

REVIEW

## The pharmacognosy of *Humulus lupulus* L. (hops) with an emphasis on estrogenic properties

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### Abstract

As the population ages, there is an ever-increasing need for therapeutic agents that can be used safely and efficaciously to manage symptoms related to postmenopausal estrogen deficiency. Endogenous estrogens, e.g.,  $17\beta$ -estradiol, of exogenous mammalian origin, e.g., horses, have long been used to manage such symptoms. There are more than 20 different classes of phytochemicals that have demonstrated affinity for human estrogen receptors *in vitro*. Some studies on exogenous estrogenic substances of botanical origin (phytoestrogens), such as standardized formulations of plant extracts with *in vitro* and *in vivo* estrogenic activity from soy (*Glycine max* Merrill.) and red clover (*Trifolium pratense* L.), suggest clinical efficacy. Few clinical data for phytoestrogens other than isoflavonoids are available. In an exhaustive review of the literature through 2003, only two clinical trials were identified that were designed to evaluate the effect of hops (*Humulus lupulus* L.) on symptoms related to menopause. Folkloric, chemical, and biological literature relating primarily to the use of hops for their estrogenic activity, and two human clinical trials, are reviewed.

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### Introduction

Women commonly suffer from a variety of symptoms brought on by chemical changes associated with menopause. Hot flashes, anxiety, insomnia, and osteoporosis are among the major complications often associated with this phase in a woman's life. Hormone replacement therapy (HRT) is effective in alleviating a number of menopausal symptoms (Grodstein et al., 1997; Henderson, 1997). Indeed, Premarin<sup>®</sup>, a naturally occurring mixture of estrogens produced from equine

urine, is commercially one of the most successful pharmaceutical preparations of all time. Its efficacy in reducing vasomotor symptoms such as hot flashes, and in curtailing osteoporosis, has been documented extensively. However, drawbacks to this form of HRT include side effects such as an increased risk of certain forms of cancer (Bolton et al., 1998; Rossouw et al., 2002; Zhang et al., 2001) and women frequently complain of abnormal bleeding, bloating, breast tenderness, weight gain, anxiety, and restlessness (Hahn, 1989; Harris et al., 1990; Rabin et al., 1999). A highly publicized,

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multi-center study by the Women's Health Initiative (WHI) was prematurely halted due to a significant increased risk of breast cancer for women receiving a crude mixture of equine estrogens plus progestin (Rossouw et al., 2002). The widespread publicity of the WHI study led many women to discontinue the use of HRT. In a recent survey of HRT users, 56% of respondents (377/670 women) were found to have discontinued the use of HRT within 8 months after the halting of the Premarin arm of the WHI trial (Ettinger et al., 2003). Among the remaining 44% who did not stop using HRT, 25.7% cited the relief of hot flashes as the primary factor in their decision. For these reasons, an alternative estrogen therapy would be a useful addition to the Pharmacopoeias of the world.

Hops, the resinous inflorescences of the twining vine *Humulus lupulus* L. (Cannabaceae), are used today primarily for their bitter and aromatic properties in the manufacture of beer. In addition, hop preparations are sold in health-food stores in the US for the treatment of anxiety and insomnia, and are sold in pharmacies in Europe (Anonymous, 2002), and formerly in pharmacies in the US (Anonymous, 1946), for these purposes. Hops have been shown to contain one of the most potent *in vitro* estrogenic substance known from the plant kingdom, ( $\pm$ )-8-prenylnaringenin (Kitaoka et al., 1998; Milligan et al., 1999). They are mentioned repeatedly in popular texts to have sedative-like activity (Blumenthal, 2000; Fluckiger and Hanbury, 1879; Gathercoal and Wirth, 1936; Schulz et al., 2001; Youngken, 1950). Thus, given the history of long-term and present use in humans with no significant adverse effects, an initial hypothesis is that hops are safe.

## Phytoestrogens

The term "estrogen" has come to refer to any substance that elicits a biological response similar to that of the principle human estrogen ( $17\beta$ -estradiol) in any hormone-related assay; likewise, the term "phytoestrogen" does not indicate a particular chemical class, but has come to include all plant-derived compounds with some form of observed estrogenic activity. The biosynthesis of  $17\beta$ -estradiol has been demonstrated in *Phaseolus vulgaris* L. (Young et al., 1977), and the principle human estrogen could therefore itself be regarded as a "phytoestrogen". The term was first used to describe the observation that certain forage plants produced effects in farm animals similar to those known for estrogen (Bickoff, 1961). The estrogenic properties of isoflavonoids were first characterized by the Australian chemists Bradbury and White (1951), who were charged with elucidating the cause of an epidemic of infertility in sheep that was ravaging their nation's wool

industry. In 1951, the team reported the results of their decade-long effort. They had traced the cause of the epidemic to genistein present in "subterranean clover" (*Trifolium subterraneum* L.) growing in pastures where the sheep grazed. Genistein had previously been identified in soy (Baker and Robinson, 1928) but its hormonal properties were unknown until the work of Bradbury and White. It is noted that many animal feeds are soy-based and should not be used for estrogen-dependent assays (Brown and Setchell, 2001).

Numerous assays currently available are designed to characterize and/or identify estrogenic activity, from *in vitro* ligand-receptor binding and whole cell assays, to *in vivo* animal models. With the independent discovery by four different research groups of a novel subtype of the estrogen receptor in 1996 (the beta-estrogen receptor, or ER $\beta$ ) in both rat (Inoue et al., 1996; Kuiper et al., 1996) and human tissues (Mosselman et al., 1996; Pink et al., 1996), a large body of research aimed at understanding the organ-specific effects of each receptor subtype has been generated. ER $\alpha$  receptors are more common in the breast, liver, CNS and uterus, whereas ER $\beta$  are expressed mainly in the intestine, prostate, ovaries, testes, and urogenital tract (Lecce et al., 2001; Pettersson and Gustafsson, 2001; Shughrue and Merchenthaler, 2001).

Many estrogenic substances have been identified from natural sources. Natural ER ligands comprise a range of chemical classes, namely (in order of highest reported potency in ER binding assays), the steroids, polyketides (zearalenones), alkylated flavanones, isoflavones, phenylbenzofurans, anthraquinones, *nor*-neolignans (hinokiresinol), napyridiomycins, benzylchromanones, flavonols, isopropylbiphenyls, dihydrochalcones, alkaloids, flavones, chalcones, stilbenes, and 1,4-diphenylbutanones (Farnsworth, 2003).

## Pharmacognosy of hops

Hops (strobiles of *H. lupulus* L., Cannabaceae) are native to Eurasia, and they have been cultivated for more than 1000 years (Behre, 1999). Distinct lineages native to temperate regions of North America also exist (Hampton et al., 2002). In addition, the species has been introduced as a cultivar to temperate regions of South America, South Africa, and Australia. The numerous common names for this plant highlight the historical significance of the species, particularly in North temperate cultures (Farnsworth, 2003; Fluckiger and Hanbury, 1879; Neve, 1991)

- Chinese/Japanese: bijuhua (beer flower), pijuhua, shemahua, xiangshehau, xianshema,
- English: hops, hop strobiles, hopp, hoppel (from Anglo-Saxon *hoppan = to climb*), hymele,

- French: houblon, vigne du Nord,
- German: Hopfen, Hopfenzapfen,
- Italian: luppolo, orticaccio, vite near,
- Latin: Strobili Lupuli, Humuli,
- Native American: Chan iyuwe, makan skithe,
- Slavic: Hmelj,
- Spanish: betiguera, hombrecillo, lúpulo, vidarra.

Traditionally used herbal medicines have typically been cultivated for hundreds of years or more and distinct genetic lineages have come to exist due to human civilization. In the case of hops, hundreds of named cultivars and many recognized chemotypes exist (Neve, 1991). Hops, as an agricultural crop, in a sense are at a higher state of evolution compared with many other botanicals. The reason for this is due largely to their coveted organoleptic properties that could readily be selected for in pursuit of the perfect beer. In essence it was bioassay-guided selection, where the bioassay was the beer drinkers' demand for certain flavors. Considering modern bioassay capabilities, similar progress should be seen over the next few decades in the agricultural perfection of other botanicals.

Hops are defined as the strobiles, or “cones” of female individuals of the dioecious species *H. lupulus* L. (Cannabaceae). Several extant hop lineages can be traced back to cuttings taken from individual plants more than a century ago (Neve, 1991). The agricultural quality control procedures in place today for hops have a long tradition dating back to at least 1603, when an act in England was passed entitled “An Act for avoiding of deceit in selling, buying or spending corrupt and unwholesome Hoppes” (Parker, 1991). In order to maintain a genetically consistent product, and to prevent seed formation, males of the species are prohibited in hop-growing areas (Neve, 1991). Growing, harvesting, drying, packaging, shipping, and storage conditions have been rigorously optimized, and usually patented, for the stable delivery of hop constituents relevant to brewing beer.

### Cannabaceae Martinov

According to the most recent International Code of Botanical Nomenclature (St. Louis Code) adopted by the Sixteenth International Botanical Congress in 2000, the currently accepted name for the family, comprised only of the two genera *Humulus* L. and *Cannabis* L., is Cannabaceae (Greuter, 2000 #1281). Other names still applied to this family are Cannabinaceae and Cannabidaceae. The genera *Humulus* L. and *Cannabis* L. are by all accounts considered to be members of the same family, and have previously been placed in the Moraceae and Urticaceae.

### *Humulus* L.

This genus arguably comprises three species. *H. lupulus* L. was first domesticated in central Europe and is currently naturalized throughout the north temperate regions of the world as well as some temperate regions in Australia, South Africa, and South America. *Humulus japonicus* Siebold & Zucc, endemic to East Asia, neither produces bitter substances nor xanthohumol (XH) derivatives (Stevens et al., 2000). There are accounts by early European settlers in North America of wild hops being present when they arrived (Burgess, 1964; Neve, 1991; Tomlan, 1992). Wild American hops readily interbred with European cultivars brought by the settlers (Neve, 1991). The names *H. americanus* Nutt., *H. neomexicanus* Rydb., *H. volubilis* Salisb., *H. vulgaris* Gilib., *Lupulus communis* Gaertn., and *L. humulus* Mill. are probably synonyms for various *H. lupulus* varieties detailed below (Small, 1978, 1980).

### *Humulus lupulus* L.

In the northern hemisphere, hop strobiles (cones) are typically harvested in late August or September, from the perennial vine, which can reach over 7 m (Burgess, 1964; Neve, 1991). The cones are immediately dried after harvest by forced hot air, and are often pressed into dense cylindrical pellets, ca. 5–8 mm in diameter and up to 25 mm long. Pelletization greatly reduces the overall surface area and therefore reduces the rate of chemical oxidation/degradation, and also provides a more compact product for shipping. Typical microscopic characters of powdered hop cones include numerous large yellow glands unique to the species (lupulin glands), abundant bracteole and leaf fragments, as well as sparsely distributed glandular and many covering trichomes (Jackson and Snowden, 1968; Neve, 1991; Yamada et al., 1998; Youngken, 1950). By noting the size of the lupulin glands, the microscopic data can also be used to determine the stage of maturity of the strobiles at the time of harvest (Jackson and Snowden, 1968; Neve, 1991; Youngken, 1950).

A number of botanical varieties and/or synonyms have been described (Anonymous, 1999; Hampton et al., 2002; Small, 1978, 1980; Stevens et al., 2000; Vanhonen et al., 2002) including:

- var. *lupulus*; European hops,
- var. *cordifolius* (Miq.) Maxim in Franch. et Sav. (= *H. cordifolius* Miq.); native to East Asia,
- var. *lupuloides* E. Small; native to the Missouri-Mississippi river basin,
- var. *brachystachyus* Zapalowicz,
- var. *neomexicanus* Nelson et Cockerell; *H. neomexicanus* Rydberg, native to the southwestern US.

In addition, over 100 hop cultivars have been named, of which some have been bred to produce over 20% (by weight) of bitter acids, while others were selected for many generations primarily for their content of volatile oil (Burgess, 1964; Neve, 1991). The reader is referred to the dendrogram presented by Seefelder et al. (2000) for a graphical representation of the genetic relationships among 90 hop cultivars. Pedigree data that are typically provided by hop producers further add to the potential reproducibility of experiments on the biological and chemical properties of hops.

## Phytochemistry

More than 1000 chemicals have been identified from hops (Anonymous, 2003; Eri et al., 2000; Farnsworth, 2003; Verzele and de Keukeleire, 1991), including natural products and their isomeric derivatives. In terms of their traditional economic value, the volatile oil and bitter acids comprise the most significant compound classes produced by hops. In addition to these, the estrogenic activity is due to prenylated flavonoids.

## Volatile oil

The essential oil of hops is known to contain a large number of volatile constituents, including simple oxidized alkanes, monoterpenes, and sesquiterpenes (Farnsworth, 2003). Eri et al. (2000) detected 286 distinct chemical entities in the chromatogram of the volatile oil, more than 100 of which were not identified. The primary volatile constituents in all cultivars were the monoterpene myrcene, and the sesquiterpenes  $\beta$ -caryophyllene and humulene, which together were shown to comprise between 57% and 82% of the volatile oil, depending on the cultivar and the method of detection (Eri et al., 2000). There is no indication in the literature that the volatile oil contributes significantly to the estrogenic activity of hop extracts.

## Bitter acids

The “bitter acids” comprise from less than 5% to upwards of 20% of the weight of mature hops (Neve, 1991; Verzele and de Keukeleire, 1991). A majority of the primary hops literature was originally published in brewing-related journals. The following brief review of the bitter acids is based largely on the work of Verzele and de Keukeleire (1991), whose thorough review of the subject matter was drawn from more than 700 papers, dating back over a century, relating to the chemistry and analysis of hops.

The hop bitter acids are classified as either “ $\alpha$ -acids” or “ $\beta$ -acids” which are, respectively, di- or tri-prenylated phloroglucinol derivatives. In addition, they each

contain a 3-, 4-, 5-, or 6-carbon oxo-alkyl side chain. The  $\alpha$ -acids were originally distinguished by the fact that they are precipitated from a crude extract of hops by the addition of lead acetate. The  $\beta$ -acids, by definition, would remain in solution. The  $\alpha$ -acids, particularly humulone (35–70% of total  $\alpha$ -acids), cohumulone (20–65%), and adhumulone (10–15%) are regarded as the most important constituents in determining the quality of hops (Neve, 1991; Verzele and de Keukeleire, 1991). Verzele and de Keukeleire (1991) noted a belief shared among many brewers that a high percentage of cohumulone (relative to humulone) is associated with low-quality hops, but also note that this conviction is not universal. Producers of hops typically provide chemical analyses along with their products, including  $\alpha$ - and  $\beta$ -acid content (expressed as percentages of the weight of plant material or concentrated extract), and often give cohumulone content (expressed as a percentage of total  $\alpha$ -acids).

The  $\alpha$ -acids occur in beers in concentrations up to 4 mg/ml and contribute to foam stability as well as imparting antibacterial properties (Verzele and de Keukeleire, 1991). While regarded as the principal “bitter acids” from hops, they perhaps paradoxically do not have a bitter taste, even at concentrations of 100 mg/ml (Verzele and de Keukeleire, 1991).

The hop  $\alpha$ -acids isomerize to the corresponding “*iso*- $\alpha$ -acid” under a variety of reaction conditions. This isomerization occurs more favorably at higher pH values and “pre-isomerized” hop extracts (usually produced by boiling the plant material, or extract thereof, with aqueous Na<sub>2</sub>CO<sub>3</sub> or K<sub>2</sub>CO<sub>3</sub> for a short time) are commercially available and are often employed by brewers to increase the concentration of the bitter *iso*- $\alpha$ -acids in the final product. The *iso*- $\alpha$ -acids are artifacts and comprise more than 80% of all hop components that occur in typical beers. They are primarily responsible for the typical bitter taste of beer and, like the  $\alpha$ -acids, provide foam-stabilizing and antibacterial properties (Verzele and de Keukeleire, 1991). A vast effort has gone into elucidating mechanisms of isomerization, oxidation, and degradation, as well as the biosynthesis, of the hop  $\alpha$ - and  $\beta$ -acids (Verzele and de Keukeleire, 1991). There is no reason to believe that the bitter acids significantly affect the estrogenic activity of hop extracts.

## Prenylated flavonoids

A mixture of prenylated, geranylated, oxidized, and/or cyclized chalcones, of which 30 have been isolated to date, is secreted by the lupulin glands along with the bitter acids and volatile oils (Milligan et al., 1999; Mizobuchi and Sato, 1994; Stevens et al., 1997, 1999a, b, 2000; Zuurbier et al., 1998). The chalcone XH is the

most abundant prenylated flavonoid in fresh and properly preserved hops, present at a concentration of ca. 0.01–0.5% (Stevens et al., 1999a,b, 2000). In a chemotaxonomic study by Deinzer et al. (Stevens et al., 2000), the compound xanthogalenol (XG) was identified as being present in hops containing germplasm from east of the Rocky Mountains in the US (*H. lupulus* var. *lupuloides* E. Small and *H. lupulus* var. *pubescens* E. Small), or East Asia (*H. lupulus* var. *cordifolius* (Miquel) Maximowicz). The authors hypothesized that methylation at the 4'-position on the chalcone nucleus is a genetically primitive trait in the species, that has been lost in European (*H. lupulus* var. *lupulus*) and southwestern US (*H. lupulus* var. *neomexicanus*) hop lineages (Stevens et al., 2000). None of the hop flavonoids associated with estrogenic or chemopreventive activity were detected in one sample of *H. japonicus* Sieb. & Zucc (Stevens et al., 2000).

It must be stressed that the principle estrogen from hops, annotated herein as “8PN”, is the 1:1 racemate, ( $\pm$ )-8-prenylnaringenin. This compound is known in the English hop literature as 8-prenylnaringenin or hopein (De Keukeleire et al., 2001). The name 8-prenylnaringenin is misleading for a variety of reasons. Naringenin is the aglycone of the flavonoid glycoside naringin, a chiral natural product, usually with an *S*-configuration at the stereocenter C2. The major estrogen from hops is a 1:1 racemate, formed along with racemic “6-prenylnaringenin” on chemical isomerization of a chalcone traditionally named 3'-prenylnaringenin chalcone, synonymous to desmethylxanthohumol (DMX) (Hänsel and Schulz, 1988; Stevens et al., 2000). A natural product that *could* properly be called 8-prenylnaringenin is known to exist, e.g., from the Fabaceous shrub native to Asia, *Sophora flavescens* Ait. This compound is known in the literature as 8-isopentenyl naringenin (Kitaoka et al., 1998) or “sophoraflavanone B” (Mizobuchi and Sato, 1994). Because it is less cumbersome than “( $\pm$ )-8-prenylnaringenin”, it may be preferable that investigators use the name “hopein” coined by Professor De Keukeleire, when referring to the estrogen from hops. By using the name hopein, it should then be understood that the compound in question is the estrogenic racemate from hops, rather than the chiral estrogen from Asian shrubs.

Even though all flavanones are ultimately derived via a chalcone intermediate, chalcones are traditionally considered as a subclass within the “flavonoids”. The fact that chalcones and flavanones have different traditional numbering systems in the natural products literature can be a source of confusion. In order to reflect their biogenetic relationship, unless explicitly stated otherwise, the same (flavonoid) numbering system is used when referring to substituents on all chalcones and flavanones throughout this manuscript.

A majority of the known flavonoids from hops can be considered as derivatives of the compound 2',4,4',6'-tetrahydroxy-3'-prenylchalcone (chalcone numbering), commonly known as DMX. With the exception of two geranylated compounds, which have only been detected by MS (Stevens et al., 1999a, b), all known constituents of hops from this series are prenylated at either the 6- or 8-position, or both. There are three known series of compounds present in hops based on this nucleus:

- the XH series (5-methoxy)(Verzele et al., 1957),
- the XG series (7-methoxy)(Stevens et al., 2000),
- the DMX series (Hänsel and Schulz, 1988; Nastainczyk, 1972).

Prior to 2003, five members of the XH series were known, namely, XH itself and four other compounds designated as XH-B through XH-E. Strictly speaking, only four members of the XH series were known, because the compound named XH-E (Stevens et al., 2000), is actually a member of the DMX series.

Verzele et al. (1957) first named XH. Forty years later Tabata et al. (1997) started a trend by proposing the trivial name “XH B” for their isolate, which can be regarded as an oxidation product of XH. Deinzer et al. continued this trend, and have defined trivial names for the compounds XH C, D, and E (Stevens et al., 2000). XH E is a diprenylchalcone lacking a 5-*O*-methyl group. While related, it is not a derivative of XH. Fig. 1 is a proposed system of nomenclature for these compounds. In this system, 7-*O*-methylchalcones are to be named in the XG series, and should be given analogous names as the corresponding “XH”. Likewise, the *O*-demethyl derivatives should be named in the “DMX” series, two of which have recently been identified for the first time (Chadwick et al., 2004). The name “XH E” would be replaced with “DMX E”, and the name “XH F” is reserved for the 5-*O*-methyl analogue. To date, XG itself has been reported in the XG series.

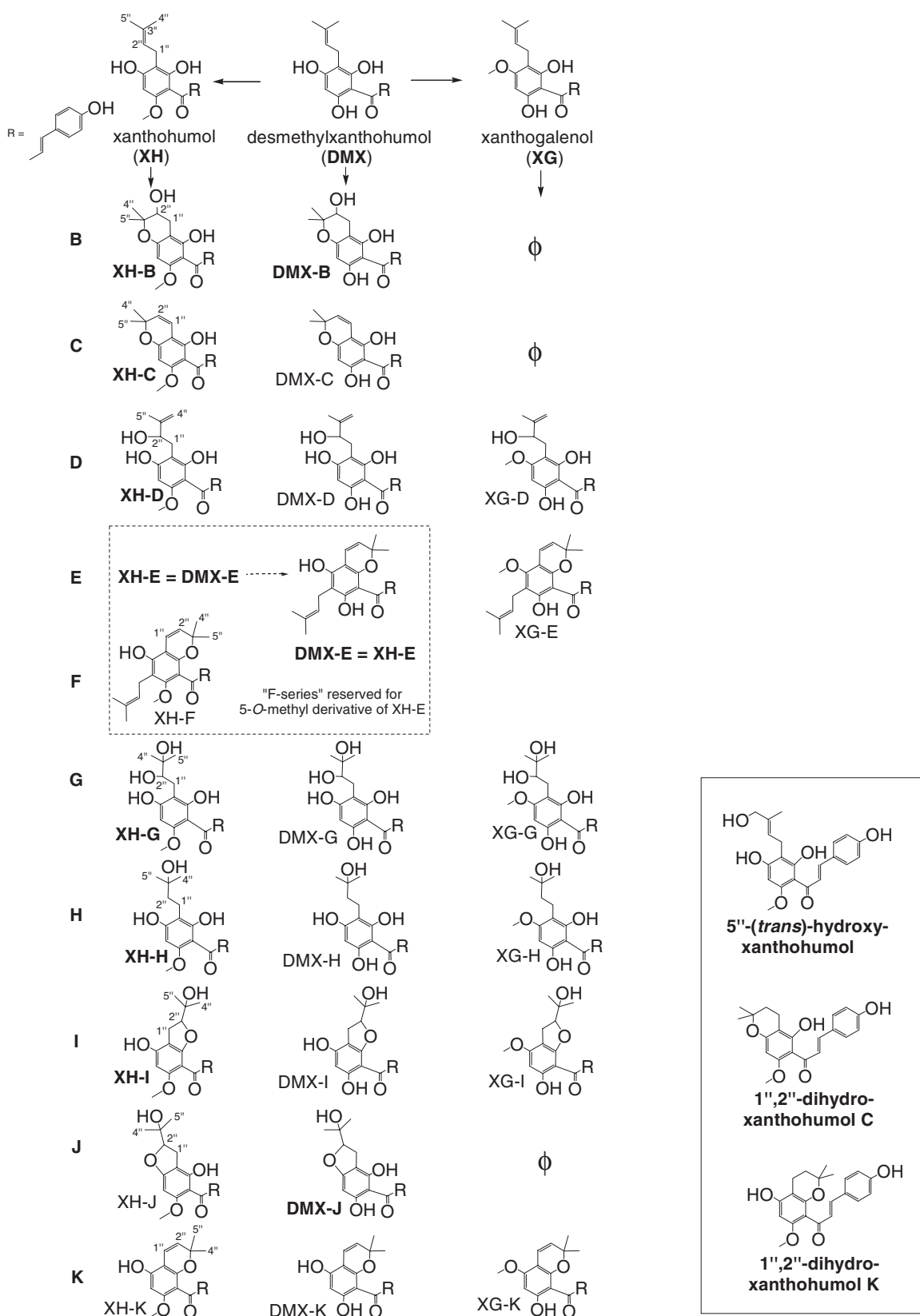
The major chalcone XH isomerizes to isoxanthohumol (IX), the reaction reportedly taking place more rapidly with increasing pH (Stevens et al., 1998, 1999a, b). The corresponding isomerization is possible in any chalcone containing a free 2'- or 6'-hydroxyl group, and in cases where there is a hydroxyl at both 2' and 6', two isomers are produced. In terms of estrogenic activity, the most significant manifestation of this isomerization event is the conversion of Nastainczyk's “pro-estrogen” (DMX) to the mixture (ca. 3:2) of 6PN and the estrogen 8PN (Hänsel and Schulz, 1988; Nastainczyk, 1972).

### Estrogenic activity

Fenselau and Talalay (1973) stated that brewery sludge baths containing ca. 30% hop extracts were

taken in Germany for the treatment of a variety of gynecological disorders. References to the traditional use of hops in the treatment of such disorders have been

identified from the US (Donsback, 1977), Iran (Zagari, 1992), Romania (Racz et al., 1980), and France (Goetz, 1990). Additionally, a number of journal articles



(Bednar and Zenisek, 1961; Fenselau and Talalay, 1973; Strenkovskaya, 1968; Zenisek and Bednar, 1960) and patents exist concerning the use of the estrogenic properties of hops, particularly for external use in cosmetics. Given what is now known about the isomeric nature of the estrogenic principles, it is likely that traditional healers had a difficult time concocting effective estrogenic formulations of hops in a reproducible manner.

Koch and Heim (1953), following up on the folk legend that women who normally live “a distance” from hop gardens regularly begin to menstruate 2 days after arriving to pick hops, reported that hops contain “the equivalent of 20–300  $\mu\text{g}$  estradiol/g”. While lacking in detail, this one-page article is apparently the first scientific confirmation that hops contain estrogenic activity in humans. Koch and Heim used a version of an *in vivo* assay with castrated female infant rodents, developed by the Nobel laureate Edward Doisy (the Allen-Doisy test), which has been used for many years to define estrogenic substances. The test substance was injected into the animal and was considered estrogenic if keratinized cells appeared and leukocytes disappeared from the vaginal smear (Hensyl, 1990). Chury (1961) reported that the estrogenic activity for a saponified ethanol extract of hops was approximately 200, 150, and 50 times those of peas, red clover, and cabbage, respectively, in a uterine weight assay in which the test substance was injected 48 h before sacrificing the animals, and its effect on uterine growth was determined. Inspired by the Koch and Heim report, Bednar and Zenisek (1961) were interested in estrogenic natural products for use in cosmetics, and concluded that, “Our discovery...could become the basis for studies of the possible use of hops in cosmetics”. Several hop-containing cosmetic preparations have been patented since that time. It was recently reported that the known estrogenic constituent 8PN, when administered subcutaneously in ethanol solution, has estrogenic activity (as measured by the effect on uterine and vaginal weights), but is 20,000-fold less potent than estradiol (Schaefer et al., 2003). It is noted that Fenselau and Talalay (1973) prepared and assayed (using a modified Allen-Doisy test, *s.c.* injection) a variety of hop extracts, but all were negative for estrogenic activity.

It was not until 1988 that the chemical structure of the estrogenic principle was established (Hänsel and Schulz,

1988). In his 1972 PhD dissertation, Nastainczyk reported the isolation of a compound he named “hops pro-estrogen,” and that it isomerized to a mixture he called “hops estrogen” (Nastainczyk, 1972). In 1988 Hänsel and Schulz reported the chemical structure of Nastainczyk’s “hops-proestrogen” as 3'-prenylnaringenin chalcone (synonymous with DMX), and that his “hops estrogen” was the mixture 8PN+6PN. They asserted without experimental data that the mixture 8PN+6PN was estrogenic and that 6PN was not estrogenic. While they did not state explicitly that 8PN is the active estrogen, Hänsel and Schulz should be given credit for correctly reporting for the first time the chemical structure of the principle estrogenic component of hops. Nastainczyk was the first to identify a pro-estrogenic substance in hops. The report by Milligan et al. (1999) may be regarded as the beginning of the modern, unambiguous understanding of the *in vitro* estrogenic activity of hops.

An estrogen-dependent human endometrial adenocarcinoma epithelial cell line containing both estrogen and progesterone receptors was established in Japan in 1985 (Nishida et al., 1985). Soon thereafter, it was discovered that alkaline phosphatase activity in these “Ishikawa cells” correlated with administration of estrogens, but not of other hormones, and that this activity could be blocked by administration of anti-estrogens (Holinka et al., 1986). This discovery provided a convenient assay for estrogens and antiestrogens in which a chromogenic phosphate monoester substrate is hydrolyzed by alkaline phosphatase, and the enzyme’s expression is readily quantified and correlated with estrogenic activity. Antiestrogenic activity is determined by coadministering the test substance with estradiol.

Milligan et al. (1999) conducted bioassay-guided fractionation using Ishikawa cells for screening. This approach led to the isolation and characterization of 8PN as the major estrogenic substance in hops and one of the most potent known plant-derived estrogens. This compound (specifically, the 2*S* enantiomer) had previously been identified as a phytoestrogen from *Anaxagorea luzonensis* A. Gray of the Annonaceae (Kitaoka et al., 1998). Subsequent investigations *in vitro* followed, and to date 8PN has generally been shown to mimic the action of 17 $\beta$ -estradiol, albeit with a lesser (10–20,000-fold) potency (Coldham and Sauer, 2001; Milligan et al., 2002, 2000; Rong et al., 2001; Zierau

**Fig. 1.** Current and proposed nomenclature for chalcones from hops.  $\phi$  indicates that the given structure cannot occur in the XG series. Compounds in boldface font have been reported in the literature, or have recently been isolated by our group. It is likely that all of the compounds in this figure, and many more related structures, will eventually be isolated from hops. A less confusing situation will arise if this naming system is implemented. The name “xanthohumol E” was previously given to a member of the desmethylxanthohumol series. Therefore, it is proposed that the name “XH F” be reserved for the true XH analogue (5-O-Me) of this compound. In cases where a new compound is a simple derivative or XH or one of its named derivatives, we suggest that the compounds be named to reflect this relationship. The 1'',2'' dihydro derivative of the XH C has been isolated, e.g., and rather than adding another letter to the XH series, the compound was simply named 1'',2''-dihydroxanthohumol C (inset below).

et al., 2002). It is a potent ligand for the ER $\alpha$  with an IC<sub>50</sub> value in the nanomolar range (Kitaoka et al., 1998), it stimulates the production of alkaline phosphatase in Ishikawa cells (Milligan et al., 1999), and stimulates the growth of estrogen-dependent MCF7 (Kitaoka et al., 1998) breast cancer cells. It was reported that 8PN has a greater affinity for the ER $\alpha$  where it is 70-fold less potent than estradiol than for ER $\beta$ , reported as 20,000-fold less potent than estradiol (Schaefer et al., 2003). Kitaoka et al. (1998) synthesized racemic 8PN, separated and assayed the enantiomers, and reported that there was no significant difference in ER binding potency between the 2R and 2S forms.

### Sedative activity

Hops are referred to in the herbal literature as having various forms of sedative activity (Blumenthal, 2000; Culbreth, 1927; Fluckiger and Hanbury, 1879; Gathercoal and Wirth, 1936; Greenish, 1909; Maisch, 1892; Meyer, 1960; Millspaugh, 1974; Schleif and Galludet, 1907; Washburn and Blome, 1927; Wilcox, 1912; Youngken, 1946). Hop preparations continue to be recommended by proponents of herbal medicine for similar indications as in the past (Blumenthal, 2000; Schulz et al., 2001). Sleep disturbances are associated with the climacteric (Polo-Kantola et al., 1998, 2001), and it is possible that the sedative properties of hops can benefit people suffering from such complaints. Wilcox noted, “hops lose their active properties on keeping” (Wilcox, 1912), while Youngken asserted that they are “unfit for use in pharmacy when they develop a valeric acid odor” (Youngken, 1950).

Reports have indicated that preparations of hops have sedative-like activity in frogs (Munch et al., 1933; Staven-Groenberg, 1928; Steidle, 1932), pigeons (Sikorski and Rusiecki, 1938), mice (Lee et al., 1993; Prokopenko et al., 1986), goldfish (Bouchardy, 1953), and golden carp (Grumbach and Mirimanoff, 1955). An understanding of the biochemical mechanism and the conclusive identification of compounds responsible for such activity have not yet been achieved. Sikorski and Rusiecki (1938) reported that both humulone and lupulone were “strongly sedative to pigeons and small birds, somewhat less active on mice”. A degradation product of these bitter acids, the five-carbon olefinic alcohol 2-methyl-3-buten-2-ol, was shown to be sedative in mice (Wohlfart et al., 1982). The oft-cited claim is that hops lose their activity with age (Rusiecki, 1938; Youngken, 1950). Wohlfart et al. (1982) showed that 2-methyl-3-buten-2-ol is nearly undetectable in fresh hops and reaches its maximum concentration after 2 years of storage at room temperature. Thus, if this compound can fully explain the sedative activity attributed to this plant, then it must be formed *in vivo* from hop

constituents, such as bitter acids, that would then be considered as “pro-drugs”, analogous to the case of the estrogenic activity. Hänsel and Wohlfart (1980) asserted that the sedative effect of hops was not due to its content of myrcene, which was since shown to have analgesic activity in mice (Lorenzetti et al., 1991; Rao et al., 1990). Despite numerous attempts to characterize the CNS-active constituents in hops, it is believed that the identity of all of the compounds responsible for sedative activity in humans, much less their biochemical mode of action, have yet to be established (Schulz et al., 2001).

### Other activities

Numerous other studies have been published citing hops to be active in specific bioassays. Hop extracts and/or compounds have been reported to be “active” or the equivalent by the authors of the given studies in the following assays: various antioxidant and/or chemoprevention (Gerhäuser et al., 2001, 2002; Liegeois et al., 2000; Masaki et al., 1995; Oyaizu et al., 1994; Stevens et al., 2002; Tagashira et al., 1995), antimicrobial, particularly against Gram positive bacteria (Grange and Davey, 1990; Langezaal et al., 1992; Matos et al., 2001; Ohsugi et al., 1997; Racz et al., 1980; Simpson and Smith, 1992; Tagashira et al., 1997) and cytotoxicity (Bae et al., 1996; Liu et al., 2001; Miranda et al., 1999; Shipp et al., 1994). Many constituents have been reported from hops (Farnsworth, 2003), among which in addition to the aforementioned estrogenic properties, many have been shown to possess other types of biological activity (Honma et al., 1998; Shipp et al., 1994; Tabata et al., 1998; Tobe et al., 1995; Tobe et al., 1997a, b; Yasukawa et al., 1995) such as potential cancer chemopreventive activity (Gerhäuser et al., 2001a; Miranda et al., 2000a) and suppression of COX-2 gene transcription (Yamamoto et al., 2000). The major chalcone XH, as well as the flavanones IX, and 6PN and 8PN show various forms of antioxidant activity *in vitro* in the micromolar range, and are weakly cytotoxic to certain cancer cell lines (Gerhäuser et al., 2001a, b; Milligan et al., 1999; Miranda et al., 2000a, b). In addition, the metabolism of prenylated chalcones and flavanones from hops *in vitro* and *in vivo* has been investigated (Nikolic et al., 2004; Nookandeh, 2004; Yilmazer et al., 2001a, b).

### Phytotherapy

Among the 73 drugs listed in section 48A (Hypnotic/sedative plant drugs) of the German Red Book (1985), 43 are formulated with hops (59%). A majority of published work on the pharmacological activity of well-defined hop preparations concerns formulations intended for their sedative properties (Gerhard et al.,

1996; Muller-Limmroth and Ehrenstein, 1977; Schmitz and Jäckel, 1998; Sun, 2003; Vonderheid-Guth et al., 2000). These studies invariably contain extracts of other plants, particularly valerian (*Valeriana officinalis* L.). Only one clinical trial specifically related to the estrogenic properties of hops has been identified (Goetz, 1990). While the author reported that the formulation (which also contained an extract of an unspecified *Crataegus* spp.) was effective in treating hot flashes, it was neither chemically nor biologically standardized by any modern standards. In another study, a morning/evening regime of polyherbal blends was clinically evaluated, where hops were a component of the evening dosing (Sun, 2003). Because the clinical formulations contained other herbs that may or may not contribute to any estrogenic activity (which was not in either case demonstrated *in vitro*), no meaningful information regarding the potential clinical efficacy of estrogenic formulations of hops can be extrapolated from these studies.

## Conclusions

Preparations of hops that contain 8PN must be considered “estrogenic”. It still is open to debate, however, whether or not they can have beneficial hormonal activity when consumed orally by humans. Whereas there are numerous animal studies and some clinical evidence that hops may contribute to the therapeutic efficacy of certain polyherbal sedative preparations, no randomized, double blind, placebo-controlled clinical trials have been identified that would support the internal use of hops for their estrogenic properties. Considering that sleep disturbances are frequently associated with menopause (Polo-Kantola et al., 1998, 2001), and that hops are not only estrogenic but also used traditionally as a sedative, it is a reasonable hypothesis that properly formulated hop preparations can have a rational place in modern medicine. In order to test this ultimate hypothesis, a dosage form must be produced. However, no officially recognized standards exist yet for estrogenic formulations of hops. It is stressed that the estrogenic principle in hops (8PN or hopein) is formed spontaneously from the chalcone DMX. This fact should be borne in mind when producing a standardized estrogenic formulation of hops. The key compounds to be monitored in the chemical standardization process are: XH (major compound/ chemopreventive), IX (weak estrogen/stability marker), DMX (proestrogen), and 8-prenylnaringenin (estrogen/stability marker).

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