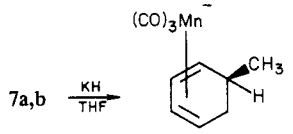
of the ring. It is presumed that **7b** arises by isomerization of **7a** through a diene hydride intermediate similar to **5**. Indeed, high-temperature ^{1}H NMR studies have verified that isomers **7a** and **7b** are in rapid equilibrium.[13] The structure of isomer **7b** has been confirmed by X-ray crystallographic analysis.[14]

Perhaps the most surprising aspect of the mechanism in Scheme II is the proposed metal-to-ring migration of the methyl group upon methylation of diene anion **2**, formally an insertion of an olefin into a metal–alkyl bond. The only other known example of a metal-to-ring alkyl migration of this type is the transfer of the ethyl group of $Cp_2Mo(Et)Cl$ to the endo side of one of the cyclopentadienyl rings upon treatment with phosphines.[15]

The conversion of **4** to **7** is remarkable in that, following methyl migration to the ring, a second endo C–H bond is activated through coordination to manganese. Thus, quantitative deprotonation of **7** by KH gives the monomethylated cyclohexadiene anion (**8**). The ultimate conversion of **2** to **8** represents formally



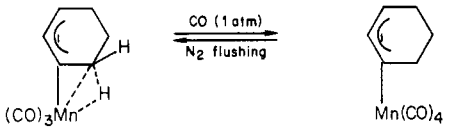
8

ν_{CO} = 1930, 1838, and 1789 cm^{-1}

an electrophilic substitution of an endo hydrogen of cyclohexadiene mediated by manganese activation. Preliminary results indicate that the monomethylated anion (8) is also highly nucleophilic and can be alkylated with methyl iodide to produce ring-dialkylated products.

A result which bears directly on the potential synthetic utility of this system is the rapid oxidative cleavage of the diene from the diene anions by oxygen. Exposure of a THF solution of **2** to 1 atm of oxygen causes immediate precipitation of a brown solid (containing MnO_2) and quantitative formation of free 1,3-cyclohexadiene which can be trapped with tetracyanoethylene and isolated as the Diels–Alder adduct.

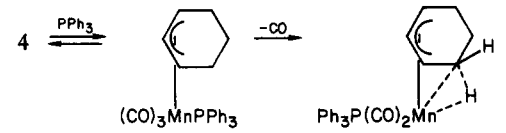
The reactions of the hydrogen-bridged species with external ligands have also been investigated. Reaction of **4** with CO in methylene chloride solution rapidly gives the π-allyl tetracarbonyl complex **9**[16] by simple C–H bond displacement. This reaction



4 9

ν_{CO} = 2020, 1945, and 1937 cm^{-1} ν_{CO} = 2055, 1987, 1970, and 1959 cm^{-1}

has parallels with the behavior of the protonated (diene)iron–L_3 species.[11b-d,17] Interestingly, the addition at 1 atm of CO pressure proceeds to only 25% completion and is readily reversible; flushing a solution of **9** with N_2 results in quantitative regeneration of starting material. Complex **4** reacts with triphenylphosphine in a similar fashion; however, the resulting π-allyltricarbonyl-



10 11

monophosphine complex **10** slowly loses CO to form the C–H bridged dicarbonylmonophosphine complex **11** which exists as a mixture of all three possible isomers.

Nucleophilic addition to the π-allyl complexes **9** and **10** has not yet been investigated; however, literature precedent suggests conversion to cyclohexene derivatives is likely.[18] Hence, the reactions reported herein should enable a series of manganese-mediated transformations for the stepwise reduction of arenes to 1,3-cyclohexadienes and possibly cyclohexenes with highly varied functionalization. Since all of the reactions leading to ring functionalization proceed exclusively either exo or endo to the metal, control of product stereochemistry is expected. Such a series should prove relatively versatile and has obvious potential synthetic utility.

The chemistry reported illustrates two novel aspects of transition-metal activation of hydrocarbon ligands. First, the activation of arenes toward addition of *2* equiv of nucleophiles to yield coordinated dienes has been demonstrated in the conversion of benzene complex **1** to the cyclohexadiene complex **2**. Secondly, although coordination of C–H bonds to transition metals has been previously observed in a limited number of systems, the transformations reported for the hydrogen-bridged manganese system represent the first clear illustration that such metal–CH interactions can be used to advantage for carrying out electrophilic substitutions at the bridged carbon. This chemistry suggests in a general way how metal-activated C–H bonds may be utilized to achieve functionalization at saturated carbon centers.

Acknowledgment is made to the donors of The Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We thank D. L. Morrison for help with initial experiments.

(13) At −10 °C isomer **7a** exhibits a ^{1}H NMR resonance at *−13.67* ppm (1 H) corresponding to the single endo hydrogen bridged to manganese. In isomer **7b** there are two endo hydrogens capable of bridging to manganese, and at −10 °C these are rapidly exchanging on an NMR time scale resulting in a two proton resonance at −6.49 ppm. Heating a mixture of **7a** and **7b** causes the two isomers to rapidly exchange and results in coalescence of the two high field bridging hydride resonances giving an average resonance at −6.44 ppm at 140 °C.

(14) Humphrey, M. B. unpublished results.

(15) Green, M. L. H.; Benfield, F. W. S. *J. Chem. Soc., Dalton Trans.* **1974**, 1324.

(16) ^{1}H NMR obtained in benzene-d_6 under 1 atm of CO by using a subtraction routine to eliminate resonances arising from **4** which is in equilibrium with **9** under these conditions: δ 4.58 (t, J = 6.5 Hz), 3.82 (t, J = 6.4 Hz), 1.90 (m), and 0.99 (m).

(17) (a) Gibson, D. H.; Vonnahme, R. L. *J. Am. Chem. Soc.* **1972**, *94*, 5090. (b) Whitesides, T. H.; Arhart, R. W.; Slaven, R. W. *Ibid.* **1973**, *95*, 5792.

(18) For examples of nucleophilic addition to the isoelectronic π-allyliron complexes, see: ref 5a and Whitesides, T. W.; Arhart, R. W.; Slaven, R. W. *J. Am. Chem. Soc.* **1973**, *95*, 5792.

Analogues for Acetoacetate as an Enzyme Substrate. Stereochemical Preferences

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The use of conformationally restricted substrate analogues is one of the few experimental options available for determining which of a number of rapidly interconverting conformers of a molecule is the actual substrate for an enzyme.[1] We report here that 2-oxocyclohexanecarboxylate (**1**), as a conformationally constrained analogue for acetoacetate (**2**), is a substrate for two enzymes that catalyze reactions of acetoacetate, acetoacetate decarboxylase (AAD),[2] and 3-hydroxybutyrate dehydrogenase

† To whom correspondence may be addressed at Department of Chemistry, Brandeis University, Waltham, MA 02254.

(1) Kenyon, G.; Fee, J. *Prog. Phys. Org. Chem.* **1973**, *10*, 381–410.

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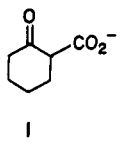
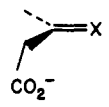


1



2, X = O
3, X = NH-Enzyme

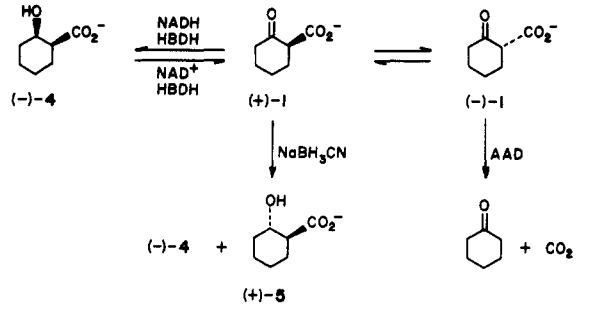
(HBDH).[3] Furthermore, we have determined the enantioselectivity of each of these enzymes for **1** and find that the stereochemical preferences are opposite.

The decarboxylation of **2** catalyzed by AAD is believed to proceed via the intermediacy of a protonated Schiff base.[4] If the transition state is to have maximum orbital overlap,[5] the conformation of this intermediate must be such that the fragmenting C–C bond is roughly parallel to the π orbitals of the C=N double bond, as depicted for structure **3**. There are two enantiomorphic conformations (conventionally designated as M and P)[6] where this geometric requirement if fulfilled; presumably only one of them is the actual substrate for AAD. By analogy, at most one enantiomer of **1** should be able to attain the required geometry and be a substrate for AAD.

We have found that AAD selectively catalyzes the decarboxylation of (−)-(2*R*)-**1** (K_M = 15 mM, based on racemic **1**, $V_{max}^{30°C}$ = 60 μmol min^{-1} (mg of protein)$^{-1}$; for the natural substrate (**2**) K_M = 10 mM, $V_{max}^{30°C}$ = 740 μmol min^{-1} (mg of protein)$^{-1}$).[7] We find that the optical activity of a solution of racemic **1** undergoing AAD-catalyzed decarboxylation rapidly increases from a small negative value characteristic of the enzyme to a plateau with a large positive value, which subsequently decreases slowly. Computer simulation of kinetic runs[8] shows that this burst in optical activity is consistent with a process by which AAD selectively converts the (−)-enantiomer of **1**, leaving (+)-**1** behind, as represented in Scheme I. Racemization of optically active **1** in aqueous solution (half-life ~10 min at pH 6, 25 °C) prevents its isolation; however, kinetic analyses show that decarboxylation of (−)-**1** is at least 12 times faster than the decarboxylation of its enantiomer.[9]

When sodium cyanoborohydride was added to an incubating solution at its peak rotation, optically active (−)-*cis*- and (+)-*trans*-2-hydroxycyclohexanecarboxylates, **4** and **5**, could be isolated.[10] Since (−)-**4** and (+)-**5** are known to correspond to the

Scheme I



S,*R* and *S*,*S* configurations, respectively,[11] (+)-**1** must have the *S* configuration at the 2 position. AAD therefore acts upon (1*R*)-2-oxocyclohexanecarboxylate. This conclusion is supported by our finding that AAD catalyzes the decarboxylation of 2-(*R*)-methyl-3-oxobutyrate.[12] If we assume that **1** and **2** behave alike in the active site of AAD, these results suggest that the *M* conformation of **3**[6] (the geometry shown) is actually undergoing decarboxylation.

We have found a different enantiomeric preference for HBDH, which catalyzes the interconversion of (−)-(1*S*)-*cis*-2(*R*)-hydroxycyclohexanecarboxylate, (−)-**4**, and (−)-(2*S*)-**1**. In the presence of HBDH, NADH reduces **1** (K_M = 6 mM, based on racemic **1**; $V_{max}^{25°C}$ = 10 μmol min^{-1} (mg of protein^{-1}); for the natural substrate (**2**) K_M = 0.8 mM, $V_{max}^{25°C}$ = 14 μmol min^{-1} (mg of protein^{-1})).[13] In the reverse direction, HBDH does not catalyze the oxidation of authentic samples of either (+)- or (−)-*trans*-2-hydroxycyclohexanecarboxylate (**5**)[14] or the (+)-cis diastereomer **4**. In a coupled enzyme study, HBDH-catalyzed oxidation of racemic **4** in the presence of AAD produces cyclohexanone, leaving behind (+)-**4**. Similarly, HBDH-catalyzed reduction of racemic **1** produces (−)-**4** exclusively. These results indicate that (+)-(2*S*)-**1** is selectively reduced with HBDH and that the (−)-cis stereoisomer, (1*S*,2*R*)-**4**, is selectively oxidized. Finally, the negative burst of optical activity that we observed when racemic **1** was incubated with NADH and HBDH[15] is consistent with this conclusion and supports the previous assignment of the 2*R* configuration to the (−)-enantiomer of **1** above. These results are also consistent with the known enantiomeric specificity of HBDH for 3(*R*)-hydroxy-2-methylbutyrate.[16]

In interpreting our data, we must recognize that one cannot be certain that conformationally restricted substrate analogues behave precisely as do the natural substrates with these enzymes. Nevertheless, in view of the conformations available to the substrate analogues **1** and **4**, and in view of the orbital constraints that are likely to govern the decarboxylation reaction, these data suggest that AAD and HBDH bind different conformations of acetoacetate. This conclusion is especially interesting in light of recent arguments that stereochemical regularities can be used as a basis for postulating the evolutionary relatedness of certain enzymes.[5,17] Naively, one might expect that if enzymes that use acetoacetate have evolved from a common precursor, their mode of binding of this substrate should have been conserved, in the same fashion as crystallographic evidence seems to suggest it has been conserved in the binding of nicotinamide cofactors.[18] Our

(2) Prepared from *Clostridium acetobutylicum* by the method of F. H. Westheimer, *Methods Enzymol.* **1969**, *14*, 231–241. We are indebted to Jerome V. Connors for his expert technical assistance in this work.

(3) From *Rhodopseudomonas spheroides*, purchased from Boehringer-Mannheim.

(4) Westheimer, F. H. *Proc. Robert A. Welch Found. Conf. Chem. Res.* **1971**, *15*, 7–50.

(5) Dunathan, H. C.; Voet, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3888–3891.

(6) These enantiomorphs can be named by use of the helicity rule for chiral conformations (Cahn, R. S.; Ingold, C.; Prelog, V. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385–415). This convention assigns helicity about a single bond on the basis of the direction from the preferred (or unique) ligand on the front carbon to the preferred (or unique) ligand on the rear carbon. Assignment of *P* (plus) or *M* (minus) helicity is based on whether the smaller dihedral angle is made by a right- or left-handed helix.

(7) Kinetic parameters were determined by the method of Westheimer (ref 2) in 50 mM phosphate buffer, pH 5.95, at 30 °C. One unit defined in ref 2 is equal to 24 IU.

(8) Plots of optical activity as a function of time were simulated by computer, using rate constants obtained from the studies mentioned in ref 7. Plots were then fitted against experimental data to obtain a value for the rate of epimerization of the α position.

(9) This conclusion was confirmed by following the loss of absorbance at λ_{max} 248 nm, corresponding to destruction of **1**.

(10) The optical activity of recovered **4** and **5** was proportional to the optical activity of the incubating solution at the time that cyanoborohydride was added, whereas reduction of a mixture of racemic **1** and denatured enzyme afforded racemic **4** and **5**. This control makes unlikely the possibility that the optical activity observed in **4** and **5** results from enzyme-induced asymmetric reduction of racemic **1**. Such asymmetric reductions by borohydride in the presence of a protein have been reported: Kosicki, G. W.; Westheimer, F. H. *Biochemistry* **1968**, *7*, 4303–4309. Sugimoto, T.; Matsumura, Y.; Tanimoto, S.; Okano, M. *J. Chem. Soc., Chem. Commun.* **1978**, 926–927.

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(12) Benner, S. A.; Rozzell, J. D.; Morton, T. H. *J. Am. Chem. Soc.* **1981**, *103*, following paper in this issue.

(13) Kinetic parameters were determined in 20 mM phosphate buffer, pH 7.4, at 25 °C by measuring the change in optical absorbance at 340 nm, corresponding to a change in concentration of NADH.

(14) Racemic **4** and **5** were resolved by recrystallization of the α-methylbenzylamine salts from acetone.

(15) Racemic **1** (35.5 mg) was reduced with NADH (122 mg) in 0.1 M phosphate buffer (5 mL, pH 5.95) with HBDH (5 mg, approximately 80 IU) in a polarimeter cell at 25 °C.

(16) Shuster, C. W.; Doudoroff, M. *J. Biol. Chem.* **1962**, *237*, 603–607.

(17) Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1–10.

(18) Stellwagen, E. *Acc. Chem. Res.* **1977**, *10*, 92–98.

data would imply that either AAD and HBDH have not evolved from a common precursor or, if they did, the original enantioselectivity was lost in the course of that evolution. In any case, we believe that this is the first case where the conformational enantioselectivity has been examined for two different enzymes catalyzing different reactions of the same substrate.

Acknowledgment. We are grateful to Professors F. H. Westheimer and R. B. Woodward, in whose laboratories this work was performed. Support of the NSF (to S.A.B. as a predoctoral fellow), the NIH (through Grants NS 14773 to T.H.M. and GM-04-71223 to F.H.W.), and the Rockefeller Foundation (to T.H.M. as an IPH Visiting Scholar) are gratefully acknowledged.

Supplementary Material Available: Kinetic data for 2-oxocyclohexanecarboxylate as an enzyme substrate (4 pages). Ordering information is given on any current masthead page.

Stereospecificity and Stereochemical Infidelity of Acetoacetate Decarboxylase (AAD)

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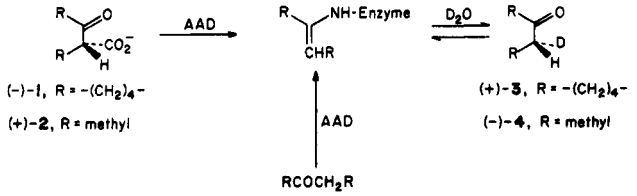
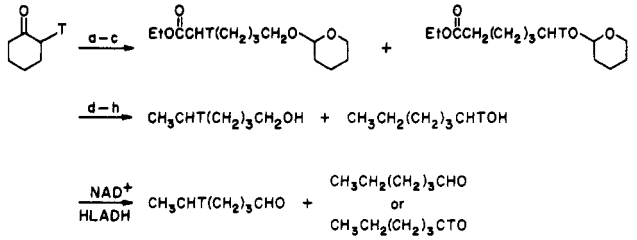
The stereospecificity observed in enzymic catalysis provides both essential clues for understanding the mechanisms of enzymic reactions[1] and opportunities for synthesizing optically active molecules that would otherwise not be easily accessible.[2] Our studies of the stereoselectivity of acetoacetate decarboxylase (AAD, EC 4.1.1.4) from *Clostridium acetobutylicum*[3] have been particularly fruitful in these respects, and we report here the following findings: (1) Decarboxylation of the two β-keto carboxylates **1** and **2** proceeds with net retention of stereochemistry; (2) AAD catalyzes the exchange of *pro-R* α-hydrogens of a variety of ketones, making it a versatile reagent for the synthesis of optically active α-deuterio ketones;[4] (3) the AAD-catalyzed exchange reaction proceeds with a small but detectable level of stereochemical infidelity, which results from competing reaction pathways at the active site.

Westheimer and co-workers[5] have shown that AAD forms a protein-bound enamine as an intermediate in the enzymic decarboxylation of β-keto carboxylates, as shown in Scheme I. When incubated with racemates of 2-oxocyclohexanecarboxylate (**1**) or 2-methyl-3-oxobutyrate (**2**), AAD catalyzes the selective decarboxylation of (−)-**1**, leaving behind (+)-**1**, and (+)-**2**, leaving behind (−)-**2**.[6] We have assigned the stereochemistries of the reactive enantiomers by determining the absolute configurations of the unreacted antipodes. Unreactive (+)-**1** has the *S* configuration;[7] likewise, we have found that unreactive (−)-**2** also has the *S* configuration by reducing it with sodium borohydride to *erythro*- and *threo*-2-methyl-3-hydroxybutyrates,[6] which have known configurations at the 2 position.[8]

Scheme I

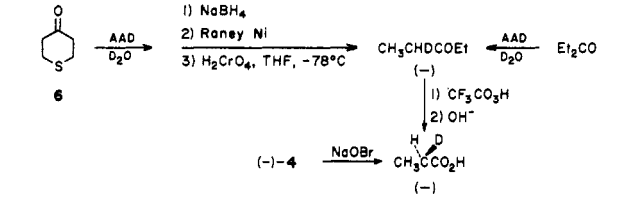


Scheme II[a]

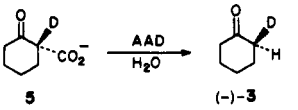


[a] (a) CF_3CO_3H, CH_2Cl_2, 0 °C; (b) EtOH, H_2SO_4, reflux; (c) dihydropyran, *p*-TsOH, benzene; (d) $LiAlH_4$, ether, reflux; (e) *p*-TsCl, pyridine, 0 °C; (g) $LiAlH_4$, ether, reflux; (h) MeOH, *p*-TsOH, reflux.

Scheme III



When AAD catalyzes the decarboxylation of racemic **5** (the 1-deuterio analogue of **1**) in H_2O, (−)-**3**, $[\alpha]^{25}_D$ −0.6° (*c* 14, ether), is recovered, which is 40% d_1.[9] Similarly, when racemic



(1)

2,4,4,4-tetradeuterio-**2** is decarboxylated in H_2O, dextrorotatory deuterated 2-butanone is recovered, while decarboxylation of racemic **2** in D_2O yields levorotatory deuterated 2-butanone. From the absolute configurations that we assign below to these chiral, deuterated ketones, we conclude that the decarboxylations of both (−)-**1** and (+)-**2** by AAD proceed with net retention of configurations, with protonation of the intermediate enamine in either case occurring on the same face from which CO_2 departed.[10]

AAD also catalyzes the exchange of deuterium or tritium into the α positions of cyclohexanone or 2-butanone to produce (+)-**3** or (−)-**4** or their tritio analogues. Three specimens of [2-^{3}H]-cyclohexanone were prepared: the first by enzymic exchange from tritiated water into cyclohexanone, the second by hydrolysis of commercial *N*-morpholino-1-cyclohexene to yield a stereorandomly labeled sample, and the third by AAD-catalyzed exchange of tritium out of the stereorandomly labeled material. These labeled

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(1) Popjak, G. *Enzymes, 3rd Ed.* **1970**, *2*, 116–214.

(2) Jones, J. B. *Tech. Org. Chem.* **1976**, *10*, 107–402.

(3) Westheimer, F. H. *Methods Enzymol.* **1969**, *14*, 231–241. We are indebted to Jerome V. Connors for his expert technical assistance in this work.

(4) AAD-catalyzed exchange of one of the methylene hydrogens of 2-butanone has been reported: Hammons, G.; Westheimer, F. H.; Nakaoka, K.; Kluger, R. *J. Am. Chem. Soc.* **1975**, *97*, 1568–1572, 4152.

(5) (a) Westheimer, F. H. *Proc. Robert A. Welch Found. Conf. Chem. Res.* **1971**, *15*, 7–50. (b) Guthrie, J. P. *J. Am. Chem. Soc.* **1972**, *94*, 7020–7024; 7024–7029. (c) Guthrie, J. P.; Jordan, F. *Ibid.* **1972**, *94*, 9132–9136; 9136–9141.

(6) A 0.75 M solution of racemic **2** was incubated in 50 mM phosphate buffer (pH 5.95) at 22 °C with 7200 IU of AAD; after 30 min the solution showed a rotation $[\alpha]_D$ −2.0°, excess sodium borohydride was added, and the reaction mixture was incubated, then acidified at 0 °C to pH 1 with HCl, filtered, lyophilized, and extracted with ether to yield *erythro*- and *threo*-2-methyl-3-hydroxybutyric acids.

(7) Benner, S. A.; Morton, T. H. *J. Am. Chem. Soc.* **1981**, *103*, preceding paper in this issue.

(8) Tai, A.; Imaida, M. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1114–1117.

(9) All isotopic purities reported herein are based on relative molecular ion intensities (corrected for ^{13}C natural abundance) in 70-eV mass spectra. Incomplete labeling of **3** from reaction 1 is a consequence of exchange of the label in **5** with solvent.

(10) The stereochemical preferences of a number of similar β-decarboxylases have been reviewed: Rose, I. A. *Crit. Rev. Biochem.* **1972**, *1*, 33–58.

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