Activated Metabolites of Carcinogenic Hydrocarbons

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Polycyclic aromatic hydrocarbons (polyarenes) are widespread environmental pollutants.[1-3] While they are believed to arise predominantly through inefficient combustion of fossil fuels and other organic matter,[1-3] there is also evidence for their biosynthesis by bacteria and higher plants.[4] Among the polyarenes to which the human population is chronically exposed are a number of highly potent carcinogens, including benzo[*a*]pyrene (BP) and dibenz[*a*,*h*]anthracene.[3] It is likely, therefore, that polyarenes may be responsible for a significant percentage of human cancer.

Identification of benzo[*a*]pyrene as the first carcinogenic constituent of coal tar of known molecular structure by Kenneway and associates in 1933[5] stimulated a great surge of interest in synthetic and theoretical polyarene chemistry. These efforts led to correlations between structure and activity which became the basis for various theories of carcinogenicity. Most prominent of these was the Pullman electronic theory[6] which used valence bond and molecular orbital (MO) methods to correlate electron densities in various molecular regions with bioactivity. However, numerous exceptions to theoretical predictions were found, and the implicit assumption that hydrocarbons are direct-acting carcinogens has gradually had to be abandoned.

The first strong evidence for the role of metabolism was the observation by Brookes and Lawley that when tritium-labeled hydrocarbons were applied to the backs of mice[7] or were incubated with mouse embryo cells,[8] a small fraction of the administered dose became covalently bound to the cellular macromolecules. The extent of binding to DNA and RNA, but not to proteins, correlated approximately with carcinogenic potency. These findings were confirmed and extended by subsequent investigators who demonstrated that binding requires metabolic activation by the P-450 microsomal enzymes.[9,10] Elucidation of the structures of these active metabolites and the metabolic pathways leading to their formation is a major triumph of modern carcinogenesis research. This story and its implications for the mechanism of cancer induction are the topic of this Account. While spatial limitations prevent citation of every publication in this active field, an effort has been made to present a balanced view of major contributions.

Metabolism

Metabolism of polycyclic hydrocarbons occurs principally on the microsomes of the endoplasmic reticulum catalyzed by the mixed-function oxygenase enzymes.[11-13] The primary metabolites are now generally accepted to be arene oxides (Figure 1).[11,14,15] These relatively unstable intermediates undergo hydration catalyzed by epoxide hydrase to *trans*-dihydro diols, rearrange spontaneously to phenols, and undergo addition of glutathione catalyzed by glutathione-*S*-transferase. The phenols and dihydro diols are excreted principally as their more water soluble glucuronic and sulfate esters,[11,16] while the glutathione conjugates undergo enzymatic degradation to mercapturic acid derivatives.[11]

The ratios of phenolic and dihydro diol products in each molecular region are dependent upon the relative stabilities of the respective arene oxide intermediates shown by Fu et al. to be predictable by MO theory.[17] In the case of benzo[*a*]pyrene, the unstable 1,2- and 2,3-oxides (**1**, **2**) rearrange spontaneously to exclusively phenolic products, the 7,8- and 9,10-oxides (**3**, **4**) which are of intermediate stability furnish both phenolic and dihydrodiol metabolites, while the relatively stable 4,5-oxide (**5**) fails to rearrange, undergoing instead enzymatic hydration to the 4,5-dihydro diol. Rearrangement of arene oxides generally occurs preferentially in one direction; e.g., isomerization of **2** and **3** furnishes only 3-HO- and 7-HO-BP, respectively, accurately predictable by MO methods.[17] The ratio of metabolites also varies with tissue, species, and whether liver microsomes or intact cells are employed for metabolic activation.[18]

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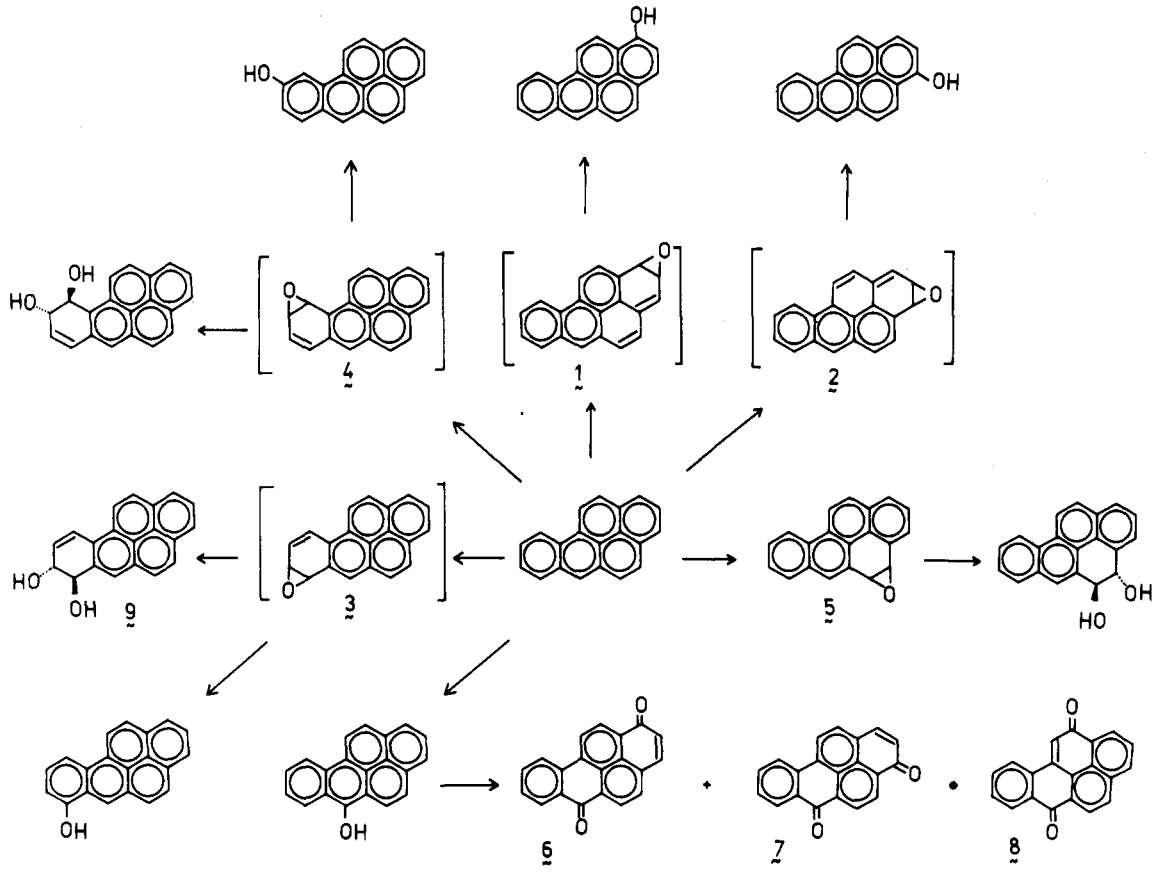

Figure 1. Metabolic pathways of BP (glutathione conjugates omitted for simplicity).

Enzymatic oxidation takes place regioselectively on only a small fraction of the available ring positions which are apparently predictable by quantum mechanical calculations.[19] However, steric and other factors may conceivably also play a role.

Further enzymatic degradation of primary metabolites also takes place. The 1,6-, 3,6-, and 6,12-quinones (6–8), which represent a major fraction of the metabolites of BP in some cells (e.g., human liver and lumphocytes),[20] have been shown by Ts'o to arise through autoxidation of the related phenols by a one-electron mechanism.[21] Other metabolic pathways include the enzymatic dehydrogenation of dihydro diols to catechols,[11,22] deoxygenation of arene oxides to the parent hydrocarbons,[23] and the reduction of arene oxides to dihydro alcohols.[11] The metabolic conversion of dihydro diols to diol epoxides will be discussed in later paragraphs.

K-Region Arene Oxides

Early studies focused on the arene oxides, assumed to be the most likely reactive intermediates capable of alkylating cellular macromolecules. Our initial goal was to devise a feasible synthesis of the unknown "K-region" arene oxides of the potent carcinogens BP and 7,12-dimethylbenz[*a*]anthracene (DMBA), i.e., BP-4,5-oxide

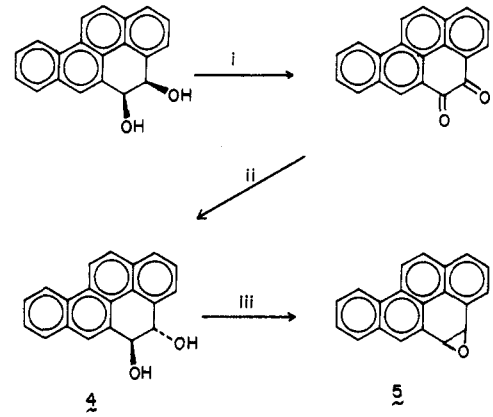

Figure 2. Synthesis of "K-region" arene oxides. Reagents: (i) pyridine–SO_3 in dimethyl sulfoxide, (ii) $LiAlH_4$, (iii) $(CH_3O)_2C\text{-}HN(CH_3)_2$.

(5) and DMBA-5,6-oxide. The "K-region" terminology derives from the Pullman theory,[6] according to which carcinogenic polyarenes were considered to be distinguished by an electron-rich bond, such as the 4,5-bond of BP, which is termed the "K-region" and excision of which leaves a fully aromatic polyarene ring system. Synthesis of **5** was accomplished[24] from BP via the *cis*-dihydro diol, the quinone, and the *trans*-dihydro diol (Figure 2). BP-4,5-oxide proved sufficiently stable to permit its isolation in pure state. This synthetic approach has proven quite general and has been employed to synthesize a wide range of arene oxides including DMBA-5,6-oxide.[25] A number of alternative syntheses

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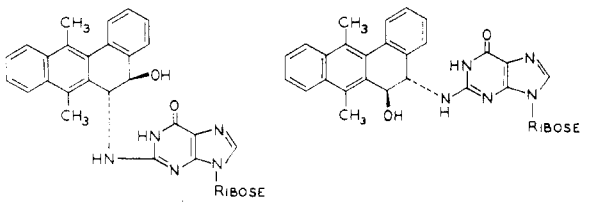

Figure 3. Structures of the guanosine adducts formed from the in vitro reaction of DMBA-5,6-oxide with poly(G); only one of each pair of diastereomers is shown.[35,36]

have also been developed.[26-29] The structures of BP-4,5-oxide and DMBA-5,6-oxide have been determined by X-ray crystallographic analysis.[30]

The K-oxides exhibit significant mutagenic activity in both bacterial and mammalian cells, while the parent hydrocarbons are generally inactive without microsomal activation.[11,31] Observed mutagenicities correlate approximately with the carcinogenicities of the parent compounds. On the other hand, carcinogenicity tests show the arene oxides to be generally less active than the parent hydrocarbons.[11,32] The 7,8- and 9,10-oxides of BP (**3** and **4**), syntheses of which have also been described,[33] proved much weaker mutagens than **5** in both bacterial and mammalian cells.[34] While activated metabolites might be expected to exhibit greater activity than their precursors, lesser activity may be the consequence of decomposition or secondary reaction prior to reaching the critical cellular target.

The strongest evidence against arene oxides as ultimate carcinogenic metabolites comes from nucleic acid binding studies. BP-4,5-oxide was allowed to react with DNA in aqueous solution, and the products were degraded enzymatically to the nucleoside level and separated on LH-20 Sephadex.[35] The elution volumes of the resulting BP–nucleoside adducts failed to correspond to those of BP–DNA adducts isolated from mouse embryo cells treated with ^{3}H-BP. Similar lack of correspondence was observed in analogous experiments with other oxides.[36,37]

The structures of the principal nucleoside adducts formed through covalent interaction of DMBA-5,6-oxide with DNA were completely elucidated by Fourier transform NMR and mass spectra and other evidence and shown to be two pairs of diastereomeric trans adducts with covalent bonding between the 2-NH_2 group of guanosine and the 5- and 6-positions of DMBA (Figure 3).[37,38]

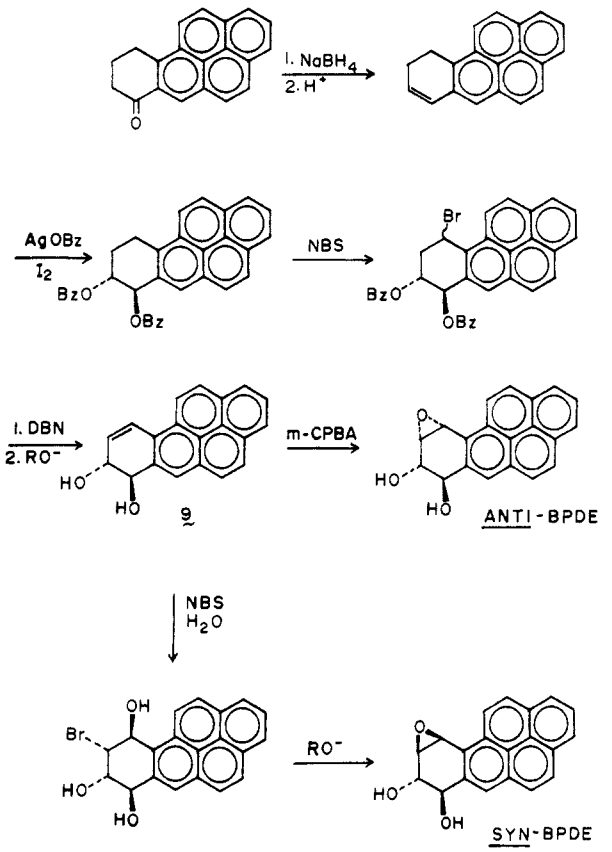

Figure 4. Synthesis of the isomeric diol epoxide metabolites of BP. Reagents: NBS = *N*-bromosuccinimide, DBN = 1,5-diazabicyclo[4.3.0]non-5-ene, m-CPBA, *m*-chloro-perbenzoic acid.

Diol Epoxide Metabolites of BP

Attention then shifted to other reactive metabolites. A vital clue was the observation of Borgen et al.[39] that a metabolite of BP tentatively identified as the 7,8-dihydro diol (**9**) was metabolized by rat liver microsomes to an intermediate which bound relatively efficiently to DNA. Sims proposed a diol epoxide structure (stereochemistry unspecified) for this metabolite.[40]

Synthesis of the isomeric diol epoxides of BP was achieved essentially simultaneously by Beland and Harvey[41] and Yagi et al.[42] via the sequence in Figure 4. Reduction and dehydration of 7-oxo-7,8,9,10-tetrahydro-BP gave 9,10-dihydro-BP. Prevost reaction with silver benzoate and I_2 followed by bromination, dehydrobromination, and methanolysis furnished the trans-dihydro diol **9**.[41-43] The bromination–dehydrobromination steps, which afforded erratic yields, were subsequently replaced by direct dehydrogenation with DDQ.[44,45] Epoxidation of **9** furnished stereospecifically the anti isomeric diol epoxide (*anti*-BPDE) in which the epoxide oxygen and the benzylic hydroxyl groups are

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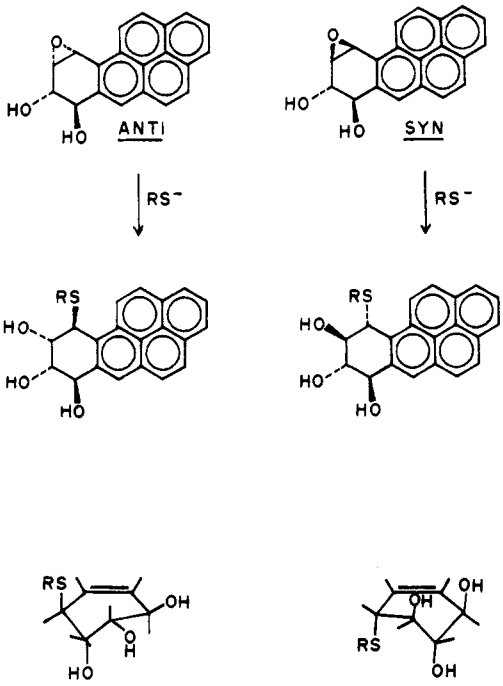

Figure 5. Reactions of *anti*- and *syn*-BPDE with Me_3CS^-.

on opposite faces of the molecule.[41,45] The syn isomer (*syn*-BPDE) which has these groups on the same face was synthesized by base-catalyzed cyclization of the bromohydrin obtained from **9**.[41,42,45]

The stereochemical assignments were established by chemical and NMR spectral evidence.[41,45] Reactions of both isomers with Me_3CS^- afforded the respective products of trans-stereospecific ring opening, effectively locking the conformations by the bulky *tert*-butyl group (Figure 5). The pair of cis vicinal hydroxyl groups in the product from the anti isomer were readily distinguished by their NMR coupling constants and by the fact that only this isomer formed an acetonide or gave a precipitate with potassium triacetylosmate. These isomer assignments were confirmed by subsequent aqueous solvolysis studies.[46,47] Resolution of the racemic (±)-7,8-dihydro diols of BP was achieved by HPLC separation of their diastereomeric (−)-menthoxyacetate and MTPA esters.[48]

The diol epoxides exhibit exceptional potency as mutagens in both bacterial and mammalian cells.[49-51] *anti*-BPDE shows generally greater activity than *syn*-BPDE in most tests; however, in strains of *Salmonella typhimurium* the syn isomer is more mutagenic.[51] *anti*-BPDE also shows greater activity in the induction of malignant transformation of mouse fibroblasts,[52] in the inhibition of replication of bacterial viruses,[53,54] and

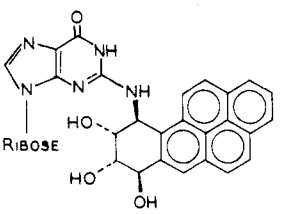

Figure 6. Structure and absolute configuration of the adduct between guanosine and *anti*-BPDE formed by reaction of the latter with DNA in human and bovine tissue.[60,61]

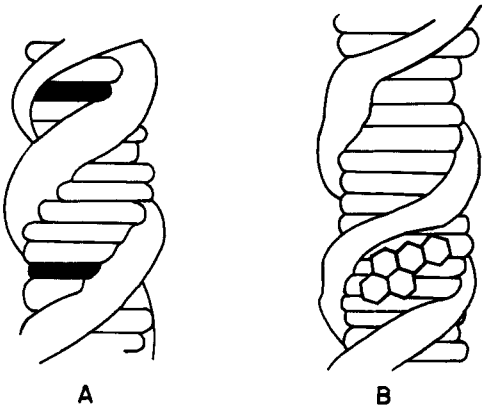

Figure 7. Schematic representation of *anti*-BPDE (A) intercalated between the base pairs of the DNA helix or (B) covalently bound to DNA and residing in a minor groove.

as a carcinogen on mouse skin.[55] However, (±)-*anti*-BPDE was only a weak carcinogen relative to BP on mouse skin, while *syn*-BPDE proved inactive under the test conditions.[56] Comparison of the tumorigenicities in newborn mouse lung of the isomers of BPDE revealed (+)-*anti*-BPDE to be considerably more tumorigenic than (−)-*anti*-BPDE, BP, and (−)- or (+)-*syn*-BPDE.[57] The (+) isomer of *anti*-BPDE was also more mutagenic than (+)-*anti*-BPDE in Chinese hamster V79 cells.[58]

The most convincing evidence for the biological importance of the diol epoxide metabolites comes from nucleic acid binding studies. The major product of reaction of *anti*-BPDE with DNA following degradation to the nucleoside level was found to be identical with the major product of metabolism and binding of BP to DNA and RNA in rodent, bovine, and human cells.[59] This adduct was shown by Fourier transform NMR and high-resolution mass spectral analysis to be a guanosine derivative covalently linked between the 2-NH_2 position and the 10-position of BP (Figure 6).[60] The absolute

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configuration of the adduct formed in cells was assigned by the exciton chirality dichroism method and shown to be derived from (+)-*anti*-BPDE.[61] Minor nucleic acid-bound products arising from reaction of *anti*-BPDE on the 7-N position of guanosine[62] and the 6-NH_2 group of adenosine[63] have subsequently also been characterized. In other studies minor metabolic products arising from *syn*-BPDE have been detected,[56,64,65] and evidence has been obtained for alkylation of ribose phosphate groups by *anti*-BPDE.[62-68]

Metabolism of BP by rat liver microsomes is shown to afford stereoselectively the (−)-7,8-dihydro diol arising from the (+)-7,8-oxide.[69] Enzymatic hydration of (+)-and (−)-BP-7,8-oxide by epoxide hydrase takes place stereospecifically at C-8 to furnish (−)-and (+)-BP-7,8-dihydro diol, respectively. Subsequent oxidation of (−)-BP 7,8-dihydro diol by the mixed function oxygenases also takes place stereoselectively to afford mainly (+)-*anti*-BPDE and a small amount of (−)-*syn*-BPDE.[69] The isomeric diol epoxides of BP are relatively unstable in aqueous media, undergoing facile hydrolysis to the related tetraols.[46,47,69,70] However, both *anti*- and *syn*-BPDE proved resistant to epoxide hydrase catalyzed hydration,[70] a factor potentially significant with respect to their carcinogenic activity.

UV fluorescence and electric linear dichroism studies of the BP–nucleic acid adducts indicate the hydrocarbon moiety to be bound externally in the minor groove approximately parallel to the DNA helix (Figure 7).[71] It appears likely that the diol epoxides intercalate prior to covalent binding to DNA.[71,72] A complicating factor is the finding that DNA catalyzes the hydrolysis of *anti*-BPDE, and the resulting tetraols also intercalate with DNA.[72] Consequences of binding of *anti*-BPDE to nucleic acids include formation of localized regions of denaturation,[65] the loss of template function of DNA in both transcription and translation by RNA polymerase,[73,74] the inhibition of replication of $\phi X174$ and

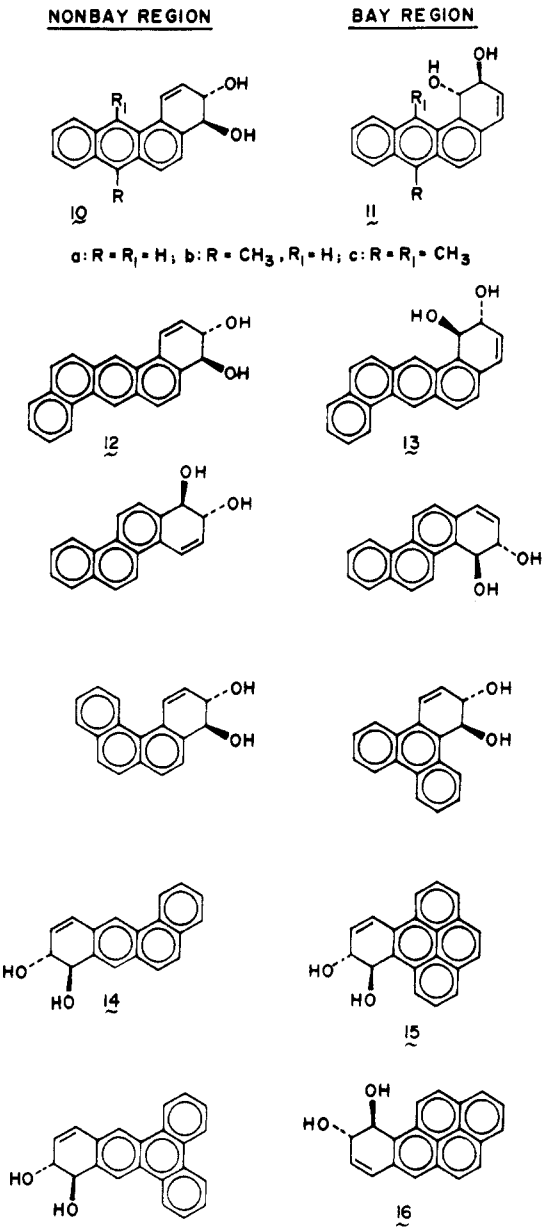

Figure 8. Examples of nonbay and bay region dihydrodiols. Epoxidation of the former afforded stereospecifically the anti diol epoxide isomers, while epoxidation of the latter gave mixtures of syn and anti isomers.

SV40 DNA viruses,[53,54] and the unwinding of supercoiled SV40 DNA.[75]

(+)-*anti*-BPDE is established on the basis of these findings, confirmed in numerous laboratories, as the first active metabolite of proven molecular structure of a carcinogenic hydrocarbon. This is consistent with both the Miller hypothesis that the active forms of carcinogens are reactive electrophiles[76] and the somatic mutation theory, an implicit assumption in most carcinogenesis research.

Diol Epoxide Metabolites of Other Polyarenes

Identification of *anti*-BPDE stimulated an outpouring of research directed toward extending these findings

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to other polyarenes and elucidating the molecular biological consequences of alkylation of DNA by diol epoxide metabolites. Syntheses of the potential oxidized metabolites of numerous structurally representative polyarenes, both carcinogenic and noncarcinogenic, have already been accomplished.[45,71-91] These include the isomeric *trans*-dihydro diols of anthracene,[77,78] phenanthrene,[77,78] triphenylene,[79] chrysene,[80] benz[*a*]anthracene (BA),[44,45,81-83] 7-methylbenz[*a*]anthracene (7-MBA),[44,45,84] DMBA,[77,85,86] dibenz[*a,h*]anthracene (DBA),[87] dibenz[*a,c*]anthracene,[88] benzo[*e*]pyrene,[79] benzo[*c*]phenanthrene,[89] dibenzo[*a,i*]pyrene,[90] dibenzo[*a,h*]pyrene,[90] and 7-methyl-BP.[91]

Epoxidation of these dihydro diols with *m*-chloroperbenzoic acid afforded the corresponding anti isomeric diol epoxides stereospecifically, except in cases where the dihydro diol function was located in a sterically crowded molecular region such as a bay region (Figure 8).[92] In these instances, both syn and anti isomers were formed, and for the 1,2-dihydro diols of 7-MBA (**11b**) and DBA (**12**) the syn isomers were predominant.[93] This difference is stereoselectivity appears to be a consequence of conformational differences. NMR and X-ray crystallographic evidence indicates the existence of the bay-region isomers (e.g., **11a**) exclusively in the pseudoaxial conformation both in solution and in the solid state; the less hindered non-bay-region dihydro diols adopt preferentially the alternative diequatorial conformation.[94] Evidently the cis-directing effect of allylic hydroxyl groups on the stereoselectivity of attack by peroxy acids[41,42,45,47] is effective only when this group is pseudoequatorial. Stereoselective syn epoxidation may be a consequence of direction of epoxidation by the pseudoaxial benzylic hydroxyl groups.[93]

Mutagenicity studies show significant activities for the majority of the dihydro diols tested in both bacterial and mammalian cells.[95] In general, maximal activities are seen for the isomeric benzo ring dihydro diols having an olefinic bond in a bay region (e.g., **9**, **10**, **12**). Comparison of the tumor-initiating activities of the various dihydro diols and their diol epoxide derivatives reveals a similar relationship.[96] Moreover, the carcinogenic activities of the isomeric dihydro diols having an olefinic bond in a bay region correlate approximately with the activities of the parent polyarenes.[96] Particularly notable is the exceptional carcinogenic activity of DMBA 3,4-dihydro diol, **10c**, which is 10-fold greater than that of DMBA,[97] generally considered the most potent carcinogenic hydrocarbon. These results are in accord with the hypothesis that the corresponding diol epoxides having an epoxide ring in the bay region ("bay region diol epoxides") are the ultimate carcinogenic metabolites of the parent compounds. While the diol epoxides themselves tend to exhibit generally weaker tumorigenicity than their dihydro diol precursors, this is assumed to be due to their relatively facile decomposition. In vivo these reactive molecules probably are formed and react within the same cell.

Diol Epoxide–Nucleic Acid Interaction

Minimal information is available concerning the covalent binding of diol epoxides other than *anti*- and *syn*-BPDE to nucleic acids. Preliminary studies indicate that the principal metabolites of 7-MBA[98] and DMBA[99] which bind to DNA in cells are the bay-region diol epoxides arising from **10b** and **10c**, respectively. An analogous bay-region diol epoxide has also been implicated as the major DNA-bound form of 3-MC.[100,101] On the other hand, the DNA-bound adducts arising from metabolism of BA in mouse skin or hamster embryo cells are shown by Sims to be formed from the anti isomeric diol epoxides of both the 3,4- and the 8,9-dihydro diols (**10a** and **14**).[102] The latter adduct was shown to involve covalent binding principally to the 2-NH_2 group of guanosine.[103] A major RNA-bound adduct also detected was tentatively identified as arising from the *anti*-8,9-diol 10,11-oxide of BA.[102] The reason the non-bay-region 8,9-dihydro diol is involved in the metabolic activation of BA but not 7-MBA or DMBA may, as suggested by Sims,[102] be due to steric inhibition of epoxidation of the 8,9-dihydro diols of 7-MBA and DMBA which are sterically constrained to be diaxial.[94]

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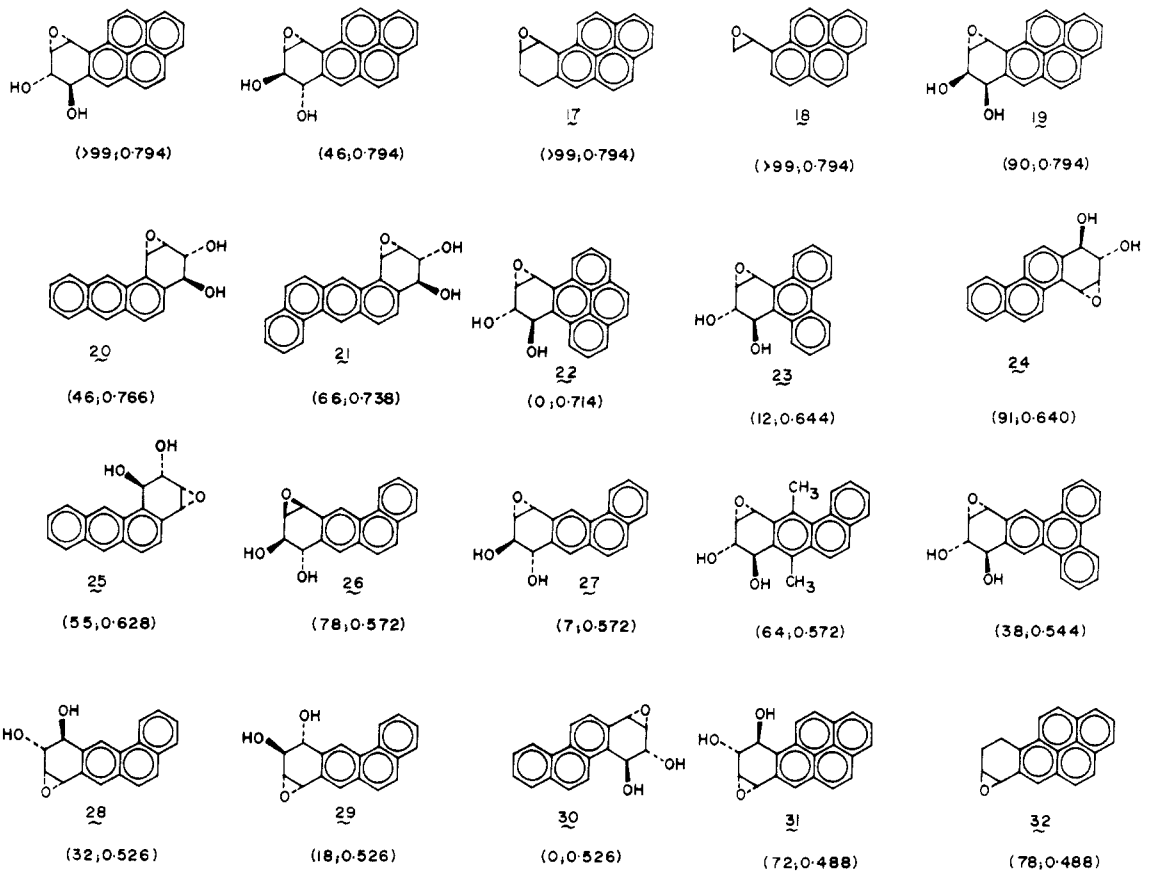

Figure 9. Inhibition of ϕX174 DNA infectivity in *E. coli* spheroplasts by diol epoxides and related compounds.[107,109] Numbers in parentheses are % inhibition and ΔE_{deloc} in β units.

This is consistent with observations that the 9,10-dihydro diols of benzo[*e*]pyrene (**15**)[104] and BP (**16**),[104] which are also diaxial, are also relatively resistant to enzymatic oxygenation. On the other hand, oxidative metabolism of the bay-region 1,2-dihydro diol of BA (**11a**) occurs primarily at the 3,4-olefinic bond,[105] indicating that axial hydroxyl groups do not necessarily inhibit metabolism in the same ring.

The regioselectivity of attack by reactive metabolites on nucleic acids and the molecular biological consequences of such interaction are poorly understood.[106] While it is established that binding of *anti*- and *syn*-BPDE, BP-4,5-oxide, and DMBA-5,6-oxide occurs preferentially on guanosine and to lesser extent on adenosine and cytosine, the relative importance of these adducts for tumor induction is unknown. Studies with the MS2 RNA and $\phi\chi$174 DNA viruses indicate that an average of one molecule of bound *anti*-BPDE sufficies to block normal phage replication,[53] leading to formation of fragmentary DNA products. Alkylation of infectious SV40 viral DNA with *anti*-BPDE gave a markedly impaired level of DNA synthesis for the first 12 h, but within 24–36 h viral synthesis returned to virtually normal.[54,107] However, the infectivity of the newly synthesized viral DNA remained depressed, suggesting that it may be somehow defective. Other studies indicate that the multiple deoxynucleoside adducts of BPDE undergo differential rates of excision during DNA repair.[108] The significance of these findings with respect to the mechanism of carcinogenesis is unknown.

What, if any, is the relationship between the comparative reactivities of diol epoxide metabolites with nucleic acids and the carcinogenic activities of the parent polyarenes? In view of the difficulties of structural analysis of tiny quantities of large numbers of hydrocarbon–nucleoside products, we utilized the *ϕX174* viral DNA bioassay developed by Weiss to provide evidence on this question.[53,109] This method is based on the principal that alkylation of the infectious viral DNA abolishes its infectivity in *E. coli* spheroplasts. Inhibition of infectivity is determined readily by counting plaque formation on agar plates. This method has the advantages of being simple and rapid and requiring only microgram quantities of precious compounds. Many of the diol epoxide derivatives tested exhibited potent antiviral activity (Figure 9). Thus, *anti*-BPDE totally inhibited viral replication, while the syn isomer showed lower activity (46%). Both 9,10-epoxy-7,8,9,10-tetrahydro-BP (**17**) and 1-oxiranylpyrene (**18**) were equally as active as *anti*-BPDE. Since **17** lacks the two hydroxy groups of *anti*-BPDE and **18** lacks also the 7,8-carbon atoms, these structural

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features apparently are not essential to reactivity. The cis isomer of *anti*-BPDE (**19**), the non-bay-region diol epoxide of BP (**31**), and 7,8-epoxytetrahydro-BP (**32**) also exhibited high activity. Thus, it appears that an epoxide function need not be in a bay region for relatively efficient alkylation of DNA. The syn isomer (not shown) was less active (46%) than the anti form (72%). In the BA series a similar pattern of activity was observed, with the anti isomers **20**, **25**, **26**, and **28** exhibiting higher activity than the syn isomers **27** and **29**. The most active BA derivative was **26** whose inhibitory activity (78%) exceeded that of the bay-region diol epoxide **20** (46%). The bay-region diol epoxides of chrysene (**24**) and DBA (**21**) also showed strong activity. While the diol epoxides of benzo[*e*]pyrene (**22**), triphenylene (**23**), and chrysene (**30**), in which the diol functions are diaxial, proved essentially inactive, the related diol epoxides **25**, and **31** showed respectable activity. Therefore, this structural feature alone is insufficient to abolish activity. In general, it appears there is no simple or obvious correlation between structure and activity. A wide range of polyarene epoxides are capable of inhibition of infectivity of $\phi X174$ viral DNA, presumably through direct alkylation of the nucleic acid, thereby blocking replication.

Many unanswered questions remain concerning the relation between DNA alkylation and tumor induction. Thus, while available evidence indicates that intercalation precedes alkylation of native calf thymus DNA by *anti*-BPDE,[69,70,73] it is not known whether intercalation is essential or incidental. Other important questions concern the regioselectivity of alkylation. Do the diol epoxide derivatives of both carcinogens and noncarcinogens exhibit similar base specificity of attack? What is the relative importance to tumor induction of alkylation on specific bases and in specific molecular regions of the nucleic acid? Other important questions concern the fidelity of transcription and the mechanism of mutagenesis. Present evidence indicates that replication of alkylated DNA does not take place past the site of the covalently bound polyarene molecule.[53] There is also evidence for differential rates of excision of the multiple deoxynucleoside adducts of BPDE during DNA repair[108] and indirect evidence for the introduction of errors during repair.[54,107] These observations suggest an important role for nucleic acid repair mechanisms.

Theories of Carcinogenesis

Invention of theories of carcinogenesis has long been a popular pastime for chemists. Early efforts focused on correlations between bioactivity and the molecular structures of the polyarenes themselves. Best known is the Pullman theory[6] which attempted to relate activity to the presence of an electron-rich "K-region" bond, presumed to combine directly with an unknown critical cellular target.

With recognition of the necessity for metabolic activation, electronic theories fell into disfavor, only to be revived and applied to the reactive polyarene metabolites.[17,19,110–114] Dipple et al.[110] pointed out an apparent correlation between the calculated relative stabilities of the carbonium ions derived from ring opening of the K-region arene oxides (calculated on the basis of their NBMO coefficients $[1 - a_{or}]$) and the carcinogenicities of the parent hydrocarbons. Following identification of *anti*-BPDE as the principal active metabolite of BP, Jerina et al.[111] drew attention to the exceptional stability of the bay-region carbocation, calculated on the basis of $\Delta E_{deloc}/\beta$, the difference in delocalization energies between the diol epoxide structure and its ionized form. They proposed that the carcinogenic potency of polyarenes is a direct function of the high reactivity of their bay-region diol epoxides as predicted by the theoretically calculated values of $\Delta E_{deloc}/\beta$. Subsequently, Smith et al.[19] attempted to correlate bioactivities with 21 MO theoretical parameters. Most satisfactory correlation was found with Q_B, an index of charge density in the bay region. The Q_B index is closely related to the reactivity numbers (N_t) proposed by Fu et al.[17] Indeed, all these parameters are interrelated [$\Delta E_{deloc}/\beta = 2(1 - a_{or})$, $Q_B = a_{or}$, and $N_t = 2a_{or}\beta$]. Osborne[112] noted that Q_B and related indexes give rise to false positives: (a) linearly annelated hydrocarbons such as hexacene yield low values of Q_B but are inactive; (b) BA, a borderline carcinogen, has a Q_B almost as low as the highly potent dibenzopyrenes and lower than that of DBA; (c) fluorenes and fluoranthenes yield very low values of Q_B, although most are weakly carcinogenic at best. He suggested an alternative index $\Sigma/a_0/$, the sum of the NBMO coefficients of all ring positions, which suffers less from these anomalies.

Support for MO theories of carcinogenesis derives mainly from the approximate correlation between theoretically predicted bay-region diol epoxide reactivities and the carcinogenicities of the parent polyarenes.[96,111] However it should be stressed that these theories predict only the relative reactivites of the carbocation intermediates rather than polyarene carcinogenicities. The only systematic study of the comparative reactivities of diol epoxide derivatives with DNA reveals no correlation with MO predictions based on $\Delta E_{deloc}/\beta$ (Figure 9).[107,109] While this lack of correlation appears to seriously undermine the validity of the quantum mechanical theories, the evidence is not entirely conclusive since it derives from indirect bioassay measurements. While the need for further investigation is evident, reactions of polyarene metabolites with nucleic acids are complex, and it is uncertain whether covalent binding to any specific base site is critical to tumor induction.

Any comprehensive theory of carcinogenesis must take into account the likelihood of molecular structural effects at each stage of enzymatic activation, detoxification, nucleic acid binding, repair, and replication. Methyl substitution in appropriate molecular regions is known to dramatically enhance or diminish carcinogenic activity dependent upon the site of substitution.[115–117] Since metabolism by microsomal enzymes

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tends to be inhibited at sites of substitution, it is reasonable to assume that methyl substitution in a ring anticipated to undergo metabolic activation should block bioactivity, while substitution elsewhere might have a contrary effect.[111] A survey of published data on the carcinogenicities of methyl- and fluoro-substituted BA and BP derivatives, while tending to support this generalization, reveals a number of notable exceptions (e.g., 2- and 3-fluoro-7-MBA and 4,7,12-trimethyl-BA are potent carcinogens).[115,116] Alternative mechanisms of metabolic activation may be operative in these cases. On the other hand, recent metabolic studies show that 7-methyl-BP and 8-methyl-BA[118] undergo significant enzymatic conversion to dihydro diols in the substituted ring. More systematic studies on these effects are required.

While the reactivity of bay-region diol epoxides is stressed in current theories, their resistance to the action of epoxide hydrase[70] may be of greater significance. *The importance of the bay region may be its ability to sterically hamper enzymatic detoxification of the epoxide function in this region.* Although K-region arene oxides, such as **5**, are able to efficiently alkylate DNA in vitro, they normally undergo rapid enzymatic hydrolysis and reaction with glutathione in vivo, preventing their alkylation of nucleic acids.

Prospects

Carcinogenesis appears to be the consequence of a rare event in which the biochemical machinery of enzymatic detoxification affords a reactive metabolite sufficiently stable to survive long enough to alkylate a critical cellular receptor, presumably DNA, in an appropriate molecular region to induce a nonlethal mutation ultimately resulting in tumor formation. While principal attention has focused on the diol epoxides, there is evidence for other activated metabolites. Thus, the 3,4-oxide is implicated as a mutagenic metabolite of cyclopenta[*cd*]pyrene,[119] a polyarene structurally incapable of forming a diol epoxide. The active form of 9-hydroxy-BP is considered to be the 4,5-oxide.[120] There is evidence that radical-cation intermediates[121] and esters of the alcohols arising from hydroxylation on benzylic sites of alkylpolyarenes[122] may function as activated metabolites in other cases. The active forms of the numerous carcinogenic polyarenes structurally incapable of forming diol epoxides[116,123] are still unknown.

While many of the details of metabolic activation and tumor initiation are still obscure, complete elucidation of the mechanisms of carcinogenesis of polyarenes now appears attainable.

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