Experimental Section

Melting points were determined on a Thomas–Hoover apparatus and are corrected. Ir spectra were recorded on a Beckman IR-9 recording spectrophotometer, nmr spectra were determined in $CDCl_3$ on a Varian A-60 spectrophotometer (TMS), and the mass spectra were run on a CEC 21-110 spectrometer with a direct-inlet system. All ether extracts were washed with water and dried over Na_2SO_4. Analytical results for the elements indicated agreed within ±0.3% of the theoretical values.

2-(1-Propenyl)-*m*-dithianes (2a–d). Method I.—To a solution of 0.4 mole of the appropriate aldehyde (**1a–d**) and 0.4 mole of 1,3-propanedithiol in 200 ml of AcOH was added dropwise 5 ml of 20% EtOH–HCl over 15 min at 20° with external cooling. After stirring for 17 hr at room temperature, the reaction mixture was poured onto ice and extracted with Et_2O. The extract was evaporated and the residual oil distilled.

Method II.—Dry HCl gas was passed into a solution of 0.4 mole of **2a–d** in 200 ml of AcOH at 10° for 1 hr and then stored at room temperature for 3 hr. The solution was then poured onto ice and extracted with Et_2O. The extracts were evaporated to give the crude product which was purified either by crystallization or distillation.

2-(2-Chloropropyl)-*m*-dithianes (3a,b,e,f). Method III.—A solution of 0.4 mole of the appropriate aldehyde and 0.4 mole of 1,3-propanedithiol in 200 ml of AcOH was saturated with dry HCl while maintaining the reaction at 25°. After stirring at room temperature, the reaction mixture was poured onto ice and extracted with Et_2O. Removal of the solvent afforded the crude product which was purified by crystallization or distillation.

1-Methyl-4,8-dithiaspiro[2.5]octanes (4a,b,e,f). Method IV.—To a solution of 0.05 mole of **3a,b,e,f** in 100 ml of anhydrous THF maintained at −20 to −30° under N_2 was added 20 ml of BuLi (3 *M* in hexane). The solution was stored at 0° for 17 hr and allowed to warm to room temperature, and 100 ml of H_2O was added and extracted with Et_2O. The extract was evaporated and the residual oil was distilled or crystallized.

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Carcinogenic Activity of Analogs of *p*-Dimethylaminoazobenzene. IX. Activity of the Quinoxaline and Indazole Analogs

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We have shown in earlier papers that replacement of the unsubstituted ring of *p*-dimethylaminoazobenzene (DAB) with pyridine[1] and quinoline[2] gave analogs with a wide range of carcinogenic activity. In later work this variation in activity among isomers has again been shown in the benzimidazole and benzthiazole series.[3]

In this paper we wish to report the preparation and testing for rat hepatocarcinogenic action of the isomeric *p*-dimethylaminophenylazoindazoles and *p*-dimethylaminophenylazoquinoxalines. All of these azo compounds are new substances. In the indazole case it is possible to attach the *p*-dimethylaminophenylazo moiety at the 3, 4, 5, 6, and 7 positions and we found we were able to make all these isomers by the usual method of diazotizing the amine and coupling it with N,N-dimethylaniline. The 5-, 6-, and 7-aminoindazoles were commercially available. The 4-aminoindazole was prepared from 2,6-dinitrotoluene by the method of Kwartler and Lucas[4] while the 5-amine was prepared from *o*-nitrobenzoic acid by the method of Aron and Elvidge.[5] The DAB analogs of indazole are listed in Table I.

In the case of the quinoxaline analogs of DAB, there are three isomers possible, *i.e.*, the 2, 5, and 6 compounds. The 5 and 6 isomers could be prepared by diazotization and coupling of the corresponding amines while it was found necessary to apply the procedure of Brown and Faessinger[6] in order to prepare the 2 isomer. 2-Aminoquinoxaline was made by the method of Tishler and coworkers.[7] 5-Aminoquinoxaline was prepared from 2,3-dinitroacetanilide by the method of Stevens and coworkers[8] while 6-aminoquinoxaline was synthesized from 4-nitro-1,2-diaminobenzene by the method of Platt and Sharp.[9] The quinoxaline analogs of DAB are listed in Table I.

Experimental Section[10]

All of the dyes but the 2-quinoxaline compound were prepared by diazotization of the amine followed by coupling with N,N-dimethylaniline. A typical procedure is given below and the dyes are listed in Table I. After applying the method below and several minor variations to the preparation of the 2-quinoxaline analog, we applied successfully the sodium coupling with nitrosodiomethylaniline to the 2-aminoquinoxaline.[6]

N,N-Dimethyl-*p*-(3-indazylazo)aniline.—3-Aminoindazole (4 g) was diazotized in 7.5 ml of concentrated HCl and 60 ml of H_2O at 0–5° with 2.1 g of $NaNO_2$. Excess nitrite was destroyed after 1 hr by addition of sulfamic acid, and coupling with 3.6 g of N,N-dimethylaniline and 6.1 g of anhydrous NaOAc in 50 ml of 50% EtOH–H_2O was allowed to proceed for 2 hr. At the end of this time the mixture was treated with excess NH_4OH. The dye was filtered, washed well (H_2O), and dried. This was dissolved in 1500 ml of C_6H_6 and chromatographed on alumina. The orange fraction eluted by C_6H_6 was concentrated and recrystallized from EtOH.

Results and Discussion

In the biological evaluation[3] DAB (Butter yellow) at the 0.06% level gave tumor incidences of 7/10 at 4 months and 9/10 at 6 months, while at the 0.3% level it gave 5/10 in 6 months. Our most active compound, BP6, at the 0.03% level gave 10/10 tumors in 2 months. BP5 gave 10/10 tumors at 4 months at this level and

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(10) All melting points were determined on a Fisher-Johns apparatus and are corrected. The C, H analyses were performed in this department on an F and M Model 185 analyzer by Mr. Daryl Sharp. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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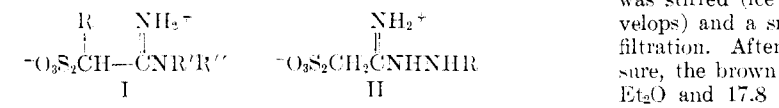
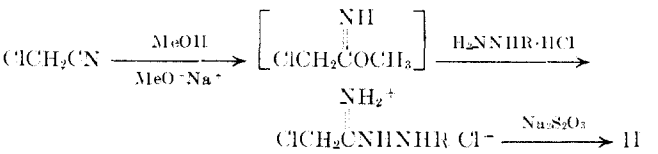
In the pursuit of antiradiation agents related to cysteamine, $HSCH_2CH_2NH_2$, Bauer and coworkers prepared a series of α-amidinium thiosulfates (I).[2,3] A number of the N-alkyl and N-aralkyl derivatives were found to possess fair to good protective activity against otherwise lethal doses of ionizing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