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

Ellis V. Brown* and Ray L. Coleman

Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506. *Received December 4, 1972*

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.

Table I

Compound	Yield, %	Mp, °C[a]	Formula[a]
N,N-Dimethyl-*p*-(1-dibenzofuryl)aniline (1)	53	159–161	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(2-dibenzofuryl)aniline (2)	60	214–215	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(3-dibenzofuryl)aniline (3)	60	256–258	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(4-dibenzofuryl)aniline (4)	90	160–161	$C_{20}H_{17}N_3O$
N,N-Dimethyl-*p*-(5-benzofuryl)aniline (5)	72	136–137	$C_{16}H_{15}N_3O$
N,N-Dimethyl-*p*-(6-benzofuryl)aniline (6)	85	127–128	$C_{16}H_{15}N_3O$
N,N-Dimethyl-*p*-(7-benzofuryl)aniline (7)	69	134–136	$C_{16}H_{15}N_3O$

[a] All melting points were determined on a Fisher-Johns apparatus and are uncorrected. Where analyses are indicated only by the symbols of the elements, analytical results obtained for carbon and hydrogen were within ±0.4% of the theoretical values.

Experimental Section

1-Aminodibenzofuran. 4-Aminodibenzofuran was synthesized according to Gilman[8] in 50% yield and acetylated in 80% yield. Bromination gave a quantitative yield of the 1-bromo compound which was hydrolyzed and converted to 1-bromodibenzofuran.[8] This material (7 g) was heated to 210° for 24 hr with cuprous bromide and ammonium hydroxide to give 6.5 g of the 1-aminodibenzofuran hydrochloride after treatment of the ether extraction of the autoclave reaction product with dry HCl. The free amine melted at 74°.[8]

2-Aminodibenzofuran. This compound was prepared from 2-iododibenzofuran by heating with ammonium hydroxide as above. The yield was 96% melting at 127°.[14]

3-Aminodibenzofuran. This compound was prepared by catalytic hydrogenation of 3-nitrodibenzofuran.

4-Aminodibenzofuran. This material was prepared in 50% yield, melting at 85°.[10]

5-Aminobenzofuran. Reduction of 5-nitrobenzofuran[15] with 40 psi of hydrogen using 10% Pd/C catalyst gave a quantitative yield of this amine as the hydrochloride melting at 253–255°.

6-Aminobenzofuran. 6-Nitrodibenzofuran[16] was reduced in the above manner and obtained as the hydrochloride salt.[17]

7-Aminobenzofuran. This amine was obtained as the hydrochloride melting at 240–250° by hydrogenation in the above manner of 7-nitrobenzofuran.[15]

N,N-Dimethyl-*p*-(*x*-dibenzofuryl)anilines. A typical procedure for all of these isomers is given. 4-Aminodibenzofuran (18.3 g, 0.10 mol) as the hydrochloride (21.9 g, 0.10 mol) was dissolved in warm water (200 ml). Concentrated hydrochloric acid (17 ml, 0.20 mol) was added and the mixture was quickly cooled by ice addition. Sodium nitrite (7.6 g, 0.11 mol) dissolved in water (50 ml) was added quickly with good stirring, and the stirring was continued for 30 min. Sulfamic acid was then added until a negative test with starch-iodide paper was observed. Sodium acetate (as a powder) was added to adjust the pH to 6–8. The mixture was filtered and transferred to a beaker, and *N,N*-dimethylaniline (14 ml, 0.11 mol) in ethanol (50 ml) was added in a steady stream with good stirring. Coupling occurred immediately and, after allowing the reaction to continue for 30 min, the solid was removed by filtration and water washed. The dried filter cake was dissolved in benzene (hot) and on cooling and filtering the 4-(*p*-*N,N*-dimethylaminophenylazo)dibenzofuran was obtained in 90% yield (29 g) (see Table I).

N,N-Dimethyl-*p*-(*x*-dibenzofuryl)anilines. These azo compounds were made in a similar manner to the dibenzofuran products above and are reported in Table I.

Biological Properties. Young male rats of the Sprague–Dawley strain, approximately 8 weeks of age and weighing 150–200 g, were distributed as equally as possible in initial body weight into groups of ten animals each. Each group was fed a diet, patterned after the "low protein, low riboflavin" diet of Miller, *et al.*,[12] to which had been added one of the azo compounds at a level of 0.03%. The composition of the basal diet per kilogram was as follows: crude casein, 120 g; cerelose, 770 g; Osborne and Mendel salt mixture, 40 g; corn oil, 50 g; Vitab (rice bran concentrate, obtained from Charles Bowman Co.), 20 g; riboflavin, 0.5 mg; vitamin A palmitate 67,500 IU.

In each experiment, groups received DAB at the 0.06 as well as at the 0.03% level. The control group received only the basal diet. All of the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment.

Typical Pathologist's Reports. 5. Regenerating nodules of hepatic parenchymal cells entrapped by wide bands of proliferating and invasive bile duct and cholangiolar elements, the latter showing marked focal anaplasia. Diagnosis: bile duct carcinoma.

7. Marked proliferation of cholangiolar and bile duct epithelium, widely invading the hepatic parenchyma and displacing hepatic cells. Tumor forms duct-like and glandular structures. Diagnosis: bile duct carcinoma.

Results and Discussion

In the biological evaluation 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 0.03% level it gave 5/10 in 6 months. Our most active compound, 7, at the 0.03% level gave 10/10 tumors in 3 months. 5 gave 10/10 tumors at 4 months at the 0.06% level and 6 gave no tumors at 6 months at the 0.06% level. The order of their carcinogenicity is 7 >> 5 >> 6 and all the dibenzofuran analogs were inactive at 8 months and the 0.06% level.

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Stereoselectivity of Cholinergic Activity in a Series of 1,3-Dioxolanes

K.-J. Chang and D. J. Triggle*

Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214.
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We have previously described[1] the utility of the 1,3-dioxolane structure as a basic nucleus of potent agonists and antagonists active at the muscarinic acetylcholine receptors of smooth muscle. 2-Methyl-4-dimethylaminomethyl-1,3-