J. Steroid Biochem. Molec. Biol. Vol. 37, No. 2, pp. 295–300, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

ESTROGEN RECEPTOR BINDING CHARACTERISTICS OF 1,11β-ETHANOESTRADIOL: EFFECT OF A 1,11β-BRIDGE ON STEROIDAL ESTROGEN

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(Received 26 June 1989; received for publication 13 June 1990)

Summary—As part of an ongoing program to develop high affinity estrogenic ligands we have synthesized the 11β-vinyl, 11β-ethyl- and 1,11β-ethanoestradiols. Because the 1,11β-ethanoestradiol had not been previously reported in the literature, the investigation of its receptor binding characteristics would provide valuable insight into the effect of 1/11β-substitution. The data obtained in this study indicate that although significant estrogen receptor affinity is present for the 1,11β-ethano derivative, the RBA values, 5–22.4%, were far less than those observed (5–300-fold less) for the corresponding 11β-ethyl and 11β-vinyl estradiols and less than those for the 1-methyl and 11β-methyl estradiols. These results suggest that the orientation that the 11β-substituent must occupy is directed away from the A-ring and that substituents in the 1–11 pocket produce a detrimental effect on receptor interactions.

INTRODUCTION

The development of radiolabeled derivatives of estrogens as potential imaging agents requires adherence to a number of stringent criteria [1–5]. Among these are the need for the parent ligand molecule to possess a receptor affinity comparable to or greater than the endogenous ligand, in this case estradiol. As part of our study to prepare and evaluate substituted derivatives of estradiol as potential radioligands, we synthesized the heretofore unreported novel bridged estrogen, 1,11β-ethanoestradiol [6]. Bridged or annelated estradiols have not been extensively evaluated and in general possess poor receptor-binding properties or low estrogenic activity [7–9]. Because we also had prepared the structurally-related 11β-ethyl- and 11β-vinylestradiols, we were in the position to compare in some detail the effect of the two carbon bridges upon the estrogen receptor binding [10, 11]. In this paper we describe in detail the synthesis of the 1,11β-ethanoestradiol and compare its RBA values to those of the structural analogs. As the results indicate, the ability of the 11β-substituent to adapt an orientation that is directed away from the A-ring is important for receptor binding.

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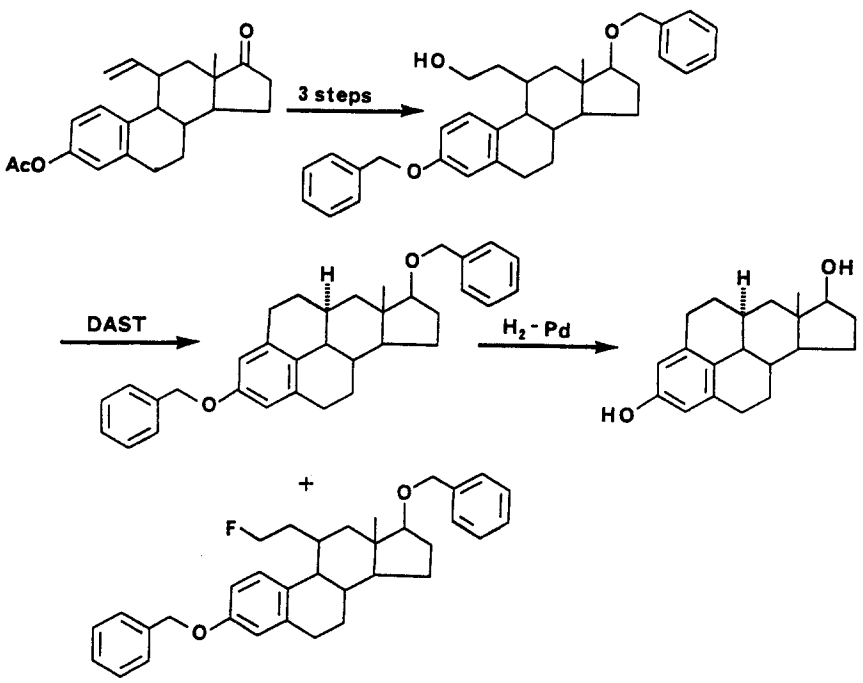
EXPERIMENTAL

Synthesis of 1,11β-ethanoestradiol

To a solution of diethylaminosulfur trifluoride, DAST, (0.15 g, 0.93 mmol) in a dicholoromethane (4 ml) cooled to −78°C was added dropwise a solution of 11β-hydroxyethylestradiol 3,17β-bis benzyl ether (0.425 g, 0.86 mmol) [10] in dichloromethane (4 ml). The reaction was warmed to room temperature; the reaction solution was washed with a saturated sodium bicarbonate solution, dried over magnesium sulfate, filtered and evaporated to dryness. The crude product was separated by chromatography on silica gel to give the 1,11β-ethanoestradiol-3,17β-bis benzyl ether which was converted to the 1,11-ethanoestradiol by catalytic debenzylation over colloidal palladium [11]. The oxide reaction mixture was purified by column chromatography to give the pure product (0.12 g mmol) in an overall yield of 60%. m.p. 212–216°C; $[\alpha]_D^{25} = -9.59°$ ($C = 1.77 \times 10^{-3}$, THF) [^{1}H]NMR (d_6-DMSO): 0.69 (s, 3H, C(18)–H), 1.06–1.72 (m, 10H), 1.78–1.92 (m, 2H), 2.02–2.16 (m, 2H), 2.44–2.64 (m, 4H), 3.48 (d, t, J = 8.0 Hz, J = 5.0 Hz, 1H, C(17)–H), 4.46 (d, H = 5.0 Hz, 1H, CH–OH), 6.28, 6.35 (2d, J = 2.0 Hz, 2H, C(2)–H, C(4)–H).

Competitive receptor binding assays. Uterine tissue from immature lambs was homogenized at 4°C in a 0.01 m Tris–1.5 mM EDTA–0.02%



Scheme 1

sodium azide, pH 7.4 buffer (TEA), and the homogenate was centrifuged at 18,000 *g* for 60 min. The supernatant was removed and the cytosol was diluted to a concentration of approximately 3 mg of protein/ml. The uterine tissue from immature female rats (21–25 days old) was homogenized at 4°C in the TEA buffer and the homogenate was centrifuged at 18,000 *g* for 60 min. The supernatant was carefully removed and the cytosol was diluted to approximately 3 mg of protein/ml.

The competitive binding assays were performed using procedures previously described [12, 13]. To the cytosol, lamb or rat uterine, containing the 10 nM [H-3] estradiol were added aliquots of the competitor solutions such that range of 10^{-5} to 10^{-10} M competitor was obtained. The solutions were incubated at 0–4°C or 25°C for 18–24 h. After 18–24 h a suspension of dextran-coated charcoal was added, followed by centrifugation. An aliquot of the supernatant was removed and the radioactivity was assayed by liquid scintillation counting. The remaining activity associated with specific estradiol binding was plotted against the competitor concentration (Fig. 1a–c). From this plot the molar concentrations of the unlabeled estradiol or steroidal competitor that reduced the specific binding of [^{3}H]estradiol could be determined. The effectiveness of the competitor was established using the ratio of the unlabeled estradiol for 50% competition to competitor concentration for 50% competition. The ratio was multiplied by 100 to obtain that relative binding affinity (RBA) at a particular temperature and cytosol source. Therefore, by definition, the RBA of estradiol under these conditions 100 [14–16].

RESULTS AND DISCUSSION

Synthesis and receptor binding affinities of the ligands

The synthesis of the 1,11β-ethano-bridged estradiol is shown in Scheme 1. The preparation of the 11β-vinyl estradiol acetate and its conversion to functionalized estradiol derivatives, including the 1,11β-ethanoestradiol, have been recently communicated [11]. The chemical aspects of these transformations which provide molecular probes for the estrogen receptor are more fully described in those communications and will not be discussed here. Instead, this study will focus on the effect that the substituent exerts upon the binding to the estrogen receptor.

The relative binding affinity (RBA) of ligands for the estrogen receptor can be estimated indirectly by competitive binding assays. The values thus obtained provide a comparison of the effectiveness of the test compound in competition for the estrogen receptor relative to the endogenous ligand estradiol. Because the values obtained in these assays reflect a combination of

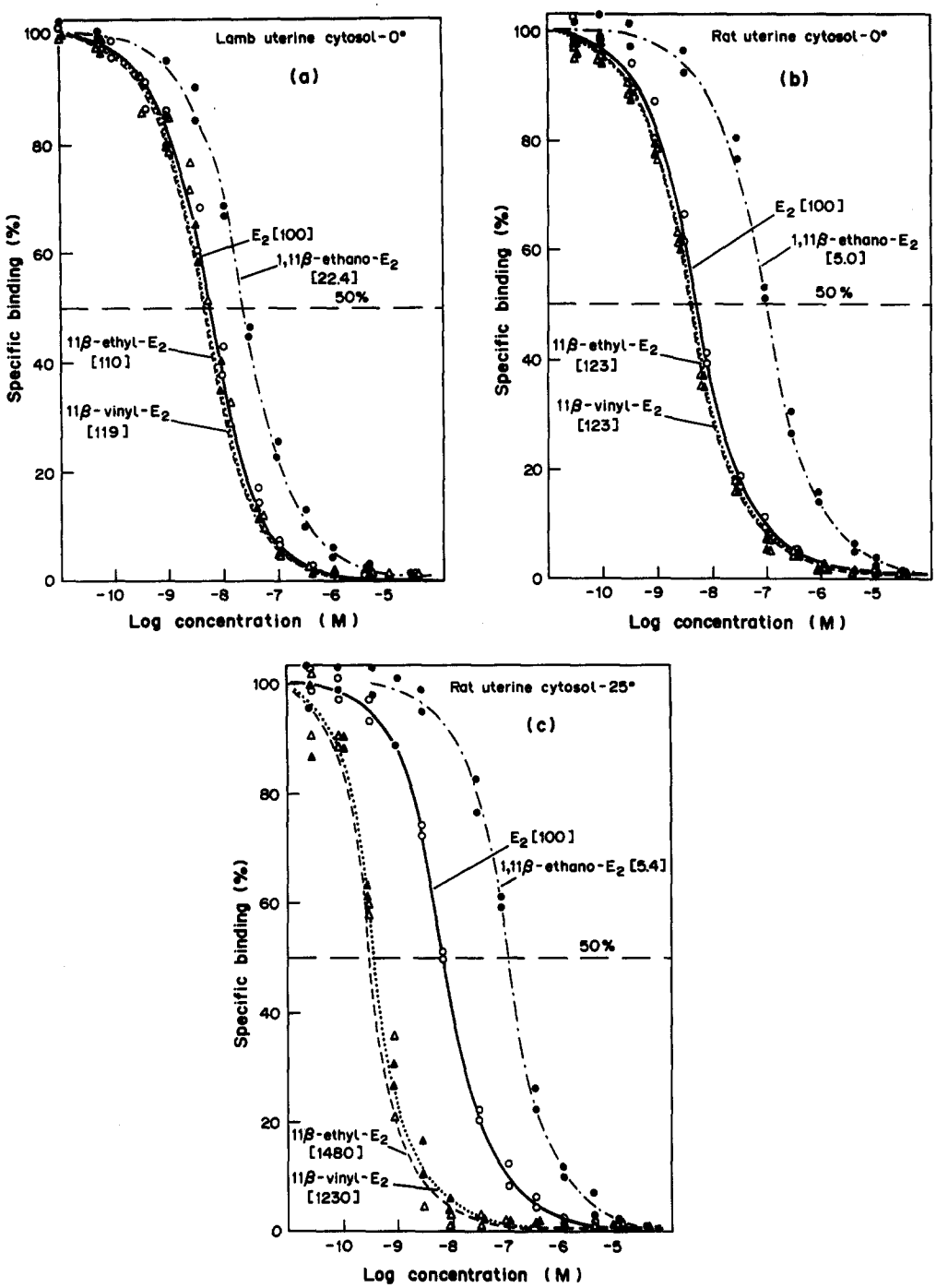


Fig. 1(a–c). Activity of the substituted estrogens to compound with [H-3]-estradiol for binding to the estrogen receptor prepared from lamb uterine cytosol (1a) and rat uterine cytosol (1b, c). Cytosol receptor preparations 1a and 1b were incubated for 18 h at 0–4°C with the indicated concentrations of the competitive estrogen and 100 mM [^{3}H]estradiol. Cytosol receptor preparation 1c were incubated for 18 h at 25°C with the indicated concentration of the competitor and 10 nM [^{3}H]estradiol. After incubation, charcoal dextran was added to absorb the unbound ligand and the radioactivity was determined with an aliquot of the supernatant. The relative binding affinities (RBA) were the concentrations of the competitor necessary to inhibit 50% of the estrogen specific binding relative to estradiol, and are indicated in the parentheses where estradiol equals 100.

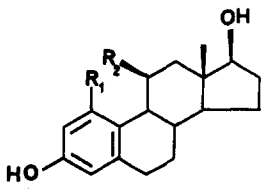
association and dissociation rates, two incubations conditions were used. Compounds which have slow dissociation rates give rise to complexes that are stable and therefore demonstrate higher RBA values at the long incubation times, at elevated temperature (25°C). Such

increases in RBA values are not observed at the lower temperature (0–4°C) because the reduction in temperature retards the dissociation of all ligands. One therefore observes primarily, but not exclusively, association effects [14]. The results of the competitive binding assays for estradiol, 11β-ethyl, 11β-vinyl and 1,11β-ethano-estradiol are illustrated in Fig. 1a–c. As can be seen, the ligands demonstrate parallel displacement curves relative to estradiol as well as identical maximal displacement at high concentrations. As such they appear to compete in a manner similar to estradiol at the estrogen receptor. The quantitative aspects of the receptor binding of the three substituted ligands differ, however. The nonbridged analogs, are slightly more potent than estradiol at 0°C in both the lamb and rat cytosol preparations. The bridged derivative has a significantly shifted displacement curve which is more pronounced in the rat uterine cytosol compared to the lamb uterine cytosol. The competitive binding effects were more vivid when the incubation was performed at 25°C as opposed to 0°C. Under these conditions, the 11β-ethyl and 11β-vinyl estradiols displayed much higher RBA values compared to estradiol as illustrated by the shift in the displacement curves. The 1,11β-ethano-bridged derivative demonstrated no such effect and its displacement curve remained to the right of estradiols.

The determination of the concentration of the ligands which effected 50% displacement of specific [^{3}H]estradiol binding permitted the calculation of the approximate RBA values. These were compiled in Table 1 and combined with the values reported elsewhere for 1-methylestradiol and 11β-methyl and 11β-ethyl estradiol [17–19]. The data in Table 1, derived from this and other studies [13–15] using different conditions, suggest two effects of the substituents upon receptor binding. The first is that the nonpolar 11β-alkyl groups markedly enhance the receptor binding affinities. This is not readily apparent when the assay is conducted at 0°C in either lamb, rat or rabbit cytosol preparations, but when the ligands were incubated at 25°C for 18 h the RBA values were dramatically increased in the rat uterine cytosols. Thus, the ligands reached equilibrium more slowly than estradiol, i.e. they slowly dissociated from the receptor. The nonpolar 1-methyl group had a deleterious effect upon estrogen receptor binding as was demonstrated by the substantially lower RBA values (15%). Whether this ligand is a slowly equilibriating estrogen is unknown. By comparison, the 1,11β-ethanoestradiol more closely resembled the 1-substituted estradiol than the 11β-substituted estrogen. The RBA value was much lower than estradiol (5–22%) and the RBA was not improved by increasing the incubation temperature to 25°C.

Several factors may contribute to the relative poor receptor binding affinity for the 1,11β-ethanoestradiol. First, in analogy to the 1-methyl estradiol, the bridge carbons extend into an area where there may be intimate ligand–receptor interaction. The additional methylene group may increase the steric hinderance and thereby further suppress the affinity as was observed in this case. The second effect, related to the 11β-substituent is that for a favorable receptor interaction, the nonpolar group must extend away from the A-ring, a conformation which is impossible for the bridged estrogen. A third effect may result from a change in A–D

Table 1. Relative binding affinities of the substituted estrogens in uterine cytosol preparations



R_1	R_2	Lamb (0°C)	Rat (0°C)	Rat (25°C)	Rabbit (0°C)
H	H	100	100	100	100
H	C_2H_5	110	123	1480 (1120)	—
H	$CH{=}CH_2$	119	123	1230	—
—CH_2—CH_2—		22.4	5.0	5.4	—
CH_3	H	—	—	—	15[b]
H	CH_3	—	124[c]	—	83[b]

[a]Ref. [13].
[b]Ref. [14].
[c]Ref. [15].

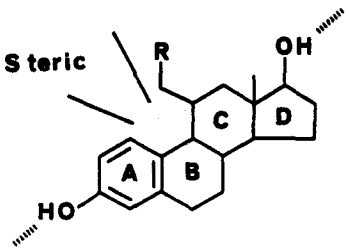


Fig. 2. Schematic representation of interactions at the 1H, 11-positions of the estradiol with the estrogen receptor.

ring conformation as a consequence of the constraint imposed by the 1,11β-bridge. A molecular model of the 1,11β-ethanoestradiol compared to that of the 1-methyl estradiol, 11β-methylestradiol or the 1,11β-dimethylestradiol indicated an alteration in the overall conformation such that the bridged steroid resembles more closely estriol or RU 16117 57,8 [20, 21] rather than moxestrol or ethynyl estradiol. In this conformation, not only is the initial association affected but the rate of dissociation is increased, thereby resulting in an overall decrease in receptor binding affinity. In all likelihood, the observed data were the result of a combination of these factors.

As a basis for radioligand development the 1,11β-ethanoestradiols may not possess much potential, however, the compound does contribute to the structure–activity relationships of the estrogens. The data obtained from this study provided additional information regarding the interaction between the A, B, C ring region and the receptor. A schematic representation of this interaction is shown in Fig. 2. The presence of substituents extending from C(1) into the C(11) region apparently produced a loss in receptor binding. This result is similar to that observed with the 1,11-methano- and 1,11-ethanoestradiol previously reported by Pitt *et al.* [7, 8]. Conversely the region that extends beyond the 11-region favorably accommodates substantial steric bulk [22]. Thus, future research will focus on the synthesis of estrogens substituted at the 11β-position and on evaluation of their interaction of the substituent with the receptor.

Acknowledgements—This work has been supported in part by Public Health Service grant 5-RO1-CA-41399. The authors acknowledge the contribution of Dr John A. Katzenellenbogen, supported by PHS-5-RO12-CA25836, for the work on the receptor binding assay and his analytical evaluation of the manuscript.

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