possibly account for the multiple NMR signals *always* observed for each ligand in mixed LO reagents in *THF*. In the final analysis, the Bertz contribution[5] admirably brings to light some of the subtleties and potential pitfalls[7a,11] associated with cuprate preparation and study. Indeed, Gilman's reagent alone has many forms.[16] The cuprate prepared from CuCN, however, just happens *not* to be one of them.

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Inactivation of General Acyl-CoA Dehydrogenase by Enantiomerically Pure (Methylenecyclopropyl)acetyl-CoA and Its Implication for This Enzyme-Catalyzed Reaction

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General acyl-CoA dehydrogenase (GAD) is a flavin-dependent (FAD) enzyme that catalyzes the oxidation of a fatty acyl-CoA to the corresponding α,β-enolyl-CoA during the first step of the fatty acid oxidation cycle.[1] When GAD is exposed to (methylenecyclopropyl)acetyl-CoA (MCPA-CoA),[2] a metabolite of hypoglycine A which is the causative agent of the Jamaican vomiting sickness,[3] time-dependent inhibition occurs with concomitant bleaching of the active-site FAD.[4] The molecular course of this inhibition is believed to proceed with an α-proton abstraction, followed by ring fragmentation and then covalent modification of the flavin coenzyme.[4] Although the crucial ring cleavage leading to inactivation has been proposed to be a direct anion-induced process, it may also be envisaged as occurring via a transient α-cyclopropyl radical intermediate.[5] Recently, we have found that this inactivation is nonstereospecific since the partition ratio of the inactivation caused by racemic MCPA-CoA is identical with that obtained from incubation with naturally derived MCPA-CoA.[5] Because the rearrangement of an α-cyclopropyl radical to the straight-chain alkyl radical is an extremely rapid process,[6] such a nonstereospecific inactivation is likely a consequence of a spontaneous ring fragmentation event induced by an α-cyclopropyl radical. However, this result contradicts an existing report in which the authors concluded that because the C_1 epimer of naturally derived MCPA-CoA showed no significant effect on the inactivation of GAD, the inactivation must be stereospecific.[7] In an attempt to resolve this stereochemical discrepancy, we have prepared MCPA-CoA in both enantiomerically pure forms and examined the inactivation of GAD by these compounds. Summarized in this paper are the results of these studies and their implication for the mechanism of the GAD-catalyzed reaction.

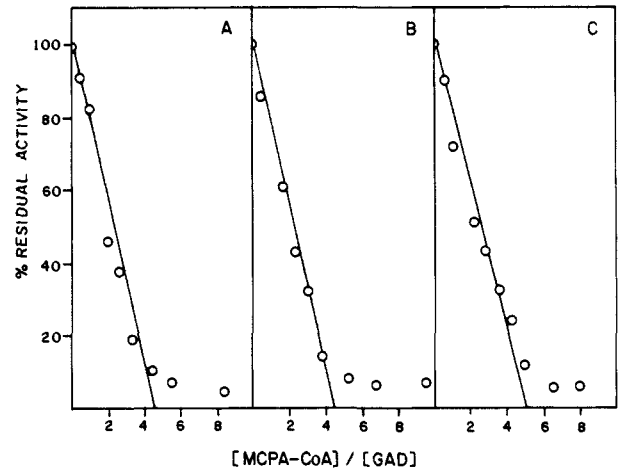
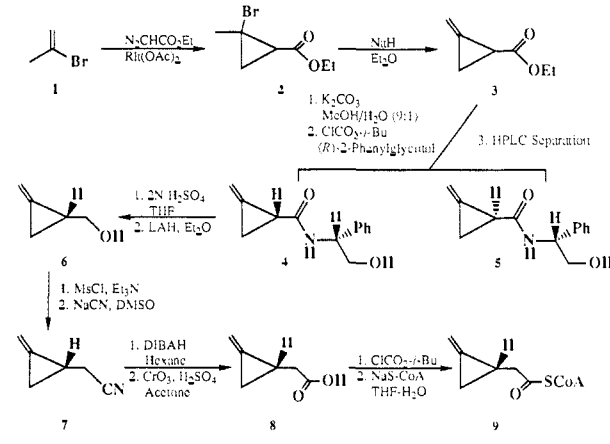


Figure 1. Effect of MCPA-CoA on the catalytic activity of GAD. The purified enzyme (16.8 nmol) in 60 mM potassium phosphate buffer (pH 7.5) was titrated aerobically with successive addition of aliquots of MCPA-CoA. The residual activity was assayed 15 min after each addition according to a procedure of Thorpe.[20] These figures show the percentage of residual activity versus the ratio of MCPA-CoA to enzyme: (A) (*R*)-MCPA-CoA; (B) racemic MCPA-CoA; and (C) (*S*)-MCPA-CoA.

Scheme I



As depicted in Scheme I, the key intermediate, ethyl (methylenecyclopropyl)formate (**3**), was prepared from 2-bromopropene (**1**) and ethyl diazoacetate by a rhodium acetate catalyzed cyclopropanation,[8] followed by a sodium hydride induced elimination (75% yield).[9] Upon hydrolysis and derivatization with (*R*)-2-phenylglycinol, compound **3** was converted to a diastereomeric mixture of amides (**4** and **5**, 72% yield) that are readily separable by flash chromatography (silica gel, 30% EtOAc/hexane).[10] Since the relative elution order of diastereomeric amides of this class by liquid adsorption chromatography has been well established,[10,11]

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compound **4**, which had a shorter retention time when passed through an HPLC silica gel column, was designated the *R,R* isomer.[12] The resolved amide **4** was subjected to acid hydrolysis and then hydride reduction, giving (*R*)-(methylenecyclopropyl)-methanol **6** in 63% yield.[13] The stereochemical assignment of this sample was further confirmed by ^{1}H NMR analysis of its Mosher ester, whose methoxyl signal showed a greater lanthanide-induced shift than that of the *S* isomer derived from **5**.[14] Chain elongation converting **6** to **8** via the cyanide intermediate **7** was accomplished in four steps, with an overall yield of 52%.[15] Condensation of **8** with isobutyl chloroformate followed by coupling to coenzyme A in aqueous THF solution (pH 8-8.5)[5,16] afforded the desired (*S*)-MCPA-CoA (**9**).[17] The corresponding *R* epimer was synthesized from compound **5** by an identical sequence. The crude MCPA-CoA was chromatographed on an HPLC Partisil-C_{18} column and eluted with 30% methanol in 50 mM potassium phosphate buffer, pH 5.3.[18] Fractions containing MCPA-CoA gave a negative result to the nitroprusside test[19] and had an A_{223}/A_{256} ratio of 0.45-0.5. After removal of methanol in vacuo, the pooled fractions were desalted by reversed-phase chromatography (eluting with water and then methanol)[18] and then lyophilized.

The effect of the MCPA-CoA isomers on the catalytic activity of GAD was analyzed by the method of successive titration used by Wenz et al.[4a,20] As shown in Figure 1, a plot of the residual activity observed under aerobic conditions versus total equivalents of MCPA-CoA added gave a partition ratio of 4.4 and 5.0 for (*R*)- and (*S*)-MCPA-CoA, respectively. These results unequivocally demonstrated that both stereoisomers of MCPA-CoA are competent inhibitors. Since the racemic mixture gave a partition ratio of 4.4 under identical conditions,[21] the aforementioned results clearly indicated that the inactivation of GAD by either epimer of MCPA-CoA follows the same course. Namely, the inactivation is nonstereospecific. Such a lack of stereospecificity of bond rupture at C_β of MCPA-CoA in the enzyme active site strongly suggests that the ring-opening step leading to inactivation is likely a spontaneous event, induced by an α-cyclopropyl radical. Since the rearrangement of α-cyclopropyl radicals to ring-opened alkyl radicals is extremely rapid, the ring cleavage may bypass the chiral discrimination normally imposed by the enzyme.[22] Thus, the mechanistic insights deduced from this study support our early notion that GAD is capable of mediating one-electron oxidation-reduction.

Note Added in Proof. Similar results were reported in a recent publication by Baldwin et al.[23]

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(12) Chromatographed on a preparative silica gel column (Spherisorb 5μ, 10 mm × 25 cm) eluting with 10% 2-propanol/CH_2Cl_2 at a flow rate of 4 mL/min, amide **4** and **5** gave a retention time of 4.5 and 5.1 min, respectively. The enantiomeric purity of the resolved **4** and **5** was determined to be greater than 99% based on the integration of the eluted peaks.

(13) This chemically synthesized racemic mixture could also be resolved at the alcohol stage (**6** and its *S* epimer) by the conversion of the alcohols to the corresponding Mosher esters followed by an HPLC separation of the diastereomeric esters with a DAICEL Chiralcel OJ column (10% 2-propanol/hexane). Attempts to resolve the racemic alcohols (**6** and its *S* epimer) by a double resolution sequence based on the lipase-catalyzed esterification and hydrolysis (Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250. Drueckhammer, D. G.; Barbas, C. F.; Nozaki, K.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 1607) were futile.

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(15) The optical rotations of compound **8** and the corresponding *R* epimer were +0.86 ($CHCl_3$, *c* 1.6) and −0.82 ($CHCl_3$, *c* 1.5), respectively.

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(17) It should be noted that the configuration assignment at the chiral center was reversed when compound **6** was converted to **7**/**8**/**9** due to the change of the priority sequence of the substituents at the chiral center.

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(19) Stadtman, E. R. *Methods Enzymol.* **1957**, *3*, 931.

(20) The GAD used in this study was purified from pig kidney to homogeneity based on a procedure described by Thorpe (Thorpe, C. *Methods Enzymol.* **1981**, *71*, 366).

(21) The partition ratio of this inactivation found in our early study was slightly lower than the value reported here.[5] Since the early racemic MCPA-CoA sample was only purified by conventional chromatography, it might still contain impurities that could inhibit GAD.

(22) It is worth noting that the inactivation caused by (*R*)-MCPA-CoA is faster than that observed for the *S* isomer. Such a rate distinction may arise from the rate difference of the α-proton abstraction step which is expected to be more sensitive to the steric environment around C_α and the binding orientation of substrate in the enzyme active site.

(23) Baldwin, J. E.; Ostrander, R. L.; Simon, C. D.; Widdison, W. C. *J. Am. Chem. Soc.* **1990**, *112*, 2021.

Asymmetric Tandem Claisen-Ene Strategy for Steroid Total Synthesis: An Efficient Access to (+)-9(11)-Dehydroestrone Methyl Ether

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The concept of the "tandem reaction sequence"[1] has currently stimulated interest as an efficient strategy for the stereocontrolled synthesis of complex molecules. Steroids have played vital roles as synthetic targets in the development of new strategies because their well-defined structures provide an opportunity to test new methods and explore their stereochemistry.[2,3] Herein we report a conceptually new, efficient strategy for the asymmetric total synthesis of (+)-9(11)-dehydroestrone methyl ether (**1**), a key intermediate for estrogens.[2,4] The key transformation is the asymmetric Claisen-ene sequence (I → II → III), which proceeds in tandem[1,5] and in a highly stereocontrolled fashion (Scheme I).

Our total synthesis starts with the preparation of the requisite *S*-*Z* allylic alcohol **2**[6] from (*R*)-glyceraldehyde acetonide **3**. Thus, the Wittig olefination[7] of methyl ketone **6**[8] derived from **3** was carried out in THF at −78 °C using [5-(trimethylsilyl)-4-pentynyl]phosphonium salt and butyllithium to afford, after desilylation (*n*-Bu_4NF), (*Z*)-enyne **7** exclusively[9] (Chart I). Methoxy-

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(2) Reviews: (a) Blickenstaff, R. T.; Ghosh, A. C.; Wolf, G. C. *Total Synthesis of Steroids*; Academic Press: New York, 1974. (b) Taub, D. In *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; John Wiley: New York, 1984; Vol. 6. (c) Groen, M. B.; Zeelen, F. J. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 465.

(3) For leading recent examples: (a) Johnson, W. S.; Lindell, S. D.; Steele, J. *J. Am. Chem. Soc.* **1987**, *109*, 5852. (b) Stork, G.; Saccomano, N. A. *Tetrahedron Lett.* **1987**, *28*, 2087. (c) Ziegler, F. E.; Wang, T. F. *J. Am. Chem. Soc.* **1984**, *106*, 718. (d) Takahashi, T.; Shimizu, K.; Doi, T.; Tsuji, J. *Ibid.* **1988**, *110*, 2674. (e) Horiguchi, Y.; Nakamura, E.; Kuwajima, I. *Ibid.* **1989**, *111*, 6257.

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(5) The tandem Claisen-ene reaction has previously been reported on racemic cyclopentenol systems: Ziegler, F. E.; Mencel, J. J. *Tetrahedron Lett.* **1984**, *25*, 127. Ziegler, F. E.; Mikami, K. *Tetrahedron Lett.* **1984**, *25*, 131.

(6) To introduce the 14*S* chirality in the Claisen product II, either (*S*)-(*Z*)-**2** or (*R*)-(*E*)-**2** is required. We employed the former in view of the easy availability of (*R*)-glyceraldehyde **3** and the high *Z* selectivity in the Wittig olefination of α-alkoxy ketones (ref 7).

(7) Streekumar, C.; Durst, K. P.; Still, W. C. *J. Org. Chem.* **1980**, *45*, 4260.

(8) The ketone **6** was prepared from **3** via the standard method [MeMgI; DMSO, $(COCl)_2$, Et_3N]; $[\alpha]^{28}_D$ +74.1°; lit. $[\alpha]^{20}_D$ +53.3°: Dumont, R. *Helv. Chim. Acta* **1983**, *66*, 814.

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