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TOLBERT L M CHEM (404)894-4043
BOTTOMLEY L A CHEM (404)-

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Title: BIOOXIDATION OF ARYLALKYL HYDROCARBONS

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

YUNG-PIN LIU, PH.D.
(301)496-5471
DIVISION OF CANCER ETIOLOGY
NATL CANCER INST, NAT INST OF HEALTH
BETHESDA, MD 20892

Sponsor issuing office

EARL C. MELVIN
(301)496-7444
GRANTS MANAGEMENT OFFICE
NAT CANCER INST, NAT INST OF HEALTH
BETHESDA, MD 20892

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GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 09/25/91

Project No. G-33-606 Center No. 10/24-6-R6345-2A0

Project Director TOLBERT L M School/Lab CHEMISTRY

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

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Title BIOOXIDATION OF ARYLALKYL HYDROCARBONS

Effective Completion Date 910630 (Performance) 910930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
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Subproject Under Main Project No. _____

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~~NOTE: Final Patent Questionnaire sent to PDPI.~~

G-33-606 Diversible

B. **Background and Significance.** For several years, the formation of a radical cation through abstraction of a single electron has been accepted as a key feature of the mechanism of for a number of mixed function oxidase systems. This step initiates a cascade of events which, in the particular case of polynuclear aromatic hydrocarbons, can lead to direct formation of covalent DNA adducts through direct nucleophilic attack or can result in formation of electrophilic agents, e. g., arene oxides, which can themselves form DNA adducts in chemically separate events.[1] Establishing the intervention of these short-lived radical cation intermediates has been more elusive, inasmuch as it involves indirect evidence such as a dependence on substrate redox potential, the formation of irreversible adducts, or the rearrangement of highly strained substrates (e. g. quadricyclene). Even less clear has been the way in which the reactivity of these highly unstable intermediates might be mediated by the structure of the solvent shell, of the enzyme, and of the substrate itself. That is, to say that radical ions are intermediates is not necessarily to provide useful mechanistic--i. e., predictive--information about how the resulting reaction cascade might partition among several competing pathways.

The work proposed here represents the convergence of two disparate observations. First, methylation of the *meso* position of certain polynuclear aromatics converts relatively docile hydrocarbons into potent mutagens and carcinogens.[2] For example, 7,12-dimethylbenzanthracene (**DMBA**) and 6-methylpyrene (**6-BP**) are among the strongest carcinogens known, in contrast to their nor-methyl derivatives, as is 3-methylcholanthrane (**3-MC**). Second, methylation of radical cations of aromatic hydrocarbons converts these reactive intermediates into extremely strong acids. 9,10-Dimethylanthracene (**DMA**), for instance, has a thermodynamic pK_a of -6!.[3] Are these two phenomena related? In order to answer this question, we must understand in exquisite detail what the chemical consequences of such methylation are and how such chemistry can be mediated by the complex nature of the enzyme active site and surrounding medium.

Much of the current effort in understanding the mechanism of PAH carcinogenesis has its origins in the work of Pullman and Pullman.[4] They associated carcinogenic activity with the presence of an electron rich "K" or "meso-phenanthrenic" region and, preferably, substitution at the "L-region" (see Figure 1). This theory became modified with the suggestion that K-region epoxides might be responsible for malignant cell growth, although K-region epoxides were gradually abandoned by most investigators, and attention turned to the vicinal diol-epoxides first described by Sims and coworkers.[5] This concept has been developed into the bay-region theory, which predicts the structure of proximate and ultimate carcinogens of more than a dozen compounds. Such diol epoxides are formed in the presence of microsomes and other cellular preparations containing cytochrome P450, and the general topic of P450-mediated oxidation of hydrocarbons has been subjected to intense study, particularly in the laboratories of Jerina,[6] Cavalieri,[7] Coon,[8] Groves,[9] Harvey,[10] and Gunsalus.[11] The bay-region theory is currently accepted by most investigators, and the role of the diol epoxides has now been demonstrated through the formation of DNA adducts which apparently correspond to those formed by direct injection of PAH's.[12]

The case of alkylated polynuclear aromatic hydrocarbons presents a special set of considerations and perhaps should be considered separately. Although methyl groups at the meso-anthracenic positions dramatically enhance carcinogenicity, ethyl groups inhibit such behavior.[1] If the ethyl group in 7-ethyl-12-methylbenzanthracene is "tied back" as in 3-methylcholanthrene,[12a] the carcinogenicity is again enhanced (see Figure 2). To account for these phenomena within the general framework of the bay-region theory, it has been common practice to view substituents as electronic "spectators", enhancing or decreasing the electrophilicity of the diol epoxide or perhaps inhibiting facile deactivation pathways but not entering directly into the metabolic pathway.[13] For example, it has been demonstrated that methylated anthracene derivatives intercalate DNA more efficiently than does anthracene itself,[14] which might provide a role for the substituent in enhancing the rate of covalent attachment of the proximate carcinogen to DNA. The sterically more demanding ethyl derivatives might have resulted in less efficient intercalation, a possibility that we have ruled out (vide infra). In any event, the assumption that methyl groups are not chemically involved presents a paradox. Microsomal oxidation of polynuclear aromatic hydrocarbons to dihydrodiol epoxides is (see Figure 2), plausibly via the primary oxidate, DMBA radical cation.[15] Since such radical cations

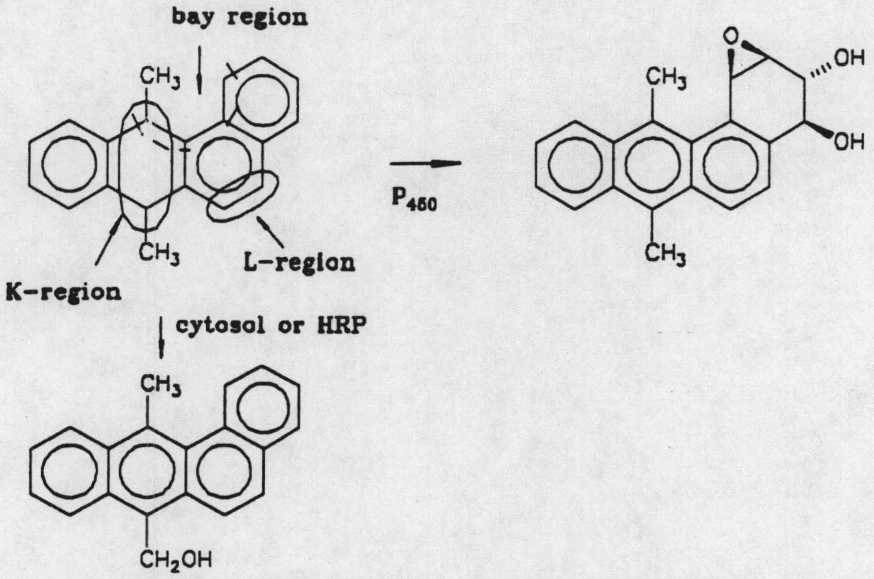


Figure 1. Active sites in 7,12-dimethylbenz[a]anthracene (**DMBA**).

are also known to be strong acids, deprotonation to form a benzylic radical leading ultimately to a 7-hydroxymethyl-12-methylbenz[a]anthracene presents a mechanistically appealing alternative which has, in fact, been observed in a number of instances.[16] Moreover, Blackburn et al discovered that DMBA specifically labelled with tritium at the 7-methyl group becomes bound to DNA in an in vivo system with partial loss of radioactivity,[17] indicating that proton loss is an important component of binding at least for this substrate. 3-MC itself undergoes side-chain oxidation as the exclusive metabolic pathway in some cases. Thus an understanding of the proton-transfer chemistry of alkylaromatic radical cations is necessary for completion of the story of PAH carcinogenesis. Conversely, if radical cations are intermediates in the epoxidation reaction itself, clearly a mechanistic rationale for the selection of this mechanistically uncoventional reaction pathway must be developed.

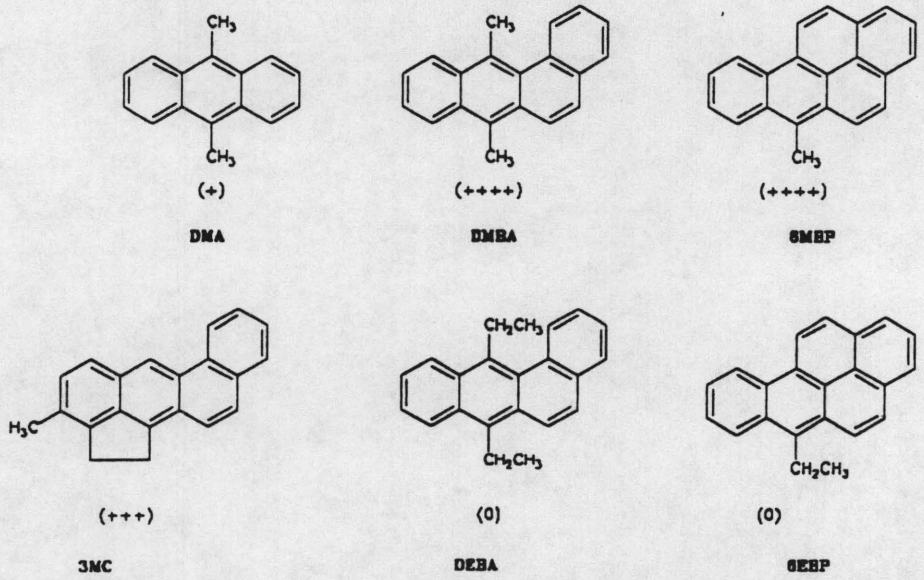


Figure 2. Representative carcinogenic and non-carcinogenic hydrocarbons.

In contrast to microsomes and their constituent membrane-bound enzymes such as cytochrome P450, non-microsomal (cytosolic) systems and their constituents such as horseradish

peroxidase do produce the products of side chain oxidation.[18] This observation provides a possible solution to the paradox, since a significant difference between these systems is the hydrophobicity of the enzyme reactive site. Proton transfer in aqueous solvents is known to occur several orders of magnitude faster than in non-aqueous solvents, and rates are dependent upon the structure of water, i. e., the presence of clustering. The observation of this solvent dependent reactivity in the face of rather overwhelming thermodynamics has coincided with a renewed interest by a number of research groups in the structural and environmental factors affecting electron and proton transfer reactions. We are intrigued by the possibility that cytochrome P450, while generating radical cations, actually decreases the rate of proton loss, contrary to one's expectation of enzymes as rate accelerators. It is intriguing to speculate that such a rate inhibition could serve the very important function of protecting against proton loss to deleterious radicals.

Even the mechanism for side-chain hydroxylation of aromatic compounds has been the subject of much controversy. Although a mechanism involving direct hydrogen atom abstraction can be written, evidence is increasing that, at least for low oxidation potential substrates, two sequential one-electron transfers are involved.[19] Again, one-electron oxidation of the hydrocarbon produces a radical cation of very high acidity. This intermediate can undergo nucleophilic attack by a number of nucleophiles, including water or superoxide, to generate ring oxidized products. Within this context, formation of arene or alkene oxides by P450 can be viewed as a special case of nucleophilic attack by Fe(IV)-O$^{\cdot}$. Alternatively, the radical cation loses a proton to form a benzylic or allylic radical. This mechanism is shown schematically in Figure 3. In fact, the direct hydrogen abstraction mechanism can be viewed as a special case in which proton transfer is so rapid as to accompany the electron transfer (i.e., $k_H H > k_{ox}$ in Figure 3). This is particularly the case for poorly oxidized substrates such as toluene, for which discrete radical cation formation is probably energetically unlikely. Typical Hammett "sigma-rho" plots for such systems have negative slopes, an indication of substantial electron transfer in the transition state.[20] The resulting benzyl radical from either pathway can react with an oxygen-centered radical (e.g., superoxide in the presence of oxygen) or undergo a second one-electron oxidation to a benzylic cation which will undergo rapid nucleophilic attack by water (or by a DNA base pair).

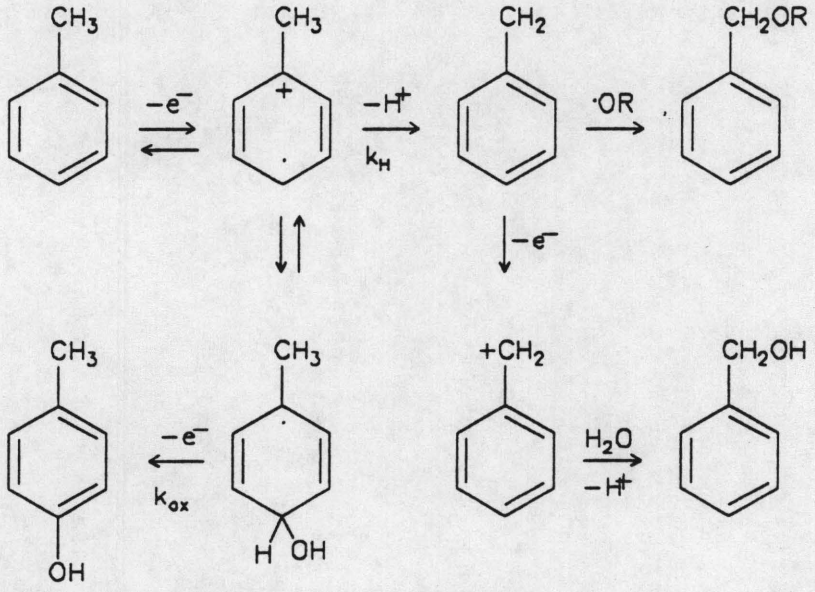


Figure 3. Radical-cation decay pathways for toluene.

Polynuclear aromatic hydrocarbons represent a well-studied group of molecules, and it is not our intention to duplicate the elegant work of Jerina, Harvey, Cavalieri, and others, which have tended to concentrate on the role of dihydrodiol epoxides in their carcinogenesis, or that of Bruice[21] and Traylor,[22] which have concentrated on the intervention of radical cations in epoxide formation by P450 model compounds. However, an understanding the the role of proton transfer

mechanisms or nucleophilic reactions following electron abstraction by oxidative enzymes represents a critical component of their enzymology. The scope of this work, therefore, is much broader, in that this effort is relevant to the oxidation of all organic molecules in which radical cations are implicated--whether through the intervention of enzymes, inorganic oxidants, or electron-deficient photosensitizers--regardless of the ultimate products of oxidation.

C. Progress Report.

That radical cations of hydrocarbons should be extremely strong acids is recognized by those familiar with the work of Weller[23] and has been quantified by Arnold[24], based upon an examination of oxidation potentials, and more recently used by Bordwell.[25] In the case of toluene radical cation, that pK_a' has been estimated at -12![24] For hydrocarbons of lower oxidation potential, the pK_a is correspondingly less negative, but even in the case of 9,10-dimethylanthracene has been estimated as -6.[24, 25] Thus a strong thermodynamic driving force exists for rapid proton loss once the radical cation is formed, leading ultimately to the product of hydroxylation.

Given the high thermodynamic acidity, one might question why this is not the exclusive pathway for one-electron oxidations. The answer is that such reactions may not always be kinetically favored. In some cases, for instance, proton transfer may occur from the weaker thermodynamic acid.[26] Thus proton transfer reactions may be inhibited by a number of factors. In nonaqueous solvents such reactions are known to be slow despite favorable thermodynamics, especially when carbon acids are involved.[27] For instance, proton loss from toluene radical cation is estimated to be four orders of magnitude slower in acetonitrile than in water.[28] For the radical cation of dimethylbenzylamine, proton loss occurs faster from the methyl group despite thermodynamically favored loss from the benzyl group, an effect attributed to unfavorable steric interactions in the latter.[29] For hydrocarbon ions and radicals where stabilization is strictly governed by p-orbital overlap, the inability of the newly-forming carbon free-valence to achieve overlap with the -orbital framework of the aromatic ring may prevent facile proton loss. The idea of a stereoelectronic effect is given support by the observation that deprotonation of p-cymene (p-isopropyltoluene) radical cation occurs from the methyl group, not the isopropyl group;[30] apparently steric effects prevent the isopropyl group from achieving the requisite planarity. More recently, this interpretation has been questioned, and the difference in reactivity ascribed to steric hindrance to approach by base rather than to a stereoelectronic effect.[30c] Indeed, in studies which space does not permit us to elaborate, we have discovered that this stereochemistry is solvent dependent, with increasing water concentration favoring from the sterically more encumbered isopropyl group.[31] For these reasons, we concentrated our further efforts on the stereochemically unambiguous *meso*-ethylanthracene case. Esr studies of 9,10-diethylanthracene and 6-ethylbenz[a]pyrene radical cations had yielded a hyperfine coupling constant indicative of a 90° alignment of the ethyl group with respect to the aromatic plane,[32] which would make deprotonation difficult in the presence of a compelling stereoelectronic effect. Indeed, our studies (see below) on ethylanthracenes indicated that this stereoelectronic effect was overwhelming, leading to a mechanistically unprecedented deethylation and forming a noncarcinogenic anthrone derivative.

The formation of bay-region diol-epoxides from **DMBA**, even in the somewhat special conditions of induced rat liver microsomes, can now be understood within this framework. Although the intervention of radical cations in oxidation by P450 is still the subject of controversy, these intermediates may be anticipated at least for substrates of low oxidation potential such as polynuclear aromatics. Such a radical cation would have been expected to undergo a deprotonation, but since cytochrome P450 is membrane bound, this hydrophobic environment may delay proton loss so that ring attack by oxidant (O=Fe(IV)) becomes competitive and expoxides form (see Figure 4). Thus the dichotomy suggested by Cavalieri[33] between one-electron oxidation and ring oxygenation is inappropriate, since radical cations are almost compulsory intermediates for substrates of such low oxidation potential. Rather, the actual dichotomy, which has provided the focus for our initial studies, is between proton loss and ring attack from a common intermediate radical cation.

In our previous proposal, we identified five specific objectives: (S1) to examine the partition between side chain oxidation and ring oxidation for a series of hydrocarbons; (S2) to examine the effect of solvent polarity and basicity, particularly of water, on this partition; (S3) to determine the stereoelectronic effect on proton loss by appropriate choice of substrate; (S4) to determine both inter- and intramolecular kinetic isotope effects for proton loss; (S5) to compare the observed partition function with that associated with both membrane-bound and water-soluble oxidases to develop a "fingerprint" for the microenvironment of biooxidation of alkylated hydrocarbons such as 7,12-dimethylbenz[a]anthracene. Our approach concentrated on product studies and electrochemical rate determination for quantification of these effects. This approach has been quite successful, allowing us to achieve objectives S1-S4 with sometimes startling and unexpected results. These "initial results" are described below, and represent work currently in print, in press, or submitted for publication. Furthermore, our "work in progress" provides us with a protocol for moving from the "what" to the "why".

1. Initial results. We concentrated our initial efforts in oxidation chemistry on 9,10-dimethylanthracene (**DMA**) and its derivatives. These hydrocarbons had the advantage of being relatively non-carcinogenic and presented no special handling problems other than the usual care in dealing with any potentially hazardous material. Optimum one-electron oxidations were obtained with tris(phenanthroline)iron(III), although more recently we have discovered that ceric ammonium nitrate (**CAN**) is more useful for spectroscopic studies. Critical factors proved to be, as anticipated, hydrocarbon and water concentration, with a 1:9 water-acetonitrile solvent system and 3 mM hydrocarbon concentration providing the optimum results. Under these conditions, oxidation of **DMA** proceeded to yield a single primary product, 9-hydroxymethyl-10-methylanthracene.[34] Oxidation of the latter produced both 9,10-bis(hydroxymethyl)anthracene and 10-methylanthracene-9-carboxaldehyde in a 2:1 ratio (see Figure 5) as well as products of further oxidation. As a result of extensive chemical electrochemical kinetic studies, we were able to develop a mechanistic scheme (see Figure 6) which has proven to be general for all the anthracene derivatives we have studied so far. The significant feature of this scheme is that upon formation of radical cation fast but reversible addition of water or added nucleophile to the *meso*-anthracenic position occurs (step A). If bond cleavage reactions (step C) make the initially formed adduct irreversible, products of nucleophilic addition result. Alternatively, (irreversible) deprotonation of the radical cation takes place (step B) to form the side-chain oxidized product. Thus the rate of deprotonation exerts a controlling influence over the partition among various products and provides the focus for our investigations. The strategy we employed was to take advantage of the symmetry of 9-methylanthracene and to use oxidation of that methyl group as a "molecular clock", comparing the reactivity when a sterically or electronically distinct moiety was attached to the opposite

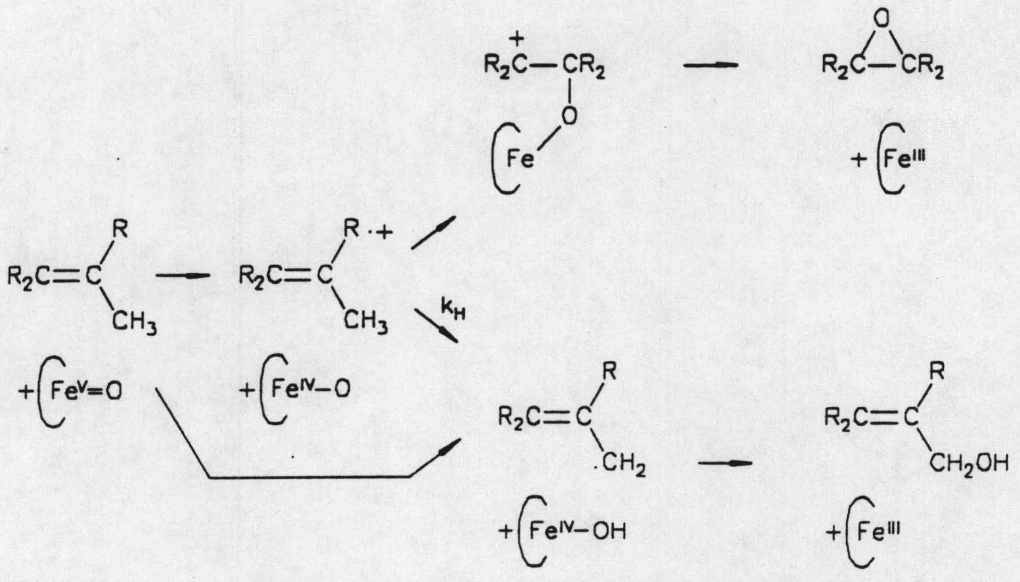


Figure 4. Epoxide vs. deprotonation pathways with Fe(V)=O oxidants.

position (C-10), or in the case of dimethylanthracene itself, when hydrogen was replaced with deuterium.

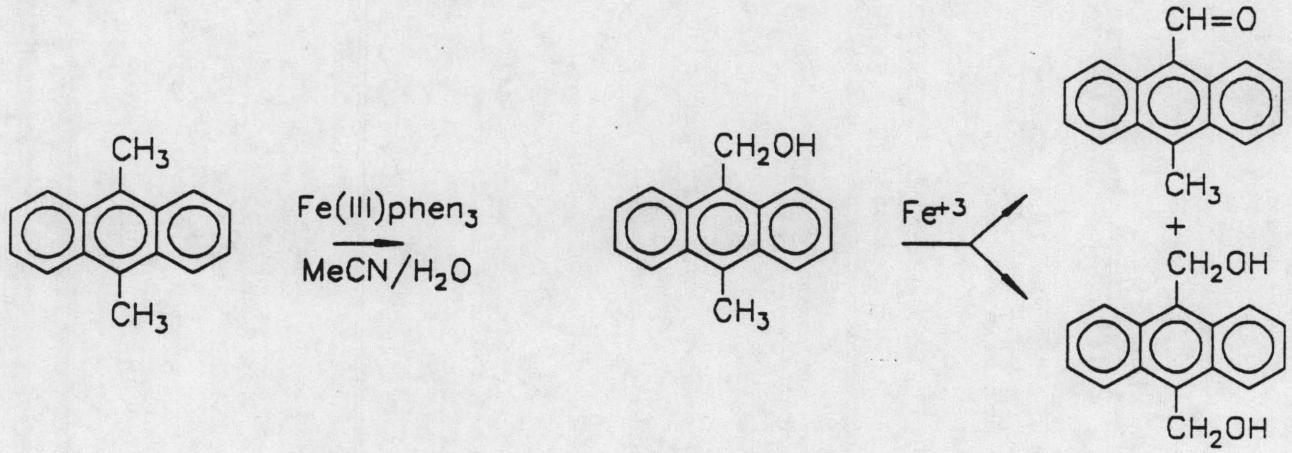


Figure 5. Oxidation pathways for DMA.

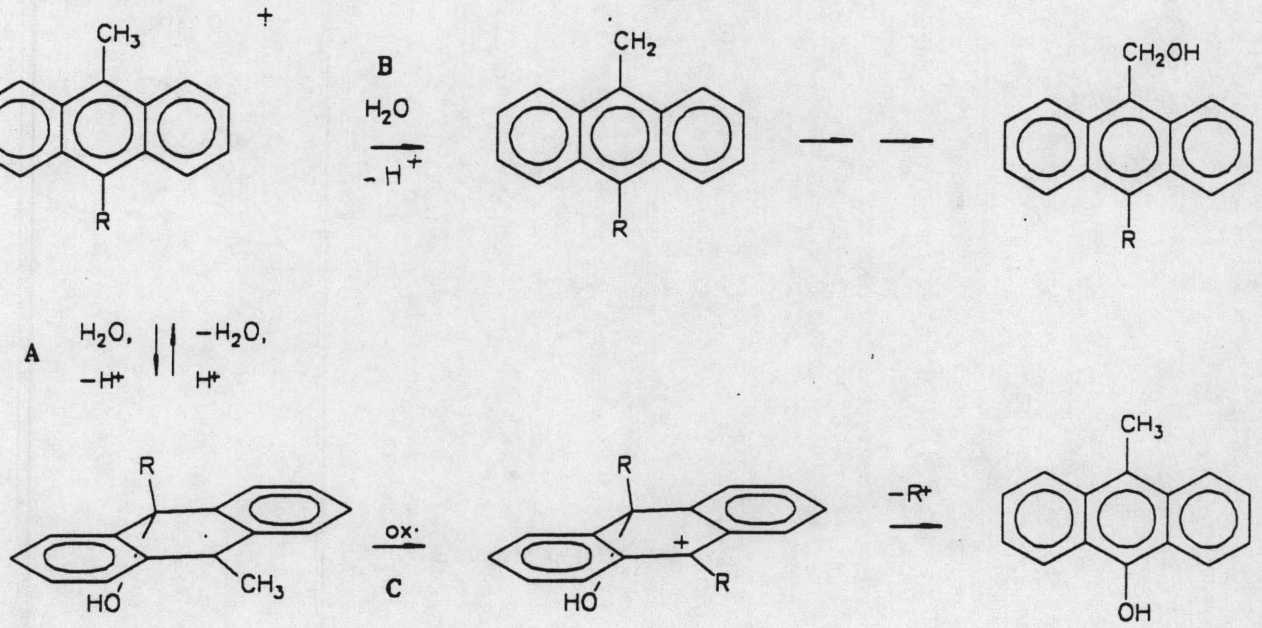


Figure 6. Oxidation pathways for methylated anthracenes.

a. Objective S4. Deuterium isotope effects. Substitution of each methyl proton in turn by deuterium also allowed us employ Hanzlik's method[35] to determine the primary kinetic deuterium isotope effect (k. d. i. e.) for the primary oxidation product as approximately 3.0.[36] This low number reflects the unsymmetrical transition state for this highly exothermic proton transfer transfer and is reminiscent of the numbers obtained by Hanzlik for toluene[35] and by White for ethylbenzene.[37] In contrast, radical cation disappearance as measured by cyclic voltammetry showed a small (or negligible) secondary deuterium isotope effect, which allowed us to separate the initial solvolysis step from the ultimate product forming step (vide infra).

b. Objective S3. The stereoelectronic effect. Chemical oxidation of 9-ethyl-10-methylanthracene (EMA) yielded several products, including 9-hydroxymethyl-10-ethylanthracene, but no products of oxidation of the ethyl group, i. e.,

9-methyl-10-(1-hydroxyethyl)anthracene (see Figure 7). In this case, the sterically more demanding ethyl group has completely shut off deprotonation via that pathway. We have only recently succeeded in synthesizing the "in-plane" equivalent 1,10-ethanoanthracene (**EA**) and are proceeding with the synthesis of 9-methyl-1,10-ethanoanthracene (**MEA**) (see Figure 15). We anticipate that in this case, facile deprotonation of the in-plane ethano group will dominate the reaction pathway, as it does for **3-MC**. In any event, we are aware of no other reaction in which simple replacement of a methyl group by an ethyl group has such a dramatic effect on product selection. The diversion of this reaction pathway into an unprecedented elimination of ethylene provided a serendipitous focus for our efforts on the role of nucleophile, as we shall see below.

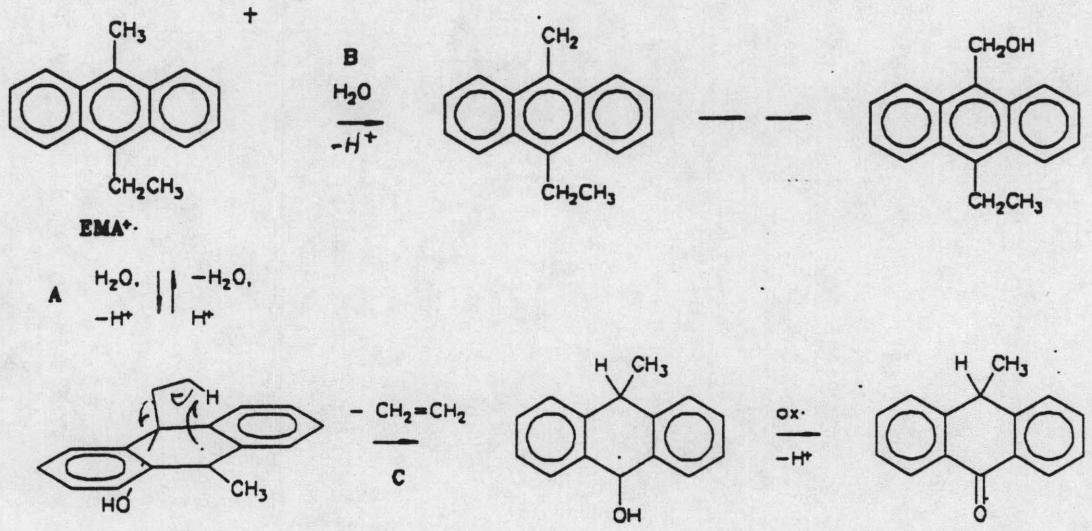


Figure 7. Oxidation of **EMA**.

c. Objective S2. The role of water. The role of water in facilitating proton loss from alkylaromatic radical cations was clearly demonstrated by cyclic voltammetry. In collaboration with Prof. Bottomley of our department, we carried out the cyclic voltammetry of 9,10-dimethylanthracene, 9,10-dimethylanthracene-d_6 9-methyl-10-ethylanthracene, and 9,10-diethylanthracene as a function of water concentration.[38] As water is added up to 10%, the reversibility of radical cation formation disappears, indicating that the intermediate radical cation is unstable relative to some other chemical process, presumably deprotonation or nucleophilic attack. Analysis of the data allowed us to determine the radical-cation decay rate as a function of water concentration (see Appendix B), which clearly showed (pseudo) first order kinetics for

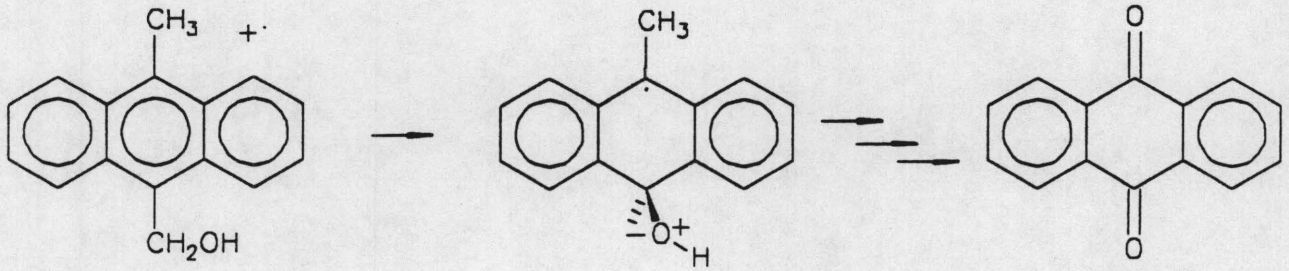


Figure 8. Internal solvolysis and ultimate oxidation of hydroxymethylanthracenes.

radical-cation deprotonation. Significantly, the rate of radical-cation disappearance under these conditions was relatively independent of structure, despite the fact that **DMA** and **DEA** undergo completely different reactions. Thus we are forced to the conclusion that the primary radical cation reaction pathway does not involve deprotonation, but rather the relatively structurally insensitive nucleophilic attack by water. Supportive of this conclusion is the fact that 9-hydroxymethyl-10-methylanthracene radical cation shows no reversibility in the CV experiment, presumably due to fast intramolecular solvolysis by the hydroxymethyl group (see Figure 8).

d. Objective S1. Side-chain oxidation vs. nucleophilic attack. An unexpected but useful result occurred in the oxidation of EMA, which allowed us to put on a firm basis the dependence of side-chain oxidation vs. nucleophilic attack on the rate of proton transfer, which in turn proved to be dependent upon the structure of water. Rather than any oxidation of the ethyl group of EMA, we were startled to observe that a new and unexpected product, 10-methylanthrone, was generated. That is, the ethyl group had been oxidatively cleaved, apparently through attack of the radical cation by water and elimination of ethylene through a seven-center transition state (see Figure 7, R = Et). *This result immediately suggests a mechanistic basis for the weak carcinogenicity of ethylated aromatics, i. e., they are oxidatively cleaved to the innocuous (i.e., excretable) anthrone derivatives.*

e. Objective S5. The nature of the oxidant. Most studies of hydrocarbon oxidations, particularly in those leading to carcinogenesis, have concentrated on microsomes, i. e., systems in which cytochrome P450 is the presumed oxidant. Conversely, rat-liver cytosol preparations can be assumed to be free of significant quantities of P450, especially since microsome formation has not been induced. Despite the absence of the presumed primary oxidant of xenobiotics, DMBA treated with these cytosol preparations shows efficient conversion to the mono- and bishydroxymethylated derivatives.[39] DMBA treated with horseradish peroxidase shows similar reactivity.[40] In our hands, EMA was efficiently oxidized to alcohol in cytosolic media but to 10-methylanthrone by microsomal preparations. Thus we were able to demonstrate unequivocally that a reaction in which a radical cation was an unambiguous intermediate could present two apparently different mechanistic results simply as a function of the hydrophilic or hydrophobic nature of the enzyme. In order to make this diversity more useful in biochemically relevant systems, expansion of these studies to flavin-containing monooxygenases (FMO) as well as more conventional cytochrome P450 models, e. g. tetraphenylporphinatoiron(V) oxide ($TPPFe^{V}=O$), will be carried out.

2. Work in progress. a. Basicity vs. nucleophilicity. Inasmuch as the solvent effect and the side-chain oxidation vs. nucleophilic attack partition are intrinsicially related, we elected to examine in more quantitative detail how the partition is affected by the nature of the nucleophile. Thus the two products from oxidation of EMA represent a paradigm for the two predominant pathways for radical cation behavior, i. e., nucleophilic attack leading to elimination of ethylene and side chain deprotonation leading to hydroxymethyl products. The competition between proton loss and nucleophilic attack was affected by

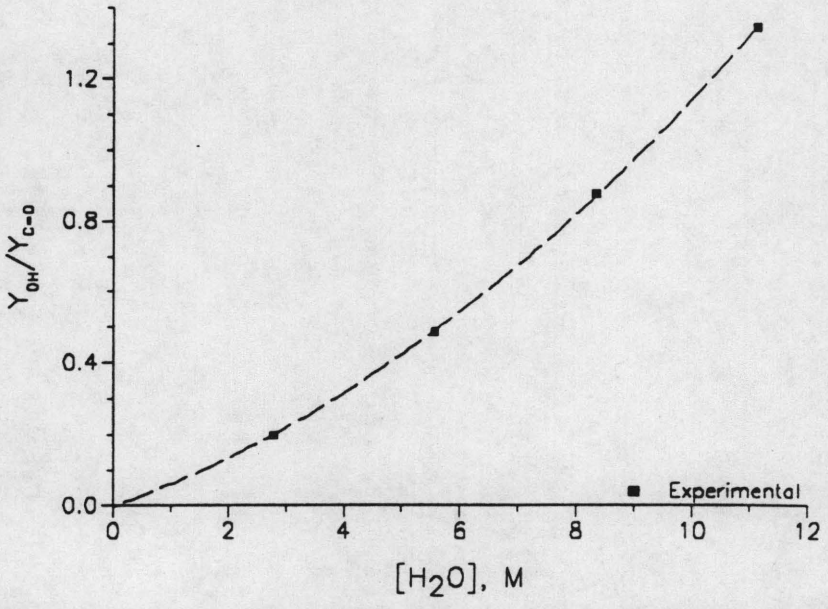


Figure 9. Ratio of deprotonation to nucleophilic attack products of EMA.

water, with increasing water concentration favoring the former (see Figure 9). With this model as our basis, we were able to derive a kinetic expression to fit the data in Figure 9, provide an estimate for the rate of deprotonation of radical cation **4**, and evaluate the role of water structure, i. e., clustering, on the deprotonation rate. Making the reasonable assumption that steps **B** and **C** in Figure 7 irreversibly lead to alcohol and anthrone, respectively, we can develop a kinetic scheme for the ratio of alcohol to ketone. In using this scheme, we assume that rate constant k_B has the following form:

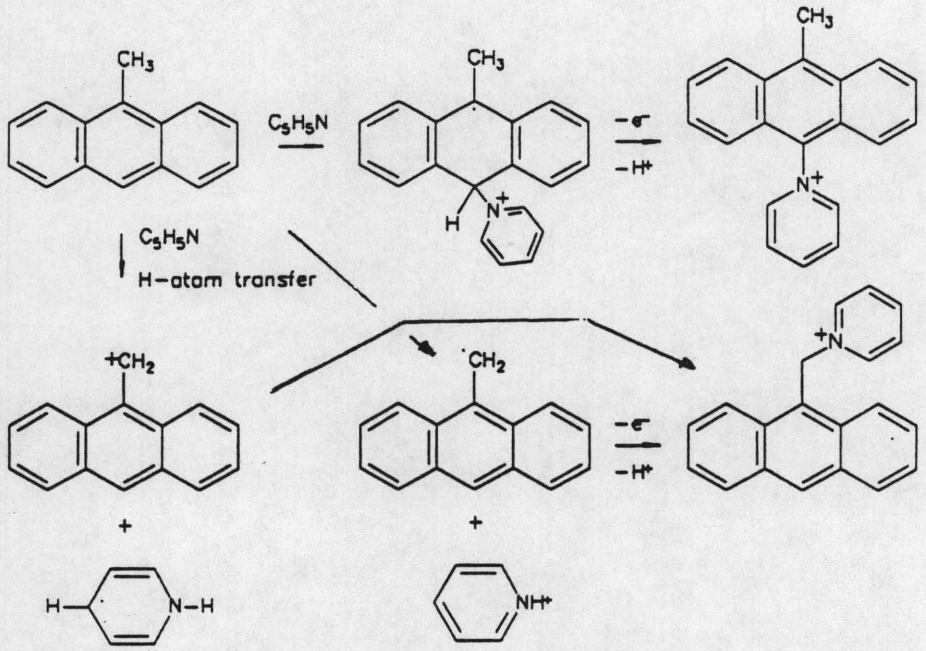


Figure 10. Pyridine adducts from 9-methylanthracene radical cations.

$$k_B = k_1 + k_2[H_2O] + k_3[H_2O]^2 \qquad [1]$$

We are able to solve for k_2 and k_3, the second and third order deprotonation rate constants, from our combined electrochemical and chemical data as well as to set upper limits for the first order rate constant:

$$k_1 = 2.0 \pm 2.0 \times 10^{-4}\ LM^{-1}s^{-1}$$
$$k_2 = 1.54 \pm .06 \times 10^{-2}\ L^2M^{-2}s^{-1}$$
$$k_3 = 1.63 \pm .06 \times 10^{-3}\ L^3M^{-3}s^{-1}$$

According to these rate constants, water monomer is a poor base. Rather, water trimer is the effective base at 10 molar (20%) water concentration, as the gas phase proton solvolysis data would indicate.[41] In 100% water, we would expect that the tetramer would dominate, as Robinson et al[22] have noted in a different system. We might emphasize in this regard that the effect of increasing water concentration on this partition simply reflects that the basicity of water increases with increasing concentration due to clustering effects, while nucleophilicity, a monomolecular phenomenon, does not.

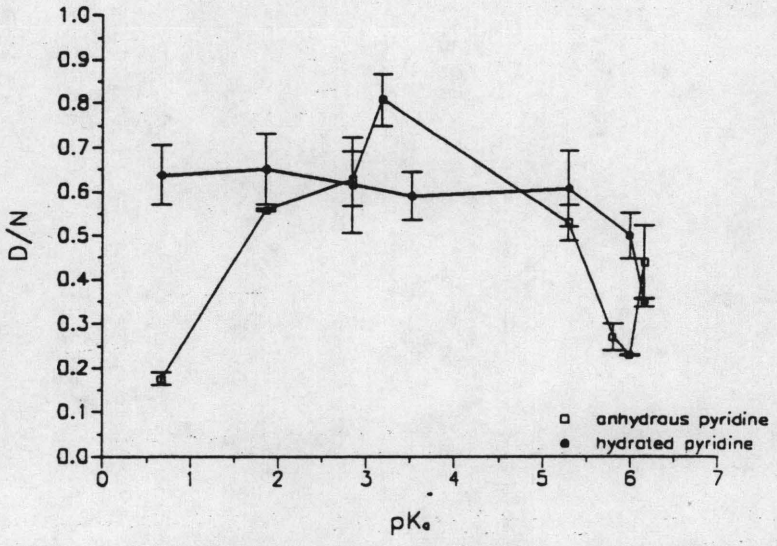


Figure 11. Dependence of deprotonation on pyridine basicity.

In order to examine proton transfer in the absence of clustering effects, we

took advantage of Cavalieri's observation that both side-chain oxidation and nucleophilic attack products result from treatment of anthracene derivatives with iodine in pyridine,[42] conditions which apparently favor formation of radical cation. Cavalieri's substrates did *not* include ones for which nucleophilic attack and deprotonation were symmetrically equivalent. Thus we turned our attention to 9-methylanthracene, for which *meso*-anthracenic attack at the nor-methyl position should lead ultimately to a ring-bonded pyridinium while side-chain attack should lead to a benzylic pyridinium. Indeed, oxidation with either iodine or Ce(IV) in chloroform led cleanly to both products, but the ratio was dominated by the nucleophilic product (see Figure 10). With **DMA**, only side-chain oxidized product, while EMA oxidation showed some ethyl cleavage but was dominated by methyl oxidation. The mechanistic scheme represented by Figure 7 still holds. Again, water exerted an important role, since this ratio increased if the pyridine was in its hydrated form. Some insight into phenomenon was provided by proton nmr of H_2O in H_2O-saturated chloroform, which exhibited a significant chemical shift upon addition of pyridine. The chemical shift behavior could be effectively modelled by assuming a dimeric equilibrium (Equation 2 and Figure 12). Thus our conclusion is that upon addition of water, the nature of the proton acceptor changes from free pyridine to a water-pyridine complex which is more efficient at proton transfer.

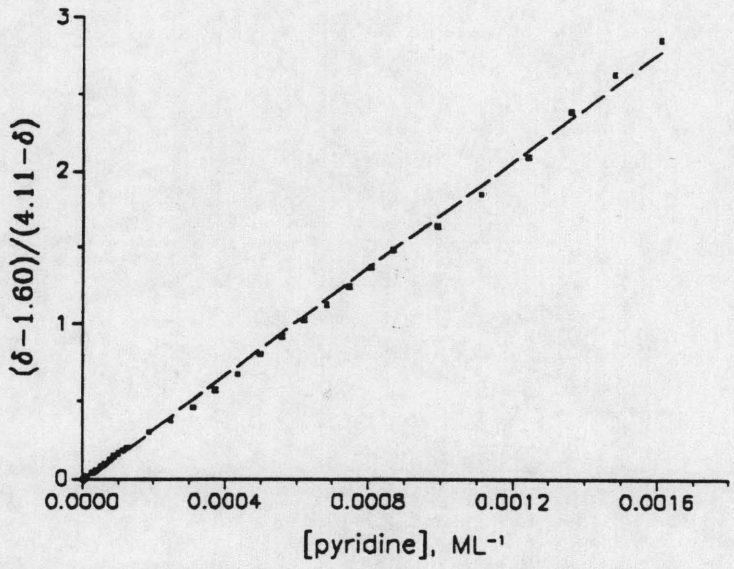


Figure 12. Observed and calculated chemical shifts.

$$H_2O + C_5H_5N \text{ -----> } C_5H_5N{\cdot}HOH \qquad [2]$$

The use of pyridine as proton acceptor provided the opportunity to examine the electronics of proton transfer more carefully by the use of substituent effects. Again, the ratio of nucleophilic to deprotonated products was a function of pyridine basicity, with a clear change in mechanism at pK_a = 5-6 for hydrated pyridine and pK_a = 3 for anhydrous pyridines, with some allowances for experimental variability (see Figure 11). The reason for this change is unknown; however, a strong candidate is the intervention of direct hydrogen atom transfer rather than proton-transfer/electron transfer (see Figure 11). That is, as the pyridine substituent becomes more electron demanding, both its basicity and the reduction potential of the resulting pyridium salt decrease, factors which at a critical point will cause a change in mechanism to hydrogen atom transfer. This phenomenon will be investigated further.

b. Spectroscopic and kinetic studies. Our initial studies relied heavily on electrochemical determination of radical cation decay rates. Although these studies have and continue to be useful in quantifying radical cation reactivity, we needed more direct methods, particularly absorption spectroscopy. Kochi and coworkers have obtained the absorption spectrum of **DMA** radical cation generated both electrochemically and photochemically.[43] Unfortunately their conditions are not readily transferable to our needs. Indeed, we have discovered that the **DMA** radical cation spectrum can be determined in trifluoroacetic acid, in which oxygen apparently acts as the electron acceptor. Generation of radical cations by this method has in fact been employed for other molecules, although the exact nature of the mechanism remains elusive.

Again, although we can determine the **DMA** radical cation spectrum by this method, its utility lies mainly in providing a ready source of stable radical cation for further spectroscopic investigations. In order to actually measure decay rates, we turned to stopped-flow spectrophotometry. We were successful in obtaining excellent spectra and decay kinetics in acetonitrile solution (see Figure 13) and repeat the decay measurements previously done electrochemically. The stopped-flow decay rates were, however, faster by almost one-hundred-fold!

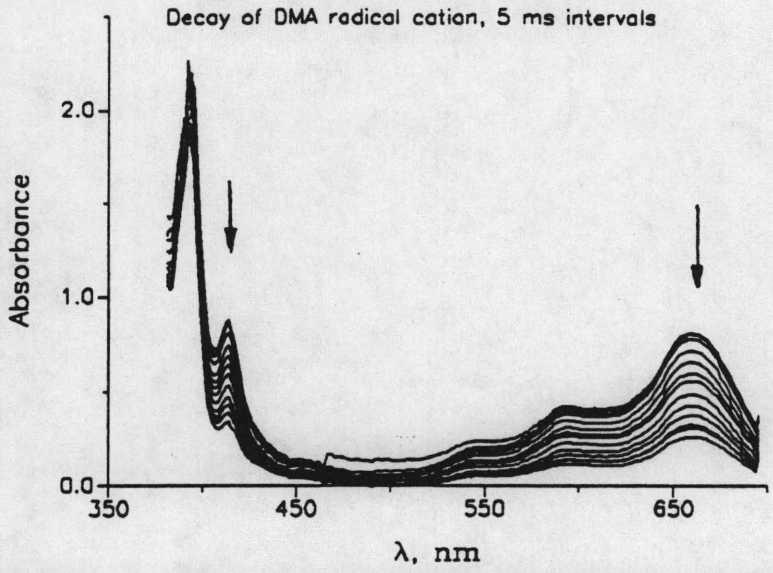


Figure 13. Spectrum and decay of DMA radical cation.

The one significant chemical difference between the electrochemical and spectrochemical measurements was the presence of .1M $LiClO_4$, the supporting electrolyte, in the electrochemical experiments. In fact, addition of .1M $LiClO_4$ to the stopped-flow solutions produced a reduction in decay rate of the correct magnitude (see Figure 14). This effect is reminiscent of the rate reduction in photopromoted proton transfer reactions[44] and is probably due to an analogous reduction of solvent activity. This result has serendipitously provided another clue to radical cation reactivity which may be important in their enzymological behavior.

3. Other results. a. DNA intercalation. Part of the controversy surrounding the significance of the side chain concerns its role in facilitating or blocking DNA intercalation, the step preceding the putative key carcinogenic event, DNA alkylation. Presumably, the strength of the DNA-PAH intercalate is the only mechanism by which an otherwise indiscriminate electrophile can distinguish this nitrogeneous material from protein or other nucleophile. In order to develop some insight into this mechanism, we examined the DNA intercalate for each of our anthracene derivatives using the method exploited by leBreton.[45] Indeed, each anthracene, in reality a derivative of 9,10-dimethylanthracene, intercalates DNA. As Table I illustrates, however, the effect of ethyl vs. methyl side chain is fairly minor. The more dramatic effect occurs upon substitution by hydroxymethyl, which in the case of 9,10-bis(hydroxymethyl)anthracene, virtually blocks intercalation. We believe this is the effect of hydrogen bonding of the hydroxymethyl group which prevents its ready insertion into the DNA base pairs. Although these results area only suggestive, they do further support the role of the ethyl groups as being a diversion of the metabolism rather than prevention of intercalation. They also raise serious questions about the role of side-chains in any intercalative DNA alkylation via hydromethyl or other hydrogen-bonding groups such as phosphates, although acetylation

Table I. Effect of side chain on fluorescence quenching of dialkylanthracenes by calf thymus DNA.

9,10-Dialkylanthracene		
R_1	R_2	K_{sv} (x 10^3M/L)
H	H	4.98
H	Me	9.59
Me	Me	29.2
Me	Et	19.5
Et	Et	10.4
Me	$HOCH_2$	2.36
$HOCH_2$	$HOCH_2$	0.92

remains a possible mechanism.

b. Nature of the side chain. Since side chain hydroxylation appears at least to be a permissive pathway in PAH activation, DNA intercalation notwithstanding, we pursued the chemical consequences of further oxidation. As we mentioned in our previous proposal, 9-hydroxymethyl-10-methylanthracene exhibits no reversibility during cyclic voltammetry, a result we ascribe to internal solvation (see Figure 8). Moreover, regardless of oxidant the ultimate product of dialkylanthracene oxidation is anthraquinone, which clearly involves carbon-carbon cleavage. How does this event occur? Although these studies are in progress, we have been able to develop some valuable clues. First, when the radical cation of hydroxymethylanthracene is an intermediate, formaldehyde is always the ultimate product. This pathway is to be distinguished from that arising from hydroxyl radical, i. e., Fenton's reagent, which does not produce formaldehyde. We do not exclude the possibility that one role for mutagenicity of hydroxymethyl aromatics is this unique production of formaldehyde, which is itself a toxin with widespread mutagenic effects.[46]

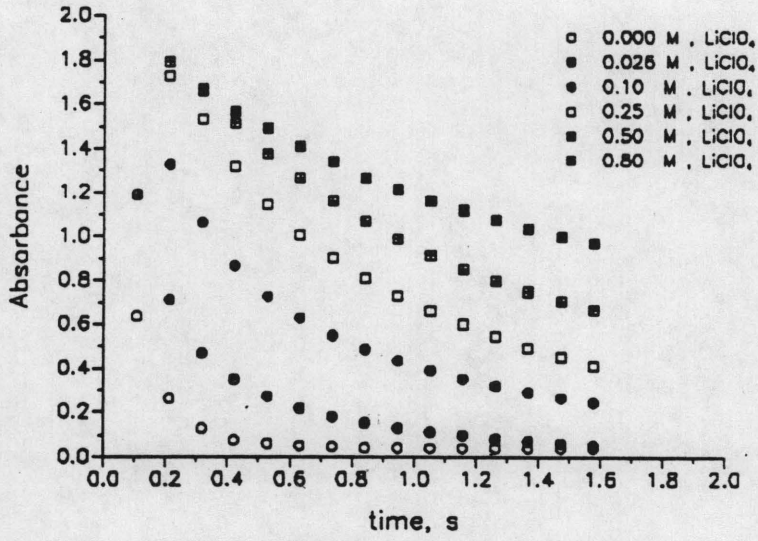


Figure 14. Effect of $LiClO_4$ on $DMA^{+\cdot}$ decay.

D. Experimental Design and Methods.

Our hypothesis is that the key factor in the partition between ring and alkyl chain oxidation is the rate of proton loss. This rate is in turn dependent upon a complex series of solvent, electronic, structural, and isotope effects. In order to deepen our understanding of this reactivity, we now require much more rigorous structural information which is now made possible by our development of facile techniques for their generation and observation. This will be accompanied by further mechanistic studies in which a strategically designed arylalkyl hydrocarbon or mixture of hydrocarbons is allowed to react with a one electron oxidant. Thus one potential product will always be a chain hydroxylated product, while the other products will reflect competitive nucleophilic attack, ring opening, or alternative proton loss. The way in which the product ratios differ as a function of these chemical changes will reveal the role of proton transfer in determining the reaction pathways. Finally, we now have kinetic methods which will enable us to directly measure rates at which the radical cations react

1. Structure. Most of the information about radical cation structure is currently available only from esr spectra. The hyperfine coupling constants for **DMA** radical cation indicate considerable delocalization of spin density into the methyl groups through hyperconjugation. MNDO calculations in our facilities and in those of Samir Farid[47] indicate that this delocalization is accompanied by CH bond stretching, as one might expect from the increased thermodynamic acidity, which also has the effect of lifting the C_3 symmetry of the methyl group. We intend to probe for the structure of the radical cation through a combination of diffraction, resonance Raman and infrared techniques.

a. Infrared absorption. The enhanced acidity and hyperconjugation produced upon radical cation formation of *meso*-methylanthracenes should have an effect upon C-H bond stretching frequencies. We have built a transmission infrared cell for simultaneous generation of radical cation and observation by Fourier transform infrared spectroscopy. We will also build a reflectance cell for increased sensitivity. Again, our deuterated anthracenes should enable us to isolate the frequencies for C-H bond stretching and determine the hyperconjugative effect on

The Bioxidation of Arylalkyl Hydrocarbons

Final Progress Report

DHHS Grant No. 5 R01 CA43806-03

Submitted by Laren M. Tolbert
School of Chemistry and Biochemistry
Georgia Institute of Technology
Atlanta, GA 30332-0400

Summary.

The case of alkylated polynuclear aromatic radical cations provides a particularly useful area for study since these radical cations can partition along two mechanistically related channels. One involves proton loss, leading to side-chain oxidation, and the other involves nucleophilic attack, leading to ring oxidation. The rate of proton loss, the kinetic acidity, is apparently the key branchpoint between these two pathways, particularly in structured environments such as those of enzyme active sites. Our aims involved a far-reaching investigation of this phenomenon, ranging from structural, electronic, and environmental effects on proton transfer from radical cations to fundamental spectroscopic studies of chemically and electrochemically generated species. Toward those aims we have accomplished the following objectives: (a) we have examined the competition between side chain oxidation and ring oxidation for a series of hydrocarbons, (b) we have observed the effect of solvent polarity and basicity, particularly of water, on this partition, (c) we have determined the stereoelectronic effect on proton loss for a meso-ethyl anthracene, (d) we have examined both inter- and intramolecular kinetic isotope effects on proton loss, and (e) we have obtained preliminary data on microsomal oxidation for a solvent-sensitive substrate, 9-ethyl-10-methylanthracene (**EMA**). In the course of this work, we have developed some useful spectroscopic and mechanistic methods which enable us to provide quantitative information on the stereoelectronic effect, and we developed methods which allowed the radical cation to form products with alternate regiochemistry.

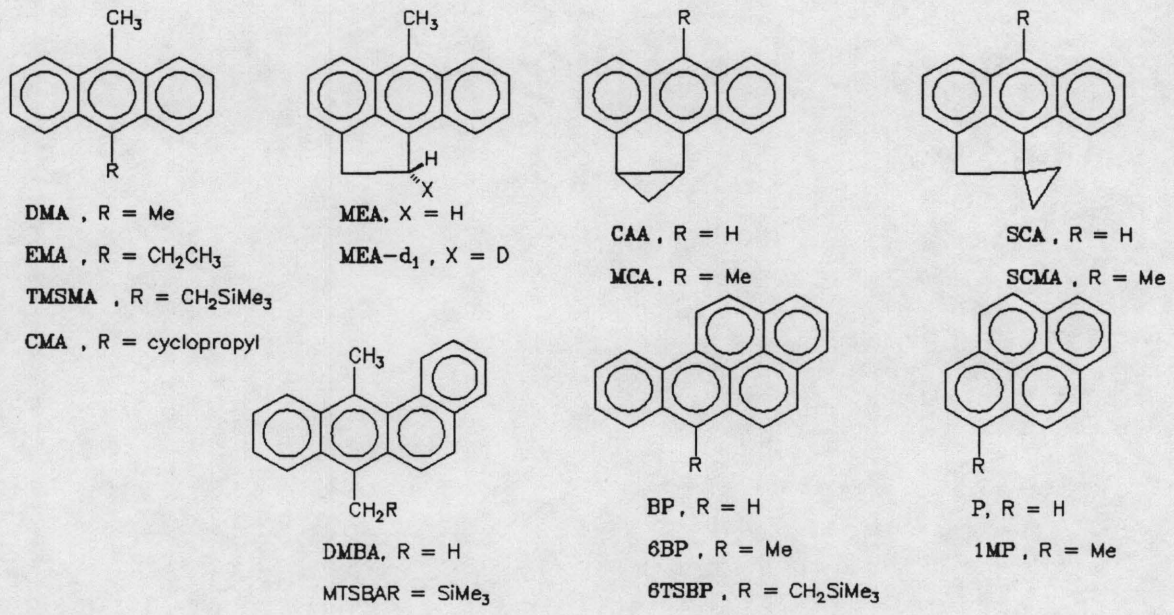


Figure 1. Substrates for kinetic and spectroscopic studies.

Results. We concentrated our initial efforts in oxidation chemistry on 9-methylanthracene (**9MA**) and its derivatives (see Figure 1). The strategy we employed was to take advantage of the symmetry of **9MA** and to use deprotonation and, therefore, oxidation of the methyl group as a "molecular clock", comparing the reactivity when a sterically or electronically distinct moiety was attached to the opposite position (C-10), or in the case of 9,10-dimethylanthracene (**DMA**), when hydrogen was replaced with deuterium. One-electron oxidations were obtained with tris(phenanthroline)iron(III) (**$Fe^{III}phen_3$**), although we discovered that ceric ammonium nitrate (**CAN**) is more useful for spectroscopic studies. For regiochemical and stereochemical products studies, we found that Cavalieri's pyridine/iodine system provides crystalline products for x-ray analysis in which the site of deprotonation or nucleophilic attack is marked by the addition of pyridine.[1] Finally, for purposes of comparison with biooxidants, we employed the P450 model tetraphenylpophinato-iron(III)/iodosobenzene (**TPPFe=O**) system. Critical factors proved to be, as anticipated, hydrocarbon and water concentration, with a 1:9 water-acetonitrile solvent system and 3 mM hydrocarbon concentration providing the optimum results with **$Fe^{III}phen_3$**. Under these conditions, oxidation of **DMA** proceeded to yield a single primary product, 9-hydroxymethyl-10-methylanthracene.[2] Oxidation of the latter produced both 9,10-bis(hydroxymethyl)anthracene and 10-methylanthracene-9-carboxaldehyde in a 2:1 ratio as well as products of further oxidation. As a result of extensive chemical electrochemical kinetic studies, we were able to develop a mechanistic scheme which has proven to be general for all the anthracene derivatives we have studied so far. The significant feature of this scheme is that upon formation of radical cation fast but reversible addition of water or added nucleophile to the meso-anthracenic position occurs. If bond cleavage reactions make the initially formed adduct irreversible, products of nucleophilic addition result. Alternatively, (irreversible) deprotonation

of the radical cation takes place to form the side-chain oxidized product. Thus the rate of deprotonation exerts a controlling influence over the partition among various products.

a. The stereoelectronic effect. Our primary goal in this area was assessing the degree to which efficient deprotonation requires the CH bond dihedral angle to be close to 0° to perpendicular (see Figure 2). Chemical oxidation of 9-ethyl-10-methylanthracene (**EMA**) yielded several products, including 9-hydroxymethyl-10-ethylanthracene, but no products of oxidation of the ethyl group, i. e., 9-methyl-10-(1-hydroxyethyl)anthracene.[3] In this case, the sterically more demanding ethyl group completely shut off deprotonation via that pathway. When **EMA** was subjected to pyridine/iodine oxidation, the single product was that resulting from oxidation/deprotonation of the methyl group, namely N-(10-ethylanthracenylmethyl)-pyridiniumiodide, although 9,10-diethylanthracene (**DEA**) slowly yielded the product of deprotonation of the ethyl group (see Figure 5). We also synthesized the "in-plane" equivalents 1,10-ethanoanthracene (**EOA**) and 9-methyl-1,10-ethanoanthracene (**MEA**) (see Figure 1). In contrast to the oxidation of **EMA**, oxidation of **MEA** by pyridine/I_2 was dominated (12:1) by the oxidation of the ethano group, despite the fact that breaking the C-H bond is at 60° to the anthracene plane rather than the optimal 90°. Similarly, **CAN** oxidation of **MEA** produced only oxidation of the ethano group.

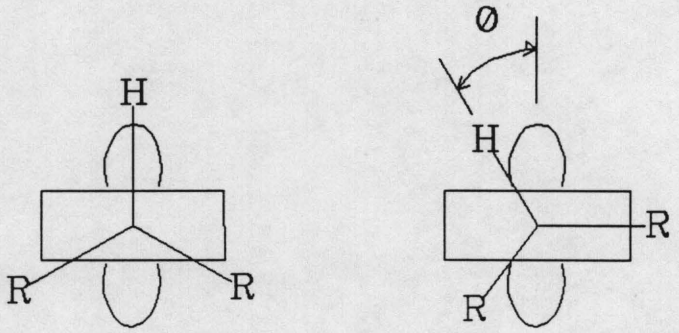


Figure 2. In-plane vs. out-of-plane conformations.

b. Solvent effects. The role of water. The role of water in facilitating proton loss from alkylaromatic radical cations was clearly demonstrated by cyclic voltammetry. We carried out the cyclic voltammetry of **DMA**, **DMA-d_6** **EMA** and 9,10-diethylanthracene (**DEA**) as a function of water concentration.[3] As water is added up to 10%, the reversibility of radical cation formation disappeared, indicating that the intermediate radical cation was unstable relative to some other chemical process, presumably deprotonation or nucleophilic attack. Analysis of the data allowed us to determine the radical-cation decay rate as a function of water concentration, which clearly showed (pseudo) first order kinetics for radical-cation deprotonation. Significantly, the rate of radical-cation disappearance under these conditions was relatively independent of structure, despite the fact that **DMA** and **DEA** undergo completely different reactions. Thus we were forced to the conclusion that the primary radical cation reaction pathway does not involve deprotonation, but rather the relatively structurally insensitive nucleophilic attack by water.

An unexpected but useful result occurred in the oxidation of **EMA**, which allowed us to demonstrate that the dependence of side-chain oxidation vs. nucleophilic attack on the rate of proton transfer is a function of the structure of water. Rather than oxidation of the ethyl group

of **EMA**, a new and unexpected product, 10-methylanthrone, was generated. The ethyl group had been oxidatively cleaved, apparently through attack of the radical cation by water and elimination of ethylene through a seven-center transition state.

We elected to examine in more quantitative detail how the partition is affected by the nature of the nucleophile, inasmuch as the solvent effect and the side-chain oxidation vs. nucleophilic attack partition are intrinsicially related. Thus the two products from oxidation of **EMA** represented a paradigm for the two predominant pathways for radical cation behavior, i. e., nucleophilic attack leading to elimination of ethylene and side chain deprotonation leading to hydroxymethyl products. The competition between proton loss and nucleophilic attack was affected by water, with increasing water concentration favoring the former (see Figure 3). With this model as our basis, we were able to derive a kinetic expression to fit the data in Figure 3, provide an estimate for the rate of deprotonation of the radical cation, and evaluate the role of water structure, i. e., clustering, on the deprotonation rate. This kinetic determination indicates that water trimer was the effective base at 10 molar (20%) water concentration, as the gas phase proton solvolysis data would indicate.[4] In 100% water, we would expect that the tetramer would dominate, as Robinson et al have noted in a different system.[5]

c. Basicity vs. nucleophilicity. Pyridine bases. In order to examine proton transfer in the absence of clustering effects, we used pyridine bases and iodine or **CAN** as oxidant, using substrates for which nucleophilic attack and deprotonation were symmetrically equivalent. Thus we turned our attention to **9MA**, for which meso-anthracenic attack at the nor-methyl position should lead ultimately to the ring-bonded pyridinium salt **MAP** while side-chain attack should lead to the benzylic pyridinium salt **AMP**. Indeed, oxidation with either iodine or Ce(IV) in chloroform led cleanly to both products, but the ratio was dominated by the nucleophilic product **MAP**, as expected (see Figure 5). Again, water exerted an important role, since this ratio increased if the pyridine was in its hydrated form. Some insight into this phenomenon was provided by proton nmr of H_2O in H_2O-saturated chloroform, which exhibited a significant chemical shift upon addition of pyridine. The chemical shift behavior could be effectively modelled by

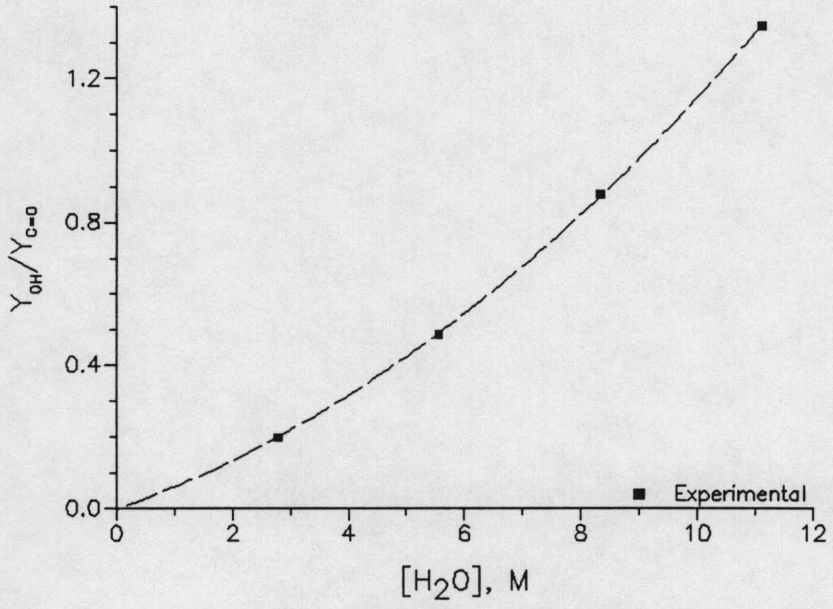


Figure 3. Ratio of deprotonation to nucleophilic attack products of EMA.

assuming a dimeric equilibrium (Equation 1). Thus our conclusion is that upon addition of water, the nature of the proton acceptor changed from free pyridine to a water-pyridine complex which is more efficient at proton transfer.

$$H_2O + C_5H_5N \text{ -----> } C_5H_5N{\cdot}HOH \qquad [1]$$

The use of pyridine as proton acceptor provided the opportunity to examine the electronics of proton transfer more carefully by the use of substituent effects on the pyridine. Again, the ratio of nucleophilic to deprotonated products was a function of pyridine basicity, with a clear change in mechanism at pK_a = 5-6 for hydrated pyridine and pK_a = 3 for anhydrous pyridines, with some allowances for experimental variability. The reason for this change is unknown; however, a strong candidate is the intervention of direct hydrogen atom transfer rather than proton-transfer/electron transfer (see Figure 4). That is, as the pyridine substituent becomes more electron demanding, both its basicity and the reduction potential of the resulting pyridium salt decrease, factors which at a critical point will cause a change in mechanism to hydrogen atom transfer.

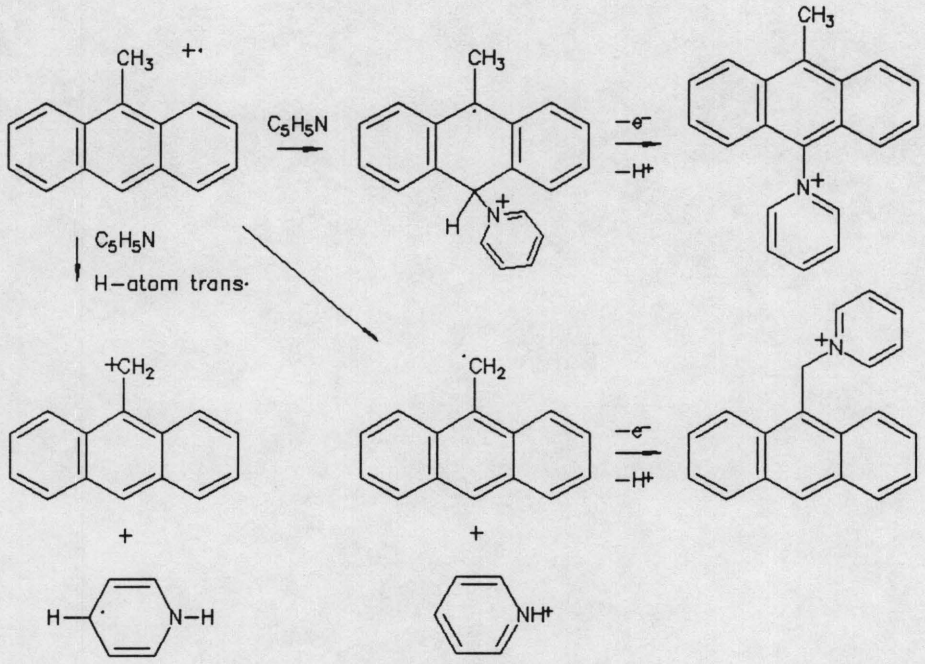
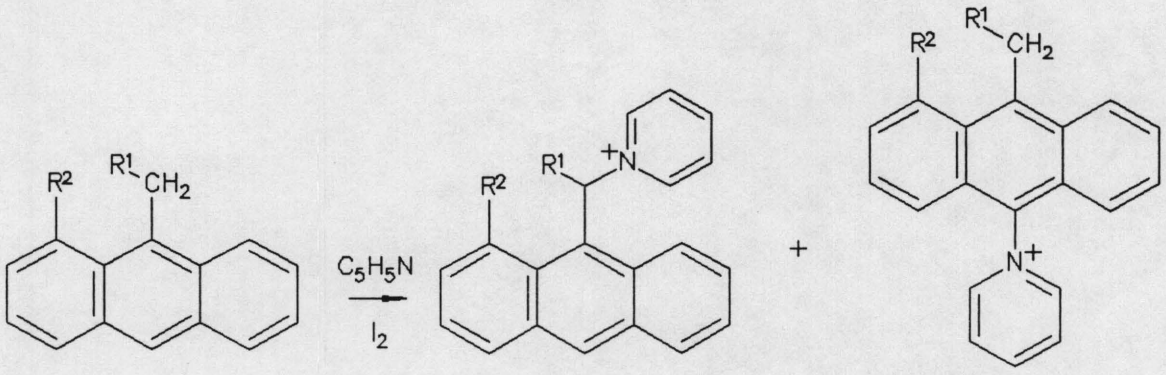


Figure 4. Pyridine adducts from 9-methylanthracene radical cations.

No side-chain oxidation was observed with **9EA**; rather, only N-[(9-anthryl)-1-ethyl)]pyridinium iodide (**AEP**)was produced (see Figure 5). In an attempt to see how replacement of a methyl proton by trimethylsilyl would mediate the "D/N" ratio, 9-(trimethylsilylmethyl)anthracene (**9TMSMA**) was synthesized and subjected to pyridine/I_2 oxidation.[6] In this case, the selectivity was completely reversed, yielding 86% of the side-chain oxidized product **AMP** and only 14% of the nucleophilic product **MAP** in which the trimethylsilyl group had been lost as well. The selectivity was also reversed by replacing methyl with a 1,10-ethano group (**EOA**), which produced a 92% yield of the side-chain oxidized product and 8% of the ring-oxidized product (see Figure 5). This dramatic difference in reactivity between **MEA** and **EMA** as well as between **EOA** and **9EA**, for which rotation of

the ethyl group by 30° could allow similar disposition of the breaking CH group within 30° of perpendicular, suggested that the stereoelectronic effect must be more compelling than we realized. We attribute this result to hyperconjugative rehybridization which places the CH_2 group more nearly perpendicular to the anthracene plane. We have confirmed this atribution by high-level calculation.[7]



9MA, R^1=R^2=H	AMP	10:90	MAP
9EA, R^1=Me, R^2=H	EAP	0:100	AEP
EOA, R^1+R^2=$-CH_2-$	AAP	76:24	EOAP
9TMSMA, R^1=Me_3Si, R^2=H	AMP	84:16	MAP

Figure 5. Pyridine/iodine oxidation of dialkylanthracenes.

d. Deuterium isotope effects. Hanzlik's method[8] relies upon the separability of primary and secondary kinetic deuterium isotope effects **P** and **S**. **DMA** was synthesized with d_1, d_2, and d_3 methyl groups, as well as with a single d_3 methyl group, to determine the isotope effect for hydroxylation. Reminiscent of the numbers for toluene[8] and for ethylbenzene,[9] but we discovered that the statistically corrected P/S ratios are dependent upon deuterium number, that is, P/S = 3.3 for **DMA**-d_6 (intermolecular), 1.5 for **DMA**-d_2 (intramolecular) and 8.8 for **DMA**-d_4 (intramolecular). This result requires that the primary and secondary deuterium isotope effects are inseparable, and suggests that significant conformational and structural changes occur upon radical cation formation, along the lines of those suggested for **MEA** above, resulting in a nonstatistical distribution of rotamers and an additional contribution to the isotope effect. In contrast to the product analysis, radical cation disappearance as measured by cyclic voltammetry showed a small (or negligible) secondary deuterium isotope effect, which allowed us to separate the initial solvolysis step from the ultimate product forming step.

e. The nature of the oxidant. In our hands, **EMA** was efficiently oxidized to alcohol in cytosolic media but to 10-methylanthrone by microsomal preparations. Thus we were able to demonstrate unequivocally that a reaction in which a radical cation was an unambiguous intermediate could present two apparently different mechanistic results simply as a function of the hydrophilic or hydrophobic nature of the enzyme.

f. Spectroscopic and kinetic studies. Our initial studies relied heavily on electrochemical determination of radical cation decay rates. More recently, we discovered that the **DMA** radical cation spectrum can be determined in trifluoroacetic acid in which dissolved oxygen apparently acts as the electron acceptor. In order to actually measure decay rates, we turned to stopped-flow spectrophotometry. We were successful in obtaining excellent spectra and decay kinetics in acetonitrile solution (see Figure 6) and repeat the decay measurements previously done electrochemically. The stopped-flow decay rates were, however, faster by almost one-hundred-fold!

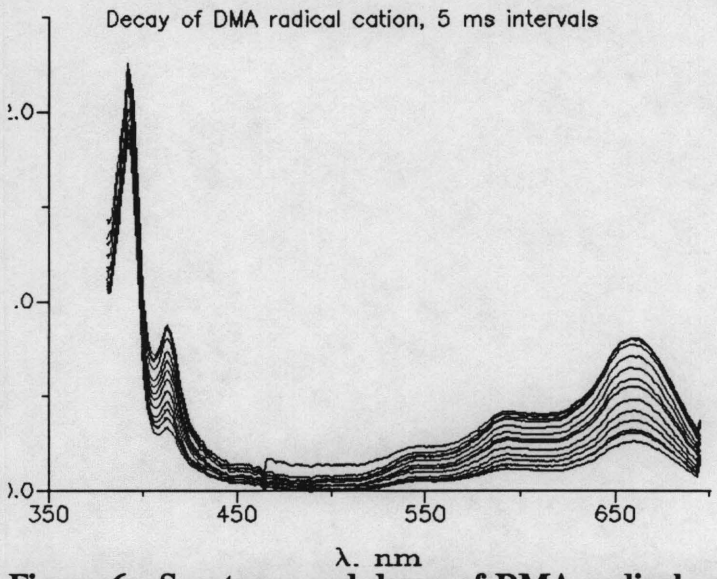


Figure 6. Spectrum and decay of DMA radical cation.

The one significant chemical difference between the electrochemical and spectrochemical measurements was the presence of .1M $LiClO_4$, the supporting electrolyte, in the electrochemical experiments. Addition of .1M $LiClO_4$ to the stopped-flow solutions produced a reduction in decay rate of the correct magnitude. Moreover, the magnitude of the effect depended on the identity of the cation (see Figure 7). This effect is reminiscent of the rate reduction in photopromoted proton transfer reactions.[10] Kochi and coworkers have observed a special (anionic) salt effect on rates of collapse of photogenerated radical ion pairs.[11] We believe our results reflect an a reduction of solvent activity, inhibiting nucleophilic attack by acetonitrile.

2. Other results. a. DNA intercalation. Part of the controversy surrounding the significance of the side chain concerns its role in facilitating or blocking DNA intercalation, the step preceding the putative key carcinogenic event, DNA alkylation. Presumably, the strength of the DNA-PAH intercalate is the only mechanism by which an otherwise indiscriminate electrophile can distinguish this nitrogeneous material from protein or other nucleophile. In order to develop some insight into this mechanism, we examined the DNA intercalate for each

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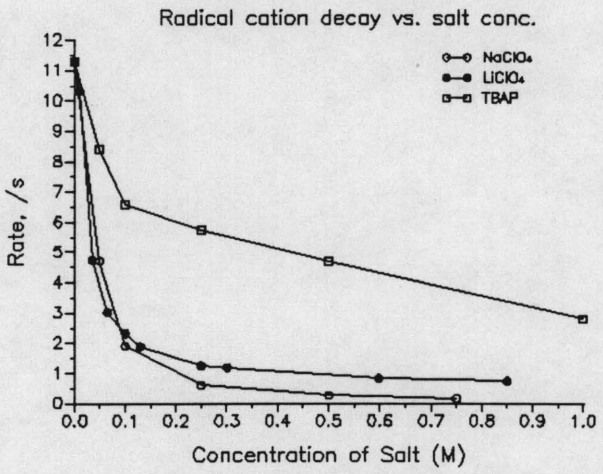

Figure 7. Effect of Cations on $DMA^{+\cdot}$ decay.

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9,10-Dialkylanthracene

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References.

1. E. Cavalieri, R. Rolt, J. Org. Chem., **1976**, 41, 2679.

2. L. M. Tolbert and R. K. Khanna, J. Am. Chem. Soc., **1987**, 109, 3477.

3. Laren M. Tolbert, Rajive K. Khanna, Leslie A. Gelbaum, Ann E. Popp, and Lawrence A. Bottomley, J. Am. Chem. Soc., **1990**, 112, 2373.

4. M. Meot-Ner, J. Am. Chem. Soc., **1986**, 108, 6189.

5. (a) Lee, J.; Griffin, R. D.; Robinson, G. W. J. Chem. Phys. **1985**, 82, 4920. (b) Lee, J.; Robinson, G. W.; Webb, S. P.; Philips, L. A.; Clark, J. H. J. Am. Chem. Soc. **1986**, 108, 6538. (c) Krishnan, R.; Fillingim, T. G.; Lee, J.; Robinson, G. W. J. Am. Chem. Soc. **1990**, 112, 1353.

6. Sarath R. Sirimanne, Zhaozhao Li, Donald R. VanderVeer, and Laren M. Tolbert*, J. Am. Chem. Soc., **1991**, 113, 1766.

7. Laren M. Tolbert, Adam Kallel, and Kendall N. Houk, J. Amer. Chem. Soc., to be submitted.

8. R. P. Hanzlik, K. Hogberg, J. B. Moon, and C. M. Judson, J. Am. Chem. Soc., **1985**, 107, 7164.

9. R. E. White, J. P. Miller, L. V. Favreau, and A. Bhattacharyya, J. Am. Chem. Soc., **1986**, 108, 6024.

10. Huppert, D.; Kolodney, E.; Gutman, M.; Nachliel, E. J. Am. Chem. Soc., **1982**, 104, 6949.

11. S. Sankararaman, W. A. Haney, J. K. Kochi, J. Am. Chem. Soc., **1987**, 109, 7824.

12. M. Abramovich, A. S. Prakash, R. G. Harvey, I. S. Zegar, and P. R. leBreton, Chem.-Biol. Interact., **1985**, 55, 39.

Publications from DHHS Support.

Sarath R. Sirimanne, Zhaozhao Li, Donald R. VanderVeer, and Laren M. Tolbert*, "One Electron Oxidation of 9-Methylanthracene and 9-(Trimethylsilymethyl)anthracene: Reversal of Cation Radical Selectivity by the Trimethylsilyl Group", J. Am. Chem. Soc., **1991**, 113, 1766.

Laren M. Tolbert, R. K. Khanna, Ann E. Popp, L. Gelbaum and Lawrence A. Bottomley, "Stereoelectronic Effects in the Deprotonation of Arylalkyl Radical Cations: meso-Ethylanthracenes", J. Amer. Chem. Soc., **112**, 2373 (1990).

Laren M. Tolbert and Rajive K. Khanna, "Dramatic Solvent and Stereoelectronic Effects in a Biomimetic Oxidation: 9,10-Dialkylanthracenes",J. Amer. Chem. Soc.,**109**, 3477, (1987).

Manuscripts in preparation.

Laren M. Tolbert, Adam Kallel, and Kendall N. Houk, "The Remarkable Structure of Toluene Radical Cation." J. Amer. Chem. Soc., to be submitted.

Laren M. Tolbert, Zhaozhao Li, Sarath R. Sirimanne, and Donald R. VanderVeer, "Chemistry of Arylalkyl Radical Cations. Deprotonation vs. Nucleophilic Attack." J. Org. Chem., to be submitted.

Sarath R. Sirimanne and Laren M. Tolbert, "Oxidative Demethylation of Methylated Anthracenes by One-electron Oxidants: Generation of Formaldehyde." J. Org. Chem., to be submitted.

Sarath R. Sirimanne and Laren M. Tolbert, "Reactions of Radical Cations of Anthracene Derivatives: Nucleophilic Attack versus Deprotonation," J. Org. Chem., to be submitted.

Sarath R. Sirimanne and Laren M. Tolbert, "Radical Cation Reactions of Substituted Anthracenes: Indirect Carbon-carbon Bond Cleavage," J. Org. Chem., to be submitted.