Conjugate Addition to Activated β-Arylacrylic Acid Derivatives. Thiolate Anions

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Abstract: The reactions of N-β-(3-indol)acryloylimidazole (**1**) with N,N-dimethylcysteamine and cysteamine have been investigated. Data are presented and interpreted as compelling evidence for the conjugate addition of thiolate anions to form transient intermediates which undergo a second nucleophilic attack at the carbonyl carbon by a second molecule of thiolate anion, and then, *via* β elimination, the corresponding β-(3-indol)acryloyl thiolester is formed. When N,N-dimethylcysteamine is the reacting thiol, the final products are the thiolester, S-β-(3-indol)-acryloyl-N,N-dimethylcysteamine, in equilibrium with the corresponding conjugate addition product. When cysteamine is the reacting thiol, the conjugated amide, N-β-(3-indol)acryloylcysteamine, obtains in yields which are pH dependent, although the S to N rearrangement of the thiolester under the same conditions is found to be quantitative. Pathways for these reactions are discussed, and a rationale is offered to explain the observation of conjugate addition by these sulfur nucleophiles *vis-á-vis* carbonyl attack by oxygen and nitrogen nucleophiles.

The β-arylacrylolyl derivatives have considerable utility in the study of reaction mechanisms involving nucleophilic displacement at carbonyl carbon, and certain of these derivatives (esters, thiolesters, and amides) have found special application in the study of proteolytic enzyme mechanisms,[2-5] since the spectrum arising from the long-wavelength π,π^* transition of the β-arylacryloyl moiety is particularly sensitive to the electronic nature and environment of the substituent attached to the carbonyl carbon.[6]

The literature pertaining to the reactions of β-arylacrylic esters[7] and β-arylacryloylimidazoles[8] gives some indication that nucleophilic attack by oxygen and nitrogen nucleophiles usually occurs at the carbonyl carbon, since the products generally are those to be expected from carbonyl attack. However, α,β-unsaturated esters and amides lacking conjugation with an aromatic group exhibit both carbonyl and conjugate modes of addition with oxygen, nitrogen, carbon, and sulfur nucleophiles.[9]

Conjugate addition to β-arylacrylolyl derivatives has been demonstrated under conditions where the products are not subject to kinetic control. For example, Hauser, *et al.*,[10] have demonstrated that ethylcinnamate undergoes deuterium incorporation at the α-carbon after long periods of refluxing in sodium ethoxide–deuterioethanol solution, presumably *via* a conjugate addition–elimination mechanism. Similarly, the reaction of ethylcinnamate with ammonia and primary amines in ethanol appears to give products derived from both modes of addition.[11]

Examples of conjugate addition to β-arylacryloyl esters occur more frequently when carbon nucleophiles are involved. The work of Hauser, *et al.*,[12-14] and others[15] demonstrates that conjugate addition is the predominant reaction of organopotassium compounds and Grignard reagents. Little information concerning the mode of addition of sulfur nucleophiles to arylacrylic esters is to be found, although cinnamic acid and cinnamaldehyde are reported to undergo conjugate addition with bisulfite ion.[16]

Factors which appear to influence the mode of addition include the nature of the nucleophile, the solvent, and steric interactions. The more reactive systems such as aldehydes, ketones, and acrylic esters and amides can react to form the thermodynamically more stable conjugate addition products with oxygen and nitrogen nucleophiles, although carbonyl addition is likely the kinetically favored product.

This paper presents experiments which are interpreted as compelling evidence for the conjugate addition of thiolate ions to high energy β-arylacrylolyl derivatives to form transient intermediates which undergo a second nucleophilic attack at the carbonyl carbon by a second molecule of thiolate ion, and then *via* β elimination result in the formation of the corresponding β-arylacryloyl thiolester as the thermodynamically more stable product.

The compounds used in this study are N-β-(3-indol)-acryloylimidazole (**1**), S-β-(3-indol)acryloyl-N,N-dimethylcysteamine (**2**), *p*-methoxycinnamyl-imidazole (**3**), N,N-dimethylcysteamine hydrochloride (**4**), and cysteamine hydrochloride (**5**).

Experimental Section

Materials. The commercially available compounds cysteamine hydrochloride (Cyclo) and N,N-dimethylcysteamine hydrochloride

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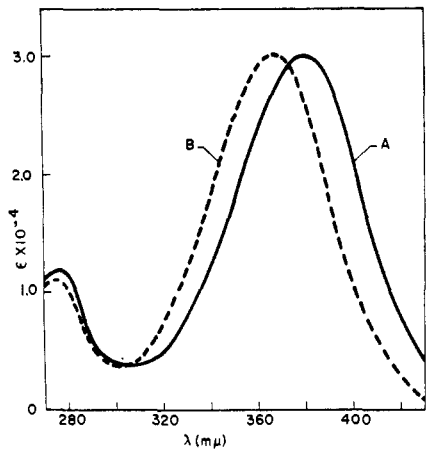


Figure 1. Curve A, the spectrum of **1**. Curve B, the spectrum of the thiolester **2** resulting from the reaction of **1** with N,N-dimethylcysteamine in pH 8.0 $P_2O_7^{2-}$ 0.1 *M* buffer.

(Aldrich) were used as received, since nmr spectroscopy indicated both compounds were of high purity. *p*-Methoxycinnamoylimidazole and N-β-(3-indol)acryloylimidazole were prepared by published procedures.[3]

S-β-(3-Indol)acryloyl-N,N-dimethylcysteamine (2). A solution of **1**, 0.100 g (4.22×10^{-4} mol), dissolved in 20 ml of acetone was added dropwise to a stirred solution of N,N-dimethylcysteamine hydrochloride, 0.100 g (7.07×10^{-4} mol), in 50 ml of 0.1 *M* pH 7.88 pyrophosphate buffer. Reaction was almost instantaneous, as evidenced by the rapid change in color from yellow to colorless. Evaporation of the acetone from the resulting solution precipitated the thiolester as a light yellow solid, which recrystallized from ether, yielding yellow prisms: mp 115–117°; uv max (H_2O) 365 mμ (ε 3.01×10^4).

Anal. Calcd for $C_{15}H_{18}N_2OS$: C, 65.69; H, 6.61; N, 10.20; S, 11.67. Found: C, 65.56; H, 6.63; N, 10.09; S, 11.50.

Preparation of S-β-(3-Indol)acryloylcysteamine (12). The thiolester was prepared by the reaction of 0.010 ml of **1**, 6.3×10^{-5} *M*, with 0.10 ml of cysteamine hydrochloride, 1.9×10^{-3} *M*, in 3.0 ml of pH 4.0 acetate buffer. The reaction at this pH is a comparatively slow, pseudo-first-order process with an apparent half-life of 15 min. The final spectrum exhibited a uv max (H_2O) at 364 mμ (ε 3.0×10^4). The thiolester obtained by this procedure was used directly in the kinetic experiments without isolation.

Kinetic Measurements. All kinetic runs were carried out on either a Cary Model 14 or a Beckman DB spectrophotometer in thermostated cell compartments. All reported spectra were recorded with the Cary 14. pH measurements were made at the end of each kinetic run to an accuracy of ±0.02 pH units with a Radiometer Model 4 pH meter. All kinetic runs were carried out in either 0.05 *M* phosphate or 0.1 *M* pyrophosphate buffers in the pH 6–9.5 range, glycine buffers were used above pH 9.5, and acetate buffers were used below pH 6.0. Quartz 1-cm cells were used throughout. The temperature was maintained at 25.0 ± 0.5° for all kinetic runs. In general, kinetic runs were carried out by mixing microliter amounts of the reactants from concentrated stock solutions with 3 ml of the appropriate buffer, such that the final concentrations of the chromophores ranged from 5×10^{-6} to 1×10^{-4}, depending on solubility and extinction coefficients. The final thiol concentrations in all cases were at least 20-fold greater than the chromophore, in order that pseudo zero-order dependencies in thiol concentration obtained. The chromophore stock solutions were made up in spectro grade acetonitrile or dimethylformamide. The thiol stock solutions were made up in distilled water within 1 week of usage, and stored at 5°. The recording of optical density changes was initiated immediately after mixing, ordinarily with, a "dead-time" of about 8–10 sec before the first optical density reading. Where the reactions were quite rapid, initial readings could be obtained after 4–5 sec.

The kinetic measurements on the S → N transfer reaction of compound **12** were carried out by transferring microliter quantities of the thiolester, prepared at low pH, to optical cells containing the desired pH buffer.

Analysis of Rate Data. All rate data, except for the complicated data pertaining to the reaction of **1** with N,N-dimethylcysteamine

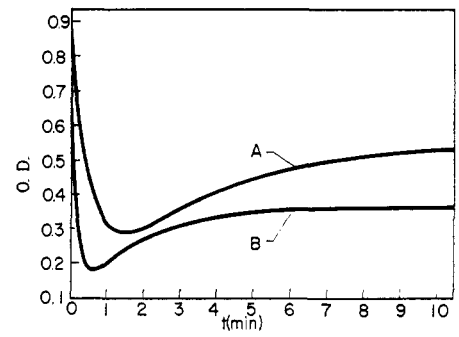


Figure 2. Time course of the optical density changes at 380 mμ accompanying the reaction of **1** with N,N-dimethylcysteamine in pH 7.88 $P_2O_7^{2-}$ 0.1 *M* buffer and 25.0°. Curve A, 3×10^{-5} *M* **1**, 1.98×10^{-3} *M* N,N-dimethylcysteamine. Curve B, 3×10^{-5} *M* **1**, 4.90×10^{-3} *M* N,N-dimethylcysteamine.

and the reaction of **1** with cysteamine at high cysteamine concentrations, give linear [ln $(OD_t - OD_\infty)$] *vs.* time plots. Second-order rate constants were obtained by dividing the slope of these plots by the concentration of the excess reactant (see Discussion section). The treatment of the complicated reaction of **1** with N,N-dimethylcysteamine is considered in detail in the Discussion section. The apparent pK_a's of N,N-dimethylcysteamine were determined by the titration of 1.00 ml of 3×10^{-1} *M* thiol dissolved in 30 ml of distilled water with 0.0984 *N* NaOH. The values obtained were 7.60 ± 0.10 and 10.75 ± 0.15 at 25°. The apparent pK_a of **1** was estimated to be 4.40 ± 0.15 at 25° by plotting the uv max of the π,π^* transition *vs.* pH, and determining the midpoint of the resulting sigmoid-shaped curve.

Results and Discussion

The spectra in Figure 1 demonstrate that the reaction of **1** with N,N-dimethylcysteamine in the pH range 6–9 results in the formation of the corresponding thiolester as the only chromophoric product. The kinetic course of this reaction in the pH range 7.5–8.5, as measured by changes in optical density, Figure 2, clearly indicates the formation of a nonchromophoric intermediate or intermediates prior to the formation of the thiolester. *Changes in optical density similar to those illustrated in Figure 2 are observable at any wavelength between 280 and 420 mμ.* The effect of thiol concentration on the optical density is also shown in Figure 2. An increase in the thiol concentration increases the apparent rate, while decreasing the thiolester yield. When a large excess of thiol is added to a solution of the thiolester, either synthesized independently or prepared from the reaction of **1** with thiol, a rapid (single exponential) partial disappearance of the thiolester spectrum occurs with a first-order dependence on thiol concentration. There is no concomitant formation of a chromophoric product (Figure 3). In a similar fashion, successive additions of thiol produce further decreases in the residual thiolester spectrum. Dilution of these samples (by a factor of 10) results in a single-exponential rate of reappearance of the thiolester spectrum, demonstrating the reversibility of this process. From these rates and equilibria, a set of apparent equilibrium constants for the assumed expression of eq 1 have been determined as a function of pH. These values are listed in Table I.

$$\text{thiolester} + \text{thiol} \overset{K_{eq}}{\rightleftharpoons} \text{product} \qquad (1)$$

$$K_{eq} = \frac{[\text{product}]}{[\text{thiolester}][\text{thiol}]}$$

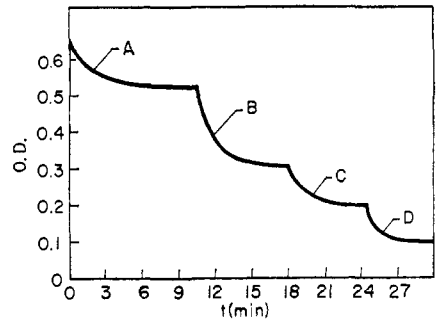


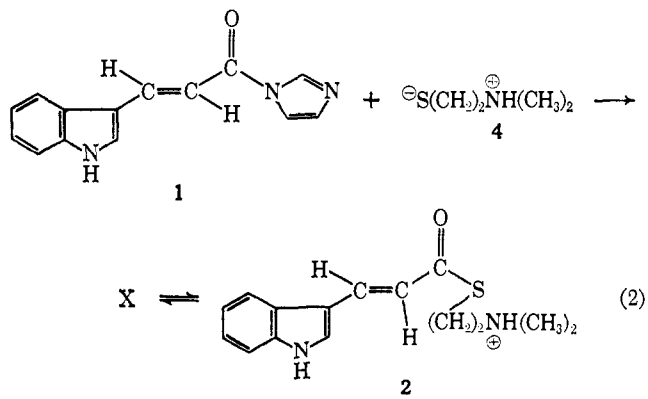
Figure 3. Time course of the optical density changes at 365 mμ accompanying successive additions of N,N-dimethylcysteamine to a solution of **2**, 2.2×10^{-5} *M*, in 0.1 *M* pH 8.07 $P_2O_7^{2-}$ buffer and 25.0°. Total N,N-dimethylcysteamine concentrations: A, 1.84×10^{-3} *M*; B, 6.68×10^{-3} *M*; C, 1.10×10^{-2} *M*; D, 2.00×10^{-2} *M*.

The rate of the first step of the reaction of **1** with N,N-dimethylcysteamine is not easily found (although initial slopes indicate a first-order thiol dependency), since the

Table I. Apparent Equilibrium Constants for the Reaction of **2** with N,N-Dimethylcysteamine

pH	$K_{eq} \times 10^{-2}$ M^{-1}
6.02	2.63
6.98	3.35
7.07	3.52
7.50	3.16
8.19	2.37
8.50	1.55
8.96	~0.95
9.00	~1.11

time-dependent optical density changes (Figure 2) suggest a kinetic scheme of at least the complexity of eq 2.



(2)

However, if it is assumed that the first step is fairly rapid with respect to the second, then, at sufficiently long times, the optical-density changes associated with the second step will be a measure of the rate of the second step with negligible perturbations from the first step. Apparent rates calculated from this portion of the data are interesting in that they are found to be numerically equal to the apparent rates obtained from the reaction of the thiolester with thiol when the same concentrations of thiol are employed. Figures 4a and b present the pH–rate profile for the forward and reverse rates, k_f and k_r, calculated from apparent rates and the equilibrium constants in Table I, assuming the relationships

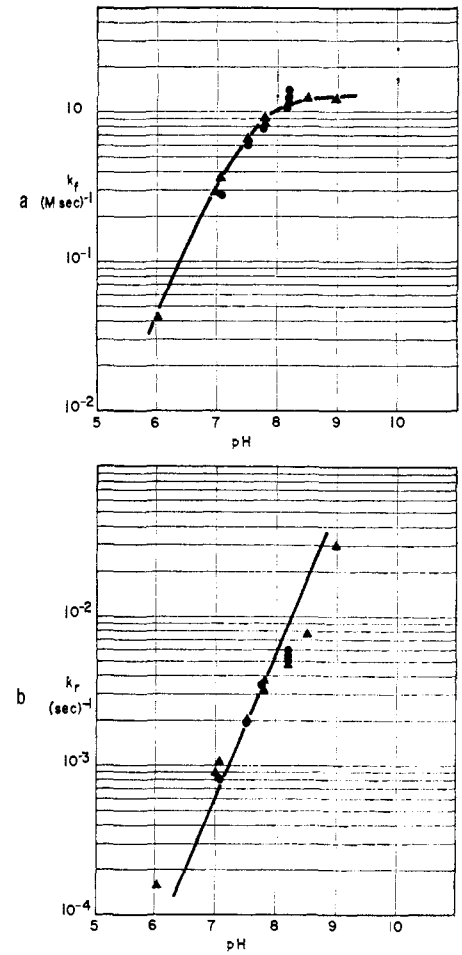
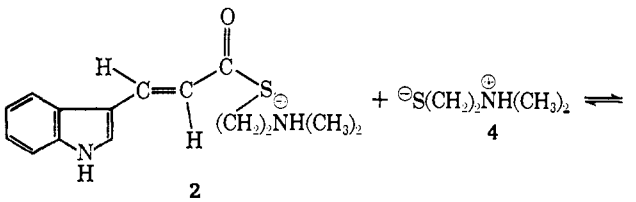


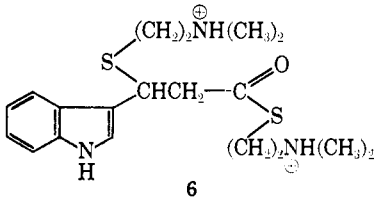
Figure 4. (a) Comparison of k_f values: ▲, calculated from the apparent rates of reaction of **2** with N,N-dimethylcysteamine and the equilibrium constants in Table I; ●, calculated from the apparent rates of appearance of **2** in the reaction of **1** with N,N-dimethylcysteamine and the equilibrium constants in Table I. The solid line is the theoretical curve for a rate dependent on an ionizable group of $pK_a = 7.6$. (b) Comparison of k_r values. The solid line is the theoretical curve calculated for a first-order dependence on hydroxide ion concentration.

$k_{app} = k_f$[thiol] $+ k_r$ and $K_{eq} = k_f/k_r$ hold for both the reaction of thiolester with thiol and for the second step of the reaction of **1** with N,N-dimethylcysteamine. *This figure clearly demonstrates the identity of the rates of these reactions over the pH range where intermediate formation is observed.* k_f and k_r are presented rather than apparent rate constants to take advantage of previously collected data. Thus, it appears that the two processes are indeed the same, since the occurrence of numerically equal rate constants over such a pH range is highly unlikely unless the two processes are identical.

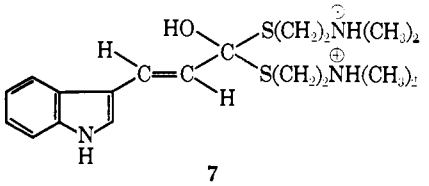
These experimental observations suggest that the reaction of the thiolester with thiol takes place by a conjugate addition mechanism (eq 3), thus accounting for the disappearance of the long-wavelength π,π^* transition characteristic of the conjugated β-arylacrylolyl system. An alternative explanation for the observed spectral changes would be the formation of a metastable tetrahedral intermediate (7) by the addition of thiol to the ketone-like thiolester carbonyl. However, the numerical identity of the rate of formation of thiolester from the reaction of **1** with N,N-dimethylcysteamine with the rate of reaction of N,N-dimethyl-



2 + $^{\ominus}S(CH_2)_2\overset{\oplus}{N}H(CH_3)_2$ ⇌ 4



6 (3)



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cysteamine with thiolester argues against such a scheme, since the two processes are different and therefore no reason exists for the identity of rates for widely varying conditions of pH and thiol concentrations.

If the reaction of N,N-dimethylcysteamine with the corresponding thiolester, **2**, does indeed occur *via* conjugate addition, then the reverse reaction, elimination to form the conjugated carbon–carbon double bond, might be expected to give rise to a primary kinetic-isotope effect when deuterium is incorporated into the methylene group α to the carbonyl group. However, if the process taking place is addition to the carbonyl, then no primary isotope effect is to be expected. When a mixture of N,N-dimethylcysteamine and the thiolester are allowed to proceed to equilibrium in 98.6% deuterium oxide and then diluted in isotopically normal buffer under conditions where the final deuterium oxide concentration is 3.3%, the apparent rate of reappearance of the thiolester spectrum when compared with a control carried out in isotopically normal water gives an uncorrected deuterium isotope effect of $k_H/k_D \simeq 1.7$. Certainly, the rate of decomposition of a tetrahedral intermediate under such conditions would not lead to an observable deuterium isotope effect, since the rate of exchange of the hydroxyl proton or deuteron would be expected to be an extremely fast if not a diffusion-controlled process.[17]

The reaction scheme in eq 4–6 is fully consistent with the experimental observations just discussed for the reaction of **1** with N,N-dimethylcysteamine, and it is offered as a rationalization for this reaction. The stoichiometrically significant intermediate in the reaction of **1** with N,N-dimethylcysteamine is presumed to be **6**, since the apparent rate of appearance of the thiolester, **2**, is experimentally identical with the process which occurs when N,N-dimethylcysteamine undergoes reaction with **2**. However, the present information is insufficient to rule out compound **8** as stoichiometrically important, since the only kinetic requirement is that both steps k_1 and k_2 be rapid relative to step k_3, k_{-3}.

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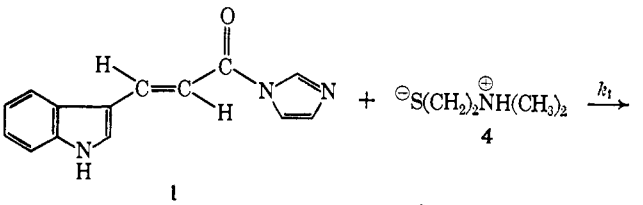
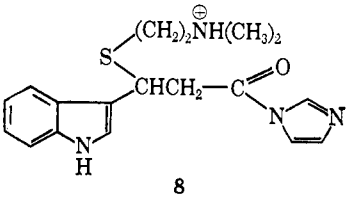
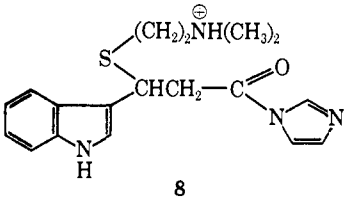


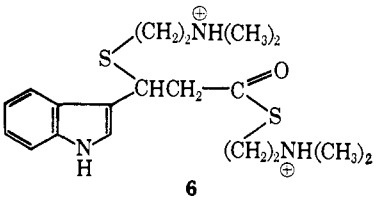
1 + 4 $\xrightarrow{k_1}$



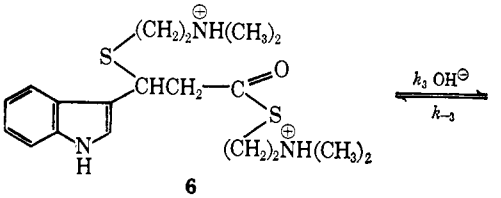
8 (4)



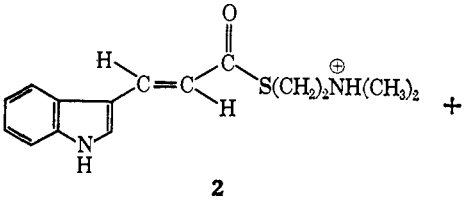
8 + 4 $\xrightarrow{k_2}$



6 + N⟨NH (5)



6 $\underset{k_{-3}}{\overset{k_3\ OH^{\ominus}}{\rightleftharpoons}}$



2 +

$^{\ominus}S(CH_2)_2\overset{\ominus}{N}H(CH_3)_2$ (6)

4

Less detailed studies on the reaction of *p*-methoxycinnamoylimidazole (**3**) with N,N-dimethylcysteamine indicate this system parallels the behavior of **1**. Here again the formation of nonchromophoric intermediates is indicated by the time course of the optical density changes, just as in Figure 2. When the apparent rate of that part of the reaction exhibiting an increase in optical density is compared with the apparent rate of reaction of the *p*-methoxycinnamoyl thiolester with N,N-dimethylcysteamine, once again, the rates are found to be experimentally identical. For example, at pH 7.88 and a N,N-dimethylcysteamine concentration of 1.98×10^{-3} *M*, the apparent rate from the second step was found to be 1.34×10^{-2} sec^{-1} and under the same conditions the apparent rate of approach to equilibrium for the thiolester reacting with N,N-dimethylcysteamine was found to be 1.27×10^{-2} sec^{-1}.

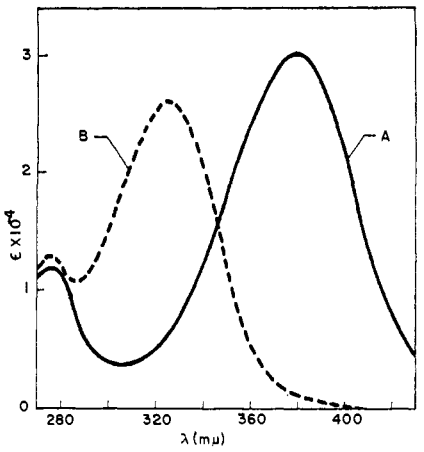


Figure 5. Curve A, the spectrum of **1**. Curve B, the spectrum of the conjugated amide, **13**, resulting from the reaction of **1** with cysteamine in pH 6.40 PO_4^{3-} 0.1 *M* buffer at 25.0°.

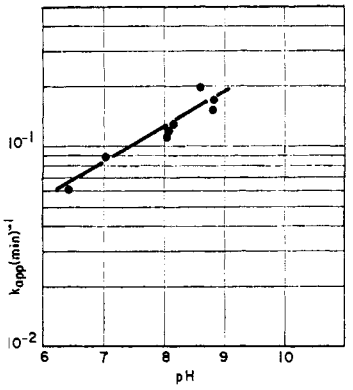


Figure 6. The apparent rate of disappearance of **1** as a function of pH for the reaction of **1** with cysteamine at 25.0° in 0.1 *M* PO_4^{3-} and $P_2O_7^{2-}$ buffers.

The reaction of **1** with excess cysteamine in the pH range 6–9 results in the formation of N-β-(3-indol)-acrylolylcysteamine (**13**) as the only observable chromophore. The spectra in Figure 5 are scans of the initial and final reaction mixtures at pH 6.4. The product is identified as the conjugated amide by comparison with the uv spectrum of N-β-(3-indol)acrylolylmethylamine,[3] λ max 320 mμ (ϵ_{max} 2.6 × 10^4 M^{-1} cm^{-1}).

In the neutral pH range (pH 6–9) the reaction of **1** with excess cysteamine gives optical-density changes (first order in thiol concentration) expressed by single-exponential rates over the entire time course of the reaction both for the disappearance of **1** and for the appearance of the product, provided that the cysteamine concentration is relatively low (but still in excess). Figure 6 shows the variation in the apparent, first-order rate constants with pH under these conditions. Repetitive uv spectrophotometric scans, during the reaction under conditions of low cysteamine concentration demonstrate a true isosbestic point between **1** and the conjugated amide product. However, the amide yield, as calculated from the initial concentration of **1** and a presumed extinction coefficient of 2.6 × 10^{-4} M^{-1} cm^{-1}, varies with pH, decreasing with increasing pH, as shown in Figure 7. The amide uv spectrum is pH independent in this pH range, and is unaffected by

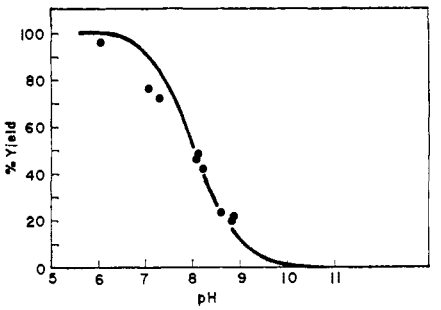


Figure 7. The yield of the conjugated amide, **13**, as a function of pH in 0.1 *M* PO_4^{3-} and $P_2O_7^{2-}$ buffers at 25.0°. The solid line is calculated for a rate dependence on an ionizable group of pK_a = 8.0.

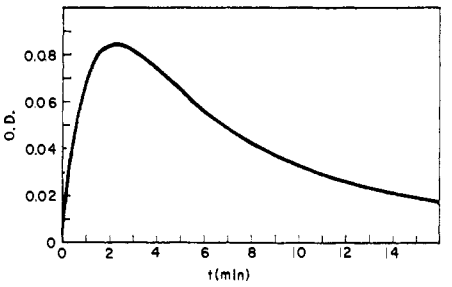


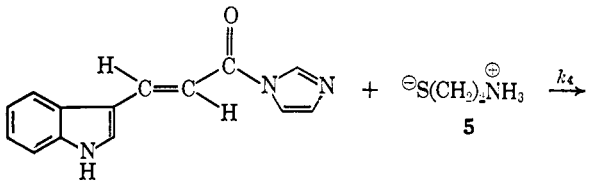
Figure 8. Time course of the optical density changes accompanying the reaction of **1** and cysteamine at the isoabsorptive point, 342 mμ, for **1** and the conjugated amide product, **13**, in pH 7.05 PO_4^{3-} 0.1 *M* buffer at 25.0°.

the presence of thiolate anion. At higher cysteamine concentrations (5–10 × 10^{-3} *M*) and at pH's of 7.5 and lower, the time-dependent optical-density changes during the reaction cannot be expressed by a single exponential. If the transient changes in optical density during the course of reaction are followed at 342.3 mμ, a wavelength where **1** and the conjugated amide have the same extinction coefficient, then the formation of one or more intermediates is clearly demonstrated, as shown in Figure 8.

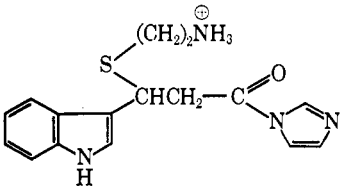
At pH 4, the reaction of **1** with cysteamine takes place at a considerably reduced rate, and the product which obtains is the thiolester, **12**. When the thiolester, synthesized in this way, is subjected to higher pH's, a more rapid, and quantitative transformation to the amide occurs, even in the presence of a large excess of cysteamine. The optical density changes accompanying this rearrangement follow apparent first-order kinetics. The pH dependence of this rearrangement over the pH range 7–10 clearly indicates a first-order dependence on hydroxide ion concentration, quite in accord with the work of Martin and Hendrick on the rearrangement of S-acetylcysteamine.[18]

The finding that the amide yield from the reaction of **1** with cysteamine is pH dependent suggests a kinetic scheme, eq 7–10, similar to the one proposed for the reaction of **1** with N,N-dimethylcysteamine (eq 4–6), but including as an additional complication the partitioning of an intermediate, **10**, between two pathways, one leading to the conjugated amide, **13**, the other leading to a nonchromophoric product tentatively assigned as structure **11**. Attempts to isolate **11**

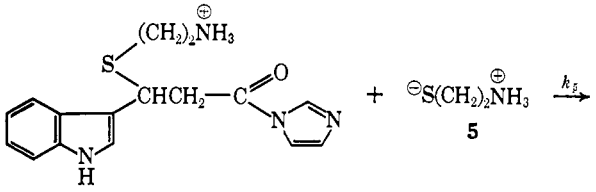
(18) R. B. Martin and R. I. Hendrick, *J. Am. Chem. Soc.*, **84**, 106 (1962).



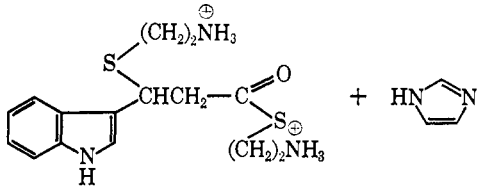
1 + $^{\ominus}S(CH_2)_2\overset{\oplus}{N}H_3$ (5) $\xrightarrow{k_4}$



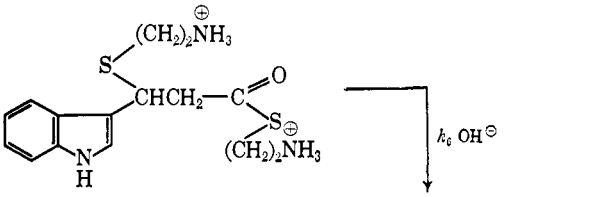
9 (7)



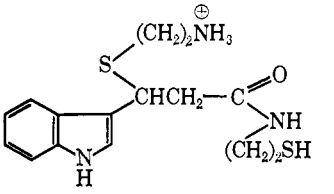
9 + $^{\ominus}S(CH_2)_2\overset{\oplus}{N}H_3$ (5) $\xrightarrow{k_5}$



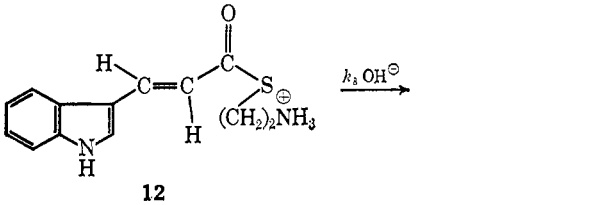
10 + HN⟨N (8)



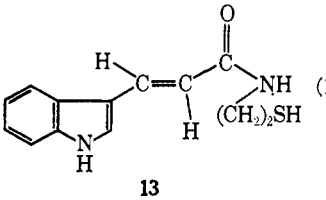
10 $\xrightarrow{k_6\ OH^{\ominus}}$



11 (9)



12 + $^{\ominus}S(CH_2)_2\overset{\oplus}{N}H_3$ (5) $\xrightarrow{k_8\ OH^{\ominus}}$



13 (10)

resulted in the isolation of a crude, amorphous material lacking uv absorption above 300 mμ, which on treatment with 2 *N* NaOH or on warming in methanol, acetonitrile, or acetone gave a material with a uv spectrum identical with that of the conjugated amide, **13**.

The choice of **10** as the partitioned intermediate is based in part on the observation of an isoabsorptive point, during kinetic runs, common to the spectrum of **1** and the conjugated amide, **13**. Thus, partitioning must take place after the slow step of this reaction, and partitioning of **1** is excluded. The thiolester, **12**, cannot be the partitioned intermediate, since experimentally the thiolester is found to rearrange quantitatively to the conjugated amide, **13**, even in the presence of a large excess of cysteamine. That the thiolester, **12**, is likely an intermediate at these pH's is indicated by the observation of a chromophoric intermediate, Figure 8, at high cysteamine concentrations, and pH's of 7.5 and lower. Only **9** and **10** remain as possibilities for the partitioned intermediate, and **10** is chosen as being the more likely on chemical grounds, although **9** cannot be ruled out.

The dependence of the conjugated amide yield on pH Figure 7, according to this scheme, is rationalized as resulting from a difference in the pH dependence of the competing steps k_6 and k_7, k_{-7}. Step k_6, leading to the nonchromophoric amide, involves N to S transfer and is expected to exhibit a first-order dependence on hydroxide ion, as is the case for the rearrangement of the conjugated thiolester and the rearrangement of S-acetylcysteamine.[18] In contrast, the step k_7, k_{-7}, involving elimination of cysteamine to form the conjugated thiolester, involves an equilibrium which, on the basis of the addition–elimination reactions of the N,N-dimethyl thiolester, **2**, with N,N-dimethylcysteamine (eq 6), should show a pH dependence paralleling the pK_a of cysteamine (pK_a = 8.3), since the reverse reaction (addition) should control the pH dependence, at least in the region of the pK_a of the nucleophile. Thus, the yield of the conjugated amide should be a function of this pK_a, as indeed it appears to be.

According to the kinetic schemes, eq 4–6 and eq 7–10, for the reactions of **1** with N,N-dimethylcysteamine and with cysteamine, respectively, the initial conjugate addition is fast relative to the displacement of imidazole from **1**. The second step in both schemes (eq 5 and 8) is required to be fast relative to subsequent steps, and in the first scheme, eq 4–6, this step is assumed to be at least comparable in rate to the first step, eq 4. The pK_a's of the conjugate acids of **1** (pK_a = 4.3, see Experimental Section) and of acetylimidazole[19] (pK_a = 3.6) as models for compounds **8** and **9**, suggest a rationale for the implied greater reactivity of the saturated acylimidazoles (**8** and **9**) with respect to **1**, since the 0.7 difference in pK_a's is undoubtedly a result of the extended system of conjugation of **1** which contributes to a lowering of the charge density on the carbonyl carbon, and thus to a lowering of the ground-state energy of the system relative to the saturated acylimidazoles.

The pH dependence of the rate of reaction of N,N-dimethylcysteamine with **2**, Figure 4a, clearly supports a mechanism for this conjugate addition reaction involving thiolate ion as the reactive nucleophile, since

(19) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272 (1959).

the experimental values of k_f fit the theoretical curve for a rate dependent on an ionizable group of $pK_a \sim 7.6$. The kinetically indistinguishable mechanism involving thiolate anion catalyzed addition of water is eliminated from consideration, since the equilibrium concentration of the adduct is thiol concentration dependent. Similarly, the thiolate anion catalyzed addition of thiol is eliminated from consideration, since the reaction kinetics would be second order in thiol concentration. The conclusion that thiolate anion is the reactive nucleophile is in accord with earlier findings involving the nucleophilic reactions of thiols at carbonyl carbon,[20] and no doubt applies to the reactions of **1** with N,N-dimethylcysteamine and with cysteamine as well.

The observation of conjugate addition in these systems as the kinetically observed first step is rather surprising. In casting about for a rationalization for this observation, Hine and Weimar's work on carbon basicity[21] comes to mind. They calculate from thermochemical data the basicities of various species toward a number of carbon acids relative to the reference base hydroxide ion. Thus, using Hine and Weimar's notation in eq 11, K_{HA}^{RA} is a measure of the R basicity

$$ROH + HA \overset{K_{HA}^{RA}}{\rightleftharpoons} RA + H_2O \qquad (11)$$

of the nucleophile A relative to its hydrogen basicity. From data given in their paper it is seen that the K_{HA}^{RA} values for thioalkoxide ion and alkoxide ion are considerably different for different carbon acids. The changes in K_{HA}^{RA}, Table II, for these two species do not vary in the same quantative way with changes in the carbon acid structure.

From the K_{HA}^{RA} values in Table II, it can be seen that the carbon basicities of the nucleophiles, A^-, relative to their hydrogen basicities, have the following qualitative orders: for thioalkoxide ions, $Me^+ \gg Ph^+ >> Ac^+$; for alkoxide ions, $Me^+ > Ph^+ \sim Ac^+$.

Even more striking is the observation that the Ac^+ carbon basicity of the thioalkoxide ion is of the order of 10^{-2} smaller than its hydrogen basicity, while the alkoxide ion has an Ac^+ basicity of the order of 10 greater than its hydrogen basicity, as might be expected from the significant resonance interaction between the carbonyl group and the alkoxy oxygen. The low basicity of the thioalkoxide ion toward Ac^+ indicates a fundamental difference between the nature of ester and thiolester electronic states as has been documented by other workers.[22,23] The K_A^R values in Table II, refer to the reaction

$$ROH + A^- \overset{K_A^R}{\rightleftharpoons} RA + OH^- \qquad (12)$$

and K_A^R measures the R^+ basicity of A^- relative to hydroxide ion. Consideration of these values allows a direct comparison of basicities. It is apparent that the CH_3^+ basicity of thioalkoxide ion is approximately 10^3 times as great as that of the corresponding oxygen species. In contrast, there is an Ac^+ basicity difference of 10^8 between alkoxide ion and thioalkoxide anion, while the Ph^+ basicities of alkoxide and thioalkoxide anion are of the same order of magnitude.

Table II. Selected Carbon Basicities[21]

R	A	K_{HA}^{RA}	K_A^R
Me^+	MeO^-	1.1×10^2	1.2×10
Me^+	MeS^-	2×10^{10}	4×10^4
Ph^+	MeO^-	1.4×10	1.5
Ph^+	MeS^-	6×10^5	1.1
Ac^+	EtO^-	6.8	6.1
Ac^+	$AcHN(CH_2)_2S^-$	5.9×10^{-2}	1.6×10^{-8}

On the basis of these data, if rates are assumed to parallel thermodynamic quantities, then the difference in the behavior of sulfur and oxygen nucleophiles regarding their modes of addition to conjugated carbonyl systems is explained quite adequately. However, a more detailed experimental inquiry into the factors influencing the relationship between carbon hybridization and nucleophile affinity would be germane to this relatively unexplored area of physical organic chemistry.

(20) J. R. Whitaker, *J. Am. Chem. Soc.*, **84**, 1900 (1962).
(21) J. Hine and D. Weimar, Jr., *ibid.*, **87**, 3387 (1965).
(22) M. Hauptschein, C. S. Stokes, and E. A. Nodiff, *ibid.*, **74**, 4005 (1952).
(23) I. Wadsö, *Acta Chem. Scand.*, **16**, 487 (1962).