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Reactivities of Radiation-protective Aminoalkylisothiuronium Salts. IV.[1] Stability of N-Ethyl Derivatives of 2-Aminoethylisothiuronium Salt

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The effect of N-substituent on the stability in an aqueous solution of AET was studied. By the substitution, both the transguanylation and the cyclization were hindered. The reactive species of N-Et-AET, and probably of N′-Et-AET, for the transguanylation is the monoionic conjugate base. N′-Et-AET, being relatively stable in the presence of less than one equivalent alkali, may be dissociated through the two-steps ionization to the neutral conjugate base, which would be considered as one of the reactive species. The degree of the transformation of N-ethylated AET derivatives was as follows; the transguanylation in the presence of 0.5 equivalent NaOH for 4 min at 15°:

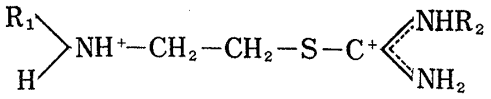
AET; 0.95, N-Et-AET; 0.84, N′-Et-AET; 0.27.

the cyclization in 3 days at 30°:

AET; 0.95, N-Et-AET; 0.36, N′-Et-AET; 0.29.

The relationship between the transguanylation and the pH drop in the solution was discussed.

The radiation-protective aminoalkylisothiuronium salt, such as 2-aminoethylisothiuronium (AET) or 3-aminopropylisothiuronium (APT) salt, is very unstable in an aqueous solution of the physiological condition, and either transguanylated or cyclized.[3] The transguanylation product, having a reactive sulfhydryl group,[4] possesses the radiation-protective ability, while the cyclization product has a little effect.[5] Accordingly, the transguanylation product has been assumed as the active form of the isothiuronium salt, and the transguanylation can be considered to be one of the reactions concerned with the protective action. If the susceptibility of the isothiuronium salt to the transguanylation is correlated with the protective ability, this reaction would be employed as an index for the chemical evaluation of the protective ability. The cyclization seems also interesting in another respect; since both the transguanylation and the cyclization have been assumed to proceed through the same cyclic intermediate, the rate of the cyclization would be related with the susceptibility to the transguanylation. The present paper dealt with the reactivities of the derivatives of AET ethylated at 2-amino group (N-Et-AET) and at nitrogen atom of thiourea moiety (N′-Et-AET). The reactivity was measured by the potentiometric titration method described in the preceding paper.[1]



	R_1	R_2
AET	H	H
N-Et-AET	C_2H_5	H
N′-Et-AET	H	C_2H_5

Chart 1

1) Part III: A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968)
2) Location: *Anagawa-4, Chiba-shi, a)* IAEA Fellow (1965—1966) from Thailand. Present Adress: *Faculty of Pharmacy, Rhya Thai Road, Bangkok, Thailand.*
3) J.X. Khym, R. Shapira and D.G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957); **80**, 3342 (1958).
4) R. Koch and W. Schwarze, *Arzneimittel-Forsch.*, **7**, 576 (1957); A. Hanaki, T. Hino and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1446 (1967)
5) R. Schapira, D.G. Doherty and W.T. Burnett, Jr., *Radiation Res.*, **7**, 22 (1957).

Experimental

Material——N-Et-AET and N′-Et-AET were synthesized in this laboratory.[6] Carbonate free NaOH solution was prepared from 50% stock solution. All the solutions were prepared from twice distilled water from all glass apparatus.

Potentiometric Titration——The potentiometric titration was carried out in a medium of 0.1N NaCl with a Radiometer TTTlc titrator and SBR2c titrigraph. In order to avoid the oxidation, the measurement was done in an atmosphere of nitrogen. The ionization constant of the isothiuronium salt was calculated from the titration curve as described previously.[7]

Rate of the Transguanylation——The rate of the transguanylation was measured at 15° in the presence of 0.5 equivalent NaOH. The detailed procedure was described in the preceeding paper.[1]

Rate of the Cyclization——An aqueous solution of AET derivatives (1.5×10^{-2}M, ionic strength 0.1 with NaCl) was kept thermostatting at 30°. After 1, 2, 3, 4, 5 and 7 days, an aliquot (5 ml) was pipetted from this solution, and mixed with 1.0 ml of 0.1N NaOH. After maintaining for 2 min, the solution neutralized with 1.0 ml of 0.1N HCl was titrated with standard NaOH at room temperature. The degree of the cyclization is represented by the following equation.

Degree of the cyclization = 1 − (equivalent of free acid determined)

Measurement of pH Drop——The measurement was carried out at 25° with a Toa–Dempa HM–5A pH meter equipped with a ESR–2T recorder. The procedure was shown in the previous paper.[8]

Results

A chemical index showing that the isothiuronium salt undergoes the transguanylation is the continuous drop of pH in the solution. In N-Et-AET, the pH drop was observed immediately after the addition of less than one equivalent alkali. The rate of hydrogen ion liberation calculated from the pH drop curve was shown in Fig. 1.[9] Con-

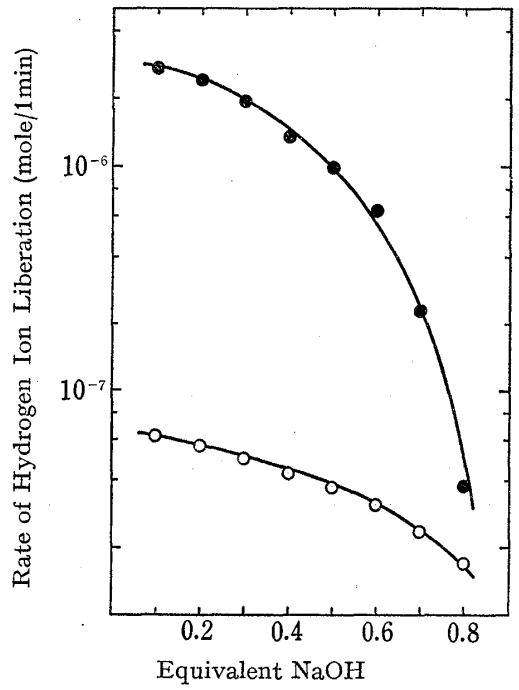


Fig. 1. Rate of Hydrogen Ion Liberation of AET and N-Et-AET

$[R]_0$: 5.00×10^{-3}M,
temperature: 25°, ionic strength: 0.1
AET: —●—● N-Et-AET: —○—○—

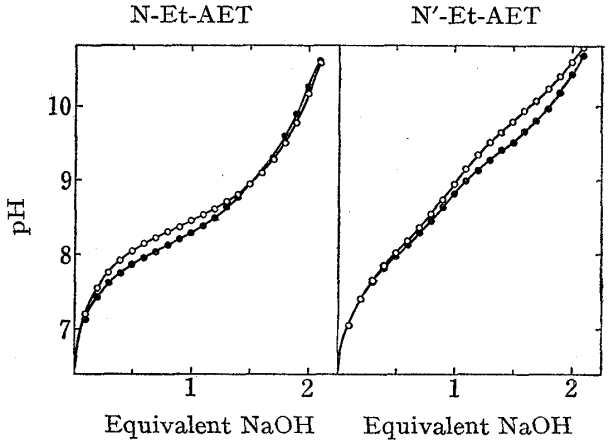


Fig. 2. Potentiometric Titration Curves of N-Et-AET and N′-Et-AET at 15°

titration speed :
N-Et-AET; —○—○— 14 sec/0.1 eq. —●—● 27 sec/0.1 eq.
N′-Et-AET; —○—○— 16 sec/0.1 eq. —●—● 35 sec/0.1 eq.
$[R]_0$: 5.00×10^{-3}M

6) T. Hino, K. Uoji, P. Xumsaeng and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), in preparation.
7) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 487 (1968).
8) A. Hanaki, T. Hanaki, K. Ōya, A. Andou, T. Hino and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 108 (1966).
9) The hydrogen ion liberated into the reaction mixtures comes from the conjugate acid RNH_3^+, that is the isothiuronium salt itself, and RNH_3^+ is ionized to the reactive species RNH_2 by adding alkali. Then, the concentration of the proton source decreases with the increasing amounts of alkali added. Therefore, the apparent rate of the hydrogen ion liberation decreased according to the increase of the equivalent alkali as shown in Fig. 1, though the transguanylation was stimulated actually by the addition of alkali.

sidering that the rate of the hydrogen ion liberation is related with that of the transguanylation, the reaction may be reduced by the N-substitution. On the other hand, N′-Et-AET did not show a detectable drop of pH in the above condition. This result might reflect as if N′-Et-AET does not undergo the transguanylation.

It has been shown that AET and APT were transguanylated during the course of the titration.[1)] When N′-Et-AET which had been titrated with 2.5 equivalents alkali was titrated again, the generation of free acid was observed. The free acid thus observed in the retitration is resulted from the generation of the sulfhydryl compound.[1)] Therefore, this finding indicates that N′-Et-AET is also transguanylated even though the pH drop can not be measured. If the transguanylation is accompanied really with the pH drop, N′-Et-AET would also lower the pH value in a different condition, probably in more alkaline medium; because the transguanylation may be promoted with the increasing of pH. In order to confirm this respect, the titration of N-Et-AET and N′-Et-AET was attempted at different speeds. The titration curve was shown in Fig. 2. The curves of both compounds are shifted to acidic side according to the decreasing speeds of the titration. In N-Et-AET, this shift was found up to 1.5 equivalents alkali of the titration curve. In N′-Et-AET, however, the shift was measured in a region between 0.5 and 2 equivalents alkali. This finding supports that N′-Et-AET is also susceptible to the transguanylation in the alkaline medium. N′-Et-AET was transguanylated unexpectedly in the presence of less than one equivalent alkali, where the pH drop could not measured. When the solution of this compound which had been titrated with one equivalent alkali was titrated again, the formation of free acid was observed. The result was given in Table I. It is correct in principle that the transguanylation is accompanied with the pH drop in the solution. A reason why the transguanylation of N′-Et-AET is not related with the pH drop may be ascribed to the buffering capacity of the isothiuronium salt. When the extent of the transguanylation is small, the liberated hydrogen ion associates again with the monoionic conjugate base and thereby pH is not decreased remarkably. The values of pH drop corresponding to 20, 50 and 90% transguanylation are calculated as 0.1, 0.3 and 1.0, respectively.[10)]

Table I. Transguanylation of N-Et-AET and N′-Et-AET during Titration with 1 Equivalent NaOH at 15°

Compound	Titration speed[a)] (sec/0.1 eq.)	Ttransguanylation (equivalent)
N-Et-AET	15	0.587
N-Et-AET	27	0.692
N′-Et-AET	16	0.098
N′-Et-AET	34	0.204

a) average speed
total concentration of AET derivative; 5.00×10^{-3}M

Table II. Transguanylation of AET, N-Et-AET and N′-Et-AET in the Presence of 0.5 Equivalent NaOH at 15°

Reaction time (min)	Degree of transguanylation[a)] (per cent) AET	N-Et-AET	N′-Et-AET
1	0.814	0.576	—
4	0.947	0.844	0.269
16	—	—	0.606

a) [transguanylation product]/[monoionic AET molecule]$_0$
total concentration of AET derivatives: 8.00×10^{-3}M

10) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), accepted.

Since N′-Et-AET was also transguanylated in the same condition as other labile isothiuronium salts, the rate of the transguanylation was estimated at 15° in the presence of 0.5 equivalent alkali. The rate was expressed as the ratio of the transguanylation product to the dissociated monoionic AET molecule, which is a reactive species. The N-substituent at 2-amino group or at thiourea moiety reduced the reaction as shown in Table II. Especially, the rate was reduced remarkably by the introduction of ethyl group into thiourea moiety, probably due to the electric effect in the formation of the activated complex.

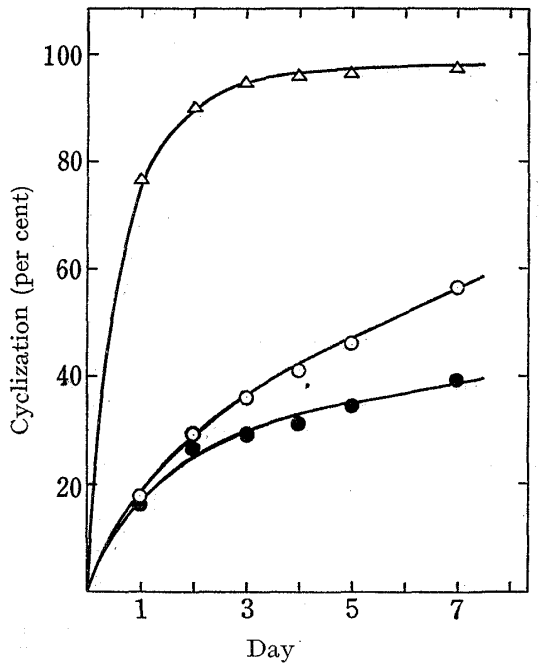


Fig. 3. Cyclization of AET, N-Et-AET and N′-Et-AET at 30°

$[R]_0$: 1.50×10^{-2}M
ionic strength: 0.1
AET: △ N-Et-AET: ○ N′-Et-AET: ●

The cyclization was delayed also by the substitution of 2-amino group and thiourea moiety. The reaction profile plotted the formation of 2-aminothiazoline derivative against the reaction time was presented in Fig. 3. Since the pH drop of the solution was observed throughout the measurement, the isothiuronium salt might be transguanylated during the storage. However, the content of the transguanylation product was very small; in the case of AET, it was less than a few per cent. The cyclization of AET was rapid extraordinarily as compared with other compounds. N′-Et-AET was more stable than N-Et-AET. Though the cyclization is observed predominantly in the acidic medium, its rate seems in parallel with the susceptibility to the transguanylation.

Discussion

We have offered that the reactive species of the isothiuronium salt for the transguanylation is the monoionic conjugate base. Since the isothiuronium salt is a dibasic acid intrinsically, the monoionic base may be further ionized in principle. Then, the reaction of the isothiuronium salt encountered during the titration may be pictured as follows.

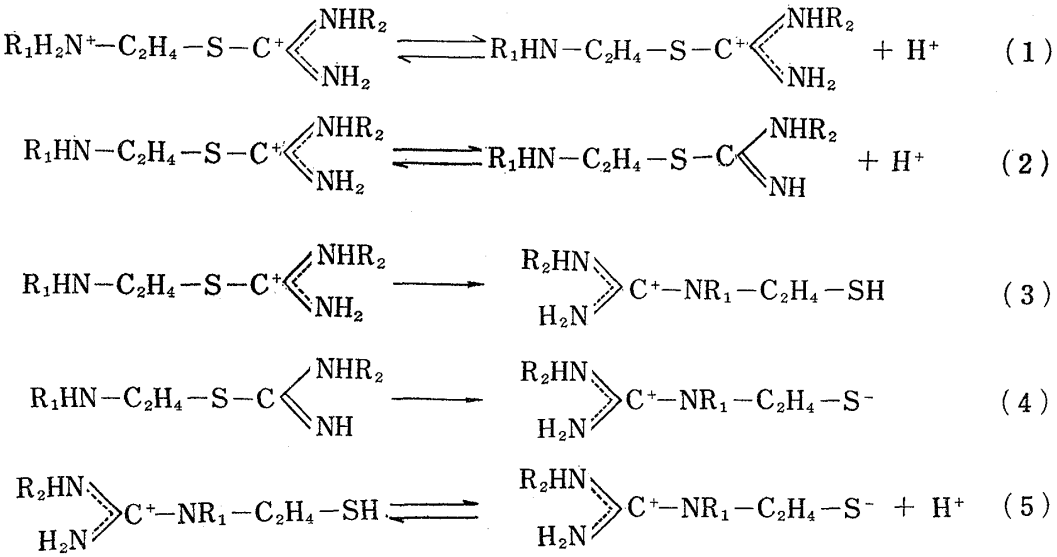


Chart 2

If the rate of the transguanylation is extremely rapid, the monoionic conjugate base may be transformed instantaneously to the sulfhydryl compound and thereby could not be encountered the second-step ionization. Therefore, the titration curve for those labile compounds behaves as if the solution contains the equimolar mixtures of the isothiuronium salt, which behaves like a monoionic acid, and the transguanylation product.

On the other hand, if the rate of the transguanylation is slow, the further ionization of the monoionic base should be considered. Then, the titration curve can be explained by the two-steps ionization of the isothiuronium salt. If those compounds are transguanylated, it might be reflected that the neutral form of AET is a reactive species. Since the pK_a values for the equilibria (1) and (2) are approximately 8 and 10, respectively, the monoionic AET is distributed throughout the range of the titration curve, while the neutral species is formed in the range over one equivalent alkali. Moreover, in the presence of between 0.5 and 1.5 equivalent alkali, where the titration curve shifts to acidic side as shown in N'-Et-AET, the monoionic base is a main component. Therefore, it is more probable that the dissociated monoionic AET is also the reactive species even in the case of the stable isothiuronium salt. The quantitative account of those problems will be presented in the next paper.

The rate of the transguanylation is assumed to be proportional to the concentration of hydroxide ion. Therefore, the rate becomes increasingly rapid with pH as far as the compound is transguanylated. The transguanylation product is dissociated in the alkaline medium, because the pK_a of the equilibrium (5) is approximately 9. If the transguanylation product is present in the reaction mixtures, its ionization may occur preferentially. Therefore, the titration curve might shift to acidic side in accordance with the increasing amounts of the transguanylation product. Thus, the pH drop may be observed as far as the transguanylation is involved.