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ENANTIOSELECTIVE REDUCTION OF ACETYLDIMETHYLPHENYLSILANE: A SCREENING WITH THIRTY STRAINS OF MICROORGANISMS

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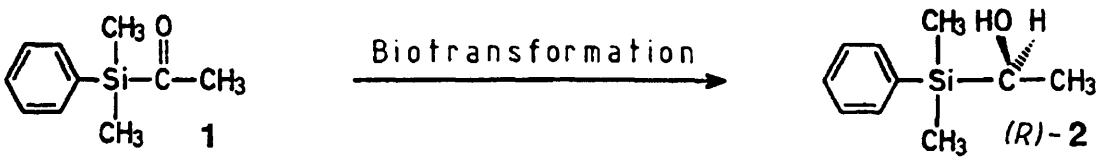
SUMMARY

Thirty strains of microorganisms (bacteria, yeasts, fungi and green algae) were tested as resting free cells for their ability to transform acetyldimethylphenylsilane (1) enantioselectively into (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-2]. The biotransformations were monitored by GC (packed OV-17 column), and the enantiomeric purities of the products isolated were determined by HPLC (cellulose triacetate column, UV detection). All microorganisms tested were found to reduce 1 enantioselectively to give (*R*)-2. Under the test conditions used, the yeast *Trigonopsis variabilis* (DSM 70714) was found to exhibit the highest specific activity (1.5 mg product x g cell wet mass^{-1} x min^{-1}), whereas the highest enantioselectivities were observed for the bacteria *Acinetobacter calcoaceticus* (ATCC 31012) (>95% ee), *Brevibacterium species* (ATCC 21860) (90% ee) and *Corynebacterium dioxydans* (ATCC 21766) (>95% ee), the yeast *Candida humicola* (DSM 70067) (90% ee), the fungus *Cunninghamella elegans* (ATCC 26269) (94% ee), as well as the cyanobacterium *Synechococcus leopoliensis* (94% ee). From the green algae tested, *Chlamydomonas reinhardii* showed the highest enantioselectivity (85% ee).

INTRODUCTION

There are many examples in the recent literature indicating the increasing importance of bioconversions as reaction steps in the synthesis of

optically active organic compounds (Schneider, 1985; Tramper et al., 1985; Yamada and Shimizu, 1988). In 1983 it was shown for the first time that biotransformations can also be useful for preparing optically active organosilicon compounds (Tacke et al., 1983). Since then a variety of stereoselective bioconversions of organosilicon substrates has been studied (Tacke and Becker, 1987; Syldatk et al., 1988). One of these reactions is the enantioselective reduction of acetyldimethylphenylsilane (**1**) into (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-**2**] using resting free cells (Syldatk et al., 1987) or immobilized cells (Stoffregen et al., 1987) of the yeast *Trigonopsis variabilis* (DSM 70714). Using resting free cells of this microorganism, (*R*)-**2** could be prepared on a preparative scale with an enantiomeric purity of 86% ee (Syldatk et al., 1987).



Here we report on the results of screening experiments with thirty different strains of microorganisms (see Table 1) which were tested for their ability to perform the conversion **1** → (*R*)-**2**. The aim of this study was (i) to improve the enantioselectivity of this biotransformation and (ii) to examine how far the ability is spread in biological systems to accept acetyldimethylphenylsilane (**1**) as a substrate for this reaction type. Compound **1** was regarded as a simple model substrate for acetylsilanes of the general type $R^1R^2R^3SiC(O)CH_3$.

MATERIALS AND METHODS

Substrate. Acetyldimethylphenylsilane (**1**) was synthesized according to the literature (Zilch and Tacke, 1986).

Microorganisms. All microorganisms listed in Table 1 were obtained from type culture collections (ATCC, DSM, IFO) and were cultivated in shake flasks at 100 rpm under the conditions described in the respective catalogues. The cyanobacterium and the green algae were gifts from Professor Wettern (Institute of Botany, Technical University of Braunschweig, FRG).

Bioconversions. After cultivating the respective microorganisms (see above), the cell wet mass (CWM) was harvested at room temperature by

centrifugation for 20 min at 6000 rpm (Centrifuge 4121, Heraeus, Osterode, FRG). 1 g of the cell wet mass was suspended in 40 ml 0.1 *M* Sörensen phosphate buffer, pH 6.8, containing 800 mg glucose, and was then incubated at 37°C and 100 rpm in a 100 ml Erlenmeyer flask. The bioconversion was started by addition of 10 mg of the substrate **1** (substrate concentration: 0.25 g x l^{-1}), and the reduction product **2** was isolated after complete conversion (GC control).

Monitoring of the bioconversions. At different times after starting the biotransformation, samples of 1 ml of the culture broth were taken and subsequently extracted with 1 ml of *n*-hexane. Quantitative determinations of **1** and **2** were performed by gas chromatographic analysis of 1 µl of these extracts using a Packard gas chromatograph, model 428 [OV-17 column, 500 mm; isothermal mode at 100°C; carrier gas nitrogen; retention time: 2.6 min (**1**) and 3.2 min (**2**)].

Determination of the enantiomeric purities. After complete conversion of the substrate **1**, the whole culture broth was extracted with 40 ml of *n*-hexane. After removing the solvent of the organic extract under reduced pressure (rotary evaporator), the residue was dissolved in 0.3 ml ethanol. 20 µl of this solution were analyzed by HPLC (Beckman, model 338) on a cellulose triacetate column (No. 50003, Merck, Darmstadt, FRG) using ethanol/H_2O(bidest) 60:40 (V/V) as the eluent (flow rate: 0.8 ml x min^{-1}). The enantiomers of **2** were detected by UV at 254 nm (retention time: 79.9 [(*S*)-**2**] and 104.1 min [(*R*)-**2**]).

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained in the screening experiments. All microorganisms tested were found to be able to reduce acetyldimethylphenylsilane (**1**) enantioselectively to give (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-**2**]. Large differences in specific activity (mg product x g cell wet $mass^{-1}$ x min^{-1}) and in enantioselectivity (% enantiomeric excess) were observed. Under the test conditions used, the yeast *Trigonopsis variabilis* (DSM 70714) exhibited the highest specific activity (1.5 mg product x g cell wet $mass^{-1}$ x min^{-1}), whereas the highest enantioselectivities (90 to >95% ee) were found with the bacteria *Acinetobacter calcoaceticus* (ATCC 31012), *Brevibacterium species* (ATCC 21860) and *Corynebacterium dioxydans* (ATCC 21766), the yeast *Candida humicola* (DSM 70067), the fungus *Cunninghamella elegans* (ATCC 26269), as well as the cyanobacterium *Synechococcus leopoliensis*. From the green algae tested, the strain *Chlamydomonas reinhardii* exhibited the highest enantioselectivity (85% ee).

Table 1

Enantioselective reduction of acetyldimethylphenylsilane (**1**) into (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-**2**]: Specific activity of the biocatalysts and enantioselectivity of the bioconversions (reaction conditions: see experimental section)

Microorganism	Type culture collection and number	Specific activity (mg product x g CWM^{-1} x min^{-1})	Enantio-selectivity (% ee)
Acinetobacter calcoaceticus	ATCC 31012	0.31	>95
Agrobacterium tumefaciens	IFO 3058	0.42	16
Arthrobacter flavescens	ATCC 13348	0.67	64
Arthrobacter paraffineus	ATCC 15591	0.19	85
Arthrobacter species	DSM 3747	0.31	75
Brevibacterium species	ATCC 21860	0.10	90
Corynebacterium dioxydans	ATCC 21766	0.31	>95
Klebsiella oxycota	ATCC 8724	0.12	63
Nocardia species	ATCC 4267	0.76	68
Pseudomonas fluorescens	ATCC 15453	0.15	70
Rhodococcus erythropolis	ATCC 11048	0.49	76
Candida albicans	ATCC 10231	0.33	86
Candida boidinii	DSM 70026	0.28	32
Candida humicola	DSM 70067	0.33	90
Candida lipolytica	DSM 1345	0.33	21
Candida utilis	DSM 2361	0.31	81
Kloeckera corticis	ATCC 20108	0.24	80
Pichia guillermondii	ATCC 46036	0.31	14
Pichia pijperi	ATCC 20129	0.35	10
Torulopsis bombicola	ATCC 22214	0.34	58
Torulopsis candidum	DSM 70590	0.28	36
Trigonopsis variabilis	DSM 70714	1.50	86
Saccharomyces cerevisiae	ATCC 22224	0.32	78
Aspergillus niger	ATCC 9020	0.05	77
Cunninghamella elegans	ATCC 26269	0.08	94
Geotrichum candidum	DSM 1240	0.32	58
Ustilago maydis	ATCC 14826	0.21	65
Synechococcus leopoliensis	SAG 1402-1	0.23	94
Chlamydomonas reinhardii	Y-1	0.003	85
Chlorella fusca	SAG 211/8b	0.67	75