Molecular structure and reactivity of Vitamin B6/salicylaldehyde containing model enzymes
by Andrew Gilchrist Sykes

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University

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Deuterium exchange of the two glycine protons in sodium bis(pyridoxylideneglycinato)cobaltate(III) is examined. Second order rate constants for exchange in a carbonate/deuterated bicarbonate buffer in D2O are determined, and activation parameters are calculated accordingly. Glycine protons exhibit differing reactivities, the faster proton exchanged roughly ten times the rate of the slow proton over a thirty degree temperature range. The difference in reactivities is attributed to greater ς-π overlap of the fast proton in the transition state, and both NMR of the complex in solution and crystallographic evidence support the different orientations of glycine protons to the neighboring pi system. Activation parameters for the fast proton are ΔH±= 9.9±2 kcal/mole and ΔS±= -28±7 e.u., and ΔH±= 14.5±1 kcal/mole and ΔS±= -17±4 e.u. for the slow proton. These energies differ in numerical magnitude from activation parameters done in a previous study.

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A thesis submitted in partial fulfillment
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Bozeman, Montana

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APPROVAL

of this thesis submitted by

Andrew Gilchrist Sykes

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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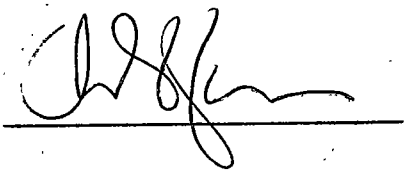
Signature 

Date 10/8/84

To my grandparents with love,

Edith Reed VanHorn
and
Bert Allison VanHorn

Dorthy O'Neil Sykes
and
Edwin Gilchrist Sykes

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ABSTRACT

Deuterium exchange of the two glycine protons in sodium bis(pyridoxylideneglycinato)cobaltate(III) is examined. Second order rate constants for exchange in a carbonate/deuterated bicarbonate buffer in D_2O are determined, and activation parameters are calculated accordingly. Glycine protons exhibit differing reactivities, the faster proton exchanged roughly ten times the rate of the slow proton over a thirty degree temperature range. The difference in reactivities is attributed to greater σ-π overlap of the fast proton in the transition state, and both NMR of the complex in solution and crystallographic evidence support the different orientations of glycine protons to the neighboring pi system. Activation parameters for the fast proton are $\Delta H^{\ddagger}$= 9.9±2 kcal/mole and $\Delta S^{\ddagger}$= -28±7 e.u., and $\Delta H^{\ddagger}$= 14.5±1 kcal/mole and $\Delta S^{\ddagger}$= -17±4 e.u. for the slow proton. These energies differ in numerical magnitude from activation parameters done in a previous study.

INTRODUCTION

Involved in the metabolism of amino acids is the cleavage of some chemical grouping to the α-carbon. Cleavage of the C_α-COOH or C_α-H bond is thermodynamically most unfavourable since pKa values of an unmodified amino acid alpha carbon lie in excess of 30, rendering ΔG° values approximately equal to 38 kcal/mole or greater. Only through some persuasive form of catalysis can the metabolism of amino acids be realized.

The original discovery leading to the interest in pyridoxal dependent biological reactions, the key to the catalysis puzzle, was made in 1934 by Paul Gyorgy of Western Reserve University.[1] This nutritional factor, which Gyorgy called vitamin B_6, was subsequently identified as pyridoxine, one of a number of closely related compounds in the B_6 grouping, pyridoxal among them.

Pyridoxal's utility as a catalytic agent became apparent when it was recognized formation of a Schiff base between the cofactor and an amino acid greatly affected rates of alpha carbon cleavages. In 1952, Metzler and Snell expanded the field even further when they published a pyridoxal-dependent, non-enzymatic

transamination in the presence of metal ions.[2] Numerous enzymatic reactions have now been reproduced using the metal-amino acid-pyridoxal model system proposed by Snell, all having the basic features shown in Figure 1.

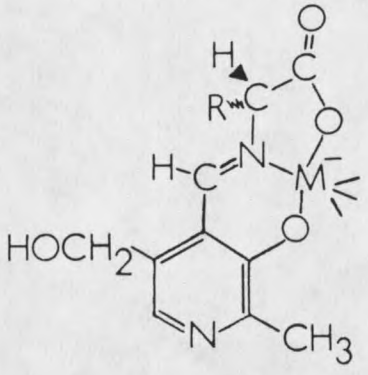

Figure 1. Non-enzymatic pyridoxal-Schiff base model.

A number of these non-enzymatic reactions, paralleling the enzymatic, biological reactions, are condensed in Figure 2, demonstrating the enormous versatility of the model system. Common in all pathways, however, is the initial loss of an electropositive substituent from the alpha carbon. Loss of the substituent and consequent formation of negative charge on the alpha carbon is relieved through two structural features of the model system. One, the conjugated pi system transfers electrons through the molecule and coordinates a proton on the pyridine nitrogen. Two, the metal ion itself is electronegative, reducing electronic

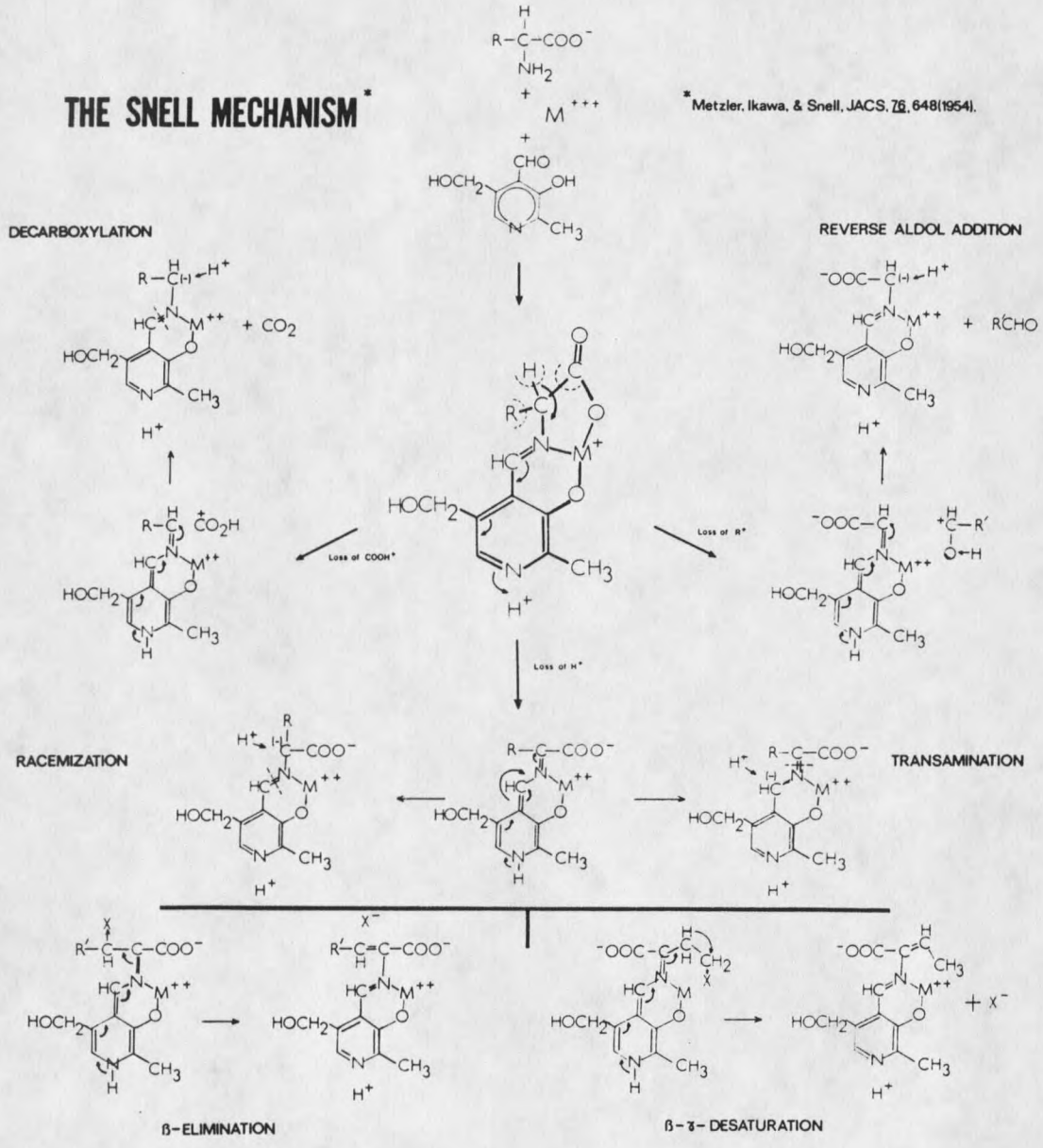

Figure 2. The Snell Mechanism.[3]

charge on the alpha carbon. Both these effects have a catalytic value, lowering the carbanionic character of the alpha carbon and increasing rates of reactions, reactions unlikely without such assistance. Furthermore, the metal ion locks the molecule into a particular constrained conformation as will be discussed further on.

Though metal ions in model systems are not obligatory for amino acid transformations (some do proceed without metal ions present), their presence has generally been found to greatly enhance reaction rates and product formation. In pyridoxal-dependent enzymes themselves, however, it is unlikely metal ions play an active role. Certainly it is the prosthetic groups of pyridoxal combined that leads to the cofactors utility. The pyridinium nitrogen, the 5-hydroxy or phosphate group, plus the carboxylic group of the amino acid all must fit or bind into the particular pattern of the enzyme in such a way as to lower the pKa value of the relevant carbon acid-whether or not in the presence of metals.

Even despite limiting evidence of metalloenzymes, the remarkable work commenced by Snell and his associates in the early 50's has led to a group of metal complexes successful in reproducing the reactions of metal-free or metal-containing biological molecules. The scope

delimiting the types and methods of non-enzymatic model reactions is now rather complete. What remains to be done is significant research activity in coordination chemistry and catalysis, and even more remains to be understood about the structural and electronic features of the complexes themselves. To provide a clearer understanding of reactivities, precise structural determinations need to be made along with further detailed kinetic studies of the various transformations as well. Helping understand the factors controlling reactivities of substituents at amino alpha carbons in complexes such as in Figure 1, then, is the general intent of this thesis.

The hypothesis of Snell concerning the mechanism of amino acid transformations emphasizes the function of the cofactor and metal ion in weakening the sigma bonds around the alpha carbon, but it does not address the question about which bond is cleaved most easily. For example, why the preferential loss of one alpha carbon substituent over another? In the late sixties, H.C. Dunathan attributed the stereochemical relationship of the sigma bond to the adjacent pi system as being responsible for determining reaction specificity.[4] Since loss of a group from the amino acid alpha carbon results in carbanion formation and the extension of the pyridoxal

imine system, an increase in delocalization energy occurs. If this gain in delocalization energy is to aid in bond breaking, a geometry placing the bond to be broken perpendicular to the pyridoxal imine system (coplanar with the pi system) is most highly favoured. Thus, transition states where sigma bond to pi system interactions are as coplanar as possible will enhance reactivities. It is expected bond breakage assisted by delocalization energy will have lower enthalpies of activation than sigma bonded substituents bonded more perpendicular to the pi system.

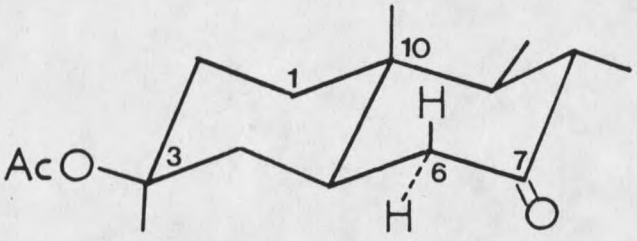

Figure 3. 3β-acetoxycholestan-7-one.

Early work done by Corey on the stereochemistry of enolization of 3β-acetoxycholestan-7-one (Figure 3) showed clear preference for bromination to occur axially at the 6-methylenic carbon.[5] Opposing this effect is the classical steric argument, more 1,3-interactions, which directs a substituent as large as bromine towards the less crowded equatorial position. Obviously, the axial

product is formed kinetically rather than for thermodynamic reasons, and it is thought in the transition state, since the enolization-ketonization process is stabilized by bonding between alpha and carbonyl carbons involving $\sigma-\pi$ delocalization, there should be a demonstrated preference for loss or gain of an axial alpha substituent. Subsequently an axial hydrogen is lost in enol formation and return of bromine occurs likewise in the more hindered axial position. This is the same phenomenon as described by Dunathan above.

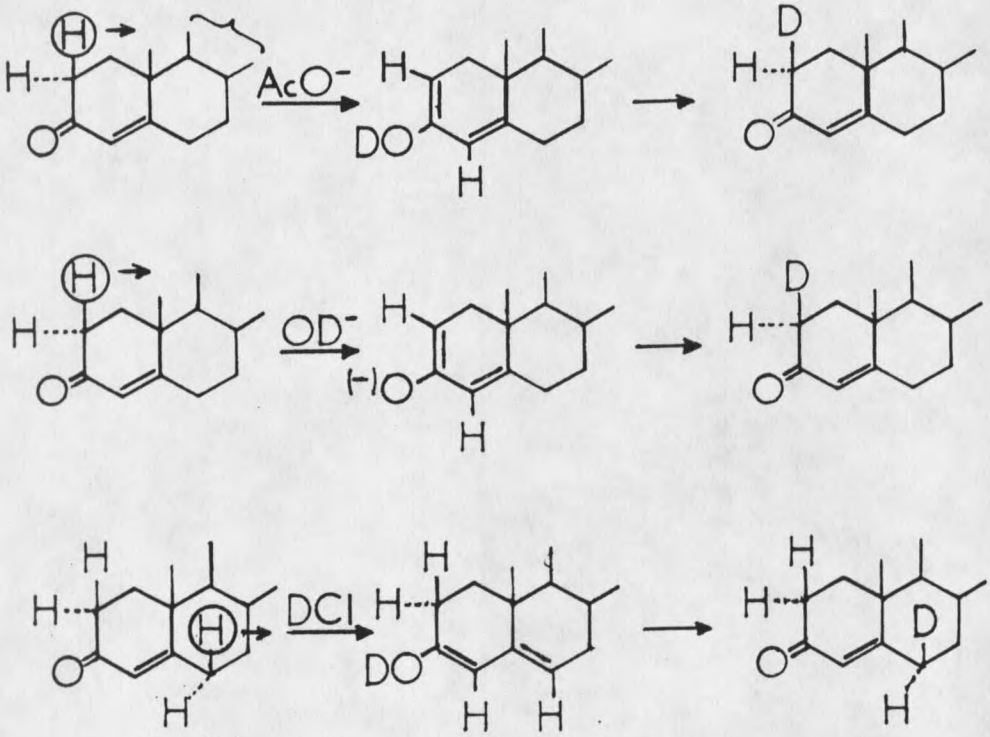

Figure 4. Favored paths of enolization and protonization of testosterone.

Malhotra and Ringold studied the kinetically controlled enolization of testosterone (Figure 4).[6] Both NaOD and CH_3COOD in D_2O catalyzed deuterium exchange at the 2-position immediately adjacent to the carbonyl group, and exchange, in both cases, occurred axial as well. Deuterium chloride catalysis resulted in the preferential loss and exchange of the C-6 proton. This change in specificity is attributed to base strength. Using strong base, CH_3COO^- or OD^-, the determining factor in the transition state is the acidity of the methylenic protons. C-2 protons next to the carbonyl being are more acidic than the C-6 protons, and at the C-2 carbon, the axial proton is more labile than the equatorial proton for reasons put forth above. With DCl catalysis however, since D_2O is the strongest base present, considerable C-H bond stretching in the transition state will lead to a greater resemblance to enol. Acidity (C-2 vs. C-6) will assume little relative importance, and stability now hinges on the respective enols themselves. It should be noted though that even with DCl catalysis, the more acidic axial C-6 proton was lost and preferentially replaced with retention of configuration.

Recent studies have focused on the kinetics of deuterium exchange between glycine protons of the alpha carbon in the following model systems (Figure 5).