Experimental

Synthesis of Primary Amides——Primary amides were prepared from carboxylic acids by way of acid chloride followed by ammonolysis as usual. Acid chlorides were prepared by conventional method using thionyl chloride or phosphorus pentachloride. Since isonicotinic acid resisted to chlorination by heating with thionyl chloride even for 49 hr, adaptation of Bosshard's method was made, in which dimethylformamide was used as a catalyst.[10]

p- and *m*-Hydroxybenzamide were prepared by heating respective ethyl esters with conc. NH_3 in an autoclave.

General Procedure for the Preparation of Nitriles (*cf.* Table I)——A solution of amide (1 part) and PPE[9] (5 parts in wt.) in chloroform was refluxed until the substrate disappeared (usually within 2 hr). The solvent was evaporated *in vacuo* and the residues was treated with 30% Na_2CO_3 solution (10 parts) with stirring and cooling for 1 hr to decompose excess of PPE. The reaction mixture was extracted with ether and extract was washed with water, dried with Na_2SO_4 and evaporated *in vacuo* to leave crude product. To separate phosphorylated impurities the above residue was purified through a column chromatography (silica gel of 10—15 parts of the product in wt.—benzene). On evaporation of the solvent *in vacuo* nitrile was obtained in practically pure state. When a product is solid of relatively high mp, such as *p*-nitrobenzonitrile, one recrystallization of the precipitate from the reaction mixture may usually be enough to give nearly pure nitrile.

10) H.H. Bosshard, R. Mory, M. Schmid and H. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).

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Reactivities of Radiation-Protective Aminoalkylisothiuronium Salts. VI.[1] Estimation of the Stability of N-Substituted Derivatives of 2-Aminoethylisothiuronium Salt from the Potentiometric Titration Curve

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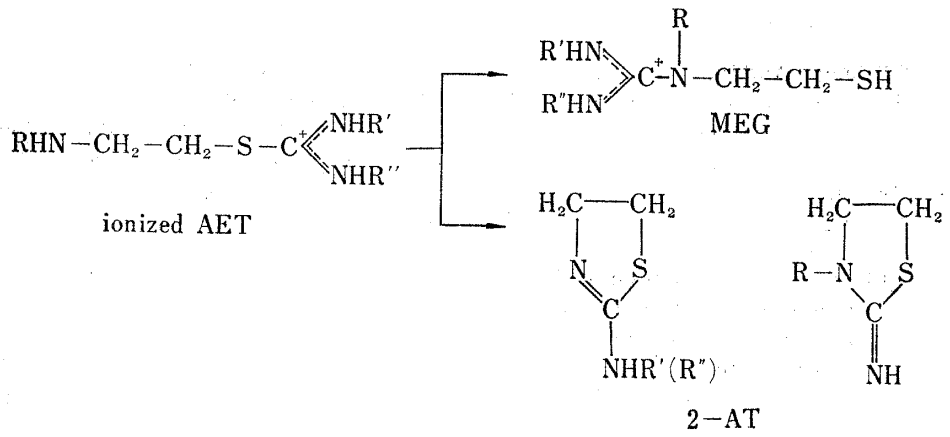
The potentiometric titration curve of 2-aminoethylisothiuronium (AET) salt, a prototype of the radiation-protective aminoalkylisothiuronium salt,[3] displays different patterns depending on the rate of alkali addition.[4] This phenomenon was explained as a result of the transformation reactions of this compound, which include both the transguanylation to yield 2-mercaptoethylguanidine (MEG) and the cyclization to 2-amino-2-thiazoline (2-AT).[5] Both the ionized AET, *i.e.*, the acid-dissociated form, and the cyclization product are able to associate with free hydrogen ion to form corresponding conjugate bases, while the transguanylation product has not an ability to associate with hydrogen ion. Therefore, when the AET solution which had been titrated with alkali was neutralized with equimolar hydrochloric acid to the alkali added, a portion of the acid corresponding to the amounts of the transguanylation product becomes surplus and was titrated as free acid.[5] The amount of free hydrogen ion thus determined is in parallel with the instability of the isothiuronium salt. The present paper concerned with the stabilities of N-substituted derivatives of AET estimated from the potentiometric titration curve.

1) Part V: A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1146 (1969).
2) Location: *Anagawa-4, Chiba.*
3) D. Doherty, W. Burnett and R. Shapiro, *Radiation Res.*, **7**, 13 (1957).
4) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 486 (1968).
5) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968).



Experimental

The potentiometric titration was done with a Radiometer TTTlc titrator and SBR2T titrigraph at 15°. An aqueous solution, 5.00×10^{-3}M, of the isothiuronium salt, synthesized in this laboratory,[6] was titrated with either 1.0 or 2.25 equivalents decarbonated NaOH at different speeds, which were controlled mechanically. When the titration had been finished, 0.1N HCl solution equimolar to the titrated NaOH was added into this solution, and then the solution was retitrated with NaOH. The detailed procedure was described previously.[5]

Results and Discussion

As reported previously, AET undergoes mainly the transguanylation and partly the cyclization during the titration, and sum of the extents of those reactions, though the ratio of the extents is varied depending on the titration speed, is equal approximately to one equivalent after the titration.[5] The rates of those reactions are expected to be affected by the N-substitution of the functional group, *i.e.*, amino group or isothiuronium moiety, of AET molecule. The titration and retitration curves of N-ethylated AETs were presented in Fig. 1 .In N-C_2H_5-AET, both curves were appeared to overlap in the region beyond 1.5 equivalent alkali. This compound might be transguanylated fairly rapidly, because the retitration curve involved approximately one equivalent free hydrogen ion indicating the quantitative transguanylation. Then, the retitration curve displays the ionization reaction of equimolar mixtures of strong acid and MEG derivatives. The overap of both curves would indicate the possibility of the same ionization reaction in this region: The ionized AET might be transguanylated rapidly to MEG, which is ionized in the second step, but not undergo the ionization of the isothiuronium moiety. In another N-ethylated derivative, substituted at isothiuronium moiety and abbreviated as N'-C_2H_5-AET, both titration curves did not overlap in any region and displayed different pattern, which indicated the possibility of different ionization reactions. The transguanylation of this compound might be slow and the ionized species might undergo further ionization at isothiuronium moiety to yield another conjugate base with its subsequent transguanylation. Then, the molecular species in the retitration mixtures may be free acid, MEG and AET unchanged during the titration, which was supported from the retitration curve. Thus, the N-substitution at isothiuronium moiety was appeared to hinder the transguanylation. In order to be valid those observations and to estimate the stability of N-substituted AETs, the titration was carried out in several derivatives and the concentration of free acid corresponding to that of the transguanylation product was determined. The results were summarized in Table I. It indicated expectedly that the reaction was retarded by the N-substitution at isothiuronium moiety and was decelerated by the bulky group. The amounts of the trans-

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

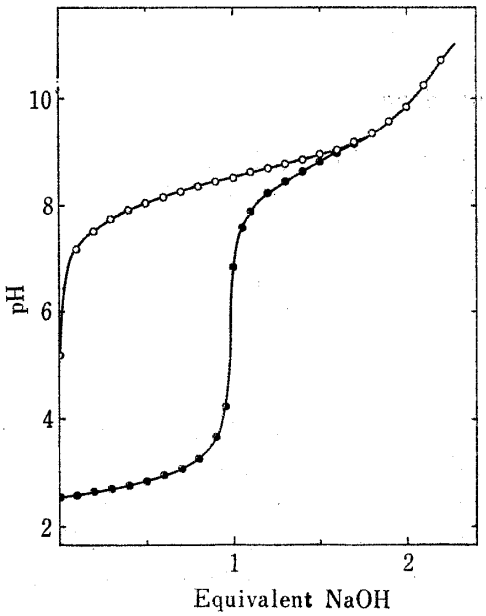


Fig. 1a. Potentiometric Titration Curve of N-C_2H_5-AET

concentration: 5.00×10^{-3}M
temperature: 15°,
ionic strength: 0.1 (NaCl)
—○—: titration curve
—●—: retitration curve
The solution was titrated with 2.25 equivalents NaOH.

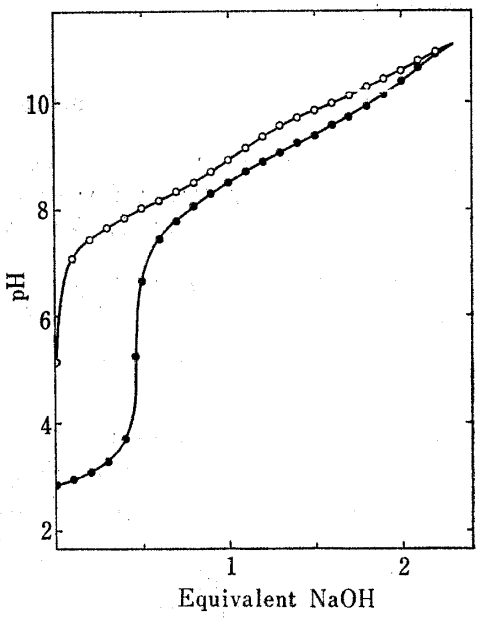
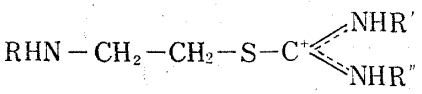


Fig. 1b. Potentiometric Titration Curve of N′-C_2H_5-AET

concentration: 5.00×10^{-3}M
temperature: 15°,
ionic strength: 0.1 (NaCl)
—○—: titration curve
—●—: retitration curve
The solution was titrated with 2.25 equivalents NaOH.

Table I. Transguranylation of N-Substituted AET with 1.0 Equivalent NaOH



R	R′	R″	Transguranylation %	Titration speed[a] sec/0.1 eq
H	H	H	72.5	16.2
			75.5	34.8
H	CH_3	H	14.5	15.4
			26.0	35.0
C_2H_5	H	H	57.0	15.0
			69.2	29.0
H	C_2H_5	H	10.0	16.8
			20.2	36.0
H	$-C_2H_4-$		44.5	16.8
			52.2	35.0
	$-C_3H_6-$		1.5	38.3
H	C_6H_5	H	13.5	21.0
			31.8	49.2

a) average titration speed from 0.2 to 1.0 equivalent NaOH

guanylation product increased with the decreasing rates of alkali addition. In any compound except AET, the extent of the cyclization was very small. A theoretical account on the titration curves of those aminoalkylisothiuronium salts will be presented in the next paper.

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