Microbiologically Modified Chiral Synthon. I. 3,8-Dioxo-4-methoxycarbonyl-9-methyl-$\Delta^{4(10)}$-octalin[1] for Formal Total Syntheses of Certain Sesquiterpenoids and Diterpenoids

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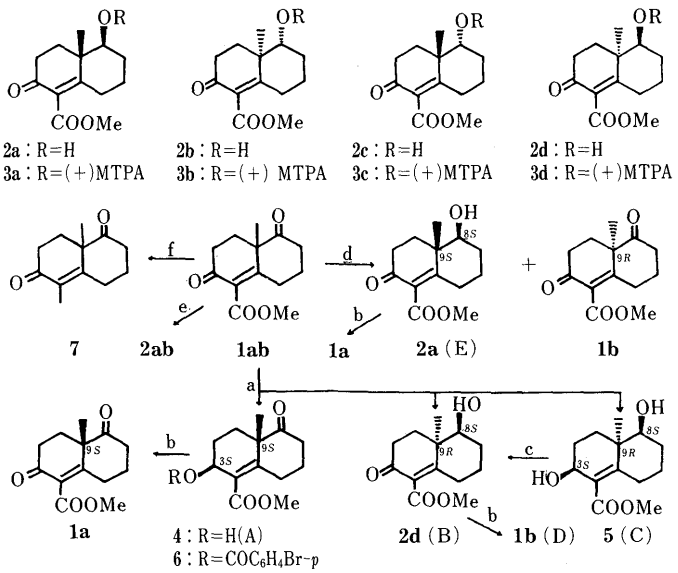
Microbiological reduction of prochiral 3,8-dioxo-4-methoxycarbonyl-9-methyl-$\Delta^{4(10)}$-octalin (1ab) with various yeasts was carried out. (+)-(8*S*)-Hydroxy-4-methoxycarbonyl-(9*S*)-methyl-3-oxo-$\Delta^{4(10)}$-octalin (2a) was desired as a chiral synthon for the formal total syntheses of certain sesquiterpenoids, *e.g.* α-costal. This ketol was obtained with high optical purity (>99% ee) when properly selected microorganisms such as *Hansenula anomala* were used. Another chiral synthon produced in high optical purity, that is, 3,8-dioxo-4-methoxycarbonyl-(9*R*)-methyl-$\Delta^{4(10)}$-octalin (1b), was also available for the formal total syntheses of *ent*-type diterpenoids *e.g.* zonarol.

Keywords 3,8-dioxo-(9*R*)-methyloctalin; (8*S*)-hydroxy-(9*S*)-methyl-3-oxooctalin; (8*S*)-hydroxy-(9*R*)-methyl-3-oxooctalin; microbiological reduction; asymmetric induction; yeast; *Hansenula anomala*; chiral synthon; sesquiterpenoid; diterpenoid

Microorganisms can be considered as microscopic reaction vessels containing numerous enzymes complete with cofactors that can potentially react with unnatural substrates and thus provide asymmetric transformations that may prove useful for synthetic applications. Asymmetric microbial reductions of carbonyl groups by various yeasts have become a useful method to obtain optically active secondary hydroxy compounds. The ability of certain enzymes to differentiate stereoheterotopic faces of a trigonal atom, such as the carbon of the carbonyl function or to distinguish two enantiotopic homomorphic groups attached to a prochiral center, can lead to asymmetric transformations resulting in enantiomerically pure products from an achiral substrate. Prochiral 4-methoxycarbonyloctalin offers the opportunity for both types of distinctions since its molecule has two trigonal carbonyl centers with stereoheterotopic faces and one prochiral tetrahedral carbon center at C(9). The enzyme-catalyzed asymmetric reduction of this type of substrate generates two or three chiral centers [C(3), C(8), C(9)], as shown in Chart 1.



a; *Kloeckera saturnus* b; Jones reagent c; MnO_2 d; *Hansenula anomala* e; $NaBH_4$ f; 1) *p*-TsOH/$(CH_2OH)_2$ 2) $LiAlH_4$ 3) CBr_4/Ph_3P 4) Bu_3SnH

Chart 1

Numerous examples[2] are known of the biological reduction of acyclic ketones using microorganisms such as yeast. There are a few reports[3] on bicyclic ketones, but an optically active bicyclic diketone (**1a** and/or **1b**) as a key synthetic intermediate has not yet been synthesized,[4a] even by the conventional asymmetric cyclization using amino acid derivatives.[4b] The optically active hydroxyoctalin (**2a**) and/or dioxooctalin (**1b**) (*ent*-type) to be derived from the corresponding dioxooctalin (**1ab**) is considered to be an essential chiral intermediate for the synthesis of optically active sesquiterpenoids and/or diterpenoids such as α-costal[5] and zonarol.[6] The present paper describes a detailed account of the work briefly reported in our communication[1] on the syntheses of either **2a** or **1b** based on the asymmetric reduction of **1ab** by a variety of yeasts.[7]

Reduction of the diketone (**1ab**) with usual fermenting baker's yeast (*Saccharomyces cerevisiae*) resulted in the formation of an inseparable mixture of products. However, the reduction using some selected yeasts, especially *Kloeckera saturnus*, was found to produce three alcohols, A ($[\alpha]_D$ +92.3°), B ($[\alpha]_D$ −77.4°) and C ($[\alpha]_D$ −168.3°), in 44%, 18% and 12% yields, respectively. The absolute structure of the main product A was determined to be as shown in Fig. 1 by the X-ray analysis of its *p*-bromobenzoate (**6**) (see Experimental).

The absolute configuration of the alcohol A was thus established to be 3*S*, 9*S* (A=**4**). The second alcohol B was oxidized with Jones reagent to provide the diketone D ($[\alpha]_D$ −85.0°), which was identical with the (9*S*)-diketone (**1a**) ($[\alpha]_D$ +62.3°) obtained by Jones oxidation of (3*S*,9*S*)-A (=**4**). Since the sign of the rotation in D was opposite to that of **1a**, the absolute configuration of D was found to be 9*R* (hence D=**1b**). The stereochemistry of C(8)-H in B was found to be equatorial, because the proton nuclear magnetic resonance (^{1}H-NMR, 400 MHz) signal due to C(8)-H appeared at δ3.679 (br s $W_{1/2}$=7.1 Hz). Therefore, the absolute configuration of B was determined to be 8*S*, 9*R* (hence B=**2d**). The manganese dioxide (MnO_2) oxidation product ($[\alpha]_D$ −105.0°) of the third alcohol C was identical with that of B mentioned above, and the α-configuration of C(3)-H was determined by ^{1}H-NMR. Thus, the absolute structure of C was found to be (3*S*,8*S*)-dihydroxy-(9*R*)-octalin (**5**). Its optical purity of >99% ee was determined

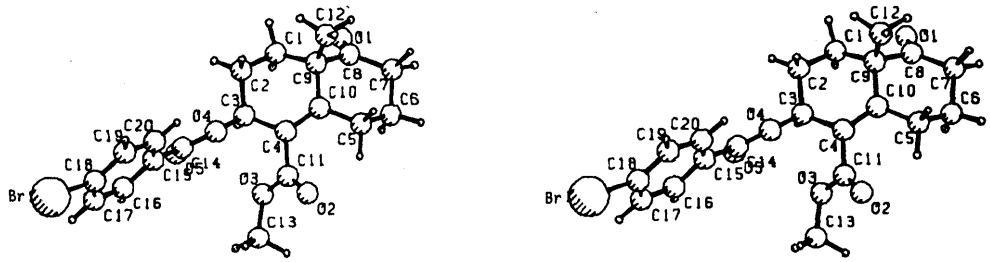


Fig. 1. Stereoview of the *p*-Bromobenzoate (**6**) of (3*S*)-Hydroxy-4-methoxycarbonyl-(9*S*)-methyl-8-oxo-$\Delta^{4(10)}$-octalin (**4**)

by comparison of the rotation of the (9*R*, 8*S*)-*cis*-ketol (**2d**) with that of an authentic sample of the above-mentioned **2d** (B). For the purpose of determining the optical purity of B, B was treated with (+)-α-methoxy-α-trifluoromethyl-phenyl-acetic acid chloride [(+)-MTPACl],[8] to give the corresponding (+)-MTPA ester (**3d**). Accordingly, the optical purity of **2d** (B) was found to be 88% ee by taking account of the height ratio of the major methoxy methyl signal of **3d** (δ3.512) to that of the minor one (δ3.480) of its enantiomer (**3c**) in the 400 MHz ^{1}H-NMR spectrum taken without any shift reagent.

With the intention of finding more selective microorganisms, we executed the reduction with a variety of yeasts and found that several yeasts actually possess the desired reduction ability. *Hansenula anomala* catalyzed asymmetric reduction of **1ab** and provided predominantly the fourth alcohol E ($[\alpha]_D$ +105.2°, δ 1.242, s, 9-CH_3) in 20% yield together with the antipodal diketone (**1b**) ($[\alpha]_D$ −13.7°) in 58% yield. The absolute structure and the optical purity of E were determined as follows. Sodium borohydride ($NaBH_4$) reduction of **1ab** gave a racemic *cis*-alcohol[9] (**2ab**), which was identical with the present reduction product E except for the rotations. Since the Jones oxidation product ($[\alpha]_D$ +119.0°) of E was identical with the (9*S*)-diketone (**1a**), including the sign of the rotation, the absolute structure of E was determined to be 8*S*, 9*S* (hence E=**2a**). For the sake of determining the optical purity of E, E and the racemate (**2ab**) were also converted as usual to the corresponding (+)-MTPA esters **3a** and **3ab**, respectively. In the case of the racemate (+)-MTPA ester (**3a** and **3b**), two ^{1}H-NMR peaks due to each angular methyl group appeared with distinctly different chemical shifts (δ 1.257 and δ 1.249). The former signal was coincident with that of **3a**, and the latter was consequently ascribed to (8*R*, 9*R*)-**3b**. Consequently, the optical purity of E was found to be more than 99% ee.

Since the absolute configuration and the optical purity of the (9*S*,8*S*)-*cis*-alcohol (**2a**) (E) were thus determined unambiguously as mentioned above, those of the (9*S*,3*S*)-*cis*-alcohol (**4**) (A) were defined on the basis of the comparison of the (9*S*)-diketone (**1a**) derived by Jones oxidation of **4** with the authentic sample of **1a** prepared from the aforegoing **2a** (E).

The asymmetric reduction of **1ab** catalyzed by *Hansenula anomala* and *Saccharomyces acidifaciens* resulted in recovery of **1b** in 58% and 75% chemical yields with 9.3% and 20.0% optical purity, respectively. The further biological reduction of the above-mentioned product of low optical purity led exclusively to the *cis*-ketol (**2a**) (>99% ee) as before and the highly optically pure diketone[10] (**1b**) (>99% ee) in 64% and 58% yields, respectively.

Thus we have established a relationship between the absolute structure and the chemical shift; the results of the asymmetric reduction of **1ab** using various yeasts are summerized in Table I.

Table I. Microbiological Reduction of 3,8-Dioxo-4-methoxycarbonyl-9-methyl-$\Delta^{4(10)}$-octalin (**1ab**)

Entry	Yeast	Chemical yield (%)	Recovery (%) as **1b**	*cis*/*trans*[a]	Optical purity (%)
1	*Hansenula anomala*	20	58	100/0	**2a** >99
2	*Hansenula anomala* NI-7572	11	74	100/0	**2a** >99
3	*Saccharomyces acidifaciens*	10	75	100/0	**2a** >99
4	*Schizosaccharomyces octosporus*	12	50	100/0	**2a** >99
5	*Kloeckera saturnus*	18	Trace	0/100	**2d** 88
6	*Candida albicans*	12	87	0/100	**2c** 44
7	*Saccharomyces cerevisiae*[b]	40	60	77/23	**2a** >99; **2d** 6
8	*Pichia membranaefaciens*	7	78	17/83	**2a** >99; **2d** >99
9	*Candida guilliermondii*	12	—	22/78	**2a** >99; **2d** 25
10	$NaBH_4$	70	—	100/0	

a) Ratios determined from the ^{1}H-NMR spectrum. *b*) Baker's yeast.

The particular features of the present asymmetric reduction are as follows. 1) The asymmetric reductions mediated by *Hansenula anomala*, *Hansenula anomala* NI-7522, *Saccharomyces acidifaciens*, and *Schizosaccharomyces octosporus*, afforded the *cis*-ketol having more than 99% ee in every case. 2) On the other hand, the asymmetric reduction catalyzed by *Kloeckera saturnus* and *Candida albicans* gave the *trans*-ketol (**2c** or **2d**) in moderate optical purity. In this case, the absolute structure of each reduction product showed the enantio relationship. 3) In the case of asymmetric reduction using baker's yeast, *Pichia membranaefaciens* and *Candida guilliermondii*, an inseparable mixture of the *cis*-ketol (**2a**) and the *trans* isomer (**2d**) was obtained, but the optical purity of the former was considerably higher in every case. 4) In all the cases, the recovered diketone (**1b**) corresponding to the starting substrate was found to be optically active and to have a (9*R*)-configuration. 5) The diketone (**1b**) that remained after in the treatment with *Hansenula anomala* and *Saccharomyces acidifaciens* was further reduced by the same yeasts yielding the optically pure diketone (**1b**) (>99% ee) and the ketol (**2a**) (>99% ee). These results prove that the diketone (**1ab**) was kinetically resolved by the yeasts.

Since the conversion of the racemic diketoester (**1ab**) to the 4-methyldiketone (**7**) was successfully achieved by the

sequential reactions, both optically active forms of **7** can be synthesized transformed from the aforegoing optically active precursors (**2a** or **1a**, and **1b**).

The optically active key intermediates (**1a** or **1b**) will be available for the syntheses of various optically active natural products, such as sesquiterpenes, *e.g.* α-costal[5] and warburgnal[11] (from **1a**), and diterpenes, *e.g.* zonarol[6] and andrographolide[9] (from **1b**).

Experimental

Melting points were measured with a Kofler micro-melting point apparatus and are uncorrected. Infrared (IR) spectra ($CHCl_3$) were measured on a JASCO A-3 spectrophotometer. All the 400 MHz ^{1}H-NMR spectra were determined on a JEOL FX 400 instrument in 5—10% w/v solutions of $CDCl_3$ with tetramethylsilane as an internal reference. Both gas chromatography-mass (GC-MS) and high-resolution mass (MS) spectrometries were carried out on a JEOL JMS-D-300 (JMA-200 data analysis system) mass spectrometer. Optical rotations were measured on a Perkin-Elmer model 241 MC polarimeter in $CHCl_3$ solution unless otherwise stated.

Asymmetric Reduction of 3,8-Dioxo-4-methoxycarbonyl-$\Delta^{4(10)}$-octalin (1ab) with *Kloeckera saturnus* i) A test tube (200 × 25 mm) containing 10 ml of culture medium comprising 5% glucose, 0.1% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.05% urea, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, 0.1% yeast extract, 0.02 ml of the mineral solution (0.1% $FeSO_4 \cdot 7H_2O$, 0.1% $MnCl_2 \cdot 4H_2O$, and 0.1% $ZnSO_4 \cdot 7H_2O$), and tap water (pH 6.5) was incubated with *Kloeckera saturnus*. The yeast was cultured at 30 °C for 3 d with continuous shaking. Then 1 ml of the seed culture was transferred to 800 ml of the same medium. After cultivation for 3 d, 400 mg of the substrate **1ab** was added, and cultivation was continued for an additional 3 d under the same cinditions. ii) The reaction mixture was filtered with the aid of celite and the filtrate was extracted with ethyl acetate. The ethyl acetate extract was dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (15 g) to give the reduction products (A, 175.6 mg, 44% yield; B, 70.4 mg, 18% yield; and C, 50.1 mg, 12% yield) from the *n*-hexane–ethyl acetate (9 : 1) eluate. A: mp 100—101 °C. MS Calcd for $C_{13}H_{18}O_4$ (M^+): 238.118, Found: 238.119. $[\alpha]_D^{26}$ +92.3° (*c* = 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 3320, 1712, 1620. ^{1}H-NMR δ: 1.405 (3H, s, 9-CH_3), 3.803 (3H, s, –$COOCH_3$), 4.444 (1H, t, *J* = 6.4 Hz, >CH(OH)). B: mp 159—160 °C. MS Calcd for $C_{13}H_{18}O_4$ (M^+): 238.118, Found: 238.119. $[\alpha]_D^{26}$ −77.4° (*c* = 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1731, 1667, 1618. ^{1}H-NMR δ: 1.279 (3H, s, 9-CH_3), 3.679 (1H, t, *J* = 5 Hz, >CH(OH)), 3.814 (3H, s, –$COOCH_3$). C: mp 99—101 °C. MS Calcd for $C_{13}H_{18}O_3$ (M^+-H_2O): 222.125, Found: 222.125. $[\alpha]_D^{26}$ −168.3° (*c* = 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3340, 1709, 1630. ^{1}H-NMR δ: 1.113 (3H, s, 9-CH_3), 3.610 (1H, t, *J* = 2 Hz, 3-CH_3), 3.775 (3H, s, –$COOCH_3$), 4.367 (1H, s, 4-CH_3).

Preparation of (3*S*)-Benzoate (6) from (3*S*)-Hydroxy-4-methoxycarbonyl-(9*S*)-methyl-8-oxo-$\Delta^{4(10)}$-octalin (4) Pyridine (0.5 ml) was added to a mixture of the ketol A (21.4 mg), *p*-bromobenzoyl chloride (30 mg) and dimethylaminopyridine (DAMP) (10 mg). Then the reaction mixture was stirred for 30 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and concentrated to give crude **6**. This was subjected to preparative thin layer chromatography (TLC) (*n*-hexane–ethyl acetate (1 : 1)) to provide the *p*-bromobenzoate (**6**) (10.9 mg, 29% yield); it was crystallized from hexane. $[\alpha]_D^{24}$ +14.6° (*c* = 1.09, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772, 1717, 1682, 1620, 1590.

Crystal Data of (3*S*)-*p*-Bromobenzoyl-4-methoxycarbonyl-(9*S*)-methyl-8-oxo-$\Delta^{4(10)}$-octalin (6) $C_{20}H_{21}BrO_5$, MW 421.3, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $D_c = 1446$ g cm^{-3}, $a = 10.525(5)$, $b = 19.267(10)$, $c = 9.548(5)$ Å, $U = 1936$ Å^3, for CuK_α = 31.3 cm^{-1}.

Crystallographic Measurements of 6 Crystals were obtained from ethyl acetate. Cell dimensions and intensity data were measured on a Philips PW 1100 diffractometer using CuK_α radiation monochromated by a graphite plate. A total of 2130 reflections were observed as above the $2\sigma(I)$ level, corresponding to about 80% of the possible number of reflections within the 2 θ range from 6° to 156°. Since a small and globular specimen of about 0.08 mm in diameter was chosen for X-ray measurements, no absorption corrections were applied for intensity data. The disagreement factor *R* for 358 Friedel pairs of $|F|$observed for *hkl* and *hk*2 reflections was 4.96%.

Absolute Structure of 6 The structure was determined by the heavy atom method and refined to an *R* value of 0.068 by the block-diagonal least-squares method. All the hydrogen atoms were included with isotropic temperature factors. Absolute configuration was determined by the anomalous dispersion method using the dispersion effect of the bromine atom for CuK_α radiation. The observed and calculated intensity ratios of the Friedel pair reflections (r_{obs} and r_{calc}, where $r_{obs} = |F(\bar{h}\bar{k}\bar{l})_{obs}|/|F(hkl)_{obs}|$ and $r_{calc} = |F(\bar{h}\bar{k}\bar{l})_{calc}|/|F(hkl)_{calc}|$), were compared for those reflections which have the intensity difference between *hkl* and $\bar{h}\bar{k}\bar{l}$ exceeding $4\sigma(I)$ and both r_{obs} and r_{calc} of which differ by more than 5% from 1. Out of the total of 82 pairs, which satisfy the above conditions, 74 pairs clearly showed a good agreement of r_{obs} with r_{calc} and the absolute configuration was determined to be S at C(9) as shown in Fig. 1.

Preparation of (+)-MTPA Ester (3d) Pyridine (0.3 ml) was added to B (20 mg), (+)-MTPACl (31.8 mg) and DAMP (10 mg), and the reaction mixture was stirred for 17 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and concentrated to give an oil; this was subjected to preparative TLC (silica gel, 20 × 20 cm, solvent, *n*-hexane–ethyl acetate (1 : 1)) to provide the (+)-MTPA ester (**3d**, 21.1 mg, 55% yield). ^{1}H-NMR δ: 1.342 (3H, s, 9-CH_3), 3.509, 3.480 (each 3H, *J* = 0.98 Hz, –OCH_3), 3.802 (3H, s, –$OCOCH_3$), 5.030 (1H, br s, >CHOMTPA). The optical purity of **3d** (and hence that of B) was found to be 88% ee.

Conversion of A into 3,8-Dioxo-4-methoxycarbonyl-(9*S*)-methyl-$\Delta^{4(10)}$-octalin (1a) Jones reagent (2 drops) was added to a stirred solution of A (30 mg) in acetone (8 ml), this mixture was cooled in an ice-salt bath for 7 min. After the addition of isopropyl alcohol and $NaHCO_3$, the reaction mixture was filtered and concentrated to an oil; this was subjected to TLC (silica gel, 20 × 20 cm; solvent, *n*-hexane–ethyl acetate (1 : 1)) to provide the diketone (**1a**). $[\alpha]_D^{26}$ +62.3° (*c* = 1.22, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1675, 1620. ^{1}H-NMR δ: 1.493 (3H, s, 9-CH_3), 3.828 (3H, s, –$COOCH_3$).

Conversion of C into (8*S*)-Hydroxy-4-methoxycarbonyl-(9*R*)-methyl-3-oxo-$\Delta^{4(10)}$-octalin (2d) A suspension of MnO_2 (84.7 mg) and diol C (37 mg) in CH_2Cl_2 was stirred at room temperature for 18 h. Then additional MnO_2 (84.7 mg) was added and the mixture was stirred again for 23 h. The precipitate was filtered off and the filtrate was evaporated to give the reaction products, which were subjected to preparative TLC (silica gel, 20 × 20 cm; solvent, *n*-hexane–ethyl acetate (1 : 1)) to provide the ketol (**2d**; 10.7 mg, 29% yield) along with some starting material (C; 14.7 mg, 38% recovery). **2d**: $[\alpha]_D^{26}$ −105.3° (*c* = 0.98, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1731, 1667, 1618. ^{1}H-NMR δ: 1.279 (3H, s, 9-CH_3), 3.679 (1H, t, *J* = 5 Hz, >CH(OH)), 3.814 (3H, s, –$COOCH_3$).

Asymmetric Reduction of 1ab with Baker's Yeast (*Saccharomyces cerevisiae*) A suspension of baker's yeast (available from Oriental Yeast Co., Ltd., 2 g) and substrate (**1ab**) (100 mg) was shaken at 30 °C for 72 h. The reaction mixture was filtered with the aid of celite, then the filtrate was extracted with ethyl acetate. The ethyl acetate extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (10 g) to give the reduction product (**2a**+**2d**, 40.5 mg, 40% yield) from the *n*-hexane–ethyl acetate (from 1 : 6 to 2 : 1) eluate.

With *Hansenula anomala* After cultivation, the crude reaction mixture was chromatographed on silica gel (16 g) to give the reaction product (E; 80.9 mg, 20% yield) along with some starting material (**1b**; 230.9 mg, 58% recovery) from the *n*-hexane–ethyl acetate (4 : 1) eluate. E: mp 155—156 °C. MS Calcd for $C_{13}H_{18}O_4$ (M^+): 238.120, Found: 238.120. $[\alpha]_D^{25}$ +105.2° (*c* = 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1730, 1670, 1618. ^{1}H-NMR δ: 1.241 (3H, s, 9-CH_3), 3.499 (1H, dd, *J* = 11.5, 4.4 Hz, 6-H). **1b**: mp 90—91 °C. MS Calcd for $C_{13}H_{16}O_4$ (M^+): 236.105, Found: 236.106. $[\alpha]_D^{25}$ −13.7° (*c* = 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1718, 1670, 1618. ^{1}H-NMR δ: 1.493 (3H, s, 9-CH_3), 3.828 (3H, s, 4-CH_3).

Preparation of (+)-MTPA Ester (3a) from E Pyridine (0.3 ml) was added to a mixture of E (15.6 mg), (+)-MTPACl (30 mg) and DAMP (10 mg). This reaction mixture was stirred for 17 h at room temperature, and was worked up and purified in the same way as in the case of B to afford E (+)-MTPA ester (**3a**) (22.5 mg, 78% yield). ^{1}H-NMR δ: 1.257 (3H, s, 9-CH_3), 3.555 (3H, d, *J* = 1.0 Hz, –OCH_3), 3.817 (3H, s, –$COOCH_3$), 4.902 (1H, dd, *J* = 11.4, 6.4 Hz, >CHOMTPA). The optical purity of **3a** was found to be more than 99% ee.

Conversion of E into the Diketone (1a) Jones reagent (2 drops) was added to a stirred solution of E (20 mg) in acetone (8 ml). This mixture was cooled in an ice bath for 7 min. The reaction mixture was worked up and purified in the same way as for D to afford 13.8 mg, 69% yield. $[\alpha]_D^{28}$ +119° (*c* = 1.38, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1658, 1615. ^{1}H-NMR δ: 1.489 (3H, s, 9-CH_3), 3.833 (3H, s, –$COOCH_3$).

(+)-MTPA Esterification of Baker's Yeast Reduction Product Pyr-

TABLE II. Fractional Atomic Coordinates and the Equivalent Isotropic Temperature Factors (B_{eq} in Å^2)

No.	Atom	x 10^5	y 10^5	z 10^5	B_{eq} Å^2
1	Br1	20253 (10)	24697 (6)	23425 (15)	7.71 (0.02)

No.	Atom	x 10^4	y 10^4	z 10^4	B_{eq} Å^2
2	C1	11015 (9)	4034 (5)	−156 (10)	5.7 (0.1)
3	C2	9752 (9)	3717 (5)	146 (10)	5.6 (0.1)
4	C3	9377 (7)	3906 (4)	1630 (8)	3.9 (0.1)
5	C4	9524 (6)	4665 (3)	1941 (7)	3.3 (0.1)
6	C5	10323 (9)	5873 (4)	1441 (10)	5.2 (0.1)
7	C6	11670 (11)	6103 (5)	1527 (12)	7.1 (0.2)
8	C7	12453 (9)	5907 (4)	211 (14)	13.0 (0.2)
9	C8	12311 (8)	5126 (5)	−91 (10)	5.5 (0.2)
10	C9	10947 (7)	4829 (5)	−135 (8)	4.7 (0.1)
11	C10	10209 (7)	5108 (4)	1138 (8)	4.0 (0.1)
12	C11	8864 (7)	4905 (4)	3247 (8)	3.9 (0.1)
13	C12	10309 (10)	5146 (7)	−1483 (10)	7.6 (0.2)
14	C13	8329 (10)	4524 (6)	5546 (9)	6.1 (0.2)
15	C14	7708 (7)	3195 (4)	2504 (10)	4.7 (0.1)
16	C15	6298 (7)	3056 (3)	2455 (10)	4.4 (0.1)
17	C16	5780 (8)	2630 (4)	3495 (10)	5.4 (0.1)
18	C17	4509 (8)	2469 (5)	3489 (10)	5.9 (0.1)
19	C18	3776 (7)	2725 (4)	2378 (11)	5.3 (0.1)
20	C19	4265 (8)	3143 (4)	1328 (9)	4.7 (0.1)
21	C20	5536 (8)	3307 (4)	1377 (9)	4.3 (0.1)
22	O1	13217 (6)	4745 (4)	−244 (8)	7.6 (0.1)
23	O2	8271 (6)	5429 (3)	3396 (6)	5.8 (0.1)
24	O3	8996 (5)	4406 (3)	4224 (5)	4.3 (0.1)
25	O4	8010 (5)	3750 (2)	1774 (5)	3.8 (0.1)
26	O5	8453 (6)	2853 (3)	3178 (8)	7.3 (0.1)

No.	Atom	x 10^3	y 10^3	z 10^3	B_{eq} Å^2
27	HC1	1164 (8)	381 (4)	65 (8)	6. (2.)
28	H′C1	1129 (9)	381 (5)	−111 (10)	9. (3.)
29	HC2	910 (7)	390 (4)	−55 (7)	5. (2.)
30	H′C2	978 (7)	318 (3)	4 (8)	4. (2.)
31	HC3	996 (7)	362 (3)	231 (9)	5. (2.)
32	HC5	983 (7)	599 (4)	240 (9)	6. (2.)
33	H′C5	984 (8)	619 (4)	68 (9)	7. (2.)
34	HC6	1178 (7)	664 (4)	161 (8)	5. (2.)
35	H′C6	1216 (8)	586 (4)	238 (9)	7. (2.)
36	HC7	1210 (10)	629 (5)	−72 (10)	10. (3.)
37	H′C7	1356 (9)	608 (5)	36 (10)	9. (3.)
38	HC12	1109 (11)	540 (6)	−187 (13)	14. (4.)
39	H′C12	1029 (10)	465 (5)	−189 (12)	13. (4.)
40	H″C12	955 (10)	549 (5)	−188 (12)	12. (4.)
41	HC13	737 (9)	454 (5)	544 (10)	10. (3.)
42	H′C13	851 (10)	405 (5)	626 (11)	10. (3.)
43	H″C13	870 (9)	498 (5)	608 (10)	9. (3.)
44	HC16	623 (8)	245 (5)	421 (8)	8. (2.)
45	HC17	421 (8)	213 (4)	427 (8)	6. (2.)
46	HC19	369 (7)	333 (4)	59 (8)	5. (2.)
47	HC20	591 (8)	362 (4)	68 (9)	7. (2.)

Centrosymmetric space group, equivalent positions and their inverted coordinates.

x	y	z
$1/2-x$	$-y$	$1/2+z-1$
$1/2+x$	$1/2-y$	$-z$
$-x$	$1/2+y$	$1/2-z$

10^5, B_{eq} and Å^2 represent $\times 10^5$, B_{eq} and Å^2, respectively.

TABLE III. Temperature Factors

No.	Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
		$U_{(ij)}$'s are multiplied by 10^4					
1	Br1	616 (5)	932 (7)	1381 (10)	−234 (6)	106 (7)	−19 (9)
		$U_{(ij)}$'s are multiplied by 10^3					
2	C1	67 (6)	71 (5)	77 (6)	−10 (5)	26 (5)	−17 (5)
3	C2	70 (6)	69 (5)	74 (6)	−14 (5)	18 (5)	−26 (5)
4	C3	42 (4)	48 (4)	58 (5)	−2 (3)	3 (4)	−1 (4)
5	C4	41 (4)	45 (3)	41 (4)	1 (3)	−4 (3)	0 (3)
6	C5	71 (6)	48 (4)	78 (6)	−1 (4)	−9 (5)	8 (4)
7	C6	100 (8)	61 (5)	108 (8)	−18 (6)	−5 (7)	−8 (6)
8	C7	128 (8)	103 (6)	264 (13)	−36 (6)	−39 (9)	43 (8)
9	C8	49 (5)	88 (7)	73 (6)	−12 (5)	−1 (5)	16 (5)
10	C9	41 (4)	86 (6)	51 (4)	−12 (4)	−1 (4)	4 (4)
11	C10	47 (4)	55 (4)	50 (4)	−4 (4)	−13 (4)	3 (4)
12	C11	54 (4)	46 (4)	48 (4)	2 (3)	−2 (4)	−3 (3)
13	C12	69 (6)	167 (11)	52 (5)	−16 (7)	−12 (5)	35 (7)
14	C13	80 (7)	106 (7)	47 (5)	6 (6)	6 (5)	−8 (5)
15	C14	54 (4)	48 (4)	76 (5)	−3 (3)	−2 (5)	4 (4)
16	C15	53 (4)	33 (3)	82 (6)	−4 (3)	14 (5)	1 (4)
17	C16	62 (5)	65 (5)	79 (6)	−3 (4)	5 (5)	21 (5)
18	C17	68 (5)	62 (5)	94 (7)	−15 (5)	18 (5)	7 (6)
19	C18	53 (4)	54 (4)	94 (7)	−10 (4)	14 (5)	−20 (5)
20	C19	59 (5)	52 (4)	69 (5)	−8 (4)	0 (5)	−3 (4)
21	C20	57 (5)	45 (4)	61 (5)	−5 (4)	4 (4)	2 (4)
22	O1	57 (4)	118 (6)	114 (6)	−3 (4)	12 (4)	9 (5)
23	O2	88 (4)	75 (4)	60 (3)	32 (4)	5 (4)	−3 (3)
24	O3	52 (3)	65 (3)	45 (3)	2 (3)	3 (3)	0 (3)
25	O4	47 (3)	43 (2)	53 (3)	−9 (2)	1 (3)	4 (2)
26	O5	64 (4)	74 (4)	138 (6)	0 (3)	−9 (4)	49 (4)
		$U_{(ij)}$'s are multiplied by 10^2					
27	HC1	8 (3)					
28	H′C1	12 (4)					
29	HC2	6 (2)					
30	H′C2	5 (2)					
31	HC3	7 (2)					
32	HC5	7 (2)					
33	H′C5	9 (3)					
34	HC6	7 (2)					
35	H′C6	9 (3)					
36	HC7	13 (4)					
37	H′C7	11 (4)					
38	HC12	17 (5)					
39	H′C12	16 (5)					
40	H″C12	16 (5)					
41	HC13	13 (4)					
42	H′C13	13 (4)					
43	H″C13	11 (4)					
44	HC16	10 (3)					
45	HC17	7 (3)					
46	HC19	6 (2)					
47	HC20	9 (3)					

Temperature factor T is in the form of

$$T=\exp\{-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)\}$$

idine (0.3 ml) was added to a mixture of (**2a**+**2d**) (15.3 mg) and (+)-MTPACl (24 mg), and the reaction mixture was stirred for 17 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with saturated aqueous NaCl, dried over $MgSO_4$ and concentrated to afford a crystalline product. This was subjected to TLC (silica gel, 20 × 20 cm; solvent, *n*-hexane–ethyl acetate (1 : 1)) to provide the less polar (+)-MTPA ester (**3a**; 12.3 mg, 43% yield) and the more polar (+)-MTPA ester (**3d**; 4.5 mg, 16% yield). **3a** ^{1}H-NMR δ: 1.258 (3H, s, 9-CH_3), 3.555 (3H, d, J=0.98 Hz, –OCH_3), 3.817 (3H, s, –$COOCH_3$), 4.902 (1H, dd, J=11.7, 4.6 Hz, >CHOMTPA). **3d** ^{1}H-NMR δ: 1.342 (3H, s, 9-CH_3), 3.511, 3.477 (each 3H, d, J=1.0 Hz, –OCH_3), 5.029 (1H, m, >CHOMTPA). The optical purities of **3a** and **3d** (and hence those of **2a** and **2d**) were found to be >99% ee and 6% ee, respectively.

Preparation of Racemic *cis*-Ketols (2a + 2b) and Their (+)-MTPA Esters

TABLE IV. Bond Lengths in Å and Bond Angles in Degrees

Atom 1	Atom 2	Length (S.T.D.)	Atom 1	Atom 2	Length (S.T.D.)	Atom 1	Atom 2	Atom 3	Angle (S.T.D.)
Br1	–C18	1.908 (8)	C1	–HC1	1.10 (8)	C2	–C1	–C9	111.4 (8)
C1	–C2	1.492 (13)	C1	–H′C1	1.05 (10)	C3	–C2	–C1	108.3 (7)
C1	–C9	1.533 (13)	C2	–HC2	1.02 (7)	C4	–C3	–C2	113.2 (6)
C2	–C3	1.516 (12)	C2	–H′C2	1.04 (7)	C4	–C3	–O4	106.3 (6)
C3	–C4	1.499 (10)	C3	–HC3	1.05 (8)	C2	–C3	–O4	106.9 (6)
C3	–O4	1.476 (9)	C5	–HC5	1.08 (8)	C10	–C4	–C3	123.9 (6)
C4	–C10	1.355 (10)	C5	–H′C5	1.08 (8)	C10	–C4	–C11	121.5 (6)
C4	–C11	1.500 (10)	C6	–HC6	1.05 (7)	C3	–C4	–C11	114.6 (6)
C5	–C6	1.488 (14)	C6	–H′C6	1.09 (8)	C6	–C5	–C10	112.3 (8)
C5	–C10	1.507 (11)	C7	–HC7	1.22 (10)	C7	–C6	–C5	112.9 (9)
C6	–C7	1.550 (17)	C7	–H′C7	1.22 (9)	C8	–C7	–C6	109.9 (8)
C7	–C8	1.539 (13)	C12	–HC12	1.03 (12)	C9	–C8	–C7	117.2 (8)
C8	–C9	1.546 (12)	C12	–H′C12	1.03 (11)	C9	–C8	–O1	120.2 (8)
C8	–O1	1.212 (12)	C12	–H″C12	1.10 (11)	C7	–C8	–O1	122.6 (9)
C9	–C10	1.540 (11)	C13	–HC13	1.01 (10)	C10	–C9	–C1	112.5 (7)
C9	–C12	1.575 (13)	C13	–H′C13	1.16 (10)	C10	–C9	–C8	108.5 (7)
C11	–O2	1.197 (9)	C13	–H″C13	1.09 (9)	C10	–C9	–C12	107.1 (7)
C11	–O3	1.346 (9)	C16	–HC16	0.90 (8)	C1	–C9	–C8	109.1 (7)
C13	–O3	1.462 (10)	C17	–HC17	1.05 (8)	C1	–C9	–C12	113.4 (7)
C14	–C15	1.509 (11)	C19	–HC19	1.00 (7)	C8	–C9	–C12	106.0 (7)
C14	–O4	1.316 (9)	C20	–HC20	0.97 (8)	O2	–C11	–C4	126.9 (7)
C14	–O5	1.210 (11)				O2	–C11	–O3	125.0 (7)
C15	–C16	1.399 (12)				C4	–C11	–O3	108.0 (6)
C15	–C20	1.391 (12)				C15	–C14	–O4	111.4 (7)
C16	–C17	1.373 (12)				C15	–C14	–O5	123.9 (8)
C17	–C18	1.401 (13)				O4	–C14	–O5	124.7 (8)
C18	–C19	1.385 (12)				C16	–C15	–C14	117.8 (7)
C19	–C20	1.375 (12)				C16	–C15	–C20	120.2 (7)
						C14	–C15	–C20	121.9 (7)
						C17	–C16	–C15	120.6 (8)
						C18	–C17	–C16	117.4 (8)
						C19	–C18	–Br1	119.7 (6)
						C19	–C18	–C17	123.2 (8)
						Br1	–C18	–C17	117.0 (6)
						C20	–C19	–C18	118.0 (8)
						C3	–O4	–C14	116.7 (6)
						C4	–C10	–C5	123.4 (7)
						C4	–C10	–C9	119.6 (7)
						C5	–C10	–C9	116.9 (7)
						C11	–O3	–C13	116.0 (6)
						C15	–C20	–C19	120.5 (7)

(3a+3b) i) A mixture of **1ab** (236 mg) and $NaBH_4$ (0.95 mg) in EtOH (2 ml) was stirred for 10 min at 0°C. This reaction mixture was extracted with ether after the addition of H_2O. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and concentrated to give a crystalline mass, which was chromatographed on silica gel (40 g) to afford the alcohols (**2a+2b**; 174 mg, 74% yield) along with the starting material (**1ab**; 43 mg, 18% recovery) from the *n*-hexane–ethyl acetate (4 : 1) eluate. ii) Pyridine (0.3 ml) was added to a mixture of (**2a+2b**) (20 mg), (+)-MTPACl (32 mg) and DAMP (10 mg). This reaction mixture was stirred for 17 h at room temperature, and was washed and purified in the same way as in the case of the preparation of the (+)-MTPA ester (**3a**) to provide the (+)-MTPA ester (**3a+3b**; 36.3 mg, 95% yield). ^{1}H-NMR δ: 1.249, 1.257 (each 3H, s, 9-CH_3), 3.498, 3.555 (each 3H, d, J=1.0 Hz, –OCH_3), 3.817 (each 3H, s, –$COOCH_3$), 4.902, 4.853 (each 1H, dd, J=11.7, 4.6 Hz, >CHOMTPA).

Screening of Various Yeasts The microorganisms described above were used to reduce the starting meterial (**1ab**). Erlenmeyer flasks (2 l) containing 800 ml of the same culture as used for the *Kloeckera saturnus* cultivation were inoculated with microorganisms; the culture was carried out at 30 °C for 4 d with continuous shaking. Then the substrate (*ca.* 400 mg of **1ab**) was added, and the culture was continued for an additional 3 d under the same conditions. Each reaction mixture was separately worked up in the same way as in the case of *Kloeckera saturnus* reduction to give the crude reaction product. Pyridine (0.5 ml) was added to a mixture of each reduction product and (+)-MTPACl (*ca.* 30 mg). The reaction mixtures were stirred for 48 h at room temperature, then worked up and purified in the same way as in the case of **3a** to afford the (+)-MTPA esters as shown in Table I.

Conversion of *trans*-Ketol B into Diketone D Jones reagent (2 drops) was added to a solution of B (20 mg) in acetone (8 ml); the mixture was cooled in an ice bath for 7 min. The reaction mixture was worked up and purified in the same way as mentioned above to afford 13 mg; 62% yield. $[\alpha]_D^{26}$ −85.0° (c=1.23, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1658, 1615. ^{1}H-NMR δ: 1.486 (3H, s, 9-CH_3), 3.833 (3H, s, –$COOCH_3$).

Conversion of 3,8-Dioxo-4-methoxycarbonyl-9-methyl-$\Delta^{4(10)}$-octalin (1ab) into 3,8-Dioxo-4,9-dimethyl-$\Delta^{4(10)}$-octalin (7) i) A mixture of **1ab** (1.142 g), ethylene glycol (736 mg) and *p*-toluenesulfonic acid (20 mg) was heated in dry benzene (20 ml), while water was removed as the benzene–water azeotrope with a Bidwell-Sterling trap. The cooled benzene solution was washed with aqueous 10% potassium carbonate, then with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residual yellow oil was chromatographed over silica gel (60 g). Elution of the column with *n*-hexane–ethyl acetate (9 : 1) afforded the diketal; 514.7 mg, 39% yield. ii) A solution of the diketal (322 mg) in ether (10 ml) was added dropwise to a stirred suspension of lithium aluminum hydride (LAH) (22.0 mg) in dry ether (10 ml) at 0°C. The mixture was stirred at room temperature for 2 h, then LAH (22 mg) in dry ether (10 ml) was added at room temperature. The reaction mixture was stirred for 1 h, then decomposed by adding H_2O (1 ml), aqueous 15% NaOH (1 ml) and H_2O (3 ml), and the product was worked up as indicated elsewhere. The yield of the crude crystals of the corresponding alcohol was 283 mg. iii) Triphenylphosphine (360 mg) was added to a mixture of the alcohol and carbon tetrabromide (533 mg) in CH_2Cl_2 (5 ml); the resulting mixture was stirred for 30 min at room temperature, then chromatographed on silica gel (10 g) to give the crude bromide from an ether fraction. iv) Tributyltin hydride (311 mg) in toluene (2 ml) was added dropwise to a mixture of the

above crude bromide in toluene (8 ml), then the reaction mixture was stirred for 30 min at room temperature, and refluxed for 30 min. Removal of the solvent gave an oily product, which was chromatographed on silica gel (10 g) to give the crude methyl derivative (123 mg). v) A mixture of the above intermediate in 80% CH_3COOH was warmed at 90 °C for 30 min. The reaction mixture was cooled, then extracted with ether and washed successively with aqueous 5% potassium bicarbonate and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (16.5 g) to yield the methyloctalin (**7**) (43.8 mg, overall yield 7.7% from diketal); ^{1}H-NMR δ: 1.430 (3H, s, 9-CH_3), 1.877 (3H, s, 4-CH_3); the NMR spectrum and TLC behavior of **7** were identical with those of an authentic sample.[12]

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