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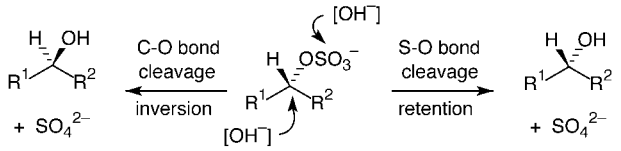
Enantioselective Stereoinversion in the Kinetic Resolution of *rac-sec*-Alkyl Sulfate Esters by Hydrolysis with an Alkylsulfatase from *Rhodococcus ruber* DSM 44541 Furnishes Homochiral Products**

Mateja Pogorevc, Wolfgang Kroutil, Sabine R. Wallner, and Kurt Faber*

Sulfatases catalyze the hydrolytic cleavage of the sulfate ester bond by liberating inorganic sulfate and the corresponding alcohol.[1] Depending on the nature of the enzyme and its catalytic mechanism, enzymatic hydrolysis of *sec*-alkyl sulfates may proceed through retention—by cleavage of the S–O bond—or inversion—by the cleavage of the C–O bond—of configuration at the chiral carbon atom (Scheme 1).[2,3]



Scheme 1. Stereochemical pathways of enzymatic sulfate ester hydrolysis.

The ability of *sec*-alkylsulfatases to effect stereoinversion during catalysis makes them prime candidates for their application in so-called enantioconvergent processes,[4] which allow the enantioselective transformation of enantiomers by opposite stereochemical pathways to furnish a single stereoisomeric product in 100% theoretical yield. Other enzymes, which potentially show this ability are a) epoxide hydrolases,[5] b) dehalogenases,[6] and c) glycosidases.[7] A limited number of alkylsulfatases have been biochemically characterized[1] but to date, these enzymes have not been applied to preparative biotransformations.[8]

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[**] We thank Degussa AG (Frankfurt) for financial support and T. Riermeier and H. Trauthwein for their valuable contributions.

  0044-8249/02/4121-4052 $ 20.00+.50/0

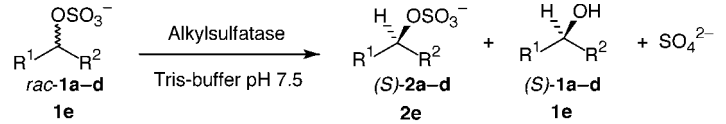
From a screening of bacteria of the genus *Actinomyces* with *rac*-2-octyl sulfate as substrate,[9] seven active strains were identified that hydrolyzed the substrate to produce 2-octanol and inorganic sulfate. The alkylsulfatase activity of the most active strain—*Rhodococcus ruber* DSM 44541—was investigated in more detail:

1) The *sec*-alkylsulfatase activity was constitutive with respect to substrate induction and was present in resting cells which were grown on a complex medium.[5a]
2) The activity could be preserved upon lyophilization in Tris-HCl buffer (pH 7.5, 10 mM) and was maintained for several months when stored at $+4$ °C.
3) The activity was associated with a soluble, monomeric protein (termed RS2) independent of any known cofactor.[10]
4) The stereochemical pathway of sulfate ester hydrolysis was found to proceed with inversion of the chiral center. Thus, when enantiopure (*R*)-2- or (*R*)-3-octyl sulfate was used as substrate, (*S*)-2- and (*S*)-3-octanol, respectively, were obtained without racemization in >99 % *ee*.
5) To evaluate the stereo- and enantioselectivity of this biocatalytic activity, the substrate tolerance and the respective enantioselectivities were investigated. The data in Table 1 reveal a clear trend: The enzyme showed excellent regioselectivity in favor of *rac-sec*-alkyl sulfates *rac*-**1a–d**[9] of medium-chain length, which were readily accepted as substrates. In contrast, *prim*-sulfate ester **1e** was not converted at all. The enantioselectivity—expressed as the enantiomeric ratio (*E* value[11]) was optimal for substrate **1b** where the relative size of substituents R^1 and R^2 was significantly different, which facilitates the chiral recognition process. Lower selectivities were observed when the functional group was gradually moved towards the center of the molecule, with R^1 and R^2 becoming similar in size to give near-symmetrical compounds *rac*-**1c**,**d**.
6) In general, the stereopreference was shown to be *R*, that is *R* configurated substrate enantiomers were transformed with inversion of configuration to give the corresponding *S* alcohols, while *S* sulfate esters remained untouched.

Almost identical results were obtained when lyophilized whole cells were used as biocatalysts, which indicates that no competing alkylsulfatase (showing different enantioselectivities or opposite stereopreference) was present. To improve the low enantioselectivity for *rac*-**1c** ($E=4.3$), a range of additives (such as metal ions, carbohydrates, and detergents) that are known to influence the chiral recognition of enzymes[12] were tested as "selectivity-enhancers". Whereas a modified polysaccharide (DEAE-dextran, $E=9.5$) and a detergent (cetyltrimethylammonium bromide, $E=30$) had a positive, but limited effect, the addition of metal ions resulted in a breakthrough: A dramatic selectivity-enhancement was achieved in the presence of Fe^{III} (5 mM), which raised the *E* value of *rac*-**1c** from 4.3 to >200, going in hand with a decrease in reaction rate.

In conclusion, a stereoselective *sec*-alkylsulfatase acting with inversion of configuration was found in *Rhodococcus ruber* DSM 44541. The remarkable feature of this biotransformation is the fact that—in contrast to the action of lipases, esterases, and proteases—the absolute configuration of the formed product and the remaining nonconverted substrate are identical to furnish homochiral *S* configurated products from a racemate. This constitutes an important step en route towards the deracemization of *sec*-alcohols by stereo- and enantioselective biohydrolysis of their corresponding sulfate esters.

Table 1. Stereoselectivities of enzymatic sulfate ester hydrolysis



Substrate	R^1	R^2	Conversion [%]	ee_P [%]	Enantioselectivity (*E*)[11]
rac-**1a**	*n*-C_5H_{11}	CH_3	3.6	–[a]	–
rac-**1b**	*n*-C_6H_{13}	CH_3	46	82	21
rac-**1c**	*n*-C_5H_{11}	C_2H_5	38	52	4.3
rac-**1d**	*n*-C_4H_9	*n*-C_3H_7	68	9	<2
1e	*n*-C_7H_{15}	H	0	–	–
rac-**1c**	*n*-C_5H_{11}	C_2H_5	33	74	9.5[b]
rac-**1c**	*n*-C_5H_{11}	C_2H_5	25	90	30[c]
rac-**1c**	*n*-C_5H_{11}	C_2H_5	9	99	>200[d]

[a] Very slow reaction. [b] Partially purified enzyme RS2 in presence of DEAE-Dextran (5 % (w/v)). [c] In presence of cetyltrimethylammonium bromide (0.2 % (w/v)). [d] In presence of Fe^{III} (5 mM).

Experimental Section

Synthesis of substrates: Alkyl sulfates **1a–e** were prepared by sulfatation of the corresponding alcohol using $NEt_3{\cdot}SO_3$ according to a known procedure.[9] In the same manner, (*R*)-2- and (*R*)-3-octyl sulfate were obtained from (*R*)-2- or (*R*)-3-octanol, respectively.

Screening for alkyl sulfatase activity: Lyophilized cells (50 mg)[5a] were rehydrated in Tris-buffer (pH 7.5, 0.1 M, 0.6 mL) for 0.5 h, *rac*-2-octyl sulfate solution was added (end concentration 22 mM, total volume 0.8 mL) and the vials were shaken for 5 days at room temperature. The conversion was determined by GC analysis after extraction of the formed alcohol with ethyl acetate using menthol as internal standard. The absence of spontaneous (nonenzymatic) hydrolysis for all substrates was proven by blank experiments in the absence of enzyme. When using whole-cell systems, a small amount of 2-octanone was formed as side product due to oxidation of 2-octanol.

Determination of enantiomeric composition: Alcohols **2a–d** were analyzed as the corresponding trifluoroacetate derivatives ($(CF_3CO)_2O$/EtOAc/60 °C/20 min) on GC (Chrompack CP7500 (cyclodextrin-B-2, 25 m × 0.25 mm, 25 μm film); Chirasil-Dex CB/G-PN (propionyl γ-cyclodextrin, 30 m × 0.32 mm; H_2)), and their absolute configuration was elucidated by co-injection using an independent reference sample.

Partial purification of *Rhodococcus* sulfatase (RS2): Cells were disrupted by using a bead mill and the cell-free extract was subjected to HIC (Phenyl Sepharose, Pharmacia) using a stepwise gradient. Active fractions were pooled and lyophilized.

Received: January 17, 2002
Revised: July 16, 2002 [Z18535]

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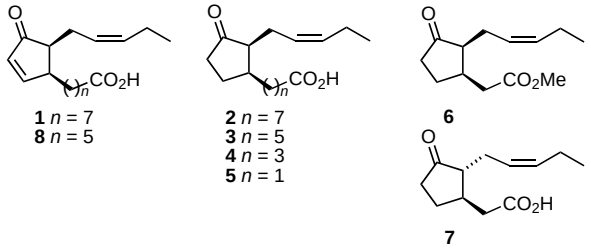
A New Synthesis Route to Enantiomerically Pure Jasmonoids**

Martin Ernst and Günter Helmchen*

Dedicated to Professor Volker Jäger on the occasion of his 60th birthday

12-Oxophytodienoic acid (12-OPDA) (**1**), ubiquitous in the plant kingdom, is the biosynthetic precursor for the jasmonoids **2**–**7**. These compounds result from **1** via the so-called octadecanoid cascade and participate as signaling compounds in a variety of processes.[1] 12-OPDA (**1**) itself originates from



1 $n = 7$
8 $n = 5$

2 $n = 7$
3 $n = 5$
4 $n = 3$
5 $n = 1$

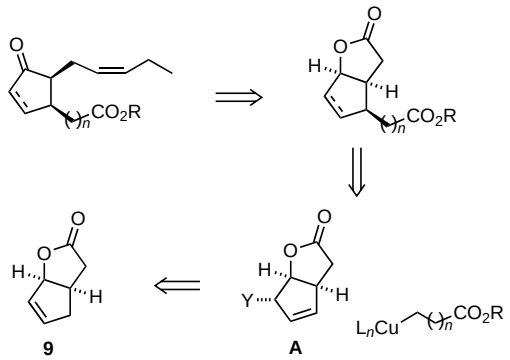
6

7

linolenic acid by oxidative cyclization. In 1997, dinor-oxophytodienoic acid (**8**), a hexadecanoid compound that is derived from hexadecatrienoic acid, was discovered and was shown to also possess pronounced biological activity.[2]

All of the jasmonoids possess an epimerizable *cis*-disubstituted cyclopentenone or cyclopentanone system. Many EPC syntheses exist for methyl epijasmonate (**6**) which is of great economical importance due to its use in fragrances.[3] For the other octadecanoids, a broadly applicable, diastereoselective route to the racemic compounds has been worked out by Crombie and Mistry.[4] The only asymmetric synthesis of enantiomerically pure **1**, presented by Grieco and Abood, employs an enzyme-catalyzed kinetic resolution.[5,15] We here report the development of an enantioselective synthetic route that could be applied to all of the jasmonoids, **1**–**8**, on the basis of a catalytic enantioselective process.

The concept of the synthesis is presented in Scheme 1 in the form of a retrosynthetic analysis. The key compound is an enantiomerically pure building block of general formula **A**



Scheme 1. General synthesis strategy for jasmonoids.

with a leaving group Y in allylic position. Herein, we could draw on studies by Roberts, Newton et al. who used the corresponding bromide, **A** with Y = Br, in the synthesis of prostaglandins, that is cyclopentanoids with *trans* configuration of the side chains.[6] They obtained the bromide by radical bromination of an isomer of lactone **9**. We wanted to introduce the carboxyalkyl side chain *cis* to the lactone function of the jasmonoids by an S_N2'-*anti*-reaction of intermediate **A** with an appropriate cuprate. Compound **A** was planned to be prepared from lactone **9** which was previously obtained in low selectivity by Pd-catalyzed asymmetric allylic alkylation of cyclopentenyl chloride using second-generation chiral phosphanyloxazoline ligands.[7]

With a third-generation phosphanyloxazoline, the ligand **L**,[8] we now obtained an enantiomeric excess of 95 % *ee* and a yield of 93 % in the allylic substitution with sodium dimethylmalonate (Scheme 2). The alkylated malonate was converted into the crystalline iodolactone whose enantiomeric purity was increased by recrystallization to > 99.9 % *ee*.[7] Dehydrohalogenation furnished the allylic lactone **9** in 70 % yield from **10**.

Bromolactone **11a** was prepared by radical bromination of **9** in CCl_4 under reflux in 60 % yield. In contrast to the racemate,[9] enantiomerically pure **11a** is an oil, and its purification by column chromatography laborious. Therefore, the series of allyl derivatives **14**–**17** was additionally prepared (Scheme 3). To access the non-natural enantiomers of the jasmonoids, *ent*-**9** was stereoselectively dihydroxylated,[10] and the resulting diol **12** was transformed into the allylic alcohol,[11] which was acylated. Both **12** and **13** as well as

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[**] This work was supported by the Fonds der Chemischen Industrie with consumables and a Kekulé scholarship for M.E. We thank Kerstin Brödner for competent experimental contributions and Dipl.-Biol. C. Bockelmann and Prof. E. Weiler, Bochum, for biological tests.

  0044-8249/02/4121-4054 $ 20.00+.50/0