Estrogenic Flavonoids: Structural Requirements for Biological Activity (43830)

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Abstract. **A systematic survey of polycyclic phenols has been performed to identify members of this chemical group with estrogenic activity. Twelve compounds were found to be able to stimulate the transcriptional activity of the human estrogen receptor expressed in cultured cells by transient transfection. These natural estrogens belong to several distinct, but chemically related classes including chalcones, flavanones, flavones, flavonols, and isoflavones. Selected examples of estrogenic flavonoids were further analyzed to determine their biological potencies and their relative affinities for binding to the estrogen receptor. These data are interpreted with respect to the molecular structure of polycyclic phenols required for hormonal activity as nonsteroidal estrogens.** [P.S.E.B.M. 1995, Vol 208]

A considerable diversity has been described of compounds that share with steroidal estrogens an ability to activate the estrogen receptor. Studies from a number of laboratories have shown that estrogenic activity is exhibited by several members of the large flavonoid family of plant secondary metabolites. It has long been appreciated that genistein and a number of related isoflavones (biochanin A, daidzein, and formononetin) are inherently estrogenic (1–7). A previous report from this laboratory recently expanded the family of phytoestrogens to include a number of other multiply hydroxylated flavonoids (8).

Flavonoids occur naturally in all plant families and can be isolated from most plant tissues, including leaves, stems, roots, flowers, and seeds (3, 9). The biological roles played by flavonoids in plants are not fully understood and do not easily account for the large chemical diversity of this family. Individual members of this group are thought to serve as natural fungicides (phytoalexins), chemical deterrents against insect and animal herbivores, regulators of plant hormones, and UV protectants (9). Since the major flower pigments are flavonoids, these compounds also play an important role supporting the reproductive success of plants that depend upon insects for pollination.

Plant flavonoids are biosynthetically derived from chalcones (10) and can be divided into several structurally related groups (Fig. 1). While most of the flavonoids found in plants are present as glycosides, only the nonconjugated (aglycone) forms appear to exert estrogen-like activity in animals. However, the aglycones can readily be released from their sugar components by acid hydrolysis (11) to reveal their latent biological activity. It is the uncharged members of this family (chalcones, flavanones, flavones, flavonols, and isoflavones) rather than the intensely colored and highly charged anthocyanidin pigments that are of interest with respect to their estrogenic activity. This study represents an extension of a previous report documenting the hormonal activity of plant flavonoids (8) and was intended to systematically analyze the structure/activity profile of estrogenic flavonoids.

Materials and Methods

Chemical Reagents. The chemicals used in this study were obtained from the following sources: Sigma Chemical Co. (St. Louis, MO) (17β-estradiol, flavone, flavanone, phloretin, chrysin, hesperetin); Aldrich Chemical Co. (Milwaukee, WI) (4-hydroxy-4′-methoxychalcone, 3-hydroxyflavone, 7-hydroxyflavone, 7,8-dihydroxy-flavone, myricetin, biochanin

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A); Steraloids, Inc. (Wilton, NH) (dienestrol, hexestrol); Fluka Chemical Corp. (Ronkonkoma, NY) (kaempferol, kaempferide, [+/−]catechin, apigenin); and ICN Biomedicals, Inc. (Costa Mesa, CA) (genistein). A number of compounds were generous gifts from R. Bednar, Merck Sharp & Dohme (West Point, PA) (4-hydroxychalcone, 4,4′-dihydroxychalcone, isoliquiritigenin, naringenin chalcone, galangin, fisetin, quercetin, morin hydrate, naringenin, taxifolin). The remaining flavonoids were aquired from Indofine Chemical Co. (Somerville, NJ). Purity of the chemicals tested for their estrogenicity was confirmed by thin layer chromatography on silica G plates (Machery-Nagel, Düren, FRG) developed in chloroform: acetic acid (9:1, v/v) or toluene:ethyl acetate:formic acid (9:7:4, v/v). These flavonoids were generally used without further purification. ICI-164,384 was a kind gift from A. Wakeling, ICI Pharmaceuticals.

Cell Culture and Transfection. The biological activity of potentially estrogenic flavonoids was determined using a transient transfection assay in HeLa cells. A wild-type, recombinant estrogen receptor cDNA was expressed from the plasmid pER-18 (Y. Wang and R. J. Miksicek, unpublished plasmid. Structures are available upon request) and stimulation of the transcriptional activity of this receptor was assessed using the estrogen-responsive reporter plasmid pERE-TK-CAT (12). Conditions used for cell culture and transfection have been previously described (7, 8). Hormones and chemicals were added directly to culture dishes at the final concentrations indicated, as 1000× stock solutions prepared in 80% ethanol. Chloramphenicol acetyl transferase (CAT) activity was measured as described by Gorman *et al.* (13) and was calculated as pmoles of chloramphenicol acetylated per min per mg of cytosolic protein. Data were compiled from over thirty independent transfection experiments and were analyzed for significance using Student's *t*-test.

Competition Binding Analysis. The relative affinities of hormonally active flavonoids were determined by their ability to compete for binding of 17β-[^{3}H]estradiol to estrogen receptor expressed in COS-7 cells using a high-copy expression vector (pCMV-ER; Wang and Miksicek). Transfection, preparation of cellular extracts, and competition binding analysis were performed as previously described (7, 8). Binding reactions were incubated for 2 hr at room temperature with 10 m*M* 17β[2,4,6,7,16,17(N)-^{3}H]estradiol (170 Ci/mmol; New England Nuclear) in the presence of increasing concentrations of unlabeled competitor, followed by the addition of dextran-coated charcoal (DCC) and further incubation for 15 min at 4°C on ice. Hormone remaining bound to the receptor was defined as radioactivity resistant to adsorption by DCC.

Results

Previous studies from this laboratory have described the use of a transient transfection assay in cultured HeLa cells to analyze the hormonal activity of known and suspected estrogens (7, 8). This assay is based upon expression of the recombinant human estrogen receptor and assessment of its transcriptional activity using an estrogen-inducible reporter plasmid. Assays of this type have been instrumental in dissecting the structural organization of the steroid receptors since they are highly sensitive, relatively rapid, and readily amenable to experimental manipulation. Using co-transfection assays with appropriate control plasmids, we have previously shown that induction of a chloramphenicol acetyl transferase (CAT) reporter gene by estrogen requires both co-expression of the estrogen receptor and the presence of a specific DNA-binding site for this receptor within the promoter of the reporter plasmid (8). This assay is responsive to physiological concentrations of 17β-estradiol, cross reacts with a wide variety of known steroidal and nonsteroidal estrogens, and is sensitive to inhibition by known estrogen antagonists (7, 8). Data using this assay to measure the activity of selected estrogens is given in Table 1.

To broaden our studies on the estrogenic activity of naturally occurring plant flavonoids, we have utilized this assay to assess the ability of additional chemically defined phenolic aglycones to activate the human estrogen receptor. Compiled results from these studies are presented in Table II through V for four independent series of hydroxyflavonoids. For simplicity, each of these compounds was tested at a single concentration (10^{-6} *M*) based on the activities of other known phytoestrogens in this system (7, 8). As shown in Table II, substantial estrogenic activity was displayed by 4,4′-dihydroxychalcone, 2′,4,4′-trihydroxychalcone (isoliquiritigenin), and 2′,4,4′,6′-tetrahydroxydihydrochalcone (phloretin) ($P < 0.005$). These data also indicate that 2′,4,4′,6′-tetrahydroxychalcone (naringenin chalcone) may possess a low level of activity ($P < 0.05$), but that 4-hydroxychalcone and 4-hydroxy-4′-methoxychalcone are apparently inactive. The biological activity of these chalcones is consistent with previous reports describing that phloretin (14) and several synthetic derivatives of chalcone (15) display estrogenic and antifertility effects when tested in animal models.

Among a large series of flavones tested (Table III), six compounds exhibited significant estrogenic activity ($P < 0.005$): 3,4′,5,7-tetrahydroxyflavone (kaempferol), 4′,5,7-trihydroxyflavone (apigenin), 4′,6-dihydroxyflavone, 4′,5-dihydroxyflavone, 6-hydroxyflavone, and 4′-hydroxyflavone. However, the

Table I. Hormonal Activity of Steroidal and Diphenylethylene Estrogens

Compound	Concentration (n*M*)	*n*	CAT activity[a]	
			Mean ± SE	Range
Ethanol	n/a[b]	15	82 ± 12	34–214
17β-Estradiol	5	15	964 ± 117[c]	435–2037
ICI-164,384[d]	100	5	43 ± 13	9–73
Dienestrol	10	6	914 ± 218[c]	371–1766
Hexestrol	10	5	800 ± 180[c]	474–1453

Note. HeLa cells were co-transfected with plasmids encoding the estrogen receptor and an estrogen-inducible CAT reporter plasmid, followed by treatment for 2 days with the indicated compound. CAT enzymatic activities were measured for the number of transfections (*n*) indicated and are expressed as the mean ± SE, with the maximum and minimum values indicated as the range.
[a] CAT activities are given as pmoles/min · mg protein.
[b] n/a = not applicable.
[c] Significantly different from the ethanol vehicle at the level of $P < 0.005$ as determined by Student's *t*-test.
[d] ICI-164,384 is a pure antagonist of the estrogen receptor developed by Wakeling and colleagues (17).

Table II. Estrogenic Activity of Chalcone Derivatives

Chalcone	Trivial name	Concentration (μ*M*)	*n*	CAT activity[a]	
				Mean ± SE	Range
Ethanol vehicle	—	n/a[b]	15	82 ± 12	34–214
4-Hydroxy	—	1	3	152 ± 99	44–350
4,4'-Dihydroxy	—	1	6	471 ± 156[c]	163–1218
4-Hydroxy-4'-methoxy	—	1	1	86	n/a
2',4,4'-Trihydroxy	Isoliquiritigenin	1	7	994 ± 219[c]	300–1801
2',4,4',6'-Tetrahydroxy	Naringenin Chalcone	1	8	156 ± 35[d]	41–301
2',4,4',6'-Tetrahydroxydihydrochalcone	Phloretin	1	7	402 ± 68[c]	124 ± 711

Note. Experiments were performed and analyzed as described in Table I to compare the biological activities of the indicated hydroxychalcones.
[a] CAT activities are given as pmoles/min · mg protein.
[b] n/a = not applicable.
[c] Significantly different from the ethanol vehicle at the level of $P < 0.005$ as determined by Student's *t* test.
[d] Significantly different from the ethanol vehicle at the level of $P < 0.05$ as determined by Student's *t* test.

magnitude of the transcriptional responses induced by the latter two mono-hydroxylated flavones was much less than that of the others. 3',7-dihydroxyflavone and 3',4',5,7-tetrahydroxyflavone (luteolin) also appeared to show activity that was weak but significantly above that of the ethanol control ($P < 0.05$). In contrast, 14 related compounds, including flavone itself were essentially devoid of activity at the concentration tested.

Only two of the flavanones tested reproducibly activated the estrogen receptor in this transfection system (Table IV). These were 4',7-dihydroxyflavanone and 4',5,7-trihydroxyflavanone (naringenin). Inactive members of this group included 3',5,7-trihydroxy-4'-methoxyflavanone (hesperetin), 3,3',4',5,7-pentahydroxyflavanone (taxifolin), and 3,3',4',5,7-flavan pentol ([+/−] catechin), as well as flavanone itself. The results shown in Table V confirm previous reports (1–7) that four isoflavones (genistein, daidzein, biochanin A, and formononetin) support statistically significant ($P < 0.005$) estrogenic responses. They also agree with the published finding that the 4'-hydroxylated isoflavones (genistein and daidzein) are inherently more active than their 4'-methoxylated counterparts (biochanin A and formononetin) (2, 7). In contrast, single determinations using 3',4',7-trihydroxyisoflavone and 4',6,7-trihydroxyisoflavone suggest that these compounds are without activity. Together, the data summarized in Tables II through V indicate that at least 12 structurally related flavonoids share with 17β-estradiol and the diphenylethylene estrogens (Table 1) an ability to stimulate the transcriptional activity of the estrogen receptor at least 4-fold above its basal level (Table VI). They also reveal a highly consistent hydroxylation pattern among polycyclic phenols with estrogenic activity, considering that Positions 4, 2', and 4' of chalcone are equivalent to Positions 4', 5, and 7, respectively, of the flavonoid ring system (Fig. 1).

Most phytoestrogens display their hormone-like activity over a concentration range of 0.1 to 10 μ*M* (7, 8). To ascertain if this is also true for the estrogenic flavonoids, an experiment was undertaken to examine the concentration dependence of the transcriptional stimulatory activity of four selected dihydroxyflavones. The dose/response curves shown in Figure 2 indicate that 4',6-dihydroxyflavone is the most potent of this subset, with an EC_{50} of approximately 0.1 μ*M*. In comparison, 4',5- and 3',7-dihdroxyflavone require progressively higher concentrations for half-maximal

Table III. Estrogenic Activity of Flavone Derivatives

Flavone	Trivial name	Concentration (μM)	n	CAT activity[a]	
				Mean ± SE	Range
Ethanol vehicle	—	n/a[b]	15	82 ± 12	34–214
Flavone	—	1	4	52 ± 11	35–85
3-Hydroxy	—	1	3	92 ± 9	81–110
4′-Hydroxy	—	1	2	237[c]	149–324
6-Hydroxy	—	1	2	287[c]	203–370
7-Hydroxy	—	1	4	93 ± 22	55–155
3′,6-Dihydroxy	—	1	2	161	93–230
3′,7-Dihydroxy	—	1	3	162 ± 30[d]	107–211
4′,5-Dihydroxy	—	1	3	389 ± 95[c]	234–563
4′,6-Dihydroxy	—	1	2	427[c]	420–434
5,7-Dihydroxy	Chrysin	1	1	50	n/a
7,8-Dihydroxy	—	1	6	72 ± 19	17–146
3′,4′,7-Trihydroxy	—	1	1	42	n/a
3,5,7-Trihydroxy	Galangin	1	4	72 ± 17	42–119
4′,5,7-Trihydroxy	Apigenin	1	6	544 ± 157[c]	179–1169
4′,7,8-Trihydroxy	—	1	1	68	n/a
3,3′,4′,7-Tetrahydroxy	Fisetin	1	4	135 ± 34	73–228
3′,4′,5,7-Tetrahydroxy	Luteolin	1	2	168[d]	114–221
3,4′,5,7-Tetrahydroxy	Kaempferol	1	5	858 ± 143[c]	321–1171
3,5,7-Trihydroxy-4′-methoxy	Kaempferide	1	3	130 ± 31	95–193
3,3′,4′,5,7-Pentahydroxy	Quercetin	1	3	75 ± 28	25–123
2′,3,4′,5,7-Pentahydroxy	Morin	1	3	67 ± 11	46–80
3,3′,4′,5,5′,7-Hexahydroxy	Myricetin	1	4	74 ± 18	48 ± 12

Note. Experiments were performed and analyzed as described in Table I to compare the biological activities of the indicated hydroxyflavones.
[a] CAT activities are given as pmoles/min · mg protein.
[b] n/a = not applicable.
[c] Significantly different from the ethanol vehicle at the level of $P < 0.005$ as determined by Student's t test.
[d] Significantly different from the ethanol vehicle at the level of $P < 0.05$ as determined by Student's t test.

Table IV. Estrogenic Activity of Flavanone Derivatives

Flavanone	Trivial name	Concentration (μM)	n	CAT activity[a]	
				Mean ± SE	Range
Ethanol vehicle	—	n/a[b]	15	82 ± 12	34–214
Flavanone	—	1	3	40 ± 13	21–66
4′,7-Dihydroxy	—	1	5	903 ± 202[c]	450–1396
4′,5,7-Trihydroxy	Naringenin	1	7	574 ± 235[c]	174 ± 1930
3′,5,7-Trihydroxy-4′-methoxy	Hesperetin	1	1	86	n/a
3,3′,4′,5,7-Pentahydroxy	Taxifolin	1	3	97 ± 28	44–139
3,3′,4′,5,7-Flavan pentol	[+/−]Catechin[d]	1	4	97 ± 16	67–140

Note. Experiments were performed and analyzed as described in Table I to compare the biological activities of the indicated hydroxyflavanones.
[a] CAT activities are given as pmoles/min · mg protein.
[b] n/a = not applicable.
[c] Significantly different from the ethanol vehicle at the level of $P < 0.005$ as determined by Student's t test.
[d] It should be noted that catechin is a flavan rather than a flavanone.

activity, while 3′,6-dihydroxyflavone shows little stimulatory activity even at 10 μM. By analogy to the flavanones tested at 1 μM (Table IV) it can be predicted that 4′,7-dihydroxyflavone will also display estrogenic activity between 0.1 and 1 μM; unfortunately, this flavone was not available for testing.

It is generally assumed that nonsteroidal estrogens exert their stimulatory effect on the estrogen receptor by binding to the same site as that occupied by steroidal estrogens such as 17β-estradiol. Supporting this presumption, we have previously shown that a variety of estrogenic flavonoids including 4,4′-dihydroxychalcone, 2′,4,4′-trihydroxychalcone, 4′,7-dihydroxyflavanone, 4′,5,7-trihydroxyflavanone, 4′,5,7-trihydroxyflavone, and 4′,5,7-trihydroxyisoflavone can compete with 17β-[^{3}H]estradiol for binding to the human estrogen receptor in extracts prepared from COS-7 cells that over express this receptor (8). Inhibition of estradiol binding required the competing flavonoid to be present at concentrations ranging from

Table V. Estrogenic Activity of Isoflavone Derivatives

Isoflavone	Trivial name	Concentration (μM)	n	CAT activity[a]	
				Mean ± SE	Range
Ethanol vehicle	—	n/a[b]	15	82 ± 12	34–214
4′,7-Dihydroxy	Daidzein	1	4	469 ± 74[c]	256–589
7-Hydroxy-4′-methoxy	Formononetin	1	3	287 ± 96[c]	177–478
3′,4′,7-Trihydroxy	—	1	1	86	n/a
4′,5,7-Trihydroxy	Genistein	1	8	988 ± 296[c]	412–2839
4′,6,7-Trihydroxy	—	1	1	63	n/a
5,7-Dihydroxy-4′-methoxy	Biochanin A	1	10	331 ± 77[c]	33–683

Note. Experiments were performed and analyzed as described in Table I to compare the biological activities of the indicated hydroxyisoflavones.
[a] CAT activities are given as pmoles/min · mg protein.
[b] n/a = not applicable.
[c] Significantly different from the ethanol vehicle at the level of $P < 0.005$ as determined by Student's t test.

Table VI. Flavonoids with Highly Significant Estrogenic Activity

- Chalcones
  - 4,4′-Dihydroxychalcone
  - 2′,4,4′-Trihydroxychalcone (isoliquiritigenin)
  - 2′,4,4′,6′-Tetrahydroxydihydrochalcone (phloretin)
- Flavones
  - 4′,5-Dihydroxyflavone
  - 4′,6-Dihydroxyflavone
  - 4′,5,7-Trihydroxyflavone (apigenin)
- Flavonols
  - 3,4′,5,7-Trihydroxyflavone (kaempferol)
- Flavanones
  - 4′,7-Dihydroxyflavanone
  - 4′,5,7-Trihydroxyflavanone (naringenin)
- Isoflavones
  - 4′,7-Dihydroxyisoflavone (daidzein)
  - 4′,5,7-Trihydroxyisoflavone (genistein)
  - 5,7-Dihydroxy-4′-methoxyisoflavone (biochanin A)

Note. This table summarizes the polycyclic phenols analyzed in Tables II through V able to support a 4-fold or greater stimulation of the transcriptional activity of the human estrogen receptor. All of these responses show significance at the level of $P < 0.005$.

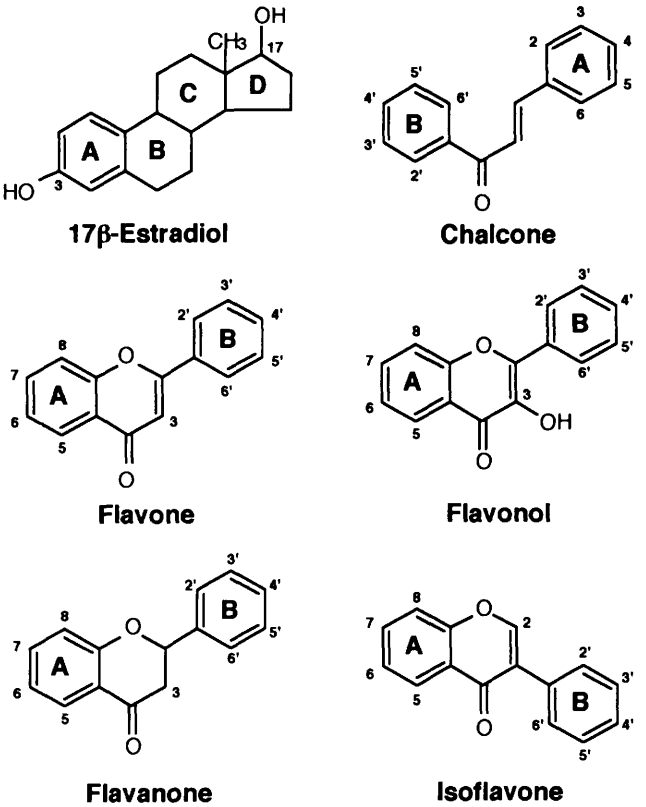

Figure 1. Structures of 17β-estradiol and classes of polycyclic phenols analyzed in this study. Also shown are the conventional numbering schemes to indicate the hydroxylation patterns of the flavonoids characterized.

10^{-7} to 10^{-5} M depending on the flavonoid in question and was not observed for nonestrogenic flavonoids such as flavone and flavanone.

To broaden these results, competition binding analysis was also used to examine the relative binding affinities of a wider range of hydroxyflavonoids. The results shown in Figure 3 confirm that 4′,5-dihydroxyflavone shares with 4′,7-dihydroxyflavanone and 4′,7-dihydroxyisoflavone (daidzein) the ability to compete with 17β-estradiol for binding to the estrogen receptor, but only when present in a 1,000- to 10,000-fold molar excess over this steroid. A slightly higher affinity was displayed by 4′,6-dihydroxyflavone, consistent with its greater potency in a transient transfection assay (Fig. 2). In contrast, little if any inhibition of estradiol binding was observed for 3′,6-dihydroxyflavone, 4′-hydroxyflavone, 6-hydroxyflavone, or 7-hydroxyflavone even when these compounds were present at a 10,000-fold excess.

These data support the conclusion that estrogenic hydroxyflavonoids exert their biological effect by interacting directly with the estrogen receptor. Extrapolating from the reported dissociation constant (K_d) of 17β-estradiol (0.3 nM), they also infer that these hydroxyflavonoids display affinities for the estrogen receptor on the order of 0.3 to 10 μM. The relationship between the estrogenic potency of selected dihydroxy-

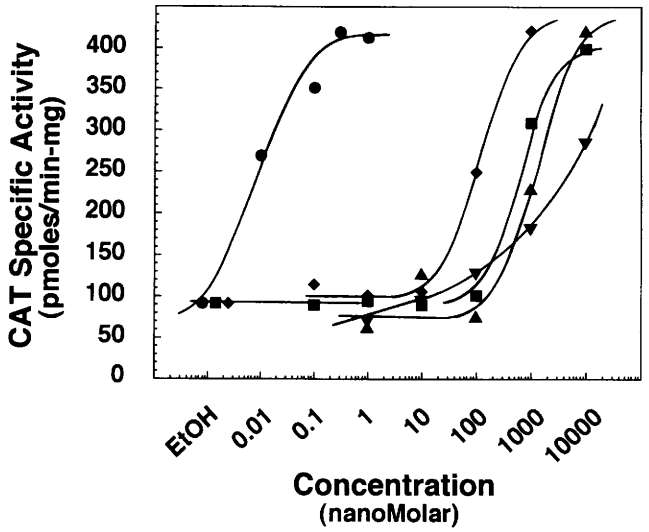

Figure 2. Concentration dependence of the transcriptional stimulatory effect of estrogenic flavonoids. HeLa cells cotransfected with the human estrogen receptor gene and the pERE-TK-CAT reporter plasmid were treated with increasing concentrations of 17β-estradiol (●), 4′,6-dihydroxyflavone (♦), 4′,5-dihydroxy flavone (■), 3′7-dihydroxyflavone (▲), or 3′,6-dihydroxyflavone (▼). CAT specific activities were measured in cell extracts following 48 hr of continuous hormone treatment. Shown are individual determinations from a representative experiment.

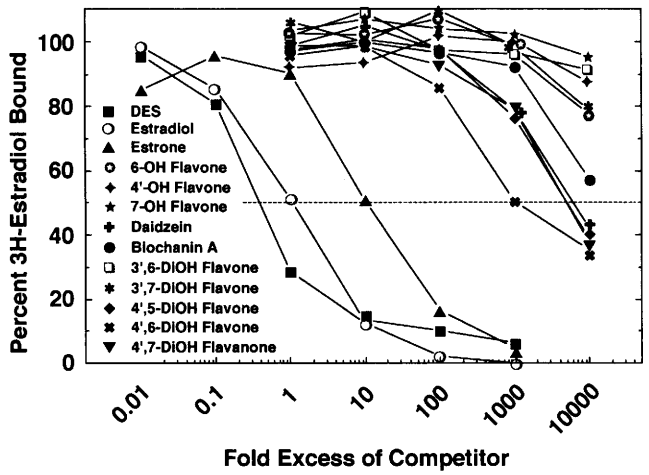

Figure 3. Competition binding analysis of estrogenic flavonoids. Extracts from COS-7 cells over expressing the human estrogen receptor were incubated for 2 hr at room temperature with 10 n*M* 17β-[^{3}H]estradiol in the presence of increasing concentrations of the unlabeled ligands shown. 17β-[^{3}H]Estradiol remaining bound to the estrogen receptor was determined as radioactivity resistant to adsorption by DCC.

flavones and their relative affinity for the estrogen receptor is shown in Figure 4. Slight differences in the uptake of these compounds or their metabolic stability may account in part for deviations of this relationship from linearity.

Discussion

The results of this study present the first systematic analysis of structure/activity relationships among

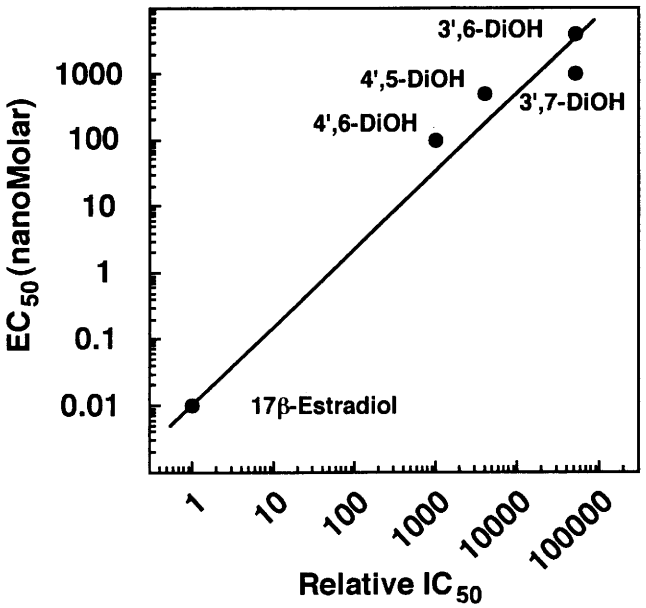

Figure 4. Correlation of estrogenic activity of dihydroxyflavones with their relative affinity for the estrogen receptor. Concentrations required for half-maximal activation of the estrogen receptor (EC_{50} values, extrapolated from Fig. 2) were plotted against the molar excess of ligand required for 50% competition of [^{3}H]estradiol binding to the estrogen receptor (relative IC_{50} values, from Fig. 3) for 17β-estradiol and the four dihydroxyflavones indicated. The line represents a least squares fit to these data (coefficient of correlation, $r = 0.983$).

estrogenic flavonoids. Summarized in Table VI are members of this family that show a highly significant ability ($P < 0.005$) to stimulate the transcriptional activity of the human estrogen receptor as assessed using a transient transfection assay in cultured cells. It is immediately evident from this compilation that the structural features that are most important with respect to estrogenic activity include the diaryl ring structure common to all flavonoids (Fig. 1) and a minimum of one hydroxyl substituent on each of these aromatic rings. Despite obvious differences in the central bridge connecting the phenolic A and B rings when the chalcones, flavones, flavanones, and isoflavones are compared there is a remarkably strong consensus in the optimal pattern of hydroxylation that gives rise to estrogenic activity. Thus, compounds with hydroxyl substituents in Positions 4′ and 7 of the flavan or isoflavan nuclei (equivalent to Positions 4 and 4′, respectively, of chalcone) are invariably estrogenic. An additional hydroxyl group in Position 5 of the flavones or isoflavones (equivalent to the Position 2′ of chalcone) are not only tolerated, but may in some cases increase estrogenic activity. While the apparent tolerance for 4′-methoxylation in biochanin A might be interpreted as an exception to the generalizations made above, it should be noted that a previous study (16) concluded that the hormone-like activity observed for biochanin A *in vivo* is likely to result from its conver-

sion to the much more potent compound genistein. This is supported by the finding that the relatively low affinity of biochanin A for estrogen receptor (Fig. 3) (2, 3, 7) does not adequately account for its estrogenic activity in cultured cells.

Among the large series of flavones analyzed, it is apparent that some flexibility exists with respect to the hydroxylation pattern of Ring A. Both 4′,5-dihydroxyflavone, 4′,6-dihydroxyflavone, and 4′,7-dihydroxyflavone (extrapolating from the behavior of the analogous flavanone) exhibit substantial estrogenicity. In contrast, there appears to be less tolerance for changes in the hydroxylation pattern of Ring B comparing the progressively reduced activities of 4′,6- and 3′,6-dihydroxyflavones (Fig. 2). The activity exhibited by 3,4′,5,7-tetrahydroxyflavone (kaempferol) indicates that hydroxylation at Position 3 is not detrimental to activity provided that the remaining pattern of hydroxylation is favorable and suggests that additional flavonols (3-hydroxyflavones) may be estrogenic. However, hydroxylations that create catechols (e.g., 7,8-dihydroxyflavone, 4′,7,8-trihydroxyflavone, 3,3′,4′,7-tetrahydroxyflavone, 3,3′,4′,5,7-pentahydroxyflavone, and 3,3′,4′,5,5′,7-hexahydroxyflavone) or that increase the number of hydroxyl substituents above 4 appear to abolish estrogenic activity. In addition, 4′-methoxylation of chalcones, flavones, flavanones, and isoflavones invariably reduces or eliminates the estrogenic activity of these compounds relative to their 4-hydroxy counterparts.

This study should serve to greatly broaden our understanding of natural plant products that possess biological activity as estrogens. There has been a recent resurgence of interest in plant estrogens both with respect to their beneficial effects and their potential to serve as human toxicants. Until recently, there has been a perception that common crop plants that represent significant sources of dietary estrogens are almost invariably members of the legume family. An important implication of this report is to question this presumption since many of the estrogenic flavonoids described above show a much broader species distribution than estrogenic isoflavones such as genistein and daidzein. Ultimately, it will be necessary to assess the distribution and content of these flavonoids among crop species that represent a significant portion of the human diet. More importantly, little is known at present about the bioavailability, absorption, metabolism, and excretion of estrogenic flavonoids in man. A thorough understanding of the beneficial and potentially deleterious effects of dietary estrogens such as the flavonoids will require questions such as these to be addressed.

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