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Structure and Tumor-Promoting Activity of Analogues of Anthralin (1,8-Dihydroxy-9-anthrone)

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Seventeen analogues of the tumor-promoting agent anthralin were tested for the same biological property by repeated skin application on mouse skin using female ICR/Ha Swiss mice, after a single application of a subcarcinogenic dose of 7,12-dimethylbenz[*a*]anthracene. Seven of the compounds tested are new compounds. They are 1,8-diacetoxy-9-anthrone, 1,8-dimyristoyloxy-9-anthrone, 1,8-dihydroxy-10-acetyl-9-anthrone, 1,8-dihydroxy-10-myristoyl-9-anthrone, 1,8,10-trihydroxy-9-anthrone, 1,8-dihydroxy-9,10-dihydroanthracene, and myristoyljuglone. All compounds were used in pure form for the bioassays. Of the 17 test compounds four showed notable tumor-promoting activity. They are 1,8-dihydroxy-10-acetyl-9-anthrone, 1,8-dihydroxy-10-myristoyl-9-anthrone, 1-hydroxy-9-anthrone, and juglone. In order to determine whether there is any relationship between tumor-promoting activity and metal chelation in this series, the chelating abilities of anthralin and of its inactive analogue 1,8-dihydroxyanthraquinone were examined using the bivalent metal ions Cu(II), Zn(II), Mn(II), Mg(II), and Ca(II). No relationship between chelation and tumor-promoting ability was found.

Anthralin (1,8-dihydroxy-9-anthrone) has been used for many years in the treatment of psoriasis and related skin diseases.[1] This compound attracted our attention because of its reported tumor-promoting activity on mouse skin.[2] In subsequent work in this laboratory, its tumor-promoting activity in two-stage carcinogenesis on mouse skin was confirmed.[3] It was also shown to be cocarcinogenic; i.e., it enhanced remarkably the carcinogenic activity of a low dose of benzo[*a*]pyrene on mouse skin when the two agents were applied simultaneously and repeatedly on mouse skin.[4] The compound is less active as a tumor promotor and cocarcinogen than the phorbol esters of croton oil, e.g., phorbol myristate acetate.[4] Because it is a comparatively simple molecule, anthralin is useful for studies on mode of action and structure–activity relationships of tumor promoters and cocarcinogens.

This report describes the tumor-promoting activity of a series of 17 analogues of anthralin. A number of these are new compounds, synthesized specifically for this study.

It has been suggested that metal chelation plays a role in chemical carcinogenesis.[5-7] As part of the present study it was, therefore, of interest to examine the chelating abilities of some representative compounds in this group. The present report includes the chelation characteristics of the tumor promoter anthralin and its biologically inactive analogue 1,8-dihydroxyanthraquinone[3] with Cu(II), Zn(II), Mn(II), Mg(II), and Ca(II) ions.

In the course of the synthesis of the acetate and myristate esters of anthralin for bioassay, it was found that hydrogen bonding of the protons of the C-1 and C-8 hydroxyl groups to the C-9 carbonyl oxygen had unusual effects on the acylation reactions of anthralin. These findings are also given in this report.

Results and Discussion

Chemistry. In the course of the synthesis of the acetate and myristate esters of anthralin, it was found that the molecule reacted to the acylating agents in an unusual manner. In previous experiments from this laboratory[8] it was found that the reaction of pyrogallol (1,2,3-trihydroxybenzene) with acyl chlorides under basic conditions (pyridine as solvent) gave a mixture of mono-, di-, and triesters; when the reaction was carried out in benzene solution, pyrogallol was acylated at C-4. Similar results with phenol esterification were obtained by others.[9] When anthrone was allowed to react with acyl chlorides in pyridine O-acylation occurred, suggesting that the enolate form of anthrone predominated. In the absence of base and in hydrocarbon solvents the keto form of anthrone is more stable than the enol form.[10]

When anthralin was allowed to react with acyl chlorides under alkaline conditions, the expected esters were not formed; instead the 10-acyl derivatives, 10-acetylanthralin (**4**) and 10-myristoylanthralin (**5**), were isolated. Under acid conditions, anthralin reacted with acetic anhydride and myristoyl chloride to give the 1,8-diesters, i.e., the diacetate **2** and the dimyristate **3**, rather than the acyl derivative.

In basic solution (pyridine), anthralin exists primarily in the enolate form with the charge being distributed between the three oxygens on C-1, C-8, and C-9 as well as on C-10 through resonance. The resonance interactions involving the three oxygens and two protons associated with them inhibit acylation at these positions, thus acylation takes place at the C-10 position via the carbanion. In acid solution anthralin exists in the keto form (1,8-dihydroxy-9-anthrone) with protonation of the C-9 carbonyl oxygen possibly disrupting intramolecular hydrogen bonding and facilitating ester formation.

Tumor-Promoting Activity. The results of the two-stage carcinogenesis experiments with 17 analogues of anthralin are summarized in Table I. Anthralin was used as a positive control. In these experiments anthralin did not result in carcinomas, as were observed in our earlier experiments.[3] The earlier experiments[3] were continued for 490 days, whereas most of the experiments shown in

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Table I. Tumor-Promoting Activity of Anthralin and Related Compounds in Mouse Skin[a]

Test compd and dose, μg	Days to first papilloma	(Mice with papillomas[b])/(total papillomas)
1,8-Dihydroxy-9-anthrone (1, anthralin), 80	68	12/22 (0)
1,8-Diacetoxy-9-anthrone (**2**), 124	322	2/3 (0)
1,8-Dimyristoyloxy-9-anthrone (3), 228		0
1,8-Dihydroxy-10-acetyl-9-anthrone (4), 15	89	6/10 (1)
1,8-Dihydroxy-10-myristoyl-9-anthrone (**5**), 300[c]	55	12/15 (2)[d]
1,8,10-Trihydroxy-9-anthrone (**6**), 85		0
1-Hydroxy-9-anthrone (7), 80[e]	154	10/12 (4)[d]
1,2-Dihydroxy-9-anthrone (**8**), 80		0
2,3-Dihydroxy-9-anthrone (**9**), 80		0
2,2′-Dihydroxybenzophenone (**10**), 75		0
2,3-Dihydroxyanthracene (11), 75		0
1-Hydroxyanthracene (12), 80[f]	486	1/1 (0)
1,8-Dihydroxyanthracene (13), 75		0
3,4-Dihydro-8,9-dihydroxy-1(2*H*)-anthracenone (14), 80		0
1,8-Dihydroxy-9,10-dihydroanthracene (**15**), 80		0
Juglone (**16**), 62	148	3/4 (2)
Acetyljuglone (17), 76	370	1/1 (0)
Myristoyljuglone (**18**), 136		0

[a] Thirty female ICR/Ha Swiss mice per group except where noted. A single treatment with 20 μg of 7,12-dimethylbenz[*a*]anthracene in 0.1 mL of acetone was followed 2 weeks later by three times weekly application of the test compounds in 0.1 mL of acetone. Duration of test 370 days except where noted. Not shown in this table are those tests in which the compounds were applied without pretreatment with initiator or with initiator alone. No skin tumors were observed with any of these tests. Skin tumors were also not observed in the no-treatment groups and the incidences of spontaneous tumors at other sites were identical with those observed in the many earlier experiments in this laboratory in which female ICR/Ha Swiss mice were used as controls. [b] Number of mice with squamous carcinoma of skin in parentheses. [c] Twenty mice; duration of test 521 days; median survival time 423 days. [d] One mouse with metastatic squamous carcinoma of lung. [e] Twenty mice; duration of test 521 days; median survival time 391 days. [f] Twenty mice; duration of test 521 days; median survival time 474 days.

Table I were terminated at 370 days which was prior to median survival time. Compounds **5**, **7**, and **12** were kept on test for 521 days; also there were only 20 animals per group with these three compounds. Not shown in Table I are those tests in which the compounds were applied without pretreatment with initiator or with initiator alone. No skin tumors were observed with any of these compounds. Skin tumors were also not observed in the no-treatment groups and the incidences of spontaneous tumors at other sites were identical with those observed in the many earlier experiments in this laboratory in which female ICR/Ha Swiss mice were used as controls.

Of the 17 test compounds, **4**, **5**, **7**, and **16** gave qualitatively noteworthy tumor-promoting activity. Compounds **2**, **12**, and **17** showed marginal activity which cannot be assessed; all the other analogues were inactive as tumor promoters.

It is difficult to rank the order of tumor-promoting activity of any series of compounds accurately because of the variety of factors which need to be considered. These include tumor multiplicity, days to first tumors, number of animals with tumors, and the incidence of squamous carcinomas of the skin. It is, therefore, not feasible to simply tabulate any one list of significance values as an index of tumor-promoting activity. The present tests were complicated by the fact that for two of the active compounds, **5** and **7**, the animal group sizes were smaller and these tests were continued for 521 days.

However, some observations on structure and tumor-promoting activity can be made. Thus, comparing compounds **1**, **4**, and **5**, all three of which are active, the 1, 8, and 9 substituents are the same and intact. Esterification of the 1 or 9 positions (**2** and **3**) results in loss of tumor-promoting activity. Also, where the three groups are intact, but the middle ring is opened as in **10**, activity is lost. The addition of an acetyl or myristoyl group on the 10 position of anthralin does not markedly affect tumor-promoting activity compared with anthralin. Compounds **11**–**15** were all inactive; it was also found in our earlier study that anthrone and 1,8-dihydroxynaphthalene are inactive as tumor promoters.[3] We conclude from these studies that the structural features of anthralin necessary for tumor-promoting activity are at least one phenolic hydroxyl group hydrogen bonded to the C-9 carbonyl oxygen and one benzylic proton at the C-10 position.

These findings suggested that metal chelation may play a role in the tumor-promoting activity of anthralin. The results of these studies are described below.

Metal Chelation of Anthralin (1) and of 1,8-Dihydroxyanthraquinone (DHAQ). These two compounds were selected for metal chelation studies because 1 is active as a tumor promoter while DHAQ is completely devoid of activity. An examination of the chelating abilities of these two ligands with biologically important metal ions was considered relevant because of the structural specificity discussed above and indications from earlier reports that chelation may play a role in cancer causation.[5,6] Anthralin (1) gave a pure solid complex only with copper $(C_{14}H_9O_3)_2Cu$. DHAQ gave solid 2:1 complexes with all five bivalent metal ions as shown in Table II. The metal–ligand mole ratios in dimethyl sulfoxide solution, determined by Job's method of continuous

Table II. Elemental Analyses and Molecular Formulas of Metal Chelates

	Molecular formula	Calcd, %			Found, %		
		C	H	Metal	C	H	Metal
DHAQ	$(C_{14}H_7O_4)_2Cu$	62.05	2.60	11.72	61.92	2.55	11.63
	$(C_{14}H_7O_4)_2Zn$	61.84	2.60	12.02	61.70	2.86	12.10
	$(C_{14}H_7O_4)_2Mn$	63.06	2.64	10.30	62.86	2.85	10.29
	$(C_{14}H_7O_4)_2Mg \cdot H_2O$	64.57	3.07	4.67	64.09	3.12	4.79
	$(C_{14}H_7O_4)_2Ca \cdot H_2O$	63.38	3.01	7.56	63.21	3.10	7.34
1	$(C_{14}H_9O_3)_2Cu$	65.43	3.51	12.36	65.33	3.54	12.21

Table III. Stability Constants of Metal Chelates of DHAQ and 1 in Dimethyl Sulfoxide[a]

Metal[b]	$\Delta\epsilon \times 10^4$	Log K
Cu 1	2.80	5.77
2	2.80	9.56
Zn 1	1.90	5.62
2	1.49	9.23
Mn 1	2.40	5.58
2	2.56	9.19
Mg 1	2.70	5.53
2	1.40	9.16
Ca 1	4.70	10.03
2	1.10	9.06

[a] These values are for the mole ratios given in the text; the concentration ranges of metal and ligands were 1.0-4.5 × 10^{-5} M and 2.0-9.0 × 10^{-5} M for DHAQ and 1, respectively. [b] 1, DHAQ; 2, 1. For DHAQ the wavelength maxima used for the different metal ions ranged from 520 to 543 nm; for 1 the same wavelength, 475 nm, was used for all five metal ions.

variation,[11] showed that DHAQ gave 1:1 metal–ligand ratios with Cu(II), Zn(II), Mn(II), and Mg(II) and a 1:2 ratio with Ca(II). Anthralin gave 1:2 mole ratios with all five metals.

The spectrophotometric method of Rossotti and Rossotti[12] was used to determine the stability constants of the complexes of all five divalent metal ions with DHAQ and 1. The values observed over wide concentration ranges are given in Table III. The stability constants for DHAQ showed the order: Cu > Zn > Mn > Mg. For anthralin the following order was observed: Cu > Zn > Mn > Mg > Ca.

Copper which forms the stronger homopolar bonds[13] also forms the most stable chelate. When the two ligands were compared using the same metal, i.e., calcium, the DHAQ–Ca chelate had the higher stability constant, demonstrating that DHAQ is a better ligand than 1. Prakash[14] has studied the crystal and molecular structure of DHAQ and concluded that this molecule is planar within the limits of experimental error. Molecular models also show that DHAQ is essentially planar, whereas 1 is puckered. This should enable DHAQ to chelate more effectively with the metal ion since the chelate ring is an unsaturated six-membered ring with considerable resonance character.

The shifts of the C=O stretching frequency ($\Delta\nu$) in the infrared absorption spectra of the metal chelates decrease in the order: Cu (29) > Zn (15) > Mn (13) > Mg (6) > Ca (4). Bellamy and Branch have also observed this shift in the position of the carbonyl absorption band to be directly related to the stability of the chelate.[15]

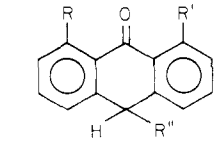
The study of the metal-chelating abilities of DHAQ and of 1 was undertaken in order to determine whether there is any clear-cut relationship between this property and tumor-promoting activity. Although the findings described here are of interest, they did not show any such relationship, and further studies in this area are underway.

Experimental Section

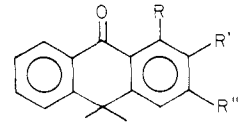
Animals. Female ICR/Ha Swiss mice (A. R. Schmidt/Sprague Dawley Inc., Madison, Wis.) were used for this experiment. They were vaccinated against ectromelia and started on test at age 8 weeks. Mice were housed on sterile wood chips (Iso-Dri; Fisher and Son, Bound Brook, N.J.), six to a cage, fed Purina Laboratory Chow and water ad libitum, and weighed regularly. The animal rooms were maintained at 22–24 °C.

Bioassay Procedure. The backs of the mice were clipped free from hair the day before the initial treatment and then as needed for the duration of the experiment. The solutions were all applied by micropipet to the dorsal skin; a single treatment with 20 μg of 7,12-dimethylbenz[a]anthracene in 0.1 mL of acetone was followed 2 weeks later by three times weekly applications of the test compounds in 0.1 mL of acetone. The dosages and duration of the experiments are given under the results below. The dosage of anthralin used was based on that used in the earlier studies.[2,3] This is also the maximum tolerable dose; higher doses were toxic and caused severe skin damage in mice. The other compounds, except compound 4, were applied at doses equimolar to that of anthralin. Compound 4 was used at a lower dose because of skin toxicity. Animals were observed regularly and the appearance of tumors was recorded; tumors greater than 1 mm in diameter were counted and recorded regularly. Only tumors which persisted for 30 days or more were counted in the cumulative totals. The results presented are based on these records. Animals bearing tumors that appeared grossly to be carcinomas were killed approximately 2 months after the tumors were clinically classified as malignant. All animals were autopsied at death and representative tumors and any gross abnormalities were excised, fixed in unbuffered 10% formalin, blocked in paraffin, stained with hematoxylin and eosin, and confirmed histologically. Included in the experimental protocol were control groups that received test compounds alone, no treatment and a positive control with anthralin as promoting agent.

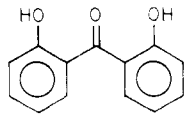
Chart I



1, R, R′ = OH; R″ = H
2, R, R′ = $OCOCH_3$; R″ = H
3, R, R′ = $OCO(CH_2)_{12}CH_3$; R″ = H
4, R, R′ = OH; R″ = $COCH_3$
5, R, R′ = OH; R″ = $CO(CH_2)_{12}CH_3$
6, R, R′, R″ = OH



7, R = OH; R′, R″ = H
8, R, R′ = OH; R″ = H
9, R = H; R′, R″ = OH



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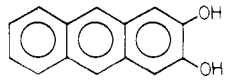

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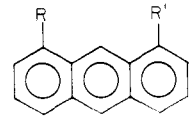
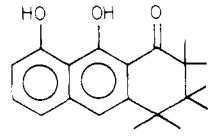


12, R = OH; R′ = H
13, R, R′ = OH



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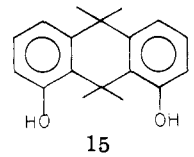
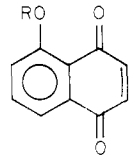


15



16, R = H
17, R = $COCH_3$
18, R = $CO(CH_2)_{12}CH_3$

Compounds 1, 10, and 16 were purchased from commercial suppliers and were used after purification and/or characterization as described below. In all cases elementary analyses were obtained (Spang Microanalytical Laboratories, Ann Arbor, Mich., and Galbraith Laboratories, Knoxville, Tenn.). Infrared absorption spectra were measured on a Perkin-Elmer Model 137 or Model 421 instrument. Ultraviolet visible absorption spectra were measured on a Cary Model 14 or a Beckman Model 25 instrument and all NMR spectra were measured on a Varian A-60A instrument. The purity of all compounds was established by melting point or boiling point, TLC and/or GC, IR and UV spectrophotometry, and NMR spectrometry.

Anthralin (1). Commercial material [ICN Life Sciences Group (K & K) Plainview, N.Y.] was purified and characterized as described before.[3]

2,2'-Dihydroxybenzophenone (10). Commercial material (Pfaltz and Bauer, Flushing, N.Y.) was pure by TLC, mp 61–62 °C (lit.[23] mp 59–60 °C) and was therefore used as such.

Juglone (16). Commercial material (Chemical Procurement Co., College Point, N.Y.) was purified by recrystallization from benzene, mp 156–158 °C (lit.[24] mp 153–154 °C).

1,8-Dihydroxy-9-anthrone Diacetate (2). This compound was prepared by heating 10 g (44.2 mmol) of anthralin in 60 mL of acetic anhydride at 120 °C for 5 h, under a nitrogen atmosphere. Upon cooling, 7.1 g of a yellow precipitate was obtained. The precipitate was washed with benzene and recrystallized twice from benzene–ethyl acetate yielding 4.1 g of the pure diacetate. Evaporation of the filtrate and column chromatography of the residue (silica gel 60, 60–230 mesh, E.M. Reagents) using benzene–ethyl acetate (10:1) as eluent afforded an additional 2.5 g of the pure acetate. The total yield was 48%: mp 210–212 °C; IR (KBr) 1760 (strong, ester carbonyl), 1660 (strong, carbonyl α to ring), 1075 cm^{-1} (strong, ester ether bond); NMR ($CDCl_3$) τ 7.6 (s, 6 H, acetate), 5.7 (s, 2 H, C-10 on ring), 3.2–2.4 (m, 6 H, aromatic); UV (CH_3OH) λ_{max} 210 nm (ϵ 11 000), 258 (11 000), 270 (7000), 310 (700), and 380 (450). Anal. Calcd for $C_{18}H_{14}O_5$: C, 69.69; H, 4.51. Found: C, 69.57; H, 4.48.

1,8-Dihydroxy-9-anthrone Dimyristate (3). Anthralin, 5.5 g (24.3 mmol), was refluxed with 14 g (65.8 mmol) of myristoyl chloride in 200 mL of toluene at 120 °C for 2 days under a nitrogen atmosphere. The brown solution was evaporated to dryness. Upon addition of petroleum ether to the viscous residue, 6.3 g of a yellow solid was obtained. The dimyristate was isolated from this yellow solid by column chromatography (200 g of silica gel 60, 60–230 mesh, E.M. Reagents) using hexane–benzene (9:1) as eluent. Recrystallization from hexane–benzene yielded 3.96 g of the purified dimyristate (26%): mp 104–106 °C; IR (KBr) 1760 (strong, ester carbonyl), 1670 (strong, carbonyl α to ring), 1140 cm^{-1} (strong, ester ether bond); NMR ($CDCl_3$) τ 8.0–9.1 (br, 54 H, methyl and methylene), 7.35 (t, 4 H, α to carbonyl), 5.75 (singlet, 2 H, C-10 on ring), 3.0–2.5 (m, 6 H, aromatic); UV (CH_2Cl_2) λ_{max} 228 nm (ϵ 6100), 270 (10 600), and 310 (2500). Anal. Calcd for $C_{42}H_{62}O_5$: C, 78.02; H, 9.59. Found: C, 77.82; H, 9.23.

10-Acetylanthralin (4). Anthralin, 6.78 g (30.0 mmol), was refluxed with 2.80 g (35.0 mmol) of acetyl chloride and 3.20 g (40.0 mmol) of pyridine in 200 mL of dry benzene. The reflux was maintained for 20 h. The solvent was removed under vacuum giving 8.9 g of an orange solid. The crude product was examined by TLC on silica gel. It was found to contain mostly **4** (R_f 0.37) and some starting material (R_f 0.51). The mixture was chromatographed (150 g of Florisil) using hexane–benzene (9:1, 1500 mL; 8:2, 1000 mL; and 5:5, 2000 mL). The 8:2 fraction contained mostly anthralin (1.13 g, 5.0 mmol). The 5:5 fraction contained 2.97 g of **4** (42%) as bright orange crystals. An analytical sample was prepared by recrystallization from benzene: mp 145–147 °C (lit.[25] mp 146 °C); IR (CCl_4) 1715 (unconjugated carbonyl), 1655 cm^{-1} (conjugated carbonyl); NMR ($CDCl_3$) τ 8.22 (s, 3 H, methyl), 4.83 (s, 1 H, benzyl), 3.17–2.35 (m, 6 H, aromatic), 2.14 (s, 2 H, phenolic); UV (ethanol) λ_{max} 223 nm (ϵ 16 000), 257 (12 000), and 385 (5000).

10-Myristoylanthralin (5). This compound was prepared by combining 5.65 g (25 mmol) of anthralin (1, mp 178–180 °C), 6.38 g (30 mmol) of myristoyl chloride, 3.00 g (37.5 mmol) of pyridine, and 175 mL of dry benzene in a 500-mL flask under a nitrogen atmosphere. The mixture was refluxed on an oil bath for 7 h. An additional 250 mL of benzene was then added and the mixture was filtered. The filtrate was washed with water (2 × 250 mL), 1 M HCl (1 × 250 mL), and water (2 × 250 mL) and then dried over anhydrous sodium sulfate. Solvent evaporation under vacuum gave 10.7 g of a light-orange crystalline material. The product was examined by TLC on silica gel (hexane–ether–acetic acid, 9.0:2.0:0.5). It was found to contain mostly product **5** (R_f 0.63) and some 1 (R_f 0.51). The mixture was chromatographed (200 g of silica gel Silic AR CC7) using hexane–benzene (9:1) as eluent. Removal of the solvent from the first 4.5 L of eluent gave 4.16 g of **5** (38%). An additional 1.04 g of **5** contaminated with some 1 was obtained in the next liter of eluent: total 5.20 g (47%). Recrystallization of **5** from hexane yielded a light yellow crystalline solid: mp 88.5–89.5 °C; IR (CCl_4) 1640 (strong, carbonyl α to ring), 1715 cm^{-1} (strong, aliphatic carbonyl); NMR ($CDCl_3$) τ 7.8–8.3 (br, 26–29 H, methyl and methylene), 4.71 (s, 1 H, benzyl), 2.30–3.25 (pair of t, 6 H, aromatic), 2.24 (s, 2 H, phenolic); UV (ethanol) λ_{max} 223 nm (ϵ 17 400), 263 (10 000), 283 (8800), and 360 (8700). Anal. Calcd for $C_{28}H_{36}O_4$: C, 77.00; H, 8.34. Found: C, 77.06; H, 8.30.

1,8,10-Trihydroxy-9-anthrone (6). 10-Bromoanthralin (2.9 g), freshly prepared by a previously described method,[25] was suspended in 150 mL of 60% aqueous acetone under nitrogen. The mixture was stirred under reflux; the solution darkened and the yellow precipitate which formed was collected (0.28 g). Water was added to the filtrate and the solution cooled to 0 °C. A second crop of the yellow precipitate was collected (1.79 g). The yellow solids were combined and recrystallized from benzene to give 1.8 g (79% yield) of yellow crystals: mp 165–167 °C; UV (*p*-dioxane) λ_{max} 368 nm (ϵ 10 200), 293 (8870), 265 (7400), and 224 (16 700); IR (KBr) 3500 (medium, hydroxyl), 3000 (medium, aromatic), 1645 (medium, carbonyl), 1620 cm^{-1} (strong, aromatic); NMR (acetone-d_6) (keto tautomer) 5.75 (singlet, 1 H), 6.80–8.0 (multiplet, 6 H), 6.90 (singlet, 1 H, –OH), and 12.1 ppm (singlet, 2 H, hydrogen-bonded phenolic OH); NMR (acetone-d_6) (enol tautomer) 6.8–8.0 (multiplet, 6 H), 7.4 (singlet, 1 H, –OH), and 7.5 ppm (singlet, 3 H, phenolic OH) (all hydroxyl proton peaks were absent in the NMR spectra after deuterium (D_2O) exchange); mass spectrum, M^+ at 242. Anal. Calcd for $C_{14}H_{10}O_4$: C, 69.42; H, 4.13. Found: C, 69.38; H, 4.11.

1,8,10-Trihydroxy-9-anthrone decomposes on silica gel TLC plates. The compound is stable in cyclohexane and benzene and is slowly oxidized to 1,8-dihydroxyanthraquinone in acetone or ethanol at 25 °C.

1,8-Dihydroxy-9,10-dihydroanthracene (15). 1,8-Dihydroxyanthraquinone diacetate was prepared as described before.[25] The product was purified by recrystallization from benzene: mp 254–255 °C (lit.[25] mp 241–242 °C). This compound was converted to 1,8-dihydroxy-9,10-dihydroanthracene by a modification of a procedure described earlier.[26] The diacetate (8.0 g, 0.025 M) was dissolved in 280 mL of glacial acetic acid and 32 g of zinc powder was added in portions with stirring. The solution was refluxed with stirring at 145–150 °C for 4.5 h. The warm solution was filtered and kept at 0–4 °C for 16 h. The solution was poured into 3 L of ice water and the resulting solution was extracted with chloroform (5 × 250 mL). The combined chloroform extracts were extracted with water until the aqueous extracts were neutral; the chloroform solution was dried over anhydrous sodium sulfate and evaporated in vacuo to obtain 6.0 g of a brown oil. The brown oil was chromatographed on a column of silica gel 60. Elution with chloroform–methanol (9.8:0.2) gave 1.1 g of a crude unstable tarry substance and further elution with chloroform–methanol (9.5:0.5) gave 3 g of a brown tar. The 3-g fraction was rechromatographed on an acid-washed Florisil column and eluted with benzene–chloroform (9:1) to obtain 1.0 g of a product identified as 1,8-dihydroxy-9,10-dihydroanthracene monoacetate, which was recrystallized: yellow crystals; mp 132.5–134.5 °C (benzene); IR (KBr) 3400 (hydroxyl) and 1740 cm^{-1} (carbonyl); UV (EtOH) λ_{max} 258 nm (ϵ 31 200), 328 (15 000), 278 (3950); NMR ($CDCl_3$) τ 3.61 (s, 3 H, acetate), 6.18 (d, 2 H, benzylic at C-10), 6.00 (d, 2 H, benzylic at C-9), 4.75 (s, 1 H, phenolic, exchangeable with D_2O), 4.45–3.50 (m, 6 H, aromatic). Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.51. Found: C, 75.32; H, 5.48.

For further structural proof 1,8-dihydroxy-9,10-dihydroanthracene monoacetate was converted to 1,8-dihydroxy-9,10-dihydroanthracene diacetate by reaction of the monoacetate with acetic anhydride–pyridine. The yellow crystalline product had

mp 140–141 °C. Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.33; H, 5.34.

1,8-Dihydroxy-9,10-dihydroanthracene was prepared from 1,8-dihydroxy-9,10-dihydroanthracene monoacetate using a special hydrolytic procedure.[19] The monoacetate (3.5 g, 0.014 M) was refluxed in 120 mL of glacial acetic acid for 3–4 min. Concentrated HCl (80 mL) was added dropwise over 20 min. The mixture was heated with stirring for an additional 5 min and then poured into 2 L of ice water. The precipitate was collected and washed with ice water until the washings were neutral and then air-dried. Successive recrystallization of the crude dark grey product (2.2 g, 76% yield) from benzene and then from EtOH–water produced yellow crystals: mp 206–207 °C; UV (EtOH) λ_{max} 259 nm (ϵ 21 400), shoulder at 253 (12 450), 272 (4470), 280 (4200); NMR (acetone-d_6) τ 6.10 (s, 4 H, benzyl), 1.85 (s, 2 H, phenolic exchangeable with D_2O). Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.23; H, 5.69. Found: C, 79.21; H, 5.64.

Myristoyljuglone (5-Hydroxy-1,4-naphthoquinone 5-Myristate) (18). Triethylamine (0.50 mL, 3.59 mmol) was added, with stirring at room temperature under nitrogen, to a solution of 0.5 g of juglone (2.87 mmol) in 50 mL of dry benzene. To this solution was added 2.5 mL (8.61 mmol) of myristoyl chloride over a period of 1 min. The resulting suspension was stirred at room temperature for 15 min and then refluxed for 22 h. After cooling and filtering this suspension, the filtrate was poured into 100 mL of ice water. The benzene layer was separated and washed with 0.5 N HCl (2 × 50 mL), H_2O (2 × 50 mL), 5% $NaHCO_3$ (3 × 25 mL), and H_2O (4 × 50 mL), and then dried over 4A molecular sieves. Benzene was removed by passing nitrogen through the solution at room temperature. The resulting solid was recrystallized from 5 mL of hexane, filtered, washed with hexane (3 × 5 mL) at −20 °C, and dried. This crude product (0.470 g, 42.8%) had an R_f 0.25 and showed a fluorescent impurity at R_f 0.08 on silica gel F_{254} TLC plates eluted with $CHCl_3$. Further purification was accomplished by fractional recrystallization from hexane–benzene (4:1). The fluorescent impurity crystallized first and was removed by filtration. The volume of the mother liquor was reduced to 4 mL and the product slowly crystallized as yellow plates. After filtration, the crystals were washed three times with hexane at −20 °C and dried at 0.1 mmHg. The pure myristoyl juglone weighed 0.110 g (10%): mp 70.5–71.0 °C; UV (ethanol) λ_{max} 338 nm (ϵ 2840), 242 (15 600) with shoulders at 227 (13 200), 248 (15 500), and 257 (10 700); IR (KBr) 2950 (strong, CH stretching), 1770 (strong, ester carbonyl), 1670 (strong, quinone), 1625 and 1600 cm^{-1} (medium, aromatic); NMR τ 8.68 (m, 25 H, aliphatic), 7.22 (t, J = 7.5 Hz, $OCOCH_2$–), 4.08 (s, 2 H, H_2 and H_3), 1.92 (m, H_6, J_{6-7} = 7.8 Hz, J_{6-8} = 1.7 Hz), 2.23 (m, H_7, J_{6-7} = 7.8 Hz, J_{7-8} = 8.0 Hz), 2.62 (m, H_8, J_{6-8} = 1.7 Hz, J_{7-8} = 8.0 Hz). Anal. Calcd for $C_{24}H_{32}O_3$: C, 74.97; H, 8.38. Found: C, 75.00; H, 8.41.

9-Anthryl Myristate. This ester was prepared as described for **5** from 2.91 g (15.0 mmol) of 9-anthrone, 4.41 g (18.0 mmol) of myristoyl chloride, and 1.80 g (22.5 mmol) of pyridine in 75 mL of dry benzene. The reflux was maintained for 7 h. The solvent was removed under vacuum to yield 6.7 g of a light yellow waxy solid. The crude product was examined by TLC on silica gel (hexane–ether–acetic acid, 9.0:2.0:0.5). The product was found to contain a new compound (R_f 0.81) and a small amount of starting material (R_f 0.51). The mixture was chromatographed on silica gel (100 g, 40–140 mesh) using hexane–benzene (9:1, 4.5 L) as eluent. Recrystallization from benzene–hexane gave 2.95 g of 9-anthryl acetate (49%): white crystalline product; mp 70.0–71.5 °C; IR (CCl_4) 1775 cm^{-1} (ester carbonyl); NMR ($CDCl_3$) τ 9.08 (br t, 3 H, terminal methyl), 8.65 (br s, 24 H, chain methylene), 7.05 (t, 2 H, methylene α to carbonyl), 1.85–2.60 (m, 8 H, aromatic protons), 1.64 (s, 1 H, proton on C-10); UV (ethanol) λ_{max} 253 nm (ϵ 97 000). Anal. Calcd for $C_{28}H_{36}O_2$: C, 83.16; H, 8.96. Found: C, 83.07; H, 8.89.

9-Anthryl Acetate. This ester was also prepared as described for **5** from 2.91 g (15.0 mmol) of 9-anthrone, 1.41 g (18.0 mmol) of acetyl chloride, and 1.80 g (22.5 mmol) of pyridine in 75 mL of dry benzene. The reflux was maintained for 7 h. The solvent was removed under vacuum giving 3.1 g of a yellow solid. The crude product was examined by silica gel TLC. It was found to contain mostly 9-anthryl acetate, R_f 0.44, and some starting material, R_f 0.51. The mixture was chromatographed on silica gel, 40–140 mesh, 100 g, using hexane–benzene (9:1, 3.5 L, and 8:2, 2.5 L, for a second fraction) as eluents. The product was obtained from the second fraction. Recrystallization from hexane–benzene gave 2.18 g (62%) of a light yellow product: mp 130.5–132.5 °C (lit.[27] mp 134–136 °C); IR (CCl_4) 1780 (ester carbonyl), 1210 cm^{-1} (COC stretching); NMR ($CDCl_3$) τ 7.40 (s, 3 H, methyl), 2.65–1.92 (m, 8 H, aromatic), 1.64 (s, 1 H, proton on C-10); UV (ethanol) λ_{max} 253 nm (ϵ 97 000). Anal. Calcd for $C_{16}H_{12}O_2$: C, 81.35; H, 5.11. Found: C, 81.34; H, 5.09.

Metal Chelation of Anthralin and 1,8-Dihydroxyanthraquinone. 1,8-Dihydroxyanthraquinone (DHAQ) was purified by column chromatography and recrystallization, mp 192–193 °C (lit.[28] mp 193–197 °C). Anthralin (1) was purified as described above. These two compounds were used for the metal chelation experiments described here. The chelates of the two compounds were prepared as follows. The ligand (2.0 mmol) was dissolved in 100 mL of warm anhydrous ethanol and 0.5 molar equiv of the metal acetate dissolved in 100 mL of anhydrous ethanol was added. The mixture was stirred at reflux under a nitrogen atmosphere for 10 min to 5 h, depending upon the metal ion used. The colored insoluble precipitates were collected by filtration, washed with 100 mL of warm anhydrous ethanol, and further purified by Soxhlet extraction with 250 mL of anhydrous ethyl ether. By this method, the pure chelates of DHAQ with Cu(II) (yield 90%), Zn(II) (yield 48%), and Mn(II) (yield 49%) and of 1 with Cu(II) (yield 48%) were obtained. In order to prepare the DHAQ chelates of Mg(II) and Ca(II), the procedure was modified. The DHAQ–Mg(II) chelate was prepared as follows. A 50-mL solution of 0.125 g of DHAQ in methanol was heated at reflux for 1 h with a 0.5 molar equiv of anhydrous magnesium chloride (0.019 g) in the presence of a catalytic amount of triethylamine. The solid product was purified by washing with hot methanol and hot benzene and dried under vacuum (yield 50%). The DHAQ–Ca(II) chelate was prepared as follows. A 125-mL solution of 0.125 g of DHAQ in methanol was stirred for 20 min with a 0.5 molar equiv of solid calcium acetate (0.044 g) at room temperature. The solid product was washed repeatedly with methanol and dried under vacuum (yield 45%). The Cu(II), Zn(II), Mn(II), Mg(II), and Ca(II) complexes of DHAQ were deep purple to red solids. These chelates are soluble in dimethyl sulfoxide, less soluble in dimethylformamide, but insoluble in other common organic solvents and water. Compound 1 gave an olive-green solid complex with Cu(II) from ethyl alcohol. Several unsuccessful attempts were made to isolate the pure chelates of 1 with Zn(II), Mn(II), Mg(II), and Ca(II). Attempts to obtain analytically pure chelates by use of other organic solvents, e.g., dioxane, acetonitrile, chloroform, dimethylformamide, dimethyl sulfoxide, acetone, etc., were unsuccessful. Elemental analyses of the metal chelates which could be obtained in pure form are given in Table II. Visible absorption spectra of the metal chelates of DHAQ and 1 were made in dimethyl sulfoxide because of low solubility in other solvents. The ratio of metal to ligand was determined by Job's method of continuous variation plots.[11] Solutions of DHAQ (1.0×10^{-4} M) and of 1 (2.0×10^{-4} M) and a metal acetate solution of identical molarity were used to prepare nine solutions in volumetric flasks varying the mole fractions of DHAQ and of 1 from 0.1 to 0.9. The absorption spectrum of each solution was then recorded from 400 to 650 nm. The stability constants were determined using eight solutions each of DHAQ (from 1.0×10^{-5} to 4.5×10^{-5} M), 1 (from 2.0×10^{-5} to 9.0×10^{-5} M), and metal acetate at the same concentrations. The absorption spectrum of each solution was recorded from 400 to 650 nm at 25 °C. All measurements were completed within 2 h after the preparation of the solutions. The IR spectrum of DHAQ showed strong intramolecular hydrogen bonding at 3050 cm^{-1} and a sharp C=O band at 1641 cm^{-1}. Another free C=O band appeared at 1680 cm^{-1}. In the metal chelates of DHAQ the band at 3050 cm^{-1} was shifted to 3450 cm^{-1}. The C=O band at 1680 cm^{-1} remained unchanged but that at 1641 cm^{-1} was shifted to shorter wavenumber 1637–1612 cm^{-1}, depending on the metal.

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Structural Requirements for Antileukemic Activity among the Naturally Occurring and Semisynthetic Maytansinoids[1a,2]

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In an effort to determine the structural requirements for the significant antileukemic, cytotoxic, antitubulin, and antimitotic activity exhibited by the novel ansa macrolide, maytansine (1), four new C-3 ester and six new C-9 ether homologues were synthesized. The biological activities of these compounds were assayed and compared to the activities of previously reported, naturally occurring maytansinoids. From the data, it is apparent that presence of the C-3 ester is necessary for significant activity, and variations in the ester group are not accompanied by marked changes in activity. However, elimination of the ester group, as in maytansinol (**7**), maysine (**8**), normaysine (**9**), and maysenine (**10**), results in a significant decrease in biological activity. Blockage of the C-9 carbinolamide via etherification markedly reduces antileukemic and cytotoxic activity and slightly reduces antitubulin activity but has relatively little effect on antimitotic acivity against sea urchin eggs. Thus, a free carbinolamide at C-9 is advantageous for optimal activity.

Earlier work in this laboratory on the potent antileukemic ansa macrolides from the genera *Maytenus* and *Putterlickia*[3] in the plant family Celastraceae led to the isolation of maytansine (**1**), maytanbutine (**2**), maytanprine (**3**), maytanvaline (**4**), maytanbutacine (**5**), maytanacine (**6**), maytansinol (**7**), maysine (**8**), normaysine (**9**), and maysenine (**10**).[4-8] Considerable biological[9-16] and chemical[17] interest in the maytansinoids continues, and maytansine (**1**) is currently undergoing clinical trials under the auspices of the National Cancer Institute. Maytansinoids **1–6** showed excellent antileukemic activity against P-388 lymphocytic leukemia in the mouse (PS) and potent cytotoxicity against cells derived from human carcinoma of the nasopharynx (KB).[18] In contrast, **7–10** showed greatly diminished activity against both PS and KB relative to **1–6**. Since **7–10** all lack the C-3 ester moiety, an ester at C-3 appears to be necessary for antileukemic activity.

In order to further investigate the structure–activity relationships among the maytansinoids, several modified C-3 esters were prepared from maytansinol (**7**).[7,8] For the current work, maytansinol (**7**) was prepared by treatment of maytanbutine (**2**) with lithium aluminum hydride in dry tetrahydrofuran at −23 °C.[19] Extensive preparative thin-layer chromatography (PTLC) of the reaction mixture yielded **7** in 40% yield.[20]

The C-3 trifluoroacetate ester **14** was prepared in 30% yield by treatment of **7** with trifluoroacetic anhydride–trifluoroacetic acid.[22] However, the most efficient method for preparation of other C-3 esters proved to be treatment

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