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Enzymatic resolution of *rac*-1,1-dimethyl-1-sila-cyclohexan-2-ol by ester hydrolysis or transesterification using a crude lipase preparation of *Candida cylindracea*

Kirsten Fritsche[1], Christoph Syldatk[1], Fritz Wagner[1], Heidi Hengelsberg[2], and Reinhold Tacke[2]

[1] Institut für Biochemie und Biotechnologie, Technische Universität Braunschweig, Konstantin-Uhde-Strasse 5, D-3300 Braunschweig, Federal Republic of Germany
[2] Institut für Anorganische Chemie, Universität Karlsruhe, Engesserstrasse, D-7500 Karlsruhe, Federal Republic of Germany

Summary. *rac*-2-Acetoxy-1,1-dimethyl-1-sila-cyclohexane (*rac*-**2**) was synthesized by esterification of *rac*-1,1-dimethyl-1-sila-cyclohexan-2-ol (*rac*-**1**) with acetic anhydride. Enantioselective hydrolysis of *rac*-**2** in aqueous solution, catalysed by a crude lipase preparation of *Candida cylindracea* (EC 3.1.1.3), led to the formation of (*S*)-**1** (95% ee). Enantioselective transesterification of *rac*-**1** with triacetin in isooctane, catalysed by the same enzyme preparation, yielded (*S*)-**2** (95% ee), which was separated by chromatography from non-reacted (*R*)-**1** (96% ee). Recrystallization led to an improvement of the enantiomeric purity of (*R*)-**1** and (*S*)-**1** up to >98% ee. Thus the enantiomers of *rac*-**1** were prepared (100 mg scale) with high enantiomeric purities by the use of two different types of enzyme-catalysed reaction.

Introduction

In the course of our systematic studies on bioconversions of organosilicon compounds, we have shown that stereoselective microbial transformations, with growing cells and with resting free and immobilized cells, may be a suitable method for preparing optically active organosilicon species (Tacke et al. 1983; Tacke 1985; Tacke and Becker 1987; Stoffregen et al. 1987; Syldatk et al. 1987, 1988a, b; Tacke and Linoh 1989). In continuation of these studies, we have also attempted to prepare optically active organosilicon compounds with free enzymes as the biocatalyst. As an example of these investigations, we report here the enzymatic resolution of *rac*-1,1-dimethyl-1-sila-cyclohexan-2-ol (*rac*-**1**) using a crude lipase preparation of *Candida cylindracea* (EC 3.1.1.3). The aim of this work was to prepare both the (*R*)- and the (*S*)-enantiomer of 1,1-dimethyl-1-sila-cyclohexan-2-ol (**1**) with high enantiomeric purity. The (*R*)-enantiomer has already been obtained on a preparative scale [yield 80%, enantiomeric purity 82% ee (ee = enantiomeric excess)] by an enantioselective reduction of 1,1-dimethyl-1-sila-cyclohexan-2-one using growing cells of the yeast *Kloeckera corticis* (ATCC 20109) (Tacke et al. 1984).

Offprint requests to: C. Syldatk or R. Tacke

Materials and methods

Substrates. *rac*-1,1-Dimethyl-1-sila-cyclohexan-2-ol (*rac*-**1**) was prepared by reduction of 1,1-dimethyl-1-sila-cyclohexan-2-one (synthesized according to Brook et al. 1975) with lithium aluminium hydride in diethyl ether [14 mmol 1,1-dimethyl-1-sila-cyclohexan-2-ol, 29 mmol $LiAlH_4$, 50 ml Et_2O, 18 h at room temperature; yield: 11 mmol *rac*-**1** (79%), white solid, mp 34–35° C].

rac-2-Acetoxy-1,1-dimethyl-1-sila-cyclohexane (*rac*-**2**) was prepared by reaction of *rac*-**1** with acetic anhydride [11 mmol *rac*-**1**, 85 mmol $(CH_3CO)_2O$, 3 h at 140° C; yield: 8 mmol *rac*-**2** (73%), colourless liquid, bp 50° C/0.13 mbar (Kugelrohr distillation apparatus, Büchi Laboratoriums-Technik AG, Flawil, Switzerland)].

Enzymatic hydrolysis of rac-2 (analytical scale). A crude lipase preparation (120 mg) of *C. cylindracea* (EC 3.1.1.3, Sigma L 1754, München, FRG) was suspended at room temperature in 5 ml of 0.1 *M* Sörensen phosphate buffer, pH 6.8 (Rauen 1964). After centrifugation of the suspension (4500 rpm, 10 min; Labofuge 1, Heraeus-Christ, Osterode, FRG), the clear supernatant was diluted (1:1.5) with the same buffer solution. The reaction was started by addition of various amounts of *rac*-**2** to 2.5 ml of the enzyme solution obtained. During conversion, the suspension was mixed by head over head rotation (12 rpm). In order to study the dependence of reaction rate and enantioselectivity on the various parameters (see Results), the conversion was stopped at different times by extracting the reaction mixture with dichloromethane. The concentration of **1** and 2-acetoxy-1,1-dimethyl-1-sila-cyclohexane (**2**) in the dichloromethane extract was measured by gas-liquid chroma-

tography (GLC) [model 436, Chrompack, Frankfurt am Main, FRG; capillary column Cp-Sil 5 CB, 10 m; temperature programme: 70–100° C, 10° C/min; carrier gas H_2, 0.5 bar; split injection; retention time 1.1 min (**1**) and 1.9 min (**2**)]. The enantiomeric purity of the samples of (*S*)-**1** was determined as described below.

Enzymatic transesterification of rac-1 (analytical scale). A crude lipase preparation (100 mg) of *C. cylindracea* [containing 6% (w/w) water, determined by Karl Fischer titration] was suspended in 2 ml of different organic solvents (see Results). After dissolving various amounts of triacetin, the conversion was started by addition of different amounts of *rac*-**1** (incubation at 30° C; head over head rotation, 12 rpm). In order to study the dependence of the reaction rate on the various parameters (see Results), the conversion was stopped at different times by cooling the suspension to 0° C and adding dichloromethane. The concentration of **1** and **2** in the solution was determined by GLC as described above. The enantiomeric purity of the samples of (*R*)-**1** was determined as described below.

Enzymatic hydrolysis of rac-2 (preparative scale). A crude lipase preparation (7.2 g) of *C. cylindracea* was suspended at room temperature in 250 ml of 0.1 *M* Sörensen phosphate buffer, pH 6.8. After centrifugation of the suspension (7000 rpm, 5 min, 15° C; RC-5, Du Pont, Bad Nauheim, FRG), the clear supernatant was diluted with 500 ml of the same buffer solution. The resulting enzyme solution was warmed to 30° C and the reaction was started by adding 567 mg (3.04 mmol) of *rac*-**2**. During the conversion the suspension was stirred magnetically. After 135 min, the reaction was stopped (degree of conversion 38%, related to total amount of *rac*-**2**) by cooling the suspension to 4° C and adding 56.7 g of swelled XAD-2 adsorber (Serva 40820, Heidelberg, FRG). The resulting suspension was stirred mechanically for 3 h at 4° C. After decanting the aqueous phase, the XAD-2 adsorber was resuspended in 600 ml of dichloromethane and the suspension was stirred for 8 h at room temperature. The XAD-2 adsorber was filtered off and washed twice with 100 ml portions of CH_2Cl_2. After combining the dichloromethane extracts and removing the supernatant aqueous phase (small volume) with a syringe, the organic layer was dried over Na_2SO_4 and the solvent was evaporated in vacuo (100 mbar) at 35° C. Both **1** and **2** were separated by column chromatography on silica gel (40 g silica gel 60, Merck 7734, Darmstadt, FRG; diameter of the column 2 cm) using *n*-hexane/diethyl ether (2:1) as the eluant (order of elution: **2**, **1**). As both **1** and **2** are volatile compounds, the column was cooled with water (15° C) during the chromatographic separation. We obtained 119 mg of (*S*)-**1** (yield 71%, related to total amount of converted substrate *rac*-**2**) with an enantiomeric purity of 95% ee and 286 mg of (*R*)-**2** (yield 81%, related to total amount of non-converted substrate *rac*-**2**) with an enantiomeric purity of 57% ee. Repeated recrystallization of (*S*)-**1** from *n*-hexane at −20° C led to a product with an enantiomeric purity of >98% ee [mp 57° C (without correction); Thermovar, Reichelt, Austria; (−)-enantiomer, determined at 589 nm in $CHCl_3$; polarimeter 241, Perkin Elmer, Überlingen, FRG]. For determination of the enantiomeric purities, see below.

Enzymatic transesterification of rac-1 (preparative scale). A crude lipase preparation (20.3 g) of *C. cylindracea* [containing 6% (w/w) water, determined by Karl Fischer titration] was suspended in 400 ml isooctane. The reaction was started by adding 300 µl (1.6 mmol) of triacetin and 200 mg (1.39 mmol) of *rac*-**1** to the magnetically stirred suspension (incubation at 30° C). After 18.5 h the reaction was terminated (degree of conversion 52%, related to total amount of *rac*-**1**) by addition of 100 ml of dichloromethane and removal of the enzyme by subsequent filtration. The enzyme was washed twice with 100 ml portions of dichloromethane and the solvent was evaporated in vacuo (100 mbar) at 35° C. Both **1** and **2** were separated by column chromatography on silica gel (20 g silica gel) as described above. We obtained 124 mg of (*S*)-**2** (yield 92%, related to total amount of converted substrate *rac*-**1**) with an enantiomeric purity of 95% ee and 73 mg of (*R*)-**1** (yield 76%, related to total amount of non-converted substrate *rac*-**1**) with an enantiomeric purity of 96% ee. Repeated recrystallization of (*R*)-**1** from *n*-hexane at −20° C led to a product with an enantiomeric purity of >98% ee [mp 58° C, determined as above; (+)-enantiomer, determined as above]. For determination of the enantiomeric purities, see below.

Determination of the enantiomeric purity of (R)-1 and (S)-1. The organic layers obtained by extraction of the respective reaction mixtures (see above and below) were dried over Na_2SO_4. After removing the solvent in vacuo (100 mbar) at 35° C and dissolving less than 1 mg of the residue in about 150 µl of dichloromethane, 100 µl of isopropyl isocyanate were added. The mixture was heated in a screw-capped vial for 30 min at 100° C. After removing the solvent and the excess isocyanate with a stream of dry nitrogen, the residue was dissolved in dichloromethane and then analysed by GLC [model 436, Chrompack; capillary column, XE-60-L-Val-L-α-PEA (see König et al. 1982), 50 m; carrier gas H_2, 1 bar; isothermal mode, 145° C; split-injection; retention time 15.7 min (derivative of (*R*)-**1**) and 15.4 min (derivative of (*S*)-**1**)] (Fig. 1).

Determination of the enantiomeric purity of (R)-2 and (S)-2. Samples of **2** obtained on a preparative scale were converted into **1** as follows: a solution of 0.7 mmol of **2** in 5 ml diethyl ether was added dropwise to a stirred solution of 2 mmol of $LiAlH_4$ in 10 ml diethyl ether at 0° C. After refluxing for 2 h, the mixture was added to 20 ml of 1 *N* hydrochloric acid and the aqueous phase was extracted twice with diethyl ether. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo (100 mbar) at 28° C. The residue (consisting of the respective enantiomers of **1**) was dissolved in dichloromethane and analysed for its enantiomeric purity by GLC as described above.

Determination of the absolute configurations. The absolute configurations were determined via the (*S*)-α-methoxy-α-trifluoromethylphenylacetates of the enantiomers of **1** using a ^1H nuclear magnetic resonance (NMR) spectroscopic correlation method (Dale and Mosher 1973).

Results

The enantiomers of 1,1-dimethyl-1-sila-cyclohexan-2-ol [(*R*)-**1** and (*S*)-**2**] were prepared as follows: starting with the racemic compound *rac*-**1**, the corresponding acetate *rac*-**2** was synthesized by reaction with acetic anhydride using a standard procedure. Enantioselective hydrolysis of *rac*-**2** in aqueous solution, catalysed by a crude lipase preparation of *C. cylindracea* (EC 3.1.1.3), led to the formation of (*S*)-**1** (reaction a) (Fig. 2). Enantioselective transesterification of *rac*-**1** with triace-

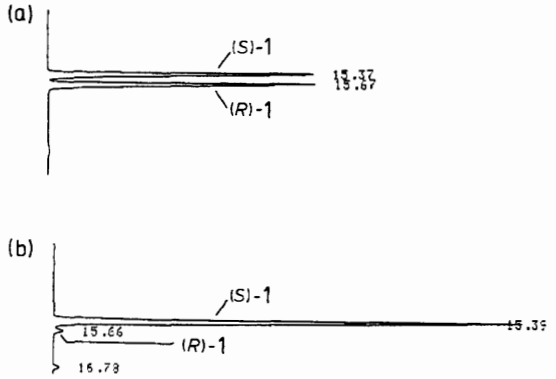

Fig. 1a, b. Determination of the enantiomeric purity of **1** by gas-liquid chromatography after derivatization with isopropyl isocyanate: chromatograms (including retention times) of the reference *rac*-**1** (**a**) and the product (*S*)-**1** (**b**) obtained by enzymatic hydrolysis of *rac*-**2**. For experimental details, see Materials and methods

tin (1,2,3-propanetriol triacetate) in isooctane, catalysed by the same enzyme preparation, yielded the acetate (*S*)-**2** (reaction b). The products of both these enzymatic conversions could be separated from the respective non-reacted substrates by column chromatography on silica gel to give the optically active products (*S*)-**1** and (*R*)-**2** (reaction a) as well as (*S*)-**2** and (*R*)-**1** (reaction b). After an additional recrystallization, (*R*)-**1** and (*S*)-**1** were isolated with enantiomeric purities of >98% ee.

Enzymatic hydrolysis of rac-2

As shown by investigations on an analytical scale, the rate and enantioselectivity of reaction a depend on the pH value, temperature and/or substrate concentration. At pH 5–7, the rate of conversion was found to be only slightly affected by the pH value, whereas at pH >7 a significant decrease of the rate was observed. Increase of the temperature from 30° C to 46° C led to a doubling of the reaction rate (measured at a degree of conversion of 48%, related to total amount of substrate *rac*-**2**), whereas the enantioselectivity decreased slightly with increasing temperature. Up to a substrate concentration of 25 mmol/l, no substrate inhibition was observed. However, studies at 2–12 mmol/l revealed a decrease of enantioselectivity with increasing substrate concentration.

Based on these results, the following conditions were chosen for a conversion of *rac*-**2** on a preparative scale (567 mg scale): pH 6.8, temperature 30° C, substrate concentration 4.1 mmol/l (for further details, see Materials and methods). The kinetics measured under these conditions are shown in Fig. 3. After terminating the reaction at a 38% conversion (related to total amount of substrate *rac*-**2**), the product (*S*)-**1** was isolated in 71% yield (related to total amount of converted *rac*-**2**) with 95% ee enantiomeric purity. Repeated recrystallization of the product led to an improvement of the enantiomeric purity up to >98% ee. The non-reacted (*R*)-**2** was isolated in 81% yield (related to total amount of non-converted *rac*-**2**) with 57% ee enantiomeric purity.

Enzymatic transesterification of rac-1

From several organic solvents (*n*-hexane, cyclohexane, *n*-heptane, *n*-octane, isooctane) tested in screening experiments, *n*-heptane and isooctane were found to be the most appropriate media (criterion: reaction rate) for the esterification of *rac*-**1** with triacetin. Compared with some other acetate

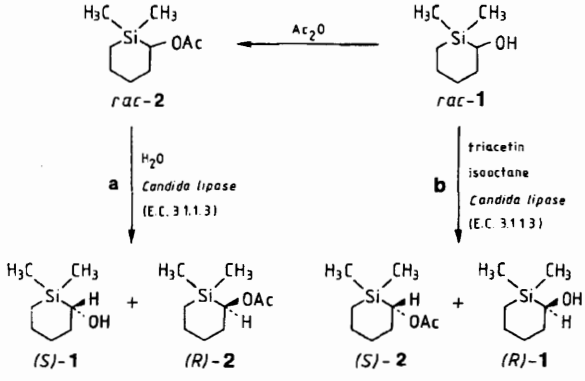

Fig. 2. Scheme showing the conversion of *rac*-1,1-dimethyl-1-sila-cyclohexan-2-ol (*rac*-**1**) to *rac*-2-acetoxy-1,1-dimethyl-1-sila-cyclohexane (*rac*-**2**) and to (*S*)-**1**, (*R*)-**2**, (*S*)-**2** and (*R*)-**1**

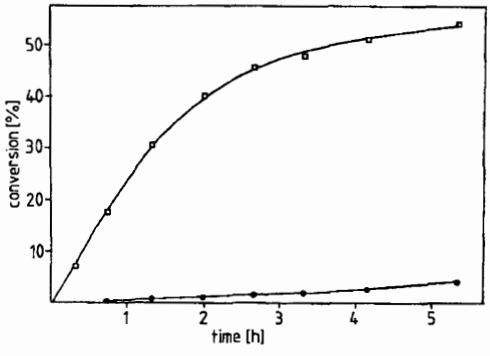

Fig. 3. Hydrolysis of *rac*-**2**: kinetics of the conversion (pH 6.8, temperature 30° C, substrate concentration 4.1 mmol/l); (□): converted (*S*)-**2**+(*R*)-**2**; (●): converted (*R*)-**2**

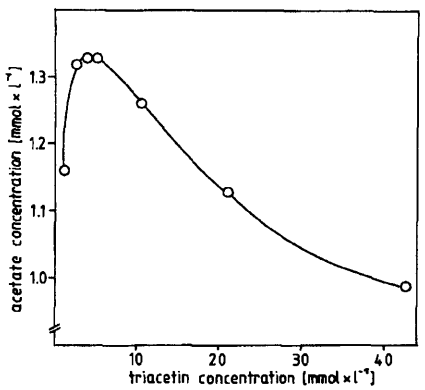

Fig. 4. Esterification of *rac*-**1**: influence of triacetin concentration on the rate of conversion (concentration of **2** formed after incubation of 3.5 mmol/l of *rac*-**1** with various triacetin concentrations for 7 h; temperature 30° C, isooctane)

sources (acetic acid, methyl acetate, ethyl acetate, isobutyl acetate, 3-methylbutyl acetate; dissolved or suspended in isooctane), triacetin gave the best results regarding the rate of conversion.

The triacetin concentration was found to have an important influence on the rate of reaction b (Fig. 4). Using a concentration of 3.5 mmol/l of *rac*-**1**, at 50% conversion (related to total amount of substrate *rac*-**1**) the highest transformation rate was observed with a triacetin concentration of 4 mmol/l (isooctane, 30° C). The kinetics of the transesterification of *rac*-**1** under the conditions used for the conversion on a preparative scale (200 mg scale) are shown in Fig. 5 (4 mmol/l triacetin, 3.5 mmol/l *rac*-**1**, isooctane, 30° C; for further details, see Materials and methods). After terminating the reaction at 52% conversion (related to total amount of substrate *rac*-**1**) the product (*S*)-**2** was isolated in 92% yield (related to total amount of converted *rac*-**1**) with 95% ee enantiomeric purity. The non-reacted (*R*)-**1** was isolated in 76% yield (related to total amount of non-

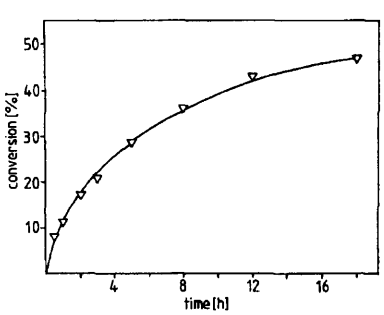

Fig. 5. Esterification of *rac*-**1**: kinetics of the conversion (temperature 30° C, 4 mmol/l triacetin, 3.5 mmol/l *rac*-**1**, isooctane)

converted *rac*-**1**) with 96% ee enantiomeric purity. Repeated recrystallization of (*R*)-**1** led to an improvement of the enantiomeric purity up to >98% ee.

Discussion

Both enantiomers of 1,1-dimethyl-1-sila-cyclohexan-2-ol [(*R*)-**1** and (*S*)-**1**] could be prepared on a preparative scale with high enantiomeric purities (96% ee and 95% ee, respectively; after recrystallization >98% ee) using two routes of enzymatic conversion, namely ester hydrolysis or transesterification, which were both catalysed by a crude lipase preparation of *C. cylindracea*. The enzyme preparation used has already been described in the literature as a potent biocatalyst which catalyses a variety of enantioselective hydrolyses of esters, esterifications of alcohols and transesterifications (Cambou and Klibanov 1984; Kirchner et al. 1985; Koshiro et al. 1985; Eichenberger et al. 1986; Langrand et al. 1986; Gillies et al. 1987).

The results described here clearly indicate that enzymatic conversions may be useful for synthetic purposes in silicon chemistry. Biotransformations with whole microbial cells have already proved their high potential for preparing optically active organosilicon compounds (Tacke et al. 1983; Tacke 1985; Tacke and Becker 1987; Stoffregen et al. 1987; Syldatk et al. 1987, 1988a, b; Tacke and Linoh 1989), and transformations with free enzymes undoubtedly constitute a very useful completion of this preparative method. From a synthetic point of view, enzymatic conversions in organic solvents (example: transesterification of *rac*-**1** with triacetin in isooctane) appear particularly promising for silicon chemistry as hydrolytically sensitive organosilicon compounds, which would undergo chemical hydrolysis in aqueous media, might also be used as substrates for enzyme-catalysed reactions.

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