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163. Tohru Hino, Katsuko Tana-ami, Kazuko Yamada, and Sanya Akaboshi : Radiation-protective Agents. I. Studies on N-Alkylated-2-(2-aminoethyl)thiopseudoureas and 1,1-(Dithioethylene)diguanidines.

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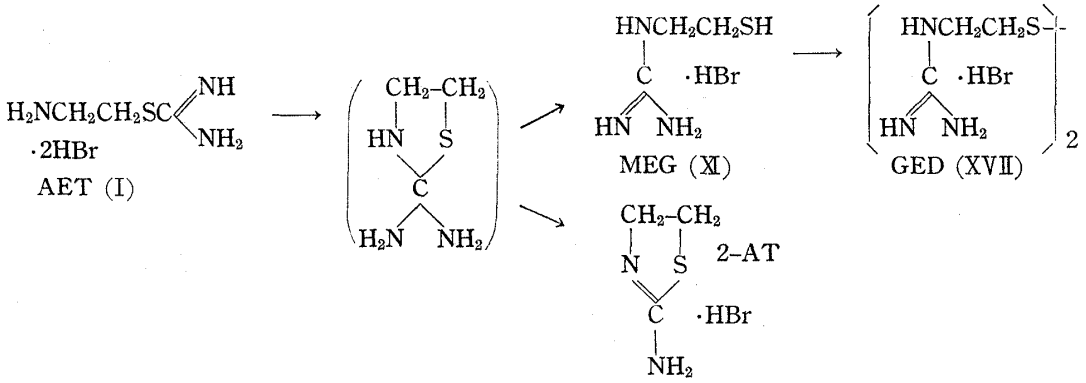
The chemical protection against radiation has been an important problem in radiation hazards and radiation biology, since Patt[1] discovered in 1949 that cysteine showed protective effect on animals against lethal dose of ionizing radiation when administrate before exposure.[2]

2-(2-Aminoethyl)thiopseudourea[3] (AET) is one of the most effective agents among a huge number of compounds[4] which were prepared and tested for radiation protective agents up to present time. Doherty[3b,5] reported that AET and its derivatives were transguanylated under neutral condition through the intramolecular rearrangement *via* cyclic intermediates to mercaptoethylguanidine (MEG) and its derivatives, and that the active form of AET *in vivo* was not AET itself but MEG. MEG is an unstable compound in air and easily oxidized to the disulfide (GED) when its aqueous solution



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1) H. M. Patt, E. B. Tyree, R. L. Strauve, D. E. Smith : Science, **110**, 213 (1949).

2) J. F. Thomas : "Radiation Protection in Mannals," 1962, Chapman and Hall; A. Hollander : "Radiation Protection and Recovery," 1960, Pergamon Press; S. Akaboshi : Yakugaku Zasshi, **83**, 1005 (1963).

3) *a*) D. G. Doherty, W. T. Burnett : Proc. Soc. Exptl. Biol. Med., **89**, 312 (1955).
b) R. Shapira, D. G. Doherty, W. T. Burnett : Radiation Research, **7**, 22 (1957).
c) D. G. Doherty, R. Shapira, W. T. Burnett : J. Am. Chem. Soc., **79**, 5667 (1957).

4) Bioassay data are tarbulated in R. Huber, E. Spode "Biologisch-Chemischer Strahlenschutz," Ein Übersicht in Tabellen I, II, III und IV 1961, 1964, Akademie-Verlag.

5) J. X. Kym, R. Shapira, D. G. Doherty : J. Am. Chem. Soc., **79**, 5663 (1957); **80**, 3342 (1958).

TABLE I. $R_1NCH_2-CH_2-S-C(=NR_3)(NHR_4)\cdot 2HX$ (R_2 on N)

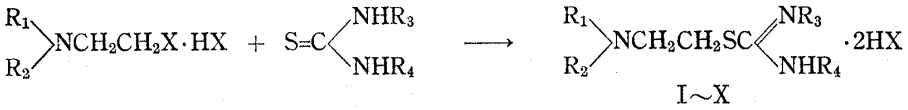
No.	R_1	R_2	R_3	R_4	X	m.p. (°C)	Recrystallizing solvent	Reported m.p. (°C)	Analytical data: Calcd. C	H	N	S	X	Found C	H	N	S	X
I	H	H	H	H	Br	191～192		193～194,[a,e] 194～195[b]	—	—	—	—	—	—	—	—	—	—
	〃	〃	〃	〃	P[i]	216～219	EtOH	235～237,[d] 231[c]	31.21	2.62	21.83	5.55		31.70	2.55	22.02	5.53	
II	CH_3	H	H	H	Br	179.5～180	〃	172～176[f]	16.28	4.44	14.24	10.87	54.17	16.40	4.15	14.49	10.28	54.60
	〃	〃	〃	〃	P[i]	188～189	MeOH	—	32.49	2.90	21.32	5.42		33.08	2.98	21.10	5.27	
III	CH_3	CH_3	H	H	Cl	181～182	EtOH	181～182,[g] 182～183,[b] 181～183[a]										
IV	C_6H_5	H	H	H	Br	204～206	〃	215～216[f]	30.27	4.23	11.77	8.98		29.85	3.88	11.34	9.03	
	〃	〃	〃	〃	P[i]	129～131	〃	163～164[f]	38.60	2.93	19.29	4.91		38.84	2.67	19.24	4.75	
V	H	H	CH_3	H	Br	212～213	MeOH	214～215[a]										
	〃	〃	〃	〃	P[i]	170～171	EtOH	—	32.49	2.90	21.32	5.42		32.69	2.89	21.66	5.42	
VI	H	H	C_6H_5	H	Br	217～218	MeOH-AcOEt	221～222[a]	30.27	4.23	11.77	8.98		30.45	3.98	11.62	9.06	
	〃	〃	〃	〃	P[i]	162～163	MeOH	—	38.60	2.93	19.29	4.91		38.85	2.59	19.28	5.23	
VII	H	H	$-CH_2-CH_2-$		Br	188～190	EtOH	121～125[a]	19.56	4.27	13.68	10.44		19.39	4.10	13.80	10.88	
	〃	〃	〃		P[i]	182～184	MeOH	—	33.84	2.84	20.89	5.31		34.02	2.40	20.58	5.11	
VIII	CH_3	CH_3	$-CH_2-CH_2-$		Cl	185～188	EtOH	183.5～184.5[h]	34.15	6.96	17.07	13.02	28.80	33.64	6.70	17.02	12.91	28.10
	〃	〃	〃		P[i]	177～178	H_2O	—	36.19	3.35	19.96	5.08		36.38	3.26	19.61	5.22	
IX	H	H	$-CH_2CH_2CH_2-$		Br	218～220.5	MeOH	—	22.44	4.71	13.09	9.99	49.77	22.93	4.68	13.03	9.85	50.27
	〃	〃	〃		P[i]	176～177	〃	—	35.01	3.10	20.42	5.19		35.31	3.06	20.19	5.34	
X	CH_3	CH_3	$-CH_2CH_2CH_2-$		Cl	190～192	iso-PrOH	—	36.92	7.36	16.15	12.32	27.25	36.61	7.01	15.56	12.23	27.29
	〃	〃	〃		P[i]	158～159	H_2O	—	37.21	3.59	19.53	4.97		37.18	3.32	19.78	4.75	

Remarks *a*) D.G. Doherty, *et al.*: J. Am. Chem. Soc., **79**, 5667 (1957).
b) R.O. Clinton, *et al.*: *Ibid.*, **70**, 950 (1948).
c) A. Schoberl, G. Hansen: Chem. Ber., **91**, 1239 (1958).
d) E.D. Bergmann, A. Kaluszyner: Rec. trav. chim., **78**, 289 (1958).
e) R. Shapira, D.G. Doherty, W.T. Burnett: Radiation Research, **7**, 22 (1957).
f) E.D. Bergmann, A. Kaluszyner: Rec. trav. chim., **78**, 309 (1958).
g) R.R. Renshaq, P.F. Dreisbach, M. Ziff, D. Green: J. Am. Chem. Soc., **60**, 1765 (1938).
h) S.O. Winthrop, G.A. Grant: Can. J. Chem., **35**, 281 (1957).
i) Picrate,

is kept at room temperature. Although the GED has been reported to be radiation-protective[3b,6] the biological assays were carried out on the solution obtained by autoxidation of an aqueous solution of MEG without isolation of the disulfide as crystalline state. Thus our current efforts were directed toward the syntheses of crystalline GED derivatives which could be submitted to biological assay, starting from N-alkylated AET derivatives, in order to test their radiation-protective activity.

The AET derivatives (I~X) used in this study were prepared in good yields from hydrohalides of N-alkylated aminoethyl halides and N-alkylated thioureas in alcohols as shown in Chart 1. The procedure was essentially the same as that of Doherty,[3] but *n*-butanol was found to be better solvent than isopropyl alcohol and ethanol in some cases (See Table Ⅵ). These AETs obtained were stable crystals in dry condition and afforded the crystalline dipicrates. The analytical data and physical constants of these AET derivatives are summarized in Table I.

The infrared (IR) spectra of these AETs in the solid state showed one or two strong absorption bands between 1600 and 1660 cm^{-1},*2 and an absorption band in the



I~X

	R_1	R_2	R_3	R_4	X
I	H	H	H	H	Br
Ⅱ	CH_3	H	H	H	Br
Ⅲ	CH_3	CH_3	H	H	Cl
Ⅳ	C_6H_5	H	H	H	Br
Ⅴ	H	H	CH_3	H	Br

	R_1	R_2	R_3	R_4	X
Ⅵ	H	H	C_6H_5	H	Br
Ⅶ	H	H	CH_2CH_2		Br
Ⅷ	CH_3	CH_3	CH_2CH_2		Cl
Ⅸ	H	H	$CH_2CH_2CH_2$		Br
Ⅹ	CH_3	CH_3	$CH_2CH_2CH_2$		Cl

Chart 1.

Table Ⅱ. Infrared and Nuclear Magnetic Resonance Data of AET Derivatives

No.	IR (KBr disk) >3100 cm^{-1}	IR (KBr disk) 1700~1500 cm^{-1}	NMR p.p.m. from DSS in D_2O
I	3330 s, 3130 s	1650 s, 1632 s, 1592 w, 1533 w	3.44[a]
Ⅱ	3345 s, 3200 s	1650 s, 1632 s, 1575 w, 1540 w	3.47,[a] 2.79(s, NCH_3)
Ⅲ	3350 s, 3160 s	1640 m, 1630 m	3.56(s), 2.97(s, NCH_3)
Ⅳ	3290 s, 3240 s, 3160 s	1660 s, 1632 s, 1605 w, 1560 m	3.52(t), 3.88(t), 7.45~7.62(phenyl)
Ⅴ	3280 s	1640 s, 1610 s, 1585 m	3.41,[a] 3.01(s, NCH_3)
Ⅵ	3270 s, 3115 s	1645 s, 1595 m, 1555 m	3.52,[a] 7.5~7.6(phenyl)
Ⅶ	3400 s, 3200 s	1610 m, 1555 sh, 1545 s, 1505 m	3.49,[a] 4.01(s, NCH_2CH_2N)
Ⅷ	3400 m, 3180 s	1565 s, 1545 s	3.57(s), 2.97(s, NCH_3), 3.98(s, NCH_2CH_2N)
Ⅸ	3250 m, 3180 m	1630 s, 1578 s	3.42,[a] 2.09(q, CCH_2C), 3.53(t, NCH_2CCH_2N)
Ⅹ	3325 m, 3180 m	1615 s, 1570 s	3.49(s), 2.00(q, CCH_2C), 2.93(s, NCH_3), 3.49(s, NCH_3), 3.49(t, $NCCH_2CN$)
A	3310 s	1640 s, 1545 m	
B	3440 s, 3380 s, 3290 s, 3230 s	1668 s, 1628 s, 1595 m, 1538 m	

Remarks: s, strong or singlet; m, medium; w, weak; t, triplet,; q, quintet
a) center of A_2B_2 spectrum,
A, Benzylisothiuronium chloride, B, Methylisothiuronium sulfate

*2 In 2-(2-aminoethyl)-imidazoline (Ⅶ) and its dimethyl derivative (Ⅷ), the strong absorption bands moved to 1565~1545 cm^{-1}. This shift would be due to the presence of a five membered ring in Ⅶ and Ⅷ, since 2-methylthio-2-imidazoline hydroiodide showed a strong absorption band at 1560 cm^{-1}.

6) E. S. Schwarz, B. Shapiro: Radiation Research, **13**, 768 (1960).

region over 3200 cm^{-1} which corresponded to N-H stretching vibration rather than N^+-H stretching (Table Ⅱ). These bands were considered to be characteristic to the thiuronium group*[3,7] in AETs, since these absorption bands were also observed in benzylthiuronium chloride and methylthiuronium sulfate. These absorption bands are analogous to those of the guanidinium group[8] and the isothiuronium salts could be written as $-S-C^+\begin{matrix}NH_2\\NH_2\end{matrix}$

The nuclear magnetic resonance (NMR) spectra of AET derivatives in heavy water are summarized in Table Ⅱ. Two methylenes between the nitrogen and the sulfur atoms of AET (I) gave A_2B_2 spectrum having a center at 3.44 p.p.m., indicating the chemical shift of both methylenes were close each other. As expected, AET derivatives (V, Ⅵ, Ⅶ and Ⅸ) having substituents at the nitrogen atoms of thiourea showed the similar A_2B_2 spectra to that of the parent AET, though signals of other methylenes were overlapped in the compound (Ⅸ). On the contrary the substitution at the amino nitrogen might affect the A_2B_2 spectrum: Methyl substitution at the amino nitrogen (Ⅱ) modified the spectrum slightly, but retained the A_2B_2 spectrum. Phenyl substitution at the amino nitrogen (Ⅳ), however, shifted the signals of the methylene adjacent to the nitrogen atom to lower field to make two separate triplets for two methylenes. In the case of dimethyl substitution at the amino nitrogen (Ⅲ, Ⅷ and X) the spectra for two methylenes changed completely and appeared as a singlet, indicating the chemical shifts of both methylene groups between the dimethylamino and the thiuronium groups were essentially the same.

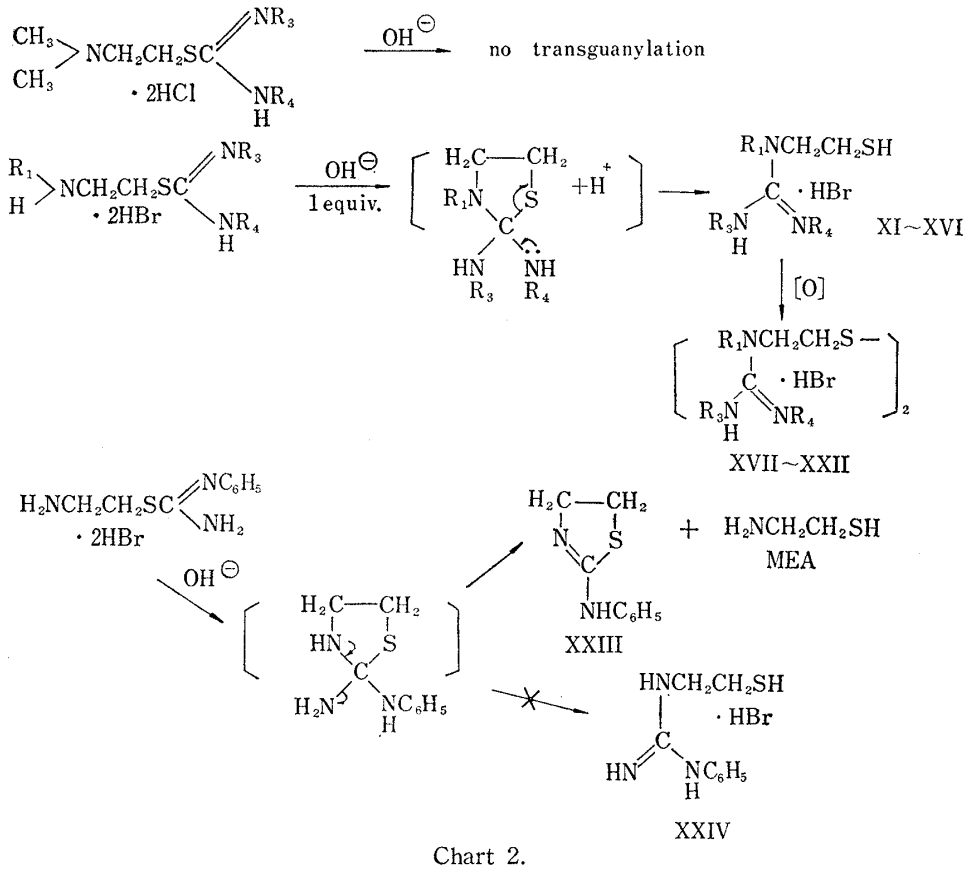


Chart 2.

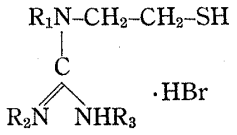
*3 Authors are grateful to Drs. H. Nishimura and M. Hashimoto for their helpful discussion on the IR spectra of the isothiuronium salts. Cf. ref. 7).

7) H. Nishimura: Yakugaku Zasshi, **84**, 930 (1964); C.G. Overberger, H.A. Friedman: J. Org. Chem., **30**, 1926 (1965).

8) T. Goto, K. Nakanishi, M. Ohashi: Bull. Chem. Soc. Japan, **30**, 723 (1957).

The AET derivatives prepared were submitted to transguanylate to MEG derivatives with one equivalent of sodium hydroxide. The reaction of these AETs with sodium hydroxide could be classified into three types as shown in Chart 2. The AETs (Ⅲ, Ⅷ and X) which possessed two methyl group at the amino nitrogen were stable toward one equivalent of sodium hydroxide, and did not transguanylate, since the formation of cyclic intermediates was not possible. The compounds (Ⅱ, Ⅳ, Ⅴ, Ⅶ, and Ⅸ) having one or two hydrogen atom on the amino nitrogen, were transguanylated as expected by this condition as the parent compound (I). However, in the case of Ⅵ, 2-aminothiazoline derivative (XXⅢ) was obtained by treating with one equivalent of sodium hydroxide and none of transguanylated product (XXIV) was obtained. With two or three equivalents of sodium hydroxide, Ⅵ gave a mixture of XXⅢ, mercaptoethylamine (MEA), and cystamine, and no MEG derivative (XXIV) was isolated. It is considered that Ⅵ could form the cyclic intermediate, but C-N bond instead of C-S bond might be cleaved to form XXⅢ even in an alkaline condition by the effect of phenyl group. All MEGs thus obtained were a viscous oil or an amorphous powder

TABLE Ⅲ.



No.	R_1	R_2	R_3	Appearance of crude product	Picrate m.p. (°C)	Analytical data							
						Calcd.				Found			
						C	H	N	S	C	H	N	S
XI	H	H	H	viscous oil	172[a,b]	36.02	3.49	16.16		36.39	3.20	15.93	
XII	CH_3	〃	〃		180~180.5[c]	33.15	3.89	23.20	8.85	33.45	3.56	23.17	8.66
XIII	C_6H_5	〃	〃	m.p. 88~90°	200~210[b]	42.45	3.80	19.80	7.56	42.52	3.65	20.09	7.52
XIV	H	CH_3	〃	viscous oil	143~144[c]	33.15	3.89	23.20	8.85	33.00	3.51	23.16	8.53
XV	〃	$-CH_2CH_2-$		〃	182~184[d]	35.29	3.77	22.45	8.57	35.38	3.54	22.72	8.26
XVI	〃	$-CH_2CH_2CH_2$		m.p. 60~64°	170~171[b]	37.11	4.15	21.64	8.26	37.14	3.93	21.45	8.16

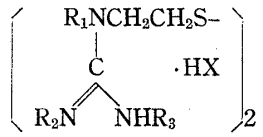
a) Flavianate, reported m.p. 170~173° (J.X. Khym, *et al.*: J. Am. Chem. Soc., **79**, 5663 (1957)).
b) Recrystallized from EtOH. *c*) Recrystallized from H_2O. *d*) Recrystallized from MeOH.

and could not be isolated as pure forms due to the air oxidation to the disulfides during the purification. The properties of the MEGs are summarized in Table Ⅲ. The NMR spectra of the crude MEGs in heavy water showed two triplets due to a large difference of the chemical shifts of two methylenes adjacent to the thiol and guanidinium groups, and were clearly distinguished from those of the corresponding AETs.

To obtain GEDs (XVⅢ~XXⅡ) the MEGs which were prepared from AETs as described above were directly oxidized by passing air into their slightly alkaline aqueous solutions. The air oxidation of XIV was so slow that XIV was still observed after 10 days' oxidation. Therefore, hydrogen peroxide was successfully used as an oxidizing agent for XIV. The hydrobromides or hydroiodides of GEDs were obtained in fair yields as stable crystals as shown in Table Ⅳ and could be submitted to bioassays. The IR spectra of GEDs were similar to those of MEGs, except the disappearance of the band due to SH stretching vibration, in the region between 4000~400 cm^{-1}.[*4] The NMR spectra, however, could distinguish GEDs from the corresponding MEGs by a lower shift of both triplets.

[*4] The stretching vibration of S-S bond was difficult to be found out in the region of 400~500 cm^{-1}, even when the spectra of GEDs were compared with those of the corresponding MEGs in this region.

TABLE IV.



No.	R_1	R_2	R_3	X	m.p. (°C)	Analytical									
						Calcd.					Found				
						C	H	N	S	Br	C	H	N	S	Br
XVII	H	H	H	Br	196.5 ~197[a,h]	18.10	4.56	21.11			18.33	4.52	21.58		
	〃	〃	〃	P[i]	239 ~240.5[b]										
XVIII	CH_3	〃	〃	Br	218[c]	22.54	5.20	19.72	15.04	37.49	23.35	4.44	19.64	15.16	37.14
	〃	〃	〃	P[i]	250 ~252[a]	33.24	3.63	23.26	8.87		33.53	3.41	23.19	9.06	
XIX	C_6H_5	〃	〃	Br	222~ 223[d]	39.28	4.76	15.27	11.65		39.55	4.42	15.44	11.43	
	〃	〃	〃	P[i]	284[e]	42.55	3.57	19.85	7.57		43.03	3.70	19.39	7.29	
XX	H	CH_3	〃	I	123[f]	18.47	4.26	16.15			18.54	4.04	15.97		
	〃	〃	〃	P[i]	178~180	33.24	3.63	23.26	8.87		33.42	3.37	23.03	8.75	
XXI	〃	CH_2-CH_2		Br	216~ 217[c]	26.67	4.92	18.66	14.24	35.49	26.90	4.70	19.01	14.02	34.73
	〃	〃	〃	P[i]	243~245[e]			22.51	8.59				22.67	8.62	
XXII	〃	$CH_2CH_2CH_2$		Br	155~ 156[g]	30.13	5.48	17.53	13.41	33.41	30.45	5.02	17.38	13.32	33.51
	〃	〃	〃	P[i]	187~ 189[e]	37.21	3.90	21.70	8.28		37.34	4.13	21.28	8.28	

a) Recrystallized from H_2O.
b) Reported m.p. 234~237°(A. Kaluszyner: Bull. Research Council Israel, **9A**, 35 (1960)).
c) Recrystallized from EtOH.
d) Recrystallized from EtOH-iso-Pr_2O.
e) Recrystallized from AcOH.
f) Recrystallized from iso-PrOH-benzene.
g) Recrystallized from iso-PrOH.
h) Reported m.p. 191~192° (E.D. Bergmann, A. Kaluszyner: Rec. trav. chem., **78**, 289 (1958)).
i) Picrate ion.

TABLE V. Infrared and Nuclear Magnetic Resonance Data of GED Derivatives (HBr salts)

No.	IR (KBr disk)		NMR (in D_2O solution) (p.p.m. from DSS as an internal standard)		
	3200 cm^{-1} (strong peaks)	1700~1570 cm^{-1}	SCH_2CN (triplet)	NCH_2CS (triplet)	Others
XVII	3430, 3300, 3245	1662, 1645, 1615	2.97	3.62	
XVIII	3340, 3170 b	1645, 1630	3.01	3.70	3.04(s, NCH_3)
XIX	3340, 3240	1658, 1630, 1603, 1572	2.87	3.93	7.3~7.6(phenyl)
XX[a]	3350, 3225	1635, 1592	2.92	3.54	2.81(s, NCH_3)
XXI	3260, b	1670, 1600	2.92	3.58	3.71(s, NCH_2CH_2N)
XXII	3430, 3280	1640	2.89	3.49	1.96(q, CCH_2C), 3.33(t, NCH_2CCH_2N)

Remarks: s, singlet; t, triplet; q, quintet; b, broad.
a) hydroiodide.

The AET (I) in aqueous solution are unstable and known to convert to 2-amino-2-thiazoline.[3c,5] This transformation was also observed during the purification or the thin-layer chromatography of AET (I). This transformation of AET derivatives will be described in the following paper.

The biological assays of the compounds described in this paper were carried out by Drs. Tamaoki and Shinoda in our institute and the details will be published elsewhere.

Experimental*5

General Procedure for the Preparation of AETs (I~X)——Equimolecular amount of substituted aminoethyl halide hydrohalide and substituted thiourea were dissolved in an appropriate solvent and the mixture was heated to reflux. At the beginning of the refluxing the mixture was a clear solution which soon became turbid and began to separate crystals. After the completion of the reaction the crystals were filtered and purified from an appropriate solvent (cf. Table I). The reaction condition and yields are

Table VI. Reaction Conditions for the Preparation of AET Derivatives

Compound No.	Solvent	Period of reflux (min.)	Yield (%)
I	iso-PrOH	30	80
II	EtOH	25	98
III	〃	24 (hr.)	99
IV	〃	40	78
V	〃	15	82
VI	〃	30	64
VII	iso-PrOH	150	90
VIII	BuOH[a)]	8 (hr.)	55
IX	EtOH	120	97
X	BuOH[b)]	5 (hr.)	75

a) No precipitate was observed during the refluxing, but crystals were separated on cooling the reaction mixture. In iso-PrOH no crystal was obtained even on cooling the mixture, but a small amount of the crystals was obtained from a residue on evaporation of the solvent.
b) Only a small amount of the product was obtained by filtration of the cooled reaction mixture. The product was mainly isolated from the residue obtained by evaporation of the solvent. In EtOH starting materials were still observed in the reaction mixture even after refluxing for 28 hr.

shown in the Table VI. The starting materials which were not commercially available were prepared by the known method as follows. N-Methylthiourea[9] was prepared from methylisothiocyanate, N-bromoethylaniline[10] from N-phenylethanolamine, N-bromoethylamine[11] from N-methylethanolamine, and hexahydropyrimidine-2-thione[12] from trimethylenediamine.

Purification of AET——AET (I) could be recrystallized once from EtOH or MeOH, but m.p. (191~192°) did not change. On repeated recrystallization from MeOH, however, the melting point of I was lowered, and it was observed by IR spectrum that the sample obtained was contaminated with 2-aminothiazoline.*6 AET (I) gave two spots by TLC using a solvent system of MeOH-AcOH-H_2O (1:1:1) on an usual silica gel plate, but I gave only one spot on the TLC plate which was prepared with silica gel and *N*/10 HCl instead of distilled water. One of the two spots was verified to be 2-aminothiazoline which was produced by decomposition of I during the ascending on the plate, and its formation was inhibited by the plate containing HCl. Other AETs were fairly stable during the purification.

Picrates of AETs——i) AET (I). AET (I) was dissolved in *N* HCl and a saturated aqueous solution of picric acid was added to the solution to afford a crude picrate, m.p. 207~209°, which gave yellow crystals, m.p. 216~219°, on one recrystallization from ethanol. Its IR spectrum was similar to that of the crude picrate and different from that of 2-aminothiazoline.*6 The crude picrate, however, gave yellow crystals, m.p. 241~242°, on repeated recrystallization from EtOH. The mixed melting point test and IR spectra proved this picrate to be identical with that of 2-aminothiazoline.*6 The reported picrates of AET, m.p. 235~237°[13] and 231°,[14] would have been the picrate of 2-aminothiazoline or the mixture of the both picrates.

*5 All melting points are uncorrected. Infrared spectra were measured with a JASCO-DS-301 spectrophotometer. NMR spectra were measured with a Varian HR-100 spectrometer, and the chemical shift were expressed by δ-value from DSS (Sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as an internal standard.
*6 cf. Part II (the following paper).

9) M. L. Moore, F. S. Crossley: Org. Syntheses, Col. Vol. III, 617.
10) W. M. Pearlman: J. Am. Chem. Soc., **70**, 871 (1948).
11) E. D. Bergmann, A. Kaluszyner: Rec. trav. chim., **78**, 309 (1958).
12) C. F. H. Allen, C. O. Edens, J. VanAllan: Org. Syntheses, Col. Vol. III, 394.

ii) Picrate of Ⅶ. A picrate, m.p. 181~184°, obtained by the first recrystallization from MeOH gave the original HBr salt (Ⅶ) when it was treated with HBr. On the other hand it was found by IR spectrum that a picrate, m.p. 198~201°, obtained by the first recrystallization from AcOH gave 2-aminoethylamino-thiazoline 2HBr*[6] when the picrate was treated with HBr. Other picrates of AETs were prepared and purified by the usual manner (cf. Table Ⅰ).

1-(2-Mercaptoethyl)guanidine (XI)——AET (Ⅰ, 282 mg.) was dissolved in *N*/10 NaOH (11 ml., one equivalent), and the solution was evaporated *in vacuo* after standing for several minutes. The viscous residue was extracted with hot iso-PrOH to remove inorganic salts, and the iso-PrOH solution was evaporated *in vacuo* to afford a crude MEG HBr as a viscous oil which could not be induced to crystallize. IR ν cm^{-1}; 2550 (ν_{SH}), 3200, 1650 (guanidinium). NMR (p.p.m. from DSS in D_2O solution) 2.72 (t, CH_2S), 3.38 (t, $-CH_2N-$) (The presence of trace of GED was also observed).

1-Phenyl-1-(2-mercaptoethyl)guanidine (XIII)——1-Phenyl AET (Ⅳ, 9.23 g.) was dissolved in *N*NaOH (29 ml., one equivalent) and H_2O (10 ml.), and precipitates began to separate from the cloudy solution. The mixture was filtered to give a crude XIII HBr (2.23 g.) as colorless crystals, m.p. 88~90°. It could be recrystallized from iso-PrOH, but gradually changed to the corresponding disulfide during recrystallizations. NMR (p.p.m. from DSS in D_2O) : 2.72 (t, $-CH_2S-$), 3.90 (t, $-CH_2N-$), 7.38~7.63 (m, phenyl). Picrate : cf. Table Ⅲ. The picrate also changed to that of the disulfide during recrystallizations.

2-(2-Mercaptoethylamino)tetrahydropyrimidine (XVI)——To an aqueous solution of the AET derivative (Ⅸ, 3.21 g. in H_2O 40 ml.) was added *N*NaOH (10 ml). After being kept the solution at room temperature for 3 hr. the mixture was evaporated *in vacuo* and the residue was extracted with hot iso-PrOH. A crude XVI HBr (2.46 g.) was obtained as a white solid, m.p. 60~64°, on evaporation of the iso-PrOH. Repeated recrystallizations from EtOH-ether gave only the corresponding disulfide. NMR (p.p.m. from DSS in D_2O) 2.73 (t, CH_2S-), 3.37 (t, $-CH_2N-$) 1.96 (quintet, $C-CH_2-C-$), 3.37 (t, $N-CH_2-C-CH_2-N$).

2-(2-Mercaptoethylamino)imidazoline (XV)——To *N*/10 NaOH (20 ml.) was added Ⅶ (0.6 g.) in H_2O and the mixture was evaporated *in vacuo* after kept at room temperature for one hr. and the residue was extracted with hot iso-PrOH. On evaporation of iso-PrOH a crude XV HBr (0.39 g.) was obtained as a viscous oil which could not be crystallized. NMR (p.p.m. from DSS in D_2O) 2.72 (t, CH_2S-), 3.40 (t, CH_2N-), 3.71 (s, $N-CH_2-CH_2-N$).

1'-Methyl-1-(2-mercaptoethyl)guanidine (XIV)——A crude XIV HBr (0.875 g.) was obtained from Ⅴ (1.77 g.) as a viscous oil in the similar way as in the case of XV. NMR (p.p.m. from DSS in D_2O) 2.71 (t, CH_2S-), 3.39 (t, CH_2N-), 2.82 (s, $N-CH_3$).

Formation of 2-Anilino-2-thiazoline (XXIII) from VI with Alkali——1'-Phenyl-AET (Ⅵ, 35 mg.) was dissolved in a NaOH solution (*N*/10 NaOH 1 ml. (one equivalent) and H_2O 2 ml.) at room temperature. Thin-layer chromatography (silica gel plate MeOH-AcOH (1:1)) of this solution showed the presence of 2-anilinothiazoline (XXIII)*[6] and Ⅵ. When the solution was kept at room temperature overnight, a small amount of precipitate was observed. The precipitates were collected, washed with H_2O, and dried to give XXIII, m.p. 158~160°. Its IR spectrum was identical with that of the authentic sample of XXIII.*[6] When two or three moles of NaOH were added to Ⅵ, cystamine and cysteamine were detected besides XXIII on silica gel TLC.

1,1'-(Dithiodiethylene)diguanidine (XVII)——To *N* NaOH (39.5 ml., one equivalent) solution was added I (10.54 g.) and the pH of the solution was adjusted to 8.0 by the addition of *N*NaOH (3 ml.). The clear solution was bubbled with air for 41 hours, and during the course of the bubbling a crude. XVII 2HBr precipitated. The crude XVII 2HBr (5.65 g., 75.6%), m.p. 195~196°, was collected by filtration and recrystallized from H_2O to afford XVII 2HBr, m.p. 196.5~197°. UV $\lambda_{max}^{H_2O}$ mμ (ε) : 245 (360).

1,1'-Dimethyl-1,1'-(dithiodiethylene)diguanidine (XVIII)——1-Methyl AET (Ⅱ, 1.97 g.) was dissolved in *N*NaOH (7 ml., one equivalent) and the solution was adjusted to pH 8.3 by the addition of *N* NaOH (2.5 ml.). The solution was oxidized (15 days) as in the case of XVII, and a crude XVIII 2HBr was obtained by the filtration as a white solid (0.94 g., 66%), m.p. 216~218° (cf. Table Ⅳ).

2,2'-Dimethyl-1,1'-(dithiodiethylene)diguanidine (XX)——To *N* NaOH (20 ml.) solution was added Ⅴ (5.21 g.) and a trace of insoluble material was removed by filtration. To the filtrate was added dropwise 3% H_2O_2 (15 ml.) at room temperature. After the completion of the addition the mixture was acidified to pH 1 with conc. HBr (2 ml.) and was evaporated *in vacuo*. The residue was extracted with hot iso-PrOH, and the iso-PrOH solution was evaporated *in vacuo* to leave a crude XX 2HBr (4.2 g., 55.8%) as a viscous oil which failed to crystallize. The crude HBr salt was dissolved in H_2O (5 ml.), and treated with a saturated aq. solution of NaI to precipitate the HI salt. The crude HI salt (4.3 g.) was collected and recrystallized repeatedly from iso-PrOH-benzene to afford XX 2HI, m.p. 123°. When Ⅴ was treated similarly to the preparation of XVII, no precipitate was observed during air oxidation and the presence of SH group was observed by Na-nitroprusside even afer 10 days oxidation.

13) E. D. Bergmann, A. Kaluszyner : Rec. trav. chim., **78**, 289 (1958).
14) A. Schoberl, G. Hansen : Chem. Ber., **91**, 1239 (1958).

1,1′-Phenyl-1,1′-(dithiodiethylene)diguanidine (XIX)——To *N* NaOH (32.5 ml.) was added Ⅳ (12.2 g.) and the pH of the solution was adjusted to 8.0 by the addition of *N* NaOH (1 ml.), and H_2O (130 ml.) was added to the solution to dissolve the separated solid. The clear solution was oxidized with air until SH test by Na-nitroprusside had become negative (24 hr.). The reaction mixture was evaporated *in vacuo* to leave a colorless oily residue which was extracted with iso-PrOH. Removal of the iso-PrOH left a crude XIX 2HBr (4.6 g., 48.9 %) as colorless crystals, m.p. 168～169°, which was recrystallized from iso-PrOH-iso-Pr_2O to afford XIX 2HBr, m.p. 220～225°.

In the similar way a crude XXI 2HBr (6.6 g., 89%, after one recrystallization) was obtained from Ⅶ (10.0 g.) by the air oxidation for 48 hr. And a crude XXII (6.4 g., 54.7%) was obtained from Ⅸ (14.5 g.) by the air oxidation for 58 hr.

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Summary

Ten kinds of N-substituted-2-(2-aminoethyl)thiopseudoureas (AETs) were prepared. 1′-Phenyl-AET (Ⅵ) gave 2-aminothiazoline derivative (XXIII) with one equivalent of alkali, while other AETs having at least one hydrogen atom at the amino nitrogen underwent intramolecular rearrangement to give MEGs. GEDs were prepared from these MEGs by mild oxidation.

The NMR and IR spectra of these compounds were also discussed.

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164. Tohru Hino, Katsuko Tana-ami, Kazuko Yamada, and Sanya Akaboshi : Radiation-protective Agents. Ⅱ.*1 The Transformation of 2-(2-Aminoethyl)thiopseudoureas to 2-Amino-2-thiazolines.

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In the preceding paper the authors described the synthesis of 2-(2-aminoethyl)-thiopseudoureas dihydrobromides (AETs) and their transguanylation reaction with a base.*1 AET was known[1] to be converted to 2-aminothiazoline hydrobromide (2-AT) in an aqueous solution *via* the same cyclic intermediate as that supposed in the case of the transguanylation.

The present paper describes the transformation of AETs described in the previous paper to 2-ATs, and discussed the effect of the substituents of AET on this transformation. 2-Aminothiazoline was obtained in good yield when AET was refluxed in an aqueous solution or a buffered solution (pH 4.5). The same transformation was also observed when its aqueous solution was kept at room temperature. The formation of 2-AT was observed by the thin-layer chromatography (TLC) after 12 hr. at room

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1) D.G. Doherty, R. Shapira, W.T. Burnett : J. Am. Chem. Soc., **79**, 5667 (1957).