follows: NMR ($CDCl_3$) δ 1.86 (br s, 3 H), 4.24 (br s, 1 H), 6.50 (m, 1 H), 6.90–7.60 (m, 9 H); IR (KBr) 3000, 1600, 1450, 1432, 741, 729, 692 μm.

Anal. Calcd for $C_{16}H_{14}$: C, 93.15; H, 6.85. Found: C, 92,84; H, 6.83.

Acknowledgment. Support by the Research Corp. and the Graduate School of the University of Kentucky is gratefully acknowledged.

Registry No. 1, 2177-45-9; (*E*)-3, 21866-70-6; (*Z*)-3, 21878-52-4; 6, 3412-44-0; 7, 22360-63-0; 8, 37634-53-0; 9, 71831-93-1; *trans*-10, 71831-94-2; *cis*-10, 71831-95-3; 12, 71831-96-4; 13, 71831-97-5; 14, 4467-88-3; 1,3-dimethyl-1-phenyl-2-butene 1-oxide magnesium bromide, 71831-98-6; 2-methyl-1,3-diphenyl-2-propene 1-oxide magnesium bromide, 71831-99-7; *trans*-1,3-diphenyl-2-propene 1-oxide magnesium bromide, 71832-00-3; *cis*-1,3-diphenyl-2-propene 1-oxide magnesium bromide, 71832-01-4; 4-methyl-3-penten-2-one, 141-79-7; *trans*-1,3-diphenyl-2-propen-1-one, 614-47-1; 3,3-diphenyl-2-propenaldehyde, 1210-39-5; *cis*-chalcone, 614-46-0; 2-methylcinnamaldehyde, 101-39-3; *trans*-cinnamaldehyde, 14371-10-9; *cis*-cinnamaldehyde, 57194-69-1; *trans*-1,1,3-triphenyl-2-propen-1-ol, 71832-02-5; 1,3,3-triphenyl-2-propen-1-ol, 21711-85-3; *trans*-2,4-diphenyl-3-buten-2-ol, 56763-56-5; *cis*-2,4-diphenyl-3-buten-2-ol, 71832-03-6; *cis*-1,3-diphenyl-2-propen-1-ol, 62839-70-7; 4-methoxy-3-buten-2-one, 4652-27-1; triphenylmethane 1-oxide magnesium bromide, 71832-04-7; diphenylmethane 1-oxide magnesium bromide, 36233-75-7; methyldiphenylmethane 1-oxide magnesium bromide, 68986-36-7; triphenylmethane, 519-73-3; 9-phenylfluorene, 789-24-2; diphenylmethane, 101-81-5; 1,1-diphenylethene, 530-48-3.

Microbial Stereodifferentiating Reduction of the Carbonyl Groups Located on the C_2 Axes of Gyrochiral Molecules[1]

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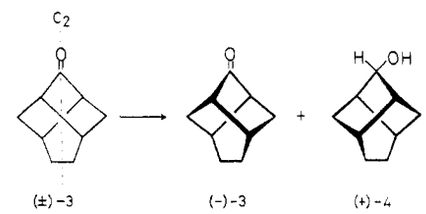
The enantiomer selectivity of *Curvularia lunata* and *Rhodotorula rubra* with respect to some C_2 ketone substrates having the C_2 axis coincident with the carbonyl axis has been examined. Both microbes were found to exhibit a marked stereodifferentiation between enantiomers of (±)-9-*twist*-brendanone (**3**), (±)-2-brexanone (**5**), (±)-D_3-trishomocubanone (**8**), (±)-bisnoradamantanone (**10**), (±)-biphenyl (**14**), and (±)-α-binaphthyl (**16**) bridged ketones, selectively reducing the *P*-C_2 ketone enantiomers (**2**).

Flanking a carbonyl group with substituents degenerates the C_{2v} symmetry inherent to the original carbonyl group to give C_s, C_2, and C_1 ketones.[2] The molecular environment around the carbonyl group of these ketones can readily be visualized when the ketones are oriented in a three-dimensional system (Figure 1). The faces around the carbonyl group are enantiotopic[3] in a C_s ketone and diastereotopic[3] in a C_1 ketone, and microbial stereodifferentiation between these faces has been well documented.[4]

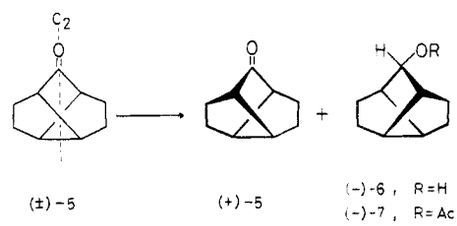
In a C_2 ketone with two identical chiral substituents flanking the carbonyl group, the faces are homotopic[3] in an internal comparison, and the only stereochemical distinction is that between enantiotopic faces in each enantiomer in an external comparison.

Our continuing interest in gyrochiral[5] cage-shaped molecules[6] has led us to study microbial stereodifferentiation between the enantiotopic faces of *M*-C_2 ketone **1**

Scheme I



Scheme II



and *P*-C_2 ketone **2**[7] (Figure 2) on which there has been no investigation reported, and this paper reports our results with *Curvularia lunata* and *Rhodotorula rubra*.

Results

Microbial Stereodifferentiating Reduction of Cage-Shaped C_2 Ketones. (±)-Tricyclo[4.3.0.0^{3,8}]nonan-9-one ("9-*twist*-Brendanone", 3) (Scheme I).[9] Monitoring the process with gas chromatography indicated that 40 h of incubation with *C. lunata* at 29 °C was enough

(1) Presented at the 26th IUPAC Congress, Sept 8, 1977, Tokyo, Japan, Abstracts p 63. For a preliminary account of this work see: Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Nishino, M.; Murakami, H.; Asao, M. *J. Chem. Soc., Chem. Commun.* **1978**, 667–8. For a review summarizing our studies on stereodifferentiating microbial reduction see: Nakazaki, M.; Chikamatsu, H. *Kagaku No Ryoiki* **1977**, *31*, 819–33.

(2) In this paper, ketones are conveniently classified according to their symmetry: C_s ketones belong to the C_s point group and have the plane of symmetry coincident with the carbonyl plane; C_2 ketones belong to the C_2 point group and have the C_2 axis coincident with the carbonyl axis; C_1 ketones have no symmetry element passing through the carbonyl axis.

(3) Mislow, K.; Raban, M. *Top. Stereochem.* **1967**, *1*, 1–38.

(4) Concise reviews can be found in: (a) Bentley, R. "Molecular Asymmetry in Biology"; Academic Press: New York, 1970; Vol. 2, pp 41–50. (b) Sih, C. J.; Rosazza, J. P. "Applications of Biochemical Systems in Organic Chemistry"; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Part 1, Chapter 3. (c) Kieslich, K. *Synthesis* **1969**, *1*, 147–57.

(5) This name is proposed to describe the symmetry of a shape which is chiral but not asymmetric; cf.: Nakazaki, M.; Naemura, K.; Yoshihara, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3278–84.

(6) For a review see: Nakazaki, M.; Naemura, K. *Yuki Gosei Kagaku Kyokai Shi* **1977**, *35*, 883–96.

(7) An inspection of the quadrant projection formula (Figure 2) should support the adequacy of our adopting *M* and *P* helicity[8] to describe these chiralities.

(8) Cahn, R. S.; Ingold, C. K.; Prelog, V. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385–415.

(9) All structural formulas in this paper are presented in their absolute configurations.

0022-3263/79/1944-4588$01.00/0

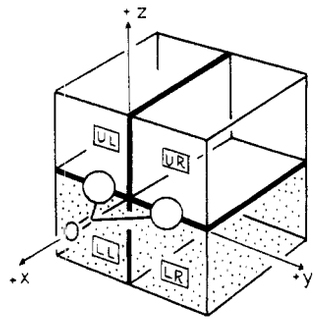


Figure 1. The quadrant orientation. Substrate ketone molecules are orientated in a three-dimensional system with the carbonyl plane on the xy plane, the carbonyl axis coincident with the x axis, and the carbonyl oxygen pointing in the $+x$ direction. Four quadrants are designated by UL (upper left), UR (upper right), LL (lower left), and LR (lower right).

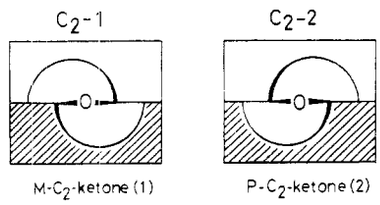


Figure 2. Two quadrant orientations (C_2-1 and C_2-2) for the enantiomers of C_2 ketones with M and P helicity.

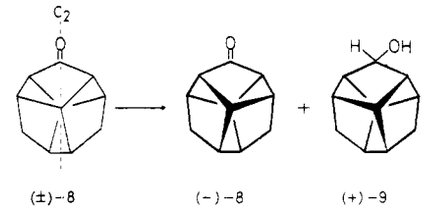
to convert half of 9-*twist*-brendanone (**3**) into 9-*twist*-brendanol (**4**). Extraction with ether followed by separation of the metabolites afforded a 30% yield of the recovered optically active (−)-9-*twist*-brendanone (**3**) (optical purity 64%)[10] and a 24% yield of (+)-9-*twist*-brendanol (**4**) (optical purity 85%).[10]

Their established absolute configurations[11] clearly indicate that the recovered (−) ketone **3** and metabolite (+) alcohol **4** have opposite configurations in their molecular frameworks.

R. rubra was found to reduce the ketone **3** more reluctantly, yielding a 78:22 mixture of **3** and **4** after a 50-h incubation at 29 °C. Separation again afforded the same metabolites, (−) ketone **3** (32% yield) and (+) alcohol **4** (12% yield), both with rather poor optical purity (10 and 53%, respectively).

(±)-Tricyclo[4.3.0.0^{3,7}]nonan-2-one ("2-Brexanone", 5) (Scheme II). Racemic 2-brexanone (**5**) was incubated with *C. lunata* for 72 h at 30 °C to give a 66:34 mixture of the ketone **5** and the alcohol **6**. The alcohol was separated by alumina chromatography, yielding (−)-2-brexanol (**6**) (12% yield) with optical purity as high as 100%.[13]

Scheme III



Scheme IV

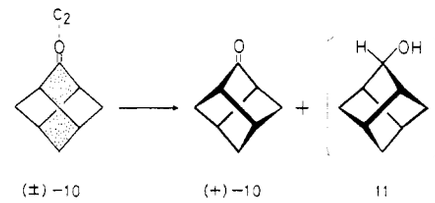
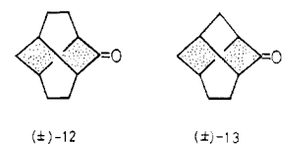


Chart I



The recovered ketone was distilled to afford a 35% yield of (+) ketone **5** (optical purity 21%).[13]

Again *R. rubra* was found to reduce this racemic ketone **5** slowly, and gas chromatography of a 67-h incubation product showed almost no indication of the alcohol **6**.

Absolute configurations of (+)-2-brexanone and (−)-2-brexanol have been recently established in our laboratory,[15] and this work unambiguously demonstrated opposite configurations in their molecular frameworks.

(±)-Pentacyclo[6.3.0.0.^{2,6}0.^{3,10}0.^{5,9}]undecan-4-one ("D_3-Trishomocubanone", 8) (Scheme III). Introduction of a carbonyl group destroys the D_3 symmetry of D_3-trishomocubane whose resolution in optically active modifications as well as absolute configuration determination has been recently reported from three laboratories.[16]

(±)-D_3-Trishomocubanone (**8**), a C_2 ketone, surprised us with its remarkably rapid rates of reduction by both *C. lunata* and *R. rubra*, yielding the metabolite alcohol **9** as a sole isolable product.

Monitoring the process with gas chromatography, we terminated the incubation with *C. lunata* (29 °C) after 3 h when formation of a 57:43 mixture of **8** and **9** was indicated. Extraction with ether and workup of the reaction mixture afforded the (−) ketone **8** (40% yield) and the (+) alcohol **9** (25% yield) with optical purity of 40 and 61%, respectively.[17]

Although *R. rubra* attacked this racemic ketone substrate **8** rapidly, a 53:47 mixture of **8** and **9** isolated from a 4-h incubation solution (30 °C) was found to furnish (−) ketone **8** (31% yield) and (+) alcohol **9** (27% yield) both with poorer optical purity (5.6 and 12.5%, respectively).

The three studies[16] carried out independently have provided the information on their absolute configurations, revealing opposite configurations in the molecular frame-

(10) We have reported a photochemical synthesis[11] of (+) ketone **3**, $[\alpha]_D$ +176° (MeOH), via (+) alcohol **4**, $[\alpha]_D$ +164° (MeOH), from *endo*-bicyclo[2.2.2]oct-5-ene-2-carboxylic acid, $[\alpha]_D$ +31.8° (MeOH). Adopting Tichý's absolute rotation value[12] $[\alpha]_D$ +50.9° (MeOH) for this unsaturated carboxylic acid permitted us to calculate absolute rotations $[\alpha]_D$ +282° (MeOH) and $[\alpha]_D$ +262° (MeOH) for the (+) ketone **3** and the (+) alcohol **4**, respectively.

(11) Nakazaki, M.; Naemura, K.; Harita, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1907–13.

(12) Tichý, M. *Tetrahedron Lett.* **1972**, 2001–4; *Collect. Czech. Chem. Commun.* **1974**, *39*, 2673–84.

(13) Intensities of the anisochronous CH_3CO signals which were observed in the NMR spectrum on addition of Eu(facam)$_3$ to a sample of (−) acetate **7**, $[\alpha]_D$ −109° (EtOH), assigned 70.5% optical purity to this specimen.[14] Since this acetate was prepared from (−) ketone **5**, $[\alpha]_D$ −201° (EtOH), via the (−) alcohol **6**, $[\alpha]_D$ −102° (EtOH), this chemical correlation furnishes absolute rotations $[\alpha]_D$ −285° (EtOH) and $[\alpha]_D$ −145° (EtOH) for (−) ketone **5** and (−) alcohol **6**, respectively. Since (−)-2-brexanol (**6**) obtained by an incubation experiment with *C. lunata* exhibited the largest $[\alpha]_D$ −157.7° (EtOH) so far, this was taken as the absolute value for the optical purity calculation, though this value is well within the experimental error for the above calculated value $[\alpha]_D$ −145° (EtOH). This automatically assigns absolute rotation $[\alpha]_D$ +311° (EtOH) for the (+) ketone **5**.

(14) Nakazaki, M.; Naemura, K.; Kadowaki, H. *J. Org. Chem.* **1978**, *43*, 4947–51.

(15) Nakazaki, M.; Naemura, K.; Kadowaki, H. *J. Org. Chem.* **1976**, *41*, 3725–30.

(16) (a) Helmchen, G.; Staiger, G. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 116–7. (b) Nakazaki, M.; Naemura, K.; Arashiba, N. *J. Org. Chem.* **1978**, *43*, 689–92. (c) Eaton, P. E.; Leipzig, B. *Ibid.* **1978**, *43*, 2483–4.

(17) Calculations of the optical purities are based on their established absolute rotation values, $[\alpha]_D$ −88.5° (EtOH) for (−)-**8** and $[\alpha]_D$ +152° (EtOH) for (+)-**9**.[16b]

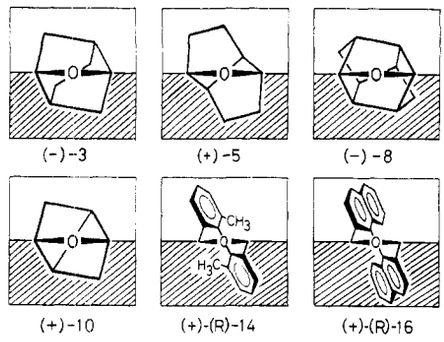


Figure 3. Quadrant projections of recovered M-C_2 ketones and the proposed C_2 ketone rule.

works of the (−) ketone **8** and the (+) alcohol **9**.

(±)-Tricyclo[3.3.0.0^{3,7}]octan-2-one ("Bisnoradamantanone", 10) (Scheme IV). C_2 ketone **10** ($C_8H_{10}O$), so far the smallest C_2 ketone whose preparation in optically active modifications and absolute configuration assignment have been accomplished recently in our laboratory,[18] was our next substrate.

Contrary to our expectation that the higher solubility of (±)-bisnoradamantanone (**10**) in the culture solution should promise a rapid microbial reduction, C_2 ketone **10** was found to resist stubbornly the attack of both *C. lunata* and *R. rubra*.

Screening various microbes proved that a strain of *C. lunata* (IFO 6299)[19] was sufficiently active toward this ketone, providing a 9:1 mixture of the recovered ketone **10** and the alcohol **11** after 10 days of incubation at 29 °C.

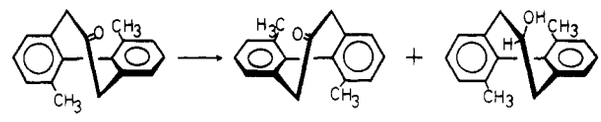
Although its meager quantity together with extremely high sublimability prevented the isolation of the metabolite alcohol **11**, there was obtained a 20% yield of the recovered (+) ketone **10** with 3% optical purity.[20]

(±)-Tricyclo[4.4.0.0^{3,8}]decan-2-one ("2-Twistanone", 12) (Chart I). Another C_2 ketone which resisted microbial reduction was found in (±)-2-twistanone (**12**)[21] whose incubations with various strains of *Saccharomyces*, *Rhizopus*, *Aspergillus*, *Curvularia*, *Rhodotorula*, *Alternaria*, and *Sporotrichum* have been found sterile in producing the metabolite alcohol even after prolonged incubation.

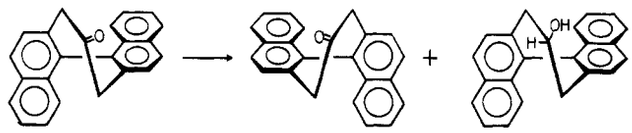
Inspection of the molecular models of C_2 ketones **10** and **12** which resisted microbial reduction reveals an interesting common molecular feature, a twist-boat cyclohexanone moiety with the C_2 symmetry axis coincident with the carbonyl axis (shown with dotting in (±)-**10** and (±)-**12**). It should be pertinent to note here that (±)-2-*twist*-brendanone (**13**)[22] which resembles **12** in this feature was also found to be immune against microbial reduction after a 43-h incubation with *C. lunata*.

Microbial Stereodifferentiating Reduction of C_2 Ketones with Axial Chirality. Figure 3 illustrates the quadrant projections of the recovered optically active C_2 ketones (−)-**3**, (+)-**5**, (−)-**8**, and (+)-**10** discussed in the preceding sections. These enantiomers having the larger part of molecule around the C_2 axis in the upper left (UL) and the lower right (LR) quadrants are classified as the M-C_2 ketone (**1**), and this means *C. lunata* and *R. rubra* preferentially reduce the enantiomer with P helicity over the one with M helicity (Figure 2).

Scheme V



Scheme VI



We presumed that the larger the difference between right and left space occupation in the C_2 molecule, the larger should be the difference between the reaction rates of its enantiomers, and biphenyl **14** and α-binaphthyl **16** bridged ketones with axial chirality seem to fulfill this requirement of molecular geometry (Figure 3).

(±)-4′,1″-Dimethyl-1,2:3,4-dibenzo-1,3-cycloheptadien-6-one (14) (Scheme V). Contrary to our experience in cage-shaped C_2 ketones, *C. lunata* was found to show disappointingly poor enantiomeric selectivity toward the atropisomeric C_2 ketone **14**, affording oily recovered ketone **14** (20% yield, optical purity 0.16%[23]) and a 15% yield of optically inactive alcohol **15**. Fortunately, however, *R. rubra* was proved to be highly enantiomer selective toward (±) ketone **14**, yielding the recovered ketone and metabolite alcohol with extraordinary high optical purity.

Incubation (30 °C) was terminated after 25 h when gas chromatography indicated a 47:53 ratio of the ketone **14** and the alcohol **15** in the culture mixture. Alumina chromatography followed by preparative TLC separated these products, yielding (+)-R ketone **14** (31% yield, optical purity 100%)[23] and (+)-S alcohol **15** (34% yield, optical purity 94%).[23]

Classical works carried out Mislow's laboratory have firmly established the absolute configuration of these atropisomers,[25] and the microbial reduction of this atropisomeric C_2 ketone **14** again confirmed our generalization that *C. lunata* and *R. rubra* selectively reduce the P-C_2 ketone enantiomer (Figure 3).

(±)-2′,1′:1,2;1″,2″:3,4-Dinaphtho-1,3-cycloheptadien-6-one (16) (Scheme VI). Trial incubations of (±)-α-binaphthyl-bridged ketone **16** with *C. lunata* were found rather discouraging, giving a poor yield of the alcohol **17**. Prolonged incubation (215 h, 29 °C), however, improved the yield. Chromatography of the metabolite products afforded a 12% yield of (+)-S alcohol **17** (optical purity 65%)[26] and the recovered (+)-R ketone **16** (46% yield, optical purity 3.3%).[26]

Reluctance of this ketone to undergo microbial reduction was further observed in *R. rubra* whose 72-h (30 °C) in-

(18) Nakazaki, M.; Naemura, K.; Arashiba, N. *J. Chem. Soc., Chem. Commun.* **1976**, 678–9; *J. Org. Chem.* **1978**, *43*, 888–91.
(19) The serial identification number of microbes issued by the Institute of Fermentation, Osaka, Japan (see Experimental Section).
(20) Calculated from the reported absolute rotation value $[\alpha]_D$ +79° (EtOH) for the ketone **10**.[6,18]
(21) Absolute configuration: ref 11 and 12.
(22) Absolute configuration: Naemura, K.; Nakazaki, M. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 888–92.
(23) Calculated on the basis of their reported maximum rotation values, $[\alpha]^{28}_D$ −628° (benzene) for (−) ketone **14** and $[\alpha]^{27}_D$ +141° (benzene) for (+) alcohol **15**.[24]
(24) Mislow, K.; Glass, M. A. W.; O'Brien, R. E.; Rutkin, P.; Steinberg, D. H.; Weiss, J.; Djerassi, C. *J. Am. Chem. Soc.* **1962**, *84*, 1455–78.
(25) Mislow, K.; O'Brien, R. E.; Schaefer, H. *J. Am. Chem. Soc.* **1962**, *84*, 1940–4.
(26) Calculated on the basis of their reported maximum rotation values, $[\alpha]^{20}_D$ +134° (benzene) for (+) ketone **16**, and $[\alpha]^{20}_D$ −634° (benzene) for (−) alcohol **17**.[27a]

Table I. Microbial Reduction of C_2 Ketones with *C. lunata* and *R. rubra*

substrate	microbe[a]	incubation period, h (T, °C)	ketone:alcohol ratio[c]	optical purity (yield), % ketone	optical purity (yield), % alcohol
(±)-3	*C. lunata*	40 (29)	51:49	63.6 (30)	84.6 (24)
	R. rubra	50 (29)	78:22	10.2 (32)	52.6 (12)
(±)-5	*C. lunata*	72 (30)	66:34	21.0 (35)	100.0 (12)
	R. rubra	67 (30)	100:0	*f*	*f*
(±)-8	*C. lunata*	3 (29)	57:43	39.7 (40)	60.6 (25)
	R. rubra	4 (30)	53:47	5.6 (31)	12.5 (27)
(±)-10	*C. lunata*[b]	240 (29)	90:10	3.0 (20)	*g*
	R. rubra	168 (30)	98:2	*f*	*f*
(±)-14	*C. lunata*	20 (29)	70:30	0.16 (20)	0 (15)
	R. rubra	25 (30)	47:53	100.0 (31)	94.0 (34)
(±)-16	*C. lunata*	215 (29)	79:21[d]	3.3 (46)	64.8 (12)
	R. rubra	72 (30)	100:0[e]	*f*	*f*

[a] IFO serial number: *R. rubra* (IFO 0889), *C. lunata* (IFO 6288) unless otherwise noted. [b] IFO 6299. [c] As determined by GLC analysis unless otherwise noted. [d] As calculated by weights of products. [e] As determined by TLC analysis. [f] The preparative experiment was not carried out. [g] The sample of the metabolite alcohol was not isolated as a pure state.

cubation with **16** showed no indication of the metabolite alcohol **17**; sparing solubility of this ketone in the culture solution should presumably be responsible for this slow reduction.

Nevertheless, the stereochemistries of the recovered (+)-*R* ketone **16** and the product (+)-*S* alcohol **17**[27] again demonstrated the *P*-C_2 ketone's predominant reduction.

It is interesting to note Mislow's generalization[28] that partially asymmetric Meerwein–Ponndorf reduction of bridged biphenyl derivatives with (+)-(*S*)-2-octanol leads to an excess of the *S* enantiomer (corresponding to our *P*-C_2 ketone) in the residual biphenyl ketone.[29]

Discussion

Table I summarizes the results of the microbial reduction of C_2 ketones with *C. lunata* and *R. rubra* reported in the present study. It will be noticed that *C. lunata* reduces cage-shaped C_2 ketones more rapidly than *R. rubra* and usually with much higher enantiomer selectivity, recommending the process as a convenient single-step research-scale method to prepare these oxygenated gyrochiral cage-shaped compounds with high optical purity and of predicable absolute configuration.

Although *C. lunata*'s superiority over *R. rubra* does not hold for the atropisomeric bridged biphenyl ketone, there distinctly remained their common stereodifferentiating attitude toward C_2 ketones: *C. lunata* and *R. rubra* both preferentially reduced the *P*-C_2 ketone over its enantiomeric *M*-C_2 ketone (the "C_2 ketone rule").

It is needless to say that this microbial reduction, when applied with due caution, will provide a simple method for determining the absolute configurations of C_2 ketones with a variety of molecular frameworks.

Being a "sideway" biological process involving unnatural substrates as well as whole living cells, factors which determine the relative reduction rates of C_2 ketone enantiomers are seemingly vast and complicated; e.g., relative rates of permeation of substrates and products into and out of the cell have to be accounted for.

Nevertheless, we are tempted to compare our "C_2 ketone rule" with the recently refined diamond lattice section of the active site of horse liver alcohol dehydrogenase[30] (HLADH) which has been one of the most extensively studied redox enzymes.

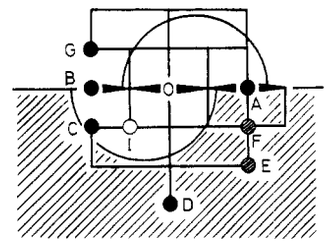


Figure 4. Overlapped quadrant projections of the *P*-C_2 ketone and the "updated" diamond lattice section[30] of the active site of horse liver alcohol dehydrogenase. Positions A–I are forbidden or undesirable locations. The qualitative order of their resistance to occupation is ● > ⊘ > ○.

Figure 4 is a schematic representation of overlapping quadrant projections of the diamond lattice section of HLADH and the *P*-C_2 ketone, the enantiomer to be preferentially reduced by *C. lunata* and *R. rubra*.

An inspection of this overlapping figure reveals that the favored *P*-C_2 ketone appears not to violate the secondly severe forbidden lattice positions E and F located in the LR quadrant section.

Experimental Section

Melting points are uncorrected. NMR spectra were determined on a JNM-60HL with Me_4Si as an internal standard (δ 0). Coupling constants are expressed as hertz, and s = singlet, d = doublet, dd = double of doublets, and m = multiplet. Optical rotations were measured with a JASCO-DIP-SL polarimeter. Circular dichroism (CD) spectra were determined on a JASCO-J-40 spectropolarimeter. GLC analyses were performed on a JGC-20K equipped with a FID using a 2 m × 3 mm inside diameter column of 10% Carbowax 20M on Chromosorb W. Preparative TLCs were carried out with silica gel 60 $PF_{254+366}$ (Merck).

General Incubation Procedure. (a) Microbe Culture. The cultures of *Rhodotorula rubra* and *Curvularia lunata* were obtained from the Institute of Fermentation, Osaka, Japan, and were identified by their IFO catalog serial numbers IFO 0889 and IFO 6288, respectively, unless otherwise specified.

(b) Culture Medium. The culture medium for these microorganisms was prepared[31] by dissolving glucose (30 g), KH_2PO_4 (1 g), cornsteep 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 1000 mL of tap water and was sterilized at 122–123 °C for 15 min before incubation.

(27) (a) Mislow, K.; McGinn, F. A. *J. Am. Chem. Soc.* **1958**, *80*, 6036–8. (b) Mislow, K.; Prelog, V.; Scherrer, H. *Helv. Chim. Acta* **1958**, *41*, 1410–3.

(28) Krow, G. *Top. Stereochem.* **1970**, *5*, 31–68.

(29) While asymmetric Meerwein–Ponndorf reduction of the (±)-bridged biphenyl ketone **14** with (+)-(*S*)-2-octanol has been reported[25] to yield the (−)-*S* ketone **14** (optical purity 34.8%) and the (−)-*R* alcohol **15** (optical purity 11.7%), the similar reduction of (±)-bridged binaphthyl ketone **16** afforded the (−)-*S* ketone **16** and (−)-*R* alcohol **17** with 34.6 and 10.2% optical purity, respectively.[27a]

(30) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 8476–82. Jones, J. B.; Beck, J. F. Ref 4b, Chapter 4.

(31) Kieslich, K. *Synthesis* **1969**, *1*, 120–34.

(c) Growth of Microorganism. Twenty-five milliliters of the culture medium in a 100-mL Erlenmeyer flask was inoculated with spores of the microbe grown on Difco YM agar slants and was shaken for 48 h at 29–30 °C on a shaker. The culture was transferred into a 500-mL Erlenmeyer flask containing 200 mL of the culture medium, and incubation was maintained for another 48 h at 29–30 °C until a sufficient mass of mycelium had developed.

(d) Feeding of Substrate. After the substrate ketone (60–125 mg/200 mL of culture) in EtOH (4–5 mL) was added to the growing culture, the incubation was continued for a suitable period.

(e) Isolation of Metabolites. At harvest, the mycelium was collected by filtration through a layer of Hyflo Super Cel and extracted with ether. The beer filtrate was also extracted with ether. The combined ether extracts were washed with water and dried (Na_2SO_4), and the solvent was evaporated to give a crude metabolite mixture.

Microbial Reduction of (±)-9-*twist*-Brendanone (3). The substrate racemic ketone **3** was prepared by following Sauers' procedure;[32] mp 142–143.5 °C (lit.[32] mp 141–143 °C) (in a sealed tube).

(a) Reduction with *C. lunata*. The racemic ketone **3** (total 200 mg) was incubated with two batches of 200 mL of *C. lunata* culture for 40 h at 29 °C. GLC of the crude metabolite mixture (217 mg) indicated that it contained the recovered ketone **3** and the alcohol **4** in a ratio of 51:49.

The mixture was taken up in *n*-pentane and chromatographed on 10 g of neutral alumina (activity 3). Elution with 150 mL of *n*-pentane followed by 150 mL of *n*-pentane–ether (9:1) afforded 73.7 mg of the crude ketone **3** and 78.9 mg of the crude alcohol **4**.

The crude ketone **3** was purified by preparative TLC followed by sublimation in vacuo (80 °C, 30 mm) to give 60 mg of (−)-9-*twist*-brendanone (**3**): 30% yield; mp 170–171.5 °C (in a sealed tube); $[\alpha]^{27}_D$ −179.3° (*c* 0.38, MeOH); optical purity 63.6% [lit.[11] mp 159–161 °C; $[\alpha]^{19}_D$ +176° (*c* 0.57, MeOH)]; CD (*c* 6.09 × 10^{-3}, isooctane) λ nm (Θ), 245 (0), 288.5 (+3.19 × 10^3), 320 (0).

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.57; H, 8.95.

The alcohol **4** was purified by preparative TLC followed by sublimation in vacuo to give 57.2 mg of (+)-9-*twist*-brendanol (**4**): 24% yield; mp 177–179 °C (in a sealed tube); $[\alpha]^{27}_D$ +221.7° (*c* 0.38, MeOH); optical purity 84.6% [lit.[11] mp 162–165 °C, $[\alpha]^{23}_D$ +164° (*c* 0.66, MeOH)].

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.07; H, 10.31.

(b) Reduction with *R. rubra*. The racemic ketone **3** (total 300 mg) was incubated with three batches of 200 mL of *R. rubra* culture for 50 h at 29 °C. The crude metabolite (350 mg) was found to contain the recovered ketone **3** and the alcohol **4** in a ratio of 78:22.

Separation and purification similar to those described for the *C. lunata* metabolite mixture afforded the following products. (a) (−)-**3**: 96.3 mg (32% yield); mp 168–171 °C (in a sealed tube); $[\alpha]^{27}_D$ −28.7° (*c* 1.9, MeOH); optical purity 10.2%. Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.19; H, 9.09. (b) (+)-**4**: 36.4 mg (12% yield); mp 176–178 °C (in a sealed tube); $[\alpha]^{27}_D$ +137.7° (*c* 0.56, MeOH); optical purity 52.6%. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.23; H, 10.21.

Microbial Reduction of (±)-2-Brexanone (5) with *C. lunata*. The substrate racemic ketone **5** was prepared by following Nickon's procedure:[33] bp 104–105 °C (26 mm); n^{26}_D 1.4950 (lit.[33] n^{26}_D 1.4951).

The racemic ketone **5** (total 400 mg) was incubated with five batches of 200 mL of *C. lunata* culture for 72 h at 30 °C. The crude metabolite (430 mg) was shown to contain the recovered ketone **5** and the alcohol **6** in a ratio of 66:34 by GLC analysis.

The mixture taken up in *n*-pentane was chromatographed on 12 g of neutral alumina (activity 3). The column was eluted with 300 mL of *n*-pentane to give 270 mg of the crude ketone **5**. Further elution with 100 mL of *n*-pentane–ether (4:1) gave 70 mg of the crude alcohol **6**.

The crude ketone was purified by preparative TLC followed by distillation to give 139 mg of (+)-2-brexanone (**5**): 35% yield; bp 100–105 °C (27 mm); n^{22}_D 1.4967; $[\alpha]^{26}_D$ +65.0° (*c* 0.64, EtOH); optical purity 21% [lit.[15] bp 116 °C (20 mm); $[\alpha]^{14}_D$ −201° (*c* 0.677, EtOH)]. Although the material was homogeneous on TLC and GLC, a satisfactory elemental analysis could not be obtained.

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 78.47; H, 8.97. MS *m/e* 136 [calcd for $C_9H_{12}O$ *m/e* 136 (M^+)].

The crude alcohol **6** was purified by preparative TLC followed by sublimation in vacuo (70 °C, 20 mm) to give 50 mg of (−)-2-brexanol (**6**): 12% yield; mp 86–87 °C (in a sealed tube); $[\alpha]^{25}_D$ −157.7° (*c* 0.34, EtOH); optical purity 100% [lit.[14] mp 84–85.5 °C; $[\alpha]^{21}_D$ −102° (*c* 0.218, EtOH)].

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.04; H, 10.21.

Microbial Reduction of (±)-D_3-Trishomocubanone (8). The racemic substrate ketone **8** was prepared by following Eaton's procedure;[34] mp 164–165 °C (lit.[34] mp 163–164 °C).

(a) Reduction with *C. lunata*. The racemic ketone **8** (total 200 mg) was incubated with two batches of 200 mL of *C. lunata* culture for 3 h at 29 °C. The crude metabolite mixture (350 mg) was analyzed by GLC to reveal its 57:43 composition of the recovered ketone **8** and the alcohol **9**.

The metabolite mixture taken up in *n*-pentane was chromatographed on 10 g of neutral alumina (activity 3). The column was eluted with 240 mL of *n*-pentane to give 96 mg of the ketone **8**. Further elution with 160 mL of *n*-pentane–ether (10:1) gave 82 mg of the alcohol **9**.

Purification of the crude ketone **8** by sublimation in vacuo (110 °C, 20 mm) yielded 79 mg of (−)-D_3-trishomocubanone (**8**): 40% yield; mp 162–164 °C (in a sealed tube); $[\alpha]^{28}_D$ −35.1° (*c* 0.857, EtOH); optical purity 39.7% [lit.[16b] mp 163–163.5 °C; $[\alpha]^{25}_D$ +83.2° (*c* 0.65, EtOH)].

Anal. Calcd for $C_{11}H_{12}O$: C, 82.46; H, 7.55. Found: C, 82.17; H, 7.58.

The crude alcohol **9** was purified by preparative TLC followed by sublimation in vacuo (120 °C, 20 mm), providing 49 mg of (+)-D_3-trishomocubanol (**9**): 25% yield; mp 165–167 °C (in a sealed tube); $[\alpha]^{28}_D$ +92.12° (*c* 0.72, EtOH); optical purity 60.6% [lit.[16b] mp 168.5–169.5 °C; $[\alpha]^{25}_D$ +143° (*c* 0.590, EtOH)].

Anal. Calcd for $C_{11}H_{14}O$: C, 81.44; H, 8.70. Found: C, 81.16; H, 8.68.

(b) Reduction with *R. rubra*. The racemic ketone **8** (total 200 mg) was incubated with two 200-mL batches of *R. rubra* culture for 4 h at 30 °C. The crude ether extract was shown to contain the ketone **8** and the alcohol **9** in a ratio of 53:47.

Purification of the metabolite mixture by preparative TLC followed by sublimation afforded the following products. (a) (−) ketone **8**: 62 mg (31% yield); mp 161–163 °C (in a sealed tube); $[\alpha]^{28}_D$ −4.96° (*c* 0.85, EtOH); optical purity 5.6%. Anal. Calcd for $C_{11}H_{12}O$: C, 82.46; H, 7.55. Found: C, 82.16; H, 7.59. (b) (+) alcohol **9**: 55 mg (27% yield); mp 166–168 °C (in a sealed tube); $[\alpha]^{28}_D$ +19.0° (*c* 0.5, EtOH); optical purity 12.5%. Anal. Calcd for $C_{11}H_{14}O$: C, 81.44; H, 8.70. Found: C, 81.37; H, 8.67.

Microbial Reduction of (±)-Bisnoradamantanone (10) with *C. lunata* (IFO 6299). The racemic substrate ketone **10** was prepared by following Sauers' procedure;[35] mp 108–110 °C (lit.[35] mp 106–110 °C) (in a sealed tube).

The substrate ketone **10** (total 500 mg) was incubated with eight batches of 200 mL of *C. lunata* (IFO 6299) culture for 10 days at 29 °C. The ether extract (540 mg), which was shown to be composed of the recovered ketone **10** and the metabolite alcohol **11** in a ratio of 9:1 by GLC analysis, was dissolved in *n*-pentane and chromatographed on 10 g of neutral alumina (activity 3). The column was eluted with 150 mL of *n*-pentane to give 310 mg of the crude ketone **10**. Further elution with 200 mL of *n*-pentane–ether (9:1) gave 100 mg of the crude alcohol **11**.

The crude ketone was purified by rechromatography on alumina followed by sublimation in vacuo (60 °C, 20 mm) to give 100 mg of (+)-bisnoradamantanone (**10**): 20% yield; $[\alpha]^{25}_D$ +2.2° (*c* 1.86, EtOH); optical purity 3% [lit.[18] $[\alpha]^{13}_D$ −55.9° (*c* 0.347, EtOH];

(32) Sauers, R. R.; Whittle, J. A. *J. Org. Chem.* **1969**, *34*, 3579–82.
(33) Nickon, A.; Kwasnik, H.; Swartz, T.; Williams, R. O.; DiGiorgio, J. B. *J. Am. Chem. Soc.* **1965**, *87*, 1613–5.
(34) Eaton, P. E.; Hudson, R. A.; Giordano, C. *J. Chem. Soc., Chem. Commun.* **1974**, 978.
(35) Sauers, R. R.; Kelly, K. W.; Sickles, B. R. *J. Org. Chem.* **1972**, *37*, 537–43.

CD (c 1.13 × 10^{-1}, isooctane) λ nm (Θ), 235 (0), 286 (+6.0 × 10^2), 312 (0).

Anal. Calcd for $C_8H_{10}O$: C, 78.65; H, 8.25. Found: C, 78.64; H, 8.06.

Preparative TLC of the crude metabolite alcohol 11 afforded 20 mg of a semisolid, but its high volatility prevented further purification.

Microbial Reduction of (±)-4′,1″-Dimethyl-1,2:3,4-dibenzo-1,3-cycloheptadien-6-one (14). The racemic substrate ketone **14** was prepared following Mislow's procedure;[24] mp 61–63 °C (lit.[24] mp 61–63 °C).

(a) Reduction with *R. rubra*. The racemic ketone **14** (total 1 g) was incubated with eight batches of 200 mL of *R. rubra* culture for 25 h at 30 °C. The crude ether extract of metabolites (980 mg), which was shown to be a 47:53 mixture of the recovered ketone **14** and the metabolite alcohol **15** by GLC analysis, was chromatographed on 25 g of neutral alumina (activity 3). Elution with n-pentane followed by n-pentane with increasing amounts of ether yielded the following 80-mL fractions: fractions 1–13 (n-pentane), fractions 14–16 (10:1 n-pentane-ether), fractions 17–25 (10:2 n-pentane–ether), fractions 26–27 (ether). The fractions were monitored by TLC, and the fractions containing the recovered ketone **14** and the alcohol **15** were combined separately.

Fractions 3–11 yielded 390 mg of the ketone **14** (39% yield) which was purified by preparative TLC to give 310 mg of (+)-4′,1″-dimethyl-1,2:3,4-dibenzo-1,3-cycloheptadien-6-one (**14**): 31% yield; mp 61–63 °C; $[\alpha]^{30}_D$ +629.5° (c 0.195, benzene); optical purity 100% [lit.[24] mp 62.5–63.5 °C; $[\alpha]^{28}_D$ –628° (c 1.0, benzene)]; CD (c 0.822 × 10^{-3}, isooctane) λ nm (Θ), 231.5 (0), 245 (+8.73 × 10^4), 281 sh (+3.77 × 10^4), 290.4 (+8.69 × 10^4), 297.4 (+11.7 × 10^4), 307 (+10.51 × 10^4), 317.5 (+5.23 × 10^4), 330 (0); NMR (CCl_4) δ 2.2 (s, 6 H, 2 CH_3), 3.17 and 3.50 (each d, each J = 15 Hz, 4 H, 2 CH_2), 7.11 (m, 6 H, aromatic H).

Anal. Calcd for $C_{17}H_{16}O$: C, 86.40; H, 6.84. Found: C, 86.47; H, 6.93.

Fractions 17–24 gave 395 mg of the alcohol **15** (40% yield) whose purification by preparative TLC provided 340 mg of (+)-4′,1″-dimethyl-1,2:3,4-dibenzo-1,3-cycloheptadien-6-ol (**15**): 34% yield; mp 78–79.5 °C; $[\alpha]^{30}_D$ +132.0° (c 0.533, benzene); optical purity 94% [lit.[24] mp 79–81 °C; $[\alpha]^{27}_D$ +141° (c 1.0, benzene)]; NMR (CCl_4) δ 1.8 (s, 1 H, OH), 1.9 (dd, J = 9 and 11 Hz, 1 H, C-5 H), 2.08 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3), 2.38 (d, J = 3 Hz, 2 H, C-7 H), 2.65 (dd, J = 6 and 12 Hz, 1 H, C-5 H), 4.02 (m, 1 H, C-6 H), 7.02 (m, 6 H, aromatic H).

Anal. Calcd for $C_{17}H_{18}O$: C, 85.67; H, 7.61. Found: C, 85.60; H, 7.68.

Further recrystallization of (+) alcohol **15** from n-hexane gave a sample which showed the following: mp 78.5–79.5 °C; $[\alpha]^{29}_D$ +142.0° (c 0.844, benzene); optical purity 100%; CD (c 0.83 × 10^3, isooctane) λ nm (Θ), 228.4 (–61.4 × 10^3), 240 (–98.8 × 10^3), 263.4 (0), 269 (+4.94 × 10^3), 273.8 (0), 276.2 (–0.84 × 10^3), 282.5 (–5.96 × 10^3), 292 (0).

(b) Reduction with *C. lunata*. Twenty hours of incubation of a total of 500 mg of the racemic ketone **14** in four 200-mL batches of *C. lunata* culture at 29 °C afforded a crude metabolite mixture which was shown to be a 70:30 mixture of the recovered ketone **14** and the metabolite alcohol **15** by GLC analysis.

Workup according to the procedure outlined for the incubation with *R. rubra* afforded (a) 100 mg of the recovered ketone **14** (20% yield) as an oil with $[\alpha]^{27}_D$ +0.99° (c 1.1, benzene) and optical purity 0.16% and (b) 75 mg of the alcohol **15** (15% yield) with mp 118–118.5 °C, $[\alpha]^{24}_D$ 0° (c 0.5, benzene). Anal. Calcd for $C_{17}H_{18}O$: C, 85.67; H, 7.61. Found: C, 85.80; H, 7.46.

Microbial Reduction of (±)-2′,1′:1,2;1″,2″:3,4-Dinaphtho-1,3-cycloheptadien-6-one (16) with *C. lunata*. The substrate racemic ketone **16** was prepared by following Mislow's procedure;[27a] mp 208–210 °C (lit.[27a] mp 207–210 °C).

The racemic ketone **16** (500 mg) in DMF (40 mL) was divided among eight 200-mL batches of *C. lunata* culture, and these culture mixtures were incubated for 215 h at 29 °C.

The process was monitored with TLC which was developed with $CHCl_3$–MeOH (50:1) to exhibit R_f 0.76 and 0.52 for the ketone **16** and the alcohol **17**, respectively.

The crude metabolite (1.2 g) was chromatographed on 30 g of neutral alumina (activity 2) to yield the following 30-mL fractions (eluent): fractions 1–7 (2:1 benzene–n-hexane), fractions 8–17 (benzene), fractions 18–25 (9:1 benzene–$CHCl_3$), fractions 26–32 (1:1 benzene–$CHCl_3$).

Monitoring with TLC indicated the fractions 4–6 were to be combined to yield 500 mg of the crude ketone **16** which was purified by preparative TLC (benzene elution) followed by rechromatography on neutral alumina (15 g, activity 2, elution with 1:1 n-hexane–benzene) to give 230 mg of (+)-(*R*)-2′,1′:1,2;-1″,2″:3,4-dinaphtho-1,3-cycloheptadien-6-one (**16**): 46% yield; mp 191–196 °C; $[\alpha]^{24}_D$ +4.5° (c 1.66, benzene); optical purity 3.3% [lit.[27a] bp 160 °C (0.005 mm); $[\alpha]^{20}_D$ +134° (c 0.40, benzene)]; NMR ($CDCl_3$) δ 3.52 and 3.68 (each d, each J = 15 Hz, 4 H, 2 CH_2), 7.0–8.0 (m, 12 H, aromatic H).

Anal. Calcd for $C_{23}H_{16}O$: C, 89.58; H, 5.23. Found: C, 89.76; H, 5.12.

The fractions 30–32 were combined to furnish 90 mg of the crude alcohol **17** whose purification by chromatography on neutral alumina (5 g, activity 2, elution with benzene) followed by preparative TLC (elution with 50:1 $CHCl_3$–MeOH) afforded 60 mg (12% yield) of (+)-(*S*)-2′,1′:1,2;1″,2″:3,4-dinaphtho-1,3-cycloheptadien-6-ol (**17**) as a hard colorless glassy material: $[\alpha]^{26}_D$ +411° (c 0.33, benzene); optical purity 64.8% [lit.[27a] glassy state, $[\alpha]^{20}_D$ –634° (c 2.2, benzene)]; CD (c 7.39 × 10^{-4}, dioxane) λ nm (Θ), 224 (0), 232 (+1.45 × 10^6), 241.5 (0), 243.7 (–2.64 × 10^4), 248 (0), 258.5 (+6.5 × 10^4), 266.3 (+7.99 × 10^4), 276 sh (+3.18 × 10^4), 282 (0), 287.5 sh (–2.40 × 10^4), 304 (–3.98 × 10^4), 311 sh (–3.62 × 10^4), 325 (–2.62 × 10^4), 338 (0); UV (c 1.48 × 10^{-4}, dioxane) λ_{max} 220 nm (ε 7.86 × 10^4), 232 (6.21 × 10^4), 267 sh (4.67 × 10^4), 306 (9.72 × 10^3); NMR ($CDCl_3$) δ 1.7 (s, 1 H, OH), 2.28 (dd, J = 10 and 12 Hz, 1 H, C-5 H), 2.75 (d, J = 3 Hz, 2 H, C-7 H), 3.02 (dd, J = 6 and 12 Hz, 1 H, C-5 H), 4.5 (m, 1 H, C-6 H), 7.2–7.6 (m, 8 H, aromatic H), 7.81 and 7.95 (each d, each J = 3 Hz, 2 H, aromatic H).

Mislow and co-workers[27a] reported that their optically active alcohol **17** resisted crystallization, and our specimen also was found to exhibit the same habit. Although we were unable to prepare an analytical sample showing satisfactory elemental analysis, comparison of its IR and NMR spectra with those of the crystalline racemic alcohol **17** established its identification.

Registry No. (±)-**3**, 69056-10-6; (–)-**3**, 69056-11-7; (+)-**4**, 57287-42-0; (±)-**5**, 71806-61-6; (+)-**5**, 69126-55-2; (–)-**6**, 68035-51-8; (±)-**8**, 66007-14-5; (–)-**8**, 61473-76-5; (+)-**9**, 61473-81-2; (±)-**10**, 71806-62-7; (+)-**10**, 71806-63-8; (–)-**11**, 71806-64-9; (±)-**14**, 69009-72-9; (+)-**14**, 56638-10-9; (+)-**15**, 69009-73-0; (±)-**15**, 71806-65-0; (±)-**16**, 71806-66-1; (+)-**16**, 71766-67-1; (+)-**17**, 35216-73-0.