Comparative Hydrolytic Rates of *N*-Substituted 6-Amino-thiouracils

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A study has been carried out on the acid and base-catalyzed hydrolysis of *N*-substituted 6-amino-thiouracils. Two series, aliphatic and halogenated aromatic *N*-substituted 6-amino-thiouracils, have been synthesized and investigated. The experimental results, obtained in this hydrolytic study, indicate that a number of factors may be involved and are rationalized in terms of steric and electronic effects.

INTEREST IN *N*-substituted 6-amino-thiouracils relative to their antitumor activity prompted this preliminary investigation of the influence of *N*-substitution on the stability of the C=S function. Although a survey of the literature indicates that a number of such compounds have been prepared (1, 2), their stability aspects are less known (3), although it would be expected that the *N*-substituted compound should be less stable than the parent unsubstituted thiouracils. Therefore, a systematic study concerning the effect of substituents on 6-amino-thiouracil upon the relationship of hydrolysis in acid and in base would be of value in the design and synthesis of sufficiently stable thiouracils for biological evaluation.

EXPERIMENTAL

Synthesis—Two series, aliphatic and halogenated aromatic *N*-substituted 6-amino-thiouracils, have been prepared by a modification of the condensation method of Carstens (4). The *N*-substituted thiourea and ethyl-cyanoacetate were added to absolute ethanol containing sodium ethoxide. The solution was refluxed with constant stirring for 4–5 hr., and then diluted with water and neutralized with acetic acid. On cooling a 60–80% yield was obtained. It was then purified by recrystallization and chromatographic techniques. The final products were identified by I.R. spectra and elemental analysis. Physical constants and analyses for all thiouracils synthesized are listed in Table I.

Kinetic Studies—Spectra of each thiouracil were taken in 0.3 *N* HCl and 0.3 *N* NaOH solution using a Beckman DB spectrophotometer equipped with a Sargent recorder. The $\lambda_{max.}$ and ϵ and hydrolytic rate constants are listed in Table II. The hydrolysis was carried out at 70° ± 0.1 in a 6 *N* HCl or 6 *N* NaOH solution, and the reaction course was followed by measuring the decrease in absorbance at $\lambda_{max.}$ utilizing a Beckman DU spectrophotometer. An accurate weight of the thiouracil was placed in a 100-ml. volumetric flask and brought to the volume with 6 *N* HCl or 6 *N* NaOH solution (previously warmed to 70°) to obtain an approximate concentration of 3×10^{-5} mole/L. The hydrolysis was carried out in a 100-ml. glass-stoppered volumetric flask which was immersed in a "Haake-Thermostate Unitherm" water bath, regulated at 70° ± 0.1. Aliquots were withdrawn periodically and diluted with water to 100 ml. and the absorbance read at $\lambda_{max.}$ In the same manner, hydrolysis at three temperatures, 70° ± 0.1, 60° ± 0.1, 50° ± 0.1, was carried out for the *N*-phenyl 6-amino-thiouracil in acid and base in order that additional data be obtained concerning the degradative pathway of these compounds.

RESULTS AND DISCUSSION

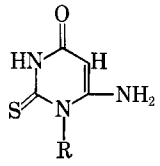
Prior to the hydrolytic study, ultraviolet absorption spectra of each thiouracil was obtained. Since the spectrum of the corresponding uracil exhibits a $\lambda_{max.}$ at approximately 260 mμ and shows little or no absorption at $\lambda_{max.}$ of the thiouracil, as shown in Fig. 1, all measurements were made at $\lambda_{max.}$ of the thiouracil. Uracil absorption causes no interference in thiouracil systems when the concentration of these thiouracils undergoing degradation is 3×10^{-5} mole/L. or less. In cases where this uracil interference is significant, particularly at higher concentration of the thiouracil, the use of two component analysis can be satisfactorily applied.

The degradation product of the *N*-substituted 6-amino-thiouracil in 6 *N* HCl or 6 *N* NaOH at 70° has been identified as the corresponding 6-amino-uracil using the thin-layer chromatographic technique. No ring cleavage was indicated in this case under the experimental condition employed.

Data obtained in this preliminary investigation indicate that the hydrolysis of these thiouracils follows a pseudo first-order rate law as shown in Figs. 2 and 3. A straight line relationship is obtained of a plot of log concentration against time. Kinetic data (Fig. 2) indicate that the aliphatic *N*-substituted 6-amino-thiouracils hydrolyze at a substantially faster rate than the corresponding

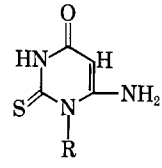
Received November 28, 1966, from the College of Pharmacy, University of Iowa, Iowa City, IA 52240
Accepted for publication January 10, 1967.
This work was supported by the American Cancer Society, Iowa Division.
Presented to the Basic Pharmaceutics Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.

TABLE I—PHYSICAL CONSTANTS AND ELEMENTAL ANALYSIS FOR *N*-SUBSTITUTED 6-AMINO-THIOURACILS



R =	Formula	M.p., °C.	Anal. C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
H	$C_4H_5N_3OS$	>360	33.55	33.54	3.52	3.64	29.34	29.06
CH_3	$C_5H_7N_3OS$	255–256	38.20	28.30	4.48	4.66	26.73	26.47
C_2H_5	$C_6H_9N_3OS$	224–225	42.09	42.08	5.29	5.02	24.54	24.30
C_3H_7	$C_7H_{11}N_3OS$	221–222	45.39	45.37	5.98	6.21	22.69	22.52
C_4H_9	$C_8N_{13}N_3OS$	219.5–220.5	48.20	48.83	6.57	6.31	21.08	21.58
ϕH	$C_{10}H_9N_3OS$	221–222	54.80	54.66	4.14	3.95	19.20	19.05
ϕCl	$C_{10}H_8ClN_3OS$	230–232	47.33	47.05	3.71	3.48	16.56	16.38
ϕBr	$C_{10}H_8BrN_3OS$	233–233.5	40.27	40.46	2.70	2.82	14.09	13.98
ϕI	$C_{10}H_8IN_3OS$	234–235.5	34.79	34.90	2.33	2.37	12.17	12.30

TABLE II—KINETIC DATA OF *N*-SUBSTITUTED 6-AMINO-THIOURACILS



R =	$\lambda_{max.}$ (mμ) in HCl	$\lambda_{max.}$ (mμ) in NaOH	ε in HCl	ε in NaOH	Rate Constant × 10^{-1}, hr.$^{-1}$, 70° in HCl	Rate Constant × 10^{-1}, hr.$^{-1}$, 70° in NaOH	Half-Life, hr.$^{-1}$, 70° in HCl	Half-Life, hr.$^{-1}$, 70° in NaOH
H	274	284	16159	10296	0.575	0.086	12.05	80.58
CH_3	278	282	17427	14287	4.830	1.440	1.43	4.81
C_2H_5	278	284	17100	13338	3.910	1.090	1.77	6.36
C_3H_7	278	284	16835	12765	3.570	0.860	1.94	8.06
C_4H_9	278	282	17114	13134	3.1220	0.69	2.15	10.04
ϕH	276	286	18396	12483	8.492	1.320	0.81	5.25
ϕCl	276	286	18288	13208	5.410	1.210	1.28	5.73
ϕBr	276	286	18178	13112	4.600	1.090	1.51	6.36
ϕI	274	284	18285	14145	2.650	0.860	2.62	8.06

parent unsubstituted 6-amino-thiouracils, as was expected. This greater stability of the unsubstituted 6-amino-thiouracils can be attributed to a resonance structure effect since this resonance structure no longer exists after *N*-substitution. It is also interesting to note (Fig. 2) that an increase in the carbon number of the aliphatic substituent increases the stability of these compounds, probably due to steric hindrance. A bulky molecule in the *N*-position would certainly hinder the nucleophilic attack (5) of water on the partially positive carbon of the C=S reaction center resulting in a slight increase in stability. Although this aliphatic effect was not studied beyond C_4, it is reasonable to believe that extension of the chain length or branching of the chain would further increase the stability of these thiouracils.

As shown in Fig. 3, the introduction of a phenyl substituent at this *N*-position also results in a significant decrease in the stability. This increase in hydrolytic rate is due probably to a diminished resonance structure of the thiouracil and an electron delocalization to the phenyl ring. The electron pair on the *N*-nitrogen can be delocalized to the benzene ring system making the reaction center more positive, increasing the rate of water molecule attack in the rate-determining step. In the absence of phenyl *N*-substitution, this electron pair can be delocalized to the reaction center making it less positive, resulting in an increase in the stability of the compound as discussed in the aliphatic series. Introduction of a halogen in the *para*-position of the phenyl group increases the stability of the thiouracils somewhat. This slight increase in stability may be attributed to a decrease of the positive charge of the reaction center by a resonance contribution of the halogenated phenyl, resulting in a push of the electron pair on the nitrogen toward the reaction center.

The kinetic data (Table II) show that rate of hydrolysis of these *N*-substituted thiouracils is considerably greater in acid than in base. This suggests that the reaction centers of the 6-amino-thiouracils in the protonated form are more positive and favor this water molecule attack in the mechanism involved. Calculation of the apparent energy of

activation, E_a, from the slope of the Arrhenius plot (Fig. 4) for *N*-phenyl-6-amino-thiouracil in acid and in base yielded 23.5 Kcal./mole and 29.7 Kcal./mole, respectively. Since this apparent energy of activation includes the heat of ionization of water (approximately 11.5 Kcal./mole), the energy of activation for the acid and base hydrolysis is 13.1 Kcal./mole and 18.2 Kcal./mole, respectively, which is a reasonable value for hydrolytic reactions of this type (6).

As previously pointed out, the protonated thiouracil species undergo a faster rate of hydrolysis suggesting that specific acid catalysis is involved. This aspect was studied by an investigation of the hydrolytic rates of *N*-phenyl-thiouracil at various acid and base concentrations. Data for acid hydrolysis are shown in Fig. 5, A. Scheme I is a possible mechanism for this acid catalyzed hydro-

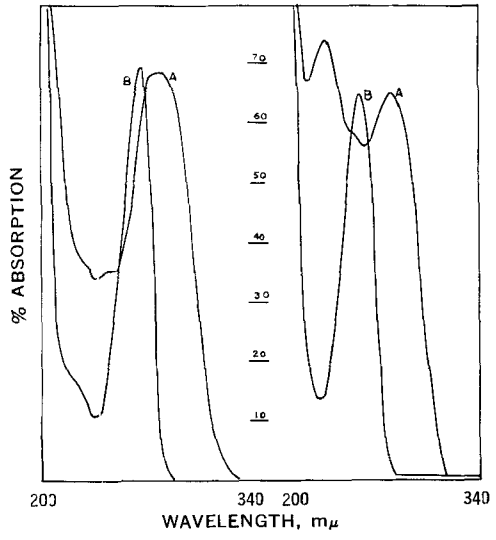


Fig. 1—U. V. spectra of 6-amino-thiouracil and 6-amino-uracil in 0.3 N HCl (lefthand curves) and 0.3 N NaOH (righthand curves). Key: A, 6-amino-thiouracil; B, 6-amino-uracil.

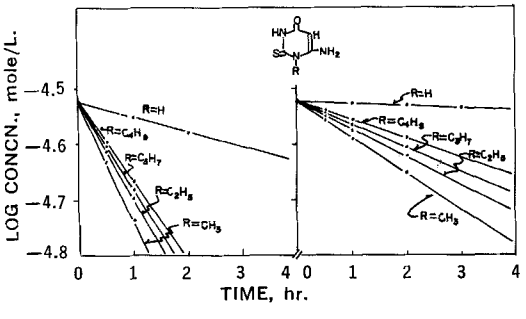


Fig. 2—Rates of hydrolysis of aliphatic N-substituted 6-amino-thiouracil in 6 N HCl (lefthand curves) and 6 N NaOH (righthand curves).

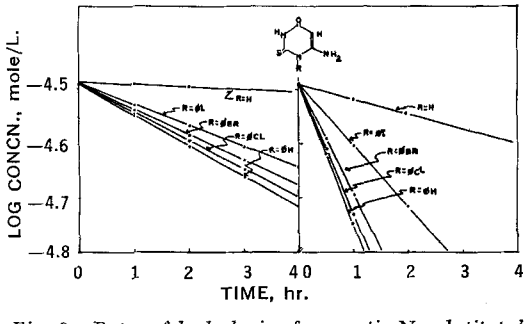


Fig. 3—Rates of hydrolysis of aromatic N-substituted 6-amino-thiouracil in 6 N NaOH (lefthand curves) and 6 N HCl (righthand curves).

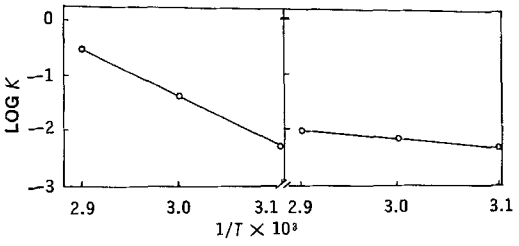


Fig. 4—Arrhenius plot of hydrolysis of N-phenyl-6-amino-thiouracil in 6 N HCl (lefthand curve) and 6 N NaOH (righthand curve).

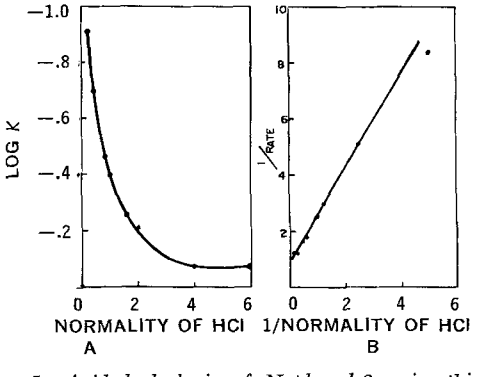
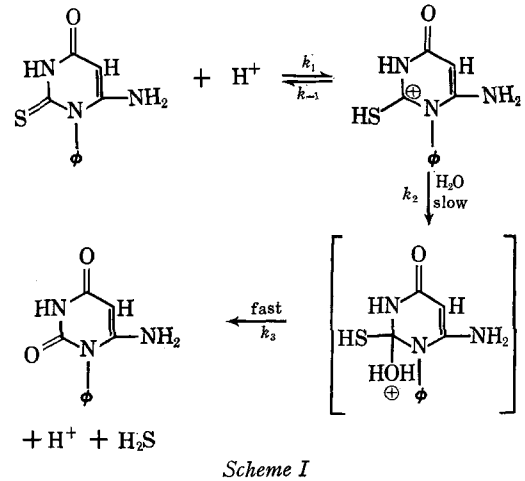


Fig. 5—Acid hydrolysis of N-phenyl-6-amino-thiouracil.



Scheme I

lytic reaction. This mechanism suggests that the thiouracils readily pick up a proton to form an intermediate which undergoes water attack and rapidly collapses to form the corresponding uracils.

Since the kinetic and mathematical aspects of such a system are adequately covered by Laidler (7), the final rate equation is listed here only for discussion purposes.

$$\text{rate} = \frac{k_1 k_2\,(\text{thiouracil})\,(H^+)}{k_1(H^+) + k_2 + k_{-1}} \qquad \text{(Eq. 1)}$$

An examination of this equation indicates that at high acid concentration the rate of hydrolysis reaches a limiting value and the rate equation becomes,

$$\text{rate} = K\,(\text{thiouracil}) \qquad \text{(Eq. 2)}$$

This is verified in Fig. 5, A, and illustrates specific hydrogen ion catalysis. At low (H^+) the rate is dependent on the equilibrium concentration of the protonated and nonprotonated thiouracil species in solution. Although only the acid hydrolysis of *N*-phenyl-thiouracil was studied, it is reasonable to assume that the other *N*-substituted derivatives would undergo a similar behavior.

At low acid concentration k_2 is small, and the rate equation then can be expressed as:

$$\text{rate} = \frac{k_2 k_1\,(\text{thiouracil})\,(H^+)}{k_1(H^+) + k_{-1}} \qquad \text{(Eq. 3)}$$

Rearrangement gives the following relationship:

$$\text{rate} = \frac{k_2\,(\text{thiouracil})}{1 + \dfrac{k_{-1}}{k_1(H^+)}} = \frac{k_2\,(\text{thiouracil})}{1 + \dfrac{K}{(H^+)}}$$

$$1 + \frac{k}{(H^+)} = \frac{k_2\,(\text{thiouracil})}{\text{rate}} \qquad \text{(Eq. 4)}$$

From Eq. 4 a straight line relationship would be obtained by plotting $1/(H^+)$ against $1/(\text{rate})$. This is shown in Fig. 5, B, suggesting specific acid catalysis.

Data for base catalysis of the *N*-phenyl-thiouracil are given in Fig. 6. No limiting of the hydrolytic rate was observed at high hydroxyl-ion concentration (6 *N*) as was seen in acid hydrolysis, suggesting that the rate is proportional to hydroxyl-ion concentration. Although the limited data available do not permit the calculation of exact rate equations, a possible mechanism for this degradation is listed in Scheme II based on the information available.

Schemes I and II are mechanisms for both acid and base hydrolysis involving a rapid polarization of the C=S or thio-group followed by the rate-determining step (8) of a water molecule attack to form a tetrahedral intermediate which rapidly collapses to form the corresponding uracil. Since water attack is postulated to be the rate-determining step, the greater the positive character of the reaction center or C=S function the faster should be the rate of hydrolysis. No bonded electrons on the *N*-nitrogen should be capable of localization toward the reaction center, such as in halogenated phenyl-*N*-substitution, which decreases the positive charge of the carbon in C=S resulting in an increase in stability.

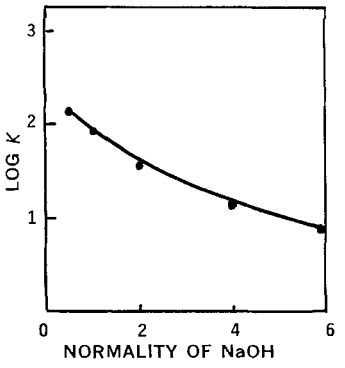
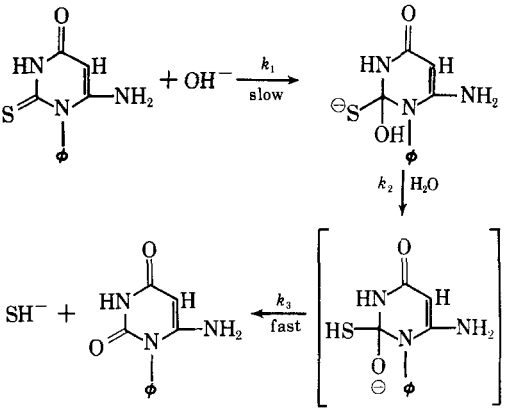


Fig. 6—Base hydrolysis of N-phenyl-6-amino-thiouracil.



Scheme II

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