and lipophilic character of these 7 compounds seems to rule out the possibility of achieving enhanced *in vivo* activity by increasing lipophilicity and cytotoxicity. Perhaps more importantly, all 7 of these compounds contain, in addition to the α-methylene-γ-lactone, either an α-methylene-δ-lactone or a conjugated side chain ester as the second functionality, and an OH or *O*-acyl group adjacent to the CH_2 of the γ-lactone grouping. Probably as a result of this, these 7 compounds all show increased rates of cysteine addition, an observation that may only be of secondary importance.

Of the compounds studied, only one more, vernolepin acetate, shares the structural features noted above for the 7 *in vivo* actives and it has not been available in sufficient quantity to allow *in vivo* testing. All of the other sesquiterpene lactones share some but not all of these properties, so it may be that the presence of the indicated structural features may be essential for *in vivo* activity among sesquiterpene lactones.

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

ALVIN SEGAL, CHARLINE KATZ, AND BENJAMIN L. VAN DUUREN*

Laboratory of Organic Chemistry and Carcinogenesis, Institute of Environmental Medicine, New York University Medical Center, New York, New York 10016

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The compound anthralin, which is used for the treatment of skin diseases, is shown to exist normally in the semiquinone form, *i.e.*, 1,8-dihydroxy-9-anthrone. In Me_2CO or MeOH the compound spontaneously dimerizes and oxidizes to 1,8-dihydroxyanthraquinone. Anthralin dimer has the structure 1,6,7,12,18,21-hexahydroxy-5,12:6,11-di-*o*-benzo[*a*,*e*]cyclooctene. Anthralin is a potent tumor-promoting agent in 2-stage carcinogenesis. Its transformation products and other related compounds were inactive in this assay.

Anthralin (dithranol) has been used for many years for the treatment of psoriasis and related skin diseases.[1] This compound is of interest also because of its reported tumor-promoting activity in 2-stage carcinogenesis[2] on mouse skin and the possible occurrence of compounds of this type in tobacco tars. Anthralin is active in the sebaceous gland suppression test[3] which was at one time thought to be indicative of carcinogenic activity. It is mutagenic in yeast[4] and has been shown by absorption spectroscopic studies to bind to deoxyribonucleic acid.[5] Clinical experience suggests that the compound is irritating to human skin at high doses.[1]

While repeating the mouse skin experiment, we discovered that the accepted tautomeric structure for anthralin[6,7] is incorrect and, furthermore, that anthralin undergoes spontaneous dimerization and oxidation in Me_2CO; acetone is the solvent of choice for chronic mouse skin bioassays with this and many other compounds. Because of our finding that anthralin degrades in acetone solution which was also the solvent used in the earlier study,[2] it was important to reexamine the tumor-promoting activity with freshly prepared solutions of pure anthralin as well as that of the transformation products, *i.e.*, anthralin dimer and 1,8-dihydroxyanthraquinone. The present report describes the clarification of the structure of anthralin, a study of its transformation products, and the bioassay of anthralin and related compounds for tumor-promoting activity on mouse skin.

(1) A. Perutz, in "Handbuch der Haut und Geschlechts-Krankheiten," Vol. 5, Jadassohn, Ed., Springer Verlag, Berlin, 1930, p 155.
(2) F. G. Bock and R. Burns, *J. Nat. Cancer Inst.*, **30**, 393 (1963).
(3) F. G. Bock and R. Mund, *Cancer Res.*, **18**, 887 (1958).
(4) B. O. Gillberg, G. Zetterberg, and G. Swanbeck, *Nature (London)*, **214**, 415 (1967).
(5) G. Swanbeck and N. Thyresson, *Acta Dermato-Venereol.*, **45**, 344 (1965).
(6) *Chem. Abstr., 7th Collective Index*, **1962–1966**, 1974S (1969).
(7) "The Merck Index," Merck and Co., Rahway, N. J., 8th ed, 1968, p 394.

Experimental Section

Animals.—Female ICR/Ha Swiss mice (A. R. Schmidt-Millerton Co., Millerton, N. Y.) were used for this experiment. They were vaccinated against ectromelia and started on test at age 7 weeks. Mice were housed on sterile wood chips (Absorb Dri, Fisher and Son, Bound Brook, N. J.), 10 to a cage, fed Purina Laboratory chow and H_2O *ad libitum*, and weighed regularly. The animal rooms were maintained at 22–24°.

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 solns were all applied by micropipette in the interscapular region; a single treatment with 20 μg of 7,12-dimethylbenz[*a*]anthracene in 0.1 ml of Me_2CO was followed 2 weeks later by 3-times weekly application of anthralin or related compds in 0.1 ml of acetone. The dosages and duration of the experiments are given under Results below. The dosage of anthralin used was based on that used in the earlier study.[2] This is also the maximum tolerable dose. Higher doses were toxic and caused severe skin damage in mice. Animals were observed regularly and the tumors recorded; tumors greater than 1 mm in diameter were counted and charted regularly. Only tumors which persisted for 30 days or more were counted in the cumulative totals. The results presented below are based on these chartings. 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 4% formalin, blocked in paraffin, stained with hematoxylin and eosin, and confirmed histologically. Included in the experimental protocol were control groups that received promoters alone, sol-

vent alone, no treatment, and a positive control with phorbol myristate acetate[8,9] as promoting agent.

Anthralin.—Commercial quality material was purified by tlc on silica gel with PhH as eluant. The compd was recryst from hexane–PhH as yellow needles: mp 178–179°, reported mp 176°;[10] uv absorption in 95% EtOH, λ_{max} (ϵ_{max}), 258 (15,000), 286 (9600), 358 (9600); reported 257 (12,000), 287 (10,000), 357 (9760);[10] ir spectrum (KBr) showed relevant peaks at 3400, 3000, 1645 (H-bonded arom C=O), 1620, 1605, 1485, 1460, and 1445 cm^{-1}; mol wt (by osmometer in $CHCl_3$), calcd, 226; found, 235; nmr spectrum (60 MHz instrument, Varian A-60A) in $CDCl_3$ (Me_4Si), 4.40 (2 H, CH_2), 6.90–7.72 (6 H, arom) and 12.27 ppm (2 H, OH). Treatment of the $CDCl_3$ soln with D_2O resulted in disappearance of the signal at 12.27 ppm; the signals at 4.40 (CH_2) and 6.90–7.72 ppm (arom H) remain in a ratio of 2:6. Anthralin in acetone-d_6 (Me_4Si) showed peaks at 4.46 (2 H, CH_2), 6.83–7.63 (6 H, arom), and 12.20 ppm (2 H, OH). Mass spectrum showed M^+ 226. Anthralin fluoresces yellow-green in MeOH, max at 523 mμ with shoulders at 492 and 555 mμ using either 260 or 387 mμ for excitation. *Anal.* ($C_{14}H_{10}O_3$) C, H.

Anthralin Dimer.—When anthralin was allowed to stand in the dark at room temp in Me_2CO (20 g in 2 l. of Me_2CO) a yellow cryst product gradually pptd. Several crops of this material were collected by filtration at roughly once a week intervals for 5 weeks. A total of 7.74 g of material, mp 235–237°, was collected in this manner. The material was purified for anal. by recrystn from PhH and $CHCl_3$, resp, mp 235–237°. The pure material gave a single spot by tlc on silica gel with PhH as solvent, R_f 0.42, compared to anthralin, R_f 0.77. With hexane–AcOH (4:1) as solvent, the dimer showed a single spot, R_f 0.47, anthralin, 0.63. 1,8-Dihydroxyanthraquinone was obtained from the filtrate as described below. The dimerization of anthralin occurs also in MeOH: mol wt, calcd 450.4; *found* by osomometer in $CHCl_3$, 438; uv absorption in EtOH λ_{max} (ϵ_{max}), 269 (26,000), 370 (26,800). Anthralin dimer does not exhibit any fluorescence. *Anal.* ($C_{28}H_{18}O_6$) C, H.

The ir spectrum of the dimer (KBr) was similar to that of anthralin in the region 3400–1460 cm^{-1} and showed small differences in the fingerprint region. The mass spectrum of anthralin dimer was identical with that of anthralin. The nmr spectrum of anthralin dimer in $CDCl_3$ (Me_4Si) (60 MHz spectrometer, Hitachi Perkin Elmer R-20A, Baron Consulting Co., Orange, Conn.); because of the insolubility of anthralin dimer, 128 scans (200 sec per scan) were taken and averaged by a computer of average transients (Hitachi A-1600 A signal averaging analyzer) and the resultant spectra were integrated. Peaks were recorded at 11.44 (4 H, phenolic OH H-bonded to benzylic O) 6.12–7.32 (12 H arom; doublet at 6.12–6.14, doublet at 6.64–6.76, triplet at 7.08, 7.16, 7.32), 4.50 (2 H, CH_2) and 1.46 ppm (2 H, benzylic OH). Peaks at 11.44 and 1.46 ppm, resp, were removed from the spectrum by D exchange after shaking the $CDCl_3$ soln with D_2O.

Anthrone.—Commercially available material (K&K Laboratories, Plainview, N. Y.) was recrystd from hexane–PhH to constant mp: pale yellow crystals, mp 158–159°, reported mp 154–155°.[11] The recrystd compd gave a single spot after tlc on silica gel (PhH), R_f 0.58; with hexane–AcOH (4:1), R_f 0.057.

1,8-Dihydroxyanthraquinone.—Commercial quality material (Winthrop Laboratories, New York, N. Y.) was used as such for the bioassays. The light orange crystals showed mp 192–193°, reported mp 193–197°.[12] On a silica gel tlc the material showed a single spot, R_f 0.62 (PhH).

1,8-Dihydroxyanthraquinone from Anthralin.—The filtrate from which anthralin dimer was removed (see above) was evapd to dryness *in vacuo*; the residue was dissolved in PhH and chromatogd on a silica gel column. An orange-colored compd was eluted with the first few PhH fractions and was recrystd from hexane–PhH, mp 192–194°, identical by mmp and R_f on a silica gel plate with 1,8-dihydroxyanthraquinone.

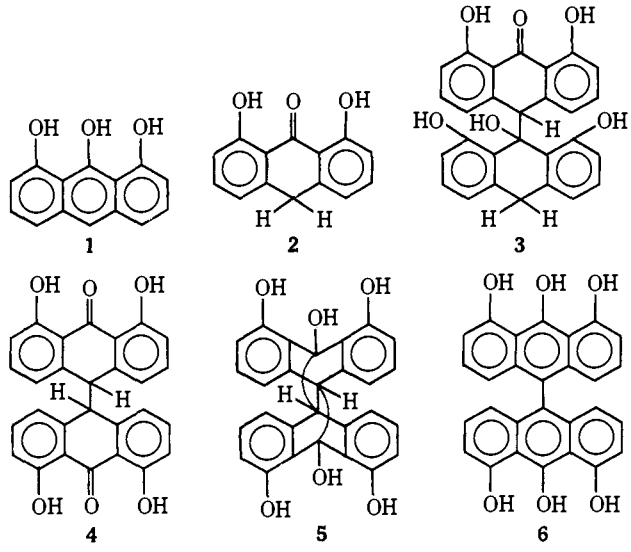
1,8-Dihydroxynaphthalene.—Commercial quality material (K&K Laboratories) was purified by recrystn from H_2O and then hexane–PhH to give colorless crystals, mp 143–144°, reported mp 145–146°.[13] Chromatography on tl plates gave a single spot only, R_f 0.21 (hexane–AcOH, 4:1).

(8) B. L. Van Duuren and L. Orris, *Cancer Res.*, **25**, 1871 (1965).
(9) B. L. Van Duuren, *Progr. Exp. Tumor Res.*, **11**, 33 (1968).
(10) D. W. Cameron and P. E. Schütz, *J. Chem. Soc. C*, 2121 (1967).
(11) K. H. Meyer, *Org. Syn.*, **8**, 7 (1928).
(12) V. V. Kozlov, *Dokl. Akad. Nauk, U.S.S.R.*, **61**, 281 (1948); *Chem. Abstr.*, **43**, 3400 (1949).
(13) Ng. Ph. Buu-Hoi and D. Lavit, *J. Chem. Soc.*, 2412 (1956).

Results and Discussion

Chemistry.—The long-accepted structure of anthralin is that shown in structure **1**, Chart I, *i.e.*, 1,8,9-trihydroxyanthracene.[6,7] However, the nmr and ir data obtained in our work indicate that the compound in the crystalline form as well as in $CHCl_3$ and Me_2CO is in the tautomeric form, *i.e.*, 1,8-dihydroxy-9-anthrone (**2**), Chart I. The integrated nmr data are quite unequivocal; the ir spectrum shows an intense peak at 1645 cm^{-1} which is in the correct region for H-bonded C=O conjugated to aromatic. In a recent report the preparation of a compound to which structure **2** was assigned, was described.[10] The melting point and spectroscopic data of that compound were identical with that found in the present work for anthralin. No comment was made about the identity of their compound with anthralin. We, therefore, conclude that anthralin is in the semiquinone form **2**.

CHART I



The observation that anthralin spontaneously undergoes dimerization and oxidation in Me_2CO is of interest since dimerization of the parent hydrocarbon anthracene and related hydrocarbons are usually light catalyzed.[14,15] The dimer was unknown and several possible structures were considered for it, structures **3**–**6**, Chart I, based on the known structures of related dimers. The ir spectrum of the dimer was similar to that of anthralin in the 3400–1460 cm^{-1} region with small differences in the fingerprint region. Although the osmometer mol wt was consistent with a dimer, the mass spectrum was identical with that of anthralin, *i.e.*, the dimer degrades *via* anthralin. It is known that compounds similar in structure to **5** degrade to the monomer when heated.[16] There was a distinct difference in the uv absorption spectra and nmr spectra of anthralin and its dimer. This change in the absorption spectrum is consistent with structure **5** by analogy to the dimer of anthracene.[14] The most useful information regarding the dimer was obtained from the nmr spectrum. Integration of the

(14) C. A. Coulson, L. E. Orgel, W. Taylor, and J. Weiss, *J. Chem. Soc.*, 2961 (1955).
(15) O. L. Chapman and K. Lee, *J. Org. Chem.*, **34**, 4166 (1969).
(16) H. B.-Laurent and R. Lapouyade, *C.R. Acad. Sci., Ser. C*, 1061 (1967).

peaks is consistent with **5**, *i.e.*, 1,6,7,12,18,21-hexahydroxy-5,12:6,11-di-*o*-benzo[*a*,*e*]cyclooctene.

Tumor-Promoting Activity.—Anthralin, anthralin dimer, and 3 related compounds were tested for tumor-promoting activity. A single dose of the initiating agent, 7,12-dimethylbenz[*a*]anthracene was followed 2 weeks later by 3-times weekly application of the promoting agent for the duration of the experiment. This is the usual 2-stage carcinogenesis experiment.[8,9] The potent tumor-promoting agent phorbol myristate acetate[8,9] was used as a positive control. The results of these experiments are shown in Table I. The animal survival was good in all cases, *i.e.*, with median survival times greater than 490 days, which was the duration of the experiment, except with the 2 potent promoting agents, anthralin and phorbol myristate acetate; with these compounds tumor deaths were responsible for shorter median survival times. The other compounds tested, *i.e.*, 1,8-dihydroxyanthraquinone, anthralin dimer, anthrone, and 1,8-dihydroxynaphthalene, did not show any promoting activity, and there were also no tumors in the group which received initiating agent and acetone alone. Thus, the tumor-promoting activity of anthralin reported earlier[2] is due to the compound itself rather than the products formed from it in acetone solution. Anthralin alone resulted in one animal with a papilloma. Past experience with tumor-promoting agents such as the phorbol esters and phenol is that these compounds when applied without initiating agent usually result in a low tumor incidence.[9] The phorbol esters derived from croton oil are by far the most potent tumor promoters known resulting in all animals tested with tumors and usually 50–60% with malignant tumors.[9] Furthermore, the animals bear multiple papillomas. In the present experiments a dose of anthralin was used which gave roughly the same tumor incidence as that observed with phorbol myristate acetate; but the tumor multiplicity is considerably lower with anthralin. Anthralin was applied at 30 times the dosage used with phorbol myristate acetate. It is, nevertheless, the second most potent tumor promoter known to date. Weaker tumor-promoting agents are phenol and the Tweens and Spans. A comparison of the potencies of the various known chemical tumor-promoting agents was made in an earlier study.[9] It is of interest that, like the phorbol esters, anthralin is a lipophilic–hydrophilic molecule.

TABLE I

TUMOR-PROMOTING ACTIVITY OF ANTHRALIN AND RELATED COMPOUNDS[a]

Primary treatment[b]	Secondary treatment and dose in μg/0.1 ml of acetone[c]	Days to first papilloma	(Number of mice with tumors)[d] (total number of tumors)
DMBA	1,8-Dihydroxy-9-anthrone, 80	59	18/94[e]
None	1,8-Dihydroxy-9-anthrone, 80	287	1/1
DMBA	1,8-Dihydroxyanthraquinone, 170		0
None	1,8-Dihydroxyanthraquinone, 170		0
DMBA	Anthralin dimer, 80		0
None	Anthralin dimer, 80		0
DMBA	1,8-Dihydroxynaphthalene, 60		0
None	1,8-Dihydroxynaphthalene, 60		0
DMBA	Anthrone, 70		0
None	Anthrone, 70		0
DMBA	Phorbol myristate acetate, 2.5	54	20/278[f]
None	Phorbol myristate acetate, 2.5	361	2/2
DMBA	Acetone		0
None	Acetone		0
No treatment[g]			0

[a] 20 ICR/Ha female Swiss mice per group. The results are at 490 days when the experiment was terminated. The median survival times were greater than 490 days except where noted. [b] DMBA: 7,12-dimethylbenz[*a*]anthracene, 20 μg in 0.1 ml of acetone once only by micropipette. [c] Promoters applied 3 times a week at doses indicated beginning 14 days after primary treatment. [d] Squamous papilloma. [e] 9 mice with squamous carcinoma; the median survival time in this group was 348 days. [f] 13 mice with squamous carcinoma; the median survival time in this group was 305 days. [g] 100 mice.

Although anthralin is a potent tumor-promoting agent on mouse skin, the available clinical evidence suggests that it does not have any deleterious effect in humans at the dosages used.

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(17) B. L. Van Duuren, A. Sivak, C. Katz, and S. Melchionne, *J. Nat. Cancer Inst.*, in press.