Reviews

Hop (*Humulus lupulus*)-Derived Bitter Acids as Multipotent Bioactive Compounds

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Hop acids, a family of bitter compounds derived from the hop plant (*Humulus lupulus*), have been reported to exert a wide range of effects, both in vitro and in vivo. They exhibit potential anticancer activity by inhibiting cell proliferation and angiogenesis, by inducing apoptosis, and by increasing the expression of cytochrome P450 detoxification enzymes. Furthermore, hop bitter acids are effective against inflammatory and metabolic disorders, which makes them challenging candidates for the treatment of diabetes mellitus, cardiovascular diseases, and metabolic syndrome. This review summarizes the current knowledge on hop bitter acids, including both phytochemical aspects, as well as the biological and pharmacological properties of these compounds.

Introduction

The cultivation of the hop plant (*Humulus lupulus* L.) has a long history. By the first century B.C., the great Roman naturalist Plinius described the plant as "the wolf of the willow" ("lupus salictarius"): wild hops grew around willows and strangled them, which could be compared to the wolf's behavior toward sheep.[1] In the eighth century A.D., hop gardens commonly surrounded monasteries, and the inflorescences were used for medicinal purposes.[2] During the Middle Ages, brewers discovered the advantage of adding hops to the brewing kettle as a natural antiseptic and flavoring agent.[3] Ever since, the hop plant has been an essential ingredient in beer brewing, and about 95% of worldwide cultivated hops is destined for brewing purposes,[4] with the remainder used largely for the production of phytomedicines and botanical dietary supplements. Young shoots are eaten in the spring as a culinary delicacy, particularly in Belgium.[2]

From a taxonomic point of view, the genus *Humulus* belongs to the family Cannabaceae of the order Urticales, but in 2003 it was incorporated in the order Rosales.[5] This genus includes the species *Humulus japonicus* Siebold and Zucc., *Humulus yunnanensis* Hu, and *Humulus lupulus* L., of which the latter is almost exclusively cultivated for brewing purposes.[6] Successful cultivation of hops requires optimal growth conditions, especially with respect to the length of day light, the summer temperature, the amount of rain, and the fertility of the soil. Therefore, hops are found in the moderate climatic zones of the Northern and Southern hemispheres, with Germany and the United States by far the largest producing countries.[4,7–9] The plant is a perennial, dioecious herb, of which the shoots start to grow during spring as bines, having stout stems with stiff hairs to aid in climbing by wrapping clockwise, from 6 m up to 18 m high. During the summer, the inflorescences of the female plants form hop cones (strobiles, hops), which secrete a fine yellow resinous powder (lupulin) in their lupulin glands.[8,10,11] Harvest occurs at the end of the summer or the beginning of autumn, when the content of lupulin is highest. Hops are collected, carefully dried to obtain a residual moisture content of less than 10%, and preferably stored in a cold room (4 °C) to minimize compositional changes. Only female plants are cultivated for brewing; moreover, in many countries it is forbidden by law to cultivate male plants in the vicinity of females. Thus, seed formation is avoided, which is believed to influence negatively hop and beer quality.[8,12,13] The bitter and aroma components in the hop cones are very sensitive to oxidation. For this reason, the hop plant is frequently processed into more stable products, such as nonisomerized aerial supercritical or liquid carbon dioxide extracts, distilled hop oil fractions, and potassium solutions of preisomerized hop acids that can be directly added to the brewing kettle (nonisomerized products) or postfermentation (hop oils, preisomerized products).[14–16] Hop extracts and hop oil fractions are also used as flavoring products in nonalcoholic beverages and foods.[10]

Since ancient times, hops have been used in folkloric medicine for their claimed anti-inflammatory, antiseptic, antidiuretic, (an)aphrodisiac, hypnotic, sedative, and stomachic properties.[2,11,17–19] Indian tribes drank hop tea to alleviate nervousness and heated a small bag of leaves to apply in cases of earache or toothache.[20] King George III slept on a pillow stuffed with hop cones to alleviate symptoms of porphyria.[2,4,21] The German Commission E approved a monograph on hops for use in mood and sleep disturbances. Similar indications are described in an ESCOP (European Scientific Cooperative on Phytotherapy) monograph.[4,10,22,23] Today, a wide range of over-the-counter preparations containing hop extracts or hop-derived products is available on the market, in particular for use in the phytotherapy of sleep disorders or pain relief and in postmenopausal therapy.[7,17,24–26]

Recently, investigators have been trying to identify the bioactive ingredients in hops and to elucidate the underlying molecular mechanisms by which they exert their activities. Much of attention has gone to the polyphenolic content of hops, and specific compounds, such as xanthohumol and 8-prenylnaringenin, have been identified as multipotent bioactive compounds (for detailed reviews, see Stevens and Page,[27] Gerhauser,[28] and Chadwick et al.[29]). Moreover, increasing evidence reveals that the so-called hop bitter acids, which represent up to 30% of the total lupulin content of hops, exhibit interesting effects on human health. In the present review, we will focus on this group of hop secondary metabolites and will start from a phytochemical characterization of the main hop acids (including purification, quantification, use in brewing, and degradation). A detailed overview will be provided of the

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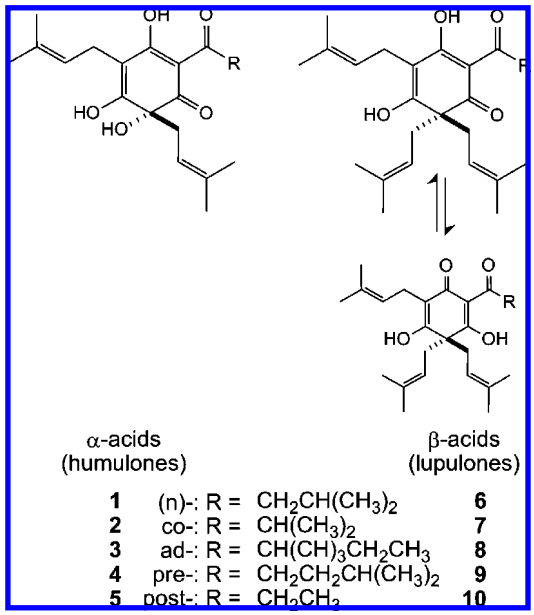
Table 1. Average Composition of Air-Dried Hops

compound(s)	percentage (m/m)
α-acids	2−17
β-acids	2−10
amino acids	0.1
ash/salts	10
cellulose-lignin	40−50
monosaccharides	2
oils and fatty acids	1−5
pectins	2
polyphenols and tannins	3−6
proteins	15
volatile oil	0.5−3 (v/m)
water	8−12

From: Benitez et al., 1997.[8]

Chart 1. Structures of Hop α-Acids and β-Acids



current evidence for the bioactivities and pharmacological properties of hop bitter acids, such as their potential to combat cancer and inflammation, effects related to metabolic syndrome, modulation of CNS activity, and bactericidal properties. Finally, the toxicological profile of the hop plant will be described comprehensively.

Hop Bitter Acids

Female hop cones contain glandular structures (lupulin glands) in which they secrete lupulin powder, rich in secondary metabolites. These metabolites can be classified as (resinous) bitter acids, volatile oil, and polyphenols, respectively (Table 1).[11] The volatile oil comprises mainly terpenoids, such as β-myrcene (30−50%), humulene (15−25%), β-caryophyllene, and farnesene, which together may account for more than 90% of the total hop oil. Lupulin-associated polyphenols include a number of prenylated chalcones, such as xanthohumol and desmethylxanthohumol, which are precursors of the isomeric flavanones isoxanthohumol and 8-prenylnaringenin, respectively. Furthermore, hop leaves contain a wide range of phenolic acids, condensed tannins, and flavonoid glycosides.[9,12,15] Also, leaves of fully grown hops contain low but detectable levels of hop acids and even prenylated chalcones, because the leaves possess a small number of lupulin glands.[30]

Hop bitter acids consist of two related series, the α-acids (or humulones) and β-acids (or lupulones), which are both characterized as prenylated phloroglucinol derivatives (Chart 1). These compounds occur as pale yellowish oils or resins that are soft and easily soluble in hydrocarbon solvents, such as hexane. Depending on the nature of the acyl side chain, five analogues can be identified: isovaleroyl in *n*-, isobutyroyl in co-, 2-methylbutyroyl in ad-, isohexanoyl in pre-, and propanoyl in postbitter acids. The relative amounts of α-acids and β-acids, as well as the concentrations of individual representatives, depend strongly on the hop variety and the conditions of growing.

Hop bitter acids are very sensitive to oxidation, thus forming a mixture of ill-defined products that are soluble in diethyl ether, but no longer in hexane (hard resins). Deterioration of hops as a function of time is accompanied by development of a strong odor that is generally not welcomed by brewers.[12] To prevent this, hops are rapidly dried after harvesting, pelleted, and stored in airtight bags, preferably at low temperatures. Today, next to the use of pellets, many beers are rendered bitter-tasting with hop extracts. The advantages of doing this are as follows: increased utilization of brewing principles, increased stability, improved uniformity, and easier handling.[14]

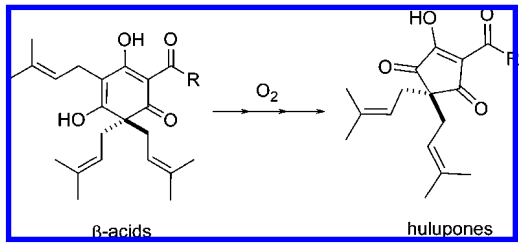
α-Acids. The α-acids are the most important constituents of hops. The acidic, salt-forming, and chelating properties of α-acids reside in their β-triketo system. The absolute structure of humulone (**1**) was established by De Keukeleire and Verzele in 1969, using a combination of chemical, spectrometric, and chiroptical techniques.[31] It appears that only the R configuration at C-6 within a fully enolized β-triketo system is formed stereoselectively in Nature (Chart 1).[9] Determination of the composition of the α-acids mixture is important, as high levels of cohumulone are generally associated with low hop quality (although there is much controversy around this topic). In general, the analogues *n*-humulone (**1**), cohumulone (**2**), and adhumulone (**3**) are the main constituents of the hop α-acids, representing 35−70%, 20−65%, and 10−15%, of the total levels, respectively. Pre- (**4**) and posthumulones (**5**) represent only a minor part of the α-acids. The relative amounts of **1** and **2** are dependent on hop variety, while the amount of **3** is fairly constant.[8] For research purposes, α-acids can be precipitated from a hop acid mixture (e.g., a liquid carbon dioxide extract) by adding lead(II) acetate, thus forming yellow-colored lead salts that can be stored for years without deterioration.[9] The exact structure of the salts is not known, but the tertiary alcohol function must be involved, as the β-acids cannot form such salts. Compound **1** can be isolated from the mixture of α-acids by complexation with 1,2-phenylenediamine followed by repeated crystallization.[32] Isolation of **2** and **3** mostly occurs via preparative HPLC.

Hop α-acids as such occur in beer in concentrations up to 4 mg/L. They improve foam stability, suppress gushing, and contribute to the preservation of beer. However, their main contribution to beer is via isomerization during the boiling of wort with hops, thereby forming the extremely bitter iso-α-acids (see below).[33]

***β*-Acids.** β-Acids are less acidic than α-acids because the tertiary alcohol function at C-6 is replaced by an extra prenyl side chain. In contrast to α-acids, for each β-acid, two different enolization patterns prevail (Chart 1). However, β-acids are mostly represented in the predominant conjugated dienolic form, which corresponds to that of the α-acids. Most hop varieties contain approximately equal levels of lupulone (**6**) and colupulone (**7**) (ca. 20−55%), next to less variable levels of adlupulone (**8**) (10−15%). Prelupulone (**9**) and postlupulone (**10**) are only present in trace quantities. β-Acids are extremely sensitive to oxidation, which is initiated by air (auto-oxidation), thus giving rise to a number of oxidized compounds and derivatives. A particular oxidative reaction leads to formation of the highly stable hulupones (Scheme 1). In contrast to β-acids that are not bitter, these compounds have a very bitter taste and can be present in beer in quantities of a few mg/L. However, β-acids as well as hulupones are of minor importance to the beer quality.[9]

Iso-α-acids and Derivatives. α-Acids are isomerized during the brewing process to the more water-soluble iso-α-acids, thereby yielding concentrations ranging from 10 up to 100 mg/L in beers. Each iso-α-acid analogue occurs as an epimeric mixture of *cis*-

Scheme 1. Oxidation of Hop β-Acids to Hulupones



and *trans*-isomers, where the stereochemical notation refers to the relative orientation of the hydroxyl at C-4 and the prenyl group at C-5 (Scheme 2A). The ratio of *cis*:*trans* depends on the reaction conditions and is typically 68:32 under normal brewing conditions. The *cis*-isomer is the more stable epimer in view of the least steric hindrance between the two large vicinal side chains.[33]

Iso-α-acids can be formed from α-acids under a variety of conditions. During the brewing process, α-acids are isomerized by boiling hops or hop extracts in the aqueous wort medium at a pH of 5.0−5.5 (Scheme 2A). In practice, a final α-acid utilization yield of 25−35% is reached in the beer. The large losses are probably due to adsorption of (iso-)α-acids on solid material or yeast cells and by further oxidative transformations.[16] In laboratory conditions, the reaction can be favored by boiling α-acids in alkaline media in the presence of divalent cations as catalysts or, alternatively, by irradiation of a methanolic solution of α-acids with UV light. This photoisomerization proceeds in fully regio- and stereoselective ways and forms exclusively *trans*-isomers.[34] Interconversion of *cis* into *trans* and vice versa, as well as conversion of iso-α-acids into their parent compounds is feasible.[9]

Iso-α-acids represent well above 80% of all hop-derived components in beer. They account for the typical bitter taste of beer and possess tensioactive properties, thereby stabilizing the beer foam, and protect beer against micro-organisms.[35] On the other hand, iso-α-acids are key ingredients in the formation of the so-called "lightstruck" flavor of beer. When exposed to light, they decompose via a series of excited states and radical-type intermediates to so-called "skunky" thiols, mainly 3-methylbut-2-ene-1-thiol (**14**) (next to dehydrohumulinic acid (**13**)) (Scheme 2B).[36,37] The reaction is initiated by riboflavin (vitamin B2) as photosensitizer, since iso-α-acids do not absorb light in the visible region. Enhanced resistance to the "lightstruck" flavor is provided by reduced derivatives of iso-α-acids. Three major types can be considered depending on the number of hydrogen atoms (dihydro, tetrahydro, hexahydro) incorporated during reduction (Scheme 3).[15] Dihydro-iso-α-acids (or rho-isohumulones)[38] are light stable, thus allowing brewers to bottle beers in clear bottles, while tetrahydro-iso-α-acids[39] and hexahydro-iso-α-acids have the extra advantage of stabilizing the beer foam.[16,33] Iso-α-acids and reduced derivates are commercially available as aqueous solutions of their corresponding potassium salts. They can be applied post-wort boiling, e.g., during lagering or even just prior to bottling, since isomerization is no longer required.[14]

Purification and Analysis of Hop Acids. Hop acids can be separated from the vegetative material by extraction with solvents of different polarity. Extraction with supercritical or liquid carbon dioxide yields, respectively, a dark green or a yellow-golden paste containing high levels of α-acids and β-acids without the more polar tannins, hard resins, and salts.[16] This type of extract serves as a suitable material for further manipulations. α-Acids can be purified by precipitation of their lead(II) salts (as described above) or by liquid−liquid extraction with aqueous carbonate, whereas β-acids need a more alkaline environment to be extracted (NaOH).[9] Iso-α-acids and reduced derivatives are commonly purified from their commercially available potassium salt solutions. Recently, efficient separation of *trans*- and *cis*-isomers by complexation with β-cyclodextrin has been described. Using a molar ratio of β-cyclodextrin to iso-α-acids from 1:1 to 1:4 results in an enriched extract of *cis*- or *trans*-isomers, respectively.[40]

Quantitative and qualitative analyses of hop acids are currently carried out by HPLC, rather than using methods based on countercurrent distribution, thin-layer chromatography, or ion-exchange chromatography. Individual hop bitter acids are obtained from extracts by (semi)preparative HPLC[41,42] coupled to UV detection,[41,43,44] mass spectrometry,[43] ^{1}H NMR spectroscopy,[45−47] or a combination of techniques. International standard extracts for α-acids, β-acids, iso-α-acids, and reduced derivatives are available for calibration purposes.[48,49]

A number of variables determine the final composition of hop bitter acid preparations. There are substantial variations in the starting material, depending on hop variety, harvest time, growing conditions, processing, and storage. Furthermore, hop bitter acids are very sensitive to oxidation and degradation. For research purposes, it is therefore essential that purification and storage of these compounds is well controlled and the relative composition of extracts must be frequently monitored. However, it is our opinion that researchers tend to underestimate this problem, and, therefore, results from different studies with the "same" compounds are frequently hard to compare. Often, the exact composition of the hop extracts is not known or not further specified. In other cases, commercially available extracts are used, with the same shortcomings. Overall, the standardization of hop extracts is to be encouraged, or individual compounds should be used.

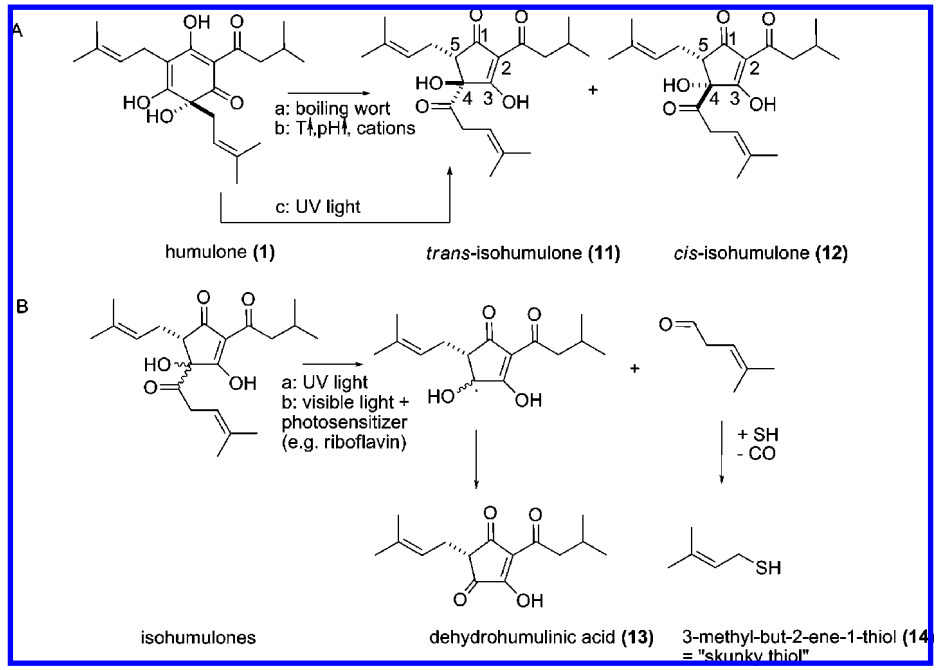
Further information on the chemistry, preparation, purification, and analysis of hop bitter acids can be found in reviews by Verzele and De Keukeleire,[9] and Ting and Goldstein.[50]

Anticancer Potential of Hop Bitter Acids

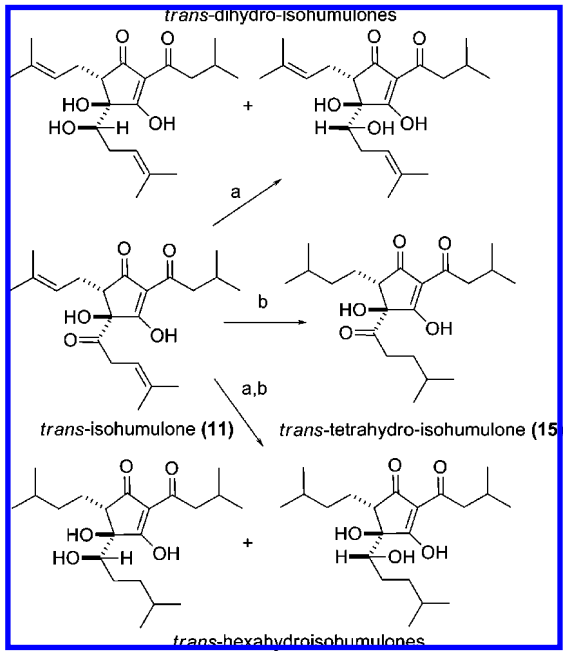
Several natural compounds, including hop bitter acids, have been identified as promising molecules for the use in cancer chemotherapy or cancer chemoprevention. Plant-derived substances may lower the risk of developing cancer by preventing metabolic activation of procarcinogens, or alternatively, they can inhibit cancer development by arresting or reversing the processes of tumor initiation, promotion, and progression.[51]

In Vitro Activity. Induction of Apoptosis. Hop bitter acids target cancer via the induction of controlled cell death (or apoptosis) in fast-growing tumor cells. The first report dates from 1997, when Tobe and co-workers reported the apoptosis-inducing properties of humulone (**1**) in promyeloid leukemia HL-60 cells. Compound **1** (1−100 μg/mL) induced DNA fragmentation into (oligo)nucleosomal units, a characteristic for apoptosis, in a time- and dose-dependent manner. Interestingly, treatment with iso-α-acids (100 μg/mL) did not induce DNA breakdown, not even after overnight incubation.[52] In later studies, scientists attempted to unravel the molecular targets of hop bitter acid-initiated apoptosis. Chen and Lin used a hop extract, consisting of 49.39% α-acids and 24.94% β-acids, which dose-dependently induced apoptosis in human leukemia HL-60 cells (IC_{50} 8.67 μg/mL) and, albeit to a much lesser extent, in human histolytic lymphoma U937 cells (IC_{50} 58.87 μg/mL).[53] Hop bitter acids activated the intrinsic mitochondrial apoptotic pathway: they disrupted the mitochondrial membrane potential and enhanced membrane permeability by altering the expression of the Bcl-2 family of proteins, consisting among others of the antiapoptotic Bcl-2 and the pro-apoptotic Bax. This finally resulted in activation of a cascade of caspases, which function as cysteine proteases, thereby causing proteolytic breakdown of structural cell proteins. Furthermore, hop bitter acids stimulated the extrinsic pathway, which involved increased expression of the death receptor Fas and its ligand, FasL.[53] In line with these results, lupulones (40 μg/mL) were reported to upregulate Fas and FasL expression in a human metastatic colon carcinoma-derived cell line (SW620 cells). Again, mitochrondrial membrane permeability was augmented in association with an altered expression of Bcl-2 and

Scheme 2. (A) Isomerization of Humulone to Isohumulones; (B) Formation of the "Lightstruck Flavor" in Beer



Scheme 3. Reduced Isohumulones (starting from *trans*-isohumulone as an example): (a) Sodium Borohydride; (b) Hydrogen/Palladium



Bax proteins.[54] A crucial role was determined for the TNF (tumor necrosis factor)-related apoptosis-inducing ligand (TRAIL)-R1 and -R2 receptors, which were upregulated and activated by lupulones (40 μg/mL) in both TRAIL-sensitive (SW480) and TRAIL-resistant colon cancer cells (SW620).[55]

Antiproliferative Activity of Hop Bitter Acids. Hop bitter acids show antiproliferative activity, thus arresting cell growth of invasive cancer cells.[54,56] Lupulone (**6**), colupulone (**7**), and a semisynthetic derivate, hexahydrocolupulone, inhibited cell growth of several human cell lines: hexahydrocolupulone was the most potent variant with a wide spectrum of activity against solid tumors and leukemias, as well as against drug-resistant cell lines (IC_{50} values in the range of 0.85 and 2.19 μM). It caused cell cycle arrest (G0/G1 phases) and affected the incorporation of precursors into their macromolecules, resulting in disrupted DNA, RNA, and protein synthesis.[56] β-Acids inhibited the cell growth of SW620 colon cancer cells (10−60 μg/mL) in a time- and dose-dependent manner.[54] Humulone (**1**) inhibited the proliferation of human leukemia U937 cells (IC_{50} 3.4 μM) and slightly induced their differentiation, as concluded from higher nitroblue tetrazolium reducing and lysozyme activities, both typical differentiation markers. Agents that inhibit proliferation and enhance the conversion of premalignant cells to differentiated cells are expected to reduce cancer development. Compound **1** enhanced the differentiation of U937 monocytes induced by vitamin D_3, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), all-*trans* retinoic acid, and tumor necrosis factor alpha (TNF-α). These effects were similar in other myelogenous leukemia cells, such as K562, HEL, KU812 erythroleukemia cells, promyelocytic leukemia HL-60 cells, monoblastic THP-1 cells, and myeloblastic ML-1 leukemia cells.[57]

Inhibition of Angiogenesis. The formation of new capillary blood vessels for the supply of oxygen and nutrients, also named angiogenesis, plays a key role in the development of malignant tumors. Shimamura and co-workers reported that humulone (**1**) dose-dependently prevented angiogenesis in chick embryo chorioallantoic membranes (CAMs), with an ED_{50} (the "effective dose" at which 50% of angiogenesis is inhibited) of 1.5 μg/CAM. Compound **1** (10 μM) inhibited tube formation by vascular lung endothelial cells from rats (RLEs) and reduced cell growth of endothelial mouse KOP2.16 cells, stimulated by basic fibroblast growth factor (bFGF), by 20%. Furthermore, 100 μM **1** suppressed the expression of vascular endothelial growth factor (VEGF), which contributes to angiogenesis, more significantly in tumor cells (Co26s) than in endothelial cells (KOP2.16).[58] Lupulone (**6**) (2.5−50 μg/mL) induced a concentration-dependent inhibition of HUVEC endothelial cell proliferation and chemotaxis toward fibronectin. Furthermore, the formation of closed capillary-like structures was reduced in a Matrigel morphogenesis assay, indicating a strong inhibitory effect on neovascularization.[59]

NO is a gaseous free radical involved in the production of VEGF, the overexpression of which induces angiogenesis and vascular hyperpermeability, and accelerates tumor development. The ethyl acetate-soluble fraction of hop cones, containing hop bitter acids, inhibited both NO production and expression of inducible nitric oxide synthase (iNOS) in RAW 264.7 mouse macrophages, stimulated by a combination of lipopolysaccharide (LPS) and

interferon-γ (IFN-γ). Lupulone (**6**) and some of its oxidative degradation products inhibited NO production, but without reducing iNOS expression.[60]

Induction of CYP-450 Enzymes. Several compounds induce the expression of detoxification enzymes of the cytochrome P450 system, which are very important in the metabolism and subsequent activation and/or inactivation of many xenobiotics including procarcinogens. As a part of a mouse diet, colupulone (**7**) (0.18%) increased the P-450 content of the liver microsomes and stimulated various phase I enzyme activities, such as those responsible for demethylation of ethylmorphine and aminopyrine and the hydroxylation of aniline and benzo[*a*]pyrene.[61] An upregulation of multiple P450 enzymes, in particular of CYP3A and CYP2B, was detected independently by western and northern blotting.[61,62] Similar results were obtained after administration of a hop hexane extract (0.33%) and crude hops (1%).[61] If hops and colupulone (**7**), in particular, are able to induce CYP-450 enzymes in species other than the mouse, then ingestion might have a significant impact on the bioactivation and/or detoxification of food-borne promutagens. However, short-term administration of **7** to the rat (0.36%) did not alter the ex vivo CYP450-mediated conversion of the promutagens aflatoxin B_1 and benzo[*a*]pyrene to their mutagenic forms, as measured in *Salmonella typhimurium* and mammalian microsomal assays.[62] Up to the present, no further studies addressing the effects of long-term administration of hops, or individual hop constituents, on in vivo CYP-450 enzyme activity have been carried out.

In Vivo Activity. In rats, adding lupulone (**6**) (0.001% and 0.005%) to the diet reduced the development of colon carcinogenesis, initiated by azoxymethane, in a dose-dependent way. Both the number of preneoplastic lesions (aberrant cryptic foci, ACF) and the total number of tumors in the colon were dramatically reduced.[54] In a similar experimental protocol, oral administration of an isomerized hop extract containing 30% iso-α-acids (0.01% or 0.05%) proved to reduce the number of ACF in the colon, as well as the prostaglandin E2 (PGE_2) levels in the mucosa.[60] Topical application of humulone (**1**) (1 mg/mouse) protected against tumor formation in mouse skin, initiated by 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted by TPA.[63] Compound **1** significantly inhibited TPA-induced epidermal cyclooxygenase-2 (COX-2) expression, for which the levels are upregulated during carcinogenesis and inflammation.[64] Additionally, orally administered lupulone (**6**) (0.01% in drinking water for 21 days) inhibited new vessel formation in mice by 50%. Neovascularization was determined by measuring the hemoglobin content of Matrigel plugs, implanted under the mouse skin, and was standardized for control plugs of mice receiving tap water containing excipient.[59]

Hop Bitter Acids in Inflammatory Disorders

Since long ago, the hop plant has been known for its anti-inflammatory properties, as American Indians (the Delaware) used hops traditionally to relieve toothache and earache.[20] Yasukawa and co-workers screened 100 edible plant extracts against TPA-induced inflammation in mice in an attempt to find new herbal anti-inflammatory compounds. They identified a hop methanolic extract as a potent inhibitor of TPA-induced ear edema and identified humulone (**1**) as the active compound. In comparison with standard drugs, humulone (ID_{50} 0.2 mg/ear) was a less effective inhibitor than the steroid hydrocortisone (ID_{50} 0.03 mg/ear) but compared well with the inhibition potency of the nonsteroidal indomethacin (ID_{50} 0.3 mg/ear).[65] Similarly, **1** was found to inhibit ear edema in mice, induced by arachidonic acid.[63]

In Vitro Activity. Cyclooxygenases (COX) are key enzymes required for the transformation of arachidonic acid to a wide range of prostanoids, including PGE_2 and thromboxane A2 (TxA_2). The COX-2 isoform is highly upregulated by cytokines at sites of inflammation, whereas, in contrast, COX-1 is constitutively expressed in many cell types, where it has homeostatic functions in gastric cytoprotection and platelet activation.[66] The so-called "COX-2 hypothesis" assumes that the gastroduodenal toxicity of traditional nonsteroidal anti-inflammatory drugs (NSAIDs), which block both COX isoforms, is mainly related to their inhibition of COX-1-dependent PGE_2 and TxA_2 formation in the gastric epithelium and platelets, while COX-2 has a major role in pain mediation, inflammation, and pyresis.[67] Specific inhibitors for the COX-2 isoform, including celecoxib (Celebrex), are used for the treatment of osteoarthritis and rheumatoid arthritis, particularly in patients at high risk of developing gastrointestinal complications.[68] From several studies, individual hop acids proved themselves interesting candidates for anti-inflammatory therapy, by selectively inhibiting COX-2 upregulation by proinflammatory mediators. Humulone (**1**) suppressed the TNF-α-dependent release of PGE_2 in murine osteoblastic MC3T3-E1 cells (IC_{50} 30 nM) and reduced COX-2 enzyme activity, mRNA expression, and promoter activity. These effects were similar for the glucocorticoid dexamethasone, but for **1**, the glucocorticoid receptor was not involved. In in vitro enzymatic assays, **1** inhibited the catalytic activity of COX-2 with an IC_{50} value of 1.6 μM, whereas COX-1 activity was not inhibited below 10 μM.[69] On screening hop bitter acid-containing formulations for COX-2 inhibition in LPS-induced mouse macrophages (RAW 264.7), a number of these emerged as strong anti-inflammatory agents with no effects on PGE_2 originating from the constitutive form of the enzyme. Furthermore, the hop acids studied left constitutively secreted COX-1 in human gastric mucosa cells unaffected, which is predictive of a low gastrointestinal toxicity.[70] Also, reduced iso-α-acids (1−20 μg/mL) inhibited PGE_2 release from LPS-stimulated RAW 264.7 macrophages in a dose-dependent manner, blocked COX-2 protein expression, but left its enzyme activity unaffected.[71] Furthermore, after a IFN-γ-combined stimulation with LPS, iso-α-acids inhibited PGE_2 production in a dose-dependent manner.[60] Independently, a standardized carbon dioxide extract from hops dose-dependently inhibited PGE_2 production in LPS-stimulated human peripheral blood mononuclear cells (PBMC), without compromising metabolic activity (IC_{50} 3.6 μg/mL). Using human blood (whole blood assay, WBA), PGE_2 production was not decreased after selective activation of COX-1 by calcimicin. In contrast, the hop extract inhibited PGE_2 concentrations in blood, pretreated with aspirin to inactivate COX-1, and supplemented with LPS to induce COX-2. Thus, the hop extract was concluded to inhibit COX-2 selectively, with a calculated IC_{50} value of 20.4 μg/mL. The large differences in IC_{50} (PBMC vs WBA) was explained by the authors by the different experimental conditions: in whole blood assays, using 50% blood, a larger concentration of plasma proteins is present and might interfere with the hop extract, when compared to the PBMC, cultured in 10% FCS.[72]

Inflammatory signaling is highly regulated by a network of transcription factors, which modulate gene transcription in response to pro-inflammatory stimuli, such as cytokines, pathogens, and oxidative stress. For example, when TNF-α triggers its cognate membrane receptor, an intracellular cascade of kinases is activated, which leads to the release of NF-κB (nuclear factor kappa B) from its inhibitor in the cytoplasm. Freed NF-κB can then translocate to the nucleus, where it initiates the formation of a functional transcriptome, leading to increased expression of cytokines, enzymes, and adhesion molecules. Furthermore, constitutive NF-κB activation is often detected in cancer. Humulone (**1**) was shown to inhibit DNA binding of NF-κB in TPA-treated mouse skin, and **1** prevented the phosphorylation and nuclear translocation of NF-κB subunits. Furthermore, **1** blocked several kinases in the TPA-stimulated activation pathway toward NF-κB, including inhibitory kappaB (IκB) kinase β (IKKβ), and the mitogen-activated protein kinases (MAPKs) ERK, p38, and JNK.[64] Independently, our research group established a dose-dependent reduction of NF-κB-dependent gene transcription by α-acids, β-acids (0.5−10 μM), and iso-α-acids (25−200 μM). The transcriptional activities of activator

protein-1 (AP-1) and cAMP-response element binding protein (CREB) were also decreased. Furthermore, several upstream activated kinases, leading to the activation of these transcription factors, were found to be blocked (Van Cleemput, M.; Heyerick, A.; Libert, C.; Swerts, K; Philippe, J.; De Keukeleire, D.; Haegeman, G.; De Bosscher, K., unpublished results).

In Vivo Activity. Our research group studied the effects of hop bitter acids in mice, in which acute inflammation was induced by subcutaneous injection of zymosan in the paw. Ip administration of 250 μg of iso-α-acids or α-acids effectively inhibited paw edema, a characteristic symptom of inflammation, and this effect was similar for administration as potassium salts in phosphate-buffered saline or neutral acids in dimethylsulfoxide, suggesting that both formulas are equally well absorbed from the peritoneal cavity (Van Cleemput, M.; Heyerick, A.; Libert, C.; Swerts, K; Philippe, J.; De Keukeleire, D.; Haegeman, G.; De Bosscher, K., unpublished results).

In contrast, Hougee and co-workers administered a carbon dioxide hop extract orally to mice (1.25 mg by oral gavage for 10 days) in which acute arthritis was induced by injection of zymosan into the knee, but failed to detect a reduction of inflammation-related symptoms. The orally administered hop carbon dioxide extract neither inhibited joint swelling nor restored the inhibited proteoglycan synthesis in the arthritic cartilage. On the other hand, upon stimulating the blood of mice with LPS ex vivo, PGE_2 production was 24% lower in samples from mice treated with the hop extract, compared to vehicle-treated mice, thus suggesting that the extract does become bioavailable. These contradictory findings can be explained by the low bioavailability of hop bitter acids after oral intake. It was suggested therefore by the authors that the dose of 1.25 mg of hops extract be increased in order to lead to a detectable reduction of parameters for inflammation.[72]

Currently, hop-containing phytomedicines are commercialized for the relief of symptoms associated with chronic inflammatory disorders, such as arthritis. In a pilot trial evaluating a commercial preparation containing reduced iso-α-acids, rosemary extract, and oleanolic acid (Meta050), a 50% decrease in pain level could be detected in patients suffering from osteoarthritis, but not in fibromyalgia patients.[64] Consistent with these findings, results from a multicenter trial of healthcare practitioners using the preparation in patients with joint discomfort showed an approximate 30% reduction in clinical symptoms of joint distress, as measured by visual analog scale.[73]

Hop Bitter Acids Improve Markers for Metabolic Syndrome

In modern western society, the prevalence of metabolic diseases is taking on epidemic proportions and implicates a high risk of mortality due to cardiovascular complications. The so-called "metabolic syndrome" is defined as a cluster of abnormalities, covering insulin resistance, central obesity, impaired glucose tolerance or type 2 diabetes, dyslipidemia, hypertension, hypercoagulability, atherosclerosis, and elevated rates of inflammatory blood markers.[74] Current treatment is based on diet, exercise, and specific lipid-altering drug therapy, supplemented with antidiabetic agents that improve some of the associated complex atherogenic parameters. Plants, as extracts or isolated pure compounds, have already been shown to play a valuable role in the prevention or treatment of lifestyle-related disorders.[75] Recently, iso-α-acids have been shown to improve health by positively influencing lipid metabolism, glucose tolerance, and body weight.

In Vitro Activity. Peroxisome proliferator-activated receptors (PPARs) are a class of nuclear receptors that are essentially involved in the regulation of fatty acid and carbohydrate metabolism. Fibrates, agonists of the α-PPAR subtype, are clinically used for the treatment of dyslipidemia, whereas the glitazones, PPAR-γ agonists, improve insulin sensitivity in type II diabetes. Positive results of hop iso-α-acids on the lipid profile in rodents suggested a direct agonistic effect on PPARs. Indeed, these compounds dose-dependently (1−30 μM) activated PPAR-α and PPAR-γ in HepG2 and CV-1 cells, respectively. Using chimeric expression plasmids in which the ligand binding domain of PPAR-α or PPAR-γ was fused to the DNA binding domain of the yeast transcription factor GAL-4, iso-α-acids bound to PPAR-α- as well as PPAR-γ, thereby increasing the transcription of a cotransfected reporter gene, containing five GAL-4 binding sites coupled to the luciferase-encoding sequence.[76] However, this result could not be substantiated in our research group when transfecting full-length receptors together with a PPRE (PPAR response element)-containing luciferase reporter in COS1L2A cells. (Van Cleemput, M.; Heyerick, A.; De Keukeleire, D.; Haegeman, G.; De Bosscher, K., unpublished results).

In Vivo Activity. In a mouse model of non-insulin-dependent diabetes (KK-A^y mice), coadministration of hop iso-α-acids improved hyperglycemia and hyperlipidemia, similar to the PPAR-γ agonist pioglitazone, but without a concomitant increase in body weight. In the liver, genes for acyl-CoA oxidase (ACO) and fatty acid translocase (FAT) were highly upregulated, resulting in an enhanced lipid metabolism. Unexpectedly, iso-α-acids increased only moderately the expression of the adipose differentiation related protein (ADRP) and lipoprotein lipase (LPL) genes, involved in lipid uptake and storage in white adipose tissue. Similar effects were observed in diet-induced obese diabetic C57BL/6N mice. Coadministration to C57BL/6N mice of a high-fat diet and an isomerized hop extract, containing high amounts of iso-α-acids, dose-dependently reduced body weight gain, improved glucose tolerance, and slightly reduced insulin resistance, compared to the control group. In white adipose tissue, apoptosis of hypertrophic adipocytes was induced, next to an increased number of small adipocytes, thus improving insulin sensitivity.[76] When feeding the mice a hop iso-α-acid-containing diet, supplemented with high amounts of cholesterol, a drastic improvement of atherosclerotic clinical parameters was observed. Indeed, an increase in plasma HDL-cholesterol and a reduction in the liver content of cholesterol and triacylglycerol were observed. Similar results were obtained feeding the animals a standard diet containing only hop iso-α-acids.[77] In general, lipid metabolism in the liver was enhanced by upregulated levels of acyl-CoA oxidase (ACO), acyl-CoA synthetase (ACS), and fatty acid transport protein (FATP) mRNA, which control cellular fatty acid uptake and peroxisomal β-oxidation, next to elevation of apoprotein CIII and lipoprotein lipase (LPL) content, crucial for the metabolism of triacylglycerol.[77–79] The changes in lipid metabolism correspond to those of the PPAR-α agonist, fenofibrate, and were not found in PPAR-α-deficient mice, thus suggesting that iso-α-acids operate via an analogous mechanism.[78,79] Iso-α-acids also inhibited absorption of dietary fat in rats, which further supports a negative effect on body weight gain. As a possible target, isomerized hop extract decreased the pancreatic triacylglycerol lipase activity in a dose-dependent manner, thus elevating the undigested lipid content in the feces.[77]

Concerning cardiovascular parameters, rats on a high-salt regimen did not develop a higher mean blood pressure when iso-α-acids were incorporated into the diet (0.3%). It was proposed that these compounds reduce oxidative stress and restore the lower levels of bioavailable NO caused by the high-salt diet. As NO is a critical messenger molecule for the kidney to maintain salt and water homeostasis, increased bioavailable NO could protect against developing hypertension.[80]

One pilot study in humans has been carried out in which oral iso-α-acids ameliorated insulin sensitivity in mild type 2 diabetic patients by decreased blood glucose and hemoglobin A1c levels.[76]

Role of Hop Bitter Acids in Osteoporosis

Osteoporosis develops when the balance between bone formation and bone resorption is disturbed, and consequently, it is considered

feasible to prevent osteoporosis by promoting bone formation or by inhibiting bone resorption. Humulone (**1**) inhibited the formation of osteoporotic lesions in dentine slices (pit formation assay) with an IC_{50} value of 5.9 nM. The ad-homologue (**3**) was equally active, while cohumulone (**2**) showed no inhibitory activity. Also, lupulone (**6**) was reported to be a strong inhibitor of bone resorption.[81–83] However, the question remains as to what extent these in vitro data are valuable in the in vivo setting. A recent paper reported that long-term administration to rats of a hop powder-enriched diet (further undefined), either alone or combined with isometric strength training, did not improve bone parameters. However, the authors concluded that the body weights were significantly lower in those rats fed with the hop diet than in the control group. Therefore, it might have been difficult to detect positive effects of hops on bone, because lower body mass is associated with lower bone mass.[84]

It should also be mentioned that recent studies have established an unequivocal relationship between osteoporosis and inflammation.[85] For example, elevated serum levels of systemic inflammation markers such as interleukin-6, TNF-α, and high-sensitivity C-reactive protein are correlated significantly with a lower bone mineral density.[86,87] Therefore, the direct anti-inflammatory effects of hop acids could also contribute in the prevention and treatment of osteoporosis.

Effects of Hop Bitter Acids on the Central Nervous System

For quite a long time, from when it was observed that hop pickers tired easily, the hop plant has been reputed to possess sedative properties.[7,88] At present, hop-based preparations, mostly in combination with valerian, are marketed widely as a natural remedy for sleeping disorders, nervousness, and insomnia.[89–91] Already by the beginning of the 20th century, the sedative activity of various hop extracts and components was shown using frogs. In general, hop extracts reduced the excitability of the striated muscles and motor nerve endings, diminished the irritability of the nervous system, and induced narcosis.[92,93] However, the effect seemed to be highly species-dependent, since in rabbits, hops caused opposite effects such as increases in body temperature and dyspnea.[93] As both hop extract, freed from hop acids, and hop essential oil were devoid of activity, as early as 1938, Sikorski and Rusiecki pointed to the hop bitter acids humulone (**1**) and lupulone (**6**) as active compounds for the observed sedative activity upon administration of hops to pigeons and small birds.[94]

Later on, in 1980, Hänsel and co-workers suggested that it was not the hop bitter acids but rather a degradation product formed during storage that is responsible for the tranquilizing properties of hops.[95–97] They reported that degradation of humulones and lupulones can result in the formation 2-methylbut-1-en-3-ol (**14**), a C_5 alcohol, by a radical-type auto-oxidation in the presence of atmospheric oxygen.[98] Both in rats[99] and mice,[100] ip administration of this decomposition product showed sedative activity at low doses and induced transient deep narcosis at higher doses. However, only hop teas and balneotherapeutic preparations were found to contain effective amounts of **14**, whereas its content in "sedative dragees" was negligible. Therefore, their effectiveness could be questionable. Still, these dragees contain high amounts of hop acids, which, according to Hänsel et al., could act as precursors of active compound **14** via a similar radical-type degradation reaction in vivo.[97]

Over the past decade, the neuropharmacological activity of hops has been reinvestigated in more detail using recent techniques including receptor binding assays. Both a carbon dioxide hop extract and an isolated α-acid fraction proved to modulate CNS activity in rats.[101] It was found that both products dose-dependently enhanced pentobarbital-induced sleeping time without influencing locomotor activity, a marker for motor behavior. In addition, antidepressant activity similar to the reference drug imipramine was observed for both hop preparations (forced swimming test), whereas no anxiolytic effects could be observed (elevated plus maze test). In contrast, oral administration of a β-acid fraction increased locomotor activity and caused a reduced percentage of animals falling asleep on pentobarbital administration. It was shown that β-acids can interfere with the GABAminergic system (GABA = γ-aminobutyric acid), leading to a general reduced neurotransmission in the CNS.[102]

In contrast to the work by Zanoli et al., reduced locomotor activities on administration of either carbon dioxide or ethanolic hop extracts to mice were observed by Schiller et al., albeit using much higher dosages. Interestingly, they did observe an increased sleeping time on treatment with a narcotic drug, and moreover, they observed a reduced body temperature, another parameter indicating sedative activity. Furthermore, by using a range of different enriched fractions, it was indicated that various components including α-acids, β-acids, and hop oil all contribute to the overall sedative activity of hops.[103]

The product Ze91019, a fixed combination of 250 mg of valerian extract and 60 mg of hop extract, has been studied extensively for its sleep-enhancing properties.[91,104–106] The hop extract concerned is produced by maceration of dried hop cones with a hydrophilic solvent [methanol/water 45% (w/w)], and therefore, the concentration of hop acids is expected to be low. Still, it is worth mentioning that components in this extract were shown to bind to serotonin and melatonin receptors.[106] Furthermore, this hop extract reduced core body temperature in mice in a highly similar way to melatonin, as the effect was also inhibited by the melatonin receptor antagonist luzindole.[107] In a four-week clinical trial with patients suffering from nonorganic insomnia, the fixed valerian hop combination product Ze91019 was demonstrated as being significantly superior to placebo in reducing the sleep latency, while the single valerian extract failed to show superiority to placebo, indicating again the importance of the addition of the hop extract.[105]

Bactericidal Activity of Hops

Initially, hops were used for prolonged storage of beer. Adding hops reduces the growth of *Lactobacillus*, the main beer contaminant, which otherwise would affect yeast performance, cause losses in ethanol yield, and form undesirable off-flavors.[108] The preservative properties of hops have been investigated for many years, and despite some reports on the antibacterial activity of hop oil,[1,109] the bitter acids seem to be the main active compounds. The target bacteria are Gram-positive species, such as *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, and *Bacillus*.[11,110–116] In contrast, Gram-negative bacteria, such as *Escherichia coli*, are either resistant or affected only by very high concentrations of hop acids. Yeast is not inhibited, which is very important for the use of hops in beer production.[114,117] Some inhibitory activity has also been reported for certain fungi, such as *Penicillium* and *Aspergillus* species.[112,118,119] In general, lupulone (**6**) has greater antimicrobial activity than humulone (**1**), which is, in turn, more active than isohumulone.[120] However, the role of iso-α-acids in beer preservation is of great value, since they represent quantitatively the main contribution of hops to beer. In all studies, hop acids may behave as either bacteriostatic substances or bactericides, depending on the conditions employed.

Shimwell noted that the antiseptic potency of hops increased at low pH, which was attributed to changes in permeability of the bacterial cell wall.[113] This hypothesis was confirmed in *Bacillus subtilis*, in which lupulone (**6**), humulone (**1**), and isohumulone caused cell wall lesions by incorporation into the cytoplasmic membrane. This activity resulted in inhibition of active transport of sugars and amino acids and, subsequently, led to inhibition of cellular respiration and synthesis of proteins, RNA, and DNA.[117] Later on, Simpson identified the mechanism by which *trans*-isohumulone (**11**) inhibits the growth of the beer-spoilage bacterium

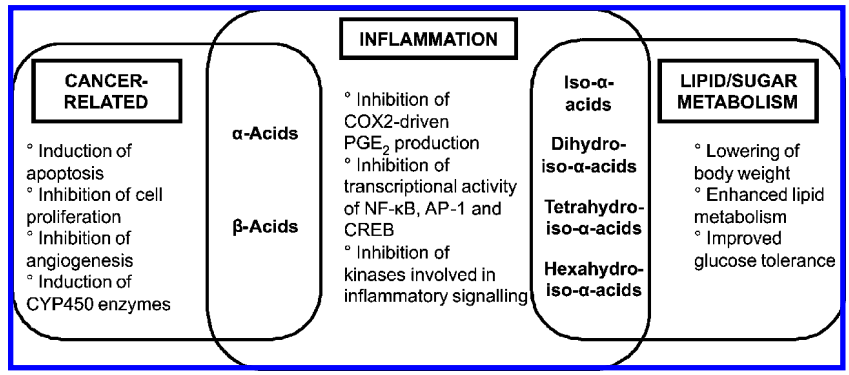


Figure 1. Overview of important biological effects of distinct groups of hop bitter acids.

Lactobacillus brevis. Apparently, the iso-α-acids act as mobile-carrier ionophores, catalyzing electroneutral influx of undissociated molecules, as well as their internal dissociation and efflux of their complexes with divalent cations such as Mn^{2+}. Consequent loss of the proton gradient inhibits the uptake of sugars and causes starvation in bacterial cells. The properties of other hop acids are similar to those of **11**, confirming a similar mechanism.[121] Since hop acids are weak acids and only undissociated forms are active, the antibacterial properties fall with higher pH values. Furthermore, the potency is enhanced by increasing the hydrophobicity of the molecules, as determined by the acyl side chain length and the number of prenyl groups.[114,122]

There are a few reports on the antibiotic properties of hops in relation to tuberculosis infection. Chin et al. demonstrated that lupulone (**6**) inhibits the growth of a virulent strain of *Mycobacterium tuberculosis* in vitro and considerably suppressed the development of tuberculotic lung lesions in mice when administrated either intramuscularly or intragastrically.[111,123] Humulone (**1**) also proved to be effective, although to a lesser extent, while the iso-α-acids were negative. A detailed study of influencing parameters showed that **6** remains active, regardless of experimental variations in pH, NaCl concentrations, and serum content.[124] Indeed, in a small-scale study in tuberculosis patients, daily oral administration of 5 g of **6** for 3 months was considered therapeutically active, without toxicity.[125] However, the most suitable method of administration and the possible development of drug resistance have not been investigated.

Limited evidence is available on the antiviral activity of hop acids. The iso-α-acids were shown to have a low to moderate antiviral activity against several DNA and RNA viruses, whereas no antiviral activity was detected for the hop β-acids.[126]

Due to their natural antimicrobial activity, hop acids have been studied for use in food preservation, e.g., to prevent contamination by *Listeria monocytogenes*.[120] Efficacy is the highest in acidic food and at lower fat content. However, it has to be mentioned that food preservation with hop acids requires quite high levels, which may impart undesirable flavors and aroma characteristics. In addition, hop acids are used in industrial ethanol production, where they represent a safe alternative to control bacteria in ethanol fermentation regardless of the process design or the disposition of produced ethanol.[108]

Hop Bitter Acids as Potent Antioxidants

Various health-promoting effects of plant compounds can be attributed to their intrinsic antioxidant activities: they neutralize cell damage caused by reactive oxygen species (ROS) and reactive nitrogen species such as free radicals, singlet oxygen, and hydroperoxides. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases of aging such as cancer, cardiovascular disease, immune system decline, diabetes mellitus, inflammation, brain dysfunction, and stress, among others. Phytochemicals may assist the body's own defense enzymes, such as superoxide dismutase and glutathione peroxidase, to scavenge or quench free radicals to protect the body against deleterious effects.

There are many in vitro assays available for determining antioxidative activities, including measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl, superoxide, or peroxynitrite radical-scavenging activities (RSA), lipid peroxidation inhibitory activity (LIA), the ferric reducing ability of plasma (FRAP), total radical trapping by antioxidants (TRAP), xanthine oxidase activity, and determination of hydrogen peroxide hemolysis. Often, a combination of methods is applied to characterize a compound as an antioxidant. Humulone (**1**) inhibited hydrogen peroxide-induced hemolysis with an IC_{50} value of 28 μM.[52] Furthermore, **1** and lupulone (**6**) were shown to be radical scavengers in the DPPH-RSA assay with IC_{50} values of 32 and 25 μM, respectively. Both compounds also inhibited lipid peroxidation (IC_{50} value of 7.9 μM for **1** and 39 μM for **6**). Interestingly, hop acids were more potent than the natural antioxidants α-tocopherol and ascorbic acid in this assay. The β-triketone moiety seems pivotal in view of its radical-stabilizing property.[127] Another report gave the following order of decrease in OH-RSA: α-acids > β-acids > dihydro-iso-α-acids > hexahydro-iso-α-acids > tetrahydro-iso-α-acids (IC_{50}: 0.21, 0.96, 1.36, 1.40, and 1.78 mg/mL, respectively). α-Acids and β-acids are potent scavengers of free radicals, whereas iso-α-acids and reduced derivatives show decreased activities in this regard. However, in terms of lipid peroxidation, the order of potency was as follows: α-acids > β-acids > iso-α-acids > tetrahydro-iso-α-acids > dihydro-iso-α-acids > hexahydro-iso-α-acids.[128]

In vivo antioxidative effects can be determined by the in situ fluorescent detection of ROS and NO in tissues or by indirect assays, such as measuring urinary NOx excretion and quantifying ROS production in the blood. Iso-α-acids have been reported to inhibit oxidative damage in rats fed a high-salt diet, thus preventing renal tissue damage. They decreased the production of ROS in renal tissues and increased bioavailable NO to basal levels. Increased ROS inactivates NO, critical for maintaining salt and water homeostasis in the kidney, thereby generating peroxynitrites, which, in turn, modify tyrosine residues of proteins to produce nitrotyrosine. Iso-α-acids in a high-salt diet, indeed, reduced the levels of renal nitrotyrosine, as detected by western blotting.[80]

Effects of Hop Bitter Acids on the Gastrointestinal Tract

Hops are used as over-the-counter products to improve gastric function. The bitterness of the hop acids is supposed to stimulate gastric secretion, similar to other bitter plant substances such as quinine. Only one study has addressed the effect of hops on gastric function, which uses the rat pylorus-ligated model. Intraorally administered hops clearly increased gastric juice volume without affecting acidity, which was not the case for intragastric administration. The effects after oral administration were similar to those obtained with carbachol, a cholinergic agonist, whereas they were

completely blocked by the cholinergic antagonist atropine. It was concluded that the increase in gastric juice volume by hops could be mediated by the cholinergic nervous system. However, since this study was carried out with an undefined hop extract, suspended in physiological saline at pH 4.5, it is not clear as to what extent these effects were caused by the bitter acid content of hops.[129]

Toxicology

In general, skin contact with hops is well tolerated. Occasional hop allergy has been reported, most frequently after long-term exposure by direct contact or inhalation, for example by hop-pickers. Allergic reactions, mostly mild, include skin symptoms, such as urticaria, dermatitis, erythema, and pruritus of the uncovered skin, as well as respiratory disorders, including rhinitis, conjunctivitis, and asthma.[2,12,17,130–133] Due to long-term uses in brewing and herbal medicine, hops are generally recognized as safe (GRAS) for oral intake.[134] In vivo toxicity studies showed that only very large doses of hop intake are toxic, causing respiratory irregularities and central respiratory depression terminating in paralysis, in frogs, pigeons, small birds, rabbits, and mice.[92,93,95] Regarding individual hop acids, intravenous injection of small doses of lupulone (**6**) stimulated respiration in rabbits and cats.[111] In mice, oral doses of 10 to 100 mg/kg body weight of **6** did not cause any adverse effects.[95] Repeated intravenously injected humulone (**1**), at doses of 1−10 mg/kg, caused hyperventilation and hyperthermia in cats. In rabbits, the effects were much weaker and also of shorter duration. Lethal doses of **1** caused an abnormally severe rigor mortis, which appeared rapidly after death, suggesting that **1** affects muscular metabolism.[135] A safety study of preisomerized hop acids revealed that the LD_{50} values of iso-α-acids and dihydro-iso-α-acids are approximately 1 g/kg body weight in the rat, when administered as single doses in a 50% corn oil solution. Long-term addition of iso-α-acids, dihydro-iso-α-acids, tetrahydro-iso-α-acids, or hexahydro-iso-α-acids to the diet (1% for 90 days) caused a reduction in body weight gain in rats, without behavioral and histopathological changes. A dose of 150 mg/kg body weight was considered to be the no-observed-adverse-effect level (NOAEL). Furthermore, tetrahydro-iso-α-acids, hexahydro-iso-α-acids, and dihydro-iso-α-acids did not cause mutagenic or genotoxic effects. In the dog, subchronic oral administration of tetrahydro-iso-α-acids and hexahydro-iso-α-acids was well tolerated with NOAEL values of 50 and 100 mg/kg body weight, respectively. Undigested material could be retrieved in the feces, suggesting a poor gastrointestinal absorption. In general, toxic effects of high doses of preisomerized hop acids were limited to the gastrointestinal tract, most probably due to irritation by these bitter compounds.[136]

There are only a small number of reports addressing the safety of hop bitter acids in humans. A daily oral administration of 5 g of lupulone (**6**) for three months was not toxic for the liver, kidney, bone marrow, or myocardium. However, each patient experienced some degree of gastrointestinal irritation, ranging from epigastric burning pain, abdominal cramping, diarrhea, nausea, and vomiting.[125] A formula containing dihydro-iso-α-acids (Meta050) (440 mg daily for eight weeks) did not result in clinically relevant changes in blood pressure, complete blood counts, or liver and kidney function. Furthermore, there was no negative impact on gastrointestinal markers normally affected by selective COX-2 enzyme inhibitors, as concluded from normal fecal calprotectin excretion.[73,137] Similar data were obtained after administration of pure dihydro-iso-α-acids (450 mg daily for 2 weeks).[73,137]

Conclusions

Through their wide range of biological and pharmacological effects, hop acids have proved interesting candidates for the treatment and/or prevention of several human disorders, including cancer, diabetes mellitus, osteoarthritis, osteoporosis, and cardiovascular disease. Hop acids may account—at least partially—for some of the health-beneficial effects of moderate beer consumption, as reported by a battery of epidemiological studies.[138–146] Especially during the past decade, a considerable amount of research has been performed in both in vitro and in vivo settings. However, there is a lack of firm conclusions, due mainly to the use of insufficiently characterized materials. The molecular targets of the hop constituents need to be better characterized, especially regarding positive effects on lipid and glucose metabolism. In addition, information concerning bioavailability, distribution, degradation, and elimination of hop acids is required for a better comprehension of their physiological concentrations and targets. In conclusion, hop bitter acid research has led to a better understanding of the effects of these compounds on health, and this knowledge has already been translated into the production of hop-derived phytomedicines and botanical dietary supplements.

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