washed with water and dried (Na_2SO_4), and the solvent was evaporated. The residue was taken up in EtOAc and the desired product crystallized from this solution upon cooling: yield 1.6 g (38%); mp 154–155 °C (from EtOAc); NMR (CD_3COCD_3) 7.19 (d, 1 H, J = 8.8 Hz, C_7 H), 6.91 (d, 1 H, J = 2.5 Hz, C_4 H), 6.78 and 6.68 (dd, 1 H, J = 8.8 and 2.5 Hz, C_6 H), 6.55 (br s, 1 H, C_3 H), 4.87–4.68 (m, 1 H, CHOH), 3.05–2.72 [m, 3 H CCH_2N and $-CH(CH_3)_2$], 1.05 ppm [d, 6 H, $-CH(CH_3)_2$]; MS m/z (relative intensity) 235 (1, M^+), 217 (1), 163 [5, M - $CH_2NHCH(CH_3)_2^+$], 147 (3), 105 (2), 72 [100, $CH_2NHCH(CH_3)_2^+$]. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

When the mother liquor was concentrated and cooled, a second crop of crystals was obtained consisting of the isomeric 2-(5-hydroxy-2-benzofuranyl)-2-(isopropylamino)ethanol (**15**): yield 0.5 g (12%); mp 158–160 °C (from acetone); NMR (CD_3COCD_3) 7.22 (d, 1 H, J = 8.8 Hz, C_7 H), 6.91 (d, 1 H, J = 2.5 Hz, C_4 H), 6.77 and 6.67 (dd, 1 H J = 8.8 and 2.5 Hz, C_6 H), 6.56 (br s, 1 H, C_3 H), 4.04–3.47 [m, 3 H, CCH_2OH and $-CH(CH_2OH)NH-$], 2.84 [septet, 1 H, $-CH(CH_3)_2$], 1.07 and 0.99 ppm [dd, 6 H, $-CH(CH_3)_2$]; MS m/z (relative intensity) 204 (71, M - CH_2OH^+), 162 (47), 147 (7), 105 (7), 101 (24), 44 (100).

(±)-1-(6-Hydroxy-2-benzofuranyl)-2-(isopropylamino)-ethanol (10). This compound was prepared as described for **9**. The crude product was chromatographed on a silica gel column eluted with chloroform with increasing amounts of MeOH. This yielded the pure product in approximately 10% yield, mp 110–111 °C (from EtOAc/ether). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

Pharmacology. Cats of both sexes, weighing between 2.6 and 3.2 kg, were starved for 24 h and pretreated with reserpin (Serpasil, 2.5 mg/mL, 5 mg/kg of body weight) 18 h before experimentation in order to provoke neuronal noradrenaline depletion. The animals were anesthetized by a pentobarbital (Mebumal, 60 mg/mL ACO) injection (30 mg/kg of weight) intraperitoneally and maintained at a constant level of anesthesia by means of a continuous infusion of sodium pentobarbital [Mebumal, 0.1 (mg/min)/kg of body weight]. Artificial respiration was given by means of a respiratory pump (Braun) connected to a tracheal cannula.

The cats were vagotomized bilaterally, and the right carotid artery was catheterized for recording of the mean arterial blood pressure via a Statham P 23 strain gauge pressure transducer. The heart rate was recorded via a Grass 7P4 Cardiotachograph, which was triggered by the blood pressure oscillations.

The pharmacological effects on the peripheral vascular resistance in one hind leg were estimated as follows: the left femoral artery was cut and both ends were reconnected via a plastic catheter loop, which was passed through a peristaltic pump (Watson Mallow) delivering a constant flow of >10 mL/min. The hind leg was thus perfused with the cat's own blood at a constant flow rate. Alterations in vascular resistance measured as changes in perfusion pressure were recorded via a Statham P23 strain gauge pressure transducer connected to the loop distal to the roller pump. The left jugular and right femoral veins were catheterized for barbiturate and test compound administration, respectively.

All recordings were made on a Grass 7D polygraph. The blood gas status and hematocrit values of the animal were controlled continuously throughout the experiment and were regarded as normal within the following ranges: pH, 7.38 ± 0.06; pCO_2, 30.7 ± 1.4 mmHg; HCO_3, 19.0 ± 1.0 mequiv/L; hematocrit, 30 ± 10%.

A standard fluid therapy (Ringer acetate, ACO, 20 mL/kg body weight, + Macrodex, Pharmacia, 5 mL/kg of body weight, infused at 0.5 mL/min) was used to compensate for the fluid loss due to the reserpine pretreatment. Maximal heart rate and peripheral vascular effects were obtained at iv and ia injection of supramaximal doses of isoproterenol (IPR).

The test compounds were infused iv for 10 min at a rate of 1.0 mL/min in five stepwise raised concentrations. Each infusion was followed by an iv and an ia injection of IPR in concentrations which in the absence of test compounds induced control responses equal to about 80% of the maximal IPR effects.

Knowing that the submaximal IPR concentration used induces a response which may be identified at the top of the linear part of the IPR dose–response graph and assuming that any antagonistic property of the test compound represents a perfectly competitive interaction with IPR at the receptor site, any decrease in the response to the IPR standard concentration will reflect a parallel rightward shift of the IPR dose–response curve.

Dissociation constants characteristic of the affinity for the receptors were calculated for agonistic (K_A) and antagonistic (K_B) effects of the test compounds.

The doses of the test compounds producing semimaximal excitatory effects on the heart rate were taken as estimates of K_A. The K_B values for antagonistic effects on heart rate and peripheral vascular dilatation were calculated according to Åblad et al.[17]

The formula used provided us with a series of K_B values, one for each dose of test compound, in every experiment. These values which theoretically should be identical with each other within one experiment were, however, widely scattered due to a possible nonlinearity of the dose–response curve segment at its extreme points (i.e., around the 20–30 and the 70–80% level). For calculation of the "mean" K_B of each experiment, a "weighing" formula[17] was used.

Acknowledgment. We thank Drs. Peder Berntsson and Arne Brändström for valuable discussions, Hans Thorin for the mass spectra, and Mrs. Anita Niklasson for help with the silica gel chromatography.

(17) B. Åblad, A. Brändström, L. Ek, and M. Sjölander, *Eur. J. Pharmacol.*, 14, 319 (1971).

Synthesis and Antineoplastic Activity of Hydroquinone Dialdehydes

Edward M. Newman, Ai Jeng Lin, and Alan C. Sartorelli*

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510. *Received December 19, 1979*

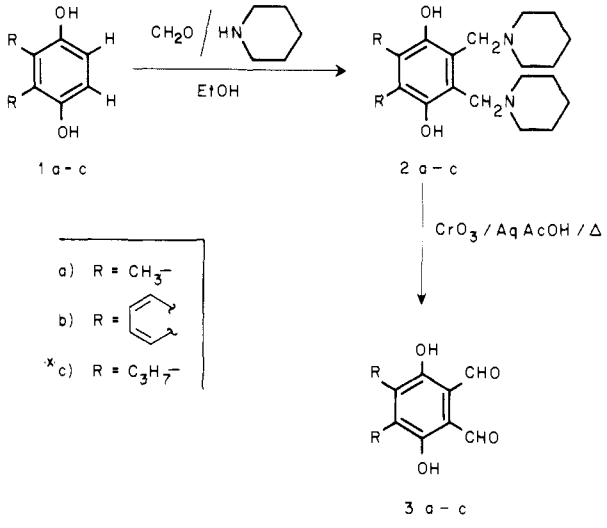
A number of hydroquinone dialdehydes and structurally related compounds were synthesized and tested for antineoplastic efficacy against transplanted murine tumors. 3,6-Dihydroxy-4,5-dimethylphthalaldehyde, 3,6-dihydroxy-4,5-dipropylphthalaldehyde, and 3,6-dihydroxy-4,5-dimethylphthalaldehyde hemialdal tetraacetate significantly prolonged the survival time of mice bearing either Sarcoma 180, Ehrlich carcinoma, or adenocarcinoma 755 ascites tumors. In addition, these agents were cytotoxic to Sarcoma 180 cells in culture at concentrations in the range of 25–30 μM.

Several α,β-unsaturated aldehydes,[1-3] α-ketoaldehydes,[4-6] and periodate oxidation products of ribonucleosides[7,8] have been demonstrated to possess antineoplastic activity against experimental murine tumors. The biochemical

(1) E. Schauenstein, *J. Lipid Res.*, 8, 417 (1967).
(2) P. J. Conroy, J. T. Nodes, T. F. Slater, and G. W. White, *Eur. J. Cancer*, 11, 231 (1975).
(3) H. M. Tillian, E. Schauenstein, H. Ertl, and H. Esterbauer, *Eur. J. Cancer*, 12, 989 (1976).
(4) F. A. French and B. L. Freedlander, *Cancer Res.*, 18, 172 (1958).
(5) M. A. Apple and D. M. Greenberg, *Cancer Chemother. Rep.*, 51, 455 (1967).
(6) L. G. Együd and A. Szent-Györgyi, *Science*, 160, 1140 (1968).

0022-2623/80/1823-0627$01.00/0

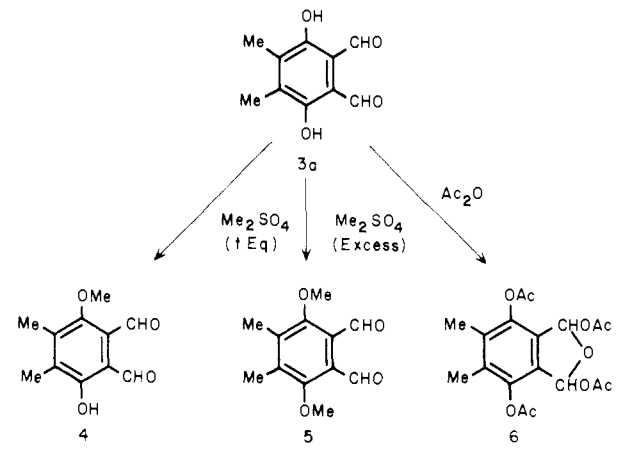
Scheme I



actions of the periodate oxidation product of 6-methylmercaptopurine ribonucleoside (MMPR-OP) have been studied in detail; this agent was shown to inhibit RNA, DNA, and protein synthesis[8,9] and has been used as an affinity label for several enzymes. In each affinity-labeled enzyme, MMPR-OP interacted with the ϵ-amino group of a lysine residue to form a Schiff base, which was trapped by sodium borohydride reduction.[10] In a similar manner, the periodate oxidation product of inosine (INOX) has been shown to form complexes with a variety of amino acids and to cross-link molecules of bovine serum albumin.[11] INOX also causes a variety of additional metabolic lesions, inhibiting ribonucleotide reductase,[12] RNA synthesis,[13] and the incorporation of radiolabeled thymidine, uridine, and amino acids into DNA, RNA, and protein, respectively.[14] INOX has undergone a phase I clinical trial, and on the basis of these tests further clinical evaluation of this dialdehyde as an antineoplastic agent is being conducted.[15]

o-Phthalaldehyde is the only aromatic aldehyde which had been reported to possess antitumor activity.[4] Aromatic aldehydes were considered to be worthy of study because they contain the chemically reactive aldehyde group with structural differences that might well lead to biochemical effects different from those of aliphatic aldehydes and periodate oxidation products of nucleosides. Because they are bifunctional, phthalaldehydes might be expected to cross-link proteins or possibly other macromolecules. For these reasons, we have concentrated on the synthesis and evaluation of phthalaldehydes. 3,6-Dihydroxy-4,5-dimethylphthalaldehyde, originally described by Cameron et al.,[16] was one of the initial compounds which we evaluated for biological activity. Since this agent possessed reasonable potency against transplanted murine ascites tumors and contained potential sites for modification, this compound was selected as the structural focus of a series of phthalaldehydes.

Scheme II



Chemistry. 2,3-Dimethyl-5,6-bis(piperidinomethyl)-1,4-dihydroquinone (**2a**) was synthesized by both of the methods described by Cameron et al.;[16] these involved treatment of duroquinone with piperidine[17] or of 2,3-dimethyl-1,4-dihydroquinone (**1a**) with piperidine and formaldehyde (Scheme I). Chromium trioxide oxidation of **2a** yielded the desired phthalaldehyde **3a**. Dihydronaphthoquinone (**1b**) was converted to the corresponding 2,3-dicarboxaldehyde **3b** using this sequence. Likewise, 2,3-dipropyl-1,4-dihydroxyquinone (**1c**) was converted to the Mannich base **2c**, which was subsequently oxidized to the phthalaldehyde **3c**.

Both the monomethyl (**4**) and dimethyl (**5**) ethers of **3a** were obtained by refluxing the hydroquinone with Me_2SO_4/K_2CO_3 in dry acetone,[18] as shown in Scheme II. Compounds **4** and **5** were moisture sensitive, a phenomenon particularly evident with **5**, and upon standing the infrared carbonyl absorption band at 1665 cm^{-1} of this compound disappeared. This was accompanied by the appearance of two broad overlapping bands at 3260 and 3390 cm^{-1}, which indicated the existence of hydroxyl groups. In a freshly sublimed sample, weak absorption occurred in the hydroxyl region and two low-intensity bands appeared in the carbonyl region in addition to the strong absorption at 1665 cm^{-1}. The NMR signal at δ 10.3 (-CHO) also disappeared upon standing, and a signal of equal relative intensity appeared at δ 6.3; these findings were consistent with hydration of the phthalaldehyde portion of the molecule.

3,6-Dihydroxy-4,5-dimethylphthalaldehyde hemialdal tetraacetate (**6**; Scheme II) was obtained by treatment of **3a** with Ac_2O in either pyridine or $AcOH/H_2SO_4$. Selective acetylation of the hydroquinone hydroxyl groups of **3a** with limited amounts of Ac_2O or AcCl failed. The acetylated derivative **6** was a mixture of isomers which were not resolved by TLC or sublimation; this mixture presumably accounted for the observed broad melting range and the

(7) W. Dvonch, H. Fletcher III, F. J. Gregory, E. H. Healy, G. H. Warren, and H. E. Alburn, *Cancer Res.*, **26**, 2386 (1966).
(8) J. P. Bell, M. L. Faures, G. A. LePage, and A. P. Kimball, *Cancer Res.*, **28**, 782 (1968).
(9) A. P. Kimball, M. J. Wilson, J. P. Bell, and G. A. LePage, *Cancer Res.*, **28**, 661 (1968).
(10) A. P. Kimball, *Methods Enzymol.*, **46**, 353 (1977).
(11) R. L. Cysyk and R. H. Adamson, *Cancer Treat. Rep.*, **60**, 563 (1976).
(12) J. G. Cory and M. M. Mansell, *Cancer Lett.*, 1, 133 (1976); J. G. Cory, M. M. Mansell, and C. S. Fox, *Proc. Am. Assoc. Cancer Res.*, **17**, 27 (1976); J. G. Cory, M. M. Mansell, and T. W. Whitford, Jr., *Cancer Res.*, **36**, 3166 (1976).
(13) J. G. Cory, S. H. Parker, and C. S. Fox, *Cancer Res.*, **38**, 815 (1978).
(14) P. G. W. Plagemann, J. C. Graff, and M. Behrens, *Cancer Res.*, **37**, 2188 (1977).
(15) J. Kaufman and A. Mittleman, *Cancer Chemother. Rep., Part 1*, **59**, 1007 (1975).
(16) D. W. Cameron, P. M. Scott, and Lord Todd, *J. Chem. Soc.*, 42 (1964).
(17) A. J. Lin, C. W. Shansky, and A. C. Sartorelli, *J. Med. Chem.*, **17**, 558 (1974).
(18) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, 1967, p 295.

Table I. Inhibition of the Growth of Sarcoma 180 Cells in Culture by Aromatic Dicarboxaldehydes and Related Compounds

compd[a]	IC_{50},[b] μM
3a	26
3b	66
3c	29
4	37
5	97
6	<25[c]
2,5-dihydroxy-3,4,6-trimethylbenzaldehyde	27
durohydroquinone	12
o-phthalaldehyde	12

[a] Tetramethyl-1,4-dihydrobenzoquinone (durohydroquinone), mp 230–233 °C (lit.[19] 233 °C), was obtained in 99% yield by reduction of duroquinone (Aldrich Chemical Co.) using 10% Pd/C as a catalyst. 3,6-Dihydroxy-4,5-dimethylphthalaldehyde (3a)[16] and 2,5-dihydroxy-3,4,6-trimethylbenzaldehyde[20] were synthesized as previously described by others. o-Phthalaldehyde (fluoroPA) was purchased from Durram Chemical Corp. [b] Compounds were tested over a range of 1–100 μM, and the IC_{50} (i.e., the concentration causing 50% inhibition of growth) was estimated from a graph of the inhibition of the increase in cell number at 72 h vs. concentration. [c] Compound 6 caused complete inhibition of growth at a level of 25 μM, which was the lowest concentration of this agent tested.

multiple NMR signals in the region of δ 2.1 and 7.2 to 7.4. Attempts to prepare a solution of **6** for evaluation of its effects on the growth of Sarcoma 180 cells in culture indicated that the compound was unstable. Therefore, the hydrolysis of **6** to the parent phthalaldehyde **3a** at ambient temperature in 10% Me_2SO–90% phosphate-buffered saline (PBS; NaCl, 8.0 g/L; KCl, 0.2 g/L; Na_2HPO_4, 1.15 g/L; KH_2PO_4, 0.2 g/L, pH 7.3) was determined by monitoring the absorbance of a solution of **6** at 400 nm. The half-life of **6** under these conditions was 9.5 min.

Biological Results. The cytotoxic activity of the hydroquinone dialdehydes against neoplastic cells was examined both in vitro and in vivo. Cytotoxicity in vitro was determined by exposing Sarcoma 180 cells in culture to the various agents over a range of concentrations from 1 to 100 μM, and data are presented as the concentration required to reduce growth to 50% of that of untreated control cells (Table I). The aromatic dicarboxaldehydes inhibited cell growth in a concentration-dependent manner. The naphthalene derivative **3b** was less potent than the two benzene derivatives **3a** and **3c**; however, at a concentration of 100 μM, all three inhibitors nearly completely abolished growth. Derivatives of **3a** in which one or both hydroxyl groups were methylated (i.e., compounds **4** and **5**, respectively) were less cytotoxic than **3a**; furthermore, the dimethyl ether **5** was less cytotoxic than the corresponding monomethyl ether **4**. The acetylated derivative (**6**) appeared to be the most cytotoxic of the derivatives of **3a** causing complete inhibition of growth at a level of 25 μM. Although **6** lacks the aldehyde residues, it seems reasonable to assume that the labile esters are hydrolyzed to yield **3a**. The fact that **6** is more potent than **3a** in vitro, together with experimental data (unpublished observations) which showed that **3a** was rapidly inactivated by components of the growth medium, suggested that **6** might serve as a protected prodrug form of **3a**. o-Phthalaldehyde and durohydroquinone, included to serve as structural analogues of **3a**, were each more toxic than **3a** to Sarcoma 180 cells in culture. A third analogue, 2,5-dihydroxy-3,4,6-trimethylbenzaldehyde, inhibited cell growth to approximately the same extent as did **3a**. It appeared reasonable to assume that the toxicity of these latter two agents to Sarcoma 180 cells in culture was the result of biochemical lesions that differed from those created by the hydroquinone dialdehydes, although this presumption remains to be demonstrated.

Table II presents the mean survival times of drug-treated groups of animals bearing transplants of Sarcoma 180 ascites tumors, expressed as a percentage of the life-span of untreated tumor-bearing control mice. Animals living 50 days or longer were excluded from these averages and tabulated separately in accordance with the suggestion of Schabel et al.[21] The two dihydroxyphthalaldehydes **3a**

Table II. Effects of Aromatic Dicarboxaldehydes and Related Compounds on the Survival Time of Mice Bearing Sarcoma 180 Ascites Tumors

compd	dose,[a] (mg/kg)/day	av Δ wt,[b] %	T/C,[c] %	50-day survivors[d]
3a	2.5	23	128	0/10
	5	6	182	3/30
	10	1	195	3/30
	15	−3	148	0/15
	20	−4	91	0/10
3b	1.25	16	81	0/5
	2.5	14	81	0/5
	5	14	93	0/10
	10	17	90	0/10
	15	0	64	1/5
	20	1	76	0/10
3c	1.25	10	88	0/5
	2.5	9	138	0/10
	5	2	205	4/20
	10	−1	178	2/25
	15	2	171	0/10
	20	−3	150	0/10
5	5	15	81	0/10
	10	15	96	0/10
	20	11	103	0/10
6	5	5	109	0/10
	10	−6	149	0/10
	20	−4	109	0/10
2,5-dihydroxy-3,4,6-trimethylbenzaldehyde	10	25	94	0/5
	20	25	84	0/5
	30	17	89	0/5
durohydroquinone	5	19	83	0/5
	10	14	85	0/5
	20	16	87	0/5
o-phthalaldehyde	0.31	15	121	0/5
	0.63	5	193	0/5
	1.25	4	176	1/10
	2.5	0	154	0/15
	5	−2	99	0/15
	10	−3	43	0/5

[a] The daily dose based on the mean weight of animals at the beginning of the experiment. Animals were treated with this level once daily for 6 consecutive days beginning 24 h after tumor implantation. [b] The change in body weight from onset to termination of treatment. Tumor-bearing control animals gained an average of 19% over the course of the treatments, which was attributed to accumulation of ascites fluid. [c] The mean survival time of treated animals not surviving 50 days as a percentage of the mean life span of control animals in a given experiment. The mean life span of tumor-bearing control animals (13 separate experiments, 65 animals) was 14.1 days. [d] The number of animals surviving at least 50 days/the total number of animals treated.

(19) L. I. Smith and F. J. Dobrovolny, *J. Am. Chem. Soc.*, 48, 1420 (1926).
(20) G. Manecke and G. Bourwieg, *Chem. Ber.*, 92, 2958 (1959).

Table III. Effects of Phthalaldehydes on the Survival Time of Mice Bearing Adenocarcinoma 755 and Ehrlich Carcinoma Ascites Tumors[a]

		adenocarcinoma 755[b]			Ehrlich carcinoma[c]		
compd	dose, (mg/kg)/day	av Δ wt, %	T/C, %	50-day survivors	av Δ wt, %	T/C, %	50-day survivors
3a	5	−2	27	4/5	21	120	0/10
	10	−13		5/5	4	125	2/10
	20	−11	138	2/5	−4	127	3/10
3c	5	−16	16	4/5	5	154	0/10
	10	−15	22	4/5	−1	202	4/10
	20	−15	35	3/5	−3	166	1/10
6	5	−7	59	3/5	4	168	2/10
	10	−15	16	4/5	−2	195	0/10
	20	−14	38	1/5	4	222	3/10

[a] Experimental details are given in the legend to Table II. [b] The mean survival time in days of untreated control mice bearing adenocarcinoma 755 was 18.6 days. [c] The mean survival time in days of untreated control animals bearing the Ehrlich carcinoma was 14.0 days.

and **3c** had significant antineoplastic activity in this test system. Thus, at daily doses of 5 and 10 mg/kg the survival time of dying animals was as much as twice that of untreated controls, and 10 to 20% of the treated animals survived at least 50 days. The 4,5-dipropyl compound **3c** had a slightly broader dose–response curve than the 4,5-dimethyl derivative **3a**, but the magnitude of the response to the two drugs was otherwise similar. In contrast, the naphthalene derivative **3b** was inactive and did not significantly increase the survival time of treated mice at any of the dosage levels employed. This finding was also true for **5**, in which the hydroxyl groups of **3a** were blocked by methylation. The hemialdal tetraacetate **6** produced a slight increase in the survival time of mice bearing Sarcoma 180; in one of two replicate experiments which were averaged, the treated group was significantly different from the control at both 5 and 10 (mg/kg)/day. *o*-Phthalaldehyde increased the life-span of tumor-bearing mice at relatively low daily dose levels [e.g., a T/C equal to 193% was attained at 0.63 (mg/kg)/day]. 2,5-Dihydroxy-3,4,6-trimethylbenzaldehyde did not increase the average survival time of tumor-bearing animals significantly above that of untreated tumor-bearing mice at any of the dose levels tested. Similar inactivity was also true for 2,5-dihydroxybenzaldehyde, 2-hydroxy-3-methylbenzaldehyde, and salicylaldehyde (data not shown). Durohydroquinone caused a decrease in life span which was not statistically significant; similar results were obtained for two other hydroquinones, **1a** and **1c** (data not shown).

The two phthalaldehydes **3a** and **3c** and the hemialdal tetraacetate (**6**), which inhibited the growth of Sarcoma 180, were tested against a variety of other transplanted murine neoplasms (Table III). Compounds **3a** and **3c** prolonged the survival time of animals bearing the Ehrlich ascites carcinoma to approximately the same extent as the mice bearing Sarcoma 180; however, **6** was considerably more active against the Ehrlich carcinoma. Adenocarcinoma 755 was exceedingly sensitive to all three drugs, and a large percentage of the treated tumor-bearing animals survived free of tumor; a number of animals died relatively early in these experiments, presumably from drug toxicity as indicated by major changes in body weight of treated animals. The survival times of mice bearing either leukemia L1210, leukemia P388, or hepatoma 129 were increased only marginally by the two phthalaldehydes **3a** and **3c** at the daily dosage level of 10 mg/kg (data not shown); no increases in the life span of mice bearing these three tumors were observed at doses of **3a** and **3c** of 5 or 20 (mg/kg)/day.

Experimental Section

Biological Methods. Cytotoxic activity in vitro was measured in Sarcoma 180 cells grown in suspension culture in Fischer's medium supplemented with 10% horse serum, 100 units/mL penicillin, and 100 μg/mL streptomycin by dilution with medium to give a cell density of 10^4 cells/mL at the beginning of each experiment. Drugs were dissolved at 10 times their final concentration in 10% Me_2SO in PBS and brought to volume with cell suspension. Control cultures contained all components except the drug (i.e., 90% medium, 9% PBS, and 1% Me_2SO). Cultures were counted in triplicate 24, 48, and 72 h later with a Coulter ZBI particle counter.

Antineoplastic activity was ascertained in mice inoculated intraperitoneally with 0.1 mL of a suspension of 10^7 cells/mL. Drugs were suspended or dissolved in 0.9% NaCl containing 5% ethanol and 1 drop of 20% aqueous Tween 80 per 12 mL at the concentration required for the highest dosage level; lower doses were obtained by serial dilution with 0.9% NaCl. Drugs were injected intraperitoneally in a volume of 0.5 mL once daily for 6 consecutive days beginning 24 h after tumor implantation. Control tumor-bearing animals received 0.5 mL of 0.9% NaCl. Each group of animals was weighed on the day following the last injection (day 7) and the difference in weight from the day of implantation of tumor (day 0) was noted and used as a measure of toxicity. Host animals were female mice of the following strains: CD-1 for Sarcoma 180 and Ehrlich carcinoma, CDF_1 for L1210 and P388 leukemias, AKD_2F_1 for adenocarcinoma 755, and C3H/Hej for hepatoma 129.

Chemical Methods. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were obtained using a Varian Model T-60A spectrometer. The chemical shifts of the protons are reported in parts per million (δ) downfield from an internal tetramethylsilane standard. IR absorption spectra of samples in KBr pellets were recorded on a Perkin-Elmer Model 257 grating infrared spectrometer. A Beckman Model 25 spectrophotometer was used to obtain UV and visible absorption spectra. Elemental analyses were performed by the Baron Consulting Co., Orange, CT.

2,3-Dipropyl-1,4-dihydrobenzoquinone (1c). 2,3-Diallyl-1,4-dihydrobenzoquinone[22] (22 g, 0.12 mol) was dissolved in 200 mL of EtOH and reduced in a Parr hydrogenator at 30 psi using 1 g of 10% Pd/C as the catalyst. Following removal of the catalyst by vacuum filtration, the solvent was evaporated under reduced pressure to yield 23 g (0.12 mol, 100%) of **1c**, mp 145–146 °C. Anal. ($C_{12}H_{18}O_2$) C, H.

2,3-Dipropyl-5,6-bis(piperidinomethyl)-1,4-dihydrobenzoquinone (2c). A mixture of **1c** (15 g, 77 mmol), piperidine

(21) F. M. Schabel, Jr., D. P. Griswold, Jr., W. R. Laster, Jr., T. H. Corbett, and H. H. Lloyd, *Pharmacol. Ther., Part A*, 1, 411 (1977).

(22) L. F. Fieser, W. P. Campbell, and E. M. Fry, *J. Am. Chem. Soc.*, 61, 2206 (1939).

(24 mL, 240 mmol), and formalin (18 mL, 230 mmol) were refluxed in 240 mL of EtOH under nitrogen for 3 h. The solvent was removed under reduced pressure, a small amount of EtOH was added, and the product was allowed to crystallize overnight. The product was collected and rinsed with ice-cold EtOH to yield 7.2 g (19 mmol, 24%) of **2c**, mp 133-134 °C. A small portion of the product was recrystallized from EtOH, mp 135-135.5 °C. Anal. ($C_{24}H_{40}N_2O_2$) C, H, N.

1,4-Dihydroxy-2,3-naphthalenedicarboxaldehyde (3b). Naphthoquinone (7 g, 44 mmol) was hydrogenated at 30 psi in 300 mL of EtOH in the presence of 1 g of 10% Pd/C as catalyst. The catalyst was removed by filtration, and to the filtrate was added formalin (15 mL, 190 mmol) and piperidine (18 mL, 180 mmol). The mixture was heated to 60 °C for 1 h under N_2. After the mixture cooled, the precipitate was collected and washed with ligroin to give 3.8 g (11 mmol, 25%) of **2b**, mp 166-168 °C dec (lit.,[16] ~160 °C dec). This material was oxidized without further purification. To the piperidine derivative (2 g, 5.6 mmol) in 20 mL of warm AcOH was added CrO_3 (1 g, 0.01 mol) in 20 mL of warm 50% aqueous AcOH. The solution was heated for approximately 10 min until an exothermic reaction occurred. The reaction mixture was poured into ice-water to yield a brown precipitate, which was collected, washed with H_2O, dried, and purified by preparative TLC ($CHCl_3$/silica gel). Recrystallization from EtOH gave 0.2 g (0.93 mmol, 16% from **2a**) of yellow crystals, mp 186-187 °C. Anal. ($C_{12}H_8O_4$) C, H.

3,6-Dihydroxy-4,5-dipropylphthalaldehyde (3c). 2,3-Dipropyl-5,6-bis(piperidinomethyl)-1,4-dihydroquinone (**2c**; 2.58 g, 6.6 mmol) in 10 mL of AcOH was heated to 80 °C and CrO_3 (1.11 g, 0.011 mol) in 10 mL of warm 50% aqueous AcOH was added at a rate that maintained the reaction temperature between 80 and 85 °C. Following this addition and completion of the resulting exothermic reaction, the mixture was poured onto ice. After the ice had melted, the resulting precipitate was collected, rinsed with ice-cold MeOH, and subjected to column chromatography on silica gel using toluene as the eluent. The toluene was evaporated under reduced pressure, taking care to not sublime the product, and the resulting bright yellow residue was crystallized from MeOH to give 0.885 g (3.5 mmol, 53%) of **3c**, mp 106.5-107.5 °C. Anal. ($C_{14}H_{18}O_4$) C, H.

4,5-Dimethyl-3-hydroxy-6-methoxyphthalaldehyde (4). A solution of **3a** (0.20 g, 1.0 mmol) in 25 mL of dry acetone in which K_2CO_3 (0.6 g, 4 mmol) had been suspended was refluxed under argon with stirring. Me_2SO_4 (0.11 mL, 1.2 mmol) was added and the mixture was refluxed for 1.5 h. Acetone-insoluble material was removed by filtration and the solvent was evaporated under reduced pressure. The residue was eluted from a column of silica gel with toluene. Material in the second peak (the first peak was unreacted **3a**) was further purified by preparative silica gel TLC developed with $CHCl_3$. The major band was extracted with acetone, the solvent was removed under reduced pressure, and the residue was stored over P_2O_5 under high vacuum. After 6 h, the product was sublimed under high vacuum at approximately 70 °C to yield 0.023 g (0.11 mmol, 11%) of **4**, mp 71-73 °C. Anal. ($C_{11}H_{12}O_4$) C, H.

3,6-Dimethoxy-4,5-dimethylphthalaldehyde (5). Compound **3a** (0.20 g, 1.0 mmol) was refluxed in 25 mL of dry acetone in which 0.6 g of K_2CO_3 had been suspended as described above for the synthesis of **4**. Me_2SO_4 (0.22 mL, 2.3 mmol) was added and the mixture was refluxed for 1 h. Additional Me_2SO_4 (0.22 mL, 2.3 mmol) was added and the reaction was continued for 2 h. The reaction mixture was cooled, filtered, and evaporated under reduced pressure. The residue was suspended in 2 to 3 mL of ice-cold acetone and filtered to give 80 mg (0.35 mmol, 35%) of **5**, mp 130 °C dec. The sample for elemental analysis was obtained by high vacuum sublimation at approximately 100 °C. Anal. ($C_{12}H_{14}O_4$) C, H.

3,6-Dihydroxy-4,5-dimethylphthalaldehyde Hemialdal Tetraacetate (6). A solution of **3a** (0.37 g, 1.9 mmol) in 10 mL of dry pyridine and 5 mL of Ac_2O (53 mmol) was stirred at room temperature overnight. The solvent and excess Ac_2O were evaporated under reduced pressure. EtOH was added and evaporated three times to remove traces of pyridine. The crystalline product was suspended in cold MeOH (5 mL), collected by vacuum filtration, and rinsed twice with cold MeOH to yield 0.6 g (1.6 mmol, 83%) of **6**, which melted over a wide range beginning at approximately 140 °C. Although the product was probably a mixture of isomers, it gave a single spot on silica gel TLC developed in either $CHCl_3$ or toluene. A sample was recrystallized from EtOH. The NMR spectrum and melting point of this product were identical with those of the nonrecrystallized product. Anal. ($C_{18}H_{20}O_9$) C, H.

Hydrolysis of **6** yielded the parent dialdehyde, **3a**. A 10^{-2} M solution of **6** in Me_2SO was immediately diluted to a concentration of 10^{-3} M with PBS, pH 7.3. At each of several time points from 0.75 to 45 min, an aliquot of the solution was diluted 10-fold in EtOH, and the absorption of the ethanolic solution at 400 nm was measured using a Gilford 2443-A rapid sampler attached to a Beckman DU spectrophotometer to monitor the appearance of **3a**.

Acknowledgment. This work was supported in part by U.S. Public Health Service Grants CA-02817 and CA-16359 from the National Cancer Institute. The authors are grateful to Dr. William Loh for assistance in obtaining some of the analytical data.

Synthesis and Antineoplastic Activity of Phenyl-Substituted Benzenesulfonylhydrazones of 2-Pyridinecarboxaldehyde 1-Oxide[1]

William Loh, Lucille A. Cosby, and Alan C. Sartorelli*

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510. *Received January 7, 1980*

A variety of derivatives of 2-pyridinecarboxaldehyde 1-oxide benzenesulfonylhydrazone, containing substituents on the benzene or pyridine rings as well as on the nitrogen atom which is bonded directly to the sulfonyl group, have been synthesized. The antineoplastic activity of these compounds has been assessed in mice bearing either leukemia L1210 or P388. The most potent agents in this series were 2,4-dimethoxy-, 3,4-dimethoxy-, and 2,4,6-trimethylbenzenesulfonylhydrazone of 2-pyridinecarboxaldehyde 1-oxide, all causing disappearance of tumors in 20-80% of leukemia-bearing mice.

Phenylsulfonylhydrazones of 2-pyridinecarboxaldehyde 1-oxide have been demonstrated by our laboratory[2-5] to possess significant antineoplastic activity against a variety of transplanted murine neoplasms. It was previously ob-

(1) Presented in part before the Division of Medicinal Chemistry at the 178th National Meeting of the American Chemical Society, Washington D.C., Sept 1979. This work was supported by U.S. Public Health Service Grants CA-02817 and CA-16359 from the National Cancer Institute.

(2) A. C. Sartorelli, K. C. Agrawal, B. A. Booth, J. Pittman, D. G. Bartholomew, and A. D. Broom, *J. Med. Chem.*, **19**, 830 (1976).

(3) K. C. Agrawal and A. C. Sartorelli, *J. Med. Chem.*, **21**, 218 (1978).

0022-2623/80/1823-0631$01.00/0