Note

Selection of Microorganisms
Producing Two Chiral CentersKoki HORIKOSHI, Akiya FURUICHI,*
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Optically active building blocks are frequently used in the synthesis of antibiotics and other biologically active materials.

Although many biological formations of one chiral center have been investigated,¹⁾ no information on two chiral center formation has been reported. The furylpropionates (Fig. 1, compounds 2 to 5) involving two chiral centers were selected because the 2-methyl, 3-hydroxy moiety in them appears in many biologically active substances, such as erythromycins,²⁾ oudemansin³⁾ and other polyoxyantibiotics.⁴⁾

A ketone compound, 2-methyl-3-(2-furyl)-3-oxopropionic acid methyl ester (compound 1), was used as a starting material to make two chiral centers in the derivatives.

The microorganisms in Table I were used to reduce the starting material. Test tubes (25 mm × 200 mm) containing 10 ml of the culture medium comprising 5% glucose, 0.1% KH_2PO_4 , 0.1% $(\text{NH}_4)_2\text{SO}_4$, 0.05% urea, 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% yeast extract and tap water (pH 7.0) were inoculated with microorganisms and cultured at 30°C for 3 days with continuous shaking. Then the substrate (5 mg of compound 1) was added to the test tube and incubated further for 3 days under the same conditions. The mixture was treated with ethyl acetate to extract the

TABLE I. LIST OF MICROORGANISMS USED

KSY-1	<i>Candida pseudotropicalis</i> IFO-0617
KSY-2	<i>Candida tropicalis</i> IFO-0587
KSY-3	<i>Endomyces magnusii</i> CCY 42-1-1
KSY-4	<i>Endomycopsis fibuligera</i> NI-7404
KSY-5	<i>Hansenula anomala</i> Jyozo Kyokai 2019
KSY-6	<i>Hansenula anomala</i> NI-7572
KSY-7	<i>Kloeckera saturnus</i>
KSY-8	<i>Lipomyces starkeyi</i> IFO-0678
KSY-9	<i>Pichia farinosa</i>
KSY-10	<i>Pichia membranaefaciens</i>
KSY-11	<i>Rhodotorula glutinis</i> IFO-0695
KSY-12	<i>Saccharomyces acidifaciens</i>
KSY-13	<i>Saccharomyces carlsbergensis</i> Jyozo Kyokai 1008
KSY-14	<i>Saccharomyces cerevisiae</i> var. <i>ellipsoideus</i> Jyozo Kyokai 2011
KSY-15	<i>Saccharomyces delbrueckii</i>
KSY-16	<i>Saccharomyces fermentati</i> IFO-0422
KSY-17	<i>Saccharomyces fragilis</i> NI-7645
KSY-18	<i>Saccharomyces pastorianus</i>
KSY-19	<i>Saccharomyces steineri</i> NI-7655
KSY-20	<i>Saccharomyces tokyo</i> Jyozo Kyokai 2013
KSY-21	<i>Saccharomyces</i> sp.
KSY-22	<i>Saccharomyces</i> sp.
KSY-23	<i>Saccharomyces</i> sp.
KSY-24	<i>Schizosaccharomyces octosporus</i>
KSY-25	<i>Schizosaccharomyces pombe</i> CCY 44-1-3
KSY-26	<i>Sporobolomyces salmonicolor</i> IFO-1038
KSY-27	<i>Torulopsis famata</i> NI-7550
KSY-28	<i>Torulopsis</i> sp. Jyozo Kyokai 17
KSY-29	<i>Saccharomyces cerevisiae</i> Kitasato Inst.
KSY-30	<i>Candida guilliermondii</i> IFO-0961
KSY-31	<i>Candida humicola</i> CCY 29-11-1
KSY-32	<i>Candida guilliermondii</i>
KSY-33	<i>Rhodotorula rubra</i> CCY 20-7-1
KSY-34	<i>Rhodotorula minuta</i> IFO-0387
KSY-35	<i>Trichosporon fermentans</i> IFO-1199
KSY-36	<i>Candida albicans</i> IFO-1060
KSY-37	<i>Candida albicans</i> YU-1200
KSY-38	<i>Candida utilis</i> IFO-0619
KSY-39	<i>Saccharomyces cerevisiae</i> 776
KSY-40	<i>Saccharomyces cerevisiae</i> (Baker's yeast)

reduced compound. The extract was dried with anhydrous MgSO_4 and concentrated *in vacuo*. The residues were chromatographed on silica gel plates (Merck, Kieselgel 60 F₂₅₄) with a solvent system of hexane-ethyl acetate (4:1, v/v) at room temperature, and detected with 10% (w/v) phosphomolybdic acid in aqueous EtOH solution.

The following microorganisms were found to reduce the starting material (compound 1): *Kloeckera saturnus* KSY-

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TABLE II. RESULT OF MICROBIOLOGICAL REDUCTION OF **1**

Microorganisms	Substrate (mg)	Chemical yield (%) (as (+)MTPA ester)	Ratio of <i>syn/anti</i>	Optical purity (% ee)
<i>Kloeckera saturnus</i> KSY-7	60	29	47/53	<i>syn</i> 2 68 <i>anti</i> 3 46
<i>Lipomyces starkeyi</i> KSY-8	49	4	47/53	<i>syn</i> 4 49 <i>anti</i> 5 23
<i>Saccharomyces delbrueckii</i> KSY-15	57	43	39/61	<i>syn</i> 4 90 <i>anti</i> 5 91
<i>Saccharomyces fermentati</i> KSY-16	45	21	40/60	<i>syn</i> 4 84 <i>anti</i> 5 86
<i>Candida humicola</i> KSY-31	58	28	36/64	<i>syn</i> 4 70 <i>anti</i> 5 65
<i>Candida guilliermondii</i> KSY-32	50	53	38/52	<i>syn</i> 4 89 <i>anti</i> 5 67
<i>Candida albicans</i> KSY-36	46	23	0/>99	<i>anti</i> 5 >99

7, *Lipomyces starkeyi* KSY-8, *Saccharomyces delbrueckii* KSY-15, *Saccharomyces fermentati* KSY-16, *Candida humicola* KSY-31, *Candida guilliermondii* KSY-32 and *Candida albicans* KSY-36. Then, reduction with these effective microorganisms using 40~60 mg of **1** was carried out under essentially the same conditions as noted above.

For the purpose of determining the stereostructure and optical purity, the reduced products were condensed with (+)- α -methoxy- α -trifluoromethyl-phenylacetic acid chloride ((+)-MTPACl)⁵ and the resulting esters were ozonolyzed and then esterified with diazomethane to yield the corresponding esters. The esters thus obtained were analyzed with a JEOL NMR (400 MHz) and their stereostructure, optical purity and the *syn*-isomer to *anti*-isomer ratio were determined by the methods described previously.⁶

The results are summarized in Table II. Usually, a mixture of 2*R*,3*R*-ester **4** and 2*S*,3*R*-ester **5** was obtained. This was readily separable by column chromatography. It is striking that the optical purities are very high and that *Candida albicans* KSY-36 produced only one compound (compound **4**) having 99% optical purity. In sharp contrast with these experiments, when *Kloeckera saturnus* KSY-7 was used, 2*S*,3*S*-ester **2** and 2*R*,3*S*-ester **3** having opposite absolute configurations to **4** and **5**, respectively, were obtained in high chemical yields with high optical purities.

In preliminary experiments using a jar fermentor (30 liters), these microorganisms reduced compound **1** and produced the corresponding reduced compounds having

two chiral centers in the same yields as those of small scale experiments. Further details on large scale production and enzymatic studies of the reduction process will be reported elsewhere.

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