We thank Dr. W. A. Kleschick for preparing compounds **5a** and **6a**.

Registry No. **1a**, 564-04-5; **1b**, 5405-79-8; **1c**, 19078-97-8; **1d**, 5340-64-7; (±)-**2a**, 87280-57-7; (±)-**2b**, 87280-40-8; (±)-**2c**, 87280-41-9; (±)-**2d**, 87280-42-0; (±)-**3b**, 87280-43-1; (±)-**3c**, 87280-44-2; (±)-**3d**, 87280-45-3; (±)-**5b**, 87280-46-4; (±)-**5c**, 87280-47-5; (±)-**5d**, 87280-48-6; (±)-**6b**, 87280-49-7; (±)-**6c**, 87280-50-0; (±)-**6d**, 87280-51-1; (±)-**9**, 87333-63-9; **11**, 59014-51-6; **12**, 87280-52-2; **13**, 87280-53-3; **14**, 87280-54-4; (±)-**15**, 87280-55-5; (±)-**16**, 87280-56-6; 2,2-dimethyl-3-hexanol, 4209-90-9; 2,2-dimethyl-3-heptanol, 19549-70-3; 2,2-dimethyl-3-octanol, 19841-72-6.

Stereochemistry of Horse Liver Alcohol Dehydrogenase Mediated Oxidoreduction of 2-Brendanone Type Cage-Shaped Tricyclic Ketones and the Related Stereoisomeric Alcohols[1]

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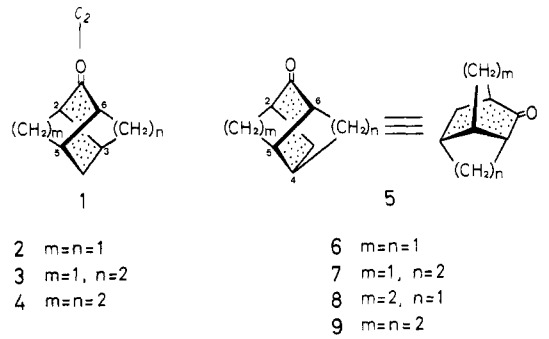
For elucidation of the origin of the inertness exhibited by 2-*twist*-brendanone type ketones **1** toward microbial and HLADH-catalyzed reductions, the stereochemistry of the oxidoreduction of 2-brendanone type ketones **5** and the related alcohols **14** was studied by preparative-scale incubation experiments. Oxidoreduction of 2-brendanone type substrates **5** and **14** by the HLADH–NAD^+ system was found to proceed with much higher rates than those of the 2-*twist*-brendanone type substrates **1** and **10**. The steric courses found in these enzymatic processes were compared with those of microbial processes.

Our continuing interests in the stereochemistry of high-symmetry chiral compounds[2] have prompted us to study the steric course of microbial reduction of C_2 ketones[3] possessing a variety of molecular frameworks, and our efforts in this direction have led us to propose a microbial "*P*-C_2 ketone rule", which summarizes the stereoselectivity exhibited by *Curvularia lunata* and *Rhodotorula rubra* toward these C_2 ketones.[4]

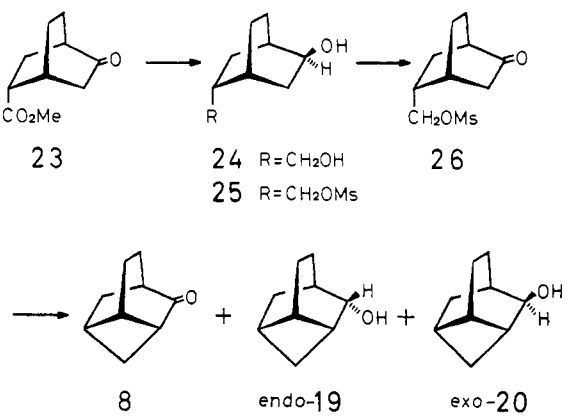
Making a striking contrast with these microbial systems, crystalline horse liver alcohol dehydrogenase (HLADH)[5] was found to exhibit a completely opposite stereoselectivity toward the same class of C_2 ketones, and we showed that another C_2 ketone rule, "*M*-C_2 ketone rule", is to be invoked for predicting the steric course in this biological system.[6]

Further extension of these studies to cage-shaped C_1 ketones[3] of various molecular frameworks has enabled us to formulate a "quadrant rule",[7,8] which summarizes the stereochemistry of these biological oxidoreductions for this type of cage-shaped ketones.[9]

Chart I



Scheme I



What perplexed us during these studies was a peculiar inertness of 2-*twist*-brendanone (**3**)[10] and 2-twistanone (**4**)[10] toward these biological oxidoreductions; we were also puzzled by our finding that the oxidoreductions of D_{2d}

(1) Presented at the 43th Annual Meeting of the Chemical Society of Japan, Tokyo, April 1981: Abstract, Vol. II, p 687.

(2) For a review of the synthesis and stereochemistry of gyrochiral cage-shaped compounds, see: Nakazaki, M.; Naemura, K. *Yuki Gosei Kagaku Kyokaishi* **1982**, *40*, 1128–1144.

(3) In this paper, we conveniently classify ketones according to their symmetry around the carbonyl center: C_1 ketones belong to the C_1 point group and have no symmetry element passing through the carbonyl center, and C_2 ketones belong to the C_2 point group and have a C_2 symmetry axis coincident with the carbonyl axis.

(4) For references concerning the microbial "*P*-C_2 ketone rule" see: Nakazaki, M.; Chikamatsu, H.; Nishino, M.; Murakami, H. *J. Org. Chem.* **1981**, *46*, 1151–1156.

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

(6) For references concerning the HLADH "*M*-C_2 ketone rule" see: (a) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Suzuki, T.; Iwasaki, M.; Sasaki, Y.; Fujii, T. *J. Org. Chem.* **1981**, *46*, 2726–2730. (b) Nakazaki, M.; Naemura, K.; Chikamatsu, H.; Iwasaki, M.; Hashimoto, M. *Ibid.* **1981**, *46*, 2300–2306.

(7) For references concerning the microbial "quadrant rule" see: Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Asao, M. *J. Org. Chem.* **1980**, *45*, 4432–4440 and also see ref 8.

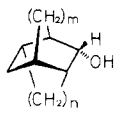
(8) The HLADH "quadrant rule": Nakazaki, M.; Chikamatsu, H.; Sasaki, Y. *J. Org. Chem.* **1983**, *48*, 2506–2511.

(9) For a review summarizing our recent studies in this field, see: Nakazaki, M. *Gendai Kagaku* **1982**, 38–47.

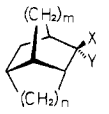
(10) For the cage-shaped substrates discussed in this paper, we have used their trivial names. Their IUPAC names are shown in parentheses: D_{2d} 2-dinoradamantanone (tricyclo[3.3.0.0^{3,7}]octan-2-one, 2-*twist*-brendanone (tricyclo[4.3.0.0^{3,8}]nonan-2-one), 2-brendanone (tricyclo[4.2.1.0^{3,7}]nonan-2-one), 2-norbrendanone (tricyclo[3.2.1.0^{3,6}]octan-2-one), 2-isobrendanone (tricyclo[3.3.1.0^{3,6}]nonan-2-one), 2-twistanone (tricyclo[4.4.0.0^{3,8}]decan-2-one), 2-isotwistanone (tricyclo[4.3.1.0^{3,7}]decan-2-one).

0022-3263/83/1948-4337$01.50/0

Chart II



10



14

11 m=n=1
anti-12 m=1, n=2
13 m=n=2

endo-isomers (X=H, Y=OH)	exo-isomers (X=OH, Y=H)
endo-15 m=n=1	exo-16 m=n=1
endo-17 m=1, n=2	exo-18 m=1, n=2
endo-19 m=2, n=1	exo-20 m=2, n=1
endo-21 m=n=2	exo-22 m=n=2

2-dinoradamantanone (**2**)[10] by the same biological ststems proceeded extremely sluggishly and with a very poor enantiomer selectivity, though their steric directions were shown to be strictly governed by the "*P*-" and "*M*-C_2 ketone rules", respectively.

A structural feature common to these three cage-shaped ketones, **2**, **3**, and **4**, is the rigid molecular framework **1** constructed by 2–5 and 3–6 diagonal bridgings across a cyclohexanone molecule in a twist-boat conformation. This observation immediately raises an interesting inquiry as to whether the observed reluctance of 2-*twist*-brendanone type ketones **1** to the microbial and HLADH-catalyzed oxidoreductions should be exhibited by 2-brendanone type ketones **5**, which differ from **1** by their 2–5 and 4–6 diagonal bridges and their boat conformation of the cyclohexanone moiety.

In this paper, we wish to report the results of our experiments that were directed to answer this question and that should provide information on the active site stereochemistry of HLADH.

Results

Preparation of the Substrates. Since information on the synthesis and stereochemistry of the substrate ketones and related alcohols has been known, except for 2-brendanone type ketones **8** and **9** and their related alcohols, our study was started from earlier preparation and the endo–exo configurational assignment of the stereoisomeric alcohols.

Preparation of (±)-2-Isobrendanone (8)[10] (Scheme I). 2-Isobrendanone (**8**) was synthesized by the ring closure of the keto mesylate **26**, which in turn was prepared from methyl 5-oxo-*endo*-bicyclo[2.2.2]octane-2-carboxylate (**23**)[11] through a sequence of routine transformations outlined in Scheme I.

Intramolecular alkylation of **26** by means of NaH in a manner similar to the preparation of the twistane skeleton[12] afforded 2-isobrendanone (**8**) (11% yield) together with a 4:1 mixture of *endo*- (**19**) and *exo*-isobrendanols (**20**) (29% yield), whose NMR spectrum exhibited two C*H*OH signals centered at δ 4.05 and 3.25. Studies of the NMR spectra of the endo–exo isomers of 2-norbrendanol (**15** and **16**)[13] and 2-brendanol (**17** and **18**)[14] have proved that the C*H*OH signals are diagnostic in assigning the endo–exo stereochemistry. Application of this to the present case allowed us to assign δ 4.05 and 3.25 signals to *endo*- (**19**) and *exo*-isobrendanols (**20**), respectively, and isolation of 2-*endo*-isobrendanol (**19**) as the sole isolable product from $LiAlH_4$ reduction (exo attack of hydride) of **8** further supported this assignment.[15]

Scheme II

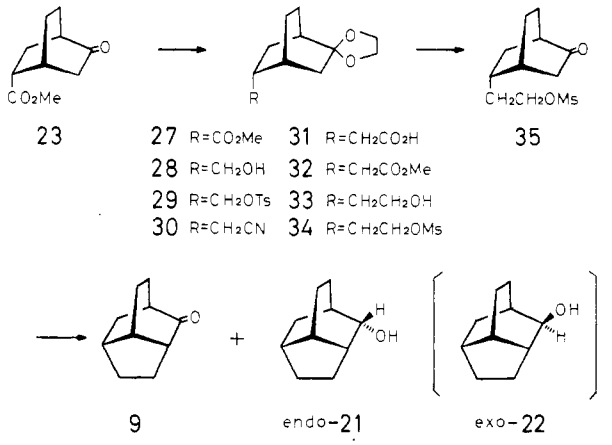


Table I. Comparison between Relative Rates[a] of HLADH-Catalyzed Reduction of 2-*twist*-Brendanone Type (1) and 2-Brendanone Type (5) Cage-Shaped Tricyclic Ketones

2-*twist*-brendanone type (1)	V_{rel}[b]	2-brendanone type (5)	V_{rel}[b]
(±)-**2**	<0.35	(±)-**6**	325
(±)-**3**	<0.26	(±)-**7**	2.5
		(±)-**8**	1.5
(±)-**4**	<0.16	(±)-**9**	1.6

[a] Reduction rates were measured at 25 °C in $^1/_{15}$ M Sørensen phosphate buffer (pH 7.0). [b] Velocities are relative to cyclohexanone; $V_{cyclohexanone} = 100$.

Table II. Comparison between Relative Rates[a] of HLADH-Catalyzed Oxidation of 2-*twist*-Brendanol Type (10) and 2-Brendanol Type (14) Cage-Shaped Tricyclic Alcohols

2-*twist*-brendanol type (10)	V_{rel}[b]	2-brendanol type (14)	V_{rel}[b]
(±)-**11**	11.4	(±)-*endo*-**15**	100
		(±)-*exo*-**16**	2
(±)-*anti*-**12**	17	(±)-*endo*-**17**	31
		(±)-*exo*-**18**	1
		(±)-*endo*-**19**	15
(±)-**13**	0	(±)-*endo*-**21**	1.7
		(±)-*exo*-**22**	0.26

[a] Oxidation rates were measured at 25 °C in $^1/_{20}$ M glycine-NaOH buffer (pH 9.0). [b] Velocities are relative to cyclohexanol; $V_{cyclohexanol} = 100$.

Preparation of (±)-2-Isotwistanone (9)[10] (Scheme II). Scheme II summarizes the sequence of transformations converting the bicyclic keto ester **23** into 2-isotwistanone (**9**).

The ring closure of the keto mesylate **35** by means of NaH[12] gave 2-isotwistanone (**9**) (21% yield) together with 2-*endo*-isotwistanol (**21**) (5.5% yield). $LiAlH_4$ reduction of **9** afforded a 7:3 mixture of *endo*-isotwistanol (**21**) (δ 3.95, doublet, J = 9 Hz) and the exo isomer **22** (δ 3.42, doublet, J = 3 Hz). Their endo–exo assignment was made again through the comparison of their NMR spectra as well as

(11) (a) Lee, R. A. *Tetrahedron Lett.* **1973**, 3333–3336. (b) White, K. B.; Reusch, W. *Tetrahedron* **1978**, *34*, 2439–2443.
(12) (a) Whitlock, Jr., H. W. *J. Am. Chem. Soc.* **1962**, *84*, 3412–3413. (b) Gauthier, J.; Deslongchamps, P. *Can. J. Chem.* **1967**, *45*, 297–300.
(13) Sauers, R. R.; Parent, R. A.; Damle, S. B. *J. Am. Chem. Soc.* **1966**, *88*, 2257–2267.
(14) Nickon, A.; Kwasnik, H. R.; Mathew, C. T.; Swartz, T. D.; Williams, R. O.; DiGiorgio, J. B. *J. Org. Chem.* **1978**, *43*, 3904–3916.
(15) For an example of stereoselective reduction of tricyclic carbonyl compounds, see: Wenzinger, G. R.; Ors, J. A. *J. Org. Chem.* **1974**, *39*, 2060–2063.

Table III. HLADH-Catalyzed and Microbial Reduction of 2-Brendanone Type Cage-Shaped Ketones (±)-2-Norbrendanone (6), (±)-2-Brendanone (7), (±)-2-Isobrendanone (8), and (±)-2-Isotwistanone (9)

substrate	biological system	incubation period, h (°C)	% conv	recovered ketone (% optical purity)	metabolite alcohol (% optical purity) *endo*	*exo*
(±)-**6**	HLADH-NAD$^+$-$Na_2S_2O_4$	2.5 (25)	56	(−)-**6** (77)	(+)-**15**-PNB[a] (72)	[b]
(±)-**6**	*C. lunata*	3.5 (30)	50	(−)-**6** (5)	(+)-**15**-PNB[a] (6)	[b]
(±)-**7**	HLADH-NAD$^+$-C_2H_5OH	96 (25)	10	(−)-**7** (0.4)	(−)-**17** (18)	[b]
(±)-**7**	*C. lunata*	13.5 (30)	60	(+)-**7** (28)	(+)-**17** (27)	[b]
(±)-**8**	HLADH-NADH[c]	48 (25)	9			
(±)-**8**	*C. lunata*	40 (30)	57	(+)-**8** (13)	(−)-**19** (14)	[b]
(±)-**9**	HLADH-NADH[c]	48 (25)	0			
(±)-**9**	*C. lunata*	69 (30)	34	(+)-**9** (20)	(−)-**21**[d] (64)	(+)-**22**[d] (21)

[a] Sign of optical rotation of the *p*-nitrobenzoate (PNB). [b] No *exo*-alcohol was detected by GLC. [c] The poor % conversion prevented carrying out preparative scale experiments. [d] GLC of crude ether extract indicated a 2.4:1 ratio for (−)-*endo*-**21**:(+)-*exo*-**22**.

Table IV. HLADH-Catalyzed and Microbial Oxidation of the Isomeric Alcohols Corresponding to 2-Brendanone Type Cage-Shaped Ketones (±)-2-*endo*- (15) and (±)-2-*exo*-Norbrendanol (16), (±)-2-*endo*- (17) and (±)-2-*exo*-Brendanol (18), (±)-2-*endo*-Isobrendanol (19), (±)-2-*endo*-Isotwistanol (21), and (±)-2-*anti*-*twist*-Brendanol (12)

substrate	biological system	incubation period, h (°C)	% conv	metabolite ketone (% optical purity)	recovered alcohol (% optical purity)
(±)-*endo*-**15**	HLADH-NAD$^+$-FMN	3.5 (25)	46	(+)-**6** (12)	(−)-*endo*-**15**-PNB[a] (9)
(±)-*exo*-**16**	HLADH-NAD$^+$-FMN	150 (25)	38	(+)-**6** (73)	(−)-*exo*-**16** (41)
(±)-*endo*-**17**	HLADH-NAD$^+$-FMN	4.5 (25)	47	(+)-**7** (29)	(+)-*endo*-**17** (27)
(±)-*exo*-**18**	HLADH-NAD$^+$-FMN[b]	113 (25)	0.4		
(±)-*endo*-**19**	HLADH-NAD$^+$-FMN	48 (25)	51	(+)-**8** (81)	(−)-*endo*-**19** (87)
(±)-*endo*-**21**	HLADH-NAD$^+$-FMN[b]	54 (25)	2		
(±)-*anti*-**12**	HLADH-NAD$^+$-FMN	12.5 (25)	62	(−)-**3** (11)	(+)-*anti*-**12** (16)
(±)-*anti*-**12**	*C. lunata*[c]	100 (30)	12	(+)-**3** (60)	(−)-*anti*-**12** (9)

[a] Sign of optical rotation of the *p*-nitrobenzoate (PNB). [b] Because of the poor % conversion, the preparative scale experiments were not carried out. [c] Oxidation experiment with the resting cells.

the assumption of exo attack of the hydride in $LiAlH_4$ reduction.

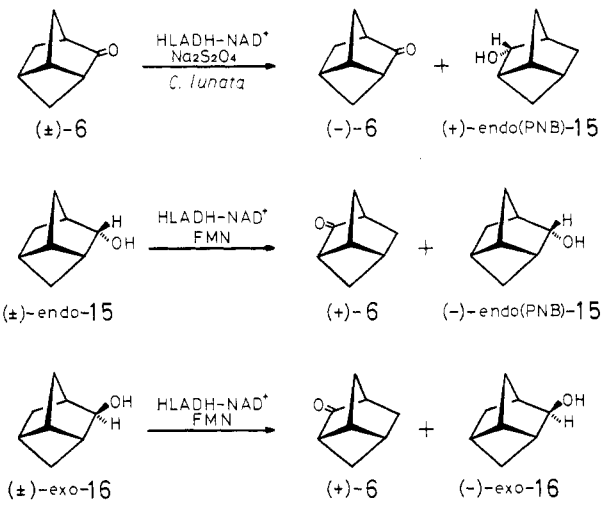
Comparative Study of Reaction Rates in HLADH-Catalyzed Oxidoreduction of 2-*twist*-Brendanone Type (1) and 2-Brendanone Type (5) Cage-Shaped Compounds (Tables I and II). Table I summarizes the kinetic data of the HLADH-catalyzed reduction of 2-*twist*-brendanone type (1) and 2-brendanone type (5) ketones. Comparison between these two series of compounds reveals that (a) the type 5 ketones are reduced much faster than the type 1 ketones and (b) (±)-2-norbrendanone (6)[10] is outstanding in its high reaction rate, which is 3 times higher than that of cyclohexanone.

Inspection of Table II, which shows the relative rates of HLADH-catalyzed oxidations of 2-*twist*-brendanol type (10) and 2-brendanol type (14) alcohols, indicates that (a) the type 14 alcohols are oxidized faster than the type 10 alcohols and (b) when comparison is made within the type 14 alcohols, the endo isomers exhibited higher rates than the exo isomers; the extreme case is to be found in 2-*endo*-norbrendanol (15) whose reaction rate is 50 times higher than that of the exo isomer 16.

Preparative-Scale Incubation Experiments. For securing information on the stereochemistry of HLADH-mediated oxidoreduction of 2-brendanone type ketones 5 and their related alcohols 14, preparative-scale incubation experiments were carried out by employing these ketones and the isomeric alcohols as the substrates.

2-Norbrendanone (6) and the Related Alcohols (Scheme III). Incubation of (±)-6 with the HLADH-NAD$^+$ system and $Na_2S_2O_4$, a recycling reagent,[16] was terminated after 2.5 h when GLC monitoring indicated a

Scheme III



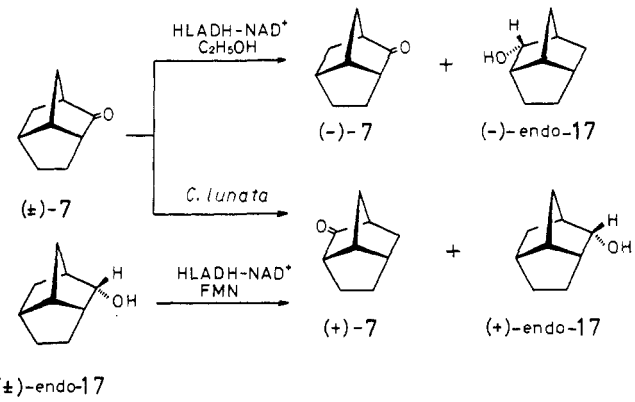
56% conversion of the substrate. Isolation of the metabolites with due precaution for their optical purity provided the recovered (−)-ketone 6 and the endo alcohol 15 whose GLC indicated no contamination from the exo alcohol 16. Although this specimen of 15 showed almost no rotation in the sodium D line region, its *p*-nitrobenzoate (PNB) was found to be distinctly dextrorotatory and its Jones' oxidation afforded the (+)-ketone 6. Our preceding studies[17]

(16) For a review of nicotinamide coenzyme recycling methods, 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 357–376.

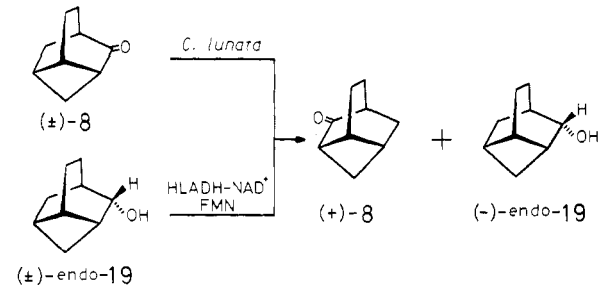
(17) The absolute configuration of 2-norbrendanone (6) was established in our laboratory.[18] The optical purity of the ketone 6 was calculated from its absolute rotation value, $[\alpha]_D$ abs. 123° (EtOH).[18] Jones oxidation of the endo (15) and the exo alcohols (16) established their absolute configurations and optical purities. The absolute rotation values of the endo (PNB)-15 and the exo alcohol 16 were estimated as 28° ($CHCl_3$) and 13.3° ($CHCl_3$), respectively (see Experimental Section).

(18) Nakazaki, M.; Naemura, K.; Kondo, Y. *J. Org. Chem.* **1979**, *44*, 16–20.

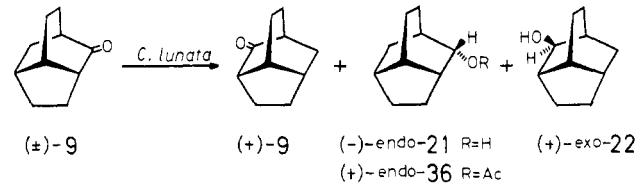
Scheme IV



Scheme V



Scheme VI



on the absolute configurations and absolute rotations allowed us to assign the structures, as shown in Scheme III, and to calculate the respective 77% and 72% optical purities.

The same metabolites, (−)-**6** and the (+)-endo (PNB)-**15**, but of lower optical purities (5% and 6%, respectively), were isolated from the ethereal extract of a 3.5-h incubation mixture of (±)-**6** with *C. lunata*.

In carrying out the HLADH-catalyzed oxidations we used an HLADH–NAD^+–FMN[5] system,[16] which was found to oxidize (±)-2-endo (**15**) much faster than (±)-2-exo alcohol (**16**), as expected from the kinetic data in the oxidative mode (Table II). Preparative-scale incubation experiments were carried out with these two isomeric alcohols, (±)-**15** and (±)-**16**, and examination of the metabolites revealed that in both cases the oxidation product was (+)-ketone **6**, though the specimens differed markedly in their optical purities (12% and 73%, respectively) (Table IV). The steric course of the biological oxidations of the isomeric 2-norbrendanols is illustrated in Scheme III.

2-Brendanone (7)[10] and the Related Alcohols (Scheme IV). The kinetic data in Table I predict that the HLADH-catalyzed reduction of 2-brendanone (**7**) should proceed much more slowly than that of 2-norbrendanone (**6**), and this prediction was verified when a 96-h incubation period was required before the HLADH–NAD^+–C_2H_5OH system[16] attained a 10% conversion of (±)-**7**.

GLC examination of the crude metabolite mixture revealed that it contained recovered ketone **7** and 2-*endo*-brendanol (**17**) with no formation of the exo alcohol **18**. The routine workup provided (−)-ketone **7** and the (−)-endo alcohol **17** and information[19] on their absolute configurations and absolute rotations permitted us to assign their structures (Scheme IV) as well as calculate their optical purities (0.4% and 18%, respectively).

When incubated with *C. lunata*, however, (±)-**7** was found to be reduced fairly rapidly, and a 60% conversion of the substrate was attained after a 13.5-h incubation. Chromatographic separation of the metabolites provided the recovered (+)-ketone **7** (28% optical purity) and the (+)-endo alcohol **17** (27% optical purity), revealing that the steric course of this microbial reduction is completely opposite to that of the HLADH system described above.

To confirm this finding, HLADH-catalyzed oxidations of (±)-2-*endo*- (**17**) and (±)-2-*exo*-brendanols (**18**) utilizing the HLADH–NAD^+–FMN system were carried out.

In contrast to the exo alcohol **18** whose oxidation proceeded so sluggishly as to discourage our efforts to isolate the expected metabolites, the (±)-endo alcohol **17** was found to be oxidized fairly rapidly. The routine workup of the metabolite mixture from (±)-**17** yielded (+)-ketone **7** (29% optical purity) and the recovered (+)-endo alcohol **17** (27% optical purity), providing the steric information on this biological oxidation as illustrated in Scheme IV.

2-Isobrendanone (8) and Related Alcohols (Scheme V). As expected from the kinetic data in Table I, reduction of 2-isobrendanone (**8**) by the HLADH–NADH system was so sluggish (9% conversion after 48-h incubation) that we were forced to abandon our attempt to isolate the reduction products.

Microbial reduction of (±)-**8** with *C. lunata*, however, was found to proceed rather smoothly (Table III), and GLC of the crude ethereal extract revealed that the endo alcohol **19** was the sole reduction product with no formation of the exo alcohol **20**. The absolute configurations of the isolated (+)-**8** (13% optical purity) and the (−)-endo alcohol **19** (14% optical purity) are shown in Scheme V.[20]

Although information on the stereochemistry of the HLADH-catalyzed oxidoreduction of 2-isobrendanone substrates could not be obtained from the reductive mode experiments (vide supra), our finding of a facile oxidation of (±)-2-*endo*-isobrendanol (**19**) provided the required information.

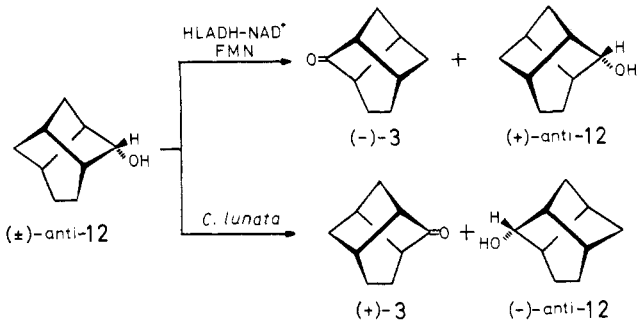
Incubation of the (±)-endo alcohol **19** with this biological system for 48 h (51% conversion) afforded a metabolite mixture containing (+)-**8** and the recovered (−)-endo alcohol **19** with 81% and 87% optical purities, respectively (Scheme V).

2-Isotwistanone (9) (Scheme VI). From the kinetic data shown in Tables I and II, it can be seen that both 2-isotwistanone (**9**) and 2-*endo*-isotwistanol (**21**) will be reluctant to undergo HLADH-catalyzed oxidoreduction. Since this was unfortunately found to be the case, we turned our attention toward the microbial reduction of the (±)-ketone **9** with *C. lunata* which, though slow, was found to give a 34% conversion after a 69-h incubation. Column chromatography of the products gave the recovered

(19) The absolute configuration of 2-brendanone (**7**) was established in our laboratory.[18] The optical purity of **7** was calculated from its absolute rotation value, $[\alpha]_D$ abs. 46.7° (EtOH).[18] Brown's oxidation of the (+)-endo alcohol **17** established its absolute configuration and optical purity. The absolute rotation value of **17** was estimated as 1.47° ($CHCl_3$) (see Experimental Section).

(20) The absolute configuration of 2-isobrendanone (**8**) was established in our laboratory.[18] The optical purity was calculated from its absolute rotation value, $[\alpha]_D$ abs. 210° (EtOH).[18] Jones oxidation of (−)-*endo*-isobrendanol (**19**) established its absolute configuration and optical purity. The absolute rotation value of the alcohol **19** was estimated as 19.6° ($CHCl_3$) (see Experimental Section).

Scheme VII



(+)-ketone **9**, the (−)-endo alcohol **21**, and a small amount of the (+)-exo alcohol **22** with 20%, 64%, and 21% optical purities, respectively. Scheme VI illustrates their absolute structure.

Assignment of the 1*R*,3*R*,6*R*,7*S* configuration to the (−)-ketone **9** was made by applying the "octant rule"[18] to the negative Cotton effect in the n → π* region of the (−)-ketone **9** spectrum. Jones' oxidation of the (−)-endo alcohol **21** and the (+)-exo alcohol **22** to (−)-**9** and (+)-**9**, respectively, established their absolute configurations (Scheme VI). Their optical purities were calculated from their absolute rotation values[21] (see Experimental Section), which were based on a large enantiomer differential shift observed when Eu(facam)$_3$[22,23] was added to a specimen of the (+)-endo acetate **36**.

2-*anti*-*twist*-Brendanol (12)[24] (Scheme VII). In contrast to the discouragingly low reaction rate observed in the HLADH-catalyzed reduction of (±)-2-*twist*-brendanone (**3**) (Table I), the kinetic data in Table II indicate that the corresponding alcohol, (±)-2-*anti*-*twist*-brendanol (**12**), will be oxidized rapidly enough to promise a successful preparative-scale experiment.

Incubation of the (±)-alcohol **12** with the HLADH–NAD$^+$–FMN system was carried out for 12.5 h before reaching a 62% conversion of the substrate, and examination of the reaction mixture revealed that the products were the (−)-ketone **3** and the (+)-anti alcohol **12** with 11% and 16% optical purity, respectively.[26]

Encouraged by this successful biological oxidation of the (±)-anti alcohol **12**, we undertook a preparative-scale incubation of (±)-**12** with the resting cells of *C. lunata*, hoping that this would provide stereochemical information on the microbial oxidoreduction of the 2-*twist*-brendane systems which had not been obtained because of the stubborn inertness of 2-*twist*-brendanone (**3**) toward the microbial reduction. The incubation was terminated after 100 h, and chromatographic separation of the products yielded the (+)-ketone **3** and the (−)-anti alcohol **12** in 60% and 9% optical purity, respectively. Scheme VII shows the opposite steric course observed in the oxidation of (±)-2-*anti*-*twist*-brendanol (**12**) by these two biological systems.

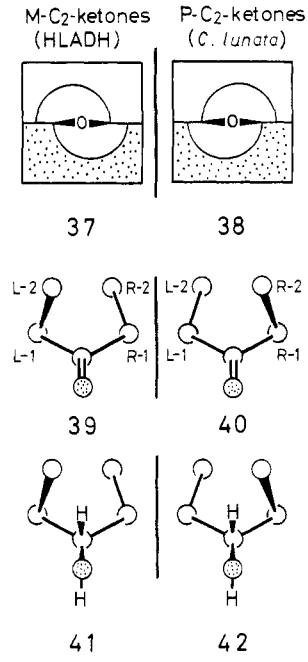


Figure 1. Quadrant orientations of *M*-C_2 and *P*-C_2 ketones and the spatial characteristics of four carbon atoms flanking the carbonyl groups.

Discussion

Tables III and IV summarize the results of these preparative-scale biological oxidoreductions of 2-brendanone type ketones **5** and the related isomeric alcohols **14**.

A survey of these tables and a comparison between the steric course of the HLADH-mediated and the microbial oxidoreductions, depicted in Schemes IV, V, and VII, reveal that the steric courses of oxidoreduction of these two biological systems are opposite, except for the case of 2-norbrendanone (**6**) (Scheme III). These opposite steric behaviors remined us of the "*M*-C_2 ketone" and the "*P*-C_2 ketone" rules which have been proposed to summarize the opposite steric courses observed in HLADH-catalyzed and microbial oxidoreductions of various cage-shaped C_2 ketone substrates.

Inspection of molecular models of the cage-shaped C_2 ketones, whose incubation experiments with the HLADH system and *C. lunata* have enable us to formulate these "C_2 ketone" rules, indicates that the C_2 ketones belonging to the same symmetry class (*M* or *P*) share a common steric characteristic in the relative disposition of four carbon atoms flanking the carbonyl group (**39** and **40** in Figure 1); the L-2 carbon atom is situated closer to the carbonyl group than the R-2 carbon atom in the *M*-C_2 ketones **37**, and the opposite is true in the *P*-C_2 ketones **38**.

Examination of molecular models of the 2-brendanone type ketones **5** and the related alcohols **14** described in this paper reveals that (a) the enantiomers which preferentially undergo oxidoreduction with the HLADH system invariable possess the common steric features of **39** or **41** and (b) the enantiomers that are preferentially oxidized or reduced by *C. lunata* possess the common steric features of **40** or **42**, except for the case of 2-norbrendanone (**6**).

This finding that the HLADH system favors the substrates having the steric characteristic around the reaction center as **39** or **41** seems to be compatible with the HLADH cubic space model recently advanced by Jones,[28]

(21) The absolute rotation values of the ketone **9**, the endo (**21**), and the exo alcohols (**22**) were estimated as 132°, 58.5°, and 21.8°, respectively ($CHCl_3$) (see Experimental Section).

(22) Eu(facam)$_3$ = tris[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]europium (III).

(23) (a) McCreary, M. D.; Lewis, D. W.; Wernick, D. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1974**, *96*, 1038–1054. (b) Goering, H. L.; Eikenberry, J. N.; Koermer, G. S.; Lattimer, C. J. *Ibid.* **1974**, *96*, 1493–1501.

(24) Sauers[25] has proposed the syn and anti designation for these diastereomers.

(25) Sauers, R. R.; Whittle, J. A. *J. Org. Chem.* **1969**, *34*, 3579–3582.

(26) The absolute configuration of 2-*twist*-brendanone (**3**) was established in our laboratory.[27] The optical purity of **3** was calculated from its absolute rotation value, $[\alpha]_D$ abs. 292° (EtOH).[27] Jones oxidation of the (+)-anti alcohol **12** to the (+)-ketone **3** established its absolute configuration and optical purity. The absolute rotation value of **12** was estimated as 213° ($CHCl_3$) (see Experimental Section).

(27) Naemura, K.; Nakazaki, M. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 888–892.

and it also suggests that the alcohol dehydrogenase of *C. lunata* must have an active site which is enantiomeric to that of HLADH at least in this steric aspect.

As for the exception found in the stereochemistry of the microbial reduction of 2-norbrendanone (**6**), we tentatively attribute this to the small size and spherelike shape inherent to this molecule which would make it difficult for *C. lunata* to differentiate between the enantiomers. This explanation seems to be supported by the poor optical purities of the recovered ketone **6** (5%) and the endo alcohol **15** (6%) (Table III) isolated from the incubation experiment of (±)-**6** with this microbe.[29]

Finally, an explanation for the reaction enhancement of the 2-brendanone type substrates **5** over the 2-*twist*-brendanone type substrates **1** observed in oxidoreduction with these two biological systems could be sought in a characteristic molecular feature inherent to the 2-brendanol type alcohols **14**; they appear to have more spacious room to accommodate the hydroxyl group and the complexing zinc atom of the alcohol dehydrogenases than the 2-*twist*-brendanol type alcohols **10**.

Experimental Section

Melting poings are uncorrected. ^1H NMR spectra were determined on a JNM-NH-100 and a JNM-C-60HL. Chemical shifts are reported as δ values in parts per million relative to internal Me_4Si ($\delta = 0$). Optical rotations were measured with a JASCO-140 polarimeter. GLC analyses were performed with a JGC-20K equipped with an FID and 2 m × 3 mm column of 10% Carbowax 20 M on Chromosorb W or 15% silicone DC QF-1 on Uniport B. Column chromatography was carried out with Woelm active alumina (neutral, activity III).

HLADH was purchased from Boehringer (Mannheim) as a crystalline suspension in phosphate buffer containing 10% ethanol. Immediately before each experiment, the enzyme suspension was freeze-dried to a powder whose average activity was found to be 1.37–1.95 U/mg.[30] $NAD^+ \cdot 3H_2O$ and $NADH \cdot 3H_2O$ were obtained from Kohjin Co., Ltd., Tokyo.

The culture of *C. lunata* (IFO 6288) was obtained from the Institute of Fermentation, Osaka.

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 $^1/_{20}$ M glycine–NaOH buffer solution (pH 9.0). The reactions were monitored by GLC, and the incubation was terminated when the monitoring indicated ca. 50% conversion (Tables III and IV).

The reaction mixture was extracted with ether, and the ethereal extract was taken up in pentane and chromatographed over alumina. Elution with pentane followed by pentane–ether (10:1) effected a clean separation of the ketone and the alcohol fractions. The eluted materials were purified by sublimation in vacuo.[32]

Microbial Reduction Procedure. The fermentation and the subsequent extraction procedures have been described previously.[33] The crude ethereal extract was chromatographed over alumina to separate the ketone and the alcohol fractions, which were purified by sublimation in vacuo.[32]

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 ketones (3.6×10^{-4} M). The oxidation runs were performed in a $^1/_{20}$ M glycine–NaOH buffer (pH 9.0) containing NAD^+ (5.0×10^{-4} M) and the substrate alcohols (3.5×10^{-4} M). The reaction was initiated by adding a 100-μL aliquot of HLADH stock solution (1 mg/1 mL in $^1/_{20}$ M Tris·HCl buffer, pH 7.4) to give a 3-mL assay solution in a 1-cm cell. The cell was incubated at 25 °C, and the reaction was followed by monitoring the absorbance change at 340 nm. For each substrate, a reference assay was performed employing cyclohexanone or cyclohexanol as the standard substrates.

Synthesis of (±)-2-Isobrendanone (8) and (±)-2-*endo*-Isobrendanol (19). (A) (±)-2-Isobrendanone (8). A solution of methyl 5-oxo-*endo*-bicyclo[2.2.2]octane-2-carboxylate (**23**)[34] (3.0 g, 16.5 mmol) in ether (25 mL) was added to a suspension of $LiAlH_4$ (0.76 g, 20 mmol) in ether (50 mL), and the mixture was refluxed for 3 h. After the mixture was cooled, water was added to the reaction mixture and a solid was filtered off. The ether layer was washed with saturated aqueous NaCl and dried ($MgSO_4$). Removal of the solvent in vacuo gave the diol **24** (2.7 g) as an oil; IR (neat) 3350 and 1020 cm^{-1}.

The keto mesylate **26** (2.47 g, 10.7 mmol) prepared from the diol **24** via **25** was treated with NaH in DMF, the same procedure used to prepare the optically active modification **8**,[18] to give a crude product (1.26 g), which was taken up in pentane and chromatographed over alumina (20 g).

Elution with pentane (400 mL) afforded a semisolid which was sublimed in vacuo (50–60 °C, 30 mm) to give (±)-2-isobrendanone (**8**) (160 mg, 11% yield), mp 146.5–150 °C (in a sealed tube) [lit.[18] (−)-**8**, mp 146–149 °C (in a sealed tube)].

Further elution with pentane–ether (20:1, 450 mL) afforded an alcohol fraction which was sublimed in vacuo (65–75 °C, 30 mm). The sublimate (430 mg, 29% yield) melted at 179–184 °C in a sealed tube and was shown to be a 4:1 mixture of 2-*endo*-(**19**) and 2-*exo*-isobrendanol (**20**) by means of GLC:IR (nujol) 3350 and 1060 cm^{-1}; NMR (100 MHz, $CDCl_3$) δ 4.05 (t, J = 6 Hz, 0.8 H, $OHCH_{exo}$), 3.25 (d, J = 6 Hz, 0.2 H, $OHCH_{endo}$), 2.8–1.00 (m, 13 H).

(B) (±)-2-*endo*-Isobrendanol (19). A solution of 2-isobrendanone (**8**) (100 mg, 0.73 mmol) in dry ether (20 mL) was added to a suspension of $LiAlH_4$ (50 mg, 1.3 mmol) in dry ether (40 mL). After the mixture was refluxed for 3 h, the routine workup gave a crystalline product (100 mg) which was sublimed in vacuo (65–75 °C, 30 mm) to give (±)-2-*endo*-isobrendanol (**19**) (90 mg, 89% yield): mp 184.5–187.5 °C (in a sealed tube); IR (KBr) 3300, 1080, and 1020 cm^{-1}; NMR (100 MHz, $CDCl_3$) δ 4.05 (t, J = 6 Hz, 1 H, $OHCH_{exo}$), 2.70–0.8 (m, 13 H).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.46; H, 10.03.

Synthesis of (±)-2-Isotwistanone (9) and (±)-2-*endo*-Isotwistanol (21). A solution of methyl 5-oxo-*endo*-bicyclo-[2.2.2]octane-2-carboxylate (**23**)[34] (21.3 g, 0.117 mol), ethylene glycol (400 mL), and *p*-toluenesulfonic acid (1 g) in benzene (1 L) was refluxed for 21 h, and the generated water was removed by means of a Dean–Stark apparatus. The routine procedure involving extraction with benzene gave a crude product, which was distilled to give methyl 5-ethylenedioxy-*endo*-bicyclo-[2.2.2]octane-2-carboxylate (**27**) (19.8 g, 75% yield): bp 138–141 °C (4 mm); IR (neat) 1730 and 1120 cm^{-1}.

Anal. Calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.02. Found: C, 63.59; H, 8.07.

A solution of the ester **27** (19.5 g, 0.082 mol) in ether (180 mL) was added to a stirred suspension of $LiAlH_4$ (4.1 g, 0.108 mol) in ether (180 mL), and the mixture was refluxed for 3 h. The routine workup gave an oil which was distilled in vacuo to give 2-(ethylenedioxy)-5-*endo*-(hydroxymethyl)bicyclo[2.2.2]octane (**28**) (15.1 g, 88% yield): bp 121–124 °C (0.35 mm); IR (neat) 3400, 1360, and 1120 cm^{-1}.

Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.05; H, 9.35.

A mixture containing the alcohol **28** (15.1 g, 0.076 mol), *p*-toluenesulfonyl chloride (30 g, 0.15 mol), and pyridine (30 mL) was allowed to stand at room temperature for 24 h. The routine

(28) Jones, J. B.; Jakovac, I. J. *Can. J. Chem.* **1982**, *60*, 19–28.

(29) In our recent preparative-scale incubation experiment of (±)-**6** with *Rhodotorula rubra* (IFO 0889), we found this microbe gave the (+)-ketone **6** instead of the (−)-enantiomer but again of a poor optical purity (4%).

(30) The enzyme activity was measured by the method of Bonnichsen.[31]

(31) Bonnichsen, R. K.; Brink, B. G. "Methods in Enzymology"; Colowick, S. P.; Kaplan, N. O., Eds; Academic Press: New York, 1955; Vol 1, pp 495–500.

(32) The structures of the previously known compounds were in accord with their IR and NMR spectra. Satisfactory elemental analysis of each compound was obtained.

(33) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Nishino, M.; Murakami, H.; Asao, K. *J. Org. Chem.* **1979**, *44*, 4588–4593.

(34) The ester **23** was prepared by the method of White et al.,[11b] bp 115–120 °C (0.4 mm) [lit.[11b] bp 100–104 °C (0.65–0.75 mm)].

procedure afforded a crude preparation of the tosylate **29** (31.2 g) (IR (neat) 1600, 1360, 1180, and 1060 cm^{-1}), which was converted into the cyanide **30** without further purification.

A solution of this crude tosylate **29** (31.2 g) and sodium cyanide (12.0 g, 0.24 mol) in DMF (30 mL) was heated at 120 °C for 11 h. After cooling, the solution was freed from a solid deposite by filtration and was extracted with ether. The extract was washed with 5% aqueous HCl, dilute aqueous Na_2CO_3, and water and then dried ($MgSO_4$). Removal of the solvent followed by vacuum distilation gave 2-(ethylenedioxy)-5-*endo*-(cyanomethyl)bicyclo-[2.2.2]octane (**30**) (11.96 g, 76% yield): bp 106–109 °C (0.07 mm); IR (neat) 2250 cm^{-1}.

Anal. Calcd for $C_{12}H_{17}O_2N$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.10; H, 8.18; N, 6.65.

A solution of the nitrile **30** (11.5 g, 55.5 mmol), NaOH (10 g, 0.18 mol), and ethylene glycol (80 mL) was heated at 160 °C for 45 h. The routine procedure involving acidification and ethereal extraction afforded a crude acid **31** (11.2 g, 89% yield), which was treated with an excess of diazomethane in ether. Removal of the solvent and the excess diazomethane left a residue which was distilled in vacuo to furnish the methyl ester **32** (9.23 g, 78% yield): bp 142–143 °C (4 mm); IR (neat) 1730 and 1160 cm^{-1}.

Anal. Calcd for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39. Found: C, 65.73; H, 8.29.

$LiAlH_4$ reduction of the ester **32** was carried out by refluxing a mixture of **32** (9.13 g, 38 mmol), $LiAlH_4$ (1.7 g, 44.7 mmol), and ether (140 mL) for 3 h. The routine procedure gave an oil which was distilled in vacuo to afford the alcohol **33** (6.73 g, 83% yield): bp 121–124 °C (0.09 mm); IR (neat) 3350 and 1040 cm^{-1}.

Anal. Calcd for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50. Found: C, 67.58; H, 9.77.

A mixture of the alcohol **33** (2.0 g, 9.42 mmol), methanesulfonyl chloride (2.3 g, 20.1 mmol), and pyridine (7 mL) was stirred at room temperature for 3 h. The mixture was poured into chilled aqueous HCl and extracted with ether. The extract was washed with dilute aqueous Na_2CO_3 and dried ($MgSO_4$). Removal of the solvent left the crude mesylate (2.3 g), which was hydrolyzed while stirring with 5% aqueous H_2SO_4 (50 mL) at room temperature for 48 h. Extraction with chloroform gave the keto mesylate **35** (1.9 g, 82% yield) (IR (neat) 1720, 1360, and 1180 cm^{-1}), which was cyclized without further purification.

To a stirred suspension of sodium hydride (2.7 g, 0.113 mol) in DMF (60 mL) a solution of the keto mesylate **35** (5.98 g, 24 mmol) in DMF (60 mL) was added, and the mixture was heated at 60 °C under an atmosphere of nitrogen for 11 h. After the unreacted sodium hydride was destroyed by dropwise addition of MeOH, the reaction mixture was poured into chilled water and extracted with ether. The extract was washed with dilute aqueous HCl and saturated aqueous NaCl and then dried ($MgSO_4$). Removal of the solvent left a residue (3.0 g) which was taken up in pentane and chromatographed over alumina (30 g).

Elution with pentane (800 mL) gave a semisolid (900 mg), which was sublimed in vacuo (50–60 °C, 30 mm) to yield (±)-2-isotwistanone (**9**) (760 mg, 21% yield): mp 91–95 °C (in a sealed tube); IR (nujol) 1720 cm^{-1}; NMR (100 MHz, $CDCl_3$) δ 1.10–2.6 (m, 14 H).

Anal. Calcd for $C_{10}H_{14}O$: C, 79.95; H, 9.39. Found: C, 79.99; H, 9.38.

Further elution with pentane–ether (20:1, 300 mL) gave a semisolid (400 mg), which was purified by preparative GLC and sublimation in vacuo (75–85 °C, 30 mm) to afford (±)-2-*endo*-isotwistanol (**21**) (200 mg, 5.5% yield): mp 147–151.5 °C (in a sealed tube); IR (KBr) 3300, 1460, 1080, and 1060 cm^{-1}; NMR (60 MHz, $CDCl_3$) δ 3.92 (d, J = 9 Hz, 1 H, $OHCH_{exo}$).

Anal. Calcd for $C_{10}H_{16}O$: C, 78.89; H, 10.59. Found: C, 78.75; H, 10.49.

$LiAlH_4$ Reduction of (±)-2-Isotwistanone (9). A solution of the ketone **9** (100 mg, 0.7 mmol) in ether (10 mL) was added to a stirred suspension of $LiAlH_4$ (50 mg, 1.3 mmol) in ether (30 mL), and the mixture was refluxed for 2 h. The usual workup gave a crude product, which was sublimed in vacuo (65–75 °C, 30 mm). The sublimate (80 mg, 79% yield) was shown to be a 7:3 mixture of 2-*endo*- (**21**) and 2-*exo*-isotwistanol (**22**) by means of GLC: mp 143–147 °C (in a sealed tube); IR (KBr) 3300, 1460, 1060, and 1040 cm^{-1}; NMR (60 MHz, $CDCl_3$) δ 3.95 (d, J = 9 Hz, 0.63 H, $OHCH_{exo}$), 3.42 (d, J = 3 Hz, 0.37 H, $OHCH_{endo}$).

Reduction of (±)-2-Norbrendanone (6) by Biological Methods. (A) HLADH-Catalyzed Reduction. HLADH (15.7 mg) was added to a phosphate buffer (1 L, pH 7.0) containing the (±)-ketone **6**[35] (203 mg, 1.66 mmol), NAD^+ (121.6 mg, 0.17 mmol), and $Na_2S_2O_4$ (17.4 g, 0.1 mol), and the reaction was allowed to proceed at 25 °C for 2.5 h to afford (a) (−)-2-norbrendanone (**6**) [35 mg, 17% yield; mp 117.5–123.5 °C (in a sealed tube); $[\alpha]^{20}_D$ −94.6° (*c* 0.95, EtOH) (77% optical purity[17]) [lit.[18] mp 126.5–128 °C (in a sealed tube); $[\alpha]^{16}_D$ +82.6° (EtOH) (67% optical purity)]], (b) 2-*endo*-norbrendanol (**15**) [65 mg, 32% yield; mp 183–185 °C (in a sealed tube); $[\alpha]^{21}_D$ ±0° (*c* 0.76, $CHCl_3$); the *p*-nitrobenzoate, mp 72.5–73.5 °C (from ethanol–water); $[\alpha]^{22}_D$ +20.2° (*c* 0.49, $CHCl_3$) (72% optical purity[17])].

Anal. Calcd for $C_{15}H_{15}O_4N$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.43; H, 5.39; N, 5.09.

The endo alcohol **15** (30 mg, $[\alpha]^{21}_D$ ±0°) was dissolved in acetone (5 mL) and treated with Jones reagent to give the (+)-ketone **6** [25 mg, mp 114–119 °C (in a sealed tube); $[\alpha]^{23}_D$ +88.8° (*c* 0.97, EtOH) (72% optical purity[17])].

(B) Reduction with *C. lunata*. A total of 400 mg of the (±)-ketone **6** was added to four batches (4 × 200 mL) of *C. lunata* culture solution, and the mixture was incubated at 30 °C for 3.5 h to give (a) the (−)-ketone **6** [90 mg, 22.5% yield; mp 122–124 °C (in a sealed tube); $[\alpha]^{23}_D$ −6.6° (*c* 1.47, EtOH) (5.3% optical purity[17])], (b) the endo alcohol **15** [146 mg, 36.5% yield; mp 183–184 °C (in a sealed tube); $[\alpha]^{27}_D$ ±0° (*c* 1.6, $CHCl_3$); the *p*-nitrobenzoate, mp 80–81 °C (from ethanol–water); $[\alpha]^{26}_D$ +1.9° (*c* 0.85, $CHCl_3$) (7% optical purity[17])].

The endo alcohol **15** (50 mg, $[\alpha]^{27}_D$ ±0°) was dissolved in acetone (2 mL) and treated with Jones reagent to afford the (+)-ketone **6** [28 mg; mp 122–124 °C (in a sealed tube); $[\alpha]^{26}_D$ +7.6° (*c* 0.55, EtOH) (6% optical purity[17])].

HLADH-Catalyzed Oxidation of (±)-2-*endo*-Norbrendanol (15). HLADH (8 mg) was added to a glycine–NaOH buffer (pH 9.0, 500 mL) containing the (±)-alcohol **15**[36] (104 mg, 0.84 mmol), NAD^+ (60 mg, 0.085 mmol), and FMN (810 mg, 1.73 mmol), and the mixture was incubated at 25 °C. The incubation was terminated for 3.5 h to give (a) the (+)-ketone **6** [25 mg, 18% yield; mp 122–124 °C (in a sealed tube); $[\alpha]^{25}_D$ +15.1° (*c* 0.57, EtOH) (12% optical purity[17])], (b) the endo alcohol **15** contaminated with 14% of the exo isomer **16** [mp 169–173 °C (in a sealed tube); $[\alpha]^{21}_D$ 0° ± 0.1° (*c* 1.2, $CHCl_3$); the *p*-nitrobenzoate: mp 84.5–85.5 °C (from ethanol–water); $[\alpha]^{26}_D$ −2.46° (*c* 0.57, $CHCl_3$) (9% optical purity[17]); NMR (100 MHz, CCl_4) δ 5.18 (m, 1 H, OCH_{exo}) (its NMR spectrum showed no contaminations with exo *p*-nitrobenzoate)].

HLADH-Catalyzed Oxidation of (±)-2-*exo*-Norbrendanol (16). HLADH (25 mg) was added to a glycine–NaOH buffer (pH 9.0, 1 L) containing the (±)-alcohol **16**[36] (220 mg, 0.56 mmol), NAD^+ (141 mg, 0.96 mmol), and FMN (1.62 g, 3.46 mmol), and the mixture was incubated at 25 °C. When GLC monitoring indicated a 23% conversion after 80 h, NAD^+ (140 mg, 0.196 mmol) and HLADH (20 mg) were added to the reaction mixture, and incubation was resumed until a 38% conversion had been achieved (70 h). The routine workup afforded (a) the (+)-ketone **6** [67 mg, 30.5% yield; mp 126–128 °C (in a sealed tube); $[\alpha]^{25}_D$ +90.3° (*c* 0.56, EtOH) (73% optical purity[17])], (b) the (−)-exo alcohol **16** [109 mg, 49.5% yield; mp 162–163 °C (in a sealed tube); $[\alpha]^{25}_D$ −5.5° (*c* 1.1, $CHCl_3$) (41.4% optical purity[17])], the *p*-nitrobenzoate [mp 106–107 °C (from ethanol–water); $[\alpha]^{28}_D$ −4.6° (*c* 0.46, $CHCl_3$); NMR (100 MHz, CCl_4) δ 4.95 (s, 1 H, OCH_{endo})].

Anal. Calcd for $C_{15}H_{15}O_4N$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.88; H, 5.50; N, 5.08.

The (−)-exo alcohol **16** (55 mg, $[\alpha]^{25}_D$ −5.5°) was dissolved in acetone (2 mL) and treated with Jones reagent to afford the (−)-ketone **6** (27 mg, 55% yield); mp 122–125 °C (in a sealed tube); $[\alpha]^{23}_D$ −50.97° (*c* 0.47, EtOH) (41.4% optical purity[17]).

Reduction of (±)-2-Brendanone (7) by Biological Methods. (A) HLADH-Catalyzed Reduction. The (±)-ketone **7**[37] (220

(35) The (±)-ketone **6** was prepared by the method of Sauers et al.;[13] mp 123–124 °C (in a sealed tube) [lit.[13] mp 126–127 °C (in a sealed tube)].

(36) The (±)-alcohols **15** and **16** were prepared by the method of Sauers et al.;[13] the endo alcohol **15** was found to be contaminated with 10% of the exo isomer **16**, mp 179–184 °C (in a sealed tube) [lit.[13] mp 184–185 °C (in a sealed tube)]; exo alcohol **16**, mp 160–164 °C (in a sealed tube) (lit.[13] mp 155–157 °C).

mg, 1.6 mmol), NAD^+ (130 mg, 0.18 mmol), and ethanol (2 mL) were dissolved in phosphate buffer (pH 7.0, 1 L). HLADH (20 mg) was added to initiate the reaction, and the mixture was shaken at 25 °C for 96 h to afford (a) the ketone **7** [135 mg, 61% yield; mp 114–115 °C (in a sealed tube); $[\alpha]^{25}_D$ −0.19° (*c* 1.93, EtOH) (0.4% optical purity[19])], (b) the (−)-endo alcohol **17** [10 mg, 5% yield; mp 171–172 °C (in a sealed tube); $[\alpha]^{25}_D$ −0.26° (*c* 0.39, $CHCl_3$) (18% optical purity[19])].

(B) Reduction with *C. lunata*. The (±)-ketone **7** (total 1.0 g, 7.38 mmol) was incubated with eight 200-mL batches of *C. lunata* culture for 13.5 h at 30 °C to give (a) the (+)-ketone **7** [180 mg, 18% yield; mp 116–119 °C (in a sealed tube); $[\alpha]^{32}_D$ +12.9° (*c* 1.17, EtOH) (28% optical purity[19]) [lit.[18] mp 116.5–118 °C (in a sealed tube); $[\alpha]^{16}_D$ +31.3° (EtOH) (67% optical purity)]]; (b) the (+)-endo alcohol **17** [400 mg, 40% yield; mp 169–173 °C (in a sealed tube); $[\alpha]^{25}_D$ +0.40° (*c* 1.23, $CHCl_3$) (27% optical purity[19])].

Brown's reagent[38] (0.9 mL) was added to a chilled and stirred solution of the (+)-endo alcohol **17** (200 mg, 1.45 mmol, $[\alpha]^{25}_D$ +0.4°) in ether (7 mL). After 30 min, water was added, and the ether layer separated, was washed with dilute aqueous $NaHCO_3$ and water, and was dried ($MgSO_4$). Removal of the solvent gave a crude product which was sublimed in vacuo to give the (−)-ketone **7** [100 mg, 50% yield; mp 112–114.5 °C (in a sealed tube); $[\alpha]^{32}_D$ −12.7° (*c* 0.99, EtOH) (27% optical purity[19])].

HLAD-Catalyzed Oxidation of (±)-2-*endo*-Brendanol (17). The (±)-alcohol **17**[39] (150 mg, 1.087 mmol), NAD^+ (93 mg, 0.129 mmol), and FMN (1.09 g, 2.3 mmol) were dissolved in glycine–NaOH buffer (pH 9.0, 750 mL). The reaction was initiated by adding HLADH (13 mg) and allowed to proceed at 25 °C for 4.5 h to afford (a) the (+)-ketone **7** [41 mg, 27% yield; mp 119–121 °C (in a sealed tube); $[\alpha]^{25}_D$ +13.6° (*c* 0.7, EtOH) (29% optical purity[19])], (b) the (+)-endo alcohol **17** [62 mg, 41% yield; mp 168–170.5 °C (in a sealed tube); $[\alpha]^{25}_D$ +0.4° (*c* 0.12, $CHCl_3$) (27% optical purity[19])].

Reduction of (±)-2-Isobrendanone (8) with *C. lunata*. A total of 225 mg (1.65 mmol) of the (±)-ketone **8** was incubated in three batches (3 × 250 mL) of the culture medium at 30 °C for 40 h to give (a) the (+)-ketone **8** [53 mg, 24% yield; mp 150–151 °C (in a sealed tube); $[\alpha]^{23}_D$ +28.0° (*c* 0.46, EtOH) (13% optical purity[20])], (b) the (−)-endo alcohol **19** [60 mg, 26% yield; mp 191–192 °C (in a sealed tube); $[\alpha]^{23}_D$ −2.75° (*c* 0.96, $CHCl_3$) (14% optical purity[20])].

HLADH-Catalyzed Oxidation of (±)-2-*endo*-Isobrendanol (19). The (±)-alcohol **19** (65.5 mg, 0.57 mmol), NAD^+ (36 mg, 0.05 mmol), and FMN (450 mg, 0.9 mmol) were dissolved in glycine–NaOH buffer (pH 9.0, 600 mL). The reaction was initiated by adding HLADH (6 mg) and allowed to proceed at 25 °C for 48 h to give: (a) the (+)-ketone **8** [10 mg, 15% yield; mp 150–150.5 °C (in a sealed tube); $[\alpha]^{23}_D$ +169.0° (*c* 0.24, EtOH) (81% optical purity[20])], (b) the (−)-endo alcohol **19** [25 mg, 40% yield; mp 194–195 °C (in a sealed tube); $[\alpha]^{23}_D$ −17.0° (*c* 0.49, $CHCl_3$) (87% optical purity[20])].

The (−)-endo alcohol **19** (17 mg, 0,12 mmol, $[\alpha]^{23}_D$ −17.0°) was dissolved in acetone (3 mL) and treated with Jones reagent to give the (−)-ketone **8** [10 mg, 59% yield; mp 150–151 °C (in a sealed tube); $[\alpha]^{22}_D$ −182.0° (*c* 0.18, EtOH) (87% optical purity[20]) [lit.[18] mp 146–149 °C (in a sealed tube); $[\alpha]^{23}_D$ −199° (EtOH) (95% optical purity)]].

Reduction of (±)-2-Isotwistanone (9) with *C. lunata*. The (±)-ketone **9** (440 mg, 2.93 mmol) was distributed to six batches of the culture solution (6 × 200 mL) of *C. lunata*, and the flasks were incubated at 30 °C for 69 h to afford (a) the (+)-ketone **9** [140 mg, 32% yield; mp 95–98 °C (in a sealed tube); $[\alpha]^{23}_D$ +26.4° (*c* 0.36, $CHCl_3$) (20% optical purity[21])], (b) the (−)-endo alcohol **21** [40 mg, 9% yield; mp 151–157 °C (in a sealed tube); $[\alpha]^{18}_D$ −37.7° (*c* 0.58, $CHCl_3$) (64% optical purity[21]); NMR (100 MHz, $CDCl_3$) δ 3.95 (d, *J* = 9 Hz, 1 H, $HOCH_{exo}$)]; (c) the (+)-exo alcohol **22** [25 mg, 6% yield; mp 143–148 °C (in a sealed tube); $[\alpha]^{20}_D$ +4.64° (*c* 0.58, $CHCl_3$) (21% optical purity[21]); NMR (100 MHz, $CDCl_3$) δ 3.42 (d, *J* = 3 Hz, 1 H, $HOCH_{endo}$)].

A specimen of the (−)-endo alcohol **21** (35 mg, $[\alpha]^{18}_D$ −37.7°) was dissolved in acetone (20 mL) and treated with Jones reagent to give the (−)-ketone **9** [10 mg, 29% yield; mp 93–94.5 °C (in a sealed tube); $[\alpha]^{21}_D$ −89.4° (*c* 0.42, $CHCl_3$) (68% optical purity[21]); CD (isooctane, *c* 8.6 × 10^{-4} mol/L) $[\Theta]_{max}$ (nm) −2.78 × 10^3 (289) (sh), −3.68 × 10^3 (298.5), −3.6 × 10^3 (307.5), −2.09 × 10^3 (317) (sh)].

A sample of the (+)-exo alcohol **22** (20 mg, $[\alpha]^{20}_D$ +4.64°) was treated with Jones reagent to give the (+)-ketone **9** [8 mg, 40% yield; mp 92.5–96 °C (in a sealed tube); $[\alpha]^{20}_D$ +28.1° (*c* 0.31, $CHCl_3$) (21% optical purity[21])].

(+)-2-*endo*-Isotwistyl Acetate (36). A solution of the (+)-ketone **9** (120 mg, 0.88 mmol, $[\alpha]^{23}_D$ +26.4°) in ether (20 mL) was added to a suspension of $LiAlH_4$ (120 mg, 3.2 mmol) in ether (40 mL). The mixture was refluxed for 3 h, and the usual workup gave a crude mixture (120 mg) which was shown by GLC analysis to be a 7:3 mixture of the endo (**21**) and exo alcohols (**22**). The mixture was taken up into pentane and chromatographed over alumina to give the (+)-endo alcohol **21** [40 mg, 33% yield; mp 151–156 °C (in a sealed tube); $[\alpha]^{21}_D$ +11.7° (*c* 0.52, $CHCl_3$)].

A sample of the (+)-endo alcohol **21** (30 mg) was treated with acetic anhydride (0.5 mL) in pyridine (1 mL), and the resulting oil was purified by preparative TLC (silica gel 60 $PF_{254+366}$ (Merck), development with $CHCl_3$). The optical purity of this specimen, $[\alpha]^{25}_D$ +19.4° (*c* 0.32, $CHCl_3$), was determined by means of the NMR method, and found to be 20%; NMR (60 MHz, CCl_4) δ 4.80 (d, *J* = 9 Hz, 1 H, $AcOCH_{exo}$).

(±)-2-*anti*-*twist*-Brendanol (12). A solution of (±)-2-*twist*-brendanone (**3**)[40] (270 mg, 2 mmol) in dry ether (20 mL) was added to a suspension of $LiAlH_4$ (110 mg, 2.89 mmol) in dry ether (50 mL). The mixture was refluxed for 4 h, and the usual workup gave a crude product (260 mg) which was chromatographed over alumina. Sublimation in vacuo gave the (±)-anti alcohol **12** (240 mg, 87% yield); mp 215–217 °C (in a sealed tube) [lit.[41] mp 188–191 °C (in a sealed tube)]; NMR (60 MHz, CCl_4) δ 3.92 (d, *J* = Hz, 1 H, $HOCH_{syn}$).

Oxidation of (±)-2-*anti*-*twist*-Brendanol (12) by Biological Methods. (A) HLADH-Catalyzed Oxidation. The (±)-alcohol **12** (253 mg, 1.83 mmol), NAD^+ (130 mg, 0.18 mmol), and FMN (1.8 g, 3.6 mmol) were dissolved in glycine–NaOH buffer (pH 9.0, 1.3 L). The oxidation was initiated by adding HLADH (20 mg), and the reaction was allowed to proceed at 25 °C for 12.5 h to give (a) the (−)-ketone **3** [83 mg, 33% yield; mp 187–188 °C (in a sealed tube); $[\alpha]^{21}_D$ −30.6° (*c* 0.71, EtOH) (10.5% optical purity[26]) [lit.[27] mp 169–171 °C (in a sealed tube); $[\alpha]^{21}_D$ −240° (*c* 0.58, EtOH) (82% optical purity)]], (b) the (+)-anti alcohol **12** [88 mg, 35% yield; mp 212–213 °C (in a sealed tube); $[\alpha]^{21}_D$ +33.7° (*c* 0.70, $CHCl_3$) (16% optical purity[26])].

The (+)-anti alcohol **12** (33.5 mg, $[\alpha]^{21}_D$ +33.7°) was dissolved in acetone (7 mL) and treated with Jones reagent to give the (+)-ketone **3** (21 mg, 63% yield); mp 187–188 °C (in a sealed tube); $[\alpha]^{23}_D$ +46.35° (*c* 0.6, EtOH) (16% optical purity[26]).

(B) Oxidation with Resting *C. lunata*. The culture medium (600 mL) inoculated with *C. lunata* was incubated at 30 °C for 48 h. The grown mycelia were collected by filtration and washed with sterilized water. The resulting wet mycelia were suspended on a 0.05 M glycine–NaOH buffer (pH 9.0, 550 mL) containing the (±)-alcohol **12** (110 mg, 0.8 mmol), and the mixture was incubated at 30 °C for 100 h to give (a) the (+)-ketone **3** [8 mg, 7% yield; $[\alpha]^2_D$ +176.4° (*c* 0.4, EtOH) (60.4% optical purity[26])], (b) the (−)-anti alcohol **12** [80 mg, 53% yield; $[\alpha]^{21}_D$ −18.5° (*c* 0.7, $CHCl_3$) (8.7% optical purity[26])].

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Registry No. (±)-**2**, 71806-62-7; (±)-**3**, 42070-80-4; (+)-**3**, 87507-31-1; (−)-**3**, 42070-71-3; (±)-**4**, 73679-80-8; (±)-**6**, 87507-32-2; (+)-**6**, 68069-47-6; (−)-**6**, 87507-33-3; (±)-**7**, 87507-34-4; (+)-**7**,

(37) The (±)-ketone **7** was prepared by the method of Nickon et al.;[14] mp 114–117 °C (in a sealed tube) (lit.[14] mp 118.5–119.5 °C).

(38) (a) Brown, H. C.; Garg, C. P. *J. Am. Chem. Soc.* **1961**, *83*, 2952–2953. (b) Brown, H. C.; Garg, C. P.; Liu, K-T. *J. Org. Chem.* **1971**, *36*, 387–390.

(39) The (±)-alcohol **17** was prepared by the method of Nickon et al.;[14] mp 163–166 °C (in a sealed tube) [lit.[14] mp 174–175 °C (in a sealed tube)].

(40) The (±)-ketone **3** was prepared by the method of Naemura et al.;[27] mp 189–190 °C (in a sealed tube) [lit.[27] mp 174–175 °C (in a sealed tube)].

(41) Nakazaki, M.; Naemura, K.; Harita, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1907–1913.

68069-46-5; (−)-**7**, 87507-35-5; (±)-**8**, 87507-36-6; (+)-**8**, 87507-37-7; (−)-**8**, 68024-12-4; (±)-**9**, 87451-48-7; (+)-**9**, 87507-38-8; (−)-**9**, 87507-62-8; (±)-**11**, 77341-13-0; (±)-*anti*-**12**, 87507-39-9; (+)-*anti*-**12**, 87507-40-2; (−)-*anti*-**12**, 87507-41-3; (±)-**13**, 77341-20-9; (±)-**15**, 87507-42-4; *endo*-**15**-(*R*)-ol, 87507-60-6; *endo*-**15**-(*S*)-ol, 87507-61-7; (+)-*endo*-PNB-**15**, 87451-49-8; (−)-*endo*-PNB-**15**, 87507-43-5; (±)-*exo*-**16**, 87507-44-6; (−)-*exo*-**16**, 87507-45-7; (−)-*exo*-PNB-**16**, 87507-59-3; (±)-*endo*-**17**, 87507-46-8; (+)-*endo*-**17**, 87507-48-0; (−)-*endo*-**17**, 87507-47-9; (±)-*exo*-**18**, 87507-49-1; (±)-*endo*-**19**, 87451-50-1; (−)-*endo*-**19**, 87507-50-4; (±)-*exo*-**20**, 87507-51-5; (±)-*endo*-**21**, 87451-51-2; (+)-*endo*-**21**, 87507-63-9; (−)-*endo*-**21**, 87507-52-6; (±)-*exo*-**22**, 87507-53-7; (+)-*exo*-**22**, 87507-54-8; (±)-*endo*-**23**, 87507-55-9; (±)-**24**, 87507-56-0; (±)-**25**, 87507-57-1; (±)-*endo*-**26**, 87507-58-2; (±)-*endo*-**27**, 87451-52-3; (±)-*endo*-**28**, 87451-53-4; (±)-*endo*-**29**, 87451-54-5; (±)-*endo*-**30**, 87451-55-6; (±)-*endo*-**31**, 87451-56-7; (±)-*endo*-**32**, 87451-57-8; (±)-*endo*-**33**, 87451-58-9; (±)-*endo*-**35**, 87451-59-0; (+)-*endo*-**36**, 87451-60-3; HLADH, 9031-72-5.

Preparation and Stereochemistry of Methylation of Some Cycloalkyl Sulfones

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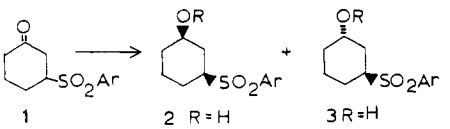
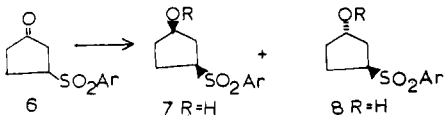
3-((4-Methylphenyl)sulfonyl)cyclohexanols (**2**, **3**), 3-((4-methylphenyl)sulfonyl)cyclopentanols (**7**, **8**), and 2-((4-methylphenyl)sulfonyl)cyclopentanols (**12**, **15**) and their tetrahydropyranyl ether derivatives were prepared. Attempted methylation of the tetrahydropyranyl ether derivatives of 2-((4-methylphenyl)sulfonyl)cyclopentanols (**13**, **16**) gave only products resulting from elimination. Methylation of the other compounds showed that cis and trans isomers generated a common set of interconverting anionic intermediates. The most stable configuration of the anion is the species that is predominantly and in some cases exclusively methylated.

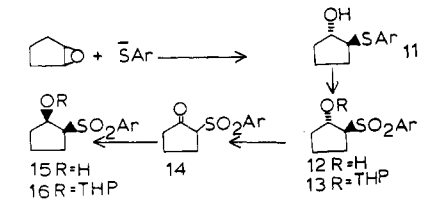
There has been much work involving α-sulfonylcarbanions. It is known that proton exchanges using a basic catalyst can occur with retention of configuration.[1-3] There have been attempts to explain the nature of stabilization and the stereochemistry of α-sulfonylcarbanions.[1-5] However, there appears to be little experimental work dealing with the stereochemistry involved in the alkylation of α-sulfonylcarbanions. We investigated the stereochemistry involved in the methylation of 1,2- and 1,3-sulfonylcyclopentanols, their tetrahydropyranyl ether derivatives,and the tetrahydropyranyl ether derivatives of 1,3-sulfonylcyclohexanols.

3-((4-Methylphenyl)sulfonyl)cyclohexanols were prepared in the following manner. 3-((4-Methylphenyl)-sulfonyl)cyclohexanone (**1**) treated with lithium aluminium



hydride led to 87% cis alcohol (**2**) and 13% of the trans isomer (**3**). Treatment of this ketone with L-Selectride (Aldrich) led to 87% of the trans and 13% of the cis alcohol. This behavior is similar to that found for the reduction of 3-*tert*-butylcyclohexanone and was of aid in assigning configuration. Reduction of 3-*tert*-butylcyclohexanone with lithium aluminum hydride gives 15% trans and 85% cis alcohols.[6] Reduction with the highly hindered lithium perhydro-9b-boraphenalyl hydride gives 72% *trans*- and 28% *cis*-*tert*-butylcyclohexanols.[6] The 3-((4-methylphenyl)sulfonyl)cyclopentanols were prepared in a similar manner. Reduction of 3-((4-methylphenyl)-sulfonyl)cyclopentanone (**6**) with lithium aluminum hydride led to 63% of *cis*- and 37% *trans*-3-((4-methylphenyl)sulfonyl)cyclopentanols (**7**, **8**). This is very similar to results reported by Richer and Gilardeau[7] for the reduction of 3-*tert*-butylcyclopentanone. Treatment of this ketone with lithium aluminum hydride led to 60% of the cis and 40% of the trans alcohols. The 1,2-sulfonylcyclopentanols were prepared as follows.





Treatment of **11** with *m*-chloroperoxybenzoic acid gave sulfone **12**. Treatment of **12** with chromium trioxide gave ketone **14** and reduction with L-Selectride gave exclusively cis alcohol **15**.

In all cases, in both the cyclopentyl and cyclohexyl sulfones, conversion of the alcohol into a tetrahydropyranyl ether derivative and subsequent regeneration of the alcohol did not lead to epimerization. The NMR spectra of all

(1) (a) Cram, D. J.; Scott, D. A.; Nielsen, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 3696. (b) Cram, D. J.; Whitney, T. A. *Ibid*, **1967**, *89*, 4651. (c) Cram, D. J.; Nielsen, W. D.; Rickborn, B. *Ibid.* **1960**, *82*, 6415.

(2) Maccagnani, G.; Montanari, F.; Taddei, F. *J. Chem. Soc. B* **1968**, 453.

(3) Block, E. "Reactions of Organosulfur Compounds"; Academic Press: New York, **1978**; pp 8–26, 36–56.

(4) (a) Wolfe, S.; Rauk, A.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1969**, *91*, 1567.

(5) Bordwell, F. G.; Vanier, N. R.; Mathew, W. S.; Henderickson, J. B.; Skipper, P. L. *J. Am. Chem. Soc.* **1975**, *97*, 7160.

(6) Brown, H. C. "Boranes in Organic Chemistry"; Cornell University Press: Ithaca, NY, 1972; pp 243–244.

(7) Richer, J. C.; Gilardeau, C. *Can. J. Chem.* **1965**, *43*, 3419.

0022-3263/83/1948-4345$01.50/0