An ESR Study of Thermal and Photo-induced Formation of Radicals from Anthralin and Acylated Derivatives

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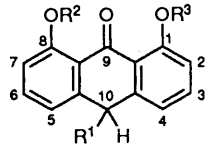
Radicals of anthralin and some anthralin derivatives generated either by thermolysis or photolysis in degassed xylene have been monitored by use of ESR spectroscopy. Thermolysis or photolysis of either anthralin or anthralin 10,10′-dehydrodimer generates the 1,8-dihydroxy-9-anthron-10-yl radical. Radical reactions are only observed during thermolysis of C-10 acyl-derivatives or during photolysis of the 1,8-diacyl derivatives. Differences in reactivity of the C-10 methylene group of the various derivatives are discussed.

Anthralin (1,8,9-trihydroxyanthracene), (**1**, $R^1 = R^2 = R^3 =$ H) has been used extensively for the topical treatment of the hyper-proliferative skin disease psoriasis.[1] However, it possesses two major undesirable side effects: skin irritancy and staining.[1] Following topical application, radical-mediated decomposition of the drug[2–5] into a mumber of oxidized products has been reported.[1,5,6] The therapeutic activity of the drug is thought to be related to radical formation in the skin.[2–5,7]



(1)

A number of derivatives (**1**, R^1, R^2, R^3 = H, acyl) of anthralin have been prepared in order to improve therapeutic effectiveness for the treatment of psoriasis. A minimum structural requirement for therapeutic activity of one free phenol group on C-1 has been postulated.[8] Esterification of the phenol groups in anthralin leads to active compounds; however, their activity may result from the formation of phenolic derivatives in the skin.[8] Substitution at C-10 also plays an important role in antipsoriatic activity, since the oxidation, essential for biological activity of the drug, is initiated at this position.[9] Substitution of both protons at C-10 has been found to suppress antipsoriatic properties,[10–11] but it is not yet clear whether a single proton at C-10 is sufficient to maintain the therapeutic activity. Although 10-acyl analogues of anthralin have been shown to be active,[12] they are also thought to be hydrolysed to anthralin in the skin.[13]

Generation of radicals from anthralin for analytical purposes has been achieved by several methods including photolysis,[14] heat-accelerated oxidation in a pure solvent,[14] peroxidation of lipids in an abundant oxygen supply[9] or with alkaline agents[5] and topical application to the skin.[2–5] However, in order to obtain high resolution ESR spectra optimal conditions are required. The present study describes the radicals generated in degassed xylene by photolysis and by thermolysis of anthralin and several of its analogues. This should serve as a basis for the interpretation of the radical reactions of these anti-psoriatic drugs in more complex systems.[3–5,15] Such spectral data provide the basis of a hypothesis concerned with radical involvement in psoriasis therapies.

Experimental

Materials.—Anthralin[15] 10-[^{13}C]anthralin (^{13}C >90% by NMR),[3] 1,8,1′,8′-tetrahydroxy-10,10′-bi-9(10*H*)anthrone[15] (10,10′-dehydrodimer), (**1**, R^1 = 1,8-dihydroxy-9-anthron-10-yl, $R^2 = R^3$ = H), 2,7-dimethyl,[15] 10-acetyl,[15] 10-propionyl,[15] 10-butyryl,[15] and 1,8-diacetyl[16] analogues of anthralin were synthesized as described elsewhere.

1,8-Dihydroxyanthraquinone was purchased from Merck. Acetonitrile and tetrahydrofuran were obtained from Fluka and were of glass-distilled grade. All other chemicals were commercial products of analytical grade.

Methods.—The radicals were generated in degassed xylene (mixture of the three isomers) either by *in situ* photolysis carried out with an Oriel 500 W mercury arc lamp at room temperature, or by *in situ* thermolysis at 120 °C. Concentrations of *ca.* 30 mg cm^{-3} in xylene were used in both cases.

The ESR spectra were recorded on a Varian E-109 Century series Mark III spectrometer equipped with an X-band klystron and 100 kHz magnetic field modulation. The microwave power did not exceed 1 mW in any experiment. The microwave frequency was measured using an HP 5342 A frequency counter and the magnetic field with a Varian E-500-2 NMR Gaussmeter calibrated with perylene cation radical in H_2SO_4 as the g-factor reference.[17] The temperature of the samples was controlled by means of a Varian E-257 variable temperature unit. Computer simulations of ESR spectra were calculated with a Varian E-900 EPR data acquisition system using Lorentzian line shape.

Analysis of anthralin derivatives before and after 1 h of either thermolysis at 120 °C or photolysis at room temperature was performed on a Waters HPLC system: a model 501 or a model 6000 A solvent delivery system; model U6K Universal Liquid Chromatograph Injector; model 480 Lambda-Max absorbance detector interfaced to a Baseline 810 data handling system with pump control (Millipore). The column used was a Zorbax CN 25 cm × 4.6 mm i.d. (Du Pont). Flow rate was 1.8 cm^3 min^{-1}. The composition of the mobile phase and the wavelength of detection were optimized for each derivative; for the 10-acetyl: 63:35 A:B, 365 nm; for the 1,8-diacetyl: 65:35 A:B, 254 nm; and for the 10-butyryl: 60:40 A:B, 365 nm (A is 0.2% trifluoroacetic

acid in Nanopure water; **B** is 5% tetrahydrofuran in acetonitrile). The anthralin derivative in xylene after reaction (150 mm^3 aliquot) was evaporated to dryness under a stream of nitrogen and made up to 10 cm^3 in chloroform:acetonitrile: acetic acid (20:80:0.1).*

Results and Discussion

Radical Reactions Occurring During Thermolysis.—Heating a solution of anthralin in xylene at 120 °C led to the observation of the ESR spectrum shown in Figure 1. This spectrum could be analysed in terms of the hyperfine splittings given in Table 1 which are in good agreement with those previously obtained from anthralin under the same conditions.[14] This spectrum could, therefore, be assigned to the 1,8-dihydroxy-9-anthron-10-yl radical (**2**) formed by hydrogen abstraction from C-10. This radical was also observed during thermolysis of the 10,10′-dehydrodimer,[14] (**1**, R^1 = 1,8-dihydroxy-9-anthron-10-yl, R^2 = R^3 = H). Under the same conditions, a solution of 10-[^{13}C]anthralin gave rise to the ESR spectrum reported in Figure 2 which could be rationalized by attributing a hyperfine splitting of 15.7 G to the 10-[^{13}C] radical [Table 1, radical (**5**)].

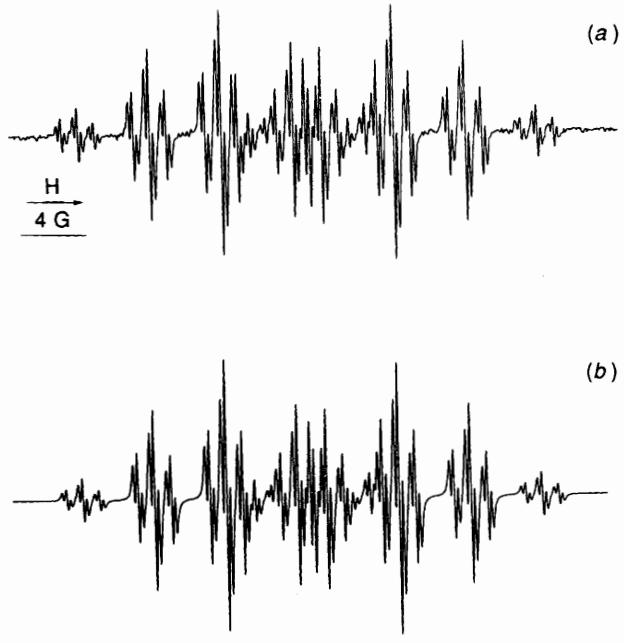
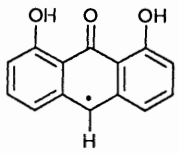
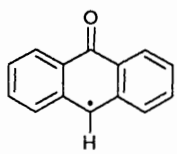


Figure 1. (*a*) ESR spectrum recorded during the thermolysis of anthralin in xylene at 120 °C. (*b*) Computer simulation using the hyperfine coupling constants given in Table 1 and a line width of 0.12 G.


(2)

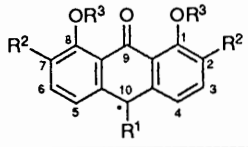

(3)

Radicals (**2**) and (**3**) can be considered as planar species by analogy with the 9-anthrone-10-yl radical (**3**).[18] This is confirmed by the low value of the hyperfine splitting of the carbon atom C-10. Indeed, such splitting is very sensitive to the geometry of the radicals. The hyperfine splitting of the carbon changes from 38.5 G for the nearly planar methyl radical to 271.6 G for the pyramidal trifluoromethyl radicals.[19–20] The hyperfine splitting associated with the carbon C-10 (15.7 G) clearly indicates a planar structure for radical (**2**). Furthermore, the assignment of the hyperfine splittings in (**2**),[14] based on theoretical calculations of spin densities in (**3**),[18] was confirmed by the ESR spectrum recorded during the thermolysis of the 2,7-dimethylanthralin derivative [radical (**4**), Table 1]. Indeed, this showed a hyperfine splitting associated with the methyl protons in the 2,7-positions equal to approximately 4 G.

During thermolysis at 120 °C, the 10-acyl derivatives of anthralin (**1**, R^1 = acyl, R^2 = R^3 = H) gave rise to ESR spectra (see Figure 3 for an example) which could be analysed according to the hyperfine coupling constants reported in the Table 1 [radicals (**6–8**)]. The hyperfine coupling constants of the aromatic as well as the hydroxy protons in the radicals (**6–8**) were almost equal to corresponding parameters in radical (**2**). The absence of hyperfine splitting around 10 G in the radicals generated from the 10-acyl derivatives indicated that the acyl group rather than the proton was present at the C-10 position. The 0.83 G hyperfine coupling associated with 3-H in radical (**6**) (Table 1) as well as the 0.40 and 0.22 G hyperfine coupling associated with 2-H and 3-H, respectively, in radical (**7**) could be unambiguously assigned to the protons of

* Bernard Martin, personal communication.

Table 1. ESR parameters of radicals generated from anthralin derivatives.



Radicals	R^1	R^2	R^3	Hyperfine splittings/G $a^H_{1,8}$	$a^H_{2,7}$	$a^H_{3,6}$	$a^H_{4,5}$	a^R_{10}	*g*-factors
(**2**)	H	H	H	0.25	4.33	1.00	4.33	10.4	2.0029[b]
(**4**)	H	CH_3	H	0.27	4.65	1.05	4.16	9.8	2.0030[b]
(**5**[a])	H	H	H	0.25	4.33	1.00	4.33	10.4 (1 H) 15.7 (1 C)	2.0029[b]
(**6**)	CH_3CO	H	H	0.24	4.35	1.06	4.35	0.83 (3 H)	2.0030[b]
(**7**)	CH_3CH_2CO	H	H	0.22	4.35	1.06	4.35	0.40 (2 H) 0.22 (3 H)	2.0030[b]
(**8**)	$CH_3(CH_2)_2CO$	H	H	0.27	4.35	1.06	4.35	0.42 (2 H)	2.0029[b]
(**9**)	$CH_3(CH_2)_2CO$	H	D	—	4.35	1.06	4.35	0.42 (2 H)	2.0029[b]
(**10**)	H	H	CH_3CO	—	3.68	1.05	3.30	11.8 (1 H)	2.0034[c]

[a] 10-[^{13}C]Anthralin. [b] Thermolysis at 120 °C. [c] Photolysis at 20 °C.

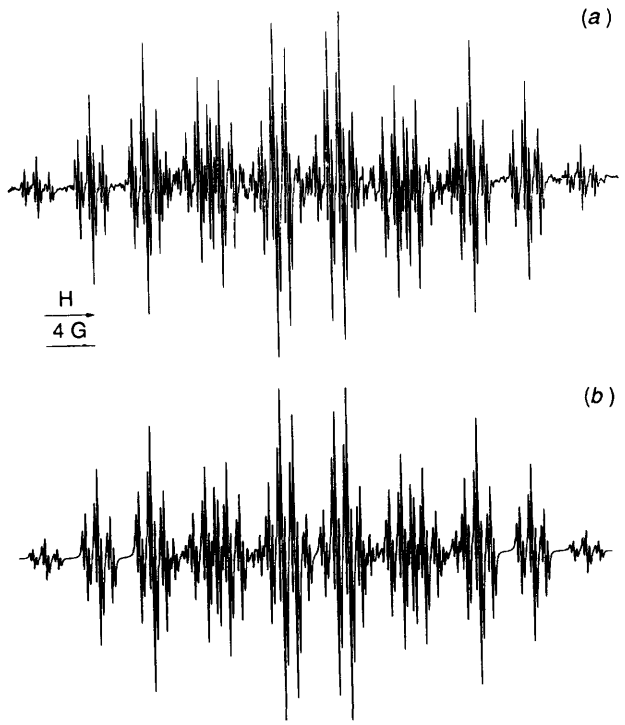


Figure 2. (*a*) ESR spectrum recorded during the thermolysis of 10-[^{13}C] anthralin in xylene at 120 °C. (*b*) Computer simulation using the hyperfine coupling constants given in Table 1 and a line width of 0.12 G.

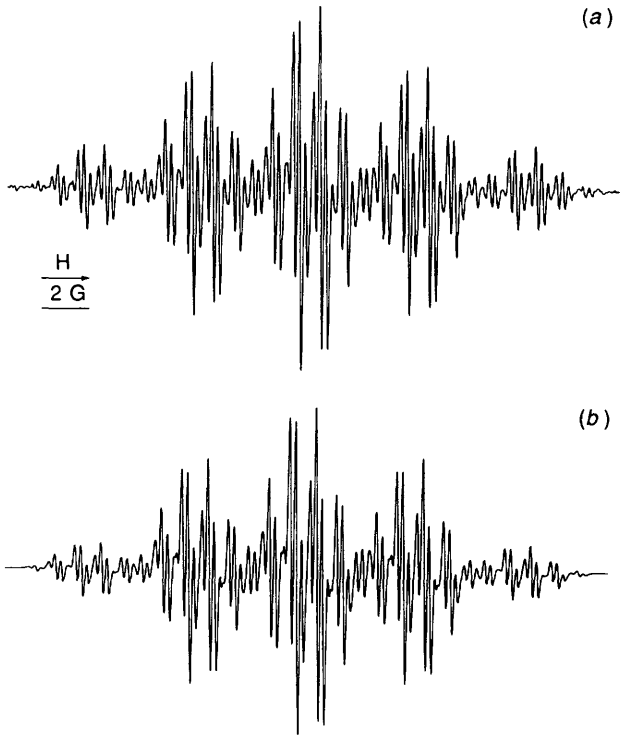


Figure 3. (*a*) ESR spectrum recorded during the thermolysis of 10-acetylanthralin in xylene at 120 °C. (*b*) Computer simulation using the hyperfine coupling constants given in Table 1 and a line width of 0.12 G.

the acyl groups. In order to attribute the smallest hyperfine splitting observed in radical (**8**), 10-butyrylanthralin was thermolysed at 120 °C in xylene in the presence of an excess of BuOD, resulting in the generation of radical (**9**). The disappearance of the 0.27 G splitting in this radical indicated

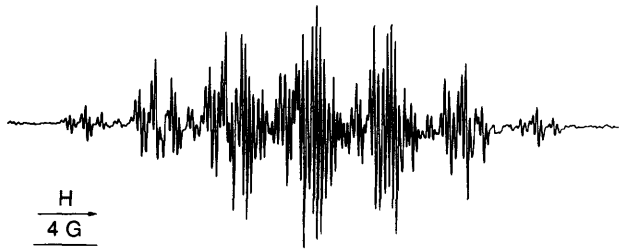


Figure 4. ESR spectrum recorded during thermolysis at 120 °C of the previously photolysed 10-acetyl analogue of anthralin in xylene.

that this splitting was associated with the C-2 hydroxyprotons. The 0.42 G splitting could consequently be attributed to the protons of the acyl group. The protons of the acyl groups resulted in the observation of hyperfine splittings in the radicals (**6**–**9**) (Table 1) due to the unpaired electron being partly delocalized over the carbonyl groups of the 1-acyl substituent. However, these splittings were small and the *g*-factors of the 10-acyl-radicals were close to that of radical (**2**) indicating that this delocalization is also small.[21]

The 10-acyl derivatives in xylene solution were not significantly degraded by heating for 1 h at 120 °C (Table 2).

Substitution of the two hydroxy groups of anthralin impaired the formation of radicals during thermolysis. Therefore, no radical was detected during the heating of the 1,8-diacetyl analogue at 120 °C. Substantial decomposition, without formation of anthraquinone or anthralin was, however, observed (Table 2).

Radical Reactions Occurring During Photolysis.—A similar, but weaker, ESR spectrum to that reported in Figure 1 was recorded during photolysis of both anthralin and its 10,10′-dehydrodimer at 20 °C. This was identical to that previously obtained by UV irradiation of anthralin either alone or in the presence of di-t-butyl peroxide.[14] No radical species could be detected during the photolysis of the 2,7-dimethyl analogue at 20 °C, probably due to the poor solubility of this compound at low temperatures. Photolysis of this derivative at 50 °C, however, generated small amounts of radical (**4**). A solution of the 2,7-dimethyl derivative heated at 50 °C in the dark did not produce any paramagnetic species.

On the contrary no ESR absorption was observed during the photolysis of the 10-acyl analogues. UV irradiation of the 10-acetyl derivative for 1 h in xylene, however, resulted in substantial decomposition of this compound as shown by the reduction of its concentration in the reaction mixture (Table 2) and also by the appearance of an extra unknown peak in the HPLC. A similar additional peak appeared in the case of the 10-butyryl derivative after exposure to UV light for 1 h, although little decomposition of the product was observed (Table 2). The amount of anthralin formed by UV irradiation of the 10-acyl derivatives was too small to allow the observation of radical (**2**) under these conditions. Nevertheless, when a solution of a 10-acyl derivative was thermolysed to 120 °C after photolysis a mixture of the radical derived from anthralin and the radical bearing the acyl group at the C-10 position was observed. Therefore, on heating to 120 °C a 10-acetyl analogue xylene solution that had previously been photolysed, an ESR spectrum (Figure 4) corresponding to a mixture of radicals (**2**) and (**6**) was obtained.

Photolysis of the 1,8-diacetyl derivative led to the observation of the ESR spectrum depicted in Figure 5 which could be analysed in terms of the hyperfine splittings reported in Table 1 [radical (**10**)]. By comparison of this data with ESR parameters of radical (**2**), the spectrum could be assigned to the radical generated by hydrogen abstraction from C-10. This radical had

Table 2. Composition of the reaction mixture after 1 h of either thermolysis or photolysis of anthralin analogues in degassed xylene.[a]

Anthralin analogues	Reaction type: Thermolysis Decomposition (%)	Thermolysis 1,8-Dihydroxy-anthraquinone (%)	Thermolysis Anthralin (%)	Photolysis Decomposition (%)	Photolysis 1,8-Dihydroxy-anthraquinone (%)	Photolysis Anthralin (%)
10-Acetyl	1	tr	—	12	tr	1
10-Butyryl	<1	tr	tr	<1	tr	tr
1,8-Diacetyl	17	—	tr	95	—	3

[a] The percentages are calculated on the basis of the original concentration.

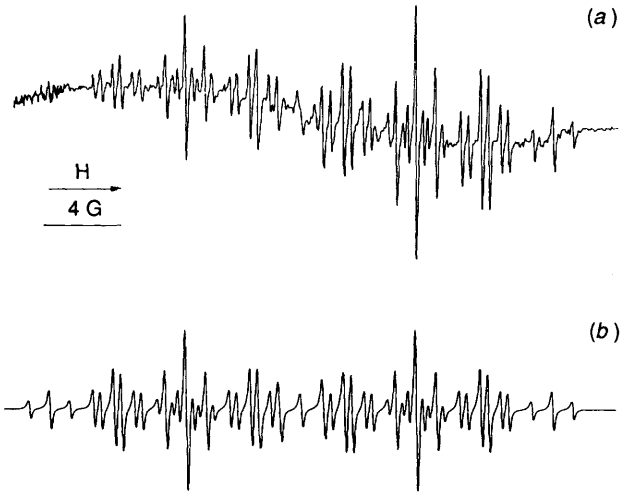
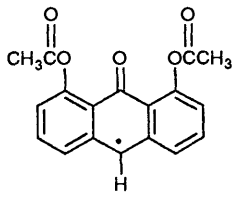


Figure 5. (*a*) ESR spectrum recorded during photolysis of the 1,8-diacetyl derivative of anthralin in xylene at 20 °C. (*b*) Computer simulation using the hyperfine coupling constants given in Table 1 and a line width of 0.11 G.



(10)

lower hyperfine splittings for aromatic protons and a higher *g*-factor than the species derived from anthralin or its 10-acyl analogues indicating that its electronic configuration was closer to that of the 9-anthron-10-yl radical (**3**)[18] than to the 1,8-dihydroxy-9-anthron-10-yl radical (**2**).

In contrast with the other derivatives investigated in the present study, most (*ca.* 95%) of the 1,8-diacetyl derivative was destroyed after 1 h of UV irradiation in xylene (Table 2). No quinone and only traces of anthralin were observed in the reaction mixture which contains, in addition, substantial amounts of unidentified compounds.

Reactivity of Anthralin Derivatives.—The 10-yl radicals could be generated by both thermolysis and photolysis from anthralin, its 10,10′-dehydrodimer and its 2,7-dimethyl derivative. The 10-yl radicals from anthralin analogues bearing acyl functions either in the 1,8 or 10 position were only observed following either photolysis or thermolysis respectively. The lack of photoreactivity of the 10-acyl derivatives was not due to the UV irradiation being quenched by the 10-acyl group since some decomposition of these derivatives occurred (Table 2). As postulated previously[22] radical formation from the 10-acyl derivatives was diminished with increasing length of the carbon chain of the 10-acyl substituent.

Radical reactions of anthralin analogues involving the protons at C-10 were affected by the substitution in both the 10 position and the 1,8 positions.[23] The reactivity of these protons depends on the tautomeric form, either keto or enol, of anthralin.[24] Although in xylene the keto form would be expected to predominate, differences in the position of equilibrium under either light or heat for 10-acyl- and 1,8-diacyl-9-anthrones cannot be excluded. Thus, we demonstrate here that C-10 acyl derivatives of anthralin can give rise to radicals which still possess an acyl substituent. This questions whether these compounds are indeed pro-drugs for anthralin as suggested.[13] It is possible that such analogues may not give rise to the same type of breakdown products as anthralin, thus, explaining the reduced staining reported, even after application of high concentrations of the substances to the skin.[11,12] Conversely substitution in the 2,7 position does not seem to modify the reactivity of the methylene group in anthralin.

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