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Enantioselective bioreduction of heptan-2-one and octan-2-one catalyzed by *Daucus carota* cells

Aliya R Chanysheva* & Vladimir V Zorin

Department of Biochemistry and Technology of Microbiological Industries,
Ufa State Petroleum Technological University, Ufa, Russia

E-mail: aliyach@mail.ru

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Synthesis of enantiomerically pure (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol by bioreduction of heptan-2-one and octan-2-one using *Daucus carota* cells has been studied. Optimization of bioreduction conditions has been carried out in the presence of various exogenous reducing agents (ethanol, isopropanol, glucose). Conditions allowing to obtain (2S)-(+)-heptan-2-ol with 91% yield and optical purity 98% *ee* and (2S)-(+)-octan-2-ol in 95% yield and > 99% *ee* have been found.

Keywords: *Daucus carota*, bioreduction, exogenous reducing agents, (2S)-(+)-heptan-2-ol, (2S)-(+)-octan-2-ol

Enantiomerically pure secondary aliphatic alcohols, in particular, (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol, are valuable intermediates in the synthesis of drugs and are also widely used in agro-industry and perfume industry[1,2].

Enantiomerically pure secondary alcohols can be obtained by enantioselective reduction of prochiral ketones using methods of enantioselective metal complex catalysis or biocatalytic reduction, where along with the microorganisms, plant cells in different forms: cell cultures, immobilized plant cells, and freshly cut plants[3-6] are widely used.

Cell suspension culture of *Nicotiana tabacum* have been used for bioreduction of heptan-2-one and octan-2-one to obtain (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol in high yield and optical purity 55% *ee* and 73% *ee*, respectively[7].

Recently, biocatalyst based on *Daucus carota* cells has been shown to enantioselectively reduce various substituted acetophenones and other ketones[8-14].

Results and Discussion

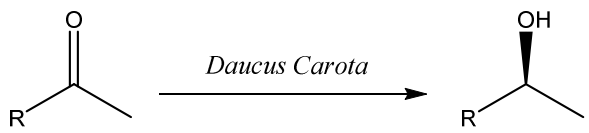
We have investigated the possibility of synthesis of optically pure (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol based on bioreduction of corresponding heptan-2-one and octan-2-one catalyzed by *Daucus carota* cells.

Heptan-2-one and octan-2-one were reduced by *Daucus carota* cells in water at room temperature for 144 hours to produce (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-olwith 67% yield (90% *ee*) and 50% (87% *ee*), respectively (Scheme I).

It is known that enzymes, reducing different carbonyl compounds, carry out this process using endogenous reducing agents – reduced coenzymes (NAD(P)H)[15,16]. Furthermore, biocatalysts require to be regenerated. There are effective methods for the regeneration of the coenzymes[17] based on the use of unexpensive, available exogenous reducing agents (ethanol, cyclopentanol, 2-propanol, sucrose, glucose etc.)[18-20].

In order to develop more efficient method for producing of (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol we studied transformation of heptan-2-one and octan-2-one catalyzed by *Daucus carota* cells in a reaction medium containing isopropanol (ethanol or glucose) in various concentrations (1-30%). Isopropanol (ethanol or glucose) are exogenous reducing agents of coenzyme $(NAD(P)^+)$[18-20], and can influence the yield and the enantioselectivity of heptan-2-one and octan-2-one reduction.

Heptan-2-one was reduced by *Daucus carota* in medium containing ethanol (2% vol) to produce (2S)-(+)-heptan-2-ol with good yield (71%) and high enantiomeric excess (97%). (2S)-(+)-Octan-2-ol was



R= C_5H_{11}, C_6H_{13}

Scheme I

obtained with 50% yield by bioreduction with *Daucus carota* in medium containing ethanol (3% vol), but with more valuable *ee* (97%).

It has been found that reduction of heptan-2-one**,** when carried out in medium containing isopropanol (2% vol), gives rise to the higher yield of (2S)-(+)-heptan-2-ol (73%) and *ee* (87%) in comparison with experiment conducted in the absence of isopropanol. *D. carota* is quite active in bioreduction of octan-2-one under the same conditions (but isopropanol concentration (3% vol), producing (2S)-(+)-octan-2-ol in excellent yield (95%) and enantiomeric excess (>99%).

It must be pointed out that increase of ethanol and isopropanol concentration (5-30% vol) in reaction medium leads to formation of (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-olin low yields, that can probably be explained by inactivation of enzyme (oxidoreductase), responsible for reduction process.

We have also studied the possibility of synthesis of optically pure (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol based on reduction of the corresponding heptan-2-one and octan-2-one in the presence of *Daucus carota* roots in medium containing glucose as reducing exogenous agent.

Heptan-2-one and octan-2-one reduction was conducted in water at room temperature for 144 hours. An equimolar amount of glucose was added to reaction mixture.

(2S)-(+)-Heptan-2-ol and (2S)-(+)-octan-2-ol were obtained with excellent *ee*s (98%) and valuable yields (91% and 60%, respectively) by reduction with *D. carota* under studied conditions.

Experimental Section

Heptan-2-one and octan-2-one are commercially available.

Racemic (2R, 2S)-heptan-2-ol and (2R, 2S)-octan-2-ol were prepared from the corresponding available ketones by reduction with $NaBH_4$.

Fresh carrots (*D. carota*) were purchased from a local market.

Gas chromatographic anylyses were performed on Khromatek-crystall-5000.2 with flame-ionization detector. Enantioselective column Astec CHIRALDEXB-PM (30m×0.25mm×0.12μm); column temperature 80°C; oven temperature 250°C, detector temperature 250°C; helium as a carrier gas, flow rate 14.2 mL/min. For more efficient chromatographic separation of enantiomeric alcohols, its acetyl derivatives were obtained.

^{1}H and ^{13}C NMR spectra were measured on Bruker AM-300 and AMX-300 spectrometeres. As internal δ (0.00) for standards served TMS ^{1}H NMR and $CDCl_3$ δ (77.0) for ^{13}C NMR spectroscopy.

GC-MS analyses were performed using GCMS-QP2010S Shimadzu. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) in the 35-500 amu range was used. The chromatographic column used for the analysis was an HP-1MS capillary column (30 m×0.25 mm×0.25 μm). The vaporizer temperature was 280°C, the ionization chamber temperature was 200°C. Helium was used as carrier gas, at a flow rate of 1.1 mL/min. The injections were performed in mode at 100-230°C at a heating rate of 20°C/min.

Polarimetric analyses was carried out on an automatic polarimeter Optical Activity Limited model AA-55.

Reduction of ketones catalyzed by *D. carota* cells

To a suspension of freshly cut *D. carota* roots (15 g) in distilled water (70 ml), kept under stirring in a conical Erlenmayer flask, heptan-2-one or octan-2-one(100mg) was added. The reaction mixture was placed in an orbital shaker (150 rpm) at room temperature. After achieving the necessary conversions suspension was filtered, and *D. carota* roots were washed three times with water. The filtrates were extracted with diethyl ether (3 × 125 mL). The organic phase was dried over Na_2SO_4, evaporated and monitored by GLC. The chemical and enantioselective purities and yields were determined by GC analyses.

Reduction of ketones catalyzed by *D. carota* cells in the presence of exogenous reducing agents

To a suspension of freshly cut *D. carota* roots (15 g) in distilled water (70 ml), kept under stirring in a conical Erlenmayer flask, heptan-2-one or octan-2-one(100mg) and exogenous reducing agents (ethanol (1-30% vol.) or 2-propanol (1-30% vol.) or glucose (equimolar amount toward substrate) was added. The reaction mixture was placed in an orbital shaker (150 rpm) at room temperature. After achieving the necessary conversions suspension was filtered, and *D. carota* roots were washed three times with water. The filtrates were extracted with diethyl ether (3 × 125 mL). The organic phase was dried over Na_2SO_4, evaporated and monitored by GLC. The chemical and enantioselective purities and yields were determined by GC analyses.

(2S)-(+)-Heptan-2-ol: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85-0.92 (t, 3H, CH_3), 1.15-1.2 (d, 3H, CH_3), 1.25-1.5 (m, 8H, CH_2), 3.7-3.8 (m, 1H, CH); ^{13}C NMR

(75 MHz, $CDCl_3$): δ 14.00 (C, CH_3), 22.58 (C, CH_2), 23.4 (C, CH_3), 25.4 (C, CH_2), 31.82 (C, CH_2), 39.26 (C, CH_2), 67.89 (C, CH); MS: *m/z* (%) 83 (11), 70(8), 55(22), 45(100), 44(12), 43 (15), 41(14).

(2S)-(+)-Octan-2-ol: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88-0.91 (t, 3H, CH_3), 1.16-1.17 (d, 3H, CH_3), 1.3-1.46 (m, 10H, CH_2), 3.75-3.77 (m, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.04 (C, CH_3), 22.55 (C, CH_2), 23.4 (C, CH_3), 25.69 (C, CH_2), 29.29 (C, CH_2), 31.8 (C, CH_2), 39.31 (C, CH_2), 67.86 (C, CH); MS: *m/z* (%) 97 (7), 70(6), 56(8), 55(21), 45(100), 44 (79), 43 (18), 41(16).

Conclusion

In this paper we studied the synthesis of enantiomerically pure (2S)-(+)-heptan-2-ol and (2S)-(+)-octan-2-ol based on bioreduction of heptan-2-one and octan-2-one catalyzed by *Daucus carota* cells. Optimization of bioreduction conditions was carried out in the presence of various exogenous reducing agents (ethanol, isopropanol, glucose). We have found conditions allowing to produce (2S)-(+)-heptan-2-ol by reduction of heptan-2-one by *D. carota* in the presence of glucose with 91% yield and 98% *ee*. Reduction of octan-2-one catalyzed by *Daucus carota* cells in the presence of isopropanol (3% vol) leads to formation of (2S)-(+)-octan-2-ol with 95% yield and > 99% *ee*.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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