IDENTIFICATION OF HUMAN HEPATIC CYTOCHROME P450 ENZYMES INVOLVED IN THE METABOLISM OF 8-PRENYLNARINGENIN AND ISOXANTHOHUMOL FROM HOPS (HUMULUS LUPULUS L.)

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ABSTRACT:

The female flowers of hops (Humulus lupulus L.) are used in the brewing of beer and are under investigation for use in dietary supplements for the management of menopausal symptoms in women. Hop extracts contain the weakly estrogenic compound isoxanthohumol (IX), proestrogenic xanthohumol, and the potent estrogen 8-prenylnaringenin (8PN). Because IX can be metabolized in the human liver to form 8PN, the specific cytochrome P450 (P450) enzymes responsible for this O-demethylation reaction were identified. In addition, the enzymes that convert IX and 8PN to their most abundant metabolites were identified because these metabolic pathways might also affect the estrogenicity of hop preparations. Specifically, the P450 enzymes that catalyze the oxidation of the prenyl side chains of IX and 8PN into trans- or cis-alcohols were investigated. Human liver microsomes and monoclonal antibodies that inhibit specific P450 enzymes were used in combination with liquid chromatography/mass spectrometry to identify the enzymes responsible for these transformations. CYP2C19 was found to catalyze the formation of both cis- and trans-alcohols of the prenyl side chain of 8PN with $K_m$ values of 14.8 ± 3.2 and 16.6 ± 4.6 M, respectively. CYP2C8 converted 8PN regioselectively to the trans-alcohol of the prenyl group with a $K_m$ of 3.7 ± 0.9 M. Finally, CYP1A2 was found to catalyze the O-demethylation of IX to generate 8PN, with a $K_m$ value of 17.8 ± 3.7 M. These results suggest that the estrogenicity of hop constituents in vivo will depend in part on metabolic conversion that may show individual variation.

The female flowers of hops (Humulus lupulus L.) are used in the brewing of beer, and extracts of these strobiles are under investigation as possible alternatives to conventional estrogen replacement therapy for menopausal women (Liu et al., 2001). Alternatives to estrogen supplements like Premarin and Prempro are needed because of safety problems associated with these products, such as increased risk of breast cancer, heart diseases, and dementia (Chlebowski et al., 2003; Culhane, 2003; Manson et al., 2003; Shumaker et al., 2003; Cushman et al., 2004). The prenylated flavanones in hops include xanthohumol, isoxanthohumol (IX), 8-prenylnaringenin (8PN), and 6-prenylnaringenin (Stevens et al., 1997). Among these, 8PN is the most estrogenic (Milligan et al., 1999, 2002).

The estrogen receptor binding activity of 8PN has been studied in vitro using the recombinant human estrogen receptors α and β and found to be similar to estradiol (Milligan et al., 2002). A mammalian cell-based transient transactivation assay showed that 8PN is a potent estrogen receptor-α selective agonist (Schaefer et al., 2003). In addition, the estrogenic activities of 8PN have been confirmed using in vivo assays of vaginal and uterine mitosis and uterotrophic response (Milligan et al., 2002).

A prenylated flavanone with a methoxy group in the 5 position (see structure in Fig. 1), IX is approximately 10-fold more abundant than 8PN in hops and hop extracts (Coldham and Sauer, 2001). In addition, IX can be metabolized by human liver microsomes (HLMs) to form the more potent estrogen 8PN (Nikolic et al., 2005). Although IX has been reported to be cytotoxic and to inhibit the proliferation of the breast cancer cell line MCF-7 (Miranda et al., 1999), IX was not found to be estrogenic in mice (Milligan et al., 2000). Overk et al. (2005) have also investigated the relative estrogenicities of IX and 8PN and reported that 8PN but not IX can activate the estrogen response element in Ishikawa cells, can significantly induce estrogen response element-luciferase expression in MCF-7 cells, and can up-regulate progesterone receptor mRNA in the Ishikawa cell line. However, both 8PN and IX can up-regulate progesterone receptor mRNA in the MCF-7 cell line. Therefore, 8PN has been shown to be more estrogenic than IX both in vitro and in vivo.

During previous studies of in vitro metabolism of 8PN using HLMs (Nikolic et al., 2004, 2005), the most abundant human liver metabo-
lites were identified as oxidation products of the prenyl group. Hydroxylation was found to occur at the terminal methyl groups to produce cis- and trans-alcohols (see Fig. 1). The trans-alcohol of 8PN, 8PN trans-prenyl alcohol (8PN-M2), was the most abundant metabolite of 8PN. In addition to 8PN, metabolism of IX by HLMs produced the hydroxylated prenyl side chain metabolites IX cis-prenyl alcohol (IX-M1) and IX trans-prenyl alcohol (IX-M2) (see structures in Fig. 1).

In this investigation, the cytochrome P450 (P450) enzymes that catalyze the oxygenation of the prenyl side of IX and 8PN in vitro were identified using monoclonal antibody (mAb) inhibitors of specific enzymes and chemical inhibitors of P450 enzymes. In addition, the kinetics of the formation of the cis-alcohol of the terminal prenyl methyl group of 8PN (8PN-M1) from 8PN and the formation of 8PN from IX were determined using recombinant human P450 enzymes. The P450 enzymes that contribute to the O-demethylation of isoxanthohumol were also identified. The identification of the P450 enzymes involved in the formation of significant metabolites of IX and 8PN should be useful in predicting potential hop-drug interactions and whether significant variation in the metabolism of IX and 8PN might occur in the population as a result of genetic variation.

**Materials and Methods**

**Chemicals, Enzymes, and Antibodies.** Racemic 8PN and IX were isolated from hops and purified by semipreparative high-performance liquid chromatography (HPLC) as described previously (see Nikolic et al., 2004 and Chadwick et al., 2004, respectively). 8PN-M1 and 8PN-M2 were synthesized as described by Nikolic et al. (2004), and IX-M2 was isolated as described by Chadwick et al. (2004). Purity was determined to be >98% based on HPLC and liquid chromatography/mass spectrometry (LC/MS) analysis. All the other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). HPLC-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA).

Pooled HLMs were purchased from In Vitro Technologies (Baltimore, MD). The total P450 content of the microsomes was 0.17 nmol/mg protein. Anti-CYP1A1, -CYP1A2, -CYP2A6, -CYP2B6, -CYP2C8, -CYP2C9*1, -CYP2C9*2, -CYP2C19, -CYP2D6, -CYP2E1, and -CYP3A4 mAb were obtained from Dr. Harry Gelboin of the National Institutes of Health (Bethesda, MD). Protein concentrations of the liver microsomes and the mAbs were determined using the method of Bradford (1976) with bovine serum albumin as a standard. Microsomes from baculovirus-infected insect cells containing human P450 reductase and human cytochrome b5 with cDNA-expressed human CYP2C8 (1.0 nmol P450/ml), CYP2C19 (1.0 nmol P450/ml), or CYP1A2 (1.0 nmol P450/ml) were purchased from BD Biosciences (San Jose, CA).

**Linearity Assays.** In preparation for inhibition and kinetics assays, the linearity of the formation of each major metabolite, including 8PN-M1, 8PN-M2, IX-M1, IX-M2, and 8PN (from IX), was investigated by incubating 8PN or IX (10 μM) with HLMs (0.5 mg/ml). The reactions were stopped at various time points up to 40 min, and the major metabolites were measured using LC/MS as described below. The formation of 8PN-M1, 8PN-M2, IX-M1, and IX-M2 was linear up to 40 min (data not shown). However, the formation of 8PN from IX was linear only during the first 15 min (data not shown).

**Inhibition of P450 Enzymes.** To identify specific P450 enzymes responsible for the formation of significant metabolites of IX and 8PN, incubations were carried out using either mAbs or chemical inhibitors of specific enzymes. All the incubations contained 10 μM 8PN or IX, 1 mg/ml human hepatic...
microsomal protein, and 1 mM NADPH in 50 mM phosphate buffer at pH 7.4 in a total volume of 0.4 ml. Either mAb inhibitors of specific P450 enzymes were added at a final concentration of 0.4 mg/ml or chemical inhibitors were used. The chemical inhibitors consisted of 10 μM omeprazole, 20 μM quercetin, 10 μM furafylline, or 10 μM sulfaphenazole (Becquemont et al., 1999; Kim et al., 2004), which selectively inhibited CYP2C19, CYP2C8, CYP1A2, or CYP2C9.

Each enzymatic reaction was preceded by a 5-min preincubation and was initiated by the addition of 10 mM NADPH. Control incubations were carried out that contained all the components except the inhibitory antibodies or compounds. All of these incubations were carried out at 37°C for 30 min, except for the IX O-demethylation reaction, which was incubated for 10 min (based on the preliminary linearity studies). Reactions were terminated by the addition of 1.6 ml of ice-cold acetonitrile/ethanol (1:1, v/v). After centrifugation to remove the precipitated proteins, the supernatants were removed and evaporated to dryness under vacuum. Each residue was reconstituted in 150 μl of HPLC mobile phase immediately before analysis using LC/MS. All the incubations using inhibitory antibodies or chemical inhibitors were carried out at least three times; means were calculated; and the mean values were compared using one-way analysis of variance (ANOVA) with the Tukey test (p ≤ 0.01).

It should be noted that before beginning the IX and 8PN studies, the inhibitory activity of the mAb was verified by incubating the HLM (protein concentration of 25 mg/ml) with or without a 10 μM concentration of each of the following standard chemical substrates: dextromethorphan, chlorzoxazone, acetaminophen, hydroxytolbutamide, 4'-hydroxy- (S)-mephenytoin, or testosterone. These compounds are substrates for CYP2D6, CYP2E1, CYP2C8, CYP1A2, CYP2C9, CYP2C19, and CYP3A4, respectively. After 30 min of incubation at 37°C, the formation of the following metabolites was measured using LC/MS: dextromethorphan, 6-hydroxychlorzoxazone, desethylamodiaquine, acetaminophen, hydroxytolbutamide, 4'-hydroxy-(S)-mephenytoin, and 6β-hydroxytestosterone, respectively. The mAbs inhibited these reactions by 90, 90, 85, 90, 80, 100, and 86%, respectively, which was adequate for the identification of enzymes that inhibited the metabolism of IX and 8PN.

Assays Using Recombinant P450 Enzymes. To confirm that the P450 enzymes identified using the inhibition screening assays catalyzed the formation of the expected IX and 8PN metabolites and to investigate the relative contribution of each enzyme, additional incubations were carried out using recombinant CYP2C8, CYP2C19, or CYP1A2. Each reaction contained 10 μM IX or 8PN, 1.0 mM NADPH, and 1 nmol/ml CYP2C8, CYP2C19, or CYP1A2 (containing coexpressed P450 reductase and cytochrome b5). Incubations were carried out for 10 min each at 37°C.

The measurement of the kinetics of the formation of 8PN-M1 and 8PN-M2 from 8PN catalyzed by HLMs has been described previously (see Nikolic et al., 2004). Briefly, 37.5 pmol/ml CYP2C8 or 10 pmol/ml CYP2C19 was incubated with 8PN at 0.625 to 100 μM. Each incubation was carried out at 37°C for 10 min. The kinetics of 8PN formation was also studied using 12.5 pmol/ml CYP2C19 and IX at concentrations from 0.625 to 100 μM. The reactions were terminated by the addition of cold acetonitrile/ethanol and prepared for LC/MS analysis as described above for the inhibition studies. Quantitative analyses of 8PN-M1, 8PN-M2, and 8PN were carried out using LC/MS as described below. Curve fitting of the kinetics data (Michaelis-Menten and Eadie-Hofstee plots) and the $K_m$ and $V_{max}$ were calculated using SigmaPlot 8.0 software (Richmond, CA).

LC/MS Analysis. The formation of specific metabolites of IX and 8PN was determined by comparison with authentic standards using LC/MS with reversed-phase HPLC separation and negative ion electrospray mass spectrometric detection. In addition, quantitative analyses of the major metabolites of IX and 8PN were carried out using LC/MS. Standard curves for these compounds were prepared using either synthetic standards or compounds isolated from hops. The LC/MS system consisted of an Agilent (Palo Alto, CA) 1100 HPLC system interfaced to a G1946A quadrupole mass spectrometer. HPLC separations were carried out using a YMC (Wilmington, NC) ODS-AQ S-3 reversed-phase column (2.0 × 150 mm, 5-μm particle size) with a 30-min
linear gradient from 35 to 70% methanol in 0.05% aqueous acetic acid, followed by a 10-min gradient from 70 to 90% methanol, and then 90 to 95% methanol in 5 min. The column was re-equilibrated for 8 min between injections. The flow rate was 0.2 ml/min; the column temperature was 30°C; and the autosampler was maintained at 4°C. During negative ion electrospray, the capillary voltage was 2.5 kV; the flow rate of the drying gas was 5.0 l/min; and the nebulizer gas pressure was 40 psig. Selected ion monitoring with a dwell time of 115 ms/ion was used to monitor the deprotonated molecules of 8PN (m/z 339.1), 8PN-M1 and 8PN-M2 (m/z 355.1), IX-M1 and IX-M2 (m/z 369.1), and the internal standard, naringenin (m/z 271.1).

**Results**

**Metabolism of 8PN by Human P450 Enzymes.** Incubations of 8PN and HLMs were carried out with each of 11 different mAbs that inhibit specific P450 enzymes to identify enzymes responsible for the formation of the most abundant metabolites 8PN-M1 and 8PN-M2. The P450 enzymes inhibited by these mAbs represent more than 75% of the P450 enzymes in the human liver. The results of these enzyme inhibition studies for 8PN metabolism are summarized in Fig. 2.

As shown in Fig. 2, CYP2C19 and CYP2C8 were primarily responsible for the formation of 8PN-M1 and 8PN-M2. In particular, only CYP2C19 catalyzed the formation of the cis-alcohol, 8PN-M1, whereas both CYP2C19 and CYP2C8 formed the trans-alcohol, 8PN-M2. These results were confirmed by the use of chemical inhibitors of P450 enzymes (Fig. 3). For example, incubation of omeprazole, which inhibits CYP2C19, with HLMs resulted in a significant reduction in formation of both 8PN-M1 and 8PN-M2. Treatment of the HLM with quercetin, which is an inhibitor of CYP2C8 but not CYP2C19, resulted in no reduction in the formation of 8PN-M1 as expected. However, no significant reduction in the formation of 8PN-M2 was observed either, although there was a downward trend (Fig. 3). This marginal inhibitory effect of quercetin on CYP2C8 was also reported by Desta et al. (2000) when studying the metabolism of cisapride.

As independent confirmation of the results from the use of selective enzyme inhibitors for the identification of P450, recombinant forms of the enzymes responsible for the formation of 8PN-M1 and 8PN-M2, CYP2C8 or CYP2C19, were incubated with 8PN, and the metabolites were characterized using LC/MS (Fig. 4). These studies showed that CYP2C8 can form trace amounts of 8PN-M1 (note that the signal for 8PN-M1 was significantly more than that of the control without enzyme, p < 0.05). Furthermore, the formation of 8PN-M2 by CYP2C8 was more efficient than that of 8PN-M1 (see Fig. 4). As indicated by the inhibition experiments, both CYP2C8 and CYP2C19 catalyze the formation of 8PN-M2 with the contribution of CYP2C19 predominating approximately 3.5-fold, based on the comparison of \( V_{\text{max}}/K_m \) for both enzymes (Table 1). Overall, CYP2C8 showed greater stereoselectivity and favored the formation of 8PN-M2 over 8PN-M1 (see Fig. 4).

Preliminary experiments were carried out to determine the linearity of 8PN-M1 and 8PN-M2 production with respect to the concentration of human CYP2C8 and CYP2C19 protein. The rate of formation of 8PN-M2 was linear for at least 10 min during the incubation of CYP2C8 (at 12.5, 25, and 50 nM) with 10 µM 8PN (data not shown). In addition, the formation of both 8PN-M1 and 8PN-M2 from 10 µM 8PN was linear for at least 10 min during incubations with human CYP2C19 (at 3.1, 6.25, and 12.5 nM) (data not shown). Therefore, 37.5 nM CYP2C8 and 10 nM CYP2C19 were incubated with 10 µM 8PN for 10 min during all the subsequent experiments.

The kinetics of the formation of 8PN-M1 and 8PN-M2 catalyzed by human CYP2C8 and CYP2C19 were determined and are shown in Fig. 5 and Table 1. The kinetics parameters were calculated using a nonlinear regression analysis fit of the data to the Michaelis-Menten equation. The \( K_m \) values (the apparent affinity constants) for the metabolism of 8PN to form 8PN-M1 and 8PN-M2 by CYP2C19 were 14.8 and 16.6 µM, respectively. Despite the similarity of \( K_m \) values, the maximum initial enzyme velocity, \( V_{\text{max}} \), for the formation of
8PN-M2 from 8PN was approximately 2-fold greater than the \( V_{\text{max}} \) for the formation of 8PN-M1 by CYP2C19. This resulted in a specificity constant (\( V_{\text{max}}/K_m \)) for the formation of 8PN-M2 by CYP2C19 that was twice that of 8PN-M1. The kinetics of 8PN-M2 formation from 8PN by CYP2C8 was also determined, and the \( V_{\text{max}} \) and \( K_m \) were both less than the corresponding values for 8PN-M2 formed by CYP2C19 (see Table 1). However, the specificity constant was approximately 30% that of CYP2C19 formation of 8PN-M2 from 8PN.

The kinetics data for the formation of 8PN-M1 and 8PN-M2 from 8PN by pooled HLMs were redrawn as Eadie-Hofstee plots. As indicated by these plots in Fig. 6, the formation of both 8PN-M1 and 8PN-M2 was nonlinear. The implications of this nonlinearity are discussed below.

Metabolism of IX by Human P450 Enzymes. The mAbs that inhibit specific P450 enzymes were also used to identify enzymes that catalyze the formation of the cis- and trans-alcohols of the terminal prenyl methyl group of IX, as well as the \( O \)-demethylation of IX to form 8PN. The results of these incubations and LC/MS assays are shown in Fig. 7 and indicate that CYP2C19 is primarily responsible for the formation of the hydroxylated metabolite IX-M2. After 30 min of incubation of IX with HLMs and an anti-CYP2C19 mAb, the formation of IX-M2 and IX-M1 was reduced 94 and 77%, respectively, compared with their formation in a control incubation that was identical except for the omission of the inhibitory antibodies. Therefore, CYP2C19 was identified as the enzyme primarily responsible for the formation of both IX-M2 and IX-M1.

Next, the P450 enzymes catalyzing the \( O \)-demethylation of IX to 8PN were identified using mAb inhibitors and then confirmed by using a chemical inhibitor. As shown in Fig. 7A, CYP1A2 was found to be the enzyme responsible for the formation of 8PN from IX based on the use of mAb inhibitors. Subsequently, treatment of HLMs with 10 \( \mu \)M furafylline, which is a selective inhibitor of CYP1A2, reduced the \( O \)-demethylation of IX by 93%, compared with control, which contained all the ingredients except the inhibitory compounds. Therefore, CYP1A2 was identified as the enzyme primarily responsible for the formation of 8PN from IX.

These results for the formation of IX metabolites were confirmed by incubating IX with recombinant human CYP1A2 and CYP2C19. As shown in Fig. 8, CYP1A2 catalyzed the \( O \)-demethylation of IX to form 8PN, and CYP2C19 catalyzed the hydroxylation of IX to IX-M1 and IX-M2. Furthermore, the formation of IX-M2 from IX by CYP2C19 was favored slightly over formation of IX-M1 (see Fig. 8B).

The kinetics of the formation of 8PN catalyzed by human CYP1A2 was investigated as shown in Fig. 9 and Table 1. The kinetic data were calculated using a nonlinear regression analysis to fit the parameters

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**TABLE 1**

Enzymatic constants of 8PN and IX metabolism

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>CYP2C8</th>
<th>CYP2C19</th>
<th>CYP1A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8PN-M1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{\text{max}} ) (pmol/min/pmol)</td>
<td>32.7 ± 2.9</td>
<td>14.8 ± 3.2</td>
<td>4.64 ± 0.49</td>
</tr>
<tr>
<td>( K_m ) (( \mu M ))</td>
<td>16.6 ± 4.6</td>
<td>72.1 ± 8.4</td>
<td>3.72 ± 0.92</td>
</tr>
<tr>
<td>Intrinsic clearance (( \mu l/min/pmol ))</td>
<td>2.21</td>
<td>4.35</td>
<td>1.24</td>
</tr>
<tr>
<td>8PN-M2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{\text{max}} ) (pmol/min/pmol)</td>
<td>4.64 ± 0.49</td>
<td>72.1 ± 8.4</td>
<td>5.47 ± 0.39</td>
</tr>
<tr>
<td>( K_m ) (( \mu M ))</td>
<td>17.77 ± 3.74</td>
<td>16.6 ± 4.6</td>
<td>3.72 ± 0.92</td>
</tr>
<tr>
<td>Intrinsic clearance (( \mu l/min/pmol ))</td>
<td>0.31</td>
<td>4.35</td>
<td>1.24</td>
</tr>
</tbody>
</table>

\( * \) Mean ± S.D. (n = 3).
of the Michaelis-Menten equation. The $K_m$ value for the metabolism of IX to form 8PN was determined to be 17.7 μM.

**Discussion**

In addition to use during the brewing of beer, hop extracts are under investigation as botanical dietary supplements for the relief of hot flashes in menopausal women because of their estrogenic properties (Liu et al., 2001). Although the most estrogenic constituent of hop preparations is 8PN, it is much less abundant than xanthohumol or IX (Coldham and Sauer, 2001). However, xanthohumol is known to cyclize to form the weak estrogen IX under the acidic conditions of the stomach, and IX can be metabolically O-demethylated to form the potent estrogen 8PN (Nikolic et al., 2005). Therefore, an understanding of the P450 enzymes responsible for the formation of 8PN from IX would be important for the interpretation of in vivo data from safety and efficacy studies. Also, the enzymes that catalyze the metabolic transformation of 8PN and IX to other specific (perhaps less estrogenic) metabolites should be identified. This information could be used to investigate potential drug-drug interactions or effects of P450 polymorphisms that might affect the safety and efficacy of dietary supplements containing hops or hop extracts.

As alternatives to estradiol or equine estrogen preparations such as Premarin for possible use by women in the management of menopausal symptoms, the activities of hop preparations containing 8PN, IX, and xanthohumol should be evaluated in vivo and in vitro. The in vitro studies reported here concern the identification of enzymes responsible for significant phase I metabolism pathways of 8PN and IX. Although these data will be useful during the design and interpretation of future in vivo studies, additional in vitro studies are required and are in progress in our laboratory to address other factors affecting the bioavailability and efficacy of hop components such as intestinal absorption, enzyme inhibition, and phase II conjugation, including glucuronidation and sulfation.

Additional issues that should be investigated concerning the in vivo activities of hop preparations include the evaluation of their selectivity toward estrogen receptor-α as compared with estrogen receptor-β, and their potential antiestrogenic effects. For example, selective estrogen receptor modulators such as tamoxifen (Fisher et al., 1998) are antiestrogens that are useful for the chemoprevention of breast cancer. Therefore, complex preparations such as hop extracts have the potential to contain both estrogenic and antiestrogenic constituents that may help relieve menopausal symptoms such as hot flashes in women while not contributing to increased risks of breast cancer.

The most abundant oxygenated metabolites of IX and 8PN are the hydroxylated products IX-M1, IX-M2, 8PN-M1, and 8PN-M2. Among these cis- and trans-hydroxylated prenyl groups, the trans-
isomers predominate, which is probably because of their lower internal energy and therefore greater stability compared with the cis-isomers. In this investigation, CYP2C19 was determined to be the P450 enzyme that was primarily responsible for the formation of the trans-hydroxylated prenyl metabolites of 8PN and IX, 8PN-M2 and IX-M2, respectively. Although CYP2C19 also catalyzed the hydroxylation of 8PN to form the cis-isomer, 8PN-M1, the specificity constant for the formation of 8PN-M2 by CYP2C19 was 2-fold greater than for 8PN-M1 (Table 1). The formation of 8PN-M2 was also stereoselectively catalyzed by CYP2C8 (Table 1), which was probably the result of the unique spatial arrangement of CYP2C8 active site amino acid residues, resulting in substrate selectivity and regioselectivity (Kerdpin et al., 2004).

The contributions of the enzymes CYP2C19 and CYP2C8 to the formation of 8PN-M2 is consistent with the Eadie-Hofstee plot shown in Fig. 6, which was obtained by incubation of 8PN with pooled HLMs. Although the plot for 8PN-M2 formation in Fig. 6 is consistent with a model describing the action of more than one enzyme, an alternative explanation that is consistent with these data would be a single enzyme containing more than one binding site. The Eadie-Hofstee plot for the formation of 8PN-M1 in Fig. 6 also indicates that either more than one enzyme or more than one binding site might be involved, even though the enzyme inhibition studies showed that only CYP2C19 catalyzed the formation of 8PN-M1 and IX-M1.

Our studies indicate that CYP2C19 and CYP2C8 are responsible, at least in part, for the hydroxylations of the prenyl side chain of 8PN and IX, and CYP1A2 catalyzes the O-demethylation of IX to form 8PN. The cis- and trans-alcohols of the prenyl side chains of 8PN and IX are the most abundant phase I metabolites of these compounds, and the formation of 8PN from IX has the potential to enhance the estrogenicity of IX. The enzymes CYP1A2 (Saito et al., 2005), CYP2C19, and CYP2C8 (Smith et al., 1998) exhibit polymorphisms in humans. Because these variants sometimes show much lower activities than the wild-type enzymes (e.g., variants of human CYP1A2 have been reported with less than 1% of the wild-type activity), such polymorphisms can be significant sources of interindividual differences in drug metabolism (Ingelman-Sundberg, 2002). Furthermore, the activities of these enzymes can be regulated by both environmental and genetic factors. Examples of environmental factors that can induce or inhibit the activities of P450 enzymes include many prescription drugs, diet, alcohol consumption, and smoking (Cranshaw and Hines, 1992; Zhang et al., 2002; Czekaj et al., 2005). In conclusion, these results suggest that the estrogenicity of hop constituents will depend in part on metabolic conversion that may show individual variation.

References
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