# Inhibition of 17β-Hydroxysteroid Oxidoreductase by Flavonoids in Breast and Prostate Cancer Cells (44237)

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*Abstract.* **Several flavonoids and isoflavonoids were found to inhibit 17β-oxidoreduction of estrogens by the purified 17β-HSOR type 1, or in cell lines expressing 17β-HSOR type 1 enzyme (T-47D breast cancer cells) or type 2 (PC-3 prostate cancer cells). The structural demands for the inhibition of estrone ($E_1$) reduction and estradiol ($E_2$) oxidation catalyzed by 17β-HSOR types 1 and 2, respectively, were not identical. Flavones, flavanones, and isoflavones hydroxylated at both the double ring (positions 5 and 7) and ring B (position 4′) were the most potent inhibitors of $E_1$ reduction in T-47D cells, and by the purified type 1 enzyme whereas flavones hydroxylated at positions 3, 5, and 7 of rings A and C, with or without a hydroxyl group in ring B, were capable of inhibiting $E_2$ oxidation in PC-3 cells. Change to flavanone structure, or hydroxylation at position 3 of ring C of flavones, or methylation of the hydroxyl group at position 4′ of ring B of flavones and isoflavones reduced or abolished their inhibitory activity on $E_1$ reduction in T-47D cells. On the contrary, hydroxyl group at position 3 of flavones (flavonol structure) markedly increased the inhibition of $E_2$ oxidation in PC-3 cells. Thus, changes in the number and location of hydroxyl groups may discriminate inhibition of $E_1$ reduction and $E_2$ oxidation. Some of the differences may be due to differences in pharmacokinetics of these compounds in T-47D and PC-3 cells. Inhibition of 17β-HSORs could lead to an alteration in the availability of the highly active endogenous estrogen, but the effects of these compounds *in vivo* cannot be predicted on the basis of these results alone. Some of these compounds (isoflavones) are estrogenic *per se,* and they may replace endogenous estrogens, whereas flavones are only very weakly estrogenic or nonestrogenic. Regarding prevention or treatment of estrogen-related diseases, apigenin, coumestrol, and genistein raise special interest.** [P.S.E.B.M. 1998, Vol 217]

The intake of weakly estrogenic isoflavonoids (phytoestrogens) is high in countries with a low incidence of estrogen-related cancers, such as breast and prostate cancer (1, 2). Phytoestrogens have been suggested to act as antiestrogens by competing with the more potent endogenous estrogens for the binding sites of estrogen receptor (ER) and to block the transactivation of estrogen responsive genes by 17β-estradiol ($E_2$). However, there is very little evidence to support this theory. Dietary estrogens representing structurally different groups (coumestans, isoflavonoids, and resorcylic acid lactones), have been shown to interact with estrogen receptor (ER) and transactivate estrogen-responsive genes (3–7). The compounds may have additive effects with $E_2$, and none of them reduced the proliferation rate of cultured breast cancer cells at concentrations below 1 $\mu M$ (3). In addition, dietary estrogens and structurally related compounds may compete with endogenous estrogens for the active site of the estrogen biosynthesizing and metabolizing enzymes resulting in altered concentrations of biologically active endogenous estrogens (8–11).

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The 17β-hydroxysteroid oxidoreductases (17β-HSORs, also known as 17β-hydroxysteroid dehydrogenases) are some of the key enzymes in estrogen biosynthesis and metabolism. The reversible reaction between a weak estrogen, $E_1$ (oxidized form) and a highly active estrogen, $E_2$ (reduced form), is catalyzed by several 17β-HSORs, expressed selectively in both steroidogenic cells as well as in some target tissues of estrogen action. Human 17β-HSOR type 1 enzyme is highly specific for estrogens and favors the reductive reaction from $E_1$ to $E_2$, and is the isoform involved in glandular $E_2$ synthesis both in humans and in rodents (12). In addition to the steroidogenic tissues, ovary and placenta, type 1 enzyme is also expressed in some estrogen target tissues, such as breast, endometrium, and prostatic urethra, in both females and males (13–17). In contrast, type 2 enzyme prefers oxidation of the active 17-hydroxy form of estrogens and androgens into their less potent 17-keto forms, and is present in both reproductive and nonreproductive organs. It is highly expressed in placenta, liver, and small intestine, and to a lesser extent in secretory endometrium, prostate, kidney, pancreas, and colon (18–20).

Both 17β-HSOR type 1 and type 2 belong to the family of short-chain alcohol dehydrogenases. The family includes both eukaryotic and prokaryotic proteins involved in the metabolism of steroids, prostaglandins, and antibiotics, as well as the flavonoid binding NodD proteins in nitrogen-fixing *Rhizobia.* Therefore, it is likely that NodD proteins and 17β-HSORs are homologs, derived from a common ancestor (21–23). Flavonoids, secreted by plants and bound to NodD proteins, are the signals by which the host plant regulates the nodulation by *Rhizobia* in plant roots. In addition, flavonoids structurally resemble the steroidal estrogens, and have been suggested to compete with mammalian sex steroids as substrates to 17β-HSORs (11, 24).

We have earlier shown that two phytoestrogens, coumestrol and genistein, and some structurally related compounds are capable of inhibiting the reduction of $E_1$ catalyzed by 17β-HSOR type 1 enzyme *in vitro* (10). However, it is possible that the inhibition could be without practical consequences when the inhibitor is also an estrogen *per se* and could replace endogenous estrogens. For instance, genistein, at concentrations only slightly below those in the serum of soy-consuming individuals, both enhanced the proliferation of estrogen-sensitive breast cancer cells and inhibited the reduction of $E_1$. Therefore, we have now further tested the structural demands for 17β-HSOR inhibition by flavonoids and isoflavonoids in order to determine the structural properties discriminating between enzyme inhibition and estrogenicity. In addition, we tested the isoform specificity of the enzyme inhibition. The inhibition of $E_1$ reduction, or $E_2$ oxidation by 17β-HSOR type 1 and type 2, respectively, was measured using the purified 17β-HSOR type 1 enzyme, and cell lines expressing 17β-HSOR type 1 or type 2 enzyme.

## Materials and Methods

**Flavonoids and Isoflavonoids.** The test compounds were purchased from the following sources: apigenin (4′,5,7-trihydroxyflavone), biochanin A (5,7-dihydroxy-4′-methoxyisoflavone), chrysin (5,7-dihydroxyflavone), kaempferol (3,4′,5,7-tetrahydroxyflavone), and quercetin (3,3′,4′,5,7-pentahydroxyflavone) from Sigma Chemical Co. (St. Louis, MO); genistein (4′,5,7-trihydroxyisoflavone) from Gibco Life Technologies Inc. (Gaithersburg, MD); daidzein (4′,7-dihyroxyflavone) from Research Biochemicals International (Natick, MA); acacetin (5,7-dihydroxy-4′-methoxyflavone), catechin (3,3′,4′,5,7-flavan pentol), flavone, flavanone, 6-hydroxyflavone, 7-hydroxyflavone, 4′-hydroxyflavanone, fisetin (3,3′,4′,7-tetrahydroxyflavone), formononetin (7-hydroxy-4′-methoxyisoflavone), galangin (3,5,7-trihydroxyflavone), kaempferide (3,5,7-trihydroxy-4′-methoxyflavone), luteolin (3′,4′,5,7-tetrahydroxyflavone), naringenin (4′,5,7-trihydroxyflavanone), and pinostrobin (5-hydroxy-7-methoxyflavone) from Carl Roth GmbH (Karlsruhe, Germany); coumestrol (2-(2,4-dihyroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid δ-lactone) from Eastman Kodak (Rochester, NY). The chemical structures of the test compounds are shown in Figure 1.

**Measurement of Reduction of Estrone by Purified Human 17β-HSOR Type 1 Enzyme.** The measurements were performed as described previously (16). Briefly, the final volume of the assay medium was 250 μl, consisting of 100 μl of purified enzyme in 50 m*M* phosphate buffer, pH 7.4, with 2 m*M* dithiotreitol (DTT), 1 m*M* EDTA and 20% glycerol, 150 μl of NADPH generating system and 3 μ*M* of test compound. After a 20-min preincubation at 37°C, 3 μCi of [$^3$H]-labeled $E_1$ (specific activity 98 Ci/mmol, Amersham International, Buckinghamshire, UK) was added to a final concentration of 0.72 μ*M,* and incubation was continued for 60 min. After incubation, unlabeled $E_2$ and $E_1$ were added as carriers. The steroids were extracted with methylene chloride and dissolved in the mobile phase. $E_1$ and $E_2$ were separated on a C18 column connected to an HPLC system equipped with an on-line beta-counter. Acetonitrile/water (35/65) was used as a mobile phase, and the flow rate was 1.2 ml/min. Reductase activities were calculated as percentages of [$^3$H]-$E_1$ converted to [$^3$H]-$E_2$. The amount of enzyme in each assay was adjusted so that the conversion of the substrate to product was 5%–30% during incubation. Product formation was proportional to incubation time up to 2 hr. In addition to $E_1$ and $E_2$, no other metabolites (17α-estradiol, 17β-estriol, 2-hydroxy- or 4-hydroxyl derivatives of $E_1$ or $E_2$) were detected. All reference steroids were purchased from Steraloids, Inc. (Wilton, NH).

**Measurement of Estrone Reduction in Cultured T-47D Cells and Estradiol Oxidation in PC-3 Cells.** T-47D breast cancer cells were kindly provided by Dr. Pirkko Härkönen, University of Turku, Turku, Finland.

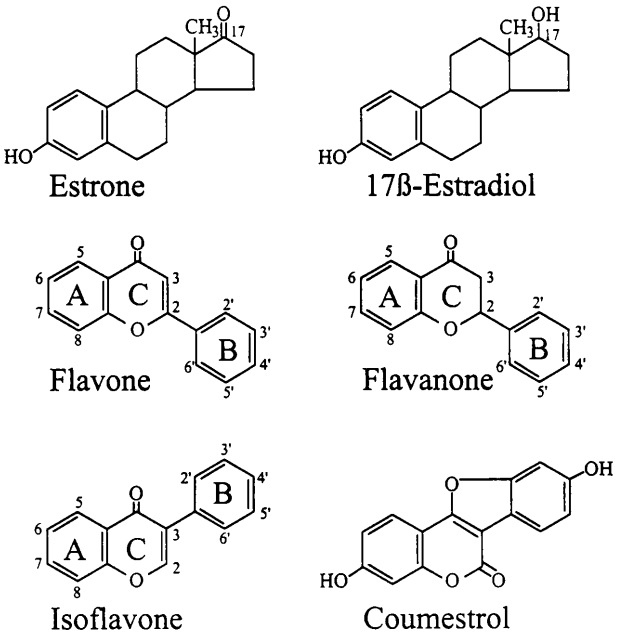


**Figure 1.** Chemical structures of estrone and classes of flavone and flavanone derivatives, isoflavones, and coumestans used in this study. Flavone derivatives: flavone, 6-hydroxyflavone, 7-hydroxyflavone, chrysin (5,7-dihydroxyflavone), pinostrobin (5-hydroxy-7-methoxyflavone), apigenin (4′,5,7-trihydroxyflavone), acacetin (5,7-dihydroxy-4′-methoxyflavone), galangin (3,5,7-trihydroxyflavone), luteolin (3′,4′,5,7-tetrahydroxyflavone), kaempferol (3,4′,5,7-tetrahydroxyflavone), kaempferide (3,5,7-trihydroxy-4′-methoxyflavone), fisetin (3,3′,4′,7-tetrahydroxyflavone) and quercetin (3,3′,4′,5,7-pentahydroxyflavone). Flavanone derivatives: flavanone, 4′-hydroxyflavanone, naringenin (4′,5,7-trihydroxyflavanone), and catechin (3,3′,4′,5,7-flavan pentol). Isoflavones: daidzein (4′,7-dihydroxyflavone), formononetin (7-hydroxy-4′-methoxyisoflavone), genistein (4′,5,7-trihydroxyisoflavone), and biochanin A (5,7-dihydroxy-4′-methoxyisoflavone). Coumestans: coumestrol (2-(2,4-dihyroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid δ-lactone).

PC-3 prostate cancer cells were purchased from American Type Culture Collection (Rockville, MD). Stock cultures were grown in phenol red-free Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Gaithersburg, MD) with antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml, Gibco BRL). The medium was supplemented with 10% fetal calf serum (FCS, Gibco BRL), and for T-47D cells also with 7.5 μg/ml of insulin and 1 n*M* 17β-estradiol, both from Sigma, (St. Louis, MO).

The enzyme activity in T-47D breast cancer cells or PC-3 prostate cancer cells was assessed as described previously (3) by determining the ability of intact monolayer cultures to convert the applied [$^3$H]-$E_1$ to [$^3$H]-$E_2$, or *vice versa* For each experiment, stock cultures of the cells were harvested by trypsinization and seeded on culture dishes (60 × 15 mm, 2 × $10^5$ cells per dish). Thereafter, the cells were cultured for 5 days in phenol red-free DMEM including antibiotics and 5% dcFCS. Medium for T-47D cells was also supplemented with 7.5 μg/ml of insulin. The medium was changed every other day. On the fifth day, growth medium was removed and 3 ml of serum free DMEM containing tested compounds was added. Control dishes, containing the vehicle alone, were run in parallel in each experiment. After a 1-hr preincubation with the test compound, [$^3$H]-labeled substrate (final concentration 2 n*M*) and nonlabeled substrate (final concentration 2 n*M*) were added, and the dishes were incubated for 4 hr. $E_1$ and $E_2$ were used as the substrates for T-47D and PC-3 cells, respectively. After incubation, 2 ml of medium was used for extraction of the steroids, and the relative amounts of $E_1$ and $E_2$ were analyzed as described above.

## Results

**Estrone Reduction Catalyzed by 17β-HSOR Type 1.** At 1.2 μ*M* concentration, several flavones and flavanones were found to inhibit $E_1$ reduction catalyzed by the purified 17β-HSOR type 1 enzyme (Table I). The most active compounds were those substituted at both rings A and B in positions 4′, 5, and 7, with or without a hydroxyl group in position 3 of ring C. Those compounds were apigenin, acacetin, naringenin, kaempferol, kaempferide, and galangin. Of all flavonoids tested, the 4′-methylated derivatives of apigenin and kaempferol, namely acacetin (5,7-dihydroxy-4′-methoxyflavone) and kampferide (3,5,7-trihydroxy-4′-methoxyflavone), were the most potent inhibitors of the purified type 1 enzyme.

In cultured T-47D cells, apigenin (4′,5,7-trihydroxyflavone), was the most effective flavonoid, showing a significant inhibition at both 1.2 and 0.12 μ*M* concentrations. As compared with the flavone, the flavanone structure (i.e., absence of the double bond between carbons 2 and 3), present in naringenin, reduced inhibition in T-47D cells, but not in assays with the purified enzyme. Methylation of the hydroxyl group at position 4′ of ring B (acacetin) also reduced inhibition in cultured cells, but not with the purified enzyme. Furthermore, absence of this hydroxyl group in chrysin, pinostrobin, fisetin, and galangin, made these compounds less inhibitory in T-47D cells. An additional hydroxyl group at position 3 of ring C (flavonol structure), present in kaempferol, kaempferide, galangin, or at position 3′ of ring B (luteolin and fisetin), further reduced the inhibition of $E_1$ to $E_2$ conversion in cultured T-47D cells and to a lesser extent also when the purified 17β-HSOR type 1 enzyme was used.

Flavone, flavanone, 6-hydroxyflavone, 7-hydroxyflavone, and 4′-hydroxyflavanone did not show any inhibition of $E_1$ to $E_2$ conversion in T-47D cells, and, with the exception of 7-hydroxyflavone, identical results were also obtained with the purified type 1 enzyme. Quercetin, a pentahydroxyflavone substituted at positions 3, 3′, 4′, 5, and 7, or the corresponding flavan pentol, catechin, showed no inhibition in either of the test systems used.

Several isoflavones were also tested. In line with our previous results (11), 1.2 μ*M* concentration of genistein (4′,5,7-trihydroxyisoflavone) significantly inhibited $E_1$ re-

**Table I.** Effect of Flavone and Flavanone Derivatives on 17β-Oxidoreduction

| Compound | Trivial name | Concentration (μM) | Conversion of [$^3$H]estrone to [$^3$H]estradiol[a] | | Conversion of [$^3$H]estradiol to [$^3$H]estrone[a] |
|---|---|---|---|---|---|
| | | | Purified 17β-HSOR type 1 | T-47D cells | PC-3 cells |
| A. Flavone derivatives | | | | | |
| Flavone | — | 1.2 | 107.3 ± 19.6 | 111.6 ± 16.1 | nd[b] |
| 6-hydroxyflavone | — | 1.2 | 95.6 ± 17.9 | nd | nd |
| 7-hydroxyflavone | — | 1.2 | 69.4 ± 5.2** | 103.3 ± 25.2 | nd |
| 5,7-dihydroxyflavone | Chrysin | 1.2 | 96.0 ± 23.9[c] | 79.8 ± 1.4*** | 75.7 ± 9.2*** |
| 5-hydroxy-7-methoxyflavone | Pinostrobin | 1.2 | 93.1 ± 11.0 | 77.9 ± 13.8 | nd |
| 4′,5,7-trihydroxyflavone | Apigenin | 1.2 | 57.7 ± 18.4*** | 22.5 ± 5.1*** | 84.4 ± 8.7*** |
| | | 0.12 | 54.2 ± 6.4** | 84.4 ± 6.6* | 94.7 ± 3.4 |
| | | 0.012 | 73.3 ± 1.2** | 105.0 ± 5.0 | 99.3 ± 6.0 |
| 5,7-dihydroxy-4′-methoxyflavone | Acacetin | 1.2 | 38.5 ± 3.8*** | 58.1 ± 7.2** | 74.9 ± 12.5*** |
| 3,5,7-trihydroxyflavone | Galangin | 1.2 | 59.1 ± 6.9*** | 109.7 ± 18.0 | 49.2 ± 5.2*** |
| 3′,4′,5,7-tetrahydroxyflavone | Luteolin | 1.2 | 111.4 ± 14.6 | 59.8 ± 5.9*** | 91.5 ± 14.5 |
| 3,4′,5,7-tetrahydroxyflavone | Kaempferol | 1.2 | 58.0 ± 20.8*** | 83.2 ± 10.5*** | 27.0 ± 5.4*** |
| | | 0.12 | 79.2 ± 10.5** | 98.4 ± 7.0 | nd |
| | | 0.012 | 137.8 ± 5.6 | 96.1 ± 7.2 | nd |
| 3,5,7-trihydroxy-4′-methoxyflavone | Kaempferide | 1.2 | 26.7 ± 7.6*** | 85.2 ± 14.3 | 32.0 ± 6.3*** |
| 3,3′,4′,7-tetrahydroxyflavone | Fisetin | 1.2 | 47.2 ± 9.4*** | 86.4 ± 6.4* | nd |
| 3,3′,4′,5,7-pentahydroxyflavone | Quercetin | 1.2 | 123.0 ± 11.8[c] | 106.0 ± 2.6[b] | nd |
| B. Flavanone derivatives | | | | | |
| Flavanone | — | 1.2 | 103.9 ± 24.8 | nd | nd |
| 4′-hydroxyflavanone | — | 1.2 | 101.9 ± 17.3 | nd | nd |
| 4′,5,7-trihydroxyflavanone | Naringenin | 1.2 | 53.0 ± 11.2*** | 67.5 ± 9.6*** | 88.7 ± 7.3 |
| 3,3′,4′,5,7-flavan pentol | Catechin | 1.2 | 120.6 ± 26.7 | nd | 98.1 ± 5.2 |

[a] Mean ± SD, control = 100.
[b] nd, not determined.
[c] Data reported earlier (11), concentration 1 μM.
*,**,***, differs significantly from control, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively (Student's $t$ test with Bonferroni correction).

duction catalyzed by the purified enzyme, and similar results were also obtained in cultured T-47D cells. Absence of the hydroxyl group at position 5, in daidzein, decreased the inhibition measured in T-47D cells, but not in assays with the purified type 1 enzyme. Similarly to that reported previously (11), biochanin A and formononetin, the methylated derivatives of genistein and daidzein, did not show any inhibition in the presence of the purified type 1 enzyme and were only very slightly inhibitory in T-47D cells. Effects of isoflavones are summarized in Table II.

**Estradiol Oxidation Catalyzed by 17β-HSOR Type 2.** Of the tested flavones, the compounds hydroxylated at both rings A and C at positions 3, 5, and 7, such as kaempferol, kaempferide, and galangin, significantly reduced $E_2$ oxidation at 1.2 μM concentration. The hydroxyl group at position 3 appeared to be critical, and its absence in chrysin, apigenin, acacetin, and luteolin decreased or completely abolished the inhibition. A hydroxyl or methoxyl group at position 4′ of ring B of flavone had no effect on the inhibitory properties of the compounds. Effects of flavones are summarized in Table I. Interestingly, genistein and coumestrol were not active in PC-3 cells (Table II).

## Discussion

In the present study we tested several hydroxylated and methoxylated flavonoids and isoflavonoids in order to determine the structural demands for the inhibition of 17β-oxidoreduction of estrogens. To estimate the enzyme specificity, we measured the inhibition of $E_1$ reduction and $E_2$ oxidation catalyzed by 17β-HSOR type 1 and type 2, respectively.

Several flavonoids and isoflavonoids were found to inhibit 17β-oxidoreduction, but the structural demands for the inhibition of $E_1$ reduction by 17β-HSOR type 1 enzyme and $E_2$ oxidation by 17β-HSOR type 2 enzyme were not identical. Changes in the number and location of hydroxyl groups of the compounds appeared to discriminate inhibition of these two enzymes. Our results indicate that hydroxylation at positions 5 and 7 is required for the inhibition of both enzymes studied, while substitution (hydroxylation or methylation) at position 4′ of ring B is critical for the inhibition of type 1 enzyme only. Furthermore, the flavonol structure (i.e., hydroxylation at position 3 of ring C) appears to have opposite effects on these two enzymes. This substitution significantly increases the inhibitory effect of the

**Table II.** Effect of Isoflavones and Coumestrol on 17β-Oxidoreduction

| Compound | Trivial name | Concentration (μM) | Conversion of [$^3$H]estrone to [$^3$H]estradiol[a] | | Conversion of [$^3$H]estradiol to [$^3$H]estrone[a] |
|---|---|---|---|---|---|
| | | | Purified 17β-HSOR type 1 | T-47D cells | PC-3 cells |
| A. Isoflavones | | | | | |
| 4′,7-dihydroxyisoflavone | Daidzein | 1.2 | 68.1 ± 10.3** | 65.6 ± 3.4*** | nd[b] |
| | | 0.12 | 76.3 ± 11.2* | 103.2 ± 5.1 | nd |
| | | 0.012 | 79.0 ± 19.1 | 110.3 ± 5.1 | nd |
| 7-hydroxy-4′-methoxyisoflavone | Formononetin | 1.2 | 97.2 ± 11.2 | 77.6 ± 2.2** | nd |
| 4′,5,7-trihydroxyisoflavone | Genistein | 1.2 | 62.8 ± 29.9***[c] | 39.4 ± 8.4***[c] | 86.9 ± 6.0 |
| | | 0.12 | 90.6 ± 38.2[c] | 88.8 ± 5.2[c] | 90.3 ± 9.7 |
| | | 0.012 | nd | 92.6 ± 10.3[c] | 103.9 ± 16.5 |
| 5,7-dihydroxy-4′-methoxyisoflavone | Biochanin A | 1.2 | 91.3 ± 28.2[c] | 85.4 ± 2.1[c] | nd |
| | | 0.12 | nd | 104.2 ± 2.1[c] | nd |
| | | 0.012 | nd | 104.2 ± 3.6[c] | nd |
| B. Coumestans | | | | | |
| 2-(2,4-dihydroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid δ-lactone | Coumestrol | 1.2 | 18.3 ± 7.5***[c] | 42.1 ± 21.3***[c] | 84.1 ± 2.1 |
| | | 0.12 | 49.0 ± 15.4**[c] | 67.4 ± 10.6***[c] | 96.8 ± 0.6 |
| | | 0.012 | 75.6 ± 27.3[c] | 91.5 ± 4.5[c] | 93.2 ± 0.4 |

[a] Mean ± SD, control = 100.
[b] nd, not determined.
[c] Data reported earlier (11).
*,**,***, differs significantly from control, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively (Student's t-test with Bonferroni correction).

compound on 17β-HSOR type 2, but decreases the effect on type 1 enzyme.

Some of the differences in the effects of the test compounds on $E_1$ reduction versus $E_2$ oxidation observed in the two cell lines may not be due solely to differences in their binding to the active site of the two enzymes. Differences in the chemical structure may also affect the uptake or metabolism in the cells, resulting in different intracellular concentrations. Furthermore, some of our findings in T-47D cells do not correlate with the results obtained using the purified 17β-HSOR type 1 enzyme. This could again be explained, at least partially, by differential metabolism of the compounds and/or ineffective uptake of the tested compounds in the cells. In T-47D cells, the methylation of the hydroxyl substituent in position 4′ of flavones reduced the inhibitory effect (apigenin and kaempferol versus acacetin and kaempferide, respectively), whereas in the presence of the purified enzyme, the opposite was found. For isoflavones, no such difference was observed, and methylation at position 4′ reduced the inhibition of $E_1$ reduction in both test systems.

In the present study, the inhibition of human 17β-HSOR types 1 and 2 has been analyzed. Previous studies have shown that some flavones and isoflavones also inhibit the 17β-oxidation of testosterone to androstenedione in genital skin fibroblasts (9), and similar results have been obtained with a bacterial 3β-hydroxysteroid dehydrogenase (3β-HSD) expressed in *P. testosteronii* (10). The structural demands for inhibition differ significantly from our findings. The most potent inhibitors of the bacterial 3β-HSD were isoflavones daidzein, genistein, biochanin A, and formononetin. Free hydroxyl group in position 7, but not in positions 4′ or 5, as well as isoflavone structure, were reported to be critical for the inhibition of the bacterial 3β-HSD, whereas flavones were considerably less potent (10). In genital skin fibroblasts, several isoflavones and coumestrol were found to inhibit the conversion of testosterone to androstenedione (9). Genistein and biochanin A were more potent than daidzein and formononetin, or coumestrol, which we found to be the most potent inhibitor of the 17β-HSOR type 1 enzyme. This suggests that in the test system used, isoflavone structure as well as the hydroxyl group in position 5 of isoflavone were critical. At the moment, it is not known which isoforms of 17β-HSOR enzymes are involved in the test system described by Evans *et al.* (9), but it is quite unlikely that the estrogen-specific 17β-HSOR type 1 enzyme would play any major role (20). Apparently, flavonoids, isoflavonoids, and coumestans inhibit several different enzymes catalyzing 17β-oxidoreduction of estrogens and androgens, but the structural demands for each enzyme inhibition may be different.

17β-HSOR type 1 is an essential enzyme for the fluctuating secretion of $E_2$ from the developing Graafian follicles of premenopausal women, but the enzyme is also suggested to have a central role in local production of $E_2$ from weak circulating precursors in the peripheral tissues of

postmenopausal women (12, 13, 15, 25, 26). Inhibition of 17β-HSOR type 1 could thus decrease the concentration of $E_2$ both in serum and in the target tissues. As intracellular $E_2$ concentration is one of the key factors regulating estrogen action, a specific 17β-HSOR type 1 inhibitor could be beneficial in the prevention and treatment of estrogen-dependent diseases. In contrast, inhibition of the type 2 enzyme reduces the conversion of $E_2$ and testosterone to their less potent 17β-keto derivatives, could lead to increased or prolonged exposure to highly active sex steroids, which in turn could lead to enhanced growth of estrogen and androgen sensitive tumors. In addition to 17β-HSORs, other important steroid metabolizing enzymes, such as P-450 aromatase and 5α-reductase, have been reported to be inhibited by flavonoids or isoflavonoids *in vitro* (8, 9). Whether any of these mechanisms, including the one described in this study, are associated with the diet-related differences in serum levels and excretion of sex steroids in humans (27, 28) remains to be established.

The inherent estrogenic properties of several isoflavonoids, flavonoids, and coumestans further complicate the evaluation of their possible beneficial and/or harmful effects on the development of estrogen-related diseases. Coumestrol and isoflavones showing significant inhibition of 17β-HSOR type 1 are also clearly estrogenic at comparable or lower concentrations (3–7), and may thus partially replace the endogenous estrogens. Flavonoids are considered to be less estrogenic, and in general micromolar concentrations of these compounds are needed for estrogenic effects (6, 7). Interestingly, apigenin, being the most potent flavone tested, significantly inhibits $E_1$ reduction at 0.12 $\mu M$, and previous results indicate that it is not estrogenic at this concentration (11).

In conclusion, we have shown that several flavonoids and isoflavonoids found in human diets inhibit 17β-oxidoreduction of estrogens. Therefore, these compounds may interfere with the biosynthesis and metabolism of the highly active endogenous estrogen, $E_2$. Analyzing systematically the structural requirements for estrogenicity and inhibition of 17β-oxidoreduction, it may be possible to develop a specific nonestrogenic 17β-HSOR type 1 enzyme inhibitor that could be useful in prevention or treatment of estrogen-related diseases. Of the compounds tested so far, apigenin, coumestrol, and genistein have given the most promising results in this respect.

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