poly(ϵ-aminomethacrylyl-L-lysine), with charged zwitterionic side chains projecting into the solvent water, shows a $k_{\min}$ of 0.0041 (compare with values in Table II), approximately 40-fold reduced with respect to its monomer. It seems unlikely that all of these different polymers spend the same fraction of time, about 98%, in conformations blocked from exposure to solvent. Furthermore, all of these polymers have intrinsic viscosities corresponding to unstructured, relatively swollen configurations. These should be relatively "porous" to solvent.

On the other hand, in the polymer matrix every [-CONH-] group is surrounded by an apolar environment built up by the backbone of the polymer, the immediate substituents of the amide group, and neighboring residues pendant from the backbone chain. In this apolar environment charged intermediates such as III and IV would be unfavored. Thus rate constants for their formation would be diminished. Furthermore, in the local neighborhood of the polymeric matrix, the local concentration of residues would be several molar, even in a relatively open conformation. In essence, then, the local solution would correspond to a relatively concentrated organic–aqueous solution. In such an environment K_w of water is decreased.[33] In addition, in the neighborhood of the polymer there will be interactions between the apolar substituents and H_2O molecules, and the structure of water would be different than in bulk solvent. This will surely affect the solvation of charged intermediates III and IV, as well as of the alkyl groups.

It seems reasonable to conclude, therefore, that the placement of an amide group in a polymeric matrix is accompanied by substantial changes in its local environment and that these can have marked effects on stability of the transition state, as well as of the ground state, in the mechanistic pathway for hydrogen–deuterium exchange.

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Mechanism of Cis–Trans Isomerization about Carbon–Carbon Double Bonds Catalyzed by Silver(I)[1]

Richard A. Johnson and Stanley Seltzer*

Contribution from the Department of Chemistry, Brookhaven National Laboratory, Upton, New York 11973.
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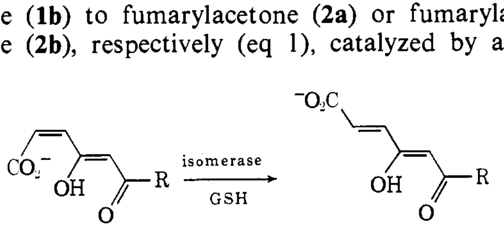
Abstract: Cis–trans isomerizations of maleylacetone, maleylacetoacetate, and similar compounds are catalyzed by enzymes and by silver ion. The possible relations between the two are discussed. Silver ion catalyzed isomerization of maleylacetone proceeds *via* a nonradical path and without vinyl proton exchange. The methyl ester (**6**) and the enol methyl ether (**7**) of maleylacetone were synthesized and the reactions of silver with these substrates are compared with that for silver and maleylacetone. The methyl ester undergoes rapid silver ion catalyzed isomerization while isomerization of the enol methyl ether is not catalyzed by silver. Solvent isotope effects, pH–rate profiles, and the lack of general acid–base catalysis suggest that a π complex between silver and the monoanion (**4**) of maleylacetone is formed. The π complex loses a proton in a preequilibrium step and could then undergo intramolecular nucleophilic attack to form a furanone intermediate, which is able to undergo facile rotation about the carbon–carbon bond that is isomerizing.

Cis–trans isomerizations about carbon–carbon double bonds are important reactions in certain biological systems.[2] Several of these reactions, catalyzed by enzymes, occur during the metabolism of aromatic amino acids and related compounds. Photocatalyzed cis–trans isomerizations are basic to visual processes, but a dark, enzyme-catalyzed trans–cis isomerization is generally required to regenerate the light-sensitive pigment.[3] Although several systems have now been characterized, the mechanism of enzyme action for any of these remains to be elucidated.

One system in which we have been interested is isomerization of maleylacetone (**1a**) or maleylacetoacetate (**1b**) to fumarylacetone (**2a**) or fumarylacetoacetate (**2b**), respectively (eq 1), catalyzed by an enzyme found in animal liver or in *Vibrio 01* bacteria.[4] Glutathione (GSH) is required as a coenzyme for



(1)

1a, R = CH_3
b, R = $CH_2CO_2^-$

2a, R = CH_3
b, R = $CH_2CO_2^-$

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(1) (a) Research performed under the auspices of the U. S. Atomic Energy Commission. (b) For a preliminary report of this work, see R. A. Johnson and S. Seltzer, *J. Amer. Chem. Soc.*, **94**, 4755 (1972).

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reaction. The discovery by Crandall and coworkers[5] that silver(I) is an excellent catalyst for converting **1** to **2** suggests the possibility that some cis–trans isomerases are metalloenzymes. It is interesting that these workers also found the following metal ions without catalytic activity: iron(II or III), zinc(II), copper(I or II), cobalt(II), aluminum(III), nickel(II), mercury(I or II), lead(II), platinum(IV), manganese(II), magnesium(II), cadmium(II), chromium(II), and sodium. Incorporation of silver itself into enzymes appears unlikely for its presence often leads to loss of activity. Although other metal ions appear not to influence the rate of conversion of **1** to **2**, the catalytic properties of these ions may be improved through coordination with a protein. Thus, it seemed worthwhile to investigate the role of silver in these reactions because of its possible application to the enzyme-catalyzed reaction.

While these studies were in progress, the *Vibrio 01* enzyme, specifically catalyzing reaction **1**, was analyzed for the presence of various metals, and as a result is presently considered not to be a metalloenzyme (*vide infra*).

Experimental Section

All inorganic salts and buffer components were analytical reagents and used as supplied. Cuprous ion used in these studies was from three sources: (1) Baker and Adamson cuprous chloride analytical reagent, (2) that prepared by reduction of copper(II) with bisulfite,[6] and (3) cuprous ion prepared *in situ* from copper(II), ammonia, and glucose.[7] Cuprous ion concentrations were determined spectrophotometrically through its *o*-phenanthroline complex.[8] Maleylacetone was prepared according to the method of Fowler and Seltzer.[9]

Kinetic studies were carried out for the most part by following the increase in optical density at 313 nm with a Datex digitized Cary 11 spectrophotometer.[10] Nonlinear least-squares programs were used to obtain rate constants from the time *vs.* optical density data that were recorded on punched cards.

Dissociation Constant of Maleylacetone in D_2O. All pH (pD) measurements were taken with an Orion pH meter, Model 801, equipped with an A. H. Thomas combination electrode (No. 4858 L-15). The pD value was obtained by adding 0.40 to the measured reading.[11] Stock solutions of anhydrous sodium acetate, acetic acid, lithium perchlorate, nitric acid, as well as maleylacetone, in D_2O, were prepared. Different ratios of sodium acetate to acetic acid were taken to provide pD's between 6.85 and 3.64. Solutions of nitric acid were used in the region of pD $\simeq 2$. Aliquots of the maleylacetone solution were added to each of the buffered and mineral acid solutions and the optical densities at 310 nm were measured. In this way 14 readings were taken over the pD 6.85–2.06 range. The total concentration of acetate was kept at 0.0125 *M* and the ionic strength maintained at 0.0125 *M* with lithium perchlorate. The optical density at 310 nm due to a 5% impurity of fumarylacetone was subtracted from each reading, and the ratio (r) of the residual optical density at each pD to that at pD 6.85 was calculated. Log $(r/(1-r))$ was plotted *vs.* pD and a straight line drawn. pK = pD at log $(r/(1-r)) = 0$. The pK_{1D} measured in this way is 4.53.

Maleylacetone Methyl Ester (6). Trimethyloxonium fluoroborate was prepared according to the method of Meerwein.[12] To 0.102 g (0.74 mmol) of 4-acetonylidenebut-2-en-4-olide[9] was added 1.5 ml of cold 1 *M* sodium hydroxide. As soon as the solid dissolved (1–2 min), the yellow solution was neutralized with cold 1 *M* nitric acid followed by the addition of 4 ml of methanol. To this solution was added 1.05 g (12.5 mmol) of sodium bicarbonate and the mixture was cooled to −33° (calcium chloride–ice bath). Trimethyloxonium fluoroborate (1.503 g, 10.2 mmol) was added with stirring over a period of 15 min. After 45 min the mixture was extracted with three 5-ml portions of chloroform at −24°. A second similar extraction was carried out at 0°. The organic phase, dried over sodium sulfate, yielded a pale yellow oil after evaporation of the solvent on a roto-evaporator. Traces of methanol were removed by repeated additions of chloroform followed by solvent evaporation. The second extract had slightly fewer side products (fumarylacetone methyl ester and maleylacetone) and contained about 90% maleylacetone methyl ester as shown by nmr: ($CDCl_3$) δ 2.15 (s, 3 H, CH_3CO), 3.80 (s, 3 H, CH_3O), 5.67 (s, 1 H, vinyl), 6.20 (s, 2 H, vinyl); (C_6D_6) δ 1.63 (s, 3 H, CH_3CO), 3.45 (s, 3 H, CH_3O), 5.18 (s, 1 H, vinyl), 5.60 and 5.88 (q, 2 H, J = 12.5 Hz, vinyl); high-resolution mass spectrum of parent peak 170.05784 ± 0.00034[13] (calcd for $C_8H_{10}O_4$, 170.05789); uv (pH 4.0) max 306 nm.

4-Hydroxy-4-methoxy-6-oxo-2-heptenoic Acid Lactone (Maleylacetone Pseudo Methyl Ester) (8). A stirred solution containing 175 ml of methanol, 3.95 g (0.0319 mol) of furylacetone,[14] 0.246 g of Rose Bengal, and 0.044 g of vanadium pentoxide was irradiated in an atmosphere of oxygen with an air-cooled, centrally positioned, 100-W incandescent lamp. The contents of the Pyrex flask was maintained at about 25°. After 4.5 hr, 750 ml of oxygen was consumed and irradiation stopped. The methanol was removed on a roto-evaporator and the residue, dissolved in ether, was washed with four 60-ml portions of 0.43 *M* ferrous sulfate solution. The brown aqueous layers were combined and extracted with ether and chloroform. The combined organic layers were dried over sodium sulfate and the solvent was removed and replaced with chloroform. The solution was treated with charcoal and filtered quickly through 30 g of neutral alumina, and the solvent was removed leaving 1.6 g of a pale yellow oil (yield 32%).

A portion was further purified by molecular distillation, bp 57° (10^{-3} mm): nmr ($CDCl_3$) δ 2.27 (s, 3 H, CH_3CO), 2.83 and 3.35 (q, 2 H, J = 15.5 Hz, CH_2 adjacent to an asymmetric center), 3.23 (s, 3 H, CH_3O), 6.32 and 7.53 (q, 2 H, J = 5.8 Hz, vinyl ring protons); uv (H_2O neutral) λ_{max} 194 nm (ϵ 7470), 273 (630); high-resolution mass spectrum[13] 170.05784 ± 0.00034 (calcd parent ion, 170.05789).

Anal. Calcd for $C_8H_{10}O_4$: C, 56.46; H, 5.92. Found: C, 56.63; H, 5.79.

Sodium *O*-Methyl-6-oxo-*cis*-2-*trans* (or *cis*)-4-heptenoate (7). Maleylacetone pseudo methyl ester (68 mg) was dissolved in 0.5 ml of 0.65 *M* sodium carbonate in D_2O. The opened anion formed immediately: nmr (D_2O, reference HOD taken as 5 ppm from TMS) δ 2.53 (s, 3 H, CH_3CO), 4.00 (s, 3 H, CH_3O), 6.07 (s, 1 H, C-5 vinyl-H), 6.55 and 7.10 (q, 2 H, J = 12.5 Hz, C-2 and C-3 vinyl-H's); uv λ_{max} (2×10^{-4} *M* NaOH) 293 nm (ϵ 11,300), 203 (ϵ 3600).

Acidification with 2.8 *M* H_2SO_4 in D_2O to pH 4.9 formed *O*-methyl-6-oxo-*cis*-2-*trans* (or *cis*)-4-heptenoic acid: nmr (D_2O, reference HOD taken as 5 ppm from TMS) δ 2.52 (s, 3 H, CH_3CO), 4.00 (s, 3 H, CH_3O), 6.12 (s, 1 H, C-5 vinyl-H), 6.50 and 7.28 (q, 2 H, J = 12.5 Hz, C-2 and C-3 vinyl-H's); uv $\lambda_{max}^{pH\ 2.63}$ 280 nm (ϵ 5600), 195 (3740).

***O*-Methyl-6-oxo-*trans*-2-*trans* (or *cis*)-4-heptenoic Acid (9).** 4-Acetonylidenebut-2-en-4-olide was added to a 0.13 *M* sulfuric acid solution in methanol. After the mixture stood for 24 hr, 65% of the starting material was converted to maleylacetone pseudo methyl ester (nmr) and the remainder to a crystalline product, mp 88.0–89.0° (methanol); uv $\lambda_{max}^{pH\ 3.94}$ 314 nm (ϵ 14,800), 235 (7300), 217 (7900); nmr ($CDCl_3$) δ 2.25 (s, 3 H, CH_3CO), 3.87 (s, 3 H, CH_3O), 5.87 (s, 1 H, C-5 vinyl-H), 6.90 and 7.20 (q, 2 H, J = 16 Hz, C-2 and C-3 vinyl H's).

Silver(I) Ion-Catalyzed Isomerization of Maleylacetone in an Esr Spectrometer Cavity. A solution containing 4.5×10^{-4} *M* maleylacetone, 9.1×10^{-4} *M* silver nitrate, 0.01 *M* acetate buffer, and 0.10 *M* lithium perchlorate (pH 4.88) with an estimated half-life for isomerization of 23 min was placed in a 0.1 × 10 × 40 mm cell in a

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(13) We thank Mr. Saltmach, Columbia University, for the mass determination.

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Varian, V 4500-10A, esr spectrometer. The region between 2900 and 3900 G was scanned slowly with a signal level of 800 and a modulated amplification of 250 over 2.5 min within 5 min after mixing the solution; a faster subsequent broad sweep from 900 to 5900 G also showed no signal.

Test for Vinyl Proton Exchange during Silver Ion-Catalyzed Isomerization of Maleylacetone. A measured amount of dioxane (0.0313 mmol) in 0.5 ml of D_2O was added to a solution of 0.138 mmol of maleylacetone in 1.5 ml of D_2O and the nmr spectrum recorded. Over a period of 20 min, three 0.100-ml portions of 1 *M* silver nitrate in D_2O were added and silver fumarylacetone precipitated during this time. The pD of the solution was maintained between 3 and 7 by addition of NaOD or DNO_3 in D_2O. The precipitate was dissolved in 2.5 ml of acetone-d_6 and silver was removed by centrifugation of silver chloride from the addition of sodium chloride in D_2O thus yielding a clear solution. The nmr spectrum was obtained through the accumulation of 22 scans.

The Catalyzed Isomerization Reaction in Acrylamide Monomer Solution. Two solutions were prepared: A, containing 5 ml of 1 *M* nitric acid, 3.65 g of trishydroxyethylamine, 49 mg of *N,N,-N′,N′*-tetramethylenediamine, and water to 10 ml; and B, containing 45 g of acrylamide, 1.2 g of *N,N′*-methylenebisacrylamide, and water to 100 ml. The silver ion-catalyzed isomerization of maleylacetone was carried out at two pH's.

Three solutions, each containing 1 ml of solution B and 5 ml of 0.02 *M* 3,3-dimethylglutarate buffer, were diluted to a final volume of 9 ml (all at pH 5.60). In addition, solution 1 contained 5.7×10^{-3} *M* silver nitrate and 1.3×10^{-4} *M* maleylacetone. Solution 2 also contained 5.7×10^{-3} *M* silver nitrate and solution 3 contained neither silver nitrate nor maleylacetone. A stream of nitrogen was passed through each solution to remove oxygen. Since none of the three solutions had gelled after 4 hr, 4 ml of an ammonium persulfate solution (final concentration 4×10^{-4} *M*) was added to solutions 2 and 3. Solution 2 gelled in less than 20 min while solution 3 had not even after 15 hr.

Four solutions, each with 1 ml of solution B, 1 ml of solution A, and 5 ml of 0.02 *M* acetate buffer, were diluted with water to a final volume of 10 ml. The final pH was 9.0. In addition, solution 4 contained 5.2×10^{-3} *M* silver nitrate and 1.2×10^{-4} *M* maleylacetone; solution 5 contained 5.2×10^{-3} *M* silver nitrate; solution 6 contained neither silver nor maleylacetone; and solution 7 contained 5.7×10^{-5} *M* ammonium persulfate. Solutions 4, 5, and 6 were deoxygenated by a stream of nitrogen. No gel had formed in 4, 5, or 6 but solution 7 gelled within 53 min without previous deoxygenation.

Test for the Effect of Light on the Rate of Silver Ion-Catalyzed Isomerization. A solution of maleylacetone (1.2×10^{-4} *M*), silver nitrate (1.14×10^{-3} *M*), and lithium perchlorate (0.10 *M*) in acetate buffer (0.02 *M*, pH 4.15) was prepared in the dark. Two aliquots were taken. One was kept in the dark except for occasional measurement of its spectrum on a Cary 14. The other was allowed to be illuminated with normal room light. The spectra of the two were compared at prescribed times.

Results and Discussion

Kisker and Crandall[5a] obtained maleylacetone through decarboxylation of maleylacetoacetate, the product of enzymatic oxidation of homogentisic acid. Although the maleylacetone from this source was impure, these workers demonstrated a pH-rate profile for its silver ion-catalyzed and noncatalyzed cis–trans isomerizations. Their results suggest that the active species in the catalyzed reaction behaves as if it were the anion of a monoprotic acid of p*K* ~ 3.5. We have verified some of these measurements on synthesized maleylacetone and in addition determined a similar silver ion-catalyzed pD–rate dependence for acetate-buffered D_2O solutions.

Solvent Isotope Effect and Kinetically Determined Dissociation Constants. Rates of silver ion-catalyzed cis–trans isomerization of maleylacetone in buffered H_2O and D_2O solutions at various pH's (or pD's) were determined and shown in Table I. The pseudo-first-order rate constants, k_1, given in the table are computer determined by fitting 200 or more points to a nonlinear least-squares program. The standard deviation of the rate constant within a run is typically about 0.5% when the reaction is followed to 99% completion. In these kinetic experiments the change of absorption at 313 nm due to the loss of maleylacetone and the appearance of fumarylacetone is followed. Thus the rate dependence on substrate concentration is clearly first order. In Table I the data are for rates at two silver ion concentrations that differ by more than a factor of 20. All pseudo-first-order rate constants, however, have been normalized to the same silver ion concentration (Table I, footnote *d*) and these rate constants are given as k_{obsd}. That this normalization leads to good agreement when the data are fitted to eq 2 (*vide infra*) demonstrates that the rate is also first order in silver ion.

Table I. Pseudo-First Order Rate Constants of Silver Ion-Catalyzed Cis–Trans Isomerization of Maleylacetone in H_2O and D_2O

pH	10^4k_1, sec^{-1}	10^4k_{obsd}, sec^{-1} [d]	pD	10^4k_1, sec^{-1}	10^4k_{obsd}, sec^{-1} [d]
2.26	0.131[a]		2.24	5.01[b]	0.233
2.25	15.5[b]	0.722[d]	2.41	7.67[b]	0.357[d]
2.41	23.0[b]	1.07[d]	2.61	10.6[b]	0.494[d]
2.66	33.4[b]	1.56[d]	3.42	1.08[c]	1.08
2.66	33.9[b]	1.58[d]	4.13	3.61[c]	3.61
3.13	3.03[c]	3.03	4.42	6.29[c]	6.29
3.64	5.85[c]	5.85	5.04	9.53[c]	9.53
3.95	6.26[c]	6.26	5.19	9.72[c]	9.72
4.36	8.10[c]	8.10			
4.90	9.32[c]	9.32[e]	5.39	8.98[c]	8.98
5.12	9.10[c]	9.10[e]	5.62	10.26[c]	10.26[f]

[a] Silver nitrate was absent. Malonic acid (0.20 *M*), adjusted with NaOH (or NaOD) to the proper pH, served as buffer. Lithium perchlorate was added to bring the ionic strength to 0.1 *M*. Initial maleylacetone concentration was 2.4×10^{-4} *M*. [b] Buffer solutions, ionic strength, and initial substrate were the same as in *a*. Silver nitrate concentration was 0.0203 *M*. [c] Acetic acid (0.02 *M*), adjusted to the proper pH with NaOH (or NaOD), served as buffer. Lithium perchlorate was added to bring the ionic strength to 0.10 *M*. The initial maleylacetone concentration was 2.24×10^{-4} *M* and the silver nitrate concentration was 9.46×10^{-4} *M*. [d] These pseudo-first-order rate constants for isomerization in the presence of 2.03×10^{-2} *M* silver ion were normalized to 9.46×10^{-4} *M* silver ion by multiplying by $(9.46 \times 10^{-4})/(2.03 \times 10^{-2})$. [e] k_{max} in H_2O solutions is chosen as the average between 9.32 and 9.10×10^{-4} sec^{-1} and used to fit the data to eq 2. [f] k_{max} in D_2O solution is chosen as 10.26×10^{-4} sec^{-1} and used to fit the data to eq 2.

If, as the previous results suggest, the rate of isomerization of varying pH's is proportional to the substrate anion concentration, then in kinetic experiments with equal silver ion concentrations, $k_{obsd} = k_{max}K/[K + (H_3O^+)]$. k_{obsd}'s are the observed pseudo-first-order rate constants for cis to trans isomerization of maleylacetone at specified pH's and silver ion concentrations and given in Table I. *K* is the dissociation constant for the monoprotic acid and k_{max} is the pseudo-first-order rate constant at a particular silver ion concentration that is observed if all substrates were in the form of monoanion, *i.e.*, when $K > (H_3O^+)$.[15] The equation can be rewritten as

$$\log [(k_{max}/k_{obsd}) - 1] = \mathrm{p}K - \mathrm{pH} \qquad (2)$$

and the data are shown plotted in this manner in Figure 1 for H_2O and D_2O solutions. At $\log [(k_{max}/k_{obsd}) - 1] = 0$, pH = p*K*. Thus $\mathrm{p}K_H = 3.43$ and

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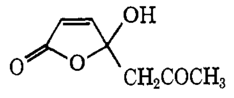
[15] The substrate is really a diprotic acid[9] and K_1 is identical here with *K*. Therefore, $k_{obsd} = k_{max}$ when $K_1 \gg (H_3O^+)$ but $K_2 \ll (H_3O^+)$.

pK_D = 4.14, which indicates an isotope effect of 5.1 on the dissociation constant. The spectrophotometrically determined dissociation constants in H_2O and D_2O are 3.95[9] and 4.53, respectively, and yield an isotope effect of 3.8.

Maleylacetone has the cyclic pseudoacid structure (3), while the anion is acyclic.[9] It would seem that



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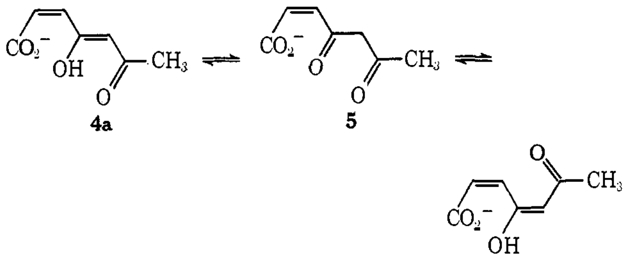
the ring-locked geometry prevents silver ion-catalyzed cis–trans isomerization. Uv and nmr spectra suggest that the anion is mainly **4a** and **4b** and that **5** might also be present. The proton nmr spectrum of the anion in aqueous solution, however, exhibits two methyl singlets of about equal intensity separated by about 5 Hz, in agreement with the notion that **4a** and **4b** are the predominant monoanionic species. Interconversion of isomers must proceed with a rate constant less than about 1.6 sec^{-1}.



4a **5** **4b**

Test for Vinyl Proton Exchange during Catalyzed Isomerization. To gain further insight into the nature of the reaction, the vinyl proton exchange during silver ion-catalyzed reaction was examined. A small amount of dioxane served as an internal standard for nmr integration of the vinyl protons in the substrate and in the product. The results, shown in Table II, indicate no proton exchange during silver ion-catalyzed isomerization in D_2O.

Table II. Nonexchange of Maleylacetone Vinyl Protons during Silver Ion-Catalyzed Isomerization in D_2O[a]

Proton peak	Average integrated peak areas: Initial spectrum[b]	Final spectrum[c]
Dioxane	52	53
Upfield vinyl proton	31	34
Downfield vinyl proton	31	34

[a] Dioxane (0.031 mmol/2 ml of D_2O solution) served as an internal standard. [b] Average of six integrals in arbitrary units. The error is ±1 unit. [c] The final spectrum is the summation of 22 scans on a CAT of fumarylacetone.

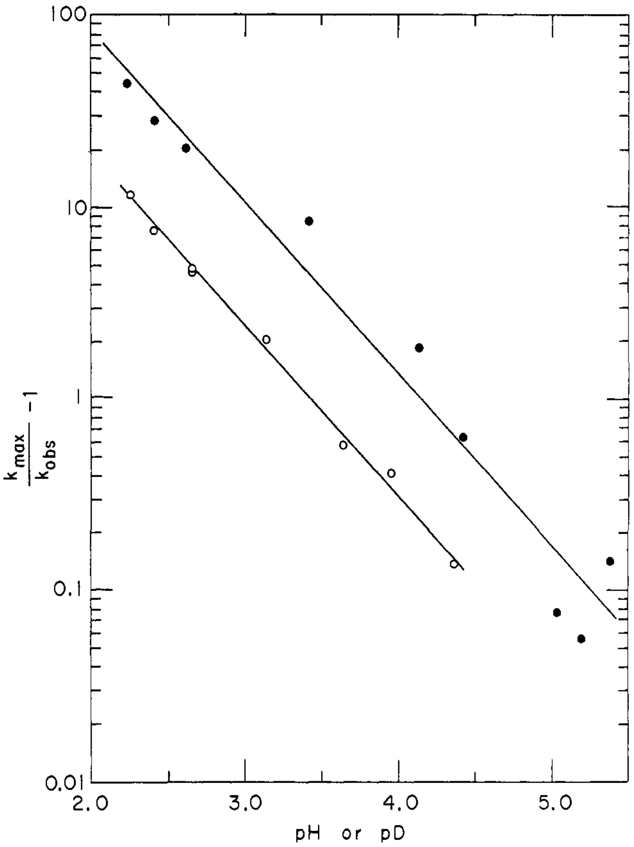
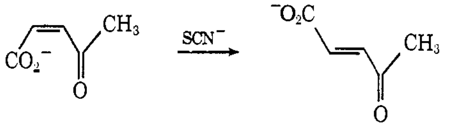


Figure 1. Pseudo-first-order rate constants for silver ion catalyzed cis–trans isomerization of maleylacetone *vs.* acidity in H_2O and D_2O solutions. Log $[(k_{max}/k_{obsd}) - 1)]$ is plotted against pH or pD to obtain K_H and K_D: (●) measurements in D_2O; and (○) H_2O solutions. The lines represent least-squares solutions obtained by fitting the data given in Table I to eq 2. Calculated slopes are: H_2O, −0.89; D_2O, −0.90.

Cu(I) Catalysis. Although silver was previously reported to be the only metal ion able to promote cis–trans isomerization, it seemed worthwhile to reinvestigate the effect of copper(I) since the properties of it and silver(I) are similar. Of three sources of copper(I) used, that prepared by heating a solution of copper(II), ammonia, and glucose in a side arm of an ultraviolet cell containing substrate and buffer seemed most reliable in our hands. Because the substrate is thermolabile, heating of the contents in only the side arm was carried out for only short periods of time and hence incomplete conversion of copper(II) to copper(I) took place. Nevertheless, the concentration of Cu(I) could be spectrophotometrically determined in parallel experiments with *o*-phenanthroline at 435 nm.[8] Moreover, it could also be shown that Cu(I) survived the conditions of the kinetic experiments if the solutions were previously deoxygenated with a stream of nitrogen.

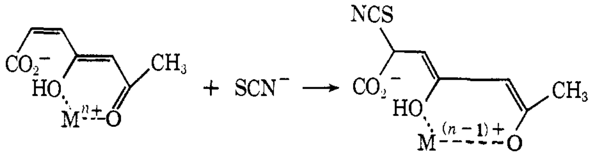
Upon mixing the substrate solution with the Cu(I)–Cu(II) solution in the pH 4–5 range, the λ_{max} shifts almost instantaneously from 313 to about 335 nm. The former maximum is due to the maleylacetone monoanion while the latter is due to the presence of a Cu(II)–monoanion complex. Upon standing, the optical density at 335 nm remains essentially unchanged over a period of 3 hr at 25°. The extinction coefficients at 335 nm for the copper(II) substrate and product complexes differ by less than 10% at this pH. Therefore aliquots of the copper(I)-substrate solutions were acidified to pH 1 and the uv spectrum was measured at 313 nm. At low pH maleylacetone is converted to **3** and its absorption at 313 nm disappears. Residual absorption at this wavelength is then due to fumarylacetone. Unfortunately, acid treatment can catalyze isomerization and makes the contribution of Cu(I) catalysis uncertain in this determination. An upper limit of about 6 M^{-1} min^{-1} for Cu(I) catalysis is obtained. It will be seen below that the effectiveness of copper(I) is not more than one-tenth that of silver(I).

Test for Simultaneous Nucleophilic and Metal Ion Catalysis. In a previous study it was shown that a strong nucleophile, thiocyanate ion, catalyzes cis–trans isomerization of *cis*-β-acetylacrylic acid, a compound which is a model for the present substrate (eq 3).[16] By analogy with previous studies,[17] the mechanism appears to involve the reversible addition of thiocyanate ion to the carbon–carbon double bond thereby allowing rotational freedom about it. Maleylacetone also undergoes thiocyanate ion-catalyzed isomerization presumably by the same mechanism.



(3)

A metal ion, chelated to the 1,3-ketoenol system of the substrate, might possibly enhance nucleophilic addition and subsequent isomerization (eq 4). That this could be a general mechanism for the role of silver ion (where water or buffer ion would be the nucleophile) in this reaction was considered. Rates of reaction for thiocyanate ion-catalyzed isomerization as well as rates for the silver ion-catalyzed reaction were measured and compared with the rate for isomerization when both thiocyanate and silver are present. The results are shown in Table III. Because copper(II) forms tighter complexes with ketoenols than does silver, the possibility that this might occur in the presence of copper(II) ion was also investigated. It is immediately apparent that a mixture of the metal and thiocyanate ions results in a poorer catalyst than the individual active component. This could be attributed to different factors. Silver and thiocyanate form complexes thereby reducing the concentrations of free thiocyanate and silver ions. If only the uncomplexed ions exhibited catalytic activity then a mixture of the two ions would exhibit a k_{cat} (the difference in pseudo-first-order rate constants for isomerization in the presence and absence of potential catalysts) lower than the sum of the individual catalytic constants. This is apparently what happens. The fraction of total silver(I) in the form of the mono, di, tri, and tetrathiocyanate complex in the absence of substrate can be calculated from the known association constants, and the fractions are shown in Table IV.[18] It appears that either the polythiocyanate–silver complexes do not bind substrate as well as free silver ion or if they do, they are virtually unreactive compared to silver ion. A similar conclusion can be reached for the copper(II) system. Free silver ion catalysis does not appear to proceed by a mechanism similar to that shown in eq 4.



(4)

Table III. Catalytic Effect of Silver(I), Copper(II), and Thiocyanate Ions on the Rate of Cis–Trans Isomerization of Maleylacetone[a]

$10^4[M^{n+}]$, M	$[SCN^-]$, M	pH	k_1, 10^{-4} sec^{-1} [g]	k_{cat}, 10^{-4} sec^{-1} [h]
		4.24	0.472[d]	
0.518[b]		4.23	0.578[d]	0.106
	0.02	4.27	0.634[d]	0.162
0.518[b]	0.02	4.26	0.618[d]	0.146
7.35[b]		4.31	4.01[d]	3.5
	0.20	4.41	4.02[e]	3.6
8.85[b]	0.20	4.40	4.52[e]	4.1
17.7[b]		4.13	10.9[f]	10.4
	0.50	4.61	7.03[f]	6.6
17.7[b]	0.50	4.59	5.33[f]	4.9
0.34[c]	0.021	3.93	0.644	0.17
	0.021	3.95	0.906	0.44

[a] At 25°; maleylacetone concentration was 8×10^{-5} M throughout and pH maintained with 0.01 M acetate buffer. [b] Silver concentration. [c] Copper(II) concentration. [d] Ionic strength adjusted with lithium perchlorate to 0.1. [e] Ionic strength equaled 0.22. [f] Ionic strength equaled 0.52. [g] The pseudo-first-order rate constant for the disappearance of substrate under the conditions listed. [h] The difference between pseudo-first-order rate constants in the presence and absence of added catalysts. All other factors held constant.

Table IV. Fractions of Total Silver Ion Existing in Thiocyanate-Complexed Forms

$10^4[Ag^+]_{tot}$, M	$[SCN^-]_{tot}$, M	Fraction: $[Ag(SCN)_n]/[Ag]_{tot}$ — n — 1	2	3	4
0.518	0.02	0.012	0.735	0.244	0.008
7.35	0.20	0.003	0.184	0.610	0.203
17.7	0.50	0.00004	0.0618	0.513	0.425

Examination for Radical Intermediates. The question of whether silver ion catalysis involves a free radical was also studied. The silver ion-catalyzed isomerization reaction was carried out in an aqueous solution of acrylamide monomer under the conditions where cis–trans isomerization proceeded with a half-time of 5 min. No polymerization occurred. Added ammonium persulfate, however, at a concentration equal to that used for substrate in these solutions rapidly polymerized acrylamide solutions of similar composition.

Silver ion-catalyzed isomerization was also allowed to proceed in an esr cavity under conditions whereby the half-life of the reactant was 23 min. No signal was detected. The rate of silver ion-catalyzed isomerization proceeding in the dark was found to be identical with that observed for a sample illuminated by normal room light. Moreover, the effect of removing bulk oxygen was studied. Shown in Table V are kinetic results for identical solutions with and without previous degassing. Rates are only slightly faster for the degassed system making it unwise to speculate about the origin of the rate difference.

The data discussed above allow us to reject isomerization mechanisms involving radicals or metal ion promoted nucleophilic catalysis. The results of at-

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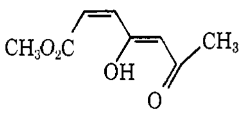
[16] K. D. Stevens and S. Seltzer, *J. Org. Chem.*, 33, 3922 (1968).
[17] S. Seltzer, *J. Amer. Chem. Soc.*, **83**, 1861 (1961).
[18] J. Bjerrum, G. Schwarzenbach, and L. G. Sillen, *Chem. Soc., Spec. Publ.*, No. 7, 40 (1958).

Table V. Effect of Air on Silver Ion-Catalyzed Isomerization of Maleylacetone

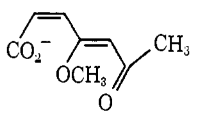
Soln	Obsd rate constant, 10^{-4} sec^{-1} Degassed system	 Normal system
A[a]	1.28 ± 0.01	1.10 ± 0.01
B[a]	1.24 ± 0.02	1.02 ± 0.02

[a] The concentrations of silver ion and substrate were 2.34×10^{-4} and 1.23×10^{-4} *M*, respectively, at a pH of 3.97.

tempted catalysis by other metal ions point to a special property of silver ion. Silver ion forms π complexes with olefins and these complexes have been shown to be more favored if a hydroxyl group is located α, β, or γ to the olefin.[19] Copper(I), however, also forms strong complexes with hydroxyolefins.[20] There appeared to be two potential hydroxyl functions in **4** that might have been important in binding silver ion; they are the 4-hydroxyl and the carboxylate group. In order to assess the relative importance of these groups two derivatives, **6** and **7**, were synthesized.



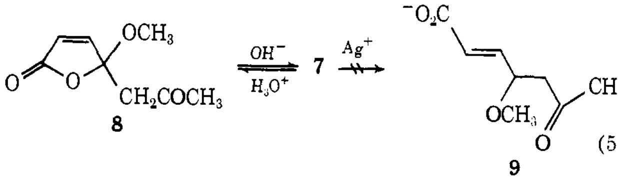
6



7

The methyl ester (**6**) was prepared through treatment of the monoanion (**4**) with trimethyloxonium fluoroborate. The enol ether carboxylate anion (**7**) was obtained through treatment of the pseudo methyl ester (**8**) with base. The structures indicated for **6**, **7**, and **8** are in agreement with nmr, uv, and mass spectral data. The uv maxima for **6** and **7** are 306 and 293 nm and are slightly lower than that for **4** which is probably a result of steric strain in the π system.

Silver ion catalyzes cis–trans isomerization of **6** but not of **7**. The predominant reaction that **7** undergoes at pH's less than 7 is to cyclize to **8** (eq 5) as shown

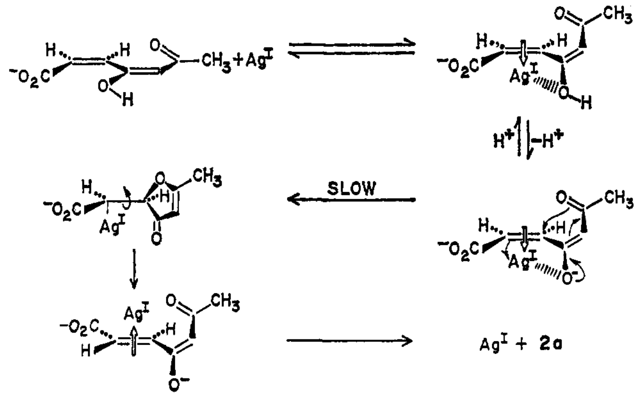


(5)

by nmr and uv. **7** appears to first undergo protonation of its carboxylate group as indicated by an almost instantaneous change in $\lambda_{\max}$ to 280 nm (ϵ 5600) and thereafter this absorption decreases in a first-order process. A more concentrated solution of **7** (~0.5 *M*), treated in the same way and followed by nmr and uv, gives the same rate of disappearance of **7** and appearance of **8** for both methods. The rate, however, is not first order at this high substrate concentration. The rate of disappearance of absorption at 280 nm is essentially the same in the absence and presence of silver ion and thus only an upper limit to any possible catalyzed cis–trans isomerization can be obtained. The product of isomerization (**9**) was prepared in another way and shown to have a maximum at 314 nm (ϵ 14,800). From the residual optical density at 314 nm of a solution of **7** treated with 0.1 *M* silver ion at pH 4, it can be estimated that the hypothetical second-order rate constant for silver ion-catalyzed cis–trans isomerization of **7** is not greater than 1/35,000 of the corresponding rate constant for the interaction of silver ion with **4**. This decrease in rate could not be due to a decrease in the silver ion π-binding ability of the substrate caused by the substitution of a methyl group for a proton for it has been shown with other hydroxyolefins that this type of substitution reduces the association constant by only a factor of about 50.[19a]

Silver ion-catalyzed cis–trans isomerization of **6** is about ten times more rapid than the corresponding isomerization with **4**. The kinetics, which have already been reported in detail,[1b] are pH dependent. Since **6** undergoes rapid hydration at pH's greater than 6, the full pH–rate profile could not be examined, but the data indicate that the kinetically active species is a silver ion complex of **6** minus a proton. The proton could be dissociated from the enol, from the hydration shell of silver, or from another site on the substrate. The fact that **7** is kinetically inactive with silver ion makes it probable that the proton is dissociated from the enol. The mechanism indicated in Chart I is most consistent with the data.

Chart I



That the enol proton is dissociated in an equilibrium prior to the rate-controlling step is supported by results of kinetic determinations in the silver ion-catalyzed isomerization of **6**. General acid–base catalysis is absent,[1b] and a solvent isotope effect, $k_{H_2O}/k_{D_2O} = 1.9$ (at pH = pD = 2.2), is observed. The isotope effect is small and is consistent with it resulting from an equilibrium effect although the magnitude alone would not be sufficient for its assignment. Because the rates in D_2O were measured at a relatively acidic pH, the ratio of proton (deuteron) dissociation constants (K_H/K_D) for the **6**-silver complex can be shown to equal the observed solvent isotope effect. Since the rate of catalyzed cis–trans isomerization is proportional to the concentration of silver and the anion of **6**, then at equal silver concentrations and for $(H_3O^+) = (D_3O^+)$, $\alpha = \gamma(1 + \beta) - \beta$ if $\alpha = K_H/K_D$, $\beta = K_H/(H_3O^+)$, and γ equals the observed isotope effect. From the previous data $1 > \beta < \gamma$.[1b] Therefore $\alpha \cong \gamma$.

As shown above (Figure 1), the pH–rate dependence

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(19) (a) D. Gray, R. A. Weis, and W. D. Closson, *Tetrahedron Lett.*, 5639 (1968); (b) R. M. Keefer and L. J. Andrews, *J. Amer. Chem. Soc.*, 71, 3906 (1949); (c) C. F. Wilcox, Jr., and W. Gaal, *ibid.*, 93, 2453 (1971).

(20) (a) R. M. Keefer and L. J. Andrews, *ibid.*, 71, 1723 (1949); (b) L. J. Andrews and R. M. Keefer, *ibid.*, 71, 2379 (1949); (c) R. M. Keefer and L. J. Andrews, *ibid.*, 71, 2381 (1949).

of the silver ion catalyzed isomerization of maleylacetone appears as if it represented the interaction of silver ion with the anion of a monoprotic acid having a kinetically determined pK of 3.43. By analogy with the corresponding catalyzed isomerization of **6**, however, the catalyzed isomerization of maleylacetone must involve a complex of silver ion and maleylacetone·minus *two* protons. In the absence of silver ion, pK_1, the dissociation of the pseudoacid to the carboxylate ion (**3** $\rightleftharpoons$ **4**), and pK_2, representing the dissociation of the enol, are 3.95 and 9.4, respectively.[9] It is expected, however, that coordination of the enol with a metal cation would decrease pK_2 by several units. The acidity of water, for example, is enhanced by a factor of 10^3 when complexed with Ag(I);[21] metals with higher charge densities would tend to increase the acidity even further. There are two other factors that will tend to contribute to a further increase of the enol hydroxyl acidity.

The first is the bonding picture currently accepted for the silver(I)–olefin complex.[22] The olefin's π orbital overlaps with a hybridized d orbital of the silver and the silver back-donates from its d orbitals to antibonding orbitals at carbon. The silver donates more than it receives which is consistent with the 4.4 ppm upfield ^{13}C shift of the olefinic carbons upon complexation with silver.[23] Consequently, the complexed silver ion would be expected to have a greater positive charge density than hydrated silver(I) and thus tend to increase the acidity of the enol by more than 10^3.

A further contribution arises from the fact that the enol is part of a chelating group. Bennett, *et al.*,[24] have recently given ample evidence to show that a hydroxyl group is rendered more acidic when it is part of a group (*e.g.*, ethylene glycol) chelating Co(III), than when the hydroxyl is part of a unidentate group as in methanol. While this chelation factor amounts to a factor of 10^3 for Co(III), it would be expected to be lower for Ag(I) complexes. The separation of pK_1 and pK_2, in the presence of silver ion, then might be small and the kinetically active species could behave as a complex of silver ion and the anion of a monoprotic acid. It is also possible that pK_2 is suppressed by silver ion to a value lower than pK_1. The observed pH–rate dependence would also be satisfied under these circumstances and essentially controlled by the fraction [**4**]/([**3**] + [**4**]).

An isotope effect of 5.1 was obtained for an apparent K_H/K_D in the silver ion–maleylacetone reaction by assuming that the rate followed a silver(I)–monoanion complex. The isotope effect is somewhat larger than that resulting from the direct determination of the pK_1 of maleylacetone in H_2O and D_2O. If pK_2' (the proton dissociation for the silver ion-complexed enol) $\geq$ pK_1, the overall isotope effect might have been expected to be $3.8 \times 1.9 = 7.2$. It is not and thus it appears that silver complexation has rendered the enol a stronger acid than the uncomplexed pseudoacid (**3**). The overall isotope effect would then essentially be controlled by K_{1H}/K_{1D}. We must caution, however, that the kinetically determined ratio of apparent dissociation constants may be subject to error since it was obtained by assuming the active species to be a monoprotic acid anion.

There is one piece of evidence, however, that appears to be at variance with the proposal that $K_2' > K_1$ and that is the pH–rate profile for the reaction of Ag(I) with **6**. It suggests that the complexed enol has a p$K_2' \geq 4$.[1b] And if this same value is to be expected in the parent system, then $K_2' \simeq K_1$. That this is not the case is shown by the slope of the pH *vs.* log ($k_{max}/k_{obsd} - 1$) plot (Figure 1). If $K_2' \simeq K_1$ a slope of close to -2 would have been expected. The observed slope (-0.9), however, is closer to minus unity or more in line with $K_2' > K_1$.[25] Enol proton dissociation constants of **4** and **6** may not be comparable because of structural differences between the two molecules.

Thus we believe the mechanism (see Chart I) can be described as an initial formation of a silver(I) complex of the monoanion of maleylacetone where the metal is bound through a π complex and to the 4-hydroxy group. If the metal requires a tetrahedral configuration, the 4-hydroxy and the 6-keto oxygens would be expected to move to opposite sides of the plane formed by C-1, C-2, C-3, and C-4. At pH's where the substrate monoanion is formed the silver ion complex would also be expected to dissociate readily to form the kinetically active silver ion π-complex–enolate ion. In the ion the oxygen at C-6 is expected to be highly nucleophilic and properly oriented for attack at C-3 leading to a 3-furanone and facile rotation about the C–C bond undergoing isomerization. In the proposed mechanism it is suggested that the silver complex of **4b** can lead to cis–trans isomerization while that of **4a** cannot. It may be, however, that the silver(I)–**4a** complex after loss of the enol proton can easily isomerize to the active silver(I)–**4b** enolate complex and thereby complete the remaining isomerization.

When these studies were initiated it was anticipated that the isomerase, active with maleylacetone and maleylacetoacetate, might contain a metal. Since that time the enzyme has been purified to homogeneity and has been found not to contain significant amounts of copper, iron, silver, nickel, zinc, manganese, molybdenum, cadmium, or cobalt. While it appears that this isomerase does not contain any metal another type of cis–trans isomerase isolated from bacteria does.[26] The mechanisms of both enzymic reactions remain to be elucidated.

Acknowledgment. The authors are indebted to Dr. Peter Wong for examination of the silver-catalyzed system by esr and to Dr. Norman Sutin for bringing ref 24 to our attention.

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(21) E. Hogfeldt and A. E. Martell, *Chem. Soc., Spec. Publ.*, No. **25**, 26 (1971).

(22) F. R. Hartley, *Chem. Rev.*, 73, 163 (1973).

(23) R. G. Parker and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 743 (1970).

(24) L. E. Bennett, R. H. Lane, M. Gilroy, F. A. Sedor, and J. P. Bennett, Jr., *Inorg. Chem.*, **12**, 1200 (1973).

(25) A referee has proposed instead that Ag(I) complexes and increases the ionization of both **3** and **4** such that K_1' (proton dissociation constant for a Ag(I)–**3** complex) is sufficiently large that it is fully dissociated at even the lowest pH (2.2) of our measurements. K_2' is also proposed to be lowered and be responsible for the kinetics following a monoprotic acid of p$K \sim 4$ and for the near minus unity slope in Figure 1. This proposal, however, is at variance with the observed isotope effect of 5.1. By analogy with **6**, a solvent isotope effect of 1.9 would have been expected for this proposal.

(26) C. R. Kepler, W. P. Tucker, and S. B. Tove, *J. Biol. Chem.*, **246**, 2765 (1971), and papers cited therein.