were chosen because these groups are essential components of proteins which are known to act as donors in molecular complex formation in biological reactions with vitamin K_1, which, e.g., are responsible for the maintenance of the normal blood coagulation.[9]

Since it is believed that the charge transfer in these complexes is accomplished by the unpaired electron of the nitrogen to the quinone group of the vitamin K_1 (or α-tocopherolquinone), this effect should be enhanced by the presence of additional *n*-butyl groups. The fact that the opposite was observed, namely that K_c decreases with the number of *n*-butyl groups present, is therefore not the result of an inductive effect, but could be explained in terms of a steric hindrance which makes the formation of the molecular complexes with the higher *n*-butyl substituted donors more difficult.

Vitamin D_3 has been known as a good electron donor in biological molecular complex formation.[12] The results of the present study where the formation constants K_c for vitamin K_1–vitamin D_3 complexes have been assessed in cyclohexane solution (see Figure 2 and Table I) confirm this observation and suggest very similar donor capabilities for vitamin D_3 and mono-*n*-butylamine.

The strong reactivity displayed by α-tocopherolquinone, which is believed to be one of the major products in the oxidation process of vitamin E, toward Ps may open up an interesting possibility of studying the oxidation process of vitamin E.

While α-tocopherolquinone reacts very rapidly with Ps, the rate constant is 2.15×10^{10} M^{-1} s^{-1} in benzene, and is even relatively reactive in its complex with indole ($K_{obsd} = 0.85 \times 10^{10}$ M^{-1} s^{-1}), the nonoxidized vitamin E shows (in benzene solution) hardly any reactivity toward Ps. Thus this drastic difference in the behavior of the oxidation product of vitamin E and vitamin E itself could be utilized for further studies of the biologically interesting autoxidation process of vitamin E, which supposedly inhibits the autoxidation of the unsaturated fatty acids.[2]

Summarizing, it can be said that these initial investigations of molecular associations in biological systems by positron annihilation techniques seem to support the feasibility of this new technique in the study of biological reactions.

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Regiospecific and Enantioselective Horse Liver Alcohol Dehydrogenase Catalyzed Oxidations of Some Hydroxycyclopentanes[1a,b]

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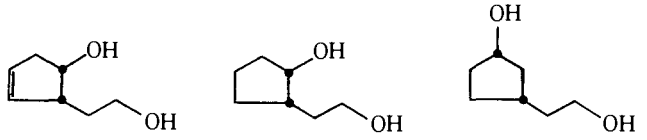
Abstract: Horse liver alcohol dehydrogenase (HLADH) has been shown to have the ability to retain its enantioselectivity while effecting regiospecific oxidation of only one of two unhindered hydroxyl groups within the same molecule. This provides a synthetically useful combination of properties which cannot be duplicated in a single step by traditional oxidation methods. All reactions were performed on a preparative (up to 1 g) scale and proceeded in good yields. HLADH-catalyzed oxidation of (±)-*cis*-2-(2′-hydroxyethyl)-3-cyclopenten-1-ol (**1**) was regiospecific for the primary alcohol group. The reaction was also enantioselective, and 1*R*,2*S*-*cis*-2-carboxymethyl-3-cyclopenten-1-ol lactone (**4**, 49% optical purity) and unchanged 1*S*,2*R*-**1** (23% optical purity) were isolated. The same regiospecificity, but no enantioselectivity, was observed with (±)-*cis*-2-(2′-hydroxyethyl)cyclopentanol (**2**) as the substrate. Both enantiomers of the prostaglandin synthon **4** were subsequently obtained (37 and 47% optical purities) via HLADH-catalyzed oxidation of the racemic hemiacetal precursor of **4**. For the 1,3 isomer of **2**, (±)-*cis*-3-(2′-hydroxyethyl)cyclopentanol (**3**), secondary alcohol regiospecificity is manifest with 3-(2′-hydroxyethyl) cyclopentanone (**9**) being the major product. The enantioselectivity of this reaction is very high, giving 3*S*-**9** and recovered 1*R*,3*R*-**3** of 97 and 70% optical purities, respectively. The regiospecificities observed were as predicted by the diamond lattice section of the active site. The model is more equivocal, but still useful, in analyzing the enantioselectivities of the above reactions.

In recent years, the requirements of synthetic chemists for reagents capable of effecting selective or asymmetric transformations have increased dramatically. Enzymes present unique opportunities in this regard, and the exploration of their properties as chiral catalysts is now receiving considerable attention.[2] One of the great synthetic attractions of an enzyme

is that the various facets of its specificity can endow it with the potential for effecting highly controlled and selective transformations in a single step which would otherwise involve several operations if more traditional reagents were used. In this paper, alcohol dehydrogenase catalyzed oxidations are explored in which regiospecificity and enantiospecificity operate concurrently. This combination is one whose asymmetric synthesis value is immediately apparent.

Several alcohol dehydrogenases, which catalyze CH(OH) $\rightleftharpoons$ C=O oxido reductions, are synthetically useful.[2] Of these, the NAD^+ [3]-dependent enzyme from horse liver, HLADH,[3] is the best documented,[2,4] but the question of whether it has the capacity to effect regiospecific oxidoreduction of only one of two or more hydroxyl or carbonyl groups within the same substrate while retaining enantioselectivity has been virtually ignored. The recently refined[2,4] diamond lattice[5] section of the active site was used to select suitable substrates for evaluating this aspect of HLADH specificity, and, in keeping with our overall desire[2,4] to extend the synthetic utility of the enzyme, the dihydroxycyclopentane compounds **1**–**3** of more organic



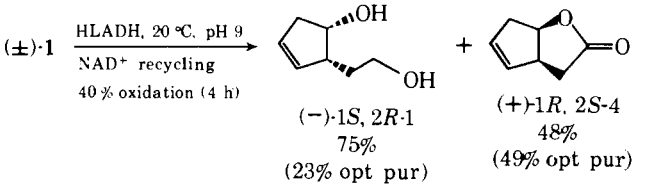
1 2 3

than biochemical interest were selected for this initial study.

Results

The racemic diol **1** was subjected to HLADH-catalyzed oxidation at pH 9 using FMN recycling[6] of catalytic amounts of the NAD^+ coenzyme. The reaction was worked up when GLC analysis showed it to be half complete.[7] Oxidation was found to be totally regiospecific for the hydroxyethyl group, and the overall process was also enantioselective to a considerable degree. The results are summarized in Scheme I.[8] The

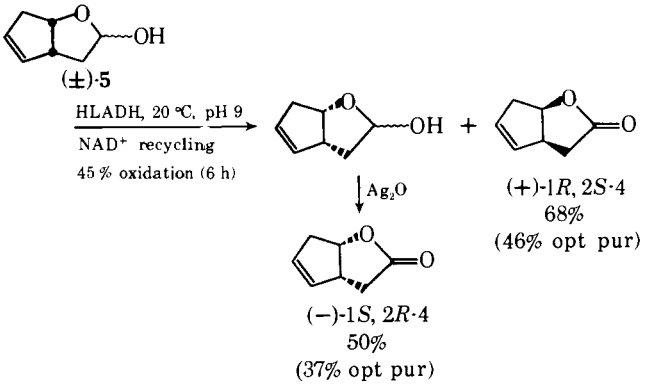
Scheme I



initially formed aldehyde did not accumulate owing to the facility with which its hemiacetal **5** underwent subsequent HLADH-mediated oxidation to the lactone **4**. A small amount (4%) of **5** was detected (by GLC) in the reaction mixture immediately following termination. However, this material was removed by the subsequent isolation and purification procedures. The optical purities and absolute configurations of the Scheme I products were assigned using literature data.[9]

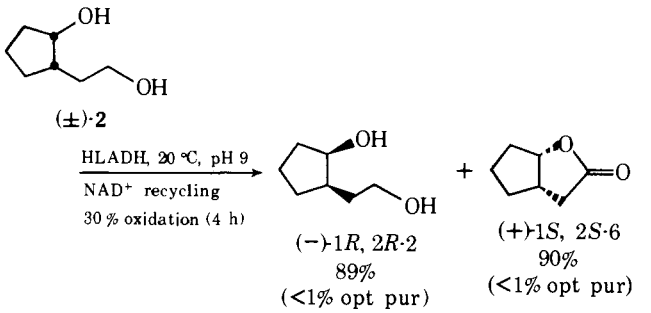
The lactone **4** and its epoxide are important prostaglandin synthons.[9-12] Disappointingly, the 1*R*,2*S* configuration of the sample of **4** obtained from the Scheme I reaction is the opposite of those of the naturally occurring prostaglandins. However, this disadvantage was overcome using the hemiacetal **5** as a substrate. HLADH-catalyzed oxidation of (±)-**5** proceeded enantioselectively as shown in Scheme II. Enantiomerically enriched 1*R*,2*S*-**4** was isolated directly from the enzyme-mediated reaction while the lactone possessing the natural 1*S*,2*R* configuration was readily obtained by silver oxide oxidation of the recovered hemiacetal 1*S*,2*R*-**5**. No oxidation of (±)-**5** occurred under the Scheme II conditions in the absence of enzyme.

Scheme II



Regiospecific enzyme-catalyzed oxidation of only the hydroxyethyl group was also observed for the saturated diol (±)-**2**. Again only a trace of the hemiacetal tautomer of the initial aldehyde product was detectable in the reaction mixture, and the products isolated (Scheme III) were unchanged diol

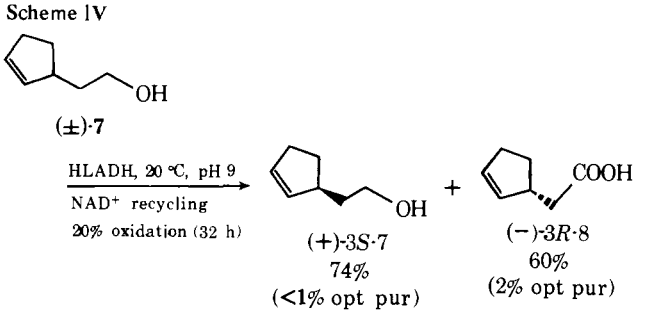
Scheme III



2 and the lactone **6**. However, in contrast to the Scheme I situation, the products were virtually racemic with the configurations shown in Scheme III being very marginally favored. The absolute configuration correlations were made using (+)-1*S*,2*S*-**2** obtained from an authentic sample of pure (−)-1*S*,2*R*-**4**.

The dramatic contrast in enantioselectivity toward two substrates, **1** and **2**, differing only by a double bond in the ring was unexpected and prompted us to examine the role played by the secondary hydroxyl group in determining the stereospecificity. The unsaturated analogue lacking this function, **7**, was found to be a much poorer substrate than either **1** or **2**. Nevertheless, HLADH-catalyzed oxidation proceeded fairly smoothly to give the products shown in Scheme IV. Again none

Scheme IV

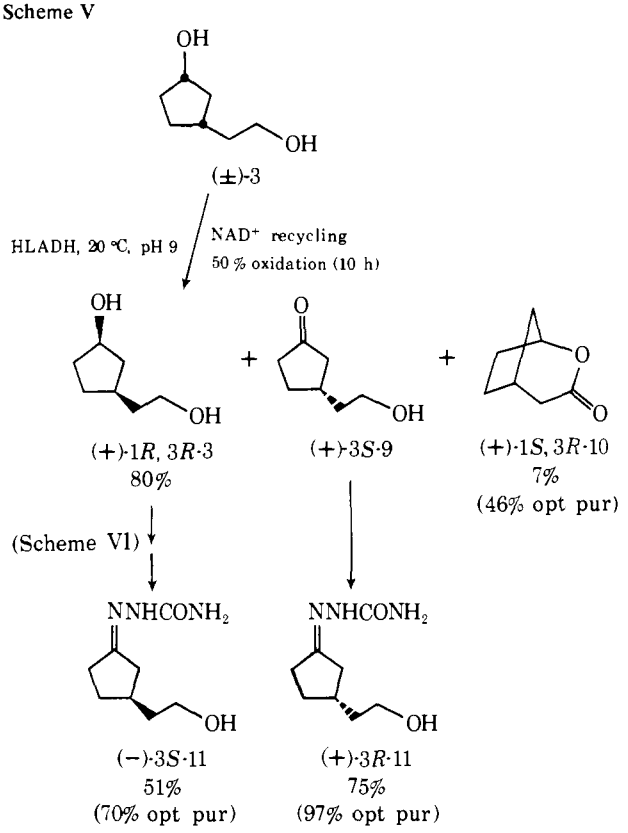


of the intermediate aldehyde was isolable. The optical purity and absolute configuration designations were based on previous literature data.[13] Thus in this case too, the degree of enantioselectivity exhibited by the enzyme is very minor.

Next, the specificity of HLADH toward the 1,3-disubstituted diol **3** was evaluated. Enzymic oxidation of (±)-**3** proceeded to 50% completion in 10 h. At that time, the reaction mixture was composed (by GLC) of unreacted diol **3** (50%), the hydroxy ketone **9** (39%), and the lactone **10** (11%). Since

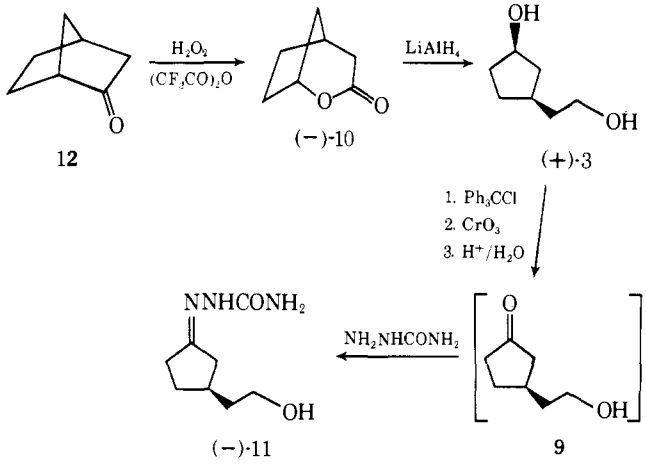
the various chromatographic techniques applied did not separate **3** and **9** well enough to permit reliable optical purities to be established, the reaction products were separated following conversion of **9** into its semicarbazone **11**. Subsequently it was also found convenient to convert the recovered diol **3** into its corresponding semicarbazone for characterization in order to avoid the optical rotation errors caused by the residual presence of ~0.8% of the highly rotating product **9**. The overall results are summarized in Scheme V.[14] The absolute configurations

Scheme V



and optical purities were assigned using reference samples of (+)-**3**, (−)-**10**, and (−)-**11** obtained from (−)-1*R*-2-norbornane (**12**, 62% optically pure[4]) by the route depicted in Scheme VI.[14] With (+)-**3**, the hydroxyethyl group regiospecificity

Scheme VI



exhibited by HLADH toward (±)-**1** and (±)-**2** is replaced by a regioselectivity for the secondary alcohol function. Furthermore, the reaction now proceeds with extremely high enantioselectivity.

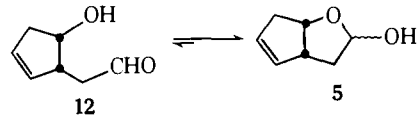
Discussion

Compounds **1**–**3** were selected as being suitable and representative organic structures for evaluating the ability of HLADH to discriminate between unhindered primary and secondary alcohol functions within the same molecule. With very few exceptions,[12b] this is not possible with chemical oxidizing agents unless protecting groups are used.

All HLADH-catalyzed oxidations were performed on synthetically significant (500 mg–1 g) quantities of each substrate. In order to reduce the coenzyme costs, catalytic amounts only of the NAD^+ required were used in conjunction with an FMN recycling system.[6] The reactions were readily monitored by GLC and were conveniently worked up using a simple continuous chloroform extraction procedure. High yields of products were obtained from each enzymic oxidation.

The unsaturated diol (±)-**1** was chosen for the first study because of its potential as a precursor of prostaglandin synthons such as **4**, in which there remains considerable current interest.[9-12,15] Analysis of **1** in terms of the diamond lattice section of HLADH (see below for details) predicted that the primary alcohol group would be oxidized exclusively. The hope also existed that oxidation would also proceed enantioselectively. The results obtained (Scheme I) were in accord with these expectations. The reaction was completely regiospecific for the primary alcohol moiety as predicted, with no 2-hydroxyethyl-3-cyclopentanone being detected. The stereoselectivity of the overall process was also significant. In fact, the enantiomeric enrichment of the 1*R*,2*S*-**4** isolated is sufficient to permit optically pure material to be obtained by recrystallization.[16] Furthermore, the optical purity of the lactone produced on the recovered diol could be raised by appropriate manipulation of the extent of reaction.[2,4]

As Scheme I implies, the intermediate aldehyde **12** did not accumulate but underwent subsequent oxidation. Under the reaction conditions, **12** would be expected to exist largely in



12 **5**

its hemiacetal form **5**, and although the aldehyde dehydrogenase activity of HLADH has been well characterized,[2] the detection of a small amount of **5** in the reaction mixture indicated that it and not **12** might be the immediate precursor of **4**.[17] That (±)-**5** was indeed a good HLADH substrate was verified by the facility with which it underwent enzyme-catalyzed oxidation to **4** (Scheme II).[18] The reaction was enantioselective in the same sense and to the same degree as for (±)-**1** (Scheme I) with the lactone **4** possessing the "unnatural" 1*R*,2*S* configuration being formed preferentially. However, silver oxide oxidation of the recovered hemiacetal yielded enantiomerically enriched **4** with the 1*S*,2*R* "natural prostaglandin" configuration. Thus both enantiomers of the important synthon **4** are now available via a single enzymic oxidation.[19]

With the saturated diol (±)-**2** as the substrate, regiospecificity for the hydroxyethyl group was again absolute (Scheme III) as predicted, but, in contrast to the marked enantioselectivity observed with the Δ^3-substrate **1**, the stereospecificity of oxidation of its fully reduced analogue **2** is marginal only. That saturation of the double bond was not wholly responsible for the loss of stereospecificity was established by subjecting the cyclopentenol **7**, in which the double bond is present but the secondary hydroxyl function is now absent, to HLADH-promoted oxidation. Again the degree of enantioselectivity observed was very limited (Scheme IV). The secondary alcohol and double bond moieties of **1** evidently have a synergistic ef-

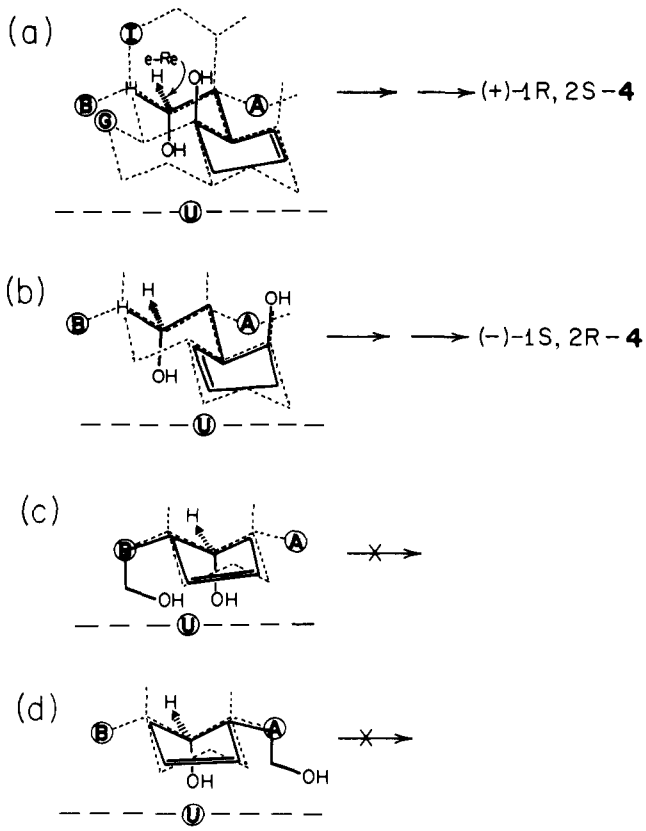


Figure 1. Diamond lattice analysis[2,4] of the regiospecificity of the diol **1**. The relevant portions of the lattice, including the forbidden or undesirable positions A, B, G, I, and U are indicated by the dashed lines. When the hydroxyethyl groups of (+)- or (−)-**1** are oriented such that removal of the *pro-R* hydrogen from the *e-Re* direction can occur as required by the model,[2] unfavorable interactions with the lattice are completely avoidable. Substrate placements meeting these requirements and leading to the observed products are depicted in (a) for (+)-**1** and (b) for (−)-**1**. In contrast, positioning of the secondary alcohol function of (+)- or (−)-**1** in a manner permitting hydride removal from the *e-Re* direction would compel a methylene group of the hydroxyethyl function to violate position B or A respectively as shown in (c) for (+)-**1** and (d) for (−)-**1**. Oxidation of the C-1 hydroxyl groups is thus precluded for both enantiomers. The situations for the enantiomers of **2** are entirely analogous.

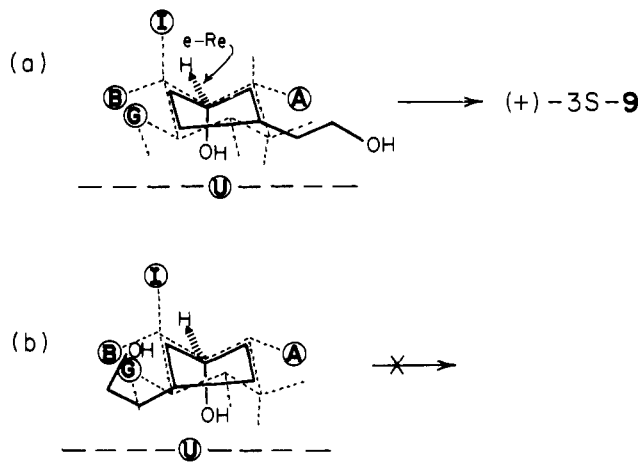


Figure 2. Diamond lattice analysis of the regiospecificity and enantioselectivity of the diol **3**. The enantiomers of **3** (solid lines) are shown superimposed on the lattice (dashed lines) in the orientations required for removal of the C-1 hydrogen from the *e-Re* direction. In (a) the hydroxyethyl group of (+)-**3** does not violate any forbidden location and HLADH-catalyzed oxidation to (−)-**9** is thus a favorable process. The other enantiomer, (−)-**3**, is not a good substrate since, as shown in (b), its hydroxyethyl group would be required to impinge severely on the highly forbidden B, G region of the lattice in the transition state.

fect on the enantiomeric specificity of HLADH within this series of substrates.

For **3**, the 1,3 isomer of **2**, diamond lattice analysis (see below) predicted that regiospecific HLADH-catalyzed oxidation of the secondary alcohol group would now be favored. As Scheme V shows, this turned out to be the case. The 1*S*,3*R* configuration assigned to (+)-**10** on the basis of the Scheme VI data was in accord with the previous literature correlation.[24] However, although the enzymic discrimination against the hydroxyethyl group was very clear-cut, it was not absolute as evidenced by the isolation of a small amount of the lactone **10**.[25] On the other hand, the enantioselectivity observed is extremely high, being virtually absolute in the case of the ketone (+)-**9** and 70% for the recovered diol (+)-**3**. The fact that the (+)-**2** isolated is of somewhat lower optical purity than that of (+)-**9** is attributable to the lower enantioselectivity of the minor oxidation pathway leading to (+)-**10**.

Diamond Lattice Section Analysis[2,4]

Diamond lattice section analyses of substrate structures and of the results obtained were performed using the updated model described previously.[2,4]

Regiospecificity. Unfavorable interactions are completely avoidable when the primary alcohol groups of (+)- and (−)-**1** are superimposed on the lattice such that *e-Re* removal of the *pro-R* hydrogen can occur. The situations envisaged are shown in Figure 1a and 1b. On the other hand, positioning of (+)- or (−)-**1** in the lattice in the required transition state-like orientation (Figure 1c and 1d) cannot be achieved without an adverse interaction of the respective hydroxyethyl groups with forbidden position B or A. The interactions of (+)- and (−)-**2** with the active site are analyzable in exactly similar terms. Regiospecific oxidation of the hydroxyethyl functions of each enantiomer of **1** and **2** was thus predicted to be a highly favored process. The experimental observations are in total accord with expectation.

The situation for the enantiomers of **3** is quite different because no interaction with a forbidden position is mandatory when either the primary or the secondary alcohol group is correctly oriented for oxidation. Using the guiding principle that, in the absence of unfavorable lattice interactions, HLADH-catalyzed oxidations of cyclic secondary alcohols occur with greater practical facility than for more flexible primary alcohol substrates,[2,26] selective transformation of the C-1 hydroxyl of (±)-**3** was anticipated[27] via substrate-lattice orientations of the type depicted in Figure 2a.[27] Again the experimental facts verified this analysis.

Enantioselectivity. When (+)- and (−)-**3** are superimposed on the diamond lattice section such that the hydride of the secondary alcohol function will be removed pseudoequatorially[30] from the *e-Re* lattice direction, each hydroxyethyl group occupies a different region of the lattice. For the (+)-1*R*,3*R* enantiomer it is situated toward the left-hand side (Figure 2b, a region in which forbidden positions B, C, G, and I are concentrated. In contrast, the hydroxyethyl group of (−)-1*S*,3*S*-**3** is directed into the much more tolerant-to-occupation right-hand region (Figure 2a).[31] Accordingly, enantioselective oxidation of 1*S*,3*S*-**3** was expected and was observed experimentally.

For **1, 2,** and **7,** the hydroxyl groups undergoing oxidation are located at the end of a relatively flexible two-methylene unit chain. Owing to the extra conformational mobility that such molecules possess, analyses via the rigid lattice section become more difficult to apply and are less likely to be unequivocal. Nevertheless, they can provide some useful indications of the degree of stereospecificity, if any, which can be anticipated. For HLADH-mediated oxidations of primary alcohols, the *pro-R* hydrogen is removed stereospecifically.[2]

When this fact is taken into account when superimposing the enantiomers of **1, 2,** and **7** on the lattice section, the cyclopentyl moieties of each stereoisomer can occupy the relatively unhindered lower right-hand region of the model as shown in Figure 1a and 1b. In all cases, at least one transition state-like orientation can be found which does not violate any forbidden or undesirable positions of the active site section, and the model thus indicates that oxidation need not be enantioselective for these substrates. The lack of enantiomeric discrimination for the (±)-**2** and (±)-**7** oxidations was therefore not surprising. The significant enantioselectivity observed in the HLADH-(±)-**1** reaction is, however, not rationalizable in terms of the current lattice section, and it is evident that minor structural changes can be of great importance in determining the stereospecificity of the enzyme with some substrates.

Experimental Section

Unless indicated otherwise, sources of materials, equipment used, analytical procedures, criteria of purity etc. were as described previously.[4] The activity of the enzyme was determined prior to use[32] and all amounts of HLADH cited refer to active enzyme.

(±)-*cis*-2-(2'-Hydroxyethyl)-3-cyclopenten-1-ol (1). A solution of *cis*-2-carboxymethyl-3-cyclopenten-1-ol lactone (**4**) (Willowbrook Laboratories, 2.70 g, 21.8 mmol) in dry tetrahydrofuran (20 mL) was added with stirring to a suspension of $LiAlH_4$ (0.83 g, 21.8 mmol) in dry tetrahydrofuran (80 mL). After refluxing the reaction for 2 h it was quenched with saturated aqueous NH_4Cl, and the precipitated oxides were filtered off and washed with water. The combined filtrates were concentrated and then continuously extracted with ether for 24 h. The other extract was dried ($MgSO_4$), evaporated, and distilled to give (±)-**1** (2.2 g, 81% yield): bp 84–87 °C (0.04 Torr) [lit.[9,16] bp 83–84 °C (0.1 Torr)]; IR ($CHCl_3$) 3623, 3401, and 1605 cm^{-1}; ^1H NMR (C^2HCl_3) δ 5.7 (2 H, m), 4.5 (1 H, m), 3.6–4.0 (4 H, broad s overlapping m), 2.2–2.9 (3 H, m), and 1.8 (2 H, m) ppm.

HLADH-Catalyzed Oxidation of (±)-*cis*-2-(2'-Hydroxyethyl)-3-cyclopenten-1-ol (1).[8] The diol (±)-**1** (500 mg, 3.9 mmol), NAD^+ (280 mg, 0.42 mmol), and FMN (δ 3.74 g, 7.8 mmol) were dissolved in 0.05 M glycine–NaOH buffer (300 mL, pH 9) at 20 °C. The enzyme (30 mg) was then added and the pH readjusted to 9.0. After 4 h (40% oxidation by GLC) the solution was continuously extracted with chloroform for 2 days. The extract was decolorized with charcoal, dried ($MgSO_4$), and evaporated to yield a pale-yellow oil (430 mg) which contained (by GLC) unreacted diol (~60%), *cis*-2-carboxymethyl-3-cyclopenten-1-ol lactone (**4**, ~40%), and a trace of *cis*-2-formylmethyl-3-cyclopenten-1-ol hemiacetal (**5**). The oil was dissolved in aqueous NaOH (pH 12) and continuously extracted with chloroform for 24 h to give, after work up and molecular distillation, 1*S*,2*R*-**1** (225 mg, 75% yield): $[\alpha]^{25}D$ −17.4° (*c* 1.9, CH_3OH) [lit.[9] $[\alpha]^{25}D$ −74° (*c* 1.0, CH_3OH)]. The aqueous phase was then acidified to pH 3 with concentrated HCl and continuously extracted for 1 day to give, after molecular distillation, 1*R*,2*S*-**4** (87 mg, 49% yield): $[\alpha]^{25}D$ +51.5° (*c* 0.8, CH_3OH) [lit.[9] $[\alpha]^{25}D$ −106° (CH_3OH)]; IR ($CHCl_3$) 1770 cm^{-1}; ^1H NMR (C^2HCl_3) δ 5.5–5.8 (2 H, m), 5.1 (1 H, m), 3.5 (1 H, m), and 2.5–2.7 (4 H, m) ppm.

(±)-*cis*-Formylmethyl-3-cyclopenten-1-ol Hemiacetal (5). The hemiacetal (±)-**5** was prepared by reduction of (±)-**4** using the literature method;[33] a 49% yield was obtained of material with bp 57–59 °C (0.15 Torr) [lit.[33] bp 44–45 °C (0.01 Torr)]; ^1H NMR (C^2HCl_3) δ 5.2–5.8 (3 H, s overlapping m), 4.8 (1 H, m), 3.3 (1 H, m), 2.5 (2 H, m), and 1.6–2.3 (2 H, m) ppm.

HLADH-Catalyzed Oxidation of (±)-*cis*-2-Formylmethyl-3-cyclopenten-1-ol Hemiacetal (5).[8] The hemiacetal (±)-**5** (500 mg, 4 mmol) was oxidized using HLADH (15 mg) under the conditions described above for the diol (±)-**1.** After 6 h at 20 °C, when ~45% of oxidation had occurred (GLC), the pH was raised to 12 and the mixture was continuously extracted with chloroform for 1 day. The oil (278 mg) obtained, which contained the unreacted hemiacetal, was dissolved in 50% aqueous ethanol (20 mL) containing silver nitrate (3 g) and 14% aqueous NaOH (10 mL) was added with stirring. After stirring for 12 h at 20 °C, the reaction mixture was filtered and the neutral contaminants were removed by chloroform extraction. The aqueous solution was then acidified with concentrated HCl and extracted with chloroform (6 × 40 mL). Evaporation and molecular distillation of the dried (K_2CO_3) chloroform extracts gave the lactone 1*S*,2*R*-**4** (136 mg, 50% yield), $[\alpha]^{25}D$ −34.6° (*c* 1.4, $CHCl_3$) [lit.[9,16] $[\alpha]^{25}D$ −93° (*c* 1, $CHCl_3$)].

The original reaction solution was then acidified to pH 3. Continuous chloroform extraction as before yielded, after molecular distillation, 1*R*,2*S*-**4** (154 mg, 68% yield), $[\alpha]^{25}D$ +43.0° (*c* 1.5, $CHCl_3$).

(±)-*cis*-2-(2'-Hydroxyethyl)cyclopentanol (2). The title compound was obtained in 57% yield by $LiAlH_4$ reduction[34] of (±)-*cis*-2-carboxymethylcyclopentanol lactone.[35] It had bp 78–83 °C (0.04 Torr) [lit.[34] bp 107–116 °C (0.4 Torr)]; IR (film) 3450–3330 cm^{-1}; ^1H NMR ($[^2H_6]$-Me_2SO) δ 4.2 (2 H, broad s), 4.0 (1 H, m), 3.5 (2 H, broad t, *J* = 6 Hz), and 1.7 (9 H, m) ppm.

HLADH-Catalyzed Oxidation of (±)-*cis*-2-(2'-Hydroxy)cyclopentanol (2).[8] The diol (±)-**2** (500 mg, 3.8 mmol) was oxidized under the conditions described above for (±)-**1.** After a 4 h reaction time (30% oxidation by GLC), the pH 12 extract yielded unchanged diol **2** (312 mg, 89% yield), $[\alpha]^{25}D$ −0.19° (*c* 3, $CHCl_3$). The subsequent pH 3 extract yielded *cis*-2-carboxymethylcyclopentanol lactone (**6**, 135 mg, 90% yield), $[\alpha]^{25}D$ −0.5° (*c* 1.3, CH_3OH) [lit.[16] $[\alpha]^{25}D$ −59.0° (*c* 1, CH_3OH)].

1*S*,2*S*-*cis*-2-(2'-Hydroxyethyl)cyclopentanol ((+)-2). The lactone 1*S*,2*R*-**4**, $[\alpha]^{25}D$ −104.3° (*c* 1, CH_3OH) (kindly provided by Dr. J. J. Partridge), was hydrogenated over Adams catalyst to give, in 87% yield, 1*S*,2*S*-*cis*-2-carboxymethylcyclopentanol lactone ((+)-**6**). The lactone (+)-**6** (860 mg, 6.8 mmol) was dissolved in dry ether (50 mL) and $LiAlH_4$ (259 mg, 6.8 mmol) added with stirring at 0 °C. After 1 h, water (0.5 mL) followed by 10% aqueous NaOH (0.4 mL) were added, and the mixture was stirred for a further 30 min at 0 °C. The solution was then dried ($MgSO_4$) and evaporated and the crude product chromatographed on silica. Ethyl acetate–benzene (1:3) elution gave the diol 1*S*,2*S*-**2** (537 mg, 61% yield), $[\alpha]^{25}D$ +32.7° (*c* 1, $CHCl_3$), +33.3° (*c* 1, CH_3OH). Anal. ($C_7H_{14}O_2$) C, H.

(±)-3-(2'-Hydroxyethyl)cyclopentene (7). Reduction[13] of (±)-cyclopenten-3-acetic acid (Aldrich) with $LiAlH_4$ gave 81% yield of (±)-**7:** bp 76–77 °C (10 Torr) [lit.[36] bp 86–87 °C (16 Torr)]; IR ($CHCl_3$) 3610, 3448, and 1610 cm^{-1}; ^1H NMR (C^2HCl_3) 5.7 (2 H, m), 3.65 (2 H, t, *J* = 8.8 Hz), 3.3 (1 H, m), 2.75 (1 H, m), and 1.1–2.5 (6 H, m) ppm.

HLADH-Catalyzed Oxidation of (±)-*cis*-3-(2'-Hydroxyethyl)-cyclopentene (7).[8] The alcohol (±)-**7** (500 mg, 4.5 mmol) was oxidized under the conditions outlined for (±)-**1** using 36 mg of enzyme. After 32 h (20% oxidation by GLC) the reaction mixture was worked up as before to yield, after molecular distillation of the pH 12 extract, unreacted **7** (296 mg, 74% yield), $[\alpha]^{25}D$ +0.57° (*c* 2.9, $CHCl_3$) [lit.[13] $[\alpha]^{25}D$ +127.4° (*c* 6.6, $CHCl_3$)]; IR and ^1H NMR as for (±)-**7.** The pH 3 extract gave, following molecular distillation, the acid **8** (51 mg, 51% yield): $[\alpha]^{25}D$ −2.7° (*c* 0.5, $CHCl_3$) (lit.[13] $[\alpha]^{25}D$ −109.2° (*c* 5.9, $CHCl_3$)); IR ($CHCl_3$) 1706 cm^{-1}; ^1H NMR (C^2HCl_3) δ 11.1 (1 H, s), 5.7 (2 H, m), 3.1 (1 H, m), and 1.3–2.6 (6 H, m) ppm.

(±)-*cis*-3-(2'-Hydroxyethyl)cyclopentanol (3). (±)-Norbornanone (**12**) was oxidized with peroxytrifluoroacetic acid.[37] After chromatographic purification on silica [ethyl acetate–chloroform (1:20) elution] and sublimation, a 47% yield was obtained of (±)-*cis*-3-carboxymethylcyclopentanol lactone (**10**): mp 61–64 °C (lit.[37] mp 64 °C; IR ($CHCl_3$) 1718 cm^{-1}; ^1H NMR (C^2HCl_3) δ 4.9 (1 H, broad s), 2.6 (3 H, m), and 1.5–2.2 (6 H, m) ppm. The lactone (±)-**10** (5.82 g, 6.2 mmol) in dry tetrahydrofuran (30 mL) was added to a stirred suspension of $LiAlH_4$ (1.76 g, 46 mmol) in dry tetrahydrofuran (150 mL). The mixture was then refluxed for 4 h, cooled, quenched with water, filtered, and the residue washed with ethanol. The filtrate was evaporated, the residue taken up in acetone, filtered, and reevaporated. Distillation gave the diol (±)-**3** (3.7 g, 62% yield): bp 100–102 °C (0.1 Torr) [lit.[38] bp 121–123 °C (0.25 Torr)]; IR ($CHCl_3$) 3390–3225 cm^{-1}; ^1H NMR (C^2HCl_3–$[^2H_6]$-Me_2SO, 1:1) δ 4.2 (3 H, s overlapping m), 3.5 (2 H, t, *J* = 6.7 Hz), and 1.0–2.0 (9 H, m) ppm.

HLADH-Catalyzed Oxidation of (±)-*cis*-3-(2'-Hydroxyethyl)-cyclopentanol (3).[8] The diol (±)-**3** (1.04 g, 7.9 mmol) was oxidized as described for (±)-**1** using HLADH (50 mg). When GLC analysis showed 50% oxidation had occurred (10 h) the mixture was continuously extracted with chloroform for 2 days at pH 7. The oil (1.01 g) obtained, which contained (by GLC) unreacted diol (50%), the ketone **9** (39%), and the lactone **10** (11%), was mixed with sodium acetate (825 mg, 6.1 mmol) and semicarbazide hydrochloride (550 mg, 4.9 mmol) in water (5 mL). The mixture was heated at 100 °C for 1 h, then kept at 20 °C for 6 h. The pH was then adjusted to 9 with 10 M aqueous NaOH and the solution continuously extracted for 2 days

with chloroform. The crystallizing oil obtained was chromatographed on silica. Ethyl acetate–chloroform (1:2) elution followed by sublimation (3×) gave the 1*S*,3*R*-lactone **10** (36 mg, 7% yield): mp 42–55 °C; $[\alpha]^{25}D$ +6.4° (*c* 0.4, CH_2Cl_2) (46% optical purity, see below). Subsequent elution with methanol–ethyl acetate (1:20) followed by molecular distillation gave unreacted 1*R*,3*R*-diol **3** (405 mg, 80% yield), $[\alpha]^{25}D$ +2.9° (*c* 4, CH_3COCH_3). This contained 0.8% (by GLC) of the highly rotating ketone 3*S*-**9** and was therefore characterized by conversion, using the Scheme VI method (see below), to the semicarbazone 3*S*-**11** (266 mg, 51% yield from diol): mp 112–125 °C; $[\alpha]^{25}D$ −37.2° (*c* 0.98, CH_3OH) (70% optical purity, see below); ^1H NMR ($[^2H_6]$-Me_2SO) δ 8.8 (1 H, s), 6.1 (2 H, broad s), 4.35 (1 H, ^2H_2O exchangeable, *J* = 5.2 Hz), 3.5 [2 H, t (with ^2H_2O added), *J* = 6 Hz], and 1.3–2.7 (9 H, m) ppm. Further elution with methanol–ethyl acetate (1:10) gave, after evaporation and one recrystallization from ethanol, the semicarbazone 3*R*-**11** (422 mg, 75% yield): mp 121–138 °C; $[\alpha]^{25}D$ +50° (*c* 1.1, CH_3OH) (97% opt. purity, see below); ^1H NMR identical with that cited above. Anal. ($C_8H_{15}N_3O_2$) C, H, N.

Absolute Configuration and Optical Purity Correlations of 10, 3, 9, and 11. The Scheme VI reactions were carried out using (−)-1*R*,4*S*-norbornanone of 62% optical purity[4] as starting material. (−)-Norbornanone [1.59 g, 14.4 mmol, $[\alpha]^{25}D$ −18.1° (CH_2Cl_2)] was oxidized to the lactone (−)-**10,** which was then reduced to the diol (+)-**2**, by the methods described above for the corresponding racemic compounds. The IR and ^1H NMR spectra of the compounds were identical with those of their racemates. The (−)-lactone 1*R*,3*S*-**10** (0.77 g, 42% yield) had mp 60–63 °C, $[\alpha]^{25}D$ −8.63° (*c* 3.3, CH_2Cl_2), which extrapolates to −13.9° for the pure enantiomer. After molecular distillation the (+)-diol 1*R*,3*R*-**3** (547 mg, 70% yield) had $[\alpha]^{25}D$ +1.13° (*c* 5.4, CH_3COCH_3) which extrapolates to +1.82° for the pure enantiomer.

The (+)-diol 1*R*,3*R*-**3** (511 mg, 3.9 mmol) and trityl chloride (1.08 g, 3.9 mmol) were reacted in dry pyridine (15 mL) at 20 °C for 2 days. The trityl ether solution was then added with stirring to chromium trioxide (2.36 g, 23.6 mmol) in dry pyridine (3.9 mL, 47.2 mmol) and methylene chloride (15 mL). After stirring for 30 min, the solution was decanted and the residual sludge washed with copious quantities of ether. The combined organic phases were washed with 5% aqueous NaOH (2 × 200 ml) and then with saturated aqueous $CuSO_4$ (4 × 100 ml). Evaporation of the dried ($MgSO_4$) solution gave the trityl ether of 3-(2′-hydroxyethyl)cyclopentanone (1.39 g, 95% yield): IR 1740 cm^{-1} (lit.[37] 1742 cm^{-1}); ^1H NMR (C^2HCl_3) δ 7.1–7.5 (15 H, m), 3.1 (2 H, 5, *J* = 6.2 Hz), and 1.2–2.4 (9 H, m) ppm. The material so obtained was heated under reflux in 70% aqueous acetic acid (30 mL) for 2 h, then cooled and diluted with water (50 mL), and the triphenyl carbinol filtered off. The filtrate was neutralized with excess 10 M aqueous NaOH, saturated with NaCl, and extracted with chloroform (6 × 100 mL). The dried ($MgSO_4$) chloroform solution was evaporated to yield the ketone 3*R*-**9** (350 mg); IR ($CHCl_3$) 3450 and 1736 cm^{-1}; ^1H NMR (C^2HCl_3) δ 3.9 (1 H, s), 3.65 (2 H, t, *J* = 6.2 Hz), and 15.–2.6 (9 H, m) ppm. This was converted directly to the semicarbazide by heating with semicarbazide hydrochloride (600 mg, 5.2 mmol) and sodium acetate (900 mg, 6.6 mmol) in water (10 mL) at 100 °C for 1 h. After keeping for a further 2 h at 20 °, the semicarbazone was isolated and characterized as described previously in the HLADH oxidation of (±)-**3** procedure. One recrystallization from ethanol–benzene afforded (−)-3*S*-**11** (162 mg, 36% yield), mp 99–106 °C, $[\alpha]^{25}D$ −32.2° (*c* 1, CH_3OH), which extrapolates to −51.9° for the pure enantiomer. The ^1H NMR spectrum was identical with that of the sample obtained enzymically.

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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 Scholar 1973–1975.

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(3) Abbreviations used: NAD^+, oxidized form of nicotinamide adenine dinucleotide; HLADH, horse liver alcohol dehydrogenase; FMN, flavin mononucleotide (riboflavin phospate).

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(8) The percentages shown in all schemes are the yields calculated taking the extent of oxidoreduction into account. All optical purities cited were calculated using the specific rotations of the pure enantiomers as the reference values. Attempts made to obtain more direct measurements of the degrees of enantiomeric enrichment via GLC or HPLC analyses of diastereomeric derivatives, or by ^1H NMR or ^{13}C NMR in the presence of various chiral shift reagents were unsatisfactory.[1b]

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(18) Support for the conclusion that formation of **4** occurs by HLADH-catalyzed oxidation of **5** is provided by the fact that there is no detectable oxidation in the absence of enzyme. Furthermore, there is no *reduction* of **5** in the presence of NADH even with very high HLADH concentrations. This suggests strongly that the **12** ⇌ **5** equilibrium lies so far to the right that the amount of **12** present under the aqueous reaction conditions is negligibly small.

(19) HLADH-catalyzed oxidation of aldehydes in their hemiacetal form has been postulated previously[20] and the ester nature of the products has been established.[20-22] However, the isolation of **4** from the Scheme II reaction has enabled the structure of the product of such a reaction to be unambiguously assigned for the first time.

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(31) The enantiospecificity of HLADH-catalyzed reductions of chiral decalone and cyclohexanone substrates[2] is also consistent with the left-hand side of the lattice section being more resistant to occupation than the right.

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