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Enantioselective microbial reduction of 1,1-dimethyl-1-sila-cyclohexan-2-one with growing cells of the yeast *Kloeckera corticis* (ATCC 20109)

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Abstract

(*R*)-1,1-Dimethyl-1-sila-cyclohexan-2-ol [(*R*)-**2**] was prepared by enantioselective microbial reduction of 1,1-dimethyl-1-sila-cyclohexan-2-one (**1**) with growing cells of the yeast *Kloeckera corticis* (ATCC 20109). At a substrate concentration of 0.5 g/l (temperature 27 °C, incubation time 16 h), (*R*)-**2** was obtained on a preparative scale in 60% yield and with an enantiomeric purity of 92% ee. Repeated recrystallization of the biotransformation product from n-hexane raised the enantiomeric purity to 99% ee.

Introduction

In the course of our studies on the synthesis of optically active organosilicon compounds using bioconversions (for recent reviews, see refs. 1–3), various strains of microorganisms (bacteria, yeasts, fungi, algae) were found to reduce the carbonyl groups of cyclic and acyclic acylsilanes stereoselectively to give the corresponding (1-hydroxyalkyl)silanes [1–8]. The enantioselective reduction of 1,1-dimethyl-1-sila-cyclohexan-2-one (**1**) with cells of the yeast *Kloeckera corticis* (ATCC 20109) is an example of this type of biotransformation (Scheme 1; for a preliminary report on this reaction, see ref. 9). Here we describe details of this conversion which leads to the formation of optically active (*R*)-1,1-dimethyl-1-sila-cyclohexan-2-ol [(*R*)-**2**].

Results and discussion

During screening experiments with various strains of microorganisms, the cyclic acylsilane **1** (synthesis according to ref. 10) was found to be reduced enantioselec-

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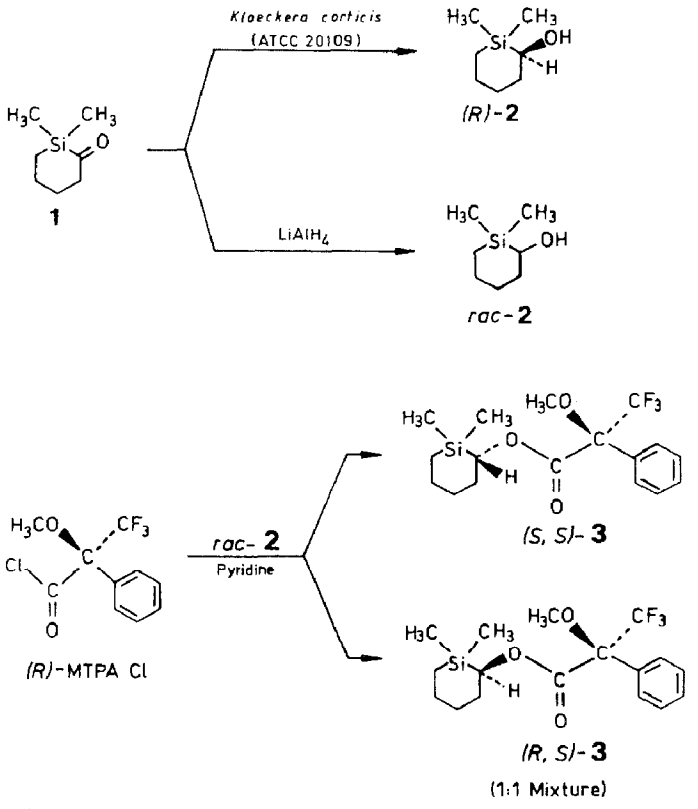
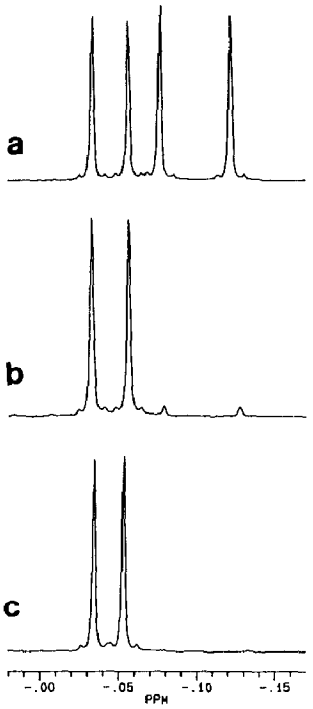


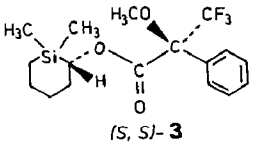
Scheme 1

tively to the optically active 1-sila-cyclohexan-2-ol (*R*)-**2** by growing cells of the yeast *Kloeckera corticis* (ATCC 20109) (Scheme 1). The biotransformation was carried out at 27 °C on a preparative scale at a substrate concentration of 0.5 g/l. After an incubation period of 16 h, the reduction was found to be almost complete (HPTLC monitoring). The biotransformation product was isolated in a yield of 60% (some product was lost during the working-up process because of its high vapour pressure) with an enantiomeric purity of 92% ee. Repeated recrystallization of the product from n-hexane at −20 °C raised the enantiomeric purity to 99% ee (Fig. 1).

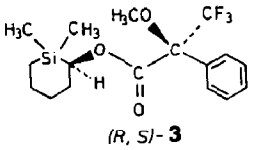
Earlier attempts to convert **1** into (*R*)-**2** with growing cells of *Kloeckera corticis* (ATCC 20109) at a substrate concentration of 1 g/l gave a yield of about 80% and an enantiomeric purity of 82% ee [3,9]. Such a dependence of enantiomeric purity on the substrate concentration has also been reported for other enantioselective microbial reductions (see ref. 5 and references therein).

The absolute configuration and enantiomeric purity of (*R*)-**2** were determined, after derivatization with (*R*)-α-methoxy-α-trifluoromethyl-phenylacetyl chloride [(*R*)-MTPA Cl], by ^{1}H NMR spectroscopic studies of the corresponding MTPA esters by the general method described in ref. 11. For purposes of comparison, racemic 1,1-dimethyl-1-sila-cyclohexan-2-ol (*rac*-**2**; available by reduction of **1** with $LiAlH_4$ in diethyl ether) and a 1 : 1 mixture of the respective diastereomeric MTPA esters (*R*,*S*)-**3** and (*S*,*S*)-**3** [available by esterification of *rac*-**2** with (*R*)-MTPA Cl in tetrachloromethane/pyridine] were prepared (Scheme 1). The MTPA esters of





(S, S)-**3**



(R, S)-**3**

Fig. 1. Partial ^{1}H NMR spectra ($SiCH_3$ groups) of the chemical (a) and biological (b) reduction products and the material (c) obtained after recrystallization of the biotransformation product. (a): (*R*,*S*)-**3**/(*S*,*S*)-**3** = 50/50; (b): (*R*,*S*)-**3**/(*S*,*S*)-**3** = 96/4; (c): (*R*,*S*)-**3**/(*S*,*S*)-**3** = 99.5/0.5.

the biotransformation product were obtained similarly. Figure 1 shows part of the ^{1}H NMR spectra of the MTPA esters of the chemical and biological reduction products and of the material obtained after repeated recrystallization of the biotransformation product.

Both enantiomers of **2** were recently prepared in high enantiomeric purity via an enzymatic resolution of *rac*-**2** [12]: (*R*)-**2** was obtained by enantioselective transesterification of *rac*-**2** with triacetin in isooctane using a crude lipase preparation of *Candida cylindracea* (E.C. 3.1.1.3), and the corresponding antipode (*S*)-**2** was prepared by enantioselective hydrolysis of *rac*-2-acetoxy-1,1-dimethyl-1-silacyclohexane in aqueous solution using the same enzyme preparation.

The results decribed in this paper and elsewhere [1–9,12] demonstrate that enantioselective biotransformations may be useful for the synthesis of optically active organosilicon compounds on a preparative scale.

Experimental

(a) Chemical syntheses

All reactions were performed in dried solvents under dry nitrogen unless otherwise indicated. Melting points were determined with a Reichelt Thermovar apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer at 400.1 and 100.6 MHz, respectively. Chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^{1}H, δ 7.25), $CDCl_3$ (^{13}C, δ 77.05) or

C_6H_6 (^1H, δ 7.15). Assignment of the ^{13}C data was supported by DEPT experiments. ^{19}F and ^{29}Si NMR spectra were recorded on a Bruker AC-200 spectrometer at 188.3 and 39.8 MHz, respectively. Chemical shifts (ppm) were determined relative to external $CFCl_3$ (^{19}F, δ 0.0) or $Si(CH_3)_4$ (^{29}Si, δ 0.0). Mass spectra were obtained with a Finnigan-MAT 8430 mass spectrometer (EI MS, 70 eV); the *m/z* values given refer to the isotopes ^1H, ^{12}C, ^{16}O and ^{28}Si. Optical rotations were determined with a Perkin–Elmer polarimeter 241.

*1,1-Dimethyl-1-sila-cyclohexan-2-one (**1**)*

This was synthesised as in ref. 10. ^1H NMR ($CDCl_3$): δ 0.16 (s, 6H; $SiCH_3$), 0.95–1.0 (m, 2H; $SiCH_2C$), 1.7–1.8 and 1.9–2.0 (m, 4H; CCH_2CH_2C), 2.35–2.45 (m, 2H; $C(O)CH_2C$). ^{13}C NMR ($CDCl_3$): δ −5.2 ($SiCH_3$), 16.2 ($SiCH_2C$), 23.9 and 28.4 (CCH_2CH_2C), 52.4 ($C(O)CH_2C$), 251.3 (C=O). ^{29}Si NMR ($CDCl_3$): δ −11.2. MS: *m/z* 142 (45%, M^+), 86 (100%).

*rac-1,1-Dimethyl-1-sila-cyclohexan-2-ol (rac-**2**)*

A solution of 1.99 g (14 mmol) **1** in 15 ml of diethyl ether was added dropwise at 0 °C (ice cooling) during 15 min to a stirred suspension of 1.10 g (29 mmol) $LiAlH_4$ in 35 ml of diethyl ether. After 5 h stirring at room temperature the mixture was added carefully (ice cooling) to 20 ml of 6*N* hydrochloric acid. The ethereal phase was separated and the aqueous layer extracted twice with 50 ml portions of diethyl ether. After neutralization of the combined organic extracts with diluted aqueous $NaHCO_3$ solution and washing with water, the organic solution was dried over $MgSO_4$. The solvent was removed under reduced pressure (260 Torr, 28 °C) and the residue purified by Kugelrohr distillation (40–50 °C/0.1 Torr) to yield 1.6 g (79%) of white crystals (by crystallization of the distillate), mp. 34–35 °C. ^1H NMR ($CDCl_3$): δ 0.07 (s, 3H; $SiCH_3$), 0.08 (s, 3H; $SiCH_3$), 0.4–0.5 and 0.7–0.8 (m, 2H; $SiCH_2C$), 1.2–1.3, 1.4–1.5, 1.6–1.7 and 1.9–2.0 (m, 6H; $CCH_2CH_2CH_2C$), 3.35 (m, 1H; $SiCH(OH)C$), OH not localized. ^{13}C NMR ($CDCl_3$): δ −7.1 ($SiCH_3$), −4.2 ($SiCH_3$), 13.0 ($SiCH_2C$), 23.8 and 26.0 ($SiCH_2CH_2CH_2C$), 35.7 ($C(OH)CH_2C$), 64.8 ($SiCH(OH)C$). ^{29}Si NMR ($CDCl_3$): δ −2.9. MS: m/*z* 144 (4%, M^+), 75 (100%). Found: C, 58.3; H, 11.2. $C_7H_{16}OSi$ (144.3) calc: C, 58.27; H, 11.18%.

*Transformation of rac-**2** into a 1:1 mixture of the diastereomeric MTPA esters (R,S)-**3** and (S,S)-**3***

30 µl of (*R*)-α-methoxy-α-trifluoromethyl-phenylacetyl chloride ((*R*)-MTPA Cl; prepared from (*S*)(−)-α-methoxy-α-trifluoromethylphenylacetic acid (Fluka 65364) as described in ref. 13) were added at room temperature to a stirred mixture of 600 µl of tetrachloromethane/pyridine (1/1, v/v) and 100 µmol of *rac*-**2**. After 24 h stirring at room temperature [complete conversion as monitored by TLC; silica gel plates (Merck, 5735), n-hexane/diethyl ether (2/1, v/v), UV detection], 24 µl of 3-dimethylamino-1-propylamine were added, and the mixture was stirred for 10 min. After addition of 15 ml of diethyl ether and 20 ml of 2% hydrochloric acid, the organic layer was separated and shaken with 20 ml of saturated aqueous Na_2CO_3 solution and then with 20 ml of water. The organic layer was dried over $MgSO_4$, the solvent removed under reduced pressure, and the residue dissolved in 0.5 ml of C_6D_6. To remove traces of diethyl ether, the solvent was again evaporated off and the residue redissolved in C_6D_6. The samples obtained by this procedure were used

for the NMR spectroscopic studies. The assignment of the absolute configuration at the carbon atoms of the sila-cyclohexane skeletons was performed by the correlation method described in ref. 11.

(*R,S*)-**3**: ^{1}H NMR (C_6D_6): δ −0.06 (s, 3H; $SiCH_3$), −0.03 (s, 3H; $SiCH_3$), 0.2–0.4, 0.5–0.7, 1.0–1.1, 1.2–1.5. 1.6–1.7 and 1.7–1.8 (m, 8H; $Si(CH_2)_4C$), 3.43 (q, ^5J(HF) = 1.2 Hz, 3H; OCH_3), 4.87 (m, X part of an ABX system, 1H; SiCH(OR)C), 6.9–7.7 (m, 5H; CC_6H_5). ^{19}F NMR (C_6D_6): δ −71.6.

(*S,S*)-**3**: ^{1}H NMR (C_6D_6): δ −0.13 (s, 3H; $SiCH_3$), −0.08 (s, 3H; $SiCH_3$), 0.3–0.4, 0.5–0.6, 1.0–1.2, 1.2–1.5, 1.6–1.8 and 1.8–1.9 (m, 8H; $Si(CH_2)_4C$), 3.46 (q, ^5J(HF) = 1.1 Hz, 3H; OCH_3), 4.93 (m, X part of an ABX system, 1H; SiCH(OR)C), 6.9–7.7 (m, 5H; CC_6H_5). ^{19}F NMR (C_6D_6): δ −71.5.

(b) Biotransformation

Slant culture

The yeast *Kloeckera corticis* (ATCC 20109) was maintained on agar slants [composition (in %): glucose (0.2), universal peptone (1), yeast extract (0.3), malt extract (1), agar (2)] and was kept at 8 °C.

Preparation of (R)-2

The yeast was precultivated for 16 h at 27 °C and 100 r.p.m. in 10 100-ml Erlenmeyer flasks, each containing 20 ml of a medium of the following composition (in %): glucose (2), universal peptone (1), yeast extract (0.5), malt extract (2). The cultures obtained were transferred to 10 2-l Erlenmeyer flasks, each filled with 400 ml of the same medium, and incubated for a further period of 24 h (27 °C, 100 r.p.m.). Then a solution of 200 mg of **1** in 0.3 ml of ethanol was added under aseptic conditions to each of the 10 flasks. After incubation for 16 h at 27 °C and 100 r.p.m., the biotransformation of **1** was almost complete (HPTLC monitoring, see below). The culture broths were combined and extracted several times with n-pentane (total amount 7 l). The combined organic layers were concentrated (→ 200 ml) in vacuo (rotary evaporator; 28 °C, 200 Torr) and then dried over $MgSO_4$. After removal of the solvent in vacuo (rotary evaporator; 28 °C, 200 Torr), the crude product was purified by Kugelrohr distillation (40–50 °C, 0.1 Torr) yielding 1.21 g (60%) of a white solid [(*R*)-**2**, enantiomeric purity 92% ee. (For determination of the absolute configuration and enantiomeric purity, see above)]. The spectroscopic properties of the purified product were identical with those of chemically prepared *rac*-**2** (see above).

The enantiomeric purity of the product obtained was improved as follows: A solution of 1.0 g of the purified biotransformation product (92% ee) in 8 ml of n-hexane was kept for several hours at −20 °C and the crystals were filtered off. The recrystallization was repeated three times under the same conditions to give 0.33 g of (*R*)-**2** with an enantiomeric purity of > 99% ee, m.p. 58 °C, $[\alpha]_{589}^{25}$ + 37 [*c* 0.99, $CHCl_3$ (Merck, 1755844)].

Monitoring of the time course of the biotransformation – analytical procedure

The progress of the conversion was monitored by HPTLC. Samples were taken and analyzed as follows: 0.2 ml of ethyl acetate were added to 1 ml of the culture broth. After 2 min shaking and subsequent centrifugation, a small sample of the

organic extract was transferred to a silica gel HPTLC plate (Merck, 5642) and the chromatogram was developed with n-hexane/ethyl acetate (7/3, v/v). After spraying of the plate with a reagent consisting of 50 ml of concentrated acetic acid, 1 ml of concentrated sulfuric acid and 0.6 ml of 4-methoxybenzaldehyde, the products showed up as a light red spot when the plate was heated for 2 min at 110 °C.

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