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Reactivities of Radiation-Protective Aminoalkylisothiuronium Salts. VIII.[1)] The Proton Ionizability and the Transguanylation Rate in N-Alkylated Derivatives of 2-Aminoethylisothiuronium Salt

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The transguanylation of N-alklated derivatives of AET was followed at 15° with the potentiometric titration method. The reactivity was reduced markedly by the substitution at the nitrogen atom of isothiuronium group. The rate was related closely with pK_2 (isothiuronium group) but not with pK_1 (ammonium group), which indicated that the proton releasing from isothiuronium group may be a limiting factor for the transguanylation. The rate increase was observed limitedly by the substitution at the nitrogen of ammonium group.

2-Aminoethylisothiuronium (AET) salt is rapidly transguanylated in a neutral or weakly alkaline medium to yield 2-mercaptoethylguanidine (MEG).[3)] In the previous paper, it is indicated that the susceptibility of AET to the transguanylation is affected considerably by modifying the structure of the compound; *i.e.*, by substituting at the nitrogen atom of ammonium or isothiuronium group.[4)] AET is intrinsically a dibasic acid and its ammonium group has to be ionized prior to the transguanylation.[5)] The monoionic conjugate base is rapidly transformed probably *via* a cyclic intermediate, in which nitrogen atom of amino group would access to be linked with carbon atom of isothiuronium group. Accordingly, if the formation of the cyclic intermediate is a rate-controlling step, the physico-chemical properties related to the electric interaction between amino and isothiuronium groups might be correlated with the reactivities of AET derivatives. In the present paper, it was described briefly some relations between the reactivity and the proton ionizability in N-alkylated derivatives of AET.

Experimental

Materials——AET derivatives were synthesized according to the procedure reported previously and used after crystallization from alcohol.[6)]

Proton Ionization Constant——Proton ionization constant was calculated from the potentiometric titration curve. The solution of 5.00×10^{-3}M AET derivatives, ionic strength 0.1 with NaCl, was titrated with carbonate free 0.1N NaOH in an atomoshere of nitrogen. The measurement was done by using a Radiometer TTTlc titrator and SBR2c titrigraph. In order to avoid the transguanylation during the measurement, the solution was titrated at low temperature, 15°, and at a rapid speed as possible. In the compounds undergone very rapid transguanylation, like AET or N-methyl-AET,[7)] the constants were estimated extraporatory.[8)] All the constants determined were the practical pK values.

[1)] Part VII: A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **18**, 1663 (1970).

[2)] Location: *Anagawa-4, Chiba.*

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

[4)] A. Hanaki, P. Xumsaeng, T. Hino and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **17**, 677 (1969); K. Uoji, K. Tsuneoka, A. Hanaki and S. Akaboshi, *ibid.*, **17**, 1742 (1969); A. Hanaki *ibid.*, **18**, 399 (1970).

[5)] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 326 (1971).

[6)] T. Hino, K. Tana-ami, K. Yamada and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 1139 (1966).

[7)] N-Methyl-AET is an abbreviation for the AET derivative methylated at amino nitrogen. The abbrevations, N′ and N″, indicate the nitrogen atoms at isothiuronium group.

[8)] A. Hanaki, *Chem Pharm. Bull.* (Tokyo), **18**, 766 (1970).

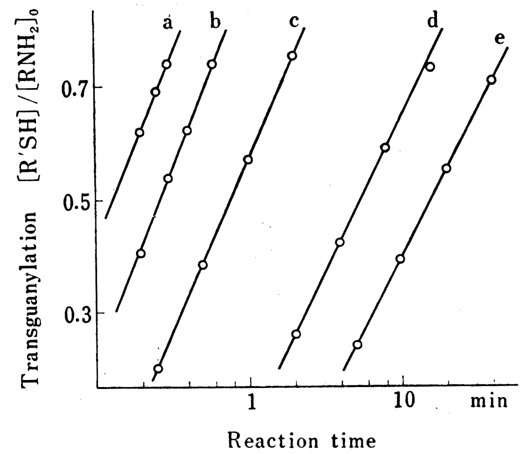

Fig. 1. Graphic Estimation of the Half Life for the Transguanylation

a : N-methyl-AET b : AET
c : N-ethyl-AET d : N'-methyl-AET
e : N, N'-dimethyl-AET
The experimental details were shown in the text.

Measurement of Transguanylation——The extent of the transguanylation was determined potentiometrically at 15°. Since the rate depended on the concentration of reactive species, *i.e.*, the conjugate base of AET abbreviated as RNH_2, the measurement was done in the following condition: the total concentration of the substrate was 8.00×10^{-3} M and the reaction was initiated by adding 0.5 equivalent NaOH. In this experiment, the initial concentration of reactive species was 4.00×10^{-3} M. The detailed procedure was described in the previous paper.[9]

Result and Discussion

The following sequence of the reactions has been postulated concerning the transguanylation;[10]

$$RNH_3^+ \rightleftarrows RNH_2 + H^+ \qquad (1)$$

$$RNH_2 \rightleftarrows R'SH \qquad (2)$$

where RNH_3^+, RNH_2 and R'SH indicate AET, its conjugate base and the transguanylation product, respectively. The rate of the transguanylation depends on the concentration of RNH_2, in which a proton of ammonium group is ionized.[9b] In addition, the rate depends somewhat upon the hydroxide ion concentration in the medium.[11] Therefore, in order to evaluate exactly the susceptibility of the compound to the transguanylation, the measurement should be done at constant pH and in the fixed concentration of the active species. Unfortunately, as predicted from Eq (1) and (2), the pH in the medium is dropped progressively as the transguanylation proceeds. Accordingly, the measurement at exactly constant pH, in the absence of buffer, is impossible as far as the potentiometric titration method presented here is employed.

An aim of the present work is to compare the reactivity of AET derivatives in relation to the structure. For this purpose, a small and definite pH variation could be allowed. The reaction was started on adding half equivalent alkali. At the start of the reaction, the pH in the medium is theoretically equal to the pK' value of the substrate concerned and the concentration of RNH_2 is 4.00×10^{-3} M. Since the half life was used as an index for the reactivity, the pH variation was within 0.3 during the measurement.[10]

The half life, estimated graphically, was listed in Table I. Provided that all the compounds are transformed by similar mechanism, the reciprocal of life time, listed as the relative value in the last column of Table I, may be proportional to the rate constant. Among those compounds, the differences in the pH effect, coming from the differences in pK_1 values, may exist but a little; the differences in the initial pH values, diversed in relatively narrow range 0.2, are covered within the pH drop 0.3 during the measurement.

The substitution by alkyl group reduced evidently the reaction rate except in the case of N-methyl-AET. By the substitution of isothiuronium group, the compound is stabilized markedly. The reactivity was related with pK_2 but not with pK_1. In Table I, the constants, pK_1 and pK_2, are due to the proton ionizations from ammonium and isothiuronium groups, respectively.[5] The pK_2 values in AET, N-methyl- and N-ethyl-AET are 9.0 or less.[11]

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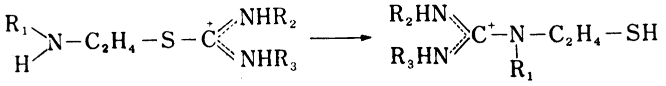
[9] a) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968); b) A. Hanaki, *ibid.*, **18**, 1653 (1970).
[10] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1146 (1969).
[11] Unpublished result.

Table I. Proton Ionization Constant and Transguanylation Rate in AET and Its Derivatives



No.	R_1	R_2	R_3	pK_1	pK_2	Half life (min)	Relative rate
1	H	H	H	7.95	—	0.27	1.0
2	CH_3	H	H	8.04	—	0.13	2.1
3	C_2H_5	H	H	8.14	—	0.75	0.36
4	H	CH_3	H	8.05	9.88	5.6	0.048
5	H	C_2H_5	H	8.03	9.86	10	0.027
6	CH_3	CH_3	H	8.12	9.60	5.2	0.052
7	H	CH_3	CH_3	7.97	10.36	16	0.017
8	CH_3	CH_3	CH_3	8.12	10.50	83	0.003

The experimental details were described in the text.

The above relation indicates that the acidity of isothiuronium group predicts the stability of the compound.

AET is transguanylated probably *via* a cyclic intermediate. For the cyclic intermediate formation, the electric interaction between amino and isothiuronium groups is considered. The bond formation between nitrogen of amino group and carbon of isothiuronium group may be concerted with the subsequent proton liberation from isothiuronium group and the bond cleavage between sulfur and carbon atoms of isothiuronium group. The proton releasing from isothiuronium group, which is evaluated from pK_2, may be a limiting factor for the reaction. The rate increase by N-methylation would be derived from the increased electric effect upon the cyclization. In the rate reduction by N-ethylation, the electric effect would be overcome by the steric effect. The steric effect exerted greatly in the stabilization of N,N′,N″-trimethyl-AET.

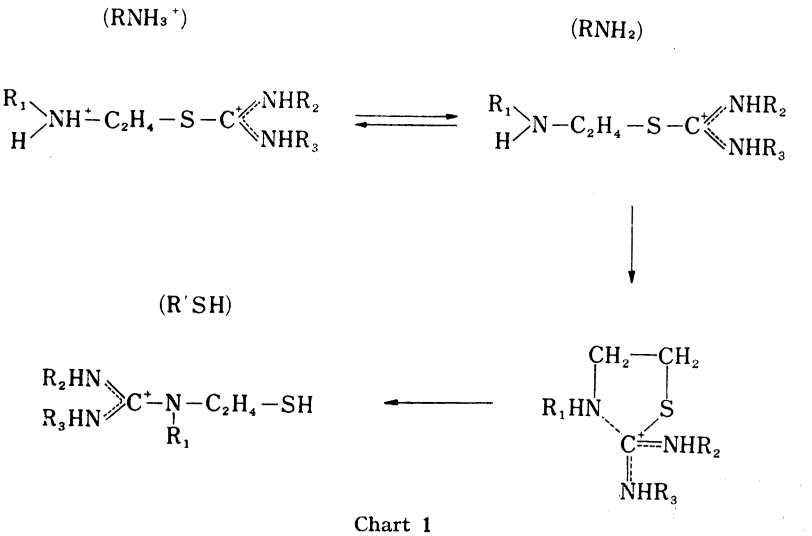


Chart 1

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