rivatives of many other sensitive amino compounds.

Dissappointingly, 1 showed no activity against the L1210 mouse tumor at 400 mg/kg by intraperitoneal injection as determined by the National Cancer Institute.

Experimental Section

All melting points were measured on a Nagle-Kopler micro hot stage. All infrared spectra were recorded on a Perkin-Elmer Model 257. All NMR spectra were recorded on a Hitachi HA-100.

2,4-Dimethoxybenzyl Isothiocyanate (3). To a stirred solution of *N,N'*-thiocarbonyldiimidazole[5] (14.95 g, 84 mmol) in dry $CHCl_3$ (100 ml) was added a solution of 2,4-dimethoxybenzylamine[3] (11.69 g, 70 mmol) in $CHCl_3$ (30 ml) over 30 min under ice–water cooling. The reaction mixture was stirred for 3 hr at room temperature and evaporated in vacuo. The residue was extracted with warm hexane repeatedly (8 × 100 ml) and the combined hexane extracts were evaporated to ca. 200 ml. The solution was passed through a short column of silica gel (10 g) and eluted with 300 ml of hexane. The combined eluate was evaporated to ca. 50 ml and cooled to −10° overnight to give 7.0 g (60%) of colorless prisms: mp 24–25°; ir ($CHCl_3$) 2080, 2175 cm^{-1} (−N=C=S); NMR ($CDCl_3$) δ 3.79 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3), 4.58 (s, 2 H, CH_2N), 6.38–6.50 (m, 2 H, Ar), 7.15 ppm (d, 1 H, Ar). Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.41; H, 5.30; N, 6.70; S, 15.30. Found: C, 57.27; H, 5.31; N, 6.64; S, 15.26.

***N*-(2,4-Dimethoxybenzyl)-*N'*-hydroxythiourea (4).** A mixture of **3** (4.18 g, 20 mmol), $NH_2OH{\cdot}HCl$ (2.78 g, 40 mmol), and $NaHCO_3$ (3.36 g, 40 mmol) in 20 ml of 75% aqueous methanol was stirred at room temperature for 1 hr. The mixture was diluted with 50 ml of water and cooled in ice–water. The precipitate was collected, washed with water, and dried to give 4.4 g of crystals: mp 105–106°. Recrystallization from ethyl acetate gave 4.0 g (83%) of prisms: mp 108–109°; ir (Nujol) 3350, 3160, 3040 cm^{-1} (OH, NH); NMR (Me_2SO-d_6) δ 3.80 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), (d, 2 H, CH_2N, J = 6 Hz), 6.44–6.60 (m, 2 H, Ar), 7.10 (d, 1 H, Ar), 7.94 (t, 1 H, J = 6 Hz, CH_2NH), 9.4 (1 H, broad, SH), 10.1 ppm (1 H, broad, −NOH). Anal. Calcd for $C_{10}H_{14}N_2O_3S$: C, 49.59; H, 5.83; N, 11.56; S, 13.21. Found: C, 49.66; H, 5.81; N, 11.54; S, 13.14.

***N*-Hydroxythiourea (1).** A solution of **4** (2.90 g, 12 mmol) and resorcinol dimethyl ether (3.31 g) in 20 ml of CF_3COOH was stirred at room temperature for 1.5 hr. After evaporation of the acid in vacuo, 20 ml of methanol was added to the residue and the mixture was evaporated in vacuo. Dry benzene (20 ml) was added to the residue and the precipitate was collected, washed with benzene, and dried. The crystals were dissolved in tetrahydrofuran (ca. 10 ml), filtered to remove insolubles, and diluted with $CHCl_3$ to afford 0.94 g (68%) of colorless prisms: mp 97–101° dec. An analytical sample was recrystallized from ether: mp 100–101° dec; ir (Nujol) 3420, 3290, 3150 (NH, OH), 1610 cm^{-1} (C=N); NMR (Me_2SO-d_6) δ 7.35 (1 H, broad, NH_2), 7.68 (1 H, broad, NH_2), 9.40 (s, 1 H, SH), 10.12 (s, 1 H, =NOH). Anal. Calcd for CH_4N_2OS: C, 13.05; H, 4.38; N, 30.43; S, 34.76. Found: C, 13.02; H, 4.37; N, 30.30; S, 34.78.

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Some Substituted Naphthazarins as Potential Anticancer Agents

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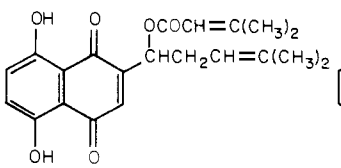
Some 2,3-bis(substituted methyl)naphthazarins and related compounds were synthesized by the Diels–Alder reaction of benzoquinone and 2,3-dimethylbutadiene followed by oxidation and substitution reactions. These compounds were prepared as potential biological alkylating agents. Screening results indicated that 1,4-diacetyl-6,7-dimethyl-4a,5,8,8a-tetrahydronaphthalene and 5,8-bis(benzoyloxy)-2,3-dimethyl-1,4-naphthoquinone possessed borderline activity against leukemia P388 and that naphthazarin diacetate possessed confirmed cytotoxicity against the cell culture of human epidermoid carcinoma of the nasopharynx.

Arnebin I (**1**), isolated from the roots of *Arnebia nobilis*, inhibits rat Walker carcinosarcoma 256.[1] This compound bears a close structural resemblance to shikonin (**2**, present in the roots of *Lithospermum erythrorhizon* Sieb et Zucc[2]) and both compounds may be biogenetically related. A crude extract prepared in this laboratory from *L. erythrorhizon* (NSC B626370) was found to possess inhibitory activity against the cell culture of human epidermoid carcinoma of the nasopharynx (9KB).



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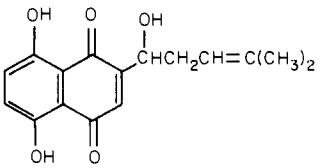

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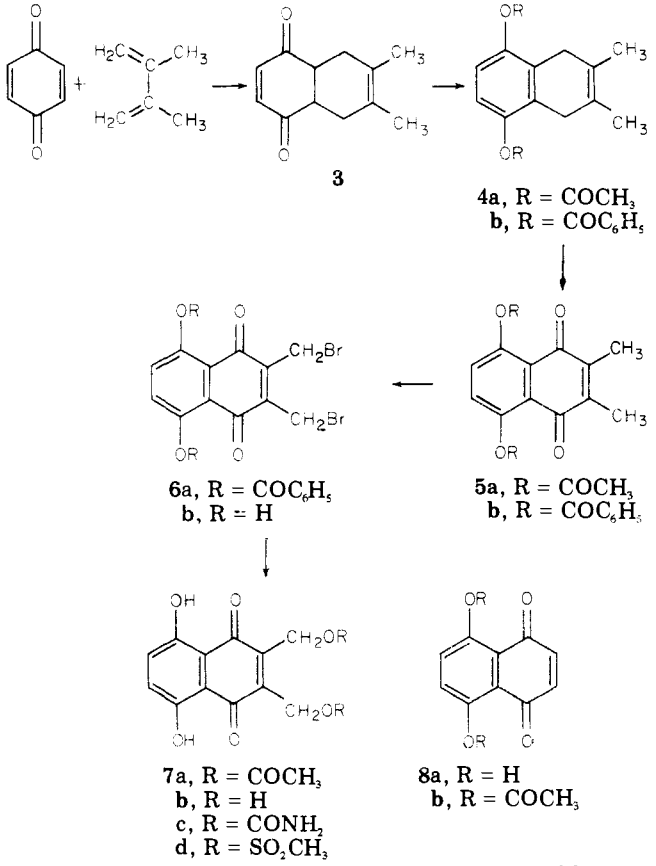
The activity of these naphthazarins, together with the fact that some antineoplastic property was noted by a number of benzoquinones and naphthoquinones substituted with one or two side chains potentially capable of biological alkylation after bioreduction,[3-7] prompted the preparation of some substituted naphthazarins for antitumor screening. This study is particularly intriguing since the naphthazarin unit is also a portion of the tetracyclic antitumor antibiotics daunomycin, adriamycin, nogalomycin,[8,9] and carminomycin.[10,11]

Chemistry. 2,3-Dimethylnaphthazarin diacetate (**5a**), prepared by Contreras[12] by the Diels–Alder adduct 4a,5,8,8a-tetrahydro-6,7-dimethyl-1,4-naphthoquinone[13-16] (**3**), was originally to be used for the preparation of 2,3-bis(substituted methyl)naphthazarins. Since the yield of **5a** was extremely low, the corresponding dibenzoate **5b** was prepared. Bromination of **5b** with *N*-bromosuccinimide in the presence of light yielded 5,8-bis(benzoyloxy)-2,3-bis(bromomethyl)-1,4-naphthoquinone (**6a**) which, upon hydrolysis, gave an almost quantitative yield of 2,3-bis(bromomethyl)naphthazarin (**6b**). The desired 2,3-bis-

(substituted methyl)naphthazarins **7a–d** were prepared from **6b** by conventional methods. For a comparison of biological activity, naphthazarin diacetate[17] (**8b**) was prepared by treatment of naphthazarin (**8a**) with acetic anhydride and sulfuric acid.



Biological Activity and Discussion. Available test results indicated that only compounds **4a** and **5b** possessed low, borderline activity against leukemia P388 (T/C 126 at 400 mg/kg and T/C 128 at 50 mg/kg, respectively) and none were active against the leukemia L1210 system. Compound **8b** possessed confirmed cytotoxicity in the 9KB test system (only 1.8 μg/ml concentration was required for 50% inhibition of growth against the cell culture of human epidermoid carcinoma of the nasopharynx) but was inactive against the leukemias L1210 and P388. None of the compounds were screened in other animal tumor systems.

Many benzo- and naphthaquinones containing analagous bis(substituted methyl) side chains were reported to possess inhibitory activity against adenocarcinoma 755 ascites cells and sarcoma 180 ascites cells in mice and to cause inhibition of nucleic acid biosynthesis as well as the activities of coenzyme Q mediated enzyme systems.[3-7] However, none of the synthetic potential bioreductive alkylating agents have been reported to possess inhibitory activity against leukemias L1210 and P388. Mitomycin C, the antibiotic which has been postulated as a bifunctionally masked alkylating agent[18] and is active only after undergoing bioreduction, exhibited outstanding inhibitory against Walker carcinosarcoma 256, Ehrlich ascites tumor, Ridgway and Wagner osteogenic sarcoma, and many other tumor systems.[19] It would appear that the current screening program does not permit a complete evaluation of compounds of this type. Also, the statement that the carbamoyl and aziridine groups are not essential for the biological action of mitomycin and can be replaced by other acyl groups without loss of activity[20,21] is perhaps more applicable to in vitro antibacterial and prophage induction tests than to anticancer evaluation.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

1,4-Bis(benzoyloxy)-5,8-dihydro-6,7-dimethylnaphthalene (4b). A mixture of 318 g of 4a,5,8,8a-tetrahydro-6,7-dimethyl-1,4-naphthoquinone[12-16] (**3**), 1500 ml of pyridine, and 435 ml of benzoyl chloride was cautiously warmed on a steam bath until a vigorous reaction took place. When this reaction had subsided, the mixture was heated at reflux for 1 hr, cooled, and diluted with 1000 ml of cold, 50% aqueous MeOH. The resulting solid product was collected by filtration and washed several times with 50-ml portions of MeOH until the filtrate was colorless. Recrystallization from $CHCl_3$–MeOH gave 636 g (95% yield) of **4b** as white needles: mp 197–200°. An analytical sample was obtained by a second recrystallization from $CHCl_3$–MeOH: mp 205–206°. Anal. ($C_{26}H_{22}O_4$) C, H.

5,8-Bis(benzoyloxy)-2,3-dimethyl-1,4-naphthoquinone (5b). To a stirred mixture of 200 g of **4b**, 60 ml of CF_3CO_2H, and 3 l. of AcOH cooled at 20° was added 210 g of CrO_3. The temperature of the reaction mixture, which rose spontaneously, was maintained at 45–50° by external cooling until the exothermic reaction was complete. It was then stirred at room temperature for 14 hr. The resulting green suspension was poured into 3 l. of H_2O and the precipitated solid collected by filtration (Celite). The solid was washed well with H_2O and EtOH and was subjected to a Soxhlet extraction using EtOH for 15 hr to remove unreacted material and side products. The remaining solid was then extracted continuously with $CHCl_3$ for 18 hr. The resulting yellow $CHCl_3$ extract was concentrated and diluted with EtOH to give 29.6 g (14% yield) of **5b**: mp 274–276°. An analytical sample was obtained as fine yellow needles by recrystallization from $CHCl_3$–MeOH: mp 275–277°. Anal. ($C_{26}H_{18}O_6$) C, H.

5,8-Bis(benzoyloxy)-2,3-bis(bromomethyl)-1,4-naphthoquinone (6a). A stirred suspension of 30 g of **5b** and 50 g of *N*-bromosuccinimide in 1500 ml of CH_2Cl_2 was irradiated with a 500-W sun lamp for 1 hr. Most of the CH_2Cl_2 (1200 ml) was distilled from the reaction mixture. To the remaining mixture was added 2500 ml of H_2O. After being stirred for 1 hr, the product was collected by filtration, washed thoroughly with CH_3OH, and dried in vacuo at 110° for 2 hr to give 39.5 g (96% yield) of **6a** as orange needles: mp 264–266°. An analytical sample was obtained as orange needles by recrystallization from CH_3CN: mp 266–267°. Anal. ($C_{26}H_{16}Br_2O_6$) C, H.

2,3-Bis(bromomethyl)naphthazarin (6b). A suspension of 20 g of **6a** in 330 ml of CF_3CO_2H and 40 ml of H_2O was heated at reflux for 70 min. The deep red solution was allowed to cool slowly and then chilled in an ice bath to complete the crystallization of **6b**. The product was collected by filtering through a sintered glass funnel and washed with cold H_2O until the filtrate was almost colorless. It was then washed with cold MeOH (2 × 50 ml). After 2 hr of drying in vacuo at 110°, 12 g (93% yield) of the product was obtained as shiny black needles: mp 181–182°. An analytical sample was prepared by recrystallization from Et_2O–C_6H_{12} as lustrous black needles: mp 185–186°. Anal. ($C_{12}H_8Br_2O_4$) C, H.

2,3-Bis(acetoxymethyl)naphthazarin (7a). A suspension of 8 g of **6b**, 14 g of silver acetate, 70 ml of $CHCl_3$, and 150 ml of AcOH was stirred at room temperature for 57 hr. $CHCl_3$ was removed in vacuo and the residue poured onto ice water saturated with NaCl. The crude **7a** and AgCl were collected by filtration (Celite) and washed well with H_2O. The solid was then added to 250 ml of $CHCl_3$ and separated from the AgCl by filtration. The solid was washed with $CHCl_3$ until the wash solution was colorless. The combined $CHCl_3$ solution was dried (Na_2SO_4) and concentrated in vacuo. Recrystallization of the residue from EtOAc gave 5.6 g (78% yield) of **7a** as fine red crystals: mp 190–192°. An analytical sample was prepared by additional recrystallization from EtOAc: mp 193–194°. Anal. ($C_{16}H_{14}O_8$) C, H.

2,3-Bis(hydroxymethyl)naphthazarin (7b). A suspension of 5.6 g of **7a** in 60 ml of CF_3CO_2H and 20 ml of H_2O was heated at reflux for 90 min. On cooling, the red reaction mixture was poured onto ice and chilled for 30 min. The resulting crude

product was collected by filtration, washed (H_2O), and dried in vacuo to give 3.8 g (92% yield) of **7b** as red crystals: mp 144–147°. An analytical sample was obtained by recrystallization from $CHCl_3$–CCl_4 as fine red needles: mp 158–160°. Anal. ($C_{12}H_{10}O_6$) C, H.

2,3-Bis[[(aminocarbonyl)oxy]methyl]naphthazarin (7c). To a gently stirred solution of 7 g of **7b** in 2000 ml of CH_2Cl_2 was added 9.1 g of NaOCN followed by 20 ml of CF_3CO_2H. Stirring was continued for 23 hr at room temperature followed by addition of 50 ml of H_2O. The mixture was stirred for 15 min and the resulting solid collected by filtration. It was washed with CH_2Cl_2 and air-dried to give 3.1 g (33% yield) of **7c** as a red powder. An analytical sample was prepared by reprecipitation from Me_2SO–$CHCl_3$ as a red powder which did not melt up to 300°. Anal. ($C_{14}H_{12}N_2O_8$) C, H, N.

2,3-Bis[[(methylsulfonyl)oxy]methyl]naphthazarin (7d). A mixture of 5.9 g of **6b** and 12.8 g of silver mesylate in 80 ml of CH_3CN was stirred at room temperature for 24 hr. The CH_3CN was removed in vacuo at 40°; the residue was triturated with 100 ml of CH_2Cl_2, filtered, and washed with 2 × 50 ml of CH_2Cl_2. The combined CH_2Cl_2 solution was concentrated to a small volume (25 ml) in vacuo, filtered from some insoluble material, and diluted with 100 ml of C_6H_6 to give 4.9 g (76% yield) of **7d** as a red powder. Depending on the rate of heating, a decomposition point of the **7d** was observed at ca. 150°. An analytical sample was prepared as a red powder by two recrystallizations from C_6H_6–CH_2Cl_2: mp 158–160°. Anal. ($C_{14}H_{14}O_{10}S_2$) C, H.

Naphthazarin Diacetate (8b). To a warm (30–40°) solution of 2.4 g of naphthazarin (**8a**) in 30 ml of Ac_2O was added six drops of concentrated H_2SO_4. The mixture was stirred overnight at room temperature and poured into 300 ml of ice water. The resulting yellow solid was collected by filtration, washed with H_2O (2 × 10 ml), and dried to give 2.4 g (70% yield) of **8b**: mp 193–195°. An analytical sample was recrystallized from H_2O–MeOH: mp 193.5–195° (lit.[17] mp 195°).

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Inhibition of Tumor Cell Transplantability by Iron and Copper Complexes of 5-Substituted 2-Formylpyridine Thiosemicarbazones

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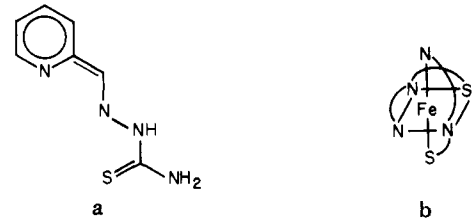
The cytotoxicity of copper and iron complexes of 5-substituted 2-formylpyridine thiosemicarbazones against Ehrlich ascites tumor cells has been measured. Brief in vitro incubation of cells and drugs is followed by implantation into host mice. Subsequent degree of tumor development is a measure of cytotoxicity. A spectrum of activities for the iron complexes is observed, starting with the least active as designated by its 5-substitution: OH < $OCOCH_3$ ~ $N(CH_3)_2$ < H < CH_3 ~ Cl ~ CF_3. The last three complexes can prevent completely tumor growth in the new host. Copper complexes of 5-H and 5-CH_3 also prevent successful tumor cell transplantation.

The possibility that metal complexes of α-*N*-formyl heterocyclic thiosemicarbazones may be cytotoxic to tumor cells has been raised recently in preliminary communications.[1,2] Experimental findings together with relevant background information are reported here in support of this proposal.

Thiosemicarbazones of 1-formylisoquinoline and 2-formylpyridine and a multitude of their derivatives have been examined for activity against a variety of transplanted animal tumors.[3,4] A number of studies have indicated that these compounds (L) can act at a molecular level by inhibiting ribonucleoside diphosphate reductase, an obligatory enzyme in the pathway of synthesis of precursors of DNA.[5-7] Furthermore, it has been hypothesized that the basis of the inhibition is the binding of iron at the active site of the enzyme (E) in an E·Fe·L complex which prevents catalysis.[8,9] In fact, Ablov and Belichuk have

Chart I. (a) 2-Formylpyridine Thiosemicarbazone and (b) Octahedral Binding of Iron by Ligand[10]



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