Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 583–588
Tetrahedron

Chemo-enzymatic enantio-convergent asymmetric synthesis of (*R*)-(+)-Marmin

Klaus Edegger, Sandra F. Mayer, Andreas Steinreiber and Kurt Faber*

Department of Chemistry, Organic and Bio-Organic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria

Received 11 July 2003; revised 1 September 2003; accepted 17 October 2003

Abstract—Asymmetric biohydrolysis of trisubstituted terpenoid oxiranes (*rac*-**1a**–*rac*-**3a**) was accomplished by employing the epoxide hydrolase activity *Rhodococcus* and *Streptomyces* spp. Depending on the biocatalyst, the biohydrolysis proceeded in an enantio-convergent fashion and gave the corresponding *vic*-diols in up to 97% ee at conversions beyond the 50%-threshold. In order to avoid a depletion of the ee of product by further oxidative metabolism, bioconversions had to be conducted in an inert atmosphere with exclusion of molecular oxygen. The synthetic applicability of this method was demonstrated by the asymmetric total synthesis of the monoterpenoid coumarin (*R*)-(+)-Marmin in 95% ee.

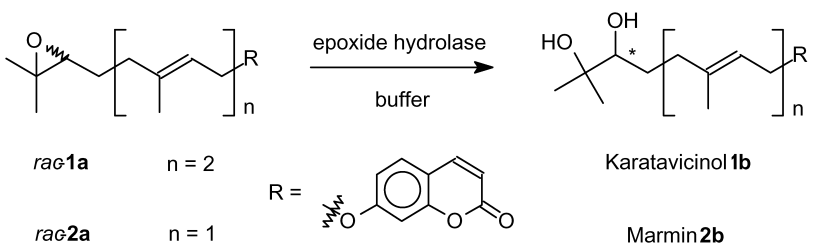
1. Introduction

A great variety of natural products possessing an isoprenylated coumarin structure of the umbelliferone family, such as Karatavicinol (**1b**) and Marmin (**2b**) have been isolated from various biological sources, such as roots, bark and fruit peel of various plants indigenous to Central Asia. They were found to be constituents of gum resins derived by incision of roots, which are applied in traditional medicine.[1,2] The sesquiterpenoid coumarin Karatavicinol[3] (**1b**) was isolated and fully characterised from *Ferula sinaica*.[1] The monoterpenoid analogue—Marmin (**2b**)[4]—was isolated from the bark of *Aegle marmelos*[4] and grapefruit peel oil[5] and was shown to be (*R*)-configurated. The (*S*)-(−)-antipode was found in *Pituranthos tridadiatus*.[6] (*R*)-(+)-Marmin has been synthesised through multistep sequences from L-glutamic acid[7] and via Sharpless epoxidation.[8] A recent more elegant approach involved a microbial 'dihydroxylation' of 7-geranyloxy-coumarin (aurapten) by *Aspergillus niger*.[9]

In light of the general nature of microbial oxidative pathways,[10,11] the latter pathway occurs presumably via epoxidation (catalysed by cytochrome P450 monooxygenases[12]) followed by hydrolytic ring-opening involving an epoxide hydrolase.[13]

2. Results and discussion

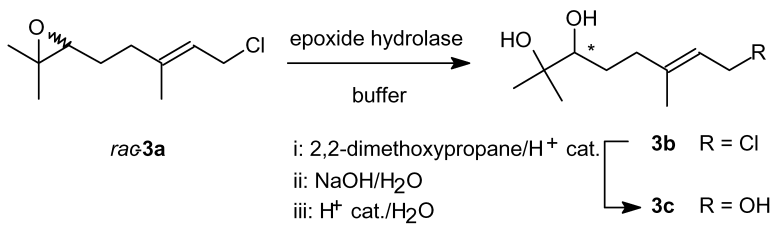
Based on the highly selective asymmetric biohydrolysis of trisubstituted oxiranes catalysed by bacterial epoxide hydrolases,[14] we envisaged that this strategy might be applicable to the synthesis of natural products bearing an isoprenoid (geranyl or farnesyl) side chain (Scheme 1). Due to the fact that many of these bioconversions proceeded in an enantio-convergent fashion,[15] the occurrence of an unwanted stereoisomer is avoided, which renders an excellent synthetic efficiency.



Scheme 1. Biosynthetic strategy for the synthesis of Karatavicinol and Marmin employing epoxide hydrolases.

Keywords: Epoxide hydrolase; Rhodococcus; Streptomyces; (*R*)-(+)-Marmin; Biotransformation.
* Corresponding author. Tel.: +43-316-380-5332; fax: +43-316-380-9840; e-mail: kurt.faber@uni-graz.at

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.10.106



Scheme 2. Biohydrolysis of building block *rac*-**3a** and synthesis of reference material **3c**.

Table 1. Biohydrolysis of *rac*-**3a**

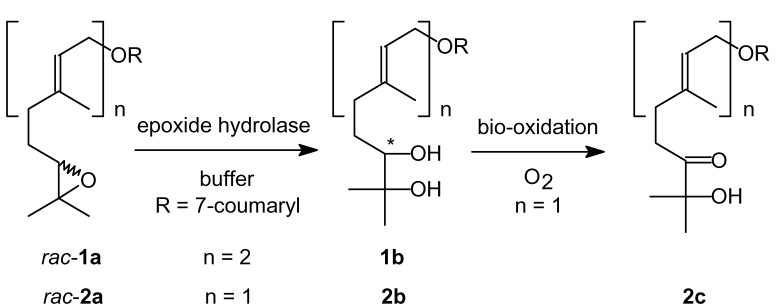
Strain	Conv. (%)	Ee (%)	
		3a	(*R*)-**3b**
Rhodococcus ruber DSM 43338	41	18	75
Rhodococcus ruber DSM 44541	49	86	79
Rhodococcus ruber DSM 44540	59	50	81
Streptomyces sp. FCC 003	64	74	93
Rhodococcus erythropolis DSM 312	60	31	83
Streptomyces venezuelae ATCC 10712	63	59	91

Our first approach was guided by the idea to create a common chiral building block bearing a terminal epoxy- or dihydroxy-functional group, which would allow to apply this strategy to a broad variety of terpenoid natural products. Thus we chose substrate *rac*-**3a** derived from geranyl chloride (**6**) by epoxidation bearing in mind that the allylic halogen moiety should allow facile coupling onto the main framework.

Oxirane *rac*-**3a** was subjected to biohydrolysis in aqueous buffer (pH 8.0) employing a range of lyophilised bacterial cells which are known to possess a broad secondary metabolism and epoxide hydrolase activity, in particular[16] (Scheme 2). A large number of strains showed good activity and produced the corresponding *vic*-diol **3b** in various amounts (data not shown). Inspection of the stereoselectivity of the reaction, however, revealed that the enantiomeric composition of diol **3b** and remaining non-converted oxirane **3a** was low to moderate (up to 70% ee) in most cases. Several strains gave sufficient stereoselectivity and produced (*R*)-**3b** in up to 93% ee (Table 1). Detailed analysis of the data (conversion, ee_P and ee_S) revealed that-depending on the strain used-mixed stereochemical pathways are found: Whereas *Rhodococcus ruber* DSM 43338, 44541 and 44540 (entries 1–3) largely followed a classic kinetic resolution pattern, enantio-convergent pathways[15] predominated with *Rh. erythropolis* DSM 312 (entry 5) and both *Streptomyces* spp. ATCC 10712 and FCC 003 (entries 4 and 6). The latter is indicated by the high ee_P (83 to 93%) at a conversion beyond 60%.

In order to investigate whether the presence of a large structural coumaryl-moiety in the side-chain would lead to enhanced stereoselectivity, substrates *rac*-**1a** and *rac*-**2a** were tested (Scheme 3). Conversion of substrate *rac*-**1a** bearing the sterically demanding sesquiterpene farnesyl-unit proved to be cumbersome and even prolonged exposure to a series of bacterial strains gave exceedingly low conversion (<5% to max 15%, data not shown). In addition, in those cases, where diol **1b** (Karatavicinol) could be detected in measurable amounts, the ee proved to be disappointingly low (<5%).

Much better results were obtained with the monoterpene analog *rac*-**2a** (Table 2). From a range of bacteria, *Rhodococcus ruber* DSM 44539 and DSM 43338 exhibited sufficient activity and formed (*R*)-**2b** (Marmin) in up to 72% ee (entries 1 and 3). Careful monitoring of the bioconversion revealed that the ee_P significantly dropped upon extended reaction times (entries 2 and 4), which is in contrast to an enantio-convergent transformation, where the ee_P should remain at a constant level.[15] When the reaction time for *Rh. ruber* DSM 43338 was extended to 216 h, the starting material *rac*-**2a** was entirely consumed and the ee of **2b** showed a mere 6%, but of the *opposite (S)-enantiomer* (entry 5). Analysis of the reaction mixture revealed that in addition to the expected biohydrolysis product **2b** a side-product was formed in ca. 20% yield. Spectroscopic analysis and comparison with independently synthesised reference material revealed that the latter was hydroxy-ketone **2c**, which was presumably formed from diol **2b** due to further oxidative metabolism. Since the initially formed diol **2b** was (*R*)-configured and prolonged exposure gave the (*S*)-enantiomer, it was deduced that the concurring bio-oxidation was (*R*)-selective.

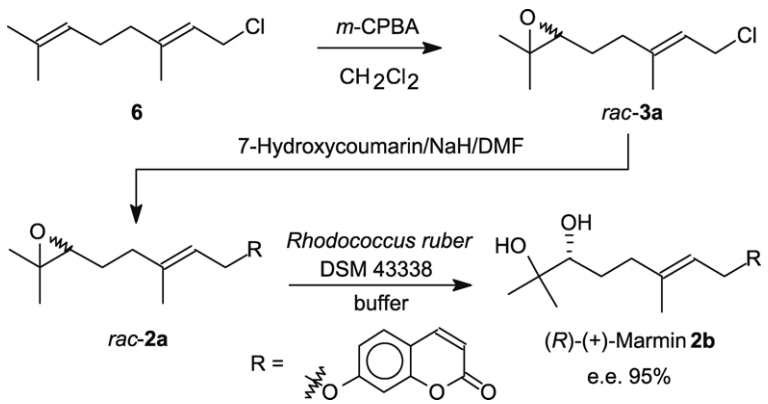


Scheme 3. Biotransformation of *rac*-**1a** and *rac*-**2a**.

Table 2. Bioconversion of *rac*-**1a**

Strain	Conditions	Time (h)	Conv.[a] (%)	ee (%)	
				1a	**1b**
Rhodococcus ruber DSM 44539	Aerobic	24	68	24	72/*R*
Rhodococcus ruber DSM 44539	Aerobic	48	87	28	62/*R*
Rhodococcus ruber DSM 43338	Aerobic	24	83	39	70/*R*
Rhodococcus ruber DSM 43338	Aerobic	48	89	44	59/*R*
Rhodococcus ruber DSM 43338	Aerobic	216	~100[b]	n.d.	6/*S*
Rhodococcus ruber DSM 43338	Ar	24	8[c]	6	97/*R*
Rhodococcus ruber DSM 43338	Ar	216	67[d]	59	96/*R*

[a] With respect to remaining epoxide *rac*-**1a**; nd=not determined.
[b] Hydroxy-ketone **1c** was formed in 20% yield.
[c] **1c** was formed in <1% yield.
[d] **1c** was formed in 1.8% yield.



Scheme 4. Chemo-enzymatic asymmetric total synthesis of (*R*)-(+)-Marmin.

In order to suppress the competing bio-oxidation, bio-hydrolysis experiments were conducted in the absence of O_2 in an Ar-atmosphere. We were pleased to see that under these conditions, the formation of hydroxy-ketone **2c** could be suppressed and that the expected biohydrolysis-product (*R*)-**2b** (Marmin) was formed in excellent ee's (up to 97%). In order to prove the synthetic value of the method, a preparative-scale experiment was conducted which gave (*R*)-(+)-Marmin in 95% ee (Scheme 4).

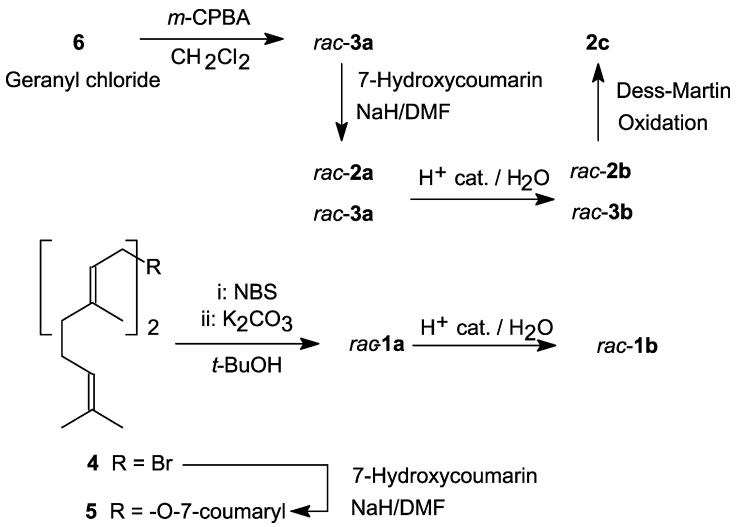
The absolute configuration of **2b** (Marmin) was revealed to be (*R*) by comparison of optical rotation values with literature data.[6] The absolute configuration of **3b** was determined by conversion of chloro-diol **3b** into triol **3c** by alkaline hydrolysis of the allylic chloride (NaOH aq.) with intermediate protection/deprotection of the *vic*-diol moiety as the corresponding acetonide. Comparison of the optical rotation of the latter material with literature data revealed its (*R*)-configuration.[17]

In summary, we have shown that the asymmetric biohydrolysis of trisubstituted terpenoid epoxides by bacterial epoxide hydrolases proceeds in an enantio-convergent fashion to furnish the corresponding *vic*-diols in high ee. This strategy offers a convenient and short access to terpenoid natural products, which was demonstrated by the asymmetric total synthesis of the monoterpenoid coumarin (*R*)-(+)-Marmin in 95% ee.

3. Experimental

3.1. General

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^{1}H) and 90 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) with $CHCl_3$ as internal standard, coupling constants (*J*) are given in Hz. TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualised by spraying with Mo-reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g L^{-1}) and $Ce(SO_4)_2\cdot 4H_2O$ (4 g L^{-1}) in H_2SO_4 (10%)] or by dipping into a $KMnO_4$ reagent (2.5 g L^{-1} $KMnO_4$ in H_2O). Compounds were purified by flash chromatography on silica gel Merck 60 (230–400 mesh). Petroleum ether had a boiling range of 60–90 °C. Enantiomeric purities were analysed on a HPLC JASCO-System equipped with a multiwavelength-detector (MD-910) and a temperature-chamber (AS-950) either on a DAICEL Chiralcel OD-H (0.46 cm d×25 cm) or a DAICEL Chiralcel AD (0.46 cm d×25 cm) column. High resolution mass spectra were recorded on a double focussing Kratos Profile Mass Spectrometer with electron impact ionization (EI,+70 eV). Optical rotation values were measured on a Perkin Elmer polarimeter 341 at 589 nm (Na-line) in a 1-dm cuvette. Solvents were dried or 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



Scheme 5. Synthesis of substrates and reference compounds.

evaporated under reduced pressure. Bacteria were obtained from culture collections, FCC stands for our in-house 'Fab-Crew-Collection'.

3.2. Syntheses of substrates

Substrates *rac*-**1a**–*rac*-**3a** were synthesised as follows (Scheme 5): Epoxidation of geranyl chloride (**6**) by *m*-chloroperbenzoic acid selectively gave *rac*-**3a**. Coupling of the coumaryl moiety using the anion of 7-hydroxy-coumarin furnished *rac*-**2a**. 7-O-Alkylation of 7-hydroxy-coumarin with farnesyl bromide (**4**) gave **5**. The side-chain of the latter was epoxidised at the terminal alkene moiety via the corresponding halohydrin (NBS/*t*-BuOH) followed by base-induced ring-closure (K_2CO_3) to give *rac*-**1a**. In order to verify the structural identity of the expected biohydrolysis products, diols **1b**–**3b** were independently synthesised in racemic form by acid-catalysed hydrolysis of epoxides *rac*-**1a**–*rac*-**3a**. Dess-Martin oxidation of *rac*-**2b** gave a sample of **2c** for proof of structure of the bio-oxidation product.

3.2.1. 7-[9-(3,3-Dimethyloxiranyl)-3,7-dimethylnona-2*E*,6*E*-dienyloxy]-chromen-2-one (*rac*-1a). To a stirred solution of 7-farnesyloxy-coumarin (**5**) (0.5 g, 1.4 mmol) in *tert*-BuOH (20 mL) and petroleum ether (20 mL), NBS (0.3 g, 1.7 mmol) was added at 0 °C. After stirring was continued for 24 h at room temperature, the bromohydrin intermediate was extracted with ether, the organic phase was dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 1:1) to give the bromohydrin in 50% yield. The latter was dissolved in MeOH (20 mL) and K_2CO_3 (0.2 g, 2.0 mmol) was added. After stirring was continued for 12 h at room temperature, solids were filtered and the solvent was evaporated. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate 1:1) to give *rac*-**1a** as a white solid (0.1 g, 86%). Mp 141–143.5 °C.

^{1}H NMR ($CDCl_3$) δ=7.64 (d, J=6.0 Hz, 1H), 7.37 (d, J=6.5 Hz, 1H), 7.27 (d, J=5.7 Hz, 2H), 6.84 (d, J=4.3 Hz, 1H), 6.25 (d, J=4.0 Hz, 1H), 5.48 (m, 1H), 5.17 (m, 1H), 4.61 (d, J=3.7 Hz, 2H), 4.13 (d, J=4.3 Hz, 2H), 2.75 (t, J=3.4 Hz, 1H), 2.14 (m, 3H), 1.77 (s, 2H), 1.64 (s, 3H), 1.57 (s, 3H), 1.31 (s, 3H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$) δ=162.2, 161.3, 155.9, 143.5, 142.2, 134.8, 128.7, 124.2, 118.6, 113.3, 113.0, 112.5, 101.6, 65.5, 64.2, 60.4, 39.5, 36.3, 27.5, 26.2, 24.9, 21.0, 16.8, 16.1.

3.2.2. 7-[5-(3,3-Dimethyloxiranyl)-3-methylpent-2*E*-enyloxy]-chromen-2-one (epoxyaurapten)[18] (*rac*-2a). NaH (0.3 g, 12.5 mmol, 60% dispersion in mineral oil) was suspended in abs. DMF (30 mL) under nitrogen and 7-hydroxycoumarin (1.0 g, 6.2 mmol) was added with stirring at room temperature. After ten minutes, *rac*-**3a** (1.1 g, 5.8 mmol) was added and stirring was continued for 12 h, until the starting material was consumed. The crude product was extracted with H_2O (300 mL) and a mixture of toluene/petroleum ether (1:1, 200 mL). The organic layers were dried (Na_2SO_4) and evaporated. The residue was flash chromatographed (petroleum ether/ethyl acetate 1:1) and recrystallised from MeOH to give *rac*-**2a** as white crystals (0.2 g, 10%). Mp 53–55 °C.

^{1}H NMR ($CDCl_3$) δ=7.62 (d, J=6.7 Hz, 1H), 7.34 (d, J=5.7 Hz, 1H), 6.81 (m, 2H), 6.22 (d, J=4.5 Hz, 1H), 5.50 (t, J=3.3 Hz, 1H), 4.59 (d, J=4.2 Hz, 2H), 2.69 (t, J=4.2 Hz, 1H), 2.22 (m, 2H), 1.77 (s, 3H), 1.69 (m, 2H), 1.28 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$) δ=162.1, 161.3, 155.9, 143.5, 141.5, 128.8, 119.1, 113.2, 113.0, 112.5, 101.6, 65.4, 63.9, 58.4, 36.3, 27.1, 24.9, 18.8, 16.8; HRMS calculated for $C_{19}H_{22}O_4$: 314.1518 [M^+]; found: 314.1509 [M^+].

3.2.3. (*E*)-3-(5-Chloro-3-methylpent-3-enyl)-2,2-dimethyloxirane (*rac*-3a). To a solution of 1-chloro-3,7-dimethyl-octa-2,6-diene (geranyl chloride, **6**) (0.5 g, 2.9 mmol) in CH_2Cl_2 (30 mL) containing K_2CO_3 (0.6 g, 6.1 mmol), *m*-CPBA (0.8 g, 4.6 mmol, Fluka 25800, 70%) was added slowly. After stirring was continued for 24 h at 0 °C, excess peracid was destroyed by shaking with aq. Na-metabisulfite solution (10%), and removal of *m*-chlorobenzoic acid by extraction with sat. $NaHCO_3$. Product was extracted with ethyl acetate, the organic phase was dried (Na_2SO_4) and evaporated. The residue

was flash-chromatographed using petroleum ether/ethyl acetate (1:1) to give *rac*-**3a** as a yellow liquid (0.4 g, 73%).

^{1}H NMR (360.13 MHz, $CDCl_3$) δ=5.54 (t, J=8.0 Hz, 1H), 4.09 (d, J=8.0 Hz, 2H), 2.69 (t, J=6.5 Hz, 1H), 2.20 (m, 2H), 1.74 (s, 3H), 1.65 (t, J=7.0 Hz, 2H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$) δ=141.6, 120.9, 63.7, 58.2, 40.7, 36.1, 27.0, 24.7, 18.7, 16.0.

3.2.4. 7-(3,7,11-Trimethyldodeca-2*E*,6*E*,10*E*-trienyloxy)-chromen-2-one (7-farnesyloxycoumarin, umbelliprenin, 5). To a stirred suspension of NaH (0.1 g, 4.2 mmol, 60% dispersion in mineral oil) in a mixture of freshly destilled anhydrous THF (30 mL) and DMSO (30 mL) under nitrogen 7-hydroxycoumarin (0.6 g, 3.7 mmol) was added at room temperature. After ten minutes, **4** (1.1 g, 3.9 mmol) was added and the mixture was refluxed for 2 h. The crude product was extracted with 0.5 M HCl (20 mL), H_2O (100 mL) and ether (100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was flash chromatographed (petroleum ether/ethyl acetate 1:1) and recrystallised from EtOAc/petroleum ether (2/98) to give **5** as white crystals (1.3 g, 98%). Mp 45–46 °C.

^{1}H NMR ($CDCl_3$) δ=7.63 (d, J=9.47 Hz, 1H), 7.36 (d, J=8.47 Hz, 1H), 6.84 (m, 2H), 6.24 (d, J=9.47 Hz, 1H), 5.47 (t, J=5.93 Hz, 2H), 5.08 (m, 1H), 4.61 (d, J=6.55 Hz, 2H), 2.1–1.9 (m, 8H), 1.77 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H); ^{13}C NMR ($CDCl_3$) δ=162.02, 161.20, 155.80, 143.29, 142.21, 135.64, 131.37, 128.51, 124.36, 123.56, 118.28, 113.08, 112.78, 112.28, 101.66, 65.54, 39.68, 39.57, 26.75, 26.19, 25.74, 17.73, 16.82, 16.08.

3.3. Synthesis of reference material

General procedure for the synthesis of reference material of diols rac-**1b**–*rac*-**3b**. Epoxide *rac*-**1a**–*rac*-**3a** was dissolved in a mixture of H_2O (5 mL) and THF (3 mL) containing 3 drops of H_2SO_4 (conc.) and stirred at room temperature for 2 h. The crude product was extracted with EtOAc, neutralised with solid $NaHCO_3$ and purified by flash chromatography (petroleum ether/ethyl acetate 1:1). Thus were obtained.

3.3.1. 7-(10,11-Dihydroxy-3,7,11-trimethyldodeca-2*E*,6*E*-dienyloxy)-chromen-2-one (*rac*-1b). Yield (30 mg, 98%) from *rac*-1a (30 mg, 0.08 mmol). ^{1}H NMR ($CDCl_3$) δ=7.64 (d, J=6.6 Hz, 1H), 7.36 (d, J=6.2 Hz, 1H), 6.83 (dd, J=6.0 Hz, 1H), 6.24 (d, J=4.7 Hz, 1H), 5.73 (t, J=4.2 Hz, 1H), 5.14 (t, J=5.5 Hz, 1H), 4.61 (d, J=4.3 Hz, 2H), 3.39 (dd, J=3.3 Hz, 1H), 1.76 (s, 3H), 1.63 (s, 3H), 1.20 (s, 3H), 1.16 (s, 3H).

3.3.2. 7-(6,7-Dihydroxy-3,7-dimethyloct-2*E*-enyloxy)-chromen-2-one (*rac*-2b). Yield (70 mg, 67%) from *rac*-2a (0.1 g, 0.3 mmol). ^{1}H NMR ($CDCl_3$) δ=7.63 (d, J=6.7 Hz, 1H), 7.36 (d, J=5.8 Hz, 1H), 6.83 (m, 2H), 6.24 (d, J=4.7 Hz, 1H), 5.74 (s, 1H), 4.62 (d, J=3.8 Hz, 2H), 3.39 (m, 2H), 2.12 (m, 3H), 1.76 (s, 3H), 1.63 (s, 3H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$) δ=162.2, 155.9, 143.6, 135.5, 128.8, 124.3, 118.8, 113.4, 101.6, 78.2, 73.0, 39.4, 36.8, 29.7, 26.5, 26.0, 23.4, 16.7, 15.9.

3.3.3. 8-Chloro-2,6-dimethyl-oct-6*E*-en-2,3-diol (*rac*-3b). Yield (0.2 g, 26%) from *rac*-3a (0.7 g, 3.7 mmol). ^{1}H NMR ($CDCl_3$) δ=4.73 (d, J=7.3 Hz, 2H), 3.95 (m, 2H), 2.1 (m, 4H), 1.67 (s, 3H), 1.22 (s, 3H), 1.18 (s, 3H).

3.3.4. 7-(7-Hydroxy-3,7-dimethyl-6-oxooct-2*E*-enyloxy)-chromen-2-one (2c). Dess Martin reagent (20 mg, 0.05 mmol) was dissolved in anh. CH_2Cl_2 (5 mL) and stirred at room temperature. *Rac*-**2b** (7 mg, 0.02 mmol) was added and stirring was continued for 12 h. The crude product was extracted with ether and sat. aq. $NaHCO_3$, the organic layer was dried (Na_2SO_4) and evaporated. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate 1:1) to give **2c** as a white solid (5 mg, 77%). Mp 59–60 °C.

^{1}H NMR ($CDCl_3$) δ=7.63 (d, J=6.9 Hz, 1H), 7.37 (d, J=5.7 Hz, 1H), 6.83 (m, 2H), 6.25 (d, J=5.4 Hz, 1H), 5.50 (t, J=4.6 Hz, 1H), 4.60 (d, J=4.4 Hz, 2H), 3.65 (s, 1H), 2.73 (t, J=5.1 Hz, 2H), 2.42 (t, J=4.5 Hz, 2H), 1.79 (s, 3H), 1.39 (s, 6H); ^{13}C NMR ($CDCl_3$) δ=213.5, 162.0, 161.3, 155.9, 143.3, 140.7, 128.8, 119.3, 113.3, 113.2, 112.6, 101.6, 76.6, 65.3, 33.8, 33.0,26.6, 17.0; HRMS calculated for $C_{19}H_{22}O_5$: 330.1467 [M^+]; found: 330.1432 [M^+].

3.3.5. (*R*)-3,7-Dimethyloct-2*E*-en-1,6,7-triol (*R*)-(3c). Biohydrolysis of *rac*-**3a** using *Rhodococcus ruber* DSM 44541 gave **3b** in 79% ee (see below). The latter material (154 mg, 748 mmol) was stirred in a mixture of 2,2-dimethoxypropane (20 mL) and cat. *p*-toluenesulfonic acid (20 mg). After 2 h, hydrolysis of the allylic chloride was affected by addition of conc. NaOH (4 M, 10 drops) and stirring was continued for 1 h. After the organic material was extracted with ethyl acetate and evaporated, deprotection of the acetonide was achieved by addition of H_2O (20 mL) containing cat. *p*-toluenesulfonic acid (20 mg). After 2 h, the solution was extracted with ethyl acetate and the pruduct was purified by column chromatography (petroleum ether/ethyl acetate 1:1) to give **3c** (74 mg, 53%) showing an optical rotation of $[\alpha]_D^{20}$+12.5 (c=0.95, $CHCl_3$, 73% ee), which nicely corresponds to literature data of $[\alpha]_D^{23}$+17.4 (c=1.4, $CHCl_3$) for the (*R*)-enantiomer.[17]

3.4. Biotransformations

3.4.1. Growth of strains. *Rhodococcus ruber* DSM 43338, DSM 44541, DSM 44540, *Rh. erythropolis* DSM 312 and *Streptomyces* sp. FCC 003 were maintained and grown on a medium as described before.[19] For *Streptomyces venezuelae* ATCC 10712 (=DSM 40230) medium #65 from DSMZ was used (http://www.dsmz.de).

3.4.2. General procedure for biotransformation. Lyophilised bacterial cells (50 mg) were rehydrated for 1 h in Tris–HCl buffer (1 mL, 0.05 M, pH=8.0) by shaking at 30 °C with 130 rpm. Racemic epoxides *rac*-**1a**–*rac*-**3a** (5 μL) were added, and the reaction was monitored by TLC. At intervals of 24 and 48 h, aliquots of 0.4 mL were withdrawn and extracted twice with EtOAc (0.4 mL each). To facilitate phase separation, the cells were removed by centrifugation. The combined organic layers were dried (Na_2SO_4) and analysed by HPLC (Table 3).

Table 3. HPLC-Data of enantioseparation

Compound	Column	Eluent[a]	Temp. (°C)	Retention time (min)
rac-**1a**	ODH	70/30	10	15.1/16.6
rac-**1b**	AD	70/30	10	12.5/13.5
rac-**2a**	ODH	90/10	10	50.4/55.6
rac-**2b**	AD	70/30	7	26.8 (*S*)/28.7 (*R*)
rac-**3a**	AD	70/30	18	7.1/7.4
rac-**3b**	AD	90/10	20	8.8 (*S*)/9.4 (*R*)

[a] Hexane/*i*-propanol.

3.4.3. Preparative-scale biotransformation. Epoxide *rac*-**2a** (100 mg, 0.3 mmol) was treated with 0.3 g of lyophilised cells of *Rhodococcus ruber* 43338 in Tris–HCl buffer (6 mL, 0.05 M, pH=8.0) as described above. After 24 h, products were extracted twice with CH_2Cl_2, the organic layer was dried (Na_2SO_4) and after evaporation the crude product was purified by flash chromatography (petroleum ether/ethyl acetate 1:1) to give 20 mg (15%) of (*R*)-(+)-Marmin *(R)*-**2b**: mp 104–109 °C, $[\alpha]_D^{20}=+9.1$ (c=2.5, MeOH, ee 95%); Lit. for (*S*)-**2b** $[\alpha]_D^{26}=-11$ (c=0.55, MeOH).[6] NMR-Spectroscopic data matched those previously reported.[6]

Acknowledgements

This study was performed within the Spezialforschungsbereich Biokatalyse and financial support by the Fonds zur Förderung der wissenschaftlichen Forschung (project no. F-115) is greatfully acknowledged. We thank R. Saf for the recording and interpretation of high-resolution mass spectra and H. Sterk and G. Uray for valuable advice in NMR-spectroscopy and HPLC-analysis, respectively.

References and notes

1. Ahmed, A. A. *Phytochemistry* **1999**, *50*, 109–112.
2. The medical use of these resins dates back to reports from the Roman empire, see: Appendino, G.; Tagliapietra, S.; Nano, G. M.; Jakupovic, J. *Phytochemistry* **1994**, *35*, 183–186.
3. Kir'yalov, N. P.; Bagirov, Y. V. *Khim. Prirodn. Soedin.* **1969**, *5*, 225–227, *Chem. Abstr.* **1970**, *72*, 55164.
4. For structure elucidation see: Chatterjee, A.; Dutta, C. P.; Bhattacharyya, S. *Tetrahedron Lett.* **1967**, *8*, 471–473.
5. Fisher, J. F.; Nordby, H. E.; Waiss, A. C., Jr.; Stanley, W. L. *Tetrahedron* **1967**, *23*, 2523–2528.
6. Halim, A. F.; Saad, H.-E. A.; Lahloub, M. F.; Ahmed, A. F. *Phytochemistry* **1995**, *40*, 927–929.
7. Yamada, S.; Oh-hashi, N.; Achiwa, K. *Tetrahedron Lett.* **1976**, *17*, 2557–2560.
8. Aziz, M.; Rouessac, F. *Tetrahedron* **1988**, *44*, 101–110.
9. Zhang, X.; Archelas, A.; Meou, A.; Furstoss, R. *Tetrahedron: Asymmetry* **1991**, *2*, 247–250.
10. Enzymatic dihydroxylation is catalyzed by dioxygenases and occurs predominantly on aromatics and alkenes which are conjugated to aromatic systems and generally does not take place on isolated alkenes, see: Boyd, D. R.; Sharma, N. D.; Allen, C. C. R. *Curr. Opin. Biotechnol.* **2001**, *12*, 564–573.
11. Holland, H. L.; Weber, H. K. *Curr. Opin. Biotechnol.* **2000**, *11*, 547–553.
12. Woggon, W.-D. *Topics Curr. Chem.* **1996**, *184*, 39–96.
13. Faber, K.; Orru, R. V. A. In *Hydrolysis of Epoxides*; 2nd ed. *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 2.
14. Steinreiber, A.; Mayer, S. F.; Saf, R.; Faber, K. *Tetrahedron: Asymmetry* **2001**, *12*, 1519–1528.
15. Faber, K.; Kroutil, W. *Tetrahedron: Asymmetry* **2002**, *13*, 377–382.
16. Steinreiber, A.; Faber, K. *Curr. Opin. Biotechnol.* **2001**, *12*, 552–558.
17. Boar, R. B.; Domps, K. *J. Chem. Soc., Perkin Trans. 1* **1977**, 709–712.
18. Bohlmann, F.; Zdero, C.; Kapteyn, H. *Liebigs Ann. Chem.* **1968**, *717*, 186–192.
19. (a) Krenn, W.; Osprian, I.; Kroutil, W.; Braunegg, G.; Faber, K. *Biotechnol. Lett.* **1999**, *21*, 687–690. (b) Osprian, I.; Kroutil, W.; Mischitz, M.; Faber, K. *Tetrahedron: Asymmetry* **1997**, *8*, 65–71. (c) Pogorevc, M.; Strauss, U. T.; Hayn, M.; Faber, K. *Monatsh. Chem.* **2000**, *131*, 639–644.