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〈Original〉

Radioprotective Effects of S-2-(ω-aminoalkylamino) ethyl Derivatives and Their Drug Toxicities.

You-Sun Kim and Suc-Won Kim

Reactor Chemistry Division Korea Advanced Energy Research Institute

Abstract

S-2-(ω-aminoalkylamino) ethyl dihydrogen phosphorothioates and S-2-(ω-aminoalkylamino) ethyl isothiuronium bromides were prepared from easily available starting compounds via convenient synthetic processes. The isothiuronium derivatives showed extreme drug toxicities as compared to that of AET, which seemed to be due to an intramolecular rearrangement of these compounds. The propyl derivative of the phosphorothioate could show better radioprotective effect than those of AET and WR-638, whereas the ethyl derivative of the equivalent drug dose revealed far less protective effect. The correlation between radioprotective effects, drug toxicities, and chemical structures were discussed through infrared spectroscopy.

I. INTRODUCTION

S-2-(ω-aminoalkylamino) ethyl dihydrogen phosphorothioates have been known as the less toxic and effective radioprotectants[1,2], the preparation of which was reported as shown in Fig. 1.[3] At this laboratory, the preparative routes for these compounds were studied according to the reported procedure of Fig. 1, but the reaction between intermediates (5) and (6) could not result the expected product (7) under the reaction conditions reported[4] and furthermore, compounds (4) were only known in literatures as a patent[5], the preparative procedures of which were not clearly reported. Alternative convenient preparations of these compounds were, therefore, studied as shown in Fig. 2 and Fig. 3, respectively. Detailed procedures were reported elsewhere[6]. The corresponding isothiuronium derivatives were not reported in literatures, the chemical structure of which could expect a radioprotective effect considering AET of isothiuronium structure, the known effective chemical radioprotectant.[7]

In this paper, the radioprotective effects of prepared derivatives were surveyed by means of ICR mouse under ^{60}Co γ-ray irradiation, and the results were compared to those of AET and WR-638 to evaluate the relative protective effects of these derivatives. In case of ethyl derivative of phosphorothioate, the reported drug dose of the corresponding propyl derivative[1] was adopted for the purpose of evaluating the correlating between chemical structure and protective effect of this derivative. The elucidation of the extreme drug toxicities observed for cases of isothiuronium derivatives were attempted with regards to their molecular structures through infrared spectroscopy. Correlations between radioprotective effects, drug toxicities, and chemical structures of these types of compounds, which may be very important to study origins of the radioprotectivity of chemical radioprotectants and to develope potent chemicals, were studied on basis of infrared spectra of derivatives concerned. Author's views along this line of study on a chemical radioprotectant were also proposed.

II. EXPERIMENTAL

1. Chemicals

The following chemicals were prepared at this laboratory according to the synthetic schemes shown in Fig. 2 and Fig. 3 : S-2-(ω-aminoethylamino) ethyl dihydrogen phosporothioate, S-2-(ω-aminopropylamino) ethyl dihydrogen phosphorothioate, S-2-(ω-aminoethylamino) ethyl isothiuronium bromide, and

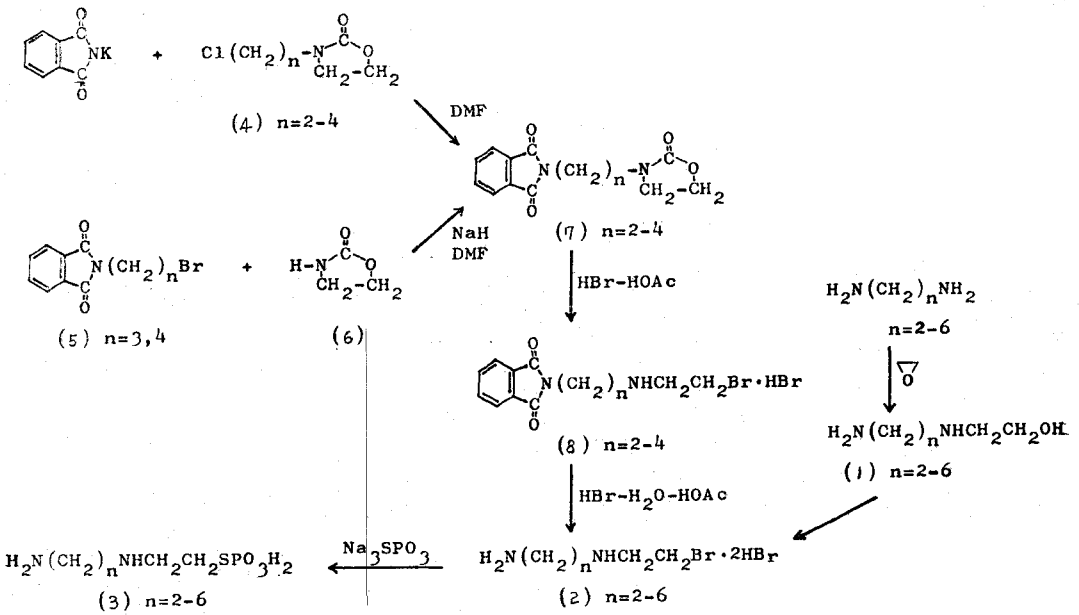


Fig. 1. Preparation of phosphorothioate derivatives.

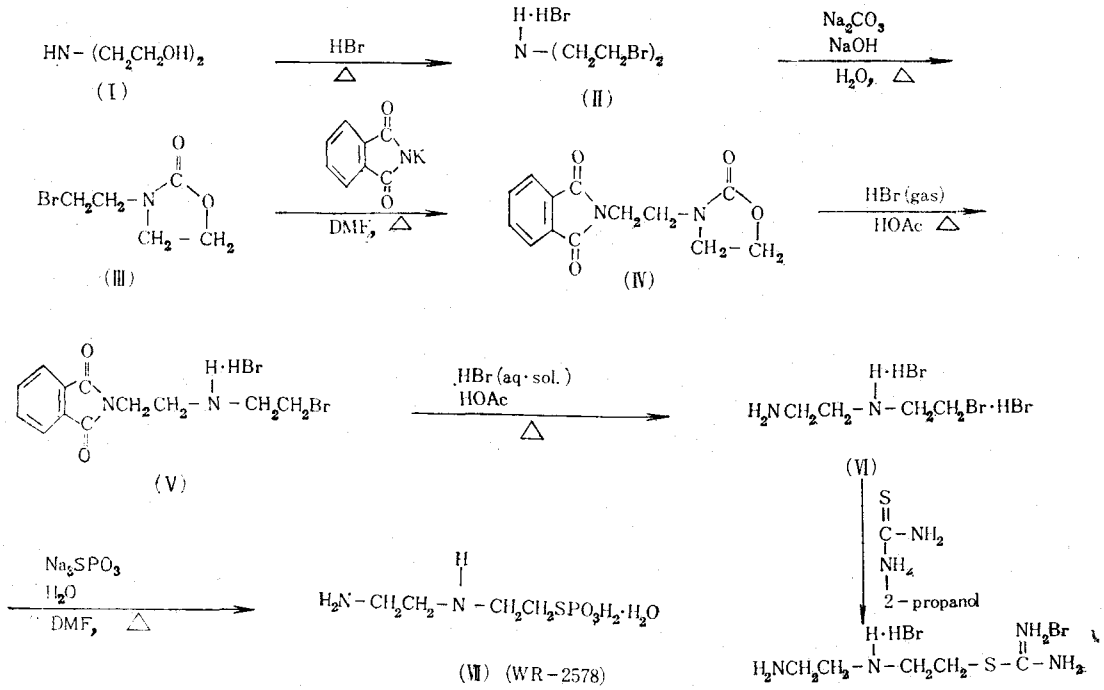


Fig. 2. Preparation of S-2-(ω-Aminoethylamino) ethyl Dihydrogen Phosphorothioate.

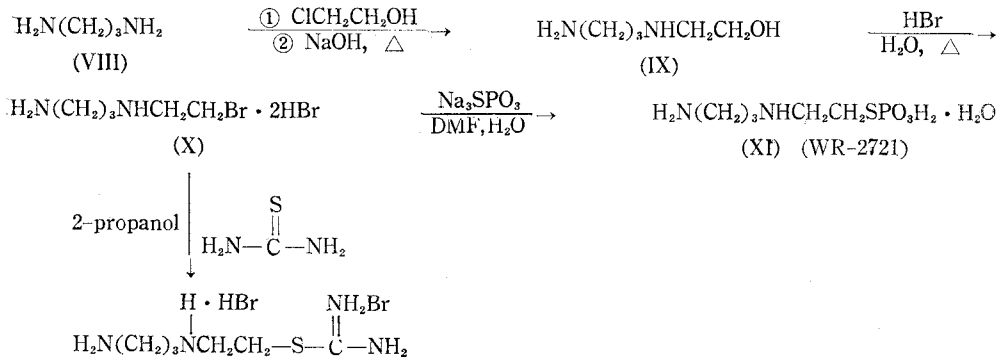


Fig. 3. Preparation of S-2-(ω-Aminopropylamino) ethyl Dihydrogen Phosphorothioate.

S-2-(ω-aminopropylamino) ethyl isothiuronium bromide. Detailed data on the preparative procedures and physical constants were reported elsewhere.[6]

2. Methods

A. Chemical toxicities

ICR male mice, 4 and 8 weeks old and weighing 25 to 35g., 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 0.2ml. portion of the solution would contain the amount of drug dose listed in the table 1. The solution was administered in the testing mice intraperitoneally and the fate of the animal was checked every day as summarized in the table 1.

B. Radioprotective effect

ICR male mice, 4 and 8 weeks old and weighing 25 to 35g. were used throughout. The prepared phosphorothioates, AET[7] and WR-638[7] were dissolved in physiological solution adjusting such drug concentration as each 0.2ml. portion of the solution would contain the amount of the drug dose listed in the table 2. The solution was neutralized to pH 7.0~7.5 before use and it was administered in a mouse intraperitoneally 15 minutes prior to the irradiation. An excessive total radiation dose, 1,100 rad. of ^{60}Co γ-ray, was adopted to differentiate fatal radiation and other deathes. The condition of irradiation, post irradiation treatments, and survival rate after 30 days were summarized in the table 2. with a listing of the total number of mouse in each group and the number of mouse that survived within three days of treatment (cheking a drug death). Results were summarized in the table 3.

C. Infrared spectroscopy

Prepared chemicals such as AET, WR-638, WR-2721, WR-2578 and isothiuronium derivatives were subjected to infrared spectroscopy via the KBr pellet sampling method. The obtained infrared spectra were partly shown Fig. 4 and Fig. 6, respectively. Characteristic frequencies of methylene groups in a molecule were particulary surveyed to confirm the presence of a cyclic structure in each compound studied.

Table 1. Chemical Toxicity of S-2-(ω-Aminoalkylamino) ethyl Isothiuronium Bromide

Chemicals*	Dose mg./25g. of Body weight	Test Animal ea.	Survival after days administered peritoneally			Remarks
			1 day	2 day	3 day	
2578-A	1.0	5	3	0	0	
2578-A	6.0	10	1	0	0	
2578-A	11.4	10	2	2	1	
2721-A	12.7	10	1	1	0	

* 2578-A; S-2-(ω-Aminoethylamino) ethyl Isothiuronium Bromide
2721-A; S-2-(ω-Aminopropylamino) ethyl Isothiuronium Bromide.

Table 2. Radioprotective Effect of Prepared Compounds in the Irradiated Mice[a]

Chemicals	Drug Dose[b] (mg./25g. of weight)	Numbers of Animal Irradiated[c]	Numbers of Survived Mice Days after being irradiated						
			3	5	10	15	20	25	30
Control	Saline sol.(0.2ml)	10	10	7	2	1	1	1	1
WR-638	12.0	10	9	9	7	7	7	7	7
AET	8.0	10	10	10	7	7	7	7	7
WR-2578	12.0	10	10	10	4	2	2	2	2
WR-2721	12.0	10	9[d]	9	9	9	9	9	9

(a) ICR Mice, 4—8 weeks old weighing 20∼25g.
(b) Administered intraperitoneally 15 minutes prior to the irradiation.
(c) Whole body irradiation. Radiation dose; 1,100 rad. of ^{60}Co γ-ray. Dose rate; 100rad./min.
(d) This was observed on that day of drug administration.

Table 3. Summary of Radioprotective Effect.

Chemicals	Drug Dose mg./25g. Body weight	Drug Death ea.	Radiation Dose** ^{60}Co γ-Ray, rad.	Radiation Death after 30 days. ea.	Remarks
WR-638	12.0	1	1,100	2	
AET	8.0	0	1,100	3	
WR-2578	12.0	0	1,100	8*	
WR-2721	12.0	1***	1,100	0	

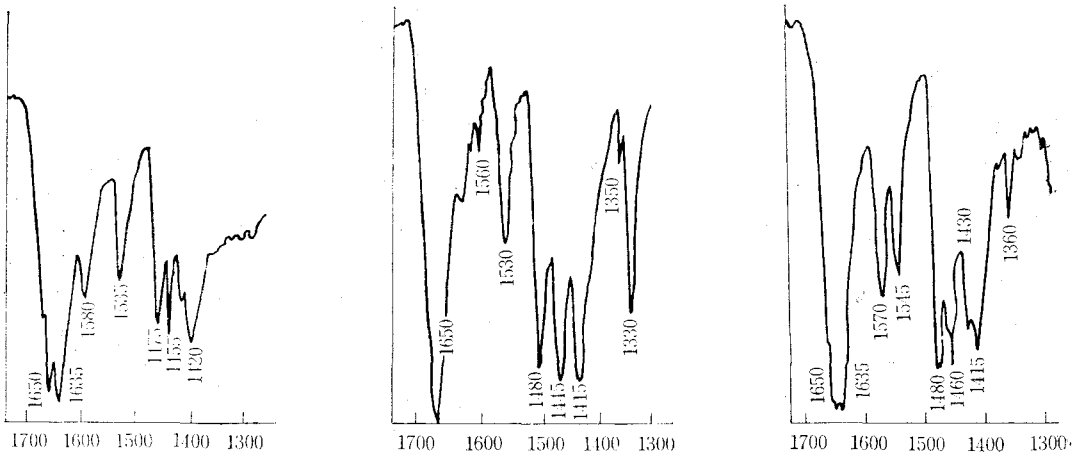
* Lit. Value is 0 when 900mg./kg. (i.e. 22.5mg./25g. weight) of the chemical was administered.
** Dose Rate; 100rad./min.
*** This was observed on that day of drug administration.

III. RESULTS and DISCUSSION

As described in the previous report[7], AET, S-2-aminoethyl isothiuronium bromide, could reveal a radioprotective effect with a slight drug toxicity, which was quite consistent to the literature concerned.[8] It was, therefore, considered that the corresponding derivatives of S-2-(ω-aminoalkylamino) ethyl could show the equivalent effect. As summarized in table 1, these derivatives were extremely toxic when they were administered intra-peritoneally in a testing mouse. Drug doses were varied from 1.0g./25g. of body weight to 11.4g., the equivalent effective dose to that of AET, but the drug toxicity was prevailed all over the adopted dose range in case of S-2-(ω-aminoethylamino) ethyl isothiuronium bromide. The similar tendency was also observed in case of S-2-(ω-aminopropylamino) ethyl isothiuronium bromide. These observations were quite unexpected judging from basic chemical structures of these compounds, which are quite similar to that of AET except the additional aminoalkyl group bound to the amino nitrogen atom of the isothiuronium part of the molecule. The infrared spectra were, therefore, examined to elucidate observed anomalous drug toxicities. As shown in Fig. 4, characteristic frequencies of methylene groups in the molecule of AET were differ from those of above derivatives.

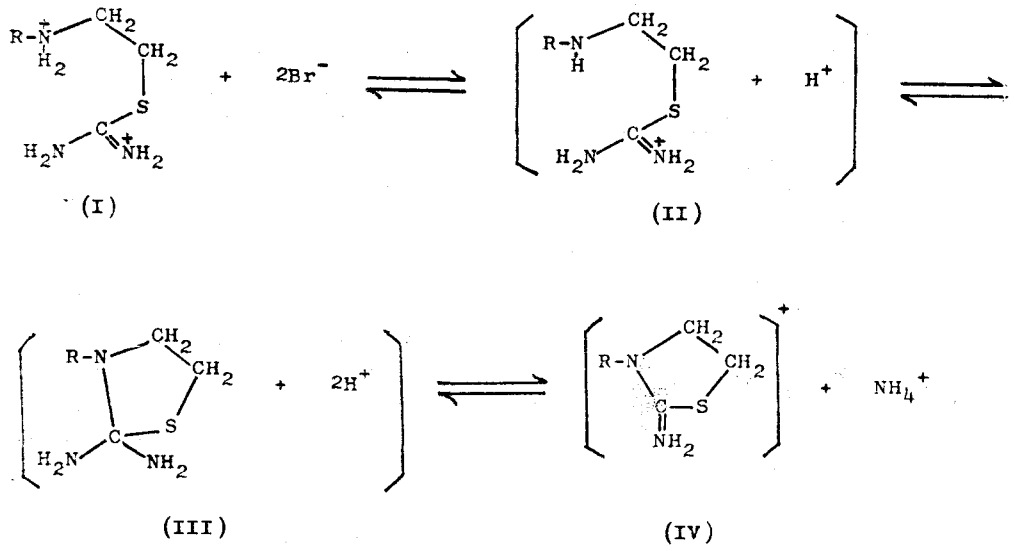
Thus, above derivatives showed strong absorption peaks at 1415∼1330cm^{-1}, which may be attributed to the presence of a cyclic structure in the molecule on basis of reported characteristic frequency in the literature[9]. An intra molecular rearrangement in the molecule to form a cyclic intermediate was, therefore, expected for these compounds as shown in Fig. 5.

In case of AET, amino part of the molecule has a primary amine structure and therefore, seemed to be less reactive to form the intermediate (IV) in Fig. 5 than those of above derivatives having a secondary



(A) AET (B) WR-2578-A (C) WR-2721-A

Fig. 4. Characteristic frequences of methylene groups in isothiuronium derivatives.



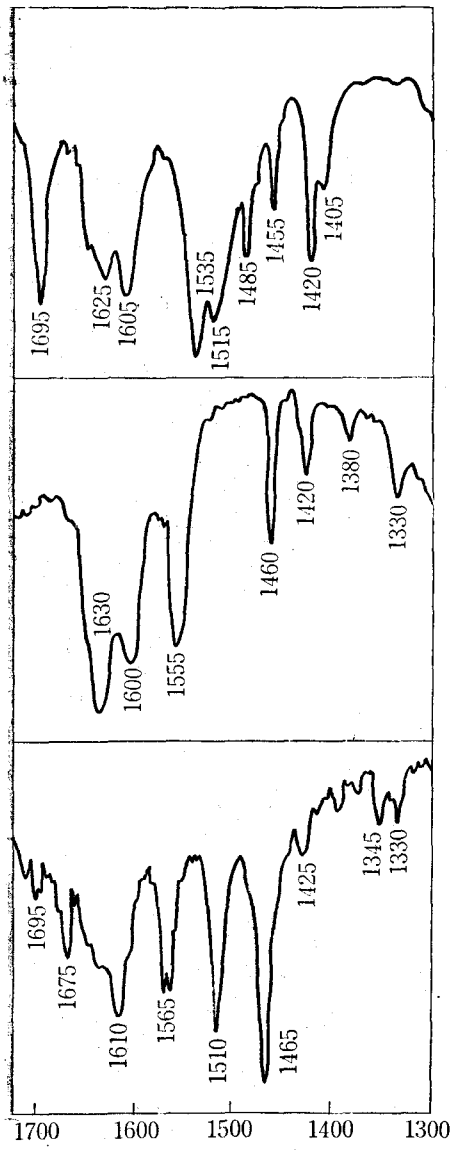
$R = -(CH_2)_n-NH_2$, $-H$(AET)

$n = 2$ & 3.

Fig. 5. Intramolecular Reaction Path of Isothiuronium Derivatives(proposed)

amine structure at the amino part in their molecules. The intermediate (III) would form intermediate (IV) liberating ammonia in an acidic aqueous solution, which is known as an extremely toxic material.[8] The observed extreme toxicity of above derivatives may be due to the formation of intermediate (IV) in the aqueous solution, which is derived from the intermedeate (III) of the cyclic structure of the original molecule. Further detailed study may be warranted to confirm these facts, but the present observations through infrared spectroscopy may be one of the powerful evidences for elucidating the anomalous toxicities of above derivatives.

Prepared S-2-(ω-aminoalkylamino) ethyl dihydrogen phosphorothioates were subjected to animal testing for evaluating their radioprotective effects as



(A) WR-2721
(B) WR-638
(C) WR-2578

Fig. 6. Characteristic Frequencies of Methylene Groups in Phosphorothioate Derivatives.

shown in table 2. WR-2721, S-2-(ω-aminopropylamino) ethyl dihydrogen phosphorothioate, could show the strongest effect than those of AET, WR-638, and WR-2578 as expected.[1] However, in case of WR-2578, S-2-(ω-aminoethylamino) ethyl dihydrogen phosphorothioate, which differ from WR-2721 in its chemical structure with regard to its alkylamino group, showed much less radioprotective effect than those of AET, WR-638, and WR-2721, when the equivalent effective drug dose to that of WR-2721 were administered in the testing animal. According to the literature[1], this chemical could show better radioprotective effect than those of AET and WR-638, when 900mg/kg (i.e. 22.5mg/25g body weight) drug dose, twice fold amount of effective drug dose of WR-2721, were administered. This discrepancy of effective drug dose between those two compounds of the resembled chemical structure are not clear enough to be understood and should be studied in details with views of confirming their chemical behaviors. Infrared spectra of WR-2721, WR-638, and WR-2578 were, therefore, examined as shown in Fig. 6. There observed absorption peaks at lower frequency range (i.e. 1,400∼1,300 cm^{-1}) in cases of WR-638 and WR-2578, whereas there are none peaks at the same range in case of WR-2721. Probably, this discrepancy of infrared absorption at the lower frequency range (i.e. an indication of the presence of cyclic structure in the molecule) would be the main origin of observed weak radioprotective effect of WR-2578 and WR-638 as compared to that of WR-2721. Further research along this line may be very important to clarify the correlations between radioprotective effect, drug toxicities, and chemical structures. Existing literatures[10] have usually ignored of these trends of chemical compounds with respects to their chemical structures, protective effects and correlations between each others. It is author's views that structural analysis of potent chemical radioprotectants as described above would be very essential to confirm the origins of radioprotective effect and drug toxicities, if any. These researches may also result a finding of a powerful chemical radioprotectant with less toxicity.

In conclusion WR-2721, WR-2578, and corresponding isothiuronium derivatives were prepared and their radioprotective effects and drug toxicities were confirmed. The correlations between radioprotective effects, drug toxicities, and chemical structures of these compounds were elucidated through infrared spectroscopy.

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초 록

S-2-(ω-aminoethylamino) ethyl dihydrogen phosphorothioate, S-2-(ω-aminopropylamino) ethyl dihydrogen phosphorothioate를 입수하기 용이한 출발화합물로부터 간편한 방식으로 합성하였다. 이들의 Isothiuronium 유도체들도 합성하였다. Isothiuronium 유도체들은 AET보다도 극심한 약독을 나타냈으며 이 약독은 분자내전위반응에 따른 고리화합물 생성에 기인하는 것으로 해석하였다. phosphorothioate 유도체들은 propyl의 경우 AET나 WR-638보다도우수한 방사성방호효과를 나타내주었으나 해당되는 ethyl유도체는 동일한 약량에서 훨씬 뒤떨어진 방호효과를 보여주었다. 이들 유도체들의 방사성방호효과, 약독 및 화학구조들 사이의 상호관계를 적외선 분광분석결과를 중심으로 하여 논의하였다.