Exceptionally High Tumor-initiating Activity of Benzo(c)phenanthrene
Bay-Region Diol-Epoxides on Mouse Skin


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ABSTRACT

Benzo(c)phenanthrene [B(c)Ph], its three metabolically possible trans-dihydrodiols, and the diastereomeric bay-region diol-epoxides derived from trans-3,4-dihydroxy-3,4-dihydrobenzo(c)phenanthrene were tested for tumor-initiating activity on mouse skin. A single topical application of 0.4 or 2.0 μmol of compound was followed seven days later by twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbolester 13-acetate for 20 weeks. B(c)Ph was found to be a weak tumor initiator on mouse skin, producing a 17 to 38% tumor incidence and 0.17 to 0.59 papillomas/mouse at the 0.4- and 2.0-μmol doses, respectively. Of the three metabolically possible trans-dihydrodiols of B(c)Ph, only trans-3,4-dihydroxy-3,4-dihydrobenzo(c)phenanthrene had significant tumor-initiating activity at the doses tested. This compound produced a 28 to 47% tumor incidence and 0.41 to 1.07 tumors/mouse at the 0.4- and 2.0-μmol doses, respectively. Comparisons of the number of papillomas observed per mouse indicated that both diastereomeric 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo(c)phenanthrenes in which the epoxide oxygen is either cis (diol-epoxide 1) or trans (diol-epoxide 2) to the benzylic 4-hydroxyl group were at least 40-fold more tumorigenic than was the parent hydrocarbon. The bay-region diol-epoxides of B(c)Ph produced 6.5 to 7.0 papillomas/mouse at the 0.4-μmol dose, and the first appearance of tumors was observed as early as six weeks after the beginning of promotion. This is the first example of high tumorigenic activity for a bay-region diol-epoxide 1 diastereomer of a polycyclic aromatic hydrocarbon, and both diastereomeric bay-region diol-epoxides of B(c)Ph are more potent tumor initiators than are the bay-region diol-epoxides derived from benzo(a)pyrene and benzo(a)anthracene. Two model compounds, 1,2-dihydrobenzo(c)phenanthrene and trans-3,4-dihydroxy-1,2,3,4-tetrahydrobenzo(c)phenanthrene, which cannot be metabolized to bay-region diol-epoxides of B(c)Ph, were found to be inactive as tumor initiators on mouse skin. These results support the concept that a bay-region diol-epoxide is a prime candidate for an ultimate carcinogenic metabolite of B(c)Ph.

INTRODUCTION

The polycyclic aromatic hydrocarbons are a large group of relatively chemically inert compounds that exert their mutagenic and carcinogenic effects only after metabolic activation to reactive metabolites by microsomal enzymes (15). During the past several years, evidence has been amassed which indicates that metabolism of polycyclic aromatic hydrocarbons to bay-region diol-epoxides is of key importance in the expression of their adverse biological properties (5, 19). These bay-region diol-epoxides can exist as diastereomeric pairs in which the epoxide group is either cis (isomer 1 series) or trans (isomer 2 series) to the benzylic hydroxyl group (Chart 1). Further, the cis and trans diol-epoxides each exist as pairs of enantiomers. It is now known that relative and absolute stereochimistries of bay-region diol-epoxides have marked effects on the expression of their mutagenic and tumorigenic activity (9). For benzo(a)pyrene (10, 17), chrysene (2), and benzo(a)anthracene (12, 22), the diol-epoxide 2 isomer is markedly more tumorigenic than is the diol-epoxide 1 isomer. The diol-epoxide 2 isomers of these bay-region diol-epoxides have their hydroxyl groups mainly in the quasi-diequatorial conformation, whereas the diol-epoxide 1 isomers have their hydroxyl groups mainly in the quasi-diaxial conformation (11, 21, 29). In the case of benzo(a)pyrene, both diastereomeric 9,10-diol-11,12-epoxide isomers 1 and 2 have their hydroxyl groups locked in the quasi-diaxial conformation (30), and neither isomer has significant tumorigenic activity.2

B(c)Ph,3 like chrysene and benzo(a)anthracene, is a tetra cyclic aromatic hydrocarbon with weak tumorigenic activity (18). There is severe steric crowding in the bay region of B(c)Ph which results in a preferred quasi-diequatorial conformation for the hydroxyl groups in the bay-region diol-epoxide isomer 1.4 This highly hindered region between C-1 and C-12 in B(c)Ph has been referred to as a "fiord region" (1). This is the first known example of a polycyclic aromatic hydrocarbon for which both diol-epoxide diastereomers prefer to have their hydroxyl groups in the quasi-diequatorial conformation. The present study was designed (a) to identify potential B(c)Ph metabolite(s) responsible for the tumorigenicity of the parent hydrocarbon and (b) to further evaluate the role of hydroxyl group conformation in the tumorigenicity of bay-region diol-epoxides.

MATERIALS AND METHODS

Chemicals. B(c)Ph (4) and the 3 metabolically possible dihydrodiols (6) were prepared as described. 1,2-H2B(c)Ph and

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3 The abbreviations used are: B(c)Ph, benzo(c)phenanthrene; 1,2-H2B(c)Ph, 1,2-dihydrobenzo(c)phenanthrene; 1,2-epoxy-1,2,3,4-tetrahydrobenzo(c)phenanthrene; B(c)Ph 3,4- and 5,6-dihydrodiol, other trans dihydrodiols of benzo(c)phenanthrene; B(c)Ph H4-3.4-diol, trans-3,4-dihydroxy-1,2,3,4-tetrahydrobenzo(c)phenanthrene; B(c)Ph diol-epoxide-1, (±)-3α,4β-dihydroxy-1,2,3,4-tetrahydrobenzo(c)phenanthrene; B(c)Ph diol-epoxide 2, (±)-3α,4β-dihydroxy-1,2,3,4-tetrahydrobenzo(c)phenanthrene; TPA, 12-O-tetradecanoylphorbol-13-acetate. All compounds are racemic mixtures where enantiomers are possible.


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The tumor-initiating activity of both B(c)Ph and B(c)Ph 3,4-di-dihydrodiol appears to be the result of metabolic conversion of these compounds to a bay-region diol-epoxide. Saturation of the 1,2-double bond in both molecules to form 1,2-H₂B(c)Ph and B(c)Ph H₂-3,4-diol resulted in compounds which were inactive as tumor initiators at both doses tested. Consistent with this proposal is the exceptional tumor-initiating activity of the diastereomeric bay-region diol-epoxides of B(c)Ph. At the 0.4- and 2.0-μmol initiating doses, both diastereomers produced an 80 to 90% tumor incidence and 6.5 to 8.1 tumors/mouse. In animals treated with the B(c)Ph 3,4-diol-1,2-epoxides, the first tumors were observed as early as 6 weeks after the start of promotion with TPA (Chart 2). Although diol-epoxides 1 and 2 showed less than a 2-fold difference in their tumor-initiating activity when the data were expressed as tumors per mouse throughout 20 weeks of promotion with TPA, it is clear that the 0.4-μmol initiating dose was already at or above the top of the dose-response curve for both compounds since a 5-fold increase in dose produced no further increase in tumor production (Table 1).

**DISCUSSION**

The bay-region theory predicts that a B(c)Ph 3,4-diol-1,2-epoxide, if formed metabolically, is a prime candidate as an ultimate carcinogenic metabolite of the parent hydrocarbon (7,...

<table>
<thead>
<tr>
<th>Initiator</th>
<th>Dose (μmol)</th>
<th>% of mice with tumors</th>
<th>Mean no. of tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(c)Ph</td>
<td>0.4</td>
<td>17</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>38</td>
<td>0.59 ± 0.18</td>
</tr>
<tr>
<td>1,2-H₂B(c)Ph</td>
<td>0.4</td>
<td>4</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>B(c)Ph 1,2-dihydrodiol</td>
<td>0.4</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>B(c)Ph 3,4-dihydrodiol</td>
<td>0.4</td>
<td>26</td>
<td>0.41 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>47</td>
<td>1.07 ± 0.37</td>
</tr>
<tr>
<td>B(c)Ph 5,6-dihydrodiol</td>
<td>0.4</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>B(c)Ph H₂-3,4-diol</td>
<td>0.4</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>10</td>
<td>0.13</td>
</tr>
<tr>
<td>B(c)Ph diol-epoxide 1</td>
<td>0.4</td>
<td>87</td>
<td>6.47</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>89</td>
<td>7.03</td>
</tr>
<tr>
<td>B(c)Ph diol-epoxide 2</td>
<td>0.4</td>
<td>80</td>
<td>7.13</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>80</td>
<td>8.12</td>
</tr>
</tbody>
</table>

*Mean ± S.E.

p < 0.05 compared to control animals according to the 4-fold contingency test described by Mainland and Murray (14).
The exceptional tumor-initiating activity of the B(c)Ph bay-region diol-epoxides is difficult to explain. Quantum mechanical calculations of the ease of carbonium ion formation as an index of chemical reactivity (8) at C-1 for the bay-region diol-epoxides of B(c)Ph (\(\Delta E_{\text{acep}}/\Delta E_{\text{acep}} = 0.600\)) predict them to be less reactive than the noncarcinogenic bay-region diol-epoxides (2) of phenanthrene (\(\Delta E_{\text{acep}}/\Delta E_{\text{acep}} = 0.858\)). It should be emphasized that, with this theoretical approach, effects due to stereochemistry of the hydroxyl groups and the oxirane ring are totally neglected (8). However, direct measurements of the solvolytic reactivity and nucleophilic susceptibility of the B(c)Ph and phenanthrene bay-region diol-epoxides confirm the low and comparable chemical reactivities of these compounds as predicted by the quantum mechanical model.\(^6\) Comparison of the half-lives of the B(c)Ph diol-epoxides (\(t_{1/2} = 25.2\) and 19.4 hr for diol-epoxides 1 and 2, respectively) with the benzo(a)pyrene diol-epoxides indicates that the former epoxides are

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torial conformation. This preferential conformation of both diol-epoxide diastereomers has quasi-diequatorial hydroxyl groups.

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Note Added in Proof. Since the submission of this manuscript, we have tested a number of benzo(a)pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carboxonium ion formation from diol-epoxides.

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Tumorigenicity of B(c)Ph Derivatives

20- to 500-fold more stable in 10% dioxane:water at pH 7.4. (It should be noted that at pH 7.4 the 4 molecules being compared are not undergoing solvolysis by the same mechanism.) In tissue culture medium, the B(c)Ph diol-epoxides are about 100 times more stable than the benzo(a)pyrene diol-epoxides (24). Although the quantum mechanical model (8) has generally proven to be quite useful (5, 21, 27), chemical reactivity is inadequate as a predictor of the high biological activity of the B(c)Ph diol-epoxides. Similarly, the simplicity of the model (8) had precluded it from being able to predict the marked difference in biological activity of (+)- and (-)-benzo(a)pyrene diol-epoxide 2 (3, 16, 26). At the present time, there is no clear explanation for the exceptionally high biological activity of the B(c)Ph diol-epoxides. Unusually high steric crowding at the bay-region of the B(c)Ph 3,4-diol-1,2-epoxides may be one of several possible reasons which contribute to its remarkable tumorigenicity. B(c)Ph is the first polycyclic aromatic hydrocarbon studied where both bay-region diol-epoxide isomers 1 and 2 possess high tumor-initiating activity. This may be the result of the preferred quasi-diequatorial conformation of the hydroxyl groups in both diastereomers. Thus, the markedly lower tumorigenic activity of isomer 1 of benzo(a)pyrene (10, 17), chrysene (2), and benzo(a)anthracene (12, 22) compared to the isomer 2 series may be due in part to the quasi-diaxial conformation of the hydroxyl groups in the isomer 1 series (11, 21, 29) for these compounds. The lack of high tumorigenic activity of the bay-region 9,10-diol-11,12-epoxides of benzo(a)pyrene is consistent with the observation that the hydroxyl groups are essentially locked in the quasi-diaxial conformation for both diastereomers (30). Thus, our studies with bay-region diol-epoxides from 5 different polycyclic aromatic hydrocarbons indicate that the conformation of the hydroxyl groups is an important factor in the expression of tumorigenic activity. In all instances, the diol-epoxide diastereomers which have the highest tumorigenic activity are those which prefer to have their hydroxyl groups in the quasi-diequatorial conformation.

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