IN VITRO BIOTRANSFORMATION OF XANTHOHUMOL, A FLAVONOID FROM HOPS (HUMULUS LUPULUS), BY RAT LIVER MICROSONES

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ABSTRACT:

Xanthohumol (XN) is the major prenylated flavonoid of the female inflorescences (cones) of the hop plant (Humulus lupulus). It is also a constituent of beer, the major dietary source of prenylated flavonoids. Recent studies have suggested that XN may have potential cancer-chemopreventive activity, but little is known about its metabolism. We investigated the biotransformation of XN by rat liver microsomes. Three major polar metabolites were produced by liver microsomes from either untreated rats or phenobarbital-pretreated rats as detected by reverse-phase high-performance liquid chromatography analysis. Liver microsomes from isosafrole- and b-naphthoflavone-pretreated rats formed another major nonpolar metabolite in addition to the three polar metabolites. As determined by liquid chromatography/mass spectrometry and 1H NMR analyses, the three major polar microsomal metabolites of XN were tentatively identified as 1) 5'-isopropyl-5'-hydroxydihydrofuran[2',3':3,4']-2,4-dihydroxy-6'-methoxychalcone; 2) 5'- hydroxyisopropyl)-dihydrofuran[2',3':3,4']-2,4-dihydroxy-6'-methoxychalcone; and 3) a derivative of XN with an additional hydroxy function at the B ring. The nonpolar XN metabolite was identified as dehydrocycloxanthohumol.

The flavonoids are some of the most ubiquitous compounds found in nature. Over 4000 different naturally occurring flavonoids have been described. Flavonoids are common substances in the daily diet and are resistant to boiling and fermentation (Kim et al., 1998). They display a wide range of biological and pharmacological properties (Hollman and Katan, 1998), including antiviral, anticancer, and antioxidant properties (Boutin et al., 1993). Several flavonoids can inhibit or activate cytochrome P450 (CYP) enzyme systems in vitro and in vivo (Nielsen et al., 1998). Flavonoids, including chalcones, have been shown to inhibit the proliferation of cancer cells and inhibit tumor growth (Miranda et al., 1999).

The hop plant (Humulus lupulus L.) is a dioecious twining perennial. The female inflorescences (cones or "hops") are used in the brewing industry to give beer its characteristic flavor and aroma. Xanthohumol (XN) is the principal flavonoid present in hop cone extracts and has a prenylated chalcone structure (Fig. 1). XN constitutes 82 to 89% of the total amount of prenylated flavonoids of different European hop varieties (Stevens et al., 1997). Substitutions on the chalcone structure have a profound influence on the antitumorogenic effects of these compounds. More specifically, studies have shown that XN is an effective antiproliferative agent in human breast cancer cells (MCF-7), colon cancer cells (HT-29), and ovarian cancer cells (A-2780) (Miranda et al., 1999). Other studies such as inhibition of CYP enzymes (Henderson et al., 2000) and induction of quinone reductase (Miranda et al., 2000) by XN suggest that XN may have promising cancer-chemopreventive properties.

Although flavonoids are abundantly present in the human diet and in animal feeds, little is known about their metabolism. Two major sites of flavonoid biotransformation are the liver and the colonic flora (Hollman and Katan, 1998). Since the CYP superfamily is abundant in the liver and small intestine, they may play a role in the metabolism of flavonoids, including XN. The metabolism of flavonoids such as naringenin, genistein, hesperetin, quercetin, chrysins, apigenin, tangeretin, kaempferol, galangin, tamarixetin, taxifolin, luteolin, myricetin, morin, and fisetin has been investigated in rat liver microsomes, but there is no information about metabolism of prenylated flavonoids (Nielsen et al., 1998; Roberts-Kirchhoff et al., 1999). Thus, in the present study, we examined the biotransformation of the prenylated chalcone, XN, in liver microsomes from rats untreated and treated with various P450-inducing agents, including phenobarbital, isosafrole, and b-naphthoflavone. The metabolites were characterized by HPLC, UV spectroscopy, liquid chromatography/mass spectrometry (LC/MS), and proton nuclear magnetic resonance (1H NMR).
Materials and Methods

Chemicals. Xanthohumol, desmethylxanthohumol, dehydroxycyclohexanohumol hydrate (DH, also referred to as xanthohumol B), and dehydroxycyclohexanohumol (DX, also referred to as xanthohumol C) were isolated and purified from hops during previous work (Stevens et al., 1997, 2000). Troleandomycin (TAO), formic acid, phenobarbital (PB), β-naphthoflavone (BNF), and NADPH were purchased from Sigma Chemical Company (St. Louis, MO). α-Naphthoflavone (ANF) and isosafrole (ISF) were from Aldrich Co. (Milwaukee, WI). Acetonitrile, ethanol, and methanol were HPLC grade from Mallinckrodt Baker, Inc. (Paris, KY). Dimethyl-d6 sulfoxide (100%) and MgCl2 were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA) and Kodak (Rochester, NY), respectively. Prazadin (SKF-525A) and 1,2-epoxy-3,3,3-trichloropropene (TCPO) were from SmithKline and French Labs (Philadelphia, PA) and Pfaltz and Bauer (Stamford, CT), respectively.

Polyfunctional antibodies against rat NADPH-cytochrome P450 reductase were purchased by M. C. Henderson according to methods previously described (Kaminsky et al., 1981; Arldies et al., 1987).

Isolation of Rat Liver Microsomes. Sixteen male Sprague-Dawley rats (165–185 g body weight) were purchased from Simonsen Company (Gilroy, CA). Animals were divided into four groups with four animals in each cage. Microsomes were prepared as described (Williams and Buhler, 1984) from pooled livers of rats that had been treated intraperitoneally with 0.9% NaCl, PB (80 mg/kg), ISF (150 mg/kg), or BNF (40 mg/kg) daily for 3 days. On the 4th day after exposure, preceded by a 24-h fasting period, the rats were anesthe-tized using CO2 and killed by asphyxiation. The washed liver microsomes, resuspended in 0.1 M potassium phosphate buffer, pH 7.4, containing 20% glycerol and 1 mM EDTA, were frozen at −80°C before use. Protein concentrations of liver microsomes were determined by the method of Omura and Sato (1964).

Xanthohumol Biotransformation by Rat Liver Microsomes. A typical 0.5-ml biotransformation incubation mixture consisted of 0.5 mg of protein, 0.1 M Tris-HCl, pH 7.4, containing 10 mM MgCl2 and 100 μM XN (dissolved in 3 μl of ethanol) as substrate. The reaction was initiated by adding 2 mM NADPH. Incubations were carried out at 37°C for 60 min with continuous shaking in a Dubnoff incubator. Control incubations were performed without the addition of NADPH or without microsomes. Reactions were terminated by adding 1.5 ml of ice-cold methanol, followed by centrifugation at 4°C. The supernatants were evaporated to dryness under nitrogen gas. The residues were redissolved in 100 μl of 75% CH3CN containing 1% formic acid and subsequently analyzed on the same day by HPLC for XN metabolites. All experiments were carried out in duplicate. One set of experiments was done with ethanol alone without microsomes.

Optimization of Protein and Substrate Concentrations for Xanthohumol Biotransformation. To obtain an appropriate concentration of microsomes for biotransformation, incubations were performed using the following microsomal protein concentrations: 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, and 2.00 mg/ml. Different concentrations of XN (10, 50, 100, 200, 400, 600, or 800 μM) also were used to obtain the optimal concentration for metabolite production.

Time Course of Biotransformation of Xanthohumol. To study the biotransformation of XN with respect to time, a slightly modified procedure was used. Liver microsomes from ISF-induced rats were used in these experiments so that all four major XN metabolites were formed. Incubations in a final volume of 250 μl were used, and reactions were stopped at 0, 10, 20, 30, 40, 50, and 60 min by adding ice-cold methanol. The samples were centrifuged and the supernatants evaporated to dryness. HPLC analysis was conducted as described below.

Incubations with CYP Inhibitors. Various CYP inhibitors were included in the incubations to give some insight into the identity of the specific enzymes involved in XN biotransformation. Mixtures were incubated at 37°C for 30 min with one of the following inhibitors, with the target enzyme and the indicated final concentration in parentheses: SKF-525 (0.5 mM; nonspecific), ANF (0.1 mM; CYP1A), TAO (0.5 mM; CYP3A), and TCPO (0.1 mM; epoxide hydrolase) before addition of XN. Inhibitor concentrations for these experiments were chosen according to those used in previous studies (Lewis, 1996). As these inhibitors and substrate were prepared in 3 μl of ethanol, an additional control incubation was included with only ethanol for each incubation to correct for any effect of the solvent on the microsomes. Carbon monoxide (100%) and antibodies raised against NADPH-cytochrome P450 reductase also were used to inhibit the microsomal biotransformation. The pretreatments with 100% carbon monoxide and rat reductase antibodies were for 15 and 30 min, respectively, at room temperature before the addition of XN. Inhibition of the reaction was expressed as the percentage decrease in metabolite production based on the peak area of the metabolite obtained from incubation with addition of inhibitor compared with that without.

HPLC and Isolation of Metabolites by HPLC. A Waters (Milford, MA) 2690 HPLC system with a 996 diode-array detector and a 4-μm Nova-Pak C18 column (3.9 × 150 mm, Waters) were used to separate metabolites. The column temperature was thermostatically maintained at 35°C. Detection was at 368 nm, with simultaneous scanning from 230 to 400 nm. Samples dissolved in 100 μl of 25% aqueous acetonitrile containing 1% formic acid were analyzed using a slight modification of the method of Nielsen et al. (1998). Metabolites were eluted with acetonitrile and water containing 1% formic acid at a flow rate of 0.8 ml/min. The initial 29% CH3CN was increased to 60% over 18 min and then to 84% over the next 10 min with a linear gradient function. At 34 min, the CH3CN was returned to 29% over 4 min, and the column was equilibrated for 10 min before the next injection. The metabolites were identified by LC/MS and 1H NMR after running a large-scale incubation with 12.1 mg of XN and a NADPH-generating system with glucose-6-phos- phate and collecting the fractions of this incubation by preparative HPLC with an Alltech (Deerfield, IL) Econosil C18 (250 × 22 mm) column at a flow rate of 11 ml/min. All isolated metabolite fractions were lyophilized and redissolved in 25% CH3CN containing 1% formic acid before LC/MS analysis.

LC/MS. LC/MS was performed with Waters 6000A pumps using a 5-μm C18 column (250 × 4.0 mm) at a flow rate of 0.8 ml/min. XN metabolites were separated with a linear solvent gradient starting from 40% CH3CN to 100% CH3CN in 1% aqueous formic acid over 30 min. At 35 min, the % CH3CN was returned to 40% in 2 min, and the column was equilibrated for 15 min before the next injection. Mass spectra were recorded on a PE Sciex API III + triple quadrupole mass spectrometer using atmospheric pressure chemical ionization (APCI) in positive mode, with an orifice voltage of +55 V, source temperature of 60°C, and 100 μl of CH3CN, and CH3CN (450°C, Ontario, Canada). Samples were introduced by loop injection or by HPLC via the heated nebulizer interface set at 500°C. The multiple ion scan mode was used for selective detection of metabolites. Daughter-ion scanning in the MS-MS mode was used to obtain structural information. The target gas in the collision cell was argon-nitrogen (9:1) at a density of ca. 1.8 × 1014 atoms cm−2. The collision energy was set at 15 V.

Nuclear Magnetic Resonance. 1H NMR spectra were recorded in dimethyl-d6 sulfoxide at room temperature on a Bruker DRX 600 spectrometer at 600 and 150.9 MHz. Dimethyl-d6 sulfoxide resonances were used as internal standard references. 1H–1H correlation spectroscopy was performed using standard pulse sequences.

Results

XN biotransformation was investigated by incubation of XN with microsomes from male Sprague-Dawley rats. Four different metabolites were detected and tentatively identified by HPLC, LC/MS, and
NMR analyses. Three major polar metabolites (M1, M2, and M3) were found with liver microsomes from either untreated or PB-pretreated rats as detected by HPLC analysis (Fig. 2A). Liver microsomes from ISF- and BNF-pretreated rats formed another major nonpolar metabolite (M4) in addition to the other three (Fig. 2B). The retention times for XN and its metabolites were as follows: XN, 17.6 min; M1, 9.3 min; M2, 11.2 min; M3, 15.2 min; and M4, 22.0 min. No biotransformation of XN occurred in microsomal incubations that did not contain NADPH (Fig. 2C). The amounts of metabolites produced by rat liver microsomes varied considerably by treatment of rats with different CYP inducers (Fig. 3). Figure 4 demonstrates the formation of XN metabolites from ISF-treated male rat liver microsomes over a 1.0-h incubation period. The ratio of the metabolite formation of all three polar metabolites decreased after 20 min of incubation, but the nonpolar metabolite, M4, showed a time-dependent increase.

Kinetic parameters were calculated according to the Michaelis-Menten equation by nonlinear least-squares regression analysis (Powell's Method). As the absorption coefficients of metabolites were unknown, the calculations were made according to an assumption that the metabolites’ UV absorbance was equivalent to XN. These apparent $K_m$ and $V_{max}$ values are presented in Table 1.

**Effects of CYP Inhibitors.** The results of XN’s biotransformation from the male rat liver microsomal incubations after treatment with various inhibitors are presented in Fig. 5. Carbon monoxide saturation of rat liver microsomes, which inhibited CYP activity, resulted in a decreased formation of metabolites [M1 (66%), M2 (23%), M3 (64%), and M4 (78%)]. The biotransformation of XN was altered with the addition of SKF-525A, a multiple type of CYP inhibitor, decreasing formation of M1, M2, M3, and M4 by 66, 41, 59, and 62%, respectively. ANF, a reversible inhibitor of CYP1A, decreased M1, M2, and M4 by 55, 17, and 82%, respectively. The effect of ANF on M3 could not be determined since ANF acted as a CYP substrate and gave a metabolite peak at the same retention time as M3. TAO, an inhibitor of CYP3A enzymes in rats and humans, inhibited M1 formation by 55% and other metabolites by an average of 21%. TCPO, an epoxide hydrolase inhibitor, decreased M1, M2, and M3 by 37% and M4 by 15%. Polyclonal antibodies against rat NADPH-cytochrome P450 reductase in the incubations caused a decrease of M1, M2, M3, and M4 by 22, 3, 30, and 33%, respectively.
Identification of Metabolites. After preparative HPLC isolation, metabolites were characterized by APCI-MS/MS and 1H NMR spectroscopy. The structure of M2 was determined by mass and NMR spectrometry. The UV spectrum showed maximum absorption at 372 nm, similar to the chalcone flavonoid XN. Its molecular mass, 370 Da, suggested that it contained an additional hydroxy group. Upon MS-MS fragmentation of the MH+ ion, loss of a water molecule, loss of the prenyl moiety, and fission of the OC-C8 bond (Stevens et al., 1997) were most prominent, giving rise to fragments with m/z 353 [371 - 18]+, 251 [A ring]+, 233 [251-H2O]+, 179 [A - C4H8O]+, and m/z 147 [B ring]+ (Fig. 6). Proton NMR analysis of M2 (Table 2) showed an OMe resonance at δ 3.89 (singlet) and two olefinic protons, H-α and H-β (broad singlet at δ 7.66, integrating for two protons). The six-proton singlets at δ 1.23 and δ 1.14 were attributed to the two methyl groups of the prenyl moiety. The B ring protons, H-2/H-6 and H-3/H-5, appeared as a set of doublets at δ 7.55 and 6.84 (J = 10 Hz). The aromatic A ring proton H-5' gave a singlet at δ 6.17. These resonances clearly indicated that the A and B rings of XN had not been oxygenated. The remaining resonances at δ 4.69 (triplet) and δ 2.97 (doublet), which showed interactions with each other in the 1H-1H correlation spectroscopy spectrum, were attributed to a -CH(O)-CH2- spin system, indicating that the prenyl substituent of XN was oxygenated and fixed in a dihydrofurano ring or a dimethylpyrano ring. The latter possibility was ruled out by proton NMR comparison with DH (compound 9 in Stevens et al., 2000). Metabolite M2 was therefore identified as 5′′-(2′′-hydroxyisopropyl)dihydrofurano[2′′,3′′:3′:4′]-2′′,4-dihydroxy-6′-methoxychalcone. The UV spectrum of M2 was determined by mass and NMR analysis. The UV spectrum of M3 appeared to be similar to that of XN, with a peak maximum at 375 nm suggesting a chalcone-like structure. The molecular mass of M3 was determined to be 370 Da by APCI-MS, indicating that M3 was also a hydroxylated metabolite of XN. MS-MS fragmentation of the MH+ ion resulted in loss of the prenyl group (see peak at m/z 315 [371 - 56]+) in Fig. 8), while RDA fragments appeared at m/z 235 and 179 [235 - 56]+. Loss of 56 mass units (C4H8) indicated that the prenyl substituent had not been oxygenated during incubation with liver microsomes. This is consistent with the metabolite’s failure to produce “dehydrated” ions (e.g., [MH - H2O]+) upon MS-MS spectra of the hydroxypropenyl derivatives, M1 and M2. Neither was the A ring metabatically modified: this piece of information was deduced from the peak at m/z 179, attributable to an RDA fragment ion also observed in MS-MS spectra of XN. Although none of the fragment ions could be related to the B ring itself because of charge retention on the A ring, it was concluded from the MS data that the oxygenation site was on the B ring (C-2 or C-3) of XN. A summary of the pathways for metabolism of XN to M2, M3, M4, and M1 with proposed intermediates is shown in Figs. 9 and 10.

**Discussion**

In this study, we characterized the in vitro metabolism of XN by rat liver microsomes. Untreated male rat liver microsomes converted XN...
to three major metabolites, namely M1, M2, and M3 (Fig. 2A). The formation of these metabolites required NADPH and was inhibited by 100% carbon monoxide and SKF-525A, confirming the role of CYP in XN biotransformation by rat liver (Fig. 5). Liver microsomes from rats pretreated with PB, an inducer of CYP2B1 and CYP2B2 (Lewis, 1996), metabolized XN to the same three major metabolites as found in untreated rats but in slightly different proportions (Fig. 3). The experiments indicated that M2 formation by liver microsomes from untreated rats followed Michaelis-Menten kinetics (Table 1) as did the production of M1 and M3, but the formation of the latter two metabolites by liver microsomes from untreated rats had much less favorable kinetic parameters than that of M2 (apparent $V_{\text{max}}/K_{m}$ relative ratios of 0.0004 and 0.00001 versus 0.0008).

On the basis of multi-wavelength HPLC, LC/MS analysis, and $^1$H NMR analysis, M1 and M2 were both identified as hydroxylated isopropylidihydrofurano derivatives of XN. Metabolite 4 was identified as the dimethylchromeno analog, DX. These metabolites are assumed to be derived from a prenyl epoxide intermediate (Fig. 9), which is in line with earlier findings that hydroxylated pyrano and furano derivatives are common fungal metabolites of prenylated flavonoids that are thought to be formed via a prenylepoxy intermediate (Tanaka and Tahara, 1997).

Liver microsomes from rats pretreated with the CYP1A inducers, ISF and BNF (Lewis, 1996), yielded the three same polar metabolites plus an additional nonpolar metabolite, designated M4. LC/MS and NMR analyses showed that M4 was identical to the structure of DX previously reported by Stevens et al. (1997). This metabolite is presumably formed by three consecutive reactions: 1) epoxidation of the prenyl group possibly catalyzed by CYP; 2) nucleophilic attack by O-4 at the C-3’ of the epoxidated prenyl group yielding the cyclic XN derivative, DH (Stevens et al., 2000); and 3) dehydration (Fig. 9).

The most significant finding in this study was that the prenyl group in the A ring of prenylchalcones is a major site for hepatic metabolism. The formation of metabolites such as M2 and M4 suggests that modification of the prenyl substituent, induced by epoxidation of the double bond, constitutes a major metabolic pathway of prenylated flavonoids (Fig. 9), although it has been reported that catechol formation on the B ring of nonprenylated flavonoids was a major site for CYP hydroxylation (Nielsen et al., 1998).

As shown in Fig. 9, we hypothesize that the formation of M1, M2, and M4 proceeds via an initial epoxidation reaction involving the prenyl group. As TCPO, an epoxide hydrolase inhibitor, did not result in a substantial inhibition of M1, M2, or M4, it seems likely that microsomal epoxide hydrolase was not involved in the formation of these metabolites. However it is possible that hydrolysis of prenyl epoxide metabolites could be catalyzed by contaminating soluble epoxide hydrolase, an enzyme not inhibited by TCPO (Moghaddam et al., 1996). Alternatively, the epoxide could be opened spontaneously in the absence of enzyme catalytic activity, which gains support from the observation that epoxidation of XN with m-chloro peroxoxygenic
acid yielded DH and DX, whereas the parent epoxide could not be isolated or detected (unpublished results). TCPO also caused a small reduction in the formation of M3, suggesting a partial inhibition of the hydroxylating CYP as shown previously by Shimada and Sato (1979).

As the CYP inhibitors used in this study did not completely inhibit metabolite formation, other rat CYPs or some other oxygenases may be involved in the epoxidation of XN (Tanaka and Tahara, 1997). Some of these enzymes are insensitive to the CYP inhibitors used in this study, which could explain why M1, M2, and M4 formation were not inhibited totally.

The amount of XN metabolites produced by rat liver microsomes was influenced by the use of various CYP inducers (Fig. 3). When compared with ISF- and BNF-treated rats, the formation of M1 and M2 was significantly higher in incubations of XN with liver microsomes of untreated rats. These results suggest that the CYP1A and CYP2B families may be involved in the formation of M1 and, to a lesser extent, M2. Metabolite 3 was produced in greater amounts by the liver microsomes of ISF-treated rats than all other groups. M4 was the only metabolite produced by the liver microsomes of ISF- and BNF-treated rats, presumably due to the involvement of the CYP1A enzymes in its formation. Thus, the CYP1A family seems to be more involved in the formation of M3 and M4 than M1 and M2. Also, the high inhibitory activity (82% inhibition) of ANF, a selective CYP1A inhibitor, toward M4 formation supports the conclusion that M4 formation was catalyzed mainly by CYP1A enzymes.

The formation of multiple XN metabolites points to the involvement of more than one CYP in the biotransformation of this plant flavonoid. The constitutive forms of CYPs could be the major catalysts for the formation of M1, M2, and M3, since all of these metabolites were produced in untreated rat liver microsomes. Studies are currently underway to provide conclusive evidence of the role of individual CYPs in the biotransformation of XN by carrying out metabolism experiments using individual CYPs, such as cDNA-expressed human CYPs.

To determine whether CYP3As were involved in the formation of M1, M2, M3, and M4, the CYP3A inhibitor, TAO, was added to the incubation mixture containing ISF-induced rat liver microsomes. TAO caused only a 55% inhibition of M1 and a 21% inhibition for M2, M3, and M4. This was in agreement with the findings of Nielsen et al. (1998), who showed that TAO does not appreciably inhibit the metabolism of flavonoids. This modest inhibition of metabolite formation by TAO indicates that CYP3As may not significantly contribute to the biotransformation of XN, with the probable exception of M1 formation.

Demethylation was not observed during the metabolism of XN by rat liver microsomal enzymes. None of the metabolites was identified as desmethyl xanthohumol by LC/MS comparison with an authentic sample isolated previously by Stevens et al. (1997). By contrast, other flavonoids with O-methyl groups have been found to undergo demethylation reactions (Nielsen et al., 1998). The presence of a prenyl group in the A ring may preclude CYP-catalyzed O-demethylation of XN, possibly due to limited acceptability of the substrate as a result of steric hindrance.

Flavonoids display a wide range of biological effects, including

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**Table 2**

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antiallergic, anti-inflammatory, antiviral, and anticarcinogenic properties. They have been used in attempts to treat a variety of human diseases (So et al., 1996). Prenylated and nonprenylated flavonoids are present in the normal diet and in herbal supplements, but information on the biotransformation of these compounds is limited. For example, these flavonoids could be metabolized by CYPs to physiologically active metabolites, and the biotransformation products could affect the activity of CYPs that are involved in carcinogen activation or detoxification.

Human exposure to XN, the principal flavonoid of hops, would be primarily through the drinking of beer or ingestion of dietary supplements containing hop extracts (Miranda et al., 2000). It was shown that XN inhibited bone resorption, and subsequently XN has been patented as a drug for osteoporosis treatment (U.S. Patent and Trademark Office, 1997). XN may be a candidate for cancer chemoprevention, as it has been reported that XN induced quinone reductase, a detoxifying enzyme, in wild-type Hepa 1c1c7 cells (Miranda et al., 2000) and inhibited the growth of human MCF-7 breast cancer cells.

Fig. 7. LC/MS-MS spectrum of M4 (dehydrocycloxanthohumol).

Fig. 8. LC/MS-MS spectrum of M3 (a hydroxyl derivative of xanthohumol).
Although XN is known to inhibit CYP1A enzyme activity (Henderson et al., 2000), the formation of M4, presumably catalyzed by CYP1A enzymes in liver microsomes of ISF- and BNF-treated rats, indicated that the XN concentration used in this study did not appreciably inhibit these enzymes. Interestingly, it was reported that the metabolite of XN, DX (referred to as M4 in the present study), at 0.1 and 1.0 μM showed significant antiproliferative activity in human breast cancer MCF-7 cells (Miranda et al., 1999).

The present findings may shed a different light on the biological activities of XN and its oxygenated derivatives (notably cancer-chemopreventive effects) because some of the oxygenated derivatives that accompany XN in hops and beer as minor flavonoids now appear to be formed from XN by liver microsome enzymes as well. Further studies are needed to clarify the mechanisms for formation of the various XN metabolites and to determine whether similar metabolites are produced by rats and humans in vivo. The biological activities of these metabolites and their involvement in cancer chemoprevention also remain to be established.

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