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Radiation Protective Agents. III.[1] N-Methylated Derivatives of S,2-Aminoethylisothiuronium Salt

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S,2-Aminoethylisothiuronium (AET) salt, a radiation-protective agent against a lethal dose of ionizing radiation, is transguanylated very rapidly in the physiological condition to 2-mercaptoethylguanidine (MEG),[4] which has been considered as an active form of this compound.[5] In weakly acidic medium, however, this compound is transformed to 2-amino-2-thiazoline (2-AT), a less effective protective agent.[5] The reactivities of AET against those transformation reactions are expected to be modified by the substituent at amino or thiourea nitrogen.[6] In this paper, we described the spectroscopic characteristics and the reactivities of several N-methylated derivatives of AET.

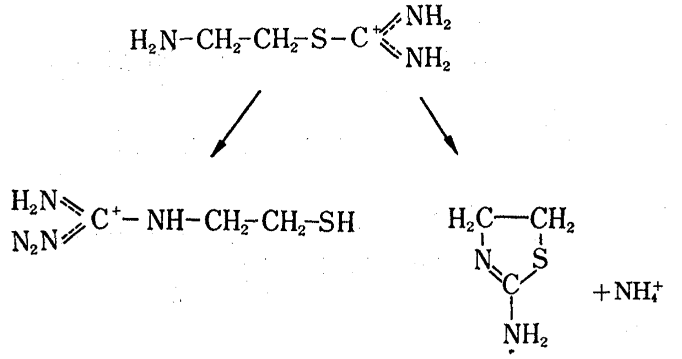

Chart 1

The N-methylated derivatives described in this paper were prepared, according to the procedure reported by Doherty,[7] from N-methylated aminoethylbromide hydrobromide and N-methylated thiourea in alcohols. Those compounds were stable in solid state, though rapidly transformed in an aqueous solution.

The characteristics of infrared absorption (IR), between 1700—1500 cm^{-1}, and nuclear magnetic resonance (NMR) spectra were shown in Table I. The IR spectra measured in nujol mull showed one or two strong absorption between 1650—1600 cm^{-1}. The band at 1650 cm^{-1} was vanished in the derivatives dimethylated at thiourea nitrogen, which was abbreviated as N',N'-dimethylated compound. Another band appeared in this region was also affected by the N'-CH$_3$ group. The compounds not having N'-CH$_3$ group showed a sharp and strong band at 1630 cm^{-1}. N'-methylated and N',N'-dimethylated compounds showed strong bands at 1610 and 1620 cm^{-1}, respectively. N'-methylated compounds did not show any band between 1540—1500 cm^{-1}. The NMR spectra showed three characteristic signals, respectively, due to $-N-CH_2-CH_2-S-$, indicating A_2B_2 pattern, $-N-CH_3$ and $N'-CH_3$ groups.

The rate of the transguanylation was measured potentiometrically at 15° in the presence of 0.5 equivalent alkali.[8] The extent of the reaction in 10 min was summarized in Table II. Both AET and N-CH$_3$-AET were transguanylated very rapidly and the reaction was com-

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[1] Part II: T. Hino, K. Tana-ami, K. Yamada, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 1201 (1966).

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

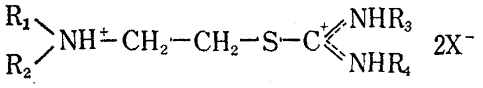
[4] A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1409 (1968).

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

[6] A. Hanaki, P. Xumsaeng, T. Hino, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **17**, 677 (1969).

[7] D.G. Doherty, R. Shapira, and W.T. Burnett, Jr., *J. Am. Chem. Soc.*, **79**, 5667 (1957).

Table I. Characteristics of Infrared Absorption and Nuclear Magnetic Resonance Spectra of N-Methylated Derivatives of AET



$$\begin{matrix}R_1\\R_2\end{matrix}\rangle NH^{+}-CH_2-CH_2-S-C\overset{+}{\leqslant}\begin{matrix}NHR_3\\NHR_4\end{matrix}\quad 2X^-$$

No.	R_1	R_2	R_3	R_4	X	IR absorption band (cm^{-1})					NMR chemical shift (ppm from DSS)		
											$N-C_2H_4-S$[a]	$N-CH_3$	$N'-CH_3$
I[9]	H	H	H	H	Br	1650 s	1632 s		1590 m	1533 w	3.44		
II[9]	CH_3	H	H	H	Br	1650 s	1632 s		1575 w	1540 m	3.47	2.79	
III[9]	H	H	CH_3	H	Br	1640 s	1610 s		1585 m		3.41		3.01
IV	CH_3	CH_3	H	H	Cl	1640 s	1630 m			1540 w	3.56	2.97	
V	H	H	CH_3	CH_3	Br		1625 s	1605 sh	1585 m	1515 w	3.42		3.06
VI	CH_3	H	CH_3	H	Br	1650 s	1613 s		1572 m		3.44	2.78	3.02
VII	CH_3	H	CH_3	CH_3	Br		1620 s	1600 sh		1515 m	3.46	2.78	3.06

a) center of A_2B_2 spectrum

pleted within 10 min. $N'-CH_3$ group appeared to reduce markedly the reaction. Those findings indicate that the transguanylation is reduced by the N'-substituent and little affected by N-substituent.

Table II. The Extent of the Transguanylation in AET Derivatives

Compound	Transguanylation in 10 min (%)
AET	100
$N-CH_3$-AET	100
$N'-CH_3$-AET	60
$N,N'-diCH_3$-AET	65
$N',N'-diCH_3$-AET	40
$N,N',N'-triCH_3$-AET	10

total concentration of AET derivatives: 8.00×10^{-3}M
NaOH added: 0.5 equivalent temperature: 15°

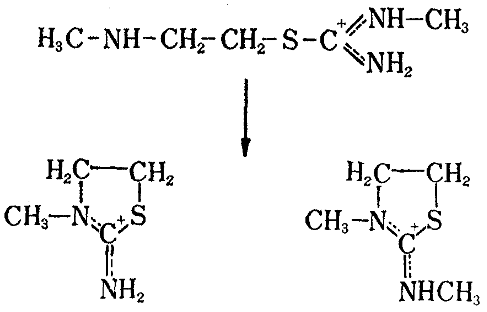


Chart 2

The AET derivatives were transformed to the corresponding 2-ATs.[1] In $N'-CH_3$-AET, both 2-AT and $2-CH_3$-2-AT were produced as the cyclization product, methylammonium and ammonium ions were splitted off, and the ratio of 2-AT to $2-CH_3$-2-AT was 2:1.[1] If the ratio of the products was not affected by the N-substituent, the same ratio would be expected in $N,N'-diCH_3$-AET. In order to determine the ratio of the cyclization products,an aqueous solution of $N,N'-diCH_3$-AET was refluxed and the NMR spectrum of the product was measured. From the intensities of N-methyl signal of methylammonium ion, which is equimolar to $3-CH_3$-2-AT, and $2,3-diCH_3$-2-AT, the ratio was calculated as 2:1.

Experimental

Preparation of AET Derivatives——Equimolar mixtures of N-methylated aminoethylbromide hydrobromide and N-methylated thiourea in EtOH or iso-PrOH was heated under reflux. The mixtures which was clear at the beginning of the reaction soon became turbid and crystals were separated gradually.[9] The reaction was continued for 75—90 min. The crystals were filtered and purified by recrystallization

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8) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2023 (1968).

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

from EtOH or EtOH–MeOH.

N,N'-diCH$_3$-AET: *Anal.* Calcd. for $C_5H_{15}N_3SBr_2$: C, 19.43; H, 4.89; N, 13.60. Found: C, 19.83; H, 4.98; N, 13.49. mp 158.5—159°.[10]

N',N'-diCH$_3$-AET: *Anal.* Calcd. for $C_5H_{15}N_3SBr_2$: C, 19.43; H, 4.89; N, 13.60. Found: C, 19.69; H, 5.01; N, 13.51. mp 212—214°.

N,N',N'-triCH$_3$-AET: *Anal.* Calcd. for $C_6H_{17}N_3SBr_2$: C, 22.30; H, 5.30; N, 13.01. Found: C, 22.44; H, 5.45; N, 12.84. mp 208—210°.

Measurements of IR and NMR Spectra——The IR spectra were measured in nujol mull with a JASCO-DS-301 spectrometer. The measurement of the NMR spectra was done in D_2O with a Varian HR-100 spectrometer.

The Rate of the Transguanylation——The rate of the transguanylation was measured potentiometrically with a Radiometer TTTIc titrator and SBR2c titrigraph. 0.8 ml of 0.1N NaOH, corresponding to 0.5 equivalent with respect to AET molecule, was added rapidly into 20 ml of 8.00×10^{-3}M AET solution, which had been thermostatted at 15°. After standing for 10 min under mechanical stir, 0.8 ml of 0.1N HCl was added into the reaction mixtures. Then, the solution was titrated with 0.1N NaOH. The alkali titer, which is equimolar to the SH compound produced as the result of the transguanylation, indicate the extent of the reaction. A detailed procedure was described in a preceeding paper.[8]

The Ratio of the Cyclization Products——The ratio of the cyclization products from N,N'-diCH$_3$-AET was measured in D_2O at room temperature with a Varian HR-100 spectrometer. An aqueous solution of N,N'-diCH$_3$-AET, 84.7 mg in 5 ml ,was refluxed until no more starting material was recognized. After the the evaporation of water from the reaction mixtures under reduced pressure, the crude products were dissolved in D_2O and applied to the NMR measurement.

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[10] All melting points described are uncorrected.

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Infrared Spectra of α-Bromoacylureas and Their N,N'-Deuterated Compounds

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This note is a continuation of the previous paper[2] dealing with the characteristic infrared absorption bands of the –CONHCONH$_2$ and –CONDCOND$_2$ groups of acylureas and deals with those of α-bromoacylureas. The infrared spectra of α-bromo-isovalerylurea and α-bromodiethylacetylurea, which were widely used as hypnotic, were reported,[3,4] but the absorptions due to the vibration of the –CONHCONH$_2$ group were not identified except a few like the C=O stretching bands. Both of these α-bromoacylureas show dimorphism and

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[1] Location: *Shimoadachi-cho, Sakyo-ku, Kyoto.*

[2] T. Uno, K. Machida, K. Hanai and Y. Saito, *Bull. Chem. Soc. Japan*, **42**, 619

[3] C.J. Umberger and G. Adams, *Anal. Chem.*, **24**, 1309 (1952).

[4] H. Satoh, *Tokyo Ika Daigaku Zasshi*, **21**, 311 (1963).