Notes

Potential Antiradiation Agents. *p*-Anisylbutyl Derivatives of *S*-2-Aminoethyl Hydrogen Thiosulfate, 2-Aminoethanethiol, the Corresponding Disulfide, and Thiazolidine†

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Received January 4, 1972

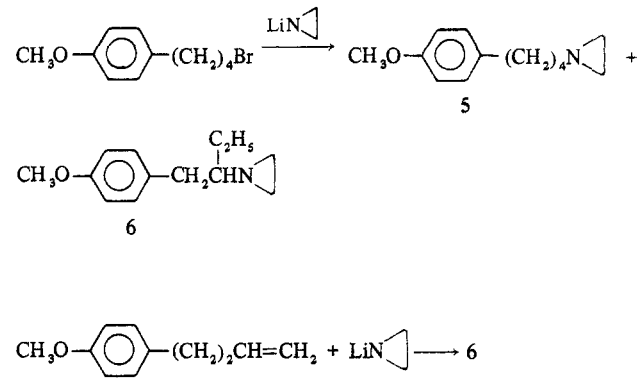
We previously have reported[1] several series of highly effective Bunte salts as antiradiation agents. Within a series of aralkyl derivatives of *S*-2-aminoethyl hydrogen thiosulfate, *S*-2-{[4-(*p*-methoxyphenyl)butyl]amino}ethyl hydrogen thiosulfate (**1**) (Table I) was markedly superior to the others. Good antiradiation effects were obtained when the drug was given either by injection or by mouth. We have published[2] a study of the effect of different sulfur-covering groups on the antiradiation properties of highly active N-substituted *S*-2-aminoethyl hydrogen thiosulfates, but modifications of the Bunte salt **1** were not considered.

Alkylation of *S*-2-aminoethyl hydrogen thiosulfate with *p*-(4-bromobutyl)anisole was used in the earlier work[1] to prepare **1**. This convenient alkylation procedure was also used for Bunte salts **2–4** by substituting *S*-3-aminopropyl hydrogen thiosulfate, *S*-2-amino-1-methylethyl hydrogen thiosulfate, and *S*-2-aminopropyl hydrogen thiosulfate for *S*-2-aminoethyl hydrogen thiosulfate.

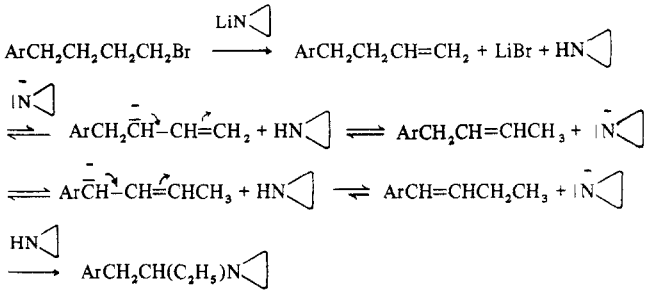
Modifications of the sulfur-covering group, however, required other intermediates; 1-[4-(*p*-methoxyphenyl)butyl]aziridine (**5**) was the common intermediate of choice for these analogs. Direct alkylation of ethylenimine with alkyl halides using a 7–10 molar excess of ethylenimine in the presence of powdered, anhydrous K_2CO_3 has been shown to be a convenient route to 1-substituted aziridines.[2] Direct alkylation also was used in the present study, but as was described earlier, it was difficult to obtain analytically pure aziridines, even though the compounds appeared homogenous when subjected to glc analysis.

Alkylation of 1-aziridinyllithium in ethylenimine–heptane as solvent was considered promising as an alternative approach to 1-alkylaziridines if an activated halide cannot be used. A high yield of pure 1-octylaziridine was obtained in this way. The reaction was completed in 1 hr at 25°, and it is particularly noteworthy for this reason. This seemingly simple modification may provide a significant practical advantage in the synthesis of some 1-alkylaziridines.[3] However, on allowing *p*-[4-bromobutyl)anisole to react with 1-aziridinyllithium in ethylenimine–hexane, 1-[4-(*p*-methoxyphenyl)butyl]aziridine (**5**) was indeed formed, but the distilled product contained about an equal quantity of another aziridine. The structure of this second product was determined by ir, nmr, and mass spectra to be 1-(α-ethyl-*p*-methoxyphenethyl)aziridine (**6**) (Scheme I). Apparently some dehydrohalogenation occurred,[3] and the system was sufficiently basic to effect migration of the double bond of *p*-

Scheme I



Scheme II



(3-butenyl)anisole, giving a substituted 2-butene (Scheme II). It is surprising that any of the 2-butene would form under such mild conditions,‡ but once formed the energetically favored substituted styrene would be the only reasonable product.

It has been well established that amines can be added to styrenes in the presence of catalytic amounts of sodium metal,[6] but it has not been determined with certainty whether the reactions proceed by an ionic or a radical mechanism. Wegler and Pieper[7] have claimed that the addition of amines to styrene is catalyzed by sodium amide, although they suggested that the amide was generated by a radical process involving styrene and metallic sodium. Razdan[8] has disputed claims of an ionic mechanism for additions of this type, and it is true that metallic sodium has been used in the reported examples of ionic addition of amide ions. Further substantiating a radical mechanism, Razdan[8] was unable to add 1-aziridinyllithium to propenylbenzene in ether solution. In our case the amide ion was generated first by using butyllithium, so that the presence of the aziridine anion was assured. However, some homologous splitting of 1-aziridinyllithium giving trace quantities of radicals has not been ruled out. In contrast to the results of Razdan, 1-aziridinyllithium has now been found to add to styrene in high yield using ethylenimine as solvent. The use of ether in the earlier case rather than ethylenimine as solvent may have prevented the addition of 1-aziridinyllithium to propenylbenzene. The addition of the aziridine

†This investigation was supported by the U.S. Army Medical Research and Development Command, Contract No. DA-49-193-MD-2306.

‡Double bond migration of this type has been shown to occur in the presence of bases such as potassium *tert*-butoxide.[4] Also, lithium in ethylenediamine has been claimed to be more effective than sodium in causing rearrangements of terminal to internal olefins.[5]

Table I. *p*-Anisylbutyl Derivatives of *S*-2-Aminoethyl Hydrogen Thiosulfate, 2-Aminoethanethiol, the Corresponding Disulfide, and Thiazolidine

No.	CH_3O–⬡–A, A	Method[a]	Recrystn solvents	Yield, %	Mp, °C	Formula[b]	Antiradiation activity[c]				
							Ca. LD_{50} mg/kg	Drug dose[d] mg/kg	Survival, %	Protect. index[e]	Rating[f]
1	$(CH_2)_4NH(CH_2)_2S_2O_3H$[g]						120	30	87		+++
2	$(CH_2)_4NH(CH_2)_3S_2O_3H$	B	EtOH	10	172–173	$C_{14}H_{23}NO_4S_2$	140	25	7	0	0
3	$(CH_2)_4NHCH_2CH(CH_3)S_2O_3H$	C	EtOH	20	153–157	$C_{14}H_{23}NO_4S_2$	240	100*	0	0	0
4	$(CH_2)_4NHCH(CH_3)CH_2S_2O_3H$	C	EtOH	35	177–179	$C_{14}H_{23}NO_4S_2$	740	75*	33	13	*h*
7	$CH_2CH(C_2H_5)NH(CH_2)_2S_2O_3H$	A	EtOH, H_2O	14	184–188	$C_{13}H_{21}NO_4S_2$	130	60	13	0	0
9	$(CH_2)_4NH(CH_2)_2SH \cdot HCl$	D	MeCN	58	143–146	$C_{13}H_{21}NOS \cdot HCl$	70	40	15[i]	0	0
10	$(CH_2)_4NH(CH_2)_2S\text{-}]_2 \cdot 2HCl$	E	DMSO	50	250–255	$C_{26}H_{40}N_2O_2S_2 \cdot 2HCl$	240	60*	83	11	+++
							215	53	60		
8	$CH_2CH(C_2H_5)X^i \cdot HCl$	F	MeCN	50	145–148	$C_{14}H_{21}NOS \cdot HCl$	125	60	0	0	0
11	$(CH_2)_4X^i \cdot HCl$	F	MeCN	65	162–165	$C_{14}H_{21}NOS \cdot HCl$	240	80	85	7	++
								40	40		
12	$(CH_2)_4X^j \cdot HCl$	G	MeCN	19	133–135	$C_{15}H_{23}NOS \cdot HCl$	150	100*	17	0	0

[a] A, RNC_2H_4 + $(NH_4)_2S_2O_3$ (see ref 2 and 12); B, 4-$MeOC_6H_4(CH_2)_4Br$ (ref 1) + $H_2N(CH_2)_3S_2O_3H$ (ref 13); C, 4-$MeOC_6H_4(CH_2)_4Br$ (ref 1) + $H_2NCH(CH_3)CH_2S_2O_3H$ or $H_2NCH_2CH(CH_3)S_2O_3H$ (ref 14); D, RNC_2H_4 + H_2S (ref 2); E, $RNH(CH_2)_2SH$ + I_2 (ref 2); F, RNC_2H_4 + (1) H_2S, (2) $HOCH_2SO_3Na$ (ref 2); G, $RNHCH_2CH_2SH \cdot HCl$ + CH_3CHO. [b] All compds were analyzed for C, H, N, and S. Compd 9 was analyzed for thiol by titration with I_2 as a substitute for S. Analytical results were within ±0.4% of theoretical values. [c] Data are given for ip administration of the compds. The antiradiation data generally represent the lowest dose of drug for which a high rate of survival was obtained. The per cent survival (30 days) of the test animals is given for the dose specified. [d] For each test 15 mice were treated with drug either 15 or 30 min preirradiation, usually with 950 rads from a ^{60}Co source. When fewer than 15 mice were used (generally 6) the drug dose is marked with an asterisk; in these cases irradiation was with 800–825 rads from an X-ray source. [e] See footnote § in the text. [f] Ratings are based on the following ranges of protective indices: 0, 0–1; +, 2–5; ++, 6–10; +++, 11–15; ++++, 16–29. The ratings are a measure of the lowest dose for which some antiradiation activity was obtained. A high survival rate and a low rating (low protective index) indicates that the compd did not protect well at doses lower than those shown. Twenty per cent survival is the smallest value used to determine ratings. [g] Ref 1. [h] A low rating is assigned because 33% was the highest survival obtained. [i] Survival of controls, 10%; irradiation was with 781 rads from a ^{60}Co source. [j] X is 3-thiazolidinyl. [i] X is 2-methyl-3-thiazolidinyl.

anion to the styrenes generated in these new systems may be the first clearly demonstrated examples of ionic addition of amines to styrenes, although radical addition of amines to styrenes in other cases is not questioned.

1-(α-Ethyl-*p*-methoxyphenethyl)aziridine (**6**) was independently synthesized by treating *p*-(3-butenyl)anisole[9] with a 0.25-molar equivalent of 1-aziridinyllithium in ethyl enimine (Scheme I). The skeletal rearrangement product **6** was the only product isolated, thereby establishing that double bond migration occurred in both reactions. Furthermore, when 1-[4-(*p*-methoxyphenyl)butyl]aziridine (**5**) was subjected to 1-aziridinyllithium in ethylenimine, the 1-substituted aziridine was recovered unchanged.

Synthesis of **6** allowed preparation of the Bunte salt (**7**) and the thiazolidine (**8**), two branched-chain analogs of **1**, by reaction with $(NH_4)_2S_2O_3$ and H_2S-$HOCH_2SO_3Na$, respectively. These compounds, though inactive as antiradiation agents, were considered important analogs because branching of the butyl group in a series of *N*-cyclohexylbutyl derivatives of *S*-2-aminoethyl hydrogen thiosulfate resulted in radioprotectors which were vastly superior to the straight-chain analog; the most active compound with a protective index § of 29 (compared with a value of 12 for **1**) was *S*-2-[(2-cyclohexylbutyl)amino]ethyl hydrogen thiosulfate, *cyclo*-C_6H_{11}-$CH(C_2H_5)$ $CH_2NH(CH_2)_2S_2O_3H$.[1]

It has now been found that the disulfide (**10**) and thiazolidine (**11**) analogs of **1** are good radioprotectors,[§,10] but that the corresponding thiol (**9**) is almost nonprotective. The high toxicity of **9** may in part explain its inactivity (ip LD_{50} = *ca.* 70 mg/kg, whereas both **10** and **11** had ip LD_{50} values of *ca.* 240 mg/kg). Interest in **1** as a radioprotector stems from its activity when given perorally (87% survival at 1000 mg/kg).[1] Administration of the thiazolidine analog (**11**) perorally also protected the mice (40% survival at 200 mg/kg, 30 min preirradiation; po LD_{50} = *ca.* 400 mg/kg). Although **11** was effective at lower doses than was the Bunte salt (**1**), survival rates greater than 50% in the po test have been obtained with **1**, but not with **11**.

The other modifications shown in Table I resulted in inactive compounds with the exception of **4**, a derivative of *S*-2-aminopropyl hydrogen thiosulfate. However, the survival rate was not sufficiently high to be of interest. In this series, therefore, the antiradiation activity of *S*-2-{[(4-*p*-methoxyphenyl)butyl]amino}ethyl hydrogen thiosulfate (**1**) has not been surpassed by the analogs described herein or homologs reported[1] earlier. The activity of the corresponding disulfide **10** and the thiazolidine **11** suggests once again that it is the 2-(substituted amino)ethanethiol which is the biologically active form of radioprotectors of this type.

Experimental Section#

1-[4-(*p*-Methoxyphenyl)butyl]aziridine (5). From 184.5 g (0.76 mole) of *p*-(4-bromobutyl)anisole,[1] 261 g (6 moles) of ethyl-

§ Protective index = (protection factor) × (LD_{50}/minimum effective dose), where doses are in mg/kg and the protection factor is 1.3 for 30% survival, 1.4 for 40% survival, etc. Testing was performed by Miss Marie M. Grenan at Walter Reed Army Institute of Research. Female albino mice 6–8 weeks old were used in the test. For the initial screening results the maximum and one-half the maximum tolerated doses were injected intraperitoneally into two groups of 15 mice each, and ten control mice were injected with the vehicle only.

#Melting points (uncorrected) were determined using a Thomas-Hoover melting point apparatus. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions are within ±0.4% of the theoretical values.

enimine, 115 g (0.84 mole) of powd, anhyd K_2CO_3, and 950 ml of abs EtOH was obtained[2] 133 g (86%) of the 1-substituted aziridine: bp 110–115° (0.3 mm); glpc 97%. The nmr spectrum was as expected. *Anal.* ($C_{13}H_{19}NO$) H, N; C: calcd, 76.05; found, 75.25.

3-[4-(*p*-Methoxyphenyl)butyl]-2-methylthiazolidine Hydrochloride (12). A soln of 9.4 g (0.034 mole) of **9** and 15 g (0.34 mole) of acetaldehyde in 100 ml of 50% aqueous MeOH was heated under reflux for 3 hr. The solvent was removed under vacuum, and the residue was treated with satd aqueous K_2CO_3. The thiazolidine base was extd into Et_2O, and the soln was dried ($MgSO_4$) and treated with dry HCl. The Et_2O, containing an oily HCl salt, was evapd leaving 4.0 g of light yellow solid which was recrystallized from MeCN to give 1.9 g (19%) of **12**: mp 133–135°. The nmr spectrum was as expected.

1-Octylaziridine. To 75 ml of cold (5°) ethylenimine was slowly added 31 ml of 1.6 *M* BuLi in heptane (1-aziridinyllithium in Et_2O has been prepd[11]). The mixt, protected from moisture, was stirred at 5° for 10 min before adding dropwise 9.7 g (0.05 mole) of 1-bromooctane. The cooling bath was removed, and the reaction was allowed to proceed for 1 hr longer. The solvent was removed under reduced pressure, and the residue (24.7 g) was extd with several portions of hexane. The combined exts were filtered through Celite and concd to give 8.7 g of liquid: glpc 88%. Distn afforded 7 g (90%) of 1-octylaziridine: bp 87° (15 mm); glpc 99%. The nmr spectrum was as expected. *Anal.* ($C_{10}H_{21}N$) C, H, N.

Reaction of *p*-(4-Bromobutyl)anisole with 1-Aziridinyllithium. To 125 ml of cold (5°) ethylenimine was slowly added under N_2 100 ml of 1.6 *M* BuLi in hexane. The mixt was stirred at 5° for 10 min before adding dropwise 20 g (0.082 mole) of neat *p*-(4-bromobutyl)anisole. The ice bath was removed, and the mixt was stirred overnight at room temp. The mixt was concd under reduced pressure, and the residue was extd with six portions of hexane. The combined exts were dried ($MgSO_4$) and concd to give 10.7 g of light yellow liquid. An additional 2.1 g of liquid was extd into Et_2O. The 10.7-g sample was distd giving the following fractions: 3.3 g, bp 80–83° (0.1 mm) and glc 60:40; 2.7 g, bp 84–89° (0.1 mm) and glc 50:50; and 3.4 g, bp 89–90° (0.1 mm) and glc 30:70. The total quantity distd was 9.4 g (56%). Ratios of the two components in the three fractions calculated from nmr spectra and based on the structural identification given below were within 5–10% of the glc ratios. A portion of the 2.7-g fraction was sepd by preparative glc (Loenco, Prepmatic; 10% SE30 column, 3/8 × 48 in.; programmed, 125–225° at 6°/min) to give 160 mg of liquid having the longer retention time, and shown by ir and nmr spectra to be the expected alkylation product, 1-[4-(*p*-methoxyphenyl)butyl]aziridine **(5)**. The component with the shorter retention time amounted to 99 mg and was shown to be 1-(α-ethyl-*p*-methoxyphenethyl)aziridine **(6)**: nmr (CCl_4) δ 6.83 (m, 4, aromatic H's), 3.68 (s, 3, CH_3O), 2.64 (d, 2, J = 6 Hz, $PhCH_2$), and 0.6–1.7 ppm [m, 10, $CH(C_2H_5)N(CH_2)_2$];** and mass spectrum (70 eV) *m/e* (rel intensity) 205 (6), 176 (7), 147 (8), 134 (8), 121 (55), 91 (16), 84 (100), 78 (28), 56 (38). *Anal.* ($C_{13}H_{19}NO$) C, H, N. Trials using a much higher ratio of 1-aziridinyllithium to aralkyl bromide did not result in any appreciable change in the ratio of the two products. None of the rearrangement product **(6)** was formed when **5** was treated with butyllithium in ethylenimine–hexane as solvent. The starting aziridine **(5)** was recovered unchanged.

1-(α-Ethyl-*p*-methoxyphenethyl)aziridine (6). To 60 ml of cold (5°) ethylenimine was slowly added under N_2 9.5 ml of a 13.3% soln of BuLi in heptane. The mixt was stirred at 5° for 15 min before adding dropwise 8.8 g (0.054 mole) of *p*-(3-butenyl)anisole.[9] The mixt was stirred for 18 hr at room temp and then concd under vacuum. The residue was extd with several portions of hexane, and the combined exts were filtered through Celite. The filtrate was concd under vacuum, and the crude oil was distd to give 6.5 g (58%) of **6**: bp 95–100° (0.1 mm); glc 99%; ir and nmr spectra were identical with the sample obtained by preparative glc (see above). A prepn using BuLi and *p*-(3-butenyl)anisole in a molar ratio of 2:1 resulted in only a 44% yield of the same product.

1-Phenethylaziridine. Freshly distd styrene (20.8 g, 0.20 mole), 30 ml of 15% BuLi, and 180 ml of ethylenimine were allowed to react as described for the prepn of **6**, except that the soln of BuLi in ethylenimine was kept at 5° for 30 min before adding the styrene. Crude liquid (30.4 g) was distd to give 24.1 g (82%) of 1-phenethylaziridine: bp 57° (1 mm) [lit.[6] bp 89° (6 mm)]; and glc 100%. The nmr spectrum was as expected.

In our work H's on α-carbons of 1-substituted aziridines are shifted upfield to *ca.* δ 2.2 ppm. The large upfield shift in the case of **6 was unexpected.

Acknowledgment. We are especially grateful for encouragement and advice from Dr. J. R. Dice and for helpful discussions with Professors G. A. Berchtold and R. E. Ireland. We are indebted to Miss Marie M. Grenan, Dr. E. A. Steck, Dr. T. R. Sweeney, and Dr. D. P. Jacobus, of Walter Reed Army Institute of Research, for the antiradiation test data, to Mr. C. E. Childs and associates for microanalyses, and to Dr. J. M. Vandenbelt and associates for spectral data.

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L-Asparaginol and Derivatives. Synthesis and Screening Data†

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The tumor growth inhibitory activity of L-asparaginase from several sources has been widely investigated.[1-6] This enzyme catalyzes the deamidation of L-asparagine, an amino acid essential for the growth of certain L-asparaginase sensitive tumors.[7] It has been found that tumors which are resistant to this enzyme synthesize L-asparagine from L-aspartic acid with the help of a synthetase.[8] In order to develop substances capable of interfering with the utilization of L-asparagine in sensitive as well as resistant tumors, the synthesis of L-asparagine analogs was undertaken.

The antitumor activity *in vivo* of *N*-carbobenzyloxy-L-asparagine has been described.[9] An analog of L-asparagine, *N*-hydroxyasparagine, was reported, although no biological data were shown.[10]

The synthesis of a series of aminoalkyl adenylates from the corresponding amino alcohols was described by Boissonas, *et al.*;[11] they were intended as inhibitors of the aminoacyl-tRNA synthetase specific for each particular amino acid. These authors did not report the synthesis of

†This investigation was supported in part by funds from the National Cancer Institute (Grant No. CA 08748), the Atomic Energy Commission (Contract No. AT (30-1)-910), the Harder Foundation Grant (T-128K) for Cancer Research from the American Chemical Society, and the American Cancer Society, Westchester Division.