Biocatalytic Asymmetric Hydroxylation of Hydrocarbons with the Topsoil-Microorganism *Bacillus megaterium*

Waldemar Adam,[†] Zoltan Lukacs,*[,†,‡] Dag Harmsen,[§] Chantu R. Saha-Möller,[†] and Peter Schreier[‡]

Institutes of Organic Chemistry and Food Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany, and Institute of Hygiene and Microbiology, University of Würzburg, Josef-Schneider-Str. 2, D-97080 Würzburg, Germany

Received November 4, 1999

A *Bacillus megaterium* strain was isolated from topsoil by a selective screening procedure with allylbenzene as a xenobiotic substrate. This strain performed the hydroxylation chemoselectively (no arene oxidation and overoxidized products) and enantioselectively (up to 99% ee) in the benzylic and nonbenzylic positions of a variety of unfunctionalized arylalkanes. Salycilate and phenobarbital, which are potent inducers of cytochrome P-450 activity, changed the regioselectivity of the microbial CH insertion, without an effect on the enantioselectivity. The biotransformation conditions were optimized in regard to product yield and enantioselectivity by variation of the oxygen-gas supply and the time of the substrate addition. The different product distributions (α- versus *β*-hydroxylated product) that are obtained on induction of cytochrome P-450 enzyme activity demonstrate the involvement of two or more hydroxylating enzymes with distinct regioselectivities in this biotransformation. An oxygen-rebound mechanism is assumed for the cytochrome P-450-type monooxygenase activity, in which steric interactions between the substrate and the enzyme determine the preferred face of the hydroxy-group transfer to the radical intermediate.

Introduction

CH oxidations belong to the most versatile reactions in organic chemistry, since potentially useful oxyfunctionalized synthons may be obtained from readily accessible hydrocarbons. Only a few conventional synthetic methods, which require harsh reaction conditions and yield racemic products, are to date available for this purpose.[1] Therefore, much effort has been previously expended to develop efficient, catalytic oxidations of hydrocarbons.[2−5] For example, in the past years, metal-catalyzed asymmetric CH oxidations have been performed by employing chiral auxiliaries[6] and optically active oxidants[7,8] to afford enantiomerically enriched products. Nevertheless, most of the time only a moderate enantioselectivity has been achieved for unactivated alkanes. In this context, biomimetic studies have been carried out to gain insight into the mechanism of the catalytic CH oxidation and to enhance its stereoselectivity.[9−11] Despite all these efforts, the development of an effective catalytic asymmetric hydroxylation of unfunctionalized hydrocarbons with broad applicability remains a challenge.

Alternatively, microorganisms have been successfully applied to the selective oxygenation of unactivated CH bonds in organic substrates.[12] So far, the major emphasis of such work has focused on the hydroxylation of steroids, terpenes and other complex natural products.[13] For these biotransformations, mostly fungi like *Rhizopus nigricans*, *Mortiella isabellina*, and *Cunninghamella elegans*, for instance, have been employed,[14,15] for which high regio- and enantioselectivities have been reported.[16−19] In contrast, the hydroxylation of xenobiotics is far more scarce because, unlike natural products, these compounds are not readily introduced into the cell for metabolism. Furthermore, many monooxygenases, particularly the well-known cytochrome P-450cam from *Pseudomonas putida*, are highly substrate-specific, and therefore, their

* To whom correspondence should be addressed. E-mail: adam@chemie.uni-wuerzburg.de. Fax: +49-931-8884756. http://www-organik.chemie.uni-wuerzburg.de.
† Institute of Organic Chemistry.
‡ Institute of Food Chemistry.
§ Institute of Hygiene and Microbiology.

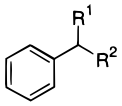
(1) Haines, A. H. *Methods for the Oxidation of Organic Compounds − Alkanes, Alkenes, Alkynes and Arenes*; Academic Press: London, 1985.
(2) Stahl, S. S.; Labinger, J. A.; Bercaw, J. E. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2180−2192.
(3) Barton, D. H. R.; Doller, D. *Acc. Chem. Res.* **1992**, *25*, 504−512.
(4) Reiser, O. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 69−72.
(5) Adam, W.; Curci, R.; D'Accolti, L.; Dinoi, A.; Fusco, C.; Gasparrini, F.; Kluge, R.; Paredes, R.; Schulz, M.; Smerz, A. K.; Veloza, L. A.; Weinkoetz, S.; Winde, R. *Chem.−Eur. J.* **1997**, *3*, 105−109.
(6) Jacobsen, E. N.; Marko, I.; Mungall, W. S.; Schröder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968−1970.
(7) Davis, F. A.; Vishwakarma L. C.; Billmers, J. M.; Finn, J. *J. Org. Chem.* **1984**, *49*, 3241−3243.
(8) Hamada, T.; Irie, R.; Mihara, J.; Hamachi, K.; Katsuki, T. *Tetrahedron* **1998**, *54*, 10017−10028.
(9) Lim, M. H.; Lee, Y. J.; Goh, Y. M.; Nam, W.; Kim, C. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 707−713.
(10) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146−153.
(11) Moro-oka, Y.; Akita, M. *Catal. Today* **1998**, *41*, 327−338.
(12) Johnson, R. A. In *Organic Chemistry−Part C−Oxidation in Organic Chemistry*; Trahanowsky, W. S., Ed.; Academic Press: New York, 1978.
(13) Holland, H. L. In *Biotechnology;* Kelly, D. R., Ed.; Wiley-VCH: Weinheim, 1998; Vol 8a.
(14) Holland, H. L. *Organic Synthesis with Oxidative Enzymes*; VCH Publishers: Weinheim, 1992.
(15) Davis, C. R.; Johnson, R. A.; Cialdella, J. I.; Liggett, W. F.; Mizsak, S. A.; Marshall, V. P. *J. Org. Chem.* **1997**, *62*, 2244−2251.
(16) Holland, H. L. *Curr. Opin. Chem. Biol.* **1999**, *3*, 22−27.
(17) Berg, A.; Rafter, J. J. *Biochem. J.* **1981**, *196*, 781−786.
(18) Schwab, E.; Bernreuther, A.; Puapoomachareon, P.; Mori, K.; Schreier, P. *Tetrahedron: Asymmetry* **1991**, *2*, 471−479.
(19) Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry: The Use of Isolated Enzymes and Whole Cell Systems in Synthesis*; Academic Press: London, 1989.

10.1021/jo991725s CCC: $19.00
Published on Web 01/11/2000

application is restricted to a limited number of closely related substrates.[12]

In the case of simple arylalkanes, the substrates of interest in the present work, the oxidation of the aromatic ring or benzylic hydroxylation is usually favored according to literature reports.[20] For instance, *P. putida* hydroxylates *p*-ethylphenol to yield the corresponding α-hydroxy phenol in an enantiomeric excess (ee) of 39% in favor of the *S* enantiomer,[14] but *Pseudomonas aeruginosa* fails to hydroxylate the sterically more demanding isobutylbenzene.[12] Another severe drawback in whole-cell CH insertions is the overoxidation of the alcohol to either the ketone or even the carboxylic acid.[12] Moreover, most of the currently known biocatalytic hydroxylations of xenobiotics with whole cells suffer from low conversions and poor enantioselectivities.

In view of these shortcomings, the purpose of the present study was to search for microorganisms that display both high chemo- and enantioselectivity, i.e., those that deliver optically pure oxyfunctionalized products without further oxidation. In this contribution, we present our results for the hydroxylation of arylalkanes by a *Bacillus megaterium* strain, which was obtained from topsoil by a selective screening procedure. We demonstrate herein for the first time that this microorganism may be used for the oxidative biotransformation of the unactivated arylalkanes **1a**−**g** to yield selectively, even on the semipreparative (mg) scale, the corresponding benzylic and nonbenzylic alcohols (no arene oxidation) in high enantiomeric excess and with low amounts, if any, of overoxidation products.



1	R^1	R^2
a	CH_3	H
b	C_2H_5	H
c	$CH{=}CH_2$	H
d	$C(CH_3)_3$	H
e	$CH(CH_3)_2$	H
f	CH_2CH_3	CH_3
g	$(CH_2)_3CH_3$	H

Results

An adequate screening procedure to select suitable soil bacteria for the biotransformation of hydrocarbons had to be developed. Allylbenzene was chosen as the selecting substrate because it possesses three different oxidation sites for its detoxification as a biologically harmful substance: the phenyl ring, the benzylic position, and the double bond. Applying various amounts (5−25 μL) of allylbenzene and glucose (0−10 g/L) to soil samples of different origin, this screening procedure resulted in three different microbial species. For their identification, they were subjected to sequencing of the first 300 base pairs of their small subunit ribosomal RNA gene (16s) by means of the Taq-cycle-DyeDeoxy-terminator technique.[21] The resulting nucleotide sequences (variable region V1−V3 of 16s-like-rRNA) were compared to sequence data of the European Molecular Biology Laboratory (EMBL) database, which disclosed that an *Arthrobacter* sp., a *Pseudomonas* sp., and a *Bacillus* sp. had been isolated. The *Bacillus* strain yielded the α-hydroxylation product (benzylic CH insertion, no epoxidation nor phenyl hydroxylation) of allylbenzene, whereas the other strains were ineffective for oxidative biotransformations. Therefore, the effective strain was more rigorously characterized and identified as a *B. megaterium* strain.

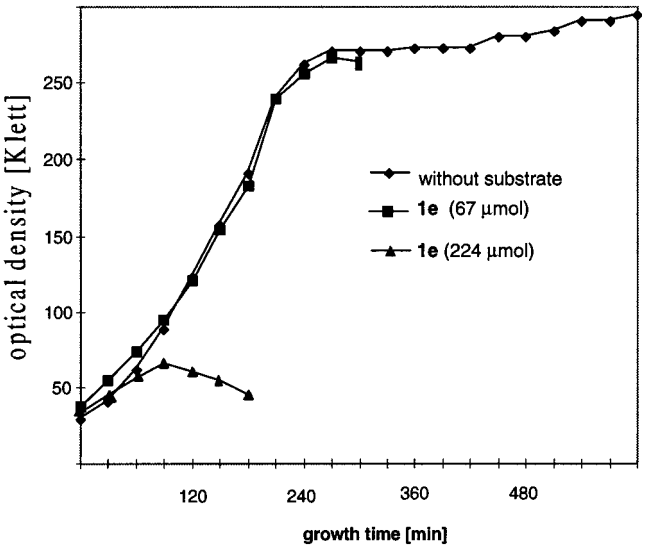
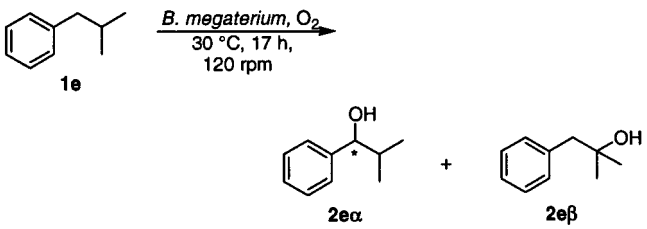


Figure 1. Growth curves of *B. megaterium* in the presence of different amounts of the model substrate **1e**.

In an effort to optimize the biotransformation conditions, the growth curve for *B. megaterium* was recorded for different substrate amounts, which were added during the early log-phase growth. For all experiments, isobutylbenzene (**1e**) was chosen as model substrate (Scheme 1).

Scheme 1. Hydroxylation of Model Substrate 1e by *B. megaterium*



Addition of 67 μmol of the substrate, which is approximately equivalent to the amount added during regular biotransformations, did not influence the growth in comparison to a culture without any substrate added (Figure 1). However, when the amount of substrate was increased to 224 μmol, cellular growth was immediately slowed and the culture rapidly died due to substance toxicity. Addition of the substrate at different times during growth led to significantly different yields of the oxidation products. Thus, substrate addition during the early log-phase, as well as early stationary phase, resulted in approximately up to 4-fold less oxidation product than during the mid-log-phase addition. Therefore, the latter conditions were chosen as the optimal time for the addition of the substrate in regard to yield (data not shown).

The influence of the oxygen-gas supply on the hydroxylation reaction was elucidated for the further optimiza-

(20) Sibbesen, O.; Zhang, Z.; Ortiz de Montellano, P. R. *Arch. Biochem. Biophys.* **1998**, *353*, 285−296.

(21) Harmsen, D.; Rothgänger, J.; Singer, C.; Albert, J.; Frosch, M. *Lancet* **1999**, *353*, 291.

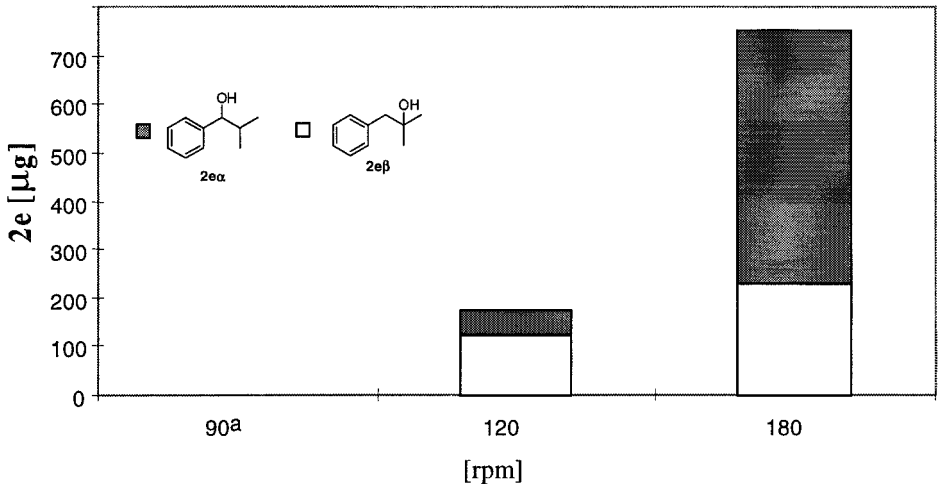


Figure 2. Dependence of the extent of hydroxylation for equal amounts of substrate **1e** by *B. megaterium* on the oxygen availability; oxygen-gas influx depends on the rotation velocity (rpm). [a] No product was observed.

tion of the biotransformation conditions. The availability of oxygen gas in the growth medium was varried by changing the rotation velocity of the incubation vessel. Clearly, no products were detected at low (90 rpm) oxygen-gas saturations (Figure 2). In contrast, relatively high oxygen-gas influx increased the hydroxylation at the α position ca. 8-fold, whereas the amount of *β* product was merely doubled.

To elucidate the scope of substrate acceptability by *B. megaterium*, several hydrocarbons were used for the biotransformation (Table 1). *B. megaterium* was incubated with ca. 100 *µ*mol of the respective hydrocarbon for 17 h, the cells were removed by centrifugation, and liquid−liquid extraction of the growth medium was carried out for product isolation. Because the products and the substrates are quite volatile, the solvent was removed by distillation through a Vigreux column to prevent loss due to evaporation. Finally, the products were submitted to gas-chromatographic analysis and mass spectrometry. The enantiomeric excess was determined by multidimensional gas chromatography (MDGC) on cyclodextrin columns. The absolute configurations were established by comparison with authentic reference samples, wherever available, and by employing the circular-dichroism-exciton-chirality method[22] (details will be published separately).

Ethylbenzene (**1a**) and propylbenzene (**1b**) both yielded solely the α-hydroxylation product. For 1-phenylethanol (**2a**), an enantiomeric excess of only 19% for the *R* alcohol was observed (entry 1), whereas 1-phenylpropanol (**2b**) displayed a much higher ee value of 74% for the *R* enantiomer (entry 2). Allylbenzene (**1c**), the screening substrate, was hydroxylated to the corresponding alcohol (*R*)-**2c** with an ee value of 70% under preservation of its double bond, which is an unexpected feature of this biotransformation since the structurally related cinnamyl alcohol was reduced by *B. megaterium* to the 3-phenylpropanol (data not shown). Mechanistically significant is the fact that ca. 4% cinnamyl alcohol was detected. The enantiomeric excess of the allyl alcohol **2c** is comparable to the one with propylbenzene (**1b**) as substrate (entry 3). A tertiary butyl group in the side chain (substrate **1d**) increased the enantiomeric excess to 91% of the *R* enantiomer; however, the conversion drops to 18% due to the greater steric hindrance (entry 4).

Branching in the propyl chain results additionally in *β*-hydroxylation products. Indeed, for isobutylbenzene (**1e**), the **2e***β* regioisomer is even preferentially formed (entry 5). This is highly unusual, because many of the reported bacterial hydroxylations of arylalkanes in the aliphatic side chain are restricted to the benzylic posi-

Table 1. Regio- and Enantioselectivities of the Oxygen-Atom Insertion into the C−H Bond of Hydrocarbons 1 by *B. megaterium*

entry	substrate	convn[a] [%]	product	selectivity [%] regio[b]	enantio[c]
1	Ph 1a	67	OH Ph 2a	100	19 (*R*)
2	Ph 1b	63	OH Ph 2b	100	74 (*R*)
3	Ph 1c	49	OH Ph 2c	96[d]	70 (*R*)
4	Ph 1d	18	OH Ph 2d	100	91 (*R*)
5	Ph 1e	43	OH Ph 2eα	31	91 (*R*)
			Ph OH 2eβ	69	
6	Ph 1f	68	OH Ph 2fα	68	38 (*R*)
			Ph OH 2fβ	32	*threo* 39 (2*R*,3*S*) *erythro* 83 (2*R*,3*R*)
7	Ph 1g	89	OH Ph 2gα	29 (4)	42 (*S*)
			Ph OH 2gβ	33 (3)	88 (*R*)
			OH Ph 2gγ	9 (3)	86 (*R*)
			Ph OH 2gδ	14 (5)	> 99 (*S*)

[a] Conversion of hydrocarbons determined by GC analysis; error limit ± 2% of the stated values. [b]Ratio of regioisomeric alcohols; in the case of ketone formation, the product distribution is normalized to 100%; the amount of the corresponding ketone is given in parentheses. [c]Enantiomeric excesses (ee values) were determined by multidimensional gas chromatography on a cyclodextrin column. [d]Small amounts (ca. 4%) of cinnamyl alcohol were observed.

(22) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy − Exciton Coupling in Organic Chemistry*; University Science Books: Mill Valley, 1983.

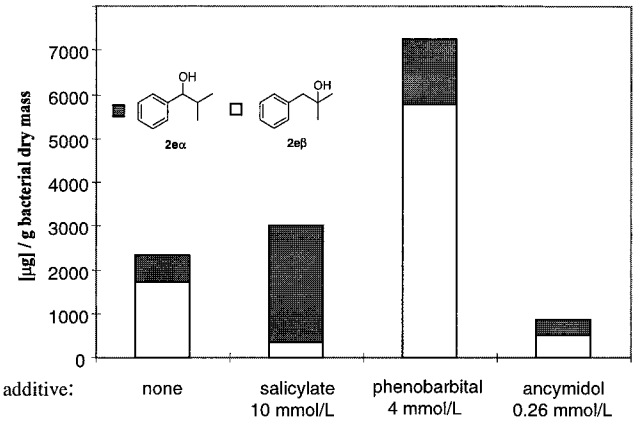


Figure 3. Effect of cytochrome P-450 inducers or repressors (additives) on the hydroxylation of equal amounts of the hydrocarbon **1e** by *B. megaterium*.

tion.[14] The enantiomeric excess of 2-methyl-1-phenylpropan-1-ol (**2e**α) was found to be 91% for the *R* enantiomer. In contrast, (1-methyl)propylbenzene (**1f**) yielded the α-hydroxylation product **2f**α in 38% ee for the *R* enantiomer, as well as the threo (39% ee) and erythro (83% ee) diastereomers of the *β*-hydroxylation product **2f***β* (entry 6). The erythro (2*R*,3*R*)-1-methyl-1-phenyl-2-propanol (**2f***β*) eluted significantly later than the other two alcohols. It is mechanistically important for this C−H insertion that the chiral starting material **1f** remained racemic throughout this biotransformation.

n-Pentylbenzene (**1g**) is especially noteworthy, as it gave all possible methylene-hydroxylation products, but no terminal oxidation of the side chain. The regioselectivity shows once more no preference for the benzylic position since the α- and *β*-regiomeric alcohols were obtained in approximately equal amounts. CH insertion at the *γ* and *δ* positions is significantly less, with a slight preference for the *δ*-methylene group (entry 7). For all regioisomers, the respective ketone products were formed in small amounts (3−5%) due to further oxidation of the alcohols (Table 1, entry 7, values in parentheses). The enantiomeric excess is very high except for the α-hydroxylation product **2g**α (42% ee). The α and *δ* hydroxylations of substrate **1g** by *B. megaterium* proceeded with a preference for the *S* enantiomers, whereas the *β* and *γ* insertions afforded the *R* enantiomers.

To assess whether monooxygenases of the cytochrome P-450 type participate in these microbial transformations, known inducers and repressors of such enzyme activity were tested on substrate **1e** (Figure 3). Addition of salicylate[23] to the growth medium effectively induced α hydroxylation, whereas *β* hydroxylation was sharply reduced. Phenobarbital, a barbiturate,[24] induced both α and *β* hydroxylation, but with a more significant increase of the *β* product. Ancymidol,[25] a cytochrome P-450 repressor, reduced the yields of both products, but more effectively that of the *β* hydroxylation. Therefore, the involvement of cytochrome P-450 enzymes seems to be likely.

Discussion

The topsoil screening process with the unfunctionalized allylbenzene (**1c**) delivered a *B. megaterium* strain for selective aliphatic CH insertions without epoxidation or phenyl hydroxylation. For the unfunctionalized hydrocarbons **1a**−**g**, *B. megaterium* gave the usually largely favored microbial benzylic hydroxylation,[14] but also the nonbenzylic positions were hydroxylated in large amounts. The use of known inducers or repressors of cytochrome P-450 activity revealed that at least two distinct enzymes are involved, one favors the benzylic hydroxylation, the other the nonbenzylic one (Figure 3). Thus, the potent inducers of cytochrome P-450 activity significantly affected the product distribution of this biotransformation. Salicylate selectively increased the amount of the α-hydroxylated product, while the barbiturate enhanced the *β* hydroxylation, in comparison to the regioselectivity found under standard conditions (no additive, Figure 3). If only one monooxygenase were responsible for all hydroxylations, then enhancement of its expression would increase all the regioisomers, but the ratio of the products would remain unchanged. The involvement of more than one enzyme is also suggested by a change in the oxygen supply of the medium (Figure 2). An increase in oxygen availability (180 rpm) shifts the ratio of α versus *β* product in the hydroxylation of the model substrate **1e** toward the benzylic position (proportionally more regioisomer **2e**α), since different oxygen concentrations in the medium affect the expression of the distinct hydroxylating enzymes on a transcriptional level and shift the product pattern of regioisomers towards the favored hydroxylation position of that particular enzyme which is transcribed preferentially. This means that for the model substrate **1e**, the observed product pattern is a result of the different cellular availability of hydroxylating enzymes with distinct regioselectivities and not a result of different selectivities of a single enzyme. In that context, it is interesting to note that cytochrome P-450 BM-3, a monooxygenase described for *B. megaterium*, is one of the few prokaryotic cytochrome P-450 enzymes inducible by barbiturates.[24,26] The known properties of this enzyme are the *ω*-1, *ω*-2, and *ω*-3 hydroxylation of fatty acids of chainlengths from C12 to C18, fatty alcohols and fatty amides, with the requirement of a polar group in the substrate to hold the methylene group to be hydroxylated close to the active center.[27,28] Since the enzymatic activity observed in the present case is also inducible by a barbiturate, but the type of substrate is completely different, it seems likely that the biotransformations described herein are carried out by cytochrome P-450 mutants, which readily accept purely hydrophobic substrates.

The enzymes involved in this oxyfunctionalization display a high enantioselectivity which is strongly dependent on the steric properties of the substrate (Table 1). This is clearly evident for substrates with branching of the aliphatic side chain (Table 1, entries 4 and 5). A sterically more demanding alkyl chain poses greater hindrance to the transfer of the hydroxy group from the pro-*S* face of the substrate and, thus, favors the insertion from the pro-*R* face. For instance, substrate **1d** with a *tert*-butyl group and **1e** with an isopropyl group are hydroxylated in an enantiomeric excess of 91%, in favor of the *R* enantiomers (Table 1, entries 4 and 5). The

(23) Shaw, G.; Kao, H.; Chiang, A. *Curr. Microbiol.* **1997**, *35*, 28−31.
(24) Narhi, L. O.; Fulco, A. J. *J. Biol. Chem.* **1986**, *261*, 7160−7169.
(25) Fuchser, J.; Zeeck, A. *Liebigs Ann. Chem.* **1997**, 87−95.
(26) Liang, Q.; Chen, L.; Fulco, A. J. *Biochim. Biophys. Acta* **1998**, *1380*, 183−197.
(27) *Cytochrome P-450*, 2nd ed.; Omura, T., Ishimura, Y., Fujii-Kuriyama, Y., Eds.; VCH Publishers: New York, 1993.
(28) Munro, A. W.; Lindsay, J. G. *Mol. Microbiol.* **1996**, *20*, 1115−1125.

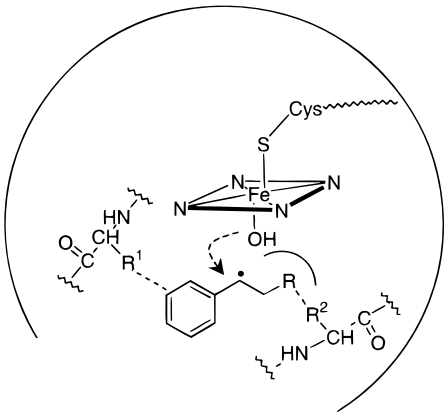


Figure 4. Oxygen-rebound mechanism for the C−H insertion of unfunctionalized hydrocarbons **1a**−**g** by *B. megaterium* monooxygenases, presumably of the cytochrome P-450 type; the substrate (R = alkyl) is aligned in the active center of the enzyme by hydrophobic interaction with amino acids (R^1, R^2 = alkyl or aryl), and the hydroxy-group transfer to the radical site is directed through the alignment of the substrate **1** in the active center by the steric interactions between the alkyl and aryl groups of the substrate and enzyme.

influence of the three-dimensional structure of the molecule on the enantiomeric excess is further demonstrated in the hydroxylation of (1-methyl)propylbenzene (**1f**). The oxidation products **2f**α and *threo*-**2f**β that display comparable chromatographic properties are both obtained with low ee values, whereas the significantly later eluting *erythro*-**2f**β diastereomer is formed in much higher enantiomeric excess (Table 1, entry 6). Therefore, the three-dimensional fit of the substrate in the active center of the enzyme is decisive in the control of the selectivity for this biotransformation. If the substrate−enzyme interaction were relatively loose, then attack from either prochiral face of the substrate would be equally facile, since it could position itself randomly in the enzyme cavity. To achieve high enantioselectivities, besides steric effects, presumably alignment of the substrate in the active center of the enzyme by hydrophobic interactions with appropriate amino acids, e.g., alanine or phenylalanine, appear to be involved (Figure 4).

For the observed monooxygenase activity in the CH insertion we assume the established catalytic cycle for cytochrome P-450 enzymes, in which the oxygen-rebound mechanism applies.[28,29] Mechanistically important is the fact that the alcohol products **2f**α and **2f**β of the chiral substrate **1f** (Table 1, entry 6) were found in moderate to high enantiomeric excesses, whereas the recovered **1f** remained racemic after the biotransformation. The lack of kinetic resolution in this oxidation signifies that the abstraction of the H atom by the oxoiron complex (ferryl intermediate) proceeds without preference of one of the substrate enantiomers. It may, therefore, be concluded that in the initial step of the CH insertion a carbon-centered radical is formed without discrimination between the different prochiral faces of the starting material. The small amount of cinnamyl alcohol, which was observed as a byproduct of the hydroxylation of allylbenzene (**1c**), lends support that radical intermediates intervene in this oxidation. In the final step, the actual oxygen insertion, the hydroxy group is transferred from the iron complex preferentially to the less hindered side of the resulting radical. In addition to the crucial steric effects, the enantioselectivity is attenuated by hydrophobic interactions between the enzyme and the substrate (Figure 4). This is evident from the sharp increase in the enantioselectivity (Table 1, entries 1 and 2) for the hydroxylations of ethylbenzene (**1a**) compared to propylbenzene (**1b**). The hydrophobic attraction between the longer alkyl chain and the appropriate amino acid (in proximity to the prosthetic group) blocks the transfer of the hydroxy group to the pro-*S* face of the radical intermediate and properly aligns the substrate in the active center of the enzyme to facilitate the hydroxy-group transfer to the pro-*R* face (Figure 4). After the hydroxy group is transferred, the original iron state is regenerated and reenters the catalytic cycle.

In regard to the sense in the *R* versus *S* selectivity, the biotransformation of pentylbenzene (**1g**) provides an unusual dependence for the four possible regioisomeric hydroxylation products (Table 1, entry 7). Thus, while the regioisomers 1-phenyl-1-pentanol (**2g**α) and 5-phenyl-2-pentanol (**2g**δ) are *S*-configured, for the **2g**β and **2g**γ regioisomers the *R* enantiomers are formed, the usual enantiomer obtained for all the other substrates employed (Table 1). This distinguished behavior cannot be explained in terms of kinetic resolution of the chiral alcohols through enantioselective oxidation, as the amounts of ketones found are too low to influence the enantiomeric excess of the alcohols. Therefore, in the case of (*S*)-alcohol formation, either different enzymes are involved or the substrate with the longer alkyl chain is positioned with the opposite orientation in the enzyme cavity to result in the observed reversed enantioselectivity.

In conclusion, we have shown that the *B. megaterium* strain employed herein as a nonpathogenic soil microorganism readily hydroxylates a variety of unfunctionalized arylalkanes selectively at the benzylic as well as non-benzylic positions, but not the phenyl ring, to yield the corresponding regiomeric alcohols in high enantiomeric excesses (up to 99% ee). Additionally, the regioselectivity of the oxidation may be altered by employing different inducers, under conservation of the absolute configuration with high enantioselectivities in the hydroxylated products. Furthermore, it was demonstrated that the stereochemical course of the hydroxylation is determined by steric effects between the enzyme and the substrate. Since for this environmentally benign process the regioselectivity may be conveniently adapted to suit different synthetic goals, further applications in organic synthesis seem promising and worthwile for the preparation of optically active oxyfunctionalized building blocks from readily available simple hydrocarbon substrates.

Acknowledgment. We express our gratitude to Dr. U. Rdest (Institute of Microbiology, University of Würzburg, Germany) for her valuable help and advice. Furthermore, we thank the Deutsche Forschungsgemeinschaft (SFB 347 "Selektive Reaktionen Metallaktivierter Moleküle") and the Fonds der Chemischen Industrie for their generous financial support.

Supporting Information Available: Experimental procedures with bacterial screening protocol, biotransformation conditions, and MDGC columns for the alcohols **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

(29) Wong, L.-L. *Curr. Opin. Chem. Biol.* **1998**, *2*, 263−268.

JO991725S