

Estrogenic activity of hop components

Status review

M. Biendl, Mainburg

This article reports on the current status of research and discusses possible consequences for beer.

In 1953, hormones were isolated from hops for the first time (1) though the active component was not definitively identified until 1999 (2). This compound was 8-prenylnaringenin (Fig. 1). The compound is not exclusive to hops. In 1998, it was isolated for the first time by a Japanese group from a tree species indigenous to Thailand (3). The estrogenic effect has meantime been proven in a multiplicity of tests both "in vitro" (2, 3, 4, 5, 6) and "in vivo" (5, 6, 7). 8-prenylnaringenin is one of the most effective phytoestrogens hitherto isolated from plants.

Effect of phytoestrogens

Phytoestrogens are compounds occurring in plants which, by interaction with the estrogen receptor, can imitate or block the effect of the female 17- β -estradiol sexual hormone and thus influence the endocrine system.

In medicine, phytoestrogens are used in hormone replacement therapy for treatment of menopause complaints and prevention of osteoporosis once endogenous 17- β -estradiol production decreases.

Moreover, generally positive attributes for prevention of chronic diseases are postulated. This assumption is primarily based on epidemiological investigations. Compared to Western countries, Asia e.g. Japan has a lower rate of chronic diseases such as cancer and cardiovascular problems. This is ascribed to the different diet. In Japan, with a diet traditionally rich in soy, up to 100 mg of phytoestrogens per day are ingested whereas intake is less than 1 mg per day in Western countries.

New scientific findings on estrogenic activity of hops are being published regularly over the last three years. This article reports on the current status of research and discusses possible consequences for beer.

Soy contains relatively high concentrations of the known phytoestrogen genistein (Fig. 1).

Phytoestrogens are also regarded as having negative connotations. A possible influence on reproductivity is being discussed. First indications stem from having observed a decidedly reduced fertility in Australian sheep after consumption of a specific clover variety. One of the causes identified was the coumestrol substance (Fig. 1). At the beginning of the 90s, it was reported that male sperm production had supposedly halved in recent decades. It is still discussed controversially whether male sperm counts actually decreased (it is, among other things, pointed out that

computer-based counting methods common nowadays generally yield lower counts than those determined under a microscope heretofore) and what role phytoestrogens have in this process. Even a positive effect on fertility cannot be excluded for the time being.

Determination of estrogen activity

The studies of Koch and Heim (1) gave hops the reputation of being "the plant richest in estrogen in our latitudes". Since that time, many groups of researchers have tried to identify the estrogen-active components. This effect has been ascribed to the following substances: β -acids, β -sitosterol, o-hydroxy dibenzoyl methane, xanthohumol, desmethyl xanthohumol.

Many of these results have meantime been challenged. E.g. in recent times, no estrogen effects were found by recognised biological test methods for xanthohumol (4, 6, 8) and also for its conversion product isoxanthohumol (4, 6) formed during wort boiling in the brewing process. 8-prenylnaringenin is currently recognised as being the estrogen-active component of hops.

Biological estrogenic activity is initiated in the target cells e.g. mammary gland or uterus by highly affine receptor proteins. For testing a substance "in vitro", such receptor cells are isolated and then the interactions compared to 17- β -estradiol measured in so-called "receptor tests" or "DNA bonding assays" (Fig. 1). Validation is car-

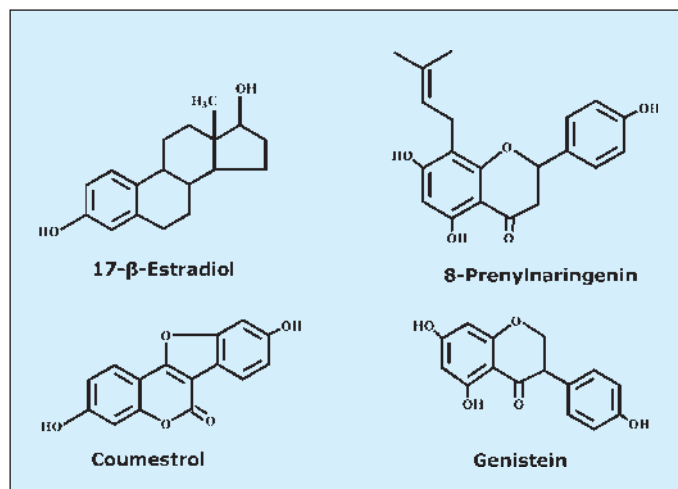


Fig. 1
Compounds with an estrogenic effect

Author: Dr. Martin Biendl, Hallertauer Hopfenveredelungsges.mBH (HVG), Auhofstr. 16, D-94048 Mainburg

Concentrations (% , air-dry) of 8-prenylnaringenin and xanthohumol in various varieties of hops (9, 12)

Hop variety	8-prenylnaringenin	Xanthohumol
Hallertau Magnum	0.0050 %	0.46 %
Hallertau Taurus	0.0045 %	0.95 %
US Galena	0.0026 %	0.35 %
Wye Challenger	0.0058 %	0.32 %

ried out by so-called “reporter gene assays” in which the activity of receptor enzymes is determined. In summarising the outcomes of the various “in vitro” tests hitherto published (2, 3, 4, 5, 6), 8-prenylnaringenin has proven to be more active than the known phytoestrogens coumestrol or genistein (factor 10 - 100). All studies also concurred that the effect of 8-prenylnaringenin is clearly lower than that of 17- β -estradiol (factor about 100).

The effect of 8-prenylnaringenin has been confirmed in several animal trials. The most frequently used test methods for “in vivo” determination of active estrogen agents are based on changes in the uterus or vagina of female rats and mice. After subcutaneous dosing of 30 mg/kg/day, rats showed an effect corresponding to a dose of 0.01 mg/kg/day of 17- β -estradiol (7). Accordingly, 8-prenylnaringenin proved to be less effective than 17- β -estradiol by a factor of 3000 in this “in vivo” trial. Another trial involving rats (6) showed a difference in activity of about 20,000 (6). A trial involving mice (5) concluded that typical estrogenic effects arise as of oral ingestion of about 15 mg of 8-prenylnaringenin per kg body weight and day, the substance having been administered in drinking water in a concentration of 100 mg/l.

■ Occurrence of 8-prenylnaringenin in hops and beer

8-prenylnaringenin levels in hops are very low. The concentration is below 0.01% and thus e.g. about 100 times lower than that of xanthohumol. There appear to be differences in 8-prenylnaringenin levels depending on hop variety but very little material has been published on this subject. Results have been published only by Rong et al. (9). 8-prenylnaringenin, as a prenylflavonoid, as well as xanthohumol is exclusively found in the lupulin glands of the hop cone and not in the leaves. The table compares the levels of 8-prenylnaringenin and xanthohumol for a number of selected hop varieties.

Due to the low concentrations, analysis of 8-prenylnaringenin is very time and labour intensive. Methods hitherto published are based on analysis by means of

GC MS (10) or HPLC MS (9, 11) while e.g. xanthohumol can be analysed much more readily using HPLC UV (12).

There appears to be no doubt that hops is the only source making a contribution to 8-prenylnaringenin present in beer. Tracking the quantity of 8-prenylnaringenin dosed with hop pellets (variety: Hallertau Magnum), *Teke* et al. (10) noted a yield of 17% in the finished beer. These authors also report on analyses of a number of beer types (17 Belgian beers, 12 beers from other countries, 3 test beers). They found a maximum 8-prenylnaringenin concentration of 0.02 mg/l. Investigations by Rong et al. (9) of 8 different Belgian beers (6 ales, 2 lagers) showed a maximum value of 0.02 mg/l. *Stevens* et al. (11) determined a level of 0.24 mg/l in an American porter from a US microbrewery whereas beers from larger breweries (4 US major brands, 4 imported beers) had clearly lower concentrations (0.07 mg/l in an imported stout, the remainder below 0.02 mg/l).

■ Has beer estrogenic activity?

If one links the very simple comparison between the value of 0.24 mg/l maximum concentration hitherto found in beer and the effective quantities of 15 and 30 mg of 8-prenylnaringenin per day and kg of body weight determined in animal trials, it would follow that humans would have to consume more than 1000 l of beer daily to achieve detectable estrogenic effects.

The outcome of these deliberations is largely in line with conclusions drawn by *Sauerwein* and *Meyer* in their studies (13) published in 1997 which involved a substrate-specific determination of estrogenic activity of beer. In this test, the substances with estrogenic activity were not singled out through structure-specific properties of the substances in question (the compound 8-prenylnaringenin had not yet been identified at that time), but through the potency of the sample as a whole (or an extract recovered from same) to dock at an estrogen receptor. The concentrations of substances with estrogenic activity found in the samples were given as 17- β -estradiol equivalents. A maximum value of 2 μ g/l was established for beer. *Sauerwein* and *Meyer*

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concluded: "For evaluation ... the daily dose of about 2 mg of estradiol-17- β effective in humans should be used as a basis: In order to get to this, about 1000 l of beer would have to be consumed daily. From this it follows that the presence of substances with estrogenic activity in beer and its raw materials can be detected analytically but the concentrations found are far below anything which would lead one to expect a hormonal effect in consumers."

Promberger et al., in their studies published in 2001 (14) on substrate and structure specificity determined by receptor tests, came to even lower 17- β -estradiol equivalents per litre of beer than Sauerwein and Meyer. A maximum value equivalent to 0.043 μ g of 17- β -estradiol per litre of beer was determined in a range of Austrian lager beers.

It can be concluded from these investigations that the estrogenic activity of hops, from an objective point of view, does not support any reservations about negative effects on beer. Reports such as on "feminisation" of beer drinkers cannot be proven scientifically.

But the following aspect is of significance: In Asia, up to 100 mg of phytoestrogens is ingested every day without observing negative concomitant phenomena (e.g. on reproductivity). On the contrary, positive effects on health are noted. With this in mind, consumption of beer could be regarded as a means of increasing ingestion of phytoestrogen which, at only about 1 mg a day, is low in Western populations compared to Asia. This is called for by some doctors and dieticians. It might thus even be conceivable that, in future, increasing 8-prenylnaringenin levels could become an objective in developing new beer types. Before implementing such strategies and campaigns, it is advisable to wait for further results from very active current research on biological activity of 8-prenylnaringenin before a definitive profile of the activity of the substance can be established.

Summary

Phytoestrogens, compounds occurring in plants, can have an influence on the human hormone system due to an interaction

with the receptor for 17- β -estradiol. As a result, positive effects on health are being postulated (prevention of cancer, osteoporosis and cardiovascular diseases, use in hormone replacement therapy) and negative effects (e.g. on reproductivity) discussed. Hops contains 8-prenylnaringenin, one of the most effective phytoestrogens hitherto known. However, the levels, less than 0.01 %, are very low. Traces of 8-prenylnaringenin found in beer can be traced back to hop raw materials. Most samples analysed had concentrations below 0.02 mg/l. Conclusions drawn from studies of various groups of researchers are in agreement that beer consumption has no hormonal effect, despite the presence of the low concentration of components with an estrogenic activity.

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