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199. Yutaka Asahi: Kinetic Study of Hydrolysis of Thioacetazone and its Related Compounds.

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It has been reported by Arita[1] and Aoki[2] that thioacetazone is hydrolyzed to *p*-acetaminobenzaldehyde, thiosemicarbazide and *p*-aminobenzaldehyde thiosemicarbazone in an artificial gastric juice. It was noticed in the previous paper[3] that the reduction wave of thioacetazone decreases gradually in an acid solution and hydrolysis of the azomethine moiety of thioacetazone could be followed by polarography. In the present work, the decomposition of thioacetazone and its related compounds has been studied kinetically by polarography and spectrophotometry.

According to Connant,[4] the reaction between carbonyl compounds and amines such as semicarbazide, hydroxylamine, oxime, phenylhydrazine, etc., is reversible and the rate of combination or decomposition depends on the concentration of oxonium ion in the solution.

This argument is prospected to apply in the case of thiosemicarbazone such as thioacetazone. Then the hydrolysis of thiosemicarbazone is shown by the equation (1):

$$\underset{(a-x)}{\mathrm{ACH{=}NB}}+\mathrm{H_2O}\ \underset{k_2}{\overset{k_1}{\rightleftharpoons}}\ \underset{x}{\mathrm{ACHO}}+\underset{x}{\mathrm{BNH_2}} \qquad (1)$$

When the initial concentration of thiosemicarbazone (ACH=NB) is a M and that after t minutes is $(a-x)M$, the rate of decomposition dx/dt is given by the equation (2), and the equilibrium constant K' is, of course, the ratio of the rate constants k_1 and k_2 as shown by the equation (3).

$$dx/dt=k_1(a-x)-k_2x^2 \qquad (2)$$

$$K'=k_1/k_2 \qquad (3)$$

Postulating the equation (2), the rate constant (k_1, k_2) can be calculated from a single time-concentration curve by mean square method (4), (5), where X, Y and Z substitute for $(a-x)$, x^2 and dx/dt, respectively.

$$k_1=(\sum YZ\cdot\sum XY-\sum XZ\cdot\sum Y^2)/[(\sum XY)^2-\sum X^2\cdot\sum Y^2] \qquad (4)$$

$$k_2=(\sum X^2\cdot\sum YZ-\sum XY\cdot\sum XZ)/[\sum XY)^2-\sum X^2\cdot\sum Y^2] \qquad (5)$$

On the other hand, the rate constant k_1 can be also calculated by the equation (7) so long as the second term of (2) is negligible in comparison with the first term of (2), that is $x\ll a$.

$$dx/dt=k_1'(a-x) \qquad (6)$$

$$k_1'=(2.303/t)\log a/(a-x) \qquad (7)$$

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1) R. Arita: Yakugaku Zasshi, **76**, 990 (1956).
2) M. Aoki: Yakugaku Kenkyu, **23**, 97 (1951).
3) Y. Asahi: This Bulletin, **11**, 930 (1963).
4) J.B. Connant, P.D. Bartlett: J. Am. Chem. Soc., **54**, 2881 (1932).

Furthermore, the equilibrium constant K is also obtained from the equation (8), where x_e is the equilibrium concentration of the product.

$$K=[\mathrm{ACHO}][\mathrm{BNH_2}]/[\mathrm{ACH{=}NB}]=x_e^2/(a-x_e) \qquad (8)$$

These theoretical considerations agree with the experimental results as shown later. In this report are described the pH profiles of rate constants and equilibrium constants for the hydrolysis of thioacetazone, their temperature dependence and the relationship between the stabilities and the constitutions of thioacetazone homologues.

Experimental

Instruments——Yanagimoto pen-recording polarograph model PA2, Beckmann pH meter model G, Beckmann pen-recording spectrophotometer model DK2 and Varian's A60 NMR spectrophotometer were used. The dropping mercury electrode had such characteristics as $m=0.826$ mg. sec^{-1}, $t=4.11$ sec., $m^{2/3}t^{1/6}=1.115$. A normal calomel electrode was used as an external reference electrode.

Materials——Thioacetazone and its related compounds were the same with those in the previous paper.[3]

Buffers——Acetate buffers containing $0.2M$ NaOAc of pH 1.38, 2.02, 3.15, 3.69, 4.15 and 5.30 at 25°; $0.1M$ phosphate buffer of pH 6.5 at 60° and 100°; $0.1M$ borate buffers of pH 7.78, 8.63 and 9.21 at 100°; $0.08N$ HCl (pH 1.16) and $0.8N$ HCl (pH 0.2) at 25° were used.

The acidity functions H_0 for 14, 23, 32 and 37% aqueous sulfuric acid were estimated to be -0.3, -0.9, -1.35 and -1.85, respectively according to Hammett.[5] The pH values of acetate and phosphate buffers at 60° and 100° were assumed to be the same at 25~30°. The pH values of borate buffers at 100°, however, were calculated from those at 30° by applying their temperature coefficients.

Polarographic Measurements of the Decompositions of Thiosemicarbazones——a) One ml. of 2 mM thioacetazone in EtOH was poured into the polarographic cell containing 9 ml. of buffer solution which was previously bubbled with N_2 gas at 25°±0.1°. The limitting current of thioacetazone at the constant potential was automatically recorded until the current reached an almost constant value.

b) For the measurement of slow decomposition of thioacetazone, ordinary polarograms (potential-current curves) of the test solutions were recorded at an every optional time.

Spectrophotometric Measurements of the Decompositions——a) Procedure for a rapid reaction: one ml. of 0.2 mM thiosemicarbazone in H_2O was mixed with 9 ml. of HCl or H_2SO_4. The absorbance of the mixture at a constant wave length was measured in a cell with 1 cm. of light path at an every optional time at room temperature, 28~32°.

b) Procedure for a slow reaction: about 1 ml. of a buffer solution containing 0.2 mM of thiosemicarbazone was heated in an ampule at 60° or 100° for a given time and diluted 10-fold with H_2O. The absorbance of the resulting solution was measured at room temperature.

Results and Discussion

Decomposition of Thioacetazone Observed by Polarography and Spectroscopy——It was reported in the previous paper[3] that thioacetazone gives two steps of two-electron reduction wave in acid media and can be determined polarographically without disturbance from *p*-acetaminobenzaldehyde and thiosemicarbazide. It was confirmed by polarography that thioacetazone decomposes to the aldehyde and the amine in acid media. Namely, the reduction waveheights of thioacetazone at -0.8 v. in 0.9 N hydrochloric acid decrease while the reduction wave of *p*-acetaminobenzaldehyde at $E_{1/2}$ -0.89 v. appears.

The decomposition of thioacetazone was also suggested by the variation of electronic spectra. In the course of acid hydrolysis of thioacetazone, the absorbance at 321 mμ decreases in place of increase of the absorbance at 240 and 293 mμ. The absorption maximum at 240 mμ was assigned to thiosemicarbazide ($\varepsilon_{\max}$ 1200) and that of 293 mμ is due to *p*-acetaminobenzaldehyde ($\varepsilon_{\max}$ 20300). A similar change was also observed in

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[5] L.P. Hammett: J. Am. Chem. Soc., **54**, 2721 (1932); **56**, 827 (1934).

the hydrolysis of S-methylthioacetazone and *p*-acetaminobenzaldehyde semicarbazone. The spectra of benzaldehyde (λ_{max} 244, 280, 328 mμ, ε_{max} 15800, 1580, 20) appear in the case of the decomposition of benzaldehyde thiosemicarbazone (λ_{max} 305 mμ). The deacetylation of *p*-acetaminobenzaldehyde was observed by the change of the spectra to those of *p*-formylanilium salt (λ_{max} 243, 280 mμ, ε_{max} 16200, 3500). The rate constant (0.008 min^{-1}) of the deacetylation in 37% sulfuric acid at about 30°, however, was far smaller than those of decompositions of thiosemicarbazones.

Treating S-methylthioacetazone with *N* sodium hydroxide at 100°, decrease of the S-methyl was observed by nuclear magnetic resonance spectra. This reaction will be studied in future.

pH Profile of the Decomposition Rate of Thioacetazone——The rate constants for the hydrolysis of thioacetazone were measured polarographically at 25° in acid solutions. The constants k_1 calculated by the equation (4) coincided with those k_1' by (7) and the equilibrium constants K calculated by (8) also agreed with those K' by (3) as shown in Table I. Therefore, the proposed equations (1~8) were recognized to be reasonable. In the following experiments, the equations (7 and 8) were utilized mainly because of their simplicity. The pH profile of the rate constants for hydrolysis of thioacetazone at 25° in pH −0.2~4 is illustrated in Fig. 1. It became evident that the rate constant k_1 is proportional to the concentration of oxonium ion in the range of pH (or H_0) −0.3~2 and is independent of H_0 below −0.9.

The rate constants k_1' for thioacetazone were also determined spetcrophotometrically by the following equation (9) derived from (7).

$$k_1' = (2.303/t)\log(\varepsilon_0-\varepsilon_\infty)/(\varepsilon_t-\varepsilon_\infty) \qquad (9)$$

where ε_t, ε_0, and ε_∞ are absorbance at a constant wave-length (305~320 mμ) after *t* minutes, in initial and final times, respectively. The pH profile of the rate constant measured in this manner at room temperature (about 30°) resembles that observed by polarography at 25°.

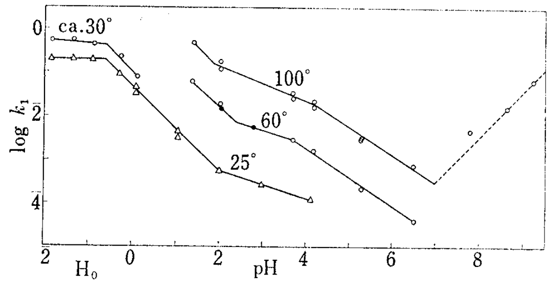

Fig. 1. pH Profiles of the Rate Constant (k_1) for the Hydrolysis of Thioacetazone

△ Polarographic determination
○ Spectrophotometric determination
• In unbuffered solution
H_0 Hammett's acidity function

Table I.

HCl	Compound	$10^3k_1'$	10^3k_1	k_2	$10^5K'$	10^5K
0.08	I	4.2	4.5	144	3.12	2.46
	II	2.5	4.3	80	5.45	3.93
	III	0.86	0.87	50	1.37	6.68
	IV	16.2	21	8	264	155
	V					63
0.8	I	42.2	45.3	158	28.7	25.8
	II	36	40	225	17.8	36.1
	III	15.7	15	177	8.5	5.67
	IV	148	166	115	144	
	V					132

The Rate Constants of Hydrolysis (k_1, k_1' : min.^{-1}), that of Combination (k_2 : $M^{-1}\cdot\text{min.}^{-1}$) and the Equilibrium Constants (K, K' : M) of Thioacetazone (I), Benzaldehyde Thiosemicarbazone (II), S-Methylthioacetazone (III), *p*-Acetaminobenzaldehyde Semicarbazone (IV) and Benzaldehyde Semicarbazone[4] (V) in 0.08*N* or 0.8*N* HCl at 25°.

It is easily assumed from these facts that the rate of hydrolysis is proportional to the concentration of the proton adduct of thioacetazone [$ACH{=}NBH^+$] in a strong acid solution. This relationship can be expressed by the equation (10), where C is total concentration of thioacetazone and its proton adduct, and K_c is the dissociation constant of the cation.

$$\text{Rate}=k_{1A}[\mathrm{ACH{=}NBH^+}]=k_{1A}C/(1+K_c/[\mathrm{H^+}]) \qquad (10)$$

When the concentration of oxonium ion [H^+] is large enough in comparison with K_c, the rate constant tends to a limiting value, k_{1A}. On the contrary, the rate is to be proportional to [H^+] at [H^+]$\ll K_c$.

The reaction steps in acid solutions are postulated as follows :

$$\mathrm{ACH{=}NB} \underset{(a)}{\xrightleftharpoons{\mathrm{H^+}}} \mathrm{ACH{=}\overset{+}{N}HB} \underset{(b)}{\xrightleftharpoons{\mathrm{H_2O}}} \mathrm{ACH(\overset{+}{O}H_2)NBH} \underset{\substack{\mathrm{H^+}\\(c)}}{\rightleftharpoons}$$

$$\mathrm{ACH(OH)NHB} \underset{(d)}{\xrightleftharpoons{\mathrm{H^+}}} \mathrm{ACH(OH)\overset{+}{N}H_2B} \underset{(e)}{\rightleftharpoons}$$

$$\mathrm{ACH({=}\overset{+}{O}H)+H_2NB} \underset{(f)}{\rightleftharpoons} \mathrm{ACHO+H_3\overset{+}{N}B} \qquad (11)$$

Since the steps (a, c, d, f) of protonation or dissociation are assumed to be very rapid and reversible, the rate determining step may be either the hydration (b) or the decomposition (e).

The rate of hydrolysis in 0.2M acetate buffer at pH 2~6 is larger than that proposed by (10). One of the reasons may be that the components of the buffer such as acetate, acetic acid and sodium chloride play a part of general acid catalyzer. But this effect is negligible at pH 2 because the rate in 0.01N hydrochloric acid is almost equal to that in 0.2M acetate buffer. At pH 4.5, a small effect of the concentration of buffer and the ionic strength was observed as shown in Table II.

TABLE II.

μ	c	10^2k_1	μ	c	10^2k_1
0.1	0.1	1.39	0.95	0.1	2.05
0.5	0.1	1.78	0.95	0.4	2.83
0.95	0.1	2.05	0.95	0.9	3.93
0.95	0.02	1.35			

Effect of Ionic Strength (μ) and the Concentration of Acetate (c) on the Rate Constant (k_1 : min.$^{-1}$) of Hydrolysis of 0.2 mM Thioacetazone at 100° and pH 4.5.

The dissociation constant pK_c of the cation [$ACH{=}NBH^+$] was estimated to be −1.1 in the previous paper,[3] so that the cation could be negligible at pH above 2. Therefore, the free base of thioacetazone (ACH=NB) will play an important role in the hydrolysis at pH 2~6. The rate constants in this region also depend on pH. The gently-sloping pH profile of log k_1 at pH 2~4 reminds of the competition of the free base with the cation in the reaction.

The hydrolysis of thioacetazone in 0.1M borate buffers of pH 7.78, 8.63, and 9.21 at 100° was observed spectrophotometrically. The rate constants increase with pH. The reaction is accelerated not only by an acid but also by a base. Consequently, thioacetazone is most stable in a neutral solution. The reaction process in an alkaline solution is postulated as follows :

$$\mathrm{ACH{=}NB} \xrightleftharpoons{\mathrm{OH^-}} \mathrm{ACH(OH)\text{-}\overset{-}{N}B} \rightleftharpoons$$

$$\mathrm{ACH(\overset{-}{O})\cdot NHB} \rightleftharpoons \mathrm{ACHO+\overset{-}{N}HB} \qquad (12)$$

The pH Profile of the Equilibrium Constants for Thioacetazone——The equilibrium constants K for the hydrolysis and recombination of thioacetazone were revealed to be constant at H_0 $-0.3\sim-1.85$ and pH $2\sim4$, and to be proportional to the concentration of oxonium ion $[H^+]$ at pH $-0.3\sim1$.

Connant[4] has studied the relationship among the equilibrium constants for hydrolysis of semicarbazones in strong acid solutions (K_A), those in weak acid solutions (K_B), those in optional acidities (K), and dissociation constants of the semicarbazones (K_c) and semicarbazide (K_R) and proposed the next equations :

$$K=K_A([H^+]+K_R)/([H^+]+K_c) \tag{13}$$

$$K_B=K_AK_R/K_c \quad \text{at} \quad [H^+]\ll K_c,\ K_R \tag{14}$$

The pH profile of the equilibrium constants for thioacetazone was explained qualitatively by the equation (13). The dissociation constant of thioacetazone (K_c) was calculated to be 1.6 since the equilibrium constants (K_A and K_B) for thioacetazone were $8\cdot10^{-4}$ and $1\cdot10^{-5}$, respectively and the dissociation constant of thiosemicarbazide (K_R) was evaluated to be $2\cdot10^{-2}$ spectrophotometrically. This constant K_c does not agree with the value (0.1) estimated by spectrophotometry. This discrepancy may be caused by erroneous determination of K_c as accurate estimation of the initial absorbance of thioacetazone in a strong acid is difficult.

Temperature Dependence of the Hydrolysis of Thioacetazone——The rate constants for hydrolysis of thioacetazone in 14% sulfuric acid of H_0 -0.3 were determined polarographically at 15, 25, 35, and 45°. A linear relationship was obtained between log k and the reciprocal of absolute temperature T. The heats of activation ΔH^* for the hydrolysis and recombination of thioacetazone were estimated to be 15.4 and 11.7 kcal./mol. from the slopes of the Arrhenius plot for log k_1 and log k_2, respectively. Their difference, 3.7 kcal./mol., corresponds to the difference of enthalpy ΔH between the original and the final systems of the decomposition. The entropy of activation ΔS^* was calculated by the next equation (15) :

$$\Delta S^*=2.303R[\log A-\log(kT/h)] \tag{15}$$

where $\log A$ is the intersept of Arrhenius plot and R, k, and h are gas, Boltzmann and planck constants, respectively. ΔS^* for the hydrolysis of thioacetazone was revealed to be -18.7 cal./deg. and ΔS^* for the recombination was estimated to be -16.7 cal./deg. The difference of ΔS between thioacetazone and its decomposition products was, therefore, evaluated to be -2 cal./deg.

On the other hand, the same ΔH and ΔS values were estimated from the relationship between the equilibrium constant K and absolute temperature T.

Hydrolysis of Some Thiosemicarbazones and Semicarbazones——The hydrolyses of benzaldehyde thiosemicarbazone (II), S-methylthioacetazone (III) and p-acetamidobenzaldehyde semicarbazone (IV) were observed polarographically in 0.8N or 0.08N hydrochloric acid at 25°. The rate constants (k_1, k_1', and k_2) and the equilibrium constant (K and K') are presented in Table I. These rate constants were proportional to the concentration of the hydrochloric acid used. The rate constants for the hydrolysis were also determined spectrophotometrically in hydrochloric acid or sulfuric acid at about 30°. The relationship between $\log k_1$ and pH (or H_0) resembles that in thioacetazone as shown Fig. 1 and Table III.

The sequence of the rate constants in a constant condition was p-acetamidobenzaldehyde semicarbazone (IV) > thioacetazone (I) > benzaldehyde thiosemicarbazone (II) > S-methylthioacetazone (III). There are intimate correlations between the rate constants

Table Ⅲ.

The Rate Constants of Hydrolysis (k_1' : min^{-1}) of Thioacetazone (I), Benzaldehyde Thiosemicarbazone (Ⅱ), S-Methylthioacetazone (Ⅲ) and *p*-Acetamidobenzaldehyde Semicarbazone(Ⅳ) and their Initial Molar Extinction Coefficients (ε_0) measured by Absorbance-Time Curves at 28~32°

H_0	$10^3k_1'$				$10^{-4}\varepsilon_0$			
	I	Ⅱ	Ⅲ	Ⅳ	I 321 mμ	Ⅱ 300 mμ	Ⅲ 316 mμ	Ⅳ 295 mμ
0.64		25				2.90		
0.2	77	90	29		3.90	2.90	3.45	
−0.3	210	150	105	380	3.75	2.65	3.50	2.60
−0.9	421	320	184	920	3.20	2.20	3.35	2.65
−1.35	509	390	310	1320	2.20	1.40	3.35	2.40
−1.85	550	620	440	1790	1.75	1.30	3.40	2.25

Table Ⅳ.

Correlation Among the Rate Constant of Hydrolysis (k_1), pK_c of NH^+, pK_c' of SH and the Chemical Shift of the Azomethine in Dimethylsulfoxide Solution (τ_1) or with Additional 10% D_2SO_4 (τ_2).

	10^3k_1	pK_c	pK_c'	τ_1	τ_2
Ⅳ	21	−1.2	13.0	2.17	1.97
I	4.5	−1.1	11.52	1.92	1.72
Ⅱ	4.3	−1.1	11.25	1.90	1.65
Ⅲ	0.87	5.77	—	1.77	1.56

k_1 and the dissociation constants pK_c as shown in Table Ⅳ. Furthermore, these constants correlate with the chemical shifts of the azomethines in proton magnetic resonance. A chemical shift (τ) refers to the electron density around the proton. It was considered from these facts that an azomethine with a low electron density is more stable than that with a high density.

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Summary

The rate constant of hydrolysis (k_1) of thioacetazone and that of recombination (k_2) of its components, the aldehyde and the amine, were estimated by polarography and spectrophotometry. The relationships between the rate constant (k_1), acidity function and the concentration of buffer show that the reaction is a general acid and base catalysed reaction. The equilibrium constants K in strong and weak acid solutions are evaluated to be $8\cdot10^{-4}$ and $1\cdot10^{-5}$, respectively. Thermodynamic data are given as follows : ΔH^* 15.4 kcal./mol., ΔS^* −18.7 cal./deg., ΔH 3.7 kcal./mol., ΔS −2 cal./deg. for the hydrolysis, ΔH^* 11.7 kcal./mol., ΔS^* −16.7 cal./deg. for the recombination. The sequence of stability for acid is S-methylthioacetazone > benzaldehyde thiosemicarbazone > thioacetazone > *p*-acetamidobenzaldehyde semicarbazone. The rate of deacetylation of *p*-acetamidobenzaldehyde is as small as one hundredth that of the hydrolysis of thioacetazone.

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