# The Effect of Schiff Base Formation on the Thiocyanate-Catalyzed Isomerization of *cis*-$\beta$-Acetylacrylic Acid. A Study of a Possible Model for the Enzyme-Catalyzed Cis–Trans Isomerization of Maleylacetoacetic Acid[1a]

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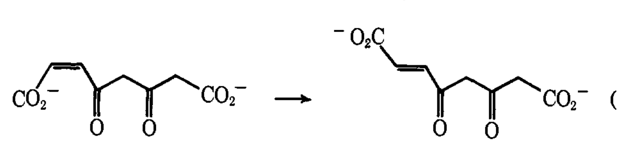
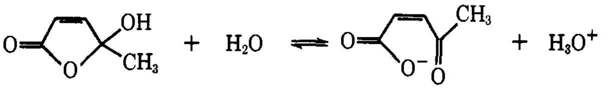
**Abstract:** A possible role for the enzyme in the cis–trans isomerization of maleylacetoacetate is suggested as providing an amino group for Schiff base formation with the substrate. As a possible model for this intermediate, the semicarbazone of *cis*-$\beta$-acetylacrylic acid has been prepared and its rate of cis–trans isomerization catalyzed by thiocyanate has been studied as a function of pH. Rate enhancement for the catalyzed isomerization is observed for the zwitterionic Schiff base as compared to the carbonyl precursor. Because of the apparently low concentration of zwitterion, however, the overall rate for semicarbazone species is slower than the rate for the original substrate. The site of semicarbazone protonation is discussed. A completely automatic system for taking kinetic measurements and entering them on IBM cards for computer processing is described.

Maleylacetoacetate (**1**), an enzymatic oxidation product of aromatic amino acids, has been shown to undergo enzyme-catalyzed cis–trans isomerization.[2] Necessary for the conversion of **1** to **2** is the coenzyme glutathione (GSH). Other sulfhydryl-containing reagents appear to activate the enzyme but cannot replace GSH.

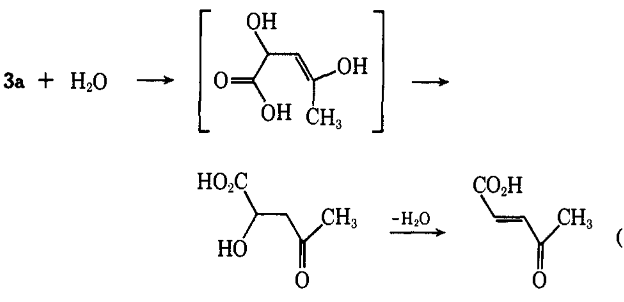


**1** **2** (1)

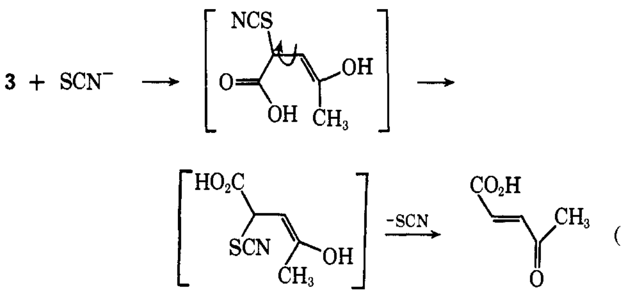
In a previous report,[3] the synthesis of a model substrate, *cis*-$\beta$-acetylacrylic acid (**3a**), was described and its cis–trans isomerization reported in a subsequent paper.[4] In approximately $10^{-3}$ *M* aqueous acid at 70°, a fraction of *cis*-acetylacrylic acid appears to isomerize by an addition–elimination mechanism as indicated by the loss and then the reappearance of the $\alpha,\beta$-unsaturated carbonyl chromophore during isomerization (eq 3).[4] Thiocyanate, on the other hand, a more efficient catalyst than water in causing cis–trans isomerization of **3**, does not appear to form an intermediate of a long enough lifetime and in large enough quantity to permit facile detection. A mechanism (eq 4) similar to that for the water-catalyzed reaction, but differing in that rotation about the carbon–carbon bond and ejection of thiocyanate ion are faster than protonation of carbon, has been suggested. The role of GSH in the enzymatic reaction has been proposed to be that of a nucleophile behaving in a way similar to thiocyanate ion in eq 4.[4]



**3a** **3b** (2)



**4a** (3)



(4)

The function of the enzyme in the conversion of **1** to **2**, however, still remains obscure. A possible role is suggested by the presence of the substituted acetoacetate group. Acetoacetate itself undergoes enzyme-catalyzed decarboxylation. The mechanism has been elegantly demonstrated to involve the formation of a Schiff base with a lysine residue of acetoacetate decarboxylase.[5] Because the Schiff base's imine group is a stronger base than the precursor carbonyl group of acetoacetate, a larger fraction is in the form of its conjugate acid. The positive charge, delocalized on the atoms $\beta$ and $\gamma$ to the carboxylate group, thus promotes decarboxylation because of the large electrostatic attraction in the zwitterion.

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(1) (a) Research carried out under the auspices of the U. S. Atomic Energy Commission; (b) this investigation was supported in part by a National Institutes of Health Special Fellowship 1 FO3 GM06392-01 from the General Medical Sciences.

(2) For a review, see W. E. Knox in "The Enzymes," Vol. 2, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1960, pp 282–289.

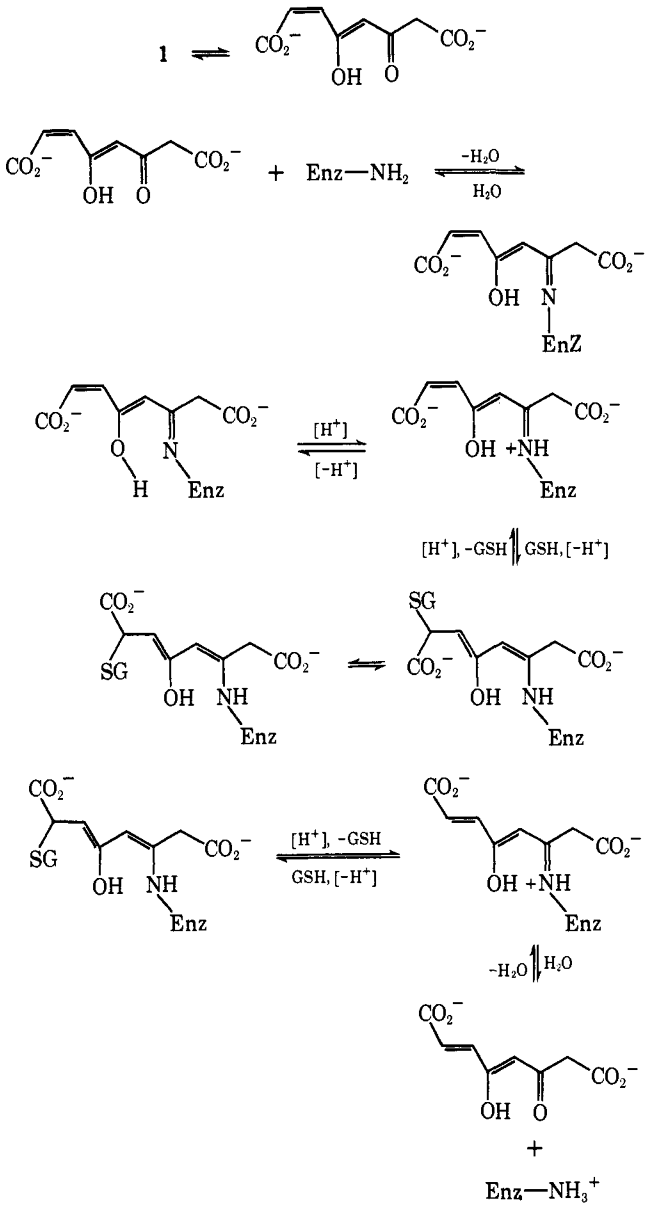
(3) S. Seltzer and K. D. Stevens, *J. Org. Chem.*, 33, 2708 (1968).

(4) K. D. Stevens and S. Seltzer, *ibid.*, 33, 3922 (1968).

(5) I. Fridovich and F. H. Westheimer, *J. Amer. Chem. Soc.*, 84, 3208 (1962); S. Warren, B. Zerner, and F. H. Westheimer, *Biochemistry*, 5, 817 (1966).

A similar type of catalysis can be suggested for maleylacetoacetate isomerase as shown in Scheme I; a parallel mechanism can also be suggested for maleylpyruvate isomerase. Here too, GSH has been shown to be required.[6] Instead of GSH, were the nucleophile, attacking the carbon–carbon double bond of the Schiff base (Scheme I), a sulfhydryl group covalently bound to enzyme, the proposed mechanism would be rendered unlikely because of the structural changes required of the protein in order to permit internal rotation of the bound substrate. Even though enzyme-catalyzed cis–trans isomerization of maleate does not require GSH but is dependent on enzyme-bound sulfhydryl groups for activity,[7] the proposed mechanism is not challenged because the formation of a Schiff base (or similar entity providing enhanced nucleophilic attack at the carbon–carbon double bond) between enzyme and substrate is held unlikely.

**Scheme I**



As a test of Scheme I we have attempted to look for Schiff base formation and amine-catalyzed cis–trans isomerization in the presence of thiocyanate ion. These studies were carried out with *cis*-β-acetylacrylic acid as a model for maleylacetoacetic acid. We report here the effect of semicarbazone formation on the rate of thiocyanate ion catalyzed cis–trans isomerization.

## Experimental Section

Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratories.

The amine derivatives used in this study were obtained from the suppliers listed below and used without further purification: imidazole, Calbiochem (grade A); pyridoxamine dihydrochloride, Mann (USP grade); guanadine hydrochloride, aniline hydrochloride, and L-lysine hydrochloride, Eastman; proline, Nutritional Biochem; morpholine, Fisher; aminoacetonitrile bisulfate, Aldrich.

**Semicarbazones of *cis*- and *trans*-β-Acetylacrylic Acid.** Either acid was dissolved in a minimum amount of water and then neutralized with 4 *N* sodium hydroxide. An equivalent amount of semicarbazide hydrochloride was added. The semicarbazone generally precipitated shortly after the semicarbazide dissolved in the stirred solution. The precipitate was filtered and washed three times with ether. The trans compound was recrystallized from ethanol, mp 203.5–204.5° (lit.[8] mp 218°). *Anal.* Calcd for $C_6H_9O_3N_3$: C, 42.10; H, 5.30; N, 24.56. Found: C, 41.93; H, 5.52; N, 24.00. The position of $\lambda_{max}$ is pH dependent. It varies from 288 nm ($\epsilon$ 24,200) at pH 2.22 to 279 nm ($\epsilon$ 23,850) at pH 5.55 in 3 *M* lithium perchlorate. Its nmr spectrum taken in DMSO-$d_6$ exhibits an AB quartet ($\tau$ 3.87, 2.81, $J_{AB}$ = 16 Hz, vinyl protons), a singlet (8.05, methyl), and a broad singlet (~3.38, position variable, NH and OH protons).

**4-Oxo-*cis*-penteneoic acid semicarbazone,** mp 106.5–107.5°, prepared by the method above, was used without further purification. *Anal.* Calcd for $C_6H_9O_3N_3$: C, 42.10; H, 5.30; N, 24.56. Found: C, 41.83, 41.83; H, 5.74, 5.28; N, 24.69, 24.42. The position of the uv maximum varies from 287.5 nm ($\epsilon$ 5214) at pH 2.16 to 268.7 nm ($\epsilon$ 15,400) at pH 7.00. Its nmr spectrum taken in dimethyl-$d_6$ sulfoxide exhibits a singlet ($\tau$ 8.03, methyl), a partially masked AB quartet (4.00, 3.61, $J_{AB}$ = 12.6 Hz, vinyl protons), a singlet (3.52, broad, probably NH's), and a very broad peak at 3.90 (probably OH). The spectrum in dimethyl-$d_6$ sulfoxide to which a few drops of $D_2O$ is added is similar to the one above except that the latter two broad peaks are absent and a water peak appears. The positions of the remaining peaks are singlet ($\tau$ 8.02, methyl) and AB quartet (3.94, 3.48, $J_{AB}$ = 12.3 Hz, vinyl). Attempted recrystallization of the *cis*-acid semicarbazone from ethanol yielded a compound exhibiting the properties of the *trans*-acid semicarbazone, mp 204.5°. *Anal.* Calcd for $C_6H_9O_3N_3$ C, 42.10; H, 5.30; N, 24.56. Found: C, 41.55; H, 5.20; N, 24.38.

**3-Methyl-6-pyridazone.** If the concentrations of *cis*-acid and semicarbazide are so low as to delay precipitation of the semicarbazone and if the resulting solution is acidified, 3-methyl-6-pyridazone is obtained instead of the semicarbazone. 4-Oxo-*cis*-penteneoic acid (342 mg) was dissolved in aqueous sodium hydroxide so that when an equivalent amount of semicarbazide hydrochloride was added the resulting pH was 5.47. The solution was concentrated without precipitation of product and was acidified with 50 ml of 0.01 *N* hydrochloric acid. A small white precipitate settled (*trans*-acid semicarbazone) and was filtered off. Continuous extraction (5 days) of the remaining solution with ether yielded 100 mg of ether-insoluble white crystals which appear to be 3-methyl-6-pyridazone monohydrate: mp 117–121.5°; uv $\lambda_{max}$ 285 nm ($\epsilon$ 2520, $H_2O$, pH 7.5) (lit.[9a] mp 119–123°; $\lambda_{max}$ 285 nm ($\epsilon$ 2200, $H_2O$)). *Anal.* Calcd for $C_5H_8N_2O_2$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.78; H, 6.15; N, 22.13. An nmr spectrum in DMSO-$d_6$, using a CAT, exhibited a singlet ($\tau$ 7.76, methyl), a very broad singlet (6.31, NH and OH), and an AB quartet (3.10, 2.59, $J_{AB}$ = 10.2 Hz, vinyl protons). After grinding and drying the crystals of the monohydrate the water of crystal-

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[6] L. Lack, *J. Biol. Chem.*, **236**, 2835 (1961); *Biochem. Biophys. Acta*, **34**, 117 (1959).

[7] W. Scher and W. B. Jakoby, *J. Biol. Chem.*, **244**, 1878 (1969); E. J. Behrman and R. Y. Stanier, *ibid.*, **228**, 923 (1957); K. Otsuka, *Agr. Biol. Chem. (Tokyo)*, **25**, 726 (1961).

[8] H. Stetter and C. W. Schellhammer, *Justus Liebigs Ann. Chem.*, **605**, 58 (1957).

[9] (a) W. G. Overend and L. F. Wiggins, *J. Chem. Soc.*, 239 (1947); 3500 (1950); (b) O. Poppenberg, *Ber.*, **34**, 3257 (1901).

lization was removed to yield a white solid, mp 144–145° (lit. 138°,[9a] 143°[9b]).

**Acid Dissociation Constant of *cis*-β-Acetylacrylic Acid Semicarbazone.** Solutions of the semicarbazone in 0.01 *M* acetic acid–sodium acetate mixtures containing 3 *M* lithium perchlorate were prepared and their pH's measured with an Orion pH meter. The optical density at 280 nm was measured on a Cary 14 as a function of pH and the observed extinction coefficients ($\epsilon_{280}$) are shown in parentheses as follows: 2.158 (4849), 3.023 (5414), 3.849 (7059), 4.099 (9128), 4.434 (10,433), 4.722 (11,243), 5.298 (12,126), 5.445 (11,832), 5.879 (11,974), 7.003 (12,403).

**Site of Protonation in Acetone Semicarbazone.** Acetone semicarbazone, prepared in the usual way, was dissolved in dimethyl-$d_6$ sulfoxide to give about a 15% solution. The nmr (A-60) spectrum of the solution at 30° was in accord with that previously reported[10] and was examined at temperatures up to 170°. A similar solution of 51.9 mg (0.451 mmol) of acetone semicarbazone and 0.3033 g of dimethyl-$d_6$ sulfoxide was acidified with 18.4 mg (0.161 mmol) of trifluoroacetic acid (Eastman White label). The nmr spectrum was examined at intervals up to 70°. Prior to these measurements the variable-temperature probe was calibrated at 60 and 160° with an ethylene glycol sample.

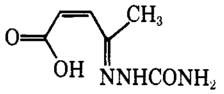
**Automatic Kinetic Measurements.** The rate of cis–trans isomerization was determined spectrophotometrically by observing the increase of absorption at 280 nm due to the appearance of *trans*-β-acetylacrylic acid semicarbazone or in some cases the loss of reactant at 270 nm. A Cary–Datex digital system, with only the pen position digitized, was connected to a Cary 11 double beam spectrophotometer. A digital clock,[11] compatible with the Cary–Datex code, was connected to the system. The system in turn was connected to an IBM 029 card punch. In this way simultaneous time and optical density readings, triggered by preset intervals in both time and optical density, were punched on IBM cards. The data entered on IBM cards were treated with a nonlinear least squares program to yield the best fit to a first-order rate law. Visual verification of the correctness of the calculated first-order rate constant was obtained by a CALCOMP 835 plotter drawing the calculated optical density–time curve and superimposing the experimental points on the graph.

## Results and Discussion

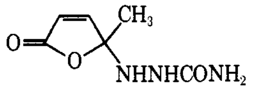
The possibility of catalysis through a Schiff base intermediate was studied by examining the effect that various nitrogenous bases have on the uv spectrum of *cis*-β-acetylacrylic acid. The formation of a considerable quantity of the Schiff base would be expected to result in the appearance of a peak at a somewhat higher wavelength than the keto acid. Alternatively, if only a small amount of Schiff base formed but was labile with respect to cis–trans isomerization and subsequent hydrolysis, then the appearance of absorption due to *trans*-β-acetylacrylic acid in the uv might be expected to form at a faster rate than that observed in the absence of amines.

Since acetals and ketals themselves do not undergo nucleophilic substitution but require an elimination–addition mechanism to exchange oxygen atoms, it would appear that they also do not react directly with amines to form Schiff bases.[12] By analogy, it is anticipated that the pseudoacid **3a** would not react with amines to form a Schiff base. For this reason the attempted reactions of *cis*-β-acetylacrylic acid with amines were carried out at pH $\simeq$ p$K_a$ + 2 where the predominant form of the substrate is **3b**, the ketocarboxylate anion. When the substrate ($\sim 4 \times 10^{-4}$ *M*), however, was mixed with imidazole (amine/substrate = a/s = 1 or 10), guanadine (a/s = 10–20), aniline (a/s = 1), pyridoxamine (a/s = 1), or proline (a/s = 10), the uv spectra were invariant with time; this suggests that even in this pH region, the rate of reaction of the substrate with these compounds at these concentrations is too slow. Similar treatment of the *trans*-acid with guanadine, aniline, or proline produced no reaction. Lysine (a/s = 10), morpholine (a/s = 10), and aminoacetonitrile (a/s = 10) caused a decrease in the initial optical density of the *cis*-acid, suggesting that Michael addition rather than Schiff base formation was occurring. The decrease in optical density might also be accommodated by the formation of a carbinolamine intermediate[13] which dehydrates very slowly, but there was no subsequent increase in optical density, however, even after 10 hr. Moreover, higher concentrations of amine and substrate when examined by nmr indicated continual loss of vinyl hydrogen absorption and lends further support to the suggestion that addition to the carbon–carbon double bond of the substrate is the predominant reaction. On mixing hydroxylamine (a/s = 10) or semicarbazide (a/s = 1) with the *cis*-acid, a rapid increase in optical density at 260 nm was observed suggesting the formation of a new chromophore. That this chromophore, in the case of the reaction with semicarbazide, was different from that exhibited by the *trans*-acid itself or from the product of *trans*-acid with semicarbazide ($\lambda_{max}$ 279 nm, pH dependent), suggested that the semicarbazone of *cis*-β-acetylacrylic acid could be isolated and its nucleophilic cis–trans-catalyzed isomerization studied.

Reaction of a saturated neutralized aqueous solution of **3a** with an equal quantity of semicarbazide hydrochloride yields the desired semicarbazone but unfortunately it isomerizes to the trans isomer upon recrystallization from ethanol. Further attempts to purify the semicarbazone were abandoned since thin layer chromatography also resulted in cis–trans isomerization. As shown previously,[3] the *cis*-acid exists as the cyclic pseudoacid, **3a**, but as the open anion, **3b**, when neutralized. This apparently is not the case with the *cis*-acid semicarbazone; the spectra are in accord with an open structure, **4a**, and not with a closed structure comparable to **4c**. The uv absorption maximum for **4c** would be expected to be near 200 nm as compared to the observed 287-nm absorption. Moreover, the observed vinyl coupling constant of 12–13 Hz is in line with a *cis*-acyclic double bond as in **4a** while the vinyl proton coupling constant for a structure as **4c** might be expected to be about 5–7 Hz.[3] Finally, the position of the methyl resonance for the *cis*-semicarbazone is almost in the identical position as the methyl for the trans compound which would not be expected if the *cis*-semicarbazone were cyclic.[3,4]



**4a**



**4c**

A different product is isolated from the reaction of *cis*-keto acid with semicarbazide if the medium is dilute and approximately 0.01 *N* in hydrochloric acid. After continuous extraction with ether, 3-methyl-6-pyridazone (**5**) is formed by acid-catalyzed cyclization of the semicarbazone and the hydrolytic loss of car-

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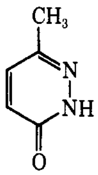
[10] G. J. Karabatsos, F. M. Vane, R. A. Taller, and N. Hsi, *J. Amer. Chem. Soc.*, **86**, 3351 (1964).

[11] Designed and constructed by the Instrumentation Division, Brookhaven National Laboratory.

[12] W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).

[13] W. P. Jencks, *J. Amer. Chem. Soc.*, **81**, 475 (1959).

bamic acid. The assignment of the structure of the product is based on elemental analysis, melting point, and uv spectra reported for it and its hydrate.[9] The



5

observed vinyl coupling constant of 10.2 Hz is also in accord with 5. *cis*-Vinyl protons in a six-membered ring have coupling constants in the range of 9.9–10.5 Hz.[14] 5 is relatively stable in aqueous solution in the presence of potassium thiocyanate as shown by the unchanging uv spectrum with time of a mixture of these compounds; the *cis*-semicarbazone behaves differently.

**Table I.** Rates of Solvent-Catalyzed Isomerization and Hydrolysis of *cis*-β-Acetylacrylic Acid Semicarbazone[a]

| pH | $10^4 k_{\mathrm{obsd}}$, sec$^{-1}$ |
|---|---|
| 3.256 | 3.49 |
| 4.016 | 1.93 |
| 4.749 | 0.518 |
| 5.310 | 0.259 |
| 5.640 | 0.216 |

[a] In 0.01 *M* acetate buffers and 3 *M* lithium perchlorate at 25°.

**Table II.** The Rate Constant Dependence on pH in the Isomerization of *cis*-β-Acetylacrylic Acid Semicarbazone[a]

| pH | $10^3 k_{\mathrm{obsd}}$, sec$^{-1}$ [b] | $10^3 k_{\mathrm{uncat}}$, sec$^{-1}$ [c] | $10^3 k_{\mathrm{cat}}$, sec$^{-1}$ Obsd | Calcd |
|---|---|---|---|---|
| 3.378 | 11.1 | 0.33 | 10.8 | 9.63 |
| 3.654 | 7.97 | 0.24 | 7.73 | 8.00 |
| 4.019 | 4.98 | 0.16 | 4.82 | 5.43 |
| 4.045 | 5.30 | 0.16 | 5.14 | 5.24 |
| 4.655 | 2.07 | 0.067 | 2.00 | 1.89 |
| 5.038 | 0.933 | 0.042 | 0.891 | 0.860 |
| 5.597 | 0.273 | 0.022 | 0.251 | 0.254 |

[a] In 0.01 *M* acetate buffers at 25°. [b] In 3 *M* potassium thiocyanate. [c] Pseudo-first-order rate constant for loss of *cis*-β-acetylacrylic acid semicarbazone in 3 *M* lithium perchlorate and in the absence of potassium thiocyanate.

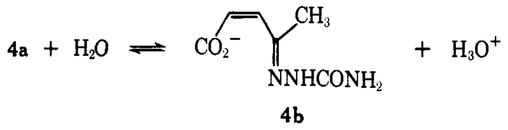
The semicarbazone of the *cis*-acid undergoes catalyzed isomerization to the *trans*-acid semicarbazone as indicated by the uv spectral changes during reaction and comparison of the final spectrum with that from an authentic sample of the *trans*-acid semicarbazone. In the absence of thiocyanate ion, the absorption at 280 nm decreases slowly to approximately half its original value even though the ratio of extinction coefficients of the *trans*- to the *cis*-acid semicarbazones at this wavelength is about 2.9:1. We assume that the considerable loss of extinction under these conditions is due to the concomitant hydrolysis of the *cis*-semicarbazone; the hydrolytic products do not contribute significantly to the absorption at 280 nm. Approximately 15% of the reactant undergoes cis–trans isomerization and the remainder hydrolyzes. In the absence of added nucleophile, the disappearance of substrate by these two paths can be treated as pseudo-first-order reactions and all observed catalyzed rates were corrected for these side reactions. To ensure that the side reactions contributed negligibly to the overall rate of substrate disappearance, the catalyzed isomerization studies were carried out in 3 *M* potassium thiocyanate. The uncatalyzed rates were studied in 3 *M* lithium perchlorate at similar hydrogen ion activities. The results are shown in Tables I and II. That the reaction is first order in potassium thiocyanate is shown by the data in Table III.

**Table III.** Catalyzed Isomerization of *cis*-β-Acetylacrylic Acid Semicarbazone. Dependence of Rate on the Thiocyanate Ion Concentration[a]

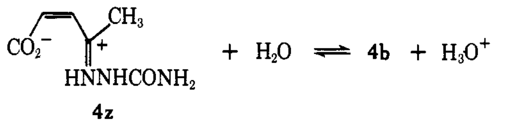
| pH | [KSCN], *M* | [LiClO$_4$], *M* | $10^3 k_{\mathrm{obsd}}$, sec$^{-1}$ | $10^3 k_{\mathrm{uncat}}$, sec$^{-1}$ [b] | $10^3 k_{\mathrm{cat}}$, sec$^{-1}$ |
|---|---|---|---|---|---|
| 4.044 | 1.0 | 2.0 | 2.20 | 0.16 | 2.04 |
| 4.062 | 2.0 | 1.0 | 3.72 | 0.16 | 3.56 |
| 4.045 | 3.0 | 0.0 | 5.30 | 0.16 | 5.14 |

[a] In 0.01 *M* acetate buffers at 25°. [b] Pseudo-first-order rate constant for loss of *cis*-β-acetylacrylic acid semicarbazone in 3 *M* lithium perchlorate and in the absence of potassium thiocyanate.

The catalyzed rate of isomerization is strongly affected by the acidity of the medium. The principle factors responsible for this behavior appear to be the acid dissociation equilibrium of the substrate over the pH range studied and the different thiocyanate ion catalyzed rates for the acid, **4a**, conjugate base, **4b**,



4b (5)



4z (6)

and zwitterion, **4z**. Although reaction 10 is kinetically indistinguishable from reactions 8 and 9, it is not explicitly considered here. It would appear that this reaction is less favored because of the very low concentration of thiocyanic acid in the pH range studied and because of its expected lower nucleophilicity. If

$$\mathbf{4b} + \mathrm{SCN}^- \xrightarrow{k_7} \text{products} \qquad (7)$$

$$\mathbf{4a} + \mathrm{SCN}^- \xrightarrow{k_8} \text{products} \qquad (8)$$

$$\mathbf{4z} + \mathrm{SCN}^- \xrightarrow{k_9} \text{products} \qquad (9)$$

$$\mathbf{4b} + \mathrm{HSCN} \xrightarrow{k_{10}} \text{products} \qquad (10)$$

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(14) O. L. Chapman, *J. Amer. Chem. Soc.*, **85**, 2014 (1963).

this kinetic scheme is adopted, the observed pseudo-first-order catalytic rate constant is given by

$$(k_{\text{cat.}})_{\text{obsd}} = \frac{k_7K + k'(\text{H}_3\text{O}^+)}{K + (\text{H}_3\text{O}^+)} \qquad (11)$$

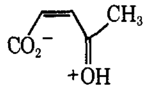
where $K = K_5K_6/(K_5 + K_6)$, $k' = (k_8K_6 + k_9K_5)/(K_5 + K_6)$, $K_5 = [\mathbf{4b}](\text{H}_3\text{O}^+)/[\mathbf{4a}]$, and $K_6 = [\mathbf{4b}](\text{H}_3\text{O}^+)/[\mathbf{4z}]$. A least squares computer program was written to find the values of $K$, $k_7$, and $k'$ which when substituted in eq 8 give the smallest standard deviation (8%) in the relative errors between observed and calculated catalytic rate constants (*cf.* columns 4 and 5, Table II) at various acidities. The values of the equilibrium and rate constants obtained from this least squares treatment are shown in Table IV. Support for this treatment is given by the close agreement between the experimentally determined p$K_a$ (4.04) and the one calculated (3.90) by using the above scheme.[15] The second-order rate constants for the corresponding reactions of thiocyanate with anion **3b** and pseudoacid **3a** are also given for comparison. On preliminary examination it appears that conversion of the carbonyl group of **3** to the semicarbazone Schiff base does not provide the anticipated increase in the rate of cis-trans isomerization.

**Table IV.** Rate and Equilibrium Constants for Thiocyanate Ion Catalyzed Cis–Trans Isomerizations. Second-Order Rate Constant for Reaction of Thiocyanate Ion with **3** and **4**

| | **3** | **4** |
|---|---|---|
| **a** + **z** | $8.3 \times 10^{-3}$ [a] | $4.2 \times 10^{-3}$ [a] |
| **b** | $5.2 \times 10^{-5}$ [a] | $2.0 \times 10^{-6}$ [a] |
| | Dissociation Constants[b] | |
| | $3.31 \times 10^{-5}$ [c] | $1.25 \times 10^{-4}$ [c] |

[a] $M^{-1}$ sec$^{-1}$. [b] $K = (\text{H}_3\text{O}^+)[\text{A}^-]/[\text{HA}]$. [c] Molar.

Ketones and aldehydes are generally less basic than their corresponding semicarbazones. Benzaldehyde is less basic than its semicarbazone by six powers of ten.[16] If **3z** is at all present in acidic solutions of **3**,[17]



**3z**

**4z** might be expected to be present in $10^6$ greater quantity, all other things being equal. Both might be expected to be highly reactive toward thiocyanate ion in undergoing catalyzed cis–trans isomerization. Judging from the basicity of the few aliphatic ketones studied, the p$K_a$'s of the protonated forms appear to be about −7.[16] The basicity is increased by 3–7 p$K$ units when, upon protonation, the positive charge can be delocalized to a large extent as in 2,6-dimethylpyrone. If such an enhancement of carbonyl basicity in **3** were to operate and if the basicity factor of $10^6$ between semicarbazone and carbonyl group were to obtain in this system, then one might except to have kinetically significant quantities of **4z** present in the pH range studied in this investigation. The fact that the second-order rate constant, $k'$, a composite of the rate constant for reaction of thiocyanate with **4a** and **4z**, is, however, less than that for the similar reaction of thiocyanate with **3a** (and perhaps **3z**) suggests that (a) the concentration of **4z** is kinetically insignificant and that **4a** is less reactive than **3a**, or (b) that the reactivity of **4z** is lower than that of **3a**. In connection with the latter proposal there is some indirect evidence that suggests that protonation of benzaldehyde semicarbazone occurs on the terminal carboxamide instead of the imino nitrogen.[18] If this zwitterion, **4z'**, were the more predominant one then it might very well be less reactive than **3a**. Karabatsos and coworkers,[10] however, suggest that semicarbazones are protonated on imino nitrogen. In particular, they noted a slight broadening of the methyl doublet in the nmr of acetone semicarbazone when dissolved in trifluoroacetic acid at room temperature. In the present study we find that the methyl doublet of acetone semicarbazone dissolved in dimethyl sulfoxide remains sharp up to at least 170°. If only 0.33 equiv of trifluoroacetic acid is added to this solution a small sharp peak appears about 13 Hz downfield from the doublet with a half-time of approximately 1 min at ambient temperature and the doublet decreases slightly in intensity. The new peak is acetone and comes about through acid-catalyzed hydrolysis of the semicarbazone by the water contaminant in the solvent. The methyl doublet in this acidified semicarbazone solution has a coalescence temperature of 66° and reverts to a doublet when cooled. Above this temperature the two methyl groups become magnetically equivalent on the nmr time scale due to the rapid rotation about the carbon–imino nitrogen bond. The acetone peak remains sharp. That this reduction in the free energy of the barrier to rotation of at least 6 kcal/mol or more takes place on acidification is best rationalized by the suggestion that protonation occurs on imino nitrogen and that p-orbital overlap between that nitrogen and carbon is weakened by the electron demand of the proton. It is also interesting to note that the results also require that in dimethyl sulfoxide transfer of a proton from protonated acetone semicarbazone to unprotonated acetone semicarbazone must also be rapid on the nmr time scale. Another mechanism for methyl exchange might be the rapid exchange of isopropylidene groups between acetone and acetone semicarbazone but this can be dismissed by the observation that the acetone peak remains sharp while the doublet coalesces. These results do not answer completely the question of the site of protonation in *cis*-β-acetylacrylic acid semicarbazone in aqueous solution. A more direct study of methyl magnetic equivalence in *cis*-β-acetylacrylic acid semicarbazone is precluded, however, by the fact that the methyl resonances in this compound and its trans isomer are singlets. Furthermore, the high lability of the cis compound toward isomerization and hydrolysis in acidic aqueous solutions makes it difficult to probe the site of protonation by other means in this medium.

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(15) The p$K_a$ for *cis*-acid semicarbazone was determined graphically from a plot of $(\text{OD}_{\text{obsd}} - \text{OD}_{\text{pH}2.1})/(\text{OD}_{\text{pH}7.0} - \text{OD}_{\text{pH}2.1})$ *vs.* pH. The dissociation constant determined this way is identical in form with the kinetically determined constant; the identity is independent of the presence of **4z**.

(16) E. M. Arnett, *Progr. Phys. Org. Chem.*, 1, 223 (1963).

(17) The demonstration of a single isosbestic point in uv spectra of **3** at different pH's[4] does not exclude the possibility of a constant [**3a**]/[**3z**] ratio. The nmr spectrum of **3**,[3] however, suggests that [3a] ≫ [**3z**].

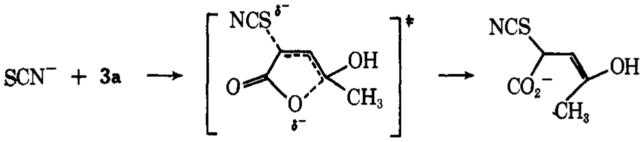
(18) R. Wolfenden and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 2763 (1961).

For the present we suggest that because of the structural resemblance, though minimal, of acetone semicarbazone to *cis*-$\beta$-acetylacrylic acid semicarbazone and the similarity of solvent polarity of water to dimethyl sulfoxide, it is most likely that protonation is on the imino nitrogen of **4a** in acidic aqueous solutions. If this is accepted it is also reasonable to propose that the proton resides on imino nitrogen in the zwitterion, **4z**, and accepting this structure there is no compelling evidence to suggest that **4z** should be less reactive than **3a** toward nucleophiles.

A question directly connected with the former case (*i.e.*, proposal (a) above) is: Does substitution of a Schiff base alone for a carbonyl group, *i.e.*, in the absence of protonation of imino nitrogen in the semicarbazone or of the keto oxygen in the precursor, affect the rate of nucleophilic attack at the $\beta$-carbon atom of the double bond? The individual catalyzed rates for **4a** and **4z** cannot be separated and therefore the reactivity of **3a** and **4a** cannot be compared directly; the reactivity of **3b** *vs.* **4b**, however, can. Conversion of the carbonyl group to the semicarbazone decreases the rate of thiocyanate attack on the anion by about a factor of 25 (Table IV). If **3a** is also 25 times more reactive than **4a** then it can be seen qualitatively that the protonated Schiff base must be substantially more reactive than **3a** in order that the combined reactivity of **4a** and **4z** be only half that of **3a** (Table IV). A more quantitative comparison of the rates cannot be made without a knowledge of the concentration of the zwitterion. Although the catalytic effect sought for in this study is here a modest one it might be enhanced further with other nitrogenous bases.

Semicarbazones are more basic than their carbonyl precursors but they are less basic than the corresponding *N*-alkyl- or -arylimines.[16] Thus, Schiff bases formed from more basic amino groups, as in the case of the lysine residue of acetoacetate decarboxylase, will have a greater fraction of its molecules in the protonated form than the corresponding semicarbazones at comparable pH's and might be expected to provide greater catalysis.

It is interesting to compare **3** with **4** in its reaction with thiocyanate ion. The 160-fold greater rate of thiocyanate-catalyzed isomerization of **3a** compared to **3b** can be ascribed to at least two possible factors. (1) The approach of two isovalent ions is not as favorable as the attack of a univalent ion on a neutral species. (2) Attack of thiocyanate $\alpha$ to the carbonyl group of **3a** is postulated to occur with ring opening, thereby providing a carboxylate ion as a leaving group while forming an enol. Anion, **3b**, however, is acyclic and reaction at pH 6 or greater leads to an enolate anion which must be unstable near neutral pH's and would appear to require protonation. Neutral acid **4a** similarly undergoes more rapid catalyzed isomerization than its conjugate base **4b**. Since both **4a** and **4b** are both acyclic the latter factor discussed above is not applicable to this pair. Thus the large rate ratio appears to be mainly controlled by electrostatics.



**Acknowledgment.** S. S. would like to express his gratitude to the National Institutes of Health for awarding him a Special Fellowship (1969–1970) and to the members of the Graduate Department of Biochemistry, Brandeis University, who have been so gracious to him while on sabbatical leave during which time this paper was prepared.