ABSTRACT:

The studies presented here were designed to elucidate the enzymes involved in the biotransformation of naturally occurring 1,8-dihydroxyanthraquinones and to investigate whether biotransformation of 1,8-dihydroxyanthraquinones may represent a bioactivation pathway. We first studied the metabolism of emodin (1,3,8-trihydroxy-6-methylanthraquinone), a compound present in pharmaceutical preparations. With rat liver microsomes, the formation of two emodin metabolites, ω-hydroxymedin and 2-hydroxymedin, was observed. The rates of formation of ω-hydroxymedin were not different with microsomes from rats that had been pretreated with inducers for different cytochrome P450 enzymes. Thus, the formation of ω-hydroxymedin seems to be catalyzed by several cytochrome P450 enzymes at low rates. The formation of 2-hydroxymedin was increased in liver microsomes from 3-methylcholanthrene-pretreated rats and was inhibited by 2-naphthoflavone, by an anti-rat cytochrome P450 1A1/2 antibody, and, to a lesser degree, by an anti-rat cytochrome P450 1A1 antibody. These data suggest the involvement of cytochrome P450 1A2 in the formation of this metabolite. However, other cytochrome P450 enzymes also seem to catalyze this reaction. The anthraquinone chrysophanol (1,8-dihydroxy-3-methylanthraquinone) is transformed, in a cytochrome P450-dependent oxidation, to aloe-emodin (1,8-dihydroxy-3-hydroxymethylanthraquinone) as the major product formed. The mutagenicity of the parent dihydroxyanthraquinones and their metabolites was compared in the in vitro micronuclear test in mouse lymphoma L5178Y cells. 2-Hydroxymedin induced much higher micronucleus frequencies, compared with emodin. ω-Hydroxymedin induced lower micronucleus frequencies, compared with emodin. Aloe-emodin induced significantly higher micronucleus frequencies than did chrysophanol. These data indicate that the cytochrome P450-dependent biotransformation of emodin and chrysophanol may represent bioactivation pathways for these compounds.

1,8-Dihydroxyanthraquinones (fig. 1) are the active compounds of many plant-derived drugs (Thomson, 1986) that are used as laxatives. Aloe and senna are the most widely used, anthraquinone-containing, herbal drugs. With the widespread use and abuse of laxatives, concern about possible toxic and tumorigenic effects induced by dihydroxyanthraquinones was provoked by the observation that the 1,8-dihydroxyanthraquinone danthron (synonym, chrysazin) produced an increased incidence of intestinal tumors in rats (Mori et al., 1985) and adenomatous hyperplasia of the caecum and liver tumors in mice (Mori et al., 1986). An increased risk for colorectal cancer in humans was reported among abusers of 1,8-dihydroxyanthraquinone-containing laxatives in one study (Siegers et al., 1993), although other studies did not confirm these findings (Kune, 1993). Furthermore, the mutagenic and genotoxic activities of naturally occurring 1,8-dihydroxyanthraquinones and some of their metabolites were discussed controversially (Bruggemann and van der Hoeven, 1984; Westendorf et al., 1990). Both positive and negative results have been obtained (Masuda et al., 1985; Masuda and Ueno, 1984). However, we recently reported that emodin, aloe-emodin, and danthron were genotoxic in mammalian cells without exogenous metabolic activation (Müller et al., 1996).

The role of biotransformation reactions, resulting in genotoxic metabolites of dihydroxyanthraquinones, is not well defined. The microsomal biotransformation of emodin (1,3,8-trihydroxy-6-methylanthraquinone) was thought to be mediated by cytochrome P450. However, in studies with rat liver microsomes, reaction rates were not determined and possible roles of specific cytochrome P450 enzymes in the generation of metabolites were not investigated (Kodama et al., 1987; Masuda et al., 1985; Masuda and Ueno, 1984; Murakami et al., 1987; Tanaka et al., 1987). The biotransformation of other 1,8-dihydroxyanthraquinoids has apparently not been studied.

After oral administration of 1,8-dihydroxyanthraquinones, intesti-
nal uptake is efficient; urinary excretion of conjugates and marked enterohepatic circulation were reported (de Witte, 1993; Hattori et al., 1993). Therefore, the cytochrome P450-dependent biotransformation of these compounds in the liver could reflect a pathway of bioactivation resulting in the formation of toxic intermediates.

Here, we attempted to elucidate the extent of cytochrome P450-dependent biotransformation of emodin and chrysophanol in rat liver microsomes and the possible involvement of particular cytochrome P450 enzymes. To define a role for anthraquinone biotransformation in the formation of genotoxic metabolites, we also investigated the genotoxicity of identified metabolites in mammalian cells.

### Materials and Methods

**Chemicals.** Emodin, aloe-emodin, and chrysophanol were purchased from Roth (Karlsruhe, Germany), in the highest purity available (>95%, as determined by HPLC). All other chemicals were obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany).

**Synthesis.** 2-Hydroxyemodin was prepared as described (Banks et al., 1978). In short, emodin was oxidized with persulfate in sulfuric acid, and the desired product was isolated by preparative TLC. This method yielded 1.00 g (6.3%) of 2-hydroxyemodin as a red crystalline solid, with a purity of >83% (as judged by HPLC with UV detection at 290 nm) [melting point, 295°C; 1H-NMR (250 MHz, dimethylsulfoxide-d_6): δ 2.4 (s, 3H, -CH_3); 7.1 (s, 1H, ArH), 7.2 (s, 1H, ArH), and 7.7 (d, J = 1.9 Hz, 1H, ArH)]; electrospray MS: m/z (relative intensity) 286 (M^+ 100%); UV (0.2% NaHCO_3/acetonitrile, 60:40): λ<sub>max</sub> (log ε) = 215 nm (4.222), 290 (4.222), and 435 (3.824)].

ω-Hydroxyemodin was prepared in a scaled-up microsomal incubation. The incubation volume was 50 ml, and all other conditions were as described above. Biosynthetic ω-hydroxyemodin was isolated by preparative HPLC (steel column, 250 × 8 mm, filled with Partisil ODS 3 resin, 5 μm; solvent A, H_2O, adjusted to pH 2 with CF_3CO_2H; solvent B, acetonitrile; linear gradient, 14% B to 100% B in 30 min; flow rate, 2 ml/min; detection wavelength, 225 nm). ω-Hydroxyemodin was obtained as a yellow solid, with a purity of >95% (as judged by HPLC with UV detection at 225 nm) [1H-NMR (250 MHz, CDCl_3): δ 2.1 (s, 2H, -CH_2-OH) and 7.2 (s, 4H, ArH)]; electrospray MS: m/z (relative intensity) 286 (M^+ 100%); UV (0.2% NaHCO_3/acetonitrile, 60:40): λ<sub>max</sub> (log ε) = 215 nm (4.103), 250 (3.824), 270 (3.845), 290 (3.865), and 435 (5.637)].

**Microsomal Incubations.** Complete incubation systems (final volume, 500 μl) contained liver microsomal protein (up to 2 mg/ml), 0.1 M phosphate buffer (pH 7.4), and an NADPH-generating system consisting of 10 mM glucose-6-phosphate, 1 mM NADP<sup>+</sup>, and 0.5 IU/ml yeast glucose-6-phosphate dehydrogenase (Werner et al., 1995). When kinetic studies were performed, the reaction was started by the addition of the NADPH-generating system and was terminated after 15 min at 37°C by extraction of emodin and products with two-ml aliquots of ethyl acetate. Substrate concentrations varied from 0.1 to 1 mM. After centrifugation, the ethyl acetate phase was concentrated under reduced pressure. The residue was dissolved in acetonitrile and analyzed by HPLC (steel column, 250 × 4 mm, filled with Partisil ODS resin, 5 μm); separation was performed by gradient elution (linear gradient from 86% H_2O, adjusted to pH 2 with CF_3CO_2H, to 100% acetonitrile in 30 min, at a flow rate of 1 ml/min). Eluting material was monitored at 225 nm (λ<sub>max</sub>): HPLC (steel column, 250 × 8 mm, filled with Partisil ODS resin, 5 μm; solvent A, H_2O, adjusted to pH 2 with CF_3CO_2H; solvent B, acetonitrile; linear gradient, 14% B to 100% B in 30 min; flow rate, 2 ml/min; detection wavelength, 225 nm).

**Animals and Treatment.** Adult male and female Wistar rats were used for all studies (Harlan-Winkelmann, Borchen, Germany); they had free access to water and a standard diet (Altromin; Harlan-Winkelmann). Cytochrome P450 induction experiments were performed, according to literature protocols, with dexamethasone, phenobarbital (Benoit et al., 1992), and 3-methylcholanthrene (Rodrigues and Prough, 1991). Twenty-four hours after the last administration of the inducers, animals were sacrificed by cervical dislocation and liver microsomes were prepared as described (Dohn and Anders, 1982; Wolf et al., 1984).

**7-Ethoxyresorufin O-dealkylation and 7-pentoxyresorufin O-dealkylation

### Table 1

<table>
<thead>
<tr>
<th>Incubation Conditions&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Formation of α-Hydroxyemodin</th>
<th>Formation of 2-Hydroxyemodin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>% of control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−NADPH</td>
<td>&lt;5</td>
<td>&lt;2</td>
</tr>
<tr>
<td>−Protein</td>
<td>&lt;4</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Heat inactivation (45°C, 3 min)</td>
<td>73 ± 19</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>Heat inactivation (100°C, 5 min)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>+CO/O_2 (80:20)</td>
<td>26 ± 7</td>
<td>16 ± 3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Incubations were performed as described in Materials and Methods, with 500 μM emodin.

<sup>b</sup> Rates of formation of α-hydroxyemodin and 2-hydroxyemodin were determined to be 23 ± 5 and 2.6 ± 0.5 nmol/min/mg protein, respectively (mean ± SD, N = 3), in liver microsomes from untreated rats.
TABLE 2  
Rates of formation of ω-hydroxymedin and 2-hydroxymedin and marker enzyme activities in liver microsomes from rats treated with different cytochrome P450 inducers

<table>
<thead>
<tr>
<th>Microsomal Preparation</th>
<th>Formation of ω-Hydroxymedin</th>
<th>Formation of 2-Hydroxymedin</th>
<th>7-Ethoxyresorufin O-Dealkylation</th>
<th>7-Pentyloxyresorufin O-Dealkylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT male rats</td>
<td>23 ± 4.5</td>
<td>2.4 ± 0.5</td>
<td>0.033 ± 0.016</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>UT female rats</td>
<td>20 ± 5</td>
<td>2.0 ± 0.9</td>
<td>0.050 ± 0.007</td>
<td>0.0021 ± 0.0002</td>
</tr>
<tr>
<td>DEX-pretreated male rats</td>
<td>15 ± 5</td>
<td>4.2 ± 2.8</td>
<td>0.027 ± 0.013</td>
<td>≤0.0021</td>
</tr>
<tr>
<td>DEX-pretreated female rats</td>
<td>17 ± 2</td>
<td>2.2 ± 1.5</td>
<td>0.016 ± 0.005</td>
<td>0.0021 ± 0.0002</td>
</tr>
<tr>
<td>PB-pretreated male rats</td>
<td>36 ± 1b</td>
<td>2.0 ± 0.3</td>
<td>0.226 ± 0.067</td>
<td>0.358 ± 0.121</td>
</tr>
<tr>
<td>MC-pretreated male rats</td>
<td>19 ± 2.5</td>
<td>9.0 ± 1.6</td>
<td>4.52 ± 1.39</td>
<td>0.045 ± 0.005</td>
</tr>
</tbody>
</table>

7-Ethoxyresorufin O-dealkylation was used as a marker activity for cytochrome P450 1A1(2) and 7-pentyloxyresorufin O-dealkylation was used as a marker activity for cytochrome P450 2B.

*α-Terbutylphenol; DEX, dexamethasone; PB, phenobarbital; MC, 3-methylcholanthrene.

**Significant increase of the formation rate in liver microsomes of pretreated male rats, compared with untreated male rats (Student’s t test), p < 0.05.

**p < 0.01.

(Burke et al., 1985) served as marker enzyme activities for the determination of microsomal cytochrome P450 1A1/2 and 2B1/2, respectively. Protein concentrations were determined by the method of Bradford (1976), using the Bio-Rad protein kit (Bio-Rad, Munich, Germany) and bovine γ-globulin as the standard.

**Inhibition of Emodin Hydroxylation by Antibodies to Cytochromes P450 1A1, 1A2, and 2B1/2.** Inhibitory anti-rat cytochrome P450 1A1, 1A2, and 2B1/2 antibodies were purchased from Daiichi (Tokyo, Japan) through Natutec (Frankfurt, Germany). The antibodies (50 μg/100 μg of microsomal protein) were preincubated with rat liver microsomes (1 mg/ml) for 30 min at room temperature. Then phosphate buffer (0.1 M, pH 7.4) and the NADPH-generating system were added, followed by emodin (final concentration, 0.75 mM) after a brief preincubation period. Final incubation volumes were 250 μl. Fifteen minutes after addition of the substrate, the reaction was stopped by extraction with ethyl acetate and product formation was quantified.

**Cell Culture.** Mouse L5178Y cells were cultured in suspension in RPMI 1640 medium supplemented with antibiotics, 0.25 mg/ml L-glutamine, 107 μg/ml sodium pyruvate, and 10% heat-inactivated horse serum (all obtained from Sigma Chemie GmbH, Deisenhofen, Germany). Cell cultures were grown in a humidified atmosphere with 5% CO2 in air at 37°C.

Genotoxicity of Anthraquinones and Metabolites in the In Vitro Micro-nucleus Test. Exponentially growing mouse L5178Y cells were treated for 4 hr with emodin, chrysophanol, the respective metabolites, or the vehicle (1% dimethylsulfoxide; as a control). After removal of the chemicals by centrifugation and medium replacement, the cells were incubated for 15 hr (expression time). The cells were then brought onto glass slides by cytospin centrifugation and were fixed with methanol (at −20°C for 1 hr). To stain nuclei and micronuclei, the slides were incubated with Hoechst 33258 (5 μg/ml, for 3 min), washed twice with calcium- and magnesium-free phosphate-buffered saline for 2 min, and mounted for microscopy. Numbers of nuclei and micro-nuclei were scored at a magnification of 1250×. Each data point represents the mean ± SD of three slides, with 2000 nuclei being evaluated on each slide. All experiments were repeated at least twice, with consistent results.

![FIG. 3. Dixon plot for the determination of Kᵢ values of α-naphthoflavone (NF) for the inhibition of 2-hydroxymedin formation in liver microsomes from 3-methylcholanthrene-pretreated male rats.](https://example.com/dixon_plot)

Data represent the mean of at least two independent incubations. The mean control activity was 3.2 nmol/min/mg. The mean Vₘₐₓ value for the calculation of the Kᵢ value was 5.1 nmol/min/mg, and the equation used was y = 0.0051x + 0.3013. The corresponding Kᵢ value was 21 μM.
Effects of inhibitory antibodies on the hydroxylation of emodin in rat liver microsomes

<table>
<thead>
<tr>
<th>Microsomal Preparation and Treatment</th>
<th>Activity$^a$</th>
<th>Formation of $\omega$-Hydroxyemodin</th>
<th>Formation of 2-Hydroxyemodin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB-pretreated male rats, +anti-rat</td>
<td>85</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>cytochrome P450 2B/1/2 antibody</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC-pretreated male rats, +anti-rat</td>
<td>100</td>
<td>80 ± 6</td>
<td></td>
</tr>
<tr>
<td>cytochrome P450 1A/1/2 antibody</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC-pretreated male rats, +anti-rat</td>
<td>100</td>
<td>51 ± 11</td>
<td></td>
</tr>
<tr>
<td>cytochrome P450 1A/1/2 antibody</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Incubations were performed as described in Materials and Methods, with the indicated variation and with 500 µM emodin, PB, phenobarbital; MC, 3-methylcholanthrene.

Results

Enzymes Involved in Emodin Hydroxylation, and Metabolite Identification. The biotransformation of emodin was studied in liver microsomes from male and female rats. In fig. 2A, a typical HPLC chromatogram obtained after an incubation of emodin with rat liver microsomes is shown. Two distinct new peaks, with retention times of 19 and 21 min, were observed. These peaks were not present in incubations without the NADPH-generating system or in incubation mixtures containing denatured microsomal proteins. Therefore, the peaks likely represent two emodin metabolites, designated M1 and M2, respectively. M1 and M2 were identified as $\omega$-hydroxyemodin (hydroxymethylmethylene) and 2-hydroxyemodin, respectively, by comparison of the electronic spectra (fig. 2B) and the mass spectra (not shown) of the metabolites with those of synthetic standards.

Hydroxylation of anthraquinones was reported to be catalyzed by cytochrome P450 (Masuda and Ueno, 1984). The formation of both emodin metabolites required NADPH and oxygen and was significantly reduced in the presence of carbon monoxide, an inhibitor of heme-dependent enzymes such as cytochrome P450. The rates of formation of both emodin metabolites were not markedly affected by heating of the microsomes at 43°C for 3 min. Heat treatment affects only the heat-labile flavin-containing monooxygenases and not cytochromes P450 (Ziegler, 1980) (table 1). These results are consistent with a major role for cytochrome P450 enzymes in the hydroxylation of 1,8-dihydroxyanthraquinones.

Effects of Various Cytochrome P450 Inducers and Inhibitors on the Rate of Emodin Hydroxylation. For elucidation of a role for specific cytochrome P450 enzymes in the hydroxylation of emodin, we determined the rates of metabolite formation with liver microsomes from dexamethasone-, phenobarbital-, or 3-methylcholanthrene-pretreated male rats (table 2). Significant increases in the rates of formation of $\omega$-hydroxyemodin and 2-hydroxyemodin were observed only with liver microsomes from phenobarbital-pretreated rats (1.6-fold) and 3-methylcholanthrene-pretreated rats (4-fold). Because phenobarbital is an inducer of cytochrome P450 2B (Benoit et al., 1992) and 3-methylcholanthrene is an inducer of cytochromes P450 1A1 and 1A2 (Rodrigues and Prough, 1991), we determined the rates of oxidation of marker substrates for cytochrome P450 2B (pentoxyresorufin dealkylation) and cytochrome P450 1A2 (ethoxyresorufin dealkylation) (table 2). No correlation between the biotransformation of the marker substrates pentoxyresorufin and pentoxyresorufin and the formation of the two emodin metabolites was observed. In addition, coinubcation of emodin and liver microsomes from untreated male rats with the cytochrome P450 3A1/2 mechanism-based inhibitor tolredomycin (Pessayre et al., 1981) or the cytochrome P450 2C competitive inhibitor sulfaphenazole (Veronese et al., 1990) did not result in significant reduction of emodin oxidation. The presence of these inhibitors reduced the biotransformation of marker substrates for these cytochrome P450 enzymes by >70% (Werner et al., 1995). Thus, emodin seems not to be a substrate for cytochrome P450 3A4/5 or cytochromes P450 2C. The rates of emodin oxidation to 2-hydroxyemodin were, however, significantly reduced in the presence of low concentrations (50 µM) of the cytochrome P450 1A2 competitive inhibitor $\alpha$-naphthoflavone (table 3). A $K_v$ value of 21 µM for $\alpha$-naphthoflavone was determined for the formation of 2-hydroxyemodin with liver microsomes from 3-methylcholanthrene-pretreated male rats (fig. 3). Formation of $\omega$-hydroxyemodin was markedly reduced (53 ± 12%) only by high concentrations (200 µM) of $\alpha$-naphthoflavone.

Inhibition of the formation of 2-hydroxyemodin by $\alpha$-naphthoflavone at low concentrations suggested the participation of the cytochrome P450 1A1 family. Therefore, incubations were performed with selective antibodies against cytochrome P450 1A1 and cytochrome P450 1A2 (with cross-reactivity against cytochrome P450 1A1) (table 3). According to the supplied information, the concentrations of the antibodies used reduced the marker activity for cytochrome P450 2B1 (testosterone 17β-hydroxylation) to 40% of uninhibited control levels and the marker activity for cytochrome P450 1A1 (7-ethoxycoumarin deethylation) by 35%. The cross-reactive anti-cytochrome P450 1A1/2 antibody reduced 7-ethoxycoumarin deethylation to 20% of uninhibited control levels and theophylline 3-demethylation (marker activity for cytochrome P450 1A2) to 27% of uninhibited control levels. Indeed, a significant reduction of the formation of 2-hydroxyemodin in liver microsomes from 3-methylcholanthrene-pretreated male rats in the presence of the anti-cytochrome P450 1A2 inhibitory antibody was observed. Because this antibody shows cross-reactivity against cytochrome P450 1A1, we performed a coincubation with an inhibitory antibody selective for cytochrome P450 1A1. This antibody has no cross-reactivity with other cytochrome P450 enzymes. Only a slight reduction in the rate of formation of 2-hydroxyemodin was found in the presence of this antibody. The presence

<table>
<thead>
<tr>
<th>Microsomal Preparation</th>
<th>$K_v$</th>
<th>$V_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td>mmol/min/mg</td>
</tr>
<tr>
<td>Untreated male rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB-pretreated male rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC-pretreated male rats</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values are given as mean ± SD (N = 3). PB, phenobarbital; MC, 3-methylcholanthrene.
of these antibodies reduced the oxidation of marker substrates for cytochrome P450 activities by >60% (table 4).

Because the highest rates of formation of ω-hydroxyemodin were found in liver microsomes from phenobarbital-pretreated rats, the participation of cytochrome P450 2B was investigated with a selective inhibitory antibody against cytochrome P450 2B. However, no reduction in the formation of ω-hydroxyemodin was observed in the presence of the antibodies (table 4). Also, we found inhibition of the formation of ω-hydroxyemodin at higher emodin concentrations (>750 μM) (data not shown).

**Enzyme Kinetics.** Kinetic parameters such as \( K_M \) and \( V_{max} \) were obtained from Lineweaver-Burke plots. Because the rates of formation of 2-hydroxyemodin in liver microsomes from untreated male rats were low even at substrate concentrations of 2 mM and could not be quantified at lower substrate concentrations, enzyme kinetic parameters were determined only with liver microsomes from 3-methylcholanthrene-pretreated male rats for enzymatic formation of 2-hydroxyemodin. As shown in table 5, \( V_{max} \) values for ω-hydroxyemodin formation were similar in microsomes from phenobarbital-treated rats and microsomes from 3-methylcholanthrene-pretreated rats, and these values were higher than those in microsomes from control rats (table 5).

**Bioactivation of Chrysophanol to Aloe-emodin by Microsomal Biotransformation.** Emodin and aloe-emodin are genotoxic in mammalian cells (Müller et al., 1996), whereas chrysophanol (fig. 1) is...
only weakly genotoxic without metabolic activation in mammalian cells (Müller et al., 1997). Cytochrome P450-dependent biotransformation of chrysophanol may yield aloe-emodin (fig. 1). Therefore, we investigated the cytochrome P450-dependent formation of aloe-emodin. Indeed, when chrysophanol was incubated under standard conditions, aloe-emodin was found to be a major metabolite (fig. 4). Again, the formation of aloe-emodin was dependent on the presence of NADPH, oxygen, and intact microsomal proteins. The rate of formation of aloe-emodin was 1.9 ± 0.9 nmol/min/mg (mean ± SD, N = 3) in liver microsomes from untreated male rats. Because a role for specific cytochrome P450 enzymes in the oxidation of the exocyclic methyl group of emodin could not be demonstrated, no attempts to characterize the role of specific cytochrome P450 enzymes in the oxidation of chrysophanol were made.

Induction of Micronuclei in Mouse Lymphoma L5178Y Cells by Anthraquinones and Their Phenolic Metabolites. The formation of micronuclei in mouse lymphoma L5178Y cells is a well-established endpoint in genotoxicity testing for chromosomal DNA damage (Miller et al., 1997). Micronucleus formation could be induced by a variety of genotoxic agents and could result in mutagenesis (Stopper and Müller, 1997). We compared the induction of micronuclei by emodin and aloe-emodin and their metabolites ω-hydroxymodin, 2-hydroxymodin, and aloe-emodin (fig. 5). With ω-hydroxymodin, no increase in the micronucleus frequency, in comparison with emodin, was observed. The micronucleus frequencies observed were significantly lower than those induced by emodin. 2-Hydroxymodin and aloe-emodin induced dose-dependent and statistically significant increases in micronuclear frequencies, compared with emodin and chrysophanol.

Discussion

In these studies, the biotransformation of 1,8-dihydroxyanthraquinones and the role of biotransformation in the formation of genotoxic metabolites from 1,8-dihydroxyanthraquinones were investigated. Using various inducers and inhibitors of cytochrome P450 enzymes, a major contribution of cytochrome P450 enzymes to anthraquinone hydroxylation was observed. From the results of in vivo experiments with enzyme inducers and those of chemical and antibody inhibition studies, a major but not exclusive contribution of cytochrome P450 1A2 to the formation of 2-hydroxymodin from emodin was indicated. Because moderate inhibition of 2-hydroxylation of emodin by an inhibitory anti-cytochrome P450 2B1/2 antibody was also observed, this cytochrome P450 family may also catalyze the formation of 2-hydroxymodin from emodin. A major role of cytochrome P450 1A1/2 in emodin 2-hydroxylation is consistent with the known substrate specificity of these cytochrome P450 enzymes. Cytochrome P450 1A2 also hydroxylates other planar polyaromatic compounds and several phenols (Stresser and Kupfer, 1997). In contrast to a major role of cytochrome P450 1A1/2 in the 2-hydroxylation of emodin, the hydroxylation of emodin at the exocyclic methyl group seems to be catalyzed by several cytochromes P450 at low rates. The oxidation of the exocyclic methyl group in chrysophanol also seems to be catalyzed by several cytochrome P450 enzymes (fig. 6).

The biotransformation reaction observed in rat liver microsomes may also be relevant for the disposition of 1,8-dihydroxyanthraquinones in vivo and may be involved in toxic effects associated with the abuse of medicines containing 1,8-dihydroxyanthraquinones. Anthraquinones are found in laxative drugs and in edible plants (Müller et al., 1997). For humans, ingestion is the usual route of intake, and high concentrations of 1,8-dihydroxyanthraquinones may be achieved locally in the intestine. In addition, absorption of ingested 1,8-dihydroxyanthraquinones from the gut and intestine may result in high concentrations of 1,8-dihydroxyanthraquinones in the liver (de Witte, 1993). Cytochrome P450 enzymes are located mainly in the villus epithelial cells and mast cells in the human small intestine (Kaminsky and Fasco, 1992). Therefore, biotransformation of ingested 1,8-dihydroxyanthraquinones may occur both in intestinal epithelial cells and in liver cells in vivo. Because the levels of cytochrome P450 1A in rats and humans are efficiently increased by emodin (Kaminsky and Fasco, 1992; Zhang et al., 1997) and cytochrome P450 1A2 is constitutively expressed in the liver (Gonzalez, 1992; Rodrigues and Prough, 1991), the cytochrome P450 1A2-dependent formation of 2-hydroxymodin from emodin may represent a relevant biotransformation pathway for this compound in vivo. The levels of cytochrome P450 1A2 differ widely among individual human subjects, although no distinct functional genetic polymorphism has been identified (Eaton et al., 1995). The large differences may be the result of the intake of efficient inducers for this cytochrome P450 enzyme with specific foods, such as charcoal-broiled meat (Felton and Knize, 1991). Therefore, individuals with high activities of this enzyme may be more efficient metabolizers of emodin to the genotoxic metabolite 2-hydroxymodin and may be more susceptible to potential adverse effects of this compound.

As shown by others and by us, some emodin metabolites and some other 1,8-dihydroxyanthraquinones have genotoxic properties. Cytochrome P450-dependent biotransformation reactions for these compounds may thus represent bioactivation reactions but may also result in detoxication. Therefore, we studied the genotoxicity of the identified metabolites in the micronucleus assay, an established test system that is highly responsive to this class of compounds (Miller et al., 1997; Müller et al., 1996; Stopper and Müller, 1997). Emodin itself is only a weak inducer of micronuclei. 2-Hydroxylation of emodin must be regarded as a bioactivation reaction, because the 2-hydroxymodin formed is significantly more potent in micronucleus induction. Moreover, the biotransformation of chrysophanol by cytochromes P450 to
yield aloe-emodin must also be regarded as a bioactivation reaction, because aloe-emodin is a much more potent inducer of micronuclei than is chrysophanol. In contrast, hydroxylation of the exocyclic methyl group in emodin may be regarded as a detoxication reaction, because the formed ω-hydroxyemodin has a lower potential for the induction of micronuclei.

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References


