Comparative Metabolic Activation in Mouse Skin of the Weak Carcinogen 6-Methylchrysene and the Strong Carcinogen 5-Methylchrysene

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ABSTRACT

We compared the metabolic activation in mouse skin of the weak carcinogen 6-methylchrysene, which lacks a bay region methyl group, and the strong carcinogen 5-methylchrysene, which has a bay region methyl group. Metabolites of 6-methylchrysene were prepared using liver homogenates and were identified by their spectral properties and by comparison to synthetic standards as dihydrodiols, hydroxymethyl derivatives, and phenols; their relative levels of formation in liver homogenates from rats and mice were dependent on inducer pretreatment. In mouse skin in vivo, the major metabolite of 6-methylchrysene was trans-1,2-dihydro-1,2-dihydroxy-6-methylchrysene (6-MeC-1,2-diol), the precursor to a bay region dihydrodiol epoxide. Its concentration was greater than that of trans-1,2-dihydro-1,2-dihydroxy-5-methylchrysene (5-MeC-1,2-diol) formed in mouse skin from 5-methylchrysene. Since 5-MeC-1,2-diol has been identified as a major proximate carcinogen of 5-methylchrysene, the further metabolism and tumorigenicity of 5-MeC-1,2-diol and 6-MeC-1,2-diol were compared. Both dihydrodiols were converted to 1,2,3,4-tetraols and to 1,2-dihydroxy metabolites to similar extents in mouse skin. However, 5-MeC-1,2-diol was significantly more active than was 6-MeC-1,2-diol as a tumor initiator on mouse skin. The formation of DNA adducts in mouse skin from 5-methylchrysene and 6-methylchrysene was compared. Both hydrocarbons gave qualitatively similar adduct patterns, but the formation of dihydrodiol epoxide type adducts was twice as great from 6-methylchrysene as from 5-methylchrysene. The results of this study indicate that the weak tumorigenicity of 6-methylchrysene compared to that of 5-methylchrysene is not due to differing rates of formation or further metabolism of their 1,2-dihydrodiols but is a likely consequence of the lower activity of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-methylchrysene compared to 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-5-methylchrysene; the unique structural feature of the latter is the presence of a methyl group and an epoxide ring in the same bay region.

INTRODUCTION

The position of methyl substitution in methylated PAH3 produced...
1.2,3,4-tetraols were extracted with ethyl acetate and purified by HPLC (System 1). The retention times of the major and minor isomers were 20 and 25 min, respectively. The MS of each isomer had a molecular ion at m/e 310. 5-MeC-1,2-diol was synthesized (13) and kindly provided by Dr. Ronald G. Harvey, University of Chicago.

1H[6-MeC-1,2-diol and 1H[5-MeC-1,2-diol were prepared metabolically using 9000 x g supernatant from livers of Aroclor-pretreated rats; 5-MeC-1,2-diol formed in this way has been characterized previously (14). These dihydrodiols were >99% pure by HPLC. 1H[6-MeC was prepared by catalytic tritium exchange (New England Nuclear, Boston, MA) of 12-bromo-6-MeC, obtained as follows. A suspension of 6-MeC (48 mg) in acetic acid (5 ml) was heated under reflux for 10 min. A solution of bromine (160 mg) in 2 ml of acetic acid was then added dropwise, and refluxing was continued for 30 min. The product separated as a crystalline material, mp 223-224°C (40 mg). NMR spectrum: δ 2.9 (s, 3H), 7.6-7.9 (m, 4H), 8.1-8.25 (m, 1H), 8.5 (s, 1H), 8.6-8.85 (m, 2H), and 9.0 (s, 1H); MS m/e (relative intensity): 322 (M*, 92.5), 320 (M+, 100), and 269 (86.3). The crude 1H[6-MeC was purified by preparative thin-layer chromatography on a 20 x 20 cm silica gel plate, using a solvent system of hexane:CH2Cl2 (80:20). Rf = 0.59. This yielded 1H[6-MeC with purity greater than 99%, 37.2 Ci/mmol. The UV spectrum, HPLC retention times and Rf value were identical to those of unlabeled 6-MeC.

6-Hydroxymethylchrysene was prepared as illustrated in Chart 24. A solution of phenylacetic acid (2.7 g, 0.02 mol; Aldrich Chemical Co., Milwaukee, WI), 1-naphthaldehyde (3.1 g, 0.02 mol; Aldrich), 5 ml of triethylamine, and 5 ml of acetic anhydride was heated with stirring at 150°C for 6 h. After cooling, the dark solution was diluted with H2O (150 ml) and acidified with 50% HCl. The resulting suspension was extracted with CH2Cl2 (2 x 150 ml). The CH2Cl2 layer was washed with H2O, dried (MgSO4), and concentrated to afford 3.0 g of 2-phenyl-3-(1-naphthyl)propenoic acid, m.p. 149-151°C. MS m/e (relative intensity): 274 (M*, 50.5) and 229 (100).

A mixture of the above acid (2.7 g, 0.01 mol), K2CO3 (2.7 g, 0.02 mol), and dimethyl sulfate (1.8 g, 0.015 mol) in 150 ml acetonitrile was heated under reflux for 4 h. The reaction mixture was then filtered, and the K2CO3 was washed several times with CH2Cl2. The organic layer was washed with H2O, dried (MgSO4), and evaporated to give the crude ester. This was purified by chromatography on silica gel with a solvent system of hexane:CH2Cl2 (80:20) to give 2.5 g (89%) of pure methyl-2-phenyl-3-(1-naphthyl)propenoate, m.p. 127-128°C. NMR spectrum: δ 3.85 (s, 3H), 6.9-7.3 (m, 7H), 7.4-7.9 (m, 4H), 8.05-8.2 (m, 1H), and 8.45 (s, 1H); MS m/e (relative intensity): 288 (M*, 56.4) and 229 (100).

A solution of this ester (1.0 g, 0.034 mol) and iodine (5 mg) in dry benzene was stirred, and dry iodine was bubbled through the solution. This was irradiated for 6 h at room temperature with a Hanovia 450-W medium-pressure mercury lamp, using a Pyrex filter. Removal of the solvent gave 800 mg of a light yellow compound which was recrystallized from ethanol to give pure 6-carboxymethoxychrysene (0.5 g, 51%), m.p. 147-148°C. NMR spectrum: δ 4.1 (s, 3H), 7.6-7.8 (m, 5H), 7.9-8.1 (m, 2H), 8.65-8.9 (m, 3H), and 8.95-9.15 (m, 1H); MS m/e (relative intensity): 286 (M*, 100), 255 (51), 227 (40), and 226 (53).

To a slurry of lithium aluminum hydride (36 mg, 0.001 mol) in ether (100 ml) at 0°C was added 6-carboxyethylchrysene (0.28 g, 0.001 mol) in ether (10 ml). The reaction mixture was allowed to stir at 0°C for 1 h. Ethyl acetate was added slowly, and the reaction mixture was acidified with 1 N HCl. The organic phase was separated, collected, washed with saturated aqueous NaCl, dried (MgSO4), and concentrated to yield 6-hydroxymethylchrysene as a white solid (0.2 g, 77%). It was recrystallized from methanol, m.p. 207°C. NMR spectrum: δ 6.5 (1H, CH); 7.4-7.8 (m, 4H), 7.95-8.1 (m, 2H), 8.13-8.3 (m, 1H), and 8.6-8.9 (m, 4H); and MS m/e (relative intensity): 258 (M*, 100) and 229 (100).

7-Hydroxy-6-MeC was prepared by a method similar to that illustrated in Chart 2A for 6-hydroxymethylchrysene. The starting materials were o-methoxyphenylacetic acid and 1-naphthaldehyde. The esterified condensation product was photocylized to give 6-carboxymethoxy-7-methoxychrysene. This product was oxidized with pyridinium chlorochromate and then reduced with hydrazine and KOH to give 7-methoxy-6-MeC. 7-Hydroxy-6-MeC was obtained by treating 7-methoxy-6-MeC with BBBr in CH2Cl2. NMR spectrum: δ 4.18 (s, 3H), 6.85 (d, 1H, J8,9 = 7.2 Hz), 7.55-7.74 (m, 2H), 7.85-7.95 (m, 2H), 8.45-8.55 (s+d, 2H), 8.55 (d, 1H), and 8.72 (d, 1H); and MS m/e (relative intensity): 258 (M*, 100).

The method used for the synthesis of 1-hydroxy-6-MeC is summarized in Chart 2B. A solution of 1-bromomethyl-4-methylnaphthalene (15) (2.34 g, 0.01 mol) and 2.6 g (0.01 mol) of triphenylphosphine (Aldrich Chemical Co.) in 50 ml of methanol was added sodium methoxide (0.1 g, 0.002 mol). The mixture was stirred at room temperature for 1 h and then diluted with H2O and extracted with CH2Cl2. The organic solution was washed with H2O, dried (MgSO4), and concentrated. The resulting oil was purified by gel filtration. To a stirred solution of this salt (0.9 g, 0.002 mol) and o-anisaldehyde (0.27 g, 0.002 mol; Aldrich Chemical Co.) in 50 ml of methanol was added sodium methoxide (0.1 g, 0.002 mol). The mixture was stirred at room temperature for 1 h and then diluted with H2O and extracted with CH2Cl2. The organic solution was washed with H2O, dried (MgSO4), and concentrated. The resulting oil was purified by silica gel chromatography with a solvent system of hexane:CH2Cl2 (80:20). This procedure gave cis- and trans-1-(4-methyl-1-naphthyl)-2-(2-methoxyphenyl)ethylene, as an oil; NMR spectrum: δ 2.6 (s, 0.9H, cis- or trans-CH3), 2.65 (s, 2.1H, cis- or trans-CH3), 3.75 (s, 0.9H, cis- or trans-OCH3), 3.85 (s, 2.1H, cis- or trans-OCH3), 6.8-7.8 (m, 10H), 7.9-8.05 (m, 1H), and 8.15-8.3 (m, 1H); and MS m/e (relative intensity): 274 (M*, 100), 259 (63.2), and 243 (39.3).

The photochemical conversion of this alkene (100 mg, 0.0036 mol) to 1-methoxy-6-MeC was accomplished in 40% yield by the procedure
described above for preparation of 6-carbomethoxychrysene. The crude product was purified by column chromatography on silica gel with a solvent system of CH₂Cl₂/hexane. 1-Methoxy-6-MeC (38 mg) was isolated as a crystalline solid, m.p. 159–160°C. NMR spectrum: δ 2.8 (s, 3H), 3.95 (s, 3H), 6.9 (d, 1H), 7.3–7.8 (m, 3H), 8.0–8.15 (m, 1H), and 8.25–8.5 (m, 5H); and MS m/e (relative intensity), 272 (M⁺, 100) and 257 (35.4).

\[ C_{16}H_{14}O \]  
Calculated: C 88.20, H 5.92  
Found: C 87.94, H 6.18

A solution of 1-methoxy-6-MeC (13 mg, 0.05 mmol) in 5 ml of CH₂Cl₂ was stirred under N₂ and boron tribromide (1 M solution in CH₂Cl₂, 0.5 ml, Aldrich Chemical Co.) was added dropwise at room temperature. The reaction mixture was stirred for 1 h, diluted with H₂O, and extracted with CH₂Cl₂. The organic solution was washed with H₂O, dried (MgSO₄), and concentrated at reduced pressure to a residue which was dissolved in methanol. Metabolites were analyzed by HPLC, using System 1. A portion was acetylated as described above for 6-carbomethoxychrysene. The resulting mixture was added to two 500-ml Erlenmeyer flasks and incubated with shaking at 37°C for 20 min, after which each flask was treated with 80 ml acetonitrile. The combined mixtures were extracted 4 times with 160 ml ethyl acetate. The ethyl acetate layers were combined, dried (MgSO₄), and concentrated to a residue which was dissolved in MeOH. Metabolites were analyzed by HPLC using System 1. A portion was acetylated as described above for 1,2-dihydroxy-6-MeC and analyzed by HPLC.

Metabolism in Vivo. For the metabolism of 6-MeC, five groups of 4 mice each were shaved and, 24 h later, 0.15 ml of an acetonol solution of [³H]6-MeC (1.7 Ci/mmol, 0.07 µmol) was applied to the shaved back of each mouse. Mice were sacrificed at time intervals, as indicated in Table 2, and the epidermal metabolites were isolated exactly as described previously (16). Metabolites were analyzed by HPLC, using Systems 1 and 2. For the comparative study of 5-MeC-1,2-diol and 6-MeC-1,2-diol, 2 groups of 4 mice were shaved and treated with either [³H]5-MeC-1,2-diol (0.05 µmol, 60 µCi/µmol) or [³H]6-MeC-1,2-diol (0.05 µmol, 105 µCi/µmol) per mouse. They were killed 2 h later, and metabolites were isolated and analyzed by HPLC using System 3.

DNA Adducts in Mouse Skin. A group of 21 mice was used. Each mouse was treated with [³H]6-MeC (0.4 µmol, 15.2 Ci/mmol) in 0.15 ml acetone. Twenty-four h later, the mice were sacrificed. DNA was isolated from the epidermis of the treated areas and hydrolyzed enzymatically to deoxyribonucleosides (17), and the hydrolysates were analyzed by Sephadex LH-20 chromatography as described previously (16, 18) and by HPLC using System 3.

Bioassay for Tumor-initiating Activity. Each group consisted of 20 female CD-1 mice obtained at age 28–35 days. At age 50–55 days, each mouse received a single initiating dose of 33 mmol of the appropriate compound in 0.1 ml acetone. Ten days later, promotion began by application of 2.5 µg 12-O-tetradecanoylphorbol-13-acetate in 0.1 ml of acetone, 3 times weekly for 20 weeks. Mice were shaved when necessary, and tumors were counted weekly. Statistical significance was evaluated using the χ² test.

RESULTS

Chart 3 is a chromatogram obtained upon reverse phase HPLC analysis of the metabolites of 6-MeC formed by incubation with liver 9000 × g supernatant from rats pretreated with Aroclor 1254. This preparation was used for the structural studies because it produces sufficient amounts of metabolites for spectral characterization. The MS of peak 1 had a molecular ion of m/e 276, which suggests the presence of dihydrodiol metabolites. From the early elution position of peak 1, we suspected that it might contain a...
mixture of dihydrodiols with their hydroxyl groups in pseudooxial conformations. Therefore, it was collected and reanalyzed using normal phase HPLC. The resulting chromatogram showed two peaks, A (88%) and B (12%). The UV spectra of peaks A and B were similar to those of 5-MeC-3,4-diol or 5-MeC-9,10-diol and 5-MeC-1,2-diol or 5-MeC-7,8-diol, respectively. Peaks A and B were each collected and treated under conditions known to cause dehydration of dihydrodiols. Dehydration of peak A gave a mixture of two chrysenols. One had a UV spectrum similar to that of 3-hydroxy-5-MeC or 9-hydroxy-5-MeC, and the other caused dehydration of dihydrodiols. Dehydration of peak A gave 7-hydroxy-6-MeC, which was identified by comparison of its UV spectrum and HPLC retention time to those of the synthetic standard. Peak 4 was identified as 6-hydroxymethylchrysene by comparison of its UV spectrum, MS, NMR spectrum, and HPLC retention time to those of the synthetic standard.

Peak 5 had a UV spectrum similar to those of 3-hydroxy-5-MeC and 9-hydroxy-5-MeC. It was tentatively identified as 3- and/or 9-hydroxy-6-MeC.

Peak 6 was identified as 1-hydroxy-6-MeC by comparison of its UV spectrum, MS, NMR spectrum, and HPLC retention time to those of the synthetic standard. Peak 7 was identified as 7-hydroxy-6-MeC by comparison of its UV spectrum and HPLC retention time to those of the synthetic standard.

The formation of metabolites of [3H]6-MeC was assessed in liver 9000 x g supernatant from mice treated with either 3-methylcholanthrene in corn oil or with corn oil alone. The results are summarized in Table 1. Data from a previous study of [3H]5-MeC metabolism carried out under identical conditions are included for comparison (20). The metabolism of [3H]5-MeC to its 1,2-dihydrodiol was greater than that of [3H]6-MeC to its 1,2-dihydrodiol in control mouse liver, but in 3-methylcholanthrene-pretreated mice these dihydrodiols were formed in similar quantities. Hydroxymethylation of [3H]5-MeC and [3H]6-MeC was similar in both uninduced and induced mice. Chrysene formation from [3H]5-MeC exceeded that from [3H]6-MeC in both uninduced and induced mice. The ratio of 1-hydroxy-6-MeC to 7-hydroxy-6-MeC was 1 in control mice but 6.6 in 3-methylcholanthrene-pretreated mice, reflecting an induction of 1-hydroxy-6-MeC formation and a suppression of 7-hydroxy-6-MeC formation in 3-methylcholanthrene-pretreated mice.

The metabolism of [3H]6-MeC was then studied in mouse skin in vivo, using the technique which we have described previously (18). The radioactivity recovered from mouse epidermis at various intervals after treatment with [3H]6-MeC accounted for percentages of the dose as follows: 0.67 h (64.4), 1 h (67.9), 2 h (47.2), and 4 h (23.2). The ethyl acetate extracts, which contained >99% of the recovered radioactivity, were analyzed by HPLC using the identified metabolites as markers. A chromatogram of the organic soluble metabolites formed after 2 h is illustrated in Chart 4. The major metabolite was 6-MeC-1,2-diol. The material eluting in 23–25 min was reanalyzed by normal phase HPLC and was resolved into two components, corresponding in retention times to 6-MeC-3,4-diol and 6-MeC-9,10-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Formation of selected metabolites of [3H]6-MeC and [3H]5-MeC by 9000 x g supernatants from control and 3-methylcholanthrene-treated mice</th>
</tr>
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<tr>
<td>[3H]6-MeC was incubated for 20 min with cofactors and 9000 x g supernatant from the livers of mice which had been treated with 3-methylcholanthrene in corn oil (75 mg/kg) 48 h prior to sacrifice or from control animals treated with corn oil only. Metabolites were analyzed by HPLC as in Chart 3 and identified by coelution of standards with radioactivity. Data from a previous study of [3H]5-MeC metabolism under identical conditions (20) are included for comparison.</td>
<td></td>
</tr>
<tr>
<td>6-MeC (nmol/mg protein)</td>
<td>5-MeC (nmol/mg protein)</td>
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<tr>
<td>Metabolite</td>
<td>Control</td>
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<tr>
<td>1,2-Dihydrodiol</td>
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<tr>
<td>7,8-Dihydrodiol</td>
<td>0.13</td>
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<tr>
<td>3,4(9,10)-dihydrodiol</td>
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<td>6,9(10)-dihydroxy-6-MeC</td>
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<tr>
<td>7-Hydroxy</td>
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<tr>
<td>9- or 3-Hydroxy</td>
<td>0.04</td>
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</table>

* 7,8-Dihydrodiol only.
METABOLIC ACTIVATION OF 6-MeC AND 5-MeC

Chart 4. Chromatogram obtained upon HPLC analysis using System 1 of the ethyl acetate extract of mouse epidermis 2 h after treatment with [3H]6-MeC. 6-HOMeC, 6-hydroxymethylchrysene; 1-OH-6-MeC, 1-hydroxy-6-MeC.

The further metabolism of 6-MeC-1,2-diol was examined using 9000 x g supernatant from the livers of Aroclor-pretreated rats. As illustrated in Chart 6, 6-MeC-1,2-diol was efficiently converted to 1,2-dihydroxy-6-MeC in this system. The metabolite was identified by comparison of its UV spectrum and HPLC retention times to those of the synthetic standard. The retention times of the diacetate derivatives of the metabolite and standard were also identical. Small amounts of material with retention times the same as those of 6-MeC-1,2,3,4-tetraols were detected. The metabolism of 5-MeC-1,2-diol under these conditions was more complex (data not shown).

The tumor-initiating activities of 5-MeC, 6-MeC, 5-MeC-1,2-diol, and 6-MeC-1,2-diol were compared. The results are summarized in Table 2. In agreement with earlier studies, 5-MeC was significantly more tumorigenic than was 6-MeC. 5-MeC-1,2-diol was also significantly more tumorigenic than was 6-MeC-1,2-diol.

DISCUSSION

In this study, we sought to determine the reasons why 6-MeC is a weak carcinogen and 5-MeC is a strong carcinogen. The
METABOLIC ACTIVATION OF 6-MeC AND 5-MeC

Chart 7. Sephadex LH-20 chromatograms of enzymatically hydrolyzed DNA from the epidermis of mice treated with (A) [3H]6-MeC and (B) [3H]5-MeC. The data in (A) were from a previous study (16) and are included for comparison. The specific activity of [3H]5-MeC used in that study was one-tenth that of the [3H]6-MeC; the data in (B) were normalized to equal specific activity.

Table 2
Comparative tumor-initiating activity on mouse skin of 5-MeC, 6-MeC, 5-MeC-1,2-diol, and 6-MeC-1,2-diol

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of tumor-bearing mice</th>
<th>Tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeC</td>
<td>66*</td>
<td>1.7</td>
</tr>
<tr>
<td>6-MeC</td>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>5-MeC-1,2-diol*</td>
<td>80*</td>
<td>2.5</td>
</tr>
<tr>
<td>6-MeC-1,2-diol*</td>
<td>25</td>
<td>0.25</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* P < 0.01 compared to control.
* P < 0.01 compared to 6-MeC.
* Synthetic and racemic.
* P < 0.01 compared to 6-MeC-1,2-diol.
* Metabolically formed; optical purity unknown.

The structural difference between the two compounds is the position of the methyl group. In 5-MeC, the methyl group is in the 4-5 bay region, and this causes an increase in the angles in the bay region and a distortion from planarity which is not likely to occur in 6-MeC (21, 22). In previous studies we have shown that 5-MeC-1,2-diol, which can form a bay region dihydrodiol epoxide with the methyl group and an epoxide ring in the same bay region, is a major proximate carcinogen of 5-MeC and that one of the dihydrodiol epoxide isomers, anti-5-MeC-1,2-diol-3,4-epoxide, is a major ultimate carcinogen (23, 24). It has been suggested that structural distortions resulting from the presence of a bay region methyl group in compounds such as 5-MeC and 7,12-dimethylbenz[a]anthracene may lead to enhanced formation of potential proximate carcinogenic metabolites such as 5-MeC-1,2-diol (10). The results of the present study demonstrate that this is not the case in the methylichrysene system. Whereas 5-MeC-1,2-diol formation exceeded 6-MeC-1,2-diol formation in mouse liver, the opposite result was observed in the target tissue, mouse epidermis. 6-MeC-1,2-diol was the major metabolite of 6-MeC, and its concentration in mouse skin was greater than the concentration of 5-MeC-1,2-diol in the skin of mice treated with 5-MeC. Thus, differing extents of formation of 1,2-dihydrodiol metabolites do not account for the greater carcinogenicity of 5-MeC compared to 6-MeC.

The results of the tumorigenicity assay show clearly that 5-MeC-1,2-diol was more tumorigenic than 6-MeC-1,2-diol. The tumorigenic activity of 6-MeC-1,2-diol was comparable to that of 5-MeC-7,8-diol (23, 24). 6-MeC-1,2-diol and 5-MeC-7,8-diol can both form bay region dihydrodiol epoxides, but neither has a methyl group in the same bay region as the epoxide ring, as in 5-MeC-1,2-diol. These comparative studies demonstrate that 5-MeC-1,2-diol is a stronger proximate carcinogen than is 6-MeC-1,2-diol.

The results of the comparative DNA binding studies of [3H]-6-MeC and [3H]-5-MeC are consistent with the lower tumorigenic activity of 6-MeC-1,2-diol than of 5-MeC-1,2-diol. Major DNA adducts are formed from 5-MeC, through its 1,2-dihydrodiol-3,4-epoxide metabolites. Minor adducts are formed from its 7,8-dihydrodiol-9,10-epoxides (16, 18). Radioactive peaks, with retention times on Sephadex LH-20 and HPLC similar to those in 5-MeC dihydrodiol epoxide adducts, were also formed from [3H]-6-MeC but in much lower concentrations. These adducts are assumed to be 6-MeC dihydrodiol epoxide-DNA adducts, and their levels in mouse skin were approximately 1/20 as great as the corresponding 5-MeC dihydrodiol epoxide-DNA adducts. Thus, although 6-MeC-1,2-diols was formed to a greater extent in mouse skin than was 5-MeC-1,2-diol, its tumorigenicity and conversion to apparent dihydrodiol epoxide-DNA adducts was far less than that of 5-MeC-1,2-diol.

We sought to explain these differences by studying the further metabolism of 6-MeC-1,2-diol. Our initial experiments were carried out using liver 9000 x g supernatant from Aroclor-pretreated rats, in order to facilitate identification of metabolites. In this system, 6-MeC-1,2-diol underwent a remarkably smooth conversion to 1,2-dihydroxy-6-MeC, as illustrated in Chart 6. Only small amounts of material with the retention times of 6-MeC-1,2-diol and 5-MeC-1,2-diol were formed from [3H]-6-MeC. Therefore, dihydroxy metabolites do not account for the greater tumorigenicity of 6-MeC compared to 5-MeC. Although 6-MeC-1,2-diol was formed to a greater extent in mouse skin than was 5-MeC-1,2-diol, its metabolism and conversion to apparent dihydrodiol epoxide-DNA adducts was far less than that of 5-MeC-1,2-diol. These studies demonstrate that 5-MeC-1,2-diol is a stronger proximate carcinogen than is 6-MeC-1,2-diol.

In previous studies we have shown that anti-5-MeC-1,2-diol-3,4-epoxide is a stronger tumorigen than is anti-5-MeC-7,8-diol-9,10-epoxide (24) and that it binds to DNA to a greater extent than does anti-5-MeC-7,8-diol-9,10-epoxide (25). These studies demonstrated that 5-MeC-1,2-diol is a stronger proximate carcinogen than is 6-MeC-1,2-diol.
led to the conclusion that the enhancing effect of a bay region methyl group on methylchrysene tumorigenicity was due to the unique tumorigenicity and greater DNA binding properties of a dihydrodiol epoxide isomer with the methyl group and epoxide ring in the same bay region. The results of the present study are in agreement with this hypothesis. They lead to the prediction that the DNA binding properties of anti-6-MeC-1,2-diol-3,4-epoxide would be low compared to those of anti-5-MeC-1,2-diol-3,4-epoxide. This prediction is currently being tested experimentally.

The structural requirements favoring tumorigenicity of methylated alternant PAH are the presence of a bay region methyl group and free peri-position both adjacent to an unsubstituted angular ring, as in 5-MeC (4, 5). In the methylchrysene series, only 5-MeC fulfills these requirements and can form a bay region dihydrodiol epoxide with the methyl group and epoxide ring in the same bay region. The results of this study provide further evidence for the importance of this unique type of bay region dihydrodiol epoxide in methylchrysene tumorigenesis and probably in methylated PAH tumorigenesis.

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