Table I. Induction of Sarcomata by Repeated Subcutaneous Administration of Benzo[a]pyrene and Related Compounds[a]

Benzo[a]pyrene derivative	Animals with tumors / number of animals	Range, days	Mean tumor induction time, days
Benzo[a]pyrene	5/5	68-80	75
6-Hydroxymethyl	10/10	64-78	70
6-Formyl	3/5	71-78	75
6-Acetoxymethyl	3/4	71-78	73
6-Benzoyloxymethyl	5/5	78-94	87
6-Bromomethyl	3/4	86-95	92
6-Chloromethyl	5/5	74-91	81
Sesame oil	0/5		

[a]The hydrocarbon (1 mg) in sesame oil (0.1 ml) was administered by subcutaneous injection to female Sprague-Dawley rats, age 50 days, on alternate days for 20 doses. The animals were examined for appearance of palpable tumors twice weekly. Tumor negative animals were observed for 200 days. They were fed a commercial ration (Purina rat chow) *ad libitum* and given tap water to drink. Tumors were fixed in 10% neutral formalin, sectioned at a thickness of 5 μ and stained with hematoxylin and eosin. Each of the compounds tested induced fibrosarcoma. The chloromethyl compound induced a histiocytic variety of fibrosarcoma. We thank Dr. Daniel Weiss, Department of Pathology, University of Kentucky, for the interpretation of histological material.

Each of the substituted benzo[a]pyrenes described in this report would be expected to be transformed to 6-hydroxymethylbenzo[a]pyrene *in vivo*. The esters and halides would be expected to form 6-hydroxymethylbenzo[a]pyrene by hydrolysis, whereas reduction of 6-formylbenzo[a]pyrene would form 6-hydroxymethylbenzo[a]pyrene. Hydroxylation of 6-methylbenzo[a]pyrene to form 6-hydroxymethylbenzo[a]pyrene was recently shown to occur in rat-liver homogenates.[6]

The fact that 6-hydroxymethylbenzo[a]pyrene is a potent carcinogen supports the hypothesis that it functions as a proximate carcinogen. Thus, compounds which would be expected to be converted to 6-hydroxymethylbenzo[a]pyrene are also carcinogenic. We therefore conclude that the relation between structure and carcinogenic activity is similar for the series of compounds related to 7-hydroxymethyl-12-methylbenz[a]anthracene and the series of compounds related to 6-hydroxymethylbenzo[a]pyrene.

Since the 6 position of benzo[a]pyrene corresponds to the 7 position of benz[a]anthracene, it would seem that compounds capable of conversion to the hydroxymethyl derivatives in the meso positions constitute proximate carcinogens for the two series.

Experimental Section

Elemental analyses were determined by Galbraith Laboratories, Knoxville, Tenn. Analytical results for the indicated elements were within ±0.4% of the theoretical values.

6-Formylbenzo[a]pyrene (1). A mixture of benzo[a]pyrene (5 g, 20 mmol) (**1**), $POCl_3$ (3.3 ml, 36 mmol), *N*-methylformanilide (5.5 ml, 40 mmol), and 5 ml of *o*-dichlorobenzene was warmed on a steam bath for 2 hr. The cooled reaction mixture was poured slowly into an aqueous solution (50 ml) of 25 g of sodium acetate. The product obtained was filtered and solvent removed by steam distillation. Filtration and drying yielded 5 g of crude product which was dissolved in hot benzene (250 ml) and filtered. Evaporation of the benzene solution under reduced pressure yielded 3.3 g of dark yellow material. Chromatography on silica gel and elution with benzene gave pure product (3.0 g, 10.7 mmol) in 54% yield, mp 205-206°.

6-Hydroxymethylbenzo[a]pyrene (2). A mixture of 6-formylbenzo[a]pyrene (1 g, 3.56 mmol) and $NaBH_4$ (150 mg, 3.96 mmol) in 200 ml of ethanol was heated to reflux for 2 hr on an oil bath. The solution was treated with acetic acid and the solvent removed completely. The solid was washed with water, filtered, and recrystallized from benzene (0.9 g, 3.19 mmol) in 89% yield, mp 232-233°. *Anal.* $C_{21}H_{14}O$.

6-Chloromethylbenzo[a]pyrene (3). Thionyl chloride (0.2 ml, 2.8 mmol) was added to a suspension of 6-hydroxymethylbenzo[a]pyrene (200 mg, 0.71 mmol) in 7 ml of dry benzene. The mixture was heated to reflux for 30 min with stirring. The solvent was removed under reduced pressure and traces of $SOCl_2$ were removed by addition of benzene and removal under vacuum. The residue was crystallized from benzene. An analytical sample was prepared by recrystallization from benzene (120 mg, 0.4 mmol) in 56% yield, mp 210-212°. *Anal.* $C_{21}H_{13}Cl$.

6-Bromomethylbenzo[a]pyrene (4). A suspension of 6-hydroxymethylbenzo[a]pyrene (500 mg, 1.77 mmol) in 30 ml of anhydrous benzene was heated under reflux with PBr_3 (0.3 ml, 3.16 mmol) for 1 hr. The solution was chilled in ice-water and excess reagent was decomposed by dropwise addition of H_2O. A crystalline material separated which was filtered, washed with benzene, and dried. Recrystallization from benzene gave an analytically pure sample (360 mg, 1.04 mmol) in 59% yield, mp 225-226°. *Anal.* $C_{21}H_{13}Br$.

6-Benzoyloxymethylbenzo[a]pyrene (5). A mixture of 6-hydroxymethylbenzo[a]pyrene (200 mg, 0.71 mmol), benzoyl chloride (1 ml, 8.6 mmol), and pyridine (4 ml) was heated on a steam bath for 2 min and then poured onto crushed ice. The oil which separated was extracted with benzene, washed with 5% $NaHCO_3$ and water, and dried. Removal of solvent gave bright yellow crystals. Recrystallization from benzene gave **5** (180 mg, 0.47 mmol) in 66% yield, mp 213-214°. *Anal.* $C_{28}H_{18}O_2$.

6-Acetoxymethylbenzo[a]pyrene (6). To 6-hydroxymethylbenzo[a]pyrene (100 mg, 0.35 mmol) was added 3 ml of dry pyridine and acetic anhydride (1 ml, 10.5 mmol) and kept at room temperature overnight to obtain a clear solution. The mixture was then poured onto crushed ice and then the product was collected by filtration. The product was found to be very soluble in cold PhH, Et_2O, and petroleum ether (bp 30-60°). It was recrystallized from small amounts of cold PhH, mp 178-179°, in high yield. *Anal.* $C_{23}H_{16}O_2$.

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Potential Antitumor Agents. 7. 4'-Diethyleneoxy Derivatives of α-(N)-Heterocyclic Carboxaldehyde Thiosemicarbazones†

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α-(N)-Heterocyclic carboxaldehyde thiosemicarbazones are potent inhibitors of the growth of a variety of transplanted rodent tumors,[1] spontaneous lymphomas of dogs,[2] and DNA viruses of the Herpes group.[3] One agent of this series, 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP), has been tested clinically and has shown weak carcinostatic potency in man.[4,5] These derivatives are strong inhibitors of the mammalian form of the enzyme ribonucleoside diphosphate reductase,[3,6,7] which catalyzes the conversion of ribonucleotides to deoxyribonucleotides. Blockade of the formation of RNA and protein also

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occurs, but these processes are considerably less susceptible to drug-induced inhibition.[8] In contrast, it was found in *Escherichia coli* B that interference with the synthesis of RNA was the primary event responsible for inhibition of growth.[9] In this microorganism, the major site of action of 1-formylisoquinoline thiosemicarbazone (IQ-1), the most potent known inhibitor of the mammalian enzyme ribonucleoside diphosphate reductase,[10] appeared to be at the level of pyrimidine nucleoside monophosphate kinase.[11] A second minor locus of action of IQ-1 in *E. coli* B appeared to be RNA polymerase.

One of the modifications of IQ-1 synthesized in initial studies of structure-activity relationships was the replacement of the terminal $-NH_2$ group of IQ-1 by a morpholino ring system.[12] This agent in *E. coli* B caused greater than 95% inhibition of the activity of pyrimidine monophosphate kinase at 2.5×10^{-5} *M*, while IQ-1 at a molar equivalent concentration produced only about 50% inhibition,[11] indicating that the introduction of a 4′-diethyleneoxy group in IQ-1 resulted in a compound (**2**) with the potential to cause greater inhibition of RNA synthesis than did IQ-1. To transpose these findings to mammalian cell systems, biochemical studies of **2** and its analogs in Sarcoma 180 ascites cells were pursued.[13] Furthermore, since compound **2** exhibited greater toxicity than IQ-1 in mice, several additional 4′-diethyleneoxy derivatives of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones have been synthesized in an effort to achieve a better therapeutic index. For this purpose, OH groups were introduced into the two most active heterocyclic ring systems (*i.e.*, isoquinoline and pyridine) of this series. Such hydroxylation of parent thiosemicarbazones has resulted in compounds with increased therapeutic indices in tumor-bearing mice.[14-16]

Chemistry. 4′-Diethyleneoxythiosemicarbazide (**1**), an intermediate for the synthesis of these substituted thiosemicarbazones, was prepared by treating methyl dithiocarbazate with morpholine (Scheme I).[17] The various heterocyclic carboxaldehydes utilized were made according to previously published procedures,[14-16] except that 4-hydroxy-1-formylisoquinoline[18] was synthesized in a better yield by benzoylating the 4-OH group of 1-methyl-4-hydroxyisoquinoline and then oxidizing the 1-CH_3 group with SeO_2 to its corresponding aldehyde. The aldehydes were allowed to react with substituted thiosemicarbazides to yield corresponding substituted thiosemicarbazones. Some data pertaining to the newly synthesized derivatives are shown in Table I.

Biological Results. The effects of active 4′-diethyleneoxy derivatives of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones on the survival time of mice bearing Sarcoma 180 ascites cells are shown in Table II. The parent compounds **2** and **5**, the 4′-diethyleneoxy derivatives of 1-formylisoquinoline and 2-formylpyridine thiosemicarbazone, respectively, and also compounds **4**, **6**, **8**, and **9** were inactive in this test system. However, compounds **3** and **7**, the 4-hydroxy analog of **2** and the 5-hydroxy analog of **5**, respectively, were found to be active antitumor agents that increased the average survival time of tumor-bearing mice to 22 and 19 days, respectively, as compared to untreated tumor-bearing control animals which survived 14.3 days. This increase in life span of tumor-bearing mice was, however, much less than was achieved by the corresponding unsubstituted thiosemicarbazones.[14-16] The results also revealed that, although ring monohydroxylation reduced toxicity of parent compounds to a great extent, the resultant derivatives still caused a fairly large percentage loss in body weight of treated animals at maximum effective daily doses. It is therefore conceivable that further reduction in toxicity, perhaps by bis hydroxylation, might result in com-

Scheme I

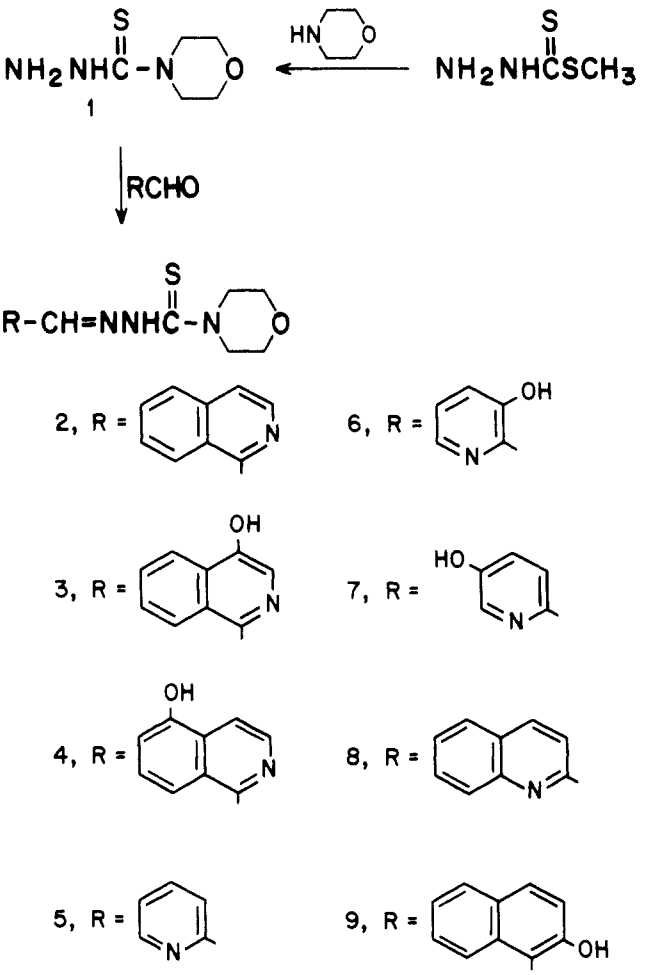

Table I

Compd	Crystn solvent	Mp, °C dec	Yield, %	Formula	Analyses
3	EtOH-H_2O	201-203	78	$C_{15}H_{16}N_4O_2S$	C, H, N
4	EtOH-H_2O	191	86	$C_{15}H_{16}N_4O_2S$	C, H, N
5	EtOH-EtOAc	150-151	74	$C_{11}H_{14}N_4OS$	C, H, N
6	EtOH-EtOAc	185-187	70	$C_{11}H_{14}N_4O_2S$	C, H, N
7	EtOH-H_2O	191-192	80	$C_{11}H_{14}N_4O_2S$	C, H, N
8	EtOH	151-152	88	$C_{15}H_{16}NOS$	C, H, N
9	EtOH	210-212	87	$C_{16}H_{17}N_3O_2S$	C, H, N

Table II. Effect of 4′-Diethyleneoxy Derivatives of α-(N)-Heterocyclic Carboxaldehyde Thiosemicarbazones on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells

Compd	Daily dosage, mg/kg[a]	Av wt, %[b]	Av survival time, days
Control		+14	14.3
3	7.5	−4	14.4
	10	−11	22.0
	20	−16	13.4
7	20	−2	17.6
	40	−19	19.0
	60	−20	6.0

[a] Administered once daily for 6 consecutive days, beginning 24 hr after tumor transplantation; each value represents results obtained with 5-20 animals. [b] Average weight change from onset to termination of drug treatment.

pounds with greater therapeutic indices.

It is known that introduction of a 4′-diethyleneoxy group in α-(N)-heterocyclic carboxaldehyde thiosemicarbazones results in derivatives that cause greater inhibition of the synthesis of RNA relative to DNA in Sarcoma 180 ascites cells than do the parent compounds;[13] this finding correlates with the observations that 4′-diethyleneoxy derivatives are considerably less potent as inhibitors of ribonucleotide reductase than unsubstituted α-(N)-heterocyclic carboxaldehyde thiosemicarbazones.‡ The pronounced inhibition of RNA synthesis in mammalian cells by 4′-diethyleneoxy compounds suggests that they may find potential use either in chemotherapy or as a biochemical tool to manipulate RNA synthesis.

Experimental Section§

4′-Diethyleneoxythiosemicarbazide (1). Methyl dithiocarbazate was prepared according to Audrieth, *et al.*,[19] by reacting potassium dithiocarbazate with CH_3I. Morpholine (16 ml, 0.18 mol) and 20 ml of H_2O were then added to 7.32 g (0.06 mol) of formed methyl dithiocarbazate. The solution was heated at 95° for 4 hr (CH_3SH is generated during the reaction). The solution was cooled and neutralized with AcOH. Needles of **1**, which were slightly pink in color, were filtered and recrystallized (EtOH, H_2O) to yield 4.4 g of white (46%) needles, mp 176–177°.

1-Methyl-4-benzoyloxyisoquinoline. To a solution of 1-methyl-4-hydroxyisoquinoline[18] (1.59 g, 0.01 mol) in 50 ml of THF was added 1.4 ml (0.01 mol) of Et_3N. An equimolar quantity of C_6H_5COCl (1.17 ml, 0.01 mol) was then added slowly with stirring. The stirring was continued for 30 min at room temperature and the mixture was filtered to remove $Et_3N \cdot HCl$. The filtrate was evaporated under vacuum and the residue was crystallized from hexane (Norit) to yield 2.0 g (76%), mp 91–92°. *Anal.* ($C_{17}H_{13}NO_2$) C, H, N.

4-Hydroxyisoquinoline-1-carboxaldehyde. To 2.63 g (0.01 mol) of 1-methyl-4-benzoyloxyisoquinoline in 50 ml of dioxane was added 1.11 g (0.01 mol) of freshly resublimed SeO_2 and the mixture was heated for 2 hr at 100°. It was then cooled and filtered through Celite and the filtrate evaporated under vacuum. To the residue was added 25 ml of 10% HCl and the solution was heated at 100° for 2 hr. The solution was cooled, filtered, and evaporated to dryness and the residue washed with ether. The HCl salt of 4-hydroxyisoquinoline-1-carboxaldehyde was utilized directly for the formation of derivatives.

4′-Diethyleneoxythiosemicarbazones. The 4′-diethyleneoxythiosemicarbazones were prepared by heating acidic solutions of corresponding aldehydes with a solution of an equimolar quantity of **1** in EtOH–H_2O. The solution was then neutralized with NaOAc, filtered, washed, and dried. Relevant data concerning these compounds are listed in Table I.

Antitumor Activity. Experiments were performed on CD-1 mice transplanted with Sarcoma 180 ascites cells. The experimental details were described earlier.[14] Mice were weighed during the course of the experiments and the percentage loss in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of the ascitic neoplasm to these agents was based on the prolongation of survival time afforded by the drug treatment.

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§Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ir absorption spectra were determined with a Perkin-Elmer Model 257 spectrophotometer and were consistent with the proposed structures. Elemental analyses were performed by Baron Consulting Co., Orange, Conn. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values.

Carcinogenic Activity of Benzofuran and Dibenzofuran Analogs of *p*-Dimethylaminoazobenzene

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Earlier reports from our laboratory have discussed the carcinogenic activity of a number of analogs of *p*-dimethylaminoazobenzene (Butter yellow). Among the rings (isomers indicated) replacing the unsubstituted benzene ring of this parent compound are pyridine and pyridine *N*-oxide (2-, 3-, 4-),[1,2] quinoline and quinoline *N*-oxide (2-, 3-, 4-, 5-, 6-, 7-, 8-),[3,4] quinoxaline (2-, 5-, 6-),[5] indazole (3-, 4-, 5-, 6-, 7-),[5] dibenzothiophene (1-, 2-, 3-, 4-),[6] benzimidazole (4-, 5-),[7] and benzthiazole[7] (2-, 4-, 5-, 6-, 7-).

In this paper we wish to report the preparation and testing for rat hepatocarcinogenic ability of the isomeric *p*-dimethylaminoazodibenzofurans and some of the isomeric *p*-dimethylaminoazobenzofurans. All of these azo compounds are new substances but all of them have been prepared by diazotization and coupling of the known aminodibenzofurans and aminobenzofurans with *N,N*-dimethylaniline. The 1-, 2-, and 4-aminodibenzofurans were prepared as described by Gilman and coworkers[8-10] with some modifications. The 3-aminodibenzofuran was prepared according to Cullinane.[11]

5- and 7-aminobenzofurans were prepared from purified 3- and 5-nitro-2-hydroxybenzaldehydes as obtained from the mixture given by the nitration of salicylaldehyde.[12] 6-Aminobenzofuran was prepared from 4-nitrosalicyaldehyde.[13] These were condensed with diethyl bromomalonate; the resulting ester was then hydrolyzed and decarboxylated to the corresponding nitrobenzofuran which was reduced with hydrogen using a Pd/C catalyst to give the desired aminobenzofurans. The isomeric *p*-dimethylaminoazodibenzofurans and benzofurans are listed in Table I.