Notes

Microbiological Transformations. 27. The First Examples for Preparative-Scale Enantioselective or Diastereoselective Epoxide Hydrolyses Using Microorganisms. An Unequivocal Access to All Four Bisabolol Stereoisomers

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Asymmetric synthesis is a question of utmost importance since it is now beyond question that most bioactive compounds will have to be prepared, or at least studied, in the form of their separate enantiomers. In this context, enantioselective preparation of epoxides constitutes a challenging and important synthetic problem, since these compounds are obviously highly valuable chiral synthons. Numerous approaches, the most famous one being the Sharpless method[1] which allows direct stereoselective oxidation of various olefins, have been explored in order to achieve the synthesis of epoxides in optically pure form. We have previously been interested in studying microbial transformations in order to prepare epoxides with high enantiomeric excesses (ees) using direct stereoselective oxygenation of olefins.[2] Other appealing ways to such chirons would be either the enantioselective hydrolysis of racemic epoxides or the diastereoselective transformation of a mixture of diastereoisomeric epoxides. This last approach could constitute a very interesting method since separation of diastereoisomers using classical techniques may, in certain cases, be extremely tedious and time consuming, leading to only low yields of pure material. However, to the best of our knowledge, no such examples have been described using conventional chemical methods.

In fact, epoxides are involved in the metabolism of a variety of aliphatic and aromatic compounds in plants and animals. They are by nature biologically toxic compounds and have to be detoxified *via* some enzymatic way. Prodigious effort has gone into the study of mammalian microsomal and cytosolic epoxide hydrases because of their likely involvement in the metabolism of toxic, mutagenic, and/or carcinogenic xenobiotics, as well as of environmental chemicals.[3] It is now known that two distinct mechanisms operate: the first one involves cytosolic glutathione transferase enzymes and the second one some epoxide hydrolase (hydrase) (EH) enzymes able to readily catalyze hydration of epoxides to the corresponding vicinal diols. These are further conjugated to afford water-soluble products.[3a] Unfortunately, this is of little preparative value since it is hardly possible to use such mammalian enzymes to produce large-scale quantities of product. Another very tempting, but almost yet unexplored strategy, would be to achieve these hydrolyses using whole-cell cultures of easily available microorganisms. Surprisingly, studies on the microbiological degradation of epoxides are very scarce. Moreover, the few known examples have been conducted essentially on the analytical scale and were often aimed toward biochemical studies of the purified epoxide hydrolase.[4a-e] Interesting results have, however, been obtained in this context,[4f-k] which suggest that the biohydrolysis of racemic epoxides could be a new tool of high preparative value to the organic chemist, eventually allowing the facile preparation of various epoxides in a high state of enantiomeric purity.

We describe here the first easy preparative access to such epoxides using microbiologically mediated hydrolysis which allows either enantioselective discrimination of a starting racemate or diastereoselective hydrolysis of a mixture of diastereoisomers. Using this technique it is now possible to separately prepare, in good yields and on a multigram scale, the optically pure 6*S* epoxide of geraniol *N*-phenylcarbamate. Similarly, this allows separate preparation of the four stereoisomers of 8,9-epoxylimonene for which the separation of the formed diastereoisomeric mixtures has not yet been properly solved with acceptable yields.[5] These are valuable key building blocks allowing the one-step synthesis of the four stereoisomers of bisabolol.

Results and Discussion

Enantioselective Hydrolysis. We have recently described the stereoselective biodihydroxylation of geraniol *N*-phenylcarbamate (1) by the fungus *Aspergillus niger* (LCP 521) and shown that the mechanism involves the stereospecific epoxidation of the 6–7 double bond, the resulting (6*S*)-2 epoxide being further processed *via* an epoxide hydrolysis which, at nearly neutral pH, must be enzymatically mediated.[2c] The presence of such an EH in this fungus prompted us to study the possibility of achieving an enantioselective hydrolysis of the racemic epoxide using this microorganism.[6]

(1) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

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(3) (a) Seidegard, J.; Depierre, J. W. *Biochim. Biophys. Acta* **1983**, *695*, 251. (b) Meijer, J.; Depierre, J. W. *Chem.-Biol. Interact.* **1988**, *64*, 207. (c) Prestwich, G. D.; Graham, S. M. G.; König, W. A. *J. Chem. Soc., Chem. Commun.* **1989**, 575. (d) Belluci, G.; Chiappe, C.; Marioni, F. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2369. (e) Bellucci, G.; Capitani, I.; Marioni, F.; Chiappe, C. *J. Chem. Soc., Chem. Commun.* **1989**, 1170. (f) Bellucci, G.; Berti, G.; Chiappe, C.; Fabri, F.; Marioni, F. *J. Org. Chem.* **1989**, *54*, 968. (g) Bellucci, G.; Chiappe, C.; Conti, L.; Marioni, F.; Pierini, G. *Ibid.* **1989**, *54*, 5978.

(4) (a) Hartmann, G. R.; Frear, D. S. *Biochem. Biophys. Res. Commun.* **1963**, *10*, 366. (b) Allen, R. H.; Jakoby, W. B. *J. Biol. Chem.* **1969**, *244*, 2078. (c) Niehaus, W. G.; Kisic, A., Jr.; Torkelson, A.; Bednarczyk, D. J.; Schroepfer, G. J. *Ibid.* **1970**, *245*, 3802. (d) Kolattukudy, P. E.; Brown, L. *Arch. Biochem. Biophys.* **1975**, *166*, 599. (e) Jacobs, M. H. J.; Van den Wijngaard, A. J.; Pentenga, M.; Janssen, D. B. *Eur. J. Biochem.* **1991**, *202*, 1217. (f) Suzuki, Y.; Imai, K.; Marumo, S. *J. Am. Chem. Soc.* **1974**, *96*, 3703. (g) De Bont, J. A. M.; Van Dijken, J. P.; Van Ginkel, K. G. *Biochim. Biophys. Acta* **1982**, *714*, 465. (h) Weijers, C. A. G. M.; De Haan, A.; De Bont, J. A. M. *Appl. Microbiol. Biotechnol.* **1988**, *27*, 337. (i) Escoffier, B.; Prome, J. C. *Bioorg. Chem.* **1989**, *17*, 53. (j) Jabbouri, S. Ph.D. Thesis, University of Toulouse, France, 1990. (k) Weijers, C. A. G. M.; De Bont, J. A. M. *Enzyme Microb. Technol.* **1991**, *13*, 306.

(5) (a) Farges, G.; Kergomard, A. *Bull. Soc. Chem. Fr.* **1969**, *69*, 4476. (b) Kergomard, A.; Veschambre, H. *Tetrahedron* **1977**, *33*, 2215.

(6) We have tested that no noticeable hydrolysis occurs in these experimental conditions using phosphate buffer without microbial cells.

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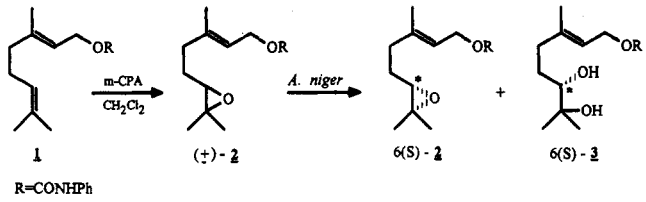
Scheme I



Since the stereochemical outcome of racemic substrate hydrolysis is known to be time dependent, we have monitored this reaction over a 22-h period. The yields and ees of the obtained residual epoxide **2** and formed diol **3** are shown in Table I. It clearly appears that *hydrolysis of rac-2 is enantioselective*, the remaining 6*S* enantiomer being obtained with an ee as high as 96% after a 3-h culture. After 22 h, all epoxide **2** was transformed leading to a 55% yield of (almost) racemic diol. Obviously, some material (i.e., epoxide **2** or diol **3**) had been consumed *via* an unknown catabolic way, which may involve oxygen incorporation (hydroxylation) steps. In order to try to limit this loss of material, we studied the same reaction under inert atmosphere (N_2) (Table I). It thus appeared that, whereas the results are quite similar for the short-time runs, an important amount of epoxide **2** (40% isolated yield) remains after 22 h when a nitrogen atmosphere is used. As shown previously, this was proven to be the (6*S*)-**2** enantiomer and showed an excellent 96% ee.

The enantiomeric excess of diol **3** has been determined using HPLC analysis of its (−)-camphanic ester. Similarly, the epoxide ee has been determined, *via* its diol, after acid hydrolysis of **2** (which, as we have shown previously, does not alter the absolute configuration at C(6)).[2b] The absolute configurations of the diols have been determined by comparison of their optical rotation sign with previously described data[2b] and confirmed by HPLC analysis (elution order comparison with known samples).

Because of the high potential value of optically pure **2** as a chiron,[7] a multigram process has been devised. In a typical experiment, 5 g of starting racemic **2** have been transformed using resting cells (suspended in 1 L of phosphate buffer at pH 7) obtained from a culture of *A. niger* grown in a 7-L fermentor jar. Prior to substrate addition, this culture was placed under nitrogen and the bioconversion was then performed for 22 h. After normal workup, this led to a 42% isolated yield (2.1 g) of remaining (6*S*)-**2**, which showed a 94% ee. The formed (6*S*)-**3** diol (2.3 g; 43% yield) showed also an enantiomeric enrichment (ee 40%).

These results indicate that the mechanism involves a preferential hydrolysis of the 6*R* epoxide **2**, leading to the 6*S* diol **3**. Thus, an enzymatically catalyzed nucleophilic water addition occurs at C(6), involving inversion of the absolute configuration at this carbon atom. Interestingly, this corresponds to a *trans* addition on the epoxide, in sharp contrast to previously described results where *cis* hydration was involved, at least in some proportion,[4f] if not exclusively.[4d]

(7) (a) Yamada, S.; Oh-Hashi, N.; Achiva, K. *Tetrahedron Lett.* **1976**, 2557. (b) Yamada, S.; Oh-Hashi, N.; Achiva, K. *Ibid.* **1976**, 2561. (c) Eschenmoser, W.; Vebelhart, P.; Eugster, C. H. *Helv. Chim. Acta* **1983**, *66*, 82. (d) Kamber, M.; Pfander, H.; Noack, K. *Ibid.* **1984**, *67*, 968. (e) Meier, H.; Vebelhart, P.; Eugster, C. H. *Ibid.* **1986**, *69*, 106. (f) Aziz, M.; Rouessac, F. *Tetrahedron* **1988**, *44*, 101. (g) Méou, A.; Bouanah, N.; Archelas, A.; Zhang, X. M.; Guglielmetti, R.; Furstoss, R. *Synthesis* **1990**, 752. (h) Méou, A.; Bouanah, N.; Archelas, A.; Zhang, X. M.; Guglielmetti, R.; Furstoss, R. *Ibid.* **1991**, 681.

Table I

expl condns[a]	time, h	recovered epoxide 2[e] ee, %	recovered epoxide 2[e] abs confign	diol 3 yield,[d] %	diol 3 ee	diol 3 abs confign
b	1	91	*S*	29	43	*S*
b	3	94	*S*	39	39	*S*
b	22		*S*	55	5	*S*
c	1.5	88	*S*	34	53	*S*
c	3	97	*S*	47	40	*S*
c	22	96	*S*	60	37	*S*

[a] All these experiments were performed in a 1-L medium containing the resting cells using 1 g of **2** as starting compound. [b] Aerobic conditions. [c] Under nitrogen atmosphere. [d] The yields were determined by sampling aliquots (2 mL) which were extracted with Et_2O (1 mL) and analyzed by HPLC. [e] Analytical yields were not significant because of heterogeneous repartition (due to partial insolubility) of the epoxide in the medium and vessel.

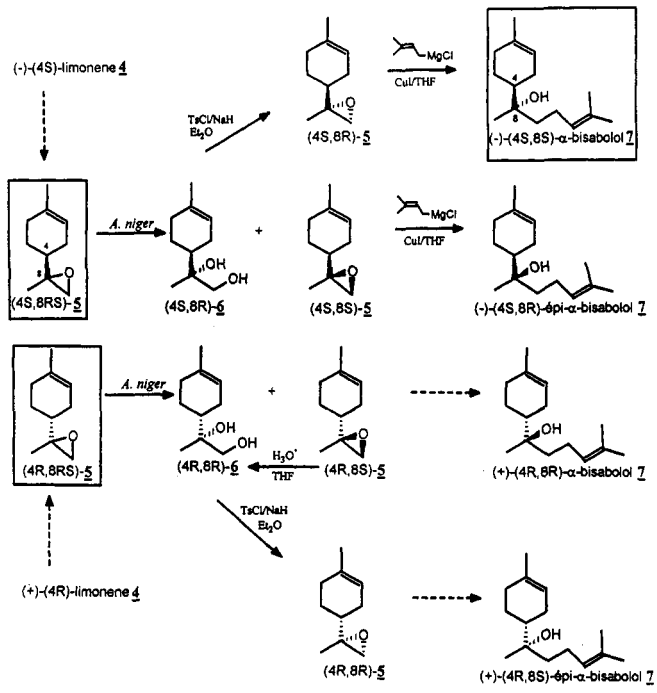
Our results also show that avoiding molecular oxygen during the bioconversion protects the remaining epoxide and/or the formed diol from further catabolism. This is particularly interesting as far as preparative-scale synthesis is concerned, since it makes it unnecessary to monitor very precisely the bioconversion ratio which varies quite rapidly during the first few hours. This drastic effect of the inert atmosphere can be due to the fact that the above-mentioned molecular oxygen dependent catabolism is suppressed. However, another explanation could be that rapid denaturation of the fungus (and therefore of the involved epoxide hydrolase) occurs in anaerobic conditions. Thus, since the hydrolysis of racemic **2** is obviously kinetically controlled, epoxide 6*R*-**2** is hydrolyzed during the first few hours where the epoxide hydrolase is active.

Diastereoselective Hydrolysis. In the course of this study, we also wondered if, using the same experimental conditions, the EH involved in these previous hydrolysis would be able to selectively hydrolyze mixtures of diastereoisomers prepared from commercially available limonene enantiomers. Therefore, we have prepared the corresponding mixtures of 8,9-epoxylimonene diastereoisomers following a method previously described by Husstedt et al.[8] This leads in three steps to the mixture of (4*S*,8*R*)-**5** and (4*S*,8*S*)-**5** epoxides starting from 4(*S*)-limonene (**4**) (ee 99%) or to the mixture of (4*R*,8*R*)-**5** and (4*R*,8*S*)-**5** epoxides starting from 4(*R*)-limonene (**4**) (ee 99%) which, as stated[5] before, are very difficult to separate.

Each one of these mixtures has been submitted to a suspension of the fungus *A. niger* (Scheme II). In either case, the hydrolysis of the epoxide moiety occurs quite rapidly, and monitoring of the reaction rate using classical GC analysis shows a transformation ratio of about 50% after 1–2 h.[6] In both cases, a mixture of two products is obtained and is shown to contain some unreacted epoxide **5** and the corresponding diol **6**. Analysis of these compounds using a chiral GC column shows that, starting from each one of these two mixtures, the remaining epoxide as well as the formed diol are of high optical and diastereomeric purity (Figure 1). This means that *the hydrolysis reaction catalyzed by the intracellular EH of this fungus is highly diastereoselective*, thus allowing straightforward separation of the two diastereoisomeric epoxide mixtures. Interestingly enough, this procedure is easily achieved on the gram (or even multigram) scale and is therefore of high preparative value, in contrast to the previously reported approaches using cytosolic or microsomic EH.

(8) Husstedt, U.; Schafer, H. J. *Synthesis* **1979**, 966.

Scheme II



Bioconversion of the (4*S*,8*RS*)-Limonene Epoxides 5. In a typical experiment, 1 g of a mixture containing (4*S*,8*R*)- and (4*S*,8*S*)-**5** obtained from 4(*S*)-limonene led to 344 mg (34.4%) of unreacted **5** and 507 mg (45%) of **6**. These products were shown to exhibit diastereomeric excesses (de) as high as 98% and 94%, respectively. However, at this stage of our work, it was not possible to determine their absolute configuration since (4*S*,8*S*)- and (4*S*,8*R*)-**5** both exhibit a negative and quite similar optical rotation value.[5b] Interestingly, these epoxides constitute key chiral building blocks allowing for the one-step synthesis of the four stereoisomers of bisabolol. All stereoisomers of bisabolol are known as natural compounds: i.e., (−)-(4*S*,8*R*)-α-*epi*-bisabolol is a natural constituent of *Citrus bergamia RISSO* essential oil,[9] and its enantiomer (+)-(4*R*,8*S*)-α-*epi*-bisabolol has been isolated from different *Abies* and *Picea* species,[10] whereas (+)-(4*R*,8*R*)-α-bisabolol is a constituent of *Atalantia monophylla* correa oil[10,11] and its enantiomer (−)-(4*S*,8*S*)-α-bisabolol is the major constituent of German camomile (*Matricaria chamomolla L* essential oil).[12] This last enantiomer is used on an industrial scale for the preparation of various skin-care creams, lotions, and ointments because of its antiinflammatory, bactericidal, and antimycotic properties.[13] In fact, the determination of bisabolol configurations led to conflicting literature which has been recently critically reviewed by Sutherland.[10] Since these absolute configurations are now well established, synthesis of bisabolol from our obtained epoxides **5** could allow determination of their absolute configuration. We therefore achieved the transformation of the unreacted epoxide **5** to the corresponding bisabolol isomer, using a copper iodide catalyzed condensation of **5** with prenylmagnesium chloride.[14] This leads to a single product (79% yield) which is shown to be (−)-(4*S*,8*R*)-*epi*-α-bisabolol **7** by comparison of its ^{13}C NMR with those of an authentic sample of (−)-(4*S*,8*S*)-α-bisabolol **7**. Owing to the fact that no inversion of the absolute configuration at C-8 occurs during this tranformation,[14] the unreacted epoxide must be the (4*S*,8*S*) enantiomer.

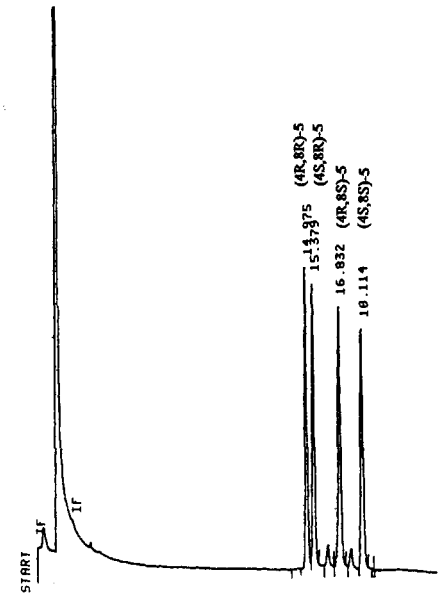


Figure 1. GC tracing of the four stereoisomers of epoxide **5** on the chiral capillary column [octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrine (T = 80 °C)].

We can assign the (4*S*,8*R*) absolute configuration to the formed diol **6** by the fact that recyclization of this diol, achieved by treatment with TsCl/NaH/Et_2O, leads to (4*S*,8*R*)-**5** (82% yield, de 94%) as shown by classical and chiral GC analysis. Since this process occurs with retention of configuration at the tertiary C-8 carbon atom,[15] the precursor diol must be of (4*S*,8*R*) absolute configuration. Interestingly enough, *this (4S,8R)-epoxide-5 leads to* (−)-*(4S,8S)-α-bisabolol* which is thus obtained (63% yield) in a state of diastereomeric purity (>95%) identical to that of the natural product.

Bioconversion of the (4*R*,8*RS*)-Limonene Epoxides 5. Similar to the mixture of epoxides obtained from 4(*S*)-limonene, the mixture of diastereomeric (4*R*,8*S*)- and (4*R*,8*R*)-**5** was submitted to the same bioconversion conditions. It was indeed interesting to check the outcome of this reaction since a combination of diastereo- and/or enantioselectivity of the EH enzyme could lead to quite different results. In particular, the possible influence of the stereogenic C-4 carbon atom on the enzyme–substrate interaction was unpredictable. In fact, as stated before, we again obtained a mixture of remaining epoxide **5** (35% yield) and of diol **6** (44% yield). The de of these products were established as being, respectively, 98% and 89%. Their absolute configurations were determined, using classical GC analysis comparison with their previously obtained diastereoisomers, as being (4*R*,8*S*)-**5** and (4*R*,8*R*)-**6**. Obviously, both of these products can lead to the corresponding bisabolol stereoisomers, thus allowing a formal unequivocal synthesis of all four bisabolol stereoisomers.

(9) Ohloff, G.; Giersch, W.; Naf, R.; Delay, F. *Helv. Chim. Acta* **1986**, *69*, 698.
(10) O'Donnel, G. W.; Sutherland, M. D. *Aust. J. Chem.* **1989**, *42*, 2021.
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(14) Carman, R. M.; Duffield, A. R. *Aust. J. Chem.* **1989**, *42*, 2035.
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Conclusion

In conclusion, these results clearly open the way to the use of a whole cell microbiological process allowing—either enantioselectively or diastereoselectively—hydrolysis of epoxide mixtures. This allows straightforward multigram preparative-scale preparation of epoxides showing very high enantiomeric purity. We have shown that, in the cases studied, the procedure can be very much improved by using anaerobic conditions. These selective hydrolyses, performed using the fungus *A. niger*, are presumably mediated by an intracellular EH enzyme which shows high enantio- and/or diastereoselectivity, as well as perfect regio- and stereoselectivity, in the course of the reaction. Interestingly, in both cases of geraniol epoxide derivatives and limonene epoxide stereoisomers a highly preferential hydrolysis of the epoxide stereoisomer bearing a 8*R* stereogenic center has occurred, an observation which is an almost general rule for mammalian microsomal EH. Due to the occurrence of such high selectivities it is now possible to achieve in a unequivocal fashion the synthesis of 6(*S*)-epoxygeraniol *N*-phenylcarbamate (**2**) as well as of the four stereoisomers of epoxide **5** which are all obtained in an excellent state of enantiomeric purity (ee 99%, due to the enantiomeric purity of the starting limonene) and a high state of diastereomeric purity (de 98%). Thus, (4*S*,8*S*)-**5** and (4*R*,8*S*)-**5** are obtained directly as remaining products of these reactions, whereas their corresponding 4*R*,8*R* and 4*S*,8*R* enantiomers may be prepared starting from the formed diols **6**. Moreover, these two last epoxide diastereoisomers are also obtainable, with only a small diminution of diastereomeric purity, *via* a simple acidic hydrolysis/recyclisation process starting from (4*R*,8*S*)-**5** and (4*S*,8*S*)-**5**. We have controlled this possibility in the case of the 4(*R*)-limonene series for which (4*R*,8*S*)-**5** (de 98%) leads to (4*R*,8*R*)-**5** (de 87%). These various experiments allow a theoretically total conversion of the starting limonene to the key building blocks of each of the four bisabolol stereoisomers. The (−)-α-bisabolol is known to be of high industrial value for the cosmetic industry. Work is in progress in our laboratory in order to explore the scope and limitations of this new preparative-scale bioconversion technique.

Experimental Section

General. Vapor-phase chromatography analyses were performed by using a classic 25-m capillary column (OV 1701) or a chiral 25-m capillary column[16] [octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin] at 80 °C for the determination of diastereo- and enantiomeric excesses of epoxide **5** [elution order: (4*R*,8*R*)-**5** t_R = 14.9 min; (4*S*,8*R*)-**5** t_R = 15.4 min; (4*R*,8*S*)-**5** t_R = 16.8 min; (4*S*,8*S*)-**5** t_R = 18.1 min]. Separation and purification of the products were achieved by flash chromatography (silica gel 60 H from Merck and solvent mixtures consisting of hexane and ether in the range of 100% hexane to 100% ether). ^1H and ^{13}C NMR spectra have been realized on a Bruker AM 100 apparatus. Natural (−)-α-bisabolol was purchased from INTERCHIM.

Biohydrolysis of 6,7-Epoxy-3,7-dimethyl-2-octen-1-yl Phenylcarbamate (2). Analytical Biohydrolysis Experiments (Table I). Analytical experiments were carried out in a 2-L fermentor jar filled with 1 L of medium (20 g of corn steep liquor, 10 g of glucose in 1 L of tap water). Before sterilization (20 min at 115 °C), 10 mL of liquid paraffin and 0.05 mL of antifoam silicone 426 R (PROLABO) were added to prevent overflowing during the growth. The medium was maintained at 27 °C, stirred at 700 rpm, and aerated with sterilized air (12 L/h). The broth was inoculated by transferring to the medium a little piece of gelose (about 1 cm² of surface) supporting the mycelium and the black spores from a 3-day-old agar slant growth of *A. niger* (LCP 521). After incubation for 40 h the mycelium was filtered off, washed with water, and then placed back in the same fermentor filled with 1 L of a pH 7 phosphate buffer (0.1 M) solution. The experimental conditions were the same as described above (27 °C, 700 rpm) but the medium was flushed with N_2 and maintained under N_2 or aerated (12 L/h). An ethanolic solution of substrate (1 g/10 mL) was added to the culture. The evolution of enantiomeric excess was followed by withdrawing samples (100 mL) at time intervals. For each sample the mycelium was filtered off and the fungal cake was washed with ether. After decantation the aqueous phase was saturated with NaCl and then extracted twice with ether. The organic layers were dried ($MgSO_4$) and evaporated in vacuum. The crude product was purified by flash chromatography before ee determination by HPLC analysis.[2b]

Preparative Biohydrolysis Experiment. The fermentation was carried out in a 7-L fermentor jar charged with 5 L of medium. Before sterilization (30 min at 115 °C) 40 mL of liquid paraffin and 0.2 mL of antifoam silicone 426 R (PROLABO) were added. The medium was maintained at 27 °C, stirred at 500 rpm, and aerated with sterilized air (60 L/h). The broth was inoculated by transferring to the medium a big piece (about 5 cm² of surface) of gelose supporting the mycelium and the black spores from a 3-day-old agar slant. After incubation for 40 h the mycelium was filtered off, washed with water, and then transferred to a 2-L fermentor containing 1 L of a pH 7 (0.1 M) phosphate buffer. The medium was stirred at 1100 rpm and maintained at 27 °C. Before addition of the racemic epoxide **2** (5 g) as a solution in ethanol (25 mL) the medium was flushed with N_2 and maintained under an atmosphere of N_2 during the bioconversion.

After incubation for 22 h the workup was carried out as described for the analytical experiments to afford 2.1 g of (6*S*)-**2** (42%) as a colorless oil and 2.3 g of (6*S*)-**3** (43%).

(6*S*,2*E*)-6,7-Epoxy-3,7-dimethyl-2-octen-1-yl Phenylcarbamate (2). The product has physical properties similar to those previously described,[2b] except: $[\alpha]^{20}_D$ = −0.93 (*c* 2.15, MeOH). Anal. Calcd for $C_{17}H_{21}NO_2$ (289.2): C, 70.54; H, 8.02; N, 4.86. Found: C, 70.39; H, 8.05; N, 4.91.

(6*S*,2*E*)-6,7-Dihydroxy-3,7-dimethyl-2-octen-1-yl Phenylcarbamate (3). The product has physical properties similar to those previously described,[2b] except: $[\alpha]^{20}_D$ = −9.9 (*c* 2.01, MeOH). Anal. Calcd for $C_{17}H_{23}NO_3$ (307.2): C, 66.40; H, 8.20; N, 4.56. Found: C, 67.58; H, 8.11; N, 4.46.

Biohydrolysis of (4*S*,8*RS*)- and (4*R*,8*RS*)-8,9-Epoxy-*p*-menth-1-ene (5). Incubation conditions are identical to those described for analytical biohydrolysis of epoxide **2** except the medium aeration was always stopped before substrate addition. The course of the bioconversion (diastereomeric epoxide proportions) was followed by withdrawing samples (2 mL) at time intervals. The samples were each extracted with ethyl acetate (1 mL), and the organic phase was analyzed by GC. The bioconversion was stopped by addition of ether (500 mL) in the fermentor when epoxide **5** exhibited de as high as 98%. The mycelium was filtered off, and the fungal cake was washed with ether. After decantation the aqueous phase was extracted twice with ether. The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo. The crude product was purified by flash chromatography to afford the desired epoxide **5** (5% ether–hexane eluent) then the diol **6** (100% ether eluent).

Biohydrolysis of (4*S*,8*RS*)-8,9-Epoxy-*p*-menth-1-ene (5). The biohydrolysis of (4*S*,8*RS*)-epoxide **5** (1 g) led to 344 mg (34%) of (4*S*,8*S*)-epoxide **5** [$[\alpha]^{25}_D$ −75.8 (*c* 1.57, EtOH) (lit.[5b] $[\alpha]^{25}_{578}$ −93 (*c* 2.8, EtOH)] and 507 mg (45%) of (4*S*,8*R*)-diol **6** [$[\alpha]^{25}_D$ −77.9 (*c* 1.99, EtOH)]. These products have physical and spectral properties similar to those described previously.[5b]

Biohydrolysis of (4*R*,8*RS*)-8,9-Epoxy-*p*-menth-1-ene (5). The biohydrolysis of (4*R*,8*RS*)-epoxide **5** (1 g) led to 350 mg (35%) of (4*R*,8*S*)-epoxide **5** [$[\alpha]^{25}_D$ +87.7 (*c* 1.39, EtOH) (lit.[5b] $[\alpha]^{25}_{578}$ +95.5 (*c* not indicated, EtOH))] and 440 mg (44%) of (4*R*,8*R*)-diol **6** [$[\alpha]^{25}_D$ +91 (*c* 1.4, EtOH) (lit.[5b] $[\alpha]^{25}_{578}$ +94.8 (*c* 6.1, EtOH))]. These products have physical and spectral properties similar to those described previously.[5b]

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Synthesis of (−)-(4*S*,8*R*)-*epi*-α-Bisabolol (7). This compound is obtained (79% yield) by a copper iodide catalyzed condensation of (4*S*,8*S*)-**5** with (3-methylbut-2-enyl)magnesium chloride according to a literature procedure:[14] $[\alpha]^{25}_D$ −67 (*c* 1.74, EtOH) (lit.[10] $[\alpha]^{27}_D$ −69 (*c* 1.3, EtOH)); ^{13}C NMR (with the corresponding peak for natural (−)-(4*S*,8*S*)-α-bisabolol in parentheses) ($CDCl_3$/TMS) δ = 17.66 (17.69), 22.34 (22.13), 23.35 (23.23), 23.96 (23.37), 24.05 (23.37), 25.73 (25.73), 26.13 (26.98), 31.12 (31.09), 39.42 (40.18), 43.39 (43.03), 74.32 (74.30), 120.85 (120.62), 124.67 (124.67), 131.65 (131.65), 133.77 (134.11); ^{1}H-NMR ($CDCl_3$/TMS) δ = 1.16 (3 H, s, C_8-Me), 1.2–2.3 (m, 21 H), 5.16 (t, *J* = 7 Hz, 1 H), 5.42 (m, 1 H).

Synthesis of (−)-(4*S*,8*S*)-α-Bisabolol (7). From 0.2 g of (4*S*,8*R*)-epoxide **5**, 0.17 g (63% yield) of (−)-α-bisabolol **7** was obtained after condensation with allylmagnesium chloride:[14] $[\alpha]^{25}_D$ −54.9 (*c* 1.57, EtOH) (lit.[10] $[\alpha]^{25}_{578}$ −59 (*c* 1.2, EtOH)); INTERCHIM product $[\alpha]^{25}_D$ −54.3 (*c* 1.27, EtOH). The ^{1}H and ^{13}C NMR spectra were identical with the spectra of the natural (−)-(4*S*,8*S*)-α-bisabolol.

Synthesis of (−)-(4*S*,8*R*)-Epoxide 5 from (−)-(4*S*,8*R*)-Diol 6. Diol **6** (370 mg, 2.18 mmol), obtained from the biohydrolysis of the (4*S*,8*RS*)-epoxide **5** and *p*-toluenesulfonyl chloride (498 mg, 2.62 mmol) were dissolved in dry Et_2O (30 mL). NaH (80% in oil, 1 g) was added in small portions. After 48 h, water was carefully added, and the aqueous phase was extracted twice with Et_2O. The organic phase was washed with water, dried ($MgSO_4$), and evaporated. Purification of the crude product by flash chromatography led to the colorless oily epoxide **5** (270 mg, 82%): $[\alpha]^{25}_D$ −95.4 (*c* 1.21, EtOH) (lit.[5b] $[\alpha]^{25}_{578}$ −88 (*c* 1, EtOH)).

Synthesis of Diol 6 from (+)-(4*R*,8*S*)-Epoxide 5. To a stirred solution of (+)-(4*R*,8*S*)-epoxide **5** (100 mg, 0.66 mmol, de 98%) in 2 mL of THF and 0.8 mL of H_2O was added a drop of concd H_2SO_4. After 1 h at room temperature, 5 mL of a saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted three times with Et_2O. The organic phase was washed with water, dried ($MgSO_4$) and then evaporated. Purification of the crude product by flash chromatography led to (4*R*,8*R*)-diol **6** (86 mg, 73%): $[\alpha]^{25}_D$ +88 (*c* 1.44, EtOH).

Synthesis of (+)-(4*R*,8*R*)-Epoxide 5 from (+)-(4*R*,8*R*)-Diol 6. Eighty mg (0.47 mmol) of diol **6**, obtained from acidic hydrolysis of (4*R*,8*S*)-epoxide **5** (de 98%), were transformed to (4*R*,8*R*)-epoxide **5** (60 mg, 84%, de 87%) using the same procedure (NaH/TsCl) as described above for the synthesis of (−)-(4*S*,8*R*)-epoxide **5**: $[\alpha]^{25}_D$ +81.6 (*c* 1.46, EtOH) (lit.[5b] $[\alpha]^{25}_{578}$ +89.2 (*c* 0.43, EtOH)).

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