Antimicrobial and Cytotoxic Properties of 9,10-Dihydrophenanthrenes: Structure–Activity Studies on Juncusol

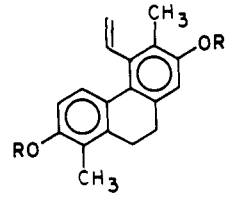
Dale L. Boger,*[†1a] Lester A. Mitscher,[†] Michael D. Mullican,[†1b] Steven D. Drake,[†] and Paul Kitos[‡]

Departments of Medicinal Chemistry and Biochemistry, University of Kansas, Lawrence, Kansas 66045.
Received February 19, 1985

The antimicrobial and cytotoxic properties of a series of 9,10-dihydrophenanthrenes structurally related to juncusol (**1a**), a postulated phytoalexin with confirmed cytotoxic properties, are detailed. Two simple 9,10-dihydrophenanthrenes, 2,7-dihydroxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2h**, desvinyljuncusol) and 2-hydroxy-3-methyl-9,10-dihydrophenanthrene (**3h**), were found to possess in vitro antimicrobial activity comparable with that of the natural product. Two 9,10-dihydrophenanthrenes substituted with quaternary ammonium salts, **2d** and **3d**, each containing a reactive benzylic dimethyl[(phenylthio)methyl]ammonio group, were found to be 10–20 times more potent than juncusol (**1a**). Confirmed in vitro cytotoxic activity that parallels antimicrobial activity was found for juncusol (**1a**), desvinyljuncusol (**2h**), 2-hydroxy-3-methyl-9,10-dihydrophenanthrene (**3h**), and the quaternary dimethyl[(phenylthio)methyl]ammonium salts **2d** and **3d** in a human lymphoblastic leukemia cell culture (CCRF-CEM, IC_{50} = nt, 9.3, nt, 0.9, and 1.4 μg/mL, respectively), B-16 mouse melanoma cell culture (IC_{50} = 12.5, 17.5, 27.7, 0.3, and 0.5 μg/mL, respectively), and L-1210 mouse lymphocytic leukemia cell culture (IC_{50} = 13.8, 10.2, 24.5, 1.3, and 3.7 μg/mL, respectively). The comparable potency and spectrum of activity of juncusol (**1a**), desvinyljuncusol (**2h**), and 2-hydroxy-3-methyl-9,10-dihydrophenanthrene (**3h**) suggest that the agents are acting as simple phenols in exerting their antimicrobial and cytotoxic effects.

Juncusol (**1a**), a highly substituted 9,10-dihydrophenanthrene isolated[2a] and identified[2] in the search for the cytotoxic constituent(s) of *Juncus roemerianus*, has been shown to possess confirmed cytotoxic activity[2a] (ED_{50} = 0.3 μg/mL against NCI 90 KB, human epidermoid carcinoma of the nasopharnyx), and recent studies have detailed its antimicrobial[3a] and antifeedant[3b] properties against organisms customarily found in the habitat of *J. roemerianus*.[4]

Herein, we detail the preparation[5] and in vitro evaluation of the antimicrobial and cytotoxic properties of a series of 9,10-dihydrophenanthrenes structurally related to juncusol (**1a**).

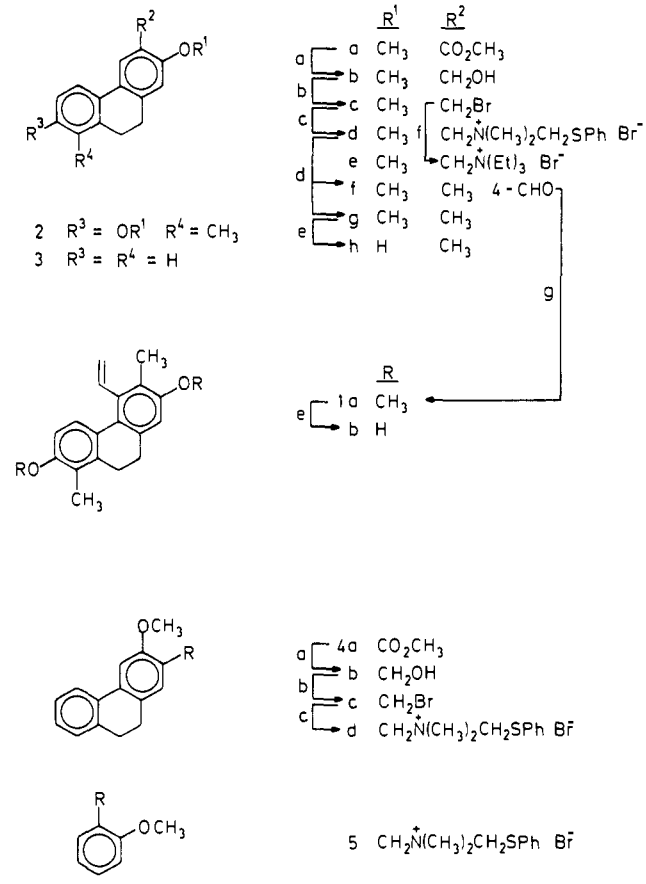


1a, R = H (juncusol)
1b, R = CH_3

Chemistry. The preparation of juncusol (**1a**) and related 9,10-dihydrophenanthrenes utilized in this study is detailed in Scheme I. Juncusol (**1a**),[5a] dimethyljuncusol (**1b**),[5a] and 9,10-dihydrophenanthrenes **2a–d,f,g**,[5a] **3a**[5b] and **4a**[5b] were prepared as previously described.[5] Demethylation (LiSEt, HMPA, 160 °C) of 2,7-dimethoxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2g**) provided 2,7-dihydroxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2h**, desvinyljuncusol).

The dimethyl[(phenylthio)methyl]ammonium salt **3d**, 2-methoxy-3-methyl-9,10-dihydrophenanthrene (**3g**), and 2-hydroxy-3-methyl-9,10-dihydrophenanthrene (**3h**) were prepared from 2-methoxy-3-(methoxycarbonyl)-9,10-dihydrophenanthrene (**3a**)[5b] by an analogous reaction sequence. Triethylammonium salt **3e** was prepared by treatment of 3-(bromomethyl)-2-methoxy-9,10-dihydrophenanthrene (**3c**) with triethylamine (CH_3CN, 25 °C, 18 h).

Scheme I[a]



[a] Key: (a) 4.0 equiv of $LiAlH_4$, THF, 0 °C, 1 h; **2b** (100%),[5a] **3b** (96%), **4b** (95%). (b) HBr(g), benzene, 25 °C, 0.25–1 h; **2c** (100%),[5a] **3c** (88%), **4c** (71%). (c) 1.0 equiv of $(CH_3)_2NCH_2SPh$, CH_3CN, 25 °C, 14–16 h; **2d** (100%),[5a] **3d** (100%), **4d** (100%). (d) 3.0–6.0 equiv of *t*-BuOK, DME, −20 to +25 °C, 4.5 h; H_2O/HOAc/THF (1:3:3), 25 °C, 16–17 h; **2f** (27%)/**2g** (28%),[5a] **3f** (20%)/**3g** (36%). (e) 3.3 equiv of EtSLi, HMPA, 160 °C, 3.5–5.5 h; **1a** (84%),[5a] **2h** (92%), **3h** (55%). (f) 2.0 equiv of Et_3N, CH_3CN, 25 °C, 18 h; **3e** (94%). (g) 7 equiv of Ph_3PCH_2, THF, 25 °C, 16 h; **1b** (77%).[5a,6a]

The dimethyl[(phenylthio)methyl]ammonium salt **4d** was prepared from 3-methoxy-2-(methoxycarbonyl)-9,10-

[†] Department of Medicinal Chemistry.
[‡] Department of Biochemistry.

0022-2623/85/1828-1543$01.50/0

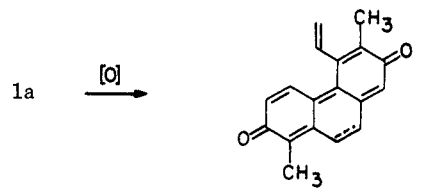
dihydrophenanthrene (**4a**)[5b] following the procedure described for **2d** and **3d**.

Dimethyl(*o*-methoxybenzyl)[(phenylthio)methyl]ammonium bromide (**5**) was prepared from *o*-methoxybenzyl bromide as previously described.[6a]

Biological Results and Discussion. The in vitro antimicrobial assays were performed with an agar-dilution/streak assay[7] against seven microorganisms: *Staphylococcus aureus* ATCC 13709, *Escherichia coli* ATCC 9637, *Salmonella gallinarum* ATCC 9184, *Klebsiella pneumoniae* ATCC 10031, *Mycobacterium smegmatis* ATCC 607, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. The results are detailed in Table I.

2,7-Dihydroxy-3,8-dimethyl-9,10-dihydrophenanthrene (desvinyljuncusol, **2h**) and 2-hydroxy-3-methyl-9,10-dihydrophenanthrene (**3h**) are comparable in potency and spectrum of in vitro antimicrobial activity with the natural product juncusol (**1a**). In each case, the corresponding methyl ether, dimethyljuncusol (**1b**), 2,7-dimethoxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2g**), or 2-methoxy-3-methyl-9,10-dihydrophenanthrene (**3g**), is virtually inactive, indicating that a free phenol is necessary for activity. The presence of the 2,7-dihydroxy-9,10-dihydrophenanthrene structure found in both juncusol (**1a**) and desvinyljuncusol (**2h**) suggests that mild oxidation to a 9,10-dihydrophenanthra-2,7-quinone or phenanthra-2,7-quinone may play a role in the observed antimicrobial and cytotoxic activity (eq 1). However, the comparable activity of phenol **3h**, which is unable to undergo a related, mild oxidation, discounts the likelihood of 9,10-dihydrophenanthra-2,7-quinone participation.

In the routine screening of intermediates en route to the preparation of **1a**/**2h**, an unexpected emergence and significant increase in antimicrobial activity was observed in the 9,10-dihydrophenanthryl methyl ethers substituted with a benzylic quaternary ammonio group.[8] [(2-Methoxy-9,10-dihydro-3-phenanthryl)methyl]triethylammonium bromide (**3e**) is equipotent with juncusol. The quaternary ammonium salts **2d** and **3d**, containing a benzylic dimethyl[(phenylthio)methyl]ammonio group, are 10–20 times as potent as juncusol and 15–30 times as potent as the parent dimethyl(*o*-methoxybenzyl)[(phenylthio)methyl]ammonium bromide (**5**). In the one case studied, moving the location of the dimethyl[(phenylthio)methyl]ammonio group on the 9,10-dihydrophenanthrene nucleus, **4d**, produced a drop in the potency and spectrum of antimicrobial activity.

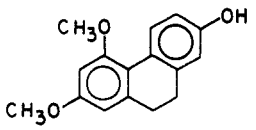


(1)

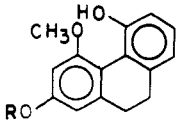
In all cases studied, the active 9,10-dihydrophenanthrenes are selective for Gram-positive, acid-fast, and fungal microorganisms and are inactive against most Gram-negative bacteria. The similarity of the spectrum of antimicrobial activity detailed in Table I and the consistent parallel potencies of the various structures suggest that they are exerting their antimicrobial activity as simple phenols or quaternary ammonium salts rather than by a mechanism unique to the structural features found in juncusol.

2,7-Dihydroxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2h**, desvinyljuncusol) and the [(9,10-dihydrophenanthryl)methyl]dimethyl[(phenylthio)methyl]ammonium salts **2d** and **3d** were tested for growth inhibitory activity in a human lymphoblastic leukemia cell culture (CCRF-CEM).[9] The results are detailed in Table II. Each compound tested showed inhibitory growth activity, thus confirming cytotoxic activity. The potency of the quaternary ammonium salts (**2d > 3d**) is 1 order of magnitude higher than that observed with desvinyljuncusol (**2h**), results that closely parallel antimicrobial activity.

Parallel studies with B-16 mouse melanoma[10] (**2d > 3d > 1a ≅ 2h ≅ 3h**) and L-1210 mouse lymphocytic leuke-



orchinol



hircinol, R = H
loroglossol, R = CH_3

(1) (a) Searle Scholar Recipient, 1981–1985. Recipient of a National Institutes of Health Career Development Award, 1983–1988 (CA 00898/01134). Alfred P. Sloan Research Fellow, 1985–1987. (b) National Institutes of Health Predoctoral Trainee, 1980–1983 (GM 07775).

(2) (a) Miles, D. H.; Bhattacharyya, J.; Mody, N. V.; Atwood, J. L.; Black, S.; Hedin, P. A. *J. Am. Chem. Soc.* **1977**, *99*, 618. (b) Miles, D. H.; Pelletier, S. W.; Bhattacharyya, J.; Mody, N. V.; Hedin, P. A. *J. Org. Chem.* **1978**, *43*, 4371.

(3) (a) Chapatwala, K. D.; De la Cruz, A. A.; Miles, D. H. *Life Sci.* **1981**, *29*, 1997. (b) *Ibid.* **1982**, *30*, 1805.

(4) Related phytoalexins, orchinol, hircinol, and loroglossol, inhibit the growth of microorganisms at a level of 3–500 μg/mL; see: (a) Fisch, M. H.; Flick, B. H.; Arditti, J. *Phytochemistry* **1973**, *12*, 437. (b) Urech, J.; Fechtig, B.; Nuesch, J.; Vischer, E. *Helv. Chim. Acta* **1963**, *46*, 2758. (c) Coxon, D. T.; Ogundana, S. K.; Dennis, C. *Phytochemistry* **1982**, *21*, 1389. (d) Gaumann, E. *Phytopathologische Z.* **1963**, *49*, 211. (e) Ward, E. W. B.; Unwin, C. H.; Stoessl, A. *Phytopathology* **1975**, *65*, 632.

(5) (a) Boger, D. L.; Mullican, M. D. *J. Org. Chem.* **1984**, *49*, 4045. Boger, D. L.; Mullican, M. D. *Tetrahedron Lett.* **1982**, *23*, 4555. (b) *Ibid.* **1982**, *23*, 4551. Boger, D. L.; Mullican, M. D. *J. Org. Chem.* **1984**, *49*, 4033.

(6) For additional total syntheses of juncusol, see: (a) Kende, A. S.; Curran, D. P. *Tetrahedron Lett.* **1978**, 3003; *J. Am. Chem. Soc.* **1979**, *101*, 1857. (b) McDonald, E.; Martin, R. T. *Tetrahedron Lett.* **1978**, 4723. (c) Schultz, A. G.; Shen, M. *Ibid.* **1981**, *22*, 1775.

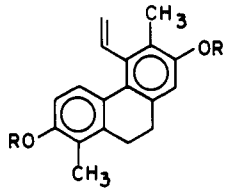
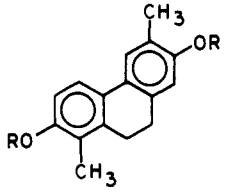
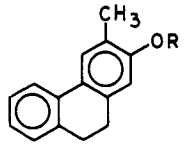
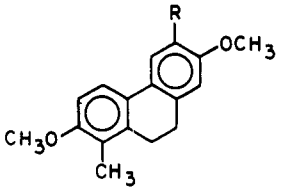
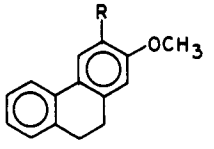
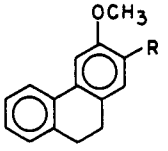
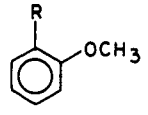
(7) Mitscher, L. A.; Leu, R.-P.; Bathala, M. S.; Wu, W.-N.; Beal, J. L.; White, R. *Lloydia* **1972**, *35*, 157.

(8) For discussions of the activity and potential modes of action of oniom salts, see: (a) Dubois, R. J.; Lin, C.-C. L.; Beisler, J. A. *J. Med. Chem.* **1978**, *21*, 303. (b) Hollstein, U. In "Burger's Medicinal Chemistry", 4th ed.; Wolfe, M. E., Ed.; Wiley: New York, 1979; Part II, p 173. (c) Bodor, N.; Kaminski, J. J.; Selks, S. *J. Med. Chem.* **1980**, *23*, 469.

(9) Foley, G. E.; Lazarus, H.; Farber, S.; Uzmar, B. G.; Boone, B. A.; McCarthy, R. E. *Cancer* **1965**, *18*, 522. Foley, G. E.; Lazarus, H. *Biochem. Pharmacol.* **1967**, *16*, 659.

(10) Donoso, J. A.; Himes, R. H. *Cancer Biochem. Biophys.* **1984**, *7*, 133. The morphology of the trypsinized B-16 cells at all test concentrations of **2d** and **3d** that were used (0.5–10 μg mL^{-1}) was different from that of the controls. They were pleiomorphic rather than essentially spherical. This observation and the remote, structural similarity of the 9,10-dihydrophenanthrenes typified by juncusol with colchicine, podophyllotoxin, and combretastatin (cf.: Hamel, E.; Lin, C. M. *Biochem. Pharmacol.* **1983**, *32*, 3864), potent mitotic inhibitors that bind to tubulin-specific sites preventing microtubule assembly, suggested that the agents under study may be cell cycle phase-specific mitotic inhibitors. DNA/cell histograms generated by a fluorescence-activated cell sorter provided no discernible evidence of cell cycle phase-specific activity for **2d** and **3d**, relative to untreated controls. We thank Dr. H. Pearce, Eli Lilly and Co., for providing us with this information.

Table I. In Vitro Antimicrobial Activity

compd[a]		MIC,[b-d] μg/mL			
		S. aureus (13709)	*K. pneumoniae* (10031)	*M. smegmatis* (607)	*C. albicans* (10231)
	1a, R = H[e] 1b, R = CH_3[f]	25 i	i i	12.5 i	i i
	2h, R = H[f] 2g, R = CH_3[f]	12.5 i	50 i	6.25 i	50 i
	3h, R = H[f] 3g, R = CH_3[f]	25 i	i i	3.12 25	6.25 100
	2d, R = $CH_2N^+(CH_3)_2CH_2SPh\ Br^-$[f]	1.56	12.5	1.56	3.12
	3a, R = CO_2CH_3[g] 3d, R = $CH_2N^+(CH_3)_2CH_2SPh\ Br^-$[f] 3e, R = $CH_2N^+(CH_2CH_3)_3\ Br^-$[g]	50 1.56 25	i 25 i	25 1.56 25	i 12.5 i
	4a, R = CH_2CH_3[g] 4d, R = $CH_2N^+(CH_3)_2CH_2SPh\ Br^-$[e]	25 6.25	i i	3.12 3.12	i i
	5, R = $CH_2N^+(CH_3)_2CH_2SPh\ Br^-$[f]	50	i	50	100

[a] Compounds 1-5 were inactive against *Escherichia coli* (ATCC 9637), *Salmonella gallinarum* (ATCC 918), and *Pseudomonas aeruginosa* (ATCC 27853). [b] See the Experimental Section for MIC (minimum inhibitory concentration) determination. [c] American type culture collection (ATCC). [d] i = inactive at the highest concentration tested. [e] Tested at 25-0.1 μg/mL. [f] Tested at 100-0.1 μg/mL. [g] Tested at 50-0.1 μg/mL.

Table II. In Vitro Cytotoxic Activity

compd	IC_{50},[a] μg/mL		
	CCRF-CEM[b]	B-16[c]	L-1210[d]
1a	nt[e]	12.5	13.8
2h	9.3	17.5	10.2
3h	nt[e]	27.5	24.5
2d	0.9	0.3	1.3
3d	1.4	0.5	3.7

[a] See the Experimental Section for IC_{50} (inhibitory concentration for 50% cell growth relative to untreated control) determination. [b] Human lymphoblastic leukemia cell culture, see ref 9. [c] B-16 mouse melanoma, see ref 10. [d] L-1210 mouse lymphocytic leukemia cell culture (ATCC CCL-219), see ref 11. [e] Not tested = nt.

mia[11] (**2d** > **3d** > **1a** ≅ **2h** ≅ **3h**) cell culture assays provided similar findings, and the results are detailed in Table II. No in vivo antitumor activity was found for **3d** in preliminary studies.[12]

The parallel potency and spectrum of in vitro antimicrobial and cytotoxic activity of juncusol (**1a**), desvinyljuncusol (**2h**), and 2-hydroxy-3-methyl-9,10-di-

(11) The L1210 ATCC 219 cell line was obtained from American Type Culture Collection.

(12) Dimethyl[(2-methoxy-9,10-dihydro-3-phenanthryl)methyl]-[(phenylthio)methyl]ammonium bromide (**3d**) exhibited no in vivo antitumor activity: 3PS31 leukemia (daily/8 days) 80/40/20/10 mg/kg (toxic), 5 mg/kg (T/C = 105), 2.5 mg/kg (T/C = 101), 1.25 mg/kg (T/C = 108). These data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

hydrophenanthrene (**3h**) suggest that juncusol exerts its effects as a simple phenol rather than by a mechanism that is embodied in the structural features of the naturally occurring material.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian FT80A spectrometer. Infrared spectra (IR) were recorded on a Beckman IR-33 or Perkin-Elmer 727 spectrophotometer. Electron impact mass spectra (EIMS) and high-resolution mass spectra (HRMS) were recorded on a Varian CH-5 or Ribermag R10-10 spectrometer. Microanalysis were performed by T. I. Nguyen on a Hewlett-Packard Model 185B CHN analyzer at the University of Kansas. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected.

All dry solvents were distilled under argon or nitrogen. Tetrahydrofuran (THF) and benzene were distilled from benzophenone ketyl. Acetonitrile was distilled from phosphorus pentoxide. All reactions involving oxygen- or moisture-sensitive materials were performed in oven-dried glassware under positive pressure of argon or nitrogen. All reaction mixtures were isolated from the atmosphere and stirred magnetically. Solutions and liquids were introduced into reaction vessels with oven-dried syringes through rubber septa.

Compounds **1a**, **1b**, **2a–d,f,g**, **3a**, **4a**,[5] and **5**[6a] were prepared and fully characterized as described elsewhere. Compounds **4b** and **4c** were prepared and characterized as described in the supplementary material.

[(2,7-Dimethoxy-8-methyl-9,10-dihydro-3-phenanthryl)methyl]dimethyl[(phenylthio)methyl]ammonium Bromide (2d). Ammonium salt **2d** was prepared as previously described:[5a] mp 173–175 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1 H, C-4 H), 7.78–7.63 (m, 3 H, aromatic), 7.41–7.27 (m, 3 H, aromatic), 6.82 (s, 1 H, C-1 H; d, J = 9 Hz, 1 H, C-6 H), 5.44 (s, 2 H, $R_3N^+CH_2SPh$), 4.96 (s, 2 H, $ArCH_2N^+R_3$), 3.89 and 3.82 (two s, 3 H each, two $ArOCH_3$), 3.22 (s, 6 H, $R_2N^+(CH_3)_2$), 2.83 (s, 4 H, $ArCH_2CH_2Ar$), 2.19 (s, 3 H, $ArCH_3$); IR ($CHCl_3$) ν_{max} 3020, 2975, 1590, 1480, 1260, 1245, 1095, 1010 cm^{-1}.

Anal. Calcd for $C_{27}H_{32}BrNO_2S$: C, 63.03; H, 6.27; N, 2.72. Found: C, 63.00; H, 6.40; N, 2.73.

2,7-Dihydroxy-3,8-dimethyl-9,10-dihydrophenanthrene (2h, Desvinyljuncusol). Following the procedure for the conversion of juncusol dimethyl ether (**1b**) to juncusol (**1a**),[5a] 2,7-dimethoxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2g**)[5a] was converted to **2h** (92%): mp 210–220 °C (dec; acetone–benzene); ^{1}H NMR (acetone-d_6) δ 8.03 (br s, 2 H, two OH), 7.41 (s, 1 H, C-4 H; d, J = 8 Hz, 1 H, C-5 H), 6.77 (d, J = 8 Hz, 1 H, C-6 H), 6.69 (s, 1 H, C-1 H), 2.71 (s, 4 H, $ArCH_2CH_2Ar$), 2.23 and 2.19 (two s, 3 H each, two $ArCH_3$); IR ($CHCl_3$) ν_{max} 3600, 3600–3100, 2955, 1591, 1275 cm^{-1}; EIMS, m/e (relative intensity) 240 (M^+, base), 239 (14), 225 (18), 181 (12), 165 (15), 152 (11); HRMS m/e 240.1139 ($C_{16}H_{16}O_2$ requires 240.1149).

Dimethyl[(2-methoxy-9,10-dihydro-3-phenanthryl)methyl][(phenylthio)methyl]ammonium Bromide (3d). A solution of 2-methoxy-3-(methoxycarbonyl)-9,10-dihydrophenanthrene[5b] (**3a**; 592 mg, 2.17 mmol) in dry THF (5 mL) was added dropwise over 25 min to a 0 °C slurry of lithium aluminum hydride (169 mg, 4.45 mmol, 4.1 equiv) in THF (5 mL) under nitrogen. The resulting mixture was stirred at 0 °C for 1.5 h. The 0 °C reaction mixture was treated sequentially with H_2O (0.10 mL), 5% aqueous NaOH (0.20 mL), and H_2O (0.10 mL) and the resulting mixture stirred at 25 °C for 1.5 h. The mixture was filtered through Celite (ether wash), and the filtrate was concentrated in vacuo. Chromatography (SiO_2, 2 × 15 cm, 40% ether–hexane eluant) afforded 528 mg (552 mg theoretically 96%) of 3-(hydroxymethyl)-2-methoxy-9,10-dihydrophenanthrene (**3b**) as a white solid: mp 80–82 °C; ^{1}H NMR ($CDCl_3$) δ 7.75–7.50 (m, 1 H, aromatic), 7.69 (s, 1 H, C-4 H), 7.22 (m, 3 H, aromatic), 6.76 (s, 1 H, C-1 H), 4.68 (d, J = 6 Hz, 2 H, $ArCH_2OH$), 3.89 (s, 3 H, OCH_3), 2.87 (s, 4 H, $ArCH_2CH_2Ar$); IR ($CHCl_3$) ν_{max} 3600, 3450, 3010, 2940, 1620, 1465, 1280, 1050 cm^{-1}; EIMS, m/e (relative intensity) 240 (M^+, base), 211 (18), 197 (10), 194 (12), 179 (51), 165 (44); HRMS, m/e 240.1142 ($C_{16}H_{16}O_2$ requires 240.1149).

Hydrogen bromide (gas) was bubbled through a 25 °C solution of 3-(hydroxymethyl)-2-methoxy-9,10-dihydrophenanthrene (**3b**; 703 mg, 2.93 mmol) in dry benzene (30 mL) for 30 min. The reaction mixture was carefully neutralized with saturated aqueous $NaHCO_3$, and the resulting mixture was extracted with ether (4 × 20 mL). The combined organic extracts were washed with saturated aqueous NaCl (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was passed rapidly through a plug of silica gel to give 781 mg (888 mg theoretical, 88%) of 3-(bromomethyl)-2-methoxy-9,10-dihydrophenanthrene (**3c**) as a white solid: mp 96–97 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (s, 1 H, C-4 H), 7.40 (m, 1 H, aromatic), 6.94 (m, 3 H, aromatic), 6.47 (s, 1 H, C-1 H), 4.36 (s, 2 H, $ArCH_2Br$), 3.65 (s, 3 H, $ArOCH_3$), 2.58 (s, 4 H, $ArCH_2CH_2Ar$); IR (KBr) ν_{max} 2950, 1610, 1465, 1280, 770 cm^{-1}; EIMS, m/e (relative intensity) 304/302 (M^+, 1/1, 10), 224 (21), 223 (base), 193 (27), 179 (24), 178 (66), 165 (36), 89 (24); HRMS, m/e 302.0274 ($C_{16}H_{15}BrO$ requires 302.0305).

The bromide **3c** (781 mg, 2.58 mmol) in dry acetonitrile (10 mL) was treated with dimethyl[(phenylthio)methyl]amine[13a] (540 mg, 3.23 mmol, 1.1 equiv) at 25 °C, and the resulting mixture was stirred at 25 °C for 16 h.[13b] The reaction solution was diluted with benzene (100 mL) and the precipitate collected by filtration (washing with benzene) to give 1.20 g (1.20 g theoretically 100%) of **3d** as a white solid: mp 163–165 °C (ethanol–ether); ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1 H, C-4 H), 7.57–7.00 (m, 9 H, aromatic), 6.82 (s, 1 H, C-1 H), 5.44 (s, 2 H, $R_3N^+CH_2SPh$), 5.00 (s, 2 H, $ArCH_2N^+R_3$), 3.90 (s, 3 H, OCH_3), 3.21 (s, 6 H, $R_2N^+(CH_3)_2$), 2.86 (s, 4 H, $ArCH_2CH_2Ar$); IR ($CHCl_3$) ν_{max} 2950, 1620, 1450, 1245, 860, 820 cm^{-1}.

Anal. Calcd for $C_{25}H_{28}BrNOS$: C, 63.82; H, 6.00; N, 2.98. Found: C, 63.80; H, 6.20; N, 2.98.

[(2-Methoxy-9,10-dihydro-3-phenanthryl)methyl]triethylammonium Bromide (3e). Following the procedure for the preparation of [(2,7-dimethoxy-8-methyl-9,10-dihydro-3-phenanthryl)methyl]dimethyl[(phenylthio)methyl]ammonium bromide (**2d**),[5a] 3-(bromomethyl)-2-methoxy-9,10-dihydrophenanthrene (**3c**) was converted to **3e** (1.3 equiv of triethylamine, CH_3CN, 25 °C, 17.5 h; 94%): mp 190–192 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1 H, C-4 H), 7.80–7.05 (m, 4 H, aromatic), 6.83 (s, 1 H, C-1 H), 4.73 (s, 2 H, $ArCH_2N^+(Et)_3$), 3.89 (s, 3 H, $ArOCH_3$), 3.52 (q, J = 7 Hz, 6 H, $-N^+(CH_2CH_3)_3$, 2.87 (s, 4 H, $ArCH_2CH_2Ar$), 1.46 (t, J = 7 Hz, 9 H, $N^+(CH_2CH_3)_3$); IR ($CHCl_3$) ν_{max} 2960, 1610, 1460, 1240, 1040 cm^{-1}.

2-Methoxy-3-methyl-9,10-dihydrophenanthrene (3g) and 3f. Following the procedure for the preparation of 2,7-dimethoxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2g**) from [(2,7-dimethoxy-8-methyl-9,10-dihydro-3-phenanthryl)methyl]dimethyl[(phenylthio)methyl]ammonium bromide (**2d**),[5a] dimethyl[(2-methoxy-9,10-dihydro-3-phenanthryl)methyl][(phenylthio)methyl]ammonium bromide (**3d**) was converted to **3f** (20%) and **3g** (36%). For **3g**: ^{1}H NMR ($CDCl_3$) δ 7.70–7.05 (m, 4 H, aromatic), 7.45 (s, 1 H, C-4 H), 6.68 (s, 1 H, C-1 H), 3.84 (s, 3 H, OCH_3), 2.82 (s, 4 H, $ArCH_2CH_2Ar$), 2.25 (s, 3 H, CH_3); IR ($CHCl_3$) ν_{max} 3010, 2950, 1610, 1580, 1220, 1140, 1050, 760 cm^{-1}; EIMS, m/e (relative intensity) 224 (M^+, base), 223 (7), 209 (46), 194 (10), 178 (26), 166 (32), 165 (66), 89 (26), 82 (22), 77 (25), 76 (20), 69 (27); HRMS, m/e 224.1191 ($C_{16}H_{16}O$ requires 224.1200).

2-Hydroxy-3-methyl-9,10-dihydrophenanthrene (3h). Following the procedure for the preparation of 2,7-dihydroxy-3,8-dimethyl-9,10-dihydrophenanthrene (**2h**), 2-methoxy-3-methyl-9,10-dihydrophenanthrene (**3g**) was converted to **3h** (55%): ^{1}H NMR ($CDCl_3$) δ 7.70–7.05 (m, 4 H, aromatic), 7.50 (s, 1 H, C-4 H), 6.64 (s, 1 H, C-1 H), 4.67 (br s, 1 H, -OH), 2.80 (s, 4 H, $ArCH_2CH_2Ar$), 2.28 (s, 3 H, $ArCH_3$); IR ($CHCl_3$) ν_{max} 3610, 3350, 3010, 2950, 1620, 1450, 1280, 1120 cm^{-1}; EIMS, m/e (relative intensity) 210 (M^+, base), 209 (24), 195 (37), 194 (18), 166 (15), 165 (40), 89 (16), 82 (17), 76 (19); HRMS, m/e 210.1042 ($C_{15}H_{14}O$ requires 210.1044).

Anal. Calcd for $C_{15}H_{14}O$: C, 85.68; H, 6.71. Found: C, 85.38; H, 6.51.

Dimethyl[(3-methoxy-9,10-dihydro-2-phenanthryl)methyl][(phenylthio)methyl]ammonium Bromide (4d). Following the procedure for the preparation of [(2,7-dimeth-

(13) (a) Grillot, G. F.; Thompson, H. G. *J. Org. Chem.* **1957**, *22*, 706. (b) Michelot, D.; Lorne, R.; Huynh, C.; Julia, S. *Bull. Soc. Chim. Fr.* **1976**, *43*, 1482.

oxy-8-methyl-9,10-dihydro-3-phenanthryl)methyl]dimethyl-[(phenylthio)methyl]ammonium bromide (**2d**)[5a] and **3d**, 3-methoxy-2-(methoxycarbonyl)-9,10-dihydrophenanthrene (**4a**)[5b] was converted to **4d** (68% overall): ^{1}H NMR ($CDCl_3$) δ 7.70–6.90 (**m**, 11 H, aromatic), 4.48 (s, 2 H, $R_3N^+CH_2SPh$), 4.15 (s, 2 H, $ArCH_2N^+R_3$), 3.83 (s, 3 H, OCH_3), 2.60 (s, 4 H, $ArCH_2CH_2Ar$); IR ($CHCl_3$) ν_{max} 3000, 1580, 1480, 1025 cm^{-1}.

Anal. Calcd for $C_{25}H_{28}BrNOS$: C, 63.82; H, 6.00; N, 2.98. Found: C, 63.43; H, 5.95; N, 3.10.

In Vitro Antimicrobial Activity. Minimum inhibitory concentrations (MIC μg/mL) were determined by the agar dilution/streak method following previously established procedures.[7]

In Vitro Antitumor Activity. The agents were tested for their toxicity to B16 (mouse melanoma) cells[10] and L-1210 (ATCC 219, mouse lymphocytic leukemia) cells[11] in culture as follows: A population of 1×10^4 cells in 0.5 mL of Dulbecco-modified Eagles medium (DMEM) containing 5% bovine serum was added to each well of a 24-place cluster dish (Costar, Cambridge, MA). The cultures were incubated at 37 °C for 48 h under 5% CO_2, 95% humidified air. The test substances, dissolved at 10 mg mL^{-1} in 95% ethyl alcohol, were added to fresh culture medium to 2× the intended test concentration. To each well of the cluster dish was added 0.5 mL of the appropriate "2×" medium so as to constitute 1 mL of medium per well having the desired concentration of test agent. The cultures were incubated for an additional 48 h, and then the number of cells per well was determined by using either a hemocytometer or a Coulter Particle Counter (Coulter Electronics, Inc., Hialeah, FL).

The L1210 cells do not adhere to the culture dish and can be separated from one another by aspirating the culture through the tip of a pipette. Thus dispersed, the cells were suspended in a total volume of 10 mL with a balanced salts solution (Isoton, Coulter Diagnostics, Hialeah, FL) and counted. The B-16 cells do attach to the plastic culture surface. The medium was removed from these cultures, and 0.2 mL of 0.01% crystalline porcine trypsin, 0.1% EDTA in divalent cation-free phosphate-buffered saline was added to each well. After 5 min at 37 °C, the culture plates were chilled on ice and 0.8 mL of phosphate buffered saline was added to each well. The number of cells in the suspensions was then determined. The number of cells per well was plotted as a function of the concentration of the test agent, and the dose that reduces the cell count to 50% of the untreated controls was determined and is reported as the IC_{50}.

Acknowledgment. This work was assisted financially by the Searle Scholars Program, The National Institutes of Health (CA 33668/42056, AI 13155, and ES 02046). We thank Sharon M. Rinzel of Eli Lilly and Co. for performing the in vitro CCRF-CEM assays and Dr. Homer Pearce for his assistance.

Registry No. **1a**, 62023-90-9; **1b**, 66447-86-7; **2a**, 85531-86-8; **2b**, 85559-36-0; **2c**, 85559-37-1; **2d**, 91551-17-6; **2f**, 69496-44-2; **2g**, 91551-21-2; **2h**, 97860-88-3; **3a**, 85531-84-6; **3b**, 97860-89-4; **3c**, 97860-90-7; **3d**, 97860-91-8; **3e**, 97877-58-2; **3f**, 97860-92-9; **3g**, 97860-93-0; **3h**, 97860-94-1; **3i**, 97860-98-5; **4a**, 85531-82-4; **4b**, 97860-95-2; **4c**, 97860-96-3; **4d**, 97860-97-4; **4i**, 97860-99-6; **5**, 69496-31-7; **6a**, 85531-85-7; **6b**, 85531-79-9.

Supplementary Material Available: Experimental details for the preparation of **4b** and **4c** and a table detailing additional in vitro antimicrobial activity of 9,10-dihydrophenanthrenes and related structures (9 compounds) examined (4 pages). Ordering information is given on any current masthead page.

Book Reviews

Modern Aging Research. Volume 5. Senile Dementia: Outlook for the Future. Edited by Jean Wertheimer and Maurice Marois. Alan R. Liss, Inc., New York. 1984. xxvii + 532 pp. 16 × 23.5 cm. ISBN 0-8451-2305-X. $68.00.

This book is the fifth in a series on Modern Aging Research. It represents the proceedings of an international conference entitled: "Senile Dementia in the Course of the Next Two Decades", which was held in Lausanne, Switzerland, May 2–4, 1983.

The editors have assembled a variety of authorities in the field of Alzheimer's disease and senile dementia, and the book reflects the broad spectrum of topics which were covered at this conference. Chapters have been subsumed under specific headings: Cerebral Biochemistry and Senile Dementia; Critical Evaluation of Senile Dementia Research; Aging of Nerve Tissue and Lesions of Alzheimer's Disease; Socio-medical Organization and Senile Dementia; Clinical Aspects of Alzheimer's Disease; Therapeutic Possibilities; Epidemiology of Senile Dementia; and Socio-political Problems of Senile Dementia. The book also includes a Forward; the Opening Address by each of the co-editors of this book; an Introduction by Dr. David Danon; and an Overview of the Conference by Sir Martin Roth. A total of 45 presentations are included in the book, representing contributors from all over the world.

This book could serve as a useful resource to individuals interested in having a general understanding of current issues of concern in the area of aging, dementia, and Alzheimer's disease. Because of the large variety of issues addressed, however, the book is not comprehensive in any one particular area. Rather, more general information and overviews have been included. Several pertinent topics were not even included in the book, such as an evaluation of drugs which are currently being used in the treatment of Alzheimer's disease and a description of animal models which might be useful for the study of aging, dementia, and Alzheimer's disease. Obviously, it was not feasible in the context of a 3-day symposium to cover all aspects of this subject in a comprehensive manner.

Since this book is an assemblance of individual manuscripts which were prepared in camera-ready format, the style throughout is not uniform. Also, a number of grammatical and spelling mistakes are evident, reflecting the consequences of attempting to hastily put together and publish the proceedings of a symposium. This is also reflected in differences in spelling of abbreviations of the same term in different chapters of the book (for example, choline acetyltransferase spelled as CAT or ChAT). While most chapters are in English, several have been included in French. Obviously, a compromise had to be reached in the interest of rapid publication of the conference's proceedings, in an attempt to provide "new" information to readers interested in the subject matter.

In summary, therefore, because of its broad and general approach, this book would not be of use to medicinal chemists interested in evaluating current knowledge in the field, as a stimulus for the development of research projects that they could undertake in the area of Alzheimer's disease and senile dementia. It would, however, as indicated above, serve as an excellent resource to provide a glimpse into the issues which currently are being discussed vis-a-vis this entire topic of investigation. As such, "Senile Dementia: Outlook for the Future" is a useful introduction to senile dementia, and does provide a good number of current references.

Israel Hanin, Ph.D.
Western Psychiatric Institute and Clinic
Department of Psychiatry
University of Pittsburgh
School of Medicine
Pittsburgh, Pennsylvania 15213