DOI:10.1021/jf8038132

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Comparative Study of Hop-Containing Products on Human Cytochrome P450-Mediated Metabolism

Brian C. Foster,*,†,§ Nikia Kearns,† John T. Arnason,†,# Ammar Saleem,†,# Carolina Ogrodowczyk,†,# and Suzanne Desjardins⊥

†Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, Ontario, Canada, §Office of Science, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada, #Ottawa-Carleton Institute of Biology, University of Ottawa, Ottawa, Ontario, Canada, and ⊥Office of Research and Surveillance, Drug Strategy and Controlled Substances Directorate, Health Canada, Ottawa, Ontario, Canada

Thirty-five national and international brands of beer were examined for their potential to affect human cytochrome P450 (CYP)-mediated metabolism. They represented the two main categories of beer, ales and lagers, and included a number of specialty products including bitter (porter, stout), coffee, ice, wheat, Pilsner, and hemp seed. Aliquots were examined for nonvolatile soluble solids, effect on CYP metabolism and P-glycoprotein (Pgp) transport, and major α- and β-hop acids. Wide variance was detected in contents of alcohol, nonvolatile suspended solids, and hop acids and in the potential to affect CYP-mediated metabolism and Pgp-mediated efflux transport. Many of the products affected CYP2C9-mediated metabolism, and only two (NRP 306 and 307) markedly affected CYP3A4; hence, some products have the capacity to affect drug safety. CYP3A4, CYP3A5, CYP3A7, and CYP19 (aromatase) inhibition to the log concentration of β-acid content was significant with $r^2 > 0.37$, suggesting that these components can account for some of the variation in inhibition of CYP metabolism.

KEYWORDS: Beer; hops; cytochrome P450 3A4; P-glycoprotein; humulone; lupulone; bitter acids

INTRODUCTION

A recognition that the Mediterranean diet contributes to reduced cardiovascular risk was one of the early drivers for the increased interest in the health benefits of food. Within this diet, foodstuffs such as vegetables, herbs, legumes, and fruits were recognized as having high secondary metabolite content and antioxidant levels. Many of these are high in antioxidant flavonoids and stilbenes. Also inherent in this diet is moderate alcohol consumption. There are several reports suggesting that low to moderate consumption of polyphenol-rich alcoholic beverages such as wine and beer may have beneficial health effects (*1*, *2*).

However, the number and range of adverse events and potential interactions being reported to health authorities and published in the scientific literature suggest that foods, herbs, or medicinal plants rich in secondary metabolites may modulate the pharmacokinetics of therapeutic products. Alcoholic beverages have potential adverse effects on the cardiovascular system (*1*), but relatively little is known of the potential of the other constituents in these products to interact with therapeutic products.

Adverse events associated with food−, natural health product−, or drug−drug interactions are a major concern to regulatory bodies, health care professionals, and patients (*3*). In 1998, death rates were 19.18% higher in patients in U.S. medicare hospitals who experienced an adverse drug reaction (ADR) and resulted in 1971 excess deaths. Hospital stays were higher with about 78000 excess patient-days. The increased charges for patients with an ADR were estimated to be about U.S. $340 million. These values do not take into consideration other health-care settings or undiagnosed events that may have occurred, including the possible development of drug resistance or nonserious adverse events. Hence, the overall impact of these interactions would be underestimated and expected to have a profound impact on health-care resources and quality of life for the consumer.

Interactions of ethanol with antidepressant drugs are well-known (*4*); those related to substances other than ethanol present in alcoholic beverages are much less so. It has been recognized that alcoholic beverages contain many constituents beyond the ethanol that have pharmacological properties (*5*). Resveratrol, a substance in red wine, is a mechanism-based inhibitor of CYP1A2- and 3A4-mediated drug metabolism (*6*, *7*). Henderson et al. (*8*) concluded that hop flavonoids are potent and selective inhibitors of CYP. They found that some hop flavonoids almost completely inhibited the 7-ethoxyresorufin *O*-deethylase (EROD) activity of CYP1A1, whereas others had a variable effect from 27 to 91% inhibition. Hidestrand et al. (*5*) in a study with light and dark beer administered to male rats noted that hepatic mRNA and protein levels of CYP4A1 were elevated in animals treated with stout but not other beers. A CYP4A-specific activity,

*Corresponding author [brian_foster@hc-sc.gc.ca; telephone (613) 957-3506; fax (613) 941-5035].

 Published on Web 05/18/2009

lauric acid 12-hydroxylase activity, was reduced ($p \leq 0.05$) in microsomes in both lager- and stout-fed rats. The relative expression of CYP2E1 and CYP2B1 as well as functional activity did not differ between beers or controls. The mean expressions of CYP1A2, CYP3A, and CYP4A apoproteins were greater in liver microsomes from stout-infused rats than in those from lager-infused rats, ethanol-infused rats, and diet controls. Although no significant differences were observed in EROD, methoxyresorufin *O*-dealkylase, midazolam, or testosterone hydroxylase activities between groups, stout-infused rats had greater hepatic microsomal erythromycin *N*-demethylase activity than other groups. Monteiro et al. (*9*) noted that lager beer, alcohol-free beer, stout beer, and xanthohumol-rich stout beer (200 μL/mL) significantly decreased aromatase (CYP19) activity. Mannering et al. (*10*) found a higher level of CYP-mediated activity in hepatic microsomes from mice fed a natural-ingredient diet than in those from mice fed a semipurified diet. This effect was attributed to the hop constituent lupulone. They also noted that the hop components did not fully account for the total inductive properties of the crude diet.

Lemos et al. (*11*) found that wine, beer, and tea, or some of their specific constituents, significantly inhibited the uptake of folic acid by Caco-2 cells. Most of these beverages, with the exception of wines (not tested), also inhibited methotrexate uptake in these cells. Ethanol, when tested separately, inhibited the uptake of both compounds. Myricetin, epigallocatechin gallate (EGCG), and isoxanthohumol markedly inhibited folic acid uptake. Myricetin and EGCG also had a concentration-dependent inhibitory effect upon the uptake of methotrexate by Caco-2 cells. Resveratrol, quercetin, and kaempferol inhibited the transport of both compounds at only the concentration of 100 μM.

Jerkovic and Collin (*12*) quantified *trans*-resveratrol and *trans*-piceid in 40 American and European hop cone samples from harvests of 2004, 2005, and 2006. The content varied greatly in the range of 0.5−12 mg/kg. The German varieties had the lowest amounts of stilbenes. The highest concentrations were found in two American low-α-acid varieties, Cascade and Willamette. A harvest year effect was observed.

Despite these studies, there has not been a systematic examination of a wide range of beer products containing hops as they affect key drug metabolism enzymes and transport proteins. Beer is categorized into three categories: ales, lagers, and specialty beers. Ale types include bitters, pale ale, and porter (including stout). Lagers can be light or colored and include Pilsners. The difference between a lager and ale is the type of yeast used in fermentation. Ale is commonly fermented at warmer temperatures. As the yeast has a tendency to flocculate at the surface before settling to the bottom it is referred to as a top fermentor. Ales are usually higher in alcohol content and will be noticeably fuller and more complex. Lager yeast, on the other hand, flocculates throughout the fermentor and then settles; therefore, it is known as a bottom fermentor. Lager yeasts need cool temperatures during fermentation. Lager styles tend to be lighter in color and taste drier and less alcoholic and complex than ales. Specialty beers are either ales, lagers, or a hybrid of the two that will contain other ingredients that cause it to not fit into a true ale or lager style.

In the present study we examined the inhibition of several human CYP enzymes by 35 types of beer representative of Canadian and international markets (**Table 1**). Six common bitter humulones of beer hops were analyzed in these products by selected ion monitoring HPLC-MS. Statistical analysis was undertaken to determine if these phytochemical markers were correlated to CYP inhibition.

MATERIALS AND METHODS

Reagents and Standards. All products were obtained locally from commercial outlets. Each product was catalogued with a Nutraceutical Research Programme (NRP) number and an intact sample archived. Acetonitrile and water (HPLC grade) were purchased from Fisher Scientific (Ottawa, Canada), whereas formic acid (99% pure) was purchased from Fluka Inc. (La Jolla, CA). The HOPS bitter acid calibration mixture containing cohumulone, 14.45%; humulone, 30.29; adhumulone, 4.66%; colupulone, 12.92%; lupulone, 10.69%; and adlupulone, 1.33% (product 8332, lot 08332-101), was purchased from Chromadex Inc. (Irvine, CA).

Nonvolatile Suspended Solids (NVSS). NVSS dry weight was determined by taking three 10 mL aliquots of each product and reducing them to constant weight in a drying oven overnight. The weight boats were reweighed, and the mean difference was determined for each product.

Human Cytochrome P450-Mediated Metabolism. Aliquots (10 μL) of stock solutions were screened for their ability to inhibit CYP3A4/A5/A7, 2C9, 2C19, and CYP19 (BD Biosciences, Mississauga, Canada) metabolism of dibenzylfluorescein (DBF), 7-ethoxy-3-cyanocoumarin (CEC), or 7-methoxy-4-trifluoromethylcoumarin (MFC) (all purchased from BD Biosciences) using an in vitro fluorometric microtiter plate assay (CytoFluor series 4000 multi-well plate reader). Briefly, assays were performed with 10 μL of product in clear-bottom, opaque-welled microtiter plates (96 wells; Corning Costar, Ottawa, Canada). Control and control blank wells contained 5% ethanol, and test and test blank wells contained the product. All wells tested contained distilled water, β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH; Sigma-Aldrich, Oakville, Canada), and the enzyme substrate DBF, CEC, or MFC (final concentrations of 1, 5, and 25 μM per well, respectively), for a total reaction volume of 200 μL. Control and test wells had active isoenzyme, whereas control blank and test blank wells contained denatured isoenzyme, both in phosphate buffer solution (PBS; 0.5 M, pH 7.4). For CYP2C9, Tris buffer (0.5 M, pH 7.5) was used instead of PBS. Comparative testing of CYPs was previously done with balanced amounts of specific activity and protein content using a Gentest insect control (*13*). For all assays, microsomes were rapidly thawed and mixed gently with the substrate solution. All microsomes were stored at −80 °C until used and were not subjected to more than two freeze−thaw cycles. All samples were prepared in triplicate with the resultant percent inhibition calculations based on the mathematical combinations for the differences in fluorescence between the test/test blank wells and the mean difference between each control and blank well. A positive control using the CYP inhibitor bifonazole (0.5 μM) (Sigma-Aldrich), ketoconazole (1.9 μM) (Calbiochem, Gibbstown, NJ), sulfaphenazole (100 μM), or tranylcypromine (100 μM) (all purchased from Sigma-Aldrich) was run with each assay. All assays were performed under reduced lighting conditions and at 37 °C. The fluorescence was measured at 485 nm excitation and 530 nm emission, with a gain of 50 for CYP3A4/A5/A7/19, at 409 nm excitation and 530 nm emission, with a gain of 80 for CYP2C9, and at 409 nm excitation and 460 nm emission, with a gain of 60 for CYP2C19.

Human P-Glycoprotein-Mediated Efflux Transport: Cell Culture and Rhodamine123 Assay Procedure, THP-1 Cell Line. Human peripheral blood monocytes from patients with acute monocytic leukemia (ATCC TIB-202; Manassas, VA) were routinely subcultured, grown, and maintained horizontally in 80 cm^2 cell culture flasks (Nunclon, Ottawa, Canada) in a Fisher Scientific 605 incubator set to 37 °C and 5% CO_2 in RPMI 1640 supplemented with 10% fetal bovine serum, 1% sodium pyruvate, 1% penicillin−-streptomycin (the above supplied by Invitrogen, Burlington, Canada), and 1% β-mercaptoethanol (Sigma-Aldrich, Oakville, Canada). Cell culture health was routinely assessed by direct observation of cultured cells via microscopy (Leica DMIL) for contamination and cell density count comparisons. Suspended cells in culture medium were centrifuged (Hettich Zentrifugen, Rotofix 32) at 300 RCF at room temperature for 10 min and then resuspended in the fresh culture medium at the concentration of 300,000 cells/978 μL for experiments.

The rhodamine 123 (Rh123) assay was carried out in the following manner. Briefly, 978 μL of THP-1 cells was plated into a 24-well cell culture plate (Falcon, Mississauga, Canada), along with 12 μL of Rh123 (final concentration of 150 ng/mL) and 10 μL of the sample production. Following an incubation time of 18 h, cells were collected in 1.5 mL

Table 1. Types of Hop-Containing Products Examined in This Study

	type	grain	dry wt (mg)	country of production
Aldaris Zelta[a]	lager	barley	407 ± 1.5	Latvia
Alexander Keith's India	ale, pale	barley	373 ± 4.8	Canada
Asahi	lager	barley	320 ± 1.9	Japan
Baltika[a]	lager	barley	389 ± 3.2	Russia
Beck's	lager	barley	367 ± 7.6	Germany
Blanche de Chambly[a]	lager	barley	375 ± 2.8	Canada
Bud Light	lager	barley	272 ± 1.7	USA[a]
Coors Light	lager	barley	285 ± 2.9	USA[a]
Creemore Springs	lager	barley	493 ± 6.6	Canada
Czechvar[a]	lager	barley	379 ± 1.2	Czech
Grolsch	lager	barley	376 ± 6.8	Holland
Guinness Extra Cold Stout	ale, porter	barley	324 ± 8.7	Canada
Hacker-Pschorr Hefe[a]	lager, Pilsner	wheat	438 ± 4.7	Germany
Holsten Maibock	lager	wheat	553 ± 5.0	Germany
Holsten Premium	lager	wheat	349 ± 2.0	Germany
Homegrown	ale, hemp	barley, hemp	452 ± 2.2	Canada
Kilkenny Irish Cream	ale	barley, hemp	309 ± 5.3	Ireland
Kokanee Glacier	lager	barley, hemp	346 ± 6.2	Canada
Labatt 50	ale	barley	354 ± 1.9	Canada
Labatt Blue	lager, Pilsner	barley	308 ± 0.2	Canada
Labatt Maximum	lager, ice	barley	449 ± 3.6	Canada
Lakeport	lager, Pilsner	barley	292 ± 2.1	Canada
Lakeport Honey	lager	barley	332 ± 5.6	Canada
Lucky Lager	lager	barley	291 ± 2.0	Canada
Mill Street Brewery[a]	ale, porter	barley, coffee	549 ± 5.9	Canada
Molson Canadian	lager	barley, coffee	305 ± 5.9	Canada
Moosehead	lager	barley, coffee	353 ± 1.2	Canada
Okocim[a]	lager	barley, coffee	425 ± 1.8	Poland
Richard's Red	lager	barley, coffee	426 ± 2.9	Canada
Tetley's English	ale	barley, coffee	319 ± 5.0	England
Traditional[a]	ale	barley, coffee	425 ± 0.8	Canada
Tuborg	lager	barley, coffee	339 ± 5.2	Denmark
Pilsner Urquell	lager, Pilsner	barley, coffee	494 ± 5.4	Czech
Wernesgruner	lager, Pilsner	barley, coffee	365 ± 3.8	Germany
Zlatorog[a]	lager	barley, coffee	368 ± 3.4	Slovenia

[a] Bottle.

microfuge tubes and washed twice with PBS (0.5 M, pH 7.4) and centrifuged at 500 RCF (Eppendorf centrifuge 5415R) for 5 min each. Following the final wash and centrifugation at 500 RCF, the supernatant was discarded and the cell pellet was resuspended in 1 mL of fresh PBS. A 300 μL aliquot of the cell suspension, in triplicate, was measured on a 96-well plate at 37 °C for reading using a fluorometric microtiter plate assay (Spectramax M5). Fluorescence was measured at 485 nm excitation and 530 nm emission.

Bitter Acids Constituents: LC-MS Method. A 10 mL aliquot of each beer sample was centrifuged at 1000*g* for 15 min at room temperature, and 1 mL of each sample was filtered through a PTFE membrane into an HPLC vial. A 90 μL aliquot of each beer sample and 0.9 mL of HOPS standardized extract were injected into an Agilent 1100 series LC-MSD system (Agilent Technologies Inc., Palo Alto, CA), equipped with an online degasser, a quaternary pump, an autosampler, a column thermostat, an atmospheric pressure chemical ionization source (APCI), and a mass selective detector (MSD). The separations were performed on a reversed phase Synergi Fusion RP-C18 column, 150 × 3.0 mm i.d., 4.0 μm particle size, (Phenomenex Inc., Torrance, CA). The flow rate of solvents (A, acetonitrile; B, formic acid 4% aqueous) was maintained at 1 mL/min while the column thermostat temperature was maintained at 60 °C and the upper pressure limit was fixed at 200 bar. The separations were achieved using a two-step gradient comprising 62% A and 38% B for 7 min, 70% A and 30% B for 5 min; the column was brought back to initial conditions in 3 min and equilibrated for 10 min before the next injection.

The APCI source was set at negative polarity to monitor the selected ions (cohumulone, 347.20; adhumulone and humulone, 361.20; colupulone, 399.30; lupulone and adlupulone, 413.30). The fragmentor was ramped from 50 to 200 for the mass range of 250−500 amu. The spray chamber conditions were as follows: drying gas (nitrogen) temperature, 200 °C; drying gas flow rate, 5 L/min; vaporizer temperature, 325 °C; nebulizer pressure, 60 psig; capillary voltage, 3500 V; corona current, 25 μA. The quantitation of hop acids was achieved through calibration curves of target compounds prepared by injecting serially diluted standardized HOPS extract.

Statistical Analysis. Pearson correlations were evaluated using S-Plus (version 8.0) software. In some cases a reduced data set was used because of uncertainties in the phytochemical analysis due to low amounts (especially adlupulone). The data set was normally distributed.

RESULTS

There are marked differences in labeled ethanol content (mean of 5.0% with a range from 3.8 to 7.1%) and dry weights of the NVSS (271.8 to 553.3 mg) of the various beer products (**Table 1**). No relationship could be seen between the different types of beer and the level of NVSS.

The α- and β-hop acids were present in a wide range of quantities among the beer samples analyzed (**Figure 1**). Cohumulone and humulone were the most abundant bitter acids and were ubiquitously present in most of the beer samples tested. The highest levels were in an ale, NRP 278; two Pilsners, NRP 304 and 305; a porter, NRP 307; and a specialty product, NRP 308. Colupulone and lupulone were present in trace amounts in most products. Colupulone levels were highest in ale NHP 278 and porter NRP 306. Lupulone was highest in both porters NRP 306 and NRP 307. Adlupulone was present only in trace amounts.

Whereas CYP2E1 results are not reported as all values were at 100% inhibition reflective of the ethanol present rather than the

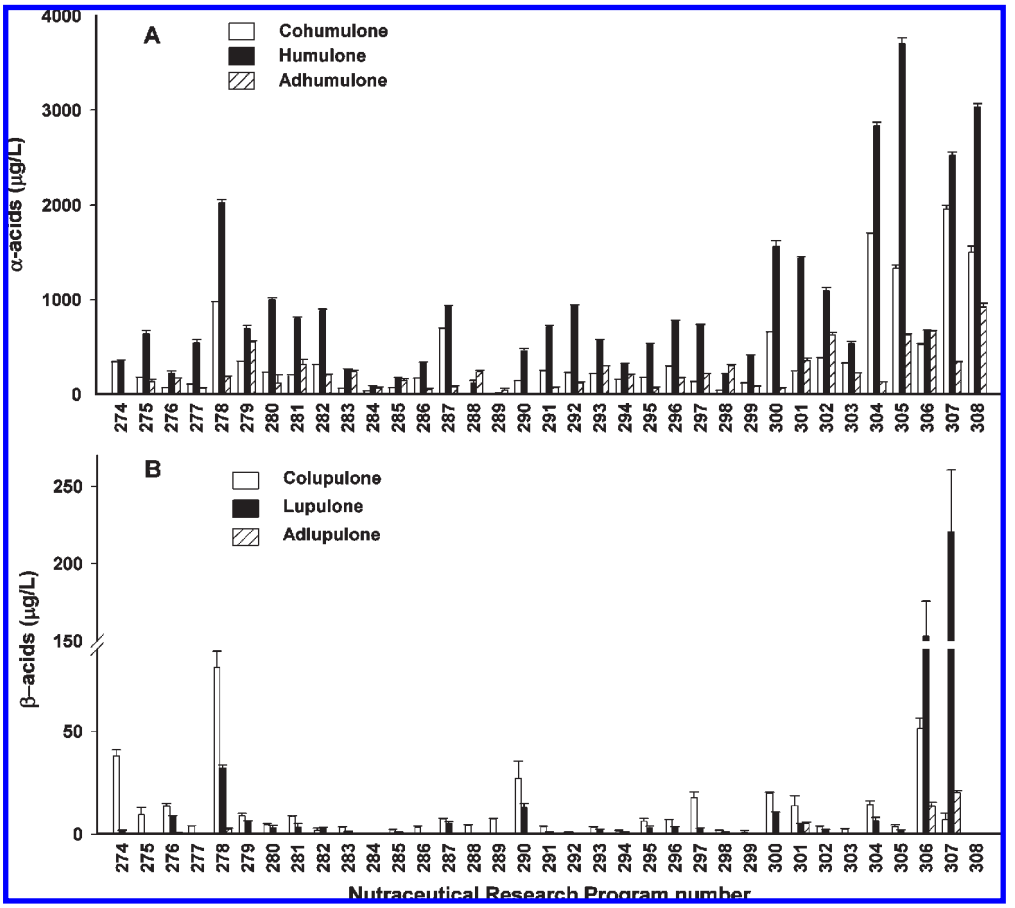

Figure 1. Determination of α-hop (**A**) and β-hop (**B**) acids in the 35 hop-containing products examined by selected ion monitoring HPLC-MS (results are mean ± SD, $n = 3$).

other constituents present in the samples, results for the other CYP isozymes examined are presented in **Table 2**. In general, most products had little or moderate (36−75%) inhibitory effect on CYP2C19, 3A4/5/7, and 19. Several of these products had a moderate (36−75%) effect on CYP2C19. Four ales, NRP 276, NRP 278, NRP 290, NRP 306, and NRP 307, had a moderate or strong (76−100%) inhibitory effect on CYP2C9 and most CYP3A isozymes. Only NRP 306 and NRP 307 had a moderate or strong inhibitory effect on CYP19. Interestingly, all products had a moderate to strong inhibitory on CYP2C9-mediated metabolism.

Ten products (ales, lagers, and porters) were selected on the basis of low, moderate, and high dry weight and α-hop acid content for P-glycoprotein analysis. The assay findings were highly variable, and representative values are reported in **Table 3**. The findings suggest only NRP 277 and NRP 307 had modest potentials to affect P-glycoprotein efflux.

A statistical analysis of the Pearson correlation between percent inhibition of CYP enzymes and the log concentration of hop acid content across beers showed many significant correlations (**Table 4**). Despite their lower amounts, the best correlations were achieved between inhibitions of CYP enzymes with the log β-acid concentration. Several of the correlations were > 0.7, suggesting that the log β-acids predict more than 50% of the variation in inhibition. In general, coefficients of determination (r^2) were > 0.37 between all β-acids and CYP 3A4, CYP3A5, CYP3A7, and CYP19. Correlations between β-acids and inhibition of 2C9 and 2C19 were not significant.

DISCUSSION

The 35 different products selected for screening in this study were representative of the major types of beers available in this region. Ethanol in the sample aliquots was balanced by control aliquots containing 5% ethanol and, with the exception of CYP2E1, did not have an inhibitory effect. Significant variation existed in the dry weights of the aliquots, but there was no apparent correlation with product type and inhibition. This was particularly noteworthy for the porters, where NRP 306 had the second highest dry weight and NRP 307 was in the lowest quartile. The correlation of CYP inhibition with the log concentration of sβ-acids, but not α-acids, suggests that β-acids may be a predictor of 3A4, 3A5, 3A7 and 19 isozyme inhibitions. Whereas correlation between key plant constituents and inhibition has not always been found, there are examples of other plant materials acting as predictors of CYP enzyme inhibition. Significant correlations were found for berberine (and CYP enzyme inhibition) in goldenseal and related alkaloid-bearing plant products (*14*) and for alkamides in diverse *Echinacea* products (*15*). Monteiro et al. (*9*) found that lager, alcohol-free, stout, and xanthohumol-rich stout beers (200 μL/mL) significantly decreased CYP19 activity. Prenylated flavonoids present in beer have also been shown to have an inhibitory effect on CYP-mediated metabolism (*16*) and should be examined further in relation to the hop acids to determine which hop constituent provides a stronger correlation that may help to predict the inhibitory potential of beer products.

The findings in this study support previously published findings that hops and some hop-derived products have the potential to affect CYP3A4- and CYP19-mediated metabolism. The results further show that some beers can moderately to strongly affect CYP2C9-, CYP2C19-, CYP3A5-, and CYP3A7-mediated metabolism. It is noteworthy that only four moderately inhibited CYP3A but 21 affected CYP3A7 activity. Clearly these two

Table 2. Mean Inhibition (Percent ± SD) of Cytochrome P450 Isozymes by Hop-Containing Products, $n = 2$, in Triplicate[a]

NRP	3A4	3A5	3A7	19	2C9	2C19
274	7.7 ± 1.9	15.5 ± 3.4	35.0 ± 3.4	20.6 ± 3.3	68.6 ± 2.0	20.2 ± 4.4
275	21.5 ± 1.5	22.7 ± 3.6	47.5 ± 5.0	14.4 ± 2.7	71.0 ± 2.6	15.2 ± 2.4
276	29.8 ± 4.6	38.5 ± 5.3	38.9 ± 3.3	30.8 ± 2.2	**83.0 ± 3.1**	39.2 ± 2.5
277	30.3 ± 0.7	18.6 ± 6.8	43.9 ± 4.3	37.7 ± 5.0	**81.9 ± 3.4**	32.3 ± 1.5
278	46.1 ± 4.0	49.0 ± 3.1	70.8 ± 1.7	34.4 ± 3.0	**96.1 ± 3.1**	63.3 ± 0.5
279	9.6 ± 4.2	18.6 ± 1.8	38.5 ± 2.4	36.7 ± 9.9	**84.0 ± 3.3**	45.6 ± 3.6
280	23.6 ± 5.5	12.6 ± 4.3	30.9 ± 6.4	37.9 ± 4.0	**84.0 ± 1.3**	42.1 ± 3.6
281	19.3 ± 1.7	15.7 ± 2.1	38.5 ± 3.0	11.3 ± 6.1	**80.2 ± 0.8**	50.4 ± 3.0
282	18.9 ± 1.9	12.3 ± 1.4	23.8 ± 3.6	1.2 ± 0.5	**80.6 ± 1.4**	43.5 ± 2.9
283	13.2 ± 2.7	10.8 ± 5.7	60.9 ± 5.9	18.7 ± 6.9	**76.7 ± 0.6**	67.6 ± 4.1
284	17.1 ± 5.5	4.4 ± 1.1	17.7 ± 0.6	18.5 ± 5.7	56.6 ± 1.3	23.6 ± 1.3
285	4.4 ± 1.9	3.9 ± 2.0	22.8 ± 3.1	23.1 ± 5.5	60.6 ± 4.1	33.7 ± 3.0
286	13.9 ± 2.5	2.0 ± 1.4	25.4 ± 4.8	13.5 ± 3.0	57.9 ± 3.7	19.9 ± 2.1
287	35.8 ± 1.5	31.2 ± 3.7	73.8 ± 4.7	28.2 ± 2.4	**98.9 ± 1.0**	72.8 ± 2.6
288	18.1 ± 0.1	17.8 ± 1.9	39.9 ± 2.1	10.4 ± 0.9	**74.5 ± 1.9**	43.6 ± 0.2
289	11.3 ± 1.0	14.3 ± 4.5	35.4 ± 1.5	9.5 ± 2.1	**89.1 ± 2.6**	30.0 ± 1.4
290	49.3 ± 2.8	14.8 ± 2.6	61.4 ± 4.9	25.3 ± 3.8	**80.4 ± 3.0**	63.7 ± 3.6
291	24.9 ± 4.4	13.2 ± 2.5	41.1 ± 0.8	22.7 ± 2.4	**84.7 ± 3.5**	37.4 ± 1.1
292	5.2 ± 3.7	9.2 ± 4.6	26.7 ± 4.4	7.0 ± 3.8	**77.1 ± 1.6**	15.9 ± 3.4
293	5.3 ± 2.2	14.9 ± 1.7	32.3 ± 2.0	12.1 ± 2.1	**74.8 ± 3.8**	19.5 ± 1.5
294	16.6 ± 1.0	14.4 ± 4.0	28.1 ± 0.3	17.2 ± 5.3	**77.8 ± 1.1**	26.3 ± 1.0
295	9.1 ± 3.1	14.7 ± 2.9	24.6 ± 7.0	14.4 ± 0.8	**64.9 ± 2.2**	20.5 ± 4.8
296	18.8 ± 4.8	11.7 ± 0.6	30.5 ± 3.2	18.9 ± 4.7	**78.7 ± 2.9**	30.5 ± 1.0
297	20.2 ± 3.5	21.5 ± 6.8	31.3 ± 2.8	15.3 ± 6.7	**77.4 ± 2.9**	36.3 ± 4.3
298	25.9 ± 5.5	27.5 ± 1.9	49.5 ± 1.5	33.6 ± 1.2	**84.5 ± 2.5**	45.9 ± 1.7
299	13.3 ± 2.5	11.4 ± 5.3	24.7 ± 5.3	15.6 ± 7.0	**76.2 ± 8.7**	50.6 ± 0.6
300	20.3 ± 0.1	14.2 ± 3.7	45.8 ± 4.8	21.6 ± 5.2	67.6 ± 1.7	47.9 ± 2.8
301	14.5 ± 4.8	17.2 ± 2.6	38.3 ± 3.4	23.3 ± 2.0	**80.5 ± 2.8**	37.5 ± 1.0
302	9.0 ± 0.8	14.8 ± 1.2	30.7 ± 3.1	19.5 ± 3.5	59.7 ± 1.2	31.1 ± 0.8
303	15.6 ± 4.7	4.4 ± 0.6	23.6 ± 0.1	24.5 ± 2.8	66.5 ± 0.1	19.4 ± 2.5
304	25.4 ± 1.3	24.1 ± 3.2	50.1 ± 4.9	26.1 ± 7.5	**87.4 ± 0.7**	45.2 ± 0.1
305	22.4 ± 2.3	12.6 ± 2.1	38.0 ± 1.9	32.4 ± 4.2	**77.1 ± 2.4**	32.9 ± 2.5
306	**77.7 ± 0.3**	**91.1 ± 1.5**	**97.8 ± 1.1**	**87.6 ± 1.6**	**96.7 ± 0.9**	**90.2 ± 0.8**
307	53.1 ± 3.0	66.1 ± 5.9	**84.4 ± 2.5**	64.7 ± 2.0	**88.8 ± 1.8**	60.9 ± 1.7
308	31.6 ± 4.2	22.2 ± 3.8	36.2 ± 1.9	23.5 ± 2.6	**75.7 ± 1.9**	56.9 ± 1.7

[a] Bold values indicate high levels of inhibition.

Table 3. Mean Inhibitory Effect (± SD) of Selected Hop-Containing Products on P-Glycoprotein-Mediated Transport As Determined by Rhodamine 123 Uptake Relative to Controls, $n = 6$

NRP	% inhibition	NRP	% inhibition
276	10.0 ± 4.3	289	7.8 ± 0.9
277	56.2 ± 12.1	304	29.8 ± 4.8
278	25.1 ± 8.9	305	25.9 ± 6.7
284	21.3 ± 0.3	306	25.5 ± 4.3
286	10.5 ± 4.3	307	38.2 ± 18.9

isozymes are not similarly inhibited by the major part of the beers tested in this study. The findings in this study are important as CYP3A and CYP2C are the two most important subfamilies involved in metabolism of many health products including drugs and natural health products. The importance of these findings is further strengthened with the moderate inhibition of rhodamine uptake by NRP 277 and NRP 307, which is generally accepted as a model to demonstrate the effect of substances on P-glycoprotein-mediated transport. This efflux transport system is a major factor in drug absorption, and the inhibition demonstrated with these selected products shows the potential of some beer products to markedly reduce drug plasma levels that could lead to decreased therapeutic efficacy and even resistance.

The findings of this study suggest that only the two porters, NRP 306 and NRP 307, may have a clinical effect with most drugs and other health products. However, the most noteworthy

Table 4. Coefficients of Determination for Percent Inhibition of Cytochrome P450 Isozymes Relative to the Individual Log Concentration of Hop Acid Content of the Product

enzyme	compound	df	r^2	p
CYP3A4	cohumulone	32	0.103	0.0639
	humulone	32	0.077	0.1125
	adhumulone	31	0.101	0.0719
	colupulone	31	0.560	10^{-5}
	lupulone	25	0.438	0.0002
	adlupulone	7	0.506	0.0317
CYP3A5	cohumulone	32	0.096	0.0747
	humulone	32	0.064	0.1498
	adhumulone	31	0.150	0.0258
	colupulone	31	0.538	10^{-5}
	lupulone	25	0.474	0.0001
	adlupulone	7	0.618	0.012
CYP3A7	cohumulone	32	0.107	0.0587
	humulone	32	0.085	0.0943
	adhumulone	31	0.114	0.0543
	colupulone	31	0.477	10^{-5}
	lupulone	25	0.456	0.0001
	adlupulone	7	0.617	0.0122
CYP19	cohumulone	32	0.149	0.024
	humulone	32	0.087	0.0905
	adhumulone	31	0.119	0.0491
	colupulone	31	0.553	10^{-5}
	lupulone	25	0.371	0.0008
	adlupulone	7	0.574	0.0181
CYP2C9	cohumulone	32	0.026	0.361
	humulone	32	0.046	0.2243
	adhumulone	31	0.133	0.0371
	colupulone	31	0.144	0.0293
	lupulone	25	0.202	0.0187
	adlupulone	7	0.316	0.1154
CYP2C19	cohumulone	32	0.039	0.262
	humulone	32	0.051	0.1973
	adhumulone	31	0.010	0.5723
	colupulone	31	0.289	0.0013
	lupulone	25	0.092	0.1244
	adlupulone	7	0.311	0.1184

findings are the high inhibitory results with CYP2C9, which mediates the metabolism of compounds such as nonsteroidal anti-inflammatory and antidiabetes agents, tamoxifen and S-warfarin. The risk of any ADR with these products would likely be additive or synergistic to existing factors and be modulated by the amount and rate at which the product was consumed. Moderate consumption over a longer time period would minimize the risk, whereas faster consumption or binge drinking would increase the potential risk. An acute inhibitory interaction with CYP and/or Pgp would increase plasma drug levels that would exacerbate the adverse side effects normally associated with the drug or health product. Chronic or repeated consumption over several days may have the same initial acute response but, with time, tolerance may develop through the induction of the associated metabolism enzymes and transport proteins. This generally leads to lower drug plasma levels that may prolong therapy or lead to therapeutic failure. In particular, the CYP2C9 interaction may have clinical significance as many of the drugs are used in the care and treatment of cardiac patients. This may also affect patient compliance, and for individuals on critical life-preserving medications, this may lead to resistance and subsequent therapeutic failure. In vitro studies have inherent

limitations associated with test substrates and methodological conditions and, hence, cannot predict clinical relevance. These studies can, however, demonstrate that the potential for an interaction exists. The clinical significance will depend on numerous intrinsic and extrinsic factors including single (acute) or multiple (chronic) uses. There are now ample reports of in vitro testing in the literature that natural health products have the potential to affect drug metabolism (*17*−*22*). Schwarz et al. (*22*) noted that St. John's wort induced CYP3A4 and Pgp through pregnane X-receptor activation. Clinical reports confirm this inductive capacity (*23*, *24*).

Some hop-containing products have the potential to affect the safety and efficacy of some therapeutic products. The relationship between inhibition and constituent levels is at best suggestive, as with many complex products combinatorial relationships may exist between these and other compounds in beer such as the as iso-α-acids and prenylated flavonoids. Further studies with additional products are warranted to extend these findings and to support the clinical evaluation required to determine the significance of these findings.

ABBREVIATIONS USED

ADR, adverse drug reaction; AUC, area under the serum concentration time curve; CEC, 7-ethoxy-3-cyanocoumarin; CYP, cytochrome P450; DBF, dibenzylfluorescein; EGCG, epigallocatechin gallate; EROD, 7-ethoxyresorufin *O*-deethylase; MFC, 7-methoxy-4-trifluoromethylcourmain; NRP, Nutraceutical Research Programme; NVSS, nonvolatile suspended solids; Pgp, P-glycoprotein; PBS, phosphate buffer solution; Rh123, rhodamine 123.

ACKNOWLEDGMENT

We gratefully note the technical assistance of Jingqin Mao and Teresa Tam.

LITERATURE CITED

(1) Fuchs, F. D.; Chambless, L. E. Is the cardioprotective effect of alcohol real?. *Alcohol* **2007**, *41*, 399–402.

(2) Romeo, J.; Warnberg, J.; Nova, E.; Díaz, L. E.; Gómez-Martinez, S.; Marcos, A. Moderate alcohol consumption and the immune system: a review. *Br. J. Nutr*. **2007**, *98* (Suppl. 1), S111−S115.

(3) Bond, C. A.; Raehl, C. L. Adverse drug reactions in United States hospitals. *Pharmacotherapy* **2006**, *26*, 601–608.

(4) Tanaka, E.; Misawa, S. Pharmacokinetic interactions between acute alcohol ingestion and single doses of benzodiazepines, and tricyclic and tetracyclic antidepressants—an update. *J. Clin. Pharm. Ther*. **1998**, *23* (5), 331–336.

(5) Hidestrand, M.; Shankar, K.; Ronis, M. J.; Badger, T. M. Effects of light and dark beer on hepatic cytochrome P-450 expression in male rats receiving alcoholic beverages as part of total enteral nutrition. *Alcohol: Clin. Exp. Res*. **2005**, *29*, 888–895.

(6) Chang, T. K. H.; Chen, J.; Lee, W. B. K. Differential inhibition and inactivation of human CYP1 enzymes by *trans*-resveratrol: evidence for mechanism-based inactivation of CYP1A2. *J. Pharmacol. Exp. Ther*. **2001**, *299* (3), 874–882.

(7) Chan, W. K.; Delucchi, A. B. Resveratrol, a red wine constituent, is a mechanism-based inactivator of cytochrome P450 3A4. *Life Sci*. **2000**, *67*, 3103–3112.

(8) Henderson, M. C.; Miranda, C. L.; Stevens, J. F.; Deinzer, M. L.; Buhler, D. R. *In vitro* inhibition of human P450 enzymes by prenylated flavonoids from hops, *Humulus lupulus*. *Xenobiotica* **2000**, *30*, 235–251.

(9) Monteiro, R.; Becker, H.; Azevedo, I.; Calhau, C. Effect of hop (*Humulus lupulus* L.) flavonoids on aromatase (estrogen synthase) activity. *J. Agric. Food Chem*. **2006**, *54*, 2938–2943.

(10) Mannering, G. J.; Shoeman, J. A.; Deloria, L. B. Identification of the antibiotic hops component, colupulone, as an inducer of hepatic cytochrome P-4503A in the mouse. *Drug Metab. Dispos*. **1992**, *20*, 142–147.

(11) Lemos, C.; Peters, G. J.; Jansen, G.; Martel, F.; Calhau, C. Modulation of folate uptake in cultured human colon adenocarcinoma Caco-2 cells by dietary compounds. *Eur. J. Nutr*. **2007**, *46*, 329–336.

(12) Jerkovic, V.; Collin, S. Occurrence of resveratrol and piceid in American and European hop cones. *J. Agric. Food Chem*. **2007**, *55*, 8754–8758.

(13) Foster, B. C.; Foster, M. S.; Vandenhoek, S.; Budzinski, J.; Gallicano, K. D.; Choudri, S.; Krantis, A.; Arnason, J. T. An *in vitro* evaluation of human cytochrome P450 3A4 and P-glycoprotein inhibition by garlic. *J. Pharm. Pharm. Sci*. **2001**, *4*, 159–167.

(14) Scott, I. M.; Leduc, R. I.; Burt, A. J.; Marles, R. J.; Arnason, J. T.; Foster, B. C. The inhibition of human cytochrome P450 by ethanol extracts of North American botanicals. *Pharm. Biol*. **2006**, *44* (5), 315–327.

(15) Modarai, M.; Gertsch, J.; Suter, A.; Heinrich, M.; Kortenkamp, A. Cytochrome P450 inhibitory action of *Echinacea* preparations differs widely and co-varies with alkylamide content. *J. Pharm. Pharmacol*. **2007**, *59*, 567–573.

(16) Gerhauser, C; Alt, A. P.; Klimo, K.; Knauft, J.; Frank, N.; Becker, H. Isolation and potential cancer chemopreventive activities of phenolic compounds of beer. *Phytochem. Rev* **2002**, *1* (3), 369–377.

(17) Foster, B. C.; Vandenhoek, S.; Hana, J.; Krantis, A.; Akhtar, M. H.; Bryan, M.; Budzinski, J. W.; Ramputh, A.; Arnason, J. T. *In vitro* inhibition of human cytochrome P450-mediated metabolism of marker substrates by natural products. *Phytomedicine* **2003**, *10*, 334–342.

(18) Zhou, S.; Gao, Y.; Jiang, W.; Huang, M.; Xu, A.; Paxton, J. P. Interactions of herbs with cytochrome P450. *Drug Met. Rev*. **2003**, *35*, 35–98.

(19) Huang, S.-M.; Leshko, L. J. Drug−drug, drug−dietary supplement, and drug−citrus fruit and other food interactions: what have we learned?. *J. Clin. Pharmacol*. **2004**, *44*, 559–569.

(20) Gaudineau, C.; Beckerman, R.; Welbourn, S.; Auclair, K. Inhibition of human P450 enzymes by multiple constituents of the *Ginkgo biloba* extract. *Biochem. Biophys. Res. Commun*. **2004**, *318*, 1072–1078.

(21) Obach, R. S. Inhibition of human cytochrome P450 enzymes by constituents of St. John's wort, an herbal preparation used in the treatment of depression. *J. Pharm. Exp. Ther*. **2000**, *294*, 88–95.

(22) Schwarz, U. I.; Hanso, H.; Oertel, R.; Miehlke, S.; Kuhlisch, E.; Glaeser, H.; Hitzl, M.; Dresser, G. K.; Kim, R. B.; Kirch, W. Induction of intestinal P-glycoprotein by St John's wort reduces the oral bioavailability of talinolol. *Clin. Pharmacol. Ther*. **2007**, *81*, 669–678.

(23) Nowack, R. Review article: Cytochrome P450 enzyme, and transport protein mediated herb−drug interactions in renal transplant patients: grapefruit juice, St John's wort—and beyond!. *Nephrology* **2008**, *13* (4), 337–347.

(24) Marchetti, S.; Mazzanti, R.; Beijnen, J. H.; Schellens, J. H. M. Concise review: Clinical relevance of drug−drug and herb−drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein). *Oncologist* **2007**, *12* (8), 927–941.

Received December 9, 2008. Revised manuscript received February 17, 2009. This research was funded by Health Canada. We acknowledge an NSERC postgraduate scholarship to C.O. and an NSERC MFA grant to J.T.A.