5j, 37549-88-5; **5k**, 40558-71-2; **5l**, 16789-51-8; **5m**, 77357-32-5; **5n**, 77341-87-8; **5o**, 77341-88-9; **5p**, 54624-35-0; **5q**, 77341-89-0; **5r**, 77341-90-3; **6h**, 110-83-8; **7a**, 533-98-2; **7b**, 3234-49-9; **7c**, 10288-13-8; **7d**, 594-34-3; **7e**, 78-75-1; *erythro*-**7f**, 5780-13-2; *threo*-**7f**, 40205-58-1; *cis*-**7g**, 33547-17-0; *trans*-**7g**, 10230-26-9; **8d**, 1192-37-6; **9a**, 106-93-4; **12a**, 77341-91-4; **12b**, 65087-61-8; **12c**, 1184-59-4; **12d**, 23042-68-4; **13a**, 1517-49-3; **13b**, 26119-76-6; **13c**, 1560-57-2; *erythro*-**14a**, 77341-92-5; *threo*-**14a**, 77341-93-6; **14b**, 77341-94-7; **14c**, 60623-79-2; *erythro*-**14d**, 77341-95-8; *threo*-**14d**, 77341-96-9; **14e**, 77341-97-0; **14f**, 77341-98-1; ethyl bromide, 74-96-4; propyl bromide, 106-94-5; allyl bromide, 106-95-6; butyl bromide, 109-65-9; isobutyl bromide, 507-19-7; phenyl bromide, 108-86-1; benzyl bromide, 100-39-0.

C_2-Ketone Rule in Horse Liver Alcohol Dehydrogenase (HLADH) Mediated Oxidoreduction[1]

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The enantiomer selectivity of HLADH with respect to various C_2 ketones and the corresponding C_2 alcohols was examined and revealed that the enzyme exhibits a marked enantiomer selectivity opposite to the microbial "(*P*)-C_2-ketone rule" found in *Curvularia lunata* and *Rhodotorula rubra*.

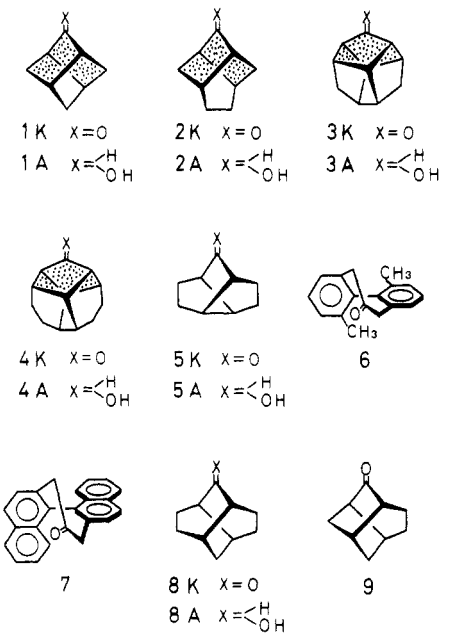
Contrary to a vast accumulation of information on the stereochemistry of microbial reduction of numerous ketones with a variety of structural features, it seems rather surprising to realize that so small an effort has been expended for exploring the microbiol stereodifferentiating reduction in cage-shaped compounds.

Our continuing interests in the synthetic studies of high-symmetry, chiral (gyrochiral), cage-shaped compounds had provided us with a considerable collection of cage-shaped polycyclic ketones, and this prompted us to investigate the expected microbial stereodifferentiating reactions toward these substrates.

To summarize the stereochemistry in microbial reduction of a large number of racemic cage-shaped C_1 ketones[2] with *Curvularia lunata* and *Rhodotorula rubra*, we have proposed[3] a "quadrant rule" whose successful applications[4] have been reported from our laboratory.

Our other efforts in studying the enantiomer selectivity of these microbes in a considerable number of C_2 ketones[2] (Chart I),[5] including D_{2d}-bisnoradamantanone (**1K**), 9-*twist*-brendanone (**2K**), D_3-trishomocubanone (**3K**), 4-C_2-methanoditwistanone (**4K**), 2-brexanone (**5K**), and the biaryl bridged ketones **6** and **7**, have been rewarded by our finding a microbial "(*P*)-C_2-ketone rule"[6] which states that both microbes selectively reduce the (*P*)-C_2-ketone enantiomers, furnishing the corresponding (*P*)-C_2 alcohol.[7]

Chart I



An interesting stereochemical characteristic common to these favored cage-shaped (*P*)-C_2 ketones **1K-4K** is the 7-bicyclo[2.2.1]heptanone framework (shown with dotting in Chart I) with a $(-90°, +20°, +55°)_2$[8] twist-boat cyclohexane moiety.

(1) A preliminary account of this work has been published: Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Sasaki, Y.; Fujii, T. *J. Chem. Soc., Chem. Commun.* **1980**, 626-7.

(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 belong to the C_1 point group and have no symmetry element passing through the carbonyl axis.

(3) (a) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Hirose, Y.; Shimizu, T.; Asao, M. *J. Chem. Soc., Chem. Commun.* **1978**, 668-70. (b) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Asao, M. *J. Org. Chem.* **1980**, *45*, 4432-40.

(4) (a) Nakazaki, M.; Chikamatsu, H.; Hirose, Y.; Shimizu, T. *J. Org. Chem.* **1979**, *44*, 1043-8. (b) Nakazaki, M.; Hirose, Y.; Shimizu, T.; Suzuki, T.; Ishii, A.; Makimura, M. *Ibid.* **1980**, *45*, 1428-35. (c) Nakazaki, M.; Chikamatsu, H.; Fujii, T.; Nakatsuji, T. *Ibid.* **1981**, *46*, 585-9.

(5) All structural formulas with (+) or (-) specification in this paper are presented in their absolute configurations.

(6) (a) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Nishino, M; Murakami, H.; Asao, M. *J. Chem. Soc., Chem. Commun.* **1978**, 667-8. (b) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Nishino, M.; Murakami, H.; Asao, M. *J. Org. Chem.* **1979**, *44*, 4588-93.

(7) Although the alcohols corresponding to the C_2-ketone precursors do not belong to C_2 point group, these are conveniently called C_2 alcohols in this paper.

(8) Klyne, W.; Prelog, V. *Experientia* **1960**, *16*, 521-3.

0022-3263/81/1946-2726$01.25/0

Table I. Relative Rates of HLADH-Catalyzed Oxidoreduction of C_2 Ketones and C_2 Alcohols[a]

substr[b]	rel rate	substr[b]	rel rate
cyclohexanone	100	cyclohexanol	100
(±)-1K	0.35	(±)-1A	11.4
(+)-(*M*)-1K (57%)	0.37		
(−)-(*P*)-1K (46%)	0.28	(+)-(*P*)-1A (52%)	5.7
(±)-2K	14.7	(±)-2A	32.3
(−)-(*M*)-2K (81%)	24.0	(−)-(*M*)-2A (80%)	47.4
(+)-(*P*)-2K (99%)	3.9	(+)-(*P*)-2A (50%)	22.7
(±)-3K	100	(±)-3A	20.25
(−)-(*M*)-3K (63%)	128	(−)-(*M*)-3A (81%)	31.8
(+)-(*P*)-3K (93%)	2.7	(+)-(*P*)-3A (23%)	11.4
(±)-4K	1.3	(±)-4A	2.2
(−)-(*M*)-4K (100%)	9.7		
(+)-(*P*)-4K (81%)	0.13		
(±)-5K	0.13	(±)-5A	0.4
(±)-6K	0.61		
(±)-8K	0.17	(±)-8A	0.0

[a] Reduction and oxidation rates were measured at 25 °C in $^1/_{15}$ M Sørensen phosphate buffer (pH 7.0) and $^1/_{20}$ M glycine–NaOH buffer (pH 9.0), respectively. [b] For the optically active substrates, their *M* or *P* helicity and optical purity are indicated in parentheses.

A natural extension of these studies led us to examine the expected C_2-ketone enantiomer selectivity of crystalline horse liver alcohol dehydrogenase (HLADH)[9] whose stereoselectivity has been extensively studied by Prelog's ETH group, leading to their proposal of the "diamond lattice section theory".[10,11]

Results and Discussion

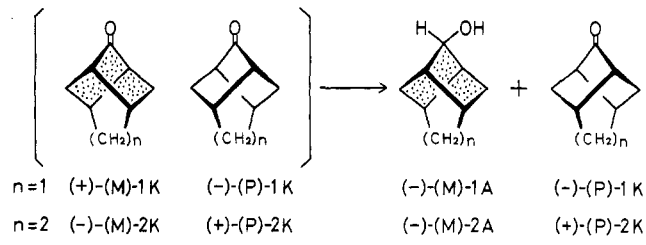
Kinetic Studies. The relative rates of HLADH-catalyzed oxidoreduction (eq 1) of the C_2 ketone and corresponding C_2 alcohol substrates are summarized in Table I.

$$>C{=}O + NADH + H^+ \rightleftharpoons >C<_{OH}^{H} + NAD^+ \qquad (1)$$

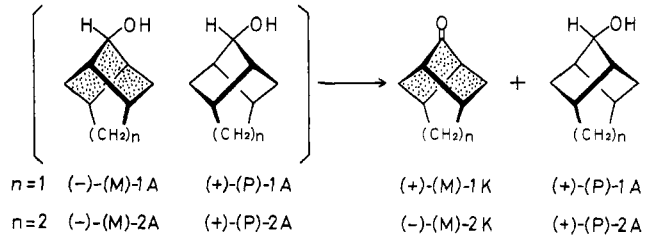
Inspection of the Table I reveals two conspicuous features: (a) the relative rate of the C_2 ketone substrates in this enzyme-mediated oxidoreduction is roughly parallel to that previously found in the microbial reduction with *C. lunata* and *R. rubra*, and (b) the enantiomeric C_2 ketones and C_2 alcohols with *M* helicity, opposite the microbial reduction, are more readily converted into the corresponding metabolites than their enantiomers.

While D_3-trishomocubanone (**3K**) was found to be outstanding in its high reduction rate, comparable to cyclohexanone and its high enantiomer differentiation ratio (ca. 50:1), a slight structural deviation from it to give 4-C_2-methanoditwistanone (**4K**) provided a sharp decline in the reaction rate. Both D_{2d}-bisnoradamantanone (**1K**) and 2-brexanone (**5K**) exhibited markedly low reaction rates, parallel to their reported reluctance in the microbial reduction.

Scheme I



Scheme II



A peculiar finding reported in our previous papers[6] is complete immunity of 2-twistanone (**8K**) and 2-*twist*-brendanone (**9**) against the microbial reduction, and this is found also to be the case in HLADH-mediated oxidoreduction as indicated by the low reaction rates (Table I) observed in the ketone **8K** and the corresponding alcohol **8A**.

Our finding of this novel "(*M*)-C_2-ketone rule" in HLADH-mediated oxidoreduction prompted us to test its applicability in the preparative-scale optical resolution of these C_2 ketones (Table II).

HLADH-Mediated Oxidoreduction in D_{2d}-Bisnoradamantanone (1K) and D_{2d}-Bisnoradamantanol (1A) (Schemes I and II). Our previous paper[6b] has reported that 10 days of incubation (29 °C) with a strain of *C. lunata* (IFO 6299) instead of our customary IFO 6288 strain was required to recover a 20% yield of (+)-(*M*)-ketone **1K** of a low optical purity (3%), and the same reluctance was also observed in the enzymatic process but with opposite stereochemical differentiation; 48 h of incubation of (±)-C_2-ketone **1K** in the phosphate buffer with HLADH and NADH at 20 °C furnished a 15% conversion which was found to be improved only to 16% after another 74 h of incubation.

Chromatography of the metabolite afforded a 25% yield of the recovered (−)-(*P*)-ketone **1K** and an 8% yield of the (−)-(*M*)-alcohol **1A** with 13% and 51% optical purities, respectively.

A test-scale recycling experiment employing 0.1 molar equiv of NAD^+ in conjuction with sodium dithionite gave a low conversion ratio (5%), preventing us from trying this approach on a preparative scale.

The enhanced enantiomer selectivity suggested by the kinetic data of the C_2-alcohol **1A** (Table I) seemed to promise that the oxidative approach should be a practical process for optical resolution.

The C_2-alcohol **1A** was incubated with HLADH and NAD^+ in the glycine–NaOH buffer until GLC monitoring indicated a 53% conversion (48 h), and separation of the metabolites furnished a 22% yield of the (+)-(*M*)-ketone **1K** and a 45% yield of the (+)-(*P*)-alcohol **1A** with 57% and 52% optical purities, respectively.

HLADH-Mediated Oxidoreduction of 9-*twist*-Brendanone (2K) and 9-*twist*-Brendanol (2A) (Schemes I and II). The kinetic data in Table I suggested that the 9-oxygenated *twist*-brendane derivatives (**2K** and **2A**) should be smoothly converted in both oxi-

(9) Abbreviations used: HLADH, horse liver alcohol dehydrogenase; NADH and NAD^+, reduced and oxidized forms, respectively, of nicotinamide adenine dinucleotide; FMN, flavin mononucleotide.

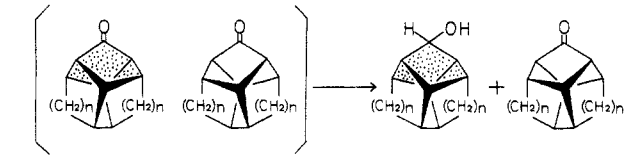
(10) (a) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119–30. (b) For a recent review, see: Jones, J. B.; Beck, J. F. "Applications of Biochemical Systems in Organic Chemistry"; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Part 1, pp 295–307.

(11) For the recent applications of the "diamond lattice section theory" in preparative-scale optical resolution, see: (a) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 8476–82; (b) Irwin, A. J.; Jones, J. B. *Ibid.* **1977**, *99*, 556–61.; (c) Irwin, A. J.; Jones, J. B. *Ibid.* **1977**, *99*, 1625–30; (d) Jones, J. B.; Goodbrand, H. B. *Can. J. Chem.* **1977**, *55*, 2685–91; (e) Davies, J.; Jones, J. B. *J. Am. Chem. Soc.* **1979**, *101*, 5405–10.

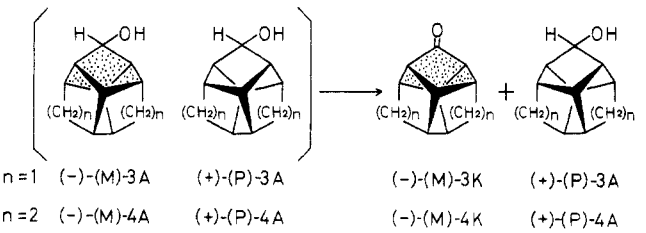
Table II. HLADH-Catalyzed Oxidoreduction of C_2 Ketones and C_2 Alcohols

	substr	coenzyme	reaction time, h	% conversion	optical purity, % (yield, %) ketone	optical purity, % (yield, %) alcohol
1	(±)-1K	NADH	48	15	13 (25), (−)-*P*	51 (8), (−)-*M*
2	(±)-1A	NAD^+	48	53	57 (22), (+)-*M*	52 (45), (+)-*P*
3	(±)-2K	NADH	6	47	90 (24), (+)-*P*	73 (33), (−)-*M*
4	(±)-2K	$NAD^+ + Na_2S_2O_4$	5	56	99 (25), (+)-*P*	63 (39), (−)-*M*
5	(±)-2A	NAD^+ + FMN	15	61	32 (45), (−)-*M*	50 (35), (+)-*P*
6	(±)-3K	NADH	2	52	93 (32), (+)-*P*	76 (40), (−)-*M*
7	(±)-3K	NAD^+ + EtOH	5	38	59 (36), (+)-*P*	81 (31), (−)-*M*
8	(±)-3A	NAD^+ + FMN	52	32	63 (18), (−)-*M*	23 (54), (+)-*P*
9	(±)-4K	NADH	70	43	81 (42), (+)-*P*	96 (35), (−)-*M*
10	(±)-4A	NAD^+ + FMN	96	40	100 (34), (−)-*M*	76 (46), (+)-*P*

Scheme III



Scheme IV



dative and reductive directions with high enantiomer selectivity.

Incubation of the (±)-C_2-ketone **2K** with HLADH and NADH in the phosphate buffer was terminated after 6 h when GLC monitoring indicated a 47% conversion, and chromatography of the metabolite afforded a 33% yield of the (−)-(*M*)-alcohol **2A** and a 24% yield of the recovered (+)-(*P*)-ketone **2K** with respective 73% and 90% optical purities.

A reductive recycling run with sodium dithionite and 0.1 molar equiv of NAD^+ was found to recover the (+)-(*P*)-ketone **2K** with an extremely high optical purity (~99%), and this can be compared with another recycling experiment using ethanol as the recycling reagent which afforded a 41% conversion with very poor enantiomer selectivity, furnishing (−)-(*M*)-**2A** and (+)-(*P*)-**2K** with respective 6.4% and 6.0% optical purities.

A recycling enzymatic oxidation of the (±)-C_2-alcohol **2A** was performed with 0.12 molar equiv of NAD^+ and 2 molar equiv of FMN; the incubation was terminated after 15 h when GLC monitoring indicated a 61% conversion to furnish a 45% yield of (−)-(*M*)-ketone **2K** with 32% optical purity and a 35% yield of the (+)-(*P*)-**2A** with 50% optical purity, demonstrating again the (*M*)-ketone superiority in the enzymatic processes.

HLADH-Mediated Oxidoreduction in D_3-Trishomocubanone (3K) and D_3-Trishomocubanol (3A) (Schemes III and IV). As its high reduction rates indicate (Table I), a 2-h incubation of (±)-D_3-trishomocubanone (**3K**) with HLADH and NADH in the phosphate buffer was found enough to give a 52% conversion, affording a 40% yield of the (−)-(*M*)-C_2-alcohol **3A** (76% optical purity) and a 32% yield of the recovered (+)-(*P*)-ketone **3K** (93% optical purity).

After an unsuccessful reductive recycling experiment with sodium dithionite to yield only 16% conversion, ethanol was employed as the recycling reagent together with 0.1 molar equiv of NAD^+. The incubation was quenched after 5 h when GLC monitoring indicated a 38% conversion, and separation of the metabolites proved this process to be a practical one, affording a 31% yield of (−)-(*M*)-**3A** (81% optical purity) and a 36% yield of (+)-(*P*)-**3K** (59% optical purity).

The kinetic data in Table I predict that, contrary to the reductive direction, the HLADH-catalyzed oxidation of (±)-D_3-trishomocubanol (**3A**) will be sluggish with poor enantiomer selectivity. When the (±)-C_2-alcohol **3A** was incubated with HLADH, NAD^+, and the recycling reagent FMN in the glycine–NaOH buffer for 52 h, there was isolated a 18% yield of the (−)-(*M*)-C_2-ketone **3K** and a 54% yield of the (+)-(*P*)-C_2-alcohol **3A**, both with rather poor optical purities (63% and 23%, respectively).

HLADH-Mediated Oxidoreduction of 4-C_2-Methanoditwistanone (4K) and 4-C_2-Methanoditwistanol (4A) (Schemes III and IV). Our recent microbial reduction of 4-C_2-methanoditwistanone (**4K**) with *R. rubra*[12] has revealed that the reaction was slow and required 238 h of incubation before a 40% conversion was achieved, furnishing a 10% yield of the (+)-(*P*)-alcohol **4A** and a 23% yield of the recovered (−)-(*M*)-ketone **4K**, both with very poor optical purities.

The same reluctance to conversion, but with a surprisingly high and opposite enantiomer selectivity, was observed in the HLADH-catalyzed oxidoreduction of the 4-oxygenated C_2-methanoditwistanes.

Incubation of (±)-C_2-ketone **4K** with HLADH and NADH in the phosphate buffer for 70 h gave a 43% conversion, and chromatography of the metabolite mixture furnished a 35% yield of the (−)-(*M*)-C_2-alcohol **4A** and a 42% yield of the recovered (+)-(*P*)-C_2-ketone **4K** with 96% and 81% optical purities, respectively.

While a reductive recycling experiment with sodium dithionite gave only a 12% conversion after 43 h of incubation, an oxidative recycling experiment with coenzyme FMN proved this process to be successful in both the yield and optical purity of the isolated metabolites; incubation of (±)-4-C_2-methanoditwistanol (**4A**) with 0.1 molar equiv of NAD^+ and 2 molar equiv of FMN for 96 h gave a 40% conversion, and separation of the metabolites furnished a 34% yield of the (−)-(*M*)-C_2-ketone **4K** and a 46% yield of the recovered (+)-(*P*)-C_2-alcohol **4A** with respective 100% and 76% optical purities.

Experimental Section

Optical rotations were measured with a JASCO-DIP-SL polarimeter, and circular dichorism (CD) spectra were taken on a

(12) Nakazaki, M.; Naemura, K.; Chikamatsu, H.; Iwasaki, M.; Hashimoto, M. *Chem. Lett.* **1980**, 1571–2.

JASCO-J-40 spectropolarimeter. GLC analyses were performed on a JGC-20K equipped with a FID and using a 2 m × 3 mm column of 10% Carbowax 20M on Chromosorb W. Preparative TLC was carried out with silica gel 60 $PF_{254+366}$ (Merck).

HLADH was purchased from Boehringer (Mannheim) as a crystalline suspension in phosphate buffer containing 10% EtOH; NAD^+ and NADH were also obtained from the same manufacturer. The enzyme suspension was freeze-dried to a powder immediately before each experiment.

All melting points were measured in sealed tubes and are uncorrected.

General Procedure for the Preparative-Scale HLADH-Catalyzed Oxidoreduction. While the reduction experiments were carried out in $^1/_{15}$ M Sørensen phosphate buffer solution (pH 7.0), the oxidation experiments were performed in 0.05 M glycine–NaOH buffer solution (pH 9.0). The reactions were monitored by GLC, and the incubations (20–25 °C) were terminated when monitoring indicated a ca. 1:1 ratio of alcohol to ketone in the reaction mixture. Extraction of the mixture with ether was followed by drying with $MgSO_4$, and removal of the solvent left a residue whose preparative TLC, when eluted with $CHCl_3$–MeOH (100:3), furnished ketone and alcohol fractions. The separated metabolites were purified through sublimation in vacuo (20–30 mm).

Kinetic Studies. The reductive runs were carried out in $^1/_{15}$ M Sørensen phosphate buffer (pH 7.0) containing NADH (1.8×10^{-4} M) and the substrate ketone (3.6×10^{-4} M for **1K–3K**, **5K**, and **8K** and 1.7×10^{-4} M for **4K**). Because of its poor solubility, the bridged biphenyl ketone **6K** was first dissolved in a minimum amount of dioxane and then brought into the buffer solution to give a 3.5×10^{-4} M solution. The oxidative runs were performed in 0.05 M glycine–NaOH buffer (pH 9.0) containing NAD^+ (5.0×10^{-4} M) and the substrate alcohol (3.4×10^{-4} M for **1A–3A**, **5A**, and **8A** and 1.75×10^{-4} M for **4A**).

The reaction was initiated by adding a 100-μL aliquot of HLADH stock solution (1 mg/1 mL in 0.05 M Tris–HCl buffer, pH 7.4) to the assay solution to make a final solution of 3 mL in a 1-cm path length cuvette.

The absorbance change was monitored at 340 nm at 25 °C, and for each compound a reference assay was performed on a solution containing the same concentration of cyclohexanone or cyclohexanol.

***D_{2d}*-Bisnoradamantanone (1K) and *D_{2d}*-Bisnoradamantanol (1A). Reduction of 1K with HLADH–NADH.** The racemic ketone **1K**[13] (mp 103–105 °C; 100 mg, 0.82 mmol) and NADH (650 mg, 0.87 mmol) were dissolved in 1000 mL of the Sørensen buffer solution. HLADH (8 mg) was then added, and the mixture was incubated at 20 °C for 48 h. Workup of the metabolite mixture whose GLC showed 15% reduction gave the following materials. (a) (−)-*D_{2d}*-Bisnoradamantanone (**1K**): 25 mg (25% yield); mp 107 °C; $[\alpha]^{18}_D$ −10.1° (*c* 0.43, EtOH); optical purity 12.8%. Anal. Calcd for $C_8H_{10}O$: C, 78.65; H, 8.25. Found: C, 78.39; H, 8.25. (b) (−)-*D_{2d}*-Bisnoradamantanol (**1A**):[14] 8 mg (8% yield); mp 119–121 °C; $[\alpha]^{22}_D$ −28.0° (*c* 0.41, $CHCl_3$); optical purity 51.4%. Anal. Calcd for $C_8H_{12}O$; C, 77.37; H, 9.74. Found: C, 76.90; H, 9.71.

Oxidation of 1A with HLADH–NAD^+. The racemic alcohol **1A**[13] (mp 119–123 °C; 200 mg, 1.16 mmol) was dissolved in 1000 mL of the glycine–NaOH buffer solution, and NAD^+ (1.12 g, 1.64 mmol) and HLADH (20 mg) were added. After 48 h at 20 °C when GLC showed 53% oxidation, the reaction was worked up to give the following materials. (a) (+)-Ketone **1K**: 43.7 mg (22% yield); mp 104–106 °C; $[\alpha]^{18}_D$ +45.2° (*c* 0.54, EtOH); optical purity 57.2%. Anal. Calcd for $C_8H_{10}O$: C, 78.65; H, 8.25. Found: C, 78.70; H, 8.30. (b) (+)-Alcohol **1A**: 89.7 mg (45% yield); mp 97–100 °C; $[\alpha]^{18}_D$ +28.1° (*c* 0.72, $CHCl_3$); optical purity 51.6%. Anal. Calcd for $C_8H_{12}O$: C, 77.37; H, 9.74. Found: C, 76.54; H, 9.77.

Jones Oxidation of (+)-*D_{2d}*-Bisnoradamantanol (1A). The (+)-alcohol **1A** (50 mg; $[\alpha]^{18}_D$ +28.1°) was dissolved in 4 mL of acetone and treated with 0.5 mL of 8 N Jones reagent at 0 °C. The routine procedure gave a crystalline product which was sublimed in vacuo [60–65 °C (20 mm)] to afford (−)-ketone **1K**: 28.9 mg (59% yield); mp 96–97 °C; $[\alpha]^{20}_D$ −40.8° (*c* 0.42, EtOH); optical purity 51.6%; CD (*c* 1.54×10^{-3} M, isooctane) $[\Theta]_{286}$ -6.89×10^3. Anal. Calcd for $C_8H_{10}O$: C, 78.65; H, 8.25. Found: C, 78.09; H, 8.28.

9-*twist*-Brendanone (2K) and 9-*twist*-Brendanol (2A). Reduction of 2K with HLADH–NADH. After the racemic ketone **2A**[16] (mp 164–169 °C; 60.2 mg, 0.44 mmol) was dissolved in 600 mL of the Sørensen buffer solution, HLADH (6 mg) and NADH (330.5 mg, 0.44 mmol) were added, and the mixture was incubated for 6 h at 21 °C. Workup of the metabolite mixture whose GLC indicated 47% reduction yielded the following metabolites. (a) (+)-9-*twist*-Brendanone (**2K**): 14.3 mg (24% yield); mp 154–157 °C; $[\alpha]^{20}_D$ +253.0° (*c* 0.27, MeOH); optical purity 90%. Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.17; H, 8.92. (b) (−)-9-*twist*-Brendanol (**2A**): 20 mg (33% yield); mp 177.5–179 °C; $[\alpha]^{20}_D$ −190.0° (*c* 0.32, MeOH); optical purity 72.5%. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.03; H, 10.08.

Reduction of 2K with HLADH–NAD^+–$Na_2S_2O_4$. The racemic ketone **2K** (100.4 mg, 0.74 mmol), NAD^+ (61.5 mg, 0.09 mmol), and $Na_2S_2O_4$ (8.7 g, 0.05 mol) were dissolved in 500 mL of the Sørensen buffer solution. HLADH (16 mg) was then added, and the mixture was incubated at 22 °C for 5 h. Workup of the metabolite mixture whose GLC indicated 56% reduction yielded the following metabolites. (a) (+)-Ketone **2K**: 24.6 mg (25% yield); mp 162–168 °C; $[\alpha]^{19}_D$ +280.0° (*c* 0.28, MeOH); optical purity 99%. Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.41; H, 9.01. (b) (−)-Alcohol **2A**: 40.0 mg (39% yield); mp 174.5–178 °C; $[a]^{23}_D$ −166.0° (*c* 0.90, MeOH); optical purity 63.4%. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.04; H, 10.23.

Oxidation of 2A with HLADH–NAD^+–FMN. The racemic alcohol **2A**[16] (mp 163–170 °C; 100 mg, 0.73 mmol), NAD^+ (60 mg, 0.088 mmol), and FMN (724 mg, 1.46 mmol) were dissolved in 500 mL of the glycine–NaOH buffer solution, and oxidation was initiated by addition of HLADH (8.5 mg). Incubation at 22 °C was terminated after 15 h when GLC monitoring indicated 61% oxidation, and workup of the metabolite mixture afforded the following materials. (a) (−)-Ketone **2K**: 45 mg (45% yield); mp 168.5–172 °C; $[\alpha]^{16}_D$ −90.1° (*c* 0.49, MeOH); optical purity 32%. Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.10; H, 8.97. (b) (+)-Alcohol **2A**: 35 mg (35% yield); mp 176.5–179 °C; $[\alpha]^{20}_D$ +131.0° (*c* 0.4, MeOH); optical purity 50%. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.23; H, 10.24.

***D_3*-Trishomocubanone (3K) and *D_3*-Trishomocubanol (3A). Reduction of 3K with HLADH–NADH.** The racemic ketone **3K**[17] (mp 163–164 °C; 62 mg, 0.387 mmol) and NADH (296.3 mg, 0.40 mmol) were dissolved in 600 mL of the Sørensen buffer solution, and HLADH (8 mg) was added to initiate the reaction. The mixture was incubated at 23 °C, and the reaction was terminated after 2 h when GLC monitoring indicated 52% reduction of the substrate ketone. Workup of the mixture gave the following materials. (a) (+)-*D_3*-Trishomocubanone (**3K**): 20 mg (32% yield); mp 163–164 °C; $[\alpha]^{22}_D$ +82.4° (*c* 0.36, EtOH); optical purity 93%. Anal. Calcd for $C_{11}H_{12}O$: C, 82.46; H, 7.55. Found: C, 82.70; H, 7.51. (b) (−)-*D_3*-Trishomocubanol (**3A**): 25 mg (40% yield); mp 167–168 °C; $[\alpha]^{21}_D$ −116.0° (*c* 0.52, EtOH); opitcal purity 76%. Anal. Calcd for $C_{11}H_{14}O$: C, 81.44; H, 8.70. Found: C, 81.64; H, 8.65.

Reduction of 3K with HLADH–NAD^+–EtOH. After the racemic ketone **3K** (96.8 mg, 0.60 mmol) and NAD^+ (44.6 mg, 0.065 mmol) were dissolved in 1000 mL of the Sørensen buffer solution, HLADH (16 mg) and EtOH (0.2 mL) were added. The reaction mixture was incubated at 22 °C until GLC monitoring indicated 38% reduction of the substrate ketone (5 h), and workup of the metabolite mixture afforded the following materials. (a) (+)-Ketone **3K**: 35 mg (36% yield); mp 163–164 °C; $[\alpha]^{23.5}_D$ +52.3°

(13) Sauers, R. R.; Kelly, K. W.; Sickels, B. R. *J. Org. Chem.* **1972**, *37*, 537–43.

(14) When coupled with the reported $[\alpha]_{D_{abs}}$ +79° of the ketone **1K**,[15] Jones oxidation of a specimen of (+)-alcohol **1A** ($[\alpha]_D$ +28.1°) to the (−)-(*P*)-ketone **1K** ($[\alpha]_D$ −40.8°; see Experimental Section) established the absolute configuration of (+)-**1A** and its $[\alpha]_{D_{abs}}$ of +54.5°.

(15) Nakazaki, M.; Naemura, K.; Arashiba, N. *J. Org. Chem.* **1978**, *43*, 888–91.

(16) Sauers, R. R; Whittle, J. A. *J. Org. Chem.* **1969**, *34*, 3579–82.

(17) Nakazaki, M.; Naemura, K.; Arashiba, A. *J. Org. Chem.* **1978**, *43*, 689–92.

(c 0.47, EtOH); optical purity 59%. Anal. Calcd for $C_{11}H_{12}O$: C, 82.46; H, 7.55. Found: C, 82.18; H, 7.46. (b) (-)-Alcohol **3A**: 30 mg (31% yield); mp 166-167 °C; $[\alpha]^{23}_D$ -122.4° (c 0.465, EtOH); optical purity 81%. Anal. Calcd for $C_{11}H_{14}O$: C, 81.44; H, 8.70. Found: C, 81.21; H, 8.58.

Oxidation of 3A with HLADH-NAD$^+$-FMN. The racemic alcohol **3A**[17] (mp 155-160 °C; 101.5 mg, 0.63 mmol), NAD$^+$ (42.7 mg, 0.063 mmol), and FMN (620 mg, 1.25 mmol) were dissolved in 1000 mL of the glycine-NaOH buffer solution. The reaction was initiated by adding HLADH (8 mg) and was allowed to proceed at 20 °C. The GLC monitoring of the process showed 32% oxidation of the substrate alcohol after 52 h of incubation. The reaction was terminated, and workup of the metabolite mixture gave the following materials. (a) (-)-Ketone **3K**: 18 mg (18% yield); mp 162-163 °C; $[\alpha]^{21}_D$ -55.3° (c 0.33, EtOH); optical purity 63%. Anal. Calcd for $C_{11}H_{12}O$: C, 82.46; H, 7.55. Found: C, 82.12; H, 7.65. (b) (+)-Alcohol **3A**: 55 mg (54% yield); mp 166-168 °C; $[\alpha]^{21}_D$ +34.6° (c 0.50, EtOH); optical purity 23%. Anal. Calcd for $C_{11}H_{14}O$: C, 81.44; H, 8.70. Found: C, 81.41; H, 8.71.

4-C_2-Methanoditwistanone (4K) and 4-C_2-Methanoditwistanol (4A). Reduction of 4K with HLADH-NADH. The racemic ketone **4K**[14] (mp 111-113 °C; 70.0 mg, 0.37 mmol) dissolved in 700 mL of the Sørensen buffer solution was treated with NADH (286 mg, 0.38 mmol) and HLADH (16 mg) at 22 °C. After 70 h when GLC monitoring indicated 43% reduction of the starting material, the incubation was terminated, and workup of the mixture afforded the following metabolites. (a) (+)-4-C_2-Methanoditwistanone (**4K**):[18] 29.4 mg (42% yield); mp 116-118 °C; $[\alpha]^{22}_D$ +206.4° (c 0.265, EtOH); optical purity 81%; CD (c 5.3 × 10^{-4} M, isooctane) $[\Theta]_{291}$ -7.64 × 10^3. Anal. Calcd for $C_{13}H_{16}O$: C, 82.93; H, 8.57. Found: C, 82.96; H, 8.59. (b) (-)-4-C_2-Methanoditwistanol (**4A**):[18] 24.5 mg (35% yield); mp 112-113 °C; $[\alpha]^{22}_D$ -362.4° (c 0.21, EtOH); optical purity 96%. Anal. Calcd for $C_{13}H_{18}O$: C, 82.06; H, 9.54. Found: C, 82.07; H, 9.60.

Oxidation of 4A with HLADH-NAD$^+$-FMN. The racemic alcohol **4A**[12] (mp 101-102 °C; 50.6 mg, 0.265 mmol), NAD$^+$ (18.7 mg, 0.0274 mmol), and FMN (272 mg, 0.55 mmol) were dissolved in 600 mL of the glycine-NaOH buffer solution. HLADH (9.7 mg) was added, and the reaction was allowed to proceed at 25 °C until GLC monitoring indicated 40% oxidation of the substrate alcohol (96 h). Workup of the metabolite mixture gave the following materials. (a) (-)-Ketone **4K**: 17 mg (34% yield); mp 111-112.5 °C; $[\alpha]^{27}_D$ -260° (c 0.10, EtOH); optical purity 100%. Anal. Calcd for $C_{13}H_{16}O$: C, 82.93; H, 8.57. Found: C, 82.91; H, 8.54. (b) (+)-Alcohol **4A**: 23 mg (46% yield); mp 111-112.5 °C; $[\alpha]^{27}_D$ +289° (c 0.155, EtOH); optical purity 76%. Anal. Calcd for $C_{13}H_{18}O$: C, 82.06; H, 9.54. Found: C, 82.11; H, 9.52.

(18) For the absolute configuration and the absolute rotation of 4-C_2-methanoditwistanone (**4K**) and the corresponding alcohol (**4A**), see ref 12.

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Registry No. (±)-**1A**, 77341-13-0; (-)-(*M*)-**1A**, 71806-64-9; (+)-(*P*)-**1A**, 77341-14-1; (±)-**1K**, 71806-62-7; (+)-(*M*)-**1K**, 71806-63-8; (-)-(*P*)-**1K**, 61826-77-5; (±)-**2A**, 77341-15-2; (-)-(*M*)-**2A**, 77341-16-3; (+)-(*P*)-**2A**, 57287-42-0; (±)-**2K**, 69056-10-6; (-)-(*M*)-**2K**, 69056-11-7; (+)-(*P*)-**2K**, 57287-43-1; (±)-**3A**, 77341-17-4; (-)-(*M*)-**3A**, 61393-99-5; (+)-(*P*)-**3A**, 61473-81-2; (±)-**3K**, 66007-14-5; (-)-(*M*)-**3K**, 61473-76-5; (+)-(*P*)-**3K**, 61473-82-3; (±)-**4A**, 77122-78-2; (-)-(*M*)-**4A**, 77122-79-3; (+)-(*P*)-**4A**, 77079-53-9; (±)-**4K**, 77079-54-0; (-)-(*M*)-**4K**, 77122-07-7; (+)-(*P*)-**4K**, 77341-18-5; (±)-**5A**, 77341-19-6; (±)-**5K**, 71806-61-6; (±)-**6K**, 69009-72-9; (±)-**8A**, 77341-20-9; (±)-**8K**, 73679-80-8.

Metalation of *o*-Halostyrene Oxides. Preparation of Benzocyclobutenols

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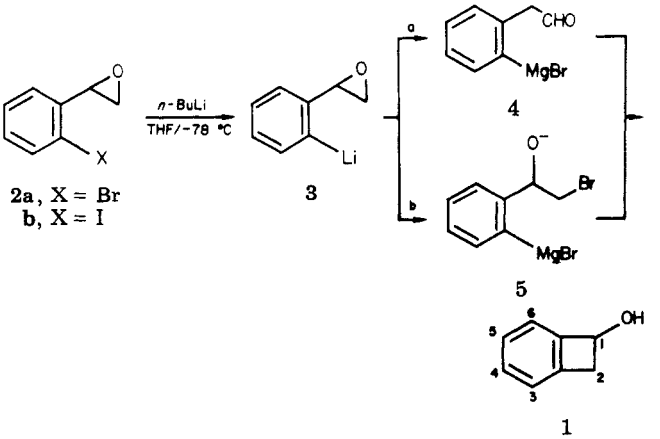
o-Bromo- and *o*-iodostyrene oxides are converted in fair to good yield to benzocyclobutenols upon treatment with *n*-BuLi and $MgBr_2$ in THF or ether at -78 °C, followed by warming to room temperature. The reaction involves initial halogen-lithium exchange followed either by $MgBr_2$-initiated opening of the epoxide function to a haloalkoxide or rearrangement of the epoxide function to a ketone or aldehyde followed by cyclization. Benzocyclobutenol formation was not successful in the case of *o*-halostilbene oxides.

We recently reported a new route to benzocyclobutenol (**1**) based on *o*-bromo- or *o*-iodostyrene oxide **2** as starting material.[1] This interconversion involved a halogen-lithium exchange between **2** and *n*-butyllithium in THF at -78 °C to generate the lithiated epoxide **3**, followed by either a $MgBr_2$-mediated rearrangement to the metalated phenylacetaldehyde **4** or an opening to the bromoalkoxide **5**, and subsequent cyclization (Scheme I). Several other *o*-halostyrene oxides were also converted into benzocyclobutenols. In some examples, including the conversion of **2** to **1**, both mechanistic pathways occurred simultaneously while in others the rearrangement route was dominant.

Scheme I



Benzocyclobutenes in general[2,3] and benzocyclobutenols (the Chemical Abstracts nomenclature for benzocyclobutenol is bicyclo[4.2.0]octa-1,3,5-trien-7-ol; for convenience the benzocyclobutenol nomenclature together with the numbering shown in structure **1** has been adopted) in particular[4] have been shown to be valuable intermediates

(1) K. L. Dhawan, B. D. Gowland, and T. Durst, *J. Org. Chem.*, **45**, 922 (1980).
(2) W. Oppolzer, *Synthesis*, 793 (1978).
(3) T. Kametani, H. Nemoto, H. Ishikawa, K. Shiroyama, H. Matsumoto, and K. Fukumoto, *J. Am. Chem. Soc.*, **99**, 3461 (1977); T. Kametani, H. Matsumoto, H. Nemoto, and K. Fukumoto, *ibid.*, **100**, 6218 (1978).

0022-3263/81/1946-2730$01.25/0