content[30] of hepatic microsomes were measured in induction experiments in order to verify that the expected response to 3MC and PB was elicited.

Acknowledgment. We thank Gary Myers for excellent technical assistance and Dr. Mike Conlon and Ned Phillips for assistance with the multiple regression analysis. This research was supported by an NIH biomedical research grant to the College of Pharmacy.

Synthesis and Antineoplastic Evaluations of 5,8-Bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones

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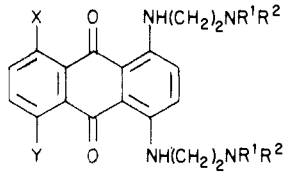
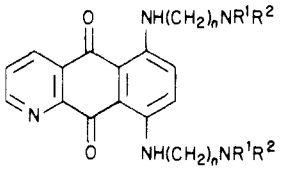
Several 5,8-bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones have been synthesized and evaluated for antitumor activity against L1210 leukemia both in vitro and in vivo. Comparisons are made to the corresponding carbocyclic analogues. One of the aza analogues showed modest in vivo activity.

Recently the synthesis and antineoplastic evaluation of a number of symmetrical 1,4-bis[(aminoalkyl)amino]-anthracene-9,10-diones related to **1** have been reported.[1]



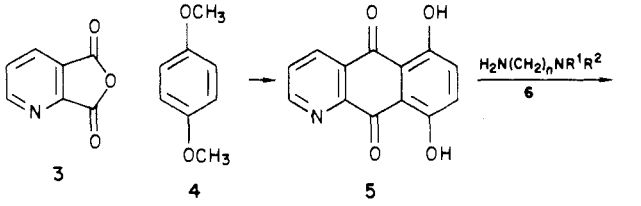


1a, X = Y = R^1 = H; R^2 = $(CH_2)_2OH$
b, X = Y = OH; R^1 = H; R^2 = $(CH_2)_2OH$
c, X = Y = H; R^1 = R^2 = CH_3
d, X = Y = H; R^1 = R^2 = CH_2CH_3

2

Compounds **1a** (AQ, ametantrone) and **1b** (mitoxantrone, DHAQ or DHAD) are representative of this relatively new class of antineoplastic compounds. In particular, **1b** has shown outstanding activity and is in clinical trials.[1c,d,h] The cardiotoxicities of both AQ and DHAQ have been studied, and AQ is reported to be 10-fold less toxic than DHAQ in a rat cardiotoxic model system.[2] It has also been reported that DHAQ on intraperitoneal administration to non-tumor-bearing mice caused a delayed lethality while **1a** (AQ) did not.[3] The 5,8-dihydroxy groups were suggested as being implicated in this lethality since **1a** did not cause delayed deaths. The myocardial effects of DHAQ and doxorubicin have recently been suggested as being quite similar in the mouse and guinea pig.[4] The search for new analogues with antitumor activity but without the undesirable cardiotoxic effects is of extreme interest.

Scheme I



3 **4** **5** **2**

a, n = 2; R^1 = R^2 = CH_3
b, n = 2; R^1 = R^2 = CH_2CH_3
c, n = 3; R^1 = R^2 = CH_3
d, n = 2 R^1 = H; R^2 = $COCH_3$

Table I. Activity of Azaanthracene-9,10-diones and Their Carbocyclic Analogues against L1210 Cells in Vitro

compd	ID_{50}, μg/mL	compd	ID_{50}, μg/mL
5	0.18	**2d**	>10
2a	0.16	**1c**	0.08
2b	2.4	**1d**	0.30
2c	1.7		

As part of a drug development program dealing with the structure–activity relationships of anthracene-9,10-diones and the preparation of less cardiotoxic compounds, we have explored the synthesis of heterocyclic analogues **2**, related to **1** in which a CH group is replaced by a nitrogen atom. It was anticipated that the ease of electron addition to an azaanthracene-9,10-dione would be more facile than the corresponding anthracene-9,10-dione. In addition, the heterocyclic models would probably be more soluble in aqueous media and more readily metabolized and excreted from the body.

A French group has recently reported that the substitution of a nitrogen atom for an aromatic CH group in an [(aminoalkyl)amino]ellipticine increased the antitumor activity for various neoplasms.[5] Several recent papers have recently appeared describing the synthesis of azaanthracene-9,10-diones that might be useful in the preparations of analogues for biological evaluation.[6] In

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(7) Raudnitz, H. *Chem. Ber.* **1929**, *62*, 938.

0022-2623/85/1828-1124$01.50/0

Table II. Activity of Substituted Azaanthracene-9,10-diones and Carbocyclic Models against L1210 Cells in Vivo

compd	dose, mg/kg	% T/C
5	100	inactive
2a	50	130
	25	120
2b	100	toxic[a]
1c	30	150[b]
	15	117

[a] These animals had a weight loss, on the average, of 2 g by day 5 of the experiment. No weight loss was observed at day 5 for other animals during the experiment. [b] Data from ref 1e.

this paper we report the synthesis and our preliminary biological studies for several 5,8-bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones **2**.

Chemistry. The synthetic procedure utilized for the preparation of compounds **2** is summarized in Scheme I.

Treatment of 2,3-pyridinedicarboxylic anhydride (**3**) with 1,4-dimethoxybenzene (**4**) in an $AlCl_3$–NaCl melt led to 5,8-dihydroxy-1-azaanthracene-9,10-dione (**5**). On heating the azaanthracenedione **5** with the appropriate diamines **6** in an aqueous medium, the 5,8-bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones **2a–c** were formed. Chromatographic purification of the crude reaction mixtures led to these products in 9–16% yields (no attempts were made to optimize the yields). On heating **5** with *N*-acetylethylenediamine in an aqueous medium the bisamide **2d** was obtained.

The carbocyclic comparative models **1c**[1e] and **1d**[1g] were prepared from quinizarin (1,4-dihydroxyanthracene-9,10-dione) and the appropriate diamines following literature procedures.

Biological Studies and Discussion

The compounds reported here have been evaluated as growth inhibitors of murine leukemic cells (L1210). These data are tabulated in Table I.

Several of the compounds were evaluated further for their ability to prolong the life span of mice innoculated with L1210 (Table II).

In the comparison of the 1-azaanthracene-9,10-diones **2a** and **2c**, in vitro activity is dependent on the length of the side chain. In addition, the comparison of **2a** and **2d** shows that the basicity of the nitrogen is also important.

In the comparison of the aza analogues to the carbocyclic 9,10-diones, **2a** and **1c** have comparable in vitro activity while **2b** is less active than **1d**.

The data suggest that the presence of the nitrogen atom in **2a** does not dramatically change the in vitro or in vivo activity in the comparison to the carbocyclic derivative **1c**.

Further synthetic studies are in progress, which will lead to different positional isomers of the azaanthracene-9,10-diones.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR was run on a Bruker WM-250 pulsed Fourier transform NMR spectrometer. TLC precoated silica gel plates (Eastman chromagram sheets with fluorescent indicator) were used to monitor reactions. For column chromatography Baker analyzed 80–200-mesh silica gel was utilized. Microanalyses were performed by Robertson Laboratories, Florham Park, NJ. Mass spectra were run on a Finnigan MAT 4610 mass spectrometer. All amines and other starting materials were obtained from Aldrich.

Synthesis. 5,8-Dihydroxy-1-azaanthracene-9,10-dione (5).[7,8] An intimately ground mixture of *p*-dimethoxybenzene (4; 2.76 g, 0.02 mol) and 2,3-pyridinedicarboxylic anhydride (3; 3.00 g, 0.02 mol) is added in portions to a magnetically stirred melt of $AlCl_3$(25 g) and NaCl (5 g) at 180 °C. When the addition is complete, the temperature of the reaction mixture is raised to 200–220 °C and held there for 10 min. The hot reaction mixture is then cautiously decomposed with 100 mL of cold water. It will be necessary to immerse the reaction vessel in an ice water bath several times during this operation. The resultant purple solution is made strongly basic with 20% NaOH. The blackish precipitate is collected by filtration and dried overnight at 80 °C.

The dried precipitate is treated with 10 mL of concentrated H_2SO_4. The mixture is diluted to 100 mL with H_2O and extracted well with toluene. An emulsion that forms during the first extraction is broken by vacuum filtration through coarse filter paper. The toluene extracts are dried over Na_2SO_4 and concentrated to give **5** (1 g, 20%) as dark red needles: mp 230–232 °C (lit.[7] mp 237 °C); NMR ($CDCl_3$) δ 12.91 (s, 1 H), 12.72 (s, 1 H), 9.17 (m, 1 H), 8.70 (m, 1 H), 7.79 (m, 1 H), 7.39 (s, 2 H): MS, *m/e* (relative intensity) 241 (100).

5,8-Bis[[2-(dimethylamino)ethyl]amino]-1-azaanthracene-9,10-dione (2a). A mixture of **5** (0.200 g, 0.83 mmol), *N,N*-dimethylethylenediamine (0.380 g, 4.31 mmol), and H_2O (8 mL) is refluxed for 15 min. The cooled reaction mixture is diluted to 50 mL with H_2O and thrice extracted with CH_2Cl_2 (50 mL). The extracts are dried over Na_2SO_4 and concentrated under vacuum. The crude residue is chromatographed (silica gel, 4:1 CH_2Cl_2/CH_3OH) and the major blue band is cut out and crystallized from ligroin (100–115 °C). This gives **2a** (0.051 g, 16%) as fine blue needles: mp 167 °C; NMR ($CDCl_3$) δ 11.20 (t, 1 H), 10.85 (t, 1 H), 9.02 (dd, 1 H), 8.69 (dd, 1 H), 7.60 (dd, 1 H), 7.30 (s, 2 H), 3.54 (dt, 4 H), 2.69 (td, 4 H), 2.35 (s, 12 H); MS, *m/e* (relative intensity) 58 (100), 381 (0.39). Anal. Calcd for $C_{21}H_{27}N_5O_2$: C, 66.12; H, 7.13; N, 18.36. Found: C, 66.04; H, 7.23; N, 18.42.

5,8-Bis[[2-(diethylamino)ethyl]amino]-1-azaanthracene-9,10-dione (2b). A mixture of **5** (0.200 g, 0.83 mmol), *N,N*-diethylethylenediamine (0.533 g, 4.60 mmol), and H_2O (8 mL) is refluxed for 8 h with stirring. The cooled reaction mixture is diluted to 50 mL with water and gravity filtered. The tarry precipitate is chromatographed on silica gel. The starting material and monosubstitution products are eluted with acetone and the disubstituted product with 9:1 CH_2Cl_2/triethylamine. The blue, disubstituted product is crystallized from ligroin (100–115 °C) to give **2b** (32 mg, 9%) as bluish-black needles: mp 141–143 °C; NMR ($CDCl_3$) δ 11.15 (t, 1 H), 10.88 (t, 1 H), 9.03 (dd, 1 H), 8.69 (dd, 1 H), 7.61 (dd, 1 H), 7.32 (s, 2 H), 3.52 (dt, 4 H), 2.81 (dt, 4 H), 2.64 (m, 8 H), 1.10 (dt, 12 H); MS, *m/e* (relative intensity) 86 (100), 437 (2.8). Anal. Calcd for $C_{25}H_{35}N_5O_2$: C, 68.62; H, 8.06; N, 16.00. Found: C, 68.91; H, 8.04; N, 16.04.

5,8-Bis[[3-(dimethylamino)propyl]amino]-1-azaanthracene-9,10-dione (2c).[9] A mixture of **5** (0.200 g, 0.83 mmol), 3-(dimethylamino)propylamine (0.424 g, 4.2 mmol), and water (8 mL) is stirred under reflux for 30 min and then poured into saturated brine (10 mol). The aqueous portion is extracted exhaustively with methylene chloride. The extracts are dried over sodium sulfate and concentrated. The residue is chromatographed on silica gel and eluted with chloroform and 10%, 20%, and finally 25% methanol in chloroform. Collection of the major blue band gives **2c** (0.39 g, 11%) as a blue solid, which is recrystallized from high-boiling ligroin to give blue needles melting at 82–84 °C: NMR ($CDCl_3$) δ 11.30 (br t, 1 H), 10.97 (br t, 1 H), 9.03 (m, 1 H), 8.69 (dd, 1 H), 7.62 (m, 1 H), 7.37 (s, 2 H), 3.53 (m, 4 H), 2.47 (m, 4 H), 2.26 (s, 12 H), 1.93 (m, 4 H); MS, *m/e* (relative intensity) 58 (100), 409 (2.0).

5,8-Bis[(2-acetamidoethyl)amino]-1-azaanthracene-9,10-dione (2d). A mixture of **5** (0.50 g, 2.07 mmol), *N*-acetylethylenediamine (1.1 g, 10.8 mmol), and water (20 mL) is stirred under reflux for 45 min and then poured into saturated NaCl solution (75 mL). The precipitated blue solid is filtered off and dried in air overnight. It is then dissolved in methanol and adsorbed onto silica gel, which is placed at the head of a column of silica gel packed in chloroform. The product is eluted first with 5% and then 10% methanol in chloroform. The major blue band

(8) The IUPAC name is 6,9-dihydroxybenzo[*g*]quinoline-5,10-dione. We use the common name, 5,8-dihydroxy-1-azaanthracene-9,10-dione.

(9) Clairol Patent; Brit. 1 134 493 (Cl. D 06p), 27 Nov 1968, U.S. Appl. 12 May 1966; *Chem. Abstr.* **1969**, *70*, 57682y.

is collected to give **2d** (0.37 g, 44%). Recrystallization from chloroform–methanol affords dark blue crystals melting at 232–233 °C; NMR ($CDCl_3$–Me_2SO-d_6) δ 11.3 (br t, 1 H), 10.93 (br t, 1 H), 9.01 (m, 1 H), 8.62 (dd, 1 H), 8.20 (s, 2 H), 8.14 (br s, 2 H), 7.75 (m, 1 H), 3.59 (m, 4 H), 3.37 (m, 10 H); MS, *m/e* (relative intensity) 59 (100), 409 (3.0).

Biological Studies. In Vitro Cytotoxicity Evaluation. L1210 murine leukemia cells are routinely maintained as suspension cultures in McCoy's 5A medium supplemented with 10% horse serum, glutamine, penicillin, and streptomycin and grown in an humidified environment of 10% CO_2, 90% air at 37 °C. To assess the in vitro toxicity, each compound was dissolved in dimethyl sulfoxide (Me_2SO) and added to 4 mL of L1210 cells (10^5 cells/tube) to attain final concentrations of 0.01, 0.1, and 10 μg of drug/mL of culture. Each drug concentration was tested in triplicate and in no case was more than 40 μL of Me_2SO added to a given culture. After 96 h of continuous exposure to the drug, the cell concentration was determined with a Coulter counter (Model ZBF, Hialeah, FL). Growth inhibition was calculated for each drug concentration with use of the following formula:

$$\% \text{ growth inhibition} = \left(1 - \frac{\text{cell number treated}}{\text{cell number } Me_2SO \text{ alone}}\right) \times 100$$

The growth inhibition data was then used to calculate the ID_{50} value (the calculated drug concentration required to inhibit cell growth by 50% of control). A compound having an ID_{50} value in excess of 10 μg/mL was considered to be inactive in our system.

In Vivo Efficacy Studies. L1210 murine leukemia cells are maintained in vivo by several intraperitoneal injections of 10^6 cells to BDF_1 mice on a weekly basis. For test purposes, mice were inoculated ip with 10^6 cells and drugs were administered as a single ip injection approximately 24 h later. Prior to injection, the drug suspended in 10% (hydroxypropyl)cellulose solution at a concentration such that 0.1 mL/10 g of body weight delivered the desired dose. Mice were observed daily for signs of toxicity and survival. When all mice had died the mean survival time (MST) for each treatment group (six mice/group) was calculated and the percent T/C determined using the following formula:

$$\% \text{ T/C} = [(\text{MST treated})/(\text{MST control})] \times 100$$

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Registry No. 2a, 96706-35-3; **2b**, 96706-34-2; **2c**, 21742-76-7; **2d**, 96706-36-4; **3**, 699-98-9; **4**, 150-78-7; **5**, 3712-11-6; *N,N*-diethylethylenediamine, 100-36-7; *N,N*-dimethylethylenediamine, 108-00-9; 3-(dimethylamino)propylamine, 109-55-7; *N*-acetylethylenediamine, 1001-53-2.

Book Reviews

Biochemical and Clinical Aspects of Pteridines. Volume 3. Cancer, Immunology, Metabolic Diseases. Edited by W. Pfleiderer, H. Wachter, and H. Ch. Curtius. Walter de Gruyter, Berlin and New York. 1984. xii + 541 pp. 17 × 24 cm. ISBN 3-11-010163-7. $100.00.

Pteridines such as tetrahydrobiopterin and neopterin are compounds of great interest and the organizers are to be commended for bringing chemists, biochemists, pharmacologists, and clinicians together yearly in St. Christoph, Arlberg, Austria. Volume 3 is mostly an update of the same areas covered in volumes 1 and 2 (volume 2 is reviewed in *J. Med. Chem.* 1984, *27*, 1375). Tetrahydrobiopterin is a cofactor for the hydroxylation of phenylalanine, tyrosine, and tryptophan and is thus an essential component in the biosynthesis of catecholamine neurohormones and serotonin. New insight into tetrahydrobiopterin synthesis from GTP is presented by Nichol et al. Sepiapterin can no longer be considered an obligatory intermediate on this pathway which can also be carried out through labile pteridine intermediates. GTP cyclohydrolase, the first enzyme in the conversion of GTP to pteridines, is well reviewed by Blau and Niederwieser and chemical aspects of tetrahydrobiopterin biosynthesis are concisely presented by Ghisla et al. Some of the other topics covered are as follows: (1) tetrahydrobiopterin metabolism in the central nervous system (Blair et al.), (2) photodecomposition of pteridines (Pfleiderer et al.), (3) chemical synthesis of biopterin (Viscontini), (4) the effect of pteridines on (a) the response of the pineal gland to light (Ebels et al.), (b) neurosecretory cells in caterpillars (L'Helias et al.), and (c) aggregation in slime molds (Tatischeff et al.). There are four papers covering HPLC and electrochemical detection of pteridines, four papers on pteridines in immunology, and 19 papers on pteridine excretion in cancer and other diseases. It is reported that T-lymphocytes stimulate macrophages to produce neopterin, a process that can be mediated by γ-interferon (Huber et al.). Although neopterin production is correlated with stimulation of the immune system, a patient lacking GTP cyclohydrolase, and thus unable to synthesize pteridines, has a normal immune response (Blau and Niederwieser). The significance of neopterin production is not clear. Mouse lymphocytes produce biopterin, hydroxymethylpterin, and formylpterin on activation by lectin (Ziegler). Neopterin was not detectable in the mouse system. Urinary neopterin is proposed as a marker for many diseases including rheumatoid arthritis, coeliac disease, ulcerative colitis, gastrointestinal carcinoma, testicle tumors ("testicle" is repeatedly misspelled "testical"), bladder cancer, lung cancer, malaria, leprosy as well as a screening test for potential blood donors. The preface states that the diagnostic value of neopterin may be regarded as a milestone in medical research. The data presented are not convincing. Regrettably, in giving authors freedom to speculate as well as to publish preliminary results, the editors of this volume go beyond acceptable limits. The following quote from the paper of Fuchs et al. titled "Urinary Neopterin Evaluation in Risk Groups for the Acquired Immunodeficiency Syndrome (AIDS)" is scientifically unsound and should be retracted because it deals with a sensitive social issue about which rumors and hysteria abound (J. W. Curran, *N. Engl. J. Med.* 1983, *309*, 609): "The reason of elevated neopterin in four out of five healthy Haitians is not easily explained, it may be a result of Voodoo-rituals common in population of Haiti. Nevertheless, neopterin elevation seems to be a sign of the special susceptibility of Haitians to develop AIDS." No documentation is given for the suggestion that Haitians have a special susceptibility to develop AIDS and, according to the literature (see J. W. Curran, loc. cit.), there is no such documentation. Errors in spelling and grammar are common throughout this book. Some sections are impossible to follow, for example (p 295), "On the other hand due to the possibility of likewise alterations of biopterin and neopterin the neopterin/biopterin ratios could be misinterpreted if only ratios differing to the normal values are attributed to disorders or malignant conditions respectively." The pteridine field deserves, and good science requires, more thoughtful, thoroughly edited and thoroughly documented work than is found in all too many of the papers included in this workshop.

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Progress in Tryptophan and Serotonin Research. Edited by H. G. Schlosberger, W. Kochen, B. Linzen, and H. Steinhart. Walter de Gruyter, Berlin and New York. 1984. xix + 889 pp. 17 × 24 cm. ISBN 3-11-009760-5. $73.00.

This book represents the proceedings of an international symposium on tryptophan research that was held in Martinsried,