# 3-(Cyclohexenonyl)-4-(4-hydroxyphenyl)-hexanes: Antiandrogenes Derived from the Estrogen Hexestrol

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Selective *Birch*-reduction of one of the phenolic rings of *meso*- or *d,l*-HES led to compounds **6a** and **6b** which exert a higher binding affinity to the androgen receptor as the respective parent HES. *In vivo* testing of **6a** and **6b** shows that **6a** has the same potency in reducing accessory sex organ weights and testosterone levels in the intact mouse as has *meso*-HES, but strongly decreased estrogenic activity. Syntheses and testing of **7** having no ethyl side chains revealed the necessity of these groups for biological activity.

**3-(Cyclohexenonyl)-4-(4-hydroxyphenyl)-hexane: Antiandrogene abgeleitet von dem Östrogen Hexestrol**

Selektive *Birch*-Reduktion eines der phenolischen Ringe von *meso*- bzw. *d,l*-HES führte zu den Verbindungen **6a** und **6b** mit einer höheren Bindungsaffinität zum Androgenrezeptor als das entspr. HES. Die *in vivo* Testung von **6a** und **6b** zeigte, daß **6a** am intakten männlichen Tier die Gewichte der akzessorische Sexualorgane und den Testosteron-Spiegel im gleichen Ausmaß wie *meso*-HES verringert, aber gleichzeitig stark reduzierte estrogene Effekte besitzt. Die Synthese und Testung von **7**, dem die Ethylseitenketten fehlen, verdeutlichte die Notwendigkeit dieser Gruppen für die biologische Aktivität.

Since the pioneering work of *Huggins* and *Hodges*[1], bilateral orchidectomy and/or application of estrogens are standard therapies for advanced prostatic carcinomas. Treatment with estrogens such as diethylstilbestrol (DES) or its diphosphate Fosfestrol, however, is associated with severe side effects like gynecomastia, cardiovascular disorder and thromboembolic effects[2].

The major antiproliferative effect of estrogens on the prostatic carcinoma is exercised indirectly via the pituitary, by suppressing LH secretion and thus decreasing testicular synthesis and secretion of testosterone (T). There is additional evidence that estrogens may have a direct effect on prostatic tissue at the cellular level which could be mediated through the estrogen receptor found in prostate and prostatic carcinoma[3].

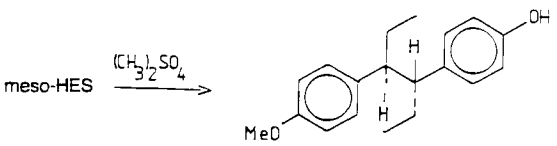
The main structural difference between estrogens and androgens is a cyclohexenone system in ring A of the latter compounds. Our aim was the development of compounds with reduced estrogenic properties but enhanced androgen receptor affinity and thereby antiandrogenic activity by reduction of one of the phenolic rings of DES.

## *Chemistry*

Selective *Birch*-reduction seemed to be the best way for synthesising compounds with a cyclohexenone system. Compounds containing a free phenolic hydroxy group in one and a methoxy-moiety in the other aromatic system can be reduced selectively in the methoxy-substituted ring by Li in liquid $NH_3$ [4]. Acid hydrolysis of the respective enol ether yields the cyclohexenone-system[5].

In the case of DES the conjugated double bond gives raise to side reactions during reduction, therefore we used hexestrol (HES), the dihydro derivative of DES. HES is a strong synthetic estrogen, too.

**1** was obtained by alkylating meso-HES (Sigma No. H 7753) with dimethyl sulfate in 10% KOH-solution.

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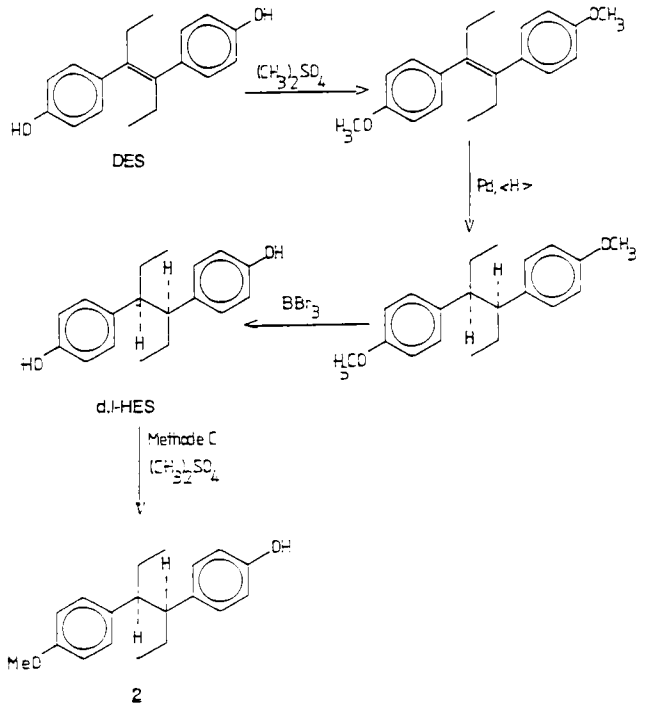
The threo from **2** was synthesized from d,l-HES which was obtained by hydrogenating dimethoxy-DES at room temp. in the presence of Pd and subsequent demethylation

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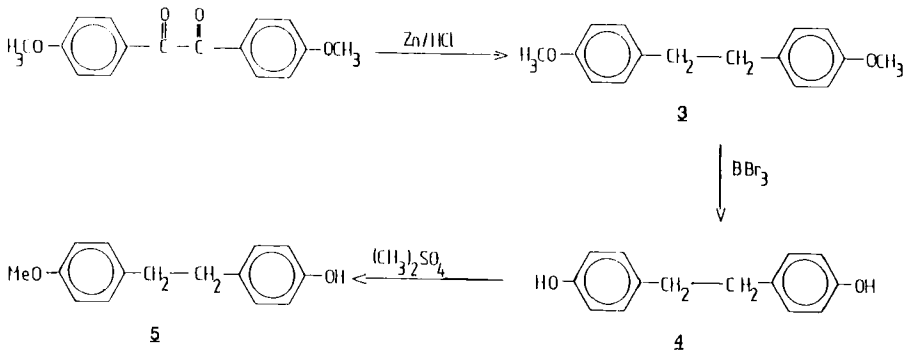
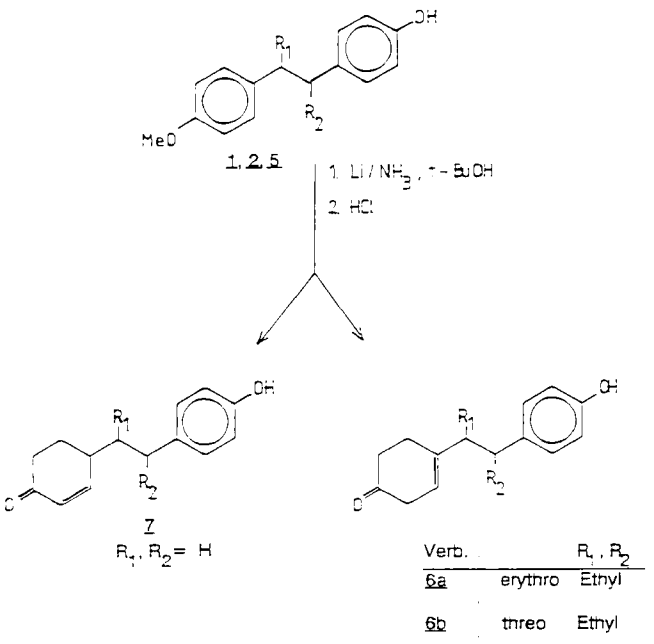



using $BBr_3$ in dichloromethane. d,1-HES was monomethylated as described above.

Reduction of 4,4'-Dimethoxybenzil to give **3** was effected by treatment with Zn/HCl. **4** was obtained after ether cleavage with $BBr_3$. Methylation of one of the two free hydroxy groups yielded compound **5**.

The monomethylethers **1**, **2**, and **5** were then reduced by Li in liquid $NH_3$ in the presence of t-butanol as proton donator. In some cases tetrahydrofuran had to be used as cosolvent to improve yields. Hydrolysis of the corresponding enol ethers was effected by treatment with 6N HCl in dichloromethane.




$^1$H-NMR spectra of **6a** and **7** showed differences in the range of 5-6 ppm. Rearrangement of β,γ-double bond during hydrolysis into α,β-position takes place only in the case of **7**. This could be demonstrated by comparison of the 250 MHz $^1$H-NMR spectra and the $^1$H-NMR literature spectrum of 2-cyclohexen-1-one[6]. On the basis of the $^{13}$C-NMR spectrum of **6a**, the structures of **6a** and **6b** could be assigned as cyclohex-3-en-1-on-4-yl-derivatives.

## *Biological Properties*

Basic requirement for hormonally active compounds is an affinity to the corresponding steroid hormone receptor. Therefore, determination of the relative binding affinitity (RBA) for the androgen receptor was tested first. Partial reduction of one phenolic ring of HES led to compounds [**6a,b**] with a tenfold enhanced affinity for the androgen receptor (Table 1) in comparison to the corresponding isomer of HES. The different configuration of **6a** (erythro) and **6b** (threo) had no major influence on binding affinity. Loss of the two ethyl side chains [**7**] strongly decreased the receptor affinity (<0.01). To gain further knowledge about the hormonal profile of these compounds their affinities to the estrogen and progesteron receptor were determined, too.

In contrast to meso and d,1-HES, **6a** as well as **6b** show only a very low affinity for the estrogen receptor (1/50 of the respective HES form). **7** had no measurable affinity for the estrogen receptor. Affinities for the progesterone receptor were either very low or not measurable.

**Table 1:** RBA of **6a**, **6b**, **7**, meso- and d,1-HES for steroid hormone receptors[a]

| compound | AR | ER | PR |
|---|---|---|---|
| **6a** (erythro) | 0.10 | 0.5 | 0.02 |
| **6b** (threo) | 0.07 | 0.06 | 0.04 |
| **7** | <0.01 | <0.01 | <0.01 |
| meso-HES | <0.05 | 27 | - |
| d,1-HES | <0.05 | 1.1 | - |

[a]RBA = ratio of the molar concentration of testosterone (AR), estradiol (ER) or progesterone (PR) and inhibitor required to decrease radioligand by 50 %, times 100. RBA of testosterone, estradiol or progesterone = 100.

In vivo activities of the new compounds were tested with regard to antiandrogenic and estrogenic properties in mice. Reduction of accesory sex organ weights in intact male animals can be caused by direct antiandrogenic effects at the receptor level or by indirect effects via a depression of T-level (antigonadotrophic activity) or by both modes.

In the intact mature mouse, **6a** reduced seminal vesicle weights and T-levels to the same extent as did the potent estrogen HES. These effects were only slightly lower than that of DES. The threo-form **6b** showed a significant inhibitory effect in all doses tested though the inhibition was weaker than that by **6a**. The decrease of the T-level by **6a** was a clear hint for its antigonadotrophic activity. **6b** affected the T-level slightly less than **6a**. In contrast to **6a** and **6b**, compound **7** showed no effect in this test, even at the 1000 µg dose.

**Table 2:** Effect of **6a, 6b, 7**, HES, and DES on the seminal vesicle weights and testosterone levels of intact mature mice (general aniandrogenic effect).

| Compound | Dose[a] (μg) | %-Inhibition | |
|---|---|---|---|
| | | Seminal vesicle weights[b] | Testosterone level |
| DES | 10 | 71* | 95 |
| | 100 | 84* | 98 |
| HES | 10 | 35* | 47 |
| | 100 | 75* | 81 |
| **6a** | 10 | 33* | 67 |
| | 100 | 71* | 84 |
| | 500 | 85* | 91 |
| | 1000 | 84* | 90 |
| **6b** | 100 | 50* | 45 |
| | 500 | 49* | 42 |
| | 1000 | 69* | 79 |
| **7** | 1000 | 8 | 0 |

[a] Dose/animal per day, administration daily for nine days sc
[b] Significant ($p < 0.01$)

The inhibition of seminal vesicle weights by meso-HES is accompanied by a strong depression of T-levels mainly due to its estrogenic effects. Therefore, it was of interest if **6a**, which shows identical antiandrogenic potency as meso-HES, exhibits such a strong estrogenic activity, too. In spite of their low ER affinities, **6a**, **6b**, and **7** show surprisingly high estrogenic properties (Table 3). However, in contrast to HES a 100-fold increased dose of **6a** had to be administered to reach an estrogenic effect comparable to that of meso-HES. At 100μg **6b** reached only 67% of the maximum effect of estrone. Consistent with the very low affinities for the ER, **7** exhibited only very low estrogenic activities.

**Table 3:** Estrogenic effect of **6a, 6b, 7**, and **HES** in the immature mouse uterine weight test

| Compound | Dose[a] (μg) | Uterine weight[b] | Estrogenic effect[c] |
|---|---|---|---|
| Control | | 11.33 ± 1.20 | 0 |
| Estrone | 0.4 | 47.25 ± 5.90 | 100 |
| **6b** | 5 | 20.65 ± 2.32 | 26* |
| | 25 | 31.63 ± 3.44 | 57* |
| | 100 | 36.13 ± 4.44 | 69* |
| **7** | 5 | 14.45 ± 2.18 | 9 |
| | 25 | 12.80 ± 4.79 | 4 |
| | 100 | 13.31 ± 3.87 | 6 |
| Control | | 17.89 ± 1.59 | 0 |
| Estrone | 0.4 | 62.41 ± 7.46 | 100 |
| **6a** | 1 | 31.70 ± 4.57 | 31* |
| | 5 | 58.10 ± 7.79 | 90* |
| | 25 | 82.70 ± 2.22 | 146* |
| | 100 | 61.05 ± 4.43 | 97* |
| Control | | 13.45 ± 0.71 | 0 |
| Estrone | 0.4 | 50.92 ± 3.04 | 100 |
| HES | 0.1 | 40.27 ± 1.61 | 72* |
| | 0.25 | 50.09 ± 1.32 | 98* |
| | 1.0 | 49.09 ± 1.92 | 95* |

[a] Dose/animal per day, subcutan in olive oil
[b] mg/100 g body wt ± SD
[c] % of estron standard
* Signifikant ($p < 0.01$)

These data reveal that partial reduction of one of the phenolic rings of HES provides a compound [**6a**] with strongly decreased estrogenic activity but retained antiandrogenic potency in intact male mice. These properties would offer the possibility of an androgen-deprivation similiar to estrogens with the advantage of reduced estrogenic activity and thereby possibly fewer side effects in the therapy of prostatic carcinoma. A detailed analysis of the tumor-inhibiting as well as the endocrinological properties of **6a** will be subject of a further publication.

## Experimental Part

Mp: Büchi 510 apparatus (uncorr.). - $^1$H-NMR Spectra: Varian EM 360 A. - IR-Spectra: Beckman Acculab 7. Mass Spectra: Varian MAT CH5. - HPLC: Altex 110 A pump; Kontron Uvikon 720 LC spectrophotometer; Column: Lichrosorb RP 18, 10 μm. - Elemental Analyses: Mikroanalyt. Laboratorium, Univ. Regensburg.

*erythro-4-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-hexane* (**1**)

KOH-solution (10%) was added to 23 mmol (5.00 g) meso-HES until a clear solution was obtained. Under stirring 5-7 drops of dimethyl sulfate were added and stirring was continued for 10 min. The monomethylether was extracted by $CH_2Cl_2$. The water layer was treated with 5-7 drops of dimethyl sulfate, stirred for 10 min and extracted with $CH_2Cl_2$. This procedure was repeated 10 - 15 times. After washing of the combined org. phases with saturated NaCl solution and drying with $MgSO_4$, the solvent was evaporated. The crude product was purified by column chromatography ($SiO_2$; $CH_2Cl_2$). Yield 71%, mp 116°C (Lit. [7]: 119-120°C), $C_{19}H_{24}O_2$ (284.4). - $^1$H-NMR (60 MHz, $CDCl_3$): δ = 0.52 (t, J = 7 Hz, 6H, $-CH_3$); 1.07-1.60 (m, 4H, $-CH_2$); 2.32-2.63 (m, 2H, -CH-); 3.80 (s, 3H, $-OCH_3$); 6.67-7.17 (m, 8H, aromat. H), 8.02 (s, 1H, -OH).

*threo-4-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-hexane* (**2**)

Yield 53 %, mp 77-78°C (Lit.[8]: 78-79°C), $C_{19}H_{24}O_2$ (284.4). - $^1$H-NMR (60 MHz, $CDCl_3$): δ = 0.72 (t, J = 7 Hz, 6H, $-CH_3$); 1.30-2.00 (m, 4H, $-CH_2$); 2.57-2.78 (m, 2H, -CH-); 3.78 (s, 3H, $-OCH_3$); 4.78 (s, 1H, -OH); 6.73-6.82 (m, 8H, aromat. H).

*2-(4-Hydroxyphenyl)-1-(4-methoxyphenyl)-ethane* (**5**)

Yield 90 %, mp 113-115°C, $C_{15}H_{16}O_2$ (228.3). - $^1$H-NMR (60 MHz, $CDCl_3$): δ = 2.98 (s, 4H, $-CH_2$), 4.03 (s, 3H, $-OCH_3$); 7.08 and 7.40 (AB, $J_{AB}$ = 9 Hz, 4H, aromat. H); 7.18 and 7.47 (AB, $J_{AB}$ = 9 Hz, 4H, aromat. H).

*1,2-Bis-(4-methoxyphenyl)-ethan* (**3**)

Zn dust (15 g) was treated with 1.50 g of $HgCl_2$ in 25 ml water and 0.5 ml conc. HCl under stirring for 5 min. After decanting the residue was washed twice with water.

The amalgamated zinc, 17 mmol (4.50 g) 4,4'-Dimethoxybenzil and 50 ml of 6N HCl were refluxed for 5 h. After the second and forth h 5 ml conc. HCl were added. After cooling, $CH_2Cl_2$ was added and the zinc separated by filtration. The org. layer was washed with saturated $NaHCO_3$ and saturated NaCl solution and dried with $MgSO_4$. After evaporation, the crude product was recrystallized from ethanol. Yield 85 %, mp 118-120°C (Lit.[9]: 123°C), $C_{16}H_{18}O_2$ (242.3). - $^1$H-NMR (60 MHz, $CDCl_3$): δ = 2.87 (s, 4H, $-CH_2$); 3.80 (s, 6H, $-OCH_3$); 6.74 and 6.88 (AB, $J_{AB}$ = 9 Hz, 8H, aromat. H).

*1,2-Bis-(4-hydroxyphenyl)-ethan* (**4**)

A solution of 20 mmol (4.85 g) **3** in 30 ml of dry $CH_2Cl_2$ was cooled to -60°C under $N_2$. Then a solution of 42 mmol (16.40 g) $BBr_3$ in 10 ml of dry $CH_2Cl_2$ was added. After 30 min the cooling bath was removed and the mixture was stirred for 2 h at room temp. After dropwise addition of 50 ml of saturated $NaHCO_3$ solution, 100 ml of EtOAc were added, the mixture stirred for 30 min and then extracted with EtOAc. The combined org. phases were washed with saturated NaCl solution and dried with $Na_2SO_4$. The solvent was evaporated and the residue recrystallized from ethanol. Yield 85 %, mp 184-186°C (Lit. [10]: 189°C), $C_{14}H_{14}O_2$ (214.3). - $^1$H-NMR (60 MHz, $CDCl_3$): δ = 2.73 (s, 4H, $-CH_2$); 6.70 and 7.02 (AB, $J_{AB}$ = 9 Hz, 8H, aromat. H).

*erythro-3-(Cyclohex-3-en-1-on-4-yl)-4-(4-hydroxyphenyl)-hexane* (**6a**)

A solution of 3.5 mmol (1.00 g) **1** in 10 ml of dry THF and 10 ml of t-butanol in 60 ml liquid $NH_3$ was treated with 170 mg-Atom (1.20 g) Li granula in portions. After each addition the blue color should disappear. Stirring was continued for 1 h at -70°C and then for 1 h at room temp.. The excess of Li was decomposed by addition of 2 ml of MeOH. After evaporation of $NH_3$, water and, if necessary, 3N HCl was added to give a clear solution. The mixture was extracted with ether. The org. phase was washed with water to remove MeOH and t-butanol.

After evaporation of the ether, the residue was dissolved in 5 ml of $CH_2Cl_2$ and cooled to 0°C. Icecold 6N HCl (5 ml) was added slowly, followed by stirring for 30 min at 0°C, 30 min at room temp. and 60 min at 40°C. The mixture was treated with water and extracted with $CH_2Cl_2$. The org. phase was washed with saturated $NaHCO_3$ solution and dried with $Na_2SO_4$. After evaporation of $CH_2Cl_2$ the residue was purified by column chromatography ($SiO_2$; $CH_2Cl_2$: EtOAc, 8:2). Yield 51 %, mp 136-137°C, $C_{18}H_{26}O_2$ (272.4), Calcd. C 79.4 H 8.82 Found C 79.5 H 9.02. - MS: (m/z) = 272 ($M^{+\cdot}$). - IR (KBr): 1720 $cm^{-1}$ (C=O). - $^1$H-NMR (250 MHz, $CDCl_3$): δ = 0.57-0.68 (m, 6H, $-CH_3$); 0.95-1.43 (m, 2H, $-CH_2$); 1.55-1.70 (m, 2H, $-CH_2$); 2.14 (dt, $J_d$ = 4 Hz, $J_t$ = 10 Hz, 1H, -CH); 2.26 (dt, $J_d$ = 3 Hz, $J_t$ = 11 Hz, 1H, -CH); 2.32 (t, J = 6 Hz, 2H, $-C(O)-CH_2-CH_2$); 2.51 (t, J = 6 Hz, 2H, $-C(O)-CH_2$); 2.94-2.95 (m, 2H, $-C(O)-CH_2-C=C-$); 5.56 (t, J = 4 Hz, 1H, -CH=C-); 6.78 and 6.98 (AB, $J_{AB}$ = 9 Hz, 4H, aromat. H).

*threo-3-(Cyclohex-3-en-1-on-4-yl)-4-(4-hydroxyphenyl)-hexan* (**6b**)

Yield 34 %, mp 88-90°C, $C_{18}H_{26}O_2$ (272.4), Calcd. C 79.4 H 8.82 Found C 78.8 H 8.34. - MS: (m/z) = 272 ($M^{+\cdot}$). - IR (KBr): 1720 $cm^{-1}$ (C=O). - $^1$H-NMR (60 MHz, $CDCl_3$): δ = 0.68 and 0.83 (t, J = 7 Hz, 6H, $-CH_3$); 1.18-2.54 (m, 10H, $-CH_2$, -CH-, $C(O)-CH_2-CH_2$); 5.38 (t, J = 4 Hz, 1H, =C-H); 6.92 and 7.14 (AB, $J_{AB}$ = 9 Hz, 4H, aromat. H).

*1-(Cyclohex-3-en-1-on-4-yl)-2-(4-hydroxyphenyl)-ethan* (**7**)

Yield 30 %, mp 108-110°C, $C_{14}H_{16}O_2$ (216.3), Calcd. C 77.8 H 7.46 Found C 78.2 H 7.37. - MS: (m/z) = 216 ($M^{+\cdot}$). - IR (KBr): 1660 $cm^{-1}$ (C=O). - $^1$H-NMR (250 MHz, $CDCl_3$): δ = 1.71-2.85 (m, 8H, alkyl H); 6.02 (d, J = 10 Hz, 1H, vinyl. H); 6.80 and 7.04 (AB, $J_{AB}$ = 8.3 Hz, 4H, aromat. H), 6.91 (d, J = 10 Hz, 1H, vinyl. H).

*Binding affinities for steroid hormone receptors*

The relative binding affinities were determined by the dextran coated charcoal method as described with some modifications [11]. 100 µl aliquots of calf uterine cytosol were incubated with 100 µl (1 nM) [$^3$H]-mibolerone (AR) for 2 h, [$^3$H]-estradiol (ER) for 16 h or [$^3$H]-R5020 (PR) for 2 h in the presence of varying concentrations of the test compounds (five or six concentrations). Incubation was stopped by adding dextran-coated charcoal. After centrifugation, the radioactivity of a 100 µl supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabelled test compounds. From this plot, the molar concentrations of unlabelled standards and of test compounds reducing radioligand binding by 50 % were determined.

*Antiandrogenic activity on intact mature male mice*

Mature male mice (23-25g; 6-7 mice/group from Ivanovas, Kisslegg, FRG) were used. The test compounds dissolved in olive oil were injected for 9 consecutive days. 24 h after the last injection, blood was collected by cardiac puncture under ether anesthesia, seminal vesicles were removed, dissected free from adhering tissue, dried overnight at 100°C and weighed.

The plasma of 3 mice (2 samples/group) was pooled and assayed for testosterone level in duplicate by radioimmunoassay.

*Testosterone radioimmunoassay*

Testosterone was assayed in plasma after centrifugation using a direct double antibody radioimmunoassay kit (DRG, Marburg, FRG).

*Estrogenic activity*

The uterotrophic activity in the immature mouse uterine weight test was determined as described [12].

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