this

depends less on the specific interactions of the anchoring group with the enzyme, but is strongly directed by the size and the conformation of the hydrocarbon part. One or several C-H bonds must have the opportunity to arrange in a proper distance to the catalytically active metal center activating the oxygen. In other words, if a specific compound is a substrate for the enzyme, that means after initial binding, the position of hydroxylation will be determined mostly by the intrinsic structure of the hydrocarbon part. Thus, we would like to modify the two former models to allow a prediction of the hydroxylation position in agreement with the mechanistic knowledge about the C-H-activation with cytochrome P-450 dependent monooxygenases, namely the radical oxygen rebound mechanism[18] or the recently published ionic "side-on" approach.[19] We propose the definition of a key distance between the oxygen directly attached to the carbocyclic system and the hydrogens at potential oxygenation positions, which is shown for the chair and the boat conformers of cyclohexyl-*N*-phenylcarbamate (**1**) (Figure 1).

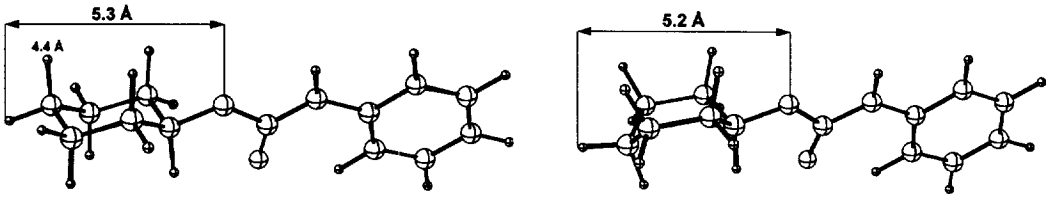


Figure 1: Chair and boat conformers of **1** (AM1[17]) depicted with distances between oxygen and hydrogens to be potentially substituted

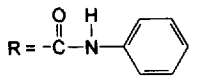
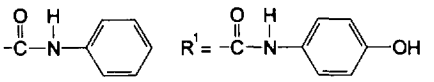
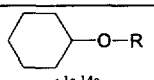
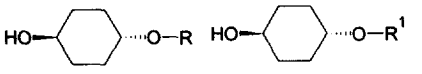
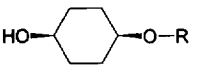
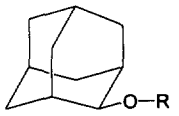
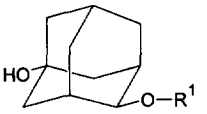
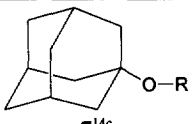
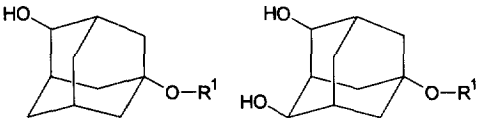
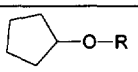
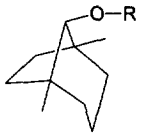
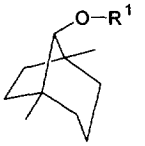
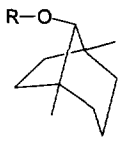
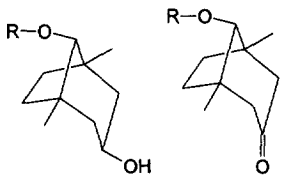
The distance between the preferentially replaced equatorial hydrogen at C(4) and the oxygen attached to the carbocyclic system amounts to 5.3 Å in the chair conformer, while for the axial hydrogen at C(4) 4.4 Å was calculated. It seems that the *trans*-H(4) is substituted stereospecifically to form **2**. The minor *cis*-product **4** could be formed by two different processes presuming that the hydroxylation occurred from the most stable chair conformation, which must not be the case considering the Curtin-Hammett principle.[20] (i) The hydroxylation is not stereospecific, giving both products **2** and **4**. (ii) The cis-H(4) is substituted giving **4**. These possibilities seem unlikely, because only *trans*-4-hydroxylation products were found after biotransformation of the conformationally rigid adamantyl-*N*-phenylcarbamates **5** and **7**, suggesting that only protons in *trans*-4-positions have been abstracted and the hydroxylation occurs stereospecifically. On the other hand, the less stable boat conformer of **1** could be hydroxylated as well. This conformer exhibits distances of 4.1 Å and 5.2 Å respectively for the equatorial and axial hydrogens at C(4) (Table). Substitution of the equatorial H(4) with retention of the configuration would give the *cis*-isomer **4**.

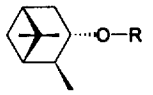
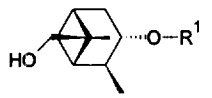
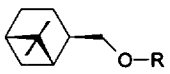
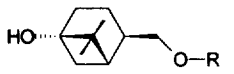
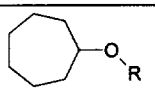
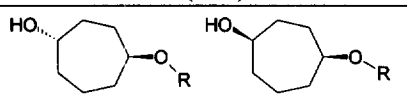
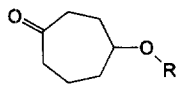
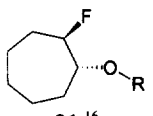
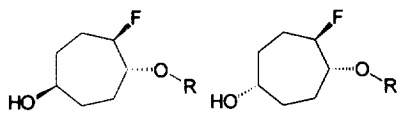
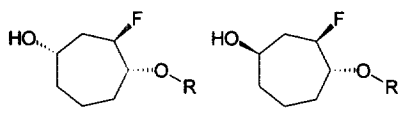
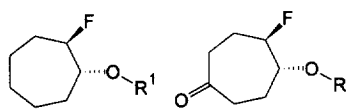
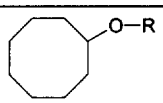
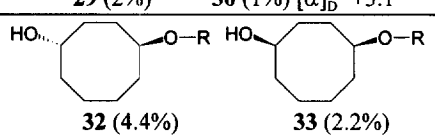
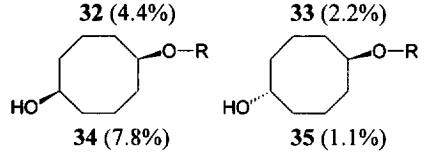
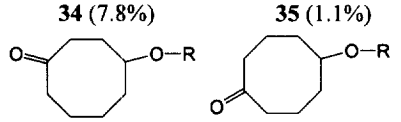
Thus, it seems that only H-atoms with a distance greater than 5 Å from the mentioned oxygen (attached to the ring) can be substituted by a hydroxyl function. This is supported by the fact that cyclopentyl-*N*-phenyl-carbamate (**10**) lacking H-atoms with this proper distance (Table) gave no hydroxylation products at all.[13c]

To prove this hypothesis, we designed the transformation of two other *N*-phenyl-carbamates with a rigid cyclohexyl part, namely the *syn*- and *anti*-isomers of bicyclo[3.2.1]oct-8-yl-*N*-phenylcarbamate **11** and **13**, respectively. Compound **11** bears the carbamate function fixed in axial position. X-Ray analysis[21] and AM1 calculations show corresponding O---H distances which are all less than 5 Å. For those in 4-positions (cyclohexane segment) 3.8 Å [*exo*-H(3)] and 4.2 Å [*endo*-H(3)] (directed into the molecule) and 4.3 Å for the *exo*-H(7) and 4.5 Å for the hidden endo-H(7) (cyclopentane segment) were calculated. In fact we did not find

any product hydroxylated in the bicyclic carbon skeleton after the biotransformation procedure. Only some traces of the phenol **12** have been detected by GC-MS measurements. On the other hand, the *anti*-isomer **13**, bearing the carbamoyl function in equatorial position gave two oxygenation products, the *trans*-hydroxylated compound **14** and its consecutive ketone **15**, likely formed by a alcohol dehydrogenase reaction. For the replaced *trans*-4-hydrogen a distance of 5.2 Å has been calculated (Table).

Table: Substrates and products of biotransformations of N-phenylurethanes with *B. bassiana*

Substrate	Products with *B. bassiana*	Distance of O to H atoms which potentially can be abstracted in [Å] in stable conformers[a)]
		
 **1**[1a,14c]	 **2** (40%) **3** (34%)  **4** (10%)	chair conformer *trans* H(4): 5.3, *cis* H(4): 4.4* boat conformer *trans* H(4): 4.1*, *cis* H(4):5.2
 **5**[14c]	 **6** (41%)	*trans* H(4): 5.3 *cis* H(4): 4.3*
 **7**[14c]	 **8** (40%) **9** (26%)	*trans* H(4): 5.3 *cis* H(4): 4.3*
 **10**[14c]	no hydroxylation product detected	envelope conformer *trans* H(3): 4.4* *cis* H(3): 3.9*
 **11**	 **12** (trace) [b)]	*endo* H(3): 4.2*[18] *exo* H(3): 3.8* *endo* H(7): 4.5* *exo* H(7): 4.3*
 **13**	 **14** (5%) **15** (1%)	*endo* H(3): 3.8* *exo* H(3): 5.2

 **16**[14c]	 **17** (43%)	H(8): 5.8-6.2
 **18**[14c]	 **19** (23%)	*endo* H(5): 5.3-6.1 H(8): 5.2*
 **20**[16]	 **22** (10%) 62% ee **23** (2%)  **24** (12%) $[\alpha]_D^{21}$+6.1° [c]	boat conformer, *trans* H(4): 5.4, *cis* H(4): 4.0 *trans* H(5): 5.2, *cis* H(5): 5.2 twist-chair conformer *trans* H(4): 5.5, *cis* H(4): 4.2 *trans* H(5): 5.5, *cis* H(5): 5.1
 **21** [16]	 **25** (24%) 82% e.e. **26** (8%) 16% e.e.  **27** (5%) 52% ee **28** (4.5%) 79% ee  **29** (2%) **30** (1%) $[\alpha]_D^{21}$+3.1°	Distance values are almost identical with those of the stable conformers of **20**
 **31**	 **32** (4.4%) **33** (2.2%)  **34** (7.8%) **35** (1.1%)  **36** (6.6%) $[\alpha]_D^{21}$-1.5° **37** (3.3%)	boat chair conformer *trans* H(4): 4.0, *cis* H(4): 5.2 *trans* H(5): 5.7, *cis* H(5): 5.8 *trans* H(6): 5.6, *cis* H(6): 4.4 crown conformer *trans* H(4): 5.7, *cis* H(4): 4.9 *trans* H(5): 6.2, *cis* H(5): 5.4 *trans* H(6): 5.6, *cis* H(6): 4.4

* No hydroxylation of this position was observed with *B. bassiana*

[a] The heats of formation were calculated by semiempirical molecular orbital calculations (AM1, MOPAC 6[17]). Frequency calculations have been carried out and all conformers were local minima on their corresponding hypersurfaces. [b] Structural assignment of **12** is assumed considering literature.[16,13b] [c] Ketone **24** is derived exclusively from the *trans*-alcohol **22** by dehydrogenase as described in the preceding publication.[16]

Other conformationally rigid polycyclic substrates such as the pinane derivatives **16** and **18**,[13c] show distance ranges of 5.2-6.2 Å for hydrogens which potentially can be substituted (Table). However, in **16** no methine or methylene hydrogen is located at a proper distance. Consequently, a carbon-hydrogen bond of the methyl group (which is generally most stable) in *anti*-position is substituted. In compound **18** the carbamoyl group is not directly fixed at the ring, and hence is more flexible. Thus, one methine and three methyl H-atoms at C(8) are in a proper distance for hydroxylation. Here the tertiary C-H bond is substituted.

Increasing the ring-size of the carbocyclic system on the one hand leads to more flexible systems, but offers, on the other hand, the opportunity to study not only the regio- and diastereoselectivity but also the enantioselectivity of *B. bassiana* mediated hydroxylations.[6c] In the preceding paper it has been shown that the hydroxylations of cycloheptyl-N-penylcarbamate (**20**) and it's racemic fluorinated analogue **21** proceed highly regioselectively at a 4-position. For the parent compound **20** one of the enantiotopic *trans*-4-hydrogens is preferentially substituted (84% de, 62% ee).

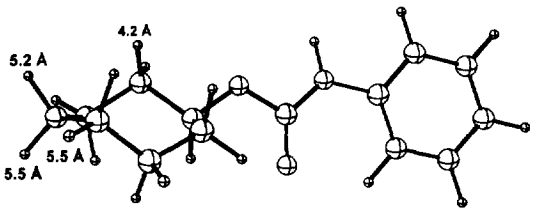


Figure 2: Twist chair conformer of **20** (AM1[17]) depicted with key distances in Å

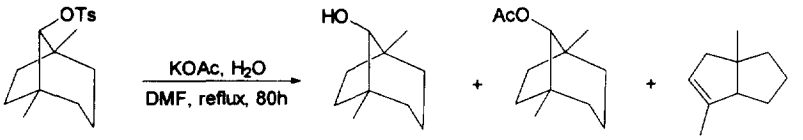
The fluorine substituent near the anchoring group lowers the regio- and diastereoselectivity, but enhances the enantioselectivity leading to partial deracemization by hydroxylation. Also with these substrates only such C-H bonds having a distance of 5.1-5.5 Å in the most stable twist-chair conformer of the cycloheptane ring (Figure 2) are hydroxylated.

Moreover, we investigated also the biohydroxylation of the very flexible cyclooctyl-*N*-phenyl-carbamate (**31**) with *B. bassiana* even though it was feared that a complicated mixture of oxygenated products would be formed. Formally **31** has four 4-methylene protons and two methylene protons in 5-position to the carbamoyl function, which all have a proper distance to the oxygen in two of the most stable conformers (Table). Moreover, there exist even more conformers of cyclooctane itself or monosubstituted derivatives having very similar energy.[22] Thus, oxygenation yielded the chiral *cis/trans*-isomeric 4-hydroxy compounds **32** and **33** and its consecutive optically active ketone **36** and the symmetric *cis*- and *trans*-alcohols **34** and **35** and the ketone **37**. That means that all possible diastereomers of the 4- and 5-regioisomers were formed. Unfortunately, it was not possible to separate all compounds and to determine the enantiomeric excess of **32**, **33** and **36**. Only the ketones **36** and **37** were isolated as pure compounds and **36** exhibited a specific rotation of $[\alpha]_D = -1.5°$.

Synthesis of the Starting Materials and Structural Elucidation of the Products.

anti-1,5-Dimethylbicyclo[3.2.1]oct-8-yl-*N*-phenylcarbamate (**13**) has been prepared from the *anti*-alcohol **39**[23] which was obtained from *syn*-1,5-dimethylbicyclo[3.2.1]oct-8-yl-tosylate (**38**)[24,25] by refluxing

with potassium acetate and small amounts of water in dimethylformamide for 80 hours to give a 69:21:10 mixture of **39**, the acetate **40** and 2,5-dimethylbicyclo[3.3.0]oct-2-ene (**41**).



38 **39** **40** **41**

No nucleophilic substitution of the tosylate **38** occurred with potassium acetate in the absence of water. Under S_N1 like conditions with potassium acetate in acetic acid **38** was transformed mainly to **41**.[23] Moreover, no reaction occurred by treatment of the parent alcohol of **38** under the conditions of the Mitsunobu inversion. The product mixture was separated, the acetate **40** treated with potassium bicarbonate in methanol to obtain another portion of **39** (87%). The carbamate **13** was synthesized (83% yield) by refluxing of **39** with phenylisocyanate at 120°C in petroleum ether for 4 hours. Analogously **11**[21] and **31** have been synthesized from *syn*-1,5-dimethylbicyclo[3.2.1]octan-8-ol or cyclooctanol, respectively.

Biotransformation of *syn*-1,5-dimethylbicyclo[3.2.1]oct-8-yl-*N*-phenylcarbamate (**11**) gave only traces of a hydroxylation product which was identified as the phenol **12** by GC-MS coupling experiment. The *anti*-isomer **13**, in contrast, gave two products, namely *anti,trans*-3-hydroxy-1,5-dimethylbicyclo[3.2.1]oct-8-yl-*N*-phenylcarbamate (**14**) and its oxidation product **15** (Table).

For **14** high resolution CI-MS with NH_3 delivered the molecular formula $C_{17}H_{23}NO_3{\cdot}NH_4^+$ and comparison of the fragmentation in the EI-mass spectra of **12** and **14** showed that the hydroxyl group is located in the aromatic ring in **12** but is attached to the bicyclic hydrocarbon skeleton in **14**. From the ^{13}C NMR spectrum which contains five signals for non-aromatic carbons it becomes obvious that the compound is symmetric and must therefore be hydroxylated in 3-position (δ = 66.2 ppm). The *trans*-configuration of the two substituents, and hence the equatorial arrangement of the hydroxyl group follows from the ^1H NMR spectrum. For the proton attached to C(3) a multiplet centered at δ = 3.93 ppm is found. The equatorial protons at C(2) and C(4) are found at δ = 1.91 ppm as a doublet of a doublet with a geminal coupling constant of 13.1 Hz and a vicinal one to the H(3) of 6.2 Hz. The axial protons at C(2) and C(4) appear as a doublet of a doublet at δ = 1.47 ppm with ^2J = 13.1 Hz and ^3J = 11.0 Hz, showing that the OH-group is tied in equatorial position. The structure of **15** was deduced from the spectroscopic data (cf. exp. part).

Biotransformation of cyclooct-1-yl-*N*-phenylcarbamate (**31**) gave four alcohols **32-35** which have been expected from our distance model and two consecutive ketones **36** and **37**. These ketones were separated from the alcohols and from each other by column chromatography. The four alcohols in contrast could not be isolated as pure single isomers. However, an enrichment in fractions containing not more than three isomers in different ratio was possible by HPLC. *Cis*-4-hydroxycyclooct-1-yl-*N*-phenylcarbamate (**33**) has been identified mainly by comparison of the ^{13}C NMR spectra of a fraction enriched with **33** and an authentic sample of this compound synthesized from commercially available *cis*-cyclooctane-1,4-diol by reaction with 0.5 equivalents of phenylisocyanate as described in the general procedure.[16]

The structures of the other alcohols **32**, **34** and **35** were assigned on the basis of 600 MHz ^1H-TOCSY experiments as well as 600 MHz ^1H and 150 MHz ^{13}C NMR data of fractions differently enriched in the

components. Successive irradiation at the frequencies of the protons neighboured to the carbamoyl function at δ = 4.86 or 4.90 or 4.96 ppm, respectively led to three ^{1}H-TOCSY sub-spectra which were compared to those of authentic samples of **33** and **34**. An authentic sample of the last one was synthesized from commercially available *cis*-cyclooctane-1,5-diol. The structure of the ketone **36** was confirmed from the fact that this compound is optically active ($[\alpha]_D^{21}$ -1.5°) and by identical NMR data with those of an authentic sample synthesized by Jones' oxidation of **33** obtained from the commercially available *cis*-cyclooctane-1,4-diol. The position of the keto group of **37** was established from the fact that only four signals appeared in the ^{13}C NMR spectrum for alicyclic carbons showing the symmetric structure of the compound.

CONCLUSION

Based on earlier knowledge[1a,13b,c,16] and new results from this work concerning the positions of *Beauveria bassiana* mediated hydroxylation of non-activated hydrocarbon positions in rigid and flexible phenylcarbamates of mono- and bicyclic alcohols we like to modify the distance-models by Fonken et al.[1] and Furstoss et al.[5a,14] We found out that hydroxylations occurred in a key distance of about 5.5 Å between the substituted hydrogens and that oxygen of the carbamate function directly attached to the carbocycle. This model takes into the account that the binding mode of the substrate to the enzyme's active site is directed mainly by the intrinsic steric and electronic properties of an electron-rich anchoring group.[5a,14,15] Likewise, the regio- and stereochemistry of hydroxylation is determined mainly by the structure of the hydrocarbon, namely by a specific distance from the anchoring group. To exclude distance variations caused by the mobility of the phenylcarbamate function (which influences the induced fit of the substrate, but does not influence the distance of the catalytically active, that means the oxygen activating, center and the potential hydroxylation position) we like to define the distance of potentially substituted hydrogens to the oxygen which is fixed at the ring and can therefore move only together with the ring carbons. This distance is, hence, independent of the specific conformations necessary for the induced fit of the anchoring group, which for a real substrate will always lead to bind to the enzyme.

EXPERIMENTAL

Analytical methods, general procedure for the preparation of N-phenylcarbamates and biotransformation conditions are described in the preceding paper.[16] Yields of products are given in % of converted substrate.

Starting Materials: Phenylisocyanate, cyclooctanol, *cis*-cyclooctane-1,4-diol and *cis*-cyclooctane-1,5-diol were purchased from Aldrich. *Syn*-1,5-dimethylbicyclo[3.2.1]octan-8-ol was synthesized as already reported.[24,25]

syn-1,5-Dimetylbicyclo[3.2.1]octyl-N-phenyl-(p-hydroxyphenyl)carbamate (**12**): Yield: trace; GC-MS: 289 ($M^{+\cdot}$, 40%), 271 (M^{+}-H_2O, 4%), 151* (M^{+}-$C_{10}H_{17}$, 8%), 135* (α-cleavage RCO-C=OR, 24%), 137* ($C_{10}H_{17}^{+}$, 10%), 109* (α-cleavage O=C-NHR +H, 28%), 107* (α-cleavage O=C-NHR -H, 39%), 93*

($C_6H_5O^+$, 100%) * Fragment-ions were assigned by according to the literature.[26] Assignment of OH group in the *para* position of **4** is deduced in analogy to literature.[13]

anti-1,5-Dimethylbicyclo[3.2.1]oct-8-yl-N-phenylcarbamate (**13**): From 0.99 g (6.43 mmol) of *anti*-dimethylbicyclo[3.2.1]octan-8-ol and 0.83 g (7.0 mmol) of phenylisocyanate: Yield: 1.45 g (83%); mp: 152°C; ^{1}H NMR: δ 0.97 (s, 6 H, CH_3), 1.25-1.63 (m, 10 H, CH_2), 4.50 (s, 1 H, CHOCO), 6.62 (br. s, 1 H, NH), 7.03 (tt, $^3J_{H,H}$ = 7.4 Hz, $^4J_{H,H}$ = 1.4 Hz, 1 H), 7.29 (tt, $^3J_{H,H}$ = 7.4 Hz, $^4J_{H,H}$ = 1.9 Hz, 2 H), 7.39 (d, $^3J_{H,H}$ = 8.1 Hz, 2 H); ^{13}C NMR: δ 19.8 (s, C-3), 21.9 (s, C-9, C-10), 34.7 (s, C-6, C-7), 39.5 (s, C-2, C-4), 44.9 (s, C-1, C-5), 90.3 (s, C-8), 118.4 (s, C-13, C-17), 123.0 (s, C-15), 128.9 (s, C-14, C-16), 138.2 (s, C-12), 153.9 (s, C-11); Anal. calcd. for $C_{17}H_{23}NO_2$ (273.4) C 74.69, H 8.48, N 5.12 found C 74.40, H 8.47, N 4.94%.

After biotransformation[16] of 0.45 g (1.65 mmol) of **13** and work up 196 mg (44%) of the starting material was recovered by column chromatography. The hydroxylation product was isolated by HPLC:

anti,exo-3-Hydroxy-1,5-dimethylbicyclo[3.2.1]oct-8-yl-N-phenylcarbamate (**14**): Yield: 15 mg (5.1%); ^{1}H NMR: δ 1.01 (s, 6 H, CH_3), 1.47 (t, $^3J_{H,H}$ = 10 and 13.2 Hz, 2 H, 2-CH_{2a}, 4-CH_{2a}), 1.60-1.68 (m, 4 H, CH_2), 1.91 (ddd, $^2J_{H,H}$ = 13.1 Hz, $^3J_{H,H}$ = 6.2 Hz, $^4J_{H,H}$ = 1.4 Hz, 2 H, 2-CH_{2e}, 4-CH_{2e}), 3.88-3.98 (m, 1 H, CH_2), 4.59 (s, 1 H, CHOCO), 6.58 (br. s, 1 H, NH), 7.02 (tt, $^3J_{H,H}$ = 7.2 Hz, $^4J_{H,H}$ = 1.4 Hz, 1 H), 7.29 (tt, $^3J_{H,H}$ = 7.4 Hz, $^4J_{H,H}$ = 2.4 Hz, 2 H), 7.38 (d, $^3J_{H,H}$ = 8.1 Hz, 2 H); ^{13}C NMR: δ 21.8 (s, C-9, C-10), 35.0 (s, C-6, C-7), 44.4 (s, C-1, C-5), 48.3 (s, C-2, C-4), 66.2 (s, C-3), 89.3 (s, C-8), 118.7 (s, C-15), 123.4 (s, C-14, C-16), 129.1 (s, C-13, C-17), 138.0 (s, C-12); HR-MS: calcd. for $C_{17}H_{23}NO_3$+NH_4^+ 307.202168, found 307.2003.

anti-3-Oxo-1,5-Dimethylbicyclo[3.2.1]oct-8-yl-N-phenylcarbamate (**15**): Yield: 3 mg (1%); ^{1}H NMR: δ 1.07 (s, 6 H, CH_3), 1.59-1.79 (m, 4 H, 6-CH_2, 7-CH_2), 2.28 and 2.51 AB spectrum (2dm, $^2J_{H,H}$ = 16.2 Hz, $^2J_{H,H}$ = 16.0 Hz, 2-CH_2, 4-CH_2), 5.06 (s, 1 H, CHOCO), 6.66 (br. s, 1 H, NH), 7.04-7.10 (m, 1 H), 7.28-7.35 (m, 2 H), 7.38-7.43 (m, 2 H); ^{13}C NMR: δ 21.7 (s, C-9, C-10), 35.1 (s, C-6, C-7), 45.6 (s, C-1, C-5), 55.2 (s, C-2, C-4), 87.6 (s, C-8), 118.6 (s, C-15), 123.7 (s, C-14, C-16), 129.1 (s, C-13, C-17), 137.7 (s, C-12), 153.3 (s, C-11), 208.9 (s, C-3); HR-MS: calcd. for $C_{17}H_{23}NO_3$ 287.152144, found 287.1535.

Cyclooctyl-N-phenylcarbamate (**31**): From 3.10 g (25 mmol) of cyclooctanol and 3.84 g (30 mmol) of phenylisocyanate: Yield: 4.45 g (72%); mp: 48-50°C; ^{1}H NMR: δ 1.42-1.59 (m, 8 H, CH_2), 1.61-1.93 (m, 6 H, CH_2), 4.94 (m, 1 H, CHOCO), 6.69 (br. s, 1 H, NH), 7.02 (t, J_{para} = 7.3 Hz, 1 H), 7.26 (t, J_{meta} = 7.9 Hz, 2 H), 7.37 (d, J_{ortho} = 8.0 Hz, 2 H); ^{13}C NMR: δ 22.8 (s, C-3), 25.3 (s, C-5), 27.1 (s, C-4), 31.6 (s, C-2), 76.1 (s, C-1), 118.5 (s, C-11, C-15), 123.1 (s, C-13), 128.9 (s, C-14, C-12), 138.2 (s, C-10), 153.3 (s, C-9); HR-MS: Calcd. for $C_{15}H_{21}NO_2$+NH_4^+ 265.1916, found 265.1893.

After biotransformation[16] of 1.2 g (4.85 mmol) of **3** and work up 304 mg (25%) of the starting material was recovered by column chromatography. The oxygenation products were isolated by HPLC:

trans-4-Hydroxycyclooctyl-N-phenylcarbamate (**32**): Yield: 42 mg (4.4%); ^{1}H NMR TOCSY: δ 1.52-1.60 (m, CH_2), 1.74-1.94 (m, CH_2), 3.84 (m,-CHOH), 4.9 (m, CHOCO), 6.69 (bs, -NH), 7.03 (t, J_{para} = 7.4 Hz), 7.28 (t, J_{meta} = 7.4 Hz, 2 H); 7.37 (d, J_{ortho} = 7.5 Hz); ^{13}C NMR: δ 71.48 (s, C-4), 75.42 (s, C-1). Strong irradiation of the proton neighboured to the carbamate function at δ 4.90 ppm afforded ^{1}H TOCSY data for all coupling protons in the eight-membered skeleton. ^{13}C signals of aromatic carbons of **32** as well as **33-37** were

not affected by aliphatic hydroxylation and showed the same chemical shifts as the starting material **3**. Alcohol **32** could not been isolated in a pure form and was contaminated with **34** and **35** in different portions in fractions.

cis-4-Hydroxycyclooctyl-N-phenylcarbamate (**33**): Yield: 21 mg (2.2%); ^{13}C NMR: δ 21.9 (s), 22.8 (s), 27.5 (s), 30.2 (s), 30.4 (s), 33.4 (s), 71.4 (s, C-4), 75.4 (s, C-1), 118.6 (s, C-11, C-15), 123.1 (s, C-13), 128.9 (s, C-12, C-14), 138.1 (s, C-10), 153.2 (s, C-8). Alcohol **33** could not been isolated in a pure form and was contaminated with **34** and **35**. The ^{13}C spectroscopic data agree with those for an authentic sample prepared from commercially available cis-cyclooctane-1,4-diol in 64% yield. For this sample the following additional data were measured: Mp 109-112°C; ^{1}H NMR ($CDCl_3$): δ 1.44-1.62 (m, 2 H, CH_2), 1.63-1.93 (m, 10 H, CH_2), 3.82 (m, 1 H, CHOH), 4.88 (m, 1 H, CHOCO), 6.83 (br. s, 1 H, NH), 7.03 (t, J_{para} = 7.3 Hz, 1 H), 7.28 (t, J_{meta} = 7.4 Hz, 2 H); HR-MS: Calcd. for $C_{15}H_{21}NO_3$+NH_4^+ 281.1865, found 281.1842.

cis-5-Hydroxycyclooctyl-N-phenylcarbamate (**34**): Yield: 74 mg (7.8%); ^{1}H NMR TOCSY: δ 1.4.5-1.76 (m, CH_2), 1.85-1.94 (m, CH_2), 3.88 (m, CHOH), 4.86 (m, CHOCO), 7.02 (t, J_{para} = 7.3 Hz), 7.23 (t, J_{meta} = 7.2 Hz), 7.37 (d, J_{ortho} = 7.5 Hz); ^{13}C NMR: δ 20.0 (s), 33.0 (s), 35.7 (s), 71.4 (s, C-5), 75.4 (s, C-1), 118.6 (s, C-11, C-15), 123.1 (s, C-13), 128.9 (s, C-12, C-14), 138.0 (s, C-10), 153.3 (s, C-9). GC-MS: m/z (%) 263 (21) [M^+], 137 (58), 120 (44), 119 (14), 93 (100),, 91 (25), 67 (84), 55 (58). Strong irradiation of the proton signal at δ 4.86 ppm (C*H*OCONHPh) resulted in ^{1}H TOCSY data for all coupled protons in the eight membered carbocyclic system. Alcohol **34** could not been isolated in a pure form and was always contaminated with **32** and **35**. The 1H, 13C and MS data agree with tose observed for an authentic sample obtained from commercially available cis-cyclooctane-1,5-diol in 58% yield. For this sample the following additional data were found: Mp 95°C; HR-MS: Calcd. for $C_{15}H_{21}NO_3$+NH_4^+ 281.1865, found 281.1853.

trans-5-Hydroxycyclooctyl-N-phenylcarbamate (**35**): Yield: 11 mg (1.1%); ^{1}H NMR TOCSY: δ 1.60-2.09 (m,-CH_2), 3.91 (m,CHOH), 4.96 (m, CHOCO); ^{13}C NMR: δ 71.3 (s, C-5), 75.1 (s, C-1). Strong irradiation of the C*H*OCONHPh proton signal at δ 4.96 ppm resulted in ^{1}H-TOCSY data for all coupled protons in the eight membered network. Alcohol **35** could not been isolated in a pure form and was always contaminated with **34** and **33**.

4-Oxocyclooctyl-N-phenylcarbamate (**36**): Yield: 63 mg (6.6%); mp 90-92°C; $[\alpha]_D^{21}$: -1.5° (c = 0.17, $CHCl_3$); ^{1}H NMR (CD_3OD): δ 1.07-1.18 (m, 1 H, CH_2), 1.47-1.78 (m, 5 H, CH_2), 2.08-2.50 (m, 6 H, CH_2), 4.76 (m, 1 H, CHOCO), 6.90 (t, J_{para} = 7.4 Hz, 1 H), 7.15 (t, J_{meta} = 7.6 Hz, 2 H), 7.30 (d, J_{ortho} = 7.6 Hz, 2 H); ^{13}C NMR: δ 22.6 (s), 28.0 (s), 28.4 (s), 30.6 (s), 39.0 (s), 40.6 (s), 74.3 (s, C-1), 118.6 (s, C-11, C-15), 120.9 (s, C-13), 129.1 (s, C-12, C-14), 137.8 (s, C-10), 152.8 (s, C-9), 216.4 (s, C-4); HR-MS: Calcd. for $C_{15}H_{19}NO_3$+NH_4^+ 279.1709, found 279.1722.

5-Oxocyclooctyl-N-phenylcarbamate (**37**): Yield: 32 mg (3.3%); ^{1}H NMR: δ 1.84-1.99 (m, 8H, CH_2), 2.41-2.51 (m, 4H, CH_2), 4.82 (m, 1H, CHCOOR), 6.67 (brs, 1H, NH), 7.04 (t, J_{para}= 7.3 Hz, 1H), 7.28 (t, J_{meta}= 7.4 Hz, 2H), 7.36 (d, t, J_{ortho}= 7.6 Hz, 2H); ^{13}C NMR: δ 21.7 (s, C-3, C-7), 32.6 (s, C-2, C-8), 42.0 (s, C-4, C-6), 73.2 (s, C-1), 118.6 (s; C-11, C-15), 123.2 (s, C-13), 129.0 (s, C-14, C-12), 138.0 (s, C-10), 152.7 (s, C-9), 216.0 (s, C-5); HR-MS: Calcd. for $C_{15}H_{19}NO_3$+NH_4^+ 279.1709, found 279.1703.

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