dependence of the 310- and 280-nm bands.

The negative 450-nm band of the 2*S*-configurated diazepines in EtOH/MeOH suggests the preference for pseudoequatorial substitution at C(2), in accordance with our PM3 results. Ring inversion in THF/CH_2Cl_2 can be brought about by a variety of factors, the most probable ones being hydrogen bonding between N^+–H and the carbonyl group of the side chain and $\pi\pi$-stacking of the side-chain aromatic with the C(5) benzene ring. Both interactions require a pseudoaxial side chain, and both are more effective in nonpolar than in polar solvents. Moreover, they result in a rather rigid, conformationally locked geometry, which is what we see in this solvent.

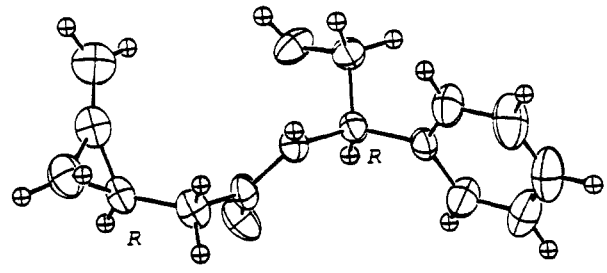

Figure 1. ORTEP drawing of the (*R*)-(−)-2-phenylglycinol amide of (*R*)-(−)-(methylenecyclopropyl)acetic acid.

Stereospecific Inactivation of the General Acyl-CoA Dehydrogenase from Pig Kidney by (*R*)-(−)-(Methylenecyclopropyl)acetyl-CoA and (*S*)-(+)-(Methylenecyclopropyl)acetyl-CoA

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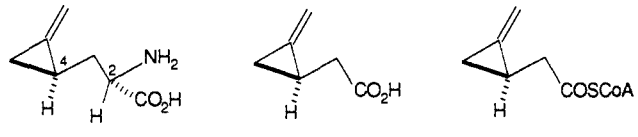
The amino acid hypoglycin isolated from the fruit of the Jamaican ackee tree (*Blighia sapida*) is metabolized in mammals to (methylenecyclopropyl)acetyl-CoA; this thioester, MCPA-CoA, elicits the biochemical derangements associated with the Jamaican vomiting sickness as it inactivates acyl-CoA dehydrogenases,[1-3]



(2*S*,4*R*)-hypoglycin (*R*)-MCPA (*R*)-MCPA-CoA

Whether the reaction of MCPA-CoA with the general acyl-CoA dehydrogenase from pig kidney (GAD; EC 1.3.99.3) is stereospecific or not remains unresolved, for the issue depends on quantitative determinations of rates of inactivation of the CoA esters of each antipode of MCPA, and such data have not previously been secured. Residual enzymic activities after inactivations by aliquots of (±)-MCPA-CoA have been reported by two groups[4,5] and compared with a third group's data for similar studies with MCPA-CoA derived from ackee fruit.[6,7] Given the sensitivity of this approach to substrate and enzyme purities and to experimental estimations of concentrations made in three different laboratories, it is not too surprising that conflicting conclusions have been reached. These attempts to resolve the issue have been limited in a more fundamental way by ignorance about the optical purity of naturally derived MCPA; a chemical correlation has established that the natural product is predominantly of *R* absolute stereochemistry,[4,8] but this correlation from (+)-hypoglycin via MCPA gave (+)-(*S*)-3-methylpentanoic acid of only 17% optical purity.[9]

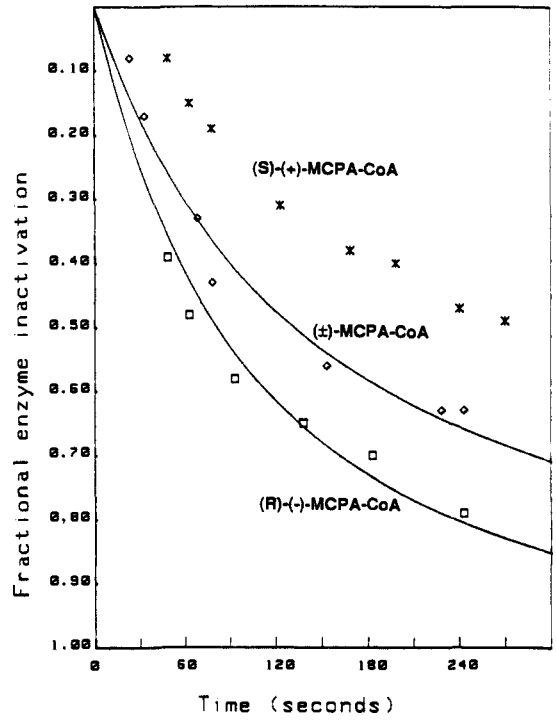


Figure 2. Loss of GAD activity (e_0, 3.1 μM) versus time for (*R*)-(−)-MCPA-CoA, (±)-MCPA-CoA, and (*S*)-(+)-MCPA-CoA (s_0, 15.5, 16.3, 15.4 μM, respectively). The theoretical curves drawn by computer are based on the Tatsunami equation[17,18] [$t = rB(\ln((1 - (1 + r)\mu(1 - x))/x))/Cs_0(1 - (1 + r)\mu) - r(\ln x)/C$, where t is time, e, e_i, and s represent concentrations of E, E_i, and S, $\mu = e_0/s_0$, r is the partitioning ratio, x is the fractional enzyme activity remaining ($1 - e_i/e_0$), and B and C are known[18] functions of rate constants] with the parameters $B = 40$, $C = 0.1$, and $r = 2.7$ ((*R*)-(−)-MCPA-CoA) or $r = 4.2$ ((±)-MCPA-CoA).

To settle this question of stereospecificity, the acid chloride of (±)-MCPA[4] was treated with (*R*)-(−)-2-phenylglycinol and the two diastereomeric amides formed were separated by HPLC on a Nucleosil 50-5 column using 3:2 ethyl acetate/2,2,4-trimethylpentane as eluant.[10,11] Slow crystallization of the first amide from 1:2 ethyl acetate/2,2,4-trimethylpentane gave monoclinic plates, mp 89–92.5 °C. The X-ray crystal structure determination[12] showed that the molecule crystallized in the

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acentric space group $P12_11$; the unit cell parameters found were a = 5.279 (2) Å, b = 10.222 (2), Å, c = 11.990 (2) Å, β = 100.11 (2)°, V = 367.0 (2) Å^3, and Z = 2. The structure found, portrayed in Figure 1, establishes the R absolute stereochemistry for the (methylenecyclopropyl)acetyl moiety in this amide.

Hydrolysis of this amide in dioxane/aqueous sulfuric acid, followed by purification of the acid through preparative GC, gave (*R*)-(-)-MCPA, $[\alpha]_D$-8° (c = 0.32, $CDCl_3$), while the second amide was similarly converted to (*S*)-(+)-MCPA, $[\alpha]_D$+9° (c = 0.56, $CDCl_3$). The two antipodes of MCPA and racemic MCPA were each converted by way of the corresponding *N*-hydroxysuccinimide esters to the respective coenzyme A esters, which were each purified by HPLC using 20 mM ammonium formate pH 5.5 buffer and a methanol gradient (20-35% over 30 min) on a C_{18} μBondapak column; after concentration under vacuum, HPLC analysis (20 mM KH_2PO_4 with 0.3 mM EDTA pH 6.0 buffer and the methanol gradient) was used to derive each CoA ester concentration from an established linear correlation of known MCPA-CoA concentration[13] with the integrated HPLC detector response at 254 nm. The concentration of the thoroughly purified GAD[14] was established through standard activity assays before and after each set of kinetic runs.[15]

Reactions of (-)-MCPA-CoA, (+)-MCPA-CoA, and (±)-MCPA-CoA with GAD at various initial substrate:enzyme ratios were monitored by following the diminution of absorbance at 446 nm and through activity assays.[15] The data thus secured establish several points: Inactivation reactions employing (*R*)-(-)-MCPA-CoA are appreciably faster than reactions utilizing (*S*)-(+)-MCPA-CoA (Figure 2). Inactivations of GAD by (*R*)-(-)-MCPA-CoA and (*S*)-(+)-MCPA-CoA are thus stereospecific, the stereospecificity[16] being manifest in different rates for inactivation of GAD by the two diastereomers. Racemic MCPA-CoA inactivates GAD at an intermediate rate. After reaction times of approximately 25 min, all three stereoisomeric forms of MCPA-CoA inactivated the enzyme to comparable extents and formed comparable product mixtures, as judged by HPLC analyses of those mixtures after gentle SDS denaturation. Reactions involving (*R*)-(-)- or (±)-MCPA-CoA as functions of time and initial concentrations may be modeled satisfactorily by using a minimal kinetic scheme for suicide inactivation,[17-19] but reactions of (*S*)-(+)-MCPA-CoA may not, indicating that a more complex kinetic situation is involved.

One possible explanation for the latter result, rate-limiting enzyme-catalyzed isomerization of (+)-MCPA-CoA to (-)-MCPA-CoA by way of a (2-methylenecyclopropylidene)acetyl-CoA, is now being tested through additional kinetic experiments with isotopically labeled and structurally related (methylenecyclopropyl)acetyl thioesters. This possibility may have a bearing as well on the biphasic kinetic behavior of the inactivation process;[7] enzyme-mediated interconversion of (-)-MCPA-CoA and (+)-MCPA-CoA in competition with inactivation could leave the slower reacting isomer to give further inactivation after the faster (*R*)-(-)-MCPA-CoA ester had been consumed.

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(12) Data collected at 23 °C by using pyrolyzed graphite monochromated Mo Kα X-radiation. A total of 4303 reflections including Bijvoet pairs were collected, with 1938 reflections having $I > 3\sigma I$. Full-matrix least-squares refinement of 221 variables gave R = 0.0398 and R_w = 0.0403 with a goodness of fit of 1.19. A check of the Fourier difference map showed that the largest peak was 0.17 e/Å^3.

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Synthesis and Reactivity of (Pentamethylcyclopentadienyl)iridium Bis(thiolate) and Thiolate Hydride Complexes

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Because metal-sulfur bonds are relatively strong, sulfur ligands are frequently used to hold metal centers together in di- and polynuclear complexes, catalysts, biological systems, and solid-state materials.[1-3] In such environments, the sulfur ligands are often relatively inert.[4] Recently, there has been growing interest in preparing mononuclear complexes with simple thiolate (M-SR) and hydrosulfido (M-SH) groups, because of the increased reactivity such ligands might exhibit toward organic substrates.[5] We report that pentamethylcyclopentadienyl-iridium complexes provide a useful system for preparing and studying such mononuclear metal-sulfur complexes. Replacement of halides and alkoxides at iridium by sulfur nucleophiles occurs in a straightforward way, leading to a series of simple, mononuclear iridium bis(thiolates), bis(hydrosulfides), and hydrido thiolates in which the structure and behavior of these ligands can be studied at a single metal center. We have completed the structural characterization of several of these materials and offer a preliminary account of their reactivity.

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