Interaction of Lignans with Human Sex Hormone Binding Globulin (SHBG)

Matthias Schöttner[a], Dietmar Ganßer[b] and Gerhard Spiteller[a]

[a] Lehrstuhl Organische Chemie I, Universität Bayreuth, Universitätsstraße 30, D-95440 Bayreuth, Germany
[b] Institut für Lebensmittelchemie, Universität Hannover, Wunstorfer Straße 14, D-30453 Hannover, Germany

Z. Naturforsch. **52c**, 834–843 (1997); received July 22/September 17, 1997

SHBG (SBP), Sex Hormone Binding Globulin, Synthesis of Lignans, Stobbe Condensation

Lignans bind to sex hormone-binding globulin (SHBG). The lignan with the highest binding affinity is (±)-3,4-divanillyltetrahydrofuran. In a double Stobbe condensation – without use of protecting groups – a wide variety of lignans with different substitution pattern in the aromatic and aliphatic part of the molecule was synthesized. These lignans were tested in a SHBG-binding assay which allowed to deduce the following relationship between structure and activity: 1) (±)-diastereoisomers are more active than meso compounds 2.) the 4-hydroxy-3-methoxy (guajacyl) substitution pattern in the aromatic part is most effective 3.) the activity increases with the decline in polarity of the aliphatic part of the molecule.

Introduction

Lignans, dimeric phenylpropanes, are typical plant constituents, are known to develop a great number of physiological activities (McRae and Towers, 1984; Nishibe 1993): Lignans have been recognized as platelet-activating factor antagonists (Coran *et al.*, 1991), display digitoxin-like activities (Hirano *et al.*, 1989) and show cytostatic effects especially on hormone dependent cancer cell lines (Hirano *et al.*, 1990).

Some lignans are able to bind to human sex hormone binding-globulin (SHBG) (Martin *et al.*, 1995; Ganßer and Spiteller, 1995), the major plasma sex steroid transport protein (Hammond and Bocchinfuso, 1995). The lignan with the highest present known affinity to SHBG is (−)-3,4-divanillyltetrahydrofuran (Schöttner *et al.*, 1997).

The influence of structural modifications on their binding to SHBG has been investigated for steroids only. These studies indicated as prerequisite for highest binding affinities the presence of a π-donor as keto or hydroxy group in position 3, a β-hydroxl group in position 17 and a planar conformation of the steroid (Cunningham *et al.*, 1981). The influence of structural variation of lignans on their binding to SHBG is subject of this paper.

Reprint requests to Prof. Spiteller.
Fax: 0049-(0)-921-552671.

This investigation required the synthesis of lignans: A wide variety of compounds in substantial quantities for tests was needed. Since isolation of lignans from natural sources is time-consuming and provides the active compounds in poor yields only, the project required the use of a versatile synthesis. Several synthetic approaches have been published (Ward, 1982). In our laboratory the synthesis of lignans by a modification of the classical Stobbe route (Matsuura and Iinuma, 1984; Batterbee *et al.*, 1969) in a variation of Daughan and Brown (Daughan and Brown, 1991) proved to be most suitable. This synthetic approach can be applied without use of protecting groups, and allows the preparation of a wide variety of lignans with different substitution pattern in the aliphatic and aromatic part of the molecules. Additionally the unsatured intermediates were suspected to show SHBG binding properties. Therefore they were also subjected to the test program. The wide variety of products available by the described synthetic approach allowed to perform a detailed study on structure-activity relationship of binding of lignans to SHBG.

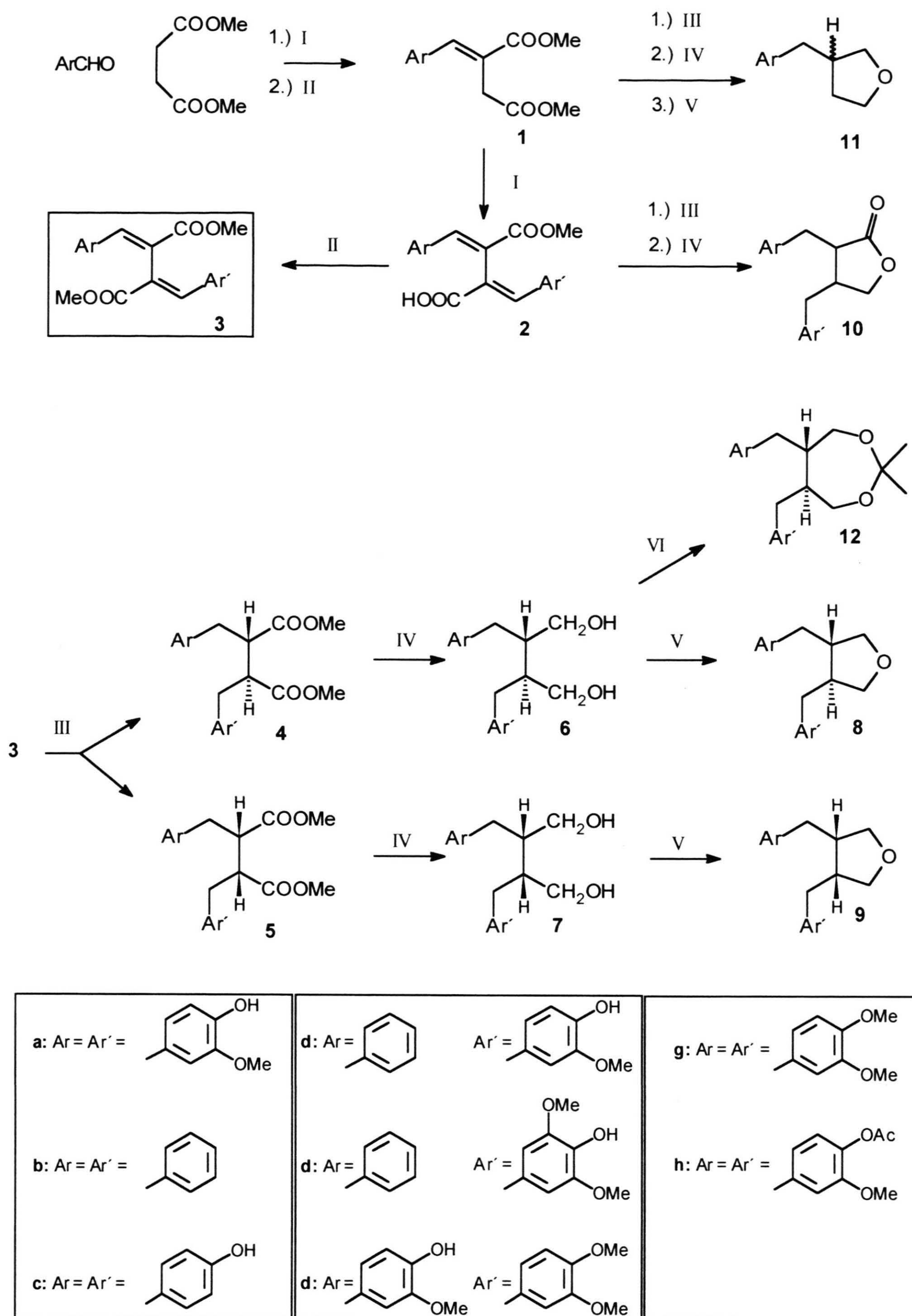
Results

The synthetic approach

(*E*)-2-(4-Hydroxy-3-methoxy-benzyliden)-dimethylsuccinate **1a** was prepared by a Stobbe condensation of dimethylsuccinate and vanillin ac-

0939–5075/97/1100–0834 $ 06.00 © 1997 Verlag der Zeitschrift für Naturforschung. All rights reserved. D

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45



Scheme 1. Synthesis of several lignans. I = LiOMe/MeOH, II = $MeOH/H_2SO_4$, III = Pd/C H_2, IV = THF $LiAlH_4$, V = $HC(OMe)_3$ H_2SO_4 MeOH, VI = $HClO_4$ acetone; (for enantiomeric pairs one enantiomer is shown only).

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

cording to Daughan *et al.* (Daughan and Brown, 1991) followed by esterfication with methanol (Scheme 1). Repetition of this reaction sequence yielded the bisbenzylidenedimethylsuccinate **3a**. After hydrogenation the diastereoisomers **4a** and **5a** (2:1) were separated by recrystallisation from MeOH/THF (1:1 v/v). The meso compound **5a** crystallized much more readily than the isomeric compound **4a,** due to its poor solubility in organic solvents. Reduction of **4a** and **5a** with $LiAlH_4$ yielded (±)-secoisolariciresinol **6a** and meso-secoisolariciresinol **7a**. (±)-3,4-Divanillyltetrahydrofuran **8a** was prepared by dehydration of (±)-secoisolariciresinol by use of acetone/$HClO_4$ as dehydration reagent (Cambie *et al.*, 1985). The acetonide **12a** was obtained as byproduct. It was separated by HPLC.

(±)-Secoisolariciresinol **6a** and meso-secoisolariciresinol **7a** were treated with MeOH/trimethylorthoformate to give (±)-3,4-divanillyltetrahydrofuran and meso-3,4-divanillyltetrahydrofuran, respectively, in quantitative yield in a reaction not previously described. The reaction of (±)-3,4-divanillyltetrahydrofuran **8a** with CH_2N_2 yielded **8f** and (±)-brassilignan **8 g**. The acetyl derivative **8h** was obtained from **8a** by treatment with AcOAc. Further variation of the aromatic substitution pattern of the lignans was achieved by using benzaldehyde, *p*-hydroxybenzaldehyde and syringaldehyde instead of vanillin.

SHBG binding test

The compounds obtained were subjected to an *in-vitro* assay described earlier (Ganßer and Spiteller, 1995). Briefly, different concentrations of a testsubstance were incubated with [1,2-^{3}H(N)]-dihydrotestosterone (^{3}H-DHT) [8.8 nM] and SHBG [9.7 nM]. SHBG is a homodimer where two molecules of SHBG are able to bind one molecule of ^{3}H-DHT (Hammond and Bocchinfuso, 1995; Joseph, 1994). The chosen ^{3}H-DHT concentration is able to saturate the protein (4.8 ± 0.3 nM bound). Nonbound ^{3}H-DHT was separated by addition of dextran-coated charcoal. The remaining activity was corrected for the non-specific binding (0.04 nM ^{3}H-DHT/nM protein) which was determined by use of a 300-fold excess of non-labelled DHT instead of a test compound. Each point represents the mean of at least three measurements.

Influence of variations in the aliphatic part of guajacyllignans on SHBG binding

The influence of variations in the aliphatic part of lignans on the binding properties to SHBG was investigated with guajacyl lignans, since it was found that all guajacyllignans inhibited the binding of ^{3}H-DHT to SHBG. Planarity is a prerequisite for an effective binding of steroids to SHBG (Cunningham *et al.*, 1981). In contrast to this finding the unsaturated compounds **1a** and **3a** developed only a very low affinity to SHBG (Fig. 1). Hydrogenation of the double bonds of **3a** results in an increase of the binding properties, whereby the (±)-diastereoisomer **4a** turned out to be more effective than the meso compound **5a**. This observation may be due to the low solubility of **5a** observed in all organic solvents.

(−)-Matairesinol (−)-**10a** and **4a** are of similar polarity, causing similar binding affinity to SHBG. An increase in polarity was achieved by the reduction of **4a** to the natural product (±)-secoisolariciresinol **6a.** This is reflected by a decrease in binding affinity to SHBG. Dehydration of the diols **6a** and **7a** produced the relatively nonpolar compounds 3,4-divanillyltetrahydrofuran **8a** and **9a**. These compounds exhibited the highest affinity of all investigated lignan derivates. Comparison between **8a** and **9a** again revealed the superiority of

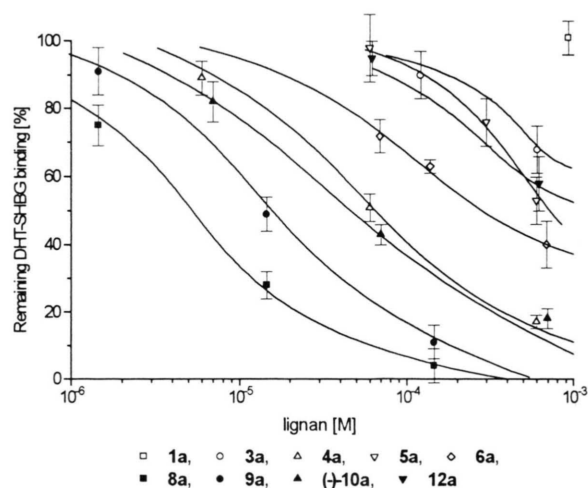


Fig. 1. Incubation of human SHBG, [1,2-^{3}H(N)]-dihydrotestosterone and synthetic lignans with variations in the aliphatic part of the molecule at different concentrations.

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

the (±)-diastereoisomer over the meso compound with respect to their binding affinity.

Influence of variations in the aromatic part of the lignans on SHBG binding

The influence of aromatic substitution pattern on the SHBG binding properties was tested by comparing compounds with an equivalent aliphatic part but different substitution pattern in the aromatic ring. The bisbenzyl dimethylsuccinates **4** and **5** with different aromatic substitution pattern are easily accessible synthetically by variation of the aldehydic compounds in the Stobbe condensation. Synthesis of **4c** in the way described above failed due to the poor solubility of compound **3c** in any organic solvent. Synthesis of the **10c** was achieved by hydrogenation of **2c** followed by reduction with $LiAlH_4$. **10c** was obtained as mixture of diastereoisomeric compounds in a ratio 2:1. The similar polarity of **10c** compared to compounds of type **4** and **5** allowed comparison of their binding properties to SHBG.

According to the data given in Fig. 2 the diastereomers **4** always showed higher affinity to SHBG than the isomeric compounds **5**. The affinity of the symmetrically substituted lignans decreased from **4a** > **4b** > **10c**.

Whether a symmetric aromatic substitution is required for an effective SHBG binding was clarified by coupling **1b** as an aromatic group with low affinity with vanillin and syringaldehyde. After the usual reaction sequence the resulting diastereoisomers **4** and **5** were separated by HPLC. The compounds **4a**, **4d** and **4e** exhibited comparable affinity to SHBG, whereby the affinity of **4e** was found to be the lowest. Obviously binding to SHBG is strongly supported by the 4-hydroxy-3-methoxy substitution pattern in one of the aromatic rings.

Further variation of the aromatic part of the molecule was achieved by derivatization of the highly active **8a** with diazomethane. The mixture of methylation products was separated by HPLC providing the compounds **8f** and **8 g**. Treatment of **8a** with pyridine and AcOAc yielded **8h**. Starting from compound **1a** the tetrahydrofuran **11a** was obtained by subsequent hydrogenation, reduction of the ester group and dehydration with trimethylorthoformate. The low affinity of **11a** to SHBG (Fig. 3) proved the importance of a second aromatic residue for SHBG binding.

The extent of SHBG binding is reduced by any variation in the aromatic part of **8a** as shown by the decline in affinity from **8a** > **8f** > **8 g** > **8 g** to **8h** (Fig. 3).

Discussion

Comparing all the tested compounds with respect to their binding affinity to SHBG, the 8–8′ coupled dimeric phenylpropane structure seems to

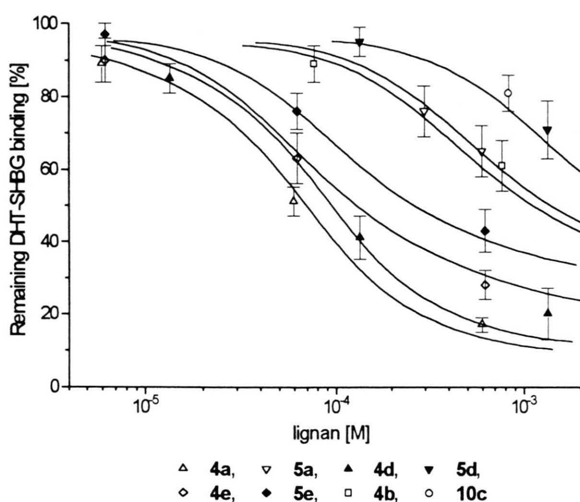


Fig. 2. Incubation of human SHBG, [1,2-^{3}H(N)]-dihydrotestosterone and lignans with various substituents in the aromatic part of the molecules in different concentrations.

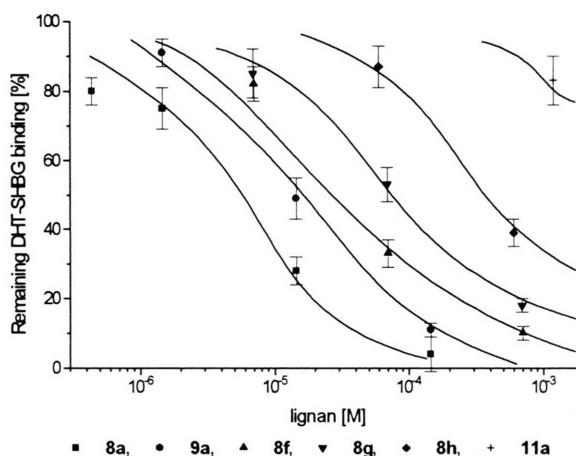


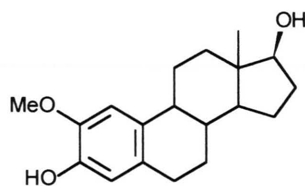
Fig. 3. Incubation of human SHBG, [1,2-^{3}H(N)]-dihydrotestosterone and different substituted monoepoxy lignans in different concentrations.

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

be a prerequisite for an effective binding to SHBG. The loss of one benzylic part of the molecule causes a total loss in activity (**11a**, **1a**). The low activity of the unsatured compound **3a** may be due to the low flexibility or for the wrong configuration of the double bond. It is apparent from the different guajacyl lignans **4a** – **10a** that the affinity to SHBG can be correlated with the polarity of the substitutents at C-9 and C-9′, resp.: The binding affinity increases with a decrease in polarity, starting from the diol **6a** via the esters **4a** and **10a** to the tetrahydrofuran derivative **8a.** The acetalic compound **12a** is exceptional. Its low affinity may be due to the bulky 7-membered ring.

The (±)-lignans **4** are always more effective than the analogous diastereoisomers **5**. This tendency is also observed with the asymmetric lignans **4d** and **4e** which are superior to **5d** and **5e**.

The most potent substitution pattern at the aromatic part of the molecule is the 4-hydroxy-3-methoxyphenyl (guajacyl-) ring. This is in agreement with the high binding affinity of 2-methoxyestradiol to SHBG (Scheme 2) (Dunn *et al.*, 1980), which is even more active than testosterone.



Scheme 2. Structure of 2-methoxyestradiol

Any variation of the aromatic substitution pattern leads to a decrease in activity. In asymmetrical lignans the aromatic part with the better binding properties is responsible for the binding affinity of the whole molecule. By comparing the achieved data the effectiveness of the substituted aromatic rings was estimated to be guajacyl > syringyl ≫ 3,4-dimethoxyphenyl ~ phenyl > 4-hydroxyphenyl ~ 4-acetoxy-3-methoxyphenyl (Scheme 3).

The concentration of lignans in female urine was found to be dependent on the menstrual cycle (Setchell *et al.*, 1980; Stitch *et al.*, 1980). Therefore, lignans discovered in urine have been assumed to represent a new class of hormonal compounds (Setchell and Adlerkreutz 1988). The binding of lignans to SHBG emphasizes these hormone-like properties.

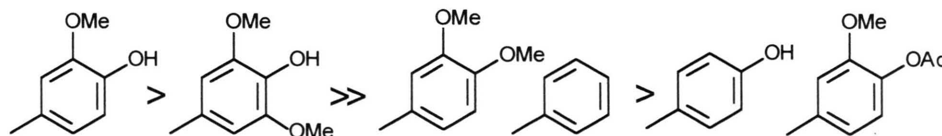
In man 58% of total testosterone is bound to SHBG (Vermeulen *et al.*, 1971). The displacement of this sex hormone by lignans should cause a change in the concentration of free and therefore active steroid hormones (Mendel, 1989). SHBG is more than a simple transport protein. Beside the steroid hormone binding site SHBG has another binding site for specific receptors on the membranes of typical hormone target tissues (reviewed by Joseph, 1994). The receptor-SHBG interaction is effected by the occupancy of the steroid binding site (Hryb *et al.*, 1990). Therefore an influence of lignans on this receptor SHBG interaction by lignans is conceivable.

The findings presented in this paper may contribute to develop a quantitative structure-activity relationship of lignans to SHBG which may allow the synthesis of even more active lignans in the future. Especially the remarkably high activity of (±)-3,4-divanillyltetrahydrofuran suggests that this compound develops benefical properties in the treatment of hormone-dependent diseases (Pugeat *et al.*, 1988), e.g. benign prostatic hyperplasia (Farnsworth, 1996).

Although lignans are present in higher concentrations than steroid hormones in blood plasma, (up to 1 μM, Adlercreutz, 1994), the pysiological relevance of their binding to SHBG is difficult to estimate and should be subject of further investigations.

Experimental

Melting points (m.p.): uncorr.; NMR: Bruker DRX 500, AM 300; EIMS: MAT 95 (Finnigan).



Scheme 3. Affinity to SHBG depending on the aromatic part of the molecules.

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

GC (H_2 at 50 kPa, 3 min 80 °C, 80 °C to 280 °C with 3 °C min^{-1}, 280 °C for 15 min) was performed on a Carlo Erba GC using a fused silica capillary column coated with DB 1 phase (30m×0.32 mm, film 0.1 μm, J&W Scientific). The retention index R_i was calculated according to van den Dool *et al.* (Van den Dool and Kratz, 1963). HPLC: (Beckman) was used with a variable wavelength detector (detector 168) using 280 nm *semiprep. HPLC-column* Spherisorb ODS-2, 5 μm (240 mm× 8 mm). Trimethylsilylation was achieved by treatment with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (Macherey & Nagel, Düren, Germany) for 8 h at room temperature. For synthesis only anhydrous solvents were used.

(E)-2-(4-Hydroxy-3-methoxy-benzyliden)-dimethylsuccinate (**1a**)

A Stobbe condensation of 21.45 g (0.14 mol) vanillin and 18.38 ml (0.14 mol) dimethylsuccinate according to Daughan (Daugan and Brown, 1991) yielded 36.8 g crude halfester. The slightly yellow product was dissolved in 400 ml dried MeOH. Then a mixture of 100 ml trimethylorthoformate, 2.5 ml H_2SO_4 (96%) and 100 ml dried MeOH was added. After refluxing for 4 h a part of the solvent was removed to reach a volume of 200 ml. This solution was added to 500 ml cooled (0 °C) half-saturated $NaHCO_3$ solution. The white precipitate was filtered and washed with water. After high vacuum drying 31.25 g crude diester was obtained. This product was recrystallized using 200 ml diethylether. Yield: 30.62 g (76%).

R_f (CH–EtOAc 2:1) = 0.57; m.p. (EtOEt): 86 °C; RI (trimethylsilylated): 2200; MS *m/z* (rel. int): 280 (M^+, 100), 248 (21), 220 (13), 189 (89), 161 (28), 145 (18); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 3.57 (s 2H H-8′), 3.70 (s 3H O**Me**); 3.78 (s 3H O**Me**); 3.85 (s 3H O**Me**); 5.87 (s 1H OH); 6.88–6.91 (m 3H H-2/H-5/H-6).

(E,E)-2,3-Bis-(4-hydroxy-3-methoxy-benzyliden)-monomethylsuccinate (**2a**)

An analogous condensation of 14.26 g (0.051 mol) **1a** and 7.75 g (0.051 mol) vanillin yielded 17.81 g (87%) of the crude monoester **2a**.

R_f (CH–EtOAc 2:1) = 0,42; RI (trimethylsilylated): 3095; MS (trimethylsilylated) *m/z* (rel. int.): 616 (M^+ 23), 601 (9), 526 (7), 499 (6), 466 (19), 437 (11), 389 (12), 331 (17), 209 (82), 73 (100); ^{1}H-NMR (500 MHz, d_4-methanol): δ (ppm) 3.67 (s 3H COOMe), 3.72 (s 3H ArO**Me**), 3.73 (s 3H ArO**Me**), 6.78 (d 2H ^3J = 8.3 Hz H-5/5′), 7.11 (m 2H H-6/6′), 7.25 (d 1H ^4J = 2 Hz H-2), 7.29 (d 1H ^4J = 2 H-2′), 7.82 (s H-7); 7.84 (s H7′).

(E,E)-2,3-Bis-(4-hydroxy-3-methoxy-benzyliden)-dimethylsuccinate (**3a**)

17 g (0,045 mol) crude monomethylester **2a** were dissolved in 400 ml MeOH. A mixture of 20 ml trimethylorthoformate in 20 ml MeOH and 1 ml H_2SO_4 (96%) was added and refluxed for 27 h. After cooling 1 g $NaHCO_3$ was added and the solvent reduced to ~50 ml. After addition of 200 ml half satured $NaHCO_3$ the aqueous solution was extracted 3 times by 200 ml CH_2Cl_2. The organic phases were combined and dried over sodium sulfate. The solvent was removed, yielding 16.75 g crude product. After recrystallization from toluene 13.76 g (0.033 mol, 74% yield) of the diester **3a** were obtained.

R_f (CH–EtOAc 1:1): 0.32; m.p. 159–161 °C (toluene); RI (trimethylsilylated): 3070; MS (trimethylsilylated) *m/z* (rel. int.): 562 (M^+, 84), 547 (5), 250 (7), 209 (100), 196 (18), 179 (17), 73 (21). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.68 (s 6H COO**Me**), 3.69 (s 6H ArO**Me**), 6.73 (2H d ^3J = 8.3 H-5), 7.01 (dd 2H ^4J = 2.1 Hz ^3J = 8.3 Hz H-6), 7.16 (d 2H ^4J = 2.1 Hz H-2), 7.84 (s 2H H-7), ^{13}C-NMR (125 MHz): δ 52.8 (COO**Me**), 56.2 (ArO**Me**), 113.4 (C-2), 116.3 (C-5), 123.9 (C-8), 126.3 (C-6), 127.8 (C-1), 144.0 (C-7), 148.9 (C-3), 150.1 (C-4), 169.6 (C-9). The assignment of the carbons was achieved by HMQC (**H**eteronuclear **M**ultiple **Q**uantum **C**orrelation) and HMBC (**H**eteronuclear **M**ultiple **B**ond **C**orrelation) experiments. The coupling constant of $^3J_{C\text{-}9\ H\text{-}7}$ = 7.2 Hz proves the (*E,E*)-configuration of **3a** (Kalinowski, 1987).

2,3-Bis-(4-hydroxy-3-methoxy-benzyl)-dimethylsuccinate (**4a, 5a**)

2.02 g (4.88 mmol) of the diester **3a** were stirred in 200 ml methanol with 200 mg Pd/C (5% Aldrich) for 1 day under 200 kpa hydrogen pressure. The solution was diluted with 200 ml THF. After

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

removal of the catalyst and solvent 1.98 g (4.73 mmol 97%) of a mixture of compounds **4a** and **5a** (2:1) was obtained. The diastereoisomeric compounds were separated by crystallization from MeOH/THF 1:1. Although **4a** was generated in excess, **5a** crystallized preferentially.

Characterization of **4a**: R_f (CH–EtOAc 1:1): 0,35; m.p. (MeOH): 159–161 °C; RI (trimethylsilylilated): 3050 (GC temperature program: 200 °C 1 min, 200–280 °C 2 K/min, 280 °C 15 min); MS (trimethylsilylated) *m/z* (rel. int.): 562 (M^+ 84), 547 (5), 250 (7), 209 (100), 196 (18), 179 (17), 73 (21); ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 2.9 (m 6H H-7 H-7′ H-8), 3.62 (s 6H COOMe), 3.75 (s 6H ArOMe), 5.48 (s 1H OH), 6.44 (d 1H J = 2 Hz H-6), 6.55 (dd 1H J = 8 Hz J' = 2 Hz H-6), 6.77 (d 1H J = 8 Hz H-5); ^{13}C-NMR (125 MHz $CDCl_3$): δ (ppm) 35.3 (C-8), 47.6 (C-7), 51.8 (COO**Me**), 55.7 (Ar-O**Me**), 111.1 (C-2) , 114.0 (C-5), 121.8 (C-6), 130.4 (C-1), 144.1 (C-4), 146.4 (C-3), 173.9 (C-9).

Characterization of **5a**: R_f (CH–EtOAc 1:1): 0,34; m.p. (MeOH–THF 1:1): 198–199 °C; RI (trimethylsilylated): 3085 (GC temperature program: 200 °C 1 min, 200–280 °C 2 K/min, 280 °C 15 min); MS: no significant difference to **4a**. ^{1}H-NMR (300 MHz): δ 2.75 (m 4H H-7/7′), 2.99 (m 2H H-8), 3.54 (s 6H ArO**Me**), 3.83 (s 6H COO**Me**), 6.60 (m 4H H-2 H-4), 6.78 (d 2H ^3J = 8 Hz H-5).

(±)-Secoisolariciresinol (**6a**)

$LiAlH_4$ reduction of 1.0 g (2.4 mmol) **4a** in THF yielded 730 mg (2.0 mmol 84%) (±)-secoisolariciresinol (**6a**).

R_f (EtOAc): 0.40; m.p. (EtOAc): 108 °C; RI (trimethylsilylated): 3050; MS *m/z* (rel. int.): 362 (M^+ 12), 344 (4), 189 (6), 137 (100), 122 (9), 94 (5); ^{1}H-NMR is in agreement with the literature (Agrawal and Rastogi, 1982).

Meso-secoisolariciresinol (**7a**)

A $LiAlH_4$ reduction of 50 mg (0.12 mmol) **4a** in THF yielded 32 mg (0.09 mmol) 75% meso-secoisolariciresinol (**7a**).

R_f (EtOAc): 0,35; m.p. (EtOAc) 110 °C; RI (trimethylsilylated): 3050; MS (trimethylsilylated) *m/z* (rel. int.): no significant difference to **6a**. ^{1}H-NMR is in agreement with the literature (Agrawal and Rastogi, 1982).

(±)-3,4-Divanillyltetrahydrofuran (**8a**)

100 mg (0.28 mmol) of (±)-secoisolariciresinol were dissolved in 50 ml MeOH. A mixture of 50 ml trimethylorthoformate and 0.5 ml H_2SO_4 was added. The mixture was stirred at room temperature over night. After neutralization with $NaHCO_3$ the solvent was reduced to ~30 ml, diluted with 150 ml diethylether and extracted with 2×50 ml brine. The organic phase was dried over Na_2SO_4. After removal of the solvent 92 mg (0.27 mmol 95%) pure 3,4-divanillyltetrahydrofuran were obtained.

R_f (CH/EtOAc 2:1): 0.38; m.p. (EtOAc): 121 °C; RI (trimethylsilylated): 2920; MS *m/z* (rel/int): 344 (M^+ 24); 137 (100), 122(18), 94 (16); UV: ε_{281} = 6560; ^{1}H-NMR (500 MHz): δ (ppm) 2.15 (m 2H H-8), 2.50 (dd 2H ^2J = 13.7 Hz ^3J = 7.9 Hz H-7_a), 2.56 (dd 2H ^2J = 13.7 Hz ^3J = 6.6 Hz H-7_b), 3.51 (dd 2H ^2J = 8.7 Hz ^3J = 5.7 Hz H-9_a), 3.81 (s 6H ArO**Me**), 3.89 (dd 2H ^2J = 8.7 Hz ^3J = 6.6 Hz H-9_b), 6.48 (d 2H ^4J = 1.9 Hz H-2), 6.57 (dd 2H ^3J = 8.0 Hz ^4J = 8,0 Hz), 6.78 (d 2H ^3J = 8.0 Hz). ^{13}C-NMR (125 MHz): δ (ppm) 39.2 (C-8), 45.0 (C-7), 55.8 (ArO**Me**), 73.3 (C-9), 111.2 (C-2), 114.2 (C-5), 121.3 (C-6), 132.3 (C-4), 144.0 (C-1), 146.4 (C-3).

Meso-3,4-divanillyltetrahydrofuran (**9a**)

Analogous to **8a** 12.3 mg meso-3,4-divanillyltetrahydrofuran **9a** were obtained by treatment of meso-secoisolariciresinol with trimethylorthoformate/H_2SO_4.

R_f (CH/EtOAc 2:1): 0.33; m.p.: 137 °C; RI (trimethylsilylated): 3050; MS (trimethylsilylated) *m/z* (rel. int.): 488 (M^+ 100), 473 (8), 261 (5), 209 (56), 179 (18), 73 (13); ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 2.48 (m 2H H-7_a, 2.54 m 2H H-8), 2.82 (m 2H H-7_b), 3.63 (dd 2H ^2J = 8.4 Hz ^3J = 5 Hz H-9_a), 3.78 (dd 2H ^2J = 8 Hz ^3J = 6 Hz H-9_b), 3.84 (s 6H ArO**Me**), 6.62 (d 2H ^4J = 1.9 Hz H-2), 6.66 (dd ^4J = 1.9 Hz ^3J = 8 Hz H-6), 6.82 (d 2H ^3J = 8 Hz H-5); ^{13}C-NMR (125 MHz; $CDCl_3$): δ (ppm) 33.0 (C-8), 43.8 (C-7), 55.8 (ArO**Me**), 72.9 (C-9), 111.2 (C-5), 114.3 (C-5), 121.2 (C-6), 132.5 (C-4), 143.8 (C-1), 146.4 (C-3).

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

(±)-5,6-Di-(4-hydroxy-3-methoxy)-benzyl-2,2-dimethyl-[1,3]dioxepane (**12a**)

(±)-Secoisolariciresinol **6a** was treated with acetone/$HClO_4$ (Cambie *et al.*, 1985). The byproduct **12a** was separated by HPLC.

HPLC: solvent CH_3CN/H_2O, 40% CH_3CN to 60% CH_3CN in 20 min, R_t = 17.9 min; R_f (CH/EtOAc 2:1): 0.40; RI (trimethylsilylated): 3060 MS (trimethylsilylated) *m/z* (rel. int.): 402 (21), 344 (12), 194 (13), 180 (19), 150 (32), 137 (100), 122 (13); ^{1}H-NMR (500 MHz, $CDCl_3$): 1.34 (s 6H C-$\mathbf{CH_3}$), 1.56 (m 2H H-8/8′), 2.58 (dd 2H ^2J = 13,9 Hz ^3J = 6.8 Hz H-$7_a/7'_a$), 2.67 (dd 2H ^2J = 13.9 Hz ^3J = 7.5 Hz H-$7_b/7'_b$), 3.40 (dd 2H ^2J = 12.3 Hz ^3J = 5.2 Hz H-$9_a/9'_a$), 3.75 (s 3H), 3.83 (dd 2H ^2J = 12.3 Hz, ^3J = 1,4 Hz ^3J = 5.2 Hz H-$9_b/9'_b$), 6.44 (d ^4J = 1.9 Hz H-2), 6.54 (dd ^3J = 8.0 Hz ^4J = 1.9 Hz H-5), 6.76 (d ^3J = 8.0 Hz H-5). ^{13}C-NMR (125 MHz; $CDCl_3$): 24.6 (C-$\mathbf{CH_3}$), 32,1 (C-8), 42.6 (C-7), 55.6 (Ar-O**Me**), 61.2 (C-9), 111.2 (C-2), 113.2 (C-5), 121.2 C-6, 143.6 (C-1), 146.3 (C-3).

(−)-Matairesinol (**10a**) was obtained from Dr. R. Kasper (Pharmazeutische Biologie, Freie Universität Berlin).

(±)-3-Vanillyltetrahydrofuran (**11a**)

Hydrogenation of **1a** (analogous **4a** and **5a**), reduction with $LiAlH_4$ in THF and a dehydation with trimethylorthoformate/H_2SO_4 yielded **11a**.

RI (trimethylsilylated): 1850; MS *m/z* (rel. int.): 208 (M^+ 64), 138 (57), 137 (100), 123 (9), 106 (8); ^{1}H-NMR (300 MHz $CDCl_3$): δ (ppm) 1.58 (m 1H H-$8'_a$), 1.97 (m 1H H-$8'_b$), 2.48 (m 1H H-8), 2.61 (d 2H ^3J = 8 Hz H-7), 3.4–4.0 (m 4H H-9/ H-9′), 3.83 (s 3H OMe), 6.64 (m 2H H-6/H-2), 6.81 (d 1H ^3J = 8.5 Hz H-5); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 32.04 (C-8′), 38.92 (C-8), 41.14 (C-7), 55.79 (C-9′), 72.85 (C-9), 111.17 (C-2), 114.28 (C-5), 121.15 (C-6), 132.64 (C-1), 143.86 (C-4), 146.41 (C-3).

(±)-2,3-Bis-benzyldimethylsuccinate (**4b**) was obtained analogous to **4a** using benzaldehyde instead of vanillin. After recrystallization from EtOAc the total yield of (±)-2,3-bis-benzyldimethylsuccinate **4b** was 10.7%.

RI: 2180; MS *m/z* (rel. int.): 326 (M^+ 4), 294 (5), 264 (5), 175 (22), 131 (69), 104 (37), 91 (100). ^{1}H-NMR (300 MHz $CDCl_3$): δ (ppm) 3.0 (m 6H H-7/8), 3.56 (s 6H COO**Me**), 7.05–7.30 (10H m Ar).

2,3 Bis(4-hydroxybenzyl)-butyrolactone (**10c**)

2a was produced by Stobbe condensation of *p*-hydroxybenzaldehyde and dimethylsuccinate. Hydrogenation and reduction with $LiAlH_4$ yielded a mixture of diastereoisomeres **10c**.

Mixture of diasteroisomers 2:1 (±): meso; MS (rel. int.): 298 (M^+ 23), 164 (10), 134 (12), 108 (7), 107 (100), ^{1}H-NMR (300 MHz, D_4-Methanol) (±)-**10c**: δ (ppm) 2.45–2.95 (m 6H H-7/8/7′/8′), 4.09 (m 2H H-9′), 6.72 (d 2H H-2/6), 6.76 (d 2H H-2′/6′), 6.93 (d 2H J = 8.2 Hz H-3/5), 7,02 (d 2H J = 8.2 Hz H-3′/5′). Meso-**10c**: δ (ppm) 3.25–2.45 (m 6H H-7/8/7′/8′), 3.92 (m 2H H9/9′), 6.69 (2H d ^3J = 8.2 Hz H-2/6), 6.80 (2H d ^3J = 8.2 Hz H-2′/6′), 6.91 (2H d ^3J = 8.2 Hz H-3/5), 7.20 (d 2H ^3J = 8.2 Hz H-3′/5′).

2-(4-Hydroxy-3-methoxy-benzyl)-3-benzyldimethylsuccinate (**4d**) was synthesized as described for **4a** using benzaldehyde, dimethylsuccinate and vanillin.

HPLC 50% aqueous MeCN isocratic R_t = 16.1 min; RI (trimethylsilylated): 2650; MS (rel. int.): 373 (19), 372 (M^+ 85), 341 (10), 309 (9), 221 (14), 209 (52), 208 (23), 178 (10), 177 (54), 137 (100), 124 (15), 91 (8); ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 2.85–3.05 (m 6H H-7/8), 3.60 (s 3H COO**Me**), 3.62 (s 3H COO**Me**), 3.79 (s 3H ArO**Me**), 5.5 (s 1H ArO**H**), 6.52 (d J = 2 Hz H-2), 6.58 (dd 1H J = 8 Hz J' = 2 Hz H-6), 6.80 (d 1H J = 8 Hz H-5), 7.07 (m 2H H-2′/6′), 7.2 (m 1H H-4′), 7.25 (m 2H H-3′/5′).

2-(4-Hydroxy-3-methoxy-benzyl)-3-benzyldimethylsuccinate (**5d**) was synthesized analogous to **5a** using benzaldehyde, dimethylsuccinate and vanillin.

HPLC: solvent CH_3CN/H_20, 50% CH_3CN isocratic, R_t = 13.2 min; RI (trimethylsilylated): 2650; MS: no significant difference to **4d**; ^{1}H-NMR (500 MHz, CDCl3): δ (ppm) 2.69–2.91 (m 4H H-7/7′), 3.2 (m 2H H-8/8′), 3.52 (s 3H COOMe), 3.53 (s 3H H-COOMe), 3.83 (s 3H H-OMe), 5.48 (s 1H OH), 6.59 (m 2H H-2/6), 6.78 (d 1H J = 8.4 Hz H-5), 7.11 (m 2H H-2′/H6′), 7.18 (m 1H H-4′), 7.24 (m 2H-3′/5′)

2-(4-Hydroxy-3,5-dimethoxy-benzyl)-3-benzyldimethylsuccinate (**4e**) was synthesized analogous to **4a** using benzaldehyde, dimethylsuccinate and syringaldehyde.

HPLC: solvent $MeOH/H_20$, 60% MeOH isocratic, R_t = 14.8 min; RI (trimethylsilylated): 2795;

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

MS (rel. int.): 402 (78), 371 (6), 239 (37), 238 (19), 207 (42), 167 (100), 91 (8); ^{1}H-NMR (500 MHz $CDCl_3$) δ (ppm) 2.84–3.06 (m 6H H-7/7′/8/8′), 3.60 (s 3H COO**Me**), 3.61 (s 3H COO**Me**), 3.78 (s 6H Ar**Me**), 7.04–7.24 (m 5H H-Ar); ^{13}C-NMR (125MHz $CDCl_3$) δ (ppm) 35.52, 35.77, 47.65, 48.02, 51.74, 51.76, 56.20, 105.58, 126.50, 128.40, 128.99, 129.56, 133.30, 138.55, 146.88, 173.75, 173.81.

2-(4-Hydroxy-3,5-dimethoxy-benzyl)-3-benzyl-dimethylsuccinate (**5e**) was synthesized analogous to **5a** using benzaldehyde, dimethylsuccinate and vanillin.

HPLC: solvent MeOH/H_20, 60% MeOH isocratic, R_t = 12.6 min; RI (trimethylsilylated): 2795; MS no significant difference to **4e**; ^{1}H-NMR (500 MHz $CDCl_3$): δ (ppm) 2.69–3.07 (m 6H H-7/7′/8/8′), 3.53 (3H s 3H COO**Me**), 3.56 (s 3H COO**Me**), 3.82 (s 6H ArO**Me**), 7.1–7.3 (m 7H Ar-H); ^{13}C-NMR (125 MHz $CDCl_3$): δ (ppm) 36.57, 36.69, 49.80, 50.16, 51.66, 51.70, 56.24, 105.38, 126.62, 128.43, 128.79, 129.13, 133.29, 138.22, 146.85, 173.88.

Derivatisation of (±)-3,4-divanillyltetrahydrofuran with diazomethane

(±)-3,4-divanillyltetrahydrofuran was treated with CH_2N_2 in diethylether yielding a mixture of different methylated products which were separated by HPLC.

(±)-3,4-Bis-(3,4-dimethoxybenzyl)-tetrahydrofuran (**8g**)

HPLC: solvent CH_3CN/H_2O, 40% CH_3CN to 60% CH_3CN in 20 min, R_t = 16.4 min; RI: 2800; MS (rel. int.): 372 (M^+ 30), 152 (97), 137 (20), 121 (24), 107 (14), 91 (9). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.18 (m 2H H-8), 2.52 (dd 2H ^2J = 13.8 Hz ^3J = 8.2 Hz H-7_a), 2.63 (dd 2H ^2J = 13.8 Hz ^3J = 6.2 Hz H-7_b), 3.52 (dd 2H ^2J = 8.7 Hz ^3J = 6.0 Hz H-9_a), 3.83 (s 6H O**Me**), 3.85 (s 6H O**Me**), 3,90 (dd 2H ^2J = 8.7 Hz ^3J = 6,6 Hz H-9_b), 6.6O (d 2-H ^4J = 2.0 Hz H-2), 6.62 (dd 2H ^3J = 8.1 Hz ^4J = 2.0 Hz H-6), 6.75 (d 2H ^3J = 8.1 Hz H-5).

3-(3,4-Dimethoxybenzyl)-4-(4-hydroxy-3-methoxy-benzyl)-tetrahydrofuran (**8f**)

HPLC: solvent CH_3CN/H_2O, 40% CH_3CN to 60% CH_3CN in 20 min, RI (trimethylsilylated): 2860; R_t = 12.4 min; MS (rel. int.): 358 (M^+ 26), 152 (77), 151 (100), 138 (27), 137 (82); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.17 (m 2H H-7), 2.46–2.62 (m 4H H-7), 3.51 (m 2H H-9_a), 3.81 (s 3H O**Me**), 3.82 (s 3H O**Me**), 3.84 (s 3H O**Me**), 3.9 (m 2H H-9_b), 6.50–6.64 (m 4H H-2/6), 6.74 (d 1H J = 8 Hz H-5), 6.80 (d 1H J = 8 Hz H-5′).

Acknowledgements

We thank Mr. K. Pöllmann and Dr. J. Reiner for NMR-Data and Dr. R. Kasper of the Department of Pharmazeutische Biologie, Freie Universität Berlin, Germany, for the generous gift of (−)-matairesinol. Furthermore we are very grateful to Kanoldt Arzneimittel GmbH for the financial support.

Adlercreutz H., Fotsis T., Watanabe S., Lampe J., Wahala K., Makela T. and Hase T. (1994), Determination of lignans and isoflavonoids in plasma by isotope dilution gas chromatography-mass spectrometry. Cancer Detect. Prev. **18**, 259–71.

Agrawal P. K. and Rastogi R. P. (1982), Two lignans from *Cedrus deodara*. Phytochemistry **21**, 1459–61.

Batterbee J. E., Burden R. S., Crombie L. and Whiting D. A. (1969), Chemistry and synthesis of the lignan (1)-cubebin. J. Chem. Soc. C **18**, 2470–7.

Cambie R. C., Clark G. R., Craw P. A. and Jones T. C., Rutledge P. S. and Woodgate P. D. (1985), Chemistry of the Podocarpaceae. LXIX. Further lignans from the wood of *Dacrydium intermedium*. Aust. J. Chem. **38**, 1631–45.

Coran S. A., Bambagiotti-Alberti M., Melani F., Giannellini V., Vincieri F. F., Mulinacci N. and Sala R. (1991), Synthetic butanolide and tetrahydrofuran lignans with platelet activating factor antagonist activity. Eur. J. Med. Chem. **26**, 643–50.

Cunningham G. R., Tindall D. J., Lobl T. J., Campbell J. A. and Means A. R. (1981), Steroid structural requirements for high affinity binding to human sex steroid binding protein (SBP). Steroids **38**, 243–62.

Daugan A. and Brown E. (1991), Lignans. 11. Preparation of (R)-(+)-β-vanillyl-γ-butyrolactone and its use in the total synthesis of natural lignans. J. Nat. Prod. **54**, 110–18.

Dunn J. F., Merriam G. R., Eil C., Kono S., Loriaux D. L. and Nisula B. C. (1980), Testosterone-estradiol bind-

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45

ing globulin binds to 2-methoxyestradiol with greater affinity than to testosterone. J. Clin. Endocrinol. Metab. **51**, 404–6.

Farnsworth W. E. (1996), Roles of estrogen and SHBG in prostate physiology. Prostate (N. Y.) **28**, 17–23.

Ganßer D. and Spiteller G. (1995) Plant constituents interfering with human sex hormone-binding globulin. Evaluation of a test method and its application to *Urtica dioica* root extracts Z. Naturforsch. **50 c**, 98–104.

Hammond G. L. and Bocchinfuso W. P. (1995), Sex hormone-binding globulin/androgen-binding protein: steroid-binding and dimerization domains. J. Steroid Biochem. Mol. Biol. **53**, 543–5.

Hirano T., Oka K., Naitoh T. and Hosaka K. (1989), Endogenous digoxin-like activity of mammalian-lignans and their derivatives. Res. Commun. Chem. Pathol. Pharmacol. **64**, 227–40.

Hirano T., Fukuoka K., Oka K., Naito T., Hosaka K., Mitsuhashi H. and Matsumoto Y. (1990), Antiproliferative activity of mammalian lignan derivatives against the human breast carcinoma cell line, ZR-75–1. Cancer Invest. **8**, 595–602.

Hryb D. J., Khan M. S., Romas N. A. and Rosner W. (1990), The control of the interaction of sex hormone-binding globulin with its receptor by steroid hormones. J. Biol. Chem. **265**, 6048–54.

Joseph D. R. (1994), Structure, function, and regulation of androgen-binding protein/sex hormone-binding globulin. Vitam. Horm. (San Diego), **49 (Steroids)**, 197–280.

Kalinowski H. (1987), Carbon 13 NMR Spectroscopy. John Wiley & Sons Inc., New York, N. Y., 730 pp.

Martin M. E., Haourigui M., Pelissero C., Benassayag C. and Nunez E. A. (1995), Interactions between phytoestrogens and human sex steroid binding protein. Life Sci. **58**, 429–36.

Matsuura S., and Iinuma M. (1985), Lignan from calyces of *Diospyros kaki*. Phytochemistry **24**, 626–8.

MacRae W. D. and Towers G. H. N. (1984), Biological activities of lignans. Phytochemistry **23**, 1207–20.

Mendel C. M. (1989), The free hormone hypothesis: a physiologically based mathematical model. Endocr. Rev. **10**, 232–74.

Nishibe S. (1993), Lignans as bioactive components in traditional medicines. Polyphenolic Phenom., 247–55. Ed.: Scalbert, A. Publ.: INRA, Paris, Fr..

Pugeat M., Garrel D., Estour B., Lejeune H., Kurzer M. S., Tourniaire J. and Forest M. G. (1988), Sex steroid-binding protein in nonendocrine diseases. Ann. N. Y. Acad. Sci. **538** (Steroid-Protein Interact.: Basic Clin. Aspects), 235–47.

Schöttner M., Ganßer D. and Spiteller G. (1997), Lignans from the roots of *Urtica dioica* and their metabolites bind to sex hormone binding globulin (SHBG). Planta Med. **63**, in press..

Setchell K. D. R., Lawson A. M., Mitchell F. L., Adlercreutz H., Kirk D. N. and Axelson M. (1980), Lignans in man and in animal species. Nature **287**, 740–2.

Setchell K. D. R. and Adlercreutz, H. (1988), Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism and biological role in health and disease. In: Role of the Gut Flora in Toxicity and Cancer (Rowland, I. R., ed.), Publ.: Acad. Press Ltd.

Stitch S. R., Toumba J. K., Groen M. B., Funke C. W., Leemhuis J., Vink J. and Woods G. F. (1980), Excretion, isolation and structure of a new phenolic constituent of female urine. Nature **287**, 738–40.

Van den Dool H. and Kratz P. D. (1963), A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chrom. **11**, 463–71.

Vermeulen A., Stoica T. and Verdonck L. (1971), Apparent free testosterone concentration, an index of androgenicity. J. Clin. Endocrinol. Metab. **33**, 759–67.

Ward R. S. (1982), The synthesis of lignans and neolignans. Chem. Soc. Rev. **11**, 75–125.

Bereitgestellt von | Technische Informationsbibliothek Hannover
Angemeldet
Heruntergeladen am | 23.11.17 12:45