Oxidation of Secondary Alcohols by Duckweed

A Biotransformation Experiment for Undergraduate Students

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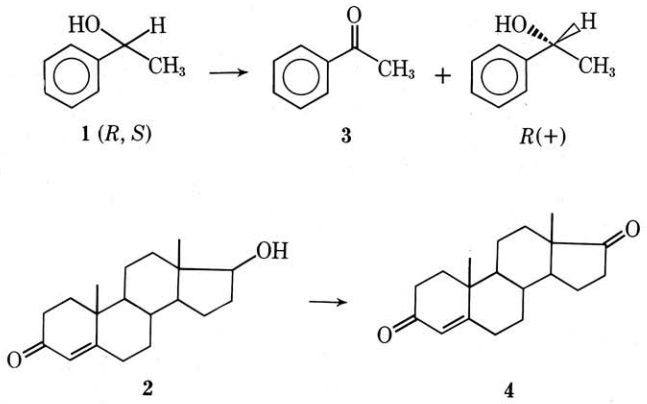
Biotransformation is the transformation of a substance A (substrate) to a substance B (product) using a biocatalyst (*1*). There are no principal differences in the outcome of a chemical reaction whether it was carried out with an inorganic, organic, or biocatalyst. However, an advantage of the biocatalyst is its high stereospecifity and regioselectivity. Biocatalysts are enzymes produced by or isolated from microorganisms, plant cells, and animal cells.

The application of microorganisms or tissue cultures for carrying out the controlled chemical reactions on a preparative scale binds the gap between organic chemists and biotechnologists.

The common aquatic weed, *Spirodela oligorrhiza* (Lemnaceae), is an ideal biocatalyst for biotransformations in the undergraduate chemical laboratory. The use of the whole plants enables one to overcome the standard difficulties caused by the specific techniques required for cultivation of tissue cultures (*2*, *3*) and is obviously safer than the use of microorganisms (*4*).

The objective of this experiment is to use the ability of *Spirodela oligorrhiza* (duckweed) to convert the secondary hydroxyl moieties into ketone groups (*5*, *6*), using 1-phenylethanol (**1**) and testosterone (**2**) as substrates, as illustrated in the reactions,



1 (*R*, *S*) **3** *R*(+)

2 **4**

Also other species (many called duckweed) in the family Lemnaceae would probably be effective in carrying out this biotransformation, as indicated by separate experiments using *Lemna minor* and *Wolffia arrhiza*.

The experiment described here is a part of a bioorganic chemistry laboratory program at the Pedagogical University in Opole. It is performed in our laboratory without isolation of the reaction products, which are determined by thin-layer or gas–liquid chromatography, using commercially available reagents as reference materials.

The stereoselectivity of the oxidation of (*RS*) 1-phenylethanol can be confirmed by the preparative separation of reaction product **3** and unreacted substrate **1** and determination of specific optical rotation of the latter.

The only disadvantage of this experiment is that two weeks are needed to complete the reaction, and thus it has to be carried out in two steps. The length of the experiment, however, can be tailored to match the time available; students may be assigned all or part of the steps as described under Experimental, or the instructor provide either the nutrient medium or the fully developed culture of plants.

Experimental

Preparation of the Nutrient Medium

The nutrient medium adapted for cultivation of cultures used directly for biotransformation was prepared according to Bollard (*7*) and contained: 0.25 g $Ca(H_2PO_4)_2 \cdot H_2O$; 0.44 g KCl; 0.493 g $MgSO_4 \cdot 7H_2O$; 1.635 g $Ca(NO_3)_2 \cdot 4H_2O$; 1 mL Gorham's microelement solution; and 0.5 mL of chelate solution in total volume of 1.0 L. The Gorham's microelement solution was prepared by dissolving 2.86 g H_3BO_3; 1.79 g $MnCl_2 \cdot 4H_2O$; 0.1 g $ZnCl_2$; 0.08 g $CuSO_4 \cdot 5H_2O$; and 0.4 g $(NH_4)_6MoO_{24} \cdot 4H_2O$ in 500 mL of distilled water.

The chelate was obtained by dissolving 3.0 g of sodium ferrate EDTA in 100 mL of distilled water.

For cultivation of parent strain the nutrient was additionally enriched with L-asparagine (200 mg/L) and glucose (10 g/L).

The nutrient medium was autoclaved in 120 °C for 10 min (a pressure cooker may be used in place of an autoclave).

Growing of the Plant

Nutrient (100 mL) in 250-mL conical flasks was inoculated with three to five plants from the parent strain (in our case the clone

originated from the collection of the Department of Botany and Physiology of Plants, Agricultural University of Wrocław). The growth was carried out at room temperature with continuous illumination (fluorescent tube) for a period up to 14 days until the whole surface area (38 cm^2) of the nutrient was covered with the plant.

Preparation of (RS) *1-Phenylethanol*

To the cooled (0 °C) solution of 12.0 g (0.1 mol) of acetophenone in 100 mL of methanol, 2.3 g (0.06 mol) of sodium borohydride was slowly added maintaining temperatures below 10 °C. The resulting solution was neutralized with concentrated hydrochloric acid, and sodium chloride was removed by filtration. The volatile components were removed in vacuo, the residue dissolved in methanol, and the solvent evaporated using a rotary evaporator. This step was repeated twice and then the residue dissolved in toluene, solid impurities were filtered off, and toluene removed in vacuo. The obtained (*RS*) 1-phenylethanol was of satisfactory purity and was used without further purification.

Transformation of (RS) *1-Phenylethanol*

To the culture of *Spirodela oligorrhiza* obtained as described above, 40 μL of 1-phenylethanol was added. The content was then stirred using magnetic stirrer and illuminated with a fluorescent tube. The intensity of stirring was regulated to prevent the plant sinking. During the experiment the plants died and their color changed from green to white. After the experiment, the plants did not reproduce when transferred into fresh nutrient.

The duckweed was then removed by filtration and the solution was extracted four times with 40-mL portions of chloroform. The combined chloroform layers were dried over anhydrous magnesium sulfate, the drying agent was filtered off, and chloroform was stripped off in vacuo. The residue was then examined by TLC and GLC.

TLC was carried out on silica gel (Merck, Darmstadt) using petrol ether–acetone–ethyl acetate (10:1:1) as eluant and iodine vapors as visualizing agent. The recorded R_f values were 0.41 and 0.26 for acetophenone **3** and 1-phenylethanol (**1**), respectively.

GLC was carried out on a N-504-Elwro (Wrocław, Poland) apparatus equipped with a 2-m × 3.5-mm glass column of 10% DEGA on Chromosorb W DMCS 80–100 mesh; temperature 150 °C. Nitrogen gas as a carrier (50 mL/min) and a flame-ionization detector were used. The recorded retention times were 120 s and 180 s for acetophenone **3** and 1-phenylethanol (**1**), respectively.

Transformation of Testosterone

The transformation was carried out in the same manner as described for 1-phenylethanol, using a solution of 40 mg of testosterone in 1 mL of ethyl alcohol as substrate. Chloroform extracts were examined by TLC and GLC.

TLC was carried out on silica gel (Merck, Darmstadt) using toluene–ethyl acetate (1:1) as eluant. After spraying with solution composed of 5 mL of concentrated sulfuric acid, 1 mL anisaldehyde, and 100 mL of ethanol, the spots were visualized by delicate heating of the plates. The recorded R_f values were 0.37 for testosterone (**2**) and 0.50 for androstendione (**4**).

GLC was carried out using a 2-m × 3.5-mm glass column of 3% QF1 on Gas-Chrom Q 100–120 mesh, a glass inlet (the presence of metallic parts causes decomposition of steroids), and a temperature 270 °C. The other conditions used as for the previous experiment. The recorded retention times were 285 s and 452 s for testosterone (**2**) and androstendione (**4**), respectively.

Preparative Separation of the Substrate and Product of Transformation of (RS) *1-Phenylethanol*

The combined chloroform extracts from 10 runs of transformation were evaporated in vacuo and separated preparatively using a 15-cm × 1.5-cm silica gel (230–400 mesh) column by means of flash chromatography. The mixture of petrol ether–ethyl acetate (8:1) was used as the eluant, while the separation process was monitored by means of TLC. The fractions containing 1-phenylethanol were combined, and the product had specific optical rotation $[\alpha]_{578}^{20} = +35°$ (c 2 in methanol), thus showing 76% of optical purity [lit. (*8*) $[\alpha]_D^{20} = +46°$ (c 5 in methanol)]. This value reflects directly the yield of biotransformation.

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