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RADICAL CATIONS IN AROMATIC HYDROCARBON CARCINOGENESIS

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Most carcinogens, including polycyclic aromatic hydrocarbons (PAH), require metabolic activation to produce the ultimate electrophilic species that bind covalently with cellular macromolecules to trigger the cancer process. Metabolic activation of PAH can be understood in terms of two main pathways: one-electron oxidation to yield reactive intermediate radical cations and monooxygenation to produce bay-region diol epoxides. The reason we have postulated that one-electron oxidation plays an important role in the activation of PAH derives from certain common characteristics of the radical cation chemistry of the most potent carcinogenic PAH. Two main features common to these PAH are: 1) a relatively low ionization potential, which allows easy metabolic removal of one electron, and 2) charge localization in the PAH radical cation that renders this intermediate specifically and efficiently reactive toward nucleophiles. Equally important, cytochrome P-450 and mammalian peroxidases catalyze one-electron oxidation. This mechanism plays a role in the binding of PAH to DNA. Chemical, biochemical and biological evidence will be presented supporting the important role of one-electron oxidation in the activation of PAH leading to initiation of cancer.

KEY WORDS: Carcinogenic activity, cellular nucleophiles, cytochrome P-450, DNA adducts, ionization potential, mechanisms of carcinogenesis, mouse skin, one-electron oxidation, peroxidases, radical cations, rat mammary gland.

ABRREVIATIONS: BP, benzo[a]pyrene; BP-C8dG, 8-(benzo[a]pyren-6-yl) deoxyguanosine; BP-N7Gua, 7-(benzo[a9pyren-6-yl) guanine; CuOOH, cumen hydroperoxide; DMBA, 7,12-dimethylbenz[a]anthracene; HPLC, high pressure liquid chromatography; HRP, horseradish peroxidase; IP, ionization potential (s); PAH, polycyclic aromatic hydrocarbon(s); MC, 3-methylcholanthrene; PHS, prostaglandin H synthase; 1,2,3,4-tetrahydroDMBA, 1,2,3,4-tetrahydro-7,12-dimethylbenz-[a]anthracene.

INTRODUCTION

Covalent binding of chemicals to cellular macromolecules, DNA, RNA and protein, is the first critical event in the multistage process leading to tumor formation.[1,2] The critical intermediates responsible for reacting with cellular macromolecules have a common unifying feature, their electrophilic character.[1,2] If the covalent bond of a polycyclic aromatic hydrocarbon (PAH) to cellular macromolecules is critical in initiating the tumor process, and little doubt exists about this assertion, the biological chemistry of PAH must fit with their carcinogenic activity. This means that the carcinogenicity of PAH must be coherent with certain common chemical properties of these compounds and the catalytic properties of the enzymes responsible for metabolic activation. This basic concept represents the essence for understanding mechanisms of carcinogenesis of PAH.

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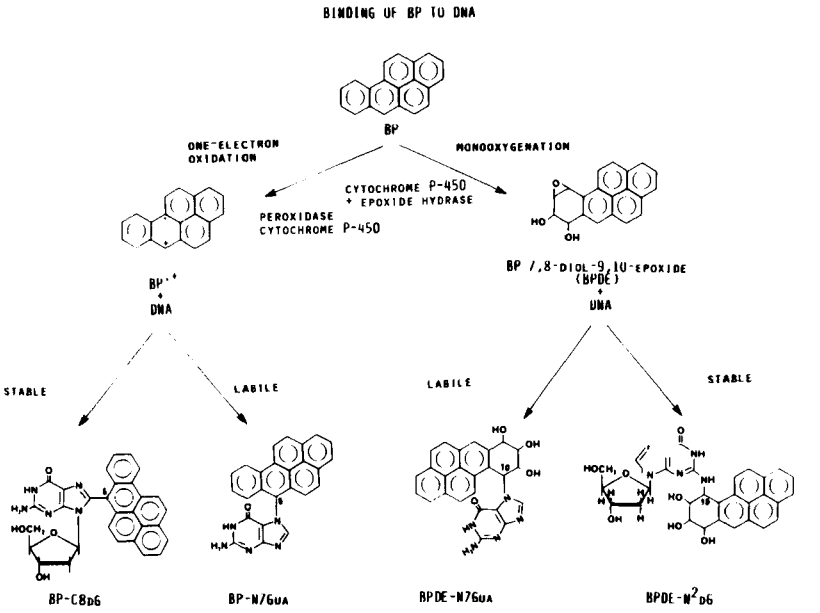

FIGURE 1. Benzo[a]pyrene-DNA adducts formed by one-electron oxidation and monooxygenation.

Most PAH are not carcinogenic per se, but need metabolic activation, which can be understood in terms of two main pathways: one-electron oxidation to yield reactive intermediate radical cations[3,4] and monooxygenation to produce bay-region diol epoxides.[5,6]

For example, one-electron oxidation of benzo[a]pyrene (BP, Figure 1) produces a radical cation with charge localized mainly at C-6. Direct reaction of this intermediate with nucleophilic groups of cellular macromolecules leads to tumor initiation. A pathway arising from monooxygenation of BP by cytochrome P-450 consists of initial formation of BP, 7,8-epoxide, followed by hydrolysis by epoxide hydrase to yield BP 7,8-dihydrodiol. A further monooxygenation produces BP 7,8-dihydrodiol-9,10-epoxide (Figure 1), that can covalently bind to cellular macromolecules.

CHEMISTRY OF PAH RADICAL CATIONS

One-electron oxidation of PAH is a coherent mechanism of activation that can account for the carcinogenic activity of the most potent PAH. This mechanism produces radical cations by removal of a π electron. Radical cations of unsubstituted and methylsubstituded PAH have been generated by iodine[7,8] and manganic acetate[9,10] oxidations with subsequent binding to a nucleophile. This is outlined in Figure 2.

Removal of one electron from the π-system generates a radical cation in which the positive charge can be localized mainly at an unsubstituted carbon atom (Path 1) or adjacent to the methyl group (Path 2). In the former case (Path 1), nucleophilic attack at the position of highest charge density generates an intermediate radical that is then further oxidized to an arenium ion with loss of a proton to complete the substitution

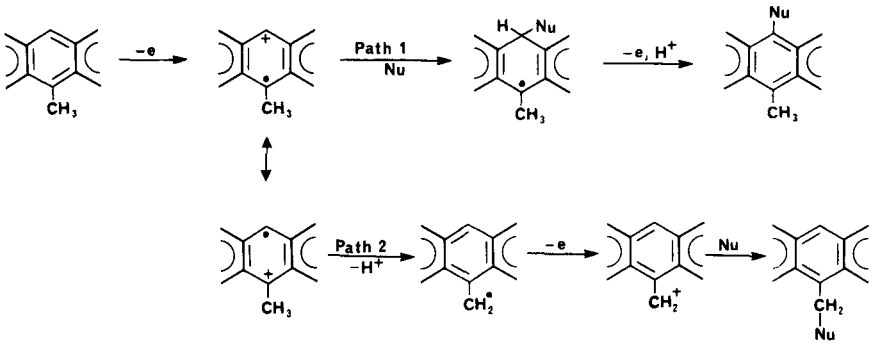

FIGURE 2. Nucleophilic trapping in radical cations of unsubstituted and methyl-substituted PAH.

reaction. When the charge is localized adjacent ot the methyl group, the first critical step is loss of a methyl proton to yield a benzylic radical (Path 2). This intermediate is rapidly oxidized to a benzylic carbenium ion with subsequent trapping by a nucleophile.

The reason we have postulated that one-electron oxidation plays an important role in the activation of PAH derives from certain characteristics of the radical cation chemistry of these compounds common to the most potent PAH, which include BP, 7,12-dimethylbenz[a]anthracene (DMBA) and 3-methylcholanthrene (MC). Three main features common to these PAH are: 1) a relatively low ionization potential (IP),

TABLE I
PHS- and HRP-catalyzed binding of PAH to DNA

Compound	IP (ev)	DNA-bound PAH (μmol/mol DNA-P)	
		PHS	HRP
Phenanthrene	8.19		3.8 ± 0.8
5-Methylchrysene	7.7	10.5 ± 1.7	1.4 ± 0.5
Benzo[e]pyrene	7.62	6.0 ± 0.5	5.1 ± 0.9
Diabenz[a,h]anthracene	7.57	13.0 ± 1.7	4.3 ± 1.0
Benz[a]anthracene	7.54	10.8 ± 1.6	4.0 ± 0.5
Pyrene	7.50	6.3 ± 1.8	2.8 ± 1.4
Anthracene	7.43	16.7 ± 1.6	8.8 ± 1.6
7-Methylbenz[a]anthracene	7.37	17.2 ± 1.3	5.6 ± 0.6
Dibenzo[a,e]pyrene	7.35	7.8 ± 0.7	
Dibenzo[a,l]pyrene	7.26	54.6 ± 3.1	
Benzo[a]pyrene	7.23	307 ± 21.4	89.2 ± 5.6
7,12-Dimethylbenz[a]anthracene	7.22	189 ± 10.3	63.9 ± 4.6
Dibenzo[a,i]pyrene	7.20	46.9 ± 5.4	
3-Methylcholanthrene	7.12	91.2 ± 9.5	60.6 ± 4.1
6-Methylbenzo[a]pyrene	7.08	89 ± 6.0	39.8 ± 5.3
Dibenzo[a,h]pyrene	6.97	111.1 ± 8.6	
Anthanthrene	6.96	33.1 ± 1.24	27.0 ± 7.1
6,12-Dimethylanthanthrene	6.68		62.0 ± 13

which allows metabolic removal of one electron with formation of a relatively stable radical cation, 2) charge localization in the PAH radical cation that renders this intermediate specifically and efficiently reactive toward nucleophiles,[3,4] and 3) optimal spatial configuration that presumably facilitates formation of appropriate physical complexes with macromolecules and favors metabolic activation.[11]

Only PAH with relatively low IP (below ca. 7.35eV) can be effectively activated by one-electron oxidation in biological systems (see below "Peroxidase catalyzed binding of PAH to DNA" and Table I).[12,13] However, low IP is a necessary, but not sufficient, factor for determining carcinogenic activity by one-electron oxidation. A second critical factor is appreciable charge localization in the radical cation, which gives it sufficient reactivity to bind with cellular nucleophiles.[3,4,7-10] Finally, several examples illustrate the requirement for a certain geometry to elicit carcinogenic activity. In general, optimum activity is found in PAH containing four to seven rings. An example of more stringent requirements concerning geometric features is the presence of the angular ring in the benz[a]anthracene series, which is necessary for eliciting carcinogenicity regardless of the ring being aromatic or aliphatic.[11,14] The angular ring may be necessary for achieving suitable intercalculation with DNA, which has been suggested to be a prerequisite for covalent binding to DNA.[15,16]

PEROXIDASE CATALYZED BINDING OF PAH TO DNA

Mammalian peroxidases, including PHS,[17-22] and cytochrome P-450[23-29] catalyze one-electron oxidation. The structures of PB-DNA adducts formed enzymatically can be used to identify the mechanism of activation (Figure 1). With one-electron oxidation, the BP radical cation is formed and binding occurs between C-6 and either the nucleophilic C-8 or N-7 of guanine.[30,31] Instead, by monooxygenation, a bond is formed between the 2-amino of guanine and C-10 of BP, after the 7,8,9,10-ring has been saturated and hydroxylated.[5,6]

A series of PAH with IP ranging from 8.19 eV to 6.8 eV was examined for their ability to bind to DNA in vitro via a horseradish peroxidase (HRP) or prostaglandin H synthase (PHS) catalyzed reaction.[12,13] A relatively low IP was required for significant binding of PAH to DNA to occur (Table I). PAH with IP below ca. 7.35 eV were active in the binding reaction, whereas those with IP at or above 7.35 eV exhibited insignificant levels of binding. These results suggest that the HRP and PHS mediated binding of PAH to DNA occurs by one-electron oxidation. These results are of particular interest because most of the strongly carcinogenic PAH have relatively low IP, and one-electron oxidation could play a role in their activation.

To study BP-DNA adducts formed by peroxidases, BP was bound to DNA in the HRP/H_2O_2 system.[30] At the end of the enzymatic reaction, the DNA was precipitated with ethanol and the ethanol-water supernatant was analyzed by high pressure liquid chromatography (HPLC) to identify BP-DNA adduct(s) lost from the DNA by depurination. The major depurinated adduct found contained BP bound at C-6 to the N-7 of guanine (Figure 1, BP-N7Gua). The DNA itself was enzymatically digested to deoxyribonucleosides to analyze the adducts stable in DNA. The major stable adduct observed by HPLC analysis contains BP bound at C-6 to the C-8 of deoxyguanoisine (Figure 1, BP-C8dG). Overall, when PB is bound to DNA by HRP, more than three times as much BP-N7Gua is isolated as the total amount of BP-DNA adducts stable in the DNA. Thus, BP-N7Gua is the predominant adduct formed when BP is bound to DNA by HRP.

Cytochrome P-450 catalyzed binding of PAH to DNA

To investigate whether cytochrome P-450 can catalyze the binding of substrates to DNA by one-electron oxidation, the ability of both liver microsomes and nuclei from uninduced and MC-induced male Wistar rats to catalyze binding of BP to DNA, including formation of the depurinated BP-N7Gua adduct, was investigated.[31] BP was bound by microsomal cytochrome P-450 to exogenous DNA, the DNA was precipitated with ethanol, and the ethanol-water supernatant was analyzed by HPLC for the BP-N7Gua adduct. Similarly BP was bound to endogenous DNA in isolated MC-induced liver nuclei, the DNA was precipitated with ethanol, and the supernatant was analyzed by HPLC for the BP-N7Gua adduct. Sufficient BP-N7Gua was formed in the binding catalyzed by MC-induced or uninduced microsomes to isolate it and confirm its identity by fast atom bombardment tandem mass spectrometry.[31] More BP-N7Gua was formed by microsomes than the total amount of adducts stable in the DNA. When BP was bound to DNA in nuclei, the amount of BP-N7Gua isolated was nine times the total amount of stable BP-DNA adducts. The identity of the BP-N7Gua adduct in nuclei was confirmed by fluorescence line narrowed spectrometry.[32]

When the specific cytochrome P-450 inhibitor 2-(4,6-dichloro-*o*-biphenyloxy)-ethylamine hydrobromide (DPEA) was added to the incubation mixtures (molar ration 10:1 with respect to BP), formation of BP-N7Gua was eliminated. This result demonstrates that the reaction is catalyzed by cytochrome P-450.

A plausible hypothesis for the formation of this adduct by this enzyme involves an electron transfer from BP that would be more likely to occur at an exposed heme edge of the enzyme (Figure 3). The activated cytochrome P-450 intermediate, $[Fe^{IV}]^{\cdot +}$-oxo group (Figure 3) is formed via two pathways, one supported by NADPH and one by cumene hydroperoxide (CuOOH).[31] Formation of the BP-N7Gua adduct would then occur by transfer of one-electron from BP to the intermediate $[Fe^{IV}]^{\cdot +}$-oxo group with formation of the BP radical cation and, concomitantly, of the Fe^{IV}-oxo group. Attack of the nucleophilic N-7 of the guanine moiety in DNA at the C-6 position of the BP radical cation would follow. The resulting covalent bond would destabilize the glycosidic link and BP-N7Gua adduct would depurinate from DNA. Further reduction of the Fe^{IV}-oxo group to Fe^{III} would oxidize the BP moiety to an arenium ion, which by loss of a proton, would produce the BP-N7Gua adduct.

This study represents the first demonstration that cytochrome P-450 catalyzes covalent binding of substrates to DNA by one-electron oxidation.

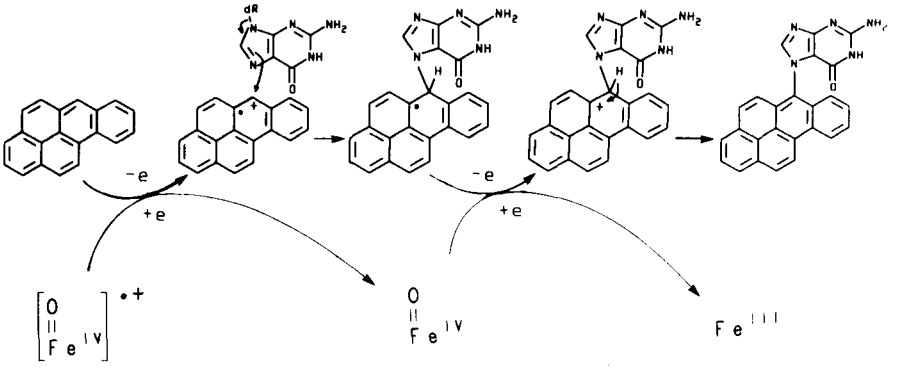

FIGURE 3. Formation of the BP-N7Gua adduct via a cytochrome P-450 catalyzed electron transfer reaction.

COMPARATIVE CARCINOGENICITY IN MOUSE SKIN AND RAT MAMMARY GLAND

PAH with high IP were tested in the two target tissues mouse skin and rat mammary gland because they were not expected to be activated by one-electron oxidation, whereas PAH with low IP (below ca. 7.35 eV) were tested because they presumably could be activated by both one-electron oxidation and monooxygenation.[14,33-35] In addition PAH with fluoro substitution at positions which block formation of diol epoxides by monooxygenation were also tested.

The compounds in Table II with IP of 7.35 eV and above were carcinogenic in mouse skin and noncarcinogenic in the mammary gland. Compound with IP below ca. 7.35 eV were generally carcinogenic in both target organs. Of the fluorinated derivatives blocking the bay-region diol epoxide (Figure 4), only 9-fluoroBP was inactive in both target tissues. BP 7,8-dihydrodiol (Figure 4), which is the proximate carcinogenic metabolite in the formation of BP diol epoxide, showed similar activity

TABLE II
Comparative carcinogenicity of PAH in mouse skin and rat mammary gland

Compound	IP (eV)[a]	Carcinogenicity in	
		Mouse skin[c]	Rat mammary gland
5-Methylchrysene	7.7	+++	−−
Dibenz[a,h]anthracene	7.57	+++	−−
Benz[a]anthracene	7.54	±	−−
7-Methylbenz[a]anthracene	7.37	+++	±
Dibenzo[a,e]pyrene	7.35	±	−−
Dibenzo[a,l]pyrene	7.26	+++++	+++++
Benzo[a]pyrene	7.23	++++	+++
Benzo[a]pyrene 7,8-dihydrodiol		++++	+++
7-Fluorobenzo[a]pyrene		++	±
8-Fluorobenzo[a]pyrene		+	+
9-Fluorobenzo[a]pyrene		−−	−−
10-Fluorobenzo[a]pyrene		−−	+
7,12-Dimethylbenz[a]anthracene	7.22	+++++	+++++
1,2,3,4-Tetrahydro-7,12-dimethylbenz[a]anthracene		+++	+++++
2-Fluoro-7,12-dimethylbenz[a]anthracene		+	−−
4-Fluoro-7,12-dimethylbenz[a]anthracene		+	++
Dibenzo[a,i]pyrene	7.20	+++	+++
10-Fluoro-3-methylcholanthrene	7.17	N.T.	++
8-Fluoro-3-methylcholanthrene	7.14	N.T.	++
3-Methylcholanthrene	7.12	++++	++++
6-Methylbenzo[a]pyrene	7.08	+++	++
Dibenzo[a,h]pyrene	6.97	++++	++
Anthanthrene	6.96	±	−−

[a]Determined from the absorption maximum of the charge-transfer complex of each compound with chloranil (12).

[b]Extremely active +++++; very active, ++++; active, +++; moderately active, ++; weakly active, +; very weakly actve, ±; inactive −−; not tested, N.T.

[c]Evaluation of mouse skin carcinogenicity is based on the results from repeated application and/or initiation-promotion obtained in our laboratory.

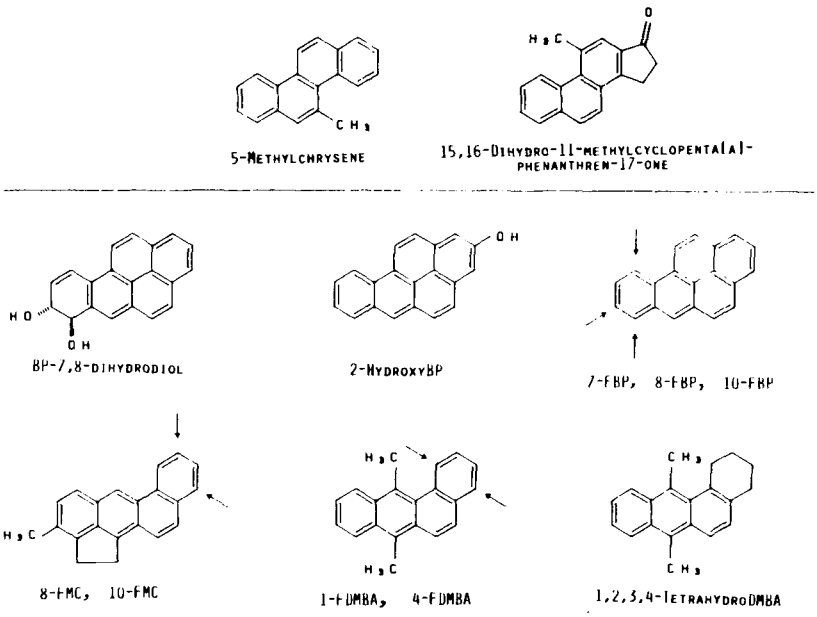

FIGURE 4. PAH tested for carcinogenic activity in which the diol epoxide pathway of activation fits or does not fit.

to the parent compound in mouse skin but was much less active than BP in rat mammary gland. 1,2,3,4-TetrahydroDMBA (Figure 4), in which formation of the bay-region diol epoxide is impossible, due to saturation of the angular benzo ring, was extremely active as a mammary carcinogen and relatively potent in mouse skin.

The compounds with high IP which were carcinogenic in mouse skin are activated by monooxygenation, because presumably one-electron oxidation of these PAH cannot occur or cannot form an intermediate specifically reactive toward nucleophiles in biological systems. The results from these experiments in mouse skin and rat mammary gland are in general consistent with activation in rat mammary gland by one-electron oxidation, whereas multiple mechanisms of activation appear to be involved in mouse skin.

Numerous experimental results concerning activation of PAH to bay-region diol epoxides have led the scientific community to think that this mechanism is almost exclusive in PAH carcinogenesin.[5,6] The bay-region theory has evolved from studies of metabolism, binding, mutagenicity and carcinogenicity of BP and has been extended to other PAH.[5,6,36] Experimental evidence demonstrates indeed that the carcinogen 15,16-dihydro-11-methylcylopenta[a]-phenanthren-17-one (Figure 4) is activated by formation of the bay-region diol epoxide.[37] Similarly, 5-methylchrysene (Figure 4) is activated by formation of the diol epoxide of the bay-region in which the methyl group is located.[38–40] Metabolism and tumorigenicity studies of fluorinated derivatives of 5-methylchrysene in which formation of the critical diol epoxide is blocked or not blocked are consistent with activation by this mechanism.[38,39]

The results of many carcinogenicity experiments with the most potent PAH do not support an important role for the diol epoxide pathway of activation. Among the

monohydroxyBPs, 2-OHBP (Figure 4) is the only potent carcinogen. It is as carcinogenic as BP by repeated application and initiation-promotion on mouse skin,[41,42] although formation of its 7,8-dihydrodiol has never been observed.[43] Initiation-promotion[44,45] and repeated application[46,47] in mouse skin by BP and BP 7,8-dihydrodiol, which is the proximate carcinogen in the diol epoxide pathway of activation, show that the two compounds have similar activity. Furthermore the (-)BP 7,8-dihydrodiol enantiomer, which is the proximate metabolite leading to the most active BP 7,8-diol-9, 10-epoxide, is not significantly more potent than BP in mouse skin,[48,49] but is more active than BP in inducing lung tumors in newborn mice.[50] When the carcinogenicity of BP and racemic BP 7,8-dihydrodiol were compared by direct application in rat mammary gland in an experiment terminated 20 weeks after treatment, BP was carcinogenic, whereas BP 7,8-dihydrodiol showed no activity.[33] In a subsequent experiment, continued for 50 weeks, BP 7,8-dihydrodiol showed some activity, but far less than the parent compound.[34] Tumor-initiating activity in mice and/or carcinogenicity in rat mammary gland were observed for BP, 7-FBP, 8-FBP and 10-FBP, whereas 9-FBP was inactive in both species.[34] In view of the fact that the metabolism of 8- and 9-FBP produces the same metabolites as BP, with the exception of the 7,8-dihydrodiol form 8-FBP and the 9,10-dihydrodiol from 9-FBP,[51] these experiments clearly indicate that the bay-region diol epoxide is not involved in the carcinogenicity of these compounds. Furthermore, the presence of 3-OHBP, which effectively inhibits the mutagenic and tumor-initiating activity of BP diol epoxide, does not significantly reduce the tumor-initiating activity of BP in mouse skin.[52]

The diol epoxide mechanism cannot play a role in the carcinogenic activity of 1-FDMBA and 4-FDMBA, in which formation of the diol epoxide is blocked by fluoro-substitution.[53–55] Similarly, the potent tumor-initiator and carcinogen 1,2,3,4-tetrahydroDMBA must be activated by another mechanism, because the angular benzo ring, in which activation to the diol epoxide would occur, is saturated (Figure 4).[35] The same rationale applies for 2-F-, 3-F- and 4-F-7-methylbenz[a]anthracene[53,56,57] and 8-FMC and 10-FMC.[34]

In conclusion, the pathway of activation involving formation of the ultimate carcinogenic diol epoxide is not the exclusive mechanism of activation of PAH, as has been reported for the last decade, but only one of the possible mechanisms that can play a role in the activation of some PAH. The various chemical, biochemical and biological experiments we have described above provide evidence that radical cations play an important role in the carcinogenic activity of these compounds. Further studies will quantitatively assess the relevance of this mechanism in the carcinogenicity of PAH.

Acknowledgements

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