An EPR Study of Free Radicals Formed by Antipsoriatic and Tumor-Promoting 9-Anthrones in Nonpolar Solvents

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · NOVEMBER 1994

Impact Factor: 3.53 · DOI: 10.1021/tx00042a024 · Source: PubMed

4 AUTHORS, INCLUDING:

Ann G Motten
National Institute of Environmental Health S…

38 PUBLICATIONS **1,063** CITATIONS

SEE PROFILE

An EPR Study of Free Radicals Formed by Antipsoriatic and Tumor-Promoting 9-Anthrones in Nonpolar Solvents

Ann G. Motten, Robert H. Sik, Colin F. Chignell, and Patrick J. Hayden*

Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

Received April 29, 1994®

Certain 9-anthrone derivatives are useful in treating psoriasis and are also known to be tumor promoters in mouse skin. Their therapeutic use is accompanied by side effects of severe skin inflammation, irritation, and staining. The precise biochemical mechanisms of therapeutic action, tumor promotion, and side effects are presently uncertain, although the corresponding 9-anthron-10-yl radicals have been proposed as important intermediates. In order to gain insight into the possible role of anthrone-derived radicals in mediating the biological effects of these compounds, in the present study free radicals from a number of anthrone derivatives were generated by thermolysis in nonpolar solvents. Hyperfine splitting constants (hfsc) of the radicals were determined by electron paramagnetic resonance (EPR) spectroscopy. The experimentally determined hfsc's were also compared with spin densities obtained by molecular calculations (MOPAC 6.0). The experimental and theoretical data were found to be consistent in all cases. The formation of 9-anthron-10-yl radicals appears to be a general phenomenon among 9-anthrones regardless of therapeutic or tumor-promoting effectiveness, although there is a trend toward easier radical formation for the more active compounds.

Introduction

Certain 9-anthrone derivatives are useful in treating psoriasis (*1*) and are also known to be tumor promoters in the two-stage model of carcinogenesis in rodent skin (*2–9*). Their therapeutic use is accompanied by dermatologic side effects of severe inflammation and irritation (*10–16*) and staining (*10, 11*). The precise biochemical mechanisms of therapeutic action, tumor promotion, and dermatologic side effects of these 9-anthrones are presently uncertain.

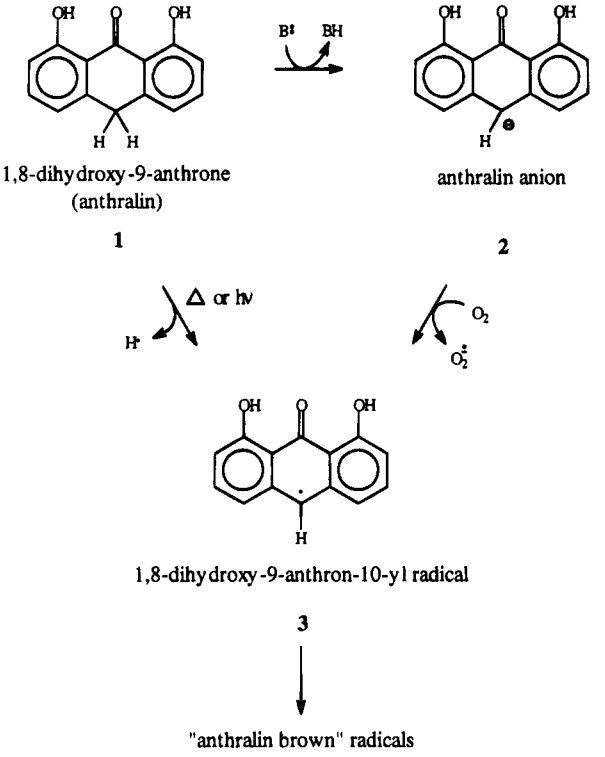
Polymeric "anthralin brown" free radical products are formed in the skin after treatment with the antipsoriatic 9-anthrone drug commonly known as anthralin (1,8-dihydroxy-9-anthrone, Scheme 1, **1**) (*17, 18*). Other free radical species including oxygen-centered radicals (*19–24*) and 9-anthron-10-yl radicals (*25–29*) are also formed during the autoxidation of anthralin. The possible role of these radicals in mediating the biological effects of the drug is uncertain and is the subject of continuing interest.

Recently, we have used the spin trapping technique and electron paramagnetic resonance spectroscopy (EPR)[1] to conduct structure/activity studies designed to provide insight into the possible role of free radicals in mediating the biological effects of the 9-anthrones. In keratinocyte cultures a correlation between the formation of anthralin brown-like secondary radicals and the antiproliferative activity of 9-anthrones or 9-anthrone dimers was noted (*30*). 9-Anthron-10-yl radicals are presumed intermediates in the formation of anthralin brown-type radicals (Scheme 1), but these radicals could not be detected by direct EPR under the conditions employed in the cellular studies (*30*).

By using the spin trapping technique, we found that the ability of 9-anthrones to undergo nonenzymatic autoxidation in aqueous media to form 9-anthron-10-yl and oxygen-centered radicals does not correlate with their known biological activities (*29, 30*). However, 9-anthrones are hydrophobic compounds, and in biological systems are likely to be concentrated in nonpolar environments such as cell membranes. Therefore, we were interested in studying the formation of radicals from 9-anthrones in nonpolar environments as well.

Previous workers have reported the nonenzymatic formation of 9-anthron-10-yl radicals from a limited number of 9-anthrones after extended time periods in

Scheme 1

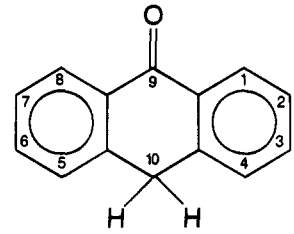


* To whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts,* October 15, 1994.

[1] Abbreviations: EPR, electron paramagnetic resonance spectroscopy; RHF, restricted Hartree Fock; AM1, Austin Model 1; hfsc, hyperfine splitting constants.

Table 1. Therapeutic and Tumor-Promoting Activities of 9-Anthrones



compound	tumor promoter	antipsoriatic	temp of radical formation (°C)	ref
(I) 1,8-dihydroxy-9-anthrone (anthralin)	yes	yes	120	2, 1
(II) 1,8-dihydroxy-3-methyl-9-anthrone (chrysarobin)	yes	yes	122	9, 1
(III) 1-hydroxy-9-anthrone	yes	yes	225	4, 1
(IV) 9-anthrone	no	no	225	3, 1
(V) 3-methyl-1,6,9-trihydroxy-9-anthrone (emodin anthrone)	no	nd[a]	220	9
(VI) 1-amino-9-anthrone	nd	no	198	1
(VII) 1,8-dichloro-9-anthrone	nd	no	260	1

[a] nd = not determined.

lipid solutions at normal temperatures (*26, 27*). Unfortunately, the EPR spectra obtained under those conditions do not reveal enough hyperfine structure to completely characterize the radicals. It has been shown, however, that thermolysis and/or photolysis of 9-anthrones produces good, high resolution EPR spectra of 9-anthron-10-yl radicals in nonpolar environments (*25, 27, 28*). Therefore, in order to investigate the nature of the primary free radicals formed by 9-anthrones in nonpolar environments, in the present study we have used thermolysis and EPR spectroscopy to generate and study free radicals from a series of 9-anthrones of known biological activities (Table 1) in nonpolar solvents. The experimental EPR parameters of the radicals were characterized, and the results were compared with molecular orbital calculations of electron spin density. It was found that all of the radicals formed have similar EPR properties and that the ability to generate 9-anthron-10-yl radicals in nonpolar solutions is not limited to the therapeutically active 9-anthrones, but is a general phenomenon, although there is a trend toward easier radical formation for the more active compounds.

Materials and Methods

Caution: *Anthrones and anthraquinones are potent skin irritants and/or tumor promoters and should be handled appropriately.* Anthralin, bianthrone, and *o*-terphenyl were obtained from Aldrich Chemical Co. (Milwaukee, WI) while xylene (as xylenes, mixed) was purchased from Mallinkrodt Chemical Co. (St. Louis, MO). These materials were used as received without further purification. All other chemicals were of reagent grade or better.

Anthrones II, III, and V–VII (Table 1) were prepared by reduction of the corresponding anthraquinones with stannous chloride and HCl in glacial acetic acid according to the method of Auterhoff and Scherff (*31*). In the case of the amino-9-anthrone, both the 1- and 4-isomers were obtained. These were separated by column chromatography on silica gel eluted with methylene chloride. 1-Amino-9-anthrone: mp 111–112 °C [lit. mp = 113–115 °C (*32*)]. 4-Amino-9-anthrone: mp 166–167 °C [lit. mp = 172–173 °C (*32*)]. Reduction of 1,8-dichloroanthraquinone also produced two isomeric 9-anthrones which were separated by column chromatography on silica gel eluted with hexane/ethyl acetate (9/1). 1,8-Dichloro-9-anthrone: mp 162–164 °C [lit. mp = 165–166 °C (*33*)]. 4,5-Dichloro-9-anthrone: mp 193 °C [lit. mp = 196–197 (*33*)]. Products were purified by recrystallization or column chromatography when necessary.

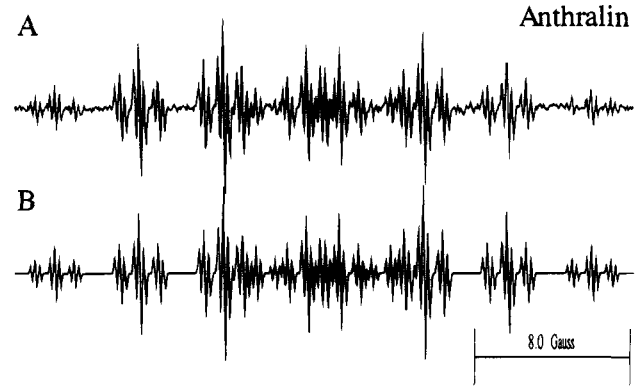


Figure 1. (A) EPR spectrum of anthralin-10-yl radical in xylene at 120 °C. Thirty-minute scan with a time constant of 1.0 s, modulation amplitude 0.033 G, receiver gain 10^5, microwave power 1.0 mW. (B) Simulation of (A) using hyperfine coupling constants in Table 2, with a linewidth of 0.042 G and a Gaussian lineshape.

Identity and purity (>98%) of synthetic compounds were confirmed by NMR, and/or mass spectrometry, TLC, and HPLC.

Electron paramagnetic resonance (EPR) spectra were recorded on an E109 Varian spectrometer equipped with a variable temperature accessory. To produce the radical, anthrone derivative samples were dissolved in xylene (anthralin: 30 mg/mL; chrysarobin: 40 mg/mL) or *o*-terphenyl (20 mg of the indicated anthrone/500 mg of *o*-terphenyl), placed in quartz EPR tubes, and heated in the cavity of the EPR instrument. For 1-aminoanthrone some spectra were also recorded on a Bruker ESP300 spectrometer, with the same sample preparation. Spectra were solved and optimized with the help of the EPR software developed in our laboratory for PC computers (*34*), and the solution for 1-aminoanthrone was found with the help of the Autosim program (*35*) also developed in our laboratory.

Restricted Hartree Fock (RHF) molecular calculations of spin density were performed on a Digital Equipment Corp. VAX computer using MOPAC 6.0 (QCPE No. 455) with the Austin Model 1 (AM1) basis set. In each calculation the geometries of the molecular structures were first optimized, and then spin densities were calculated using the ESR function.

Results and Discussion

When anthralin dissolved in nitrogen-gassed xylene was heated to 120 °C, a well resolved EPR spectrum was observed (Figure 1A). Radical formation from anthralin began rather abruptly at approximately 120 °C. Once radical formation began, the EPR signal intensity in-

Table 2. Hyperfine Splitting Constants and Spin Densities of 9-Anthron-10-yl Radicals[a]

compound	position 1	2	3	4	5	6	7	8	10
1,8-dihydroxy-9-anthrone	0.25 (OH)	4.33	1.00	4.33	4.33	1.00	4.33	0.25 (OH)	10.40
(anthralin)[b]	[0.091]	[0.284]	[−0.061]	[−0.301]	[−0.297]	[−0.060]	[0.279]	[0.089]	[0.527]
1,8-dihydroxy-3-methyl-9-	0.23 (OH)	4.21	1.00 (CH3)	4.33	4.52	0.89	4.35	0.25 (OH)	10.54
anthrone (chrysarobin)[b]	[−0.100]	[−0.305]	[0.070]	[0.330]	[0.335]	[0.069]	[−0.320]	[−0.102]	[−0.596]
1-hydroxy-9-anthrone	0.27 (OH)	4.72	0.93	4.74	2.68	0.74	3.30	1.17	10.97
	[−0.093]	[−0.298]	[0.064]	[0.317]	[0.290]	[0.048]	[−0.294]	[−0.077]	[−0.070]
9-anthrone	1.06	3.54	0.90	3.05	3.05	0.90	3.54	1.06	11.96
	[0.078]	[0.314]	[−0.050]	[−0.303]	[−0.293]	[−0.049]	[0.307]	[0.078]	[0.624]
3-methyl-1,6,8-trihydroxy-9-	0.23 (OH)	4.54	0.96 (CH3)	4.54	4.30	0.18 (OH)	3.47	0.18 (OH)	10.65
anthrone (emodin anthrone)	[−0.093]	[−0.336]	[0.054]	[0.355]	[0.304]	[0.054]	[−0.276]	[−0.036]	[−0.619]
1-amino-9-anthrone	0.35 (N)	5.04	1.25	5.04	2.89	0.91	3.84	1.01	11.02
	0.49 (2H)	[−0.087]	[−0.367]	[0.080]	[0.378]	[0.043]	[0.299]	[−0.070]	[−0.637]
1,8-dichloro-9-anthrone[c]	nd[d] (Cl)	3.39	1.17	3.84	3.84	1.17	3.39	nd[d] (Cl)	11.98
	[−0.061]	[−0.292]	[0.042]	[0.324]	[0.278]	[0.041]	[−0.255]	[−0.050]	[−0.566]

[a] Brackets indicate calculated electron spin densities. Spin densities may be either positive or negative. Thus, the absolute values should be compared without regard to the sign. The calculated spin densities are for the substituent on the indicated carbon except in the case of the hydroxy substituents, in which case the value reported is that of the carbon atom itself. [b] In xylene. [c] In the minimized MOPAC structure, the large chlorines force the ketone group out of plane by about 25°, and slight side-to-side asymmetry in the minimized structure causes a difference in corresponding spin densities (i.e., positions 1 and 8 should be equal but are slightly different). This asymmetry is not reflected in the observed splitting constants and may reflect a broad minimum in the minimization procedure. [d] nd = no splitting detected.

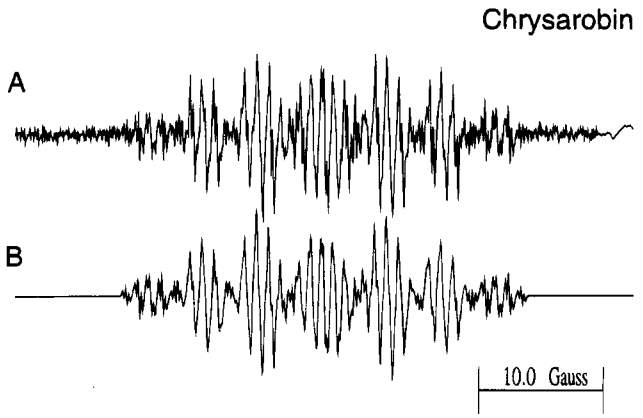


Figure 2. (A) EPR spectrum of chrysarobin-10-yl radical in xylene at 120 °C. Thirty-minute scan with a time constant of 1.0 s, modulation amplitude 0.033 G, receiver gain 10^5, microwave power 1.0 mW. (B) Simulation of (A) using hyperfine coupling constants in Table 2, with a linewidth of 0.042 G and a Gaussian lineshape.

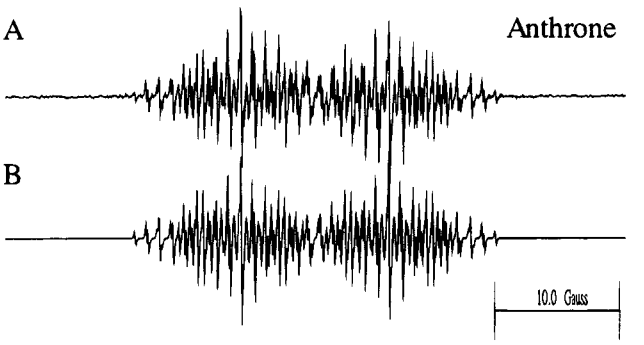


Figure 3. EPR spectra of 9-anthrone in *o*-terphenyl. (A) 9-Anthron-10-yl radical at 225 °C. Ten-minute scan with a time constant of 0.128 s, modulation amplitude 0.033 G, receiver gain 5×10^3, microwave power 1.5 mW. (B) Simulation of (A) using hyperfine coupling constants in Table 2, with a linewidth of 0.070 G and a 56% Gaussian lineshape.

creased linearly with increasing temperature up to the highest temperature obtainable with the apparatus (>260 °C using *o*-terphenyl as solvent). The signals were stable with respect to time at any give temperature. We interpret the temperature profile for radical formation as indicating a minimum activational energy requirement for radical production followed by steady state conditions of radical formation and decay increasingly favoring production of the radical over decay as the temperature continued to increase. The hyperfine splitting constants (hfsc) of the radical (Table 2) are similar to those previously reported by Davies and co-workers (*25*) and Lambelet and co-workers (*28*). The simulated spectrum is shown in Figure 1B. RHF molecular calculations of the spin densities (Table 2) agreed with the hyperfine assignments of previous workers (*25, 28*), predicting the correct ordering of splittings in positions 1 and 3 ($a_1 < a_3$), as noted previously by Devolder and Goudmand (*36*).

Under the same conditions EPR spectra were also obtained from 1,8-dihydroxy-3-methyl-9-anthrone (chrysarobin) (Figure 2A). Hyperfine splitting constants were assigned to this radical based on the rank ordering of the calculated spin densities (Table 2). None of the other anthrones produced radicals under these conditions. Therefore, we attempted to find another set of conditions that would allow radical formation.

Devolder and Goudman (*36*) have reported that the 9-anthron-10-yl radical may be generated in *o*-terphenyl by thermolysis of 9-anthrone between 200 and 250 °C. We obtained a similar EPR spectrum under their conditions (Figure 3A). The EPR parameters of the 9-anthronyl radical (Table 2) are similar to those reported by Devolder and Goudman (*36*). Thereafter, radicals were successfully generated in *o*-terphenyl from 1,8-dichloro-, 1-hydroxy-, 1-amino-, and 3-methyl-1,6,8-trihydroxy-9-anthrone (emodin anthrone), by thermolysis above 200 °C (Figure 4, panels A, and C, and Figure 5, panels A and C, respectively). Additionally, the experimental hfs constants for these radicals were determined and their spin densities were calculated (Table 2).

The splitting constants (Table 2) for each of the eight anthronyl radicals consisted of one large hydrogen splitting at the 10-position, splittings of several gauss at the 2, 4, 5, and 7 positions, and smaller splittings at positions 1, 3, 6, and 8. No splittings from Cl substituents were observed, and splittings from hydroxyl hydrogens were quite small (~0.25 G). These splitting patterns were consistent with the anthronyl radical structure containing a carbon-centered radical at the 10-carbon and only

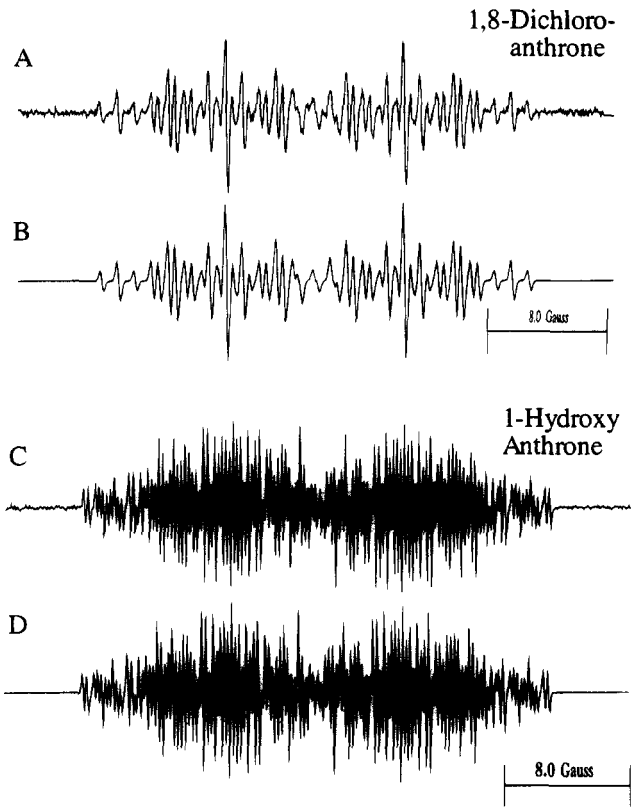


Figure 4. EPR spectra of 1,8-dichloro-9-anthrone and 1-hydroxy-9-anthrone radicals in *o*-terphenyl. (A) 1,8-Dichloro-9-anthron-10-yl radical at 260 °C. Sixteen-minute scan with a time constant of 0.5 s, modulation amplitude 0.033 G, receiver gain 2.5×10^4, microwave power 0.5 mW. (B) Simulation of (A) using hyperfine coupling constants in Table 2, using a linewidth of 0.042 G with a Gaussian lineshape. (C) 1-Hydroxy-9-anthron-10-yl radical at 225 °C. Thirty-minute scan with a time constant of 1.0 s, modulation amplitude 0.033 G, receiver gain 10^5, microwave power 1.0 mW. (D) Simulation of (C) using hyperfine coupling constants in Table 2, using a linewidth of 0.042 G with a Gaussian lineshape.

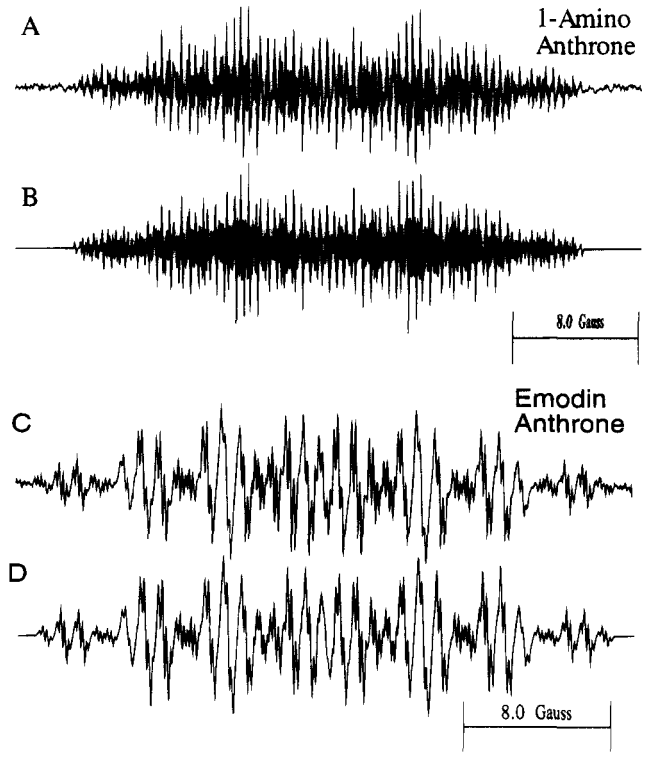


Figure 5. EPR spectra of 1-amino-9-anthrone and 3-methyl-1,6,8-trihydroxy-9-anthrone in *o*-terphenyl. (A) 1-Amino-9-anthron-10-yl radical at 198 °C. Twenty-minute scan with a time constant of 2.6 s, modulation amplitude 0.05 G, receiver gain 10^5, microwave power 2.0 mW. (B) Simulation of (A) using hyperfine coupling constants in Table 2, using a linewidth of 0.042 G with a Gaussian lineshape. (C) 3-Methyl-1,6,8-trihydroxy-9-anthron-10-yl radical at 220 °C. Eight-minute scan with a time constant of 0.25 s, modulation amplitude 0.033 G, receiver gain 5×10^4, microwave power 0.5 mW. (D) Simulation of (C) using hyperfine coupling constants in Table 2, using a linewidth of 0.074 G with a 61% Gaussian lineshape.

one hydrogen atom located on that carbon (Scheme 1). Observed hyperfine splittings were assigned to positions 1–8 by comparison with the calculated spin densities, with all molecular orbital calculations performed on structures corresponding to a radical centered on the bridging carbon in the 10 position (Scheme 1). Reasonable structures could also be drawn with the unpaired electron located closer to the oxygen(s). However, the close correspondence of the experimentally obtained hyperfine splittings to the spin densities calculated for a radical located at the 10 position (Table 2) confirms that all anthronyl radicals studied share the same basic electronic structure and spin distribution corresponding to the 10-yl radical structure. There is no discernible trend in the individual hyperfine splitting constants or calculated molecular properties that would distinguish between the active and inactive anthrones. We conclude that the basic electronic structure of these radicals is a general property of both active and inactive anthrones alike.

All of the compounds produced 10-yl radicals upon thermolysis in nonpolar solvents. It was interesting to note, however, that the compounds could be divided into two main groups: those that formed the radical at approximately 120 °C, and those that required much higher temperatures approaching 200 °C or higher. The two most potent radical producers are compounds possessing the 1,8-dihydroxy structure. If these groups play an important role in allowing radical formation by providing additional resonance stabilization for the radical species, then it may be expected that the compounds possessing only one such group, or lacking these groups, would exhibit a decreased propensity to produce radicals. However, it is difficult to rationalize the results obtained with emodin anthrone, which possesses the 1,8-dihydroxy structure and an addition phenolic group as well, but requires relatively high temperatures to initiate radical formation. The reason for the resistant behavior of this compound toward radical formation is not readily apparent.

Of the seven derivatives examined, the two therapeutically active anthrones anthralin and chrysarobin easily formed anthronyl radicals at temperatures just over 120 °C. Four derivatives known to have either no therapeutic or tumor-promoting activity (Table 1) require much higher temperatures (~200 °C) for anthronyl radical formation. 1-Hydroxy-9-anthrone, which is reported to be both therapeutically active (*1*) and tumor-promoting (*4*), also requires higher temperatures to initiate radical formation. In this regard it is noteworthy that the tumor-promoting ability of 1-hydroxy-9-anthrone was found to be considerably weaker than that of anthralin (*4*). No quantitative data or rank ordering of the antipsoriatic efficacy of therapeutically active 9-anthrones was found in the literature. Thus, there is not a clear and unambiguous division in the activation energy required for radical formation between active and inactive anthrones,

although there appears to be a trend toward easier radical formation in the more active species.

Conclusions

The formation of anthronyl radicals is a general phenomenon among anthrones in both aqueous (*29*) and nonaqueous solutions regardless of therapeutic or tumor promoting effectiveness. Similar electronic structure and hyperfine splitting constants were found in all of the anthronyl radicals studied. It would be interesting to know if the appearance of side effects is also general, as some of the side effects (i.e., skin irritation) may very well be associated with anthronyl radical formation, or with concomitantly formed oxygen radicals. There is a trend toward greater difficulty in radical formation in nonpolar solutions among the less active anthrones, but not a clear division into active and inactive species. It will be interesting to determine whether the trend observed in the present study for the ease of radical formation by thermolysis of 9-anthrones is reflected in a similar trend toward oxidizability of 9-anthrones by oxidative membrane enzymes such as prostaglandin H synthase or cytochromes P450. Studies designed to address this question are ongoing.

References

(1) Krebs, A., Schaltegger, H., Sr., and Schaltegger, A., Jr. (1981) Structure specificity of the antipsoriatic anthrones. *Br. J. Dermatol.* **105**, Suppl. 20, 6–11.

(2) Bock, F. G., and Burns, R. (1963) Tumor-promoting properties of anthralin (1,8,9-anthratriol). *J. Natl. Cancer Inst.* **30**, 393–398.

(3) Segal, A., Katz, C., and Van Duuren, B. L. (1971) Structure and tumor-promoting activity of anthralin (1,8-dihydroxy-9-anthrone) and related compounds. *J. Med. Chem.* **14**, 1152–1154.

(4) Van Duuren, B. L., Segal A., Tseng, S.-S., Rusch, G. M., Loewengart, G., Maté, U., Roth, D., Smith, A., Melchionne, S., and Seidman, I. (1978) Structure and tumor-promoting activity of analogues of anthralin (1,8-dihydroxy-9-anthrone). *J. Med. Chem.* **21**, 26–31.

(5) Männistö, P. T., Vaissi, L., Mustakallio, K. K., Viluksela, M., Kosma, V.-M., and Collan, Y. (1984) Tumor-producing activity of dithranol (anthralin) and two of its 10-acyl analogs in the dorsal skin of female NMRI mice. *J. Pharmacol. Exp. Ther.* **229**, 255–260.

(6) Viluksela, M., Puotunen, E., Newman, A. J., and Männistö, P. T. (1986) Tumor-producing and skin-irritating activity of dithranol (anthralin) and its 10-acyl analogues in SENCAR mice. *Carcinogenesis* **7**, 1755–1760.

(7) DiGiovanni, J., and Boutwell, R. K. (1983) Tumor promoting activity of 1,8-dihydroxy-3-methyl-9-anthrone (chrysarobin) in female SENCAR mice. *Carcinogenesis* **4**, 281–284.

(8) DiGiovanni, J., Kruszewski, F., and Chenicek, K. J. (1987) Studies on the skin tumor promoting actions of chrysarobin. In *Nongenotoxic Mechanisms in Carcinogenesis, Banbury Report 25* (Butterworth, B., and Slaga, T. J., Eds.) Cold Spring Harbor Laboratory, pp 25–39, Cold Spring Harbor, NY.

(9) DiGiovanni, J., Kruszewski, F. H., Coombs, M. M., Bhatt, T. S., and Pezeshk, A. (1988) Structure–activity relationships for epidermal ornithine decarboxylase induction and skin tumor promotion by anthrones. *Carcinogenesis* **9**, 1437–1443.

(10) Mustakallio, K. K. (1980) Irritation and staining by dithranol (anthralin) and related compounds: II. Structure–activity relationships among 10-meso-substituted acyl analogues. *Acta Derm.-Venereol. (Stockholm)* **60**, 169–171.

(11) Mustakallio, K. K. (1981) Irritation, staining and antipsoriatic activity of 10-acyl analogues of anthralin. *Br. J. Dermatol.* **105**, Suppl. 20, 23–27.

(12) Mustakallio, K. K., Martinmaa, J., Vilvala, R., and Halmekoski, J. (1984) Free radicals and the treatment of psoriasis with special reference to dithranol. *Med. Biol.* **62**, 155–158.

(13) Juhlin, L. (1981) Factors influencing anthralin erythema. *Br. J. Dermatol.* **105**, Suppl. 20, 87–91.

(14) Männistö, P., Havas, A., Haasio, K., Hanhijärvi, H., and Mustakallio, K. (1984) Skin irritation by dithranol (anthralin) and its 10-acyl analogues in 3 animal models. *Contact Dermatitis* **10**, 140–145.

(15) Kingston, T., and Lowe, N. J. (1986) Factors influencing anthralin irritancy. *Br. J. Dermatol.* **115**, Suppl. 31, 80–81.

(16) Kemény, L., Csató, M., and Dobozy, A. (1989) Pharmacological studies on dithranol-induced irritative dermatitis in mice. *Arch. Dermatol. Res.* **281**, 362–365.

(17) Shroot, B., and Brown, C. (1986) Free radicals in skin exposed to dithranol and its derivatives. *Arzneim.-Forsch.* **36** (II), 1253–1255.

(18) Fuchs, J., and Packer L. (1989) Investigations of anthralin free radicals in model systems and in skin of hairless mice. *J. Invest. Dermatol.* **92**, 677–682.

(19) Bruce, J. M., Kerr, C. W., and Dodd, N. J. F. (1987) Formation of superoxide during the auto-oxidation of anthralin (1,8-dihydroxy-9-anthrone). *J. Chem. Soc., Faraday Trans. 1* **83**, 85–89.

(20) Müller, K., Wiegrebe, W., and Younes, M.. (1987) Dithranol, active oxygen species and lipid peroxidation *in vivo*. *Arch. Pharm. (Weinheim)* **320**, 59–66.

(21) Müller, K. (1988) Active oxygen species and structure specificity of antipsoriatic anthrones. *Arch. Pharm. (Weinheim)* **321**, 385–389.

(22) Müller, K., and Kappus, H. (1988) Hydroxyl radical formation by dithranol. *Biochem. Pharmacol.* **37**, 4277–4280.

(23) Dabestani, R., Hall, R. D., Sik, R. H., and Chignell, C. F. (1990) Spectroscopic studies of cutaneous photosensitizing agents—XV. Anthralin and its oxidation product 1,8-dihydroxyanthraquinone. *Photochem. Photobiol.* **52**, 961–971.

(24) Müller, K., and Gürster, D. (1993) Hydroxyl radical damage to DNA sugar and model membranes induced by anthralin (dithranol). *Biochem. Pharmacol.* **46**, 1695–1704.

(25) Davies, A. G., Hawari, J. A-A., and Whitefield, M. (1983) Generation and E.S.R. spectrum of the 1,8-dihydroxy-9-anthron-10-yl radical. *Tetrahedron Lett.* **24**, 4465–4468.

(26) Ducret, F.,. Lambelet, P., Löliger, J., and Savoy, M.-C. (1985) Antipsoriatic drug action of anthralin: Oxidation reactions with peroxidizing lipids. *J. Free Radicals Biol. Med.* **1**, 301–306.

(27) Lambelet, P., Ducret, F., Löliger, J., Maignan, J., Reichert, U., and Shroot, B. (1990) The relevance of secondary radicals in the mode of action of anthralin. *Free Radical Biol. Med.* **9**, 183–190.

(28) Lambelet, P., Prior, E., and Löliger, J. (1990) An ESR study of thermal and photo-induced formation of radicals from anthralin and acylated derivatives. *J. Chem. Soc., Perkin Trans. 2*, 1509–1513.

(29) Hayden, P. J., and Chignell, C .F. (1993) Detection and characterization of 9-anthron-10-yl radicals formed by antipsoriatic and tumor-promoting 9-anthrones in aqueous buffers. *Chem. Res. Toxicol.* **6**, 231–237.

(30) Hayden, P. J., Free, K. E., and Chignell, C. F. (1994) Structure–activity relationships for the formation of secondary radicals and inhibition of keratinocyte proliferation by 9-anthrones. *Mol. Pharmacol.* **46**, 186–198.

(31) Auterhoff, H., and Scherff, F. C. (1960) The dianthrones of pharmaceutically interesting hydroxyanthraquinones. *Arch. Pharm. (Weinheim, Ger.)* **293**, 918–925.

(32) Bradley, W., and Maisey, R. F. (1954) Reduction of anthraquinone derivatives with alkaline dithionite. *J. Chem. Soc.*, 274–278.

(33) House, H. O., Ghali, N. I., Haack, J. L., and VanDerveer, D. (1980) Reactions of the 1,8-diphenylanthracene system. *J. Org. Chem.* **45**, 1807–1817.

(34) Duling, D. R. (1994) Simulation of multiple isotropic spin trap spectra. *J. Magn. Reson. B* **104**, 105–110.

(35) Duling, D. R., Motten, A. G., and Mason, R. P. (1988) Generation and evaluation of isotropic ESR spectrum simulations. *J. Magn. Reson.* **77**, 504–511.

(36) Devolder, P., and Goudmand, P. (1978) ESR of anthronyl radicals. Evidence of a temperature dependent phenyl twist angle in the 9-phenylanthronyl radical. *Chem. Phys.* **35**, 307–315.