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-methylanthaquinone), a compound present in pharmaceutical preparations. With rat liver microsomes, the formation of two emodin metabolites, ω-hydroxymodin and 2-hydroxymodin, was observed. The rates of formation of ω-hydroxymodin were not different with microsomes from rats that had been pretreated with inducers for different cytochrome P450 enzymes. Thus, the formation of ω-hydroxymodin seems to be catalyzed by several cytochrome P450 enzymes at low rates. The formation of 2-hydroxymodin was increased in liver microsomes from 3-methylcholanthrene-pretreated rats and was inhibited by α-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-methylanthaquinone) is transformed, in a cytochrome P450-dependent oxidation, to aloe-emodin (1,8-dihydroxy-3-hydroxymethylanthaquinone) as the major product formed. The mutagenicity of the parent dihydroxyanthraquinones and their metabolites was compared in the in vitro micronucleus test in mouse lymphoma L5178Y cells. 2-Hydroxymodin induced much higher micronucleus frequencies, compared with emodin. ω-Hydroxymodin 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). 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; Tanaka et al., 1987). The biotransformation of other 1,8-dihydroxyanthraquinoids has apparently not been studied.

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-methylanthaquinone) was thought to be mediated by cytochrome P450. However, in studies with rat liver microsomes, reaction rates were not compared with emodin. ω-Hydroxymodin 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.

After oral administration of 1,8-dihydroxyanthraquinones, intesti-
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; \textsuperscript{1}H-NMR (250 MHz, dimethylsulfoxide-\textit{d}_{6}): \delta 2.4 (s, 3H, -\textit{CH}_3), 7.1 (s, 1H, Aromatic), 7.2 (s, 1H, ArH), and 7.7 (d, J = 1.9 Hz, 1H, ArH); electrospray MS: \textit{m/z} (relative intensity) 286 (M\textsuperscript{+}, 100%); UV (0.2% NaHCO\textsubscript{3}/acetonitrile, 60:40): \textit{A}_{max} (log \textit{e}) = 215 nm (4.222), 290 (4.222), and 435 (3.824)].

\(\omega\)-Hydroxyemodin was prepared in a scaled-up microsomal incubation. The incubation volume was 50 ml, and all other conditions were as described above. Biosynthetic \(\omega\)-hydroxyemodin was isolated by preparative HPLC (steel column, 250 × 8 mm, filled with Partisol ODS III resin, 5 µm; solvent A, H\textsubscript{2}O, adjusted to pH 2 with CF\textsubscript{3}CO\textsubscript{2}H; solvent B, acetonitrile; linear gradient, 14% B to 100% B in 30 min; flow rate, 2 ml/min; detection wavelength, 225 nm). \(\omega\)-Hydroxyemodin was obtained as a yellow solid, with a purity of >95% (as judged by HPLC with UV detection at 225 nm) \textsuperscript{1}H-NMR (250 MHz, CDCl\textsubscript{3}): \delta 2.1 (s, 2H, \textit{CH}_2-OH) and 7.2 (s, 4H, ArH); electrospray MS: \textit{m/z} (relative intensity) 286 (M\textsuperscript{+}, 100%); UV (0.2% NaHCO\textsubscript{3}/acetonitrile, 60:40): \textit{A}_{max} (log \textit{e}) = 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\textsuperscript{+}, 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 1-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 Partisol ODS resin, 5 µm); separation was performed by gradient elution (linear gradient from 86% H\textsubscript{2}O, adjusted to pH 2 with CF\textsubscript{3}CO\textsubscript{2}H, to 100% acetonitrile in 35 min, at a flow rate of 1 ml/min). Eluting material was monitored at 225 nm (M1) and 290 nm (M2), using a Hewlett-Packard 1040 diode-array detector. Metabolite formation was quantified by comparison of the peak areas with calibration curves for authentic materials. Recovery of substrates in control samples varied from 60 to 70%. Formation rates were corrected for recovery.

In inhibition studies with troleandomycin, the inhibitor was preincubated with microsomes and the NADPH-generating system for 10 min at 37°C. The competitive inhibitors \(\alpha\)-naphthoflavone and sulphanilamide were preincubated with microsomes for 5 and 10 min, respectively, at 37°C. After addition of the NADPH-generating system and the substrate, incubations were performed as described above.

Animals and Treatment. Adult male and female Wistar rats were used for all studies (Harlan-Winkelman, 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; Wof et al., 1984).

7-Ethoxyresorufin O-dealkylation and 7-pentoxyresorufin O-dealkylation
Effects of various cytochrome P450 inhibitors on the hydroxylation of emodin in
rat liver microsomes

<table>
<thead>
<tr>
<th>Microsomal Preparation and Treatment</th>
<th>Activitya</th>
<th>Activityb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formation of ω-Hydroxymedin</td>
<td>Formation of 2-Hydroxymedin</td>
</tr>
<tr>
<td>Untreated/PB-pretreated male rats</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Untreated male rats</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Untreated male rats + Troleandomycin (100 µM)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Untreated male rats + Sulfaphenazole (50 µM)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Untreated male rats + α-Naphthoflavone (20 µM)</td>
<td>100 ± 8</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>Untreated male rats + α-Naphthoflavone (50 µM)</td>
<td>100 ± 10</td>
<td>82 ± 1</td>
</tr>
<tr>
<td>Untreated male rats + α-Naphthoflavone (100 µM)</td>
<td>75 ± 8</td>
<td>55 ± 9</td>
</tr>
<tr>
<td>MC-pretreated male rats + α-Naphthoflavone (50 µM)</td>
<td>96 ± 6</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>MC-pretreated male rats + α-Naphthoflavone (50 µM)</td>
<td>82 ± 13</td>
<td>42 ± 11</td>
</tr>
<tr>
<td>MC-pretreated male rats + α-Naphthoflavone (70 µM)</td>
<td>69 ± 15</td>
<td>37 ± 11</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.

b Rates of formation of ω-hydroxymedin and 2-hydroxymedin were determined to be 5 ± 2 and 2.6 ± 0.5 nmol/min/mg protein, respectively, in liver microsomes from untreated rats and 1.7 ± 1.1 and 2.0 ± 0.3 nmol/min/mg protein, respectively, in liver microsomes from phenobarbital-pretreated rats; rates of formation of ω-hydroxymedin and 2-hydroxymedin in liver microsomes from 3-methylcholanthrene-pretreated rats were determined to be 19 ± 3 and 9 ± 1 nmol/min/mg protein, respectively (all given as mean ± SD, N = 3).

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.
of formation of ω-hydroxymedin and 2-hydroxymedin 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 (pentoxysorulin dealkylation) and cytochrome P450 1A(2) (ethoxyresorufin dealkylation) (table 2). No correlation between the biotransformation of the marker substrates ethoxyresorufin and pen-toxysorulin and the formation of the two emodin metabolites was observed. In addition, coincubation of emodin and liver microsomes from untreated male rats with the cytochrome P450 3A1/2 mechanism-based inhibitor tolreamycin (Pessaye 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-hydroxymedin were, however, significantly reduced in the presence of low concentrations (50 μM) of the cytochrome P450 1A1/2 competitive inhibitor α-naphthoflavone (table 3). A Kᵣ value of 21 μM for α-naphthoflavone was determined for the formation of 2-hydroxymedin with liver microsomes from 3-methylcholanthrene-pretreated male rats (fig. 3). Formation of ω-hydroxymedin was markedly reduced (53% ± 12%) only by high concentrations (200 μM) of α-naphthoflavone.

Inhibition of the formation of 2-hydroxymedin by α-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 4). 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-hydroxymedin 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-hydroxymedin was found in the presence of this antibody. The presence

### Effects of inhibitory antibodies on the hydroxylation of emodin in rat liver microsomes

<table>
<thead>
<tr>
<th>Microsomal Preparation and Treatment</th>
<th>Activity</th>
<th>Formation of ω-Hydroxymedin</th>
<th>Formation of 2-Hydroxymedin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-pretreated male rats, + anti-rat cytochrome P450 2B/1/2 antibody</td>
<td>85%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>MC-pretreated male rats, + anti-rat cytochrome P450 1A1 antibody</td>
<td>100%</td>
<td>80% ± 6</td>
<td></td>
</tr>
<tr>
<td>MC-pretreated male rats, + anti-rat cytochrome P450 1A2 antibody</td>
<td>100%</td>
<td>51% ± 11</td>
<td></td>
</tr>
</tbody>
</table>

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

### Microsomal Preparation and Treatment

- **PB-pretreated male rats**: + anti-rat cytochrome P450 2B/1/2 antibody
- **MC-pretreated male rats**: + anti-rat cytochrome P450 1A1 antibody
- **MC-pretreated male rats**: + anti-rat cytochrome P450 1A2 antibody

### 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 ω-hydroxymedin (hydroxymethylmedin) and 2-hydroxymedin, 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 monoxygenases 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

### Kinetic parameters V₉₉ and Kₘ for the hydroxylation of emodin in liver microsomes from untreated, phenobarbital-pretreated, and 3-methylcholanthrene-pretreated male rats

<table>
<thead>
<tr>
<th>Microsomal Preparation</th>
<th>ω-Hydroxymedin*</th>
<th>2-Hydroxymedin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kₘ (μM)</td>
<td>V₉₉ (nmol/min/mg)</td>
</tr>
<tr>
<td>Untreated male rats</td>
<td>22 ± 4</td>
<td>23 ± 11</td>
</tr>
<tr>
<td>PB-pretreated male rats</td>
<td>97 ± 40</td>
<td>46 ± 9</td>
</tr>
<tr>
<td>MC-pretreated male rats</td>
<td>196 ± 49</td>
<td>44 ± 10</td>
</tr>
</tbody>
</table>

* 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

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**Fig. 4.** HPLC separation of an incubation mixture of chrysophanol with liver microsomes from untreated male rats, with UV detection at 225 nm (A), and electronic spectra of the substrate and metabolite, with reference to the synthetic compounds (B).

**Fig. 5.** Micronucleus induction by 1,8-dihydroxyanthraquinones in mouse L5178Y cells treated with emodin, ω-hydroxyemodin, or 2-hydroxyemodin (A) or chrysophanol or aloe-emodin (B).

Significant increases in micronucleus frequencies with the metabolites, compared with the substrates (emodin and chrysophanol), were obtained (Student’s $t$ test). *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.001$; ****, $p < 0.0001$. 

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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 dependent on the presence of 2-hydroxyemodin. Indeed, when chrysophanol was incubated under standard conditions, aloe-emodin was observed. Therefore, we investigated the cytochrome P450-dependent formation of aloe-emodin (fig. 5). With 2-hydroxyemodin, 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-Hydroxyemodin and aloe-emodin induced dose-dependent and statistically significant increases in micronucleus frequencies, compared with emodin and chrysophanol.

Discussion

In these studies, the biotransformation of 1,8-dihydroxyanthraquinones and their 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-hydroxyemodin 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 polycyclic aromatic 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 inducers (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-hydroxyemodin 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 charbroiled meat (Feltin and Knize, 1991). Therefore, individuals with high activities of this enzyme may be more efficient metabolizers of emodin to the genotoxic metabolite 2-hydroxyemodin 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 (Müller 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-hydroxyemodin formed is significantly more potent in micronucleus induction. Moreover, the biotransformation of chrysophanol by cytochromes P450 to

![Diagram](image-url)
yield aloe-emodin may 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 strand breaks by active oxygen. Free radical and hydrogen peroxide from 2-hydroxyemodin, a direct-acting mutagen, and DNA yield aloe-emodin must also be regarded as a bioactivation reaction, and DNA strand breaks by active oxygen. Toxicol Lett 67:149–156.


