The Mechanism of Hemiacetal Decomposition. Substituent Effects in Breakdown of Substituted Benzaldehyde Ethyl Hemiacetals

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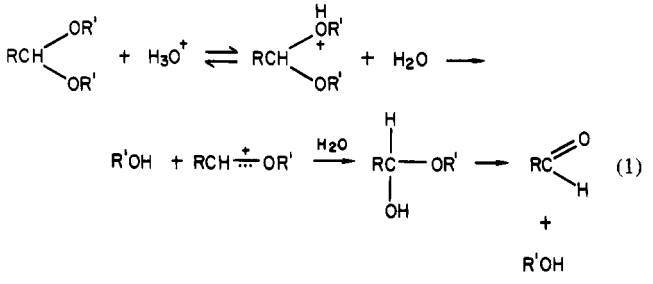
Abstract: A series of substituted benzaldehyde ethyl hemiacetals has been generated from the corresponding substituted benzaldehyde ethyl salicyl acetals. Rate constants have been determined for decomposition of the hemiacetals in H_2O at 30 °C. From the magnitude of the rate constants it is clear that in hydrolysis of the corresponding diethyl acetals formation of an oxocarbonium ion is the rate-determining step at all pH values. The pH–log k_{obsd} profiles for hemiacetal breakdown have slopes of −1.0, 0, and 1.0, indicating hydronium ion, water, and OH^- catalysis, respectively. The Hammett ρ value for hydronium ion catalysis is −1.9 in contrast to the value of −3.25 for hydrolysis of substituted benzaldehyde diethyl acetals. The Hammett plots for these compounds cross at $\sigma = -0.5$. Thus, hemiacetal breakdown will only be rate determining with strongly electron-donating substituents in the phenyl group. The D_2O solvent isotope effect k_D/k_H is 1.7 for hydronium ion catalyzed hemiacetal breakdown. Changing the solvent from H_2O to 50% dioxane–H_2O (v/v) reduces the rate of hydronium ion catalyzed hydrolysis of the diethyl acetals by a factor of 30, whereas the effect is only twofold with the corresponding hemiacetal. Thus, it is probable that the hemiacetal reaction involves proton transfer and charge dispersal in the transition state. The pH-independent hemiacetal decomposition has a ρ value of +0.35 and a D_2O solvent isotope effect (k_{H_2O}/k_{D_2O}) of 2.0, thus indicating that a proton transfer is taking place either internally or through solvent molecules to the leaving group. In contrast to the large difference in rate constants in the hydronium ion catalyzed reaction for benzaldehyde ethyl hemiacetal and formaldehyde ethyl hemiacetal ($>10^3$), the benzaldehyde derivative decomposes less than eightfold faster in the pH-independent reaction. There is very likely considerable proton transfer from the hemiacetal –OH group in the tranisition state of the pH-independent reaction of the benzaldehyde ethyl hemiacetals, whereas less proton transfer from that group is required in the hydronium ion catalyzed reaction.

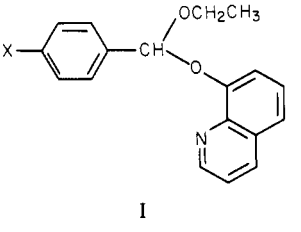
In the hydrolysis of acetals proceeding by an A1 mechanism, preequilibrium protonation of the acetal is followed by unimolecular breakdown of the conjugate acid to a resonance stabilized carbonium ion (eq 1).[1,2] The oxocarbonium ion then reacts with



(1)

H_2O to give a hemiacetal, which in turn decomposes to aldehyde and alcohol. It has long been assumed that breakdown of the protonated acetal is rate determining and that the subsequent reactions are fast. However, it has recently been suggested that a hemiacetal intermediate may build up in the hydrolysis of relatively reactive substituted benzaldehyde diethyl acetals.[3,4] This suggestion was based on experiments in which the acetal was allowed to partially hydrolyze at low pH followed by a rapid increase in pH which resulted in further production of aldehyde, although acetal breakdown had presumably been quenched. Employing this method, Finley et al.[4] reported rate constants for breakdown of some hemiacetals at ~pH 7. The accuracy of such measurements will, of course, depend on the extent of intermediate buildup during the initial phase of the reaction, which is dependent on the relative rates of acetal and hemiacetal breakdown. Direct measurement of the rate constants would be preferable since the reactions would not then be subject to the above uncertainties and since an extensive mechanistic investigation could then be achieved. However, the direct synthesis of hemiacetals of aromatic aldehydes is difficult, presumably because of a very unfavorable equilibrium for addition of alcohol to the aldehyde carbonyl.

Hemiacetals have been detected in the hydrolysis of highly strained cyclic acetals[5] and epoxy ethers.[6] Capon et al.[7] were able to measure rate constants for breakdown of the hemiacetal derived from α-acetoxy-α-methoxytoluene where the leaving group is carboxyl. It should also be possible to directly measure rate constants for hemiacetal breakdown in the hydrolysis of unsymmetrical acetals with which appearance of both the initial leaving group and the aldehyde product can be followed in cases where the rate constants for these processes are widely separated. Measurement of rate constants for decomposition of a series of acetals and their corresponding hemiacetals would not only allow a complete description of acetal hydrolysis reactions with assurance of the rate-determining step but would also allow one to obtain pH–rate constant profiles and structure-reactivity data for the product-forming step. Knowledge of the mechanism of breakdown of hemiacetals is not only of importance in its own right but also because of the insight it would provide into the breakdown of structurally similar tetrahedral intermediates in ester hydrolysis. Acetals meeting the above criteria are those of types I and II. In



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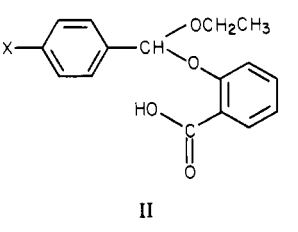

II

both cases the initial leaving group (the phenol) departs with extreme rapidity due to metal ion catalysis with I[8] and intramolecular general-acid catalysis with II.[9] A hemiacetal (III) is

(1) Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264.
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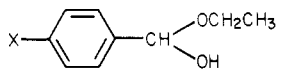

III

then generated which is identical with that produced in hydrolysis of the appropriately substituted benzaldehyde diethyl acetals. When the methyl acetal analogues of these compounds were first studied,[8,9] the rates were followed by monitoring the fast phenol release. In the present work we have studied the relatively slow aldehyde formation, i.e., hemiacetal decomposition (although stopped-flow measurements are still required), and we have determined electronic effects due to variation of the X substituent of III in the hydronium ion, hydroxide ion, and uncatalyzed reactions.

Experimental Section

Materials. The diethyl and mixed acetals were synthesized as previously described.[10,11]

Benzaldehyde 2-(Carbomethoxy)phenyl Ethyl Acetal: bp 120 °C (0.01 mm); n^{22}_D 1.5467. Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.32; H, 6.29. Found: C, 71.20; H, 6.64.

4-Chlorobenzaldehyde 2-(Carbomethoxy)phenyl Ethyl Acetal: bp 170 °C (0.2 mm); n^{22}_D = 1.5536. Anal. Calcd for $C_{17}H_{17}ClO_4$: C, 63.65; H, 5.31. Found: C, 63.67; H, 5.57.

3-Chlorobenzaldehyde 2-(Carbomethoxy)phenyl Ethyl Acetal: bp 135 °C (0.01 mm); n^{22}_D = 1.5517. Anal. Calcd for $C_{17}H_{17}ClO_4$: C, 63.65; H, 5.31. Found: C, 63.88; H, 5.47.

4-Nitrobenzaldehyde 2-(Carbomethoxy)phenyl Ethyl Acetal: bp 185 °C (0.02 mm); n^{23}_D = 1.5648. Anal. Calcd for $C_{17}H_{17}NO_6$: C, 61.63; H, 5.14; N, 4.23. Found: C, 61.65; H, 5.26; N, 4.11.

4-Chlorobenzaldehyde Ethyl 8-Quinolyl Acetal: bp 160 °C (0.02 mm); n^{22}_D = 1.6109. Anal. Calcd for $C_{18}H_{16}ClNO_2$: C, 68.90; H, 5.10; N, 4.47. Found: C, 68.61; H, 5.34; N, 4.44.

Stock solutions of the salicyl acetals were made by dissolving 30–50 mg of acetal–ester in 10 mL of 90/10 EtOH–H_2O (v/v) containing 1 M OH^- and allowing 2 h for the ester to hydrolyze. Quinolyl acetal stock solutions were 5×10^{-3} M in EtOH. The dioxane used for kinetic studies in 50% dioxane–H_2O was spectral grade (Mallinckrodt) and was refluxed over sodium borohydride for at least 3 h and freshly distilled prior to use. The D_2O (99.8%) was obtained from Bio-Rad.

Kinetic Measurements. The rates of reaction of the acetals were measured at 30 °C, μ = 0.1 M (KCl), in water or 50% dioxane–water (v/v) by using a Beckman Model 25, Pye Unicam SP8-100, or Durrum D110 stopped-flow spectrophotometer. The rate of appearance of aldehyde was monitored at 260 nm for all the acetals, while the rate of salicylic acid release was followed at 303 nm. Formation of 8-hydroxyquinoline or metal ion–8-hydroxyquinoline complex was measured at 260 nm.

In a typical experiment, 10–15 μL of acetal stock solution was injected into 3 mL of reaction solution maintained at 30 °C. In rate measurements carried out with the stopped-flow spectrophotometer, 100–150 μL of acetal stock solution was mixed in one syringe with either 15 mL of 0.01 M KOH solution (salicyl acetals) or 15 mL of 0.002 M *N*-ethylmorpholine buffer at pH 8.0 (quinolyl acetal). The other syringe contained the appropriate buffer. Buffer solutions used in the nonmetal ion catalyzed hydrolysis of the quinolyl acetal contained 2×10^{-5} M EDTA as a precaution against trace metal ion in buffer or salt. Rate constants were determined in either HCl solutions or in 0.02 M total buffer. Hemiacetal breakdown is buffer catalyzed. However, it was shown in buffer dilution experiments that 0.02 M buffer does not have an experimentally significant effect on the observed rate constants. No correction was made for possible buffer–metal ion complexation. The reactions followed pseudo-first-order kinetics for at least 4 half-lives. Reaction mixture pH values were measured with a Radiometer Model 22 pH meter. The glass electrode gives the correct pH reading in concentrated dioxane–H_2O mixtures.[12]

Results

In Figure 1 is shown a plot of log k_{obsd} vs. pH for hydrolysis of *p*-methoxybenzaldehyde diethyl acetal in H_2O at 30 °C, μ = 0.1 M with KCl. The plot is linear with a slope of −1.0. The value of the second-order rate constant k_H is 1800 M^{-1} s^{-1}; k_H (k_{obsd}/a_H) was determined from the best fit line to the experimental points

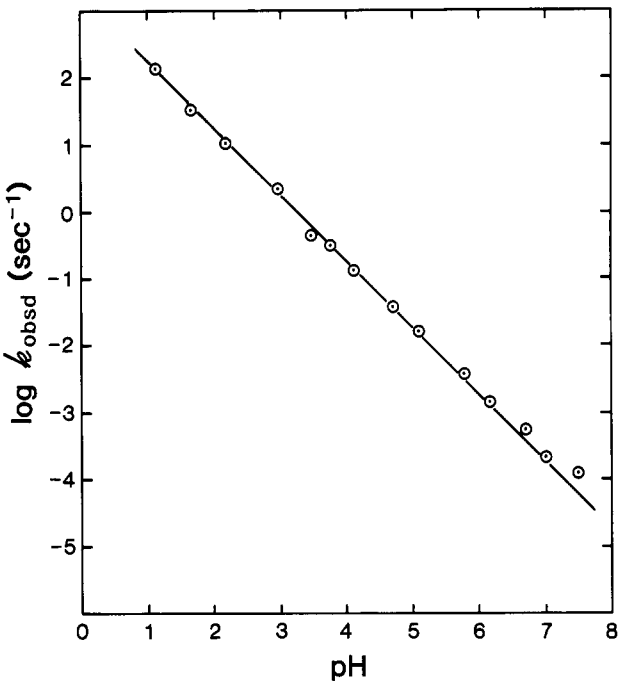

Figure 1. A plot of log k_{obsd} vs. pH for hydrolysis of *p*-methoxybenzaldehyde diethyl acetal in H_2O at 30 °C and μ = 0.1 M with KCl.

Table I. Rate Constants for Hydrolysis of Substituted Benzaldehyde Diethyl Acetals in H_2O at 30 °C (μ = 0.1 M)

substituent	k_H, M^{-1} s^{-1}	substituent	k_H, M^{-1} s^{-1}
p-OCH_3	1800	*m*-Cl	17.5
p-CH_3	613		0.59[a]
H	203	*p*-NO_2	0.78
p-Cl	72.3		

[a] 50% dioxane–H_2O (v/v).

in Figure 1. Similar linear plots were also obtained for all of the substituted benzaldehyde diethyl acetals in the series, and rate constants are given in Table I. The Hammett ρ value (slope of a plot of log k_H vs. σ)[13] is −3.25.[14]

Metal ion catalyzed hydrolysis of *p*-chlorobenzaldehyde ethyl 8-quinolyl acetal proceeds by a two stage mechanism. At pH 7 and with low concentrations of Co^{2+} or Zn^{2+}, the measured reaction at 260 nm is first-order. A plot of the observed pseudo-first-order rate constant vs. metal ion concentration is linear and yields a second-order rate constant for the metal ion assisted reaction which was separately shown to be pH independent. As the metal ion concentration is increased, the reaction seen at 260 nm becomes non-first order and finally splits into two first-order reactions. The faster reaction (release of 8-hydroxyquinoline) is metal ion dependent, and dividing k_{obsd} by the metal ion concentration yields the same second-order rate constant as found at low metal ion concentration. The slower reaction (aldehyde formation) is independent not only of the concentration of the metal ion but also of the identity of the metal ion. The second-order rate constants for metal ion assisted reactions of *p*-chlorobenzaldehyde ethyl 8-quinolyl acetal at 30 °C, μ = 0.1 M, are Co^{2+} (1.0×10^3 M^{-1} s^{-1}), Zn^{2+} (1.8×10^3 M^{-1} s^{-1}), and Cu^{2+} (5×10^6 M^{-1} s^{-1}).

In Figure 2 is shown a plot of log k_{obsd} vs. pH for release of salicylic acid from *m*-chlorobenzaldehyde ethyl salicyl acetal in 50% dioxane–H_2O (v/v) at 30 °C. The plateau in the profile represents either general-acid catalysis by the unionized carboxyl group or a transition from a hydronium ion catalyzed reaction of the unionized species to a faster hydronium ion catalyzed

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(14) The ρ value in 50% dioxane–H_2O for an extensive series of meta- and para-substituted benzaldehyde diethyl acetals is −3.35.[10]

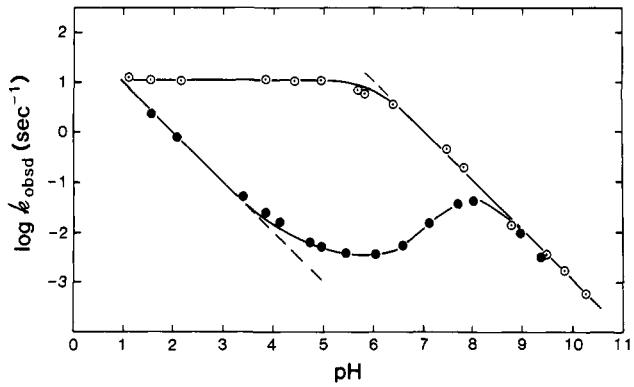


Figure 2. A plot of log k_{obsd} vs. pH for release of salicylic acid from *m*-chlorobenzaldehyde ethyl salicyl acetal at 30 °C in 50% dioxane–H_2O (v/v) (μ = 0.1 M) (⊙) and formation of *m*-chlorobenzaldehyde (●).

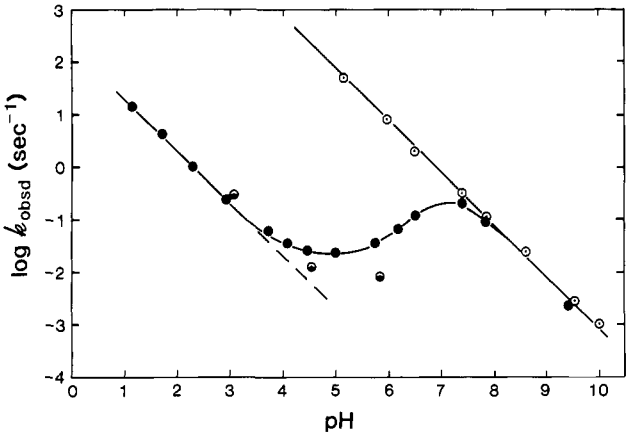


Figure 3. A plot of log k_{obsd} vs. pH for release of salicylic acid from *m*-chlorobenzaldehyde ethyl salicyl acetal at 30 °C in H_2O (μ = 0.1 M) (⊙) and formation of *m*-chlorobenzaldehyde (●) in H_2O and D_2O (◒).

Table II. Rate Constants for Release of Salicylic Acid from Substituted Benzaldehyde Ethyl Salicyl Acetals in H_2O at 30 °C (μ = 0.1 M)

substituent	k_0, s^{-1}	$10^{-7}k_2$, M^{-1} s^{-1}	pK_{app}
H		6.50	
p-Cl		2.79	
m-Cl		0.751	
	11.5[a]	1.06[a]	5.97[a]
p-NO_2	60	0.034	3.76

[a] 50% dioxane–H_2O (v/v).

reaction of the ionized species. These possibilities are kinetically equivalent and either eq 2 or eq 3 fits the observed data, where

$$k_{obsd} = k_0 a_H/(K_a + a_H) \quad (2)$$

$$k_{obsd} = k_2 a_H K_a/(K_a + a_H) \quad (3)$$

k_0 is the rate constant for intramolecular general acid catalysis, k_2 is the second-order rate constant for hydronium ion catalyzed reaction of the ionized species, and K_a is the dissociation constant of the carboxyl group. The plateau in the profile at low pH could also be observed in reactions of *p*-nitrobenzaldehyde ethyl salicyl acetal at 30 °C in H_2O. However, with the other more reactive acetals in H_2O, salicylic acid release could only be measured at pH >5, and therefore only k_2 (or the kinetically equivalent k_0/K_a) could be determined. In Figure 3 is shown the plot of log k_{obsd} vs. pH for release of salicylic acid from *m*-chlorobenzaldehyde ethyl salicyl acetal at 30 °C in H_2O. Values of the rate constants for salicylic acid release are given in Table II. In Figure 4 a plot of log k_2 vs. σ, the Hammett substituent constant, is presented. The value of ρ is −3.09.

Also shown in Figures 2 and 3 are plots of log k_{obsd} for the slower second reaction (aldehyde formation) vs. pH. There are hydro-

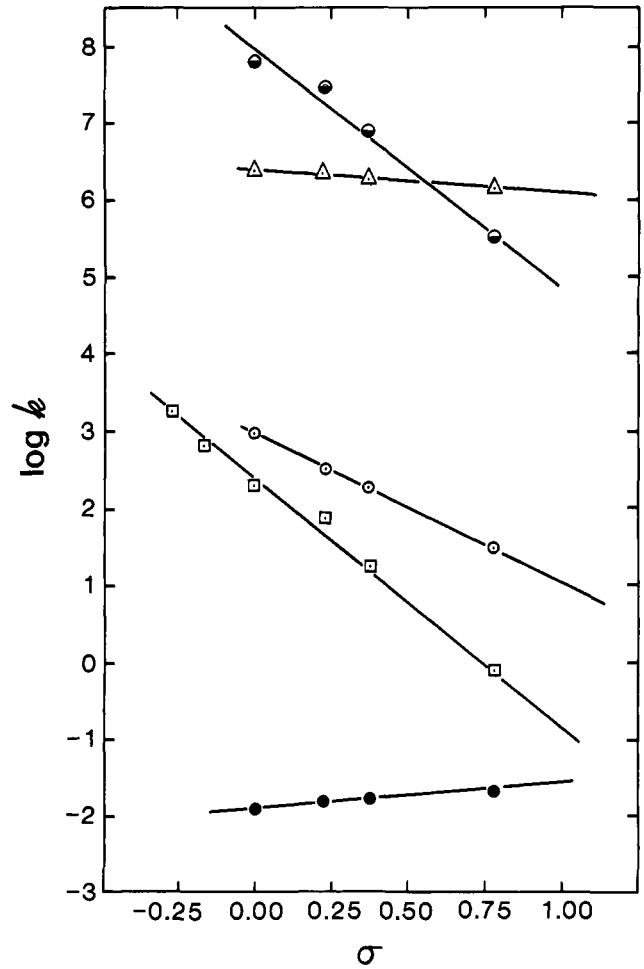


Figure 4. Plots of the logarithms of the rate constants in H_2O at 30 °C (μ = 0.1 M) vs. σ for release of salicylic acid (k_2) from substituted benzaldehyde ethyl salicyl acetals (◒), hydronium ion catalyzed hydrolysis (k_H) of substituted benzaldehyde diethyl acetals (□), hydronium ion catalyzed breakdown (k_H) of substituted benzaldehyde ethyl hemiacetals (⊙), pH-independent breakdown (k_0') of substituted benzaldehyde ethyl hemiacetals (●), and hydroxide ion catalyzed breakdown (k_{OH}) of substituted benzaldehyde ethyl hemiacetals (△).

Table III. Rate Constants for Aldehyde Formation from Substituted Benzaldehyde Ethyl Salicyl Acetals in H_2O at 30 °C (μ = 0.1 M)

substituent	$10^{-2}k_H$, M^{-1} s^{-1}	$10^{-6}k_{OH}$, M^{-1} s^{-1}	$10^2k_0'$, s^{-1}
H	9.36	2.47	1.26
p-Cl	3.52	2.34	1.63
m-Cl	1.97	2.00	1.83
	0.98[a]		0.35[a]
	3.42[b]		
p-NO_2	0.312	1.42	2.39

[a] 50% dioxane–H_2O (v/v). [b] D_2O.

nium ion and hydroxide ion catalyzed reactions and also a pH-independent reaction, eq 4 being followed. Rate constants for the

$$k_{obsd} = k_H a_H + k_{OH}(OH^-) + k_0' \quad (4)$$

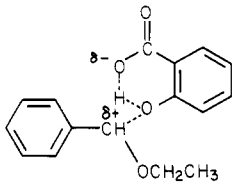
pH-independent reaction k_0' are twofold slower in D_2O than in H_2O in reaction of the *m*-chloro-substituted compound. Similarly shaped plots were obtained for the other substituted benzaldehyde ethyl salicyl acetals in the series.

Rate constants for aldehyde formation from the substituted benzaldehyde ethyl salicyl acetals are given in Table III. The logarithms of k_H are plotted vs. σ in Figure 4 (ρ = −1.90), and also in Figure 4 log k_0' is plotted vs. σ (ρ = +0.35) as is log k_{OH} (ρ = −0.3).

Discussion

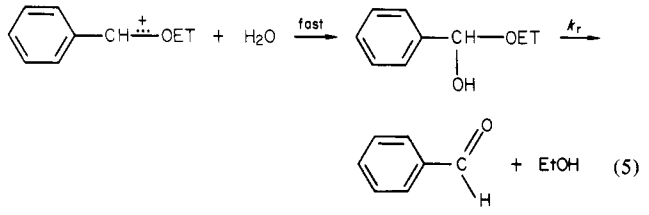
The reports that a hemiacetal intermediate may build up at certain pH values in the hydrolysis of substituted benzaldehyde diethyl acetals[3,4] lead to important questions concerning the hydrolysis reactions; among these are the following. (1) What is the extent of hemiacetal buildup? (2) How does it depend on pH? (3) Is there a change in rate-determining step in the hydrolysis reactions as pH is changed, from breakdown of the protonated acetal to decomposition of the hemiacetal? Question 3 can be answered from inspection of the log k_{obsd} vs. pH profiles for the formation of aldehyde. In Figure 1 it can be seen that in hydrolysis of *p*-methoxybenzaldehyde diethyl acetal such a plot is linear with a slope of −1.0. This shows that a change in rate-limiting step does not occur. Since at pH >4 hemiacetal decomposition is water and hydroxide ion catalyzed, breakdown of the acetal must be rate determining at all pH values in the hydrolysis reaction. Identically shaped profiles were found for hydrolysis of all substituted benzaldehyde diethyl acetals in H_2O, as had been found in 50% dioxane–H_2O in previous work.[10]

The answers to the above questions 1 and 2 depend upon a detailed knowledge of the pH–rate constant profiles for hemiacetal decomposition. Such profiles should be obtainable in cases where the initial leaving group of the acetal departs very rapidly in comparison to the second. This can be accomplished by employing metal ion catalysts in cases where the initial leaving group contains a metal ion complexing functional group[8] as with I, or a group which can act as an intramolecular general acid (II).[9] It has previously been found that the Cu^{2+}-assisted reaction of 4-nitrobenzaldehyde methyl 8-quinolyl acetal in H_2O proceeds in two stages, and it was postulated that these reactions correspond to the pH-independent release of 8-hydroxyquinoline and subsequent hemiacetal hydrolysis.[8] *p*-Chlorobenzaldehyde ethyl 8-quinolyl acetal also hydrolyzes in H_2O by a two-stage mechanism at relatively high metal ion concentrations. These reactions must also correspond to formation of metal ion–phenol complex (breakdown of the acetal) and formation of aldehyde (breakdown of hemiacetal). In reactions of benzaldehyde salicyl acetals the neighboring carboxyl group participates as an intramolecular general acid (IV).[9] Kinetically equivalent reactions involving



IV

preequilibrium formation of a conjugate acid can be conclusively ruled out since one of the rate constants in the scheme would then be required to be greater than a rate constant for a diffusion-controlled reaction.[9] The initial step is very rapid and is monitored directly by following appearance of salicylic acid spectrophotometrically. The hemiacetal that results from reaction of the carbonium ion intermediate with H_2O will be identical with that produced in the hydrolysis of the appropriately substituted benzaldehyde diethyl acetal. Thus, following the subsequent much slower appearance of aldehyde (eq 5) allows precise determination



(5)

of the rate constants for hemiacetal breakdown. The slower reaction in hydrolysis of I (X = Cl) proceeds at a rate which is equal to that for aldehyde formation from *p*-chlorobenzaldehyde ethyl salicyl acetal, as must be the case for reactions proceeding through a common intermediate. Thus, either approach will yield

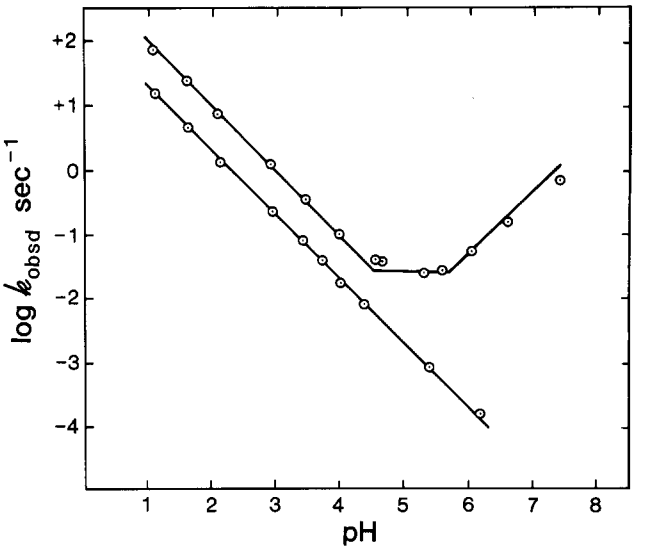


Figure 5. A plot of log k_{obsd} vs. pH for breakdown of benzaldehyde ethyl hemiacetal in H_2O at 30 °C (μ = 0.1 M with KCl). For comparison purposes the plot of log k_{obsd} vs. pH for hydrolysis of benzaldehyde diethyl acetal under the same conditions is also shown (lower line).

the same results. A detailed study was, however, only carried out with the salicyl acetals since those reactions are less complex than the metal ion catalyzed reactions. Aldehyde formation is catalyzed by hydronium ion, H_2O, and hydroxide ion, giving slopes of −1.0, 0, and +1.0, respectively, in the pH–log k_{obsd} profiles. In view of the shape of the profiles and the magnitude of the rate constants reported in Table III, there is no doubt that the second reaction (aldehyde formation) represents hemiacetal breakdown rather than reaction of the oxocarbonium ion with water. The latter reaction should not be hydronium ion catalyzed and would proceed with rate constants much greater than those observed.[15]

A typical pH–log k_{obsd} profile is presented in Figure 5 for hemiacetal breakdown in the hydrolysis of benzaldehyde ethyl salicyl acetal, and for comparison purposes the profile for hydrolysis of benzaldehyde diethyl acetal is also shown, illustrating the slower hydrolysis of the diethyl acetal. From the second-order rate constants for hydronium ion catalysis in the two reactions, the maximum percent buildup of hemiacetal in the hydrolytic reactions of the diethyl acetals in H_2O can be calculated.[16] These percentages are as follows: H, 14%; *p*-Cl, 13%; *m*-Cl, 7%; *p*-NO_2, 2%. Since at pH >4 the decomposition of hemiacetals becomes pH independent and then with increasing pH becomes hydroxide ion catalyzed, the hemiacetals would not build up at pH >5. Therefore, the calculated percentages for the hydronium ion catalyzed reactions represent the maximum buildup at any pH. Only a very small fraction of hemiacetal is ever present, smaller than has previously been estimated by less precise experiments.[3,4] It may however be noted in Figure 4 that plots of log k_H vs. σ for the diethyl acetals and hemiacetals will cross at σ = −0.5. Thus, with strongly electron donating substituents hemiacetal breakdown will become the observed step at low pH.

There have been two reports of general-acid catalysis in hydrolysis of substituted benzaldehyde dialkyl acetals in cases where the reactions were followed by monitoring appearance of aldehyde.[17,18] In these cases the question arises as to whether buildup of hemiacetal might have influenced the observation since hemiacetal breakdown would be general acid–general base catalyzed. Very weak buffer acid catalysis was reported in hydrolysis of benzaldehyde diethyl acetals; an 0.8 M concentration of cacodylic acid enhances the rate of hydrolysis of benzaldehyde diethyl acetal by ~12%.[17] Larger catalytic effects were observed in hydrolysis

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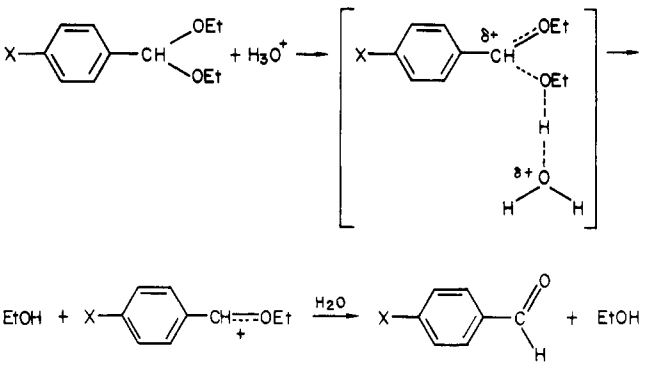
(16) The equations employed are given in: Frost, A. A.; Pearson, R. G.; "Kinetics and Mechanism", Wiley: New York, 1965; pp 166–169.
(17) Jensen, J. L.; Herold, L. R.; Lenz, P. A.; Trusty, S.; Sergi, V.; Bell, K.; Rogers, P. *J. Am. Chem. Soc.* **1979**, *101*, 4672.
(18) Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1971**, *93*, 1701.

of substituted benzaldehyde di-*tert*-butyl acetals.[18] In the latter case buffer acid effects, with one exception,[19] were studied at pH values above 5.4 where hemiacetals react rapidly[20] (benzaldehyde di-*tert*-butyl acetal was studied in phosphate buffers at pH 6.5–7). Hemiacetal buildup might, however, become significant below pH 4.7 with the benzaldehyde derivative and below pH 4 with the *p*-chlorobenzaldehyde acetal due to the enhanced rate of acetal breakdown.[22] Appearance of aldehyde was also followed in studies of benzaldehyde methyl phenyl acetals.[23,24] The intramolecular reactions of benzaldehyde salicyl acetals[9,25] were followed by watching appearance of the phenolic leaving group. The conclusions regarding general-acid catalysis in reactions of benzaldehyde acetals have not been influenced by hemiacetal buildup, but the present results point out that unless the general-acid catalysis is intramolecular the difference in rate constants at pH <5 for acetal and hemiacetal breakdown might not be large, and consequently, rate constants for both species should be obtained.

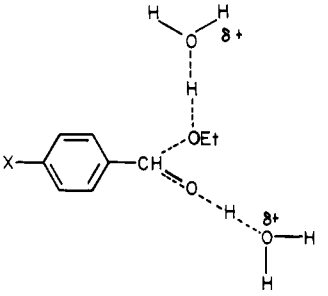
The Hammett ρ value for hydronium ion catalyzed hydrolysis of the substituted benzaldehyde diethyl acetals (−3.25) in H_2O is considerably more negative than for hydronium ion catalyzed decomposition of the corresponding hemiacetals (−1.90). Increases in electron withdrawal by substituents in the aldehyde portion of the molecule will lower basicity and also decrease the ease of C–O bond breaking. Thus, the less negative ρ in reactions of the hemiacetals indicates that basicity is not as important as with the acetals and/or that bond breaking has not proceeded to as great an extent in the transition state. This interpretation is supported by the fact that changing the solvent from H_2O to 50% dioxane–H_2O (v/v) reduces the second-order rate constant k_H for the acetals by a factor of 30, whereas the less polar solvent decreases k_H for hemiacetal breakdown only twofold (there is no hemiacetal buildup in 50% dioxane–H_2O in hydrolysis of dialkyl acetals).[26] The rate of acetal hydrolysis is markedly reduced in 50% dioxane–H_2O because of the relative difficulty of forming the conjugate acid and oxocarbonium ion intermediates. Thus, the small effect of solvent in hemiacetal breakdown implies a transition state that is much less polar than in acetal hydrolysis, in accord with the respective ρ values.

An A1 mechanism for hydrolysis of the diethyl acetals, involving a conjugate acid intermediate, would occur as in eq 1. An alternative in which there is partial proton transfer is shown in eq 6, although protonation of the leaving group by hydronium ion must be nearly complete in the transition state.[27] A transition state for hemiacetal breakdown in accord with the ρ value and



(6)

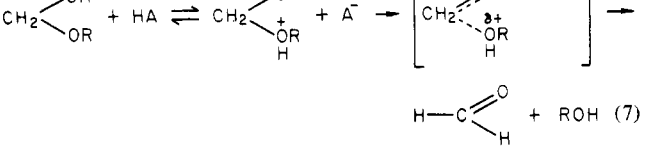
solvent effects would involve proton transfer. Basicity of the



V

acetals and corresponding hemiacetals should be approximately the same. However, partial abstraction of a proton from the hemiacetal –OH group would allow additional stabilization of the developing carbonium ion beyond that which is possible in breakdown of a diethyl acetal. Therefore, bond breaking will be easier in hemiacetal decomposition, and, as a consequence, there may be less proton transfer from hydronium ion to the leaving group in the transition state. That proton transfer is occurring in the transition state is suggested by the D_2O solvent isotope effect (k_D/k_H = 1.7 for *m*-chlorobenzaldehyde ethyl hemiacetal), a value definitely not in accord with an A1 mechanism. Solvent isotope effects (k_D/k_H) of >2.7 typically have been found in hydronium ion catalyzed hydrolysis of diethyl acetals including the substituted benzaldehyde derivatives.[10] On the other hand, D_2O solvent isotope effects (k_D/k_H) near unity (1.0–1.5) have been observed in the hydronium ion catalyzed hydrolysis of acetals subject to general-acid catalysis in which there is proton transfer in the transition state.[24,29,30] Thus, the ρ values, solvent effects, and D_2O solvent isotope effects all indicate that transition-state structure is considerably different in hydronium ion catalyzed breakdown of the substituted benzaldehyde diethyl acetals and corresponding ethyl hemiacetals even though values of k_H are not greatly different.

While k_H differs by only a factor of 5 with benzaldehyde diethyl acetal and benzaldehyde ethyl hemiacetal, k_H for breakdown of formaldehyde methyl hemiacetal is 2600 times larger than for hydrolysis of formaldehyde dimethyl acetal.[21] Funderburk et al.[21] suggested a mechanism for general-acid-catalyzed decomposition of formaldehyde hemiacetals involving preequilibrium protonation followed by proton abstraction from the –OH group (eq 7). This



(7)

mechanism differs from that of V only in the extent of protonation

(19) *p*-Chlorobenzaldehyde di-*tert*-butyl acetal was studied in acetate buffers at pH 4.90. However, the rate constant at zero buffer for the hydronium ion catalyzed reaction is 10-fold less than k_{obsd} at pH 4.90 for *p*-chlorobenzaldehyde ethyl hemiacetal.[20]

(20) Changing the leaving group has little effect in pH-independent hemiacetal breakdown.[21]

(21) Funderburk, L. H.; Aldwin, L.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 5444.

(22) Capon, B. *Pure Appl. Chem.* **1977**, *49*, 1001 suggested that hemiacetal buildup might be important in hydrolysis of benzaldehyde di-*tert*-butyl acetal at pH <4.6 at low buffer concentration, and our calculations support this suggestion.

(23) Anderson, E.; Capon, B. *J. Chem. Soc. B* **1969**, 1033.

(24) Capon, B.; Nimmo, K. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1113.

(25) Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1973**, *95*, 6437.

(26) The reactions of substituted benzaldehyde methyl *cis*-2-carboxycyclohexyl acetals and benzaldehyde bis(*cis*-2-carboxycyclohexyl) acetal have been studied in 50% dioxane–H_2O.[11] With these acetals it is clear that hemiacetals do not influence the observed rate constants from (1) the shapes of the pH–rate constant profiles, (2) the lack of buffer catalysis, and (3) the magnitude of the observed rate constants, which are much too low to allow hemiacetal buildup even though carboxyl group participation occurs. This is due to the effect of solvent; the carboxyl pK_a's are elevated so that participation occurs at pH values where hemiacetal breakdown is rapid.

(27) The weak general acid catalysis reported by Jensen et al.[17] in hydrolysis of benzaldehyde diethyl acetal in H_2O, in which a 0.8 M concentration of cacodylic acid increased k_{obsd} by approximately 12%, would correspond to a Brønsted α of 0.9.[28] These authors failed to detect any general-acid catalysis in hydrolysis of *m*-chlorobenzaldehyde diethyl acetal. In this laboratory significant general-acid catalysis was not observed with any of a series of substituted benzaldehyde diethyl acetals in 50% dioxane–H_2O.[10] Whether the mechanism involves a conjugate acid (A1) or involves proton transfer that is nearly complete in the transition state is immaterial for the present argument.

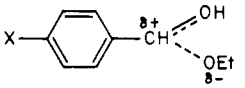
(28) Fife, T. H.; Anderson, E. *J. Org. Chem.* **1971**, *36*, 2357.

(29) Fife, T. H.; Jao, L. K. *J. Am. Chem. Soc.* **1968**, *90*, 4081.

(30) Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1969**, *91*, 7163.

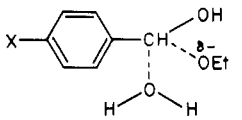
of the leaving group in the transition state. However, a large effect of solvent polarity would be expected for the mechanism of eq 7 because of formation of a conjugate acid intermediate. With formaldehyde hemiacetals all of the driving force for leaving group expulsion would arise from protonation of the leaving group and proton abstraction from the hydroxyl group, and therefore, complete protonation might be required. On the other hand, in the case of benzaldehyde derivatives the aryl substituent can provide considerable assistance to leaving group departure, and therefore bond breaking will be more facile. The value of k_H for benzaldehyde ethyl hemiacetal at 30 °C is >10^3-fold larger than that of formaldehyde ethyl hemiacetal (25 °C). Thus, substituent groups at the reaction center have a large influence in these reactions on rate and very likely on the relative extent of proton transfer from the –OH group and to the leaving group in the transition state. Less proton transfer from the hemiacetal –OH group of benzaldehyde ethyl hemiacetal would be required to attain the transition state because of the great stability of a developing oxobenzyl carbonium ion in comparison to an oxoformyl carbonium ion (the rate difference for hydrolysis of benzaldehyde diethyl acetal and formaldehyde dimethyl acetal is 10^6 and is attributable primarily to the difference in oxocarbonium ion stability).

The most interesting feature of the pH–rate constant profiles is the pH-independent region from pH 4 to 6. This must represent an uncatalyzed or water-catalyzed reaction of the neutral species. The Hammett ρ value for this reaction is +0.35, in contrast to the highly negative value for the hydronium ion catalyzed reaction. Increased electron withdrawal would hinder C–O bond breaking and consequently produce a negative influence on the ρ value. Therefore, a simple unimolecular decomposition of the neutral species (VI) is not occurring. A unimolecular breakdown of this



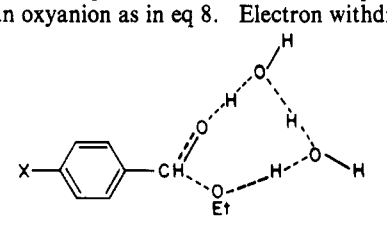
VI

type would be energetically very unfavorable because of the poor leaving group. Nucleophilic assistance by a water molecule (VII)

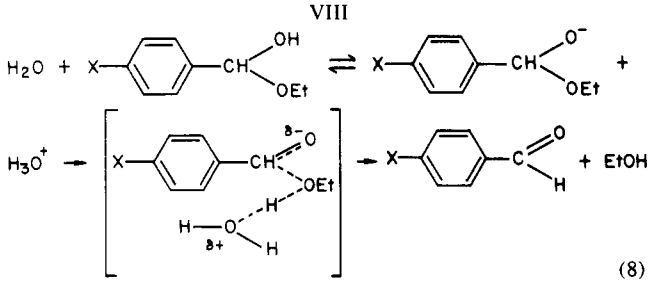


VII

would be aided by electron withdrawal, and in a reaction where the leaving group is poor ρ might be positive. However, the pH-independent reaction is slower in D_2O than in H_2O (k_{H_2O}/k_{D_2O} = 2.0) for the *m*-chloro-substituted derivative. Therefore, it is probable that proton transfer is taking place which could be either totally concerted with bond breaking (VIII) or proceed via a reaction in which one proton transfer occurs in a preequilibrium step to give an oxyanion as in eq 8. Electron withdrawal would

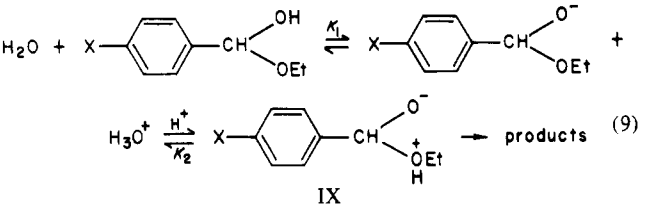


VIII



(8)

aid removal of a proton from the –OH group but hinder protonation of –OEt and C–O bond breaking. Thus, the ρ value might be close to zero in either scheme. If proton transfer to the leaving group were a preequilibrium process, then the D_2O solvent isotope effect would depend upon the effect of D_2O on the dissociation constants of the neutral species and the zwitterion (eq 9). The



(9)

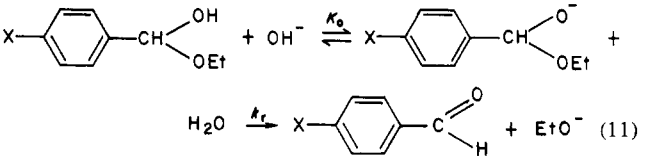
IX

concentration of reactive species IX would be given by eq 10. Acid

$$IX = (K_1/K_2)S_T \quad (10)$$

dissociation constants are reduced in D_2O as compared to H_2O, and this effect can be greater for weaker acids. Therefore, an isotope effect could arise in the stepwise mechanism of eq 9 since the zwitterion will be a strong acid. However, conversion of the hemiacetal to IX should have a ρ close to zero, and formation of products from IX should have a small negative ρ value, thereby giving a ρ for the overall reaction that is slightly negative, whereas the measured value is positive. The mechanisms involving concerted proton transfer and C–O bond breaking would be consistent with the general-base catalysis that has been reported in hemiacetal breakdown[4,21] and with the evidence for proton transfer in the transition state in the hydronium ion catalyzed reaction. A concerted mechanism would avoid the energetically unfavorable zwitterion IX. The mechanism of eq 8 is in accord with the mechanism preferred by Funderburk et al.[21] for the general-base-catalyzed breakdown of hemiacetals of formaldehyde. In contrast to the large difference in rate constants in the hydronium ion catalyzed reaction for benzaldehyde ethyl hemiacetal and formaldehyde ethyl hemiacetal (>10^3), the benzaldehyde derivative decomposes less than eightfold faster in the pH-independent reaction. Thus, the aryl substituent is of greatly reduced influence in the latter reaction, and the transition state is, therefore, very likely one in which there is considerable proton transfer from the hemiacetal –OH group. Proton transfer from that group, with consequent greater assistance to leaving group departure by oxygen, would reduce the need for resonance interaction between the aryl substituent and the developing carbonium ion.

The ρ for hydroxide ion catalyzed hemiacetal breakdown is –0.3. Finley et al.[4] previously noted that the effect of substituents in this reaction is small although they reported that the Hammett plot was U-shaped. The reaction very likely proceeds as in eq 11. Thus, the calculated second-order rate constant is a composite



(11)

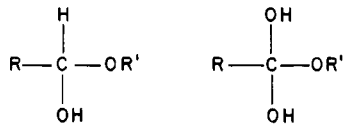
constant K_0k_r, and the ρ value will reflect substituent effects on both the equilibrium constant and the rate constant for the rate-determining step. Electron withdrawal would assist the ionization step, thereby producing a positive influence on ρ. The ρ for the equilibrium step (K_0) would be expected to be ~1.1.[31] The bond-breaking step would be retarded by electron withdrawal. Thus, it is probable that substantial bond breaking is occurring in the transition state in view of the observed magnitude of ρ (–0.3), from which ρ in the –1.4.[32] step can be calculated to be approximately –1.4.[32] A β_{lg} value of –1.1 was found for OH^--catalyzed breakdown of formaldehyde hemiacetals, indicating

(31) Stewart, R.; Van der Linden, R. *Can. J. Chem.* **1960**, *38*, 399. (The ρ for ionization of trifluoroacetophenone hydrates is 1.1).

(32) This may be compared with ρ = –2.24 for the analogous equilibrium for elimination of OH^- from anionic benzaldehyde hydrates (Greenzaid, P. *J. Org. Chem.* **1973**, *38*, 3164).

a late transition state.[21] There is a 10^3 difference in k_{OH} for benzaldehyde ethyl hemiacetal and formaldehyde ethyl hemiacetal.

There is a marked resemblance between a hemiacetal and a tetrahedral intermediate formed in hydrolysis of esters (-H is



replaced by -OH or in the general case of a nucleophilic reaction by X). The mechanisms found for decomposition of hemiacetals should therefore be applicable to breakdown of tetrahedral intermediates. The breakdown step is normally rapid in OH^--catalyzed ester hydrolysis, attack of OH^- being rate determining.[33,34] However, the techniques employed in the present study allow the easy and accurate determination of rate constants for hemiacetal breakdown, which should permit an approach to this and other important problems.

Acknowledgment. This work was supported by research grants from the National Institutes of Health.

(33) Bruice, T. C.; Benkovic, S. J. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966.

(34) However, in certain intramolecular nucleophilic reactions of benzoate esters with poor leaving groups, decomposition of the tetrahedral intermediate is the rate-determining step, and general-base catalysis has been found.[35-37] These reactions have Brønsted β coefficients of 1.0, indicating that a proton-transfer step is rate determining. In contrast, β values of 0.2-0.6 have been reported in general-base-catalyzed decomposition of formaldehyde hemiacetals,[21] indicating that proton transfer is concerted with bond breaking in those reactions.

(35) Fife, T. H.; DeMark, B. R. *J. Am. Chem. Soc.* **1976**, *98*, 6978.

(36) Fife, T. H.; Bambery, R. J.; DeMark, B. R. *J. Am. Chem. Soc.* **1978**, *100*, 5500.

(37) Fife, T. H.; DeMark, B. R. *J. Am. Chem. Soc.* **1979**, *101*, 7379.

Enzyme-Catalyzed Organic Synthesis: NAD(P)H Cofactor Regeneration by Using Glucose 6-Phosphate and the Glucose-6-phosphate Dehydrogenase from *Leuconostoc mesenteroides*[1]

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Abstract: Glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*) and glucose 6-phosphate comprise a useful system for regeneration of reduced nicotinamide cofactors for use in enzyme-catalyzed organic synthesis. This enzyme is approximately equally active in reduction of NAD and NADP. It is commercially available, inexpensive, stable, and easily immobilized. Glucose 6-phosphate can be prepared in quantity by hexokinase-catalyzed phosphorylation of glucose by ATP (coupled with ATP regeneration) or by other methods. The operation of this regeneration system is illustrated by syntheses of enantiomerically enriched D-lactic acid (0.4 mol, enantiomeric excess 95%) and (*S*)-benzyl-α-d_1 alcohol (0.4 mol, enantiomeric excess 95%) and by a synthesis of *threo*-D_s(+)-isocitric acid (0.17 mol). Factors influencing the stability of NAD(P)(H) in solution have been explored.

Enzyme-catalyzed reactions that require reduced nicotinamide cofactors (NADH, NADPH) are not widely used in preparative chemistry.[2,3] Although many of these reactions are potentially valuable in synthesis, the cofactors required are expensive and significantly unstable in solution and can be used economically in stoichiometric reactions only on a small scale. A number of procedures for regenerating the reduced cofactors have been proposed and tested.[4] Of these, the use of formate and formate dehydrogenase (EC 1.2.1.2) to reduce NAD to NADH is, in principle, one of the most practical,[5,6] although the enzyme used in this procedure (from *Candida boidinii*) is still expensive when purchased commercially. This formate dehydrogenase does not reduce NADP,[7] although formate dehydrogenase from *Clostridium thermoacticum* does do so.

This paper describes a regeneration system based on dehydrogenation of glucose 6-phosphate (G-6-P) catalyzed by glucose-6-phosphate dehydrogenase (G-6-PDH, G-6-P, NAD(P) oxidoreductase, EC 1.1.1.49, from *Leuconostoc mesenteroides*). The G-6-P required can be prepared by several procedures. The most convenient for syntheses carried out on a ~1 mol scale is the hexokinase-catalyzed phosphorylation of glucose with ATP, with coupled acetate kinase catalyzed regeneration of ATP by using acetyl phosphate. The preparations of acetyl phosphate[8] and glucose 6-phosphate[9] have been described previously. For larger scale work, routes based on conversion of starch and phosphate to glucose 6-phosphate (using phosphorylase *a* and phosphoglucose mutase) may also prove useful. The advantages of the G-6-PHD from *Leuconostoc mesenteroides* as a catalyst for use in organic synthetic procedures are that it is almost equally effective in reducing NAD and NADP[10,11] and that it is stable,

(1) Supported by the National Institutes of Health, Grant GM 24025.

(2) Suckling, C. J. *Chem. Soc. Rev.* **1974**, 384-407; Suckling, C. J.; Wood, H. C. S. *Chem. Br.* **1979**, *15*, 243-8.

(3) Jones, J. B.; Beck, J. F. In "Applications of Biochemical Systems in Organic Chemistry"; Jones, J. B.; Perlman, O.; Shih, C. J., Eds.; Wiley-Interscience: New York, 1976; pp 107-401; Jones, J. B. In "Nezymic and Nonenzymic Catalysis", Dunnill, P.; Wiseman, A.; Blakebrough, N., Eds.; Ellis Harwood Ltd., 1980; pp 58-83.

(4) Wang, S. S.; King, C.-K. *Adv. Biochem. Eng.* **1979**, *12*, 119-146; Baricos, W.; Chambers, R.; Cohen, W. *Enzyme Technol. Dig.* **1975**, *2*, 39-53.

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(6) Wichmann, R.; Wandry, C.; Bückmann, A. F.; Kula, M. R., Abstracts, VIth International Fermentation Symposium, July 1980, London, Ontario, National Research Council, Ottawa, Canada; Abstract F-12.1.24 (P), p 125.

(7) Andreesen, J. R.; Ljunghdahl, L. G. *J. Bacteriol.* **1974**, *120*, 6-14. In principle, the NAD and NADP cofactor systems can be coupled by using nicotinamide nucleotide trans-hydrogenase (EC 1.6.1.1.); Colowick, S. P.; Kaplan, N. O.; Neufeld, E. F.; Ciotti, M. M. *J. Biol. Chem.* **1952**, *195*, 95-105.

(8) Lewis, J. M.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1979**, *44*, 864-5.

(9) Pollak, A.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1977**, *99*, 2366-7.

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