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ENZYMES IN ORGANIC SYNTHESIS—29[1]

PREPARATIONS OF ENANTIOMERICALLY PURE *cis*-2,3- AND 2,4-DIMETHYL LACTONES VIA HORSE LIVER ALCOHOL DEHYDROGENASE-CATALYZED OXIDATIONS[2]

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Abstract—Further examples of the broad applicability of horse liver alcohol dehydrogenase-catalyzed oxidations of *meso*-diols as a route to chiral lactones of asymmetric synthetic value are described. The acyclic meso substrates, *cis*-2,3-dimethyl- and -diethylbutane-1,4-diols, and *cis*-2,4-dimethylpentane-1,5-diol, are stereospecifically oxidized in good yields to the corresponding enantiomerically pure γ- and δ-lactones. The oxidation of *cis*-3,4-bis(hydroxymethyl)thiacyclopentane is similarly stereospecific. For each *meso*-diol the oxidation takes place with a net stereospecificity for the hydroxymethyl groups attached to the *S*-centers, with the initially formed hydroxyaldehydes undergoing further enzyme-catalyzed oxidations via their hemiacetal forms to produce lactone products directly.

The asymmetric synthetic opportunities provided by the chiral catalytic properties of enzymes are becoming increasingly recognized.[4] Alcohol dehydrogenases, which catalyze oxidoreductions of the type depicted in eqn (1)†, are among the most useful enzymes for

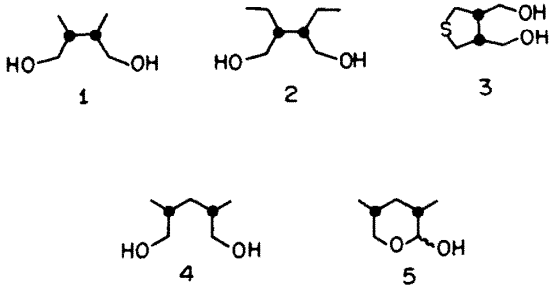
$$H^+ + \rangle C{=}O + NADH \rightleftharpoons \rangle CH(OH) + NAD \quad (1)$$

organic synthetic purposes, with the commercially available enzyme from horse liver being the best documented. HLADH is an extremely versatile alcohol dehydrogenase that has a well defined and predictable specificity[4a,5] and that is capable of effecting highly stereospecific oxidoreductions on a broad structural range of aldehyde, ketone and alcohol substrates.[4a,b,k,5,6] Of particular asymmetric synthesis value is the ability of HLADH to effect stereospecific transformations of symmetrical substrates such as *meso*-compounds. This has been demonstrated for cyclic *meso*-diol[6g] and -diketone[6h] oxidoreductions. In this paper we report further on the generality of such transformations and the extension of the method for the stereospecific oxidation of acyclic *meso*-diols to enantiomerically pure lactones.

RESULTS

Synthesis of substrates. The substrates evaluated were the *meso*-diols **1–4** and the hemiacetal (±)-**5**. They were obtained by literature methods or by unexceptional routes that are described in the Experimental.

†Abbreviations used: NAD and NADH, oxidized and reduced forms respectively of nicotinamide adenine dinucleotide coenzymes; HLADH, horse liver alcohol dehydrogenase; FMN, flavin mono nucleotide (riboflavin phosphate).



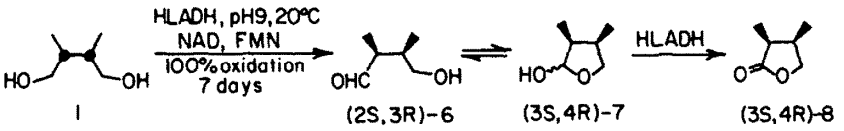
HLADH-Catalyzed oxidations of **1–5**. The *meso*-diols **1–4** and the hemiacetal (±)-**5** were each good substrates. Their rates of HLADH-catalyzed oxidations relative to that of the standard reference cyclohexanol are recorded in Table 1. The substrates were subjected to preparative-scale, enzyme-catalyzed, oxidation at pH 9 using FMN to effect recycling [6i] of the catalytic quantities of the NAD coenzyme employed. For the *meso*-diols **1–4**, enantiopically specific or selective oxidations of the hydroxymethyl groups attached to *S*-centers occurred. In every case the initial intermediate hydroxyaldehyde product underwent further *in situ* oxidation to a considerable extent via its hemiacetal form to give the corresponding lactone product directly. This is illustrated in Scheme 1 for the 2,3-dimethyl substrate **1** for which the overall HLADH-catalyzed oxidation sequence is **1**→**6**→**7**→**8**.

The situation is analogous for the remaining diols **2-4**. The hemiacetal **5** was oxidized under similar conditions, with the reaction being terminated after <50% oxidation owing to the racemic nature of the substrate. The overall results for the oxidations of **1-5** are summarized in Scheme 2. The structures of the optically active lactones **8**, **9**, **11** and **12** were confirmed by comparison with the racemic materials obtained by silver carbonate oxidations[7] of the corre-

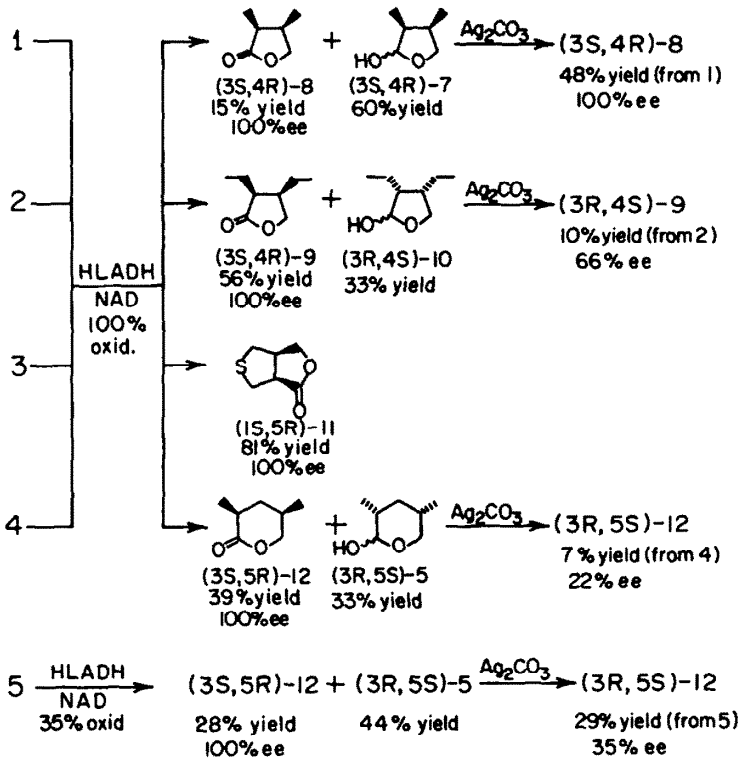
Table 1. Relative rates of HLADH-catlayzed oxidations of 1–5

Substrate	Relative Rate[a]
cyclohexanol	100
1	31
2	23
3	17
4	30
(±)-5	25

[a]Oxidation rates were measured spectrophotometrically at 25°C in 0.1M NaOH-glycine buffer (pH 9) with [S] = 10^{-2} - 10^{-4}M and [NAD] = 5×10^{-4}M



Scheme 1.



Scheme 2.

sponding *meso*diols **1**, **2** and **4** and sodium borohydride reduction[8] of the anhydride precursor of **3**. The hemiacetals **5**, **7** and **10** isolated from the enzymic reactions were oxidized directly with silver carbonate to the corresponding lactones, which were then characterized (Scheme 2).

Enantiomeric excess determinations. The ees of the Scheme 2 lactones **8**, **9** and **12** were established by treatment with methyl lithium followed by ^{1}H NMR examination of the diols obtained in the presence of Eu(tfc)$_3$.[9] The thiacyclopentane lactone **11** was desulfurized to the dimethyl lactone **8** prior to reaction with methyl lithium. The diols obtained from the corresponding racemic lactones were used as reference standards. The $\Delta\Delta\delta$ peak separations observed for the diastereotopic Me proton resonances of the reference diols (±)-**13**-**15** are recorded in Table 2.

Absolute configuration determinations. The absolute configurations of the optically active Scheme 2 lactones were established by degradations to known compounds as follows: (+)-(3*S*, 4*R*)-**8** to (+)-(2*S*)-**17**,[10] (−)-(3*R*, 4*S*)-**9** to (−)-(3*S*, 4*S*)-**18**,[11] (+)-(1*S*, 5*R*)-**11** to (+)-(3*S*, 4*R*)-**8** and (+)-(3*S*, 5*R*)-**12** to (−)-(2*S*)-**21**.[10] The correlation reactions are summarized in Scheme 3.

DISCUSSION

The preparations of the substrates **1**–**5** were achieved without difficulty and each may be regarded as a convenient and readily available starting material The *meso*-diols **1**–**4** and the hemiacetal (±)-**5** were excellent substrates of the enzyme (Table 1), with their relative rates of oxidation being well above the level regarded as the practical limit† for preparative-scale reactions.

†Good preparative-scale results are generally assured with oxidation rates > 5% of that of the standard reference substrate cyclohexanol.

The synthetic HLADH-catalyzed oxidations were performed on up to ~2 g of substrate. Further scaling up of each reaction to 10 g or more presents no problem if greater quantities of the lactone products are required.[6g] The usual simple experimental procedure and work-up gave good recoveries of pure product materials. The reaction conditions have not been optimized. Higher yields of the lactones will be obtained by allowing the oxidations to proceed beyond the current reaction periods, when more complete oxidation of the hemiacetal intermediates can occur. The course of each reaction was monitored by GLC. In the case of (±)-**5**, the traditional 50%-of-reaction termination point for oxidation of racemates was not accurately identified by the GLC monitoring procedure and the reaction was in fact stopped after ~35% of actual oxidation had occurred.

The NMR method used for the determination of the ees of the optically active lactones **8**, **9**, **11** and **12** permitted accuracies of ±3% to be achieved with ease. The absolute configuration determinations of Scheme 3 were also straightforward.

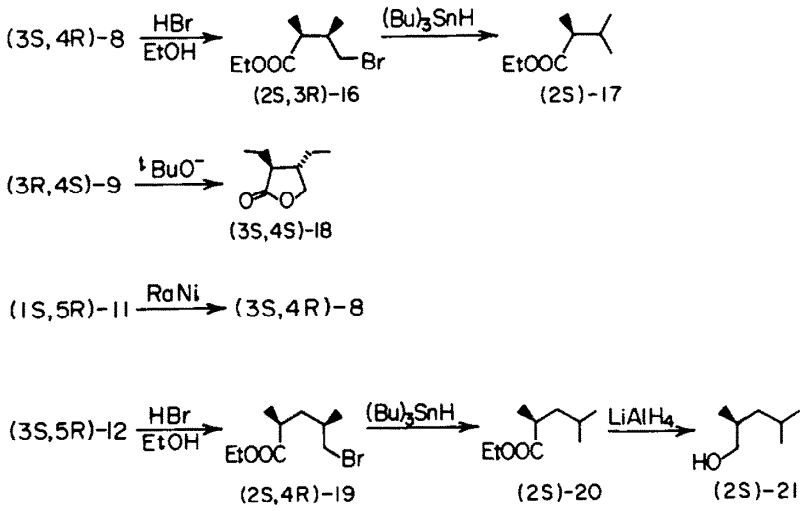
In the enzyme-promoted oxidations of the diols **1**–**4**, the lactone products reflect a net stereospecific oxidation of the hydroxymethyl group adjacent to the *S*-center in each case. There are two ways in which this overall stereospecifiity can arise. The enzyme may operate stereospecifically on the *S*-hydroxymethyl groups of **1**–**4** directly, thereby giving rise to a hemiacetal intermediate with 3*S*-chirality. Alternatively, if the enzyme does not discriminate in its initial oxidation of the enantiotopic hydroxymethyl groups of the *meso*-diols, it can operate stereospecifically on the (3*S*)-enantiomers of the intermediate hemiacetals. The final chirality may also reflect a synergistic combination of the two specificities. For the conversion of **1** and **3** the Scheme 2 data show that the stereospecificity selection is made in the initial oxidation step, with only the *S*-center hydroxymethyl groups being oxidized. This follows from the exclusive formation of (1*S*, 5*R*)-**11**

Table 2. Enantiomeric shift differences for diastereotopic methyl groups of diols (±)-**13**–**15**

Lactone	Diol[a]	$\Delta\Delta\delta$[b] (ppm)
(±)-**8**	$HOC(Me)_2CH(Me)CH(Me)CH_2OH$ (±)-**13**	0.08
(±)-**9**	$HOC(Me)_2CH(Et)CH(Et)CH_2OH$ (±)-**14**	0.07
(±)-**12**	$HOC(Me)_2CH(Me)CH_2CH(Me)CH_2OH$ (±)-**15**	0.23

[a]obtained by treatment of corresponding lactone with methyl lithium

[b]between gem-dimethyl protons in the presence of 0.2–0.4 equivalents of Eu(tfc)$_3$



Scheme 3.

in very high yield from **3** and that both the lactone **8** and hemiacetal **7** products of the oxidation of **1** have the *same* (3*S*, 4*R*) absolute configuration. In contrast for the transformations of **2** and **4** the stereospecific discrimination is manifest in the second oxidation step, with the unreacted hemiacetals (3*R*, 4*S*)-**10** and (3*R*, 5*S*)-**5** respectively leading to lactones of *opposite* chirality to the (3*S*, 4*R*)-**9** and (3*S*, 5*R*)-**12** products isolated directly. The ability of HLADH to select between the hemiacetal enantiomers with 3*S*-preference was confirmed by its catalysis of the oxidation of (±)-**5** to optically pure (3*S*, 5*R*)-**12**. Within the experimental error limits, the enantiomeric purities of the Scheme 2 products indicate that the stereospecific discriminations by HLADH occur virtually exclusively in either the first or second step, and are not attributable to a combination of enantiotopic and enantiomeric selectivity in the sequential oxidations.

The ready access provided by HLADH-mediated oxidation of acyclic *meso*-diols to enantiomerically pure chiral lactones cannot be matched by the traditional methods currently available. The lactones of Scheme 2 have considerable chiral synthon value. For example, **12** is an attractive intermediate for multistriatin,[12] methynolide[13] and monensin[14] and **11** for some antileukemic lignan lactones.[15] The thiadiol **3** was also included in this study since it provided a further example of using cyclic sulfide moieties to provide the ring structures favored by HLADH in its substrates [4a] followed by excision of the sulfur atom to give the corresponding ring-opened product of predetermined chirality.[6g] This is illustrated by the smooth conversion of (1*S*, 5*R*)-**11** to (3*S*, 4*R*)-**8** by treatment with Raney Ni (Scheme 3).

Cubic active-site section analysis of stereospecificity

The stereospecificities observed in the Scheme 2 reactions, and the stage at which the enzymic discrimination of enantiotopic groups or enantiomers occurs, are readily interpreted using the HLADH active-site section based on cubic-space descriptors.[5c] The cubic space model used in the following analyses is the extended section reported in reference 6*g*.

The analysis for the *cis*-2,3-dimethyl diol **1**, for which HLADH exhibits enantiotopic specificity for the *S*-center CH_2OH group, is shown in Fig. 1.† For its higher homolog, the *cis*-2,3-diethyl diol **2**, the presence of ethyl groups at both chiral centers removes much of the clear-cut *S*- over *R*-preference of Fig. 1 because intrusion of part of either ethyl group into limited access regions of the section cannot be avoided in any orientation that would lead to the oxidation. The more unfavorable binding modes of **2** compared with **1** are reflected by a reduced oxidation rate for the diethyl substrate (Table 1). Although marginal preference of *S*-center oxidation is still indicated by the model for the initial oxidation of **2**, the predominance of (3*S*, 4*R*)-enantiomeric discrimination deduced from the Scheme 2 results for the subsequent hemiacetal **10** oxidation step is unequivocally supported by the cubic section analysis. This is summarized in Fig. 2.

For the 2,4-dimethyl diol **4**, either of the enantiotopic hydroxy-methyl groups can be correctly located at the oxidation site such that the remainder of the molecule occupies fully allowed space. The lack of *S*- versus *R*-center group discrimination in the oxidation of **4** inferred from the experimental results (Scheme 2) is thus explained. The complete enantiomeric specificity in subsequent oxidation of the racemic hemiacetal **5** formed in the initial step, and verified by the independent examination of HLADH-catalyzed oxidation of (±)-**5** itself, is also in accord with the cubic active-site model predictions. The preferential binding mode open to (3*S*, 5*R*)-**5**, but precluded for its unreactive (3*R*, 5*S*)-enantiomer, is illustrated in Fig. 3.

The above analyses provide further demonstrations of the validity of the cubic section method for analyzing the specificity of HLADH-catalyzed oxidoreductions of its substrates. The model has proven applicable to all transformations investigated to date. It is recommended with confidence for routine use

†The cubic section analysis of the thia-diol **3** oxidation, which is also enantiotopically *S*-center specific, parallels that described previously for other cyclic *meso*-diols.[5c,6g]

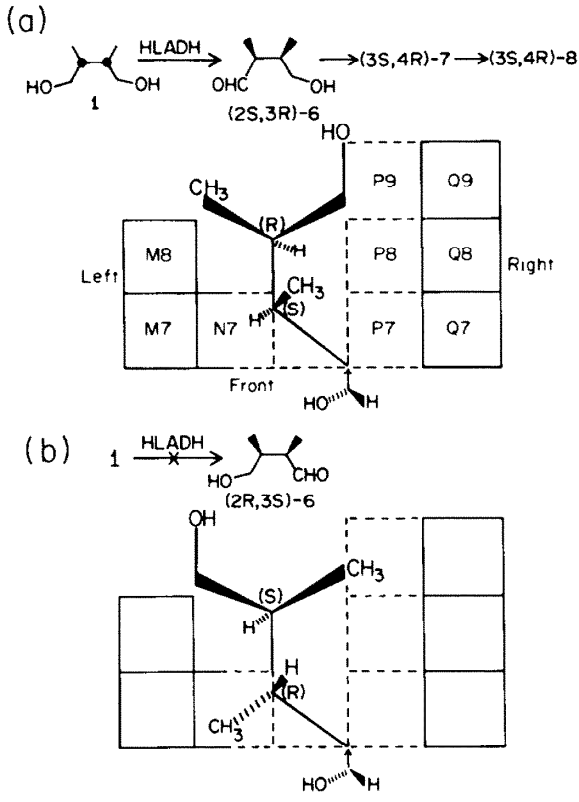


Fig. 1. Cubic active-site section analysis of the stereospecificity of HLADH-catalyzed oxidation of the 2,3-dimethyl diol **1**. The model and analytical procedure used and the alphanumeric designations of the cube locations are as described previously.[5c,6g] In this figure the top elevation perspective is employed to depict a representative "best orientation" of the substrate at the active site. Cubes bounded by solid lines are "forbidden" regions where substrate binding is precluded, as are the front and underneath portions of the section, due to their being occupied by enzymic aminoacid residues or by coenzyme. Cubes bounded by broken lines are "limited" regions where substrate binding is possible, but not favored as a result of their proximity to forbidden space. The open areas are "allowed" zones where the substrate is freely accommodated. For oxidation to occur the alcohol group must locate at the oxidation site, identified by the arrow (↑). In HLADH-mediated primary alcohol oxidations the pro-*R* hydrogen is always abstracted.[5c] (a) With the (2*S*)-hydroxymethyl group correctly oriented at the oxidation site the remainder of the substrate structure can all be accomodated in allowed active-site regions, as shown. Productive ES-complex formation is therefore favored and oxidation to (2*S*, 3*R*)-**6** takes place readily. The hemiacetal (3*S*, 4*R*)-**7** also binds favorably and thus its subsequent oxidation to the (3*S*, 4*R*)-lactone **8** likewise proceeds smoothly. (b) All orientations of **1** that would permit oxidation of the *R*-center alcohol function require parts of the molecule to locate in forbidden space. In the orientation depicted, which is the least unfavorable, the (*R*)-methyl group must be positioned in forbidden cubes **B1** or **U(B1)**. Productive ES complex formation is thus precluded and oxidation cannot occur.

and for predicting the structural- and stereospecificity of the enzyme towards new or potential substrate structures.

EXPERIMENTAL

The sources of enzyme and chemicals, and the general methods, criteria of purity and instrumentation used were as described previously.[6g] HLADH quantities refer to mg of active enzyme.[6g]

Preparations of meso-*diols* **1–4**

cis-2,3-*Dimethyl*-1,4-*butanediol* (**1**) was obtained by the method of McCasland and Proskow,[16] b.p. 85° (0.1 mm Hg) (lit.[16] b.p. 127–128° (9 mm Hg)), IR (film) 3400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.1 (6H, d, J = 8 Hz), 2.0 (2H, m), 3.6 (4H, d, J = 6 Hz) and 3.8 (2H, s, OH) ppm.

cis-2,3-*Diethyl*-1,4-*butanediol* (**2**), prepared by the general method of Kuhn *et al.*,[17] had b.p. 106° (0.1 mm Hg) (lit.[17] b.p. 130–132° (8 mm Hg)), IR (film) 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.1 (6H, t, J = 7 Hz), 1.5 (4H, m), 3.7 (4H, m) and 4.5 (2H, s, OH) ppm.

cis-3,4-*Bis(hydroxymethyl)thiacyclopentane* (**3**) was obtained by reducing *cis*-3,4-dicarboxythiacyclopentane anhydride[18] with $LiAlH_4$ according to the general procedure of Bailey and Johnson.[8] It had m.p. 94–95° (lit.[18] m.p. 90–92°), IR ($CHCl_3$) 3390 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.42–3.20 (8H, m) and 3.64–3.96 (4H, m) ppm.

cis-2,4-*Dimethyl*-1,5-*pentanediol* (**4**). A mixture of $LiAlH_4$ (4.4 g, 110 mmol) and lithium hydride (0.8 g, 100 mmol) was added portionwise with stirring to *cis*-2,4-bis(hydroxymethyl)cyclopentene bis-*p*-toluenesulfonate[19] (20 g, 46 mmol) in dry ether (300 mL). The suspension was then refluxed for 24 hr and then cooled to 0°. Water (6 mL) was then added cautiously with stirring followed by 15% NaOH aq (6 mL) and then water (18 mL). The mixture was

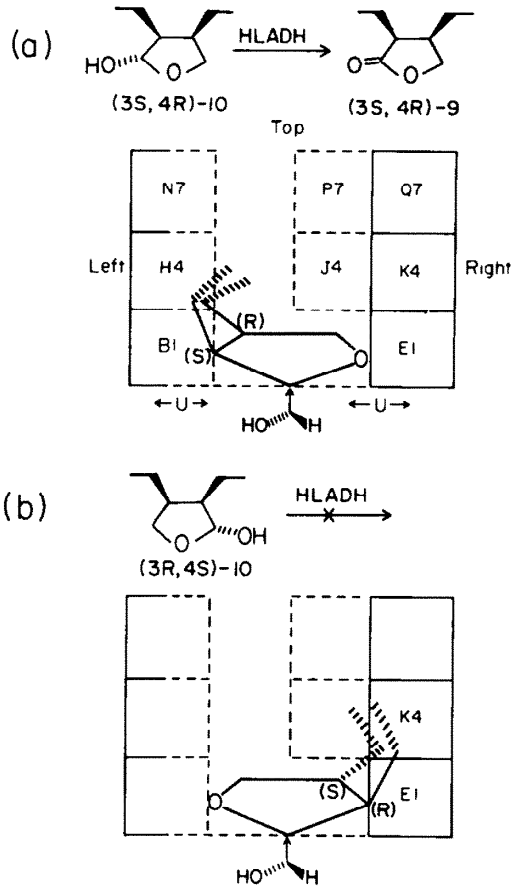


Fig. 2. Cubic active-site section analysis of the hemiacetal **10** oxidation stage of the HLADH-catalyzed transformation of **2** to **9**. The substrate orientations are depicted from the front elevation perspective.[5c] The initial oxidation of the diol **2** to the aldehyde tautomer of **10** is largely nonstereospecific (see text). Of the stereoisomers that are epimeric at the carbon (C-2) bearing the OH group, only those with the OH function anti to the Et groups allow oxidation to take place. Oxidation is precluded when all groups are syn, because the Et groups cannot avoid heavily forbidden space underneath the section if the CH(OH) moiety is to be positioned at the oxidation site. (a) In this orientation all portions of the substrate can locate in allowed or limited cubes and a productive ES-complex leading to (3*S*, 4*R*)-**9** can form. (b) Oxidation of (3*R*, 4*S*)-**10** is ruled out because the orientation required for oxidation cannot avoid placing the (*R*)- and (*S*)-ethyl groups into forbidden cubes **E1**, **K4** and **E3** respectively.

stirred for 20 min at 20° and filtered. The filtrate was dried ($MgSO_4$) and evaporated. The crude *cis*-2,4-dimethylcyclopentene obtained was ozonized directly in MeOH–CH_2Cl_2 (1:4 v/v) at −78° until the soln turned blue. $NaBH_4$ (2g, 40 mmole) was then added with vigorous stirring. The solvent was removed by rotoevaporation and the residue dissolved in water and stirred for 1 hr at 20°. The aqueous mixture was extracted with EtOAc (2 × 30 mL), and the organic soln dried ($MgSO_4$), evaporated, and Kugelrohr-distilled to give *cis*-**4** (2.5 g, 41% yield) b.p. 95° (0.4 mm Hg) (lit.[20] b.p. 130–132° (5 mm Hg)), IR (film) 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (6H, d, J = 7 Hz), 1.8 (4H, m,), 2.9 (2H, s, OH) and 3.4 (4H, d, J = 5 Hz) ppm.

cis-3,5-*Dimethyltetrahydropyran*-2-*ol* ((±)-**5**). Diisobutylaluminum hydride (11.9 mmol, 8.5 mL of 1.4 M soln in hexane) was added dropwise with stirring under N_2 to (±)-**12**,[21] (1.16 g, 9.06 mmol) in hexane (50 mL) at −78°. Stirring was continued for 3 hr at −78° and the reaction was then quenched by the cautious addition of water (3 mL). The mixture was allowed to warm to 0° and sufficient 6N HCl to dissolve the gelatinous ppt was added. The mixture was extracted with ether (5 × 25 mL) and the ether soln dried ($MgSO_4$) and evaporated. Kugelrohr-distillation followed by MPLC purification on silica gel (EtOAc–hexane (1:9) elution) afforded the title hemiacetal (±)-**5** (690 mg, 60% yield) b.p. 60° (1.0 mm Hg), IR (film) 3340 cm^{-1}; ^1H NMR (CCl_4) δ 0.87 (6H, s), 1.13–2.0 (4H, m), 2.83–5.0 (3H, m) and 4.28 (1H, s, OH) ppm. (Found: C 64.84; H, 10.91. Calc for $C_7H_{14}O_2$: C, 64.58; H, 10.84%).

Preparations of racemic lactones (±)-**8**, **9**, **11** and **12**

Lactones (±)-**8**, -**9** and -**12** were prepared by oxidation of the corresponding diols by the procedure of Fetizon[7] and (±)-**11** by $NaBH_4$ reduction of its precursor anhydride using the general method of Bailey and Johnson.[8] Lactone (±)-**12** was also prepared by the $NaBH_4$ reduction of anhydride method[21] (see above). Their properties were as follows: from **1** in 90% yield,(±)-**8**, b.p. 45° (0.25 mm Hg), IR (film) 1775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.01 (3H, d, J = 6 Hz), 1.15 (3H, d, J = 6 Hz), 2.33–2.87 (2H, m), 3.98 (1H, d of d, J = 3, 9 Hz) and 4.33 (1H, d of d, J = 6, 9 Hz) ppm. (Found: C, 62.92; H, 8.98; Calcd for $C_6H_{10}O_2$; C, 63.00; H, 8.83%). From **2** in 98% yield (±)-**9** b.p. 59° (0.2 mm Hg) (lit.[11] b.p. 107–108° (10 mm Hg)), IR (film) 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0 (6H, t, J = 7 Hz), 1.6 (4H, m), 2.2 (2H, m), 3.8 (1H, t, J = 8 Hz) and 4.4 (1H, *t*,

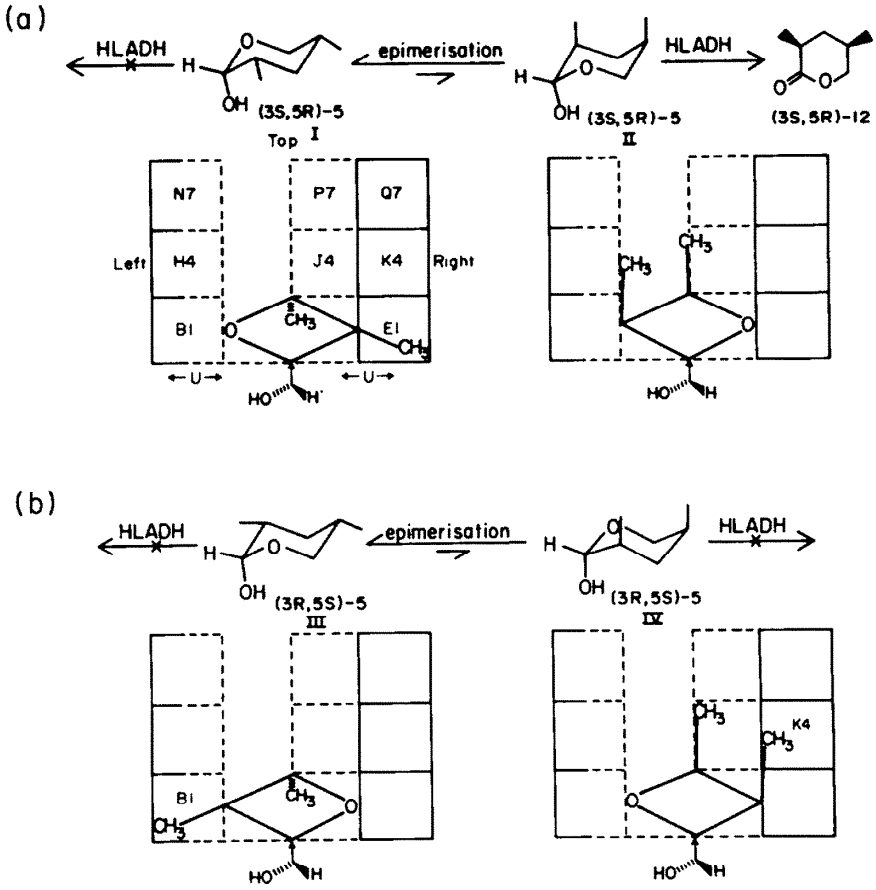


Fig. 3. Cubic section analysis of hemiacetal (±)-**5** oxidation. The substrate orientations are again shown from the front elevation perspective of the cubic model. Only the hemiacetal conformers with axially directed hydroxyl groups can orient correctly at the oxidation site.* Each conformer is analyzed separately. (a) Oxidation via the thermodynamically preferred conformation I is not permitted since the (*S*)-center Me substituent would intrude into forbidden location **E1**. For the all-axial conformer II, all groups can reside in allowed space in a productive ES complex and transformation to the lactone (3*S*, 5*R*)-**12** takes place smoothly. (b) Neither epimeric axial-OH conformer of (3*R*, 5*S*)-**5** can orient satisfactorily when the CH(OH) function is at the oxidation site. For conformation III, the (3*R*)-Me substituent violates forbidden region **B1** while for IV, it cannot avoid the **K4**, **5** intersection, also a forbidden location. Accordingly, no productive ES complex can form and oxidation of the (3*R*, 5*S*)-enantiomer does not occur.

J = 8 Hz) ppm; from *cis*-3,4-dicarboxythiacyclopentane[18] in 50% yield, (±)-**11** b.p. 110° (0.3 mm Hg), IR (film) 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.64–3.70 (6H, m), 4.16 (1H, d of d, J = 9 Hz) and 4.44–4.82 (1H, m) ppm; from **4** in 94% yield (±)-**12** b.p. 60° (0.75 mm Hg) (lit.[21] b.p. 107–109° (12 mm Hg)), IR (film) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0 (3H, d, J = 6 Hz), 1.25 (3H, d, J = 7 Hz), 1.5–2.9 (4H, m), 3.87 (1H, d of d, J = 8, 11 Hz) and 4.33 (1H, d of d, J = 2.5, 11 Hz) ppm.

Relative rates of HLADH-catalayzed oxidations of diols **1–4** *and hemicetal* (±)-**5**

The general HLADH kinetic assay method[6c,22] was used, monitoring the change in the 340 nm absorption of NAD. The assays were performed at 25° at pH 9.0 using the following stock solns: HLADH, 1 mg mL^{-1} in 0.05 M Tris-HCl buffer, pH 7.4; NAD, 10 mg mL^{-1} in 0.1 M glycine–NaOH buffer, pH 9.0. For each substrate the reference essay was performed under the same conditions on a cyclohexanol soln of the same concentration. The rates observed, relative to cyclohexanol = 100, are recorded in Table 1.

Preparative-scale HLADH-catalyzed oxidations of meso-*diols* **1–4**

Oxidation of meso-*diol* **1** *to lactone* (3S, 4R)-**8**. *cis*-**1** (1.4 g, 12 mmol), NAD (0.72 g, 1.1 mmol) and FMN (9.7 g, 20 mmol) were dissolved in 0.1 M glycine–NaOH buffer (pH 9.0, 500 mL) in a 1L-Erlenmeyer flask. The pH of the soln was then readjusted to 9.0 with 15% NaOH. Aq. HLADH (50 mg) was added and the mixture kept at room temp (20°). The pH was periodically adjusted, to 9.0 as the reaction proceeded. The mixture turned from its initial clear orange to an opaque, almost black, color as the oxidation progressed. The course of the oxidation was monitored periodically by GLC analysis of $CHCl_3$ extracts (5 × 5 mL) of small aliquots (5 mL). When no starting diol remained (7 days) the mixture was adjusted to pH 12 and then continuously extracted with $CHCl_3$ for 2 days. The aqueous soln was retained for re-extraction (see below). The dried ($MgSO_4$) chloroform *basic extract* was evaporated and the residue chromatographed on silica gel (100 g) to effect separation of traces of unreacted diol from hemiacetal products. Elution with EtOH: hexane (1:4) gave the hemiacetal (3*S*, 4*R*)-**7** (0.83 g, 60% yield) which was oxidized

directly with Ag_2CO_3 on Celite[7] to give, after Kugelrohr-distillation, (3*S*, 4*R*)-*cis*-3,4-dimethyltetrahydrofuran-2-one (**8**, 0.68 g, 48% yield from **1**, 100% ee), $[\alpha]_D^{20}$ +39.9° (*c* 4, $CHCl_3$). The aqueous enzymic soln retained above was acidified to pH 2 with 6N HCl and reextracted continuously with $CHCl_3$ for 2 days. Evaporation of the dried ($MgSO_4$) *acid extract* $CHCl_3$) solution followed by Kugelrohr-distillation gave additional (3*S*, 4*R*)-lactone **8** (0.2 g, 15% yield, 100% ee) $[\alpha]_D^{20}$ +40.0 (*c* 11, $CHCl_3$).

The oxidations of diols **2–4** were effected similarly. The results are summarized below. The physical and spectral properties of all optically active lactones **8**, **9**, **11** and **12** were identical to those reported above for the racemic compounds.

Oxidation of meso-*diol* **2** *to* (3S, 4R)- *and* (3R, 4S)-**9**. *cis*-**2** (1.2 g, 8.2 mmol), NAD (720 mg, 1.1 mmol), FMN 9.72 g, 20.3 mmol) and HLADH (35 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 500 mL) at 20° for 4 days gave, from the *basic extract*, the hemiacetal (3*R*, 4*S*)-**10** (0.4 g, 33% yield) which on Ag_2CO_3 oxidation yielded (3*R*, 4*S*)-*cis*-**9** (0.12 g, 10% yield from **2**, 66% ee), $[\alpha]_D^{20}$ −30.4° (*c* 2, $CHCl_3$). The *acidic extract* afforded the (3*S*, 4*R*)-lactone **9** (0.67 g, 56% yield, 100% ee), $[\alpha]_D^{20}$ +46.3° (*c* 10.8, $CHCl_3$).

Oxidation of the meso*diol* **3** *to* (1S, 5R)-**11**. *cis*-**3** (2.3 g, 15.5 mmol), NAD (1.5 g, 2.3 mmol), FMN 18 g, 38 mmol) and HLADH (60 mg) in 0.1 M glycine NaOH buffer (pH 9.0, 1L) at 20° for 7 days gave, from the *acid extract* only, (1*S*, 5*R*)-*cis*-**11** (1.81 g, 81% yield, 100% ee) b.p. 80° (0.05 mm Hg), m.p. 50-52°, $[\alpha]_D^{25}$ +88.8° (*c* 1.4, $CHCl_3$). (Found: C, 50.15; H, 5.49; S, 22.45. Calc for $C_8H_8O_2S$: C, 50.00; H, 5.55; S, 22.22%).

Oxidation of meso-*diol* **4** *to* (3S, 5R)-**12**. *cis*-**4** (1.8 g, 14 mmol), NAD (1.5 g, 2.3 mmol), FMN (18 g, 38 mmol) and HLADH (50 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 1L) at 20° for 3 weeks yielded, from the *basic extract*, the hemiacetal (3*R*, 5*S*)-**5** (0.6 g, 33% yield) which on Ag_2CO_3 oxidation gave (3*R*, 5*S*)-**12** (0.12 g, 7% yield from **4**, 22% ee), $[\alpha]_D^{20}$ −8.5° (*c* 1.9, $CHCl_3$). The *acidic extract* gave the (3*S*, 5*R*)-lactone **12** (0.7 g, 39% yield, 100% ee) $[\alpha]_D^{20}$ +39.1° (*c* 10, $CHCl_3$).

Preparative-scale HLADH-catalyzed oxidation of the hemiacetal (±)-**5**

The oxidation was performed by the general procedure described above for **1**→**8** except that, because of the racemic nature of the substrate, the reaction was terminated when GLC analysis indicated ~50% of reaction had occurred. (In fact, the assay was not accurate due to incomplete extraction of starting diol and only ~35% of oxidation had taken place.) (±)-**5** (500 mg, 3.8 mmol), NAD (600 mg, 0.9 mmol), FMN (7.2 g, 15.2 mmol) and HLADH (40 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 400 mL) at 20° for 10 days afforded, from the *basic extract*, recovered (3*R*, 5*S*)-**5** (220 mg, 44% yield) $[\alpha]_D^{25}$ +4.7° (*c* 1.2, $CHCl_3$) that on oxidation with Ag_2CO_3 gave (3*R*, 5*S*)-**12** (142 mg, 29% yield from (±)-**5**, 35% ee), $[\alpha]_D^{25}$ −14.0° (*c* 1.2, $CHCl_3$). The *acid extract* yielded the (3*S*, 5*R*)-lactone **12** (140 mg, 28% yield, 100% ee) $[\alpha]_D^{25}$ +35.1° (*c* 0.8, $CHCl_3$).

Enantiomeric excess determinations

The ees of all optically active lactones **8**, **9** and **12** were determined by reacting each with excess MeLi and examining the ^1H NMR spectra of the diastereotopic methyl peaks of the diols **13–15** obtained in the presence of 0.1–0.4 equiv of $Eu(tfc)_3$.[9] The ee of (1*S*, 5*R*)-**11** was measured on the (2*S*, 3*R*)-lactone **8** obtained after RaNi desulfurization (see below). The $\Delta\Delta\delta$ separations observed for the reference diols (±)-**13–15** obtained from the corresponding racemic lactones are recorded in Table 2.

Absolute configuration determinations

The correlations are summarized in Scheme 3. They were obtained as follows:

(3S, 4R)-cis-3,4-*Dimethyltetrahydrofuran*-2-*one* (**8**). Dry HBr was bubbled into a stirred soln of the (+)-(3*S*, 4*R*)-lactone **8** (465 mg, 4.1 mmol, 100% ee) in EtOH (30 mL) at 0° for 1 hr. The resulting soln was stirred for 12 hr at 20° and then poured into saturated brine (50 mL) and extracted with ether (3 × 20 mL). The ethereal soln was washed with $NaHCO_3$ aq (2 × 20 mL) and then dried ($MgSO_4$) and evaporated. The oily residue was purified by MPLC on silica gel (EtOAc:hexane (1:20) elution) to give **16** (700 mg, 78% yield) b.p. 60–65° (0.5 mm Hg), $[\alpha]_D^{20}$ +18.4° (*c* 16.7, $CHCl_3$) IR (film) 1720 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.18 (3H, d, J = 5 Hz), 1.24 (3H, d, J = 8 Hz), 1.40 (3H, t, J = 7 Hz), 2.2 (1H, m), 2.6 (1H, m), 3.5 (2H, d of d, J = 6 Hz) and 4.2 (2H, q, J = 7 Hz) ppm. (Found: C, 42.92; H, 6.67; Br, 35.60. Calc for $C_8H_{15}O_2Br$: C, 43.47; H, 6.78; Br, 35.81%). Tri-n-butyltin hydride (800 mg, 2.7 mmol) in dry benzene (4 mL) was added dropwise with stirring during 10 min at 20° to the (+)-**16** (460 mg, 2 mmol) in dry benzene (5 mL) and stirring was continued overnight. The benzene was then removed by rotary evaporation and the residue purified by column chromatography on silica gel (CCl_4 elution) to give **17** (270 mg, 94% yield), $[\alpha]_D^{20}$ +9.9° (*c* 4.5, $CHCl_3$) (lit.[10] $[\alpha]_D^{25}$ +8.1° (neat)), IR (film) 1725 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.0 (6H, d, J = 6 Hz), 1.1 (3H, d, J = 6 Hz), 1.3 (3H, t, J = 7 Hz), 2.0 (2H, m) and 4.1 (2H, *q*, J = 7 Hz) ppm.

cis-(1S, 5R)-3-*Oxa*-7-*thiabicyclo*[3.3.0]*octan*-2-*one* (**11**). W-2 Raney Ni[23] (1 g) was added to the (+)-(1*S*, 5*R*)-lactone **11** (130 mg, 0.9 mmol) in ethanol (20 mL) and the mixture heated under reflux for 2 hr. The mixture was then cooled, filtered, and the RaNi washed with EtOH (4 × 5 mL). The ethanol solution was evaporated and the residue Kugelrohr-distilled to give the (3*S*, 4*R*)-lactone **8** (81 mg, 80% yield, 100% ee) b.p. 90° (10 mm Hg) $[\alpha]_D^{25}$ +42° (*c* 0.5, $CHCl_3$).

cis-(3R, 4S)-3,4-*Diethyltetrahydrofuran*-2-*one* (**9**). The (−)-(3*R*, 4*S*)-lactone **9** (100 mg, 1.4 mmol, 66% ee) in t-BuOH (10 mL) containing t-BuOK (100 mg, 0.9 mmol) was heated under reflux under N_2 for 2 hr to give a mixture of **9** and **18** (1:4). The t-BuOH was removed by rotary evaporation and 2N HCl (10 mL) added. The mixture was then extracted with ether (3 × 20 mL) and the ether soln dried ($MgSO_4$) and evaporated to give a light brown oil (quant. recovery), a portion of which was purified by GLC (3% QF-1 on Chromasorb column, 180°) to give *trans*-**18** (8 mg) $[\alpha]_D$ −28.8° (*c* 0.6, $CHCl_3$) (lit.[11] $[\alpha]_D^{25}$ −41° (*c* 10.3, $CHCl_3$)); IR and NMR spectra were identical with those of (±)-**9** recorded above.

cis-(3S, 5R)-3,5-*Dimethyltetrahydropyran*-2-*one* (**12**). Dry HBr was bubbled into a stirred soln of the (+)-(3*S*, 5*R*)-lactone **12** (0.96 g, 7.5 mmol, 100% ee) in dry EtOH (30 mL) for 2.5 hr at 0°. The stirring was continued at 20° for 3 days. Saturated brine (50 mL) was then added and the mixture extracted with ether (3 × 30 mL). The ether soln was washed with $NaHCO_3$ aq (30 mL) and then dried ($MgSO_4$) and evaporated. The residue was Kugelrohr-distilled to give ethyl (2*S*, 4*R*)-*cis*-**19** (1.6 g, 96% yield), b.p. 80° (4 mm Hg), $[\alpha]_D^{20}$ +3.9° (*c* 16, $CHCl_3$), IR (film) 1735 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.0 (3H, d, J = 6 Hz), 1.2 (3H, d, J = 7 Hz), 1.3 (3H, t, J = 7 Hz), 1.6 (3H, m), 2.5 (1H, q, J = 8 Hz), 3.4 (2H, d, J = 8 Hz) and 4.1 (2H, q, J = 7 Hz) ppm. Tri-n-butyltin hydride (2.1 g, 7.4 mmol) in dry benzene (5 mL) was added dropwise with stirring at 20° to the (+)-bromoester **19** (1.5 g, 6.3 mmol) in dry benzene (25 mL). After stirring for 12 hr the mixture was refluxed for 24 hr and the solvent then evaporated. The residue was purified by column-chromatography on silica gel (CCl_4 elution). Compound **20** (1 g, 6.3 mmol) obtained was dissolved directly in dry THF (20 mL) and added slowly to a stirred slurry of $LiAlH_4$ (0.24 g, 8 mmol) in dry THF (30 mL) at 0°. The suspension was stirred for a further 12 hr at 20°C and lithium hydride (80 mg, 10 mmol) and $LiAlH_4$ (36 mg, 0.9 mmol)[19] added to complete reduction of any unchanged bromide. After refluxing for 30 min the mixture was cooled to 0°. Water (0.4 mL) was added cautiously with stirring, followed by 15% NaOH aq (1 mL). After filtration

the solvent was dried ($MgSO_4$) and evaporated. The oily product was column-chromatographed on silica gel ($CHCl_3$ elution) to give (2*S*)-**21** (340 mg, 47% yield) b.p. yield) b.p. 40° (0.1 mm Hg), $[\alpha]_D^{20} - 3.9°$ (*c* 3.3, $CHCl_3$) (lit.[10] b.p. 157° (760 mm Hg), $[\alpha]_D^{25} - 1.1°$ (neat)), IR (film) 3460 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.9 (3H, d, $J = 6$ Hz), 0.95 (6H, d, $J = 7$ Hz), 1.4–2.0 (5H, m) and 2.5 (2H, d, $J = 5$ Hz).

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