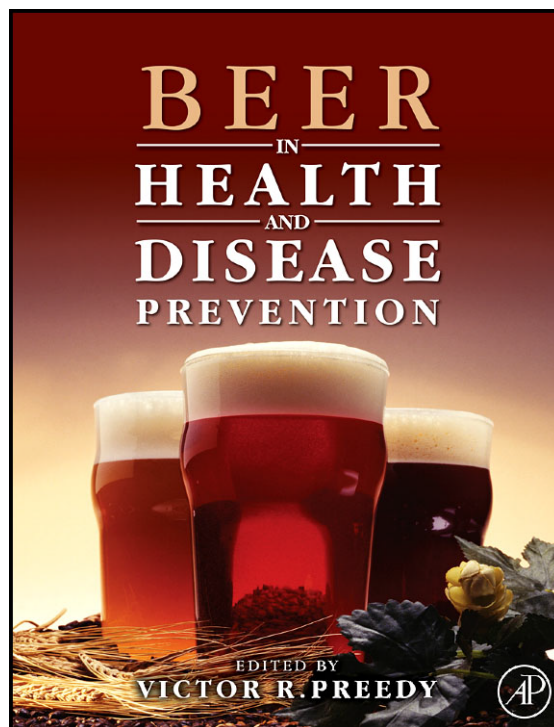


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12

Phenolic Compounds in Beer**Clarissa Gerhäuser** German Cancer Research Center, Heidelberg, Germany**Hans Becker** Pharmacognosy and Analytical Phytochemistry, University of Saarland, Saarbrücken, Germany**Abstract**

Beer is a complex alcoholic beverage made from barley (malt), hops, water and yeast. It is a good source of phenolic antioxidants. The majority of phenolic constituents of beer are derived from malt (70–80%), whereas about 20–30% are derived from hops. Phenolic constituents of beer represent a large structural variety and belong to the classes of simple phenols, benzoic- and cinnamic acid derivatives, coumarins, catechins, di-, tri- and oligomeric proanthocyanidins, (prenylated) chalcones and flavonoids. The structural diversity of these phenolic components that have been identified and quantified in beer will be summarized.

Introduction

Beer is one of the most consumed alcoholic beverages. Beer is rich in nutrients including carbohydrates, amino acids, minerals, and vitamins, but also provides a variety of non-nutrient components including phenolic compounds (Piendl, 1989). In Germany, beer is brewed according to the Beer Purity Law from 1516 only from barley, hops and water, with the addition of yeast. Beer brewing is a complex process that allows variation in multiple parameters during brewing which influence the type and quality of beer. These include the variety of barley and the malting process, temperature and pH during mashing, sparging, boiling, the variety of hops added during wort boiling, and yeast fermentation.

The phenol content of beer was determined as 500–1,000 mg/l (Leupold and Drawert, 1981). About 70–80% of beer polyphenols are malt-derived (Knorr, 1978; De Keukeleire, 2000). Barley polyphenols undergo changes during the malting and brewing process and are less well characterized than phenolic compounds from hops (Moll *et al.*, 1984). Hops (*Humulus lupulus* L.) is added to beer in small quantities for aroma and bitterness and to provide antifungal and antibiotic properties (Mizobuchi and Sato, 1985; Verzele, 1986; Stavri *et al.*, 2004). These characteristics are mainly attributed to their content of α -acids like

humulone, which are converted during brewing to the bitter-tasting iso- α -acids. However, the dried hops cones also contain a large quantity of polyphenols (4–14%) which account for about 20–30% of the polyphenols found in beer (Knorr, 1978; Stevens *et al.*, 1998; De Keukeleire *et al.*, 1999; Taylor *et al.*, 2003).

Description of Structural Classes of Phenolic Beer Constituents

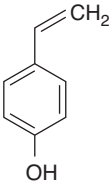
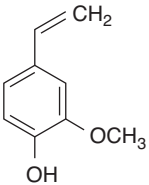
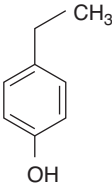
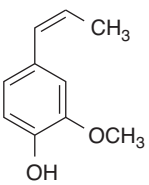
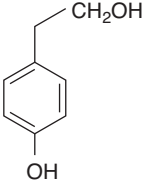
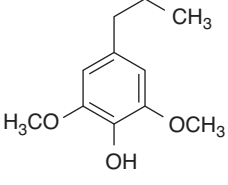
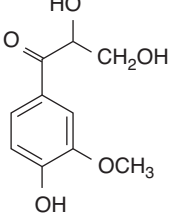
Phenolic constituents of beer cover a large structural variety and belong to the classes of simple phenols, benzoic- and cinnamic acid derivatives, coumarins, catechins, di-, tri- and oligomeric proanthocyanidins, (prenylated) chalcones and flavonoids. This chapter will summarize the structural diversity of these components that have been identified and quantified in beer. Each class of phenolic beer constituents will be described, mean quantities measured in beer sample will be summarized, and chemical structures of the beer constituents will be given. A detailed description of biological activities will be found elsewhere in this handbook.

Simple phenols

Simple volatile phenols in beer contribute to the typical beer aroma. They either have an unpleasant “phenolic” smell, or are characterized by a pleasant vanillin, clove-like spicy or smoky odor. Levels of simple phenols in beer are generally low (Table 12.1).

The aromatic alcohol tyrosol (**5**), which is formed by fermentation of the amino acid tyrosine (Tressl *et al.*, 1976), was detected in exceptionally high concentrations up to 40 mg/l. Levels in alcohol-free beers were considerably lower than in standard beers (2.78 ± 3.12 mg/l vs. 11.83 ± 3.12 mg/l). This was attributed to differences in the duration of fermentation and yeast strains employed in brewing alcohol-free beers (Bartolomé *et al.*, 2000). Interestingly, tyrosol (**5**) was identified also as a major phenolic constituent of olive oil, with levels in the range of

Table 12.1 Summary of simple phenols and their contents in beer

	Simple phenols	(mg/l)
1	4-Vinyl phenol 	Up to 0.15 ^{a,b}
2	4-Vinyl guaiacol 	Up to 0.55 ^{a,b} ; 0.68 ^c
3	4-Ethyl phenol 	Up to 0.01 ^a
4	4-Propenyl guaiacol (isoeugenol) 	Up to 0.04 ^a
5	Tyrosol 	Up to 40 ^a ; 11.8 ± 4.03 ^d ; 2.9 ± 5.95 ^e
6	4-Propyl syringol 	Up to 0.2 ^a
7	2,3-Dihydroxy-1-guaiacylpropane-1-one 	0.034 ± 0.011 ^d

^a Summarized in Alt (2001).

^b Highest levels in German dark bock beer (Tressl *et al.*, 1976).

^c High levels in German Weissbier (Madigan *et al.*, 1994).

^d Mean ± standard deviation from eight beers (Spanish, Danish, German) (Bartolomé *et al.*, 2000).

^e Mean ± standard deviation from 23 Italian beers (Floridi *et al.*, 2003).

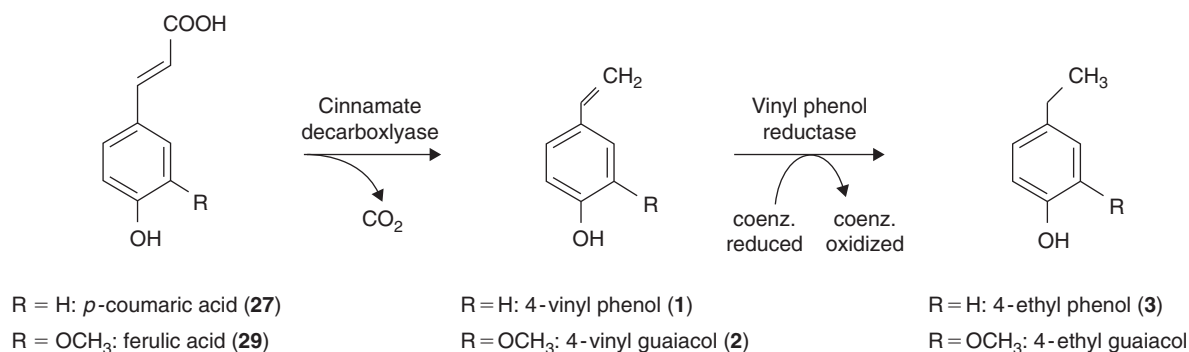


Figure 12.1 Scheme for the formation of simple phenol derivatives from cinnamic acids during beer brewing. *Source:* Modified from Vanderhaegen *et al.* (2003).

3.9–8.75 mg/l (Romani *et al.*, 2001). Although only weakly antioxidative, it was effective in preserving cellular antioxidant defences, probably by intracellular accumulation (Di Benedetto *et al.*, 2006).

Other simple phenols are formed either during yeast fermentation by enzymatic decarboxylation or by thermic decomposition of phenolcarboxylic acids (Tressl *et al.*, 1976). An example for the formation of 4-vinyl phenol (1), 4-vinyl guaiacol (2) and 4-ethyl phenol (3) from *p*-coumaric (27) or ferulic acid (29) (see section “Cinnamic acid derivatives”) is depicted in Figure 12.1 (modified from Vanderhaegen *et al.*, 2003).

Typically, levels of 4-vinyl guaiacol (1) are below the flavor thresholds (0.2–0.3 mg/l) in lager, ale and stout, but higher contents were measured in German Weissbier (wheat beer), which has a strong phenolic character (Madigan *et al.*, 1994).

Benzoic acid derivatives

Occurrence and quantitative levels of 10 benzoic acid derivatives were reported in beer in various studies, as summarized in Table 12.2. In addition, four related aldehydes were detected, which could be either reduction products of benzoic acids or oxidation products of phenols. Quantification was achieved by high performance liquid chromatography (HPLC) with diode array, coulometric array or fluorimetric detection.

Highest benzoic acid levels were measured for salicylic acid (8) and *p*-hydroxybenzoic acid (10) in an Italian study by Floridi *et al.* (2003). In addition, relatively high levels of vanillic acid (16) and vanillin (20) were reported in a German beer (Achilli *et al.*, 1993). Levels of *p*-hydroxybenzoic acids (10) and (16) were lower in alcohol-free beers than in standard beers (*p*-hydroxybenzoic acid: 0.073 ± 0.019 mg/l vs. 0.092 ± 0.03 mg/l; vanillic acid 0.35 ± 0.09 mg/l vs. 0.48 ± 0.11 mg/l), whereas the concentration of vanillin (20) was higher in alcohol-free beers (0.048 ± 0.033 mg/l vs. 0.028 ± 0.009 mg/l). Losses were partly explained by the procedures for the dealcoholization process (Bartolomé *et al.*, 2000). Notable, levels of benzoic acid derivatives varied

quite considerably between different studies. This might either be due to variations in types and origin of beers under investigation, or due to differences in sensitivity and specificity of the chromatographic method chosen for quantitative determinations. Further, most phenolic acids in beer are present as bound forms and only a small portion can be detected as free compounds (Nardini and Ghiselli, 2004). As an example, a content of 0.63 mg/ml of vanillic acid (16) was measured in a non-hydrolyzed beer sample, whereas a twofold increase to 1.2 mg/ml was determined after alkaline hydrolysis (Nardini *et al.*, 2006). Depending on the degree of hydrolysis during sample preparation, this might also lead to variations in quantitative levels.

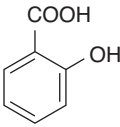
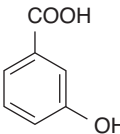
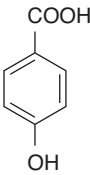
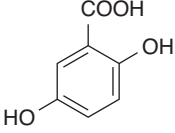
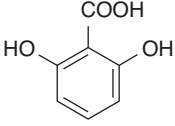
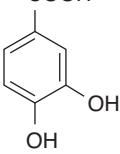
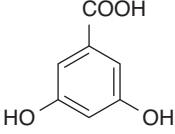
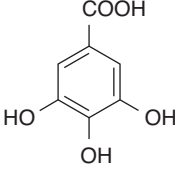
Phenylacetic acid derivatives

Only two phenylacetic acid derivatives, *p*-hydroxyphenylacetic acid (22) and homovanillic acid (23) were detected in low quantities in various beer samples (Table 12.3). *p*-Hydroxyphenylacetic acid (22) might represent a metabolic breakdown product of proanthocyanidins (see section “Proanthocyanidins”) or of flavonoles like kaempferol (see section “Flavonoles”) and has been detected in human fecal water (Jenner *et al.*, 2005). Homovanillic acid (23) is known as a major metabolite of the neurotransmitter dopamine (3,4-dihydroxyphenethylamine) (Knight and Haymond, 1977). Dopamine has been detected in beer, although at very low concentrations in a range of 0.0005–0.015 mg/l (mean: 0.004 ± 0.003 mg/ml, equivalent to 28 ± 20 nM) (Duncan and Smythe, 1982).

Cinnamic acid derivatives

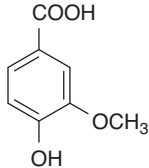
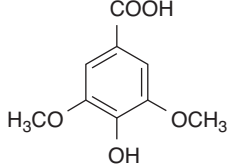
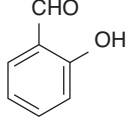
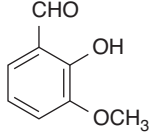
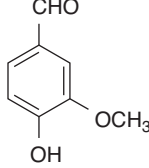
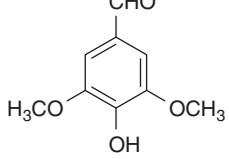
Ferulic acid (29) is the main phenolic acid in barley, malted barley and beer (Table 12.4). It is found in the cell walls of the aleurone layer of the barley kernel. Generally, 90% of ferulic acid is present in barley in bound form as an ester with arabinoxylan polymers, and only about 10% are present in free form (Szwajgier *et al.*, 2005). Alkaline hydrolysis increased the detectable levels of ferulic acid

Table 12.2 Summary of benzoic acid derivatives and their contents in beer

<i>Benzoic acid derivatives</i>			<i>(mg/l)</i>
8	Salicylic acid	2-Hydroxybenzoic acid	 $2.87 \pm 1.56^a; 0.16 \pm 0.23^b$
9	<i>m</i> -Hydroxy benzoic acid	3-Hydroxybenzoic acid	 0.32 ± 0.21^a
10	<i>p</i> -Hydroxy benzoic acid	4-Hydroxybenzoic acid	 $16.8 \pm 11.0^a;$ $0.36 \pm 0.39^b; 0.09 \pm 0.03^c$
11	Gentisic acid	2,5-Dihydroxybenzoic acid	 0.38 ± 0.31^a
12	γ -Resorcylic acid	2,6-Dihydroxy benzoic acid	 0.92 ± 0.46^a
13	Protocatechuic acid	3,4-Dihydroxybenzoic acid	 $0.84 \pm 1.66^a; 0.15 \pm 0.15^b$
14	α -Resorcylic acid	3,5-Dihydroxybenzoic acid	 0.35 ± 0.60^a
15	Gallic acid	3,4,5-Trihydroxybenzoic acid	 $0.59 \pm 0.63^a; 2.9^d$

(Continued)

Table 12.2 (Continued)

Benzoic acid derivatives			(mg/l)
16	Vanillic acid	4-Hydroxy-3-methoxybenzoic acid 	0.74 ± 0.25 ^a ; 0.52 ± 0.33 ^b ; 3.6 ^e ; 0.48 ± 0.11 ^d ; 0.85 ^f ;
17	Syringic acid	3,5-Dimethoxy-4-hydroxybenzoic acid 	0.24 ± 0.10 ^a ; 0.07 ± 0.11 ^b ; 0.5 ^c , 0.23 ^f
18	Salicylaldehyde	2-hydroxybenzaldehyde 	n.d.
19	<i>o</i> -Vanillin	2-Hydroxy-3-methoxybenzaldehyde 	1.6 ^e
20	Vanillin	4-Hydroxy-3-methoxybenzaldehyde 	0.14 ± 0.19 ^b ; 0.03 ± 0.01 ^d
21	Syringic aldehyde	3,5-Dimethoxy-4-hydroxybenzaldehyde 	0.7 ^e

n.d: not determined.

^aMean ± standard deviation from 23 Italian beers (Floridi *et al.*, 2003).

^bMean ± standard deviation from 10 beers (Dutch, German, Czech, Belgian, French, Italian, Austrian) (Jandera *et al.*, 2005).

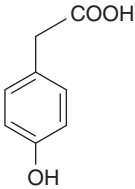
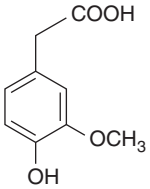
^cMean ± standard deviation from eight beers (Spanish, Danish, German) (Bartolomé *et al.*, 2000).

^dMean of Israeli beer samples (Gorinstein *et al.*, 2000).

^eIn one German beer (Achilli *et al.*, 1993).

^fHighest content of three beers (Italian, Austrian, German) (Nardini and Ghiselli, 2004).

Table 12.3 Summary of phenylacetic acid derivatives and their contents in beer

<i>Phenylacetic acids</i>			<i>(mg/l)</i>
22	4-Hydroxy-phenylacetic acid		0.61 ^a ; 0.33 ± 0.48 ^b ; 1.2 ^c
23	Homovanillic acid (3-methoxy-4-hydroxy-phenylacetic acid)		0.58 ± 0.11 ^d

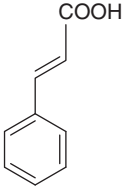
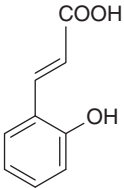
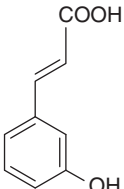
^aHighest content of three beers (Italian, Austrian, German) (Nardini and Ghiselli, 2004).

^bMean ± standard deviation from 10 beers (Dutch, German, Czech, Belgian, French, Italian, Austrian) (Jandera *et al.*, 2005).

^cIn one German beer (Achilli *et al.*, 1993).

^dMean ± standard deviation from 23 Italian beers (Floridi *et al.*, 2003).

Table 12.4 Summary of cinnamic acid derivatives and their contents in beer

<i>Cinnamic acids</i>			<i>(mg/l)</i>
24	Cinnamic acid		n.d.
25	<i>o</i> -Coumaric acid <i>trans</i> -2-Hydroxycinnamic acid		1.73 ± 3.74 ^a
26	<i>m</i> -Coumaric acid <i>trans</i> -3-Hydroxycinnamic acid		0.23 ± 0.33 ^a

(Continued)

Table 12.4 (Continued)

Cinnamic acids			(mg/l)	
27	<i>p</i> -Coumaric acid	<i>trans</i> -4-Hydroxycinnamic acid	<chem>OC(=O)/C=C/c1ccc(O)cc1</chem> 	1.36 ± 0.71^a ; 0.77 ± 0.15^b ; 2.1^c ; 0.83 ± 0.93^d
28	Caffeic acid	3,4-Dihydroxycinnamic acid	<chem>OC(=O)/C=C/c1cc(O)c(O)cc1</chem> 	0.57 ± 0.34^a ; 0.07 ± 0.03^b ; 0.2^e ; 0.09 ± 0.13^d
29	Ferulic acid	4-Hydroxy-3-methoxycinnamic acid	<chem>OC(=O)/C=C/c1cc(O)c(OC)cc1</chem> 	2.4 ± 0.87^a ; 1.3 ± 0.6^b ; 2.3^e ; 6.8^c ; 2.9 ± 2.3^d ; 6.5^f ; $1.6 - 3.4^g$
30	Sinapic acid	3,5-Dimethoxy-4-hydroxycinnamic acid	<chem>OC(=O)/C=C/c1cc(OC)c(O)c(OC)c1</chem> 	0.15 ± 0.15^a ; 0.09 ± 0.04^b ; 0.84^e ; 0.38 ± 0.41^d
31	Chlorogenic acid	3-O-caffeoylquininic acid	<chem>OC(=O)[C@@H]1[C@H](OC(=O)/C=C/c2cc(O)c(O)cc2)[C@H](O)[C@@H](O)[C@H]1O</chem> 	0.9 ± 0.49^a ; 0.26^e ; 0.68 ± 0.94^d

n.d: not determined.

^a Mean \pm standard deviation from 23 Italian beers (Floridi *et al.*, 2003).

^b Mean \pm standard deviation from eight beers (Spanish, Danish, German) (Bartolomé *et al.*, 2000).

^c Mean of Israeli beer samples (Gorinstein *et al.*, 2000).

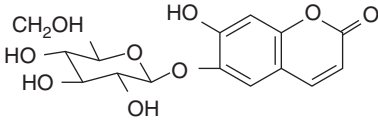
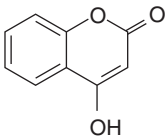
^d Mean \pm standard deviation from 10 beers (Dutch, German, Czech, Belgian, French, Italian, Austrian) (Jandera *et al.*, 2005).

^e Highest content of three beers (Italian, Austrian, German) (Nardini and Ghiselli, 2004).

^f In one German beer (Achilli *et al.*, 1993).

^g In four Danish lager beers (Andersen *et al.*, 2000).

Table 12.5 Summary of coumarins and their contents in beer

Coumarins		(mg/l)
32	Esculin (6,7-dihydroxycoumarin-6-O- β -d-glucopyranoside)	3.6 (fresh), 1.8 (aged)*
		
33	4-Hydroxycoumarin	23.2 (fresh), 13.9 (aged)*
		

* In Czech pilot fresh and aged beer; not detectable in commercial beer samples (Jandera *et al.*, 2005).

(**29**) in a German beer about 11-fold. Also, sinapic acid (**30**) levels were 1,382% higher after hydrolysis than in non-hydrolyzed beer (0.2 ± 0.02 mg/l vs. 3.0 ± 0.34 mg/l; Nardini and Ghiselli, 2004). In contrast, *p*-coumaric acid (**27**) is present in beer mainly as free form (69.8% of total) (Nardini *et al.*, 2006).

Interestingly, esterified ferulic acid is less susceptible than free ferulic acid (**29**) to undergo thermal or metabolic decarboxylation to 4-vinyl guaiacol (**2**), therefore preventing undesirable aroma development (Szwajgier *et al.*, 2005).

Nardini *et al.* (2006) compared bioavailability and metabolism of various phenolic acids in 10 healthy subjects after consumption of 500 ml beer. Plasma samples were collected before 30 and 60 min after beer consumption. Free acids as well as sulfate and glucuronide metabolites were determined. The study indicated that phenolic acids from beer were absorbed from the gastrointestinal tract. Whereas *p*-coumaric acid (**27**) was present in plasma equally in non-conjugated and conjugated form, vanillic (**16**), caffeic (**28**) and ferulic (**29**) acid were present mainly as conjugated forms (sulfates > glucuronates). Overall, it was concluded that monohydroxy derivatives were less conjugated than dihydroxy derivatives. Total plasma levels were higher after 30 min than after 60 min and were in the range of 8 ng/ml plasma for caffeic acid (equivalent to $0.05 \mu\text{M}$) to 21 ng/ml for ferulic acid (equivalent to $0.11 \mu\text{M}$). The total level of 4-hydroxyphenylacetic acid (**22**) present mainly in non-conjugated form (>70%) reached maxima of 1.4 and $1.17 \mu\text{M}$ after 30 and 60 min, respectively. It was suggested that these high levels are partly due to postabsorption metabolism of other compounds present in beer, including tyrosine and phenolic acids (Nardini *et al.*, 2006), as well as kaempferol and proanthocyanidins (Jenner *et al.*, 2005).

Coumarins

Coumarins are lactones of *o*-hydroxylated cinnamic acid precursors. There is only little information on the occurrence of coumarins in beer (Table 12.5).

High levels of coumarins have been described in several plant families including Poaceae. Consequently, umbelliferon (7-hydroxycoumarin), one of the most common coumarins, has been detected in malt by paper chromatography (Solomakhina *et al.*, 1978). Jandera *et al.* (2005) detected relatively high levels of esculin (**32**) and 4-hydroxycoumarin (**33**) in sweet and hopped wort as well as in Czech pilot beer samples (up to 23.2 mg/l of 4-hydroxycoumarin (**33**) in fresh beer), but not in commercial beer samples. Levels were about twice as high in fresh beer as in aged beer.

Flavan-3-ols (catechins)

Catechins (flavan-3-ols) are an important group of dietary antioxidants. One of the best sources for monomeric catechins is green and black tea. It was estimated that consumption of one cup of green tea provides about 100–200 mg of epigallocatechin gallate (Zaveri, 2006). Several epidemiological studies have shown beneficial effects of green tea in cancer, cardiovascular and neurological diseases (Zaveri, 2006).

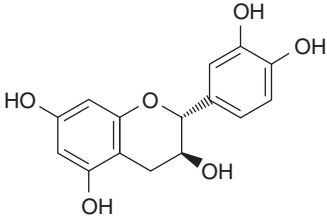
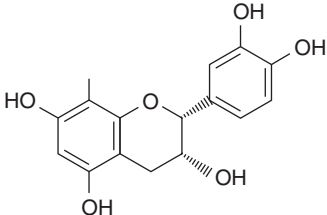
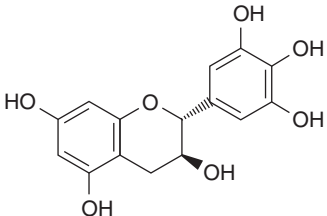
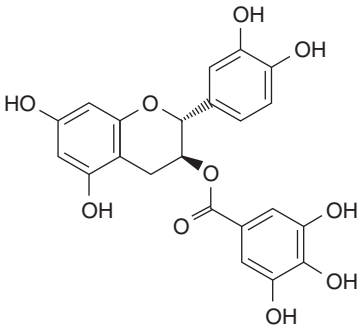
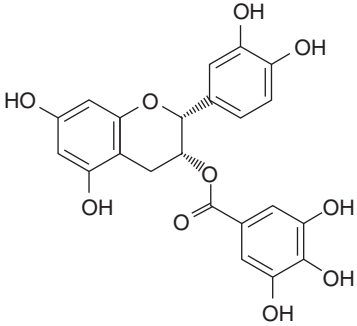
Beer also contains catechins derivatives, although in much lower quantities than tea (Table 12.6).

Catechin derivatives in beer include monomers like (+)-catechin (**34**) and (–)-epicatechin (**35**), but also their esters with gallic acid, that is catechin gallate (**37**) and epicatechin gallate (**38**). Beer catechins are derived both from barley/malt (catechin and gallo catechin (**36**)) as well as from hops (catechin and epicatechin). Fractionation of 300 l unstabilized German beer led to the isolation of 3'-*O*-methylcatechin (**39**), which had not been described in beer before (Alt, 2001). Further fractionation yielded a catechin glucoside (**40**) and its nicotinic ester (**41**) as a novel natural product (Alt, 2001; Gerhauser *et al.*, 2002b). Quantitative levels of these compounds were not determined. The catechin glucoside (**40**) has also been identified in barley and malt (Friedrich and Galensa, 2002).

Proanthocyanidins

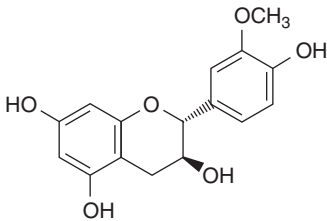
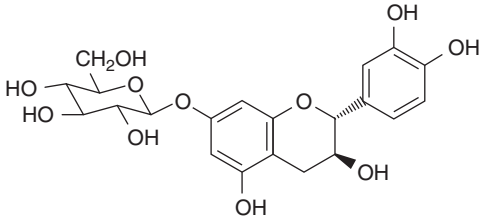
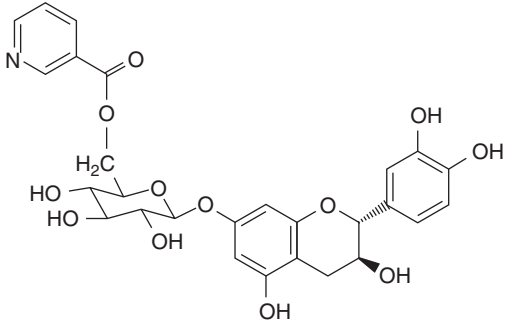
Proanthocyanidins are di-, tri- and oligomeric condensation products of flavan-3-ol monomers. Dimeric

Table 12.6 Summary of flavan-3-ols and their contents in beer

<i>Flavan-3-ols (catechins)</i>		<i>(mg/l)</i>
34	(+)-Catechin 	5.4 ^a ; 0.18 ± 0.42 ^b ; 0.46 ± 0.19 ^c ; 0.2–3.7 ^d
35	(-)-Epicatechin 	Up to 1.1; 0.76 ± 0.33 ^b ; 0.65 ^e
36	Gallocatechin 	n.d. ^f
37	Catechin gallate 	5–20 ^g
38	Epicatechin gallate 	5–20 ^g

(Continued)

Table 12.6 (Continued)

Flavan-3-ols (catechins)		(mg/l)
39	3'-O-methylcatechin	n.d. ^f
		
40	Catechin-7-O-β-d-glucopyranosid	n.d. ^f
		
41	Catechin-7-O-β-(6''-O-nicotinoyl)-β-d-glucopyranosid	n.d. ^f
		

^aIn one German beer (Achilli *et al.*, 1993).

^bMean ± standard deviation from 10 beers (Dutch, German, Czech, Belgian, French, Italian, Austrian) (Jandera *et al.*, 2005).

^cMean ± standard deviation from eight beers (Spanish, Danish, German) (Bartolomé *et al.*, 2000).

^dIn four Danish lager beers (Andersen *et al.*, 2000).

^eMean of Israeli beer samples (Gorinstein *et al.*, 2000).

^fn.d.: not determined, identified after isolation from unstabilized beer (Gerhauser *et al.*, 2002b).

^gSummarized in Alt (2001).

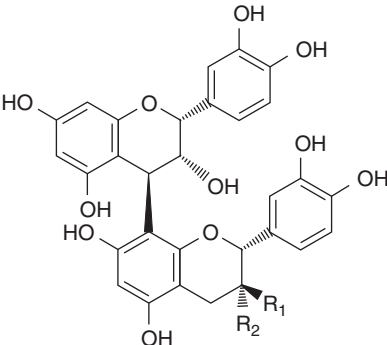
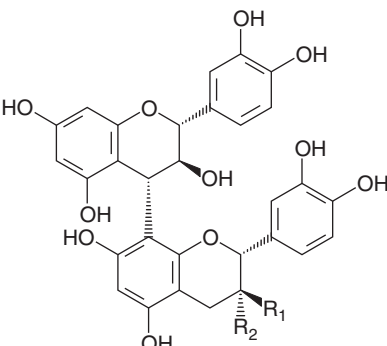




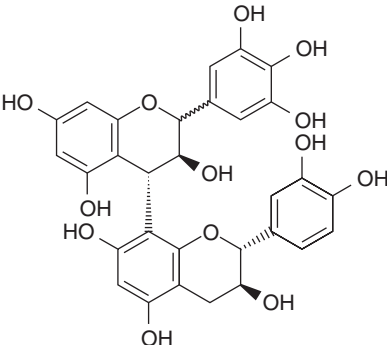
proanthocyanidins of the so-called B-type have one 4 → 6 or 4 → 8 interflavan linkage, whereas in A-type proanthocyanidins, the monomer subunits are further linked by an unusual second ether linkage. C-type proanthocyanidins are trimers composed of three flavan-3-ol subunits with 4 → 6 or 4 → 8 bonds (Santos-Buelga and Scalbert, 2000).

Early interest in proanthocyanidins during the brewing process was mainly related to their haze forming properties, which seem to increase with increasing molecular weight (McMurrough and Baert, 1994). Only recently, proanthocyanidins in foods and beverages are also of interest in nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health in reducing the risk of chronic diseases such as cardiovascular diseases and cancers (reviewed in Santos-Buelga and Scalbert, 2000). Most important dietary sources of procyanidins are chocolate, apples, blueberries and nuts, whereas their content in vegetables is generally low (Gu *et al.*, 2004;

Prior and Gu, 2005). Gu *et al.* (2004) determined the levels of proanthocyanidins in beer as 23 mg/l. These levels were about 13-fold lower than in red wine and 23-fold lower than in grape juice. The total amount of beer proanthocyanidins was divided into 4 mg/l monomers, 11 mg/l dimers, 3 mg/l trimers, 4 mg/l tetra- to hexamers, whereas higher mers were not detectable. In contrast, the proanthocyanidins of red wine consisted of about 28% mono- to trimers, 37% tetra- to decamers and 35% > 10 mers.

Proanthocyanidins are divided into various classes, depending on their monomer composition: procyanidins are composed of catechin and epicatechin monomers, whereas prodelphinidins additionally consist of gallo catechin and epigallo catechin subunits. It is generally accepted that prodelphinidins are more important for beer haze formation than procyanidins (McMurrough and Baert, 1994). In beer, both procyanidins and prodelphinidins have been detected (Table 12.7). It is suggested that B-type procyanidin dimers B1 (42),

Table 12.7 Summary of proanthocyanidins and their contents in beer

	Proanthocyanidins		(mg/l)
42	Procyanidin B1 (epicatechin-(4β→8)-catechin)	(42) R ₁ = OH, R ₂ = H (43) R ₁ = H, R ₂ = OH	n.d. ^a
43	Procyanidin B2 (epicatechin-(4β→8)-epicatechin)		n.d. ^a
44	Procyanidin B3 (catechin-(4α→8)-catechin)	(44) R ₁ = OH, R ₂ = H (45) R ₁ = H, R ₂ = OH	0–3.6 ^b ; 2.1 ^c ; 5/76.0 ^d
45	Procyanidin B4 (catechin-(4α→8)-epicatechin)		n.d. ^{a,c}
46	Prodelfinidin B3 (gallocatechin-(4α→8)-catechin)	(46)  =  (47)  = 	0–4.5 ^b ; 2.7 ^c
47	Prodelfinidin B9 (epigallocatechin-(4α→8)-catechin)		n.d. ^e

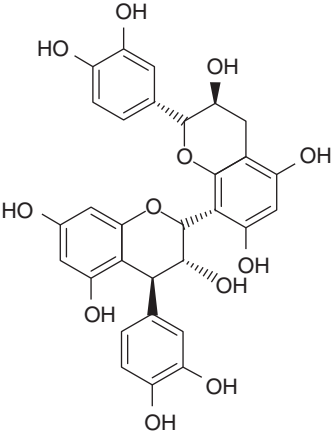
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Table 12.7 (Continued)

Proanthocyanidins		(mg/l)
48	Procyanidin C2 (catechin-(4 α →8)-catechin-(4 α →8)-catechin) (48) R ₁ = R ₂ = H (49) R ₁ = R ₂ = OH	n.d. ^{c,f}
49	Prodelfinidin C2 (gallocatechin-(4 α →8)-gallocatechin-(4 α →8)-catechin)	n.d. ^f
50	<i>ent</i> -Epigallocatechin-(4 α →8, 2 α →O→7)-catechin	n.d. ^g
51	<i>ent</i> -Epigallocatechin-(4 α →6, 2 α →O→7)-catechin	n.d. ^g

(Continued)

Table 12.7 (Continued)

	Proanthocyanidins	(mg/l)
52	2,3- <i>cis</i> -3,4- <i>trans</i> -2-[2,3- <i>trans</i> -3,3',4',5,7-Pentahydroxyflavan-8-yl]-4-(3,4-dihydroxyphenyl)-3,5,7-trihydroxybenzopyran	n.d. ^g
		
	Total proanthocyanidins	62.3 ^h ; 23 ⁱ

n.d: not determined.

^a Identified after isolation (Delcour, 1988).

^b In four Danish lager beers (Andersen *et al.*, 2000).

^c In Irish lager storage beer (McMurrough and Baert, 1994).

^d Free/bound at bottling in a French beer sample (Moll *et al.*, 1984).

^e Identified after isolation from Belgian pilsner beer (Delcour and Tuytens, 1984).

^f Summarized by Delcour and Vercruyse (1986).

^g Identified after isolation from unstabilized German beer (Gerhauser *et al.*, 2002b).

^h Mean of Israeli beer samples (Gorinstein *et al.*, 2000).

ⁱ Including 4 mg/l monomers (catechin, epicatechin) (Gu *et al.*, 2004).

B2 (43), B3 (44) and B4 (45) are mainly derived from hops, whereas prodelphinidin B3 (46), prodelphinidin B9 (47) and the trimer prodelphinidin C2 (49) have been isolated and structurally elucidated from barley and malt (Delcour and Tuytens, 1984). In a recent study by Gerhauser *et al.* (2002b), two novel A-type proanthocyanidins were isolated for the first time from beer: *ent*-epigallocatechin-(4 α → 8, 2 α → O → 7)-catechin (50) and *ent*-epigallocatechin-(4 α → 6, 2 α → O → 7)-catechin (51). Compound (52) also belongs to the class of proanthocyanidins, but is probably formed during the brewing process (Alt, 2001; Gerhauser *et al.*, 2002b).

Chalcones

Chalcones are biosynthetic precursors of flavonoids. Prenylated flavonoids with one or more isoprene units attached to the flavonoid core structure are present only in a limited number of plant families, of which about 80% belong to the Moraceae–Cannabinaceae (with *Humulus lupulus*), Leguminosae and Asteraceae (Stevens *et al.*, 1999). Beer is the major dietary source of prenylated flavonoids (Yilmazer *et al.*, 2001).

In spite of its relatively low levels in commercial beers (Table 12.8), the prenylated chalcone xanthohumol (53) has gained considerable interest because of its potential

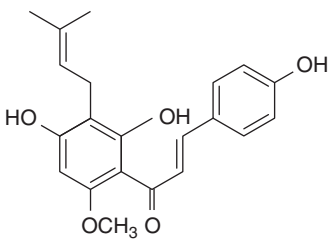
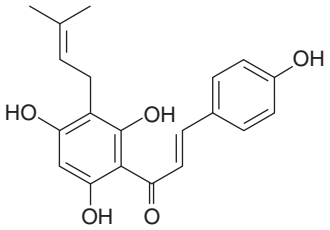
cancer chemopreventive properties (Gerhauser *et al.*, 2002a; reviewed in Gerhauser, 2005). Levels up to 1.2 mg/l have been reported in stout and porter-style beers (Walker *et al.*, 2004). Recently, attempts have been made to increase the amount of xanthohumol in beer by modifications in the brewing process. Generally, xanthohumol is isomerized to the flavanone isoxanthohumol (55, see section “Flavanones”) during wort boiling. Wunderlich *et al.* (2005) used xanthohumol-enriched hops products combined with a late hops dosage during wort boiling to effectively increase the xanthohumol content in beer. Interestingly, xanthohumol was found to form stable complexes with several high molecular weight substances in a size range of 300–600 kDa. In an experimental setup, dark beers with more than 10 mg/l xanthohumol were achieved by usage of roasted malt or special xanthohumol-enriched roasted malt extracts (Wunderlich *et al.*, 2005).

Flavanones

In beer, four prenylflavanones – isoxanthohumol (55), 6- and 8-prenylnaringenin (58, 57) and 6-geranylnaringenin (59) were identified (Table 12.9).

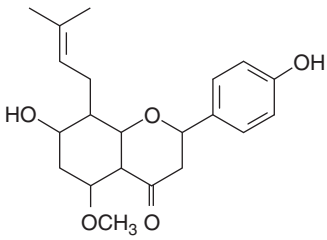
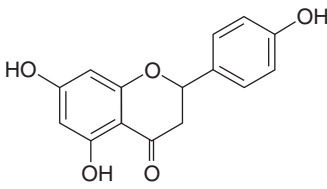
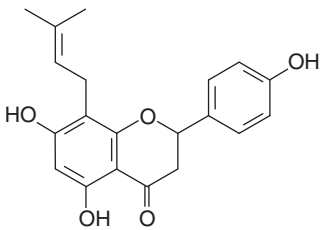
The most abundant flavonoid in hopped beers is isoxanthohumol (55), ranging from 0.04 to 3.44 mg/l (Stevens

Table 12.8 Summary of chalcones and their contents in beer

<i>Chalcones</i>		<i>(mg/l)</i>
53	Xanthohumol 	0.14 ± 0.21 (range: 0.002–1.2) ^a
54	Desmethylxanthohumol 	Traces only ^a

^aMean ± standard deviation from 11 beers (United States and imported); highest levels were found in stout and porter-style beers (Stevens *et al.*, 1999; Walker *et al.*, 2004).

Table 12.9 Summary of flavanone derivatives and their contents in beer

<i>Flavanones</i>		<i>(mg/l)</i>
55	Isoxanthohumol 	1.02 ± 0.94 (0.04–3.44) ^a
56	Naringenin 	Traces ^b
57	8-Prenylnaringenin 	0.05 ± 0.07 (0.001–0.24) ^a ; 0.005 ± 0.006 ^c

(Continued)

Table 12.9 (Continued)

Flavanones		(mg/l)
58	6-Prenylnaringenin	0.12 ± 0.16 (0.001–0.56) ^a
59	6-Geranyl naringenin	0.01 ± 0.02 (0–0.074) ^a
60	5-Methyl-6''-dimethyl-4'',5''-dihydropyrano[2'',3'',7,8]naringenin	Traces ^b
61	Taxifolin (flavanonol)	Up to 1.0 ^d

^aMean ± standard deviation from 11 beers (United States and imported); highest levels are found in stout and porter-style beers (Stevens *et al.*, 1999; Walker *et al.*, 2004).

^bIdentified after isolation from unstabilized beer (Gerhauser *et al.*, 2002b).

^cMean ± standard deviation from 32 beers (Belgian and imported) (Tekel *et al.*, 1999).

^dAlt (2001).

et al., 1999). As mentioned above, isoxanthohumol is formed by isomerization of xanthohumol during the brewing process. Although present only in very small quantities in beer, 8-prenyl naringenin (57) has attracted substantial attention because of its potent estrogenic activity. 8-Prenyl naringenin (57) has been identified as one of the most potent phytoestrogens, based on several *in vitro* and *in vivo* animal investigations (summarized in Gerhauser, 2005). The effect of 8-prenyl naringenin was described as almost pure estrogen agonistic activity with a relative binding affinity of about

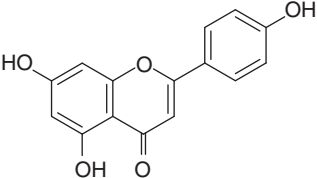
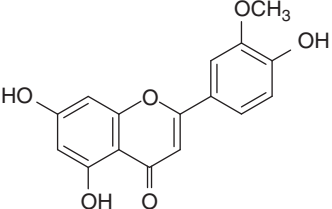
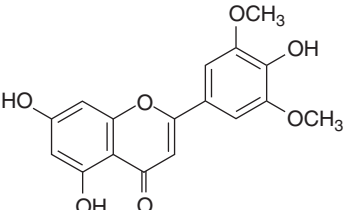
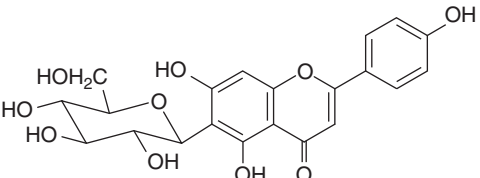
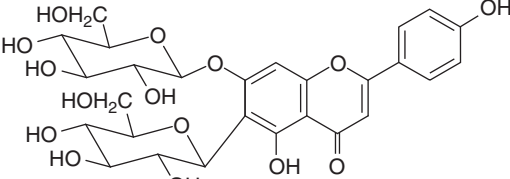
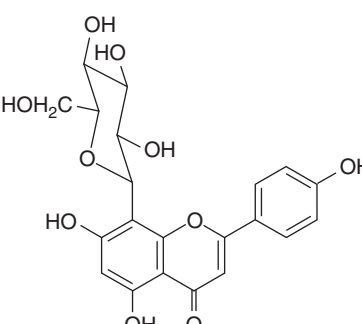
10% of that of the hormone 17β-estradiol, which is binding to its receptors in extremely low concentrations.

Flavones

Table 12.10 summarizes a number of flavones that were isolated from unstabilized beer (Alt, 2001; Gerhauser *et al.*, 2002b).

Apigenin (62), chrysoeriol (63) and tricetin (64) are aglycones, whereas compounds (65)–(67) are apigenin derivatives

Table 12.10 Summary of flavones and their contents in beer

	Flavones	(mg/l)
62	Apigenin	n.d.*
		
63	Chrysoeriol	n.d.*
		
64	Tricin	n.d.*
		
65	Apigenin-6-C-glucoside (saponaretin)	n.d.*
		
66	Apigenin-6-C-glucoside-7-O-glucoside (saponarin)	n.d.*
		
67	Apigenin-8-C-glucoside (vitexin)	n.d.*
		

*n.d.: not determined, identified after isolation from unstabilized beer (Alt, 2001; Gerhauser *et al.*, 2002b).

belonging to the C-glucosylflavone group, with a glucoside moiety linked via a C—C bond to the flavone structure. Most likely, these flavone derivatives in beer are derived from barley, since C-glycosides are typically found in the leaves of cereals including barley (Herrmann, 1970). Also, Seikel and Bushnell (1959) reported that saponarin (**66**) was the principal flavonoid identified in barley leaves. Hydrolysis of the 7-O-glucosidic bond of saponarin (**66**) yielded an equilibrium mixture of saponaretin (**65**) and vitexin (**67**). Chrysoeriol (**63**) was isolated from barley grains by Bhatia *et al.* (1972). Tricin (**64**) occurs at high concentrations in the leaves of grass species and has been isolated from rice bran (Harborne and Williams, 1976).

Flavonoles

In contrast to the occurrence of flavones, flavonoles are less likely to occur in cereals (Jende-Strid, 1993). Flavonoles like quercetin (**70**) and kaempferol (**68**) and their respective glycosides are typical constituents of fruits (with the exception of citrus fruits) and vegetables and have also been identified in hops (Hermann, 1970).

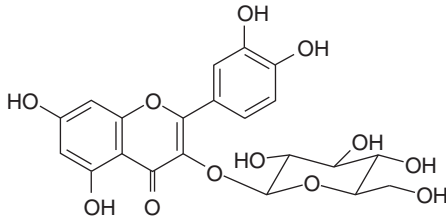
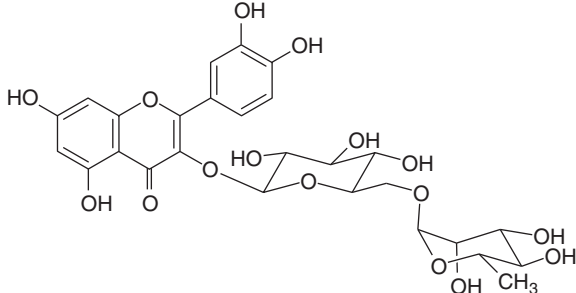
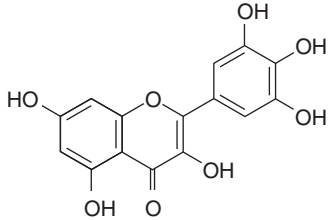
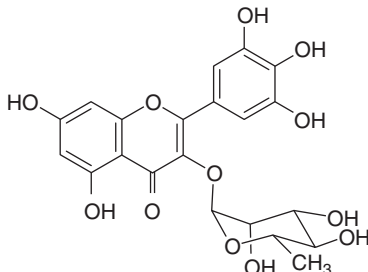
Flavonole derivatives have not been extensively determined in beer. They were mostly detected as O-glycosides, and their concentrations are comparatively low (Table 12.11). Relatively high amounts of kaempferol (**68**) were measured in one German beer (Achilli *et al.*, 1993).

Table 12.11 Summary of flavonols and their contents in beer

Flavonols		(mg/l)
68	Kaempferol	16.4 ^a
69	Afzelin (kaempferol-3-O-rhamnoside)	Up to 1.0 ^b
70	Quercetin	0.95 ^c , up to 10 ^b
71	Quercitrin (quercetin-3-O-rhamnoside)	Up to 2.3 ^b

(Continued)

Table 12.11 (Continued)

Flavonols		(mg/l)
72	Isoquercitrin (quercetin-3-O-glucoside)	Up to 1.0 ^b
		
73	Rutin (quercetin-3-O-rutinoside)	1.8 ^a ; 0.58 ± 0.41 ^d
		
74	Myricetin	n.d.
		
75	Myricitrin (myricetin-3-O-rhamnoside)	n.d.
		

n.d.: not determined.

^aIn one German beer (Achilli *et al.*, 1993).

^bSummarized in Alt (2001).

^cMean of Israeli beer samples (Gorinstein *et al.*, 2000).

^dMean ± standard deviation from 10 beers (Dutch, German, Czech, Belgian, French, Italian, Austrian) (Jandera *et al.*, 2005).

Miscellaneous compounds (indole-based compounds, lignans)

Although not of phenolic nature, we have added three indole-based compounds, which have been isolated and identified from beer. Tryptophol (77) is the only one of the indole-based compounds that has been quantified in beer so far. Bartolomé *et al.* (2000) compared tryptophol

concentrations in alcohol-free and standard beers and found slightly lower levels in the alcohol-free beers (0.24 ± 0.20 mg/l) than in the standard beers (Table 12.12). It was suggested that the presence of this substance in beer is related to the action of yeasts on wort amino acids during the brewing process. Lately, the conversion of tryptophan to tryptophol (77) by *Saccharomyces cerevisiae* has

Table 12.12 Miscellaneous phenolic beer constituents

	Miscellaneous compounds	(mg/l)
76	Indole-3-carboxylic acid	n.d. ^a
77	Indole-3-ethanol (tryptophol)	0.37 ± 0.4 ^b
78	1-Methyl-1,3,4,9-tetrahydropyrano[3,4b]indole	n.d. ^a
79	4-Ketopinoresinol (lignan)	n.d. ^a
80	Syringaresinol (lignan)	n.d. ^a

^an.d.: not determined; identified after isolation from unstabilized beer (Gerhauser *et al.*, 2002b).

^bMean ± standard deviation from eight beers (Spanish, Danish, German) (Bartolomé *et al.*, 2000).

been investigated by Dickinson *et al.* (2003) and involves a deamination and a decarboxylation step.

Indole-3-carboxylic acid (76) is a biotransformation product of the indole alkaloid gramine (Figure 12.2), which is found in barley and has defence-related functions (Digenis, 1969). Compound (78) was described for the first time from a natural source and is probably formed non-enzymatically during beer brewing (Alt, 2001).

The lignans 4-ketopinoresinol (79) and syringaresinol (80) were isolated from unstabilized beer (Alt, 2001). So far, the occurrence of lignans in beer has not been described.

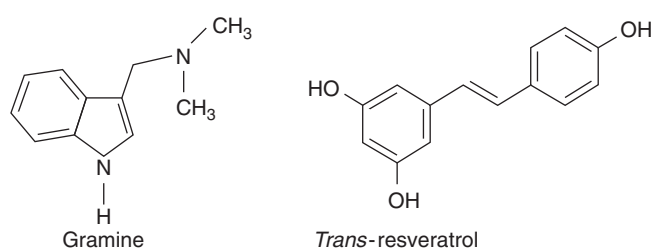


Figure 12.2 Chemical structures of the indole alkaloid gramine from barley and the stilbene *trans*-resveratrol found in hops cones.

Although information on lignans from barley is limited, we speculate that malt is the primary source for lignans in beer. Generally, flaxseeds are recognized as the best dietary source of lignans, but recently cereal bran, especially rye or wheat bran, has also been described to contain considerable amounts of lignans (Begum *et al.*, 2004). Dietary lignans are of interest, since they are converted by the fecal microflora to so-called mammalian lignans enterolactone (ENL) and enterodiol which possess phytoestrogenic properties.

Two recent reports suggest the presence of stilbenes like resveratrol (Figure 12.2) in hops. Hops with lower α -acid content were shown to contain higher total amounts of stilbenes (Callemien *et al.*, 2005; Jerkovic *et al.*, 2005).

Since stilbenes also act as phytoestrogens, albeit weaker than 8-prenylnaringenin (57), the detection of these two groups of plant compounds – lignans and stilbenes – in beer and beer constituents, respectively, might partly contribute to our understanding of the proposed estrogenic properties sometimes ascribed to beer (Koch and Heim, 1953; Milligan *et al.*, 1999).

Summary Points

- Beer is a very complex beverage and contains various classes of polyphenolic compounds from simple phenols to complex oligomeric proanthocyanidins.
- The composition and levels of phenolic compounds in beer vary strongly in dependence of the raw materials utilized, the brewing process and type of beer, and also change during storage. Alcohol-free beer usually has lower levels of phenolic constituents due to losses during the dealcoholization process.
- Reported values for constituents in beer are relatively low, with some exceptions. However, it should be noted that amounts of polyphenols measured in beer often only account for the free compound, although it has been shown that most of the compounds occur in beer in bound form. The reported values probably underestimate actual levels.
- Polyphenols that occur in beer in relatively high concentrations are: the phenol tyrosol, the benzoic acid derivative *p*-hydroxybenzoic acid, the cinnamic acids *p*-coumaric and ferulic acid, (+)-catechin and (–)-epicatechin, the proanthocyanidin dimers procyanidin B3 and prodelphinidin B3 and the flavanone isoxanthohumol.
- With the exception of prenylated flavonoids, beer constituents are also found in fruits, vegetables, cereals and other beverages, often in considerably higher amounts.
- Due to the high complexity and variability in the polyphenolic pattern, biological activities attributed to beer consumption cannot be linked to one particular class of constituents.

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