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# SYNTHESIS OF OPTICALLY ACTIVE HYDROXYESTERS USING BIOCATALYSTS

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*Optically active hydroxyesters were prepared using the fungus* Aspergillus niger *and the yeast* Sacharomyces cerevisiae. *Substrate is converted highly specifically into optically active isomers with 95% purity. The R-hydroxyester was isolated by asymmetric reduction. (R,R)-Tartaric acid of 60% optical purity was used as an inductor.*

**Key words:** aggregation pheromones, grain beetles, synthesis, biocatalysts, *Aspergillus niger*, *Sacharomyces cerevisiae*, asymmetric reduction.

Grain beetles (*Coleoptera, Cucujidae*) are agricultural pests. The aggregation pheromones of these insects are a mixture of macrolide components, cucujolides with chiral centers [1]. Research has shown [2] that one of the factors leading to species-specificity of grain-beetle pheromones is the chirality.

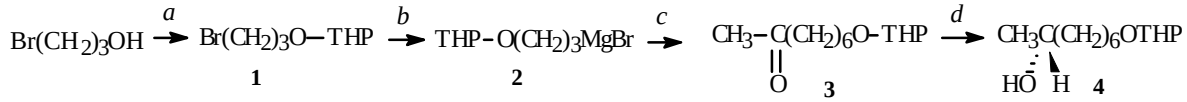
Therefore, we propose various approaches to the synthesis of optically active synthons of both R- and S-configuration. The chiral center was introduced into the synthon structures using microorganisms as biocatalysts.

Microorganisms represent a large group of living organisms with a wide spectrum of metabolic processes owing to the variety of enzymes they contain. It is not always necessary to isolate the enzymes from the bulk microorganism because the substrate may be transformed chemically into product by the intact microorganism cells that contain the enzyme complex. Therefore, undesired enzymes can be excluded from the process by regulating the temperature and pH [3].

We studied the reduction of ketones into alcohol with previously grown fungus *Aspergillus niger* and yeast *Sacharomyces cerevisiae*.

The fungus was maintained under optimal growth conditions. The biomass was separated from the culture liquid by centrifugation and then used in the experiment.

Substrate for biotransformation by *A. niger* was synthesized by the following scheme:




a) 2,3-dihydropyran (DHP), HCl, $Et_2O$; b) EtMgBr/THF; c) 5-chloro-2-pentanone; d) *A. niger*, 27°C, 10 d.

The bioconversion was performed in aqueous medium and in culture liquid. The degree of conversion was 60%; optical purity of the R-isomer, 95%.

The S-enantiomer **8** was obtained by treating **7** with *S. cerevisiae*. The yeast growth conditions were identical to those for growing the fungus.

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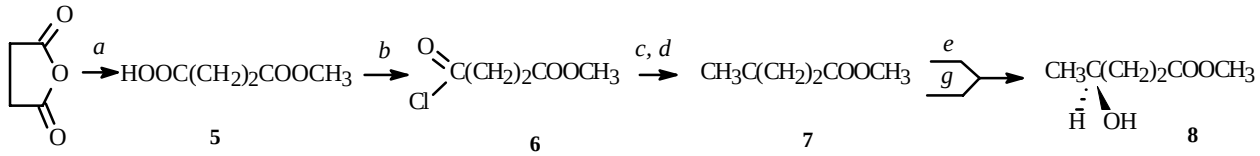
Substrate **7** for the biotransformation by yeast was synthesized by the following scheme:




a) MeOH; b) $PCl_5$; c) MeMgI/$Et_2O$; d) $CdMe_2/C_6H_6$; e) *S. cerevisiae*, 35°C, 7 d; g) $NaBH_4$, R,R-tartaric acid, -5°C, 24 h.

$\beta$-Carbomethoxypropanoic acid (**5**) was synthesized by reacting methanol and succinic anhydride. Treatment with $PCl_5$ converted the acid to the acyl chloride **6** in 70% yield. Ketoester **7** was prepared by reacting an organocadmium compound with **6**.

The methyl ester of ketovaleric acid was treated with yeast and periodically stirred at 35°C for 7 d. The degree of conversion was 20%. The optical purity of the S-isomer was 95%.

We attempted to prepare optical isomers via a chiral synthesis. Reduction of the ketone in alcohol with a suspension of $NaBH_4$ and the chiral inductor (R,R)-tartaric acid by the literature method [4] gave the R-isomer of **8** in 60% optical purity.

Thus, the biotransformation of asymmetric synthons was studied for the synthesis of chiral macrolides by *A. niger* and *S. cerevisiae*. The results have showed that substrate is converted highly specifically to product by the microorganisms and forms synthons with 95% optically pure R- and S-configurations.

The highest degree of conversion of substrate into product was observed with *A. niger* micelles and reaction in culture medium.

## EXPERIMENTAL

GLC was performed in an LKhM-8MD instrument over a column (1 m × 3 mm) packed with 15% Carbowax 20M on Chromaton N-AW-DMCS and a Chrom 5 instrument over a column packed with 5% XE-60 Chromaton N-AW-DMCS with He carrier gas. PMR spectra were obtained on a Varian XL-200 NMR spectrometer at working frequency 200 MHz in $CCl_4$ with HMDS internal standard. Optical rotation was measured on a POLAMAT polarimeter in $CHCl_3$. Column chromatography used Chemapol L 100/250 silica gel with elution by ether—hexane (2:1). Analyses for all compounds agreed with those calculated.

**1-(Tetrahydropyran-2-yl)oxy-3-bromopropane (1).** 3-Bromopropan-1-ol (27.8 g, 0.2 mole) and HCl (5 drops, conc.) were placed in an Erlenmeyer flask, cooled to 0°C, treated dropwise with 2,3-dihydropyran (18.4 g, 0.22 mole) over 30 min, stirred for 16 h at room temperature, diluted with ether (50 mL), washed with NaOH (100 mL, 1 N) and NaCl solution (100 mL), and dried over $MgSO_4$. Solvent was removed at reduced pressure. The residue was vacuum distilled, bp 102°C/7 mm. Yield 36.1 g (81%).

**1-(Tetrahydropyran-2-yl)oxy-7-oxooctane (3).** Ethylmagnesium bromide was prepared from metallic Mg (0.92 g, 0.04 mole) and ethylbromide (4.36 g, 0.04 mole). The solution was heated, treated dropwise with **1** (8.43 g, 0.04 mole), stirred and boiled for 5 and 6 h at room temperature, cooled (0°C) and treated dropwise with 5-chloro-2-pentanone (4.82 g, 0.04 mole), and stirred for 4 h. The mixture was decomposed with HCl (20 mL, 1 N). The organic layer was separated. The aqueous layer was extracted with ether (3×25 mL). The ether extracts were dried over $MgSO_4$. Solvent was removed at reduced pressure. The residue was vacuum distilled. The distilled fractions were purified by column chromatography, $R_f$ = 0.37. Retention time 2.8 min (GLC). bp 98°C/8 mm. Yield 4.91 g (57%). PMR (δ, ppm, J/Hz): 4.06 (1H, m, OCH–O), 3.2-3.8 (4H, m, O–$CH_2$), 2.50 (2H, t, J = 6.4, $CH_2$–C=O), 2.04 (3H, s, $CH_3$–C=O), 0.8-2.1 (14H, m, $CH_2$).

**1-(Tetrahdyropyran-2-yl)oxy-7-hydroxyoctane (4).** Moist mycelium of *A. niger* (8.77 g) and **3** (600 mg) were stored in culture medium (100 mL) at 27°C and periodically stirred over 10 d. The temperature was maintained using a thermostat. The solution was filtered and extracted with ether. The extract was dried over $MgSO_4$. The solvent was removed. Yield 0.31 g (60% conversion) of crude product. The reaction product was analyzed using GLC at 160°C, retention time 0.5 min. Optical purity of the R-isomer, 95%, $[\alpha]_D^{23}$ +10.5 (*c* 0.31, $CHCl_3$).

**$\beta$-Carbomethoxypropionic acid (5).** A mixture of succinic anhydride (200 g, 2 mole) and methanol (97 mL, 2.4 mole) was boiled on a water bath for 20 min and stirred for 15 min. When the mixture became homogeneous it was placed on a steam

bath for 20-25 min. The excess of methanol was removed at reduced pressure (water aspirator). The residue was poured into a marble dish for crystallization. The reaction product was dried in a desiccator to constant mass for 5 d, mp 56-64°C, 166.14 g (70%).

**β-Carbomethoxypropionylchloride (6).** Acid **5** (49 g, 0.37 mole) was treated with portions of ground $PCl_5$ (80.2 g, 0.39 mole) with stirring and cooling. The reaction was very violent and exothermic. The reaction mixture was stirred for 4 h. $POCl_3$ was removed at reduced pressure (water aspirator). The residue was distilled, bp 76-77°C/7 mm, yield 23.84 g (42.6%).

**Methyl Ester of Ketovaleric Acid (7).** $CH_3MgI$ was prepared from $CH_3I$ (19.88 g, 0.14 mole) and metallic Mg (3.22 g, 0.14 mole) in absolute ether (100 mL), cooled (0°C), treated with portions of previously calcined $CdI_2$ (27.54 g, 0.0752 mole), stirred at room temperature for 1.5 h, cooled (0°C), and treated dropwise with **6** (20.83 g, 0.14 mole). The reaction mixture was stored at this temperature for 12 h. The excess of organocadmium reagent was decomposed with $H_2SO_4$ (9 mL, 10%). The resulting two layers were separated. The aqueous layer was extracted with benzene (3×50 mL). The combined extracts were dried over $MgSO_4$. The solvent was removed. The residue was vacuum distilled. The product was further purified by column chromatography with elution by ether—hexane (5:1), $R_f$ = 0.4, bp 70-72°C/4 mm, yield 5.08 g (28.3%). PMR (δ, ppm, J/Hz): 3.63 (3H, s, $COOCH_3$), 2.64 (2H, d, J = 4.1, $CH_2$), 2.47 (2H, d, J = 4.1, $CH_2$), 2.13 (3H, s, O=C–$CH_3$).

**Methyl Ester of 4-Hydroxyvaleric Acid (8).** a. A suspension of $NaBH_4$ (1.26 g, 0.02 mole) and R,R-tartaric acid (4.5 g, 0.03 mole) in THF (100 mL) was boiled for 4 h, cooled to 0 to -5°C, treated dropwise over 5 min with **7** (2 g, 0.02 mole) in THF (10 mL), held at this temperature for 29 h using a thermostat, cooled, and decomposed with ethylacetate (50 mL) and HCl (25 mL, 1 M). The organic layer was separated, washed with $NaHCO_3$, and dried over $K_2CO_3$. The solvent was removed under reduced pressure. The residue was vacuum distilled. The product was purified by column chromatography, bp 60-62°C, yield 0.6 g. PMR (δ, ppm, J/Hz): 3.71 (1H, m, CH), 3.61 (3H, s, $COOCH_3$), 2.35 (2H, t, J = 6.7, $CH_2CO$), 1.67 (2H, m, $CH_2$), 1.15 (3H, d, J = 6.6, $CH_3$). The degree of conversion was 30%; optical purity of the R-isomer, 60%; $[\alpha]_D^{23}$ +45.5° (*c* 1.22, $CHCl_3$).

b. Compound **7** (540 mg) was treated with *S. cerevisiae* biomass (80 g) and glucose (0.04 g). The mixture was stored and periodically stirred at 35°C for 7 d. The temperature was maintained using a thermostat. The reaction mixture was filtered. The filtrate was treated with $(NH_4)_2SO_4$ and extracted with ether. The extract was dried over $MgSO_4$. The solvent was removed. The degree of conversion was 20%; optical purity of the S-isomer, 95%; $[\alpha]_D^{23}$ -44.8 (*c* 0.06, $CHCl_3$).

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