Synthesis of 5,12-Naphthacenequinones with Boric Anhydride.—Compound **1** was treated with a slight excess of **2a**,[11] **2b**,[12] **2c**,[12] and **2d**[13] in the presence of a 50% mol excess of B_2O_3 at 190° for 2 hr. The solid mass was pulverized and extracted with several portions of boiling H_2O, filtered, washed with EtOH, dried, and recrystallized. Table I lists the pertinent data for the compounds.

1-Amino-6,11-dihydroxy-5,12-naphthacenequinone (3f).—Compound **3d** (2 g) was hydrolyzed by refluxing in 20 ml of concd HCl for 2 hr. When cool, a reddish crystalline product was obtained and recrystallized.

3-Dimethylaminophthalic Acid.—A solution of 10.55 g of 3-nitrophthalic acid and 10 ml of formalin in 160 ml of EtOH was reduced under 3 atm of H_2 in the presence of 0.5 g of PtO_2 until the theoretical amount was absorbed. The filtered solution was evaporated *in vacuo*, and the solid recrystallized from EtOH as yellow crystals: mp 138–140°; yield 6.5 g (65%). *Anal.* ($C_{10}H_{11}NO_4$) C, H, N.

3-Dimethylaminophthalic Anhydride (2e).—3-Dimethylaminophthalic acid (3 g) was heated at 150–160° for 0.5 hr, cooled, and the product recrystallized from C_6H_6: mp 140–142°; yield 2.4 g (89%). *Anal.* ($C_{10}H_9NO_3$) C, H, N.

Synthesis of 6,11-Dihydroxy-1-dimethylamino-5,12-naphthacenequinone (3e).—An intimately ground mixture of 7.6 g of **2e** and 6.4 g of **1** was added portionwise during 1 hr to a molten mixture of 53.5 g of anhyd $AlCl_3$ and 11.7 g of NaCl at 150°. The temperature was then raised to 220°, and maintained for 0.5 hr. When cool, the fused mass was pulverized with a mixture of 500 ml of H_2O and 500 ml of concd HCl, and the mixture refluxed for 4 hr to decompose the complex. After cooling, an ash-free product was obtained, and recrystallized from DMF plus a small amount of H_2O. *Anal.* ($C_{26}H_{17}NO_4$) C, H, N.

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Reduction of 1-(4-Dimethylaminobenzylidene)indene[1a,b]

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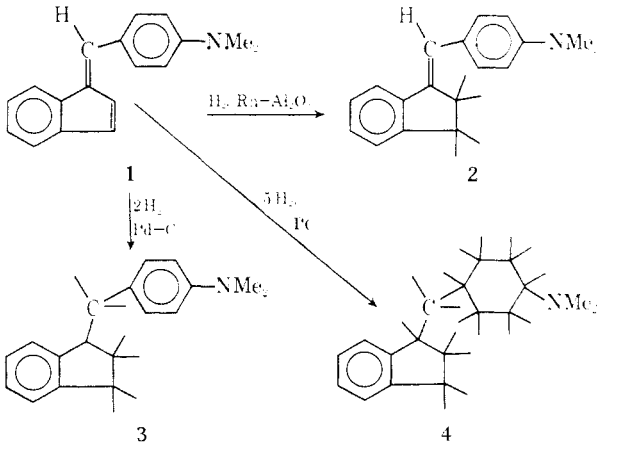
The synthesis of 1-(4-dimethylaminobenzylidene)-indene (**1**) was reported[2] recently as a result of our continuing search for compounds which have antitumor activity. Compound **1** was found to have definite effect against the Walker 256 tumor in rats, but the rats which recovered from their tumors sometimes developed mammary tumors, an effect noted particularly in female rats.[3] Further investigation revealed that tumors developed also in healthy rats treated with **1**.[4]

In an attempt to diminish or exclude the carcinogenic effect and at the same time retain the antitumor activity, various reduced derivatives were prepared by the catalytic hydrogenation of **1** (Scheme I). Based

Scheme I



on the amount of H_2 consumed in each reaction, structures **2**, **3**, and **4** represent the expected products. Analyses confirmed the postulated structures. Changes in the nmr spectra in going from **1** to **2**, **3**, and **4** are in agreement with those expected for the structures shown.

These compounds were tested against the Walker tumor by the single i.p. dose method. Compound **2** showed a slight antitumor activity, but **3** and **4** showed no antitumor effect. We conclude that the conjugated double bond system is necessary for antitumor activity in compounds of this type.

Experimental Section[5]

α-1-Indanylidene-*N*,*N*-dimethyl-*p*-toluidine (2).—Compound **1** (5.0 g, 0.02 mol) in 100 ml of EtAc was hydrogenated over 0.5 g of 5% Rh–Al_2O_3. The reaction stopped after *ca.* 1.5 mol of H_2/mol of **1** had been absorbed. The catalyst and solvent were removed and the residue was recrystallized from *i*-C_4H_{11} and MeOH. A 54% yield of a pale yellow solid, mp 123°, was recovered. *Anal.* ($C_{18}H_{19}N$) C, H.

α-1-Indanyl-*N*,*N*-dimethyl-*p*-toluidine (3).—Compound **1** (5.0 g, 0.02 mole) in 100 ml of EtAc was hydrogenated over 0.5 g of 5% Pd–C. The reaction stopped after exactly 2 mol of H_2/mol of **1** had been absorbed. The catalyst and solvent were removed and the residue was recrystallized from MeOH. An almost 100% yield of an off-white crystalline solid was obtained, mp 36.5–37.0°. *Anal.* ($C_{18}H_{21}N$) C, H.

4-(1-Indanylmethyl)-*N*,*N*-dimethylcyclohexylamine (4). Compound **1** (20 g, 0.08 mol) in 200 ml of HOAc was hydrogenated over 0.5 g of Adams' Pt(PtO_2). The reaction stopped when

(1) (a) This investigation was supported in part by Public Health Service Research Grants CA-03717-09-10. (b) Presented before the Division of Organic Chemistry at the 29th Southeastern Regional Meeting of the American Chemical Society, Tallahassee, Florida, December, 1968. (c) To whom inquiries should be addressed.

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5 mol of H_2/mol of **1** had been absorbed. The catalyst and solvent were removed. The residue was distilled *in vacuo* and a colorless liquid, bp 125–130° (0.5 mm), was collected, $n^{25}D$ 1.5302. *Anal.* ($C_{18}H_{27}N$) C, H. A picrate melted at 162–164°. *Anal.* ($C_{24}H_{30}N_4O_7$) C, H.

Nmr.—The nmr spectra of **1–4** were obtained on a Varian-A60 spectrometer in $CDCl_3$ (Me_4Si). Confirmation of the expected structures were provided through analysis of the changes in the spectra in going from **1** to **4**. The CH group connecting the indene and benzene rings couples with the protons of the aromatic ring of indene to produce a complicated multiplet. The reduction of the double bond between the two rings removes this coupling effect and a single peak for the four protons of the aromatic ring of indene is observed at δ 7.12 in the spectra of **3** and **4**. The absence of this peak in the spectrum of **2** is evidence for the retention of the double bond between the rings in this compound.

Uv-Visible Spectra.—These spectra were as expected for the structures given in Scheme I.

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Synthesis of Additional Arylhydroxamic Acids Which Inhibit Nucleic Acid Biosynthesis *In Vitro*[1]

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Prompted by the observation[2] that salicylhydroxamic acid selectively inhibits the synthesis of deoxyribonucleic acid (DNA) in Ehrlich ascites tumor cells *in vitro*, 11 arylhydroxamic acids were synthesized earlier and their activities were assessed.[3] Six were shown to possess varying degrees of selectivity in the test system. One of these, 4-hydroxybenzoylhydroxamic acid, has now been shown to possess significant antitumor activity *in vivo*.[4] In BDF_1 mice bearing the L-1210 leukemia, daily administration of 400 mg/kg per day intraperitoneally for 9 days increased the survival times of the animals 36% to 57% in four experiments, with no deaths due to toxicity of the compound. The present report is concerned with the synthesis and biological evaluation of additional arylhydroxamic acids (HAs) as regards their effects on biosynthesis of DNA, ribonucleic acid (RNA), and protein.

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Experimental Section[5]

Chemical.—Each of the compounds listed in Table I was prepared from the corresponding Me or Et ester by the well-known reaction with excess NH_2OH in basic solution.[6,7] In each case the base was NaOH and the solvent was MeOH or H_2O, or a combination of these depending upon the solubility of the individual ester. In the case of a phenolic compound, an extra equivalent of NaOH was used, while I, X, and XI, which are sensitive to oxidation in basic solution, were prepared in a N_2 atmosphere.

With the exception of I, IX, XIX, and XX, the required esters were obtained commercially. Methyl 3,5-diisopropylsalicylate, bp 142–143.5° (7 mm), was prepared in 35% yield by the HCl-catalyzed esterification of the corresponding acid in MeOH [lit.[8] bp 146° (7 mm), yield 15%]. Methyl 2-bromo-3,4,5-trimethoxybenzoate was synthesized by the bromination[9] of 3,4,5-trimethoxybenzoic acid followed by esterification using MeOH and anhydrous HCl. The compound exhibited bp 162–163.5° (2 mm), mp 31–32°, and gave an acceptable elemental analysis [lit.[10] bp 160–161° (2 mm), mp 34–36°]. Methyl 3,5-dichlorobenzoate was also prepared by the MeOH–HCl technique. The purified product melted at 58–60° (lit.[11] mp 58°). Methyl 3,4,5-trimethoxyphenylacetate was prepared by the H_2SO_4-catalyzed esterification of the corresponding acid, and was isolated and used as a viscous oil without further purification.

Biological.—These methods were as described in the corresponding section of the previous report.[3] The parameters investigated were (a) relative potency against DNA and RNA synthesis as measured by least-squares analysis of dose–response data; (b) slopes of the regression lines; (c) relative selectivity for DNA synthesis; (d) reversibility of the DNA and RNA inhibitory action upon removal of the inhibitor; and (e) effect upon preformed DNA and RNA, *i.e.*, depolymerization, to an acid-soluble form, of thymidine-methyl-^{3}H or uridine-5-^{3}H, respectively, which had been incorporated into nucleic acid of the cells prior to exposure to each inhibitor.

Results

The concentrations of each active compound which conferred 50% and 90% inhibition (IC_{50} and IC_{90}) of DNA synthesis in Ehrlich ascites tumor cells *in vitro* are shown in Table II. When the inhibitor and isotopic precursor were added simultaneously to the cell suspension, the slopes of the regression lines were numerically similar, with greater variations occurring after the 1 hr preincubation period. Compounds I and VI were of similar potency after the 1-hr preincubation as compared with no preincubation; compounds II, IV, V, VII, VIII, and IX were more active after 1 hr.

Four compounds suppressed RNA synthesis immediately upon contact with the cells, and the extent of inhibition was virtually the same as that obtained on DNA synthesis. Table III shows the IC_{50} and IC_{90} concentrations of these compounds when added to the cells simultaneously with the isotopic precursor. The relative potency and slopes of the regression lines of compounds V, IX, and XI were quite similar. Compound I was more active and the slope of the regression line was greater.

Figure 1 shows a comparison of inhibitory action of each of the 11 active compounds at a single (10^{-3} *M*)

(5) The melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. The elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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