Mutagenicity of Diol-Epoxides and Tetrahydroepoxides of Benz(a)acridine and Benz(c)acridine in Bacteria and in Mammalian Cells


ABSTRACT

The mutagenic activities of benz(a)acridine, benz(c)acridine, and a number of their derivatives, including 12 epoxides and diol-epoxides, were examined in bacterial and mammalian cells to determine the importance of bay-region activation of azapolycyclic aromatic hydrocarbons. In Salmonella typhimurium strain TA98, the diastereomeric bay-region 3,4-diol-1,2-epoxides of benz(c)acridine, in which the benzylic hydroxyl group is either cis (diol-epoxide 1) or trans (diol-epoxide 2) to the epoxide oxygen, had equivalent mutagenic potency (250 to 300 His* revertants/nmol) while in S. typhimurium strain TA100, diol-epoxide 1 induced 5100 His* revertants/nmol and was approximately twice as active as was diol-epoxide 2. In Chinese hamster V79 cells, the diol-epoxide 2 isomer of benz(c)acridine 3,4-diol-1,2-epoxide was approximately twice as mutagenic (4.5 8-aza-guanine-resistant colonies/10^5 surviving cells/nmol) as diol-epoxide 1. In all three test systems, the bay-region diol-epoxides of benz(c)acridine were from 1 to 4 orders of magnitude more mutagenic than were the non-bay-region diol-epoxides in which the epoxide was in the 3,4-, 8,9-, or 10,11-positions of the molecule. With the benz(a)acridine diol-epoxides, the 3,4-diol-1,2-epoxide 2 diastereomer was 2 to 4 times more active than was the 3,4-diol-1,2-epoxide 1 diastereomer in the bacterial systems, but both diastereomers had less than 10% of the activity of their benz(c)acridine counterparts. The bay-region tetrahydro-1,2-epoxides of both benzacridines were exceptionally mutagenic, and the benz(c)acridine epoxide was 4- to 11-fold more active than was the benz(a)acridine epoxide. The bay-region diol-epoxide diastereomers of benz(c)acridine and its bay-region tetrahydro-epoxide were not metabolized to nonmutagenic products by highly purified epoxide hydrolase (EC 3.3.2.3). Metabolic activation experiments using the cytochrome P-450-dependent monooxygenase system and the dihydrodiol precursors of the bay-region diol-epoxides were consistent with the intrinsic mutagenicity data, in that benz(c)acridine 3,4-dihydrodiol was metabolized to mutagenic products to a greater extent than was benz(a)acridine 3,4-dihydrodiol. The benz(c)acridine 1,2-, 5,6-, 8,9-, and 10,11-dihydrodiols were not metabolically activated to mutagenic metabolites. These results provide initial evidence that the bay-region theory can be extended to certain azapolycyclic aromatic hydrocarbons and indicate that the position of the nitrogen heteroatom can markedly affect mutagenic activity.

INTRODUCTION

Polycyclic aromatic hydrocarbons are a large, widespread, and well-characterized class of environmental carcinogens formed during the incomplete combustion of organic matter (7, 10). Under appropriate combustion conditions, nitrogen may be incorporated into the aromatic ring systems to form nitrogen heterocycles. Significant concentrations of these compounds have been identified in coal-derived fuels (27), urban air particulates (25, 29), and aquatic sediments (2, 32), and several of the azaaromatics are known carcinogens (10, 15).

Numerous investigations over the past 8 years have indicated that bay-region diol-epoxides (Chart 1) play a predominant role in mediating the mutagenic and carcinogenic activity of at least 12 unsubstituted and alkyl-substituted polycyclic aromatic hydrocarbons (8, 14, 21, 39). These diol-epoxides are formed in mammalian cells via oxidation of the angular benzo-ring of the hydrocarbon to form an arene oxide with a bay-region double bond, hydration of the arene oxide to a trans-dihydrodiol,2 and finally oxidation of the olefinic double bond to form the diol-epoxide. Cytochrome P-450-dependent monoxygenases catalyze the 2 oxidative reactions, and another microsomal enzyme, epoxide hydrolase, catalyzes diol-epoxide formation (19).

Despite the voluminous literature on the metabolic fate and biological activity of polycyclic aromatic hydrocarbons and their metabolites, few, if any, comparable studies have been reported for the nitrogen heterocycles. We have therefore undertaken an evaluation of the mutagenic potential of 2 nitrogen heterocycles, B(a)ACR and B(c)ACR, as well as a number of their epoxide derivatives (Chart 1). The choice of these 2 heterocycles was predicated on: (a) their presence in our environment; (b) the reported carcinogenicity of several of their methyl derivatives; (c) the expectation that several diol-epoxides and related derivatives of both heterocycles could be unequivocally synthesized; and (d) the availability of a large number of comparable derivatives of BA, the analogous polycyclic aromatic hydrocarbon. Thus, we

2 The abbreviations used are: dihydrodiol, trans-dihydroxydihydro derivatives of benz[a]acridine, benz[c]acridine, or benz[a]anthracene, in which the hydroxyl groups are either at the 1,2-, 3,4-, 5,6-, 8,9-, or 10,11-positions; B(a)ACR, benz[a]acridine; B(c)ACR, benz[c]acridine; BA, benz[a]anthracene; H-1,2-epoxide, 1,2-epoxy-1,2,3,4-tetrahydrodibenzo[a]anthracene; H-3,4-epoxide, 3,4-epoxy-1,2,3,4-tetrahydro derivative of benz[a]acridine or benz[c]acridine; H-3,4-dihydrodiol, 3,4-dihydrodiols of benz[a]acridine or benz[c]acridine; H-3,4-dihydrodiol, benz[a]anthracene; DMSO, dimethyl sulfoxide; 3,4-diol-1,2-epoxide 1, (±)-3«,4-epoxide, 3,4-epoxy-1,2,3,4-tetrahydro derivatives of benz[a]acridine, benz[c]acridine, or benz[a]anthracene; diol-epoxide diastereomer, in which the benzylic hydroxyl and the epoxide oxygen are cis; 3,4-diol-1,2-epoxide 2, (±)-3α,4β-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]acridine, benz[c]acridine, or benz[a]anthracene diol-epoxide diastereomer, in which these groups are trans; other diol-epoxides are similarly designated; α and β, relative stereochemistry, and all compounds are racemic mixtures where enantiomers are possible.
Mutagenicity of Benzacridine Epoxides

MATERIALS AND METHODS

Mutagenicity Assays with Bacteria. Strains TA98 and TA100 of histidine-dependent Salmonella typhimurium (20) were obtained from Dr. B. Ames, University of California, Berkeley, Calif., and were cultured as described (34). Intrinsic mutagenicity was assessed by incubating the epoxides (added in 15 μl of DMSO) with 2 × 10^6 bacteria suspended in 0.5 ml of phosphate-buffered saline (5 mm potassium phosphate:150 mm sodium chloride, final pH 7.0) for 5 min at 37° before addition of top agar. Mutagenesis experiments with microsomes as the source of monooxygenase activity were based on the procedure described by Ames et al. (1), and the exact composition of the incubations has been described (35). Experiments were initiated by addition of the test compound in 15 μl of acetone:DMSO: NH₄OH (95:5:0.03). Incubations with the purified monoxygenase system were also conducted as described previously (35). Mutations to histidine-independent growth were assessed by counting the macroscopic colonies of bacteria after a 2-day incubation of the plates at 37°.

Colonies were counted on a 3M Model 620 colony counter interfaced with a Honeywell 6080 computer which was programmed to provide individual mutation frequencies, their mean before and after background subtraction, S.E., and coefficients of variation. Experimental values were obtained from 3 replicates, while background mutation frequencies were determined from 6 replicates. Coefficients of variation averaged 10% and rarely exceeded 15%. At least 2 separate experiments were performed with each compound. None of the derivatives caused any discernible (x1 and x100 magnification) irregularities in the background lawn and were thus considered to be nontoxic to the bacteria at the doses tested.

Mutagenesis Assays with Mammalian Cells. Line V79-6 of Chinese hamster cells (6) was a generous gift of Dr. E. H. Y. Chu, University of Michigan, Ann Arbor, Mich. The cells, which appear to be devoid of the enzymes that metabolize polycyclic hydrocarbons, were cultured as described previously (34). Assays assessing the cytotoxicity and intrinsic mutagenicity of the diol-epoxides (added in 20 μl of DMSO) were adapted from the procedure of Chu (6) and were performed as described previously (34). Resistance to the lethal effects of 8-azaguanine was used as the mutagenic marker, and a 3-day interval between exposure of the cells to the epoxides and treatment with the purine analog was optimal, providing that cell survival was at least 35% of control values.

In each of at least 2 separate experiments for each compound, 4 and 16 replicate dishes were used to assess cell survival and 8-azaguanine resistance, respectively. The spontaneous mutation frequency for 8 experiments averaged 0.36 ± 0.19 (S.E.) 8-azaguanine-resistant colonies/10^6 surviving cells and never exceeded a value of 1.3.

RESULTS

The dose-response relationships for the inherent mutagenicity of the bay-region epoxides of BA, B(a)ACR, and B(c)ACR in strains TA98 and TA100 of S. typhimurium are shown in Chart 2. Although large differences in mutagenic potency are apparent, all of the compounds exhibited linear or nearly linear dose-response relationships, and most of the epoxides induced higher numbers of histidine autotrophs in strain TA100. Mutation frequencies, expressed as His^+ revertants/nmol of epoxide, were calculated from these data, as well as from additional experiments with these and the other epoxides. The results are summarized in Table 1.

Among a total of 21 epoxides, the bay-region tetrahydroepoxides were the most mutagenic derivatives of BA, B(a)ACR, and

Mature male Long-Evans rats treated with the polychlorinated biphenyl mixture Aroclor 1254. The cytochromes P-450 induced by Aroclor 1254 represent a mixture of the major hemoproteins induced by phenobarbital and 3-methylcholanganthrene (22, 23). This cytochrome P-450 mixture was purified from the microsomes as described (23). NADPH-cytochrome c reductase (43) and epoxide hydrolase (24) were purified from liver microsomes of immature male Long-Evans rats as described.

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A. W. Wood et al.

Chart 2. Intrinsic mutagenic activity of the angular benzo-ring diol-epoxides and tetrahydroepoxides of BA, B(a)ACR, and B(c)ACR in strains TA98 (A) and TA100 (B) of S. typhimurium. Approximately $2 \times 10^8$ bacteria of either strain were treated with the indicated amounts of the epoxides as described in "Materials and Methods." Each value represents the average number of histidine revertant colonies observed per plate in 3 replicate determinations. Spontaneous mutation frequencies of 36 ± 1 (S.E.) and 170 ± 10 his$^+$ revertants/plate for strains TA98 and TA100, respectively, have been subtracted from the data.

Table 1

<table>
<thead>
<tr>
<th>Derivative</th>
<th>His$^+$ revertants/nmol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BA</td>
</tr>
<tr>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td>H$_4$-1,2-epoxide</td>
<td>1,800</td>
</tr>
<tr>
<td>H$_4$-3,4-epoxide</td>
<td>450</td>
</tr>
<tr>
<td>1,2-Diol-3,4-epoxide 1</td>
<td>5</td>
</tr>
<tr>
<td>1,2-Diol-3,4-epoxide 2</td>
<td>170</td>
</tr>
<tr>
<td>3,4-Diol-1,2-epoxide</td>
<td>380</td>
</tr>
<tr>
<td>3,4-Diol-1,2-epoxide 2</td>
<td>590</td>
</tr>
<tr>
<td>8,9-Diol-10,11-epoxide 1</td>
<td>10</td>
</tr>
<tr>
<td>8,9-Diol-10,11-epoxide 2</td>
<td>20</td>
</tr>
<tr>
<td>10,11-Diol-8,9-epoxide 1</td>
<td>ND</td>
</tr>
<tr>
<td>10,11-Diol-8,9-epoxide 2</td>
<td>30</td>
</tr>
</tbody>
</table>

$^a$ND, not determined.

$^b$Previously shown (34) to have <5% of the mutagenic activity of BA 3,4-diol-1,2-epoxide 1 in S. typhimurium TA100.

B(c)ACR toward S. typhimurium strains TA98 and TA100. Comparison of the mutation frequencies for BA H$_4$-1,2-epoxide and B(a)ACR H$_4$-1,2-epoxide indicates that the presence of a nitrogen atom at position 7 diminished mutagenic activity about 2- and 6-fold in strains TA98 and TA100, respectively. In contrast, when the nitrogen atom was in position 12 [B(c)ACR], mutagenic activity relative to BA H$_4$-1,2-epoxide was enhanced approximately 2-fold in strain TA98 and remained essentially the same in strain TA100. As expected, based on predicted chemical reactivity (13, 39), the non-bay-region tetrahydro-3,4-epoxides of BA and B(c)ACR had significantly less mutagenic activity than did their bay-region counterparts, but the diminution in activity was considerably greater with the B(c)ACR epoxide. Thus, while BA H$_4$-3,4-epoxide had 20 to 25% of the activity of BA H$_4$-1,2-epoxide, B(c)ACR H$_4$-3,4-epoxide had only 0.4 to 3% of the activity of B(c)ACR H$_4$-1,2-epoxide. The H$_4$-3,4-epoxide of B(a)ACR was not synthesized.

Seven of the 8 diol-epoxides derivable from the 4 non-K-region dihydrodiols of B(c)ACR were synthesized and tested for mutagenic activity. As shown in Table 1, the bay-region 3,4-diol-1,2-epoxide diastereomers were from 1 to 4 orders of magnitude more mutagenic than were their non-bay-region counterparts in which the epoxide was in the 3,4-, 8,9-, or 10,11-positions. Comparison of the mutagenic activities induced by the B(c)ACR diol-epoxides with those induced by their BA counterparts indicates that the presence of nitrogen at position 12 resulted consistently in a diminished mutagenic response. With the bay-region diol-epoxides, this decrease ranged from 35% (dihydro-
ide 1 isomer, strain TA98) to 76% (dil-epoxide 2 isomer, strain TA100). Presence of nitrogen at position 7 had an even more profound effect on bay-region dil-epoxide mutagenicity, since the diastereomeric 3,4-diol-1,2-epoxides of B(a)ACR had <3% and <10%, respectively, of the mutagenic activity of their BA and B(c)ACR dil-epoxide counterparts. Comparison of the mutagenic activities of the bay-region dil-epoxides of BA, B(a)ACR, and B(c)ACR with their respective bay-region tetrahydroepoxides indicates that substitution of hydroxyl groups for hydrogens at the 3- and 4-positions results in a significantly greater loss of mutagenic activity in the N-heterocycles compared to that in the hydrocarbons.

The mutagenic activity of 17 epoxides of BA, B(a)ACR, and B(c)ACR in Chinese hamster V79 cells is summarized in Table 2. Consistent with results in the bacterial cells, the bay-region dil-epoxides of B(c)ACR were 2 orders of magnitude more mutagenic than were the non-bay-region dil-epoxides and were significantly (20- to 40-fold) more mutagenic than were the bay-region dil-epoxides of B(a)ACR. Interestingly, B(c)ACR 3,4-diol-1,2-epoxide 2 had about twice the mutagenic activity of its diastereomer in the mammalian cells, while it was only one-half as mutagenic as its diastereomer in S. typhimurium TA100. Also consistent with the bacterial mutagenicity data was the observation that a bay-region tetrahydroepoxide was the most mutagenic derivative of each of the 3 parent compounds and that B(c)ACR H4-1,2-epoxide was more active than were the H4-1,2-epoxides of B(a)ACR (12-fold) and BA (2-fold). The relative activity of the compounds to induce cell killing paralleled their mutagenic activity.

Previous studies have shown that the bay-region and non-bay-region tetrahydroepoxides of several polycyclic hydrocarbons are metabolized by epoxide hydrolase, while the corresponding diol-epoxides are not metabolized by this enzyme (31, 34–36, 40). The results summarized in Table 3 indicate that, like their BA counterparts, the B(c)ACR 3,4-diol-1,2-epoxide diastereomers are also not metabolized by epoxide hydrolase. However, B(c)ACR H4-1,2-epoxide was also refractory to epoxide hydrolase under conditions that resulted in the efficient metabolism of the H4-1,2-epoxides of BA and B(a)ACR. Whereas 5 units of epoxide hydrolase reduced the mutagenic activity of BA H4-1,2-epoxide by over 50%, 25 units of the enzyme failed to reduce the mutagenicity of B(c)ACR H4-1,2-epoxide. The low mutagenicity of the other epoxides precluded the analysis of the effect of epoxide hydrolase on their activity under comparable conditions.

The capacity of B(c)ACR and the 5 possible trans-dihydrodiols of B(c)ACR to be metabolically activated by hepatic microsomes from Aroclor-treated rats to products mutagenic to S. typhimurium TA100 is shown in Chart 3. B(c)ACR 3,4-dihydrodiol, the immediate metabolic precursor of the B(c)ACR bay-region diol-epoxides, was at least 5 times more active than were B(c)ACR and the other dihydrodiols and was the only compound tested whose mutagenic activity was clearly dependent on the amount of cytochrome P-450 in the incubation mixture. None of the compounds had any intrinsic mutagenic activity.

The mutagenic activities in strain TA100 of S. typhimurium of the metabolic products formed by incubation of BA, B(a)ACR, and B(c)ACR and their 3,4-dihydrodiols with a cytochrome P-450-dependent monooxygenase system are shown in Chart 4. In Chart 4A, 20 nmol of compound were incubated with varying amounts of hepatic microsomes from Aroclor 1254-treated rats for 5 min. The metabolic products of BA 3,4-dihydrodiol induced about 900 His<sup>+</sup> revertants per nmol of cytochrome P-450 and were significantly more mutagenic than were the metabolites formed from the other 5 compounds. The metabolic products formed from B(c)ACR 3,4-dihydrodiol had 20% of the mutagenic activity (180 His<sup>+</sup> revertants/nmol of cytochrome P-450) observed when BA 3,4-dihydrodiol was the substrate. In both cases, the 3,4-dihydrodiol was metabolically activated to a greater extent than was the parent compound. No significant increase in mutation frequency was observed with B(a)ACR 3,4-dihydrodiol, and BA was the only parent hydrocarbon which was activated to mutagenic products by the microsomes.

Chart 4B summarizes the results of an analogous experiment in which the same 6 compounds were incubated with a highly purified and reconstituted cytochrome P-450-dependent monooxygenase system for 10 min. Comparison of the data in Chart 4A and B indicates that, per nmol of cytochrome P-450 and per min of incubation, the purified monooxygenase system was 5- to 10-fold more active than were the microsomes for the activation of the 3,4-dihydrodiols of BA and B(c)ACR. Low but significant (p<0.02) activation of B(a)ACR 3,4-dihydrodiol was observed with the purified monooxygenase system when 0.1 nmol of cytochrome was used.

**Table 2**

Mutagenic activity of 17 dil epoxides and tetrahydroepoxides of BA, B(a)ACR, and B(c)ACR in Chinese hamster V79 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>BA</th>
<th>B(a)ACR</th>
<th>B(c)ACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>H4-1,2-epoxide</td>
<td>22</td>
<td>4.5</td>
<td>52</td>
</tr>
<tr>
<td>H3-4-epoxide</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1,2-Diol-3,4-epoxide 1</td>
<td>0.06</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1,2-Diol-3,4-epoxide 2</td>
<td>0.11</td>
<td>ND</td>
<td>0.02</td>
</tr>
<tr>
<td>3,4-Diol-1,2-epoxide 1</td>
<td>2.6</td>
<td>0.10</td>
<td>2.4</td>
</tr>
<tr>
<td>3,4-Diol-1,2-epoxide 2</td>
<td>13.5</td>
<td>0.12</td>
<td>4.5</td>
</tr>
<tr>
<td>8,9-Diol-10,11-epoxide 1</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
</tr>
<tr>
<td>8,9-Diol-10,11-epoxide 2</td>
<td>ND</td>
<td>ND</td>
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<td>10,11-Diol-8,9-epoxide 1</td>
<td>ND</td>
<td>ND</td>
<td>0.33</td>
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<tr>
<td>10,11-Diol-8,9-epoxide 2</td>
<td>ND</td>
<td>ND</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*ND, not determined.
*Previously shown to have <1% of the mutagenic activity of BA 3,4-diol-1,2-epoxides (34).

**Table 3**

Effect of highly purified epoxide hydrolase on the mutagenic activity of several dil-epoxide and tetrahydroepoxide derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of control activity with the following epoxide hydrolase unit&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>BA H4-1,2-epoxide</td>
<td>0.050</td>
</tr>
<tr>
<td>BA H4-1,2-epoxide</td>
<td>0.225</td>
</tr>
<tr>
<td>B(c)ACR H4-1,2-epoxide</td>
<td>0.050</td>
</tr>
<tr>
<td>B(c)ACR 3,4-diol-1,2-epoxide 1</td>
<td>0.150</td>
</tr>
<tr>
<td>B(c)ACR 3,4-diol-1,2-epoxide 2</td>
<td>0.225</td>
</tr>
</tbody>
</table>

<sup>3</sup>Amounts of the epoxides were selected from linear portions of their dose-response curves with strain TA100 of S. typhimurium.

P<br>nmol of styrene glycol formed from styrene oxide per min at pH 8.7 and 37°. The enzyme was added to the bacteria in a volume of 0.3 ml 1 min before the addition of the epoxides.
The bay-region diol-epoxides and tetrahydroepoxides of B(a)ACR were from 1 to 4 orders of magnitude more mutagenic than were their non-bay-region counterparts. These results, and the demonstration that B(c)ACR 3,4-dihydrodiol was metabolically activated to mutagenic products to a greater extent than were B(c)ACR and the other 4 possible dihydrodiols of B(c)ACR by the cytochrome P-450 monooxygenase system of the hydrocarbon. With C-1 carbocations formed from ring opening of a bay-region diol-epoxide or tetrahydroepoxide of BA, B(a)ACR, or B(c)ACR, a resonance structure with the positive charge at position 7, but not position 12, is possible. Since a positive charge on the nitrogen heteroatom is energetically destabilizing, one would expect that a bay-region epoxide of B(a)ACR would not be as reactive as those from BA or B(c)ACR. A similar argument can be used to rationalize the relatively low mutagenic activity of the non-bay-region tetrahydro-3,4-epoxide of B(c)ACR relative to its BA counterpart. In the case of ring opening of a 3,4-epoxide, resonance structures would include assignment of the positive charge to position 12, but not to position 7.

Although the introduction of a nitrogen heteroatom at position 12 of BA either enhanced or had no significant effect on the mutagenic activity of the bay-region tetrahydroepoxide, a significant inhibitory effect was, with one exception, noted with the bay-region diol-epoxides. The explanation for this observation is not apparent, but it is consistent with studies with polycyclic hydrocarbon epoxides which indicated that the presence of benzo-ring hydroxyl groups adjacent to the epoxide can have an inhibitory effect on mutagenic activity (33, 34, 36, 40). Previous studies have shown that the orientation of these hydroxyl groups is also a determinant of biological activity (4, 5, 28, 37, 38).

Relatively little information is available on the metabolism of the benzacridines. Comparison of the metabolism of 7-methylbenz[c]acridine and benzo(a)pyrene by rat liver microsomes has revealed similarities with respect to turnover number, Michaelis constants, and inducibility (12). Individual metabolites of 7-methylbenz[c]acridine were not reported, although a more recent study from the same laboratory has demonstrated the formation of the 5,6-dihydrodiol and the 7-hydroxymethyl derivative (3). The data in Charts 3 and 4 indicate that B(c)ACR 3,4-dihydrodiol is metabolized to mutagenic products, presumably the 3,4-diol-1,2-epoxide diastereomers, by the cytochrome P-450-dependent monooxygenase system. However, BA 3,4-dihydrodiol is activated from 5 to 9 times more efficiently than is B(c)ACR 3,4-dihydrodiol. Since this difference is somewhat greater than the 2- to 4-fold difference in intrinsic mutagenicity of the bay-region diol-epoxides of BA and B(c)ACR in S. typhimurium TA100 (Table 1), it would appear that the presence of the nitrogen atom in position 12 may retard oxidative metabolism or shift it away from the 1,2-position. Interestingly, recent studies have shown that a relatively small percentage of BA 3,4-dihydrodiol is converted to cyclic hydrocarbon carcinogenicity can be extended to certain azapolycyclic aromatic hydrocarbons.

Perhaps the most striking structure-activity relationship deduced from the study of the derivatives of BA, B(a)ACR, and B(c)ACR is that the position of the nitrogen heteroatom in the aromatic ring system has a profound effect on the biological activity of the epoxides. In all mutagenic test systems studied, the bay-region diol-epoxides and tetrahydroepoxides of B(c)ACR (N-12) are substantially more active than are the analogous derivatives of B(a)ACR (N-7). While a complete explanation is not possible at the present time, these observations are consistent with qualitative resonance arguments and with Hückel and perturbational molecular orbital calculations (data not shown) used to predict the relative ease of carbonation formation from diol-epoxides and tetrahydroepoxides (9, 13). Ring opening is facilitated by the capacity of the hydrocarbon to delocalize the positive charge of the carbonation throughout the r-electron system of the hydrocarbon. With C-1 carbonations formed from ring opening of a bay-region diol-epoxide or tetrahydroepoxide of BA, B(a)ACR, or B(c)ACR, a resonance structure with the positive charge at position 7, but not position 12, is possible. Since a positive charge on the nitrogen heteroatom is energetically destabilizing, one would expect that a bay-region epoxide of B(a)ACR would not be as reactive as those from BA or B(c)ACR. A similar argument can be used to rationalize the relatively low mutagenic activity of the non-bay-region tetrahydro-3,4-epoxide of B(c)ACR relative to its BA counterpart. In the case of ring opening of a 3,4-epoxide, resonance structures would include assignment of the positive charge to position 12, but not to position 7.

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BA 3,4-diol,1,2-epoxide by rat hepatic microsomes (30). The studies with purified epoxide hydroxase in the present study (Table 3) clearly indicate that the presence of the nitrogen heteroatom in position 12 can inhibit the metabolism of an azapolycyclic tetrahydroepoxide. B(c)ACR H4-1,2-epoxide is the first of over 6 tetrahydroepoxides tested which is apparently not azapolycyclic tetrahydroepoxide. B(c)ACR H4-1,2-epoxide is the 

Tumorigenicity studies have generally indicated that B(a)ACR and B(c)ACR are very weakly, if at all, carcinogenic while BA has been metabolized by microsomal epoxide hydrolase. 

Note Added in Proof

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The results of the present study, the substituted benz[a]cradines have been significantly more tumorigenic than the substituted benz[a]cradines. Tumorigenicity studies with many of the compounds described in this study are in progress.

REFERENCES

6. Chu, E. H. Y. Induction and analysis of gene mutations in mammalian cell strains TA-98 and TA-100, respectively, while in the same experiment BA 3,4-diole (K-region oxide) induced 41 and 530 His+ revertants in strains TA-98 and TA-100, respectively.

Mutagenicity of Benzacridine Epoxides


Note Added in Proof


A. W. Wood et al.


Mutagenicity of Diol-Epoxides and Tetrahydroepoxides of Benz(a)acridine and Benz(c)acridine in Bacteria and in Mammalian Cells


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