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論 文

Drug Toxicities of S-2-(W-aminoalkylamino)ethyl and S-2, W-diaminoalkyl Isothiuronium Bromides and their Potent Radioprotective Effects

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=Abstract=

S-2, W-diaminopentyl isothiuronium bromide and relevant thiophosphate derivative were prepared starting from both phthalimide and l-ornithinic acid. Drug toxicities of the prepared isothiuronium bromide and S-2-(W-aminoalkylamino) ethyl isothiuronium bromides were tested through ICR male mice, 4 and 8 weeks old and weighing 25~35 g. The former compound was found to be less toxic than those of latter compounds. This difference of drug toxicities seemed to be originated from their chemical structures, which were examined through IR Spectrometry Radioprotective effects of these compounds were discussed through relevant research data and diaminopentyl derivatives are considered to be a potent radioprotectant with low drug toxicity.

Introduction

In continuation of previous researches[1,2], the preparation of low toxic radioprotectant was studied with special regards to low drug toxicity with effective radioprotective effect. Recent literatures[3] reported that S-2-W-Diaminoalkyl dihydrogen phosphorothioates and related derivatives had shown relatively low drug toxicity and high radioprotective effect with less amount of drug dose as compared to S-2-(W-aminoalkylamino)ethyl derivatives, which had been known as the most effective radioprotectant with less drug toxicity[4]. In this study a simple and convenient preparation of S-2, W-diaminoakyl derivatives was attempted starting from easily available materials on basis of the reported preparation scheme[3]. The preparative scheme starting from phthalimide had been suffered from the formation of optical isomers at the later stages of the preparation than the step V in the Fig. 1, which was very troublesome to separate each optical isomer formed. It was, therefore, considered to be feasible to start the preparation using the definite compound of the fixed optical structure. L-ornitinic acid was used as the starting material and the preparation of S-2, W-diaminopentyl derivatives of L-structure was easily achieved with high yields. The detailed of preparative study will, however, be reported in elsewhere. During the course of this research, isothiuronium bromide derivative could show less drug toxicity as compared to the corresponding S-2-(W-aminoalkylamino) ethyl derivatives, which seemed to be very promising to be applied as the potent radioprotectant. As previous report

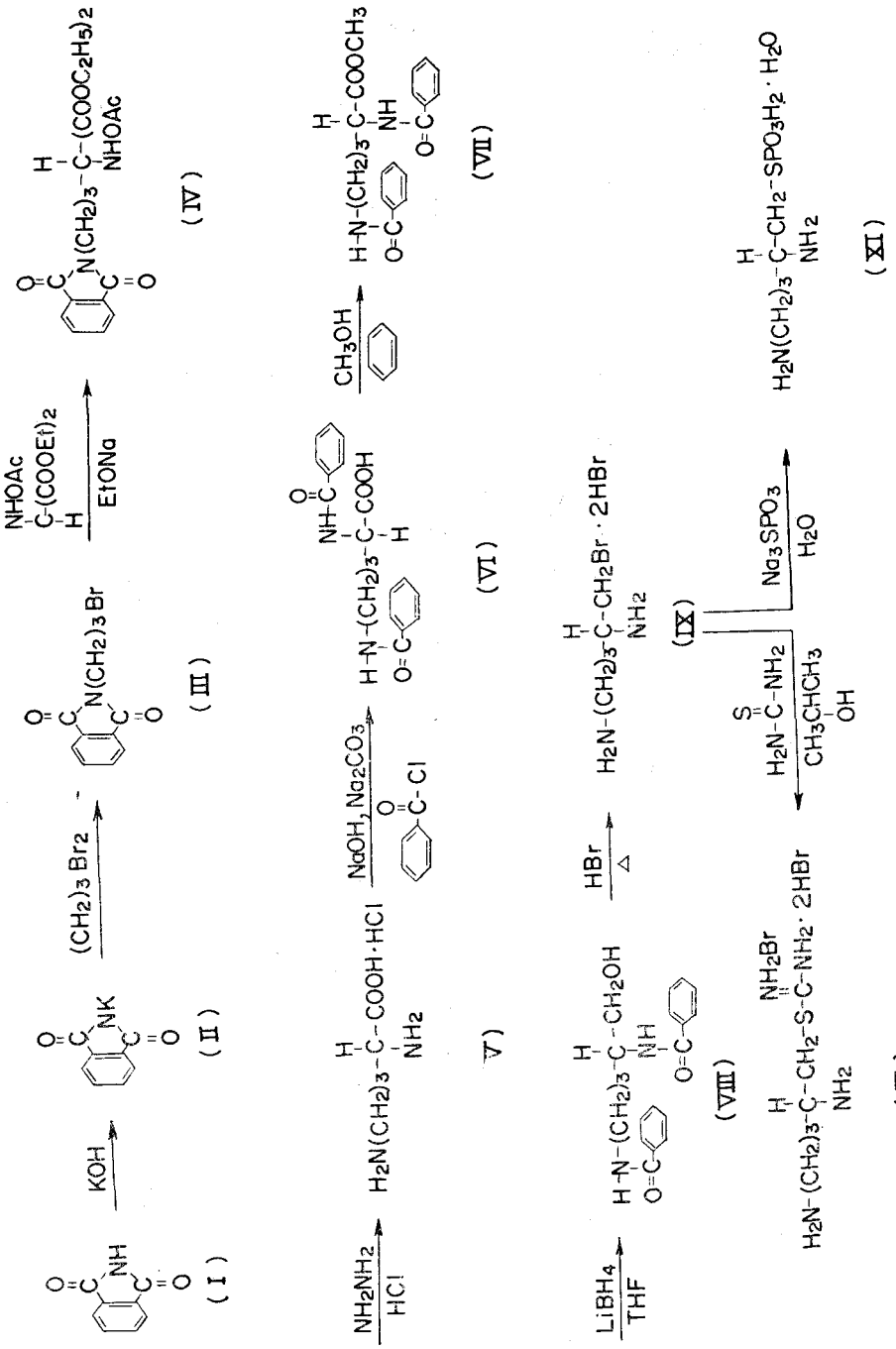

Fig. 1. Preparation of S-2,5-Diaminopentyl Isothiuronium Bromide and Dihydrogen Phosphorothioate.

Table 1. Chemical Toxicity of S-2, 5-Diaminopentyl Isothiuronium Bromide

Run No.	Dose mg/25 g of Body weight	*Test Animal ea.	Survival after days administered peritoneally Remarks		
			1 day	3 day	5 day
1	control (0.2 ml saline)	5	5	5	5
2	8.0	5	5	3	3
3	2.5	5	5	5	5

* ICR Mice: 4~8 weeks old weighing 25~27g.

had stated[1], isothiuronium bromides of AET and others were known to have effective radioprotect effects but they were suffered from relatively high drug toxicities[1]. It was, therefore, considered worthwhile to examine the origin of the less drug toxicity of S-2, W-diaminopentyl isothiuronium bromide through IR spectrometry, which may be able to clarify the correlation between the drug toxicity and chemical structure. The result of this study may further be extended to ascertain the reported high radioprotective effect of this series of compounds with less amount of drug dose from the view point of the chemical structure of the compound itself. In this study drug toxicities of isothiuronium bromides of S-2, W-diaminopentyl and S-2-(W-aminoalkylamino) ethyl series were investigated by means of ICR mouse and results were correlated with their chemical structures through their IR spectra. Radioprotective effects of these compounds were also evaluated on basis of both reported data and testing data in this laboratory.

Experimental

1. Chemicals

The following chemicals were prepared at this laboratory according to the preparative scheme shown in Fig. 1: S-2, W-diaminopentyl dihydrogen phosphorothioate and S-2, W-diaminopentyl isothiuronium bromide. In order to confirm the optical purity of products, L-ornithine hydrochloride (Alfa Products, U.S.A.) was used at the stage of V in Fig. 1. Detailed data on the preparative procedure, and physical constants were reported elsewhere[5]. Isothiuronium bromides of S-2-(W-aminoalkylamino) ethyl series were prepared as reported in the previous paper[2].

2. Methods

A. Drug Toxicities

ICR mice, 4 and 8 weeks old and weighing 25 to 35 g, were used throughout. The prepared isothiuronium derivatives were dissolved in physiological saline solution and pH of the solution was adjusted to 7.0~7.5 by means of 5% sodium hydroxide solution. The drug concentration of the solution was adjusted as each 0.2 ml. portion of the solution contained the amount of drug dose listed in tables 1 and 2. The solution was administered in the testing mice intraperitoneally and fate of the animal was checked every day as summarized in tables 1 and 2.

B. Infrared Spectrometry

Prepared chemicals such as S-2, W-diaminopentyl isothiuronium bromide S-2-(W-aminoalkylamino) ethyl isothiuronium bromides were subjected to infrared spectrometry via KBr pellet sampling method. The obtained infrared spectra were partly shown in Fig.

Table 2. Chemical Toxicity of Isothiuronium Bromide Derivatives

Test Animal: ICR Mice; 4~8 weeks old weighing 25~27 g

Chemicals*	Dose mg/25 g of Body weight	Test Animal ea.	Survival after Days Administered Peritoneally Remarks		
			1 day	2 day	3 day
1. Control	(0.2 ml Saline Sol.)	10	10	10	10
2. AET	8.0	10	10	10	10
3. 2578-A	3.0	5	2	2	2
4. 2578-A	6.0	10	1	0	0
5. 2578-A	11.4	10	2	2	1**
6. 2721-A	12.7	10	1	1	0
7. Diamino deriv.	8.0	5	5	3	3
8. Diamino deriv.	2.5	5	5	5	5

*2578-A: S-2-(ω-Aminoethylamino) ethyl isothiuronium bromide.
2721-A: S-2-(ω-Aminopropylamino) ethyl isothiuronium bromide.
Diamino deriv.: S-2, 5-Diaminopentyl isothuronium bromide.
**Survived for 12 days after being irradiated under 1,100 rad. of γ-ray, ^{60}Co.

Table 3. Characteristic Frequency of Methylene Group in the Molecules of S-2(W-aminoalkylamino) ethyl and S-2, 5-Liaminopentyl Isothiuronium Bromides

Substance*	C-H deformation		
	I	II	III
AET	1,475	1,455(w)	1,420(w)
WR-2578-A	1,480	1,445(s)	1,425(s)
WR-2721-A	1,480	1,460(s)	1,415(s)
Diamino deriv	1,480	1,450-40(w)	1,420(w)

* AET: S-2 aminoethyl isothiuronium bromide
WR-2578-A: S-2(W-aminoethylamino) ethyl isothiuronum bromide
WR-2721-A: S-2(W-aminopropylamino) ethyl isothiuronium bromide
Diamino deriv: S-2, 5-Diaminopentyl isothiuronium bromide.

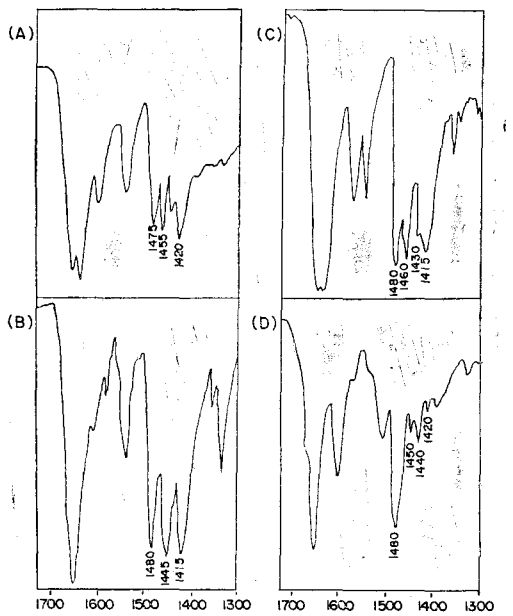

Fig. 2. Characteristic Frequences of Methylene Groups in Isothioronium Derivatives.
(A) AET (B) WR-2578-A (C) WR-2721-A
(D) $NH_2(CH_2)_3—CH(NH_2)—CH_2—S—C(=NH)—NH_2 \cdot 2HBr$

2 and the characteristic frequency of the C-H deformation of the methylene group of each compound was summarized in Table 3, in order to confirm the presence of a cyclic structure in each compound studied.

C. Radioprotective Effect

ICR male mice, 4 and 8 weeks old and weighing 25 to 35 g, were used throughout. The prepared compounds were dissolved in physiological saline solution adjusting such drug concentration as each 0.2 ml portion of the solution contained the amount of the drug

	Compounds	Test Dose mg/kg	30-day survival %
	$NH_2CH_2CH_2$-SH	200	100
I	$NH_2CH_2CH_2$-S-C(=NH_2Br)-NH_2·HBr	320	100
	$NH_2CH_2CH_2$-SPO_3H_2·Na	500	100
II	$NH_2CH_2CH_2$-N(H)-CH_2CH_2-SPO_3H_2·H_2O	900	100
	$NH_2CH_2CH_2CH_2$-N(H)-CH_2CH_2-SPO_3H_2·H_2O	500	100
III	L-$NH_2CH_2CH_2CH_2$-C(NH_2)(H)-CH_2-SPO_3H_2·H_2O	200	100
	L-$NH_2(CH_2)_4$-C(NH_2)(H)-CH_2-SH·2HCl	25	90

※ Irradiated in mice against lethal γ-radiation : 950 rad. (^{60}Co)

Fig. 3. Characteristics of Major Radioprotective Compounds reported.[1~3]

dose listed in Fig. 3. The solution was neutralized to pH 7.0~7.5 before use and it was administered in a mouse intraperitonealliy 15 minutes prior to the irradiaton. The condition of irradiation and survival rate after 30 days were summarized in Fig. 3. For S-2, W-diaminopentyl derivatives the relevant radioprotective data were cited from the reported literature[3], due to the local inavailability of testing radioprotective effect of this series of compounds in this laboratory.

Results and Discussion

As shown in Table 1, S-2, W-diaminopentyl isothiuronium bromide has shown less drug toxicity as compared to those of S-2-(W-aminoethylamino)ethyl isothiuronium bromide, drug toxicities of which were prevailed all over the adopted dose range as reported in the previous paper[2]. The drug toxicity was comparable with that of AET, which was known as a potent radioprotectant[6]. These observations were quite interesting judging from basic chemical structures of these compounds which are quite similar with each other except the additional aminoalkyl group bound to the amino nitrogen atom of the isothiuronium part of the molecule. The infrared spectra of these compounds were, therefore, examined to elucidate observed anomalous drug toxicities. As shown in Fig. 2, characteristic frequencies of methylene groups in the molecule of AET or S-2, W-diaminopentyl isothiuronium bromide were differ from those of S-2-(W-aminoalkylamino) ethyl isothiuronium bromides. In Table 3, C-H deformation frequencies of methylene groups in each molecule were summarized for reference.

Thus, S-2-(W-aminoalkylamino)ethyl derivatives showed strong absorption peaks at 1,460~1,415 cm^{-1}, which may be attributed to the presence of a cyclic structure in the molecule on basis of reported characteristic frequencies in the literature[7]. In cases of AET and S-2, W-diaminopentyl isothiuronium bromide, absorption peaks at the correspon-

R=—$(CH_2)_n$—NH_2, —H(AET & Diaminopentyl Derivative)
n=2 & 3
R′=—H, —H(AET), —$(CH_2)_3NH_2$ (Diaminopentyl Derivative)

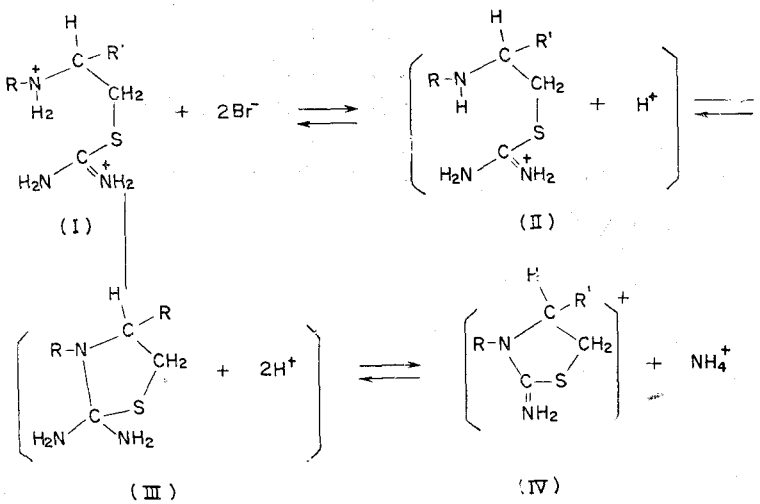

Fig. 4. Intramolecular Reaction Path of Isothiuronium Derivatives (proposed).

ding frequencies were quite weak, which may duly explain the poor formation of a cyclic structure in these molecules, An intramolecular rearrangement in the molecule to form a cyclic intermediate was, therefore, highly expected for S-2-(W-aminoalkylamino)ethyl derivatives, whereas this tendency was quite negligible in cases of AET and S-2, W-diaminopentyl isothiuronium bromide.

In the molecule of AET or S-2, W-diaminopentyl derivative, the amino part of the molecule has a primary amine structure and there fore, seemed to be less reactive to form the intermediate(Ⅳ) in Fig. 4 than those of S-2-(W-aminoalkylamino)ethyl derivatives having a secondary amine structure at the amino part in their molecules. The intermediate(Ⅲ) would form the intermediate(Ⅳ) liberating ammonia in an aqueous solution, which is known as an extrimely toxic material[6]. The observed less toxicity of S-2, W-diaminopentyl derivative may be due to the poor for mation of the intermediate(Ⅳ) in the aqueous solution, which may easily derived from the intermediate(Ⅲ) of the cyclic structure of the original molecule. Further detailed study may be necessary to assure these facts, but the present observations through infrared spectrometry may be one of the powerful evidences for elucidating the less drug toxicity of S-2, W-diaminopentyl derivative. Because of unavailablity of chemicals for preparing numbers of S-2, W-diaminoalkyl derivatives, diaminopentyl derivatives were only prepared and studied as described hereto, but the tendency on the observed less drug toxicity of the compound may equally be noticed through all types of compounds of the S-2, W-diaminoalkyl structure judging from their chemical structures of the primary amine nature at the amino part of molecules.

For radioprotective effects of compounds of present interests, the recent literature[3] reported that S-2, W-diaminoalkyl derivatives could show high radioprotective effects with less amount of drug doses as compared with that of WR-2721, a model radioprote-

ctive agent[8]. Referring to this reported data, test results[1,2] in this laboratory were summarized as shown in Fig. 3. Compounds were classified group Ⅰ, Ⅱ and Ⅲ according to their chemical structures. Compounds of the group Ⅲ showd the least amount of the effective drug dose, whereas compounds of the group Ⅱ showed the highest amount of the effective drug dose. It had been known that compounds of the group Ⅰ had been suffered from their drug toxicities when effective drug doses listed in Fig. 3 were administered in the testing animal, whereas compounds of the group Ⅱ showed no drug toxicity, though their effective doses are very high as compared to those of the group Ⅰ compounds. Compounds of the group Ⅱ had therefore been known as the most effective radioprotectant and considered as a model radioprotectant. Considering this trend of the existing radioprotectants, compounds of the group Ⅲ may be one of the promising model radioprotectant, since their effective drug doses are very low when compared to those of the group Ⅱ compounds and drug toxicities of the group Ⅲ compounds such as S-2, W-diaminopentyl isothiuronium bromide are far less than those of the group Ⅱ compounds such as S-2-(W-aminoalyl amino)ethyl isothiuronium bromides as described in the present paper. Further detailed researches may be necessary to assure these aspects of existing radioprotectants, but it is the author's recommendation that compounds of the group Ⅲ type may be very prospecting as an effective radioprotectant of the less drug toxicity.

In conclusion S-2, W-diaminopentyl isothiuronium bromide showed less drug toxicity as compared to those of S-2-(W-aminoalkylamino)ethyl isothiuronium bromides, which may be one of the strong evidence of indicating the feasibility of this type of compounds as a potent radioprotectant of the less drug toxicity.

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S-2, -(W-aminoalkylamino)ethyl 및 S-2, W-diaminoalkyl Isothiuronium Bromide의 藥毒性과 放射線障害防護 效果

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=초 록=

S-2, W-diaminopentyl isothiuronium bromide 및 그 thiophosphate 유도체는 phthalimide 또는 l-ornithine 酸으로부터 出發하여 合成되었다. 合成한 isothiuronium bromide 및 S-2-(W-aminoalkylamino)ethyl isothiuronium bromide의 藥毒性을 體重 25-35 g, 4~8週生 ICR 생쥐를 使用하여 檢査하였다. 그 結果 前者의 化合物이 後者의 것들보다 藥毒性이 弱하다는 것이 判明되었다. 이와같은 藥毒性의 差異는 化合物들의 化學構造의 差에 起因하는 것으로 보였으며 IR 分光分析法으로 그 內容이 檢討되었다. 이들 化合物의 放射線障害防護效果에 關하여 現在까지의 關聯된 硏究문헌에 대한 고찰을 하였으며 diaminopentyl 유도체들은 藥毒性이 비교적 낮고 좋은 放射線障害防護化合物인 것으로 사려된다.