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The Use of Microorganisms in Oraganic Synthesis. V. Microbiological Asymmetric Reduction of Methyl 2-Methyl-3-(2-thienyl)-3-oxopropionate

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Microbiological asymmetric reduction of methyl 2-methyl-3-(2-thienyl)-3-oxopropionate (**3**) by various yeasts was carried out. When *Kloeckera saturnus* was used, the C_3-(*S*)-alcohols **4c** and **4d** were obtained, whereas other yeasts such as *Lipomyces starkeyi, Saccharomyces fermentati,* and *Sporobolomyces salmonicolor* produced the isomeric C_3-(*R*)-alcohols **4a** and **4b**. On the other hand, when *Endomycopsis fibligera, Hansenula anomala,* and *Candida albicans* were used, the C_2-(*S*)-reduction products **4b** and **4c** were obtained.

Keywords——α-methyl β-keto ester; α-methyl β-hydroxy ester; asymmetric reduction; microbiological reduction; yeast

In the previous paper,[1] we reported that microbiological asymmetric reduction of the α-methyl β-keto ester **1** having a furan ring on the β-position gave the 3*R*-alcohols (**2a**+**2b**) or the 3*S*-alcohols (**2c**+**2d**) depending upon the microorganisms used, and the reduction products had high optical purities (**2a** >99% e.e., **2b** 98% e.e., **2c** 83% e.e., and **2d** 73% e.e.). Chromatographic separation of the *syn*[2]-isomer (**2a** or **2c**) and *anti*[2]-isomer (**2b** or **2d**) was found to proceed quite smoothly. Moreover, large-scale cultivation of **1** was possible.

These comounds, particularly the *syn*-isomer **2a**, should be useful building blocks for the synthesis of polyoxomacrolide antibiotics and related natural products.

We next investigated the effect of changing a furan ring to a thiophene ring on the chemical and optical yields, and the ratio of *syn*/*anti*-isomers in the above asymmetric reduction, because a thiophene ring is readily convertible to various functionalized groups.[3]

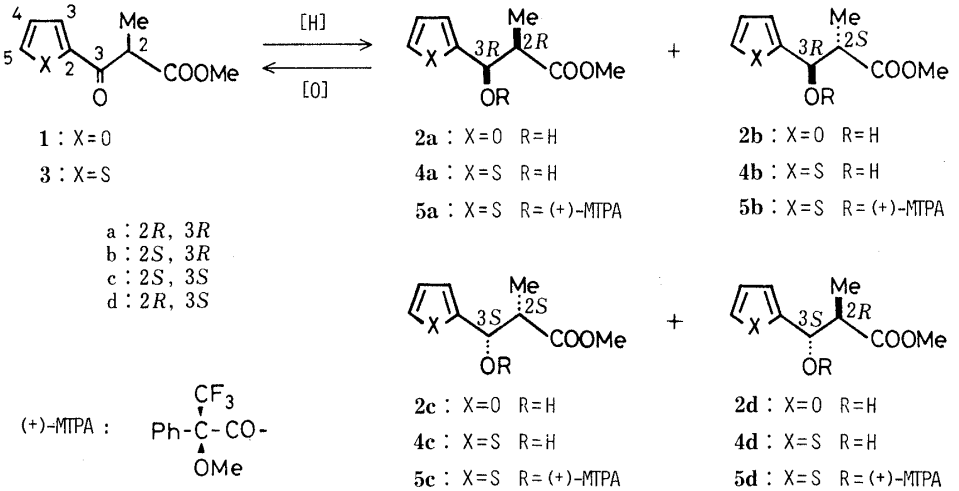
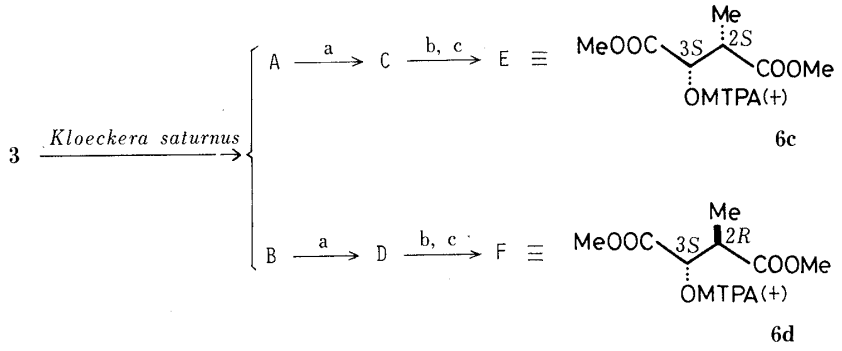


Chart 1

The present paper deals with an asymmetric reduction of methyl 2-methyl-3-(2-thienyl)-3-oxopropionate (**3**) with various yeasts, separation of the products, and determination of the stereostructure and optical purity of each product.

Reformatsky reaction of 2-thiophene carboxaldehyde and methyl bromoacetate in the presence of activated Zn in dry benzene quantitatively gave the β-hydroxy esters **4a**—**4d**, which were oxidized with pyridinium dichromate (PDC) in CH_2Cl_2 to give the starting β-keto ester **3** in 87% yield. Reduction of **3** with actively fermenting baker's yeast (*Saccharomyces cerevisiae*) did not proceed at all. However, it became apparent that microorganisms able to reduce the substrate **3** did exist when the preliminary screening method reported previously by us[1] was applied to the present case. In fact, various yeasts such as *Endomycopsis fibligera, Hansenula anomala, Kloeckera saturnus, Lipomyces starkeyi, Saccharomyces fermentati, Sporobolomyces salmonicolor,* and *Candida albicans* were found to be effective for the asymmetric reduction of **3**. Initially, reduction of **3** with *Kloeckera saturnus* cells, which can be obtained on a large scale,[4] was carried out and the alcohols produced were subjected to silica gel chromatography. In this case, also, the isomers were found to be separable by simple chromatography into two optically active alcohols A (236 mg; 17% yield, $[\alpha]_D^{27.5}$ −9.08° (*c*= 5.1, $CHCl_3$)) and B (298 mg; 21% yield, $[\alpha]_D^{27.5}$ −26.36° (*c*=5, $CHCl_3$)). For the purpose of determining the stereostructure and optical purity, A and B were separately converted to the corresponding (+)-α-methoxy-α-trifluoromethylphenylacetates[5] ((+)-MTPA esters) C and D, which were subjected to ozonolysis. Subsequent esterification with CH_2N_2 yielded the corresponding diesters E and F, respectively. The 400 MHz NMR spectra of E and F were found to be identical with those of the authentic (+)-MTPA esters **6c** (*syn*; 2*S*, 3*S*)[6] and **6d** (*anti*; 2*R*, 3*S*),[6] respectively, which reveals that the absolute configuration of E and hence A and C is 2*S*, 3*S* (therefore A=**4c**, C=**5c**) and that of F and hence B and D is 2*R*, 3*S* (therefore B=**4d**, D=**5d**).



a: (+)-MTPACl/pyridine b: 1) O_3/CH_2Cl_2, 2) 30% H_2O_2 c: CH_2N_2

Chart 2

Next, in order to prepare the four possible stereoisomers (**4a**—**4d**), the Reformatsky reaction products (**4a**—**4d**) were directly converted into the corresponding (+)-MTPA esters (**5a**—**5d**). The signals due to the four ester methyl protons appeared in distinctly different fields (δ 3.502, 3.571, 3.617, and 3.693) in the 400 MHz nuclear magnetic resonance (NMR) spectra without adding any shift reagent. The signals at δ 3.617 and 3.693 could be ascribed to **5c** (2*S*, 3*S*) and **5d** (2*R*, 3*S*) by direct comparison with those of the authentic specimens obtained above. Then, the β-keto ester **3** was reduced with $Zn(BH_4)_2$, which is known to give predominantly *syn*-isomers[7] **4a** and **4c**. The reduction products were converted to the (+)-MTPA esters as usual. Only two peaks appeared in the ester methyl region, as expected, and one of the peaks was identified as that of **5c**. Consequently the other peak (δ 3.571) could be

TABLE I

Entry	Microorganisms	Substrate **3** (mg)	Chemical yield (%) (as (+)-MTPA ester)	*syn*/*anti*	Optical yield (% e.e.)
1	*Kloeckera*[a] *saturnus*	1400	38[b]	44/56	*syn* **4c**: 85 *anti* **4d**: 79
2	*Endomycopsis fibligera*	46	8	82/18	*syn* **4c**: 93 *anti* **4b**: 24
3	*Hasenula anomala*	47	5	41/59	*syn* **4c**: 86 *anti* **4b**: 57
4	*Kloeckera saturnus*	46	29	49/51	*syn* **4c**: 88 *anti* **4d**: 61
5	*Lipomyces starkeyi*	51	16	68/32	*syn* **4a**: 69 *anti* **4b**: 49
6	*Saccharomyces fermentati*	48	5	26/74	*syn* **4a**: 86 *anti* **4b**: 96
7	*Sporobolomyces salmonicolor*	48	17	35/65	*syn* **4a**: 88 *anti* **4b**: 95
8	*Candida albicans*	50	4	26/74	*syn* **4c**: 61 *anti* **4b**: 92

a) Data obtained with yeast cells.
b) Isolated yield.

ascribed to the other *syn*-isomer **5a**. Thus, the remaining unassigned peak at δ 3.502 should be due to the other *anti*-isomer **5b**. The relation of the signals and the stereostructure was thus established. From the small peaks due to the isomers present in **5c** and **5d**, the optical yields of **5c** and **5d** were calculated as 85% e.e. and 79% e.e., respectively.

Now that we had a means to identify the reduction products, further screening experiments were undertaken using about 50 mg each of **3** and the yeasts which had been proved to be effective by the preliminary screening experiments. The chemical yield (as (+)-MTPA ester), optical purity (% e.e.) and the ratio of *syn*-/*anti*-isomers are summarized in Table I.

The main features of the present results are as follows. 1) When *Kloeckera saturnus* was used, the C_3-(*S*)-alcohols **4c** and **4d** were obtained, whereas other yeasts such as *Lipomyces starkeyi, Saccharomyces fermentati*, and *Sporobolomyces salmonicolor* produced the isomeric C_3-(*R*)-alcohols **4a** and **4b**. 2) When *Endomycopsis fibligera, Hansenula anomala*, and *Candida albicans* were used, alcohols having both absolute configurations, C_3-(*R*)-**4b** and C_3-(*S*)-**4c**, were obtained although the yields were very poor. 3) Both the chemical and optical yields were rather unsatisfactory compared with those in the asymmetric reduction of **1** having a furan ring. However, much better results are expected to be obtained by carrying out large-scale cultivation of **3**, as observed in the case of **1**.

Experimental

Melting points were measured with a Kofler micro melting point apparatus and are uncorrected. Infrared (IR) spectra (CCl_4) were measured on a JASCO A-3 spectrometer. NMR spectra were measured either on a JEOL FX-60 spectrometer or a JEOL FX-400 instrument. Spectra were taken in $CDCl_3$ with Me_4Si as an internal reference. Gas chromatography-mass spectroscopy (GC-MS) was carried out on a Hitachi RMU-6M mass spectrometer, and high-resolution mass spectra were taken with a Hitachi M-80 GC-MS spectrometer. $[\alpha]_D$ was measured on a Perkin-Elmer model 241 MC polarimeter.

Reformatsky Reaction of 2-Thiophenecarboxaldehyde and Methyl Bromoacetate——A mixture of 2-thiophenecarboxaldehyde (4.75 g), methyl bromoacetate (8.48 g), and activated Zn dust (prepared from Zn 4.15 g) in dry PhH (50 ml) was refluxed for 1 h with stirring. Reformatsky reaction products (**4a**—**4d**) were quantitatively obtained by the

usual work-up of the reaction mixture. IR ν_{max} cm^{-1}: 3500, 1720, 1735 (sh). *syn*; **4a**+**4c**, NMR δ: 3.697 (3H, s, COOMe), *anti*; **4d**+**4d**, NMR δ: 3.735 (3H, s, COOMe). The ratio of *syn*-(**4a**+**4c**)/*anti*-(**4b**+**4d**) was found to be 1:1.39 from the integrated intensities of the ester methyl signals in the 60 MHz NMR spectra.

Preparation of Methyl 2-Methyl-3-(2-thienyl)-3-oxopropionate (3)——A suspension of the above crude Reformatsky products (5 g) and pyridinium dichromate (29 g) in CH_2Cl_2 (50 ml) was stirred for 5 d at room temperature, then worked up as usual. The product was chromatographed on silica gel (200 g) to give a homogeneous oil **3** (4.329 g, 87% yield) from the *n*-hexane–ethyl acetate (19:1) eluate. *Anal.* High-resolution MS. Calcd for $C_9H_{10}O_3S$ (M^+; *m/e*): 198.035. Found: 198.031. IR ν_{max} cm^{-1}: 1670, 1740. NMR δ: 1.512 (3H, d, J=7.1 Hz, 2-Me), 3.706 (3H, s, COOMe), 4.262 (1H, q, J=7.1 Hz, 3-H), 7.144 (1H, dd, $J_{4',5'}$=5.1 Hz, $J_{3',4'}$=3.7 Hz, 4′-H), 7.694 (1H, dd, $J_{4',5'}$=5.1 Hz, $J_{3',5'}$=1.1 Hz, 5′-H), 7.785 (1H, dd, $J_{3',4'}$=3.7 Hz, $J_{3',5'}$=1.1 Hz, 3′-H).

Asymmetric Reduction of 3 with *Kloeckera saturnus*——A suspension of *Kloeckera saturnus* cells[4] (100 g), sucrose (100 g) and the substrate **3** (700 mg) in H_2O (400 ml) was shaken at 30 °C for 45 h. The asymmetric reduction of **3** (finally 1.4 g of **3**) was carried out twice on the same scale. The reaction mixture was worked up and purified in the same way as reported previously[1] to afford two fractions on silica gel chromatography eluted with *n*-hexane–ethyl acetate (9:1) eluate. The first product A (236 mg, 17% yield) and the second product B (298 mg, 21% yield) were each a homogeneous oil. A: *Anal.* High-resolution MS. Calcd for $C_9H_{12}O_3S$ (M^+; *m/e*): 200.051. Found: 200.050. $[\alpha]_D^{27.5}$ −9.08° (*c*=5.1, $CHCl_3$). IR ν_{max} cm^{-1}: 3520, 1720, 1735 (sh). 400 MHz NMR δ: 1.238 (3H, d, J=7.1 Hz, 2-Me), 2.891 (1H, qq, J=7.1 Hz, $J_{2,3}$=4.4 Hz, 2-H), 3.075 (1H, d, J=4.4 Hz, 3-OH), 3.700 (3H, s, COOMe), 5.308 (1H, t, J=4.4 Hz, $J_{2,3}$=4.4 Hz, 3-H), 6.952 (1H, dd, $J_{3',5'}$=1.5 Hz, $J_{3',4'}$=3.9 Hz, 3′-H), 6.976 (1H, dd, $J_{3',4'}$=3.9 Hz, $J_{4',5'}$=4.9 Hz, 4′-H), 7.244 (1H, dd, $J_{3',5'}$=1.5 Hz, $J_{4',5'}$=4.9 Hz, 5′-H). B: A part of B was converted to the corresponding 3,5-dinitrobenzoate, which was recrystallized from EtOH to give pale yellow needles. mp 92.5—93 °C. *Anal.* Calcd for $C_{16}H_{14}N_2O_8S$: C, 48.73; H, 3.58; N, 7.10; S, 8.13. Found: C, 48.57; H, 3.64; N, 7.02; S, 8.10. B: $[\alpha]_D^{27.5}$ −26.36° (*c*=5, $CHCl_3$). IR ν_{max} cm^{-1}: 3500,1720, 1735 (sh). 400 MHz NMR δ: 1.121 (3H, d, J=7.3 Hz, 2-Me), 2.886 (1H, qq, J=7.3 Hz, $J_{2,3}$=7.9 Hz, 2-H), 3.173 (1H, d, J=5.1 Hz, 3-OH), 3.740 (3H, s, COOMe), 5.029 (1H, dd, $J_{2,3}$= 7.9 Hz, J=5.1 Hz, 3-H), 6.970 (1H, dd, $J_{3',4'}$=3.5 Hz, $J_{4',5'}$=4.9 Hz, 4′-H), 6.997 (1H, dd, $J_{3',4'}$=3.5 Hz, $J_{3',5'}$= 1.2 Hz, 3′-H), 7.282 (1H, dd, $J_{3',5'}$=1.2 Hz, $J_{4',5'}$=4.9 Hz, 5′-H).

Preparation of the (2*S*, 3*S*)-(+)-MTPA Ester C and Conversion of C into E by Ozonolysis——i) Pyridine (0.3 ml) was added to a mixture of A (55 mg) and (+)-MTPACl[5] (83 mg), and the reaction mixture was stirred for 137 h at room temperature. The reaction mixture was worked up and purified in the same way as reported previously[1] to provide the (2*S*, 3*S*)-(+)-MTPA ester C (83 mg, 73% yield) as a homogeneous oil. 400 MHz NMR δ: 1.323 (3H, d, J=7.1 Hz, 2-Me), 3.617 (3H, s, COOMe), 6.520 (1H, d, $J_{2,3}$=7.1 Hz, 3-H). The optical purity of C and hence A was found to be 85% e.e.

ii) Ozone was passed through a solution of C (73 mg) in CH_2Cl_2 (6 ml) under dry ice–acetone cooling for 30 min, then 30% H_2O_2 aq. (2 ml) was added to the ozonolyzed poduct and the reaction mixture was stirred for 20 min at room temperature. The reaction mixture was treated in the same way as reported previously[1] to afford the dimethyl ester E (9 mg). The 400 MHz NMR spectrum of E was identical with that of the authentic (2*S*, 3*S*)-(+)-MTPA ester **6c**.[6]

Preparation of the (2*R*, 3*S*)-(+)-MTPA Ester D and Conversion of D into F by Ozonolysis——i) (+)-MTPA esterification of B (54 mg) afforded D (103 mg, 91% yield) as a homogeneous oil in the same way as in the case of C. 400 MHz NMR δ: 1.075 (3H, d, J=7.3 Hz, 2-Me), 3.693 (3H, s, COOMe), 6.358 (1H, d, $J_{2,3}$=10.4 Hz, 3-H). The optical purity of D and hence B was found to be 79% e.e.

ii) Ozonolysis and subsequent esterification of D (93 mg) provided the dimethyl ester F (35 mg) in the same way as in the case of E. The 400 MHz NMR spectrum of F was identical with that of the authentic (2*R*, 3*S*)-(+)-MTPA ester **6d**.[6]

Preparation of Four (+)-MTPA Esters (5a+5b+5c+5d)——(+)-MTPA esterification of the Reformatsky products (**4a**+**4b**+**4c**+**4d**; 156 mg) gave a mixture of four (+)-MTPA esters (**5a**+**5b**+**5c**+**5d**; 281 mg, 87% yield). **5a**+**5c**: 400 MHz NMR δ: 1.179, 1.323 (each 3H, d, J=7.1 Hz, 2-Me), 3.571, 3.617 (each 3H, s, COOMe), 6.494 (1H, d, $J_{2,3}$=8.3 Hz, 3-H), 6.520 (1H, d, $J_{2,3}$=7.1 Hz, 3-H). **5b**+**5d**: 400 MHz NMR δ: 1.054, 1.075 (each 3H, d, J= 7.1 Hz, 2-Me), 3.502, 3.693 (each 3H, s, COOMe), 6.358 (1H, d, $J_{2,3}$=10.4 Hz, 3-H), 6.394 (1H, d, $J_{2,3}$=10.7 Hz, 3-H).

Preparation of Racemic *syn*-Alcohols (4a+4c) and Their (+)-MTPA Esters (5a+5c)——i) $Zn(BH_4)_2$ reduction of **3** (145 mg) gave the *syn*-alcohols (**4a**+**4c**; 140 mg, 96% yield) as a homogeneous oil in the same way as reported previously[1] The ratio of *syn*-(**4a**+**4c**)/*anti*-(**4b**+**4d**) was found to be 52.6:1 by comparison of the integrated intensities of ester methyl signals in the 400 MHz NMR spectra.

ii) (+)-MTPA esterification of the above $Zn(BH_4)_2$ reduction products (27 mg) afforded the *syn*-(+)-MTPA esters (**5a**+**5c**; 39 mg, 69% yield) as a homogeneous oil in the same way as in the case of C. *Anal.* High-resolution MS. Calcd for $C_{19}H_{19}F_3O_5S$ (M^+; *m/e*): 416.090. Found: 416.090. 400 MHz NMR δ: 1.179, 1.323 (each 3H, d, J=7.1 Hz, 2-Me), 3.571, 3.617 (each 3H, s, COOMe), 6.494 (1H, d, $J_{2,3}$=8.3 Hz, 3-H), 6.520 (1H, d, $J_{2,3}$=7.1 Hz, 3-H).

Screening Experiments with Various Yeasts——Screening was carried out in the same way as reported previously,[1] and the results are summarized in Table I.

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References and Notes

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