Stereoselective Horse Liver Alcohol Dehydrogenase Catalyzed Oxidoreductions of Some Bicyclic [2.2.1] and [3.2.1] Ketones and Alcohols[1a,b]

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Abstract: The asymmetric synthesis potential of horse liver alcohol dehydrogenase (HLADH) with respect to some 2-oxy-bicyclic [2.2.1] and [3.2.1] substrates has been examined. Preparative-scale (up to 1 g) HLADH-catalyzed oxidoreductions of (±)-2-norbornanone (**1**), (±)-bicyclo[3.2.1]-2-octanone (**5**), and (±)-*exo*-2-norbornanol (**11**) have been found to proceed enantioselectively and with very high epimeric specificity. HLADH-catalyzed reductions of (±)-**1** give rise to (−)-(1*R*,4*S*)-**1** and (+)-(1*S*,2*R*,4*R*)-*endo*-2-norbornanol of up to 66% optical purity. Similarly, (±)-**5** yields enantiomerically enriched (−)-(1*R*,5*R*)-**5** and (−)-(1*S*,2*S*,5*S*)-*exo*-bicyclo[3.2.1]-2-octanol (83% optically pure), while HLADH-mediated oxidation of (±)-**11** leads to (+)-(1*R*,2*R*,4*S*)-**11** and (+)-(1*S*,4*R*)-**1** of 71 and 68% optical purities, respectively. Furthermore, the optical purities of the products can be manipulated by varying the extent of the reaction. The stereospecificities observed are all interpretable in terms of an updated diamond lattice model of the active site. This enzymic approach is the most convenient single-step process for preparing research-scale quantities of enantiomerically enriched 2-oxynorbornanes and 2-oxy[3.2.1]octanes. In addition, enantiomerically pure samples of each stereoisomer of the 2-hydroxy- and 2-ketonorbornanes have been prepared by classical procedures.

The potential of enzymes for effecting asymmetric syntheses and resolutions has been widely recognized. Nevertheless, despite the large number of examples of such applications which have been documented,[2] considerable scope remains with respect to the exploitation of enzymes as chiral catalysts. Alcohol dehydrogenases, which catalyze selective and stereospecific oxidoreductions of the type represented in eq 1,[3]

$$>C{=}O + NAD(P)H + H^+ \rightleftharpoons >CH(OH) + NAD(P)^+ \quad (1)$$

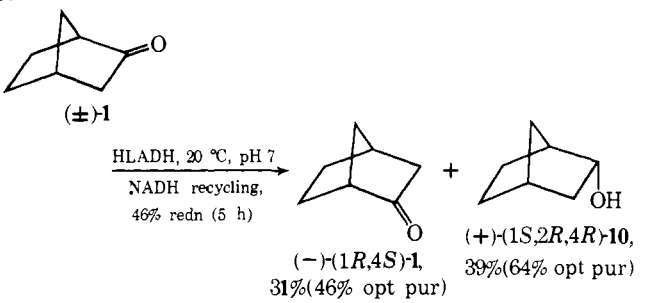
are of particular asymmetric synthesis value. The oxidoreductase of most current utility is HLADH; it is a commercially available enzyme of well-defined and predictable stereospecificity and a broad structural range of aldehydes, ketones, and alcohols have been identified as substrates.[2] However, the enzyme's specificity with respect to many structures of organic chemical interest remains to be delineated. We have now examined the specificity of HLADH toward some bicyclic [2.2.1] and [3.2.1] compounds and have found the HLADH approach to be the most rapid and convenient single-step method yet developed for preparing substantially enantiomerically enriched 2-oxynorbornanes or 2-oxybicyclo[3.2.1]octanes from their racemic precursors. Furthermore, all the data are compatible with the updated[2] diamond lattice section of the active site.

Results

Bicyclic [2.2.1] and [3.2.1] Ketone Specificity of HLADH. The relative rates of HLADH-catalyzed reductions of a range of potential substrates of this type are summarized in Table I. Of the bridged bicyclic ketones evaluated, (±)-**1** and (±)-**5** were clearly the best substrates and the stereochemistries of their enzyme-catalyzed reductions were therefore examined in some detail under preparative-scale conditions.

HLADH-Catalyzed Reduction of (±)-2-Norbornanone (1). Racemic 2-norbornanone was subjected to HLADH-catalyzed reduction using sodium dithionite recycling[4] of the nicotinamide coenzyme. The reaction was terminated when ~50% of the starting material had been reduced. The residual norbornanone and norbornanol product were then isolated and characterized as their DNP and AP derivatives, respectively. The results are summarized in Scheme I.[5] *endo*-2-Norbornanol (**10**) was produced exclusively; none of the exo epimer could be detected. The absolute configurations shown were assigned on the basis of literature correlations.[6b] Owing to the uncertainties in the literature values[6-8] of the specific rotations of the pure enantiomers of 2-norbornanones and 2-norbornanols and their derivatives, it was necessary to resolve them before the optical purities[9] of the HLADH-derived samples of (−)-**1** and (+)-**10** of Scheme I could be established. The enantiomerically pure reference compounds required were prepared from racemic *exo*-2-norbornanol (**11**) as outlined in Scheme II. The specific rotations cited were measured in chloroform solution. (±)-*endo*-2-Norbornanol (**10**) was a much less satisfactory starting material for the Scheme II type of resolution.

Scheme 1

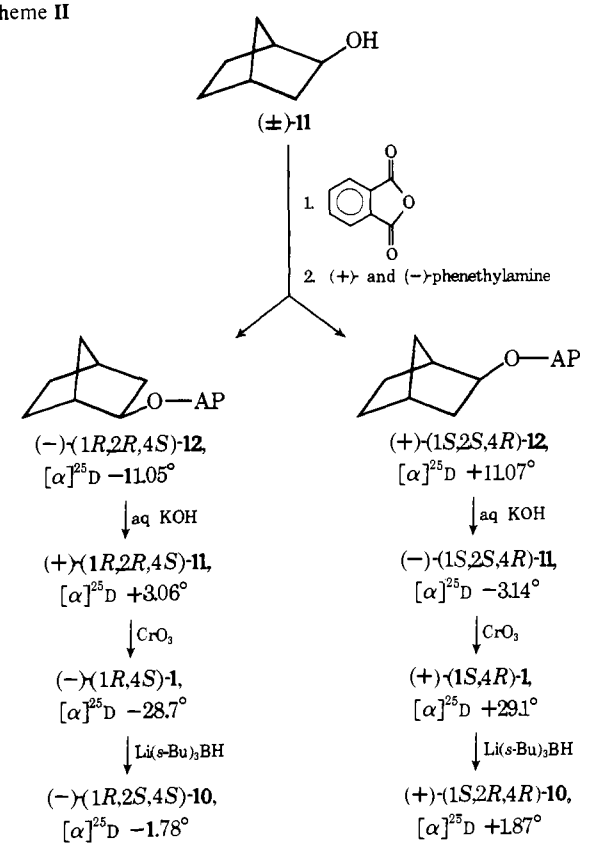


As for all enantioselective enzymic processes,[2] the degree of enantiomeric enrichment of the Scheme I products can be manipulated by varying the extent of reduction. This aspect is illustrated in Table II.

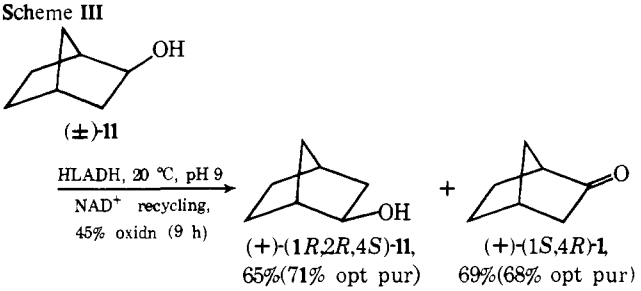
HLADH-Catalyzed Oxidation of (±)-*exo*-2-Norbornanol (11). FMN recycling[10] of the NAD^+ cofactor was employed when (±)-**11** was subjected to HLADH-mediated oxidation. The reaction was terminated at the 45% of reaction point and the alcohol and ketone products were again characterized as their AP and DNP derivatives. The overall results are given in Scheme III. The absolute configurations shown are based on previous literature proofs;[6b,7] the optical purities of (+)-**11** and (+)-**1** were calculated from the Scheme II data.

HLADH-Catalyzed Reduction of Bicyclo[3.2.1]-2-octanone (5). The reduction of racemic **5** in the presence of HLADH and with sodium dithionite recycling of NADH was terminated after 25% of the reaction had occurred. The product alcohol was separated from residual ketone by conversion to its AP

Scheme II

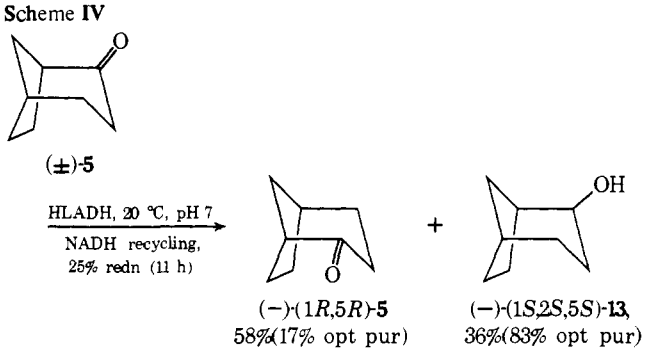


Scheme III



derivative followed by hydrolysis. The results obtained are shown in Scheme IV. The *exo*-bicyclo[3.2.1]-2-octanol

Scheme IV



product was of very high epimeric integrity. NMR and IR analysis indicated any contamination by the endo epimer to be <3%. The absolute configurations of (−)-**5** and (−)-**13** were assigned, and the optical purity of (−)-**13** was calculated on

Scheme V

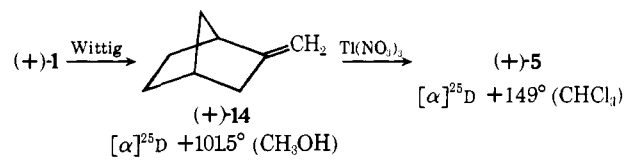
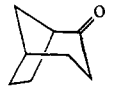
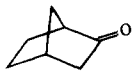
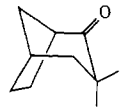
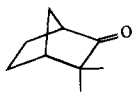
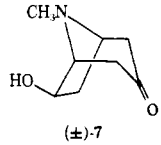
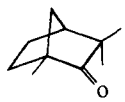
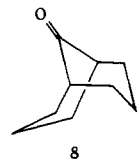
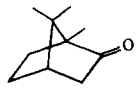
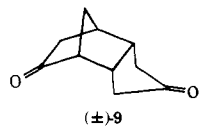
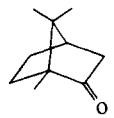


Table I. Relative Rates[a] of HLADH-Catalyzed Reduction of Some Bridged Bicyclic Ketones

Substrate	Rel rate	Substrate	Rel rate
	100	 (±)-5	36
 (±)-1	26	 (±)-6	0.015
 (±)-2	0.001	 (±)-7	0.09
 (−)-3	0	 8	0.015[b]
 (+)-4	0	 (±)-9	0.025
 (−)-4	<0.1		

[a] Reduction rates were measured at 25°C in 0.1 M phosphate buffer, pH 7. Compounds **6**, **7**, and **9** were kindly provided by Drs. J. Stothers, Y. Y. Lin, and P. Deslongchamps, respectively. [b] Measured by Dr. K. P. Lok.

Table II. Effect of Extent of Reaction[d] on Optical Purities in HLADH-Catalyzed Reductions of (±)-2-Norbornanone (**1**)

% redn	% opt purity of (−)-**1**[a]	% opt purity of (+)-**10**[b]
22	13	*c*
46	46	64
77	66	22

[a] Isolated as the 2,4-dinitrophenylhydrazone. [b] Isolated as the acid phthalate. [c] Insufficient material for characterization. [d] For reactions carried out as indicated for Scheme I.

the basis of literature data.[11,12] A revised [α]D value for (+)-**5**, obtained as shown in Scheme V, was used to determine the optical purity of the enzyme-derived sample of (−)-**5**.

Discussion

This investigation of the bridged bicyclic substrate specificity of HLADH was prompted by our interest in (a) extending the asymmetric synthesis utility of the enzyme and (b) evaluating the ability of the recently refined[2] diamond lattice section of the active site to rationalize the behavior of such substrates. Cyclohexanone was used as the reference substrate.[13] Of the bicyclic [2.2.1] and [3.2.1] ketones examined (Table I), rates of reduction rapid enough to make preparative-scale reactions feasible were observed for (±)-**1** and (±)-**5**.

In order to provide clear demonstrations of the practicability of applying HLADH as a chiral oxidoreduction catalyst, each

enzymic reaction was performed using synthetically significant amounts (up to 1 g) of substrates. To enable the preparative-scale oxidoreductions to be carried out as economically as possible, catalytic amounts only of the nicotinamide coenzyme NAD^+ were used in conjunction with the convenient and inexpensive recycling agents of sodium dithionite (reductive-mode regeneration of NADH)[2,4] and FMN (oxidative-mode regeneration of NAD^+).[2,10] The HLADH-mediated reduction and oxidation reactions were effected in solutions of pH 7 and 9, respectively; these values are the optimal ones when all the factors are taken into account.[2] Each reaction was monitored by GLC and terminated as soon as it had proceeded to the desired extent. The ketone and alcohol products were easily extracted by continuous chloroform extraction but the chromatographic (column, thin layer, and liquid) isolation and separation methods applied initially all proved unsatisfactory and were eventually abandonded in favor of classical purification techniques. The [2.2.1] ketones and alcohols were isolated and characterized as their DNP and AP derivatives. In order to minimize errors due to optical fractionation on repeated recrystallization, the optical purities cited in the norbornane series are based on the specific rotations of once-recrystallized materials.[14] In the [3.2.1] series, the AP derivative of (−)-**13** used in the separation procedure was hydrolyzed back to the alcohol before its specific rotation was measured.

Norbornane Substrates. HLADH-catalyzed reduction of racemic 2-norbornanone (Scheme I) proceeded very smoothly and the reaction was worked up when the alcohol:ketone ratio was 46:54. That the 2-norbornanol product was exclusively (>99%) endo was established by comparisons of its ^1H NMR and ^{13}C NMR spectra with those of authentic samples of *endo*-(**10**) and *exo*-2-norbornanol (**11**). The endo stereoselectivity of the HLADH-mediated reduction of **1** is much superior to that of lithium aluminum hydride[15] and higher or equivalent to the hindered lithium trimethoxyaluminum hydride[16a] and tri-*sec*-butyl borohydride[16b] reagents.

The absolute configurations of the Scheme I compounds and their derivatives were assigned on the basis of their correlation with (−)-fenchone.[6b] However, the literature data[6-8] on the specific rotations of the relevant norbornanes were inconsistent. Consequently, the Scheme II resolutions were carried out in order to provide the enantiomerically pure reference compounds needed for the optical purity calculations. Confirmation that the Scheme II enantiomers obtained were optically pure was provided by the close agreement between the specific rotations of (+)- and (−)-**1**, -**10**, and -**12** with the values predicted by isotope dilution experiments[6a] and from previous chemical correlation[6c] and resolution[7] studies. The current results show the previously obtained samples of (+)-**1**-DNP[8] and of (+)- and (−)-**11** (and the corresponding AP's[7]) to have been of 70 and 77% optical purity, respectively. Furthermore, it is now apparent that Jones oxidation of 2-norbornanols can result in partial racemization to the extent of 11% for the endo[6b] and 39% for the exo[7] enantiomer.

Enzyme-catalyzed resolutions are traditionally terminated after 50% of reaction has occurred since this is the point at which optically pure compounds will be obtained if the stereospecificity is absolute. The HLADH-catalyzed reduction of (+)-**1** was worked up at the ~50% stage for this reason and, as Scheme I shows, very satisfactory levels of enantiomeric enrichment were achieved. Furthermore, when an enzyme-mediated process is enantioselective rather than enantiospecific, the degrees of enantiomeric enrichment attainable can often be regulated by judiciously controlling the extent of reaction. For example, with (±)-**1** as the substrate (Table II) late termination of the reduction produces recovered ketone and product alcohol of high and low optical purities, respectively. Early quenching has the opposite effect. A lower yield of the more optically pure material must be accepted as an inevitable consequence of such an operation.

While no *exo*-alcohol was formed during HLADH-catalyzed reduction of (±)-**1**, the enzyme was found to be highly enantioselective when racemic *exo*-2-norbornanol (**11**) was the substrate, and both the yields and degrees of enantiomeric enrichment of the (+)-**1** and (+)-**11** products isolated were good (Scheme III).

Reduction of Bicyclo[3.2.1]-2-octanone [(±)-5]. The HLADH-mediated reduction of (±)-**5** (Scheme IV) was not quite as rapid as that of (±)-**1**. The reaction was terminated after 25% of conversion in order to increase the enantiomeric enrichment of the bicyclo[3.2.1]-2-octanol product. Comparisons of its IR and ^1H NMR [with and without $Eu(thd)_3$] spectra with those of an authentic endo/exo mixture[17] indicated virtually exclusive (>97%) formation of the exo isomer **13**.[18]

The absolute configuration assignment and determination of optical purity of (−)-**13** presented no difficulty since the pure enantiomer had been fully characterized.[11] However, although the designation of the recovered ketone as (−)-**5** was equally straightforward,[12,17] its reported[11] specific rotation was found to be 11% low. As for the analogous 2-norbornanone enantiomers, this error is again attributable to partial racemization during Jones oxidation of the hydroxy precursor. The preferred $[\alpha]_D$ value of enantiomerically pure **5** used in calculating the optical purity of the recovered ketone of Scheme IV was obtained via the Scheme V homologation route. The sample of (+)-2-norbornanone used for this purpose was 96% optically pure; the specific rotations quoted in Scheme V have been corrected for this fact. This reaction sequence also provides additional confirmation for the absolute configuration assignment of Scheme IV.

Conclusions and Prognosis

The classical techniques for resolving the compounds discussed above are tedious and require the expenditure of large amounts of time and materials. In contrast, the HLADH-catalyzed oxidoreduction approach is direct and convenient. It is currently the quickest research-scale method of preparing 2-oxygenated bicyclo[2.2.1]heptanes and bicyclo[3.2.1]octanes of high epimeric and optical purity. Although all the epimers and enantiomers of these compounds are not enzymically producible, most of the remaining stereoisomers can be readily prepared from the Scheme I, III, and IV products by simple chemical oxidoreductions.

The degrees of enantiomeric enrichment reported do not represent the maximum values attainable by the enzymic technique. The individual optical purities observed in the representative experiments described can be enhanced considerably by altering the extent of reaction or by subsequently subjecting partially resolved material to a further enzyme-catalyzed oxidoreduction. These operations can be repeated until the desired level of optical purity is reached.[2,19]

Diamond Lattice Section Analysis. The stereochemical results observed are interpretable in terms of the diamond lattice section approach[20a] using the composite model shown in Figure 1.

Analysis of Bicyclo[3.2.1]-2-octanone Reductions. The enantio- and stereoselectivity of the HLADH-catalyzed reduction of (±)-**5** is very readily interpreted using the diamond lattice model. The analysis is summarized schematically in Figure 2. The conformational constraints imposed by the bridged structure of **5**, coupled with the requirement (Figure 1) that the hydride delivery be from the e-Re direction, makes formation of an exo epimer product obligatory.[21] Only for the (+) enantiomer orientation [Figure 2 (a)] leading to the observed product (−)-**13** are unfavorable lattice interactions avoided.

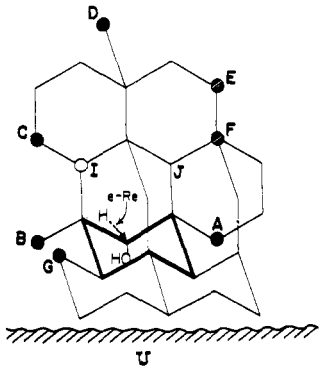


Figure 1. Updated diamond lattice section of the active site of HLADH.[2] Positions A-I, and underneath the lattice (U), are forbidden or undesirable locations in the sense that oxidoreduction is precluded or severely retarded if they are occupied by any part of a potential substrate. The qualitative order of their resistance to occupation is ● > ⊘ > ○. A cyclohexanone (or related) substrate is considered to orient preferentially in the "flat" position (shown in heavy lines) such that equatorial delivery of H to the Re[20b] face of the carbonyl occurs. HLADH is thus termed an e-Re alcohol dehydrogenase. The geometry indicated at the reacting H ··· C-OH center represents the "alcohol-like" transition state envisaged for the oxidoreduction process. A detailed discussion of this lattice and how to apply it is given in ref 2. The use of molecular models is considered essential when analyzing or interpreting substrate behavior in diamond lattice terms.

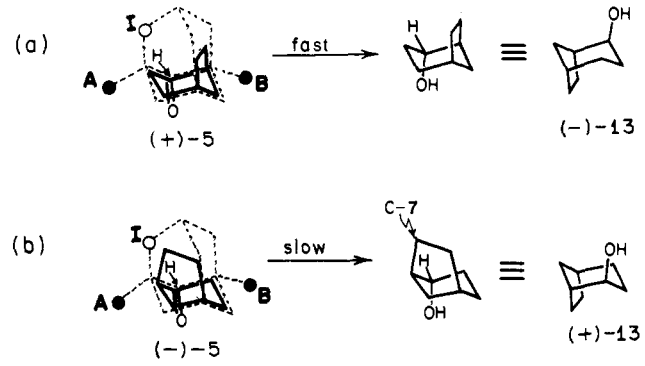


Figure 2. Schematic representation of the orientations of the enantiomers of bicyclo[3.2.1]-2-octanone (**5**) in their preferred "flat" positions within the diamond lattice section of HLADH. Delivery of H to the carbonyl group from the e-Re direction ensures the formation of an *exo*-alcohol. In (a) orientation of (+)-**5** as shown does not place any substituents at undesirable positions. Reduction to the observed product (−)-**13** is thus facile. The corresponding orientation of the enantiomeric ketone (−)-**5** is shown in (b). Here, C-7 is required to locate close to the unsatisfactory position I and reduction in this mode is not favored. Formation of (+)-**13** is thus a relatively slow process.

The diamond lattice section also accounts for the fact that the substrate activity of the 3,3-dimethyl[3.2.1] derivative (±)-**6** is negligibly low (Table I). Orientation of either enantiomer of **6** in the Figure 2 manner would place an equatorial methyl substituent in one or other of the forbidden A or B positions, thereby precluding HLADH-catalyzed reduction. The ketone **8** is a poor substrate for similar reasons. However, the diamond lattice does not give any indication as to why **7** is not a good substrate; the adverse effect here may be due to having the polar nitrogen atom, or its conjugate acid, forced into the hydrophobic active site environment.

Analysis of 2-Oxygenated Norbornane Oxidoreductions. Interpreting the stereospecificity of HLADH toward its 2-oxynorbornane substrates is less clear-cut. For 2-norbornanone (**1**), orientation of the two enantiomers in the diamond lattice with the carbonyl group correctly positioned can be achieved in four ways. These are depicted in Figure 3. All of the carbons of (+)- and (−)-**1** are acceptably situated in the Figure 3 (a) and (b) orientations leading to the *endo*-alcohol products (+)- and (−)-**10** actually produced (Scheme I). On the other hand, formation of the epimeric *exo*-alcohols would require the substrate enantiomers to be oriented as shown in Figure 3 (c) and (d). The Figure 3 (d) situation would not be a favored one since C-6 of (−)-**1** would be placed at I. This explains the absence of (+)-**11** in the product. At first sight, no forbidden or undesirable lattice positions appear to be violated by the Figure 3 (c) orientation. However, while only a few lattice positions, for example, A and B, are identifiable as being clearly forbidden, the overall data on cyclohexanone substrates[2] show that *any* substitution of the cyclohexane ring will reduce the rate of HLADH-catalyzed reduction. When this factor is taken into account, the fact that (−)-**11** is not formed becomes understandable since in the Figure 3 (c) superposition, C-6 of (+)-**1** is required to locate close to the somewhat restricted lattice position J.[22]

The enantioselective formation of the *endo*-alcohol (+)-**10** in preference to (−)-**10** (Scheme I) is rationalizable in a similar manner. The C-4 bridgehead carbon of (−)-**1** projects into the unfavorable area at the left-hand side of the lattice (the C, I, G region) in the transition state preceding (−)-**10** while in the (+)-**1** orientation leading to the predominant enantiomer (+)-**10**, C-4 is located in the unrestricted hydrophobic area

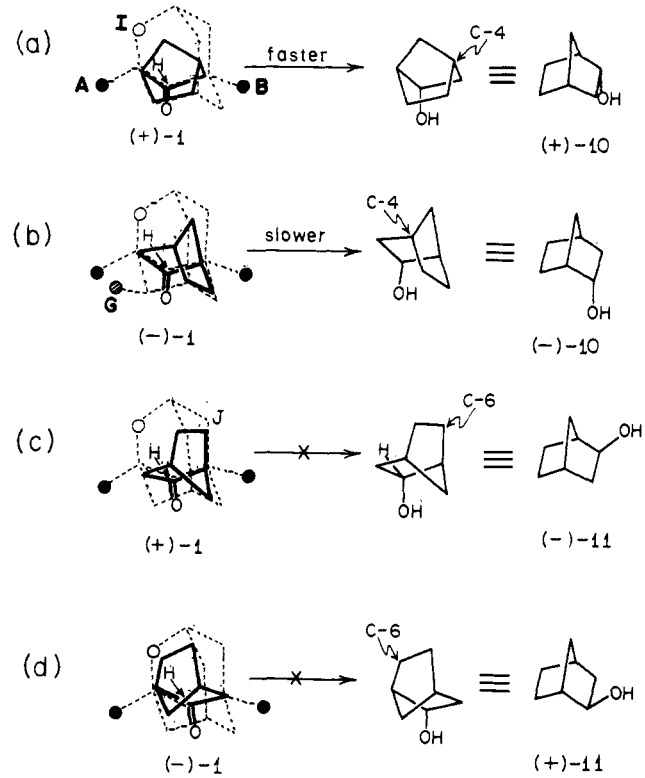


Figure 3. Diamond lattice section analysis of the stereochemical course of HLADH-catalyzed reduction of the 2-norbornanone (**1**) enantiomers. The orientations shown are considered to be those of the "alcohol like" transition states which would be involved, with H being delivered from the e-Re direction of Figure 1. There are no unfavorable lattice interactions when (+)- or (−)-**1** are positioned as shown in (a) and (b), respectively. Reductions to the *endo*-alcohols (+)- and (−)-**10** are thus permitted processes. The predominant formation of the (+) enantiomer of **10** (cf. Scheme I) is thought to be due to the preference of C-4 for its unhindered location in (a) over its position in (b) in which it approaches the forbidden A, I, C region of the lattice. The discrimination of HLADH against *exo*-alcohol, (+)- or (−)-**11**, formation is accounted for by the unfavorable interactions of C-6 with lattice positions J and I, as represented in (c) and (d), respectively.

situated above and to the right of the preferred "flat" cyclohexane position of Figure 1.

The zero or negligibly low substrate activities of the other [2.2.1] ketones of Table I are easily accounted for since Figure

3 type orientations of each enantiomer of **2**, **3**, and **4** place methyl group(s) at either or both the forbidden positions A and B. The diketone **9** is likewise difficult to accommodate without violating an undesirable lattice position.

Interpretation of the enantioselectivity observed in the oxidation (Scheme III) of (±)-*exo*-2-norbornanol (**11**) is also straightforward. Oxidation of (+)-**11**, for which C-6 is required to be situated very close to the occupation-resistant position I, is slow [cf. Figure 3 (d)]. On the other hand, the HLADH-catalyzed conversion of the other enantiomer, (−)-**11**, to (+)-**1** is more rapid since C-6 is positioned adjacent to J [cf. Figure 3 (c)]. Although as discussed above, J is a less than ideal lattice location, it is a much more tolerant one than I.[2]

The diamond lattice section was originally derived[20a] from data on simple mono- and bicyclic six-membered ring ketone substrates.[2] However, it is now evident that, provided it is recognized that forbidden lattice positions vary in their degree of resistance to occupation, the model can be used for interpreting and predicting HLADH stereospecificity for substrates whose carbon skeletons do not conform exactly to the rigidly defined lattice. Further data on the applicability of the diamond lattice analysis to HLADH substrates of previously unevaluated structural types will be presented shortly.

Experimental Section

Melting points were determined on a Thomas-Hoover or Fisher-Johns apparatus and are uncorrected. IR and NMR spectra were recorded for all compounds prepared. For each the spectra were unexceptional and were in total accord with the structures assigned; complete spectral details are available in ref 1b. UV absorptions were monitored with a Cary 16 spectrophotometer equipped with a G-2500 recorder. Unless specified otherwise optical rotations all refer to $CHCl_3$ solutions and were measured with a Perkin-Elmer 141 polarimeter. GLC analyses were performed on an F&M 400 unit equipped with a flame ionization detector using 1 m × 3 mm columns of 2% QF-1 on Chromosorb G. Elemental analyses were by Gygli Microanalytical Laboratory, Toronto, Ontario. The bicyclic compounds used for which no preparation is cited were either gifts (Table I) or were purchased from Aldrich. NAD^+ and NADH were purchased from Sigma and HLADH from Worthington. The activity of each batch of enzyme was determined[24] prior to use and the amounts of HLADH quoted are in milligrams of active enzyme.

Survey of Bridged Bicyclic Ketone Specificity of HLADH. Assays were performed at 25 °C in 0.1 M potassium phosphate buffer pH 7 solutions 1.75×10^{-4} M in NADH. The assay solution concentrations of the substrates **1–9** varied from 10^{-2} M for the most soluble to 10^{-4} M for the least soluble. For each compound evaluated, a reference assay was performed on a solution containing the same concentration of cyclohexanone. The reductions were initiated by adding a 10–100-μl aliquot of HLADH stock solution (2 mg/ml in 0.05 M Tris-HCl buffer, pH 7.4) to the assay solutions to make a final volume of 3 ml in a 1-cm path length cuvette. The absorbance change was monitored at 340 nm at 25 °C and each run was performed in duplicate. The rates of reduction of each substrate relative to cyclohexanone at the same concentration are recorded in Table I.

Preparation of Racemic 2-Oxynorbornanes. (±)-*exo*-2-Norbornanol (**11**), mp 126–127 °C (lit.[7] mp 127.6–128.5 °C), was obtained by hydrolysis of its formate ester[25] or directly from norbornene by hydroboration.[26] (±)-2-Norbornanone (**1**), mp 94–95 °C (lit. mp 97–98 °C,[25] 90–91 °C[27]), was prepared by Jones oxidation of the above *exo*-formate.[25] Reduction of (±)-**1** with lithium trimethoxyaluminum hydride[16a] gave (±)-*endo*-2-norbornanol (**10**), mp 147–148 °C (lit.[7] mp 152–153 °C).

Resolution of (±)-*exo*-2-Norbornanol (11). (a) (+)-(1*R*,2*R*,4*S*)-11. (±)-*exo*-2-Norbornanol (**11**) was converted[7] (71% yield) into its acid phthalate (±)-**12**, mp 101–102 °C (lit. mp 98.6–99.7 °C,[7] 102–103 °C[27]). (+)-Phenethylamine (67.2 g, 0.55 mol) was added to a solution of (±)-**12** (144 g, 0.55 mol) in acetone (1.5 l.) and the mixture heated on a steam bath for 10 min and allowed to cool. The salt obtained was recrystallized (ten times) from acetone to give material (16.2 g, 15%) of constant rotation, $[\alpha]^{25}D$ −4.71° (*c* 1.2). The above salt was dissolved in warm chloroform (200 ml) and the solution washed with an excess of 2 N hydrochloric acid and then with water. The dried ($MgSO_4$) chloroform solution was evaporated and the residue recrystallized from ethyl acetate–petroleum ether (bp 30–60 °C) to give (−)-(1*R*,2*R*,4*S*)-**12** (10.2 g, 94% yield): mp 92–93 °C; $[\alpha]^{25}D$ −11.05° (*c* 1.4) [lit.[7] mp 89.3–90.3 °C; $[\alpha]^{25}D$ −8.49° (*c* 10)]. Anal. ($C_{15}H_{16}O_4$) C, H. Hydrolysis of (−)-**12** as described by Winstein and Trifan[7] gave (in 89% yield) the (1*R*,2*R*,4*S*)-*exo*-alcohol (+)-**11**: mp 126–127 °C; $[\alpha]^{25}D$ +3.06° (*c* 3) [lit.[7] mp 126–126.6 °C; $[\alpha]^{25}D$ +2.44° (*c* 10)]. Anal. ($C_7H_{12}O$) C, H.

(b) (−)-(1*S*,2*S*,4*R*)-11. The mother liquors obtained above from the first four recrystallizations of the (±)-**12** (+)-phenethylamine salt were combined and evaporated. The resulting solid was dissolved in chloroform (800 ml) and washed with excess 2 N hydrochloric acid and then with water. The dried ($MgSO_4$) chloroform solution was evaporated to give a dark red oil (111 g) which was allowed to react with (−)-phenethylamine (52 g) in acetone (900 ml). The salt obtained was recrystallized (six times) to give 26.6 g (24% yield) of material of $[\alpha]^{25}D$ +4.78° (*c* 1.2). The previously described procedure was then followed to convert this to (+)-(1*S*,2*S*,4*R*)-**12** (8.5 g, 47% yield): mp 92–93 °C; $[\alpha]^{25}D$ +11.07° (*c* 1.6) [lit.[7] mp 89–90.2 °C; $[\alpha]^{25}D$ +8.45° (*c* 10)]. Anal. ($C_{15}H_{16}O_4$) C, H. Hydrolysis of (+)-**12** yielded 92% of (−)-(1*S*,2*S*,4*R*)-**11**: mp 126–127 °C; $[\alpha]^{25}D$ −3.14° (*c* 3.1) (lit.[7] mp 126–126.8 °C; $[\alpha]^{25}D$ −2.41°). Anal. ($C_7H_{12}O$) C, H.

(+)-(1*S*,4*R*)- and (−)-(1*R*,4*S*)-2-Norbornanone (1). Chromium trioxide (3.0 g, 30 mmol) was added with stirring to dry pyridine (4.75 g, 60 mmol) in dry methylene chloride (75 ml). After 15 min, (−)-**11** (560 mg, 5 mmol) in methylene chloride (20 ml) was added and the mixture stirred for 30 min at 20 °C. The supernatant was decanted and the black residue washed by decantation with ether (4 × 30 ml). The combined organic phases were washed with saturated aqueous copper sulfate, dried ($MgSO_4$), and evaporated to give (+)-(1*S*,4*R*)-**1** (360 mg, after two sublimations): mp 97–98 °C; $[\alpha]^{25}D$ +29.1° (*c* 1.5).

The (−) enantiomer (1*R*,4*S*)-**1** was prepared in 83% yield in an identical manner from (+)-**11** (3.7 g); it had mp 97–98 °C; $[\alpha]^{25}D$ −28.7° (*c* 2.2) (lit.[7] mp 95.5–96.2 °C; $[\alpha]^{25}D$ −15.73°).

(+)-(1*S*,2*R*,4*R*)- and (−)-(1*R*,2*S*,4*S*)-*endo*-2-Norbornanol (10).[28] Tri-*sec*-butylborane[29] (1.05 ml, 4.2 mmol) in ether was added at 20 °C under nitrogen to a stirred solution of lithium trimethoxyaluminum hydride[16a] (4.2 mmol) in tetrahydrofuran (4.4 ml). The reaction mixture was stirred for a further 30 min at 20 °C and was then cooled to −78 °C and (+)-**1** (230 mg, 2.1 mmol) in tetrahydrofuran (3 ml) added with a syringe. Work-up[16a] followed by sublimation gave (+)-(1*S*,2*R*,4*R*)-**10** (125 mg); mp 151–152 °C; $[\alpha]^{25}D$ +1.87° (*c* 1.3). (−)-(1*R*,2*S*,4*S*)-**10** was similarly prepared in 87% yield from (−)-**1** (320 mg); the sublimed product had mp 150–151.5 °C; $[\alpha]^{25}D$ −1.78° (*c* 2.8) (lit.[7] mp 151.2–152.5 °C; $[\alpha]^{25}D$ −1.89°).

HLADH-Catalyzed Reduction of (±)-2-Norbornanone (1). Racemic **1** (250 mg, 2.27 mmol) and NAD^+ (200 mg, 0.3 mmol) were dissolved at 20 °C in 0.1 M potassium phosphate buffer (500 ml, pH 7) which was also 0.1 M in sodium dithionite[4] (8.6 g needed). The reaction was initiated by the addition of HLADH (15 mg) and was monitored by GLC. After 4 h at 20 °C, when GLC analysis showed ~50% reduction, the reaction mixture was continuously extracted with chloroform for 2 days. The dried ($MgSO_4$) extract was evaporated to give an oily solid (213 mg) containing (by GLC) 46% of **10** and 54% of **1**.

The crude product (105 mg) and phthalic anhydride (65 mg) in dry pyridine (1 ml) were heated under reflux for 4 h. Work-up followed by recrystallization from ethyl acetate–petroleum ether (bp 30–60 °C) gave the (−)-acid phthalate of (+)-**10** (52 mg): mp 98.5–99.5 °C; $[\alpha]^{25}D$ −3.2° (*c* 1.5) [lit.[7] mp 98.3–99.7 °C; $[\alpha]^{25}D$ −4.97° (*c* 10)]. Neither the melting point nor the $[\alpha]^{25}D$ changed on further recrystallization.

The remaining initial product (105 mg) was dissolved in warm methanol (5 ml) containing 6 N hydrochloric acid (0.6 ml) and 2,4-dinitrophenylhydrazine (102 mg) and the mixture warmed (2 min) on a steam bath. The cooled solution was filtered and the red solid recrystallized from ethanol to give the DNP of (−)-**1** (56 mg): mp 130–131 °C; $[\alpha]^{25}D$ −23.8° (*c* 2.8) [lit.[8] [optically impure (+) enantiomer] mp 129–131 °C; $[\alpha]^{25}D$ +30°, which extrapolates to −51.3° for the DNP of optically pure (−)-**1**].

The results of reactions terminated after 22 and 77% reduction are given in Table II.

HLADH-Catalyzed Oxidation of (±)-*exo*-2-Norbornanol (11). Racemic **11** (1 g, 8.9 mmol) was dissolved in 0.05 M glycine–NaOH buffer (500 ml, pH 9) and NAD^+ (560 mg, 0.85 mmol), FMN[10] (6.28 g, 13 mmol), and HLADH (23 mg) were added successively. The pH of the solution was readjusted to 9 with aqueous sodium hydroxide and the mixture kept at 20 °C until GLC analysis showed ~50% oxidation (9 h). The mixture was then continuously extracted with chloroform for 2 days, and the extract dried ($MgSO_4$) and evaporated to give a semisolid (1 g) containing **11** and **1** in the ratio 55:45 (GLC).

The above product (500 mg) and phthalic anhydride (360 mg) in dry pyridine (10 ml) were heated under reflux for 4 h. The cooled solution was diluted with benzene and washed with excess 1 N sulfuric acid. Evaporation of the dried ($MgSO_4$) organic solution followed by recrystallization from ethyl acetate–petroleum ether (bp 30–60 °C) gave the (−)-acid phthalate of (+)-**11** (417 mg): mp 91–92 °C; $[\alpha]^{25}D$ −7.8° (*c* 1.9) (71% optically pure).

The remainder of the crude product (500 mg) was dissolved in methanol (20 ml) containing 10 N hydrochloric acid (2 ml) and 2,4-dinitrophenylhydrazine (400 mg) and the DNP of (+)-**1** was isolated as before. The once recrystallized product (410 mg) had mp 129–130 °C; $[\alpha]^{25}D$ +5.1° (*c* 3) (68% optically pure).

(+)-(1*S*,5*S*)-Bicyclo[3.2.1]-2-octanone (5). Triphenylmethylphosphonium bromide[30] (17.9 g, 0.05 mol) in dry dimethyl sulfoxide (50 ml) was added under nitrogen to sodium hydride (1.2 g, 0.05 mmol) in dimethyl sulfoxide (25 ml).[31] To this was added a solution of (+)-**1** (2.8 g, 25 mmol, 96% optically pure) in dimethyl sulfoxide (20 ml). The mixture was heated at 50 °C for 3 h and was then poured into water (120 ml) and extracted with redistilled pentane (4 × 5 ml). The combined organic phases were washed with 50% aqueous dimethyl sulfoxide (30 ml) and then with brine (3 × 30 ml), dried ($MgSO_4$), and concentrated carefully to 20 ml. Two severe fractional distillations yielded (+)-(1*S*,4*R*)-2-methylenenorbornane (**14**, 1.15 g): bp 122 °C (760 Torr); $[\alpha]^{25}D$ +97.4° (*c* 1.7 in MeOH) [lit.[32] bp [of (±)-**14**] 123 °C (755 Torr)].

Thallium trinitrate trihydrate[33] (3.7 g, 8.3 mmol) in methanol (20 ml) was added with stirring at −10 °C to (+)-**14** (900 mg, 8.3 mmol) dissolved in methanol (25 ml). After being stirred at −10 °C for 30 min the mixture was filtered and concentrated. Ether (50 ml) was added and then 2 N hydrochloric acid (50 ml), the resulting mixture was shaken well, and the phases were separated. The aqueous solution was reextracted with ether (3 × 50 ml) and the combined ether layers were dried ($MgSO_4$), and evaporated. The oil obtained was adsorbed onto a silica column (15 × 2.5 cm), washed with benzene, and eluted with ether–benzene (1:6). The remaining trace impurities were removed by treatment with charcoal followed by steam distillation and sublimation to give (+)-**5**, mp 120–123 °C, $[\alpha]^{25}D$ +142.9° (*c* 2.6), which extrapolates to +149° for the pure enantiomer (lit.[11] $[\alpha]^{25}D$ +130°).

HLADH-Catalyzed Reduction of (±)-Bicyclo[3.2.1]-2-octanone (5). Racemic **5** (1 g, 8.1 mmol), NAD^+ (100 mg, 0.15 mmol), and sodium dithionite (9.5 g, 55 mmol) were dissolved in 0.1 M potassium phosphate buffer (500 ml, pH 7). HLADH (28 mg) was then added and the reaction allowed to proceed at 20 °C. After 11 h, when GLC analysis showed that 25% reduction had occurred, the mixture was worked up in the usual way via chloroform extraction. The oily product obtained was mixed with phthalic anhydride (352 mg) and dry pyridine (20 ml) and the mixture refluxed for 4 h, then cooled, and diluted with chloroform (300 ml). The chloroform solution was washed with excess saturated aqueous copper sulfate, dried ($MgSO_4$), and evaporated to an oil which was chromatographed on a short (15 × 2.5 cm) silica column. Ether–benzene (1:100) elution yielded the unreacted ketone contaminated with phthalic anhydride. This mixture was dissolved in 15% ethanolic potassium hydroxide (30 ml), heated for 30 min on a steam bath, and then steam distilled. The distillate was saturated with sodium chloride and extracted with ether (4 × 50 ml). Evaporation of the ether solution, followed by two sublimations, gave (−)-**5** (436 mg): mp 126–130 °C; $[\alpha]^{25}D$ −23.4° (*c* 1.3) (17% optically pure).

Further elution of the silica column with ether–benzene (1:20) gave the acid phthalate of (−)-**13** as a semisolid. This was heated with 25% aqueous potassium hydroxide at 50 °C for 5 min and then steam distilled. Extraction of the sodium chloride saturated steam distillate with ether (4 × 50 ml) followed by two sublimations yielded (−)-**13**[34] (90 mg): mp 190–194 °C; $[\alpha]^{25}D$ −14.1° (*c* 0.8) (lit.[11] mp 198–200 °C; $[\alpha]^{25}D$ −16.9°).

References and Notes

(1) (a) This work was supported by the National Research Council of Canada. (b) Abstracted from the Ph.D. Thesis of A. J. Irwin, University of Toronto, 1975. (c) Ontario Graduate Fellow, 1972–1973; National Research Council of Canada Scholarship Holder, 1973–1975.

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(3) Abbreviations used: $NAD(P)^+$ and NAD(P)H, oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide (phosphate); HLADH, horse liver alcohol dehydrogenase; DNP, 2,4-dinitrophenylhydrazone; AP, acid phthalate, FMN, flavin mononucleotide; $Eu(thd)_3$, tris(2,2,6,6-tetramethyl-3,5-heptanedionato)europium.

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(5) The extent of oxidoreduction has been taken into account in all calculations of percentage yields.

(6) (a) J. A. Berson and S. Suzuki, *J. Am. Chem. Soc.*, **81**, 4088 (1959); (b) J. A. Berson, J. S. Walia, A. Remanick, S. Suzuki, and D. Willner, *ibid.*, **83**, 3986 (1961); (c) J. A. Berson and A. R. Remanick, *ibid.*, **86**, 1749 (1964).

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(9) All optical purities cited in this work were calculated using the specific rotations of the pure enantiomers as reference values. Attempts to measure enantiomeric enrichment directly using GLC or HPLC analyses of a variety of diastereomeric derivatives, or by ^1H NMR or ^{13}C NMR in the presence of chiral and achiral shift reagents, were unsuccessful.[1b]

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(13) In our experience, preparative-scale reactions are practicable for carbonyl substrates whose relative rates of reduction are >1% that of cyclohexanone.

(14) In fact, the optical properties of these compounds did not change on repeated recrystallization with the exception of (−)-**1**-DNP; its rotation did increase marginally after several recrystallizations.

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(16) (a) H. C. Brown and H. R. Deck, *J. Am. Chem. Soc.*, **87**, 5620 (1965); (b) H. C. Brown and S. Krishnamurthy, *ibid.*, **94**, 7159 (1972).

(17) A. A. Youssef, M. E. Baum, and H. M. Walborsky, *J. Am. Chem. Soc.*, **81**, 4709 (1959).

(18) It is interesting to note that in the enzymic and chemical reductions of (±)-**5**, the hydride equivalents are preferentially delivered to opposite sides of the carbonyl group (endo and exo approaches, respectively). In contrast, in HLADH-mediated and chemical reductions of 2-norbornanone [(±)-**1**], approach of the hydride equivalent from the same (exo) direction predominates.

(19) Cf. J. B. Jones and P. W. Marr, *Tetrahedron Lett.*, 3165 (1973).

(20) (a) V. Prelog, *Pure Appl. Chem.*, **9**, 119 (1964); (b) V. Prelog and G. Helmchen, *Helv. Chim. Acta*, **55**, 2581 (1972), summarized in ref 2, pp 485–489.

(21) Even if the hydride were delivered from the axial direction for (+)- or (−)-**5**, or if nonchair conformations were involved, formation of either of the *endo*-alcohol enantiomers would be precluded since substrate orientations violating forbidden or undesirable lattice positions would be involved in each case.

(22) This is the position occupied by the axial methyl group during HLADH-catalyzed reduction of (2*S*)-methylcyclohexanone.[2] Although occupation of J is preferable to that of its equatorial counterpart A, it is evidently less than ideal since the rate of reduction of (±)-2-methylcyclohexanone is between 10^{-2} and 10^{-3} that of cyclohexanone.[2] Even when allowance is made for the presence of the inactive 2*R* enantiomer (factor of 2) and the low population of the axial conformer of the 2*S* substrate (factor of 15[23]), the consequence of requiring a methyl group to locate at J is a rate of reduction for 2-methylcyclohexanone that is more than 30 times slower than that of cyclohexanone itself.

(23) The conformational free-energy difference between an axial and equatorial methyl group in 2-methylcyclohexanone is 1.6 kcal mol^{-1}; E. L. Eliel, "Stereochemistry of Carbon Compounds", McGraw-Hill, New York, N.Y., 1962, p 240.

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(27) G. Komppa and S. Beckmann, *Justus Liebigs Ann. Chem.*, **512**, 172 (1934).

(28) Preparation of the *endo*-alcohol enantiomers by resolution of (±)-**10** as described for (±)-**11** was not practical. Following recrystallization of the (+)-phenethylamine salt of the acid phthalate of (±)-**10** to constant rotation, (+)-(1*R*,2*S*,4*S*)-**10** acid phthalate, mp 100–101 °C, $[\alpha]^{25}D$ +5.1° (*c* 0.8 in $CHCl_3$) [lit.[7] mp 98.3–99.7 °C, $[\alpha]^{25}D$ +5.0° ($CHCl_3$)], was obtained in only 0.15% yield.

(29) G. F. Hennion, P. A. McKusker, E. C. Ashby, and A. J. Rutkowski, *J. Am. Chem. Soc.*, **79**, 5190 (1957).

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(34) The carbinol C–H ^1H NMR peak at δ 3.75 ppm (in C^2HCl_3) was shifted to 12.6 in the presence of $Eu(thd)_3$. No other peaks or shifts indicative of the

endo-alcohol were detectable. The infrared spectra[17] also failed to reveal the presence of any significant amount of endo isomer. The reference spectra used in drawing this conclusion were obtained on a mixture of the *endo*- and *exo*-alcohols, with the former predominating, prepared by lithium aluminum hydride reduction of (±)-**5**.[17] On addition of $Eu(thd)_3$ to C^2HCl_3 solutions of this mixture, the overlapping carbinol ^1H NMR peaks at δ 3.5–3.8 (broad, endo) and 3.75 (sharp, exo) ppm gave well-separated peaks in the 12–15-ppm region. The endo peak was shifted by δ 1–1.1 ppm more to lower field than the corresponding exo resonance; their relative integrated intensities were 77:23, respectively.

Secondary Deuterium Kinetic Isotope Effects and the Intervention of Nonclassical Ions in the Solvolysis of *exo*-Norborn-2-yl Bromobenzene-*p*-sulfonate

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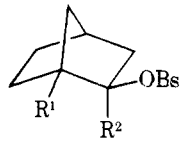
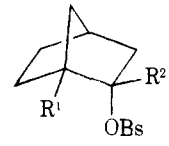
Abstract: 1-Deuterio- and 1,2-dideuterio-*exo*-norborn-2-yl bromobenzene-*p*-sulfonates have been prepared, and the deuterium kinetic isotope effects have been measured in 80% aqueous ethanol (k^H/k^D = 1.081, 25.5 °C, and 1.192, 25.4 °C, respectively) and buffered acetic acid (1.051 and 1.173, respectively, 29.4 °C). These results are in accordance with a mechanism involving bridging at the transition state for ionization which leads to the nonclassical norbornyl cation. The results are incompatible with a mechanism involving direct ionization to interconverting classical cations.

Since the classic kinetic investigation of the solvolysis of *exo*-norborn-2-yl bromobenzene-*p*-sulfonate (**1a**) by Winstein and Trifan,[1] the reaction has been studied in finer detail stereochemically,[2] with isotopic labels,[3,4] and very extensively by considering the effect of structural modifications.[5] These developments have been to support or refute Winstein's view[1,2,6] that, whereas *endo*-norborn-2-yl brosylate (**2a**) reacts

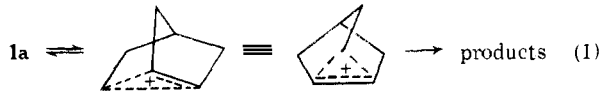


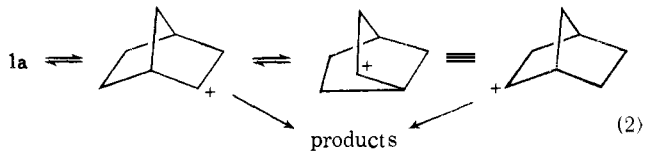
1a, $R^1 = R^2 = H$
b, $R^1 = H$; $R^2 = {}^2H$
c, $R^1 = {}^2H$; $R^2 = H$
d, $R^1 = R^2 = {}^2H$



2a, $R^1 = R^2 = H$
b, $R^1 = {}^2H$; $R^2 = H$
c, $R^1 = R^2 = {}^2H$

without anchimeric assistance,[7] the exo isomer **1a** ionizes with the nucleophilic assistance of the C(1)–C(6) σ bond to give the stabilized intermediate nonclassical cation **3**, which undergoes further reaction (eq 1). The conflicting interpretation that **1a**





reacts without anchimeric assistance, but by a mechanism involving rapidly interconverting enantiomeric classical cations (eq 2) has been cogently expressed.[8]

Although investigations on diversely substituted norbornyl derivatives have produced many experimental results and interpretations, extrapolation to the parent has required presumptions which have not been universally accepted. Consequently, the norbornyl problem has remained controversial and is still one of the central problems of organic chemistry upon which new techniques and theories continue to be tested.[9-11]

More recently, the norbornyl cation has been generated in very acidic media at low temperatures and has been studied spectroscopically.[12] Olah and his colleagues assert that the cation has a nonclassical structure, but their interpretation of the spectra has been repudiated.[8a,13]

The structural information, however, is about a cation rendered stable by an extremely nonnucleophilic environment which is very unlike that of the intermediate in the solvolysis of **1a**. Furthermore, even if the intermediate in the solvolysis is bridged (nonclassical), the extent of bridging at the transition state for ionization is still conjectural. This question can be answered in principle by a kinetics method, but an analysis of the rates of analogues[14] is not a sufficiently subtle probe.

To the extent that the Born–Oppenheimer approximation is a good one, isotopic substitution within a molecule does not modify its potential energy hypersurface.[15a,16] Consequently, although the substitution of protium by deuterium affects the vibrational energy levels of a molecule and of its transition state for a reaction, and therefore the rate of the reaction, such substitution does not affect the mechanism of the reaction.

The use of secondary kinetic isotope effect measurements for elucidating reaction mechanisms has been reviewed.[15-17] An α-deuterium secondary kinetic isotope effect (α-kie) of ca. 1.22 (25 °C) is expected for the solvolysis of a simple secondary alkyl arenesulfonate without nucleophilic assistance.[15,17-19] This value is reduced as nucleophilic assistance increases, regardless of whether the assistance is intramolecular (neighboring group participation) or bimolecular (S_N2), and in the limit is ca. 1.00.[15b,c,20] This attenuation of the α-kie has been attributed to the approaching nucleophile causing the C–H(^{2}H) vibrations in the ground state to transform into transition-state vibrations with no changes, or only compensating changes, in the magnitudes of the force constants.[15a,16a] It follows from this model that the extent of the reduction of the α-kie from ca. 1.22 (25 °C) is a (nonlinear) measure of the closeness of some atom or group to the α carbon at the transition state for the departure of the leaving group.[15c]

Shiner[15b,19] has argued that the maximal α-kie of 1.22 (25