Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 1519–1528

TETRAHEDRON: *ASYMMETRY*

Biocatalytic asymmetric and enantioconvergent hydrolysis of trisubstituted oxiranes

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Received 21 May 2001; accepted 4 June 2001

Abstract—Asymmetric biohydrolysis of trialkyl oxiranes (±)-**1a**–**3a** using the epoxide hydrolase activity of whole bacterial cells proceeded in an enantioconvergent fashion and thus led to the corresponding (*R*)-configurated vicinal diols **1b**–**3b** in up to 97% enantiomeric excess (e.e.) as the sole product. The mechanism of this enantioconvergence was investigated by ^{18}O-labelling experiments and it was found that both enantiomers were hydrolysed with opposite regioselectivity.

1. Introduction

The asymmetric hydrolysis of epoxides using (bio)-chemical catalysts provides a convenient access to non-racemic vicinal diols. For monosubstituted oxiranes, the Jacobsen Co^{II}-salen catalyst[1] gives the best selectivities, whereas microbial epoxide hydrolases are the biocatalysts of choice for more sterically demanding disubstituted analogues.[2] A particular feature of the latter processes is the fact that (particularly for 2,3-disubstituted oxiranes) the asymmetric biohydrolysis proceeds in an enantioconvergent fashion, which leads to the formation of a single enantiomeric vicinal diol as the sole product.[3] The mechanistic reason for such a 'deracemisation' is based on the fact that both oxirane enantiomers are attacked with opposite regioselectivity, which is facilitated by the steric similarity of both adjacent oxirane carbon atoms.

For more sterically demanding trisubstituted epoxides, however, enantioconvergent hydrolysis is rather unlikely, since it would require a nucleophilic attack on a sterically congested carbon atom. Although such a pathway has been observed in mechanistic studies,[4] no preparative-scale reaction has been reported to date. The reported data on the asymmetric biohydrolysis of trisubstituted oxiranes show that these sterically demanding substrates are not easily accepted by microbial epoxide hydrolases.[5,6] For instance, (±)-1-methylcyclohexene oxide was hydrolysed by resting cells of *Corynebacterium* C12,[7] *Rhodotorula glutinis* CIMW 147[8] and an isolated epoxide hydrolase from *Rhodococcus erythropolis* DCL 14.[9] In each case, the reaction proceeded via kinetic resolution producing two enantiomers (i.e. formed diol and unreacted epoxide) possessing opposite configuration. In contrast, diastereoisomeric limonene oxides derived from either (+)- or (−)-limonene were hydrolysed in a diastereoconvergent fashion to give a single enantiomeric diol.[10,11] The only enantioconvergent transformation of a trisubstituted oxirane was reported for (±)-10,11-epoxyfarnesol using *Helminthosporium sativum* to give the (*S*)-diol as the sole metabolite.[12] In order to provide a mechanistic explanation for the enantioconvergence, an enzymatic *cis*-hydration was assumed, which (in view of the nowadays generally accepted mechanism of action for epoxide hydrolases[13]) is extremely unlikely and warrants a more detailed investigation.

Herein, we present our results on the biocatalytic hydrolysis of trisubstituted epoxides (±)-**1a**–**3a** using the epoxide hydrolase activity of whole bacterial cells.

2. Results and discussion

Epoxides (±)-**1a**–**3a** were prepared via Wittig reaction from commercially available phosphonium salts via the corresponding alkenes **1**–**3** (Scheme 1). Due to their volatility, the latter were not isolated and the crude

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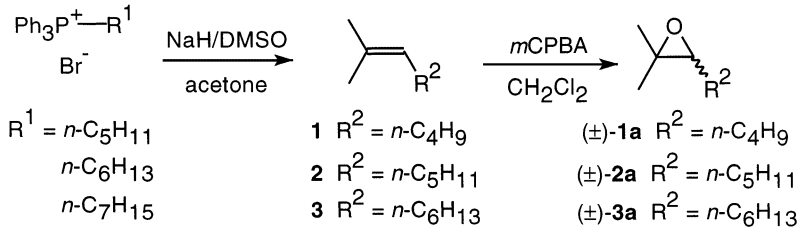
PII: S0957-4166(01)00256-7



Scheme 1. Synthesis of substrates (±)-**1a**–**3a**.

product was directly subjected to epoxidation using *m*-chloroperbenzoic acid. In order to validate the outcome of the bioreactions, spontaneous hydrolysis of substrates (±)-**1a**–**3a** was proven to be negligible (<2% within 48 h).

A screening for biocatalytic activity was performed by using whole bacterial cells in Tris-buffer at pH 8.0 and 30°C for 48 h (Scheme 2). With one exception (Table 1, entry 8) diols **1b**–**3b** were isolated as the sole product of the biotransformation. Their structural identity was confirmed by co-injection on GLC with the racemic diols (±)-**1b**–**3b** obtained via acid catalysed hydrolysis of the corresponding epoxides. Enantiomeric purities of products from the biohydrolysis were determined by GLC on a chiral stationary phase. In line with previous observations,[3] diols **1b**–**3b** were (*R*)-configured and were obtained in moderate to excellent enantiomeric excesses.

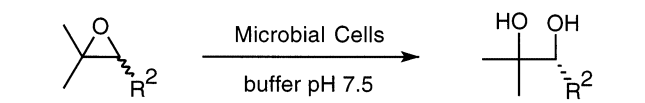
Absolute configurations of non-reacted epoxides and formed diols were determined by co-injection on GLC using independently synthesised reference material (Scheme 3). Diol (*S*)-**4** was obtained in 87% e.e. by asymmetric dihydroxylation of 2-methylocta-2,7-diene according to Sharpless.[26] The latter material was hydrogenated (Pd/C, H_2) to furnish (*S*)-**2b** without loss of enantiomeric purity. Products (*S*)-**1b** and (*S*)-**3b** were obtained from alcohol (*S*)-**5**, which was synthesised from (*S*)-**4** via (i) protection of the diol as the acetonide, followed by (ii) oxidative cleavage of the terminal C=C bond (O_3) and (iii) reductive work-up of the ozonide ($LiAlH_4$). Removal of the primary alcohol moiety by mesylation and nucleophilic displacement by hydride ($LiAlH_4$) and deprotection of the *vic*-diol moiety gave (*S*)-**1b**. C2-Chain extension of (*S*)-**5** was achieved by standard methodology (halogenation of the primary alcohol moiety via the mesylate, followed by nucleophilic displacement with EtMgBr and deprotection of the acetonide) to yield (*S*)-**3b**. Reference material for (*S*)-**1a**–**3a** was obtained by treatment of diols (*R*)-**1b**–**3b** (formed during the biotransformation) with triflic anhydride in pyridine to give the corresponding epoxides of opposite configuration.

In contrast to the majority of asymmetric biotransformations (where the absolute configuration of the stereogenic centre always remains unaffected throughout the reaction), the enzymatic hydrolysis of epoxides may take place with different regioselectivity via attack at either one of the oxirane carbon atoms which may be different for both enantiomers (Scheme 4). As a consequence, four stereochemical pathways (denoted via their apparent first-order relative rate constants k_1 through k_4) are possible. For trisubstituted oxiranes possessing only a single stereogenic centre, two pairs of pathways are possible, which proceed either via inversion (though attack at the stereogenic centre, k_2, k_3) or via (apparent) retention of configuration (via attack at the fully substituted oxirane carbon atom, k_1, k_4), and their relative value determines the stereochemical outcome of the reaction. The following parameters are used to describe the characteristics of these reactions: (i) The enantioselectivity (defined as the relative reaction rate of enantiomers) is determined as $E=(k_3+k_4)/(k_1+k_2)$ or reciprocal value, and the (ii) regioselectivity for each enantiomer is expressed as $\alpha_S=k_2/k_1$ and $\alpha_R=k_4/k_3$. Relative rate constants were calculated from progress curves based on datasets of $e.e._S$, $e.e._P$ and conversion versus time by employing the computer program '*EntCon*'.[14] The validity of the data obtained was verified by ^{18}O-labelling experiments (see below).

The data depicted in Table 1 reveals the following features of the process.

(i) In general, [for exceptions see (iii)], (*R*)-diols were formed from the (faster reacting) corresponding (*S*)-epoxides via attack at the less congested C(3) stereocentre leading to inversion of configuration ($k_2>k_1$ and $k_2>k_4$). As a consequence, it can be concluded that the slower reacting epoxide possessed (*R*)-configuration.

(ii) Two *Rhodococcus* strains (previously denoted as *Nocardia*,[15] entries 3 and 4) were shown to prefer the (*R*)-epoxide with opposite regioselectivity by attacking the fully substituted oxirane C-atom, thus forming (*R*)-diols with retention of configuration ($k_4>k_3$ and $k_4>k_2$).[16]



(±)-**1a** R^2 = *n*-C_4H_9 (*R*)-**1b** R^2 = *n*-C_4H_9
(±)-**2a** R^2 = *n*-C_5H_{11} (*R*)-**2b** R^2 = *n*-C_5H_{11}
(±)-**3a** R^2 = *n*-C_6H_{13} (*R*)-**3b** R^2 = *n*-C_6H_{13}

Scheme 2. Asymmetric biohydrolysis of epoxides (±)-**1a**–**3a**.

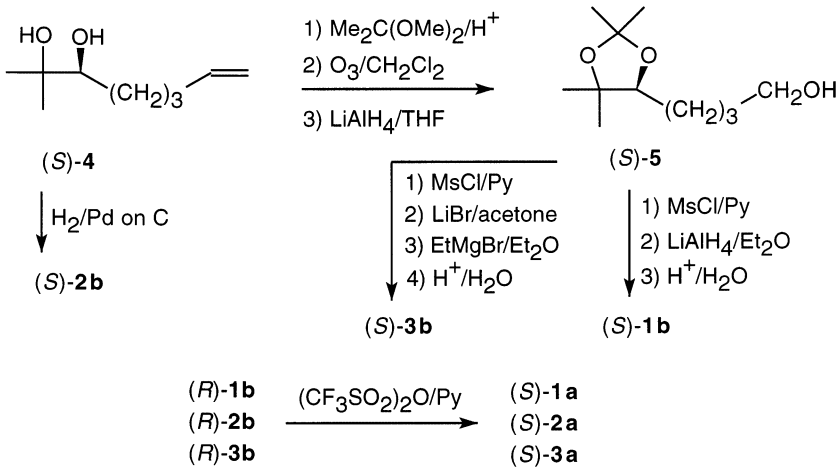
Table 1. Selectivities of asymmetric biohydrolysis of (±)-**1a**–**3a**

Entry	Microorganism	Substrate	Conversion (%)	Diol		Epoxide		Relative rate constants[a]				Enantioselectivity (E^b)
				Abs. Config.	E.e. (%)	Abs. Config.	E.e. (%)	k_1	k_2	k_3	k_4	
1	*M. paraffinicum* NCIMB 10420	(±)-**1a**	76	*R*	89	*R*	99	2	100	1	5	17
2	*R. ruber* DSM 43338	(±)-**1a**	49	*R*	86	*R*	9	1	25	1	19	1.3
3	*R. ruber* SM 1789	(±)-**2a**	85	*R*	85	*S*	92	1	15	9	27	2.3
4	*R. ruber* SM 1790	(±)-**2a**	80	*R*	83	*S*	91	1	18	12	80	4.7
5	*S. lavendulae* ATCC 55209	(±)-**2a**	49	*R*	91	*R*	51	1	61	3	49	1.2
6	*S. lavendulae* ATCC 55209	(±)-**3a**	60	*R*	97	*R*	23	1	126	1	99	1.3
7	*M. paraffinicum* NCIMB 10420	(±)-**3a**	90	*R*	83	*R*	93	1	723	24	151	4.2
8	*R. ruber* CBS 717.73	(±)-**2a**	46	*R*	55	*R*	99	1	−4.5	−2.2	−0.8	n.a.

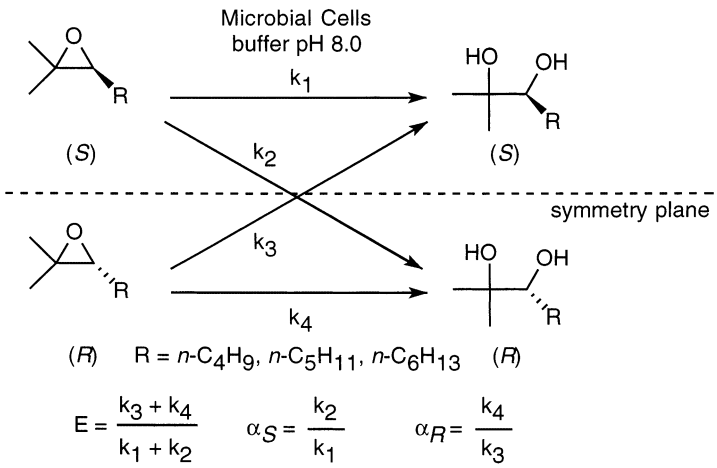
[a] Calculated from progress curves of $e.e._S$, $e.e._P$ and c versus time using computer program '*EntCon*' (see Ref. 14).

[b] Defined as the ratio of relative rates of fast versus slow reacting enantiomer; $E=(k_3+k_4)/(k_1+k_2)$ or the respective reciprocal value.

n.a., not applicable; due to enzymatic oxidation of diol (*R*)-**2b** leading to the formation of 2-methyl-2-hydroxyoctan-3-one (ca. 20%), the kinetics could not be applied, as indicated by negative k-values.



Scheme 3. Synthesis of reference materials and determination of absolute configuration.



Scheme 4. Stereochemical pathways of biohydrolysis.

(iii) In all cases, both oxirane enantiomers were hydrolysed with opposite regioselectivity ($k_2>k_1$ and $k_4>k_3$), thus forming the same enantiomeric diol in an enantioconvergent fashion.

(iv) Most remarkably, both enantiomers of substrates were hydrolysed at comparable rates, leading to low enantioselectivities [$(k_1+k_2)\approx(k_3+k_4)$]. In contrast, the regioselectivities were significantly higher, which resulted in good optical purities of products ($k_2\gg k_1$; $k_4\gg k_3$).

(v) The calculation of the relative rate constants from the hydrolysis of (±)-**2a** using *Rhodococcus ruber* CBS 717.73 gave negative k-values (entry 8). Thus, the reaction cannot be explained by the four pathways depicted in Scheme 4 and a more complex metabolism has to be considered. Indeed, detailed investigation of this reaction revealed that diol (*R*)-**2b** (formed during hydrolysis) was further enzymatically oxidised at the *sec*-alcohol moiety to furnish the corresponding hydroxyketone (2-hydroxy-2-methyloctan-3-one) in ~20% yield. Since this latter pathway showed some enantioselectivity for (*S*)-**2b**, the kinetics of Scheme 4 could not be applied.

The preparative applicability of this reaction regarding an optimal chemical and optical yield of product(s) can only be assessed by kinetic analysis, the importance of which is demonstrated along three selected examples.

Case I (entry 5, Fig. 1): Hydrolysis of (±)-**2a** by *Streptomyces lavendulae* ATCC 55209 proceeds without noticeable enantioselectivity ($E=1.2$) and, as a consequence, the time-course of conversion is completely steady and does not show any drop near half-way of the reaction. In accordance with that, the e.e.$_S$ remains very low throughout the whole process and does not exceed a value of ~10%. It is impossible to perform this reaction as a kinetic resolution. On the other hand, the regioselectivities are high ($\alpha_S=61$, $\alpha_R=16$) and show a matching opposite trend for both enantiomers. Thus, the enantiomeric composition of (*R*)-**2b** remains high throughout the whole reaction and complete deracemisation can be achieved.

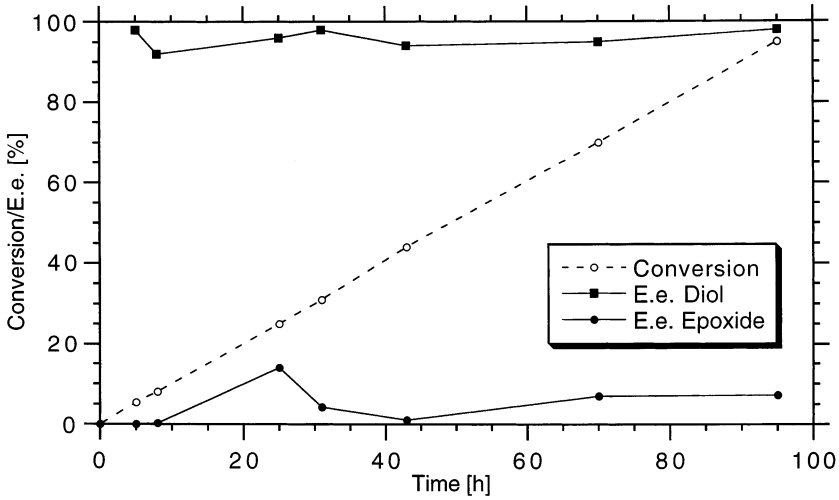


Figure 1. Time-course of hydrolysis of (±)-**2a** using *S. lavendulae* ATCC 55209.

Case II (entry 3, Fig. 2): On the contrary, *R. ruber* SM 1789 shows some enantioselectivity for (*R*)-**2a** ($E=2.3$), as indicated by a drop in the rate of the reaction at near to 50% conversion. As a consequence, the remaining non-reacted epoxide (*S*)-**2a** can be obtained in good e.e. beyond 70% conversion. In this case, kinetic resolution would be feasible, albeit at reduced chemical yields of epoxide. Again, the regioselectivity for both substrate enantiomers is opposite but rather low ($\alpha_S=15$, $\alpha_R=3$). The $e.e._P$ is rather low at the onset of the reaction (caused by the low regioselectivity of the fast reacting (*R*)-enantiomer) but it increases steadily towards the end, when the hydrolysis of the slow reacting (*S*)-enantiomer (occurring with better regioselectivity) takes over. Overall, deracemisation is possible and the best results are obtained at high conversion.

Case III (entry 1, Fig. 3): As indicated by a sharp drop in reaction rate at 50%, substrate (±)-**1a** is hydrolysed by *Mycobacterium paraffinicum* NCIMB 10420 with good enantioselectivity ($E=17$) and at this point the $e.e._S$ has already reached >95%. Due to the large difference in reaction rates of enantiomers, a conversion of ~50% is already reached within 2.5 h, but the completion of the second half of the process takes considerably longer (>100 h). Due to the high regioselectivity of the faster reacting (*S*)-enantiomer ($\alpha_S=50$), the $e.e._P$ is high at the onset of the reaction but is gradually depleted by the low regioselectivity of the slow (*R*)-enantiomer ($\alpha_R=5$).

Typically, this process can conveniently be run in a kinetic resolution mode leading to (*R*)-**1a** and (*R*)-**1b** in >95% e.e. at about 50% conversion. Deracemisation, however, is hampered by the long reaction times of (*R*)-**1a**.

In order to elucidate the reason for the enantioconvergence and to prove the validity of the calculation of relative rate constants, experiments were performed in ^{18}O-labelled water using substrate (±)-**2a**. All four diastereomers (with respect to the ^{18}O-label) of diol **2b** were analysed by GC–MS on a chiral stationary phase using electron-impact ionisation (Scheme 5).

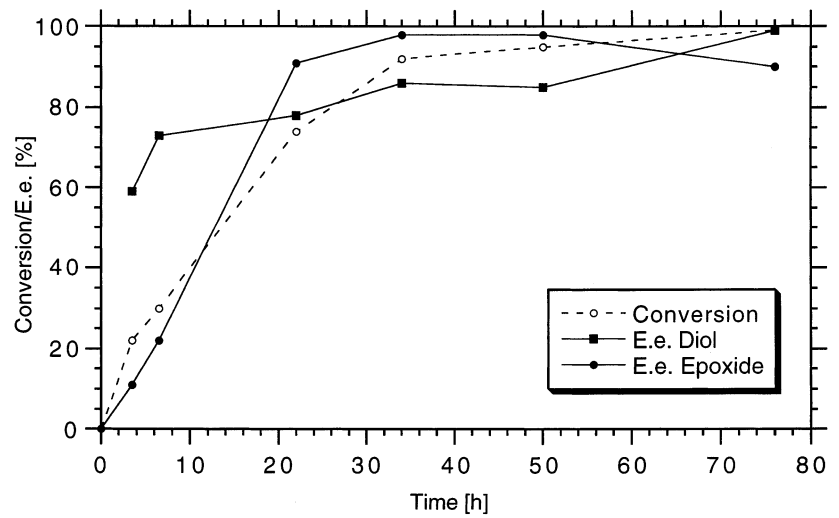


Figure 2. Time-course of hydrolysis of (±)-**2a** using *R. ruber* SM 1789.

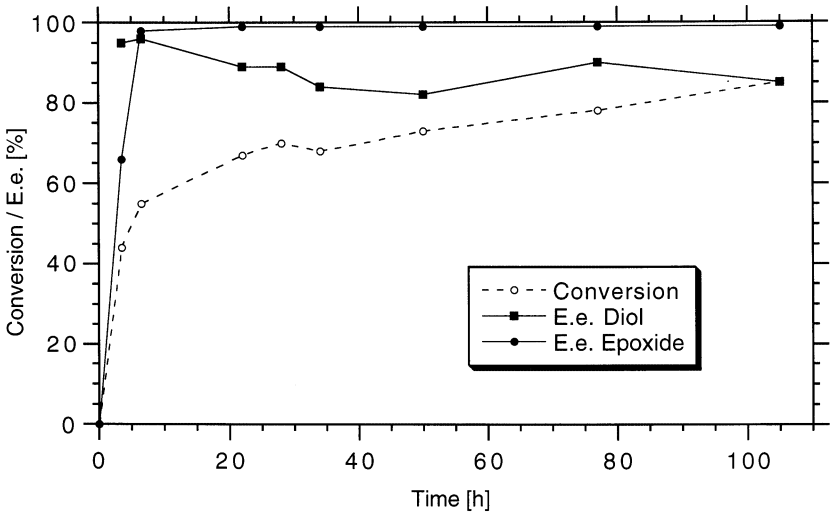
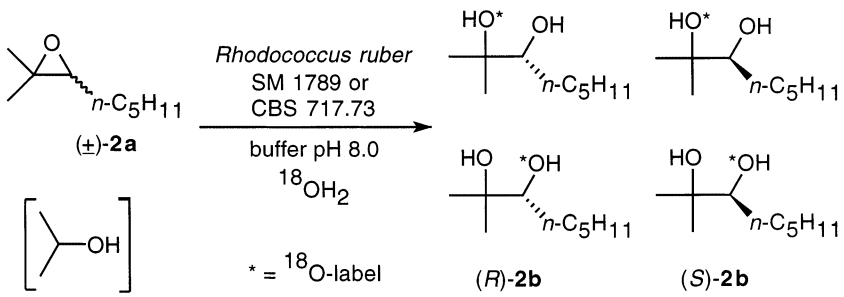


Figure 3. Time-course of hydrolysis of (±)-**1a** using *M. paraffinicum* NCIMB 10420.



Scheme 5. ^{18}O-Labelling experiments.

The number of oxygen atoms incorporated during enzymatic hydrolysis was determined by using the $[M-CH_3]^+$ signal, since the relative intensity of M^+ was rather small. Both *R. ruber* SM 1789 and CBS 717.73 gave exclusively a signal for 147 Da, indicating a clear preference for the incorporation of a single oxygen atom from $^{18}OH_2$,[4,17] no trace of double incorporation ($M=149$ Da) was found.

The regioselectivity of the *O*-incorporation was determined by using the $^{16}O/^{18}O$ ratio of the smaller fragment resulting from glycol cleavage, i.e. $[(CH_3)_2\text{-C-OH}]^+$ (Table 2). The corresponding signal for the matching heavy fragment $[HO\text{-CH-}n\text{-}C_5H_{11}]^+$ was very small. The data revealed the following.

(i) Both microorganisms exhibited the same predominance for ^{18}O-incorporation at the C(3) atom in the case of the (*S*)-diol (Table 1, entries 3 and 8). From the experimentally determined enantiopreference and the stereochemical outcome of the biotransformations, it can be concluded that $k_3>k_1$. Thus, the (*R*)-epoxide is predominantly transformed with inversion to the (*S*)-diol. For *R. ruber* SM 1789, the measured ratios of regioselectivity are in good agreement with the calculated values, i.e. k_3/k_1 is 12:1.0 (exp.) and 9:1 (calcd). For *R. ruber* CBS 717.73, k_3/k_1 (exp.) is 4.6:1.0. Due to further oxidative metabolism of (*R*)-**2b**, the corresponding calculated values could not be obtained.

(ii) For the (*R*)-diol, opposite regioselectivities were observed with both strains: *R. ruber* SM 1789 predominantly attacked the C(2) atom (entry 3, $k_4>k_2$). Thus, it can be concluded that the (*R*)-epoxide is hydrolysed with retention to furnish the (*R*)-diol. Again, the measured ratio of regioselectivity corresponds nicely to the calculated values, i.e. k_2/k_4 is 1.0:2.2 (exp.) and 1.0:1.8 (calcd). On the contrary, *R. ruber* CBS 717.73 exhibited a preference for incorporation at C(3) (entry 8) and, as a consequence, $k_4<k_2$. Thus, the (*S*)-epoxide is transformed with inversion to the (*R*)-diol and k_2:k_4 equals 1.0:1.8.

In order to test the practical applicability of the process, a preparative-scale experiment was performed: When (±)-**2a** (597 mg) was subjected to lyophilised cells of *R. ruber* SM 1789 (2.13 g) over a period of 140 h, (*R*)-**2b** (547 mg) was obtained as the sole product with 79% e.e. in 92% yield.

Table 2. Relative distribution of $^{16}O/^{18}O$-label in the small fragment $[(CH_3)_2\text{-C-OH}]^+$

Strain	(*R*)-**2b**[a]	(*S*)-**2b**[b]
Rhodococcus ruber SM 1789	1.0:2.2	12:1.0
Rhodococcus ruber CBS 717.73	1.8:1.0	4.6:1.0

[a] Corresponding to the ratio of k_2/k_4.
[b] Corresponds to the ratio of k_3/k_1.

3. Conclusion

In summary, it was shown that the biohydrolysis of the racemic trialkyl epoxides (±)-**1a**–**3a** using the bacterial epoxide hydrolase activity from *Rhodococcus* and *Streptomyces* spp. proceeds in an enantioconvergent fashion. Due to its simplicity and high efficiency, this process is feasible on a preparative scale and provides an elegant method for the preparation of non-racemic long-chain 2-methyl-2,3-diols, which constitute the chiral moieties of numerous natural products derived from isoprenoid pathways. The applicability of this method for the asymmetric synthesis of bioactive terpenoids, such as Juvenile hormone analogues, is currently under investigation.

4. Experimental

4.1. General remarks

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^{1}H) and 90 (^{13}C) MHz, a Bruker DMX Avance 500 at 500 (^{1}H) and 125 (^{13}C) MHz or a Varian XL-200 at 200 (^{1}H) and 50 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) with $CHCl_3$ as internal standard [δ 7.23 (^{1}H) and 76.90 (^{13}C)], coupling constants (J) are given in Hz. ^{13}C NMR multiplicities were determined by using a DEPT pulse sequence.

TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualised by spraying with vanillin/H_2SO_4 conc. (5 g/L) (detection I), or by dipping into a $KMnO_4$ reagent (2.5 g/L in H_2O) (detection II). Compounds were purified either by flash chromatography on silica gel Merck 60 (230–400 mesh) or, for volatile substances, by Kugelrohr distillation. Petroleum ether had a boiling range of 60–90°C. GC analyses were carried out on a Varian 3800 gas chromatograph equipped with FID and either an HP1301 or an HP1701 capillary column (both 30 m, 0.25 mm, 0.25 μm film, N_2). Enantiomeric purities were analysed on a Varian 3800 gas chromatograph equipped with FID, using a CP-Chirasil-DEX CB column (25 m, 0.32 mm, 0.25 μm film, H_2). High-resolution mass spectra were recorded on a double-focussing Kratos profile mass spectrometer with electron-impact ionisation (EI, 70 eV). A Shimadzu GC-14A directly coupled to the MS was used for sample introduction.

Solvents were dried and freshly distilled by common practice. For anhydrous reactions, flasks were dried at 150°C and flushed with dry argon just before use. Organic extracts were dried over Na_2SO_4, and then the solvent was evaporated under reduced pressure. $H_2{}^{18}O$ (94.5% isotope content) was purchased from Promochem (Germany). *n*-Pentyl-, *n*-hexyl- and *n*-heptyltriphenylphosphonium bromide, as well as α- and β-AD-mix, were purchased from Aldrich. *m*-Chloroperbenzoic acid (*m*-CPBA, 70%, Fluka) and NaH suspended in mineral oil (60%, Aldrich) were used. Biotransformations were performed with lyophilised bacterial cells; strains were obtained from culture collections. SM numbers refer to the culture collection of the Institute of Biotechnology, Graz University of Technology. All strains were grown as previously described.[18–21]

4.2. Synthesis of substrates and reference materials

4.2.1. General procedure for Wittig reaction and epoxidation of alkenes to furnish (±)-1a, (±)-2a and (±)-3a. Epoxides (±)-**1a**, (±)-**2a** and (±)-**3a** were synthesised via a two-step sequence described in Method A. Due to the high volatility of alkenes **1**, **2** and **3**, they were directly converted into the corresponding epoxides **1a**, **2a** and **3a** without isolation. Only alkene **2** was isolated to prove the outcome of the first step.

4.2.2. Method A. NaH (60%, 1.13 g) was washed with pentane and suspended in DMSO (35 mL). The mixture was heated at 80°C for 0.5 h and then cooled to rt. *n*-Alkyltriphenylphosphonium bromide (46.1 mmol) in DMSO (60 mL) was added during a period of 0.5 h. After stirring the reaction mixture for a further 0.5 h, acetone (2.57 g, 44.26 mmol) was added and stirring was continued for 12 h. The reaction was quenched by addition of water (700 mL) and pentane (100 mL). The phases were separated and the aqueous phase was extracted with pentane (3×100 mL). The combined organic phase was dried (Na_2SO_4), evaporated and cooled (0°C, 12 h) to precipitate residual phosphonium salts, which were removed by filtration. The liquid residue was dissolved in CH_2Cl_2 (300 mL), K_2CO_3 (9.5 g, 68.7 mmol) and *m*-chloroperbenzoic acid (10.0 g, 40.6 mmol, 70%) were added and the suspension was stirred for 3 h. After the reaction had reached completion, the white suspension was filtered. The resulting solution was treated with $Na_2S_2O_5$ (200 mL, 10%). After phase separation, the organic phase was washed with sat. $NaHCO_3$ (3×200 mL), dried and evaporated. Kugelrohr distillation afforded (±)-**1a**, (±)-**2a** and (±)-**3a**. The following compounds were thus obtained.

4.2.3. 2-Methyl-2-octene 2. *n*-Hexyltriphenylphosphonium bromide (8.2 g, 19.2 mmol) and acetone (1.5 mL, 20.4 mmol) were used as described in method A. The pentane extract was subjected to Kugelrohr distillation to afford 0.71 g (28%) of **1** as a colourless liquid; R_f (petroleum ether) = 0.78 (detection II); bp (Kugelrohr) 95–100°C (400 mbar); ^{1}H NMR (360.13 MHz, $CDCl_3$):[22] δ 0.89 (3H, t, J = 7.0), 1.26–1.60 (6H, m), 1.60 (3H, s), 1.69 (3H, d, J = 1.0), 1.97 (2H, m), 5.13 (1H, m); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.06 (q), 17.56 (q), 22.69 (t), 25.67 (q), 28.08 (t), 29.67 (t), 31.66 (t), 125.02 (d), 131.04 (s).

4.2.4. 2-Methyl-2,3-epoxyheptane (±)-1a. Method A was employed using *n*-pentyl-triphenylphosphonium bromide (17.3 g, 41.8 mmol) and acetone (3.2 mL, 43.6 mmol). Work-up and Kugelrohr distillation afforded (±)-**1a** as a colourless liquid (1.30 g, 40%); R_f (petroleum ether/EtOAc, 5:1) = 0.75 (detection I); bp (Kugelrohr) 110–115°C (38 mbar); ^{1}H and ^{13}C NMR data matched those previously reported.[23]

4.2.5. 2-Methyl-2,3-epoxyoctane (±)-2a. Method A was employed using *n*-hexyl-triphenylphosphonium bromide (18.2 g, 42.5 mmol) and acetone (3.25 mL, 44.3 mmol). Work-up and Kugelrohr distillation afforded (±)-**2a** as a colourless liquid (3.10 g, 49%); R_f (petroleum ether/EtOAc, 5:1) = 0.72 (detection I); bp (Kugelrohr) 140°C, (32 mbar); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 0.89 (3H, t, J = 7.1), 1.25 (3H, s), 1.30 (3H, s), 1.31–1.55 (8H, m), 2.69 (1H, t, J = 5.9); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.03 (q), 18.75 (q), 22.65 (t), 24.96 (q), 26.23 (t), 28.85 (t), 31.73 (t), 58.24 (s), 64.62 (d).

4.2.6. 2-Methyl-2,3-epoxynonane (±)-3a. Method A was employed by using *n*-heptyl-triphenylphosphonium bromide (17.6 g, 39.8 mmol) and acetone (3.05 mL, 41.5 mmol). Work-up and Kugelrohr distillation afforded (±)-**3a** as a colourless liquid (3.40 g, 43%); R_f (petroleum ether/EtOAc, 5:1) = 0.68 (detection I); bp (Kugelrohr) 145°C (20 mbar); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 0.84 (3H, t, J = 6.8), 1.21 (3H, s), 1.26 (3H, s), 1.28–1.50 (10H, m), 2.66 (1H, t, J = 5.9); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.07 (q), 18.73 (q), 22.60 (t), 24.93 (q), 26.51 (t), 28.88 (t), 29.19 (t), 31.82 (t), 58.18 (s), 64.59 (d).

4.3. Synthesis of 1,2-diols (±)-1b, (±)-2b and (±)-3b

Diols (±)-**1b**, (±)-**2b** and (±)-**3b** were obtained by acid catalysed hydrolysis of the corresponding racemic oxiranes **1a**, **2a** and **3a** (0.2 M in water/THF 1/1, three drops of 6N H_2SO_4). Extractive work-up and flash chromatography (petroleum ether/EtOAc 5/1) gave pure diols **1b**, **2b** and **3b**. The following compounds were thus obtained.

4.3.1. 2-Methylheptane-2,3-diol (±)-1b. Hydrolysis of **1a** (0.14 g, 1.09 mmol) afforded **1b** as a colourless oil (0.12 g, 76%); R_f (petroleum ether/EtOAc, 1:1) = 0.25 (detection I); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 6.9), 1.11 (3H, s), 1.16 (3H, s), 1.26–1.55 (6H, m), 2.31 (1H, s), 2.46 (1H, s), 3.32 (1H, d, J = 8.2); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.08 (q), 22.75 (t), 23.10 (q), 26.53 (t), 29.02 (t), 31.42 (t), 73.25 (s), 78.65 (d). The non-racemic reference material was obtained according to the reported procedure.[24]

4.3.2. 2-Methyloctane-2,3-diol (±)-2b. Hydrolysis of **2a** (0.15 g, 1.05 mmol) afforded **2b** as a colourless oil (0.11 g, 64%); R_f (petroleum ether/EtOAc, 5:1) = 0.16 (detection I); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 0.86 (3H, t, J = 6.8), 1.11 (3H, s), 1.17 (3H, s), 1.36–1.55 (8H, m), 2.30–2.34 (2H, br s), 3.33 (1H, d, J = 9.1); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.10 (q), 22.68 (t), 23.11 (q), 26.50 (t), 26.54 (q), 31.70 (t), 31.91 (t), 73.23 (s), 78.68 (d).

4.3.3. 2-Methylnonane-2,3-diol (±)-3b. Hydrolysis of **3a** (0.15 g, 1.05 mmol) afforded **3b** as a colourless oil (0.15 g, 90%); R_f (petroleum ether/EtOAc, 1:1) = 0.35 (detection I); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 0.86 (3H, t, J = 6.0), 1.15 (3H, s), 1.20 (3H, s), 1.24–1.57 (10H, m), 2.02–2.18 (2H, br s), 3.36 (1H, d, J = 6.3); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ 14.26 (q), 22.82 (t), 23.30 (q), 26.70 (q), 26.96 (t), 29.53 (t), 31.90 (t), 32.02 (t), 73.36 (s), 78.84 (d).

4.3.4. 2-Methyl-2-hydroxyoctan-3-one. Diol **2b** (85 mg, 0.53 mmol) was dissolved in CH_2Cl_2 (3 mL) and Dess–Martin periodinane oxidant (0.3 g, 0.71 mmol) was added. After stirring the mixture for 3 h the solution was diluted with Et_2O (7 mL) and sat. aqueous $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4) and evaporated to yield 2-methyl-2-hydroxyoctan-3-one (45 mg, 54%) after flash chromatography (pentane/Et_2O, 3:1); R_f (petroleum ether/EtOAc, 1:1) = 0.62 (detection I); ^{1}H NMR (503.03 MHz, $CDCl_3$):[25] δ 0.83 (3H, t, J = 5.1), 1.30 (6H, s), 1.18–1.96 (4H, m), 2.46 (2H, t, J = 7.3), 3.77 (1H, s); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 13.80 (q), 22.35 (t), 23.33 (t), 26.38 (q), 31.27 (t), 35.34 (t), 68.05 (s), 214.53 (s). High-resolution MS for $C_9H_{18}O_2$: calcd 158.1291, found 158.1307.

4.4. Determination of absolute configuration

Abolute configurations of the biotransformation products were determined by co-injection on chiral GC with reference material (Table 3). This was independently synthesised as follows: Sharpless dihydroxylation of 2-methyl-octada-2,7-diene with α-AD-mix was previously described to give (*S*)-**4** (e.e. 87%).[26] Hydrogenation of (*S*)-**4** using Pd/C (5% w/w) in dry EtOH under a hydrogen atmosphere gave (*S*)-**2b** (e.e. 88%). Diol (*S*)-**4** was transformed into the corresponding acetonide (2,2-dimethoxypropane, cat. IR 120 H^+-form, rt) which led after ozonolysis and reductive work-up (dry CH_2Cl_2, O_3, $LiAlH_4$ in THF) to alcohol (*S*)-**5** (spectroscopic data are given below). Mesylation of (*S*)-**5** (MsCl, pyridine, CH_2Cl_2), reduction ($LiAlH_4$, Et_2O) and deprotection (H_2SO_4 cat., H_2O) led to (*S*)-**1b** (e.e. 90%). Bromination of (*S*)-**5** (MsCl, pyridine, CH_2Cl_2 followed by LiBr in acetone) was followed by chain extension via a Grignard reaction (EtMgBr, Et_2O). After deprotection, (*S*)-**3b** was obtained in 95% e.e. Diols (*R*)-**1b**, (*R*)-**2b** and (*R*)-**3b** (e.e. >90%) were

Table 3. GC retention times of compounds on a chiral stationary phase

Compound	Conditions	Retention time [mm], (absolute config.)
1a	12 psi, 60°C (*iso*)	3.38 (*S*), 3.63 (*R*)
2a	12 psi, 60°C (*iso*)	7.29 (*S*), 7.84 (*R*)
3a	12 psi, 80°C (*iso*)	5.11(*S*), 5.43 (*R*)
lb	12 psi, 110°C (*iso*)	4.56 (*S*), 5.03 (*R*)
2b	12 psi, 120°C (*iso*)	3.45 (*S*), 3.81(*R*)
3b	12 psi, 125°C (*iso*)	5.65 (*S*), 6.35 (*R*)
4	12 psi, 125°C (*iso*)	3.49 (*S*), 3.85 (*R*)

obtained from biotransformations. Treatment of these diols with trifluoromethanesulfonic anhydride in pyridine led to (*S*)-**1a**, (*S*)-**2a** and (*R*)-**3a** (e.e. >90%) via inversion of configuration.

(*S*)-5,6-Isopropylidenedioxy-6-methylheptan-1-ol [(*S*)-**5**]: R_f (petroleum ether/EtOAc, 1:1) = 0.45 (detection I). ^{1}H NMR (503.03 MHz, $CDCl_3$):[27] δ 1.08 (3H, s), 1.24 (3H, s), 1.32 (3H, s), 1.41 (3H, s), 1.43–1.64 (6H, m), 1.95 (1H, br. s), 3.65–3.85 (3H, m).

4.5. General procedure for the biocatalytic hydrolysis of (±)-1a, (±)-2a and (±)-3a

Lyophilised cells (50 mg) were rehydrated in Tris-buffer (1 mL, 0.05 M, pH 8.0) for 1 h, (±)-epoxides **1a**, **2a** and **3a** (5 μL) were added and the mixture was shaken at 30°C at 130 rpm. At intervals of 3.5, 6.5, 22, 33.5, 49.5, 76.5 and 105 h, aliquots of 0.1 mL were withdrawn and extracted twice with EtOAc (0.1 mL each). To facilitate phase separation, cells were removed by centrifugation. The combined organic layers were dried (Na_2SO_4) and analysed by GC.

Optical rotation values were as follows: (*R*)-**1b**: $[\alpha]_D^{20} = +27.5$ (*c* 2.69, $CHCl_3$); (*R*)-**2b**: $[\alpha]_D^{20} = +24.9$ (*c* 1.15, $CHCl_3$); (*R*)-**3b**: $[\alpha]_D^{20} = +24.4$ (*c* 1.95, $CHCl_3$).

4.5.1. Preparative-scale hydrolysis of (±)-2a. Lyophilised cells of *R. ruber* SM 1789 (2.13 g) were rehydrated in Tris-buffer (100 mL, 0.05 M, pH 8.0) for 1 h. The epoxide (±)-**2a** (597 mg) was added in one portion and the mixture was agitated on an orbit shaker at 120 rpm at 30°C. After 140 h the suspension was extracted with CH_2Cl_2 (3×30 mL), dried (Na_2SO_4) and evaporated. Flash chromatography furnished (*R*)-**2b** (547 mg, 92%) as the sole product with e.e. of 79%.

4.5.2. General procedure for the biocatalytic hydrolysis of (±)-2a in $H_2{}^{18}O$. A cell-free enzyme extract was obtained by cell-disruption in a bead-mill (Vibrogen, glass beads 0.3 mm diameter (10 g) in Tris-buffer (0.05 M, pH 8.0, 12 mL), four shaking–cooling cycles) followed by centrifugation for 2 h at 37,000 *g*. The supernatant was then lyophilised. Racemic epoxide (±)-**2a** (4 μL) was hydrolysed using rehydrated lyophilised cell-free extract (20 mg) in $H_2{}^{18}O$ (0.3 mL, 94.5% isotope content) by shaking the mixture at 30°C at 130 rpm. After 4 and 15 h, samples were withdrawn (0.15 mL) and extracted twice with EtOAc (each 0.15 mL). To facilitate phase separation, cells were removed by centrifugation. The combined organic layers were dried (Na_2SO_4) and analysed by GC and GC/MS as described above.

Acknowledgements

This research was performed within the Spezialforschungsbereich Biokatalyse (Project No. F-104) and financial support by the Fonds zur Förderung der wissenschaftlichen Forschung and the Austrian Ministry of Science is gratefully acknowledged.

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16. Due to the absence of a stereogenic centre, the exact stereochemical nature of the attack at C(2) (i.e. inversion or retention) cannot be elucidated for this type of substrate.
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