1β and, presumably, the 1α epimers. Sublimation at 35° (0.08 mm) afforded 55 mg (81%) of decalol **53**: mp 52–54°; λ_{max}^{film} 2.91 (OH), 7.19, 7.28, 7.94, 8.69, 8.98, 9.42, 9.74, 10.20, 10.34, 10.61, 10.73, and 14.57 μ; $\delta_{TMS}^{CCl_4}$ 3.25 (H-1), 1.73 (OH), 1.00 (angular CH_3), 0.98 (angular CH_3), and 0.84 ppm (CH_3 doublet, J = 5 Hz).

The analytical sample, mp 54–55° (for the enantiomer, lit.[40] mp 56–57°), was secured after an additional sublimation.

Anal. Calcd for $C_{15}H_{28}O$: C, 80.29; H, 12.58. Found: C, 80.5; H, 12.5.

Reduction of (+)-Valeranone (19).—A 15-mg sample of (+)-valeranone (**19**) in 5 ml of dry ether was stirred with 30 mg of lithium aluminum hydride for 3 hr at room temperature. The mixture was treated with 0.06 ml of water and 0.05 ml of 10% aqueous sodium hydroxide, stirred for several hours, and filtered. The solvent was removed from the filtrate under reduced pressure affording 14 mg (90%) of an alcohol mixture containing 63% 1β-decalol **53** and 37% 1α epimer identified by peak enhancement[56] with the previously prepared mixture.

(+)-Valeranone (19).—A solution of 20 mg of decalol **53** in 10 ml of acetone was cooled to 0° and treated dropwise with 0.3 ml of Jones reagent.[44] Isopropyl alcohol was added to destroy the excess reagent, and the product was isolated with ether[42a] affording 19 mg (95%) of (+)-valeranone (**19**) identified by comparison with the sample prepared previously *via* angular methylation.

Registry No.—**15,** 5090-56-2; **16,** 5195-62-0; **17,** 5195-63-1; **18,** 5195-64-2; (+)-valeranone (**19**), 17414-58-3; **20,** 17408-81-0; **21,** 17408-82-1; **22,** 5090-58-4; **24** (R = Me), 17408-83-2; **24** (R = H), 17408-77-4; **25,** 5195-65-3; **26,** 5090-59-5; **27,** 17414-60-7; **32,** 17408-61-6; **33,** 17448-31-6; **34,** 10208-75-0; **35,** 17408-63-8; **36,** 10208-77-2; **37,** 17414-62-9; **39,** 10208-73-8; **40,** 17408-64-9; **40** dinitrophenylhydrazone, 17408-65-0; **43,** 17378-42-6; **44,** 10253-25-5; **45,** 17408-66-1; **45** dinitrophenylhydrazone, 17408-67-2; **46,** 17408-68-3 **47,** 17408-69-4; **50,** 17408-70-7; **51,** 17408-71-8; **52,** 17408-72-9; **53,** 17408-73-0 **54,** 17408-74-1; **55,** 17408-75-2; **56,** 17408-76-3.

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Thiocyanate-Catalyzed *cis–trans* Isomerization of *cis*-β-Acetylacrylic Acid. A Model for Maleylacetoacetic Acid[1]

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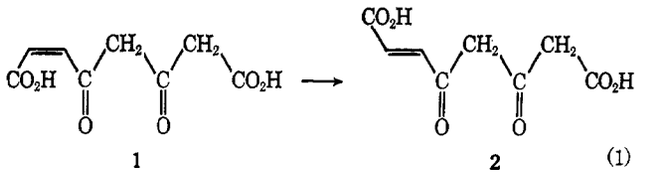
The kinetics of *cis–trans* isomerization of *cis*-β-acetylacrylic acid, a model for maleylacetoacetic acid, has been measured in the absence of any catalyst and in the presence of thiocyanate ion over five powers of ten in acid concentration. A kinetic scheme is proposed to account for the dependence of the rate on thiocyanate and hydronium ion concentrations. Rate constants have been obtained by fitting the data to the kinetic scheme. The similarities and differences of this system to the enzymatic system are discussed.

Homogentisic acid, an intermediate in the oxidation of aromatic amino acids such as tyrosine and phenylalanine, has been shown to undergo further oxidation to 4-maleylacetoacetic acid (4,6-dioxo-*cis*-2-octenedioic acid, **1**) in animal liver[2] and bacterial cells.[3] From these same extracts, an enzyme has been isolated which together with glutathione (GSH) catalyzes the *cis–trans* isomerization of 4-maleylacetoacetic acid to 4-fumarylacetoacetic acid (**2**, eq 1).[2,3] Also present is another enzymatic system which catalyzes the specific hydrolysis of **2** to fumaric and acetoacetic acids.



1 **2** (1)

Similar to the metabolism of aromatic amino acids is the bacterial oxidation of nicotinic acid. Soluble extracts of *Pseudomonas fluorescens* catalyze the oxidative conversion of nicotinic acid into N-formylmaleamic acid through the intermediacy of 2,5-dihydroxypyridine.[4] Here too, there is an enzyme present in these extracts which catalyzes the *cis–trans* isomerization of the maleic acid product to fumaric acid. It also appears that thiol groups are necessary for isomerase activity.[5]

As an extension of our earlier interest in the mechanism of the catalyzed *cis–trans* isomerization of maleic acid,[6] it was decided to investigate possible models for the enzyme–coenzyme-catalyzed isomerization of 4-maleylacetoacetic acid. 4-Maleylacetoacetic acid has not been synthesized or isolated in pure form.[2] Its high lability suggested the synthesis of a more stable model substrate, *cis*-β-acetylacrylic acid, **3**, possessing what appears to be the same functional groups necessary for facile isomerization. Its preparation is described elsewhere.[7] We report here the kinetics of the spontaneous and thiocyanate ion catalyzed isomerization of *cis*-β-acetylacrylic acid.

Results and Discussion

The uv and nmr spectra of *cis*- and *trans*-β-acetylacrylic acid have been reported.[7] Nmr spectra indicate that in neutral or basic media the *cis* acid exists as an open anion, **4**, but in acidic solution the acid has

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

(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) P. J. Chapman and S. Dagley, *J. Gen. Microbiol.*, **28**, 251 (1962).

(4) E. J. Behrman and R. Y. Stanier, *J. Biol. Chem.*, **228**, 923 (1957).

(5) K. Otsuka, *Agr. Biol. Chem.* (Tokyo), **25**, 726 (1961).

(6) S. Seltzer, *Chem. Ind.* (London), 1313 (1959); *J. Amer. Chem. Soc.*, **83**, 1861 (1961).

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

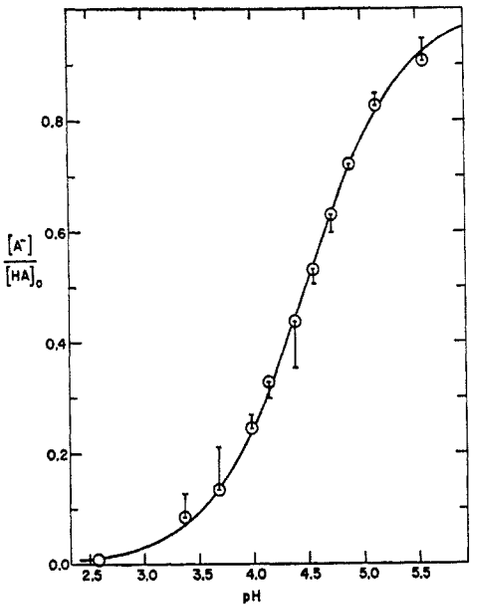
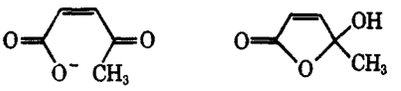


Figure 1.—Degree of dissociation *vs.* pH for *cis*-β-acetylacrylic acid. Open circles refer to measurements at 237 mμ while the vertical lines of error flags refer to measurements at 195 mμ. The line is the theoretical curve for a monoprotic acid with a pK_a of 4.48.

the cyclic pseudo-acid structure, **5**.[7] Since it was desirable to measure the kinetics of isomerization at



4 **5**

various hydrogen ion concentrations, a determination of the ionization constant of the substrate was necessary. The acid absorbs strongly at 195 mμ, while the anion has maxima of about equal intensity at 198 and 240 mμ. The optical densities due to acid and conjugate base in buffered solutions were measured at both 195 and 237 mμ. The concentration of anion and acid can be determined from the optical density at only one wavelength if the total concentration is known and if only two species are assumed. The ratio of anion to initial acid present *vs.* pH is shown plotted in Figure 1. The line represents the theoretical curve for a monoprotic acid with a pK_a of 4.48. That only two species, **4** and **5**, are present is shown by the isosbestic point at 210 mμ. The average deviation of optical densities at 210 mμ between pH 2.4 and 6.3 is about ±1% from the mean of 14 points.

Uncatalyzed Isomerization.—In the course of studying the conditions for the preparation of *cis*-β-acetylacrylic acid from the ethyl pseudo-ester, nmr spectra revealed rapid concurrent hydration and isomerization of the *cis* acid at pH 3 and 100°.[7] Repetitive scans in the ultraviolet region of an aqueous solution of *cis* acid at 69.5° and pH 3.3 further support this interpretation. As the *cis* acid (ϵ_{195} 9850, ϵ_{220} 1130) disappears in the early part of the reaction, very little *trans* acid (ϵ_{220} 12,230) appears. Both the *cis* and the *trans* acids appear to undergo slow hydration. The reactions can be summarized by eq 2 and 3 where C and

$$C \xrightarrow{k_1} T \xrightarrow[H_2O]{k_2} H \qquad (2)$$

$$C \xrightarrow{k_3} H' \qquad (3)$$

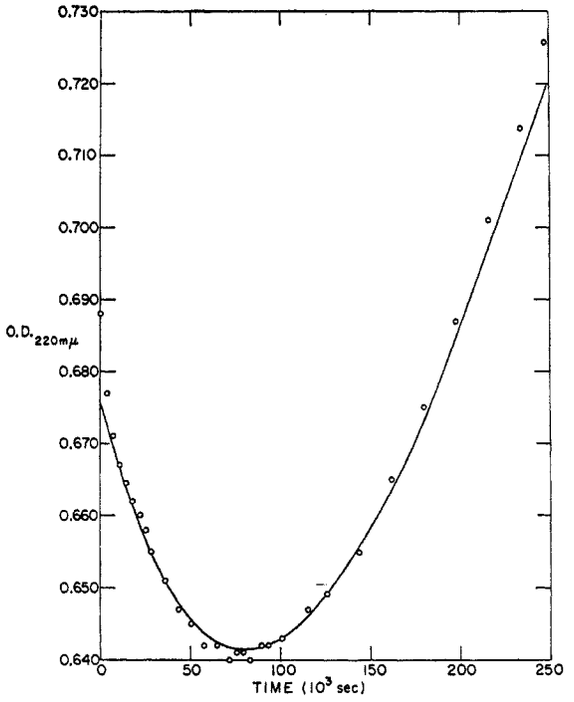


Figure 2.—The change in optical density at 220 mμ with time for a solution containing 4.20 × 10^{-4} *M cis* acid and 1.06 × 10^{-5} *M trans* acid at 69.5° and pH 3.3.

T refer to *cis*- and *trans*-β-acetylacrylic acid, respectively, and the H's refer to hydrated acids; k_1, k_2, and k_3 are pseudo-first-order rate constants, since water is undoubtedly a reactant in these reactions. If the optical density at 220 mμ (λ_{max} for the *trans* acid) is continually monitored for a solution of the *cis* acid at pH 3.3 and 69.5°, the optical density is observed to decrease very slowly and, after about 23 hr, the absorbance *increases* slowly (see Figure 2). If, however, the optical density at which the *cis* absorbs much more strongly than the *trans*, *viz.* 195 mμ, is examined the optical density falls more rapidly than in the previous experiment. The observed rate constant for the disappearance of the *cis* acid under these conditions is about (8 ± 1) × 10^{-6} sec^{-1}.[8] Moreover, the loss of *trans* acid itself, under the same conditions, but in separate kinetic runs, was determined from the change in optical density at 220 mμ. The rate of loss of the *trans* acid is less than one fourth the total rate of loss of the *cis* acid. The decrease in the optical density at 220 mμ of a solution containing the *cis* acid initially requires the *trans* acid to be lost at a faster rate than it is produced in the early part of the reaction. This and the slow hydration rate of the *trans* acid suggest that less than 20% of C goes directly to T; the remainder of C hydrates directly. If, now, an equilibrium exists between *trans* and hydrated acids that is not wholly on the side of hydrated acid it might be expected that some *trans* acid will be formed from the hydrated acid, *i.e.*

$$H \text{ or } H' \xrightarrow{k_{-2}} T + H_2O \qquad (4)$$

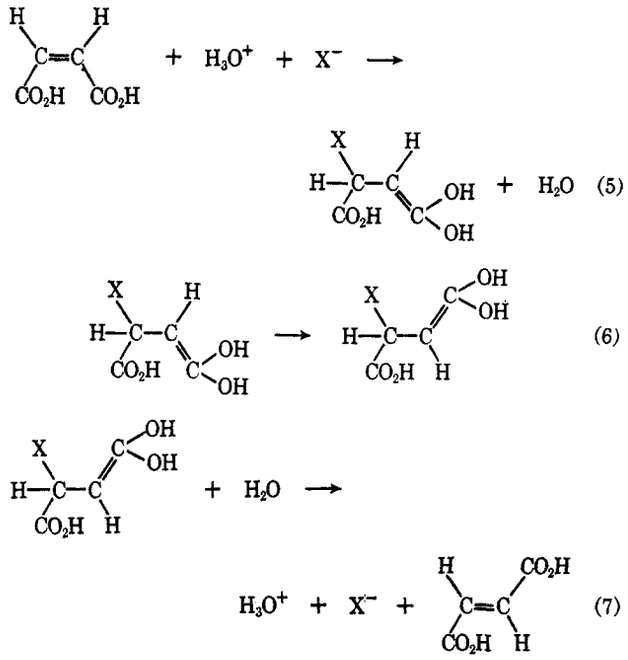
(8) The observed rate constant by this method is approximate. At the beginning of the reaction it equals the sum of k_1 and k_2. Because the *trans* acid contributes to the total optical density, the observed rate constant for the disappearance of the *cis* acid will have contributions from k_2 and k_{-2}.

This would explain the increase in the optical density at 220 mμ after 23 hr.

Assuming that H and H′ behave identically, the usual differential equations, expressing the rate of change in the concentrations of C, T, H, and H′, can be written from examination of eq 2–4. A least-squares program, KNTCS10, was written to find the values for k_1, k_2, k_{-2}, and k_3 needed to obtain the best fit for representative data shown in Figure 2. A fuller description of KNTCS10 is given in the Appendix. The rate constants were found to have the following values: k_1, $9.48 \times 10^{-9}\ M^{-1}\ \text{sec}^{-1}$; k_2, $1.83 \times 10^{-8}\ M^{-1}\ \text{sec}^{-1}$; k_{-2}, $4.24 \times 10^{-7}\ M^{-1}\ \text{sec}^{-1}$; k_3, $1.07 \times 10^{-7}\ M^{-1}\ \text{sec}^{-1}$. Incorporation of these rate constants into the calculation of the optical density at selected times leads to the parabolic curve in Figure 2. The standard deviation between observed and calculated optical densities is about ±0.5%.

As predicted from the gross features of the change in optical density with time, the *cis* acid hydrates about ten times faster than it isomerizes when water is the catalyst. The sum of these two rate constants, k_3 and k_1, multiplied by the concentration of water yields the pseudo-first-order rate constant, $6.5 \times 10^{-6}\ \text{sec}^{-1}$, for loss of *cis* acid under these conditions; agreement between this value and that obtained by a less precise method[8] is fairly good. Water adds about six times faster to the *cis* acid than to the *trans* acid under these conditions.

Thiocyanate-Catalyzed Isomerization.—In the absence of the isomerase, glutathione catalyzes the *cis–trans* isomerization of maleylacetoacetate. A stable coenzyme–substrate addition product has not been isolated. In contrast to this, fumarylacetoacetate slowly forms a stable product with GSH which is presumably the product of addition to the carbon–carbon double bond of the fumaryl group.[2] The role of glutathione as a coenzyme in the enzymatic isomerization has been suggested to involve the addition of the glutathiyl radical, GS·, to the carbon–carbon double bond of maleylacetoacetate followed by rotation about the central carbon–carbon bond and then loss of (GS·).[2] The mechanism is similar to the bromine atom catalyzed isomerization of maleic acid.[9] The reaction with glutathione, however, need not involve radicals. The isomerization of maleic acid can be catalyzed by mineral acid but there is a strong dependence of the rate on the nature of the anion of the acid.[10] The greater the nucleophilicity of the anion the faster is the rate. In fact the most active catalyst encountered by Nozaki and Ogg was potassium thiocyanate itself in the absence of added acid. They suggested the following mechanism. The mechanism is supported by the earlier observation of Horrex that no deuterium is introduced on the carbon–carbon double bond of fumaric acid if deuterium chloride is the catalyst.[11] Absence of exchange is also noted when thiocyanate is the catalyst.[6] That an addition intermediate, as shown in eq 5, is formed is further supported by the observation of an inverse secondary α-deuterium isotope effect in the isomerization of maleic-2,3-d_2 acid.[6]



Both the mercaptide anion and bicovalent sulfur in the conjugate base and acid of glutathione would be expected to be efficient nucleophiles.[12] Hence, it was of interest to study the effect of a nucleophilic catalyst on the isomerization. As a simplification we have studied the thiocyanate-catalyzed isomerization of *cis*-β-acetylacrylic acid. The product of this reaction can be easily identified as the *trans* acid by its characteristic uv and nmr spectra. In Table I are shown the

TABLE I

EFFECT OF TEMPERATURE ON THE THIOCYANATE ION CATALYZED ISOMERIZATION OF *cis*-β-ACETYLACRYLIC ACID[a]

Temp, °C	pH	$10^3 \times$ [KSCN], M	$10^4 \times k_{obsd}$, sec^{-1}	$10^2 \times k_2$, $M^{-1}\ \text{sec}^{-1}$
69.5	3.15	1.0	1.84	18.4
69.5	3.16	1.0	1.80	18.0
69.5	3.17	1.0	1.83	18.3
48.3	3.17	2.25	1.32	5.87
48.3	3.18	0.502	0.298	5.94
48.3	3.13	1.0	0.583	5.83
48.3	3.11	1.0	0.590	5.90
25.0	3.11	1.0	0.129	1.29
25.0	3.11	1.0	0.146	1.46
25.0	3.12	1.0	0.144	1.44

[a] The concentration of substrate in these experiments was $9.24 \times 10^{-4}\ M$ except for the last two runs where it was $9.50 \times 10^{-4}\ M$.

observed pseudo-first-order rate constants which were obtained by a nonlinear least-squares fit of the observed spectrophotometric data[13] and the calculated second-order rate constants at three different temperatures. Since the kinetic runs shown here are all approximately at the same hydrogen ion concentration it is of interest to obtain over-all activation constants for the reaction at this pH. The enthalpy of activation is 11.2 kcal/mol while the entropy of activation is −29 eu. The entropy appears to be normal for an ion–dipole reaction while the enthalpy seems to be quite low. Assuming that the water molecule is the

(9) F. R. Mayo and C. Walling, *Chem. Rev.*, **27**, 351 (1940).
(10) K. Nozaki and R. Ogg, Jr., *J. Amer. Chem. Soc.*, **63**, 2583 (1941).
(11) C. Horrex, *Trans. Faraday Soc.*, **33**, 579 (1937).
(12) E. C. Kooyman in "Organosulfur Chemistry," M. J. Janssen, Ed., Interscience Publishers, New York, N. Y., 1967, pp 2–6.
(13) S. Seltzer, *J. Amer. Chem. Soc.*, **83**, 2625 (1961).

corresponding nucleophile in the uncatalyzed isomerization, the second-order rate constant at comparable pH and 69.5° is 9.5×10^{-9} M^{-1} sec^{-1}. Thiocyanate is a more effective catalyst than water by a factor of about 10^7. It is interesting that thiocyanate ion is only about $10^{4.8}$ more effective than water as a nucleophile toward saturated carbon.[14]

The isomerization reaction was studied over a 100-fold range of thiocyanate ion concentration as shown in Table II. A plot of log k_{obsd} *vs.* log [KSCN] gives a good straight line with a slope of 0.97 indicating first-order dependence on potassium thiocyanate. Similarly, the rate of isomerization was studied over a 450-fold

TABLE II

EFFECT OF THIOCYANATE CONCENTRATION ON THE RATE OF *cis*-β-ACETYLACRYLIC ACID ISOMERIZATION[a]

[KSCN], M	pH	$10^4 k_{obsd}$, sec^{-1}	$10^2 k_2$, M^{-1} sec^{-1}
1.0×10^{-3}	3.12	0.144	1.44
1.0×10^{-2}	3.13	1.30	1.30
2.0×10^{-2}	3.12	2.64	1.32
4.0×10^{-2}	3.12	5.22	1.31
1.0×10^{-1}	3.16	12.6	1.26

[a] The initial concentration of *cis*-β-acetylacrylic acid was 9.50×10^{-4} M throughout and the temperature was 25.0°. In all of these runs the sum of the acetic acid and sodium acetate concentrations was 0.01 M, and lithium perchlorate was added to bring the ionic strength to 0.1.

variation in substrate concentration (Table III). The change in the observed rate constant over this range is about 1.5. Unfortunately the pH over this range varied somewhat from one run to the next, and consequently the rate constants are not exactly comparable. As will be shown below, the rate increases with increasing acidity. If the observed rate constants in Table III

TABLE III

EFFECT OF REACTANT CONCENTRATION ON THE RATE OF *cis*-β-ACETYLACRYLIC ACID ISOMERIZATION[a]

[*cis* acid], M	pH	$10^4 k_{obsd}$, sec^{-1}	$10^2 k_2$, M^{-1} sec^{-1}
2.1×10^{-5}	3.23	1.21	1.21
9.50×10^{-4}	3.13	1.30	1.30
1.90×10^{-3}	3.13	1.33	1.33
2.85×10^{-3}	2.95	1.52	1.52
9.50×10^{-3}	2.71	1.81	1.81

[a] Potassium thiocyanate was 0.010 M and the temperature 25.0°. The sum of acetic acid and sodium acetate concentrations was 0.01 M, and lithium perchlorate was added to make the ionic strength 0.1.

are all corrected to pH 3.13, they deviate from the mean by less than ±1%, indicating that the reaction is also first order in *cis* acid, at least at pH 3.13.

That the rate is little affected by a change in the ionic strength is shown in Table IV. At pH 3 the reaction is characteristic of an ion–dipole reaction and is expected to be fairly insensitive to the polarity of the solvent.[15]

The effect of hydrogen ion concentration on the rate is shown in Table V. In the pH range 5.7–3.0 the ratio of the concentrations of cyclic pseudo-acid (**5**)

(14) A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 11.

(15) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley & Sons, Inc., New York, N. Y., 1953, pp 135–142.

TABLE IV

EFFECT OF IONIC STRENGTH ON THE RATE OF THE CATALYZED ISOMERIZATION AT 25° [a]

μ	pH	$10^2 k_2$, M^{-1} sec^{-1}
0.05	3.18	1.32
0.10	3.15	1.24
0.20	3.01	1.29

[a] In all runs the substrate and potassium thiocyanate concentrations were 9.24×10^{-4} M and 1.0×10^{-2} M, respectively. The sum of the acetic acid sodium acetate concentrations was 1.0×10^{-2} M. The desired ion strength was obtained by adding lithium perchlorate.

TABLE V

EFFECT OF pH ON THE THIOCYANATE ION CATALYZED ISOMERIZATION OF *cis*-β-ACETYLACRYLIC ACID AT 25° [a]

10^4[*cis* acid], M	pH	$10^2 \times k_2$, M^{-1} sec^{-1}
6.75[b]	5.70	0.0523
9.50[b]	5.23	0.147
4.75	4.91	0.207
19.0[b]	4.60	0.368
9.5	4.44	0.410
9.5	4.19	0.550
9.5	4.00	0.609
9.5	3.78	0.754
9.5	3.45	1.01
9.5	3.13	1.31
8.72	2.93	1.52
8.72	2.70	1.83
8.72	2.31	2.54
8.72	2.04	3.29
8.72	1.81	4.03
8.72	1.67	4.31
8.72	1.48	4.83
8.72	1.05	5.84
8.72	0.38	8.03

[a] Kinetics were measured in acetic acid–sodium acetate buffers (total concentration, 0.01 M) where feasible. Perchloric acid was added in the more strongly acid region. Lithium perchlorate was added to adjust the ionic strength to 0.1. The last run, of course, had an ionic strength greater than 0.1. [b] The concentration of potassium thiocyanate was 1.0×10^{-1} M. In all other runs the concentration was 1.0×10^{-2} M.

to opened *cis* anion (**4**) changes significantly. As might be expected, the rate of catalyzed isomerization is different for these two species. If catalysis were solely due to thiocyanate anion and not its conjugate acid, the rate constant would reach a limiting value at pH's lower than 3.0. Eventually the rate would be expected to decline at still higher acid concentration for thiocyanate would be converted into thiocyanic acid. As can be seen from Table V, the rate continues to increase at higher than 10^{-3} M acid and indicates that another protonated species is formed which reacts more rapidly than its unprotonated form. This protonated catalytic species appears to be thiocyanic acid. The reactions of *cis* anion (A^-), *cis* acid (HA), thiocyanate ion (S^-), and thiocyanic acid (HS) are summarized by eq 8–10 where HT and T^- refer to the

$$S^- + A^- \xrightarrow{k_8} S^- + T^- \quad (8)$$

$$S^- + HA \xrightarrow{k_9} S^- + HT \quad (9)$$

$$HS + HA \xrightarrow{k_{10}} HS + HT \quad (10)$$

trans acid and its conjugate base. There appears to be no general acid or general base catalysis when the buffer is acetic acid–sodium acetate. The second-order

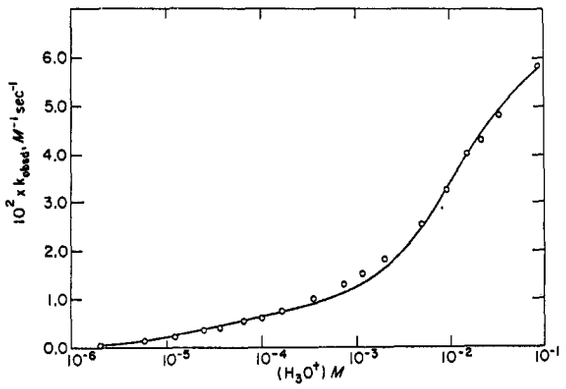


Figure 3.—The observed second-order rate constant of thiocyanate catalyzed *cis-trans* isomerization of *cis*-β-acetylacrylic acid *vs.* hydronium ion activity. The points shown represent experimental observations; the line is the theoretical curve.

rate constant is relatively constant over a sevenfold variation in the total buffer concentration (Table VI). The rate of reaction proceeding by eq 8–10 is given by

$$\text{rate} = \frac{k_8K_aK_{11} + k_9K_{11}(H_3O^+) + k_{10}(H_3O^+)^2}{(H_3O^+)^2 + K_a(H_3O^+) + K_{11}(H_3O^+) + K_aK_{11}}[S]_0[A]_{total}$$

where the dissociation constants are defined as[16]

$$K_a = \frac{(H_3O^+)[A^-]}{[HA]} \text{ and } K_{11} = \frac{(H_3O^+)[S^-]}{[HS]} \quad (11)$$

TABLE VI

EFFECT OF BUFFER CONCENTRATION ON THE RATE OF CATALYZED ISOMERIZATION AT 25° [a]

[Buffer], M	pH	10^2k_2, M^{-1} sec^{-1}
0.00960	4.04	0.661
0.0192	4.10	0.654
0.0288	4.12	0.658
0.0672	4.15	0.718

[a] The substrate and potassium thiocyanate concentrations were 8.72×10^{-4} M and 1.00×10^{-2} M.

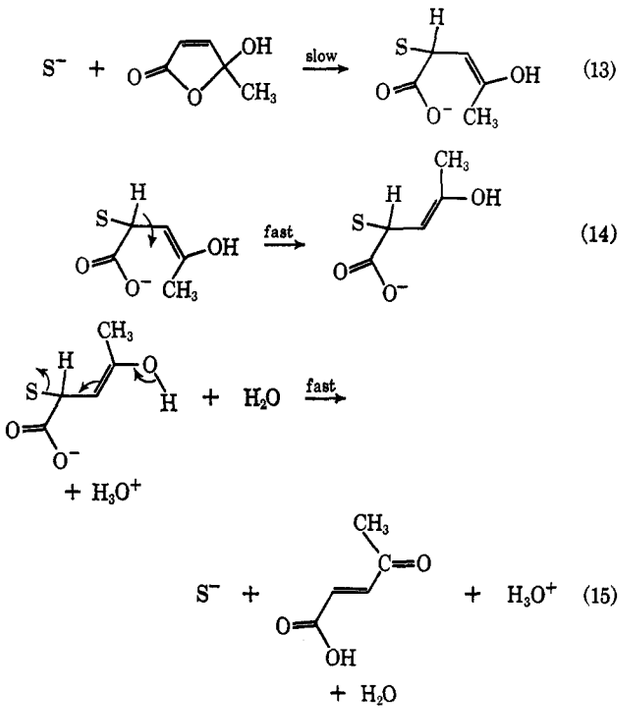
As discussed above, K_a was found to be 3.31×10^{-5} M^{-1}. Thiocyanic acid is known to be a strong acid.[17] Its pK_a has recently been found to be 0.97 ± 0.7 at 26°.[18] The observed second-order rate constant can be written as

$$k_{obsd} = \frac{k_8K_aK_{11} + k_9K_{11}(H_3O^+) + k_{10}(H_3O^+)^2}{(H_3O^+)^2 + K_a(H_3O^+) + K_{11}(H_3O^+) + K_aK_{11}} \quad (12)$$

The best fit of the data in Table V to eq 12 requires the equilibrium and rate constants to have the following values: k_8, 5.2×10^{-5} M^{-1} sec^{-1}; k_9, 8.3×10^{-3} M^{-1} sec^{-1}; k_{10}, 6.5×10^{-2} M^{-1} sec^{-1}; K_{11}, 1.2×10^{-2} M^{-1}. The fit of calculated and experimental data is shown in Figure 3. The standard deviation of the relative errors between calculated and observed k_{obsd}'s is under 9%. The major factor responsible for this error is probably the inability of the Model G pH meter to measure the pH precisely. It should be mentioned that the dissociation constant of thiocyanic acid derived from these studies is just barely outside the error limit and tends to support the reaction scheme in eq 8–10. Another protonated species (H_2A^+) might be postulated to explain the increased rate at pH's lower than 3. However, we have not been able to detect any nmr or uv spectral change of the *cis* acid in strong acidic solution. Moreover, substitution of H_2A^+ for HS in the kinetic scheme appears to lead to a poorer fit of the experimental data. In addition inclusion of both HS and H_2A^+ and their equilibria into an alternative kinetic scheme leads to a calculated ionization constant for the hypothetical species, H_2A^+, that appears to be unreasonable.

By analogy with the thiocyanate-catalyzed isomerization of maleic acid, we write the following general mechanism. That ring opening itself is not rate controlling is suggested by the nmr spectrum of the substrate at pH ≃ pK_a. Only one very sharp methyl peak is observed in spite of the fact that an aqueous solution containing only **4** has its methyl peak shifted 0.59 ppm downfield from that of a solution containing only **5**.[7] At this pH, reversible ring opening takes place with $k \geq 2 \times 10^3$ sec^{-1}. No addition product builds up during isomerization, supporting the contention that the addition is the slow step. In very weakly acidic media the rate constant, k_8, is that for the combination of two negatively charged species. As expected it is smaller than k_9, the rate constant for attack of an anion on a dipole. It is somewhat surprising that the second-order rate constant for attack of thiocyanic acid on the *cis* acid is larger than k_9. This may be due to simultaneous proton transfer or hydrogen bonding between thiocyanic acid and the incipient negatively charged carboxyl group as the sulfur atom adds to the carbon–carbon double bond.

Near neutral pH, the isomerization reaction of GSH with maleylacetoacetic acid in the absence of enzyme proceeds about 4000 times faster than the comparable isomerization of *cis*-β-acetylacrylate (**4**) catalyzed by



(13) (14) (15)

(16) The equilibrium constants shown are hybrid equilibrium constants and used here for convenience. K_a was determined from hydrogen ion activities and concentrations of anion and acid. Since the concentrations of substrate and catalyst are generally low in all runs and the ionic strength constant, these equilibrium constants are expected to be constant.

(17) T. Suzuki and H. Hagisawa, *Bull. Inst. Phys. Chem. Res.* (Tokyo), **21**, 601 (1942); *Chem. Abstr.*, **43**, 2074 (1949).

(18) J. H. Boughton and R. N. Keller, *J. Inorg. Nucl. Chem.*, **28**, 2851 (1966).

thiocyanate.[19] This rate ratio appears to be mainly the result of a difference in the electrophilicities of the natural and model substrates. The natural substrate having two additional carbonyl groups would appear to have a more electrophilic carbon–carbon double bond than the model compound. It can be estimated[20] that the "spontaneous" rate of natural substrate isomerization at 10° and pH 3 is about seven times faster than the isomerization of the model compound at about the same pH but at about 70°.[21] If we assume a factor of 10 in rate for a 25° increment in reaction temperature, it is estimated that the model compound isomerizes spontaneously about 1700 times more slowly than the natural substrate at the same temperature and pH. It might be anticipated that GSH and thiocyanate might be almost equally effective in catalyzing the isomerization of the natural substrate.

Although the molecular weight of the enzyme is not yet known the rate of the enzyme-catalyzed maleylacetoacetate isomerization appears to be very much faster than the thiocyanate-catalyzed isomerization of *cis*-β-acetylacrylic acid. Moreover, the enzymatic reaction exhibits optimal rate at pH 8.5.[2] Assuming that a similar equilibrium exists between cyclic (maleylacetoacetic acid) and noncyclic (maleylacetoacetate) forms, this optimal rate represents reaction with the noncyclic form and should be compared in rate to k_8, the smallest of the three rate constants. The enzyme appears to be doing much more than holding nucleophile (GSH) and substrate in juxtaposition. Work is currently in progress to determine possible roles for the enzyme.

Experimental Section

Reagents.—*cis*-β-Acetylacrylic acid was prepared by the method reported previously.[7] Acetic acid, sodium acetate, potassium thiocyanate, lithium perchlorate, and perchloric acid were reagent-grade chemicals.

Dissociation Constant.—Fourteen acetic acid–sodium acetate buffer solutions containing lithium perchlorate were prepared to yield solutions in which the sum of acetic acid and sodium acetate concentrations was 0.01 *M* and the ionic strength 0.10 when diluted with the stock *cis*-β-acetylacrylic acid solution. A Beckman Model G pH meter with internal miniature electrodes was used to read the pH's of each solution. The meter was standardized with pH 4 and 7 buffers. Uv spectra were recorded for each of these buffer solutions containing 7.50×10^{-5} *M* *cis*-β-acetylacrylic acid. The reference uv cell contained only buffer solution. Spectra were recorded on a Cary 14 spectrophotometer at 25°. In acidic media the acid exhibits the following molar extinction coefficients: ϵ_{195} 9850; ϵ_{237} 280. In neutral media the following molar extinction coefficients were observed: ϵ_{195} 3960; ϵ_{237} 4200. The ratio (α) of the concentrations of anion to the total added *cis* acid was determined at both 195 and 237 mμ by eq 16.

$$OD_\lambda = [HA]_{total}\{\epsilon_\lambda^{HA}(1 - \alpha) + \epsilon_\lambda^{A^-}(\alpha)\} \quad (16)$$

The optical densities at the isosbestic point (210 mμ) *vs.* pH are as follows [pH (OD_{210})]: 2.41 (0.310); 3.37 (0.313); 3.39 (0.315); 3.68 (0.318); 3.98 (0.305); 4.14 (0.323); 4.37 (0.306); 4.55 (0.308); 4.72 (0.313); 4.89 (0.309); 5.14 (0.309); 5.57 (0.312); 6.25 (0.308); 6.34 (0.309); av OD, 0.311 ± 0.004.

Kinetics.—The same buffers that were used to measure the dissociation constant were used for the most part in the kinetic studies. In runs with no thiocyanate present both the disappearance of *cis* acid and the appearance of *trans* acid were determined by continually recording the optical density at 200 and 220 mμ, respectively. Water from a thermostated bath was circulated through the thermostatable cell jacket. The cell compartment, however, had water at 25.0° circulated through it to ensure no thermal drift in the monochromator. The temperature of the contents of the uv cell was measured with a thermocouple which had been previously calibrated against an NBS thermometer. At temperatures above 25°, the contents in the quartz cell were cooled in ice and the cell was sealed by allowing the heated quartz to collapse together under atmospheric pressure. This ensured that solvent was not lost during kinetic measurement. This precaution was not taken when the reaction temperature was 25°; normal ground stoppered cells were used in this case. In runs containing thiocyanate the appearance of the *trans* acid had to be measured because of the strong absorption below 230 mμ due to thiocyanate ion. In these runs the optical density at 250 mμ was generally recorded. The reaction time was taken from the speed of the recorder chart. This was generally 4 in./hr.

Nmr spectra were recorded on a Varian A-60 spectrometer.

Registry No.—Thiocyanate ion, 302-04-5; *cis*-β-acetylacrylic acid, 2833-21-8; 4-maleylacetoacetic acid, 5698-52-2.

Acknowledgments.—The authors would like to thank Dr. R. W. Dodson for his interest in this study and Dr. W. C. Hamilton for helpful discussion.

Appendix

Equations 17–19 are derived from eq 2–4; [A] = the sum of H and H′ concentrations. The observed optical density is given by eq 20 where the ε's refer to the extinction coefficients at λ. Equation 21 is obtained by differentiating eq 20 with respect to t and substitution of eq 17–19. The slope at an individual point (OD_i, t_i) was obtained in two ways which are shown in eq 21.

$$-\frac{d[C]}{dt} = (k_1 + k_3)[C][H_2O] \quad (17)$$

$$\frac{d[T]}{dt} = k_1[C][H_2O] - k_2[T][H_2O] + k_{-2}[A] \quad (18)$$

$$\frac{d[A]}{dt} = k_2[T][H_2O] + k_3[C][H_2O] - k_{-2}[A] \quad (19)$$

$$OD_\lambda = \epsilon_C[C] + \epsilon_T[T] + \epsilon_A[A] \quad (20)$$

$$\text{slope} = \frac{d(OD_\lambda)}{dt}$$

$$\text{slope} = \epsilon_C(k_1 + k_3)[C][H_2O] + \epsilon_T(k_1[C][H_2O] - k_2[T][H_2O] + k_{-2}[A]) + \epsilon_A(k_2[T][H_2O] + k_3[C][H_2O] - k_{-2}[A]) \quad (21)$$

The first, a rather crude method, finds the slope generated by the two points (OD_{i+1}, t_{i+1} and OD_{i-1}, t_{i-1}) that bracket OD_i, t_i. The second method fits the experimental points to a general polynomial equation[22] and then evaluates the first derivative at each point. In this case a tenth-order polynomial was found to provide the best fit.

A least-squares program, KNTCS10, was written to calculate the rate constants needed to give the minimum squared relative difference between experimental and

(19) S. W. Edwards and W. E. Knox, *J. Biol. Chem.*, **220**, 79 (1956).

(20) W. E. Knox and S. W. Edwards, *ibid.*, **216**, 489 (1955).

(21) It should be recalled that the major pathway for isomerization of the model compound is conversion to hydrated acid followed by dehydration. The over-all rate of conversion of *cis* to *trans*, however, is used here for comparison.

(22) We thank Dr. S. Ehrenson for making available his General Polynominal Least Squares (GPLS) program.

calculated slopes at each point. Similar results were obtained by the two methods of obtaining the slope. During this iterative procedure, concentrations were calculated by numerical integration.

Another least-squares program, BESTFIT, was written to obtain the values for k_8, k_9, k_{10}, and K_{11} which would give the minimum squared relative difference between experimental and calculated k_{obsd} in eq 12.

Cycloaddition Reactions of Isocyanates. The Reaction of Aryl Isocyanates with N,N-Dimethylformamide

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Heating aryl isocyanates in N,N-dimethylformamide produces the previously unreported pentaaryl-1,3,6,8,10-pentazaspiro[4.5]decane-2,4,7,9-tetraones **4**. The formation of **4** involves N-aryl-N′-dimethylformamidines **1** as well as the 2:1 cycloadducts **2** of aryl isocyanates and **1** as intermediates.

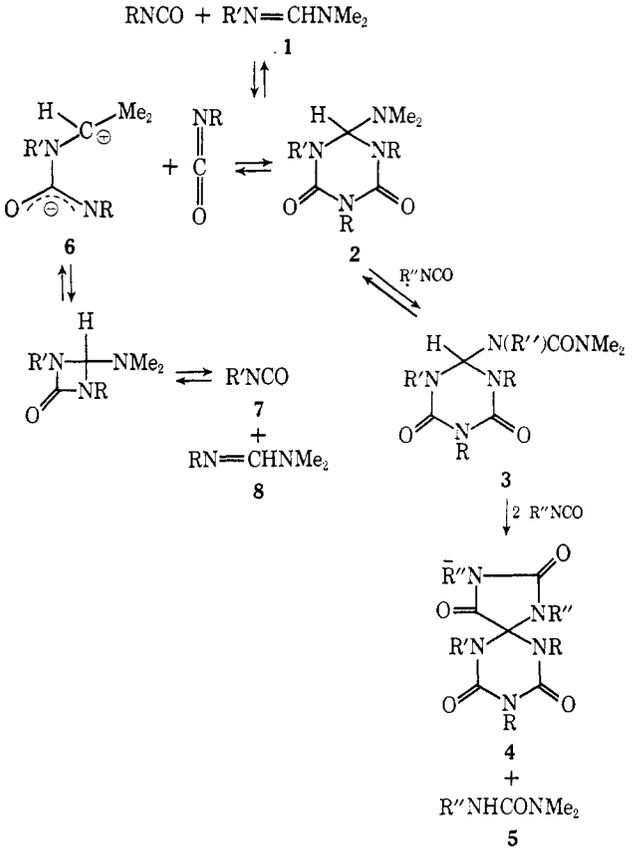
The reaction of aryl isocyanates with N,N-dimethylformamide (DMF) reportedly produces N-aryl-N′-dimethylformamidines (**1**)[1-3] or triaryl isocyanurates,[3] the cyclic trimerization products of aryl isocyanates, depending upon the reaction conditions. In addition to **1** substantial amounts of high-melting solid materials are formed on heating equimolar quantities of aryl isocyanates and DMF at 150° (Table I). As discussed below, these products are assigned the spiro structure **4**.[4,5]

The phenyl compound (**4**, R, R′, R″ = C_6H_5) was also obtained when N-phenyl-N′-dimethylformamidine (**1**, R′ = C_6H_5) was heated with excess phenyl isocyanate (Scheme I). Thus, it is suggested that **1** is an intermediate in the formation of **4**. We have recently shown that 2:1 adducts can be obtained from arylsulfonyl isocyanates and C=N double bond containing substrates,[6] and a similar reaction may account for the formation of **4**. When a mixture of 2 equiv of phenyl isocyanate and 1 equiv of **1** (R′ = C_6H_5) is kept at room temperature for 5 days, a 78.5% yield of the 2:1 adduct **2** is obtained.[7]

The 2:1 adduct **2**, upon heating with 3 equiv of phenyl isocyanate, produces the spiro compound **4** and N-phenyl-N′-dimethylurea **5** (R″ = C_6H_5). Heating **2** (R, R′ = C_6H_5) with *p*-tolyl isocyanate at 150° gives spiro compound **4** (R, R′ = C_6H_5; R″ = 4-$CH_3C_6H_4$) in low yield. The structure of the mixed spiro compound follows from the nmr spectrum, which shows only two methyl signals at δ 2.22 and 2.42 ppm, and a relative intensity of methyl protons to aryl protons of 6:23.

The formation of the 2:1 adducts of aryl isocyanates and N-aryl-N′-dimethylformamidines indicates that the linear 1:1 adducts **6** can be intercepted by a second isocyanate molecule to afford **2**. As a side reaction cyclization of **6** can occur, as indicated by the isolation

SCHEME I



of 1,2 cycloadducts and exchange products **7** and **8**. The latter are formed almost exclusively when the generated new isocyanate (R′NCO) is removed by distillation.[8]

The formation of the exchange products **7** and **8** leads to a great variety of products in the reaction of aryl isocyanates and N-aryl-N′-dimethylformamidines, in which both aryl groups are different. For example, **7** could intercept **6** or form a 1:1 adduct with the starting formamidine. Likewise, **8** could form a 1:1 adduct with the starting isocyanate. In view of the ambident character of **6** and the possibility of addition across the C=N or C=O bond in the isocyanate, four isomeric 2:1 adducts are visualized, discounting the possibility

(1) M. L. Weiner, *J. Org. Chem.*, **25**, 2245 (1960).
(2) J. L. Neumeyer, *J. Pharm. Sci.*, **53**, 1539 (1964).
(3) A. Jovtscheff and F. Falk, *J. Prakt. Chem.*, [4] **13**, 265 (1961).
(4) An asymmetric aryl isocyanate trimer structure was erroneously assigned to these compounds in the abstracts of the General Meeting of the Gesellschaft Deutscher Chemiker, 1967.[5]
(5) H. Ulrich, *Angew. Chem. Intern. Ed., Engl.*, **6**, 1000 (1967).
(6) H. Ulrich, B. Tucker, and A. A. R. Sayigh, *J. Amer. Chem. Soc.*, **90**, 528 (1968).
(7) The formation of 2:1 adducts of phenyl isocyanate and N-phenyl-N′-dimethylformamidine has also been observed by Dr. R. H. Richter of the Carnegie-Mellon University. We are grateful to Dr. Richter for communicating this information to us.
(8) H. Ulrich, B. Tucker, and A. A. R. Sayigh, *Angew. Chem. Intern. Ed. Engl.*, **7**, 291 (1968).