FREE RADICALS DERIVED FROM BENZO[a]PYRENE

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SUMMARY

At least four different free radicals can be formed from benzo[a]pyrene under different reaction conditions, namely the 6-oxybenzo[a]pyrene radical, the benzo[a]pyrene anion and cation radicals and a radical from heated benzo[a]pyrene. The formation and esr spectra of these radicals have been studied with the aim of clarifying the nature of the radical species involved under different reaction conditions. Additionally the reactivity of the 6-oxybenzo[a]pyrene and the benzo[a]pyrene cation radicals towards several phenolic antioxidants have also been investigated.

INTRODUCTION

There has been considerable recent interest (1-4) in the possibility that free radicals are involved as intermediates in the metabolism of benzo[a]pyrene (BaP). In particular the cation radical of benzo[a]pyrene ($BaP^{\cdot +}$) has been implicated in the covalent binding of BaP to DNA in the presence of I_2 (2). Model studies have also indicated that $BaP^{\cdot +}$ may be produced in vivo by a transition-metal containing hydroxylase enzyme (5). The 6-oxybenzo[a]pyrene (6-oxyBaP) radical has also been identified on incubation of BaP with rat liver homogenates and has been postulated as an intermediate in the covalent binding of 6-hydroxy BaP to DNA (3,4). Other radical forms of BaP are apparently formed on heating (6) or on reduction with alkali metals (7).

A systematic comparison of the esr properties of these radical species has not been performed which has unfortunately led to some confusion concerning the species present under a given set of conditions. In this paper a fully resolved esr spectrum of the 6-oxy BaP radical has been obtained and analyzed and the conditions under which cationic BaP radicals can be observed have been more fully investigated. The results for all radical forms of BaP are then compared to show that at least four different radical species are produced under different conditions.

EXPERIMENTAL

All chemicals used were commercial samples of the highest available purity and were used as received. Solvents were stored over molecular sieves and degassed before use. Samples were prepared at room temperature, using standard vacuum techniques, in glass esr tubes. U.V. irradiations were carried out with a high pressure Hg lamp using glass filters. Esr spectra were recorded on a Varian E-15 spectrometer using a dual sample cavity, g-values and field calibrations were obtained using a perylene radical anion sample as a secondary standard (8-9).

RESULTS AND DISCUSSION

6-Oxy BaP. The 6-oxy BaP radical has been identified as the esr signal produced when BaP is stirred with albumin or incubated with rat liver homogenates (3,4). The same signal is also produced when BaP solutions are irradiated with light in the presence of O_2 or when 6-hydroxy BaP is autooxidized or chemically oxidized with ceric sulfate (10,11). There seems little doubt that the radical species produced is the same in all cases, however, the reported esr spectra have not been fully analyzed. In an effort to obtain a more fully resolved spectrum irradiations of BaP solutions were carried out in the presence of a 100% O_2 atmosphere followed by several freeze-pump-thaw degassing cycles. Under these conditions we were able to generate better resolved esr spectra of 6-oxy BaP. Fig. 1 shows the spectrum obtained in CH_2Cl_2, it was analyzed in

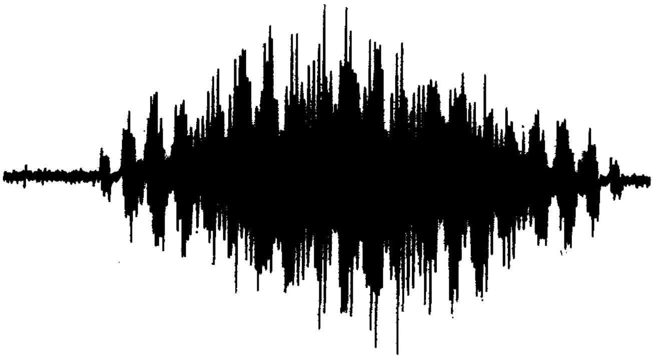

Figure 1: The esr spectrum of 6-oxy BaP generated by irradiation of BaP in a 100% O_2 atmosphere followed by degassing. The overall width of the spectrum is 25.92 G.

Table I

SUMMARY OF ESR RESULTS FOR BaP RADICALS

System	Radical Species	g-factor[a]	Analysis[b]
BaP/CH_2Cl_2/$h\nu$	6-oxy BaP	2.00341	0.110(1), 0.260(1), 1.097(1), 1.102(1), 1,243(1), 1.615(1), 2.591(1), 2.95 (1), 4.15 (1), 5.15 (1), 5.65 (1)
Heated BaP	?		1.135(2), 1,562(2), 2.362(2), 5.196(2), 5.460(2), 8.271(1)[c]
BaP/K/DME	BaP$^{\cdot-}$	2.00275[d]	0.34 (1), 0.39 (1), 0.53 (1), 1.85 (1), 1.99 (1), 2.49 (1), 2.56 (1), 2.67 (1), 3.05 (1), 4.05 (1), 5.94 (1)[e]
BaP/I_2/CH_2Cl_2	BaP$^{\cdot+}$	2.0031	
BaP/$FeCl_3$/CH_3NO_2	BaP$^{\cdot+}$	2.0027	
BaP/H_2SO_4	BaP$^{\cdot+}$	2.0026	
BaP/TFA/TFAn/CH_3NO_2	BaP$^{\cdot+}$	2.0027	
BaP/$AlCl_3$/CH_3NO_2	BaP$^{\cdot+}$	2.0026	
BaP/$Tl(OOCF_3)_3$/TFA	?	2.0029	

(a) All g factors are ±0.0001 or better, (b) numbers in parentheses refer to the number of equivalent protons, (c) splitting constants from reference 6, (d) g factor measured in Na/THF, (e) splitting constants from reference 7.

terms of the expected eleven different hyperfine splittings (see Table I). The seven smallest splitting constants were determined from the wings of the experimental spectrum and are believed accurate to ± 0.005 Gauss. The four largest splittings were arrived at by trial and error comparison of simulated spectra and as such cannot be reported with absolute certainty. The final simulated spectra was in good agreement with the experimental spectra when due account was taken of the large amount of overlap present in the center of the spectrum and the possible slight overmodulation of the experimental spectrum. Because of overlap the gross features of the spectra can be appreciably changed by only small changes in the splitting constants, thus it was noted that in solvents such as $CHCl_3$, $C_2H_4Cl_2$, CCl_4 and CS_2 the spectra were somewhat changed. The g factors were as expected for a phenoxy radical varying slightly from 2.00341 in CH_2Cl_2 to 2.00350 in CS_2.

$BaP^{\dot{+}}$. Poorly resolved esr spectra of BaP in conc H_2SO_4 have been attributed to the cation radical (3,12), BaP-I_2 solutions have also been reported to give an esr signal in the frozen state which has also been assigned to the cationic species (2). In our studies several oxidizing systems have been used with both BaP and other compounds such as perylene (PER) and 1,2,4,5-tetramethoxybenzene (TMB) which produce readily identifiable esr spectra (8,13).

Upon oxidation with I_2 in CH_2Cl_2 or with $FeCl_3$ in CH_3NO_2 BaP gave a broad single line spectrum in the fluid state, PER also gave a broad single line on I_2 oxidation but gave a poorly resolved spectrum on $FeCl_3$ oxidation, the g factors of these species are consistent with hydrocarbon radicals (see Table I). TMB on the other hand gives a resolved spectrum on I_2 oxidation which is readily identified as $TMB^{\dot{+}}$ (13). The intensities of the esr signals from I_2 oxidation of the hydrocarbons are strongly temperature dependent increasing rapidly with decreasing temperature, they are also dependent on the concentrations of hydrocarbon and I_2. The results suggest that one or more equilibria exist in solution possibly involving charge-transfer complexes and/or radical ion pairs. These equilibria are dependent on solvent, temperature and concentration, which may explain why previous workers were only able to observe a signal in the frozen state.

The oxidizing systems of conc H_2SO_4, $AlCl_3$-CH_3NO_2, Trifluoroacetic acid-trifluoroacetic anhydride-CH_3NO_2 (1:1:0.1), $Tl(OOCF_3)_3$-CH_3NO_2 each oxidized PER and TMB to their respective cation radicals as evidenced by their characteristic esr spectrum. BaP only produced a poorly resolved spectrum at best in each of these oxidizing systems. In view of the expected non-equivalence of the 12 protons in $BaP^{\dot{+}}$ it is perhaps not too surprising that only a poorly resolved signal can be obtained. Since the g factor in each of the oxidizing systems is as expected for a hydrocarbon radical (see Table I) and is definitely different from 6-oxy BaP and since other hydrocarbon cation radicals are undoubtedly produced in these systems one can attribute with some degree of confidence the spectrum from BaP as a cationic species.

Interestingly when $Tl(OOCF_3)_3$ in TFA is used as an oxidant a well resolved signal is obtained from BaP (see fig. 2). Unfortunately both anthracene (14) and perylene do not produce esr spectra of their respective cation radicals in this media. The spectrum from BaP is therefore probably not due to $BaP^{\dot{+}}$ but either to a complexed $BaP^{\dot{+}}$ species (14) or a secondary reaction product.

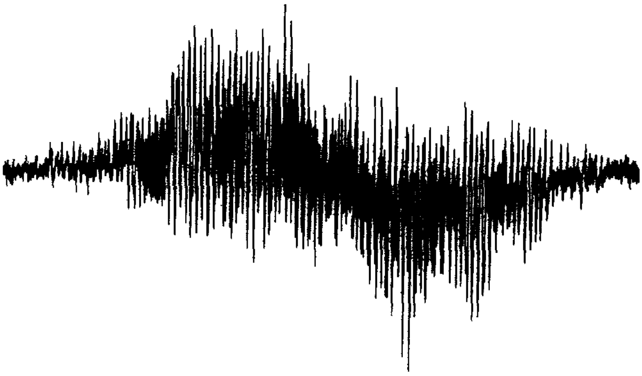

Figure 2: The esr spectrum produced upon oxidation of BaP with $Tl(OOCF_3)_3$ in TFA. The overall width is again approximately 25 G.

<u>$BaP^{\dot{-}}$</u> . This radical species is produced when BaP is reduced by alkali metals in ethereal solvents. As such this species is not likely to be of any significance to the metabolism of BaP, however, because of the alternant nature of the hydrocarbon the anion and cation radicals should be very similar. Robinson (7) has analyzed the $BaP^{\dot{-}}$ spectrum in terms of the splitting constants shown in Table I. We have remeasured the spectrum in Na/THF to obtain the g value sho and have also qualitatively observed a similarity between an incompletely

resolved $BaP^{\dot{-}}$ spectrum and the $BaP^{\dot{+}}$ spectrum obtained as described in the preceding section.

Heated BaP. An analysis of the esr spectrum which is obtained when BaP is heated in vacuum at 220° has been reported (6). A comparison of this analysis with that of the 6-oxyBaP (see Table I) indicates conclusively that these species are not the same, as had been previously suggested (10). The species responsible for the esr spectrum of heated BaP was hypothesized to be derived from an azuleno[1,2,3-cd]phenalene (6). It is interesting to note that azuleno[5,6,7-cd]phenalene has since been synthesized and shown to be carcinogenic (15).

Radical Species from BaP and their Reactions with Phenolic Antioxidants.

The results discussed in the previous sections indicate that there are at least four different radical species which may be derived from BaP, namely, the 6-oxyBaP, $BaP^{\dot{+}}$, $BaP^{\dot{-}}$ and the species from heated BaP. Evidence for the occurrence of 6-oxyBaP and $BaP^{\dot{+}}$ as metabolic intermediates has been cited (2-5), it is also possible that the heated BaP radical may come in contact with lung tissue, $BaP^{\dot{-}}$, however, is unlikely to be produced in vivo.

In view of recent reports concerning the protective effect of antioxidants against chemical carcinogens (15, 17) the direct reaction between the 6-oxyBaP and $BaP^{\dot{+}}$ radicals and certain phenolic antioxidants was investigated. In these experiments solutions of the antioxidants butylated hydroxy anisole (BHA), bis-(3-methyl-4-hydroxy-5-t-butylphenyl)sulfide (Ethyl 736) and 3,3',5,5'-tetra-t-butyl-4,4'-biphenol (TBP) in CH_2Cl_2 were added to solutions of the 6-oxyBaP radical or the BaP-I_2 system also in CH_2Cl_2. The addition of the antioxidant solutions did not result in an appreciable decrease in the BaP radical signals except when a large excess of the antioxidants was used. These results indicate that the direct reaction between the antioxidants and BaP radicals is unlikely to play an important role in the protective action of these antioxidants.

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