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Enantioselective reduction of acetyldimethylphenylsilane by *Trigonopsis variabilis* (DSM 70714)

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Summary. Growing and resting cells of the yeast *Trigonopsis variabilis* (DSM 70714) can be used for the enantioselective reduction of the organosilicon compound acetyldimethylphenylsilane (*1*) to give optically active (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-*2*] in good yields. The enantiomeric purity of the isolated product was determined to be 62–86% ee depending on the substrate concentration used. Both substrate and product caused an inhibition of the reaction at concentrations higher than 0.35 and 0.5 g/l, respectively. Besides, higher substrate and product concentrations led to increased formation of the by-product 1,1,3,3-tetramethyl-1,3-diphenyldisiloxane. Considering the limiting substrate and product concentrations, it was possible to use the same biomass at least 5 times without significant loss of enzyme activity. 3-Methyl-3-phenyl-2-butanone (*5*) and acetyldimethylphenylgermane (*7*), which represent carbon and germanium analogues of *1*, were also found to be accepted as substrates by *Trigonopsis variabilis* (DSM 70714). The reduction rates of the silicon (*1*) and germanium compound (*7*) were much higher than the transformation rate of the corresponding carbon analogue *5*.

Introduction

The great efficiency of biotransformation steps in asymmetric organic synthesis has been sufficiently appreciated in numerous reviews (e.g. Leuenberger 1984; Leuenberger and Kieslich 1984; Sih and Chen 1984; Schmidt-Kastner and Egerer 1984; Sariaslani and Rosazza 1984; Whitesides and Wong 1985). There are many examples of compounds accessible by bioconversions, which are not available so far by chemical methods (resolution of racemates via diastereomeric derivatives, asymmetric chemical synthesis, stereospecific reactions) at all or only at much higher costs (Sih and Rosazza 1976; Jones 1985). One important application of biotransformations is the preparation of optically active compounds for the use as drugs, agrochemicals or as feed and food stuffs (Rétey and Robinson 1982).

In recent years there has been increasing interest in optically active organosilicon compounds with respect to their use as reagents in asymmetric organic synthesis (Larson and Torres 1985) and as drugs in experimental pharmacology (Sheldrick et al. 1985; Tacke and Zilch 1986a, b). Several methods have been employed to prepare optically active silicon compounds including resolution through separation of diastereomers, kinetic resolution, asymmetric synthesis, as well as stereospecific reactions starting from resolved compounds (Corriu and Guérin 1982; Corriu et al. 1984). Recently, it was shown for the first time that biotransformations are also useful for preparing optically active organosilicon compounds: Some microorganisms were found to reduce carbonyl groups of various organosilicon substrates stereoselectively to give the corresponding optically active alcohols (Tacke et al. 1983; Tacke et al. 1984; Tacke et al. 1985; Tacke and Zilch 1986a; Syldatk et al. 1986). One of these bioconversions is the enantioselective reduction of acetyldimethylphenylsilane (*1*) to give (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-*2*]. The aim of the present work was to optimize this biotransformation process and to develop reproducible procedures to obtain (*R*)-*2* in high yield and with high enantiomeric purity at low costs. It was of interest to find

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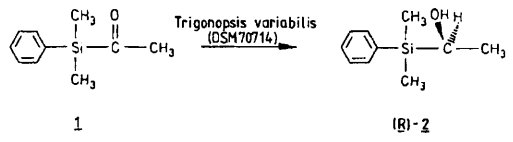
out, which sideconditions have to be fulfilled for a complete and highly enantioselective reduction of *1* and which influence *1* and *(R)-2* have on the microorganisms.



1 (R)-2

Materials and methods

Microorganisms. One hundred yeasts were tested for their ability to reduce the substrate acetyldimethylphenylsilane (*1*) to give the corresponding alcohol (1-hydroxyethyl)dimethylphenylsilane (*2*). All microorganisms were obtained from public type culture collections (ATCC, CBS, DSM).

Medium and culture conditions. The microorganisms were cultivated under aerobic conditions at pH 6.8 and 27°C in a medium containing 0.5% yeast extract, 2% malt extract, 1% peptone and 2.5% glucose.

Screening experiments were carried out in shaking flasks (100 ml) containing 20 ml of medium. The microorganisms were incubated at 100 rpm for 24—48 h before 20—50 mg of substrate *1* were added to the growing cells. For a preparative scale, the selected microorganism was cultivated in shaking flasks (2000 ml) containing 500 ml of medium in the same way before adding the neat substrate to the culture broth (→substrate concentration 0.2—0.5 g/l). Batch cultivations were carried out in a G20 bioreactor (Braun-Melsungen AG, Melsungen, FRG) equipped with flat blades. Physiological activity of the cells was monitored using a pH-electrode, a pO_2-electrode, as well as oxygen and carbon dioxide analyzers (Maihak, Hamburg, FRG). During the cultivations a constant pH value of pH 6.8 was maintained by titration with a 10% aqueous solution of NaOH. The time course of the cultivations was followed by measuring the yeast dry mass and the glucose concentration. Cells for the resting cell experiments were harvested by centrifugation at 20000 rpm with a Padberg centrifuge type 41 (Carl Padberg GmbH, Lahr, FRG) after the C-source had been consumed.

Synthesis of substrate 1 and its racemic reduction product rac-2: Acetyldimethylphenylsilane (*1*) was synthesized as described in the literature (Zilch and Tacke 1986c).

rac-(1-Hydroxyethyl)dimethylphenylsilane (*rac-2*): A solution of 10 mmol *1* in 10 ml of dry ether was added dropwise to a stirred slurry of 20 mmol $LiAlH_4$ in 50 ml ether during 10 min at room temperature. After stirring at room temperature for 1 h, the mixture was cooled and poured into 50 ml of 6 N aqueous HCl at 0°C. The organic layer was separated, washed with dilute aqueous $NaHCO_3$, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue purified by Kugelrohr distillation (70°C, 0.1 Torr) and column chromatography [silica gel 60 (Merck AG, Darmstadt, FRG), ether/n-hexane 1:1.5 (V/V)] to give a colourless liquid (yield 90%). ^{1}H-NMR ($CDCl_3$; WM 400-spectrometer, Bruker): $\delta = 0.32$ (s, 6H, $SiCH_3$), 1.25 (d, $J = 7.6$ Hz, 3H, CCH_3), 3.66 (q, $J = 7.6$ Hz, 1H, $SiCH(OH)CH_3$), 7.1—7.7 (m, 5H, SiC_6H_5), OH not located. MS (70 eV; CH7-spectrometer, Varian MAT): m/z = 165 (44%, $M^+ - CH_3$), 135 (100%, $M^+ - CH(OH)CH_3$). Anal. ($C_{10}H_{16}OSi$) C, H.

Biotransformations. Biotransformations of the substrate acetyldimethylphenylsilane (*1*) by growing cells of the yeast *Trigonopsis variabilis* (DSM 70714) were carried out in a bioreactor by adding the neat compound to the culture medium under sterile conditions in three portions 16, 28 and 29 h after start of the cultivation (see Fig. 1B); the respective substrate concentrations were 0.5 g/l. Experiments with resting cells were carried out under various conditions in 250 ml shaking flasks containing 50 ml of 0.1 M Sörensen phosphate buffer, 1.0% glucose, 25.0 g yeast wet mass, and various concentrations of acetyldimethylphenylsilane (*1*). The yeast wet mass was washed twice with 0.1 M Sörensen phosphate buffer (pH 6.8) before use.

Analytical procedure. After various times of incubation, 1 ml of the culture suspension was extracted with n-hexane or ethyl acetate. Qualitative detection of substrate *1* and product *2* was performed by thin layer chromatography on silica gel plates (No. 5554, Merck AG, Darmstadt, FRG) with ether/n-hexane [1:1.5 (V/V)]. *1* and *2* were detected by spraying the plates with 4-methoxybenzaldehyde (Krebs et al. 1967). Quantitative determinations of *1* and *2* were carried out by gas chromatography using a Packard gas chromatograph model 428: Column OV17 (500 mm), isothermal mode at 100°C.

Preparative isolation and purification of the biotransformation product (R)-2. The whole culture broth was extracted twice with ethyl acetate. After removing the solvent with a rotary evaporator, the crude product was purified by column chromatography on silica gel 60 (Merck AG, Darmstadt, FRG) with ether/n-hexane [1:1.5 (V/V)] as the eluent, followed by Kugelrohr distillation in vacuo. Purification by preparative layer chromatography on silica gel plates (No. 5745, Merck AG, Darmstadt, FRG) was also successful. The spectroscopic properties of the purified product were identical with those of *rac-2*, which was obtained by chemical synthesis (see above).

Enantiomeric purity and absolute configuration of (R)-2. The enantiomeric purities of the biotransformation products were determined by ^{1}H-NMR measurements after derivatisation with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride according to Mosher's method (Dale et al. 1969). These determinations were performed with the purified biotransformation products (after chromatography and distillation). The absolute configuration of the excess enantiomer was determined by a ^{1}H-NMR correlation method described in the literature (Dale and Mosher 1973). The specific rotation of pure (*R*)-2 (100% ee) was calculated to be $[\alpha]_{546} = +24°$ (c = 1, $CHCl_3$). This value was obtained by extrapolation from the optical rotations of different samples with known enantiomeric purities as determined by Mosher's method.

Results

Screening experiments

One hundred yeasts were tested for their ability to transform acetyldimethylphenylsilane (*1*) into (*R*)-(1-hydroxyethyl)dimethylphenylsilane [(*R*)-*2*]. The racemic reduction product *rac-2*, which was prepared by chemical synthesis as described

above, was used as reference in these experiments. Most of the yeasts were able to reduce *1* *Trigonopsis variabilis* (DSM 70714) showed the best results concerning the reaction rate, the isolation and the purity of the product *2* with respect to the formation of biogenic by-products, and was therefore used in the experiments described in this paper.

Biotransformation by growing cells

Figure 1 shows the time course of the cultivation of *Trigonopsis variabilis* (DSM 70714) in a bio-

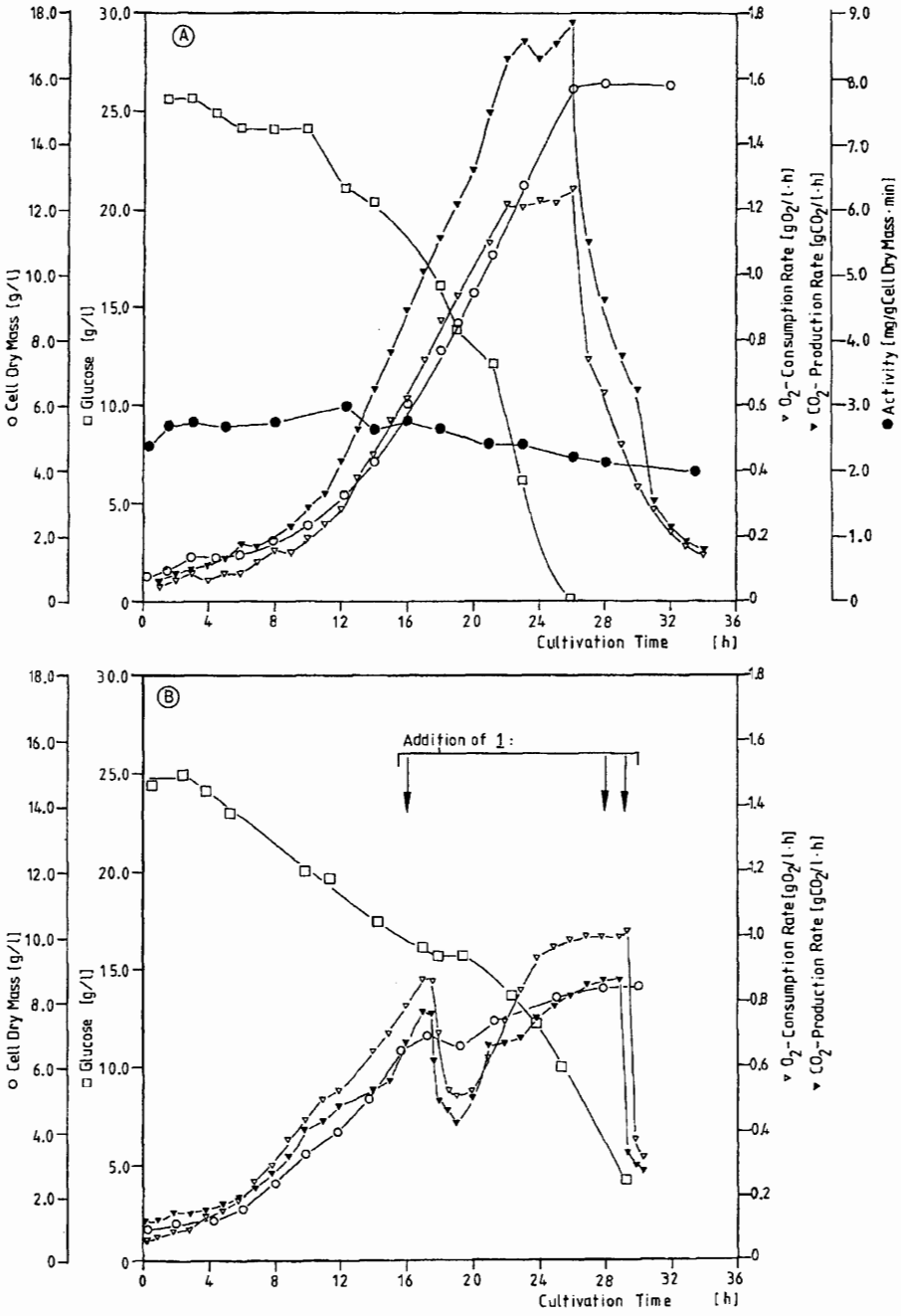


Fig. 1. Time course of the cultivation of *Trigonopsis variabilis* (DSM 70714) in a 20 l bioreactor without addition of acetyldimethylphenylsilane (*1*) (**A**) and with addition of three portions of 0.5 g/l *1* (neat compound) 16, 28 and 29 h after the start of the cultivation (**B**) (conditions: medium containing 0.5% yeast extract, 2% malt extract, 1% peptone, and 2.5% glucose; pH 6.8, 27°C, 400 rpm, 0.5 V/V min)

reactor without addition of *1* (Fig. 1A) and with addition of 0.5 g/l of neat *1* 16, 28 and 29 h after the start of the growth (Fig. 1B). The addition of the substrate had an inhibiting effect on the O_2-consumption and on the growth of the microorganism. While the first biotransformation with growing cells was complete after only 20 min (see Fig. 2), a second transformation took 60 min. The third one, following 1 h after the second bioconversion, required even 4 h. The crude product, obtained by extraction of the culture broth, was found to contain many biogenic by-products. After purification the biotransformation product (*R*)-*2* could be isolated with a yield of 54% and an enantiomeric purity of 84% ee.

Biotransformation by resting cells

Figure 1A shows the growth of *Trigonopsis variabilis* (DSM 70714) without addition of *1*. At several points of time samples were taken to test the enzymatic activity of the cells as a function of their physiological age under resting cell conditions. Since there was no difference in the enzymatic activity of cells from different growth phases, biomass from the late exponential phase of growth was used for the following experiments. Figure 3 shows the biotransformation of *1* by growing cells in a bioreactor in comparison with the bioconversion by resting cells in a phosphate buffer containing only 1% glucose for the co-enzyme regeneration. Since only a slight difference in specific activity was observed, resting cell experiments were used to optimize the reaction conditions. With regard to the transformation rate of *1*, a temperature of 37°C and a pH value in the range of 6.0—8.0 were found to be optimal conditions. A linear correlation was observed between the biomass concentration and the reaction rate. In addition, the glucose concentration was found to play an important role: For the complete reduction (gaschromatographic control) of 0.5 g/l substrate 3 g/l glucose had to be added per gram cell dry mass per liter.

In experiments with varying substrate concentrations, *1* was found to have an inhibiting effect on the enzymatic activity of the cells at substrate concentrations higher than 0.35 g/l (see Fig. 4).

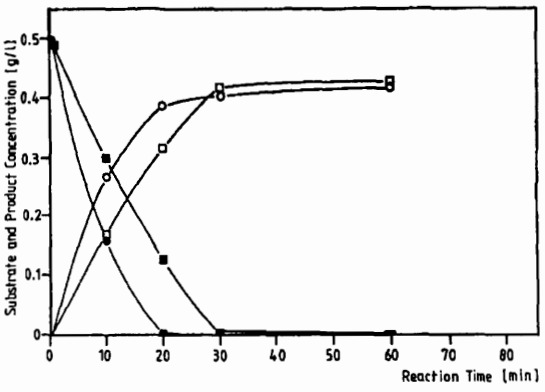


Fig. 3. Time course of the reduction of acetyldimethylphenylsilane (*1*) (closed symbols) and the formation of (1-hydroxyethyl)dimethylphenylsilane (*2*) (open symbols) by growing (○) and resting cells (□) of *Trigonopsis variabilis* (DSM 70714) (reaction conditions: substrate concentration 0.5 g/l, 25.0 g/l yeast wet mass, 500 ml shaking flasks containing 100 ml medium; conditions for growing cells: 0.5% yeast extract, 2% malt extract, 1% peptone, and 2.5% glucose; pH 6.8, 27°C, 100 rpm; conditions for resting cells: Sörensen phosphate buffer, pH 6.8, 27°C, 1% glucose, 100 rpm)

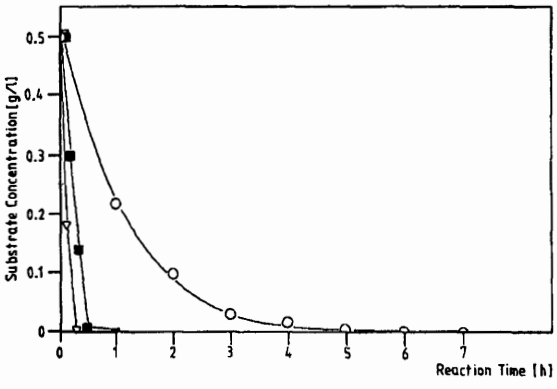


Fig. 2. Time course of the reduction of acetyldimethylphenylsilane (*1*) by growing cells of *Trigonopsis variabilis* (DSM 70714). The neat substrate was added in three portions to the culture broth (respective substrate concentration 0.5 g/l) 16 (▽), 28 (■) and 29 h (○) after the start of the cultivation (conditions: see Fig. 1)

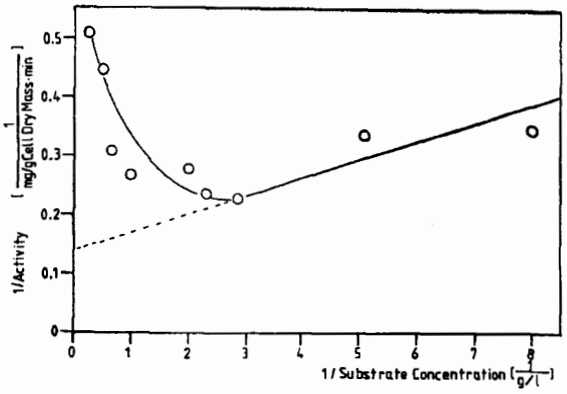


Fig. 4. *Lineweaver Burk* plot of the substrate concentration against the specific reduction activity (*1*→*2*) of resting cells of *Trigonopsis variabilis* (DSM 70714)

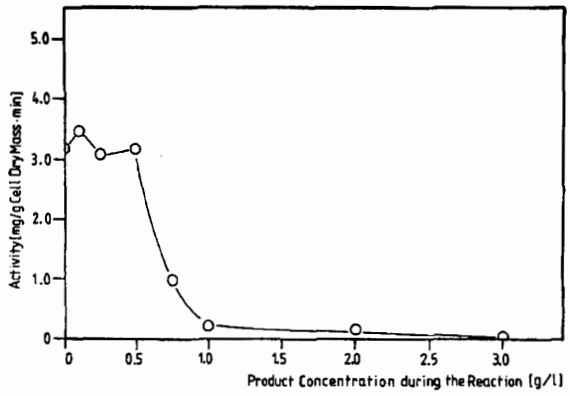
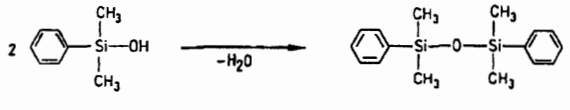


Fig. 5. Dependence of the specific reduction activity (*1*→*2*) of resting cells of *Trigonopsis variabilis* (DSM 70714) on the product concentration (reaction conditions: Sörensen phosphate buffer, pH 6.8, 27°C, 1% glucose, 25 g/l yeast wet mass)

This effect, which had already been indicated by the growing cell experiments in the bioreactor (see Figs. 1B and 2), is irreversible and nearly complete at substrate concentrations higher than 1 g/l. Product concentrations higher than 0.5 g/l also caused inhibition (see Fig. 5).

At substrate concentrations of 1.0 g/l or higher, not only the reaction velocity dropped rapidly, but also the by-product 1,1,3,3-tetramethyl-1,3-diphenyldisiloxane (*4*) could be monitored by gas chromatography (by comparison with an authentic sample) and isolated from the reaction mixture. The disiloxane *4* is a condensation product of dimethylphenylsilanol (*3*), the formation of which may be explained by a cleavage of the Si—C bond of the Si—C(O)CH_3 unit of *1*. Even after incubating *1* with heat-treated cells (→inactivation of the enzymes by heating the cells for 90 min at 60°C), the disiloxane *4* could be isolated indicating that its formation is probably not an enzymatic but rather a chemical one.



3 4

Considering the inhibiting substrate and product concentrations, it was possible to use free resting cells at least five times for the reduction of *1* without significant loss of activity, if they were separated from the reaction mixture by centrifugation and washed with phosphate buffer before the next biotransformation. Moreover, it was possible to store the cells by freezing at −32°C without significant inactivation.

As can be seen from Table 1, the substrate concentration was found to play an important role for the enantioselectivity of the biotransformation. Enantiomeric purities between 62 (substrate concentration 1.0 g/l) and 86% ee (substrate concentration 0.25 g/l) were observed; the enantiomeric purity increased with decreasing substrate concentration.

The crude product isolated from the reaction mixture of the resting cells by extraction with ethyl acetate contained much less biogenic by-products than in the case of growing cells; isolation and purification were much easier. Using a substrate concentration of 0.25 g/l, (*R*)-*2* was obtained in a yield of 70% and an enantiomeric purity of 86% ee.

Preliminary studies have shown that 3-methyl-3-phenyl-2-butanone (*5*) and acetyldimethylphenylgermane (*7*) are also accepted as substrates by *Trigonopsis variabilis* (DSM 70714) to give the reduction products *6* (not isolated; structural proof by gaschromatographic comparison with an authentic racemic sample of *6*) and *8* (isolated as the (*R*)-configurated enantiomer; structural proof by elemental analysis and spectroscopic investigations). Compounds *5* and *7* represent a carbon and a germanium analogue, respectively, of ace-

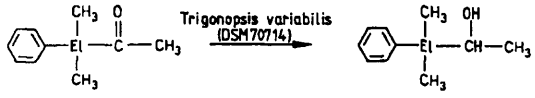
Table 1. Dependence of the enantiomeric purity [enantiomeric excess (% ee)] of the reduction product (*R*)-*2* on the concentration of the substrate acetyldimethylphenylsilane (*1*) (reaction conditions: 0.1 M Sörensen phosphate buffer, pH 6.8, 27°C, 2.5% glucose, 25.0 g/l yeast wet mass)

Substrate concentration [g/l]	Enantiomeric excess of (*R*)-*2* [% ee]
1.0	62
0.5	68
0.35	77
0.25	86

Table 2. Specific activities of resting cells of *Trigonopsis variabilis* (DSM 70714) concerning the transformation of acetyldimethylphenylsilane (*1*) and its carbon (*5*) and germanium analogue (*7*). The substrates were added as neat compounds (reaction conditions: 0.1 M Sörensen phosphate buffer, pH 6.8, 27°C, 2.5% glucose, 25.0 g/l yeast wet mass, substrate concentration 0.35 g/l)

Substrate	El	Activity [mg/g cell dry mass·min]
1	Si	4.2
5	C	0.2
7	Ge	2.4

tyldimethylphenylsilane (*1*). As can be seen from Table 2, the highest specific activity of the yeast was observed in the case of the silicon compound *1*, and the lowest specific activity for the carbon analogue *5*. These investigations were carried out with resting cells using substrate concentrations of 0.35 g/l. Systematic studies of the biotransformations *5*→*6* and *7*→*8* are in progress.



5,7 6,8

Discussion

The results presented in this paper demonstrate that biotransformations can be used for the preparation of optically active organosilicon compounds. By analogy to the enantioselective reduction of many organic ketones (R_3C—CO—CR_3) by various yeast species, acetyldimethylphenylsilane (*1*) with its R_3C—CO—SiR_3 unit is transformed by *Trigonopsis variabilis* (DSM 70714) into the reduction product (*R*)-*2*. Although a carbonyl group attached to silicon differs substantially in its properties from a carbonyl unit bound to carbon (Brook 1968; Armitage 1982), the CO group of *1* is accepted by the microorganism in a "normal manner".

The growth of *Trigonopsis variabilis* (DSM 70714) is inhibited by the addition of *1* to the culture medium at a concentration of 0.5 g/l as one can see from Fig. 1. The experiments with resting cells and varying substrate concentrations showed that *1* and *2* also have an inhibiting effect on the enzymatic reduction activity of the yeast at concentrations higher than 0.35 and 0.5 g/l. Using substrate concentrations of *1* of 1.0 g/l or higher, the by-product 1,1,3,3-tetramethyl-1,3-diphenyldisiloxane (*4*) is produced. The enantiomeric purity of the product *2* also depends on the substrate concentration used. The highest ee values were obtained at low substrate concentrations. Such dependence of enantiomeric purity on substrate concentration has already been reported in the literature for related reductions (Matzinger and Leuenberger 1985; Sih and Chen 1984; Sih et al. 1984).

There is probably more than one enzyme in *Trigonopsis variabilis* (DSM 70714) involved in the reduction of *1*, which are characterized by different K_M-values and stereoselectivities as is the case in the reduction of β-ketoesters by *Saccharomyces cerevisiae* (Sih et al. 1984).

Considering these results and the fact, that resting cells can be reused five times without significant loss of activity for the reduction of *1*, these experiments seem to be an appropriate basis for a continuous process with immobilized resting cells. Such a process with low substrate and product concentrations could be more advantageous than a batch process with respect to the enantioselectivity of the biotransformation. The use of immobilized resting cells seems to be interesting for the reduction of acetyldimethylphenylsilane (*1*) and structurally related silicon and germanium compounds on a preparative scale, because immobilized cells often show greater stability than resting free cells and can be reused to catalyze enzymatic reactions for several weeks without significant loss of activity; however, the reaction speed is often reduced as compared with resting free cells (Klein and Wagner 1986; Chibata et al. 1983). It might therefore be interesting to investigate the effect of immobilization on the reaction velocity of the bioconversion *1*→(*R*)-*2*, on the stability of the biocatalyst, as well as on the yield and enantiomeric purity of the biotransformation product in comparison with the results obtained with free resting cells. Concerning the preparative aspects of this biotransformation, positive results with immobilized cells would represent a greater efficiency of the process because the separation of the cells from the reaction mixture and the isolation and purification of the product is, in general, much easier than with processes that use growing or free resting cells, respectively. We will soon report on the results of these experiments.

Furthermore, we are investigating the microbial reduction of other acetyltriorganylsilanes $R^1R^2R^3Si(CO)CH_3$ and acetyltriorganylgermanes $R^1R^2R^3Ge(CO)CH_3$ ($R^1=R^2\neq R^3$, $R^1\neq R^2\neq R^3$) at the moment, which are structurally related to *1* and *7*, respectively (Tacke et al. 1985), to learn about the influence of the substituents attached to silicon and germanium on reaction speed and stereoselectivity. It is of interest to see whether the stereochemistry of these biotransformations is in agreement with Prelog's rule (Prelog 1964). Furthermore, we are studying other microbial and enzymatic reaction types with regard to their applicability on elementorganic substrates, such as esterase (Syldatk et al. 1986) and amidase reactions, which have been described to reveal wide substrate specifity (e.g.: Whitesides and Wong 1985;

Kasai et al. 1984; Schmidt-Kastner and Egerer 1984); Yonaha and Soda 1986).

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