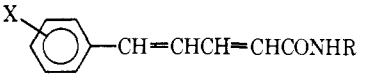
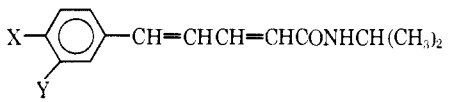
TABLE I
5-PHENYL-2,4-PENTADIENAMIDES



No.	X	R	Mp, °C[a]	Yield purified, %	Reaction cond[a]	Purifica solvent	Formula	Analyses[b]
1	H	$NHCSNH_2$	220–221	9	A	EtOH–H_2O	$C_{12}H_{13}N_3OS$	C, H, N
2	H	$N(CH_3)OCH_3$	68–69	25	A	EtOH–H_2O	$C_{13}H_{15}NO_2$	C, H, N
3	H	$CH_2CH_2N(C_2H_5)_2$	164–165	87	B	*i*-PrOH	$C_{17}H_{24}N_2O \cdot C_7H_6O_3$[b]	C, H, N
4	H	$N(CH_2CO_2C_2H_5)COCH{=}CHCH{=}CHC_6H_5$	198–199	8[d]	A	EtOH–H_2O	$C_{25}H_{29}N_3O_7$	H, N; C[c]
5	H	$N[CH_2CH_2N(C_2H_5)_2]COCH{=}CHCH{=}CHC_6H_5$	190–192	6[d]	A	MeCN	$C_{28}H_{33}N_3O_2$	C, H, N
6	3,4-Cl_2	$NHCOCH_3$	260–262	26	C	DMF–H_2O	$C_{23}H_{22}Cl_2N_2O_2$	C, H, N
7	3,4-Cl_2	$NHCO_2C_2H_5$	190–192	16	D	EtOH–H_2O	$C_{24}H_{24}Cl_2N_2O_3 \cdot 0.5H_2O$	C, H, N; Cl[e]
8	3,4-Cl_2	$NHSO_2C_6H_4$-*p*-CH_3	209–210	16	E	EtOH	$C_{26}H_{24}Cl_2N_2O_3S$	C, H, N
9	3,4-Cl_2	$N(CH_2CH_2OH)COCH{=}CHCH{=}CHC_6H_3$-3,4-$Cl_2$	196–198	21[f]	B	...	$C_{29}H_{29}Cl_3N_2O_3$	H, N; C[c]
10	3,4-Cl_2	$(CH_3)_2NHCOCH{=}CHCH{=}CHC_6H_3$-3,4-$Cl_2$	211–213	15	C	EtOH–H_2O	$C_{26}H_{24}Cl_4N_2O_2$	C, H, N

[a] A, pyridine at room temperature for 1–3 days; B, $CHCl_3$ at room temperature for 1–2 days; C, C_6H_6 at room temperature for 1–3 days; D, C_6H_6 under reflux for 3 hr; E, THF under reflux for 16 hr. [b] $C_7H_6O_4$ = 2,4-dihydroxybenzoic acid. [c] C: calcd, 71.75; found, 72.16. [d] Absence of an exchangeable proton determined *via* infrared spectra in $CHCl_3$–D_2O allows assignment as the N,N′ rather than the N,N derivative. [e] C: calcd, 54.77; found, 54.30. [f] Absence of carbonyl absorption above 1660 cm^{-1} and the presence of only one labile proton (D_2 exchange) allow assignment of the N,N′ structure. [g] Melting points (corrected) were taken in open capillary tubes in a Thomas–Hoover capillary melting point apparatus. [h] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [i] Cl: calcd, 20.97; found, 21.08.

various 5-phenyl-2,4-pentadienamides for antimalarial evaluation.[3] Surprisingly, none of them, including Ia, was active against normal strains of *Plasmodium berghei* in mice.[3] Subsequently, the pentadienamides Ia–e have been evaluated against *P. gallinaceum* in the chick.[4] Using 9–12-day-old chicks and a standard



Ia, X = Cl; Y = H
b, X = Cl; Y = Cl
c, X = CH_3; Y = H
d, X = Br; Y = H
e, X = C_6H_5; Y = H

inoculum of *P. gallinaceum*, a consistently uniform disease, fatal to 100% of the untreated control birds within 72–96 hr, was produced. In this test, as in the mouse test,[5,6] the antimalarial activity of candidate substances was assessed by comparing the maximum survival times of treated and untreated animals. None of the pentadienamides (Ia–e) exhibited activity against *P. gallinaceum* when administered in a single subcutaneous dose of 240 mg/kg. The apparent discrepancy between earlier reports[2] and results of the current investigations remains unexplained.

Before it was confirmed that these materials lacked appreciable effects against *P. berghei* and *P. gallinaceum*, it was deemed of interest to vary the nitrogen functionality in this system. The derivatives described in Table I were prepared by condensation of a 5-phenyl-2,4-pentadienoic acid chloride with the desired amine or hydrazine derivative under known conditions. None was active against normal strains of *P. berghei* when administered to mice in a single subcutaneous dose of 640 mg/kg.[5,6]

(3) L. M. Werbel, N. Headen, and E. F. Elslager, *J. Med. Chem.*, **10**, 366 (1967).

(4) Antimalarial studies utilizing *P. gallinaceum* in chicks were carried out under the auspices of the Walter Reed Army Institute of Research, and test results were supplied through the courtesy of Dr. David P. Jacobus.

(5) Antimalarial screening against *P. berghei* was carried out by Dr. Leo Rane of the University of Miami, and test results were supplied through the courtesy of Dr. David P. Jacobus of the Walter Reed Army Institute of Research.

(6) For a description of the test method see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

Carcinogenic Activity of Analogs of *p*-Dimethylaminoazobenzene. VI. Activity of the Benzimidazole and Benzthiazole Analogs[1]

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In previous reports[2,3] we have shown that the unsubstituted ring of *p*-dimethylaminoazobenzene (DAB) can be replaced by pyridine and pyridine N-oxide and thus we have obtained a number of compounds with varying degrees of carcinogenic activity. The interesting results observed in the pyridine series led us to investigate the isomeric *p*-dimethylaminophenylazoquinolines and their corresponding N-oxides.[4] In this new series, we have the possibility of attaching the azo linkage to either the pyridine or benzene rings of the quinoline nucleus and thereby preparing compounds which can be considered pyrido analogs of DAB or benzo analogs of the previously prepared pyridine azo compounds. As might have been anticipated from the results obtained in the pyridine series, the 4-substituted isomer was the most active of the compounds substituted on the pyridine side of the quinoline nucleus. However, the high activity of the 5- and 6-substituted compounds was quite surprising and in contrast to the lack of activity in the 7- and 8-substituted compounds.

In this paper we wish to report the preparation and testing for carcinogenic activity of a number of *p*-dimethylaminophenylazobenzimidazoles and -benzthiazoles. N,N-Dimethyl-*p*-(4-benzimidazolylazo)aniline and N,N-dimethyl-*p*-(5-benzimidazolylazo)aniline have been prepared by Montanari.[5] So far we have been

(1) Presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968.

(2) E. V. Brown, *et al.*, *Cancer Res.*, **14**, 22 (1954).

(3) E. V. Brown, *et al.*, *ibid.*, **14**, 715 (1954).

(4) E. V. Brown, R. M. Novack, and A. A. Hamdan, *J. Natl. Cancer Inst.*, **26**, 1461 (1961).

(5) F. Montanari, *Boll. Sci. Fac. Chim. Ind. Bologna*, **11**, 4066 (1953).

TABLE I

Compd	Code	Mp, °C	Formula
p-Dimethylaminoazobenzene	DAB	*a*	*a*
3′-Methyl-*p*-dimethylaminoazobenzene	3′-MeDAB	*a*	*a*
N,N-Dimethyl-*p*-(2-benzthiazolylazo)aniline	BT-2	130–132	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(4-benzthiazolylazo)aniline	BT-4	209	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(5-benzthiazolylazo)aniline	BT-5	171	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(6-benzthiazolylazo)aniline	BT-6	159	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(7-benzthiazolylazo)aniline	BT-7	150–151	$C_{15}H_{14}N_4S$
N,N-Dimethyl-*p*-(4-benzimidazolylazo)aniline	BI-4	215–216[b]	*b*
N,N-Dimethyl-*p*-(5-benzimidazolylazo)aniline	BI-5	215[b]	*b*

[a] See ref 6. [b] See ref 4.

unable to prepare N,N-dimethyl-*p*-(2-benzimidazolylazo)aniline from 2-aminobenzimidazole either by the normal diazotization and coupling or the procedure of Brown and Faessinger.[6]

The 2-, 4-, 5-, 6-, and 7-benzthiazole analogs of DAB were all prepared from the corresponding amines and their melting points and formula are listed in Table I.

Experimental Section

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.

4-Aminobenzimidazole was prepared according to van der Want.[7] Both this compound and 5-aminobenzimidazole were diazotized and coupled with N,N-dimethylaniline using the procedure reported by Montanari[5] to produce the benzimidazole dyes previously reported. This method and several minor modifications were then applied to 2-aminobenzimidazole but we did not obtain any dye. We then applied the sodium coupling with *p*-nitrosodimethylaniline as applied successfully to other 2-amino heterocycles by Brown and Faessinger[6] but again there was no evidence of azo dye formation.

4-, 6-, and 7-nitrobenzthiazoles were separated from the mixture formed on nitration of benzthiazole.[8,9] 7-Nitrobenzthiazole was also prepared by the method of Ward.[10] 5-Aminobenzthiazole was obtained according to Spieler.[11] All the nitrobenzthiazoles were reduced in the usual manner with $SnCl_2$–HCl. The azo compounds were prepared by diazotization and coupling of these amines. A typical procedure is given below and the various dyes prepared are listed with melting point and formula in Table I.

N,N-Dimethyl-*p*-(4-benzthiazolylazo)aniline.—4-Aminobenzthiazole (13.4 g) was diazotized in 14 ml of concentrated HCl and 150 ml of H_2O at 0–5° with 6.3 g of $NaNO_2$. Excess nitrite was destroyed after 1 hr by addition of urea, and coupling with 10.8 g of N,N-dimethylaniline and 12 g of anhydrous NaOAc in 100 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 to give 19.8 g of crude azo compound. This was dissolved in 1500 ml of C_6H_6 and chromatographed on alumina. The red fraction eluted by C_6H_6 was concentrated and recrystallized from EtOH. See 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.

Results and Discussion

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. On the other hand, 3′-MeDAB at 0.03% gave 5/10 in 4 months and 9/10 in 6 months. Our most active compound, BT-6, at 0.03% gave 5/10 in 1 month and 10/10 in 2 months. BI-4 gave 10/10 in 2 months at 0.03%, while BT-7 gave 10/10 in 3 months at this level. BT-2 at 0.06% and BT-4, BT-5, and BI-4 at 0.03% gave no tumors in 6 months at which time the experiment was terminated. The order of their carcinogenicity is BT-6 > BI-4 > BT-7 > 3′-MeDAB > DAB > BI-5, BT-4, BT-5, BT-2. The first two compounds mentioned, which produced multiple tumor nodules verified macroscopically and microscopically in 2 months or less, are certainly among the most powerful rat hepatocarcinogens ever reported.

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.

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(7) G. M. van der Want, *Rec. Trav. Chim.*, **67**, 45 (1948).
(8) R. P. Veltman, *J. Gen. Chem. USSR*, **30**, 1394 (1960).
(9) E. R. Ward and W. H. Poesche, *J. Chem. Soc.*, 2825 (1961).
(10) E. R. Ward and C. H. Williams, *ibid.*, 2248 (1965).
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(12) J. A. Miller and E. C. Miller, *Advan. Cancer Res.* **1**, 339 (1953).

Phosphorus–Nitrogen Compounds. X.[1,2] Sulfur Analogs

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Since 4,4,4′′-phosphinylidynetrissemicarbazide[2] (I) has shown confirmed activity against one tumor test system[3] it was advisable to prepare a related sulfur-con-

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(2) For the previous paper of this series see L. A. Cates, *J. Med. Chem.*, **11**, 382 (1968).

(3) Against Walker 256 carcinoma (im). Data from CCNSC. Confirmation defined according to *Cancer Chemotherapy Rept.*, **25**, 1 (1962).