Tetrahedron, 1963, Vol. 19, pp. 701 to 706. Pergamon Press Ltd. Printed in Northern Ireland

A SILVER-CATALYZED *CIS-TRANS* ISOMERIZATION[1]

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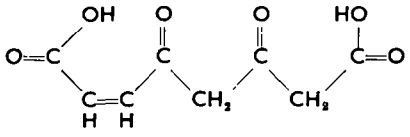
(*Received* 24 *October*; *revised form* 8 *December* 1962)

Abstract—A silver (Ag^+) catalyzed conversion of 4-maleylacetoacetate and of maleylacetone to their *trans* isomers has been demonstrated in dilute aqueous solutions at 25°. Parallel increases in the degree of catalysis, in the ionization of adjacent (maleyl) carboxyl group, and in the enolization of the carbonyl group adjacent to the double bond accompanied elevation of the pH from 1 to 6. The conversion of maleic to fumaric acid was not catalyzed by Ag^+.

RESULTS AND DISCUSSION

ALTHOUGH 4-maleylacetoacetate is the immediate product of the oxidation of homogentisic acid in the presence of the purified oxidase prepared from mammalian liver,[3] disilver fumarylacetoacetate is isolated when the enzymatic reaction mixtures are deproteinized and treated with $AgNO_3$.[4] Similarly, sodium maleylacetone, derived by



4-maleylacetoacetic acid

decarboxylation of disodium maleylacetoacetate when lyophilized enzymatic incubation mixtures are extracted with warm ethanol, yields silver fumarylacetone when precipitated with $AgNO_3$.[5] These observations led to the recognition that the *cis-trans* conversions were due to the presence of $AgNO_3$.

The experiments described in this report establish the catalytic role of silver ion in this reaction, the conditions favorable to catalysis, and the failure of other metallic ions to act as catalysts.

To avoid precipitation of the silver salts of maleylacetoacetate and maleylacetone, the *cis-trans* isomerization was studied in 0·005 M solutions of the *cis*-isomers in the presence of 0·0005 M $AgNO_3$. A comparison of the spontaneous and the silver catalyzed isomerization of Na maleylacetone is shown in Fig. 1 in which the data are plotted on semi-logarithmic coordinates. (Identical results were obtained with 4-maleylacetoacetate.) It may be seen that although the spontaneous reaction follows first order kinetics, the Ag-catalyzed reaction deviates from first order kinetics by a progressively excessive reduction in velocity during the later stages of the reaction

[1] Supported by Public Health Service Grant RG-3193.
[2] Lederle Medical Student Research Fellow in 1959.
[3] W. E. Knox and S. W. Edwards, *J. Biol. Chem.* **216**, 479 (1955).
[4] R. G. Ravdin and D. I. Crandall, *J. Biol. Chem.* **189**, 137 (1951).
[5] D. I. Crandall, R. C. Krueger, F. Anan, K. Yasunobu and H. S. Mason, *J. Biol. Chem.* **235**, 3011 (1960).

interval. These deviations do not contraindicate first order kinetics in our view but appear to correlate with a loss of Ag^+ indicated by the discoloration of the reaction mixture by a brown colloidal precipitate containing Ag^+ but not β-diketonic acid. It arises presumably from impurities in the maleylacetone and maleylacetoacetate.

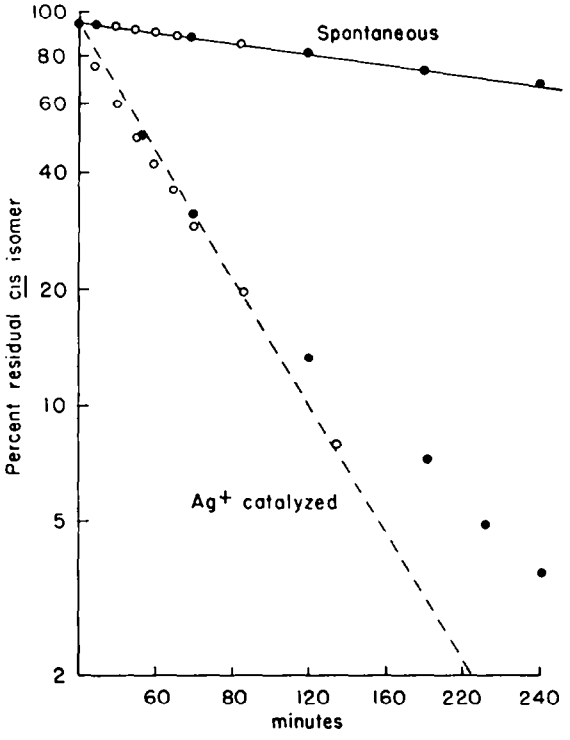


FIG. 1. *Cis-trans* isomerization of 0·0045 M Na maleylacetone in the presence (- - -) and in the absence (——) of 0·00045 M $AgNO_3$ at pH 7·0 (o) and at pH 4·7 (●) at 25°.

The apparent initial first order velocity constants of the catalyzed and the spontaneous reaction were 1·93 and 0·16% per minute respectively under the conditions of the experiment. Under the same conditions there is no catalysis of the reaction by $Al(NO_3)_3$, $CdSO_4$, $Co(NO_3)_2$, $CuCl$,[6] $CuSO_4$, $CrCl_3$, $FeSO_4$, $Fe(NO_3)_3$, $HgNO_3$,[6] $Hg(C_2H_3O_2)_2$, $MgSO_4$, $MnSO_4$, $NaNO_3$, $NiSO_4$, $Pb(OH)C_2H_3O_2$, $PtCl_4 \cdot 2HCl$,[7] and $ZnSO_4$ at a concentration of 5×10^{-4} M.

The silver catalyzed isomerization is abolished at pH 1 and is maximal at pH 5 as shown in Fig. 2. To this there is a closely parallel dependency of ionization of the maleyl carboxyl group on pH, as shown by the acid titration curve of Na maleylacetone in Fig. 3.

The pK of the maleyl carboxyl group was estimated to lie between pH 4·0 and 4·1 by titrating 5·00 mg of sodium maleylacetone with standard HCl using a glass

[6] Although insoluble or unstable in aqueous solution, these substances formed soluble complexes in the presence of the *cis*-diketonic acids.

[7] Neutralized to pH 4 before use.

electrode. This material contained 85% of the theoretical β-diketone moiety determined colorimetrically with *o*-phenylenediamine[4] and required 87% of the theoretical amount of HCl for back-titration. In spite of these indications of impurity, the data clearly showed that the carboxyl group is titrated between pH 3 and 6.

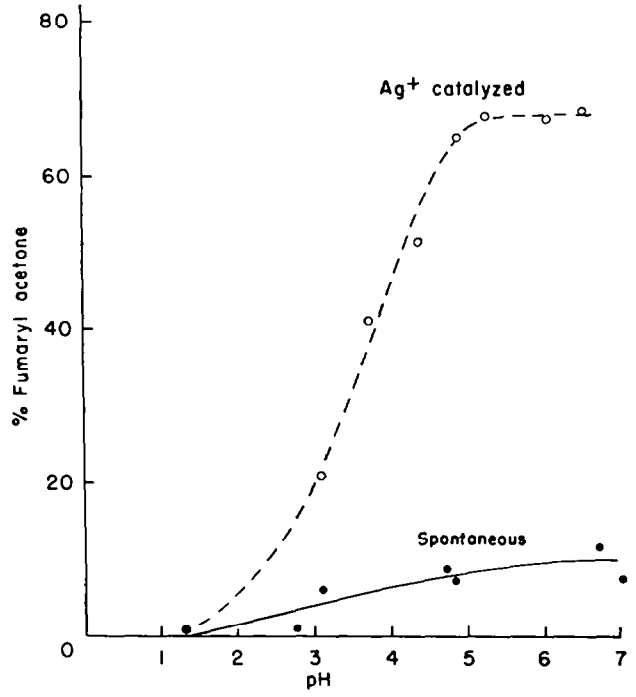


FIG. 2. Effect of pH on the isomerization of 0·0046 M Na maleylacetone in the presence (- - -) and in the absence (——) of 0·00045 M $AgNO_3$ when incubated 45 min at 27°. Medium: pH 1·3 = 0·05 N HNO_3; the higher pH's were produced with dilute acetic acid and 0·01 M acetate buffers.

Enolization of the adjacent carbonyl group also appeared to exhibit a parallel dependency on pH with the Ag^+ catalyzed isomerization. The pH range of enolization of the maleyl carbonyl group was estimated to lie between 2 and 5 from the data in Fig. 4 in which variations in the molar extinction of maleylacetoacetate and of maleylacetone with pH are compared at 315 mμ. As the pH is increased from 2 to 5 the U.V. absorptions of both compounds undergo a similar increase which represents enolization of the β-diketone group[8] but not enolate ion formation due to the failure of enols to ionize appreciably below pH 8·0. The pronounced difference in the absorption curves of the two compounds between pH 5 and 10 more probably reflects a change in the carbonyl group at position 3 of maleylacetoacetate (position 2 of maleylacetone) which is nearer to the carboxyl group lost on conversion of the former to maleylacetone by decarboxylation.

In view of the foregoing considerations it is impossible to distinguish between terminal carboxylate anion formation and/or enolization of the adjacent carbonyl group as adjuvants of the silver catalyzed isomerization of the maleyl double bond.

[8] W. E. Knox and S. W. Edwards, *J. Biol. Chem.* **216**, 489 (1955).

We assume that these adjacent groups are involved in the catalysis since the "argentation" of *cis*-2-butene does not result in isomerization[9] and since two other metal ions (Cu^+ and Hg^{++}) which form olefin complexes[10] do not catalyze this *cis-trans* isomerization.

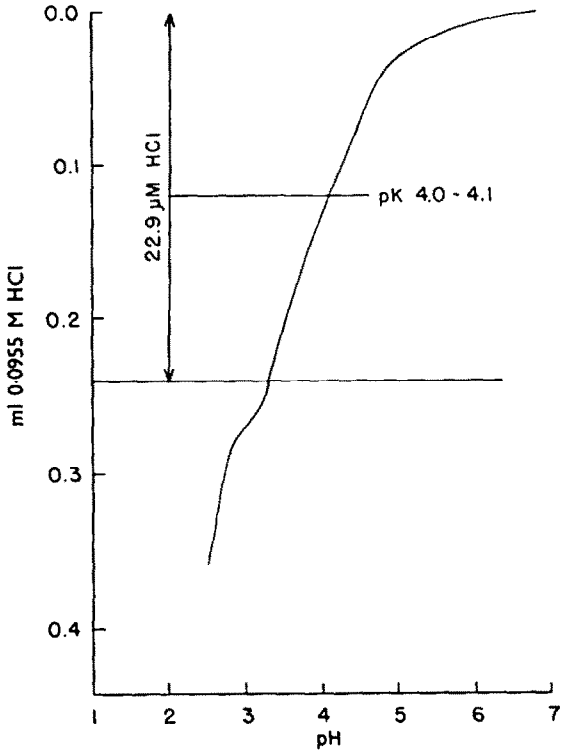


FIG. 3. Titration of 22·9 micromoles of Na maleylacetone dissolved in 3·00 ml H_2O with 0·0955 M HCl using a glass electrode.

Failure of Ag^+ to catalyze the isomerization of maleic to fumaric acid was indicated by the absence of pH change when solutions of 0·02 M maleic acid were incubated at pH 6·1 at 25° in the presence of 0·002 M $AgNO_3$. Conversion to fumarate would have caused the pH to drop to 4·4 as a consequence of the difference in the second dissociation constants of maleic and fumaric acids.[11] The failure of Ag^+ to isomerize maleic acid suggests that the β-diketone groups of maleylacetone and maleylacetoacetate are required for their silver catalyzed *cis-trans* isomerization. There is some indication, however, that the maleyl carbonyl (enol) component of the diketone group rather than the "acetone" (or acetoacetate) carbonyl is implicated in this reaction, namely, (1) the failure of decarboxylation of maleylacetoacetate at position 1 to affect the isomerization rate although this should alter the reactivity of the carbonyl at position 3, and (2) the proximity of the C_5 enol to the maleyl double bond.

[9] S. Winstein and H. J. Lucas, *J. Amer. Chem. Soc.* **60**, 836 (1938).

[10] L. N. Ferguson, *Electron Structures of Organic Molecules*, pp. 36–38, Prentice Hall, New York (1952).

[11] G. Dahlgren, Jr. and F. A. Long, *J. Amer. Chem. Soc.* **82**, 1303 (1960).

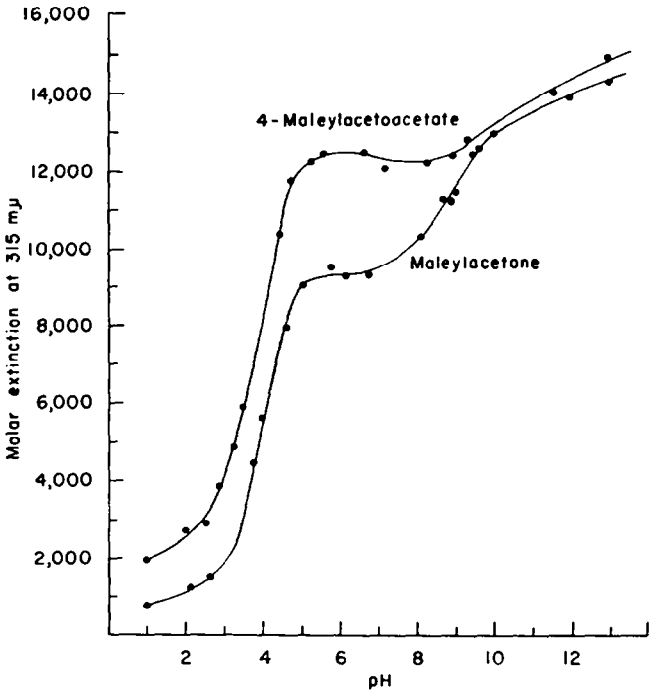


FIG. 4. Effect of pH on ultraviolet absorption by maleylacetoacetate and maleylacetone at 315 mμ.

EXPERIMENTAL

Preparation of chemicals. Electrolyte-free homogentisate oxidase was prepared by a modification of the method of Ravdin and Crandall[4] in which rat livers were homogenized with two volumes of H_2O (instead of buffer) during which the pH was maintained at 7·0 by adding 2 N NaOH. The ethanol-precipitated oxidase (prepared later in this procedure) was freed of glutathione by resuspension in ice-cold 32 per cent ethanol[5] and centrifugation prior to final resuspension in water and adjustment to pH 7·0. This step was included to inactivate the maleylacetoacetate isomerase present in this oxidase preparation. The oxidase was then incubated with homogentisate in a medium containing 0·025 M bicarbonate buffer as the sole electrolyte in equilibrium with 5% CO_2/95% O_2(pH 7·0). At the end of the incubation the bicarbonate was decomposed by acidification to pH 4·0 with HNO_3 under red. press. The reaction mixture was then readjusted to pH 7·0 with NaOH and consisted of protein, Na maleylacetoacetate and $NaNO_3$. It was lyophilized immediately and stored over P_2O_5 *in vacuo* at −20°. Maleylacetoacetate solutions were prepared from redissolved portions of this lyophilate by deproteinizing with 0·266 ml of 5% Na tungstate and 1·20 ml of 0·1 N HNO_3 per 100 mg of lyophilate. The filtrates were tungstate- and protein-free. Attempts to isolate solid maleylacetoacetate from these filtrates were unsuccessful. Their maleylacetoacetate content was determined spectrophotometrically.[8]

Na maleylacetone was prepared from the lyophilate by extraction with warm absolute ethanol and precipitation with anhydrous chloroform as previously described.[5] During the extraction, decarboxylation of the maleylacetoacetate has been demonstrated to occur.[5] The solid Na maleylacetone at this stage was 70% pure according to analysis for β-diketone content with an *o*-phenylenediamine reagent[5] which had been standardized with pure Ag fumarylacetone. It was then redissolved in ethanol, filtered, and reprecipitated with $CHCl_3$ resulting in an estimated purity of 85%.

Maleic acid (m.p. 137–139°) and solutions of maleic acid prepared from maleic anhydride (m.p. 53°) (both obtained from Matheson, Coleman and Bell) and H. M. Chemical Co. fumaric acid (m.p. 297–299°) were used. All inorganic salts were reagent grade.

Analyses. Isomerization of 0·005 M solutions of maleylacetoacetate and maleylacetone was

followed spectrophotometrically by measuring the concomitant increase in absorption at 315 mμ of aliquots of the reaction mixture. The molar extinctions of maleyl- and fumarylacetoacetic acids are 1,000 and 16,500 respectively at pH 1 and 315 mμ.[8] The corresponding values for maleyl- and fumarylacetone under these conditions are approximately 1,000 and 13,500 respectively.

One hundred-fold dilution of aliquots of *cis-trans* reaction mixtures with 0·1 N HNO_3 prior to the measurement of absorption at 315 mμ not only provided the necessary pH for determination of the extent of isomerization in a given aliquot as indicated above, but also stopped the isomerization reaction and decomposed any complexes which may have formed between metallic ions and the diketonic acids thus preventing extraneous U.V. absorption. None of these metallic ions absorb appreciably in the U.V. and it was therefore unnecessary to remove them from the reaction mixture by precipitation or other means. At this wavelength 0·1 N HNO_3 has appreciable absorption for which a correction must be made.

At pH 13 both maleylacetoacetate and maleylacetone[6] exhibit high molar extinction coefficients at 315 mμ (16,000 and 15,000 respectively) due to a pH-dependent enolization of the β-diketone group thus providing a means for estimating the degree of enolization at intermediate pH values between 1 and 13.