THE INHIBITION OF PROLINE RACEMASE BY A TRANSITION STATE ANALOGUE: Δ-1-PYRROLINE-2-CARBOXYLATE

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Summary: Δ-1-Pyrroline-2-carboxylate is a potent inhibitor of proline racemase from Clostridium sticklandii. It is proposed that Δ-1-pyrroline-2-carboxylate acts as an analogue of the transition state of the catalyzed reaction. 4,5-Imidazoledicarboxylate, 2,3-pyridinedicarboxylate, and 2-benzimidazolecarboxylate, when present at concentrations equal to that of the substrate, L-proline, failed to inhibit proline racemase.

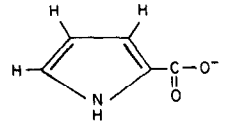
A thermodynamic treatment suggests that during the course of an enzymatic reaction the substrate must be bound to the enzyme much more tightly than in the initial Michaelis complex. A corollary of this proposition suggests that a nonreactive analogue which resembles the structure assumed by the substrate in the transition state should be an extraordinarily potent enzyme inhibitor. The investigation of suitable inhibitors which may function as transition state analogues can thus be used to help confirm the characteristics of a postulated transition state for an enzymatically catalyzed transformation. Conversely, the structural features of an inhibitor that acts as a transition state analogue may be used to deduce features of the catalytic site of an enzyme. [See Wolfenden (1) for a detailed description of analogue approaches to the structure of the transition state in enzyme reactions.]

Cardinale and Abeles (2) found that pyrrole-2-carboxylate (I) was an extremely potent inhibitor of proline racemase from Clostridium sticklandii. When present at a concentration 1/160 that of the substrate, L-proline (II), I causes a 50% inhibition of the racemase. This effectiveness of I as an inhibitor of proline racemase has been attributed (1,3) to its structural similarity to a postulated transition state involved in the racemization of L (II) or of D-proline (IV). The postulated transition state, which is planar about the C-2 atom, is represented below by III.

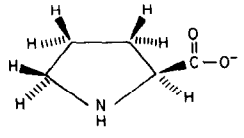
Although I and III both possess planar C-2 atoms bonded to NH and to carboxylate groups, I and III differ in the overall geometries of the heterocyclic rings and in the basicities of the heterocyclic nitrogen atoms. The aromatic pyrrole ring of I is planar while III is saturated at the C-3, 4, and 5 atoms and should therefore exist in a nonplanar, puckered, conformation. The pyrrole nitrogen of I is nonbasic [The pKa of pyrrole itself is

-0.27 (4) and, in fact, pyrroles are preferentially protonated at a ring carbon in strong acid (5)], while the nitrogen of III would be anticipated to be a relatively strong base. To the extent that the ring conformation and the basicity of the nitrogen atom are important in binding a transition state to the active site, a compound embodying these additional structural features of III would be expected to be a more potent inhibitor of proline racemase than is I.

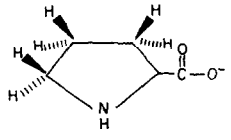
Δ-1-Pyrroline-2-carboxylate (V) was chosen as a model compound to determine the importance of these structural features in binding a transition state analogue to proline racemase. The C-3,4,5 atoms of the heterocyclic ring of V are saturated and the basicity of the nitrogen atom [estimated pKa 8-9 (6)] approximates that of proline (pKa 10.6) and that expected for III. The inhibitory effects of 4,5-imidazoledicarboxylate, 2,3-pyridinedicarboxylate, and 2-benzimidazolecarboxylate were investigated to gain additional insight into the character of the active site of proline racemase.



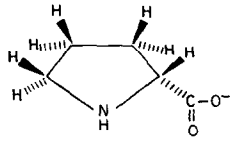
I



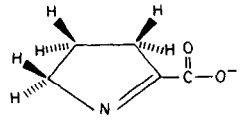
II



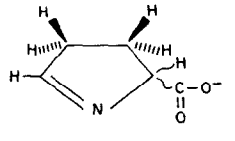
III



IV



V



VI

Materials and Methods

All pmr spectra were recorded on a Jeolco MH-100 spectrometer in D_2O solution. Sodium-3-(trimethylsilyl)-1-propane sulfonate was used as an internal standard. Optical rotations were determined with a Bendix Model 1169 Electronic Polarimeter at 589 nm. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The chemical analysis was performed by Spang Microanalytical Laboratories.

Frozen cells from a 75 l, 24 hr culture of C. sticklandii grown accord-

ing to the directions of Wright and Stadtman (7) were obtained from Truett Laboratories, Dallas, Texas. Proline racemase was isolated and partially purified according to published procedures (2,8). The enzyme assays were carried out as described by Cardinale and Abeles (2) except that dithiothreitol was substituted for mercaptoethanol.

L-Proline and dithiothreitol were obtained from Sigma. Pyrrole-2-carboxylic acid, 4,5-imidazoledicarboxylic acid, and 2,3-pyridinedicarboxylic acid were obtained from Aldrich. 2-Benzimidazolecarboxylic acid and o-aminobenzaldehyde were prepared by published procedures (9,10). 2-Keto-5-aminovaleric acid was prepared and characterized as the hydrochloride salt according to procedure of Hasse and Wieland (11). The salt was recrystallized three times and stored at -20° in a vacuum dessicator over $NaOH/P_2O_5$, mp 105-107°, lit. 113° (11). Anal. Calc'd for $C_5H_8O_3NCl$: C, 35.81; H, 5.97; N, 8.37. Found: C, 35.67; H, 5.79; N, 8.48. Dinitrophenylhydrazone-HCl derivative, mp 219-224° (decomposition), lit. 222-224° (decomposition) (12). Δ-1-Pyrroline-2-carboxylic acid (V) was formed by the spontaneous cyclization of the 2-keto-5-aminovaleric acid in neutral aqueous solution (11,13). The pmr spectrum of V dissolved in D_2O (multiplet 3.68 δ; multiplet 2.62 δ; multiplet 1.92 δ; relative integral, 1:1:1) is virtually identical to that of the Δ-1-pyrroline ring of myosmine (14) (multiplet ~3.68 δ; multiplet ~2.62 δ; multiplet ~1.72 δ; relative integral, 1:1:1) and the Δ-1-piperideine ring of the nemertine toxin anabaseine (15) (multiplet, 3.86 δ; multiplet 2.65 δ; multiplet 1.72 δ; relative integral, 1:1:2). From the pmr spectra it was concluded that Δ-1-pyrroline-2-carboxylate was the predominant species present when the 2-keto-5-aminovaleric acid was dissolved in neutral aqueous buffer. Reaction of a solution of V with o-aminobenzaldehyde resulted in the formation of a characteristic yellow adduct, presumably the dihydroquinazolinium derivative (16,17).

It has been suggested (18) that Δ-1-pyrroline-2-carboxylate (V) may be in equilibrium with its tautomer, Δ-1-pyrroline-5-carboxylate (VI). Such an equilibrium would introduce the possibility that any observed effect of V on proline racemase could be due to the formation of VI. A variety of experimental observations (2,17,19), however, indicate that V and VI are not in equilibrium under the assay conditions. For example, Meister has found that V will not support the growth of an E. coli mutant that will grow in the presence of either L-proline or VI (17).

Results and Discussion

The results summarized in Table I show that Δ-1-pyrroline-2-carboxylate (V) and pyrrole-2-carboxylate (I) are about equally effective as inhibitors of proline racemase. These results indicate that the shape of the five mem-

TABLE I

Inhibition of Proline Racemase*

Inhibitor (M)	% Inhibition		
	Δ-1-Pyrroline-2-carboxylate	Pyrrole-2-carboxylate	Pyrrole-2-carboxylate (Data of Cardinale & Abeles, ref. 2)
5.7×10^{-2}	100	---	---
5.7×10^{-3}	100	100	98
3.6×10^{-4}	44	52	50
5.7×10^{-5}	9	---	---

*L-Proline concentration was 5.6×10^{-2} M.

bered ring (saturated at C-3,4,5 as in V or aromatic as in I) is unimportant in the binding of these analogue inhibitors. The results in Table I also indicate that the basicity of the heterocyclic nitrogen atom is unimportant in controlling binding to the active site of proline racemase. This observation is surprising because it implies that neither a protonated, conjugate acid form of the heterocyclic nitrogen atom, nor a nucleophilic nitrogen possessing a nonbonded pair of electrons is a determining factor in the binding. Cardinale and Abeles (2) has previously concluded that the inhibition of proline racemase required compounds that could potentially serve as bidentate ligands, even though pyrrole-2-carboxylate was the most potent inhibitor detected.

2-Benzimidazolecarboxylate, 2,3-pyridinedicarboxylate, and 4,5-imidazoledicarboxylate, when present at concentrations equal to that of saturating substrate, all fail to inhibit proline racemase. This observation indicates that the active site of proline racemase has a rather stringent steric requirement. [Cardinale and Abeles (2) also found that pyridine-2-carboxylate (α-picolinic acid) was an ineffective inhibitor.] Furthermore this observation is consistent with the previous conclusion that the potential to function as a bidentate ligand is an unimportant structural feature of those compounds which are extremely effective inhibitors of the racemase.

The data presented here indicates that the critical structural feature

in I and V which leads to marked inhibition of proline racemase is the planar conformation about C-2. We conclude that these observations were most consistent with the view that both I and V act as nonreactive analogues of the transition state of the proline racemase catalyzed epimerization.

Acknowledgements

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