Lignans Interfering with 5α-Dihydrotestosterone Binding to Human Sex Hormone-Binding Globulin

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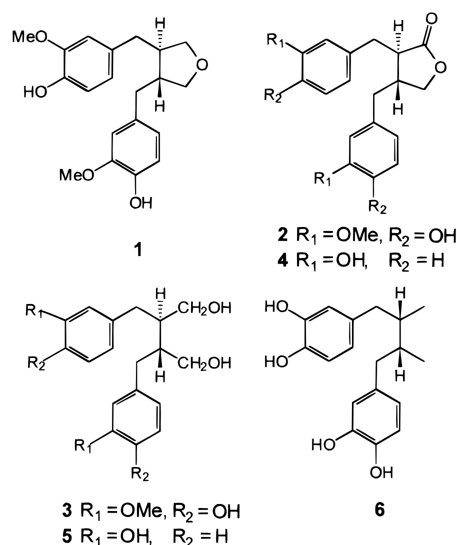
The natural lignans (−)-3,4-divanillyltetrahydrofuran (**1**), (−)-matairesinol (**2**), (−)-secoisolariciresinol (**3**), (±)-enterolactone (**4**), (±)-enterodiol (**5**), and nordihydroguaiaretic acid (NDGA) (**6**) reduce the binding of ^{3}H-labeled 5α-dihydrotestosterone (DHT) to human sex hormone-binding globulin (SHBG). (−)-3,4-Divanillyltetrahydrofuran (**1**) has the highest binding affinity ($K_a = 3.2 \pm 1.7 \times 10^6$ M^{-1}) of all lignans investigated so far; the reversibility of its binding and a double reciprocal plot suggest a competitive inhibition of the SHBG−DHT interaction. Increasing hydrophobity in the aliphatic part of the lignans (butane-1,4-diol− butanolide− tetrahydrofuran structures) leads to higher binding affinity. In the aromatic part, a 3-methoxy-4-hydroxy substitution pattern is most effective for binding to SHBG.

Sex hormone binding globulin (SHBG) is the major plasma sex steroid carrier protein. It has a very high affinity to androgenic and estrogenic steroid hormones. The most preferred ligand of SHBG is the androgen 5α-dihydrotestosterone.[1,2] The association constants for steroid hormones are 2×10^9 M^{-1} for 5α-dihydrotestosterone, 1×10^9 M^{-1} for testosterone, and 5×10^8 M^{-1} for estradiol.[3]

The discovery of receptors for SHBG on typical steroid hormone target tissues demonstrates that SHBG is more than a simple transport protein.[4,5] It was shown to mediate hormone action by a hormone−SHBG−membrane-receptor complex by the generation of the *second messenger* cAMP inside the cells.[6] The presence of SHBG and dihydrotestosterone (DHT) stimulates the growth of the prostate cancer cell line ALV-41.[7] SHBG was shown to be an allosteric protein in which the protein−receptor interaction depends on the occupancy of the steroid binding site and the kind of bound ligand.[8] Therefore, nonsteroidal ligands of SHBG are of great interest.

Lignans, naturally occurring secondary plant metabolites consisting of two phenylpropanoid units, are suggested to have a beneficial effect on hormone-dependent cancer.[9] Their concentration in human plasma strongly depends on nutrition. Vegetarian Finnish women have plasma concentrations of up to 3.7 nM of matairesinol (**2**), 8.8 nM of enterodiol (**5**), and 1 μM of enterolactone (**4**).[9] In Australian postmenopausal women, concentrations of total enterolactone (**4**) and enterodiol (**5**) of up to 2 μM have been reported.[10]

The link to the beneficial effects of lignans may be their binding affinity to SHBG. The two lignan metabolites, enterolactone (**4**) and enterodiol (**5**), and the naturally occurring lignan, nordihydroguaiaretic acid (NDGA, **6**), are able to reduce the binding of estradiol and testosterone to SHBG.[11] Effects of (−)-secoisolariciresinol[12] (**3**) and (−)-3,4-divanillyltetrahydrofuran[13] (**1**) on the binding of DHT to SHBG were reported previously. The affinity of (−)-matairesinol (**2**) to SHBG is described in this paper for the first time. The most potent lignan in this respect was recognized to be the monoepoxylignan (−)-3,4-divanillyltetrahydrofuran (**1**).



The high binding affinity of (−)-3,4-divanillyltetrahydrofuran necessitated further quantitative investigations on its binding properties to SHBG. For positive control, the lignans NDGA, enterolactone, and enterodiol, which have a known affinity to SHBG, were included in the study. An in vitro charcoal assay, as described previously, was used.[12] The testing conditions were verified by determination of the association constant [0.98×10^9 M^{-1} (4 °C)] of SHBG to DHT by

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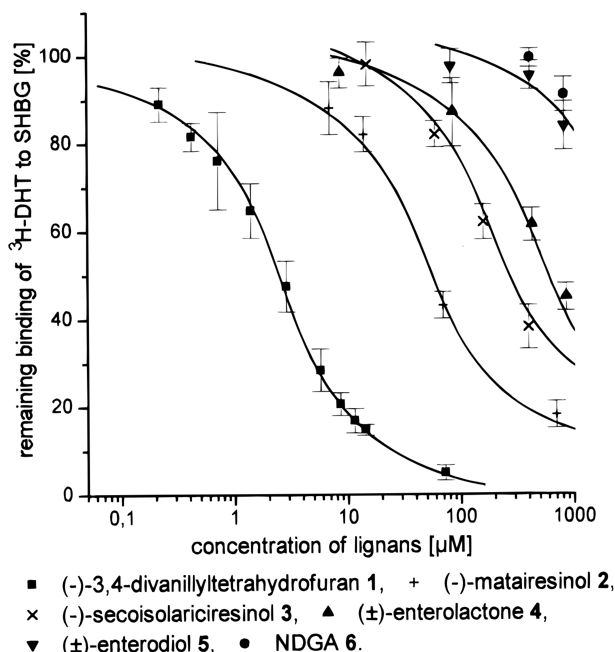

Figure 1. Effect of lignans on binding of DHT to SHBG. Each point is the average of a minimum of three experiments.

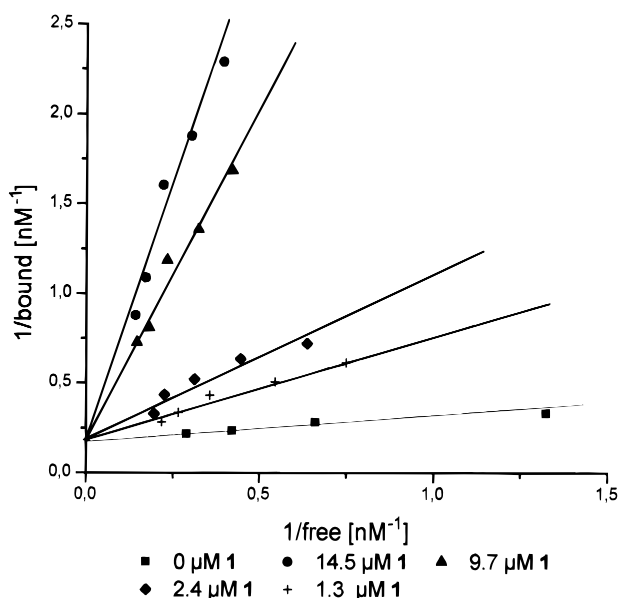

Figure 2. Double reciprocal plot of (−)-3,4-divanillyltetrahydrofuran (**1**) with ^{3}H-DHT (8.8 nM; 7.0 nM; 5.28 nM; 4.4 nM; 3.52 nM). Each point is the mean of three experiments.

Scatchard. The slightly lowered value as compared to literature[14,3] (1.4×10^9 M^{-1}, 2.1×10^9 M^{-1}) may be due to the storage of SHBG at −18 °C.

The effect of the lignans to the binding of DHT to SHBG was shown to be dose dependent (Figure 1). The binding affinity increases from NDGA (**6**) < enterodiol (**5**) < enterolactone (**4**) < (−)-secoisolariciresinol (**3**) < (−)-matairesinol (**2**) < to (−)-3,4-divanillyltetrahydrofuran (**1**) (Figure 2). The IC_{50} values are 620 μM for enterolactone (**4**), 230 μM for (−)-secoisolariciresinol (**3**), 52 μM for (−)-matairesinol (**2**), and 2.6 μM for 3,4-divanillyltetrahydrofuran (**1**) (9.7 nM SHBG; 8.8 nM ^{3}H-DHT). The very high binding affinity of **1** to SHBG is remarkable. The binding of SHBG was shown to be reversible by addition of ^{3}H-DHT to a SHBG−**1** complex.

A double reciprocal plot suggests that **1** interferes with DHT−SHBG binding by competitive inhibition (Figure 2). The K_a of **1** was calculated from its relative binding activity (RBA) compared to DHT using the following equation: $K_a = K^*/[(1/RBA) \times (1 + R) - R]$,[14,15] where K* is the association constant of DHT (0.98×10^9 M^{-1}), and R is the ratio of bound-to-free radiolabeled DHT at 50% displacement (R = 0.2, under these conditions). It turned out to be $3.2 \pm 1.7 \times 10^6$ M^{-1} at 4 °C.

Regarding the binding affinity of the different lignans, conclusions on the structural requirements of SHBG to lignans can be drawn. The 4-hydroxy-3-methoxy-substituted benzene ring (guajacyl group) enhances the binding of lignans to SHBG. This is impressively demonstrated by the 12-fold increasing affinity of (−)-matairesinol (**2**) relative to (±)-enterolactone (**4**). The same effect is reflected by the binding of (±)-enterodiol (**5**) and (−)-secoisolariciresinol (**3**) to SHBG. This structure−activity correlation is emphasized by 2-methoxyestradiol, which is six times more active than estradiol.[16] An increase in polarity of the aliphatic part of the molecule reduces the affinity to SHBG, which is demonstrated by comparing 3,4-divanillyltetrahydrofuran, (−)-matairesinol, and (−)-secoisolariciresinol (factor 88:4:1) or (±)-enterolactone with (±)-enterodiol.

All the above-mentioned conclusions resemble criteria that have been established for steroids:[17] Binding activity to SHBG is highest in steroids with an electronegative functional group at C-3, a −OH-function at C-17, and a relatively planar configuration.

The low affinity of NDGA seems to contradict these observations, but there are at least three structural differences making it hard to compare NDGA directly to the other lignans: (a) the different stereochemistry at C8; (b) the deficiency of a π-donor substituent at C9; and (c) the 3,4-dihydroxy substitution of the benzene ring may be too polar for the SHBG steroid binding site, which has been characterized as hydrophobic.[18]

The conditions of the in vitro experiment of 9.8 nM SHBG and 8.8 nM DHT are comparable to the concentrations of SHBG and SHBG-binding steroids in healthy men: SHBG 10−30 nM,[19] DHT 2 nM, and testosterone 25 nM,[20] whereby the association constant of testosterone to SHBG is less than half of the K_a of DHT.[3] The low IC_{50} value of **1** (2.6 μM) in the in vitro assay makes an influence on the steroid−SHBG balance conceivable even under physiological conditions.

Experimental Section

Test Compounds. (−)-Secoisolariciresinol (**3**) was isolated from *Urtica dioica* L. roots. [12] 3,4-Divanillyltetrahydrofuran (**1**) by dehydration of (−)-secoisolariciresinol with H_2SO_4/trimethylorthoformate[13] and identified by ^{1}H-NMR and ^{13}C-NMR data in accordance with literature reports. [21] (±)-Enterodiol (**5**) and (±)-enterolactone (**4**) were generous gifts from Prof. M. Metzler and E. Jacobs (Lebensmittelchemie, Universität Karlsruhe, Germany). (−)-Matairesinol (**2**) was received from Dr. R. Kasper (Pharmazeutische Biologie, Freie Universität Berlin). NDGA was purchased from Fluka (Neu-Ulm, Germany).

SHBG. SHBG from blood serum of pregnant women was enriched by affinity chromatography on concanava-

lin A−sepharose and $(NH_4)_2SO_4$ precipitation as described previously.[12]

Charcoal Assay. Testing conditions were as previously reported [12] with minor modifications. Briefly, human SHBG (9.7 nM) was incubated with [1,2-^{3}H(N)]-dihydrotestosterone (NEN, Dreieich, Germany, sp. act. 45.5 Ci/mmol) (8.8 nM) and varying concentrations of the lignan under investigation for 3 h at 4 °C. Nonbound ^{3}H-DHT was removed by the addition of dextran-coated charcoal (5 min). The remaining activity was corrected for nonspecific binding, which was measured by using a 300-fold excess of unlabeled DHT instead of the lignan. The double reciprocal plot and Scatchard analysis was gained by varying of the ^{3}H-DHT concentrations from 3.5 nM to 8.8 nM.

Determination of SHBG Concentration. The concentration of SHBG in the in vitro binding assay was determined by an immunoenzymometric assay (SR1, BioChem ImmunoSystems GmbH, Freiburg, Germany). The value of 9.7 nM was in good agreement with the one calculated by saturation of SHBG with ^{3}H-DHT in the charcoal assay.

Determination of Reversibility of the Binding of 3,4-Divanillyltetrahydrofuran. We incubated 2.9 μM 3,4-divanillyltetrahydrofuran (**1**) with 9.7 nM SHBG for 6 h. In one sequence of three experiments, 8.8 nM ^{3}H-DHT and **1** were added simultaneously. In another sequence of three experiments, the ^{3}H-DHT was added after 3 h to the SHBG-**1** complex. In both sequences the inhibition of ^{3}H-DHT binding to SHBG was 49 ± 5%.

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