[Chem. Pharm. Bull. 18(6) 766—770 (1970)]

UDC 547.496.3.04 : 615.246.9.011.5

Ionization Constants of 2-Aminoethyl- and 3-Aminopropyl-isothiuronium Salts and Their Transformation Products

Akira Hanaki

National Institute of Radiological Sciences[1]

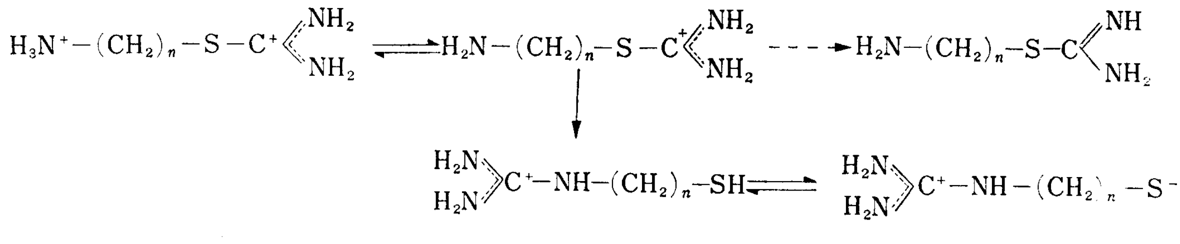
(Received November 20, 1969)

The ionization constants of 2-aminoethylisothiuronium (AET) and 3-aminopropylisothiuronium (APT) salts, dibasic acid intrinsically, were determined potentiometrically. Those isothiuronium salts were extremely unstable and underwent the transguanylation, during the titration, through the conjugate base to the sulfhydryl compounds, which were ionized in the second step. For the determination of the ionization constant, the titration curve was treated as an equimolar mixture containing two acids. From the temperature dependence of the ionization constant, measured at 5°, 15°, 25° and 35°, was calculated the enthalpy of the ionization, which led to the evaluation of the entropy. The thermodynamic functions of those compounds at 25° were as follows:

	ΔF kcal/mole	ΔH kcal/mole	ΔS cal/mole·deg
AET	10.2	13.8	12
APT	11.8	13.8	7

The thermodynamic functions for the transguanylation and the cyclization products from those isothiuronium salts were also determined.

2-Aminoethylisothiuronium (AET) salt, a protective agent against a lethal dose of ionizing radiation, was shown to be unstable in an aqueous solution of the physiological condition and undergo either transguanylation to produce the sulfhydryl compound, which has been postulated to be an active form of this protective agent, or cyclization to yield 2-amino-2-thiazoline (2-AT).[2] 3-Aminopropylisothiuronium (APT) salt, another protective agent, undergoes the transguanylation but not the cyclization in the same condition. Both the transguanylation and the cyclization are considered to be closely connected with the ionization of the isothiuronium salt, because the ionized product, *i.e.*, the conjugate base, of the isothiuronium salt is an only species susceptible to those transformation, especially to the transguanylation.[3] Because of the instability of the conjugate base, the determination of the ionization constant, which might be necessitated for the elucidation of the reaction mechanism, has not hitherto been performed. The pH value of the solution is dropped progressively during the



AET : $n=2$ APT : $n=3$

Chart 1. Ionization Reaction of Aminoalkylisothiuronium Salt

---

[1] Location: *Anagawa-4, Chiba.*

[2] J.X. Khym, R. Shapira and D.G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957); **80**, 3342 (1958).

[3] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1409 (1968).

titration, which is explained as a result of the transguanylation of the conjugate base.[4] In order to determine a reliable constant, the titration should be carried out at a rapid speed as possible not to occur the hydrogen ion liberation. In the previous paper, it was described that the patterns of the titration curves of AET and APT was affected by the rates of alkali addition and the principle for the determination of the ionization constant of those unstable compounds was given.[5] The present paper is concerned with the reliable ionization constants of AET and APT, as well as those of their transformation products, and the thermodynamic functions, enthalpy and entropy, of the ionization reaction.

Experimental

Materials——AET, APT and 2-amino-2-penthiazoline (2-PT), the cyclization product from APT, were synthesized according to the procedure reported previously.[6] 2-Amino-2-thiazoline (2-AT), the cyclization product from AET, was purchased from Aldrich Chemical Company, Inc., and used after recrystallization from EtOH. The solution of the transguanylation products, mercaptoethylguanidine (MEG) and mercaptopropylguanidine (MPG), were prepared just prior to use by neutralizing the AET and APT solutions, respectively, with 1.5 equivalents KOH at room temperature. NH_4Cl, analytical grade, was desiccated *in vacuo* over P_2O_5 to constant weight. All the solutions were prepared from twice distilled water from all glass apparatus.

Method——The ionization constant was determined potentiometrically at 5°, 15°, 25° and 35° in a medium of 0.1N KNO_3. The titration was performed with a Radiometer TTTlc titrator and SBR2c titrigraph. The glass electrode 202C and 202B were used, respectively, for the measurements at 5° and 15°, and at 25° and 35°. The instrument was standardized at an appropriate temperature with the phthalate and the phosphate buffers before and after the measurement. For the determination of the ionization constant, the 5.00×10^{-3}M solution of the isothiuronium salt or the transformation products was titrated with carbonate free 0.1N KOH in a 50 ml water-jacketted cell. Purified nitrogen gas, once passed through 0.1N KNO_3, was bubbled into the test solution, which was equilibrated at an appropriate temperature by circulating the thermostatted water through the jacket during the entire measurement. Since both AET and APT were apt to undergo the transformation during the titration, the delivery of standard KOH from the buret of the titrigraph was adjusted to a rapid speed as possible; *i.e.*, chart speed=40 mm/min and pen speed =60%/min. For other compounds, the speed was kept slow; *i.e.*, chart speed=10 mm/min and pen speed =30%/min.

Calculation——In the calculation of the ionization constant, equations (1) and (2) were applied.

$$pK = pH + \log [HA^+]/[A] + \log f_1 \qquad (1)$$

for MEG, MPG, 2-AT, 2-PT and NH_4^+

$$pK = pH + \log [H_2A^{2+}]/[HA^+] + \log f_2/f_1 \qquad (2)$$

for AET and APT

In the present case, ionic strength, I, was kept below 0.1 and Davies' equation (3) was used for the estimation of activity coefficient f_z.[7]

$$-\log f_z = 0.5\, z^2 \frac{\sqrt{I}}{1+\sqrt{I}} - 0.3\, I \qquad (3)$$

The activity coefficient of the zwitter ion, *i.e.*, the conjugate base of MEG or MPG, was taken as unity.[8]

Results and Discussion

Titration Curve

Since the transguanylation was initiated by adding alkali and proceeded in parallel with the pH drop of the solution,[9] the titration should be carried out at a rapid speed as possible

---

[4] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1146 (1969).

[5] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 486 (1968).

[6] T. Hino, K. Tana-ami, K. Yamada and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 1193, 1202 (1966).

[7] C.W. Davies, "Ion Association," Butterworth, London, 1962.

[8] S.P. Datta and A.K. Czrbowski, *Trans. Faraday Soc.*, **54**, 1179 (1958).

[9] The pH drop corresponding to the 50% transguanylation, $\Delta pH_{50\%}$, was calculated to be 0.3 at any point on the titration curve below 1 equivalent alkali.[4]

in order to determine a reliable constant. The average titration speeds from 0.2 to 0.8 equivalent alkali were 10 sec and 8 sec, respectively, for AET and APT. Nevertheless, it appeared that the pH drop, proportional to the amounts of alkali added, was observed in the entire region below 1.0 equivalent alkali. This fact suggests that, in order to calculate the reliable ionization constant of the isothiuronium salt, one should have regard to the participation of the ionization of the transguanylation product in the titration curve. Certainly, APT was transguanylated very rapidly; the conjugate base has a short life and was transguanylated quantitatively during the titration with 2 equivalents alkali. On the other hand, in AET, which has been postulated to be cyclized in weakly acidic or neutral medium,²⁾ the extent of the transguanylation during the titration was generally less than 100%.¹⁰⁾ Both reactions, proceed rapidly, are considered to advance through the conjugate base.⁴⁾ Since the cyclization competes probably with the transguanylation, the extent of the latter reaction could not reach to 100%. In accordance with the elevation of the temperature, the ratio of the extents of the cyclization to the transguanylation increased, though the rates of both reaction should increase. When the solution containing one acid is titrated, pK_a is reduced and thereby the pH of the solution titrated becomes lowered with the increasing degrees of temperature. Then, the increase in the ratio described above may be ascribed to the difference of the pH dependence between both reactions. Probably the lower pH might not be inconvenient for the cyclization as compared with the transguanylation. Thus, the titration curve of AET involves the ionizations of AET itself, the transguanylation and the cyclization products. However, the initial part of the curve may be explained from the ionization of AET molecule, because this compound has to be transformed through the conjugate base.

Formation of the Transguanylation Product

When excess alkali was added all at once into the solution, AET as well as APT, was appeared to undergo the complete transguanylation. The apparent pK_a of this product calculated from the titration curve decreased with the increasing amounts of alkali added. An example in APT was shown in Table 1. In any case, one equivalent of sulfhydryl group was detected potentiometrically. The apparent pK_2 of APT, which corresponds to the pK_a of MPG as explained below, was calculated to be 9.38 at 25°. Those facts indicate the possibility that the increasing amounts of alkali may force the formation of other sulfhydryl compounds which show the lower pK_a than that of the transguanylation product, and thereby the apparent pK_a from the titration curve gives a lower value. Though the higher concentration of hydroxide ion is convenient for the complete transguanylation, the rate is reversed when the amount of alkali added is more than 1.5 equivalents.¹¹⁾ For those reasons, the preparation of the sulfhydryl compound was carried out by adding 1.5 equivalents alkali into the corresponding isothiuronium salt.

Table 1. Apparent pK_a of the Transformation Product from APT by Various Amounts of KOH

Equivalent KOH	Apparent pK_a	Equivalent KOH	Apparent pK_a
1.5	9.37	2.5	9.26
2.0	9.32	3.0	9.21

The titation was carried out at 25°.

Ionization Constant

The second ionization constant K_2 of the isothiuronium salts examined here, dibasic acid intrinsically, could not be determined because of the extreme instability of their conjugate

---

10) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968).
11) A. Hanaki, unpublished data.

bases. The transformation of the conjugate base proceeds rapidly during the titration, and the ionization constant of the product is calculated like a constant in the second step equilibrium. Thus, the isothiuronium salt did undergo only one step ionization, and the solution could be considered to contain equimolar mixtures of the isothiuronium salt and the transformation product. The ionization constants of those two dissociable groups were calculated by solving the simultaneous equation (4):

$$A[\mathrm{H}^+]^2/K_1=(2-A)K_2+(1-A)[\mathrm{H}^+] \qquad (4)$$

$$A=a[R]_0-([\mathrm{OH}^-]-[\mathrm{H}^+])/[\mathrm{R}]_0$$

where *a* and $[R]_0$, both are variable, represent equivalent alkali and the total concentration of the isothiuronium salt, respectively. Providing that the curve behaves like a monobasic acid below 0.5 equivalent alkali, K_1 could be determined more simply. This treatment may be valid and useful for the rapid determination of K_1 for those unstable compounds, which undergo apparently two ionization reactions; one is due to the mother compound itself and another is due to the species produced through the conjugate base of the former. In any treatment, since the pH drop was progressed during the titration, the real pK_a should be estimated extraporatory as illustrated in Fig. 1. In AET, since the reaction was rather complicated, involving both the transguanylation and the cyclization, pK_1 was estimated estraporatory by the simplified method. For this reason, the constant of AET may be less accurate as compared with that of APT.

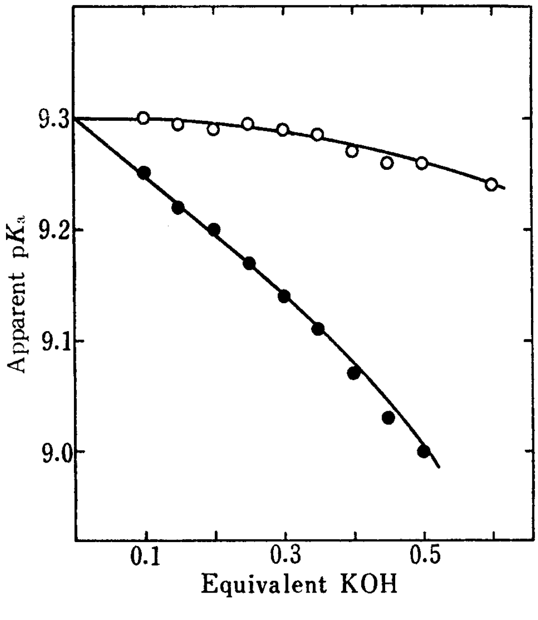


Fig. 1. Estimation of pK_1 of APT at 5° from Several Calculated Data

○ : calculated as dibasic acid
● : calculated as monobasic acid

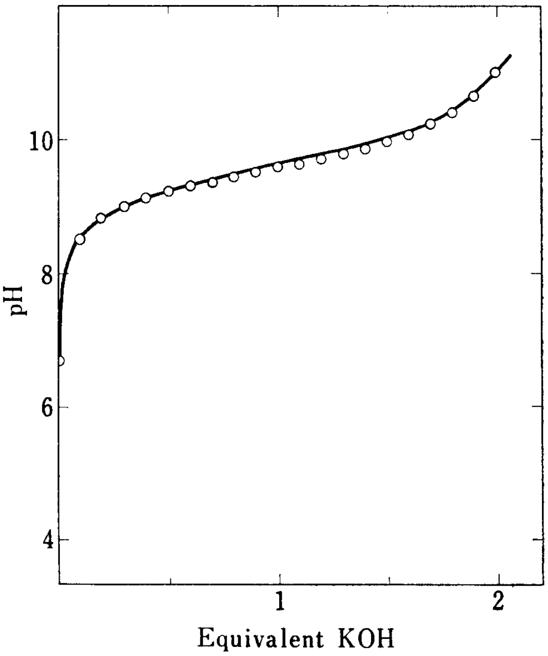


Fig. 2. Titration Curve of APT at 5°

Solid line indicates the calculated curve by using pK_1 9.30 and pK_2 9.68.

As described above, the titration curve of the isothiuronium salt was composed of two ionization reactions; one is due probably to the ammonium group of the isothiuronium salt and another to the sulfhydryl group of the transguanylation product. In order to confirm this respect, a titration curve of a dibasic acid calculated by using pK_1 9.30 of APT and pK_2 9.68 of MPG prepared was compared with the experimental curve of APT at 5°. The result was shown in Fig. 2. The calculated and experimental curves were appeared to be fairly coincided and this fact indicated the validity of the above treatment.

The ionization constants of the transguanylation products, MEG and MPG, and the cyclization products, 2-AT and 2-PT, were determined also potentiometrically. In order to elevate the reproducibility and the accuracy of the constant, the titration was done at a slow rate. The ionization constants calculated were summarized in Table II.

Table II. Ionization Constants at Various Temperatures

Compound	Temperature			
	5°	15°	25°	35°
AET	8.10	7.75	7.41	7.04
APT	9.30	8.94	8.54	8.26
MEG	9.05	8.85	8.66	8.52
MPG	9.68	9.43	9.28	9.13
2-AT	9.32	9.01	8.73	8.50
2-PT	10.66	10.33	9.96	9.71
NH_4^+	9.86	9.55	9.20	8.94

Thermodynamic Functions

From the pK_a values at four different temperatures, enthalpy was determined by using van't Hoff equation (5). The accuracy of those values is, in any case, not extremely high as

$$-d \ln K/d(1/T) = \Delta H/R \qquad (5)$$

compared with that of the calorimetric data, but the calorimetric method could not be employed in the present case because of the instability of the compound during the measurement. The method employed in this paper may give the most reliable value for the heat of ionization of the labile compound. From the enthalpy and free energy changes, entropy was calculated by equation (6).

$$-RT \ln K = \Delta H - T\Delta S \qquad (6)$$

Those thermodynamic functions summarized in Table III furnish a most valuable guide to the behavior of the molecule concerned, and to the nature of the group ionizing at various pH values.

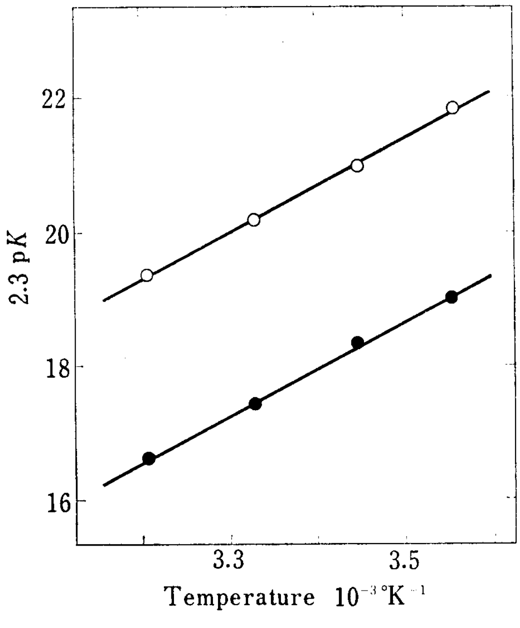


Fig. 3. Estimation of Enthalpy by using van't Hoff Equation

● : AET, ○ : APT

Table III. Thermodynamic Functions for Ionization at 25°

Compound	ΔF kcal/mole	ΔH kcal/mole	ΔS cal/mole·deg
AET	10.2	13.8	12
APT	11.8	13.8	7
MEG	11.8	7.0	−16
MPG	12.7	7.4	−18
2-AT	11.9	11.4	− 2
2-PT	13.5	12.5	− 3
NH^+_4	12.5	12.3	− 0.7