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STEROID BINDING TO THE CYTOSOLIC ESTROGEN RECEPTOR FROM RAT UTERUS. INFLUENCE OF THE ORIENTATION OF SUBSTITUENTS IN THE 17-POSITION OF THE 8β- AND 8α-SERIES

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Summary—The exact chemical and sterical requirements in the 17-position of 8β- and 8α-estrogens for an effective binding to the cytosolic receptor of immature rat uterus were investigated by competition experiments under non-equilibrium conditions. Oxygen or nitrogen functions with free electron pairs seem to be of essential importance in the 17-position. In contrast to 17α-methyl-, -vinyl- or -ethinyl-substituents a 17α-ethyl group strongly disturbs receptor binding. Also the introduction of a quasi equatorial allene or a 17β-ethinyl group reduces receptor binding. In comparison to the 8β-estrogens, the 8α-derivatives always showed lower, but still significant receptor binding and similar response to changes of substituents in the 17-position.

INTRODUCTION

The interaction of an estrogenic steroid with its cytoplasmatic receptor has to fulfil some basic requirements. Besides the existence of an aromatic A-ring with a phenolic OH group in the 3-position of the steroid a further functional group in the D-ring is essential. In the case of the naturally occurring estrogens, this substituent either consists of a 17-keto group (estrone) or a 17β-OH group (estradiol), while a further OH-substituent may occupy the 16α-position (estriol). The essential importance of an oxygen function within the D-ring has been demonstrated already by several authors [1–5]. However there is only poor information available on the precise sterical and electronical requirements for a good interaction between the steroid and its receptor. A more detailed knowledge of the range of possible variations could be highly valuable for drug design in pharmacology, as well as for designing better fluorescence labelled steroids for receptor detection in histology. In order to get such information we systematically varied the structure of estrogenic steroids in the following ways: (1) by changing the position of the 17-OH group; (2) by adding different aliphatic substituents in the 17-position; (3) by replacing the 17-OH group by other polar groups, and (4) by changing the geometry of the molecule using steroids of the (unnatural) 8α-series. The binding of these steroids to the uterine estrogen receptor of immature rats was determined by the thin layer gel chromatography method of Toepert *et al.*[6].

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EXPERIMENTAL

All reagents were commercial products of p.a. grade. Column chromatography was done with silica gel 60, 35–70 mesh, from Merck; the plates for preparative thin layer chromatography were from Schleicher and Schuell (Silical G 1505/LS 254, 20 × 40 cm). The spectra were recorded on the following instruments: NMR: HR 90 (Bruker) and HX 100 (Varian). i.r.: Perkin–Elmer 580, u.v.: Carry 14 (Varian): MS: CH-7A (Varian) and Micromass 7070 (Vacuum Generators). The NMR data were expressed on the δ-scale. Melting points were determined on a Tottoli apparatus of Buechi, Switzerland, and are uncorrected. If not stated otherwise crystallizations were done from hexane–acetone.

Materials

1,3,5(10)-Estratrien-3,17β-diol (estradiol) (**1**), 1,3,5(10)-estratrien-3,17α-diol (**2**), 8α-estra-1,3,5(10)-trien-3,17β-diol (**3**), 17α-ethinyl-1,3,5(10)-estratrien-3,17β-diol (**5**), 17α-ethinyl-8α-estra-1,3,5(10)-trien-3,17β-diol (**7**), 17α-methyl-1,3,5(10)-estratrien-3,17β-diol (**9**), 17α-ethyl-1,3,5(10)-estratrien-3,17β-diol (**10**), 1,3,5(10)-estratrien-3-ol (**12**), 1,3,5(10)-estratrien-3,17β-diol-3-methylether (**16**), 1,3,5(10)-estratrien-17-one (estrone) (**18**), 8α-estra-1,3,5(10)-trien-17-one(8α-estrone) (**19**), 1,3,5(10)-estratrien-3,17β-diol-17-mono-acetate were gifts from Schering AG, Berlin.

Preparation of cytosolic receptor

Female Hagemann rats (23–24 days) were decapitated without anaesthesia. The following steps were all done at 4°C. The fresh uteri were collected in ice-cold 0.25 M sucrose solution, dried on filter

paper, washed with Tris–EDTA buffer (10 mM Tris–HCl, pH 7.5, 1.5 mM EDTA), dried again, cut into small pieces with scissors and suspended with 0.6 ml Tris–EDTA buffer per gram of tissue. The suspension was homogenized with a Potter–Elvehjem apparatus (400 rpm, 6 times 2 strokes with intervals of 1 min between each 2 strokes, ice cooling). After adjusting the concentration to 1 ml Tris–EDTA buffer per gram of tissue the homogenate was centrifuged for 90 min at 105,000 *g*. The supernatant under the fatty coat was sucked off with Pasteur pipettes and frozen in aliquots at −18°C. Storage time of the frozen samples did not exceed 1 year. The receptor preparation contained 6–8 nmol binding sites per liter as determined by Scatchard analysis with [6,7-^{3}H]estradiol.

Competitive receptor binding assay by thin layer gel chromatography was done as described by Toepert *et al.*[6] with Sephadex G 100 superfine, using 10 mM Tris–HCl buffer pH 7.5 with 1 mM NaN_3. Cytochrome c from horse heart (Boehringer Mannheim) and ferritin from horse spleen (K & K Laboratories, NY) were used as markers. Equal volumes of cytosolic receptor preparation and a mixture of [6,7-^{3}H]estradiol (final concentration 5×10^{-8} M) with the competing steroids in 90% Tris–EDTA buffer (see above) and 10% ethanol were incubated for 1 h at 4°C. Each steroid was investigated at 5 different concentrations, covering the concentration range around the level for 50% inhibition of tracer binding, except the low binding steroids (RCF_{50} value > 100) which were investigated at least twice at 5×10^{-6} M. After applying 10 μl samples onto the thin layer gel chromatography plate, separation was done at 4°C, and stopped when the ferritin spot reached 8 cm distance from the starting position (approx 2.5 h). Non-specific binding was determined by incubation of tracer with heat treated receptor preparation (30 min, 40°C) and did not exceed 2%. Radioactivity was counted on a Tri-Carb KB 2450 scintillation counter (Packard). Competition curves were evaluated by unweighed linear regression in a logit–log plot. Relative Competition Factors at 50% competition (RCF_{50}-values) are the amounts of the compounds under investigation, necessary to produce 50% inhibition of tracer binding divided by the amount of estradiol necessary to produce the same effect. The threshold values of estrogenic activity were determined according to Allen and Doisy [8] by subcutaneous injection.

Preparation of the steroids

The preparation of 17β-hydroxyamine-1,3,5(10)-estratrien-3-ol (**26**), 17β-methoxyamino-1,3,5(10)-estratrien-3-ol (**28**), 17-hydroxyimino-8α-estra-1,3,5-(10)-trien-3-ol (**30**), 17-methoxyimino-8α-estra-1,3,5(10)-trien-3-ol (**31**), 17β-isopropylideneamino-*N*-oxy)-1,3,5(10)-estratrien-3-ol (**34**), and 17β-(isopropylideneamino-*N*-oxy)-8α-estra-1,3,5(10)-trien-3-ol (**35**) is described elsewhere [7]. [6,7-^{3}H]Estradiol, 42 Ci/mmol was obtained from the Radiochemical Centre, Amersham.

8α-Estra-1,3,5(10)-trien-3,17α-diol (**4**) was prepared similar to the method of v. Gorp *et al.*[9]: (**3**) was treated with $Pb(OAc)_2$ and acetic acid anhydride in DMF, giving *8α-estra-1,3,5(10)-trien-3,17β-diol-3-monoacetate* (**36**), m.p. 128–130°C, which subsequently was reacted with methanesulfonic acid in pyridine, giving *17β-mesyloxy-8α-estra-1,3,5(10)-trien-3-ol-3-acetate* (**37**), m.p. 146–148°C, NMR ($CDCl_3$: 0.95, s, 3H, 2.26, s, 3H, 2.99, s,3H, 4.51, t, J = 7.5 Hz, 1H; i.r. (KBr): 3040–2870, 1756, 1610, 1581, 1492, 1328, 1205 cm^{-1}.

Reaction of (**37**) with K-acetate in DMF and methanesulfonic acid and subsequent hydrolysis with KOH in methanol–water gave *8α-estra-1,3,5(10)-trien-3.17α-diol* (**4**), m.p. 211°C, NMR (pyridine-d_5): 0.80, s, 3H, 3.84, d, J = 5 Hz; i.r. (KBr): 3420, 3260, 3020–2840, 1618, 1588, 1497 cm^{-1}.

17β-Ethinyl-1,3,5(10)-estratrien-3,17α-diol (**6**) was prepared according to Kanojia *et al.*[10, 11], m.p. 201–203°C, NMR ($CDCl_3$): 0.92, s, 3H, 2.52, s, 1H; i.r. (KBr): 3400, 3308, 3280, 3020–2870, 2100, 1610, 1580, 1495 cm^{-1}.

17β-Ethinyl-8α-estra-1,3,5(10)-trien-3,17α-diol (**8**) was prepared similar to the procedure of van Dijk *et al.*[12]: (**7**) was reacted with benzoylchloride in pyridine, giving *17α-ethinyl-8α-estra-1,3,5(10)-trien-3,17β-diol-3-benzoate* (**38**), m.p. 167–168.5°C, NMR ($CDCl_3$): 1.03, s, 3H, 3.05, s, 1H, 6.20, t, J = 2 Hz, 1H, 6.9–7.2, 3H, 7.4–7.7, m and 8.2, d of a d, J = 2 Hz and 8 Hz, 5H; i.r. (KBr): 3600, 3520, 3280, 3270, 3060, 3015, 2960–2840, 2100, 1730, 1595, 1581, 1495 cm^{-1}; u.v. (methanol): 216 (ε = 21,100), 229 (20,700), 260 (4020), 265 (2750), 275 (3220), 282 (2080) nm.

(**38**) was dehydrated with $POCl_3$ and 2,4-lutidine in benzene to *17α-ethinyl-8α-estra-1,3,5(10),16-tetraen-3-ol-3-benzoate* (**39**), m.p. 167–168.5°C. NMR ($CDCl_3$): 1.03, s, 3H, 3.05, s, 1H, 6.20, t, J = 2 Hz, 1H, 6.9–7.2, 3H, 7.4–7.7, m, and 8.2, d of a d, J = 2 Hz and 8 Hz, 5H; i.r. (KBr): 3620, 3060, 2990–2860, 1735, 1602, 1588, 1499 cm^{-1}; u.v. (methanol): 220 (ε = 23,200), 229 (27,200), 265 (3790), 275 (3360) nm.

(**39**) was converted with *p*-nitroperbenzoic acid in dichlormethane to *16α,17α-epoxy-17β-ethinyl-8α-estra-1,3,5(10)-trien-3-ol-3-benzoate* (**40**), m.p. 212.5°C; NMR ($CDCl_3$): 1.10, s, 3H, 7.4–7.7, m, and 8.13, d of a d, J = 2 Hz and 8 Hz, 5H; i.r. (KBr): 3255, 3040–2860, 1732, 1727, 1600, 1588, 1498 cm^{-1}; u.v. (methanol): 216 (ε = 18,700), 230 (20,300), 265 (3860), 275 (3310), 281 (2150) nm.

(**40**) was reduced with lithium-triethyl-borohydride in THF at 60°C to *17β-ethinyl-8α-estra-1,3,5(10)-trien-3,17α-diol* (**8**), m.p. 200–205°C; NMR ($CDCl_3$): 1.03, s, 3H, 2.52, s, 1H, 6.5–7.1, 3H; i.r. (KBr): 3420, 3320, 3020–2850, 1610, 1585, 1500 cm^{-1}; u.v. (methanol): 217 (ε = 7480), 228 (5360), 281 (2040). 286

(1860) nm; u.v. (0.1 N NaOH in methanol–H_2O): 241 ($\varepsilon = 9520$), 298 (2590) nm.

Compounds (**11**), (**13**), (**14**) and (**15**) were obtained according to the method of van Dijk *et al.*[13] starting with compound (**5**) instead of the 3-methylether (mestranol). $LiAlH_4/AlCl_3$ reduction gave 19-*nor*-*pregna*-1,3,5(10),17(20),20-*pentaen*-3-*ol* (**14**), m.p. 128–129°C; NMR (pyridine-d_5): 0.90, s, 3H, 4.70–5.00, m, J = 4 Hz, 2H; i.r. (KBr): 3540, 2945–2880, 1955, 1610, 1580, 1500 cm^{-1} u.v. (methanol): 217 ($\varepsilon = 8240$), 221 (7800), 229 (5550), 281 (2070), 287 (1890) nm, $[\alpha]_D = +40{,}4°C$ ($CHCl_3$).

Isomerization of (**14**) with butyllithium according to Mühlstädt[14] gave a pure sample of 17*β*-*ethinyl*-1,3,5(10)-*estratrien*-3-*ol* (**13**), m.p. 185°C; NMR ($CDCl_3$): 0.83, s, 3H, 2.07, d, J = 2 Hz, 1H; i.r. (KBr): 3510, 3300, 3030–2850, 2105, 1612, 1580, 1495 cm^{-1}; $[\alpha]_D = +122.2°C$ ($CHCl_3$), and a further sample which according to the NMR data was a 1:1 mixture of (**13**) and 17*α*-*ethinyl*-1,3,5(10)-*estratrien*-3-*ol* (**15**); NMR ($CDCl_3$): 0.77, s, 3H, (C-13 methyl of (**15**)), 0.83, s, 3H, (C-13 methyl of (**13**)), 2.07, d, J = 2 Hz, 1H (17*β*-ethinyl of (**13**)), 2.13, d, J = 2.5 Hz, 1H (17*β*-ethinyl of (**15**)); $[\alpha]_D = +33.4°$ ($CHCl_3$).

For preparation of compound (**17**), 8*α*-estradiol was treated with benzylchloride and K_2CO_3 in DMF for 1 h at 120°C, giving 8*α*-*estra*-1,3,5(10)-*trien*-3,17*β*-*diol*-3-*monobenzylether* (**41**) as an oily product. NMR ($CDCl_3$): 0.88, s, 3H, 3.66, t, J = 8 Hz, 1H, 5.02, s, 2H, 6.65–7.15, 3H, 7.2–7.5, 5H; i.r. (KBr): 3540, 2940–2865, 1610, 1578, 1500 cm^{-1}; u.v. (methanol): 227 ($\varepsilon = 10{,}500$), 247 (510), 259 (730), 265 (840), 272 (1530), 278 (2030), 287 (1920) nm.

Conversion of (**41**) according to Engelfried and Schenck[15] gave 8*α*-*estra*-1,3,5(10)-*trien*-3,17*β*-*diol*-3-*benzylether*-17-*methylether* (**42**), m.p. 117–119°C; NMR ($CDCl_3$): 0.88, s, 3H, 3.22, t, J = 8 Hz, 1H, 3.36, s, 3H, 5.02, s, 2H, 6.65–7.15, 3H; i.r. (KBr): 2925–2855, 2820, 1605, 1585, 1500 cm^{-1}; uv (methanol): 217 ($\varepsilon = 11{,}000$), 247 (536), 253 (563), 259 (767), 265 (1080), 272 (1540), 278 (2070), 285 (1950) nm.

(**42**) was debenzylated by hydrogenation with Pd/C (10%) in THF/methanol, yielding 8*α*-*estra*-1,3,5(10)-*trien*-3,17*β*-*diol*-17-*monomethylether* (**17**); m.p. 214°C; NMR ($CDCl_3$): 0.88, s, 3H, 3.24, t, J = 8 Hz, 1H, 3.36, s, 3H; i.r. (KBr): 3300, 2930–2870, 2840, 1620, 1585, 1500 cm^{-1}, u.v. (methanol): 217 ($\varepsilon = 7440$), 228 (5280), 281 (2100), 287 (1920) nm; u.v. (0.1 N NaOH in methanol–H_2O): 241 ($\varepsilon = 9620$), 298 (2680) nm.

8*α*-*estra*-1,3,5(10)-*trien*-3,17*β*-*diol*-*diacetate* (**43**) was obtained from reaction of (**36**) (see above) with acetic acid anhydride in pyridine; m.p. 76–77.5°C (from diethylether); NMR ($CDCl_3$): 0.93, s, 3H, 2.04, s, 3H, 2.26, s, 3H, 4.64, t, J = 8 Hz, 1H; i.r. (KBr): 3040–2840, 1765, 1746, 1738, 1612, 1588, 1494 cm^{-1}; u.v. (methanol): 216 ($\varepsilon = 9650$), 262 (600), 268 (820), 275 (860), 287 (50) nm.

Hydrolysis of (**43**) in methanol/aqueous K_2CO_3 at −10°C gave 8*α*-*estra*-1,3,5(10)-*trien*-3,17*β*-*diol*-17-*monoacetate* (**21**), m.p. 150.5°C (from methanol/water), NMR ($CDCl_3$): 0.93, s, 3H, 2.06, s, 3H, 4.65, t, J = 8 Hz, 1H; i.r. (KBr): 3430–3000–2680, 1738, 1710, 1610, 1588, 1500 cm^{-1}, u.v. (methanol): 218 ($\varepsilon = 7580$), 221 (7430), 228 (5440), 281 (2120), 286 (1920) nm; u.v. (0.1 N NaOH in methanol–H_2O): 241 ($\varepsilon = 9790$), 298 (2720) nm.

17-*Hydroxyimino*-1,3,5(10)-*estratrien*-3-*ol* (estrone oxime) (**29**) was prepared according to Matkovics *et al.*[16]. 17*β*-Amino-1,3,5(10)-estratrien-3-ol (**22**) was prepared by reduction of (**29**) with sodium in ethanol according to [17]. 17*β*-*Amino*-8*α*-*estra*-1,3,5(10)-*trien*-3-*ol* (**23**) was prepared in the same manner as (**22**) from 17-hydroxyimino-8*α*-estra-1,3,5(10)-trien-3-ol (**30**); m.p. 276°C (from methanol, decompn); NMR (pyridine-d_5): 0.79, s, 3H; i.r. (KBr): 3350–3280, 2900–2860, 2690–2600, 1580, 1490 cm^{-1}.

17*β*-*Hydroxyamino*-8*α*-*estra*-1,3,5(10)-*trien*-3-*ol* (**27**) was obtained as hydrochloride from hydrolysis of [35] in methanol/1 N HCl according to [7] as an oily product, which showed the same R_f-value in TLC as (**26**). NMR (pyridine-d_5): 1.29, s, 3H, 3.60, t, J = 9 Hz, 1H.

17*β*-*Formamido*-1,3,5(10)-*estratrien*-3-*ol* (**32**) was obtained from reaction of (**22**) in pyridine with a prereacted mixture of formic acid and acetic acid anhydride, and subsequent hydrolysis of the phenolic ester group with aqueous K_2CO_3, m.p. 228–229°C (decompn); NMR (pyridine-d_5): 0.77, s, and 0.80, s, together 3H (2 rotamers of C-13 methyl?), 4.2–4.5, m, J = 9 Hz, 1H, 8.3–8.6, m, 2H; i.r. (KBr): 3380, 3280, 3060, 3020–2870, 1670, 1632, 1612, 1588, 1540, 1502 cm^{-1}.

17*β*-*Formamido*-8*α*-*estra*-1,3,5(10)-*trien*-3-*ol* (**33**) was prepared in the same manner as (**32**) from (**23**), m.p. 285°C (from methanol, decompn); NMR (pyridine d_5) 0.86, s, and 0.89, s, together 3H (2 rotamers of C-13 methyl?), 4.1–4.5,m,J = 9 Hz,1H, 8.3–8.65, m, 2H); i.r. (KBr): 3385, 3280, 3020–2865, 1668, 1620, 1585, 1528, 1500 cm^{-1}.

3-*Hydroxy*-1,3,5(10)-*estratrien*-17-*one*-17-*hydrazone* (**24**) was prepared according to Robinson and Finckenor[18] from estrone (**18**), m.p. above 280°C (from methanol, decompn) NMR (pyridine-d_5): 0.91, s, 3H; i.r. (KBr): 3300, 3280, 2930–2870, 1660, 1610, 1505 cm^{-1}; u.v. (methanol): 219 ($\varepsilon = 12{,}200$), 229 (8340), 281 (1930), 286 (1760) nm; u.v. (0.1 N NaOH in methanol–H_2O): 239 ($\varepsilon = 9590$), 298 (2470) nm.

3-*Hydroxy*-8*α*-*estra*-1,3,5(10)-*trien*-17-*one*-17-*hydrazone* (**25**) was prepared in the same manner as (**24**) from 8*α*-estrone (**19**); m.p. 281–283°C (from methanol, decompn); NMR pyridine-d_5): 1.01, s, 3H; i.r. (KBr): 3400–3110, 2960–2840, 2700–2600, 1660, 1620, 1582, 1500 cm^{-1}; u.v. (methanol): 217 ($\varepsilon =$ 13,100) 228 (9100), 281 (2070), 286 (1950) nm; u.v. (0.1 N NaOH in methanol–H_2O): 240 ($\varepsilon = 11{,}200$), 298 (2650) nm.

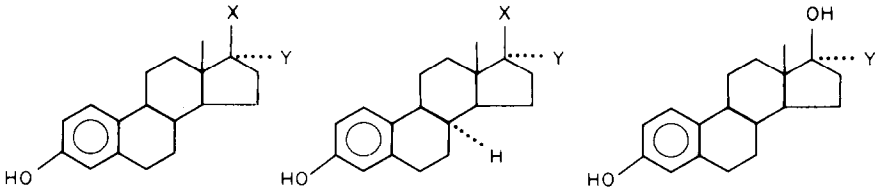

Table 1

X	Y	Ref. comp. A	RCF_{50}	Allen–Doisy Test s.c.	Ref. comp. B	RCF_{50}	Allen–Doisy Test s.c.
OH	H	**1**	≡1	0.25–0.50 μg	**3**	2	0.3–1.0 μg
H	OH	**2**	5	100 μg	**4**	13	30–100 μg
OH	C≡CH	**5**	1	0.3 μg	**7**	1.5	0.3–1.0 μg
C≡CH	OH	**6**	15	100–300 μg	**8**	>100	300 μg

RESULTS

Tables 1–5 show the RCF_{50} values and the Allen–Doisy threshold values for the indicated compounds. 8α-Estrogens always have lower receptor binding as compared with the coresponding 8β-compounds. Furthermore, variations in the 17-position show the same effects within the 8α- and 8β-series. The 17-OH group is more effective for receptor binding in the 17β-position than in the 17α-position (Table 1, **1** and **3** vs **2** and **4**). In the case of 17-ethinyl-estradiol derivatives receptor binding is not disturbed, when the ethinyl group is introduced in the 17α-position (Table 1, **5** and **7** vs **1** and **3**). However, a strong negative effect is observed after introduction of an ethinyl group in the 17β-position (Table 1, **6** and **8** vs **2** and **4**). The same tendencies are also reflected by the corresponding results of the Allen–Doisy test.

The comparison of 17β-estradiol derivatives substituted in the 17α-position reveals, that methyl (**9**), vinyl (**11**), and ethinyl (**5**) moieties rarely exert an influence on the RCF_{50} values. In contrast a 17α-ethyl-substituent (**10**) causes a significant decrease of receptor binding (see Table 2). The same holds true for the Allen–Doisy test: Here the threshold value for 17α-ethyl-estradiol is more than

Table 2

Y	Ref. compd C	RCF_{50}	Allen–Doisy Test s.c.
H	**1**	≡1	0.25–0.50 μg
CH_3	**9**	1.5	0.3–1.0 μg
C≡CH	**5**	1	0.3 μg
$CH{=}CH_2$	**11**	1.5	0.3–1.0 μg
CH_2CH_3	**10**	16	300–1000 μg

Table 3

X	Y	Ref. compd A	RCF_{50}	Allen–Doisy Test s.c.
H	H	**12**	50	30 μg
C≡CH	H	**13**	>100	1000–3000 μg
$=C=CH_2$		**14**	>100	100–300 μg
H	C≡CH	**15**	22†	300–1000 μg‡

†The RCF_{50}-value for (**15**) is derived from a 1:1 mixture of the two isomers (**13**) and (**15**), the RCF_{50}-value for the mixture was 44.
‡1:1 mixture of (**13**) and (**15**).

Table 4

X	Y	Ref. compd A	RCF_{50}	Ref. compd B	RCF_{50}
OH	H	**1**	≡1	**3**	2
=O		**18**	7	**19**	>47
OCH_3	H	**16**	14	**17**	>100
OAc	H	**20**	45	**21**	>100

Table 5

X	Y	Ref. comp. A	RCF_{50}	Allen–Doisy Test s.c.	Ref. comp. B	RCF_{50}	Allen-Doisy Test s.c.
NH_2	H	**22**	>100	>1000 μg	**23**	>100	
$=N{-}NH_2$		**24**	60		**25**	>100	
NH–OH	H	**26**	8	300–1000 μg	**27**	>100	
$NH{-}OCH_3$	H	**28**	60				
=N–OH		**29**	8	100 μg	**30**	>100	1000 μg
$=N{-}OCH_3$		**31**	46				
NH–CHO	H	**32**	>100		**33**	>100	
$^{\oplus}N{=}C(CH_3)(CH_3)$ $^{\ominus}O$	H	**34**	>100	100 μg	**35**	>100	

2 orders of magnitude higher than those for the 17α-methyl, -vinyl or -ethinyl derivatives. This striking effect of the 17α-ethyl group cannot be explained solely by the size of this group. The extraordinary properties of 17α-ethyl-estradiol (**10**) are discussed in a following paper [19] on the basis of i.r. and NMR spectroscopic data.

Substitution of the OH-group in the 17-position by hydrogen as in (**12**) demonstrates the essential function of the OH-substituent and reveals the remaining residual contribution to receptor binding of a steroid containing only a phenolic OH group in the 3-position of the A-ring. Introduction of substituents in this deoxy compound shows the influence of different 17-substituents without the additional effect of the 17-OH-group (Table 3). Again there is no negative effect of a 17α-ethinyl substituent (**15**), whereas the quasi-equatorial allene group (**14**) and also the 17β-ethinyl group (**13**) exert a deteriorating effect on receptor binding as compared with the unsubstituted compound (**12**).

In order to see whether the receptor binding of the steroid is affected via the oxygen or the hydroxyl proton of the 17β-OH-group, this OH group of estradiol was oxidized, methylated and acetylated. These derivatives (**18**/**19**), (**16**/**17**), (**20**/**21**) showed in the 8β-series, as well as in the 8α-series lower receptor binding, than estradiol (**1**) or 8α-estradiol (**3**) itself (see Table 4). Comparison of these derivatives with the 17-deoxy compound (**12**) reveals that the keto compound (**18**) and the methylether compound (**16**) still show better receptor binding than the deoxy compound (**12**). This indicates at least a contribution of the oxygen to the receptor binding, possibly involving its free electron pairs.

Therefore we tested other 17-substituents with free electron pairs for contributions to the receptor binding. The RCF_{50}-values for several model compounds in the 8β- and 8α-series are listed in Table 5. The low receptor binding of the 17β-amino compounds (**22, 23**) and the 17-hydrazino compounds (**24, 25**) can be explained by a nearly complete protonation of the nitrogen at pH 7.5. In contrast, the oximes (**29, 30**), the 17β-hydroxylamino compounds (**26, 27**) as well as the corresponding methylethers (**31** resp. **28**) have lower pK_a-values, and consequently should not be protonated at pH 7.5 [20–23]. The non-etherized compounds of the 8β-series (**26** and **29**) show RCF_{50}-values comparable to that of estrone (**18**). The RCF_{50}-values of the etherized derivatives (**31** and **28**) however, correspond to the value of (**12**).

One of the reasons for the reduced binding of the etherized derivatives could be the greater volume of the 17-substituents as compared to the non-etherized molecules. It also could be that free rotation of the substituents is hindered, thus fixing the free electron pairs of the nitrogen in an unfavorable position for interactions with a receptor group. Similar reasons may also account for the low receptor binding of the formamides (**32, 33**) and the nitrones (**34, 35**). It might be noteworthy that the nitrone (**34**) has an Allen–Doisy value similar to that of the oxime (**29**) despite of its high RFC_{50}-value. We assume that the nitrone is metabolized slowly to the hydroxylamine (**26**) and possibly further to the oxime (**29**).

DISCUSSION

The investigations about steroid receptor interactions described above were done by means of competition experiments with 1 h incubation at 4°C. Having in mind the results of the kinetic investigations of Ellis and Ringold[24] as well as of Bouston and Raynaud[25] one must suppose that under the chosen conditions thermodynamic equilibrium is not achieved. There is some evidence that under physiological conditions the transformation of the 4S receptor into the 5S receptor complex and the subsequent translocation into the nucleus occurs more rapidly than the achievement of equilibrium between the hormone and its receptor [26–28]. Therefore measurements under non-equilibrium conditions may reflect even better the *in vivo* effects of steroids than true equilibrium constants.

8α-Steroids always show lower receptor binding than the analogous (natural) 8β-steroids. The surprisingly moderate decline indicates that the steroid skeleton probably takes up a conformation close to that of the 8β-isomers. Thus the distance between the oxygen atoms at C-3 and C-17 would remain nearly equivalent. Various authors considered this parameter an important criterium for receptor binding [1, 3, 5, 29].

Support for the assumption that substituents at the D-ring with electron donor properties may participate in receptor binding can be derived in the *O*-series (see **16** vs **13** or **12**, and also results of Bergink *et al.*[30]) as well as in the *N*-series. Here the basicity of the substituent should not be so strong that the free electron pair of the hetero atom is protonated at physiological pH, as e.g. in the case of 17β-aminoestradiol (**22**). The apparently contradictory observation of Terenius [4], that the amino derivative of hexestrol binds nearly as well to the receptor as hexestrol itself, may be explained by the fact that we have here an aniline derivative with only low basicity, which exists as a free base at physiological pH.

A possible counterpart at the estrogen receptor site should be a proton donating group. Recently Ikeda[31] has shown that a thiol group forms a covalent disulfide bond when an estrogen with an activated disulfide in position 17 is offered. Such a thiol group could also play the role here postulated for the interaction with the free electron pair of the 17-substituent.

Apart from the importance of the electron donor property of the steroid an additional participation of the hydroxyl proton cannot be excluded as all studied steroids without the hydroxyl proton showed lower receptor binding. However, the remarkably good

receptor binding of estrone (**18**) may indicate a relatively small hypothetical contribution from an OH-proton at the 17-substituent, although conversion of estrone to estradiol under assay conditions cannot be ruled out. Since additional 17α-substituents in estradiol derivatives—except in 17α-ethyl estradiol (**10**)—affect the receptor binding only slightly, it appears that the receptor does not interact with the α-side of the steroid. This hypothesis is further supported by the comparable RCF_{50}-values of 8α-estradiol (**3**), where the 17β-OH-group is placed directly "above" the plane (in β-direction) formed by the A-ring and B-ring of the steroid. The fact that 17α-estradiol (**2**) shows a lowered but still significant receptor binding, which is highly superior to the binding of the 17-deoxy-compound (**12**), could indicate some flexibility in the conformation of the interacting group of the receptor.

It is not likely that 17α-OH compounds bind to the receptor "upside down" after a rotation around the C-3–C-17 axis now like a "normal" 17β-OH steroid. In this case substitution of the 17α-OH compounds (**2** and **4**) with 17β-ethinyl groups (**6** and **8**) should not cause a decrease in receptor binding, because these ethinyl substituents would point "downwards", after rotation of the molecule around the C-3–C-17 axis, as in the epimeric compounds (**5**) and (**7**). However, 17β-ethinyl-substituents always reduce receptor binding (c.f. (**6**) vs (**2**) and (**8**) vs (**4**)).

A further surprising observation is that introduction of a 17α-ethinyl group (**15**) into the 17-desoxy compound (**12**) results in an improved receptor binding (Table 3) and that 17α-ethinyl-8α-estradiol (**7**) shows an equal or rather lower RCF_{50}-value (better receptor binding) than 8α-estradiol (**3**) itself (Table 1). The RCF_{50}-values reported in the literature for 17α-ethinyl-estradiol (**5**) indicate the same trend (e.g. 0.5 [32], 0.6 [33], 0.88 [34], 0.85 [35]), when compared with estradiol. A tentative interpretation could be that the extended 17α-ethinyl group causes a preorientation of the steroid as it approaches the receptor, which favors rapid receptor binding. This should affect the association velocity constant, which under the conditions chosen here is reflected by the observed RCF_{50}-values.

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