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Note

Reduction of Alkyl (2-Oxocyclohexyl)acetates by Baker's Yeast

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Baker's yeast reduction of methyl and ethyl (2-oxocyclohexyl)acetates proceeded with enantio- and diastereo-selectivity, affording the corresponding (2*S*)-*trans*-alcohols (major), (2*S*)-*cis*-alcohols (minor), and the unaltered (1*S*)-ketones with high optical purity.

Key words: yeast-reduction; γ-ketoester; kinetic resolution; asymmetric reduction

In work on naturally occurring polycyclic lactones, Pirkle and Adams[1] have prepared the enantiometrically pure *cis*- and *trans*-γ-lactones of (2-hydroxycyclohexyl)-acetic acid by resolving their racemic precursors with (*R*)-(+)-1-(1-naphthyl)ethylamine or (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate as a chiral-conversion agent.

We reported in 1990[2] preliminary findings that yeast-reduction of methyl 2-oxocyclohexylacetate (**1**) afforded the respective 2*S*-*trans*-alcohol, one of the precursors of those lactones. We now report the results and related data in further detail.

In contrast with β-keto esters,[3a] some of the alkali metal salts of γ- and δ-keto acids,[3b,3c,3d] γ- and δ-keto esters having a sulfur-containing substituent on the α- or β-carbon relative to the ketone carbonyl[3e,3f] which have been reported to be reduced with high enantioselectivity, simple γ-keto esters have been claimed to be poor substrates for reduction by baker's yeast.[4] However, we found that reduction of one of the simple γ-keto esters, methyl (2-oxocyclohexyl)acetate ((±)-**1**), proceeded smoothly with high enantio- and diastereoselectivity.

Substrate (±)-**1**[5] was first incubated with baker's yeast for two days, the progress of the reaction being followed by capillary GC, using benzoic acid as an internal standard. **Fig. 1** shows the time-course for the reduction of ketone (±)-**1** by baker's yeast. It is apparent that the reaction was almost complete within 24 hours. The relative proportions of the reduction products (*cis*:*trans*=1:3) at the 24-hour point are in marked contrast to those of the lower homolog, (±)-2-oxocyclohexanecarboxylate (a β-keto ester) which has been reported to exclusively afford the corresponding *cis*-hydroxy-ester in 65% yield.[6]

The absolute stereochemistry of the reduction products was assigned as shown in **Fig. 2** on the basis of the following facts: The *trans*-hydroxy-ester (the main product) had a positive optical rotation value, showing that it had a (1*R*, 2*S*)-absolute structure[7] as indicated in structure (+)-**2**. This structure was supported by the transformation of (+)-**2** into (−)-*trans*-lactone ((−)-**4**) of known (1*R*, 2*S*)-configuration.[1] The recovered (−)-ketone gave (+)-*trans*-lactone ((+)-**4**), the enantiomer of the foregoing (−)-lactone, by reduction with $NaBH_4$ and subsequent lactonization. The ketone accordingly had an (*S*)-configuration ((−)-**1**). The *cis*-hydroxy-ester (the minor product) had a positive rotation value, showing that it had a (1*S*, 2*S*)-configuration, since it was transformed into the corresponding (−)-*cis*-lactone ((−)-**5**) with a known absolute structure.[1,8]

The stereochemistry of the products shows (1) that the (1*R*)-substrate was reduced faster than was the (1*S*)-enantiomer and (2) that reduction of the carbonyl group proceeded according to the so-called Prelog rule.[9]

Analytical data for the reduction products are summarized in the Table. These results indicate that not only the enantiomeric excess (ee) of both the diastereomeric products, but also the substrate specificity of the reduction were quite high. Thus, enantio- and diastereo-selective reduction and kinetic resolution were achieved.

The reduction of ethyl (2-oxocyclohexyl)acetate ((±)-**1′**)[10,11] proceeded similarly, although ee of the recovered ketone ((−)-**1′** was slightly lower (89.8% ee) than that obtained with the methyl ester.

Fujisawa and co-workers reported[12] in 1991 that yeast-reduction of methyl or ethyl 4-oxo-3-tetrahydrothiopyranylacetate, the 5-thiacyclohexyl analog (i.e. a γ-ketoester having a sulfur-substituent on the β-carbon relative to the ketone carbonyl) of our substrate, was completed within a day to afford an optically pure

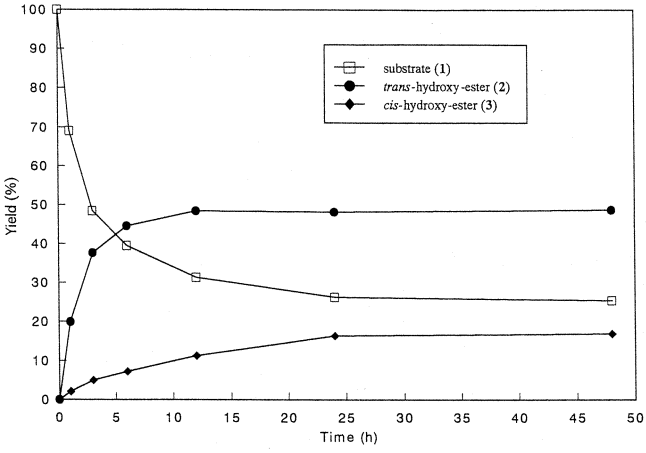

Fig. 1. Time-Course Plot for the Reduction of Methyl (2-oxocyclohexyl)acetate with Baker's Yeast.

Abbrevation: ee, enantiomeric excess

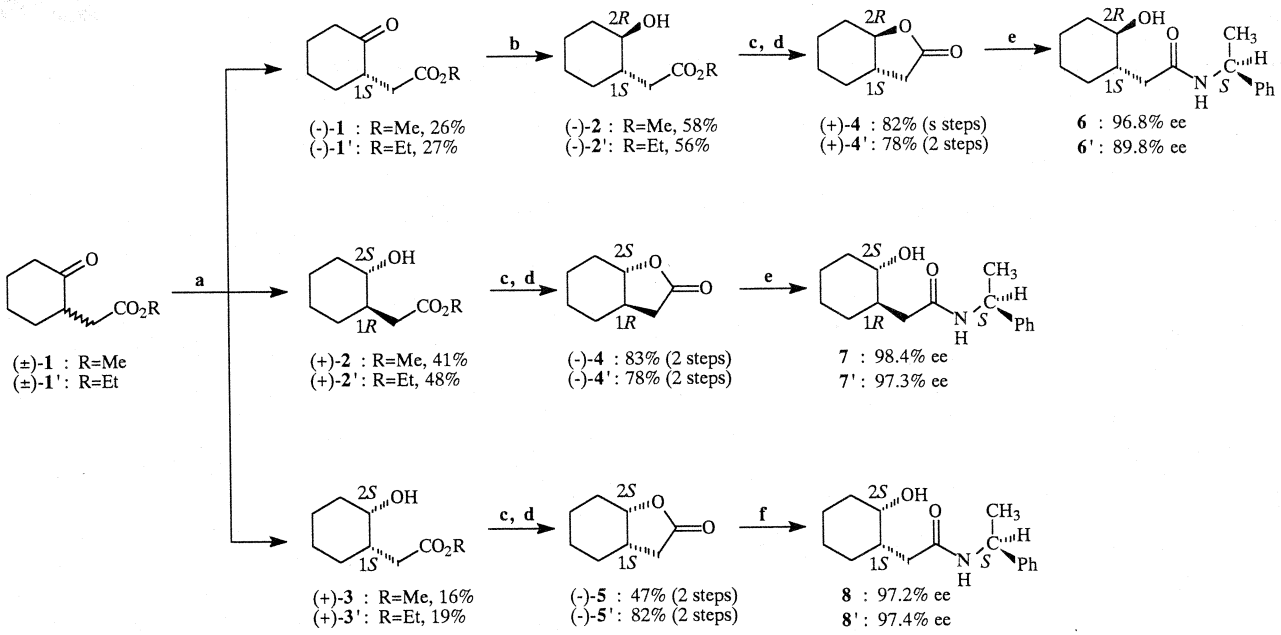

Fig. 2. Determination of the Absolute Configurations and Enantiomeric Excess Values of the Yeast-reduction Products.
a: baker's yeast, sucrose, H_2O, 2 days; b: i) $NaBH_4$, ROH; ii) 2 M HCl; c: i) NaOH, aq. ROH; ii) 5% HCl; d: *p*-TsOH·H_2O, C_6H_6; e: (*S*)-1-phenylethyl-amine, ether, rt; f: (*S*)-1-phenylethylamine, xylene, reflux

Table Baker's Yeast Reduction of (2-Oxocyclohexyl)acetates

R	Yield (%)[a]	Substrate:*cis*:*trans*[b]	% ee (substrate, *cis*, *trans*)[c]
CH_3	83	28:19:53	96.8 97.2 98.4
C_2H_5	94	28:18:54	89.8 97.4 97.3

a. Isolated yield
b. Determined by GC analysis
c. Determined by HPLC analysis of the corresponding acetamides

cis- and *trans*-*S*-alcohol mixture in nearly equal amounts; in this case, the ketone substrate was not recovered, in contrast with our result.

In conclusion, the yeast-mediated reaction of alkyl (2-oxocyclohexyl)acetates proceeded with concomitant kinetic resolution to afford alkyl (1*S*)-(2-oxocyclohexyl)-acetates, alkyl (1*R*, 2*S*)-*trans*-(2-hydroxycyclohexyl)-acetates and alkyl (1*S*, 2*S*)-*cis*-(2-hydroxycyclohexyl)-acetates of high purity.

The yeast-reduction method is of greater advantage than the chemical resolution method[1] for preparing optically active γ-lactones or their precursors in that it needs no expensive resolving agent nor troublesome multi-step process for the resolution.

Further investigation of the reduction of alkyl (2-oxocyclopentyl)acetates and utilization of the enantiomerically enriched products for the synthesis of optically active compounds are in progress.

Experimental

Column chromatography was performed on silica gel (Wakogel C-300, 0.045–0.075 mm particle size). IR spectra were determined with a Shimadzu FTIR-8100, while ^{1}H-NMR spectra were recorded with a JNM-EX400 FT-NMR. Specific rotation values were determined with a Horiba SEPA-200 instrument. GC was done with a Shimadzu GC-14A instrument with a flame ionization detector in a silica capillary column (Shimadzu Techno-Research CBP-20-M-25; 0.25 mm × 25 m). The column temperature was programmed from 100°C to 230°C at a rate of 5°C/min and then held at 230°C. The flow rate of the carrier gas (N_2) was 50 ml/min. HPLC analyses were carried out in a pre-packed Shimadzu STR ODS-II column (4.6 mm × 250 mm, UV detection at 254 nm, MeOH:H_2O:CH_3CN=4:7:1 at 0.7 ml/min) with a Shimadzu LC-6AD instrument fitted with a UV-VIS detector (Shimadzu SPD-6AV). Dry baker's yeast of "saf-instant" brand from S. I. Lesaffre (France) was used. Mass spectra were determined with a Hitachi M-80B spectrometer at 20-eV ionizing irradiation.

Baker's yeast reduction of (±)-**1**. A mixture of water (500 ml), sucrose (60 g) and baker's yeast (28 g) was shaken at 30°C for 10 min. A solution of the substrate (3.404 g, 20.0 mmol) in ethanol (10 ml) was then added, and the mixture was shaken at 30°C for 48 h. The mixture was filtered through Celite, and the filter cake was successively washed with water and acetone. The filtrate and washings were combined and concentrated. The residue was extracted with ether, the ethereal solution

being washed with brine, dried over Na_2SO_4, and concentrated to yield a crude mixture (3.40 g) which was analyzed by gas chromatography. The peak No., retention time (min), and percentage of each integrated peak area were as follows: **A**, 10.1, 27.9%; **B**, 12.1, 18.6%; and **C**, 12.3, 53.5%. The retention time for each peak coincided with that of an authentic sample: **A** with **1**,[13] **B** with **3**,[13,14] and **C** with **2**.[13,15] The mixture was chromatographed over silica gel (eluting with 3:1 hexane-EtOAc) to give (1*S*)-(−)-**1**[16] (893 mg, 26% yield, $[\alpha]_D^{20}$ −31±2° (*c* 1.328, $CHCl_3$); (1*S*, 2*S*)-(+)-**3**[16] (534 mg, 16% yield, $[\alpha]_D^{20}$ +18±1° (*c* 2.046, $CHCl_3$)); and (1*R*, 2*S*)-(+)-**2**[16] (1.402 g, 41% yield, $[\alpha]_D^{20}$ +29±1° (*c* 1.556, $CHCl_3$)).

Baker's yeast reduction of (±)-**1′**. In a similar manner, (±)-**1′** (3.686 g, 20.0 mmol) was reduced by baker's yeast to give a crude mixture (3.77 g) which was analyzed by gas chromatography. The peak No., retention time (min), and percentage of each integrated peak area were as follows: **E**, 10.7, 28.3%; **F**, 12.6, 18.4%; and **G**, 12.8, 53.3%. The retention time of each peak coincided with that of a racemic authentic sample: **E** with **1′**,[13] **F** with **3′**,[13] and **G** with **2′**.[13] The mixture was chromatographed over silica gel (eluting with 3:1 hexane-EtOAc) to give (1*S*)-(−)-**1′**[16] (1.007 g, 27% yield, $[\alpha]_D^{23}$ −28±1° (*c* 1.701, $CHCl_3$)); (1*S*, 2*S*)-(+)-**3′**[16] (692 mg, 19% yield, $[\alpha]_D^{23}$ +15±2° (*c* 1.572, $CHCl_3$)); and (1*R*, 2*S*)-(+)-**2′**[16] (1.783 g, 48% yield, $[\alpha]_D^{23}$ +26±2° (*c* 1.572, $CHCl_3$)).

The absolute configuration of (−)-**1′** was assigned by comparing the sign for the optical rotation with the literature value,[17] and those of (+)-**3′** and (+)-**2′** were assigned by converting the corresponding lactones in a similar manner to that for the methyl ester.

(*1S, 2R*)-(−)-**2**.[16] (−)-**1** (751 mg, 4.41 mmol) in methanol (35 ml) was reduced with $NaBH_4$ (84 mg, 2.2 mmol) to give (−)-**2** (439 mg, 58% yield, $[\alpha]_D^{20}$ −33±3° (*c* 0.694, $CHCl_3$)) and a mixture of the *cis*-hydroxy ester and its lactone derivative (256 mg).

(*1S, 2R*)-(−)-**2′**.[16] In a similar manner (−)-**1′** (986 mg, 5.35 mmol) was converted to (−)-**2′** (559 mg, 56% yield, $[\alpha]_D^{23}$ −25±2° (*c* 1.502, $CHCl_3$)), (1*S*, 2*S*)-ethyl *cis*-(2-hydroxycyclohexyl)acetate[16] (246 mg, 25% yield), and the lactone of (1*S*, 2*S*)-*cis*-(2-hydroxycyclohexyl)-acetic acid[16] (76 mg, 10% yield).

(*1S, 2R*)-(+)-*trans*-**4**.[16] To a solution of (−)-**2** (390 mg, 2.27 mmol) in methanol (20 ml) was added NaOH (750 mg, 18.8 mmol) in water (2 ml). The mixture was refluxed for 4 h and concentrated. After the usual work-up, the free (−)-acid (318 mg, 89% yield, $[\alpha]_D^{20}$ −48±2° (*c* 1.764, EtOAc)) was obtained as colorless crystals. This acid (302 mg, 1.91 mmol) was lactonized according to the procedure of Pirkle and Adams[1] to afford (+)-**4** (248 mg, 92% yield, $[\alpha]_D^{20}$ +88±1° (*c* 2.044, $CHCl_3$)) as a colorless solid.

(*1S, 2R*)-(+)-*trans*-**4′**.[16] In a similar manner, except that ethanol was used instead of methanol, (−)-**2′** (352 mg, 1.89 mmol) was converted to the free acid (263 mg, 88% yield, $[\alpha]_D^{23}$ −47±2° (*c* 1.019, EtOAc)) as colorless crystals. This was converted to (+)-**4′** (196 mg, 89% yield, $[\alpha]_D^{23}$ +80±2° (*c* 1.372, $CHCl_3$)) as a colorless liquid.

Determination of the enantiomeric excess of the (+)-*trans-lactone*. The (±)-*trans*-lactone ((±)-**4**)[1,13,18] was converted to the diastereomeric amide according to Cromwell and Cook[19] by using (*S*)-1-phenylethylamine. The converted diastereomeric mixture gave two well-separated peaks by HPLC with an area ratio of 1:1.

(+)-**4** (123 mg, 0.878 mmol) was similarly converted to the amide (**6**). HPLC analysis of the amide gave two peaks in a 98.4:1.6 ratio. Thus, the samples of (1*S*, 2*R*)-(+)-**4** and (1*S*)-(−)-**1** were of ≧96.8% ee.

In an analogous manner (+)-**4′** (125 mg, 0.889 mmol) was converted to the amide (**6′**). HPLC analysis of the amide gave two peaks in a 94.9:5.1 ratio. Thus, the samples of (1*S*, 2*R*)-(+)-**4′** and (1*S*)-(−)-**1′** were of ≧89.8% ee.

(*1R, 2S*)-(−)-*trans*-**4**.[16] (+)-**2** (1.305 g, 7.58 mmol) was hydrolyzed to the corresponding free (+)-acid (1.058 g, 88% yield) as pale yellow crystals by a procedure similar to that used for the enantiomer, $[\alpha]_D^{20}$ +47±2° (*c* 2.270, EtOAc). The acid (1.036 g, 6.55 mmol) was converted to (−)-**4** (867 mg, 94% yield, $[\alpha]_D^{18}$ −93±1° (*c* 2.034, $CHCl_3$)) as a colorless solid by a procedure similar to that used for (+)-**4**.

(*1R, 2S*)-(−)-*trans*-**4′**.[16] In a similar manner, except that ethanol was used instead of methanol, (+)-**2′** (681 mg, 3.66 mmol) was converted to the free acid (487 mg, 84% yield, $[\alpha]_D^{23}$ +48±2° (*c* 1.196, EtOAc)) as a colorless solid. This (465 mg, 2.94 mmol) was converted to (−)-**4′** (385 mg, 93% yield, $[\alpha]_D^{23}$ −92±2° (*c* 1.235, $CHCl_3$)) as a colorless solid.

Determination of the enantiomeric excess of the (−)-*trans-lactone*. (−)-**4** (160 mg, 1.14 mmol) was converted to the amide (**7**) by a procedure similar to that used for the diastereomeric amide from (±)-**4**. HPLC analysis gave two peaks in a 99.2:0.8 ratio. Thus, the samples of (1*R*, 2*S*)-(−)-**4** and (1*R*, 2*S*)-(+)-**2** were of ≧98.4% ee.

In an analogous manner, (−)-**4′** (142 mg, 1.01 mmol) was converted to the amide (**7′**). HPLC analysis gave two peaks in a 98.65:1.35 ratio. Thus, the samples of (1*R*, 2*S*)-(−)-**4′** and (1*R*, 2*S*)-(+)-**2′** were of ≧97.3% ee.

(*1S, 2S*)-(−)-*cis*-**5**.[16] (+)-**3** (489 mg, 2.84 mmol) was converted via the corresponding acid to (−)-**5** (186 mg, 2 steps, 47% yield, $[\alpha]_D^{19}$ −52±1° (*c* 2.074, $CHCl_3$)).

(*1S, 2S*)-(−)-*cis*-**5′**.[16] Similarly, (+)-**3′** (463 mg, 2.48 mmol) was converted to (−)-**5′** (284 mg, 2 steps, 82% yield, $[\alpha]_D^{22}$ −51±2° (*c* 1.510, $CHCl_3$)).

Determination of the enantiomeric excess of the (−)-*cis-lactone.* The (±)-*cis*-lactone ((±)-**5**)[1,13,18] was converted to the corresponding diastereomeric amide mixture by using (*S*)-1-phenylethylamine. The converted diastereomeric mixture gave two well-separated peaks by HPLC with an area ratio of 1:1.

(−)-**5** (125 mg, 0.891 mmol) was converted to the amide (**8**) by a procedure similar to that used for the diastereomeric amide from (±)-**5**. HPLC analysis gave two peaks in a 98.6:1.4 ratio. Thus, the samples of (1*S*, 2*S*)-(−)-**5** and (1*S*, 2*S*)-(+)-**3** were of ≧97.2% ee.

Similarly (−)-**5′** (128 mg, 0.913 mmol) was converted to the amide (**8′**). HPLC analysis gave two peaks in the ratio of 98.7:1.3. Thus, the samples of (1*S*, 2*S*)-(−)-**5′** and (1*S*, 2*S*)-(+)-**3′** were of ≧97.4% ee.

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