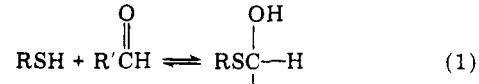
Acyl Substituent Effects on Thiohemiacetal Equilibria

Marc S. Kanchuger and Larry D. Byers*

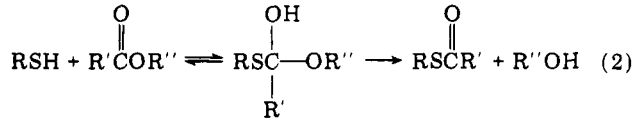
Contribution from the Department of Chemistry, Tulane University, New Orleans, Louisiana 70118. *Received December 1, 1978*

Abstract: The equilibrium constants for thiohemiacetal formation between a series of aldehydes and two thiols (glutathione, pK_a = 9.1, and *p*-nitrothiophenol, pK_a = 4.4) were measured. Thiohemiacetal formation and aldehyde hydration are equally sensitive to electron-withdrawing substituents on the aldehyde. The equilibrium constant for thiohemiacetal formation, K_s (where glutathione is the thiol), is related to the Taft polar substituent constant: $\log K_s = 1.65\sigma^* + 1.41$, $r = 0.986$. The interval estimator of ρ^* (at 90% confidence) is 1.47–1.83. Thiohemiacetal formation with *p*-nitrothiophenol is also correlated reasonably well with σ^* by the same Taft equation obtained with glutathione. The relative stabilities of thiohemiacetals are independent of the basicity of the thiol. For all the aldehydes tested, however, the thiohemiacetal is stabilized over the aldehyde hydrate by 4.3 (±0.2) kcal/mol (25 °C). The ρ^* value for formation of the thiohemiacetal anion is estimated to be 3.0. This provides a useful index for interpreting the ρ^* value obtained for the rate of acyl transfers to thiolates in terms of the resemblance of the transition state to the anionic tetrahedral intermediate.

The addition of a thiol to a carbonyl group was first reported in 1885.[1] Since then thiohemiacetal and thiolhemiketal formation has been shown to play an important part in many organic and biological reactions. For example, thiohemiacetals are intermediates in a variety of enzymic reactions and many naturally occurring and synthetic antiproteolytic aldehydes inhibit thiol proteases via the formation of thiohemiacetals.[2,3] The kinetics and thermodynamics of thiohemiacetal formation have been extensively studied. A particularly attractive feature of this reaction (1) is its analogy to the formation of the neutral tetrahedral intermediate in thiolysis of esters (reaction 2).



(1)



(2)

Guthrie[4,5] and Fastrez[6] have recently shown the utility of comparing addition reactions, such as aldehyde hydration, to the estimation of the relative free energy of the tetrahedral intermediate in acyl transfer reactions, such as hydrolysis of esters. Since it is often difficult, if not impossible, to directly detect the tetrahedral intermediate in acyl transfer reactions the techniques of Guthrie and Fastrez are particularly valuable in providing mechanistic insight into such reactions. The dependence of the equilibrium constant for reaction 1 (R′ = CH_3–) on the pK_a of the thiol has been investigated by Jencks and co-workers[7,8] and they have found that this equilibrium is insensitive to the basicity of the thiol. However, there has been no systematic study of the dependence of thiohemiacetal formation on the electronic effects of the acyl substituent (R′). This study was undertaken to investigate the relationship between the electron-withdrawing ability of the acyl substituent and the equilibrium constant for thiohemiacetal formation.

Experimental Section

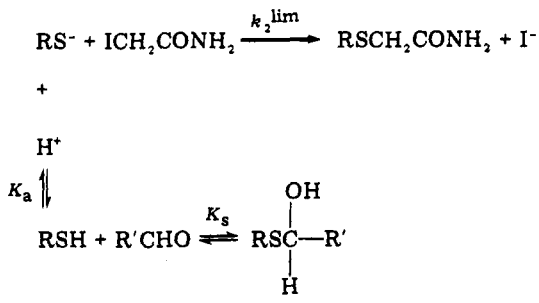
Materials. Glutathione, iodoacetamide, DL-glyceraldehyde, DL-glyceraldehyde 3-phosphate (diethyl acetal), and iodoacetate were obtained from Sigma Chemical Co. Glycoaldehyde phosphate (diethyl acetal) was obtained from Calbiochem, aminoacetaldehyde (dimethyl acetal) was obtained from Chemalog, and all other aldehydes were obtained from Aldrich Chemical Co. The acetals were converted to the free aldehydes by mixing with Dowex-50 (H^+ form) for 3–5 min at 100 °C. *N*-Acetamidoacetaldehyde was prepared by the method of Lewis and Wolfenden.[9] Aldehydes [acetaldehyde, benzaldehyde, butanal, isobutyraldehyde, isovaleroaldehyde, and pyruvaldehyde (methylglyoxal)] were redistilled under nitrogen immediately before use. The concentration of all aldehydes was determined by titration either following bisulfite addition or following oxidation by hydrogen peroxide.[10] *p*-Nitrothiophenol was recrystallized from ether–hexane (1:1) before use. Spectrophotometric measurements were carried out in a Beckman DU spectrophotometer modified with an update Model 122 digital display log converter amplifier. ^1H NMR spectra were recorded on a JEOL 100-MHz spectrophotometer.

Methods

The equilibrium constants for the formation of the thiohemiacetals were determined by a direct spectrophotometric method where RSH is *p*-nitrothiophenol or indirectly where RSH is glutathione. Addition of *p*-nitrothiophenol to the aldehydes was monitored by the decrease in absorption at 412 nm due to the thiolate anion (ϵ_{412} 1.36×10^4 M^{-1} cm^{-1}). The reactions were carried out in buffered solutions at pH 4–5. The equilibrium constants for formation of the aldehyde adducts with glutathione (K_s) were determined kinetically by the dependence of the observed rate constant for glutathione (GSH) alkylation on the aldehyde concentration. The alkylation reaction was followed by removing aliquots from the reaction mixture (GSH, aldehyde, and alkylating reagent) at various times and diluting the aliquot into a cuvette containing 5×10^{-4} M 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at pH 7.5. DTNB reacts rapidly with free thiols to produce 2-nitro-5-thiolbenzoic acid which absorbs at 412 nm (ϵ_{412} 1.36×10^4 M^{-1} cm^{-1}).[11] There is negligible reaction of the 2-nitro-5-thiolbenzoate product with the alkylating reagent under these conditions. Thionitrobenzoate was found to be about 30 times less reactive than glutathione toward iodoacetamide. The dilution of the alkylating reagent into the DTNB solution (~20–60-fold dilution) thus not only reduces the rate of reaction of glutathione with the alkylating reagent but also minimizes the reaction of the chromophore, thionitrobenzoate, with the alkylating reagent. The final absorbance reading at 412 nm remains constant (for several minutes), which further indicates the lack of reaction of the chromophore with the alkylating reagent under these assay conditions. This indirect kinetic evaluation of K_s is illustrated in Scheme I (where iodoacetamide is the alkylating reagent). k_2^{lim} is the limiting rate constant for the reaction of the thiolate with the alkylating reagent. Various alkylating reagents were employed, depending on the pH of the medium. In the case of glutathione, the k_2^{lim} values were on the order of 10, 100, and 1000 M^{-1} min^{-1} for the reaction with chloroacetamide, iodoacetate, and iodoacetamide, respectively (μ = 0.4 M, 25 °C). The rate constants were determined under pseudo-first-order conditions.

0002-7863/79/1501-3005$01.00/0

Scheme I



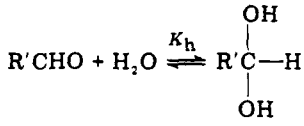
Under pseudo-first-order conditions ([alkylating reagent] ≫ [thiol]) the disappearance of thiol is a first-order process characterized by an observed rate constant, k_{obsd}, which is a function of k_2^{lim}, pH, K_a, and K^{obsd}:

$$k_{obsd} = \frac{k_2^{lim}[\text{alkylating reagent}]}{\left(1 + \frac{[H^+]}{K_a}\right)(1 + K^{obsd}[\text{aldehyde}]_{total})} \quad (3)$$

K^{obsd} can be evaluated by measuring k_{obsd} at various concentrations of aldehyde ([aldehyde] ≫ [thiol]). The ratio of k_{obsd} in the absence of aldehyde, k^0_{obsd}, to k_{obsd} in the presence of aldehyde, k^+_{obsd}, is, thus, a linear function of the total aldehyde concentration:

$$k^0_{obsd}/k^+_{obsd} = 1 + K^{obsd}[\text{aldehyde}]_{total} \quad (4)$$

The observed association constants, K^{obsd}, differ from the true association constants, K_s, because the total aldehyde exists as the free aldehyde (which can bind to the thiol) and the hydrated aldehyde:



$$K_s^{obsd} = K^{obsd}(1 + K_h) \quad (6)$$

The observed association constant must also be corrected for the degree of dissociation of the thiol at the pH in which the reaction was carried out. The equilibria which occur under the conditions in which the thiohemiacetal formation is measured are summarized in Scheme II. The observed association constant, K^{obsd}, is thus a function of these equilibria. Since the pK_a for proton dissociation from the thiohemiacetal adduct (i.e., pK_T) is greater than 12 for the various aldehydes tested (see Discussion section) and the thiohemiacetal formation constants were all studied at pH ≤ 8.3, dissociation of a proton from the thiohemiacetal is insignificant under our experimental conditions. Thus, the observed association constant and the true association constant, K_s, are related as follows:

$$K^{obsd} = \frac{[\text{thiohemiacetal}]_{total}}{[\text{thiol}]_{total}[\text{aldehyde}]_{total}} \quad (7a)$$

$$= \frac{[TH] + [T^-]}{([RSH] + [RS^-])([A] + [H])}$$

$$\approx \frac{[TH]}{([RSH] + [RS^-])([A] + [H])} \quad (7b)$$

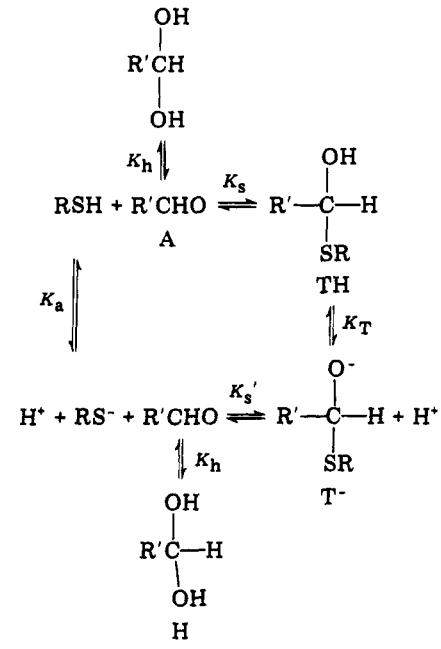
$$= \frac{K_s}{(1 + K_a/[H^+])(1 + K_h)} \quad (7c)$$

or

$$K_s = K^{obsd}(1 + K_a/[H^+])(1 + K_h) \quad (7d)$$

Thus, knowledge of K_a for the particular thiol, K_h for the particular aldehyde, and the K^{obsd} for the particular pair provides a basis for the evaluation of the true association constant for thiohemiacetal formation.

Scheme II



The association constants, K_s, were found to be independent of the aldehyde and thiol concentrations assuming a 1:1 stoichiometry consistent with formation of a thiohemiacetal adduct.

In the time course for the kinetic determinations of K_s (usually less than 10 min) there was negligible oxidation of the aldehyde or further reaction of the thiohemiacetal as indicated by the first-order kinetics (i.e., linearity of ln [free thiol] as a function of time) in the presence of aldehyde. Methylglyoxal is known to undergo an intramolecular Cannizzaro reaction in the presence of glutathione[12] but this reaction (which results in the formation of *S*-lactoylgluthathione) is slower than the alkylation rate of glutathione under our experimental conditions even in the presence of the highest methylglyoxal concentration tested.

Results

The observed equilibrium constants for thiohemiacetal formation, K^{obsd}, were measured as described under Methods. Correction of this value to the true association constant, K_s, requires a knowledge of the hydration constant and the acid dissociation constant of the thiol (eq 7d). The pK_a of *p*-nitrothiophenol (4.4) was determined by spectrophotometric titration in buffered solutions under the same conditions used for monitoring thiohemiacetal formation (μ = 0.4 M, 25 °C). The pK_a value is consistent with that reported by Jencks and Salvensen[13] (pK_a = 4.50, μ = 1 M, 25 °C). The pK_a of the thiol group of glutathione (9.1) was determined from the pH dependence of the rate of alkylation (μ = 0.4 M, 25 °C) and agrees with the microscopic pK_as of 8.93 (amino group protonated) and 9.08 (amino group unprotonated) which were determined by ^{1}H NMR (μ = 0.3–0.4 M, 25 °C).[14]

The hydration equilibrium constants, K_h, were taken from the literature, where available (Table I). Greenzaid et al.[15] have shown (based on seven aliphatic aldehydes) a good correlation between the Taft σ^* value for the acyl substituent and log K_h with ρ^* = 1.70 (±0.07). Extending this correlation to include additional aldehydes (some listed in Table I) we obtain the following Hammett-Taft relationship:

$$\log K_h = 1.68\sigma^* - 0.033 \quad (8)$$

The correlation coefficient is 0.998 and the 90% confidence interval for the slope (ρ_h^*) is 1.61–1.75. Equation 8 does not take into account the steric effect of the substituents (E_s).

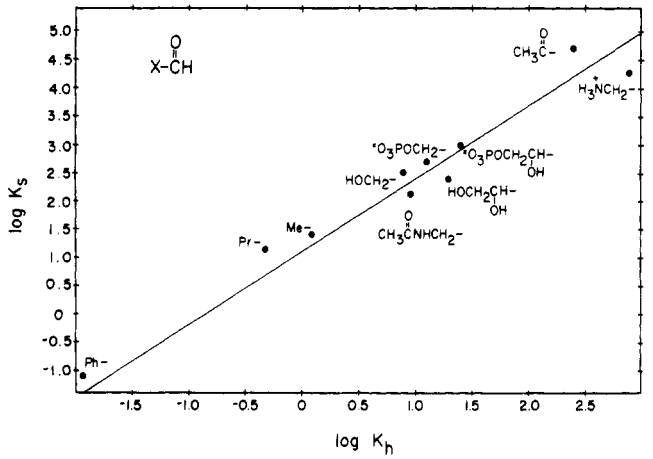


Figure 1. Correlation of thiohemiacetal formation between various aldehydes and glutathione (K_s) with hydrate formation (K_h). The equation providing the best least-squares fit of the data is $\log K_s = 1.13 \log K_h + 1.34$. The correlation coefficient is 0.98 and the interval estimator of the slope (90% confidence) is 0.98–1.28.

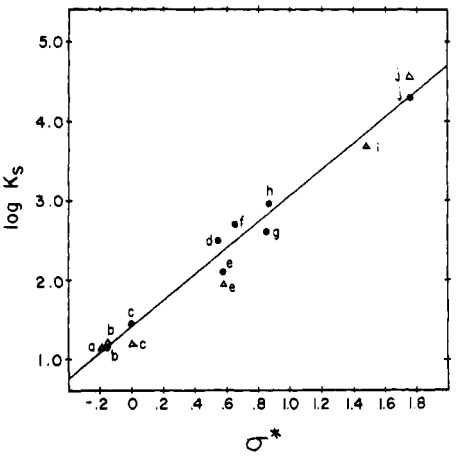
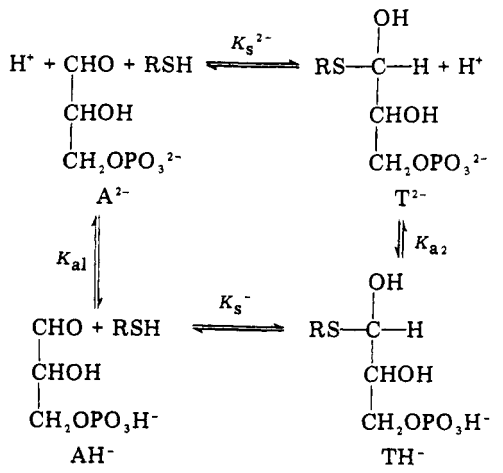


Figure 2. Taft plot of the dependence of the logarithm of the equilibrium constant for thiohemiacetal formation (K_s, M^{-1}) on σ^*. The various aldehyde substituents (XCHO) are (a) (X = isopropyl), (b) (X = *n*-propyl), (c) (X = CH_3–), (d) (X = $HOCH_2$–), (e) (X = $CH_3C(O)NHCH_2$–), (f) (X = $^{2-}O_3POCH_2$–), (g) (X = $HOCH_2CH$–), (h) (X = $^{2-}O_3POCH_2CHOH$), (i) (X = $^{-}HO_3POCH_2CHOH$), and (j) (X = $^{+}H_3NCH_2$–). The line is a least-squares fit for the points obtained with glutathione as the thiol (●). The ρ^* value is 1.65. The points obtained with *p*-nitrothiolphenol (△) are also indicated.

Scheme III



However, Greenzaid et al.[15] find a better correlation when the steric effects of the acyl substituents on the aldehyde are not treated as a separate parameter in the correlation (cf. Bell[16]). The relative insignificance of the steric effect is seen in the good correlation of thiohemiacetal formation ($\log K_s$) with aldehyde hydration ($\log K_h$) (Figure 1). The thiol glutathione (γ-glutamylcysteinylglycine) is sterically more bulky than water. Nevertheless, the aldehydes with bulky substituents (e.g., glyceraldehyde and glyceraldehyde 3-phosphate) correlate with the less bulky aldehydes. Steric effects are expected to be more significant in thiohemiacetal formation with glutathione than in hydration but the bulky aldehydes do not show any significant negative deviation from the correlation line. Furthermore, aldehydes with ionic substituents (e.g., glyceraldehyde 3-phosphate, phosphoglycolaldehyde, and aminoacetaldehyde) do not deviate from the line, indicating that ionic interactions between the aldehydes and glutathione do not contribute to the formation of the thiohemiacetal adduct. Aromatic aldehydes show a significant deviation in linear free energy relationships between hydration equilibria and the Taft polar substituent constant.[17] For example, benzaldehyde hydration shows a negative deviation from the correlation of $\log K_h$ for aliphatic aldehydes with σ^* amounting to 4.1 kcal/mol, which reflects the resonance stabilization of the carbonyl group (i.e., conjugative interaction with the aromatic ring).[17] The same effect is seen in thiohemiacetal formation since benzaldehyde does not deviate from the correlation line in Figure 1.

The equilibrium constants for thiohemiacetal formation (with glutathione and *p*-nitrothiophenol) with the aliphatic aldehydes correlate well with the Taft polar substituent constants (Figure 2). Thus, with glutathione the Taft equation ($\log K_s = \rho^*\sigma^* + C$) is

$$\log K_s = 1.65\sigma^* + 1.41, \; r = 0.986 \qquad (9)$$

and the interval estimator of the slope (ρ^*) at 90% confidence is 1.47–1.83. There is somewhat more scatter in the points obtained with *p*-nitrothiophenol but they fit reasonably well to eq 9. Indeed, a plot of $\log K_s$ (with glutathione) vs. $\log K_s$ (with *p*-nitrothiophenol) yields a least-squares slope of 0.98 and an intercept of 0.17. Thus, within experimental error, K_s appears to be independent of the thiol and the sensitivity of K_s to σ^* also appears to be independent of the thiol. In fact, both thiohemiacetal formation and aldehyde hydration are equally sensitive to the inductive effects of the acyl substituent (cf. eq 8 and 9).

The K_s values were found to be pH independent when the acyl substituent does not possess an ionizing moiety. However, in the case of glyceraldehyde 3-phosphate and phosphoglycoaldehyde the K_s value was found to decrease as the pH was raised from 6.0 to 8.3 (by 56% in the case of glyceraldehyde 3-phosphate). This reflects the dependence of the apparent σ^* value of the phosphate substituent on pH. This is summarized in Scheme III for the 2-phospho-1,2-ethanediol substituent (of glyceraldehyde 3-phosphate). A knowledge of the ρ^* value for thiohemiacetal formation provides a basis for the estimation of the σ^* values for the two ionic species of this aldehyde. Glyceraldehyde 3-phosphate is an important metabolite which is involved in at least six different enzymic reactions (viz., glyceraldehyde 3-phosphate dehydrogenase, triose-phosphate isomerase, transaldolase, aldolase, tryptophan synthase, and possibly methylglyoxal synthase[18]). Thus, a knowledge of the two σ^* values (of the monoanionic and the dianionic species) for the 2-phospho-1,2-ethanediol substituent is useful in mechanistic studies on these enzymes. These σ^* values can be estimated from the pH dependence of the K_s value of glyceraldehyde 3-phosphate described in Scheme III. A knowledge of any three of the parameters in this cycle determines the value of the forth parameter. The observed equilibrium constant for

thiohemiacetal formation, at any given pH, is

$$K^{obsd} = \frac{\left(1 + \frac{[H^+]}{K_{a2}}\right) K_s^{2-}}{\left\{\left(1 + \frac{[H^+]}{K_{a1}}\right) + \left(1 + \frac{[H^+]}{K'_{a1}}\right) K_h\right\}\left(1 + \frac{K_a}{[H^+]}\right)} \quad (10)$$

K_s^{2-} and K_s^- are related by the relationship

$$K_s^- = K_s^{2-}\left(\frac{K_{a1}}{K_{a2}}\right) \quad (11)$$

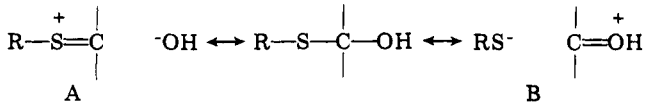
Equation 10 contains six variable parameters, which makes fitting the data (K_s^{obsd} as a function of $[H^+]$) to unique values of these parameters virtually impossible. However, three of these parameters can be determined independently. K_a, the dissociation constant of the thiol, can be measured by titration of the thiol (pK_a = 9.1 for glutathione and 4.4 for *p*-nitrothiolphenol at μ = 0.4 M, 25 °C). The value of K_h (the hydration equilibrium constant of glyceraldehyde 3-phosphate (dianionic species)) has been determined by Trentham et al.[19] to be 29.4 ($\mu \sim 0.2$ M, 20 °C, measured at pH 8.6) and by Kirschner[20] to be 25.6 ($\mu \sim 0.5$ M, 25 °C, measured at pH 8.5). Thus, an average value of K_h = 27.5 was used in these calculations. K'_{a1}, the acid dissociation constant of the hydrated aldehyde, can be determined by direct titration of glyceraldehyde 3-phosphate since this aldehyde exists as ≳96% in the hydrated form in aqueous solution. Titration at an ionic strength of 0.4 M (25 °C) yields a pK'_{a1} value of 6.2. Thus, eq 10 reduces to the three-parameter equation (where the thiol is glutathione)

$$K^{obsd} = \frac{\left(1 + \frac{[H^+]}{K_{a2}}\right) K_s^{2-}}{\left\{1 + \frac{[H^+]}{K_{a1}} + (1 + 10^{(6.2-pH)})27.5\right\}(1 + 10^{(pH-9.1)})} \quad (12)$$

The K^{obsd} values were determined over the range 6 ≤ pH ≤ 8.3 and the data analyzed by a nonlinear least-squares fit to eq 12. In this way a pK_{a1} = 5.5 and pK_{a2} = 6.6 were determined. The lower value of pK_{a1} with respect to pK_{a2} and pK'_{a1} is reasonable because of the greater electron-withdrawing effect of the free aldehyde substituent compared to that of the thiolhemiacetal and hydrated species. The values of K_s^- and K_s^{2-} are indicated in Table I. The σ^* value for the dianionic substituent can be calculated from eq 8 since the K_h value is known (27.5). This yields a σ^* value of 0.88. The σ^* value for the monoanionic substituent, calculated from eq 9 (based on a K_s^- value of 7.47×10^3), is 1.49.

Discussion

The Taft ρ^* value for thiohemiacetal formation is 1.65 (±0.18) with glutathione and is essentially the same with *p*-nitrothiophenol, which is about 4000 times less basic than glutathione. It has been observed that the equilibrium constant for thiohemiacetal formation (with acetaldehyde) is also independent of the basicity of the thiol.[7,21] This contrasts with the dependency of hemiacetal formation on the pK_a of the oxygen acid, which has been attributed to hydrogen bonding of the oxygen acid with the solvent which is lost upon hemiacetal formation.[22] The greater hydrogen bonding of alcohols and carboxylic acids with water than of thiols is also consistent with the recent kinetic studies of Hupe et al.[23] For a given aldehyde, the equilibrium constant for thiohemiacetal formation (K_s) is greater than the equilibrium constant for hydration (= $K_h/55.6$ where the water concentration is taken into account). Thus, for the ten aldehydes listed in Table I, the thiohemiacetal adduct with glutathione is 4.3 (±0.2) kcal/mol more stable than the corresponding hydrate. This stabilization is independent of the basicity of the thiol. For example, with acetaldehyde (σ^* = 0) thiohemiacetal formation with *p*-nitrothiophenol (pK_a = 4.4) is more favorable that water addition (pK_a = 15.74) by 3.9 kcal/mol. This has been attributed,[7] in part, to the double bond–no bond resonance stabilization of the thiohemiacetal:



and is consistent with the inverse solvent isotope effect ($K_{H_2O}/K_{D_2O} \approx 0.44$)[7] on thiohemiacetal formation. The double bond–no bond resonance model is also consistent with the smaller (by ~10%) inverse secondary deuterium equilibrium isotope effect on thiohemiacetal formation than on aldehyde hydration.[9] However, the ρ^* value for thiohemiacetal formation is essentially the same as the ρ^* value for water addition to aldehydes. If substantial double bond–no bond resonance exists in the thiohemiacetal it is expected that ρ^* should be *less* for thiohemiacetal formation than for *gem*-diol formation because electron-withdrawing substituents will more effectively stabilize the free aldehyde relative to their stabilizing effect on the contributing resonance structures A and B. Differences in polarizability and electronegativity between sulfur and oxygen can also be partly responsible for the greater stability of the thiohemiacetal than the *gem*-diol but these factors are expected to increase the ρ^* value for thiol addition relative to that for water addition, if they are significant. There are many factors which are likely to contribute to the greater carbon basicity of thiols relative to that of water and alcohols. Nevertheless, the higher affinity of thiols for carbonyl groups compared to that of water is not manifested in any significant alteration in the sensitivity to acyl substituents (inductive and field effects). As pointed out by Sander and Jencks[22] and by Hupe et al.[23] the greater carbon basicity of thiols than of alcohols (both thermodynamic and kinetic) is largely a reflection of differential solvation of the attacking atom in the ground state and in the product (or transition state). This is consistent with the similarities of the ρ^* values for thiohemiacetal formation and for hydration.

Insofar as thiohemiacetal formation can be considered a model for formation of the tetrahedral intermediate in thiolysis of esters (or a model for breakdown of the tetrahedral intermediate in hydrolysis of thiol esters) a comparison of the ρ^*_{nuc} value (ρ^* for the rate of the acyl transfer reaction) with ρ_s^* (ρ^* for thiohemiacetal formation equilibria) can be a useful index of the resemblance of the reactants in the transition state to the tetrahedral intermediate. Such a rate-equilibria correlation for thiol addition to carbonyl groups is dependent on the ionic character of the tetrahedral addition complex. Thiolates are about 10^{10} times more reactive than thiols in acyl transfer reactions, although acid-catalyzed thiol addition is known.[24] As pointed out by Guthrie,[5] thiolate attack on esters involves an anionic tetrahedral intermediate and acid-catalyzed thiol attack most likely involves formation of a neutral, uncharged, tetrahedral intermediate. These intermediates are modeled by the neutral (TH) and anionic (T^-) thiohemiacetals which are related by the thermodynamic cycle illustrated in Scheme II. From Scheme II it can be seen that the equilibrium constant for formation of the anionic thiohemiacetal ion is related to the equilibrium constant for formation of the neutral thiohemiacetal, K_s, by the relationship

$$K_s' = \left(\frac{K_T}{K_a}\right) K_s \quad (13)$$

Table I. Equilibrium Constants for Thiohemiacetal Formation (25 °C, μ = 0.4 M)

R	σ^* [a]	K_h [b]	glutathione		*p*-nitrothiophenol	
			K_s^{obsd}, M^{-1} [c]	K_s, M^{-1} [d]	K_s^{obsd}, M^{-1} [c]	K_s, M^{-1} [d]
$(CH_3)_2CH$-	−0.19	0.43[j]			10 (±1)	14.3
$CH_3CH_2CH_2$-	−0.115	0.48[j]	10 (±1)	14.8	11 (±1)	16.3
CH_3-	0	1.26[k]	12 (±1)	27	7 (±0.4)	15.8
$HOCH_2$-	0.555	7.9[e]	36 (±3)	320		
$CH_3C(O)NHCH_2$-	0.585[e]	8.95[l]	13 (±1)	129	8.7 (±0.4)	86.6
Ph-	0.60	0.011[m]	0.08 (±0.03)	0.08		
$^{2-}O_3POCH_2$-	0.655[f]	11.7[e]	38 (±3)	483		
$HOCH_2CHOH$-	0.806[e]	21[n]	18 (±2)	396		
$^{2-}O_3POCH_2CHOH$-	0.876[e]	27.5[o]	33 (±2)	940 (K_s^{2-})		
$CH_3C{=}O$	[g]	548 > K_h > 56[p]	200 (±10)[q]	(53 400)[r]	85 (±6)	(22 700)[r]
$^{-}HO_3POCH_2CHOH$-	1.49[h]	295[h]		7 470[h] (K_s^-)	17.6 (±0.9)	5200
$^{+}H_3NCH_2$-	1.76[i]	839[e]	23 (±2)	19 320	49 (±4)	41 200

[a] Values from R. W. Taft, "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, 1956, unless otherwise indicated. [b] Equilibrium constant for hydration (based on water concentration = 1.0). [c] Observed association constant, corrected for ionization of the thiol. [d] Equilibrium constant for RCHO + R'SH thiohemiacetal (corrected for thiol ionization and aldehyde hydration). [e] Calculated from eq 8. [f] Estimated from the relationship $0.49\sigma^* + 0.555 = 0.876$ where 0.876 is the σ^* value for the 1-phospho-1,2-ethanediol substituent, 0.555 is the substituent constant for CHOH, and the insulation effect of this moiety is assumed to be the same as that for a methylene moiety (= 0.49; J. Shorter, "Correlation Analysis in Organic Chemistry", Clarendon Press, Oxford, 1973, p 10). [g] A meaningful value of σ^* for this substituent is difficult to obtain owing to the hydration of this substituent [$CH_3C{=}O \rightleftharpoons CH_3C(OH)_2$-]. This hydration equilibrium will depend on the nature of the C-1 substituent [i.e., -CHO, $-CH(OH)_2$, or $-(OH)C(SR)H$]. [h] Estimated for the pH dependence of K_s with glyceraldehyde 3-phosphate and glutathione (see text). [i] Based on the method of Fastrez[6] and a pK_a of β-alanine of 3.60. [j] From Greenzaid et al.[15] [k] Average (±18%) of the values from Greenzaid et al.[15] and Lewis and Wolfenden[9] (corrected for a solvent isotope effect, H_{H_2O}/K_{D_2O}, of 0.8; see Barnett and Jencks[8] and R. L. Schowen in "Isotope Effect on Enzyme-Catalyzed Reactions", W. W. Cleland, M. H. O'Leary, and D. B. Northrop, Eds., University Park Press, Baltimore, Md., 1977, pp 64-99). [l] Lewis and Wolfenden,[9] corrected for a solvent isotope effect of 0.80. [m] Greenzaid.[17] [n] From IR data of C. A. Swenson and R. Barker, *Biochemistry*, **10**, 3151-3154 (1971). [o] Average (±8%) of the values of Trentham et al.[18] and Kirschner.[19] [p] The ^1H NMR spectrum of pyruvaldehyde (in D_2O) shows no evidence of any free aldehyde. The substituent exists as 67% keto form ($CH_3C{=}O$) and 33% hydrated form [$CH_3C(OH)_2$-] based on the chemical shift assignments (CH_3- group) of Y. Pocker, J. E. Meany, B. J. Nist, and C. Zodorjin, *J. Phys. Chem.*, **73**, 2879-2882 (1969). However, this equilibrium will depend on the nature of the C-1 substituents. The limiting values for K_h were estimated from eq 8 based on $\sigma^* = 1.65$ (100% $CH_3C{=}O$) and $\sigma^* \approx 1.06$ [100% $CH_3C(OH)_2$-] = σ^* for $ClCH_2$- [pK_a ($ClCH_2CO_2H$) = 2.86, pK_a[$CH_3C(OH)_2CO_2H$] = 2.6]. [q] A value, based on the UV spectrum, about 25% larger than this has been reported [R. Vince, S. Daluge, and W. B. Wadd, *J. Med. Chem.*, **14**, 402-404 (1971); D. L. Vander Jagt, L-P. B. Han, and C. H. Lehman, *Biochemistry*, **11**, 3735-3740 (1972)]. [r] Based on a K_h value of 266 assuming an effective $\sigma^* = 1.46$ [67% $CH_3C{=}O$ and 33% $CH_3C(OH)_2$-] for the substituent in the hydrated aldehyde; $\sigma^*_{eff} = 1.46 = 0.67(1.65) + 0.33(1.06)$.[p]

or

$$\log K_s' = \log K_s + \log K_T - \log K_a \quad (14a)$$

$$\rho_s^{*\prime}\sigma^* + C' = \rho_s^*\sigma^* + C + \rho_T^*\sigma^* + C_T + pK_a \quad (14b)$$

$$= (\rho_s^* + \rho_T^*)\sigma^* + C + C_T + pK_a \quad (14c)$$

Thus, $\rho_s^{*\prime}$, the Taft ρ^* value for addition of the thiolate to the aldehyde to form the thiohemiacetalate, T^-, is the sum of the ρ^* value for thiohemiacetal formation and the ρ^* value for ionization of the thiohemiacetal, ρ_T^*.

The pK_a of the thiohemiacetal will depend on the electronic effects of both the acyl substituent and the thiol. The pK_a of the thiohemiacetal adduct of acetaldehyde and seven thiols ($2.7 \leq pK_a \leq 10.3$) is fairly insensitive to the thiol pK_a. Thus, based on the data of Gilbert and Jencks,[21] the pK_a of the thiohemiacetal, pK_T, is related to the pK_a of the thiol by the relationship

$$pK_T = 0.16pK_a + 11.1 \quad (15)$$

when the acyl substituent is a methyl group ($\sigma^* = 0$). The sensitivity of pK_T to acyl substituents on the aldehyde moiety (ρ_T^*) can be estimated by the following method.

The ρ^* value for the acid dissociation of alcohols (XCH_2OH) is 1.32:[25]

$$pK_a(XCH_2OH) = -1.32\sigma^* + 15.7 \quad (16)$$

Assuming that the substitution of an RS moiety for a H does not significantly alter the sensitivity of the pK_a to the electron-withdrawing ability of X, the ρ_T^* should not be altered:

$$pK_T = pK_a\,(XC(SR)HOH) = -1.32\sigma^* + C \quad (17)$$

When $\sigma^* = 0$ the pK_T value is given by eq 15. Thus, $C = 0.16pK_a + 11.1$. Therefore

$$\log K_T = 1.32\sigma^* - 0.16pK_a - 11.1 \quad (18)$$

Substitution of $\rho_T^* = 1.32$ and $C_T = -(0.16pK_a + 11.1)$ into eq 14c yields the parameters of the Taft equation for formation of the thiohemiacetalate anion (from the aldehyde and the thiolate):

$$\log K_s' = 2.97\sigma^* + 0.84pK_a - 9.7 \quad (19)$$

Thus, with acetaldehyde ($\sigma^* = 0$) the equilibrium constant for formation of the thiohemiacetalate with the glutathione anion is expected to be $\sim 10^{-2}$ M, or less favorable than thiohemiacetal formation by a factor of ~1600.

The ρ^* for formation of the thiohemiacetalate is, as expected, larger than the corresponding ρ^* value for thiohemiacetal formation. This is similar to the results obtained with addition of H_2O (or -OH) or HCN (or ^-CN) to aromatic aldehydes. Thus, Greenzaid[17] found a 98% greater ρ value for hydroxide addition compared to water addition and Ching and Kallen[26] found a 48% greater ρ^+ value for cyanide addition compared to HCN addition to substituted benzaldehydes.

Equation 19 provides a useful basis for the estimation of the dependence of the relative energies of anionic tetrahedral intermediates (in acyl transfer reactions to and from thiols) on the electron-withdrawing ability of the acyl substituents (σ^*) and on the basicity of the thiol (pK_a). Substitution of any group (e.g., RO-, RNH-, or RS-) for the hydrogen on the acyl carbon (e.g., in thiolysis of oxygen esters, amides, and thioesters) for the hydrogen on the acyl carbon will affect the stability of the anionic tetrahedral intermediate but should have little, if any, effect on the sensitivity of the stability of this

intermediate to electronic effects on the acyl group or to the basicity of the thiol nucleophile. Thus, substitution of groups for hydrogen on the acyl carbon is expected to alter the constant term in eq 19 but have little effect on the coefficients of σ^* or pK_a. Thus, for a fixed acyl group the Brønsted coefficient for the formation of the anionic tetrahedral intermediate (β_{eq}) for acyl transfer from alcohols, thiols, or amines to thiols is expected to be ~0.8. Hupe and Jencks[27] have shown that the β_{nuc} value for acyl transfer from esters and thiol esters to thiolates is 0.3 for reactions in which formation of the anionic tetrahedral intermediate is the rate-limiting step. This suggests that the thiolate has lost ~37% (= 0.3/0.8) of its negative charge in going from the ground state to the transition state. Similarly, a comparison of the ρ^* for the rate of nucleophilic attack of thiolates on acyl compounds with the equilibrium ρ^* value (2.97) should provide a useful index of the structural similarity between the transition state and the anionic tetrahedral intermediate.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We also thank Dr. Jack Kirsch and the referees for several valuable comments.

References and Notes

(1) E. Baumann, *Chem. Ber.*, **18**, 258–263 (1885).
(2) C. A. Lewis, Jr., and R. Wolfenden, *Biochemistry*, **16**, 4890–4895 (1977).
(3) P. I. Clark, G. Lowe, and D. Nurse, *J. Chem. Soc., Chem. Commun.*, 451–453 (1977).
(4) J. P. Guthrie, *J. Am. Chem. Soc.*, **95**, 6999–7003 (1973).
(5) J. P. Guthrie, *J. Am. Chem. Soc.*, **100**, 5892–5904 (1978).
(6) J. Fastrez, *J. Am. Chem. Soc.*, **99**, 7004–7013 (1977).
(7) G. E. Lienhard and W. P, Jencks, *J. Am. Chem. Soc.*, **88**, 3982–3995 (1966).
(8) R. E. Barnett and W. P. Jencks, *J. Am. Chem. Soc.*, **91**, 6758–6765 (1969).
(9) C. A. Lewis and R. Wolfenden, *Biochemistry*, **16**, 4886–4890 (1977).
(10) J. F. Walder, "Formaldehyde", Reinhold, New York, 1964, pp 486–487.
(11) G. Ellman, *Arch. Biochem. Biophys.*, **82**, 70–77 (1959).
(12) S. S. Hall, A. M. Doweyko, and F. Jordan, *J. Am. Chem. Soc.*, **100**, 5934–5939 (1978).
(13) W. P. Jencks and K. Salvensen, *J. Am. Chem. Soc.*, **93**, 4433–4436 (1971).
(14) D. L. Rabenstein, *J. Am. Chem. Soc.*, **95**, 2797–2803 (1973).
(15) P. Greenzaid, Z. Luz, and D. Samuel, *J. Am. Chem. Soc.*, **89**, 749–756 (1967).
(16) R. P. Bell, *Adv. Phys. Org. Chem.*, **4**, 1–29 (1966).
(17) P. Greenzaid, *J. Org. Chem.*, **38**, 3164–3167 (1973).
(18) D. J. Hopper and R. A. Copper, *Biochem. J.*, **128**, 321–328 (1972).
(19) D. R. Trentham, C. H. McMurray, and C. I. Pogson, *Biochem. J.*, **114**, 19–24 (1969).
(20) K. Kirschner, *J. Mol. Biol.*, **58**, 51–68 (1971).
(21) H. F. Gilbert and W. P. Jencks, *J. Am. Chem. Soc.*, **99**, 7931–7947 (1977).
(22) E. G. Sander and W. P. Jencks, *J. Am. Chem. Soc.*, **90**, 6154–6162 (1968).
(23) D. J. Hupe, D. Wu, and P. Shepperd, *J. Am. Chem. Soc.*, **99**, 7659–7662 (1977).
(24) A. R. Fersht, *J. Am. Chem. Soc.*, **93**, 3504–3515 (1971).
(25) S. Takahashi, L. A. Cohen, H. K. Miller, and E. G. Peake, *J. Org. Chem.*, **36**, 1205–1209 (1971).
(26) W-M. Ching and R. G. Kallen, *J. Am. Chem. Soc.*, **100**, 6119–6124 (1978).
(27) D. J. Hupe and W. P. Jencks, *J. Am. Chem. Soc.*, **99**, 451–464 (1977).

The Timing of the Proton Transfer Process in Acid-Catalyzed Carbonyl Addition. Evidence for a Preassociation Mechanism for Catalysis of Carbinolamine Formation from Acethydrazide and *p*-Chlorobenzaldehyde[1]

J. M. Sayer* and Carl Edman

Contribution from the Department of Chemistry, University of Vermont, Burlington, Vermont 05405. *Received September 27, 1978*

Abstract: General acid catalysis of carbinolamine formation from acethydrazide ($CH_3C(O)NHNH_2$) and *p*-chlorobenzaldehyde in aqueous solution probably occurs by a "preassociation" mechanism that involves rate-determining attack of the nucleophile on the aldehyde in the presence of the acid catalyst in a termolecular encounter complex, and rate-determining diffusion apart of the protonated carbinolamine and the conjugate base of the catalyst, in limiting cases of strongly and weakly acidic catalysts, respectively. Unlike amines of even slightly greater basicity, acethydrazide does not add to *p*-chlorobenzaldehyde by a mechanism involving a kinetically significant *free* zwitterionic carbinolamine ($T^{\pm}$). The data are most consistent with a mechanism in which there is a small amount of stabilization of the transition state for amine attack, and of the initial product of this attack, by hydrogen bonding of oxygen to strongly acidic catalysts. Evidence in support of the proposed mechanism includes (1) the absence in the pH–rate profile of a break at low pH corresponding to a change from rate-determining hydronium ion catalyzed protonation to uncatalyzed formation of $T^{\pm}$, (2) the absence of any detectable effect of increased solvent viscosity (50% aqueous glycerol) on the rate constants for catalysis by heterocyclic ammonium ions, and (3) a nonlinear Brønsted plot for general acid catalysis, with limiting slopes of ca. 0.11 for strongly acidic and ≥0.8 for weakly acidic catalysts. In contrast, the triazolium ion catalyzed reaction of methoxyamine (CH_3ONH_2) with *p*-chlorobenzaldehyde, which is known to involve rate-determining diffusion-controlled protonation of *free* $T^{\pm}$, is inhibited by a factor of approximately 12 in 50% aqueous glycerol.

The addition of weakly basic nitrogen nucleophiles to substituted benzaldehydes occurs in many cases by the initial formation of a highly unstable zwitterionic carbinolamine intermediate, $T^{\pm}$, that is trapped by rate-limiting diffusion-controlled proton transfer (k_a) from hydronium ion or a general acid catalyst at pH values greater than 1.0 (Scheme I, left-hand side).[2,3]

In order for a mechanism involving rate-determining protonation of free $T^{\pm}$ to be significant, $T^{\pm}$ must be sufficiently short lived that it reverts to starting materials faster than it

0002-7863/79/1501-3010$01.00/0