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Isolation and potential cancer chemopreventive activities of phenolic compounds of beer

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Abstract

Beer contains a variety of phenolic compounds. During the brewing process, some of these compounds are removed by polyvinylpolypyrrolidone (PVPP) to prevent haze formation. We have analyzed the phytochemical composition of a PVPP residue as well as of unstabilized beer and isolated a total of 51 compounds. Eight structures were identified as novel, i.e., 2-(4′-hydroxyphenyl)-3,5-dihydroxybenzoic acid (**6**), 2′-(4″-hydroxyphenyl)isoferulic acid ester (**12**), 1,2,5,7-tetrahydroxyanthraquinone (**23**) and 4,7-dihydroxy-5-(2′,4′,6′-trihydroxyphenyl)-indan-1,2-dione (**24**) from the PVPP residue, and catechin-7-O-β-(6″-O-nicotinoyl)-β-D-glucopyranoside (**41**), *ent*-epigallo-catechin-(4α →8, 2α →O→7)catechin (**44**), *ent*-epigallocatechin (4α →6, 2α →O→7)catechin (**45**) and 2,3-*cis*-3,4-*trans*-2-[2,3-*trans*-3,3′,4′,5,7-pentahydroxyflavan-8-yl]-4-(3,4-dihydroxyphenyl)3,5,7-trihydroxybenzopyran (**46**) from the unstabilized beer. Most of the compounds were tested for potential cancer chemopreventive activities in *in vitro* test systems detecting a modulation of carcinogen metabolism (inhibition of phase 1 cytochrome P450 1A (Cyp1A) activity, induction of NAD(P)H:quinone oxidoreductase (QR) activity) and anti-inflammatory mechanisms (inhibition of lipopolysaccharide (LPS)-mediated induction of inducible nitric oxide synthase (iNOS), inhibition of cyclooxygenase 1 (Cox-1) activity). 1,2,5,7-Tetrahydroxyanthraquinone (**23**) and xanthohumol (**25**), a prenylated chalcone derived from hop, were identified as the most potent compounds and were additionally tested for inhibition of chemically-induced preneoplastic lesions in an *ex vivo* mouse mammary gland organ culture model (MMOC). Importantly, both agents inhibited lesion formation with halfmaximal inhibitory concentrations (IC_{50}) of 0.1 and 0.02 μM, respectively. Our results demonstrate that beer is an interesting source of potential cancer chemopreventive agents and should be further investigated with this respect.

Abbreviations: CD – concentration required to double the specific activity of QR; Cox-1 – cyclooxygenase 1; Cyp – cytochrome P450; IC_{50} – halfmaximal inhibitory concentration; HPLC – high pressure liquid chromatography; iNOS – inducible nitric oxide synthase; LPS – lipopolysaccharide; MMOC, mouse mammary gland organ culture; NO – nitric oxide; PG – prostaglandin; PVPP – polyvinylpolypyrrolidone; QR – NAD(P)H:quinone oxidoreductase; TLC – thin layer chromatography; VLC – vacuum liquid chromatography.

Introduction

Beer and wine are the most consumed alcoholic beverages. Whereas moderate consumption of red wine may have beneficial health effects designated as 'French paradoxon' (Chèze et al., 2001) little is known about the influence of moderate consumption of beer on health. The health promoting activity of red wine is usually contributed to its high content in polyphenols. This is reflected by the fact that some producers of red

wine meanwhile provide information on quantitative polyphenol analyses on the label. Beer has not yet been investigated with the same intensity. However, some recent results indicate that polyphenols from beer might also exert health beneficial effects.

According to the Beer Purity Law from 1516, traditional German beer is made from barley, hops, and water, with the addition of yeast. During storage of beer, haze may be formed by interaction of proteins and polyphenols. In order to prevent haze formation and thus extend the shelf live of beer, polyphenols are partly removed from beer by treatment with polyvinylpolypyrrolidone resin (PVPP). PVPP can be regenerated with hot sodium hydroxide solution. Ultrafiltration of the sodium hydroxide solution generates a residue (PVPP retentate), which contains beer components removed from beer, as well as degradation products. In 1997, we initialized a project to analyze the composition of this waste product, which is generated in large quantities, and to evaluate its potential health beneficial effects. Since the initial results were not very encouraging, we extended the project to unstabilized beer. During activity-guided fractionation of beer, we identified components from hops as interesting potential chemopreventive agents, and therefore finally also included hop extracts in our studies.

The PVPP residue contained mostly degradation and rearrangement products of genuine beer constituents. A summary of the isolated compounds including yields is given in Table 1. The compounds belonged to five structural classes, i.e., benzoic acid and cinnamic acid derivatives, acetophenones, flavonoids and a series of miscellaneous phenolic compounds. Benzoic acid derivatives might result from β-cleavage of cinnamic acids. Novel compounds included a benzoic acid derivative, 2-(4′-hydroxyphenyl)-3,5-dihydroxybenzoic acid (**6**, Figure 1), as well as a complex cinnamic acid derivative, 2′-(4″-hydroxyphenyl)isoferulic acid ester (**12**, Figure 1). We were most astonished to identify an anthraquinone derivative in the PVPP residue, 1,2,5,7-tetrahydroxyanthraquinone (**23**, Figure 1), a class of compounds which is neither known for hop nor for barley and which has not yet been detected previously in beer. Also, it cannot be explained as a degradation product from any known substance. An additional novel product was 4,7-dihydroxy-5-(2′,4′,6′-trihydroxyphenyl)-indan-1,2-dione (**24**, Figure 1).

Fractions and isolated compounds were tested for their potential cancer chemopreventive activities in various enzyme- and cell culture tests. These test systems included antioxidant effects (scavenging of 1,1-diphenyl-2-picrylhydrazyl-, hydroxyl-, peroxyl- and superoxide anion radicals), modulation of carcinogen metabolism (inhibition of phase 1 cytochrome P450 1A (Cyp1A) enzyme activity, induction of phase 2 NAD(P)H:quinone reductase (QR) activity), and anti-inflammatory mechanisms (inhibition of inducible nitric oxide synthase (iNOS) induction, inhibition of cyclooxygenase 1 (Cox-1) activity).

We could demonstrate that phenolic compounds from beer display good antioxidant activity, especially *vs.* the highly reactive hydroxyl radicals involved in lipid peroxidation processes. A summary of the antioxidant potential is given in Gerhauser et al. (2001).

Material and methods

Isolation of compounds

PVPP residue and unstabilized beer were obtained from Karlsberg Brewery, Homburg, Germany.

PVPP-residue. We started with seven liters of an alkaline sodium hydroxide solution (pH 13.5) containing polyphenols removed from PVPP resin. The solution was brought to pH 3.5 with concentrated hydrochloric acid and extracted stepwise with a total of 35 l ethylacetate. After evaporation of the solvent, 6.01 g of a brownish residue were obtained. This residue was dissolved in MeOH/Cl_2CH_2 (1:1) and separated on a Sephadex LH 20 column with MeOH/Cl_2CH_2 (1:1), followed by MeOH/Cl_2CH_2 (3:1) and MeOH. According to TLC spotting, 11 fractions were combined. Further fractionation was achieved by vacuum liquid chromatography (VLC) mainly on diol-modified silicagel and elution with hexane/ethylacetate or ethylacetate/methanol mixtures. Final separation was achieved by HPLC under similar conditions.

Structure elucidation of isolated compounds was based on NMR spectrometry: one dimensional ^{1}H-NMR spectra were obtained at 400 MHz and ^{13}C-NMR at 100 MHz with an AM 400 Bruker instrument (Karlsruhe), two dimensional spectra were recorded on a DRX-500 Bruker spectrometer. Detailed structure information is described by Alt (2001) and will be published elsewhere.

Unstabilized beer. 300 l beer in portions of 10 l were poured over columns (5 × 80 cm) filled with Sephadex LH 20 which had been equilibrated with 5% ethanol

Table 1. Polyphenolic constituents of the PVPP retentate.

	Compound name	Yield
	Benzoic acid derivatives	
1	4-hydroxy benzoic acid	4.4 mg
2	protocatechuic acid	25.9 mg
3	vanillic acid	4.1 mg
4	gallic acid	3.5 mg
5	syringic acid	4.7 mg
6	2-(4'-hydroxyphenyl)-3,5-dihydroxybenzoic acid (n)	3.8 mg
	Cinnamic acids	
7	cinnamic acid	1.8 mg
8	*p*-coumaric acid	2.5 mg
9	caffeic acid	7.1 mg
10	ferulic acid	92.7 mg
11	sinapic acid	19.7 mg
12	2′-(4″-hydroxyphenyl)isoferulic acid ester (n)	1.3 mg
	Acetophenone derivatives	
13	4-hydroxy-acetophenone	2.0 mg
14	4-hydroxy-3-methoxy-acetophenone	2.5 mg
15	4-hydroxy-3,5-dimethoxy-acetophenone	10.5 mg
16	4-hydroxy-2,6-dimethoxy-acetophenone	0.5 mg
	Miscellaneous compounds	
17	3′-methoxy-4′-hydroxy-phenyl-acetic acid	(traces)
18	succinic acid	5.3 mg
19	glutaric acid ethylester	2.0 mg
20	3-hydroxy-3-methyl-glutaric acid	1.7 mg
21	4-hydroxydihydrofuran-2-on	2.3 mg
22	indol-3-carboxylic acid	1.5 mg
23	1,2,5,7-tetrahydroxyanthraquinone (n)	23 mg
24	4,7-dihydroxy-5-(2′,4′,6′-trihydroxyphenyl)-indan-1,2-dione (n)	31 mg
	Flavonoid derivatives (compare unstabilized beer)	
26	isoxanthohumol	1.5 mg
31	apigenin	2.0 mg
32	chrysoeriol	1.2 mg
33	tricin	55.2 mg

(n) represents novel compounds

in water. Polyphenols were retained (McMurrogh and Baert, 1994), whereas carbohydrates, proteins and salts were washed off the column. Polyphenols were then eluted with a mixture of acetone/methanol (3:1). 23.7 g raw material were obtained and subjected to ultrafiltration with a cut off of 4 kDa. After freeze-drying, we obtained 8.7 g ultrafiltrate. The residue was extracted with ethylacetate and yielded 4.8 g. The combined fractions (13.3 g) were separated as described for the PVPP residue.

In vitro *test systems of potential chemopreventive activity*

Experimental details of all test systems utilized in this study are summarized in Gerhauser et al. (2002a). Inhibition of Cyp1A (EC 1.14.14.1) enzymatic activity and induction of QR (EC 1.6.99.2) in cultured Hepa1c1c7 cells were assayed as described by Crespi et al. (1997) and Gerhauser et al. (1997), monitoring the dealkylation of 3-cyano-7-ethoxycoumarin to 3-cyano-7-hydroxycoumarin and the NADPH-dependent menadiol-mediated reduction of MTT [3-

Table 2. Polyphenolic constituents of unstabilized beer.

	Compound name	Yield
	Flavonoid derivatives	
	Chalcones	
25	xanthohumol	4.5 mg
	Flavanones	
26	isoxanthohumol	177.8 mg
27	naringenin	2.1 mg
28	8-prenyl-naringenin	3.9 mg
29	6-prenyl-naringenin	5.8 mg
30	5-methyl-6″-dimethyl-4″,5″-dihydropyrano[2″,3″,7,8]naringenin	5.2 mg
	Flavones	
31	apigenin	3.7 mg
32	chrysoeriol	1.2 mg
33	tricin	31.0 mg
34	apigenin-6-C-glucoside	9.7 mg
35	apigenin-6-C-glucoside-7-O-glucoside	11.5 mg
	Flavan-3-ols	
36	catechin	935 mg
37	epicatechin	6.1 mg
38	gallocatechin	14.8 mg
39	3′-O-methylcatechin	15.8 mg
40	catechin-7-O-β-D-glucopyranosid	58.4 mg
41	catechin-7-O-β-(6″-O-nicotinoyl)- β-D-glucopyranosid (n)	5 mg
	Proanthocyanidins	
42	procyanidin B 3	47.7 mg
43	prodelphinidin C	22.7 mg
44	*ent*-epigallocatechin-(4α →8, 2α →O→7)catechin (n)	101.7 mg
45	*ent*-epigallocatechin (4α →6, 2α →O→7)catechin (n)	4.3 mg
46	2,3-cis-3,4-trans-2-[2,3trans-3,3′,4′,5,7-pentahydroxyflavan-8-yl]-4-(3,4-dihydroxyphenyl)3,5,7-trihydroxybenzopyran (n)	6.2 mg
	Miscellaneous compounds	
22	indol-3-carboxylic acid	2.0 mg
47	1-methyl-1,3,4,9-tetrahydropyrano[3,4b]indol	1.5 mg
48	2-(4′-hydroxyphenyl)ethanol	12.5 mg
49	4-ketopinoresinol	13.7 mg
50	syringaresinol	1.8 mg
51	3-acetoxy-propan-1,2-diol	3.1 mg

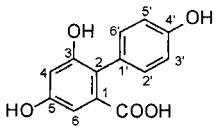
(n) represents novel compounds

(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] to a blue formazan, respectively. Inhibition of Cox-1 (prostaglandin G/H synthase, EC 1.14.99.1) activity was measured with a modification of the system described by Jang et al. (1997), measuring oxygen consumption during the conversion of arachidonic acid to prostaglandins (PGs). Inhibitory activity on the LPS-induced expression of iNOS in murine Raw 264.7 macrophages was quantified via the Griess reaction as described previously (Ding et al., 1988; Heiss et al., 2001).

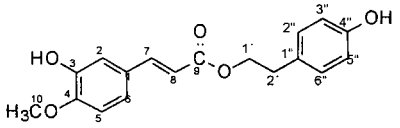
Table 3. Summary of potential chemopreventive activities.

No.[a]	Cyp1A Inhibition IC_{50}-A [μM][b]	QR induction CD [μM][c]	IC_{50}-T [μM][b]	Inhibition of iNOS induction IC_{50}-A [μM][b]	IC_{50}-T [μM][b]	Cox-1 inhibition IC_{50}-A [μM][b]
23	0.07	10.3	23.9	33.8	>50	7.2
25	0.02	1.7	7.4	12.9	>50	16.6
26	0.3	6.5	29.9	21.9	>50	>100 (36)
27	0.2	>50 (1.5)	>50	>50 (10)	>50	164.6
28	0.07	15.5	>50	>50 (15)	>50	27.1
29	0.09	15.4	>50	>50 (33)	>50	113.7
30	1.6	19.6	>50	>50 (24)	>50	>100 (0)
31	0.02	>38.6 (0.9)	38.6	17.5	>50	>100 (22)
33	0.13	>50 (1.9)	>50	>50 (7)	>50	>100 (38)
34	>5 (31)	>50 (1.5)	>50	>50 (0)	>50	>100 (10)
35	>5 (1)	>50 (1.6)	>50	>50 (0)	>50	>100 (9)
36	>5 (0)	>50 (1.0)	>50	>50 (3)	>50	5.2
37	>5 (0)	>50 (0.9)	>50	>50 (16)	>50	7.5
38	>5 (8)	>50 (1.2)	>50	>50 (17)	>50	45.3
39	>5 (40)	>50 (1.4)	>50	>50 (0)	>50	24.8
40	>5 (0)	>50 (1.1)	>50	>50 (0)	>50	>100 (8)
47	1.5	33.9	>50	>50 (20)	>50	>100 (5)
49	2.2	6.0	34.7	33	>50	>100 (15)

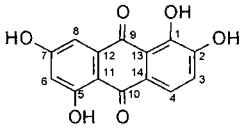
[a]Compounds **1**, **2**, **3**, **4**, **5**, **7**, **8**, **9**, **10**, **11**, **13**, **15**, **22**, **24**, **42**, **43**, **44**, and **46** were not active at the highest concentrations tested, i.e. 5 μM for Cyp1A inhibition, 50 μM for QR induction and inhibition of iNOS induction, respectively, and 100 μM for Cox-1 inhibition. Compounds **6**, **12**, **14**, **16**, **17**, **18**, **19**, **20**, **21**, **32**, **41**, **45**, **48**, **50**, and **51** were not tested.
[b]Halfmaximal inhibitory concentration for enzyme activity (A) or cell viability (T for toxicity). Values in parentheses indicate the percentage of inhibition at the indicated concentration.
[c]Concentration required to double the specific activity of QR. Values in parentheses indicate the fold induction at the indicated concentration.



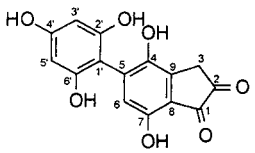
2-(4`-Hydroxyphenyl)-3,5-dihydroxybenzoic acid (**6**)



2`-(4"-Hydroxyphenyl)ethylisoferulic acid ester (**12**)

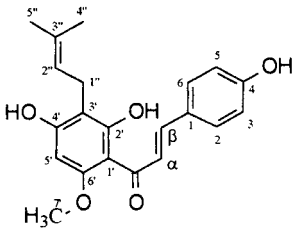


1,2,5,7-Tetrahydroxyanthraquinone (**23**)

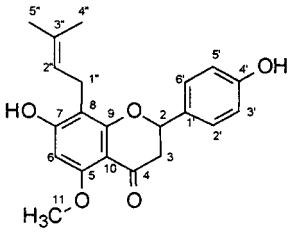


4,7-Dihydroxy-5-(2`,4`,6`-trihydroxyphenyl)-indan-1,2-dione (**24**)

Figure 1. Novel compounds isolated from the PVPP regeneration residue.



Xanthohumol (**25**)



Isoxanthohumol (**26**)

Figure 2. Xanthohumol and isoxanthohumol, prenylated flavonoids derived from hops.

Results

Isolation of compounds

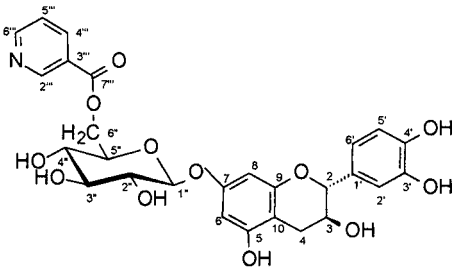
As a result of this project, 51 compounds were isolated from a PVPP residue and unstabilized beer. The PVPP residue yielded 28 compounds (Table 1), four of which represent hitherto undescribed compounds. Similarly, 28 compounds were isolated from the unstabilized beer (Table 2). Of these, four compounds are also novel structures.

The polyphenols isolated from unstabilized beer were mostly flavonoids and proanthocyanidins and are summarized in Table 2. We detected the chalcone xanthohumol (**25**, Figure 2), the main prenylated flavonoid of hop cones, together with its isomerization product, the flavanone isoxanthohumol (**26**, Figure 2), which is formed during the brewing process. Catechin (**36**) was the major constituent of unstabilized beer, whereas the isomeric epicatechin (**37**) was only a minor compound. Within this group of flavan-3-ols, catechin-7-O-β-(6″-O-nicotinoyl)-β-D-glucopyranoside (**41**, Figure 3) was identified as a new natural product. Five proanthocyanidins were isolated from the beer extract: two known compounds, procyanidin B 3 (**42**) and prodelphinidin C (**43**), and three new natural products, *ent*-epigallocatechin-(4α $\rightarrow$8, 2α $\rightarrow$O$\rightarrow$7)catechin (**44**, Figure 3), *ent*-epigallocatechin (4α $\rightarrow$6, 2α $\rightarrow$O$\rightarrow$7)catechin (**45**, Figure 3), and 2,3-*cis*-3,4-*trans*-2-[2,3-*trans*-3,3′,4′,5,7-pentahydroxyflavan-8-yl]-4-(3,4-dihydroxy phenyl)3,5,7-trihydroxybenzopyran (**46**, Figure 3). The latter compound has been described in its permethylated form by Steynberg et al. (1990) as a reaction product of procyanidin B3 in an alkaline buffer system (Na_2CO_3/$NaHCO_3$).

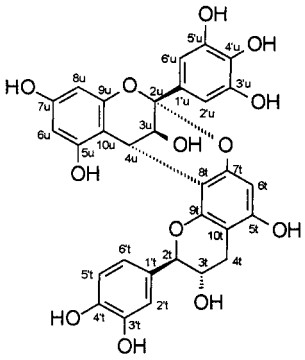
Cancer chemopreventive activity

Modulation of carcinogen metabolism. We evaluated the potential of beer constituents to modulate phase 1 and 2 carcinogen metabolism as a mechanism of cancer prevention in the initiation phase of carcinogenesis. Often, pro-carcinogens have to be metabolically activated to become ultimate carcinogens. We selected Cyp1A, which is e.g. involved in the activation of carcinogens from grilled meat, as a model phase 1 enzyme, and measured the potential of beer constituents to inhibit its enzymatic activity. Phase 1 metabolites are generally further metabolized and detoxified during phase 2 metabolism by conjugation to endogenous ligands. These conjugation reactions render them more water-soluble and enhance excretion. Consequently, induction of phases 2 enzymes contributes to chemopreventive activity. QR is not catalyzing a conjugation reaction, but it is induced coordinately with other phase 2 enzymes and contributes to detoxification by reduction of reactive quinones to less reactive hydroquinones.

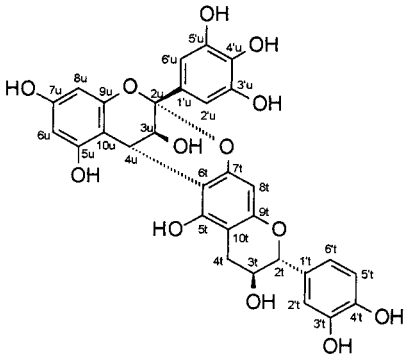
With the exception of 1,2,4,7-tetrahydroxyanthraquinone (**23**), which was identified as a potent inhibitor of Cyp1A with an IC_{50} (halfmaximal inhibitory concentration) of 0.07 μM and also induced QR activity with a CD value (concentration required to double the specific activity of QR) of 10.3 μM, none of the tested constituents isolated from the PVPP residue was able to modulate the activity of these enzymes at the highest concentrations tested (5 μM for Cyp1A activity and 50 μM for QR induction) (Table 3). On the other hand, the chalcone xanthohumol (**25**) and the flavanones (**26**)–(**30**) isolated from beer potently inhibited Cyp1A activity, with IC_{50}s in the range of 0.02 μM–1.6 μM. These compounds were also inducers of QR activity, and we



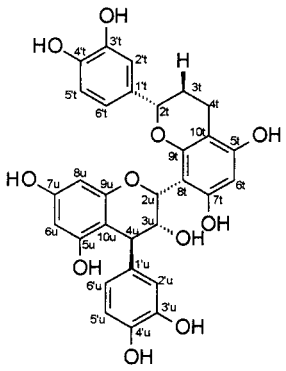
Catechin-7-O-(6''-O-nicotinoyl)-β-D-glucopyranoside(**41**)



ent-Epigallocatechin(4α→8, 2α→O→7)catechin (**44**)



ent-Epigallocatechin(4α→6, 2α→O→7)catechin (**45**)



2,3-cis-3,4-trans-2-[2,3-trans-3,3′,4′,5,7-pentahydroxyflavon-8-yl]-4-(3,4-dihydroxyphenyl)-3,5,7-trihydroxybenzopyran (**46**)

Figure 3. New compounds from unstabilized beer.

determined CD values from 1.7 μM for xanthohumol (**25**) to 19.6 μM for (**30**). 8-Prenylnaringenin (**27**) induced QR activity only 1.5-fold at 50 μM. Generally, the tested flavones (**31**)–(**35**) and flavan-3-ols (**36**)–(**40**), but also the proanthocyanidins (**42**)–(**46**) were less active in both test systems. As an exception, apigenin (**31**) potently inhibited Cyp1A activity with an IC_{50} of 0.02 μM, whereas its glucosides (**34**) and (**35**) completely lost potential to inhibit this enzyme. Chrysoeriol (**32**) was also a good Cyp1A inhibitor with an IC_{50} of 0.13 μM. In addition, 1-methyl-1,3,4,9-tetrahydropyrano[3,4b]indol (**47**) and 4-ketopinoresinol (**49**) displayed moderate activity in Cyp1A inhibition and induced QR activity with CD values of 33.9 μM and 6.0 μM, respectively (Table 3).

Anti-inflammatory mechanisms. It is estimated that 10% of all cancer cases are related to chronic inflammatory processes. Chronic inflammation and infections stimulate the inducible form of nitric oxide (NO) synthase (iNOS) and consequently, enhance the generation of NO. Generally, NO is an important signaling molecule, long-term elevated levels of NO, however, have been linked to early steps in carcinogenesis. Prostaglandins (PGs) are hormone-like endogenous mediators of inflammation and are formed from arachidonic acid by Cox-1 and the inducible form Cox-2. They enhance cell proliferation and stimulate the formation of new blood vessels (angiogenesis) and tumor invasiveness. Since PG levels and the expression of Cox-2 are often elevated in tumor tissue in comparison to normal tissue, inhibitors of Cox-1 and preferentially Cox-2 activity are regarded as promising chemopreventive agents.

We utilized sheep seminal vesicle microsomes as a source of Cox-1, and, for selected compounds, also human recombinant Cox-2 to determine Cox-inhibitory potential. Inhibition of iNOS induction was

measured in LPS-stimulated murine macrophage cultures. As summarized in Table 4, only five of all tested compounds were able to moderately inhibit iNOS induction, i.e., the novel anthraquinone derivative (**23**), xanthohumol (**25**), its cyclization product isoxanthohumol (**26**), apigenin (**31**) and 4-ketopinoresinol (**49**), with IC_{50}s in the range of 12.9 μM–33.8 μM. With respect to inhibition of Cox-1 activity, catechin (**36**), epicatechin (**37**), the anthraquinone (**23**), xanthohumol (**25**), 3′-O-methylcatechin (**39**) and 8-prenylnaringenin (**28**) were the most potent compounds, with IC_{50}s of 5.2, 7.5, 7.2, 16.6, 24.8 and 27.1 μM. Based on these results, catechin (**36**), the anthraquinone (**23**), xanthohumol (**25**), and 8-prenylnaringenin (**28**) were also tested for their potential to inhibit Cox-2 activity. At a test concentration of 100 μM, (**36**) and (**25**) inhibited Cox-2 activity 70 and 83%, respectively, and we determined an IC_{50} of 41.5 μM for xanthohumol (**25**). (**23**) and (**28**) were significantly less potent and inhibited Cox-2 activity only 6 and 46%.

Of all compounds tested, xanthohumol (**25**) was identified as the most promising lead compound for cancer chemoprevention. In addition to the described activities, it demonstrated various anti-proliferative mechanisms, including anti-estrogenic properties, inhibition of DNA polymerase α and induction of apoptosis and cell differentiation (Gerhauser et al., 2002b). As a first demonstration of chemopreventive potential in a more complex test system, xanthohumol (**25**) and the anthraquinone (**23**) were additionally tested in an organ culture model using mouse mammary glands (MMOC), as a link between short-term *in vitro* and long-term *in vivo* carcinogenesis models. Both compounds were significantly more potent inhibitors of chemically induced pre-neoplastic lesions, with IC_{50}s of 0.02 and 0.1 μM, respectively, than the positive control *t*-resveratrol found in red wine, which displayed an IC_{50} value of 4.2 μM.

Conclusion

Components of the PVPP residue were characterized and analyzed for the first time. They demonstrated little potential to modulate carcinogen metabolism or to inhibit the formation of inflammatory mediators. Protective effects of beer with regard to coronary heart disease prevention have been reported before (Denke, 2000; Brenner et al., 2001). The present *in vitro* results might stimulate animal and human studies to further investigate the value of beer as a source of dietary antioxidants and potential cancer chemopreventive agents and its application in cancer prevention. Nevertheless, it should be considered that high alcohol intake is a risk factor for various types of cancer.

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