TABLE I

Compd	Code	Yield, %	Mp, °C	Formula
N,N-Dimethyl-*p*-(3-indazylazo)aniline	3IN	55	245–247	$C_{15}H_{15}N_5$
N,N-Dimethyl-*p*-(4-indazylazo)aniline	4IN	25	201–203	$C_{15}H_{15}N_5$
N,N-Dimethyl-*p*-(5-indazylazo)aniline	5IN	45	265–267	$C_{15}H_{15}N_5$
N,N-Dimethyl-*p*-(6-indazylazo)aniline	6IN	29	194–197	$C_{15}H_{15}N_5$
N,N-Dimethyl-*p*-(7-indazylazo)aniline	7IN	14	204–207	$C_{15}H_{15}N_5$
N,N-Dimethyl-*p*-(2-quinoxalylazo)aniline	BP2	11	177–179	$C_{16}H_{15}N_5$
N,N-Dimethyl-*p*-(5-quinoxalylazo)aniline	BP5	18	180–182	$C_{16}H_{15}N_5$
N,N-Dimethyl-*p*-(6-quinoxalylazo)aniline	BP6	25	174–176	$C_{16}H_{15}N_5$

6IN gave 10/10 tumors at 5 months at the same level. The order of their carcinogenicity is BP6 > BP5 > 6IN > DAB and all the other compounds of Table I were inactive at the 0.03% level after 8 months of testing.

Acknowledgment.—The authors are indebted to Dr. Daniel L. Weiss, Department of Pathology, University of Kentucky College of Medicine, for the microscopic evaluation of the tumors.

α-Amidrazonium Thiosulfates as Potential Antiradiation Agents[1]

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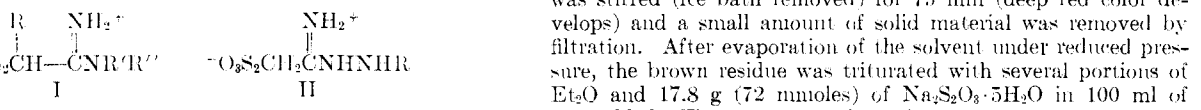
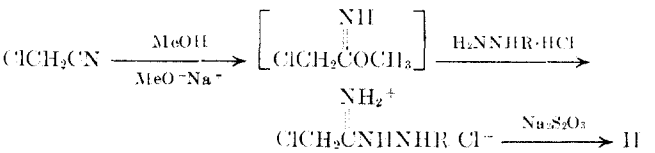
In the pursuit of antiradiation agents related to cysteamine, $HSCH_2CH_2NH_2$, Bauer and coworkers prepared a series of α-amidinium thiosulfates (I).[2,3] A number of the N-alkyl and N-aralkyl derivatives were found to possess fair to good protective activity against otherwise lethal doses of ionizing radiation in mice,[3] and further analogs in the area of N-heteroaralkyl-substituted α-amidinium thiosulfates have also been reported.[4]



I II

We now wish to describe the synthesis and biological activity of a related series of potential antiradiation agents, the α-amidrazonium thiosulfates (II). This new compound class maintains the basic criteria for radiation protection, namely a basic functional group separated from a thiol, or potential thiol group, by two or three carbon atoms.[2] It was hoped that the second basic moiety present in the amidrazones would lead to drugs with superior therapeutic ratios compared to the presently available materials.

The synthetic route to the α-amidrazonium thiosulfates was adapted from the amidinium procedure previously described.[2,3] Base-catalyzed addition of MeOH to chloroacetonitrile[5] afforded methyl α-chloroacetimidate, which was directly converted into the N-substituted α-chloroacetamidrazonium chloride by the addition of the hydrazine hydrochloride. Treatment of the intermediate salts with aqueous sodium thiosulfate gave the corresponding α-acetamidrazonium thiosulfates as deeply colored solids, which melted with decomposition (Table I).



Attempts to employ salts of hydrazine, simple alkylhydrazines or aralkylhydrazines in the reaction scheme have been unsuccessful.

To date several of the α-amidrazonium thiosulfates have been evaluated for radiation protection.[6] These preliminary results have revealed only "slight" activity[7] (Table I).

Experimental Section[8]

The synthetic procedures for compounds III and IV are described as representative examples of the conversion of chloroacetonitrile to α-acetamidrazonium thiosulfates. Compounds V–VII were prepared similarly with yields and physical constants collected in Table I.

ω-Phenyl-α-acetamidrazonium Thiosulfate (III).—To a stirred (N_2 atmosphere), cold (ice bath) solution of methyl α-chloroacetimidate [prepared from 5.4 g (72 mmoles) of chloroacetonitrile and 0.16 g (7.0 mg-atoms) of Na as described by Schaefer and Peters[5]] was added in small portions 11.4 g (79 mmoles) of phenylhydrazine hydrochloride. The resulting yellow mixture was stirred (ice bath removed) for 75 min (deep red color develops) and a small amount of solid material was removed by filtration. After evaporation of the solvent under reduced pressure, the brown residue was triturated with several portions of Et_2O and 17.8 g (72 mmoles) of $Na_2S_2O_3 \cdot 5H_2O$ in 100 ml of H_2O added. The mixture was heated at reflux 55 min and filtered hot, and the filtrate was refrigerated overnight to afford 2.5 g (13%) of yellow crystalline product, mp 151–152° dec. Recrystallization (1:1 EtOH-H_2O) gave analytically pure gold-colored crystals, mp 152–153° dec.

ω-(*p*-Nitrophenyl)-α-acetamidrazonium Thiosulfate (IV).—To a stirred (N_2 atmosphere), cold (ice bath) solution of methyl α-chloroacetimidate [prepared from 5.2 g (69 mmoles) of chloroacetonitrile and 0.15 g (6.5 mg-atoms) of Na as previously described] was added 14.1 g (74 mmoles) of *p*-nitrophenylhydrazine hydrochloride. The resulting red-brown mixture was stirred at room temperature for 27 hr and the solid (13.5 g, mp 240–250° dec), presumed to be the intermediate ω-(*p*-nitrophenyl)-α-chloroacetamidrazone hydrochloride, was collected by filtration. A 5.0-g aliquot was directly stirred with 11.9 g (48 mmoles) of $Na_2S_2O_3 \cdot 5H_2O$ in 50 ml of H_2O for 16 hr at room temperature to afford 4.8 g of red-brown solid, mp 176–177° dec. The material

(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2923.

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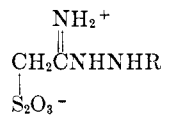
(6) Antiradiation screening tests were performed at Walter Reed Army Institute of Research, Washington, D. C., under the direction of Dr. J. P. Jacobus.

(7) "Slight" denotes 1–25% survival of mice in standard antiradiation screening tests (see Table I).

(8) Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Ir spectra were obtained on all pure compounds and were in accordance with the proposed structures.

TABLE I

α-ACETAMIDRAZONIUM THIOSULFATES



Compd	R	% yield	Mp, °C dec	Formula[a]	Antiradiation act. Approx LD_{50}, mg/kg	Drug dose, mg/kg[d]	30-day survival, %[e]
III	C_6H_5	13	152–153	$C_8H_{11}N_3O_3S_2$			
IV	p-$O_2NC_6H_4$	37	178–179	$C_8H_{10}N_4O_5S_2$[b]	150	80	20
V	o-$CH_3OC_6H_4$	19	136–137	$C_9H_{13}N_3O_4S_2$	110	25	13
VI	p-FC_6H_4	19	143–144	$C_8H_{10}FN_3O_3S_2$			
VII	EtO_2CCH_2	17	143–144	$C_6H_{13}N_3O_5S_2$[c]	750	400	13

[a] Analytical results obtained for C, H, and N were within ±0.4% of the theoretical values unless listed otherwise. [b] N: calcd, 18.3; found, 17.8. [c] C: calcd, 26.6; found, 26.1. [d] Compounds were suspended in a physiological saline solution containing 0.3% carboxymethylcellulose and 0.1% Tween 80 and administered intraperitoneally to mice, which were subjected to lethal radiation of 950 R. [e] No survival among control mice.

was triturated with boiling absolute EtOH and dried to give 2.9 g (37%) of orange product, mp 178–179° dec.

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5-Nitro-8-quinolinols and Their Copper(II) Complexes. Implications of the Fungal Spore Wall as a Possible Barrier against Potential Antifungal Agents[1]

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A hypothesis was proposed by Gershon, *et al.*,[2,3] which suggested that the fungal spore wall acted as a barrier against certain potential antifungal agents. If the geometry and charge distribution of a molecule are not compatible with geometry and distribution of charge around the periphery of the holes in the fungal spore wall, the compound cannot penetrate the wall and cause toxic reactions in the spore. It was deduced from the shapes and dimensions of the Cu(II) chelates of substituted 8-quinolinols that the holes cannot be circular but may be elliptical or hexagonal.

If the hypothesis is sound, and the explanation of the nontoxicity of certain compounds is due to the long axes being greater than the major axes of the spore holes, alteration of a secondary axis of the compound should not cause the derivative to become toxic. Bis(5-nitro-8-quinolinolato)copper(II) was shown to be nontoxic to five fungi, *Aspergillus niger*, *Trichoderma viride*, *Aspergillus oryzae*, *Myrothecium verrucaria*, and *Trichophyton mentagrophytes*.[2] The explanation was that its long axis was greater than the diameter of the holes in the spore walls and penetration of the spores could not be effected. Consequently, any 7-substituted 5-nitro-8-quinolinol Cu(II) complexes should also be nontoxic to the same fungi. To test this, the Cu(II) bischelates of 7-fluoro-, 7-chloro-, 7-bromo-, and 7-iodo-5-nitro-8-quinolinol were prepared and screened against the same five fungi.

Although the chloro ligand[4] was previously prepared by a Skraup synthesis, the present preparation was obtained by treatment of 5-nitro-8-quinolinol[5] with NaOCl. The bromo[6] and iodo[7] compounds were also prepared from 5-nitro-8-quinolinol. The respective copper(II) complexes were prepared from the ligands by treatment with cupric acetate in aqueous MeOH, or aqueous MeOH containing DMF.

The data characterizing the new compounds are contained in Table I. All of the compounds were screened for antifungal activity in shake culture against the spores of the five fungi previously mentioned, according to published methods.[8]

The data of Table II show that the 7-substituted 5-nitro-8-quinolinols possess significant antifungal activity but weaker than that of the parent compound, 5-nitro-8-quinolinol.[2] The Cu(II) bischelates were all inactive. Thus, these results were found to be consistent with our hypothesis.[2,3]

It should be mentioned that in certain cases the freshly prepared chelate caused inhibition of mycelial development. On repeated boil-up of the chelate with DMF, the inhibitory effect was eliminated. Upon cooling the DMF solution, a chelate was obtained which was inhibitory. When both the soluble and insoluble chelates were decomposed with H_2S and the Cu(II) removed, followed by recovery of the ligands, gas chromatography of the trimethylsilyl derivatives indicated that both chelates appeared to possess the same component parts. We cannot interpret this observation properly, but a reasonable explanation may be that in the formation of the chelate, a small amount of *cis*

(1) This work was supported in part by the U. S. Public Health Service, Grant No. AI-05808.

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