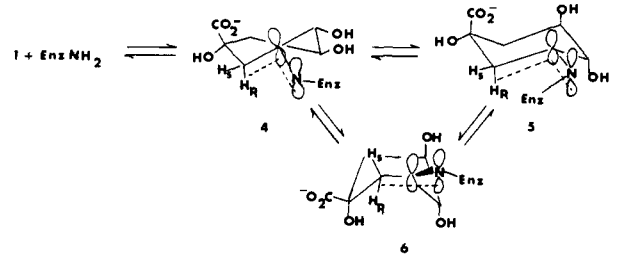
Table I. Rates of Deuterium Incorporation from D_2O in Sodium Benzoate 0.237 *M*, pH 7.0 at 34.40°, Ionic Strength = 1.44

Compound	k_S[a] (hr^{-1})	k_S/k_R[b]
1	$(2.12 \pm 0.06) \times 10^{-3}$	6.65
Methyl ester of 1	$(2.05 \pm 0.07) \times 10^{-3}$	6.70

[a] Pseudo-first-order rate constant for deuterium incorporation in the pro-*S* position at C-2. [b] Relative rate of incorporation of deuterium into pro-*S* and pro-*R* positions.

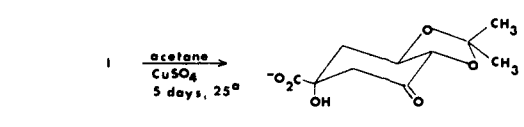
rates of deuterium incorporation from D_2O at C-2 from **1** and its methyl ester. These results are shown in Table I. Clearly, masking the carboxylate function with a methyl group changes neither the stereochemistry nor the rate of enolization. Thus, binding of the carboxylate to the enzyme, while important, cannot explain the reversed stereochemistry of the biological elimination. The pro-*S* stereoselectivity of the nonenzymatic enolization and the resulting anti elimination must then derive from the stereoelectronic necessity for overlap between the n orbitals of the carbonyl oxygen of **1** and the σ orbitals of the adjacent axial proton.[8] In the most stable conformation of **1**[9] only the pro-*S* proton meets this axial requirement. Since a Schiff-base is the electronic counterpart of a ketone, these same considerations would predict that the enzyme Schiff-base substrate complex would also undergo anti elimination from a conformation of the substrate such as **1**. That a syn elimination occurs strongly implies that the substrate undergoes a conformational change during the enzyme catalyzed process.

There are three distinct conformational changes that can occur, **4**, **5**, and **6**, any one of which will lead to the required



overlap. Skew-boat conformation **4** differs from **5** and **6** in important ways; namely, the OH groups remain diequatorial, and this conformer can be generated from enzyme and **1** by movement of only the carboxyl-bearing carbon and those carbons adjacent to it. These distinguishing features allow an experimental test for the reactive substrate conformation.

We have synthesized the isopropylidene derivative (**7**) from **1** by the route shown below. The ketal (**7**) was obtained as a crystalline solid, mp 124–127, from ethyl acetate.[7] The superposability (except for the methyl resonances at 2.04 and 2.20 ppm) of the NMR spectrum of **7** with that of **1** shows the identity of the ring hydrogen coupling constants in both compounds and assures that the cyclohexane ring of **7** has a conformation which is identical with **1**.[9]



Models show that **7** unlike **1** cannot assume the conformations represented by **5** and **6** since the diequatorial hydroxyl groups at C-4 and C-5 are locked by the fused five-membered ketal ring.

Ketal **7** at 0.3 *M* is a reactive substrate for dehydroquinase and reacts with the enzyme at a rate 0.553 that of the natural substrate **1** in 0.033 *M* Tris at pH 7.4. That prior hydrolysis of **7** to **1** does not occur under the reaction conditions is shown by the fact that treatment of **7** with a solution of the enzyme at pH 7.8 for 12–13 hr produces more than 99% of the elimination product with the fused ketal ring intact; only a trace of the hydrolysis product can be detected by NMR at this pH. The rate of elimination of **7** is slower than that of the natural substrate **1**, and an equimolar mixture of **1** and **7**, each at 0.3 *M*, reacts only 0.714 times as fast as **1**, i.e., **7** inhibits the enzymatic elimination of **1**. Since a conformational change is required in this reaction and since the only conformational change available to **7** is that corresponding to **4**, we propose that the OH groups at C-4 and C-5 remain diequatorial throughout this reaction.

While this work was in progress, Haslam and coworkers proposed that the reactive conformation of dehydroquinate was the skew-boat **6**.[4] Since this conformation has diaxial OH groups at C-4 and C-5, it cannot be the reactive conformation in light of the present evidence. That the carboxyl group at C-1 should become more nearly axial during this conformational change is in complete accord with the evidence that this carboxylate is necessary for substrate reactivity. We believe that it is this carboxylate group which together with the ketone carbonyl engages the enzyme and sets into play the conformational change to **4** which results in the syn elimination. Such a conformational change in the substrate may be necessary to produce a corresponding conformational change in the enzyme which is necessary to align the proton abstracting base on the enzyme or any other groups necessary for the reaction to proceed. We expect to comment on these features in a future publication.

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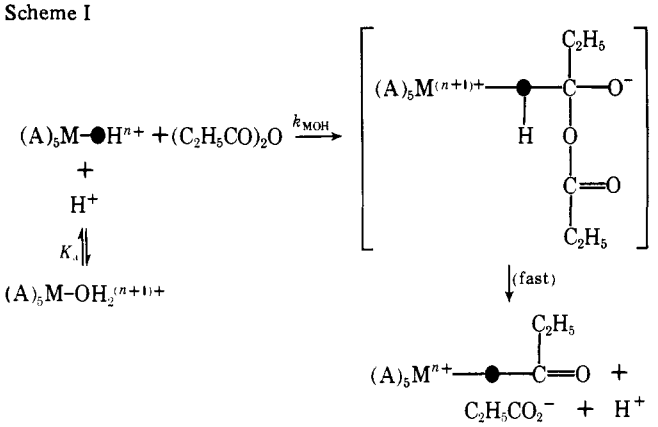
Metal Hydroxide Promoted Hydrolysis of Carbonyl Substrates

Sir:

The recently reported hydration of CO_2 by "inert" metal hydroxides of the type $(NH_3)_5MOH^{2+}$ (M = Co,[1] Rh,[2] Ir[2])

Scheme I



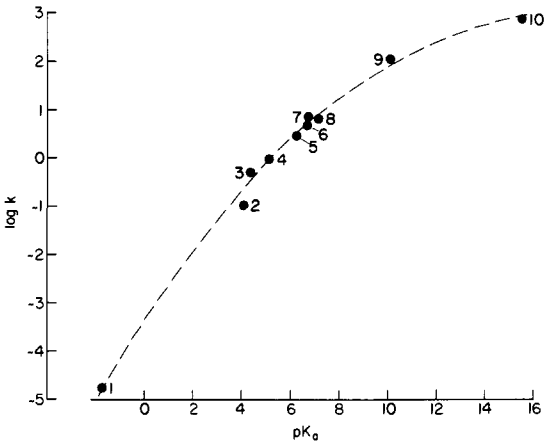


Figure 1. Brönsted plot for the reaction of propionic anhydride with H_2O (1), $Cr(H_2O)_5OH^{2+}$ (2), $Ru(NH_3)_5OH^{2+}$ (3), $Cr(NH_3)_5OH^{2+}$ (4), $Co(NH_3)_5OH^{2+}$ (5), $Ir(NH_3)_5OH^{2+}$ (6), $Rh(NH_3)_5OH^{2+}$ (7), *trans*-$Co(NH_3)_4NO_2OH^{+}$ (8), $Co(CN)_5OH^{3-}$ (9), and OH^- (10) at 25° and μ = 1.0 ($NaClO_4$). Rate data were obtained as follows: (1), (6), (7), (8), and (10), excess nucleophile, pH-stat titration of propionic acid; (4) and (5), excess complex ion, spectrally (480 and 320 nm, respectively), pH-stat; (2) chromatographic separation, analysis of product $Cr(H_2O)_5OCOC_2H_5^{2+}$; (3) spectrally (320 nm), pH-stat, excess propionic anhydride; (9) borax buffer (0.025 *M*, pH 9.65–9.55), change thymolphthalein indicator followed at 600 nm using Durrum stopped-flow spectrophotometer.

to give $(NH_3)_5MOCO_2H^{2+}$, and the condensation of $Co(en)_2OH(H_2O)^{2+}$ with acetylacetone to give $Co(en)_2acac^{2+}$,[3] has demonstrated the ability of metal-bound hydroxide to add rapidly to carbonyl substrates. This facility has interested us for some time, but certain features associated with analyzing the kinetic and equilibrium data for the above reactions and practical difficulties in extending them to a variety of metal hydroxides has prompted us to look at the inherently more simple hydrolysis of carbon and phosphate esters and anhydrides.[4]

In this note we summarize our results on the hydrolysis of propionic anhydride. The results given below show that, (1) hydrolysis is effected by the M-OH, and not the $M-OH_2$, species; (2) hydrolysis involves direct attack of M-OH on the anhydride with little or no general base component; (3) the rate of reaction is related to the pK_b of the M-OH group and is largely unaffected by additional factors associated with the electronic configuration of the metal ion, the overall charge on the complex, or on the other ligands; (4) the more acidic the metal-aquo ion the more effective is the M-OH species as a hydrolyzing agent compared to OH^- at the same pH.

Treatment[5,6] of propionic anhydride with $(NH_3)_5CoOH^{2+}$ (0.01 *M*) in aqueous solution at pH 8.0, 25.0°, μ = 1.0 ($NaClO_4$), results in the rapid ($t_{1/2}$, 20 sec) formation of $(NH_3)_5CoOCOC_2H_5^{2+}$ (Calcd for the perchlorate salt: C, 8.65; H, 4.61; N, 16.83. Found: C, 8.87, H, 4.95; N, 16.66. ϵ_{501}, 77.5; ϵ_{350}, 67 at 25° μ = 1.0 ($NaClO_4$)) according to the equation $(NH_3)_5CoOH^{2+} + (C_2H_5CO)_2O \rightarrow (NH_3)_5CoOCOC_2H_5^{2+} + C_2H_5CO_2^- + H^+$. Spectroscopic rate data (319 nm) obtained under pH-stat control (pH, 5.5–8.5) fit the rate law

$$k_{obsd} = k_{CoOH}K_a[Co]_T/(K_a + [H^+]) + k_{H_2O}[H_2O] + k_{OH}[OH^-]$$

with the first term corresponding to the reaction of the hydroxo complex (pK_a (aquo) 6.35; k_{CoOH}, 3.1 M^{-1} sec^{-1}), Scheme I,[7] and the latter two terms to the water and hydroxide promoted reactions, respectively. The latter terms were independently established by pH-stat titration (pH 6.0–8.5) in the absence of metal ion, (k_{H_2O}, 1.8×10^{-5} M^{-1} sec^{-1}; k_{OH}, 750 M^{-1} sec^{-1}). An experiment carried out using 1 g of ^{18}O-labeled $[(NH_3)_5CoOH_2]Br_3$ (1.5 atom % ^{18}O) dissolved in normal water (10 ml, pH ~9) gave on treatment with excess propionic anhydride 93% retention of ^{18}O label in the isolated $[(NH_3)_5CoOCOC_2H_5]Cl_2$ demonstrating retention of the metal-oxygen bond during hydrolysis. Also, addition of 2.25×10^{-4} mol of propionic anhydride to a large excess of $(NH_3)_5CoOH^{2+}$ (100 ml, 0.0412 *M*, pH 6.32, 6.58, 25°) resulted in the recovery (Dowex 50Wx2 ion exchange, 1 *M* $NaClO_4$, pH ~2) of 5.2, 5.1% (two experiments) of $(NH_3)_5CoOCOC_2H_5^{2+}$ demonstrating that at least 95% of the metal promoted reaction results in the propionate complex; i.e., within experimental error no general base catalysis by the metal hydroxide occurs. Activation parameters (13, 25, 38°) for the various reaction paths ($\Delta H^{\ddagger}$ (kcal mol^{-1}), 9.3 (H_2O), 7.4 (OH^-), 7.0 (CoOH); $\Delta S^{\ddagger}$ (cal deg^{-1} mol^{-1}), −49 (H_2O), −20 (OH^-), and −33 (CoOH)) show that the variation in rate is largely controlled by the entropy factor.

Spectroscopically collected rate data for a wide variety of metal hydroxides, Figure 1, follow rate laws similar to that given above

$$k_{obsd} - k_{hyd} = k_{MOH}K_a'[M]_T/(K_a' + [H^+])$$

with K_a' corresponding to the independently measured acidity of the aquo complex. The results, given as a Brönsted plot in the figure, fall on a fairly smooth curve which includes HO^- and H_2O. Thus, *the rate constant* (k_{MOH}) *is simply related to the* pK_a *of the aquo complex,* even though the overall charge varies from +2, +1, to −3, the metal varies from a first-row (Co, Cr) to a third-row (Ir) transition element, and the coordination sphere includes a variety of ligands (H_2O, NH_3, NO_2^-, CN^-). Also, excluding water, the small slope of the curve, β = 0.20–0.25, results from the fact that the M-OH promoted reaction is not especially sensitive to the pK_a of the bound water molecule. Thus k_{MOH} varies by only a factor of 10^2 for a 10^6 change in acidity. A similar slope of 0.18 obtains for the $(NH_3)_5MOH^{2+}$ (M = Co, Rh, Ir) and HO^- promoted hydration of CO_2,[1,2] and this may well be a general result; log $k_{MN} \simeq 0.2pK_a + C_N$.

To us these results were somewhat unexpected and we feel they may have important implications. *The relative insensitivity of* k_{MN} *to* pK_a *suggests that a metal bound nucleophile* (*M–N*) *is far superior in a rate sense to the free nucleophile* (*N*) *at pH's where the latter is largely protonated,* and this may have some relevance to metal catalyzed enzyme processes where hydration (carbonic anhydrase[8]) phosphorylization (pyruvate kinase, DNA polymerase, cre-

atine kinase),[9] or hydrolysis (carboxypeptidase)[10] is involved. These results provide the first direct support for the often quoted hypothesis that a prime function of the metal ion may be to provide useful concentrations of the nucleophile at biologically acceptable pH's. Also, if the above relationship turns out to be a general one[11] it allows the relative efficiencies of different metal ions to be calculated from a knowledge of the pK_a's of the metal-conjugate acid (e.g., water, amines, phosphates, thiols, etc.), and furthermore from a knowledge or estimate of the bimolecular rate constant for the reaction in the absence of the metal (k_N) the rate for the corresponding metal induced reaction (k_{MN}) may be evaluated.

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Delineation of Interactions between Specific Solvent and Solute Nuclei. A Nuclear Magnetic Resonance Solvent Saturation Study of Gramicidin S in Methanol, Dimethyl Sulfoxide, and Trifluoroethanol

Sir:

Detailed information about solute-solvent interactions can aid in the elucidation of molecular conformation in solution and in the development of general concepts of solution structure. Interactions between specific nuclei of the solvent and solute can be detected by NMR solvent saturation experiments, in which intensity changes in the solute spectrum are monitored while resonances of the solvent are saturated. Intensity perturbations of solute resonances result from intermolecular nuclear Overhauser effects[1-4] (NOE's) and from transfer of saturation[5-8] between exchangeable nuclei of the solute and solvent. We have recently demonstrated the application of this technique to studies of the peptide hormones angiotensin II[9] and oxytocin[10] in water. Here we show that with nonsymmetric solvents such as methanol and trifluoroethanol (TFE) preferential interactions between the solute and specific functional groups of the solvent can be detected.

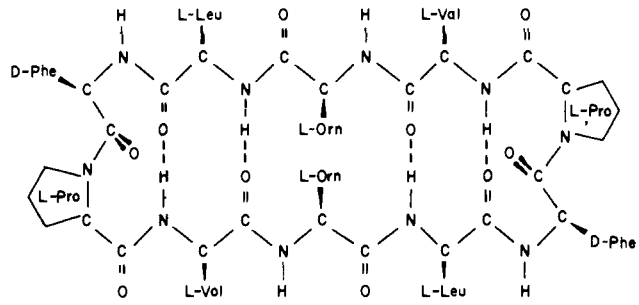

Figure 1. Conformation of gramicidin S.

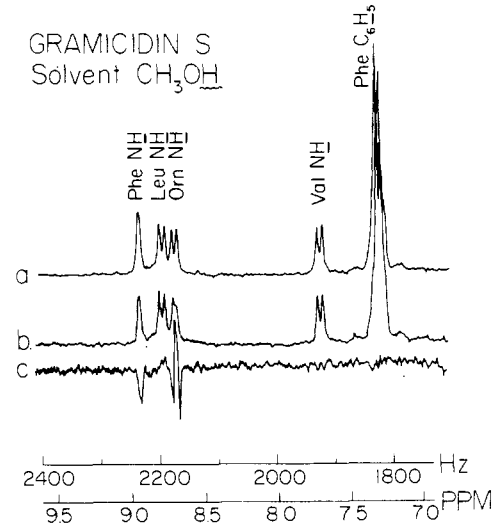

Figure 2. Solvent saturation study of gramicidin S (5% w/v) in methanol at 30 ± 1°C showing the effect of saturation of the solvent O*H* resonance. Spectra were measured at 250 MHz by correlation spectroscopy[15,16] (250 scans/spectrum; 1.6 sec/scan): (a) with off-resonance irradiation 4000 Hz to low field of the CH_3OH peak, (b) with saturation of the CH_3OH peak, and (c) the difference spectrum (spectrum b − spectrum a) amplified three times. Chemical shifts are relative to internal Me_4Si.

To illustrate the type of information which is obtained from solvent saturation experiments in organic solvents, we present a study of gramicidin S in methanol, dimethyl sulfoxide (Me_2SO), and TFE. In each of these solvents the preferred conformation of this cyclic decapeptide antibiotic is the antiparallel pleated sheet structure shown in Figure 1.[11-13] The NH groups of Leu and Val are internally hydrogen bonded to amide carbonyls; the peptide NH's of Phe and Orn are exposed to the solvent.

Figure 2 depicts a typical solvent saturation experiment in methanol. When the CH_3OH resonance is saturated, the intensity of the gramicidin S Phe N*H* peak is decreased by 24 ± 2%. This transfer of saturation results from rapid proton exchange between the methanol OH and Phe NH groups. No significant change in the intensities of the other resonances was measured. The signal with dispersion character in Figure 2c at the Orn N*H* resonance position results from partial decoupling of this proton from its $C^{\alpha}H$, whose chemical shift is the same as that of the methanol O*H*. Because of this effect no attempt was made to measure changes in the intensity of this resonance. Deuterium exchange experiments[12] indicate that the exchange of this proton is significantly slower than that of the Phe NH proton, but several orders of magnitude faster than that of the hydrogen bonded Leu and Val NH protons.