Biocatalytic preparation of chiral alcohols by enantioselective reduction with immobilized cells of carrot

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The ability of immobilized cells of *Daucus carota* to reduce enantioselectively organic foreign substrates has been examined. The immobilized plant cells reduced, with excellent enantioselectivity, prochiral ketone substrates such as keto esters, aromatic ketones and heterocyclic aromatic ketones, leading to the corresponding chiral secondary alcohols with an enantiomeric purity of 52–99% ee in a chemical yield of 30–63%.

The two biotransformation systems with bakers' yeast and lipases have widely been used as a useful means for obtaining chiral synthons or optically active compounds.[1] Plant cell cultures have been assumed to be a biochemical system which will transform, enantioselectively, important foreign synthetic substrates, as well as natural substrates (or secondary metabolites).[2-4]

For some years now we have been investigating the biotransformation of organic xenobiotics, *e.g.* ethyl 3-oxobutanoate[3] and acetophenone[4] by immobilized plant cell cultures. Recently we showed that the biotransformation of acetophenone with immobilized carrot (*Daucus carota*) cells, unlike the analogous transformations with immobilized tobacco and *Gardenia* cells, proceeded primarily by an enantioselective reduction pathway to give (*S*)-α-phenethyl alcohol with an enantiomeric purity of 99% ee in a chemical yield of 54%.[5,6] In conjunction with our efforts to investigate the possibility of using plant cell cultures in the same manner as bakers' yeast and lipases, as a reagent in organic synthesis, we have carried out the enantioselective reduction of synthetically important foreign substrates including the keto esters **1–4**, the aromatic ketones **5–9** and the heterocyclic aromatic ketones **10–12** by immobilized *D. carota* cells entrapped in calcium alginate beads. Their corresponding optically active alcohol products are an important chiral synthon (**1a** and **2a**) for the synthesis of natural products[7] or are a useful chiral auxiliary (**10a–12a**) for the resolution of carboxylic acids.[8]

Results and discussion

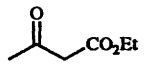
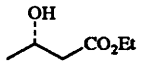
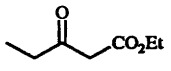
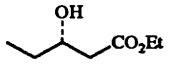
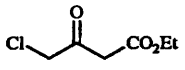
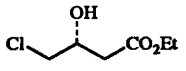
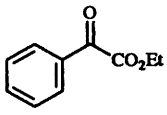
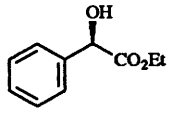
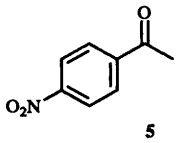
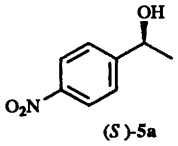
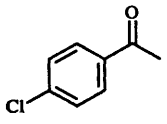
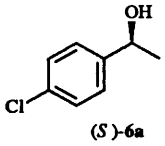
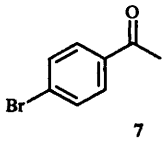
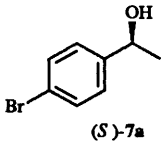
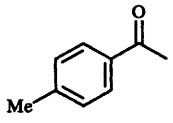
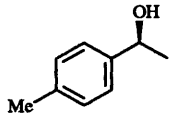
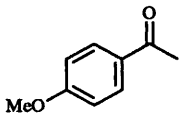
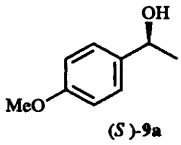
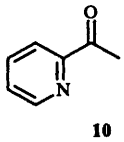
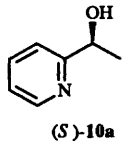
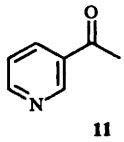
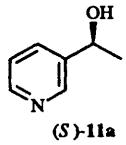
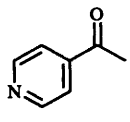
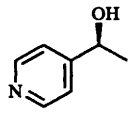
The bioreduction of β-keto esters **1** and **2** with immobilized *D. carota* cells (IDCC) proceeded enantioselectively to give their corresponding hydroxy esters (*S*)-**1a** and (*S*)-**2a**, respectively, having a high enantiomeric purity of 98 and 90% ee. A similar bioreduction of 4-chloro-3-oxobutanoate **3** with IDCC reached 100% conversion within 5 h, but the enantiomeric purity of the alcohol product (*R*)-**3a** was lower than expected, 52% ee. The enantioselective reduction of the aromatic α-keto ester **4** was complete within 24 h, the hydroxy ester (*R*)-**4a** of 95% ee being obtained.

As exemplified in Table 1, the enantioselective bioreduction of the aromatic ketones **5–9** to produce the corresponding chiral alcohols **5a–9a** by IDCC was examined. IDCC enantioselectively reduced the five acetophenone derivatives to afford the corresponding (*S*)-alcohols **5a–9a** with an excellent enantiomeric purity of 96–99% ee in a chemical yield of 30–63%. The bioreduction of **5–7**, possessing an electron-withdrawing group at the aromatic ring, proceeded at a faster rate than did that of **8** and **9**, having an electron-donating group at the aromatic ring. For example, the bioreduction of **7** reached 100% conversion within 12 h, while that of **9** required 5 days to reach a maximum conversion of 57%. Next, IDCC bioreduction of the heterocyclic aromatic ketones **10–12** was carried out, in order to further test the reducing ability of IDCC cells. As was expected, these three IDCC reductions were also highly enantioselective, (*S*)-heterocyclic aromatic alcohols **10a–12a** of 96–99% ee being produced in reasonable chemical yields.

Many workers have studied the microbial reduction of the keto esters **1–4** by bakers' yeast.[9-16] For the bakers' yeast reductions of **1**[9,11,13-16] and **4**,[15,16] (*S*)-**1a** and (*R*)-**4a** were formed with a high enantiomeric purity (84–97% ee), while similar reductions of **2**[11-13,16] and **3**[10,11,13,16] led to the alcohol products, (*R*)-**2a** and (*S*)-**3a**, of low ees (40–65.5%). It is of interest to note that the present IDCC reduction of **2** and **3** produces **2a** and **3a** with the opposite stereochemistry at the asymmetric carbon. The chemical yields of the alcohol products **1a–4a** obtained by bakers' yeast reduction were somewhat higher than those obtained by IDCC reduction. A chemical reagent, BINAP-Ru catalyst, under an initial hydrogen pressure of 100 atm reduced **1** to (*S*)-**1a** of 99% ee in a yield of 99%.[17] Bakers' yeast reductions of the 4-substituted acetophenones **5–9** were recently carried out, and the corresponding (*S*)-alcohols with an enantiomeric purity of 82–96% ee were produced.[18] More recently, chemical reductions of the ketones **6**, **8** and **9** were investigated by using $LiAlH_4$ modified with chiral ethanolamines.[19] The ee (70–86%) of the (*R*)-alcohol products obtained by the chiral aluminium reagent was lower than those produced by the present IDCC system, although the aluminium hydride reduction proceeded at a faster rate than did the IDCC one. Three different acetylpyridines **10–12** were reduced to the corresponding (*S*)-alcohols **10a–12a** in a chemical yield of 18–36% by bakers' yeast.[20] The enantiomeric purity of (*S*)-**10a** and (*S*)-**12a** isomers was high, 96% ee, but that of (*S*)-**11a** was lower, 67% ee.

In conclusion, IDCC could behave efficiently as an asymmetric reducing agent, converting several prochiral ketones into the corresponding chiral secondary alcohols in reasonable chemical yields and in high enantioselectivities. Although the present IDCC reduction system has not been optimized, these results indicate that plant cell cultures are accessible, like bakers' yeast and lipases, as a reagent in organic synthesis.

Table 1 Enantioselective reduction of keto esters, aromatic ketones and heterocyclic aromatic ketones by immobilized carrot cells

Substrate	Product	Time	Conversion (%)	Yield[a] (%)/ee[b] (%)
 **1**	 (*S*)-**1a**	15 h	100	38/98
 **2**	 (*S*)-**2a**	15 h	100	50/90
 **3**	 (*R*)-**3a**	5 h	100	42/52
 **4**	 (*R*)-**4a**	24 h	100	35/95
 **5**	 (*S*)-**5a**	15 h	100	63/99
 **6**	 (*S*)-**6a**	14 h	98	56/99
 **7**	 (*S*)-**7a**	12 h	100	56/98
 **8**	 (*S*)-**8a**	4 day	100	35/99
 **9**	 (*S*)-**9a**	5 day	57	30/96
 **10**	 (*S*)-**10a**	2 day	75	38/99
 **11**	 (*S*)-**11a**	6 day	91	36/96
 **12**	 (*S*)-**12a**	2 day	100	30/97

[a] The % yield refers to isolated alcohol product. [b] The ee does not change at lower % conversions.[6]

Experimental

General

IR spectra were determined on a Fourier transform Perkin-Elmer 1720 IR spectrometer. ^{1}H NMR spectra were obtained on a Fourier transform Hitachi R-1500 (60 MHz) spectrometer or a Bruker AMX-R400 (400 MHz) spectrometer in $CDCl_3$ solutions, using Me_4Si as an internal standard. Column chromatography was performed with 70–230 mesh silica gel (Merck Kieselgel 60 Art. No. 7734) and 230–400 mesh silica gel (Merck Kieselgel 60 Art. No. 9385). Gas chromatography was carried out on a Hitachi G-3000 chromagraph equipped with a TC-WAX 30 m × 0.25 mm column (GL Sciences). Optical rotations were measured on a Horiba SEPA-200 high-sensitivity polarimeter; $[\alpha]_D$ values are given in units of 10^{-1} deg cm^{-2} g^{-1}. HPLC analyses were performed on a Waters 510 liquid chromatograph. Each reaction product was analysed by capillary GLC. The conversion ratios were determined on the basis of the peak areas of ketone substrates and alcohol products. The enantiomeric purities (% ee) of **1a**–**12a** were determined by the HPLC analyses in comparison with racemic ones.

Cultivation of carrot cells

Cells of *D. carota* in suspension were cultivated in Murashige and Skoog's (MS) medium[21] containing naphthylacetic acid (2 ppm), kinetin (0.1 ppm) and sucrose (3%) as previously described.[22]

Preparation of immobilized carrot cells

Cells of *D. carota* in suspension (150 g) were immobilized with 5% aqueous sodium alginate (Kanto Chemical) (600 cm^3) and 0.6% aqueous $CaCl_2$ according to the procedures described previously.[23] The resulting IDCC beads, *ca.* 4–5 mm diam., after being kept for 30 min were washed with MS medium and then stored in the same medium.

Bioreduction of ketone substrates with IDCC

IDCC beads prepared from a suspension of *D. carota* cells (150 g) as described were added to freshly prepared MS medium (1000 cm^3) containing naphthylacetic acid (2 ppm), kinetin (0.1 ppm) and sucrose (3%), and the medium was shaken for 2 days. A ketone substrate (182–215 mg) was then added to the precultured MS medium containing IDCC beads and the mixture incubated at 25 °C on a rotary shaker. The reaction mixture was filtered, and IDCC beads were washed with MS medium. The filtrate (the cultured medium from IDCC beads) and washings were combined and extracted continuously with diethyl ether employing a liquid–liquid extractor. Work-up of the extracts gave a crude product (112 mg for **1**, 134 mg for **2**, 126 mg for **3**, 102 mg for **4**, 158 mg for **5**, 138 mg for **6**, 129 mg for **7**, 97 mg for **8**, 132 mg for **9**, 150 mg for **10**, 107 mg for **11**, 89 mg for **12**), which was purified by silica gel column chromatography. Elution with hexane–ethyl acetate (**1a**–**3a**, **5a**–**8a**, **11a**, **12a**) or with hexane–CH_2Cl_2 (**4a**, **9a**, **10a**) gave the purified products. Analytical samples were further purified by vacuum distillation with a Kugelrohr distillation apparatus.

Ethyl (*S*)-3-hydroxybutanoate 1a. (*S*)-**1a** was obtained in 38% yield (79 mg) from **1** (203 mg), $[\alpha]_D^{20}$ +42.33 (*c* 2.87, $CHCl_3$) {lit.,[13] $[\alpha]_D^{24}$ +12.3 (*c* 1.26, $CHCl_3$); lit.,[14,16] $[\alpha]_D^{22}$ +42.25 (*c* 2.37, $CHCl_3$)}. The IR and ^{1}H NMR spectra of (*S*)-**1a** were identical with those of racemic **1a** and with those of (*S*)-**1a** reported previously.[13]

HPLC analysis of its benzoate ester: column Chiralcel OB 4.6 × 250 mm (Daicel); eluent hexane–propan-2-ol (9:1); flow rate 0.5 cm^3 min^{-1}; detection λ 220 nm: t_R 9.0 (0.9%) and 11.5 min (99.1%).

Ethyl (*S*)-3-hydroxypentanoate 2a. (*S*)-**2a** was obtained in 50% yield (107 mg) from **2** (211 mg), $[\alpha]_D^{20}$ +30.86 (*c* 1.82, $CHCl_3$) {lit.,[24] $[\alpha]_D^{22}$ +20.64 (*c* 3.85, $CHCl_3$); lit.,[12] $[\alpha]_D^{22}$ −13.44 (*c* 1.2, $CHCl_3$) for (*R*)-isomer; lit.,[13] $[\alpha]_D^{29}$ −31.2 (*c* 0.93, $CHCl_3$) for (*R*)-isomer}. The IR and ^{1}H NMR spectra of (*S*)-**2a** were identical with those of racemic **2a** and with those of (*R*)-**2a** reported previously.[13]

HPLC analysis of its benzoate ester: Chiralcel OB; hexane–propan-2-ol (1:4); 0.5 cm^3 min^{-1}; 220 nm: t_R 11.7 (4.8%) and 16.1 min (95.2%).

Ethyl (*R*)-4-chloro-3-hydroxybutanoate (3a). (*R*)-**3a** was obtained in 42% yield (86 mg) from **3** (200 mg), $[\alpha]_D^{20}$ +12.13 (*c* 1.91, $CHCl_3$) {lit.,[24] $[\alpha]_D^{22}$ +5.22 (*c* 6.12, $CHCl_3$); lit.,[10] $[\alpha]_D^{23}$ −11.7 (*c* 5.75, $CHCl_3$) for (*S*)-isomer; lit.,[13] $[\alpha]_D^{29}$ −17.6 (*c* 0.93, $CHCl_3$) for (*S*)-isomer}. The IR and ^{1}H NMR spectra of (*R*)-**3a** were identical with those of racemic **3a** and with those of (*S*)-**3a** reported previously.[13]

HPLC analysis of its MTPA ester: Lichrosorb SI100-5 4.6 × 250 mm (GL Sciences); hexane–diethyl ether (20:1); 0.5 cm^3 min^{-1}; 220 nm: t_R 58.7 (23.7%) and 67.2 min (76.3%).

Ethyl (*R*)-mandelate (4a). (*R*)-**4a** was obtained in 35% yield (77 mg) from **4** (215 mg), $[\alpha]_D^{20}$ −125.27 (*c* 4.32, $CHCl_3$) {lit.,[15] $[\alpha]_D^{22}$ −125.41 ($CHCl_3$); lit.,[16] $[\alpha]_D^{21}$ −125.26 (*c* 4.79, $CHCl_3$)}. The IR and ^{1}H NMR spectra of (*R*)-**4a** were identical with those of racemic **4a**.

HPLC analysis of (*R*)-**4a**: Chiralcel OB; hexane–propan-2-ol (30:1); 0.5 cm^3 min^{-1}; 220 nm: t_R 32.9 (2.5%) and 35.6 min (97.5%).

(*S*)-1-(4-Nitrophenyl)ethanol (5a). (*S*)-**5a** was obtained in 63% yield (133 mg) from **5** (207 mg), $[\alpha]_D^{20}$ −27.27 (*c*. 2.42, $CHCl_3$) {lit.,[25] $[\alpha]_{546}$ +35 (*c* 2, EtOH) for (*R*)-isomer}. The absolute configuration of (*S*)-**5a** was estimated by analogy with $[\alpha]_D$-values of (*S*)-**6a**–(*S*)-**9a**. The IR and ^{1}H NMR spectra of (*S*)-**5a** were identical with those of racemic **5a**.

HPLC analysis of its benzoate ester: Chiralcel OB; hexane–propan-2-ol (9:1); 1.0 cm^3 min^{-1}; 254 nm: t_R 40.7 (0.4%) and 49.7 min (99.6%).

(*S*)-1-(4-Chlorophenyl)ethanol (6a). (*S*)-**6a** was obtained in 56% yield (122 mg) from **6** (212 mg), $[\alpha]_D^{20}$ −48.82 (*c* 3.13, $CHCl_3$) {lit.,[19] $[\alpha]_D^{20}$ +30 (*c* 1, $CHCl_3$) for (*R*)-isomer}. The IR and ^{1}H NMR spectra of (*S*)-**6a** were identical with those of racemic **6a**.

HPLC analysis of (*S*)-**6a**: Chiralcel OB; hexane–propan-2-ol (30:1); 0.1 cm^3 min^{-1}; 220 nm: t_R 115.7 (99.6%) and 139.7 min (0.4%).

(*S*)-1-(4-Bromophenyl)ethanol (7a). (*S*)-**7a** was obtained in 56% yield (113 mg) from **7** (202 mg), $[\alpha]_D^{20}$ −40.72 (*c* 3.46, $CHCl_3$). The absolute configuration of (*S*)-**7a** was estimated by analogy with $[\alpha]_D^{20}$ −48.82 of (*S*)-**6a**. The IR and ^{1}H NMR spectra of (*S*)-**7a** were identical with those of racemic **7a** and indistinguishable from those of (*S*)-**6a**.

HPLC analysis of (*S*)-**7a**: Chiralcel OB; hexane–propan-2-ol (30:1); 0.1 cm^3 min^{-1}; 220 nm: t_R 122.4 (99.2%) and 152.5 min (0.8%).

(*S*)-1-(4-Methylphenyl)ethanol (8a). (*S*)-**8a** was obtained in 35% yield (65 mg) from **8** (182 mg), $[\alpha]_D^{20}$ −55.4 (*c* 1.97, $CHCl_3$) {lit.,[19] $[\alpha]_D^{20}$ +39 (*c* 1, $CHCl_3$) for (*R*)-isomer}. The IR and ^{1}H NMR spectra of (*S*)-**8a** were identical with those of racemic **8a**.

HPLC analysis of (*S*)-**8a**: Chiralcel OB; hexane–propan-2-ol (9:1); 0.5 cm^3 min^{-1}; 220 nm: t_R 14.1 (99.6%) and 16.7 min (0.4%).

(*S*)-1-(4-Methoxyphenyl)ethanol (9a). (*S*)-**9a** was obtained in 30% yield (64 mg) from **9** (208 mg), $[\alpha]_D^{20}$ −50.6 (*c* 3.64, $CHCl_3$) {lit.,[19] $[\alpha]_D^{20}$ +45 (*c* 1, $CHCl_3$) for (*R*)-isomer}. The IR and ^{1}H NMR spectra of (*S*)-**9a** were identical with those of racemic **9a**.

HPLC analysis of (*S*)-**9a**: Chiralcel OB; hexane–propan-2-ol (9:1); 0.5 cm^3 min^{-1}; 254 nm: t_R 25.2 (98.1%) and 37.6 min (1.9%).

(*S*)-1-(2-Pyridyl)ethanol (10a). (*S*)-**10a** was obtained in 38%

yield (76 mg) from **10** (196 mg), $[\alpha]_D^{20}$ −35.6 (*c* 4.31, $CHCl_3$) {lit.,[8] $[\alpha]_D^{20}$ −26.4 (*c* 1.34, $CHCl_3$); lit.,[20] $[\alpha]_D$ −25.1 (*c* 1.5, $CHCl_3$)}. The IR and ^1H NMR spectra of (*S*)-**10a** were identical with those of racemic **10**, which was prepared from **10** by treatment with $NaBH_4$.[26]

HPLC analysis of its MTPA ester: Lichrosorb SI-100; hexane–diethyl ether (20:1); 1.0 cm^3 min^{-1}; 254 nm: t_R 80.3 (0.5%) and 90.9 min (99.5%).

(*S*)-1-(3-Pyridyl)ethanol 11a. (*S*)-**11a** was obtained in 36% yield (73 mg) from **11** (198 mg), $[\alpha]_D^{20}$ −57.28 (*c* 3.94, $CHCl_3$) {lit.,[8] $[\alpha]_D^{20}$ −53.5 (*c* 1.09, $CHCl_3$); lit.,[20] $[\alpha]_D$ −39.0 (*c* 0.8, $CHCl_3$)}. The IR and ^1H NMR spectra of (*S*)-**11a** were identical with those of racemic **11a**, which was prepared from **11** by treatment with $NaBH_4$.[26] The ^1H NMR spectrum was in good accordance with that of (*R*)-**11a** reported previously.[27]

HPLC analysis of (*S*)-**11a**: Chiralcel OB; hexane–propan-2-ol (9:1); 0.5 cm^3 min^{-1}; 254 nm: t_R 18.1 (97.9%) and 35.5 min (2.1%).

(*S*)-1-(4-Pyridyl)ethanol 12a. (*S*)-**12a** was obtained in 30% yield (61 mg) from **12** (200 mg), mp 63–65 °C (lit.,[26] mp 63–65 °C); $[\alpha]_D^{20}$ −43.9 (*c* 2.2, MeOH) {lit.,[8] $[\alpha]_D^{20}$ −43.0 (*c* 1.24, MeOH); lit.,[20] $[\alpha]_D$ −43.8 (*c* 1, EtOH); lit.,[28] $[\alpha]_D^{20}$ +46.7 (*c* 0.51, EtOH) for (*R*)-isomer}. The IR and ^1H NMR spectra of (*S*)-**12a** were identical with those of racemic **12a**, which was prepared from **12** by treatment with $NaBH_4$.[26]

HPLC analysis of (*S*)-**12a**: Chiralcel OB; hexane–propan-2-ol (30:1); 0.5 cm^3 min^{-1}; 254 nm: t_R 101.3 min (98.6%) and 155.2 min (1.4%).

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