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Reduction of acetophenones with methyl fluorines and a bulky group on the aromatic ring using microorganisms and related enzymes

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ABSTRACT

Whole-cell yeasts and mold-catalyzed reduction of two fluorinated acetophenone derivatives with very bulky substituents on *ortho* position of aromatic ring, (±)-1′-(2-*tert*-butyl-2-methyl-1,3-benzodioxol-4-yl)-2′,2′-difluoroethanone and (±)-1′-(2-*tert*-butyl-2-methyl-1,3-benzodioxol-4-yl)-2′,2′,2′-trifluoroethanone were examined. On the former substrate, *Geotrichum candidum* NBRC 5767 showed high *re*-facially selective attack of hydride, while with *Pichia angusta* JCM 3620, complementary *si*-facially selective attack proceeded. *G. candidum* NBRC 5767 was revealed to be potent biocatalyst which provides (1′*S*)-alcohols from both substrates in a highly facially selective manner. Some unknown reductases were suggested responsible for those reductions, other than so far having been reported acetophenone reductase and trifluoromethyl ketone reductase from *G. candidum*, comparing the results obtained by applying those enzymes.

1. Introduction

Asymmetric reduction of ketones with cultured whole-cell biocatalysts, such as strains of *Geotrichum candidum* (NBRC 4597 and 5767), have been studied intensively [1–3]. Their activity and enantioselectivity in asymmetric reduction are extremely variable. For example, *G. candidum* NBRC 4597 reduces acetophenone (**1**) and 2′-fluoroacetophenone (**3**) to yield (*S*)-**2** and (*R*)-**4** in the same enantiofacial selectivity. The enantiofacial preference changes, with increasing the number of fluorine atoms on the methyl groups. When using difluoro derivative **5** as the starting material, the enantioselectivity in **6** is lost and, eventually, in 2′,2′,2′-trifluoroacetonphenone (**7**), the selectivity was completely inversed to give (*S*)-**8** [4,5].

When starting with (±)-**9**, which contains a unique acetal on the aromatic ring, the reduction with *G. candidum* NBRC 4597 was very slow. This result agreed well with studies showing that the introduction of a bulky substituent on the *ortho* position suppressed reduction by this microorganism [6]. The enantiofacially selective reduction, however, was proceeded by applying cultured cells of strain *G. candidum* NBRC 5767 [7] to give mainly (2*S*,1′*S*)- and (2*R*,1′*S*)-**10a**. Based on the results from ketones **1**, **3**, and **5**, the effect of the fluorine-containing substituents in **9** and **11** on reactivity and stereoselectivity was investigated. This report presents the difference in selectivity of the cultured whole-cell catalyzed reduction of trifluoromethyl ketone **9** and difluoromethyl ketone **11** from eight yeasts and fungi strains, including *G. candidum*. Scheme 1.

2. Experimental

IR spectra were measured as films for oils or KBr disks of solids on a Jasco FT/IR-410 spectrometer, and as ATR on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ at 270 MHz on a Jeol JNM EX-270 or at 400 MHz on an Agilent 400-MR spectrometer. ^{13}C NMR spectra were measured in $CDCl_3$ at 68 MHz on a Jeol JNM EX-270 or at 100 MHz on an Agilent 400-MR or at 125 MHz on an Agilent INOVA-500 spectrometer. HPLC data were recorded on Jasco MD-2010 or SHIMADZU SPD-20A multi-channel detectors. Merck silica gel 60 F_{254} thin-layer plate (1.05715, 0.25 mm thickness) was used for thin-layer chromatographic analysis. Merck silica gel 60 F_{254} thin-layer plates (1.05744, 0.5 mm thickness) and silica gel 60 (spherical and neutral; 100–210 μm, 37560-79) from Kanto Chemical Co., Inc. were used for preparative thin-layer chromatography and column chromatography, respectively. Yeast strains are available from Japan Collection of Microorganisms; Riken Bioresource Center, Planning

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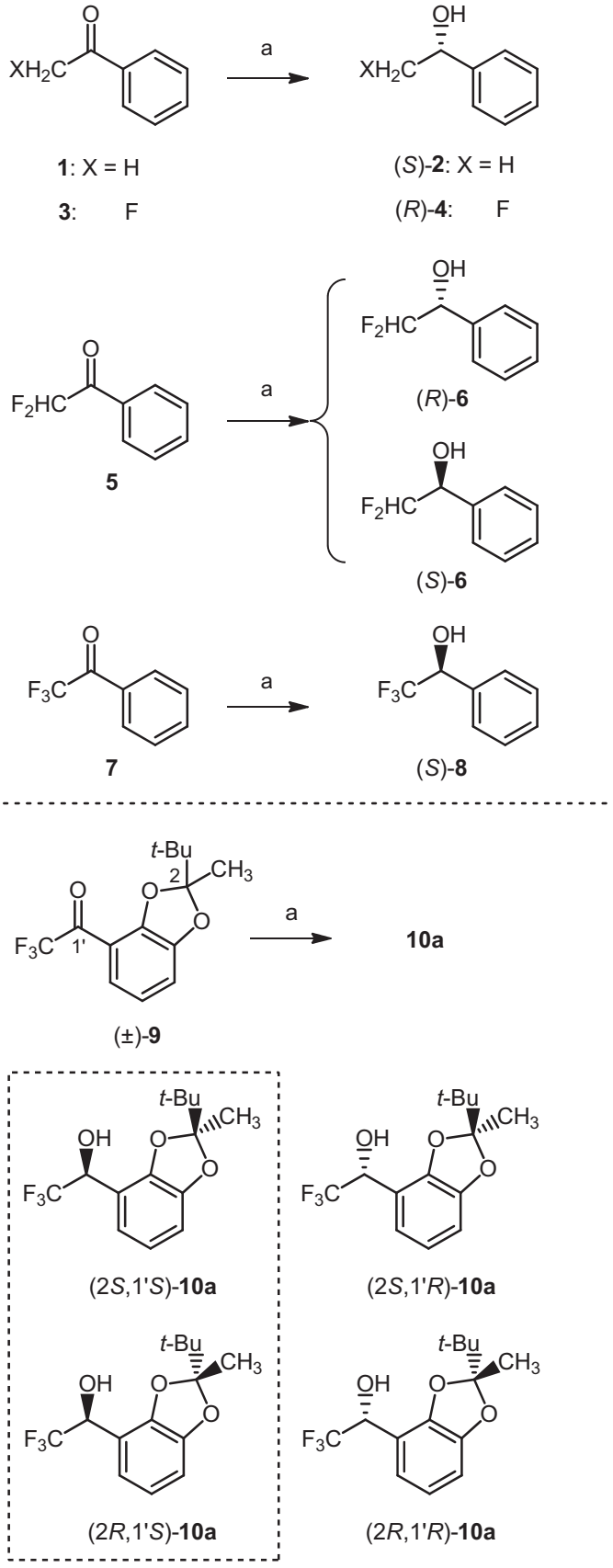

Scheme 1. Reagents and conditions: (a) *Geotrichum candidum*.

Section, Research Promotion Division, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan, and to NITE Biological Resource Center; Department of Biotechnology, National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan. Peptone, malt extract and yeast extract were purchased from Kyokuto Pharmaceutical Industrial Co., Ltd.

*2.1. (±)-1′-(2-tert-Butyl-2-methyl-1,3-benzodioxol-4-yl)-2′,2′-difluoroethanone (**11**)*

In a similar way for the preparation of **9** [7], a solution of lithiated form of 2-*tert*-butyl-2-methyl-1,3-benzodioxole (1.00 g, 5.2 mmol), which was made with *n*-butyllithium (2.6 M in hexane, 3.0 mL, 1.5 equiv.) and TMEDA (1.1 mL, 1.5 equiv.) was treated with F_2HCCO_2Et (1.4 mL, 13 mmol, 2.5 equiv.) at −50 °C. Similar workup [7] and the purification of the residue by silica gel column chromatography (100 g) by the elution with hexane/AcOEt = 40:1 to 20:1 provided ketone **11** (1.17 g, 83%) as yellow oil. ^{1}H NMR: δ 1.08 (s, 9H, *tert*-Bu), 1.62 (s, 3H, Me), 6.48 (t, $J_{2',F}$ = 53.6 Hz, 1H, H2′), 6.85 (dd, $J_{5,6}$ = 8.2 Hz, $J_{6,7}$ = 7.6 Hz, 1H, H6), 6.94 (dd, $J_{5,7}$ = 1.4 Hz, 1H, H7), 7.36 (dd, 1H, H5); ^{13}C NMR: δ 20.2, 24.4, 39.6, 109.1 (t, $J_{2',F}$ = 989.4 Hz, C2′), 111.6, 113.3, 120.4, 121.4, 126.0, 149.3, 149.3, 184.8 (t, $J_{1',F}$ = 96.8 Hz, C1′), the signals 24.4 included totally three carbons; IR: 2978, 1709, 1460, 1142, 854, 725 cm^{-1}.

2.2. (2R,1′R*)-(±)-1′-(2-tert-Butyl-2-methyl-1,3-benzodioxol-4-yl)-2′,2′-difluoroethanol (**12a**) and (2R*,1′S*)-(±)-1′-(2-tert-butyl-2-methyl-1,3-benzodioxol-4-yl)-2′,2′-difluoroethanol (**12a**)*

To a solution of **11** (15.9 mg, 0.059 mmol) in EtOH (590 μL) were treated with $NaBH_4$ (6.7 mg, 0.176 mmol, 3.0 equiv.) at room temperature. The same workup and the purification of the residue by preparative TLC [developed with hexane/AcOEt = 4:1] to afford (2*R**,1′*R**)-**12a** and (2*R**,1′*S**)-**12a** (15.0 mg, 94%) as pale yellow oil as a mixture. The diastereomeric ratio between (2*R**,1′*S**)- and (2*R**,1′*R**)-**12a** was determined to be 3:2 judging from their NMR spectrum: δ 5.88 for (2*R**,1′*S**)- and δ 5.92 for (2*R**,1′*R**)-**12a**. A small portion was separated by a preparative HPLC [column, Kanto Chemical Co., Inc. Mightysil 60, 1 cm × 25 cm; hexane/AcOEt = 15:1); flow rate 5 mL/min]: t_R (min) = 6.6 [(2*R**,1′*R**)-**12a**], 6.8 [(2*R**,1′*S**)-**12a**] and the former was isolated in pure state. (2*R**,1′*R**)-**12a**: ^{1}H NMR: δ 1.05 (s, 9H, *tert*-Bu), 1.55 (s, 3H, Me), 4.86 (ddd, $J_{1',2'}$ = 4.1 Hz, $J_{1',F}$ = 9.8, 13.9 Hz, 1H, H1′), 5.92 (dt, $J_{2',F}$ = 55.8 Hz, 1H, H2′), 6.70–6.83 (aromatic, 3H); ^{13}C NMR: δ 20.0, 24.5, 39.4, 70.7 (t, $J_{1',F}$ = 99.8 Hz, C1′), 108.4, 115.1 (t, $J_{2',F}$ = 976.0 Hz, C2′), 116.5, 119.6, 121.2, 124.4, 145.8, 148.1, the signals 24.5 included totally three carbons. (2*R**,1′*S**)-**12a**: ^{1}H NMR: δ 1.05 (s, 9H, *tert*-Bu), 1.55 (s, 3H, Me), 4.93 (dt, $J_{1',2'}$ = 4.3 Hz, $J_{1',F}$ = 10.6 Hz, 1H, H1′), 5.88 (dt, $J_{2',F}$ = 55.9 Hz, 1H, H2′), 6.70–6.83 (aromatic, 3H); ^{13}C NMR: δ 20.0, 24.4, 39.5, 69.8 (q, $J_{1',F}$ = 101.2 Hz, C1′), 108.3, 115.2 (t, $J_{2',F}$ = 976.0 Hz, C2′), 116.5, 119.0, 121.2, 124.3, 145.8, 148.0, the signals 24.4 included totally three carbons. HPLC [column, Daicel Chiralcel OJ-H, 0.46 cm × 25 cm; hexane/*i*-PrOH = 40:1; flow rate 0.5 mL/min]: t_R (min) = 18.3 [(2*R*,1′*R*)-**12a**], 20.4 [(2*S*,1′*S*)-**12a**], 22.4 [(2*R*,1′*S*)-**12a**], 30.5 [(2*S*,1′*R*)-**12a**]. The assignment of relative and absolute configurations as above were described in detail, in Sections 2.7 and 2.8.

*2.3. Screening of microorganisms for the reduction of **9** and **11***

The microorganisms from stock culture samples were incubated in glucose medium [8–10] [containing glucose (5.0 g), peptone