Stereoselective Reduction of Symmetrical Diketones with Biological Methods. Microbial Reduction of *cis*-2,7-Decalindione

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Synopsis. The *pro-S* group of two enantiotopic carbonyl groups in *cis*-2,7-decalindione was preferentially reduced with *Rhodotorula rubra* to give (−)-(7*S*,9*R*,10*S*)-7-hydroxy-*cis*-2-decalone of high optical purity.

In the course of our investigations of the stereo-differentiating reduction of various carbonyl compounds with biological systems, two completely opposite enantiomer selectivities toward C_2-ketones,[1] which were found in a microbial reduction and a horse liver alcohol dehydrogenase (HLADH)-mediated reduction, have led us to propose the microbial "*P*-C_2-ketone rule"[2] and the HLADH "*M*-C_2-ketone rule"[3] to summarize their respective stereochemical behaviors.

Among our further studies on the symmetrical diketones,[4,5] we found that one of two enantiotopic carbonyl groups was preferentially reduced and the hydrogen attack occurred from the *Re*-face of its carbonyl plane.[5] These findings encouraged us to develop a useful methodology for asymmetric syntheses.[6] For instance, it is theoretically possible by utilizing biological stereoselectivity toward prochiral diketone, *cis*-2,7-decalindione (**1**),[7] to transfer the total amount of starting material into one chiral isomer of four molecules, enantiomers of *cis,trans*-ketol **2**[8] and of *cis,cis*-ketol **3**.[8] The HLADH-mediated reduction of **1** did indeed show differentiations between enantiotopic *pro-R* and *pro-S* carbonyl groups,[9] and diastereotopic faces of the carbonyl plane affording (−)-(7*S*,9*S*,10*R*)-7-hydroxy-*cis*-2-decalone (**2**)[10] with a good yield and high optical purity. Starting from **2**, optically pure (+)-twistane was synthesized in short steps.[5b,11]

Coupled with the opposite enantiomer selectivities toward C_2-ketone, we expected to find the opposite enantiotopic group selectivities toward the diketone **1** in the HLADH-mediated reduction and the microbial reduction.

Results and Discussion

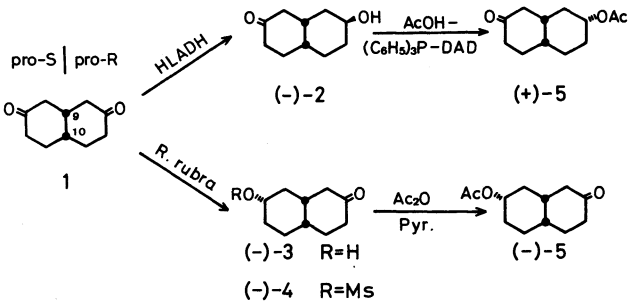
Microbial Reduction of Diketone 1. The preliminary test incubation of **1** both with *Rhodotorula rubra* and *Curvularia lunata* revealed that these microbes rapidly reduced **1** giving a ketol as the major product and sluggishly reduced it into diol(s) during further incubation. Monitoring by means of GLC indicated that the metabolite ketol was found to be *cis,cis*-ketol **3**, one of the diastereomers of (−)-*cis,trans*-ketol **2** obtained from an HLADH-mediated reduction.

Preparative-scale incubation with *R. rubra* was carried out for 68 h at 30°C. During this period, 85% of the diketone was consumed. After an extraction of the metabolite, alumina chromatography provided a 59% yield of crystalline (−)-ketol **3**, $[\alpha]_D^{25}$ −2.3°. Contrary to *R. rubra*, *C. lunata* was found to show a poor enantiotopic group selectivity, affording a 21% yield of optically inactive ketol **3**.[12]

In the ^{1}HNMR spectrum, a singlet signal at δ 3.0 ppm due to methyl protons of $-OSO_2CH_3$ exhibited by the (±)-keto mesylate **4** of (±)-ketol **3**, was clearly separated ($\Delta\Delta\delta$ 0.04 ppm) with an addition of a chiral shift reagent, Eu(facam)$_3$.[13] In case of (−)-**4** prepared from (−)-ketol **3**, such an enantiomer differential shift was not observed to give any information about the optical purity of (−)-ketol **3** as to be 100% within experimental error.

To secure information on the absolute configuration of (−)-ketol **3**, we attempted to relate it to (−)-ketol **2**, whose absolute configuration had been established as being (7*S*,9*S*,10*R*) in our labolatory.[5b]

By the method of Mitsunobu,[14] an inversion of the configuration at C-7 of (−)-ketol **2** using triphenylphosphine, diethyl azodicarboxylate, and acetic acid in tetrahydrofuran, gave (+)-keto acetate **5**, $[\alpha]_D^{24}$ +41°. This was found to be an enantiomer of (−)-**5**, $[\alpha]_D^{24}$ −40°, prepared from (−)-ketol **3** by a treatment with acetic anhydride in pyridine. Thus, the absolute configuration of (−)-ketol **3** was determined as being a (7*S*,9*R*,10*S*)-configuration.[15]



These results clearly show that *R. rubra* preferentially catches the *pro-S* carbonyl group and the hydrogen attack occurs from the *Re*-face of the carbonyl plane. The demonstrated enantiotopic group selectivity of *R. rubra* is completely opposite to that found in the HLADH-mediated process which selectively reduced the *pro-R* carbonyl group.

Experimental

Melting points are uncorrected. ^{1}HNMR spectra were determined on a JNM-60-HL and JNM-NH-100 with Me_4Si as an internal standard (δ=0). Optical rotations were measured with a JASCO-DIP-140 polarimeter. CD spectra were determined on a JASCO-J-40 spectropolarimer. GLC analyses were performed on a JGC-20K equipped with a FID using a 1 m×3 mm inside diameter column of 10% Carbowax 20M on Chromosorb W. Column-chromatography was carried out with Woelm active alumina (neutral, activity III).

Preparation of 1. The diketone **1** was prepared by the method of Anderson and Barlow,[16] mp 63—64°C (lit,[16] mp 62—64°C).

Cultured Broth. *(a). Microbe Culture*: The cultures of *R. rubra* (IFO 0889) and *C. lunata* (IFO 6288) were obtained from the Institute of Fermentation, Osaka, Japan. *(b). Culture medium*:[17] The culture medium was prepared by dissolving glucose (30 g), KH_2PO_4 (1 g), corn-steep liquor (10 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $NaNO_3$ (2 g), $FeSO_4 \cdot 7H_2O$ (0.02 g), K_2HPO_4 (2 g), and KCl (0.5 g) in 1 L of tap water and was sterillized at 123 °C for 15 min. *(c). Growth of Microbes*: 25 mL of the culture medium in a 100-mL Erlenmeyer flask was inoculated with spores of the microbe on Difco MY agar slants and was shaken for 48 h at 30 °C on a shaker. The culture was transferred into a 500mL Erlenmeyer flask containing 200mL of the culture medium, incubation was maintained for another 48h at 30 °C until a sufficient mass of mycelium had developed.

Microbial Reduction of 1 with *R. rubra*. An aquous solution (40 mL) containing 1.5 g (9 mmol) of **1** was divided into eight batches of the culture broth of *R. rubra* (each 200 mL) and the mixture was incubated at 30 °C for 68 h. After the mycelium was collected by filtration through the layer of Hyflo Super Cel and extracted with chloroform, the beer filtrate was also extracted with the same solvent. The combined extracts were washed with water and dried over anhydrous magnesium sulfate. The removal of the solvent in vacuo left a metabolite mixture (1.4 g) which was analyzed by GLC to reveal that it was a 15:5:78:2 mixture of **1**, **2**, **3**, and diol(s).

The mixture was taken up in benzene and chromatographed upon 50 g of alumina. Elution with 200 mL of benzene–chloroform (10:1) afforded 50 mg of **1**. Further elution with 200 mL of benzene–chloroform (1:1), followed by 600 mL of chloroform, gave 880 mg (59% yield) of (−)-ketol **3**; mp 88—89 °C (from pentane); $[\alpha]_D^{25}$ −2.3° (*c* 1.6, $CHCl_3$); CD (*c* 2.4×10^{-3} mol dm^{-3}, MeOH) [θ] 290 nm −380; IR (KBr) 3400, 1690, 1060 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ= 3.6 (m, 1H, 1/2*W* 24Hz, C-7-H).

Found: C, 71.45; H, 9.57%. Calcd for $C_{10}H_{16}O_2$: C, 71.39; H, 9.39%.

(−)-Keto Mesylate 4. To a solution of (−)-ketol **3** (460 mg, 2.7 mmol) in pyridine (5 mL) was added methanesulfonyl chloride (600 mg, 5.2 mmol) at 0 °C. The mixture was allowed to stand overnight in a refrigerator. The reaction mixture was diluted with ice-water and extracted with dichloromethane. The extract was washed with dil hydrochloric acid and water, then dried over anhydrous magnesium sulfate. After the removal of the solvent in vacuo, the residue (620 mg) was recrystallized from pentane-dichloromethane to give 420 mg (62% yield) of (−)-keto mesylate **4** as flaks; mp 99—100 °C; $[\alpha]_D^{25}$ −24.4° (*c* 1.7, $CHCl_3$); IR (KBr) 1700, 1350, 1170, 938 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ=4.65 (m, 1H, 1/2*W* 23Hz, C-7-H), 3.0 (s, 3H, S-CH_3).

Found: C, 53.57; H, 7.39; S, 12.98%. Calcd for $C_{11}H_{18}O_4S$: C, 53.64; H, 7.37; S, 13.02%.

(−)-Keto Acetate 5. To a solution of (−)-ketol **3** (103 mg, 0.6 mmol) in pyridine (1 mL) was added acetic anhydride (0.1 mL). The mixture was allowed to stand overnight. The reaction mixture was poured into ice-water and extracted with ether. The ethereal layer was washed with 5% hydrochloric acid and water followed by drying over anhydrous magnesium sulfate. The removal of the solvent gave 110 mg (90% yield) of (−)-keto acetate **5**; mp 82—83 °C (from pentane); $[\alpha]_D^{22}$ −40.0° (*c* 0.54, $CHCl_3$); IR (KBr) 1730, 1705, 1250, 1050 cm^{-1}; ^{1}H NMR (60 MHz $CDCl_3$) δ=4.75 (m, 1/2*W* 22 Hz, C-7-H), 2.0 (s, 3H, O-CH_3).

Found: C, 68.52; H, 8.69%. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63%.

Microbial Reduction of 1 with *C. lunata*. The diketone **1** (total 1.5 g, 9 mmol) was incubated for 68 h at 30 °C in eight batches. The metabolite mixture containing **1**, **2**, **3**, and diol(s) in a ratio of 8:9:80:3 was worked up according to the procedure described on the *R. rubra* reduction to afford 317 mg (21% yield) of (±)-ketol **3**; mp 101.5—102.5 °C (from pentane); $[\alpha]_D^{23.5}$ ±0° (*c* 1.33, $CHCl_3$).

Found: C, 71.51; H, 9.58%. Calcd for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59%.

The (±)-keto mesylate **4**; mp 78—79 °C (from pentane–dichloromethane); $[\alpha]_D^{24}$ ±0° (*c* 1.2, $CHCl_3$).

Found: C, 53.57; H, 7.39%. Calcd for $C_{11}H_{18}O_4S$: C, 53.64; H, 7.37%.

(+)-Keto Acetate 5. To a solution of (−)-ketol **2** obtained from HLADH mediated reduction ($[\alpha]_D^{24}$−16.4°,[5b] 170 mg, 1 mmol), triphenylphosphine (510 mg, 2 mmol), and acetic acid (120 mg, 1 mmol) in dry THF (4 mL) was added dropwise a solution of diethyl azodicarboxylate (340 mg, 2 mmol) in dry THF (2 mL). After being stirred at room temperature for 48 h, the reaction mixture was analyzed by GLC to reveal that 76% of **2** was inverted into keto acetate **5**. The solvent was removed under reduced pressure to afford a syrupy product which was taken up into ether (50 mL). An insoluble material was filtered off and the filtrate was concentrated. The resultant residue was taken up into pentane (150 mL) and a soluble portion was washed with aq sodium hydrogencarbonate. It was then chromatographed over alumina (5 g). Elution with pentane–ether (10:1) (250 mL) gave 55 mg (26% yield) of (+)-keto acetate **5**; mp 82—83 °C (from pentane); $[\alpha]_D^{24}$ +41° (*c* 0.73, $CHCl_3$).

Found: C, 68.58; H, 8.72%. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63%.

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8) According to the stereochemical naming of decalols, we named these ketols *cis,cis* and *cis,trans*. First *cis* means a *cis* relationship between C-9 and C-10 hydrogens, and second *cis* or *trans* means a *cis* or *trans* relationship between C-7 and C-9 hydrogens. See, a) T. Cohen, M. Malaiyandi, and J. L.

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