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# Oxidative Hydrolysis of Scoparone by Cytochrome P450 CYP2C29 Reveals a Novel Metabolite

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**Regioselective 7-demethylation of scoparone is regularly employed as an indicator of phenobarbital-like induction of rat liver cytochrome P450 isoform CYP2B1, e.g., by the antiepileptic drug phenytoin. After induction with phenobarbital and phenytoin, a new reaction sequence catalyzed by Cyp2c29 was identified in mouse liver microsomes. Cyp2c29-dependent 6-demethylation of scoparone resulted in the formation of isoscopoletin, an intermediate which is susceptible to further oxidation. This subsequent oxidation was also catalyzed by Cyp2c29 with a $K_m$ of 30,31 $\mu$M and a $V_{max}$ of 3,41 $\mu$M/min · $\mu$M P450, and resulted in the formation of the new metabolite 3-[4-methoxy-*p*-(3,6)-benzoquinone]-2-propenoate. This novel metabolite is the product of two consecutive oxidation reactions, proceeding over isoscopoletin to a putative lactone which is accessible to immediate hydrolysis, due to the onium character of the ring oxygen. This opening of the lactone ring corresponds to an oxidative hydrolysis. Differential oxidation of scoparone can be used as a sensitive indicator for distinguishing between different cytochrome P450 isoforms.** 

***Key Words:*** **cytochrome P450; scoparone; phenytoin; phenobarbital; oxidative hydrolysis; Cyp2c29; CYP2B1; mouse; liver.**

The P450 monooxygenases represent a multigenic family, mainly localized in the endoplasmic reticulum of hepatocytes (1). These enzymes are involved in oxidative and reductive metabolism of endogenous and exogenous compounds, such as steroids and xenobiotics; many P450 monooxygenases are inducible specifically by these compounds (2).

The antiepileptic drugs phenobarbital and phenytoin are potent inducers of rat liver CYP2B1 (3, 4). In mouse liver, phenobarbital and phenytoin act as inducers of the families Cyp2b, Cyp2c, and Cyp3a (5, 6). In contrast to rat liver, the main microsomal isoform induced by phenytoin in mouse is Cyp2c29 (3, 7, 8). Phenytoin has also been shown to be a substrate for murine Cyp2c29 in a previous study (3).

Rapid, highly specific enzymatic assays are essential for the functional characterization of novel P450 isoforms. The demethylation of aminopyrine (9) or the dealkylation of pentoxyresorufins (10, 11) have been commonly used to analyze the induction of P450 in hepatic microsomes. Because of the broad substrate specifity of some P450's, these assays are often markers for several different P450 isoforms (12). In order to confirm differential induction of specific P450 isoforms by phenobarbital or phenytoin, enzymatic assays with distinctive substrate specifity are required.

The coumarin derivative scoparone and its demethylation products isoscopoletin (6-demethylation) and scopoletin (7-demethylation) are natural occurring in roots and barks of plants like belladonna and oleander, and function as inhibitors of plant growth (13). These compounds exert good fluorescence at 345 nm which predetermines them for use in enzymatic assays. The regioselective O-demethylation of the coumarin derivative scoparone has been used as an indicator for phenobarbital-like induction of CYP2B1 in rat liver (14). Whereas untreated rats catalyze the 6-demethylation of scoparone almost exclusively, 7-demethylation of scoparone is strongly increased after phenobarbital treatment. In the present study, we demonstrate that scoparone oxidation catalyzed by rat CYP2B1 and murine Cyp2c29 can be employed to differentiate between the P450 induction profiles of phenytoin and phenobarbital in these species. The oxidation of scoparone by murine Cyp2c29 reveals a characteristically different pathway from that of rat CYP2B1,

Abbreviations used: amu, atomic mass units; CYP, Cyp; P450, cytochrome P450; [MBQ]-propenoate, 3-[4-Methoxy-*p*-(3,6)-benzoquinone]-2-propenoate; MS, mass spectrometry; PBS, phosphate buffered saline; PVDF, polyvinylidendifluoride.

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indicating a new reaction sequence leading to a previously unknown metabolite in scoparone oxidation. This is the first report of an oxidative hydrolysis reaction mediated by a cytochrome P450.

## MATERIALS AND METHODS

*Animals.* Ten male Sprague–Dawley rats (150–200 g), and 10 male C57B1/6J mice (25–30 g) were fed for 2 weeks with solid standard diet (Eggersmann, Rinteln, FRG) supplemented with 2000 ppm (rats) or 500 ppm (mice) phenytoin and tap water *ad libitum*. Ten animals received fluid diet of tap water supplemented with 500 mg phenobarbital/1 for 1 week. This resulted in a daily consumption of 80 mg/kg · d of either drug. The drug serum levels of rats were 5 $\mu$g/ml, those of mice 15 $\pm$ 3 $\mu$g/ml. Ten animals were used as controls. The animals were kept under a 12 h light/dark cycle at a temperature of 22°C.

*Chemicals.* Scoparone (6,7-dimethoxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin), and isoscopoletin (6-hydroxy-7-methoxycoumarin) were purchased from Roth (Karlsruhe, FRG), phenytoin (sodium salt), and phenobarbital (sodium salt) from Sigma (München, FRG). All other reagents were from commercial sources at the highest purity available.

*Preparation and characterization of liver microsomes.* Ten- to twelve-week-old animals were sacrifized as described previously (15). After perfusion with PBS, livers were immediately removed. Microsomal fractions were obtained by differential centrifugation. Briefly, liver homogenates were centrifuged at 750*g*, 4300*g*, and 25,000*g* prior to a final spin at 105,000*g* for 90 min (3, 16). 10 mM Tris–HCl, pH 7.4 containing 0.32 mM sucrose, 1 mM EDTA, and 0.5 mM 1,4-dithiothreitol was used as homogenization buffer. P450 content of microsomal fractions was measured by spectrophotometry (17).

*Immunoblotting with polyclonal IgG-fraction against mouse liver Cyp2c29.* Microsomal samples (5 $\mu$g protein) from phenytoin-, phenobarbital-, and nontreated liver from mice were separated via 10% SDS–PAGE. The proteins were transferred to a PVDF membrane (Immobilon P, Millipore, Eschborn, Germany) and probed with a polyclonal anti mouse liver Cyp2c29 IgG-fraction (dilution 1:2000) (3). Protein signals were visualized by enhanced chemoluminescence (ECL, Amersham, Freiburg, Germany) and quantificated by densitometry (BioRad, München, Germany). Negative controls omitting the first antibody generated no signal.

*Assay for measurement of oxidation of scoparone and isoscopoletin.* Scoparone O-demethylation and isoscopoletin oxidation by liver microsomes equivalent to 1 $\mu$M P450 were tested in 100 mM Tris–HCl buffer, pH 7.6, containing 4 mM NADPH, 80 $\mu$M scoparone, and 2 mM $MgCl_2$. The incubations were carried out for 0, 3, 6, and 10 min at 37°C under gentle shaking (18). The reaction was stopped by the addition of 10% (v/v) 3 M trichloroacetic acid (TCA). For determination of blanks protein was added after incubation. A stop-time-assay was used for determination of the dependency of reaction velocity from substrate concentration and proved to be linear under the experimental conditions.

*Immunoinhibition of oxidation of scoparone and isoscopoletin.* To verify Cyp2c29 specificity of the reaction, scoparone O-demethylation and isoscopoletin oxidation in phenytoin-treated mouse liver microsomes were analyzed in presence of polyclonal anti mouse liver Cyp2c29 IgG-fraction (0, 0.1, 0.5, and 1 mg) and preimmune IgG to give a total IgG level of 1 mg/mg protein. Preincubation was carried out at 25°C for 30 min. Incubation was carried out subsequently for 10 min as described.

*High performance liquid chromatography.* Separation of scoparone and its metabolites was performed on a Nova-Pak C18 column heated to 30°C (150 $\times$ 3.9 mm, 5 $\mu$m particle size; Waters, Eschborn, Germany) with a Waters Separations Module 2690 and detected with the Waters 996 PDA-detector operating at 345 nm. The mobile phase consisted of 80% 50 mM ammonium acetate buffer, pH 4.25, and 20% acetonitrile (v/v) (18) with flow rates adjusted to 1 ml/min.

*Kinetic data.* Kinetic parameters of mouse liver Cyp2c29 for scoparone and isoscopoletin were evaluated graphically in the v/[S]v-plot (19), using substrate concentrations of 5, 10, 20, 40, and 80 $\mu$M and P450 amounts of 100, 200, and 400 pmol per assay. To inhibit scoparone and isoscopoletin oxidation, phenytoin was added as substrate in concentrations of 0.05, 0.2, 1, and 5 mM.

*Spectrometry.* UV/VIS-spectra were recorded in the range of 250–500 nm (Perkin Elmer Lamba-2, Überlingen, FRG) using ethyl acetate as solvent. Mass spectra were recorded using a MAT 312 mass spectrometer operating with electron impact ionization, EI, at 70 eV, connected with data unit SS 200 (Finnigan Mat, Bremen, FRG). The temperature of the ion-source was 220°C [*m/z* 208 $M^{\oplus}$; *m/z* 69 (basis peak), 92% rel. intensity].

## RESULTS

### *Scoparone Oxidation*

Scoparone is metabolized in a P450-dependent pathway in both rat and mouse liver by O-demethylation in the 7- and 6-positions, resulting in the formation of scopoletin or isoscopoletin, respectively. Whereas microsomes of untreated rats and mice showed no difference in scoparone O-demethylation, the ratio of isoscopoletin:scopoletin being about 5:1 in both cases (Figs. 1A, 2A, and Table I), the microsomes of drug-treated animals revealed a species dependent difference in the demethylation of scoparone.

Treatment of rats with 80 mg/kg · day phenobarbital increased microsomal scoparone 7-demethylation to form scopoletin at about 56-fold higher levels when compared to untreated controls. This resulted in a isoscopoletin: scopoletin ratio of 0.5:1 (Fig. 1B, Table I). Rats treated with 80 mg/kg · day phenytoin increased microsomal scoparone 7-demethylation about 20-fold, leading to an isoscopoletin:scopoletin ratio of approximately 1:1 (Fig. 1C, Table I). The specific 7-demethylation originates from induction of CYP2B1, as described by Müller-Enoch *et al.* (18).

Treatment of mice with 80 mg/kg · day phenobarbital led to a sixfold increase in the formation of isoscopoletin by 6-demethylation, resulting in an isoscopoletin:scopoletin ratio of 9:1 (Fig. 2B, Table I). Phenytoin-treatment caused an 18-fold increase of 6-demethylation, leading to an isoscopoletin:scopoletin ratio of 12:1 (Fig. 2C, Table I). This increase of 6-demethylation of scoparone by either phenobarbital or phenytoin corresponds to the induction of Cyp2c29 after treatment of mice with either drug as confirmed by immunoblotting using a polyclonal IgG-fraction against mouse liver Cyp2c29 (20) (Fig. 3). Cyp2c29 was induced sevenfold by phenobarbital and 17-fold by phenytoin, respectively, revealing Cyp2c29 to be the main enzyme involved in this oxidation.

In addition to increased formation of isoscopoletin in mouse liver, an additional peak at the retention time of 3.25 min revealed the formation of a previously un-

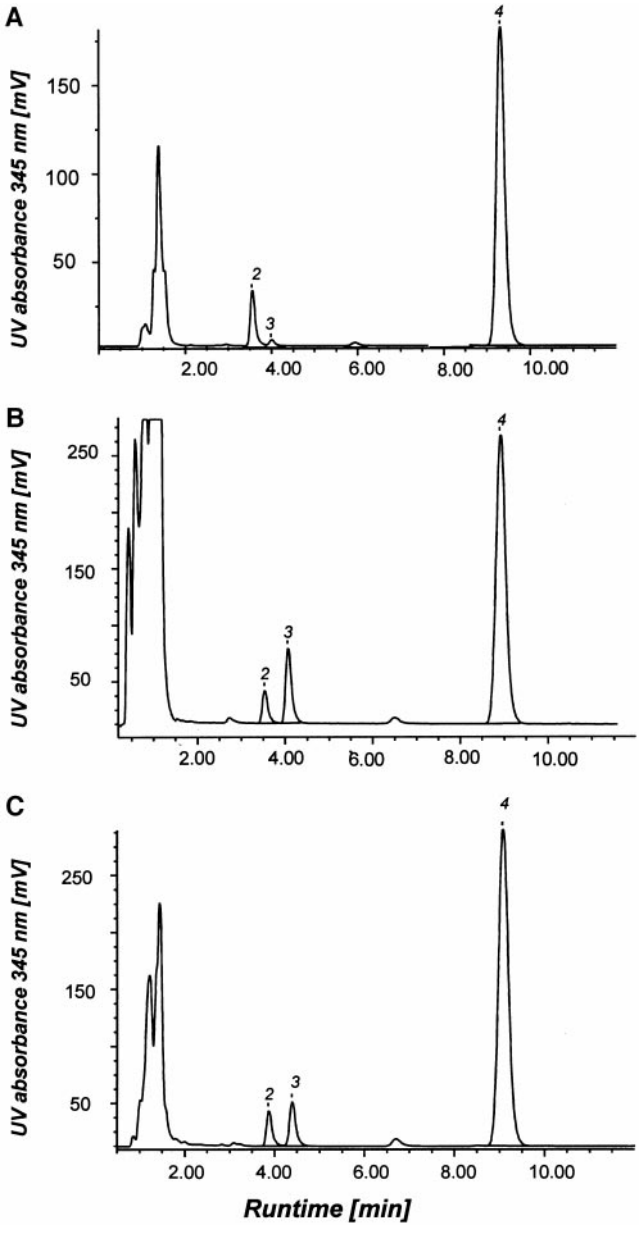


**FIG. 1.** Regioselective O-demethylation of scoparone in rat liver microsomal fractions. HPLC-chromatograms of assays containing 80 $\mu$M scoparone and 1 $\mu$M P450. Incubations were carried out as described under Materials and Methods. (A) Supernatant of untreated control microsomes. (B) Supernatant of microsomal fraction obtained from livers treated with phenobarbital. (C) Supernatant of microsomal fraction obtained from rat livers treated with phenytoin. (2) isoscopoletin, (3) scopoletin, (4) scoparone.

identified product (peak *1*, Figs. 2B and 2C). The retention time, shorter than those of the other metabolites of scoparone-O-demethylation, points to a more hydrophilic character of this product.

*Identification of the New Metabolite*

The novel product was collected via HPLC and examined by UV/VIS spectroscopy and mass spectrometry. The UV/VIS spectrum is characterized by three bands, an intensive band at 305 nm due to a methoxy group, a weak band at 425 nm and a shoulder at 281 nm indicating a *p*-chinoid structure (21). Mass spectrometry revealed that the molecular ion of the novel compound is at *m/z* 208 ($M^+$), corresponding to the base peak. The fragment peaks of the new product, *m/z* 193 (loss of $^{\bullet}CH_3$), *m/z* 180 (loss of CO), *m/z* 165 (loss of $^{\bullet}CH_3$ and CO), and *m/z* 147 (loss of $^{\bullet}CH_3$, CO, and $H_2O$), match only to the structure

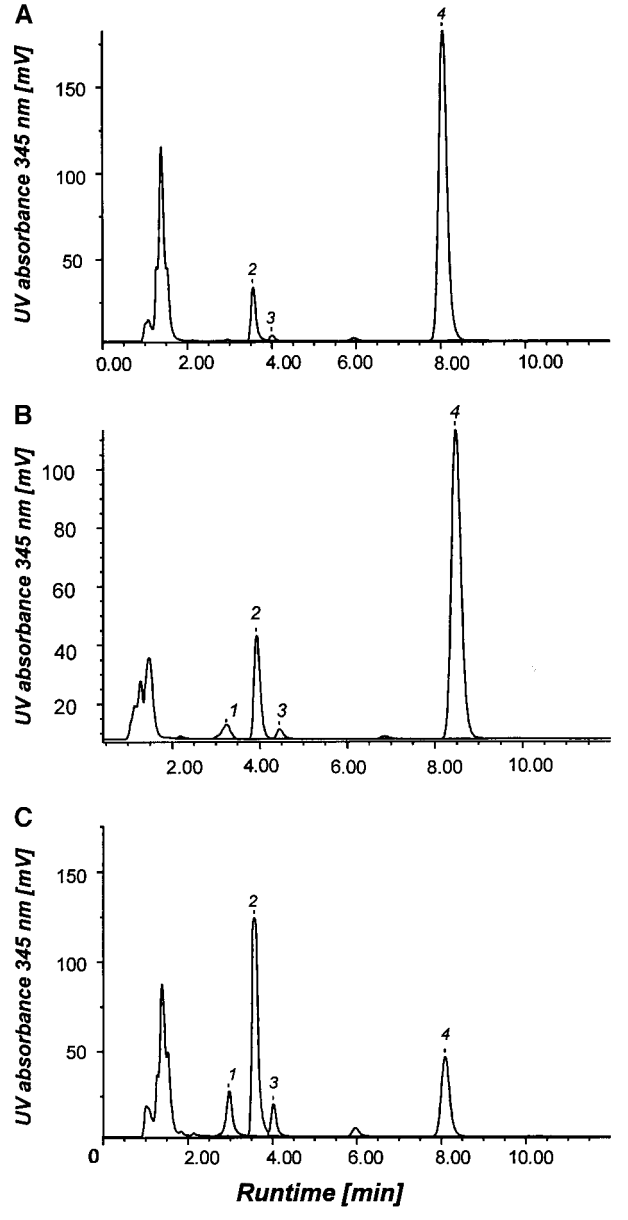


**FIG. 2.** Oxidation of scoparone in mouse liver microsomal fraction forming isoscopoletin and [MBQ]-propenoate. HPLC-chromatograms of the assays as described in legend of Fig. 1. (A) Supernatant of untreated control microsomes. (B) Supernatant of microsomal fraction obtained from livers treated with phenobarbital. (C) Supernatant of microsomal fraction obtained from mouse livers treated with phenytoin. (1) [MBQ]-propenoic acid, (2) isoscopoletin, (3) scopoletin, (4) scoparone.

**TABLE I**

Enzymatic Activities of Scoparone Demethylation by CYP2B1 in Rat Liver Microsomes and by Cyp2c29 in Mouse Liver Microsomes

| | Controls | | Phenobarbital | | Phenytoin | |
|---|---|---|---|---|---|---|
| | Rat | Mouse | Rat | Mouse | Rat | Mouse |
| Ratio | | | | | | |
| Isoscopoletin/Scopoletin | 5:1 | 5:1 | 0.5:1 | 9:1 | 1:1 | 12:1 |
| Isoscopoletin formed | | | | | | |
| pmol/min · pmol P450 | 0.69 | 0.69 | 1.16 | 1.20 | 1.20 | 2.42 |
| pmol/min · mg protein | 158 | 130 | 915 | 788 | 530 | 2318 |
| enrichment factor | — | — | 5.8 | 6.1 | 3.4 | 18 |
| Scopoletin formed | | | | | | |
| pmol/min · pmol P450 | 0.13 | 0.13 | 2.12 | 0.14 | 1.35 | 0.19 |
| pmol/min · mg protein | 30 | 24 | 1673 | 92 | 597 | 182 |
| enrichment factor | — | — | 55.6 | 3.8 | 20 | 7.6 |

of 3-[4-methoxy-*p*-(3,6)-benzoquinone]-2-propenoic acid ([MBQ]-propenoic acid) (Fig. 6). This structure would reflect the hydrophilic character of the new metabolite resulting from opening the lactone ring of one of the coumarin derivatives. Under the conditions used in the assay, at pH 7.6, [MBQ]-propenoic acid is present as [MBQ]-propenoate.

*Formation of the New Metabolite*

In order to clarify the mechanism of formation of the new metabolite [MBQ]-propenoate, time dependent oxidation of scoparone, isoscopoletin, and scopoletin were compared. Incubations of phenytoin-induced mouse liver microsomes with 80 $\mu$M of each of the three compounds revealed that [MBQ]-propenoate is formed effectively from isoscopoletin, to a lesser extent from scoparone, and not at all from scopoletin (Fig. 4). The use of phenobarbital-treated mouse liver microsomes resulted in exactly the same pattern of [MBQ]-propenoate formation. The generation of [MBQ]-propenoate from scoparone and isoscopoletin showed a clear linear dependency on increasing amounts of induced mouse liver microsomes and did not occur in the absence of NADPH. Moreover the reaction could be completely inhibited by the presence of CO, indicating that these reactions are P450-dependent.

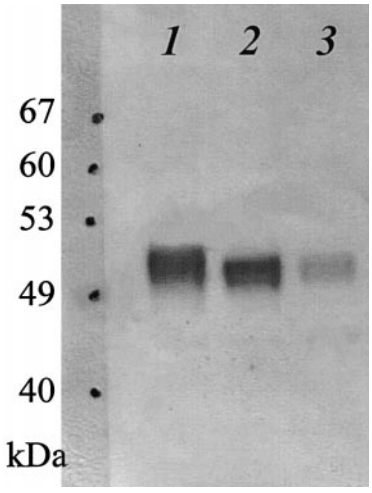


**FIG. 3.** Induction of Cyp2c29 by phenytoin and phenobarbital in mouse liver. Immunoblot using 5 $\mu$g of microsomal protein from phenytoin-treated (lane 1), phenobarbital-treated (lane 2), and untreated mice (lane 3), separated via 10% SDS–PAGE. Marker enzymes: bovine serum albumin (67 kDa); catalase (60 kDa); glutamate dehydrogenase (53 kDa); fumarase (49 kDa); aldolase (40 kDa) (31). The proteins were incubated with a polyclonal IgG-fraction against mouse liver Cyp2c29 (dilution 1:2000). Immunoreaction was by enhanced chemoluminescence (ECL). The immunopositive proteins were quantitated using a BioRad model 620 densitometer.

*Kinetic Parameters of Isoscopoletin Oxidation*

In order to determine the kinetic parameters for formation of [MBQ]-propenoate by oxidation of isoscopoletin, the dependency of reaction velocity on substrate concentration was analyzed graphically in the v/[S]-versus-v plot (plot not shown). The values of the kinetic parameters are $V_{max}$ = 3.41 $\mu$M/min · $\mu$M P450, and $K_m$ = 30.31 $\mu$M, the latter being three times higher than the $K_m$ of 6-demethylation of scoparone to isoscopoletin (22). In comparison with mouse liver, formation of [MBQ]-propenoate in rat liver was always in the range of the limit of the detection sensitivity, even after induction with phenobarbital or phenytoin (Figs. 1B and 1C).

*Evidence of Cyp2c29 as the Scoparone Oxidating Enzyme*

In order to estimate whether both subsequent oxidations, the 6-demethylation of scoparone and the oxidation of isoscopoletin, are catalyzed by Cyp2c29, reactions were inhibited with either an anti-Cyp2c29 IgG fraction or the substrate phenytoin (Fig. 5). Immunoinhibition assays with increasing amounts of the anti-

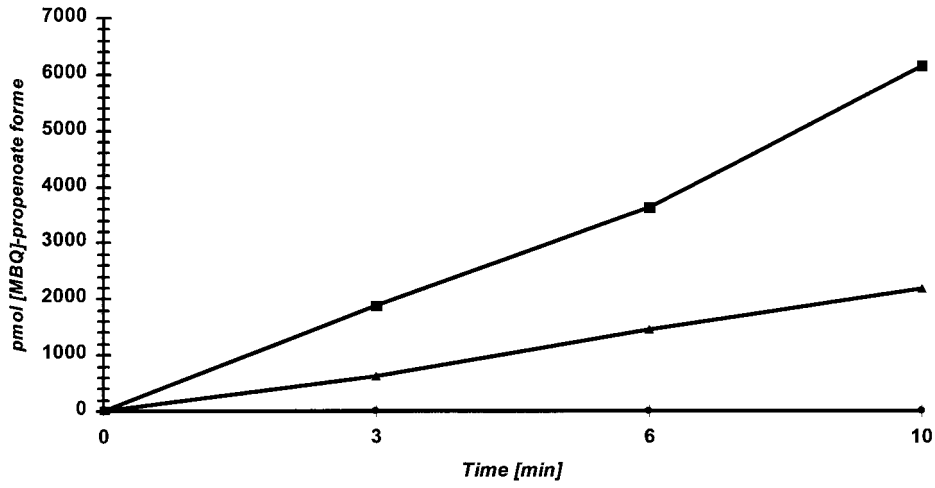


**FIG. 4.** Dependency of the formation of [MBQ]-propenoate from substrate. Scoparone (▲) isoscopoletin (■), or scopoletin (●) in concentrations of 80 $\mu$M were incubated for 0, 3, 6, and 10 min at 37°C with 1 $\mu$M P450 derived from phenytoin-induced mouse liver microsomes. All other conditions were as described in the legend of Fig. 1 ($n$ = 3).

Cyp2c29 IgG-fraction indicated a substantial decrease in product formation of both scoparone metabolites, isoscopoletin, and [MBQ]-propenoate. Formation of [MBQ]-propenoate was inhibited to 30% of normal activity (Fig. 5). As expected, isoscopoletin formation was reduced in the presence of anti-Cyp2c29 IgG-fraction as well. However, this inhibition was less than that of [MBQ]-propenoate and resulted in the retention of 75% of normal activity (Fig. 5). The specificity of the IgG fraction recognizing only Cyp2c29 is demonstrated in Fig. 3 and previous work (15, 20). When using phenytoin as inhibitor, formation of both metabolites, isoscopoletin from scoparone, and [MBQ]-propenoate from isoscopoletin, clearly decreased with increasing inhibitor concentrations, proving that both oxidative reactions could be inhibited by phenytoin (data not shown).

## DISCUSSION

Demethylation of scoparone in mouse liver, catalyzed by Cyp2c29, reveals a previously unknown biochemical pathway after treatment with either pheno-

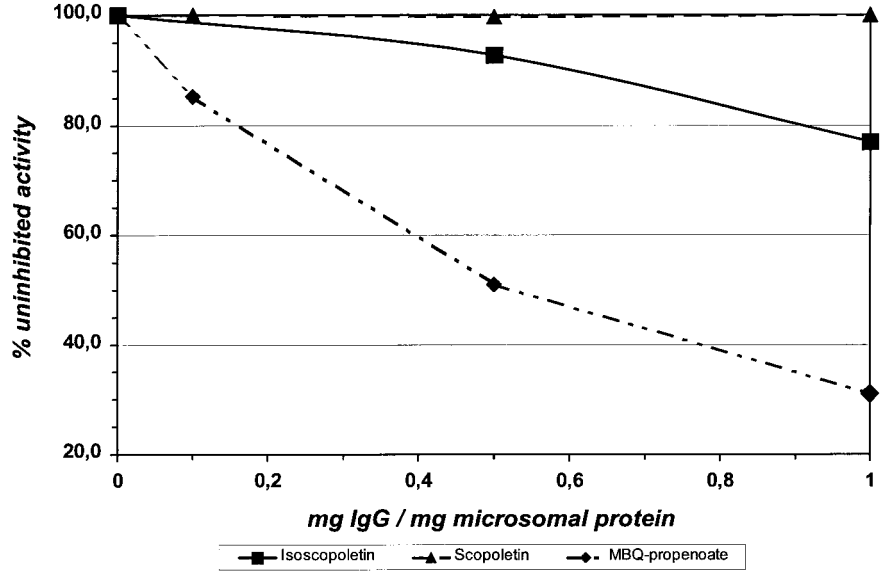


**FIG. 5.** Decrease of formation of isoscopoletin and [MBQ]-propenoate in mouse liver microsomes caused by an anti-Cyp2c29 IgG fraction (see Fig. 3). Immunoinhibition of scoparone oxidation and isoscopoletin hydroxylation was analyzed using liver microsomes from phenytoin-treated mice in presence of 0, 0.1, 0.5, and 1 mg IgG fraction. Preimmune IgG was added to each incubate to give a total IgG level of 1 mg per reaction. Incubations contained 1 mg microsomal protein and the incubation time was 10 min. The uninhibited point was set to 100% ($n$ = 3).

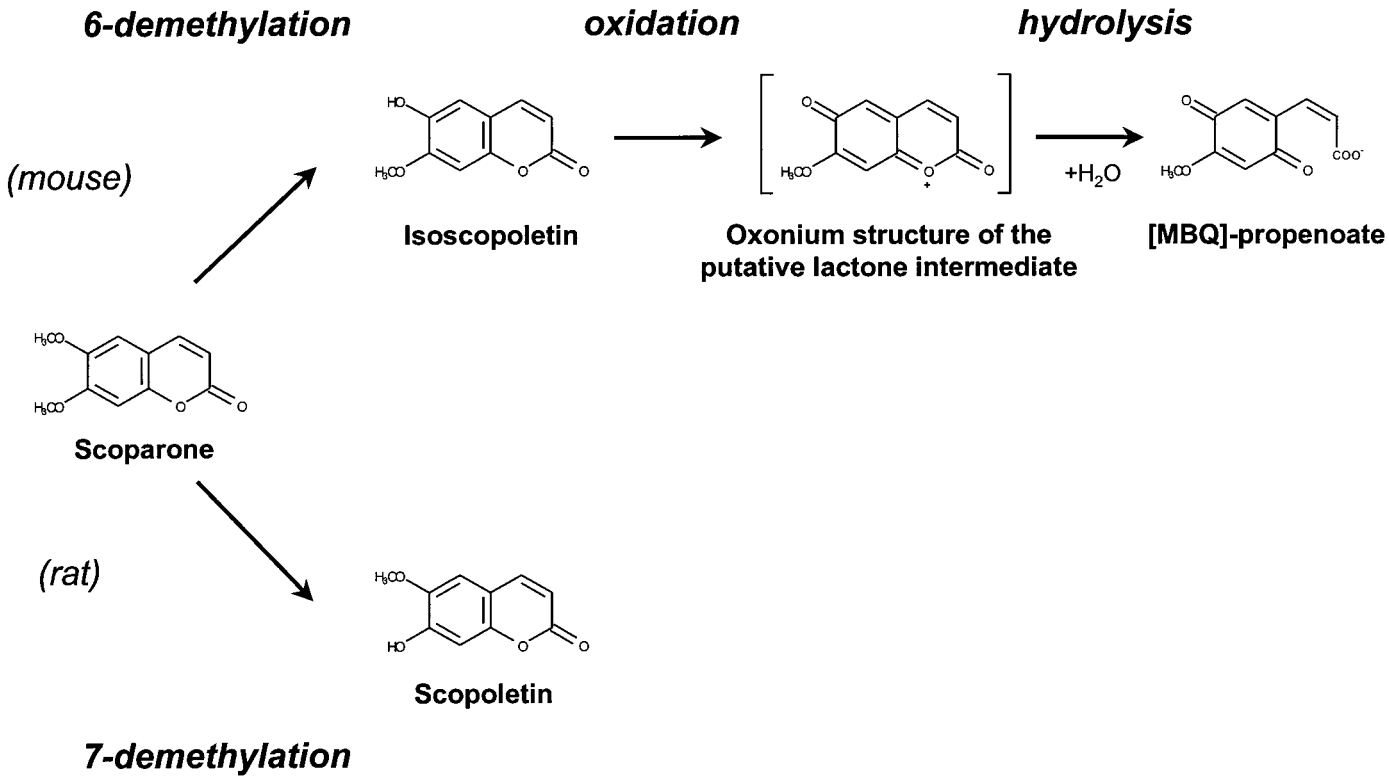


**FIG. 6.** Regioselective scoparone O-demethylation reactions in rat and mouse liver microsomes after treatment with phenytoin or phenobarbital. 6-Demethylation of scoparone and oxidative hydrolysis of isoscopoletin by Cyp2c29 are the major pathway in mouse liver, while 7-demethylation by CYP2B1 is the major pathway in rat liver.

barbital or phenytoin. In contrast to the increased 7-demethylation of scoparone forming scopoletin by CYP2B1 in rat liver (Fig. 1, Table I) (18), Cyp2c29 catalyzes a more complex reaction sequence in mouse liver microsomes. In the first reaction scoparone is demethylated by oxidation at position 6 and isoscopoletin is formed (Fig. 6, Fig. 2, Table I). In a subsequent reaction, the isoscopoletin derived from scoparone oxidation is further oxidized by Cyp2c29 at the same position with a higher $K_m$ forming [MBQ]-propenoate (Fig. 6). As expected, initial [MBQ]-propenoate formation is faster with isoscopoletin as substrate than with scoparone at the same concentration (Fig. 4). Scoparone has a lower $K_m$ value and must be transformed first to isoscopoletin before the latter reaches a sufficiently high concentration to be oxidized as a competing substrate. No reaction occurred with scopoletin as substrate (Fig. 4).

Cyp2c29-mediated oxidation of isoscopoletin causes the opening of the lactone ring, as explained by hydrolysis of the putative oxonium intermediate, resulting in the generation of [MBQ]-propenoate (Fig. 6). This intermediate is most likely a lactone with a 1,4-quinoidal structure with an onium oxygen in the lactone ring, making it highly accessible to immediate hydrolysis (Fig. 6). The putative lactone intermediate is completely hydrolyzed, forming [MBQ]-propenoate, as identified by UV/vis-spectroscopy and mass spectrometry. Spontaneous opening of the lactone ring of isoscopoletin could be excluded by examining its stability in the absence of enzyme. Furthermore, a mechanism that involves P450-mediated hydroxylation of C4 of the lactone ring, as generally proposed for ester cleavage (23, 24), was excluded in same way as is the possibility of an enzyme catalyzed direct hydrolysis of the lactone ring of isoscopoletin, as no mass signal derived from a putative hydroquinone was detectable in the mass spectrum. The oxidation of isoscopoletin followed by the immediate hydrolysis of the putative intermediate, can thus be defined as oxidative hydrolysis of isoscopoletin.

Oxidative hydrolysis is a novel metabolic pathway for P450-mediated reactions. It is generally assumed that P450 enzymes catalyze direct substrate hydroxylations, dealkylations, or hydroxy group oxidations (25). A P450-mediated cleavage of acyl- or pyridine esters is in some way similar to the oxidative hydrolysis presented here (23, 24, 26), but, as stated above, we couldn't observe any hint for a C4-hydroxylation of the coumarin residues. Furthermore, a conceivable stepwise oxidation mechanism, starting at C6, would not allow final C4-oxidation necessary for lactone ring opening, due to the oxidation status of the heme-iron. To support our theory of P450-based scoparone oxida-

tive hydrolysis in the formation of [MBQ]-propenoate, we demonstrate here that murine Cyp2c29 is sufficient for these catalytic reactions. Moreover, addition of an IgG-fraction against Cyp2c29 to the incubation mixture inhibited isoscopoletin oxidation forming [MBQ]-propenoate almost completely, whereas scoparone oxidation forming isoscopoletin was reduced to 75% (Fig. 5). These findings correlate well with the hypothesis that only Cyp2c29 catalyzes the oxidation of isoscopoletin, whereas more than one P450 is involved in the demethylation of scoparone in mice (14, 27). The lack of [MBQ]-propenoate formation in induced rat liver is most likely due to the predominant induction of CYP2B1, which catalyzes the 7-demethylation of scoparone to scopoletin (18). Isoscopoletin, formed by the concurrent 6-demethylation, does not reach concentrations necessary for effective oxidation and subsequent hydrolysis.

To our knowledge, this is the first report describing an oxidative hydrolysis reaction mediated by a cytochrome P450. As evidenced by our results, such an oxidative hydrolysis seems to be mediated by CYP2C in mice. CYP2C enzymes represent a major portion of CYP's in liver, metabolizing a wide range of substrates with diverse chemical structures (28). To explain unusual substrate and product specificities of CYP2C's, extensive studies concerning structure–function relationships have been done. CYP2C9 is a good example in interacting differently with chemical diverse substrates like (S)-naproxen, neutral phenytoin, phenytoin anion, and progesterone (29, 30). Obviously interaction energies of the key amino acid residues representing the catalytic binding site, Arg 97, Leu 102, Arg 105, Asn 202, Asp 293, and Gly 296, differ between these substrates. To obtain first information about differences between CYP2C9 binding site residue and Cyp2c29, we aligned both enzymes, revealing 50% sequence mismatch from AA97 to AA108, while Leu 102, Asp 293, and Gly 296 were consensus (data not shown). We conclude, that perhaps CYP2C9 will aid us in predicting the residues important for scoparone oxidation of Cyp2c29, although the rank order of interaction energies of Cyp2c29 binding sites and the possible substrates is unclear so far.

The 7-demethylation of scoparone in induced rat liver and its 6-demethylation in induced mouse liver, indicate that scoparone represents a potentially useful tool and a good chemical substrate for the independent detection of either CYP2B1- or Cyp2c29-induction. Moreover, the formation of different reaction products may be used as a specific and sensitive indicator not only for induction of P450 isoforms within one species but also for distinguishing between P450 isoforms specifically induced by definite compounds between species. This novel oxidative hydrolysis pathway of scoparone might be useful in the characterisation of specific drug effects or metabolic activities exerted by P450's in a wide range of model systems, independent of possible drug interactions.

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