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3-HYDROXY-19-NOR-17α-PREGNA-1,3,5(10)-TRIENE-21,17β-CARBOLACTONE AS INHIBITOR OF 17β-HYDROXYSTEROID DEHYDROGENASE TYPE 2

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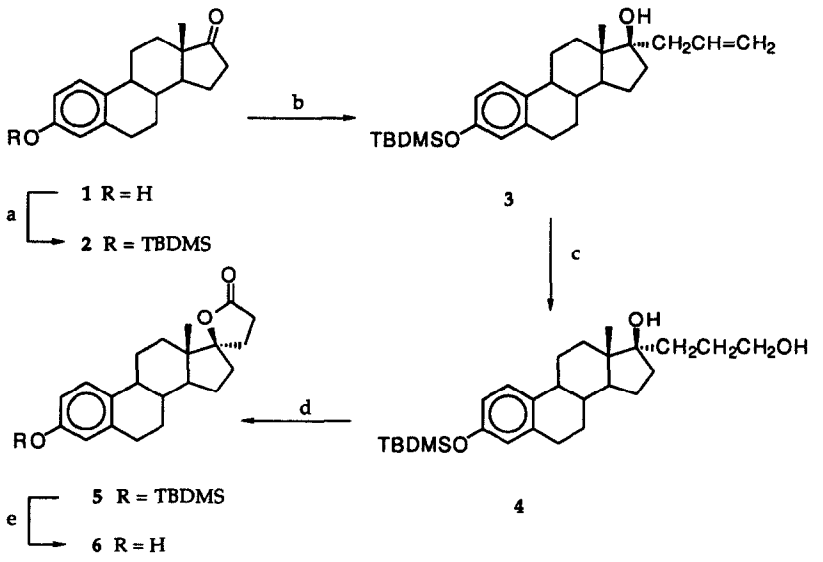
Abstract: Introducing a spiro-γ-lactone at position 17 of an estradiol nucleus provokes a potent inhibition of 17β-hydroxysteroid dehydrogenase (17β-HSD) type 2. Synthesis of such a compound, namely **6** (3-hydroxy-19-nor-17α-pregna-1,3,5(10)-triene-21,17β-carbolactone), was performed in five steps, starting with estrone. Inhibition analysis of this compound, the first inhibitor of 17β-HSD, using human placental microsomes and 4-androstene-3,17-dione as substrate shows a Ki value of 0.25 μM.

The enzyme 17β-HSD is involved in the biosynthesis of active steroid hormones. The widespread distribution of 17β-HSD activities in rat and human tissues clearly indicates the importance of this enzyme in peripheral sex steroid formation [1]. In human placenta, the largest source of 17β-HSD [1], it is known that a cytosolic 17β-HSD (designated type 1) is responsible for the interconversion of estrone (E_1) and estradiol (E_2) [2-6] and a microsomal 17β-HSD (designated type 2) is responsible for the interconversion of E_1 and E_2 as well as that of 4-androstene-3,17-dione (Δ^4-dione) and testosterone (T) [7,8]. Inhibitors or inactivators of 17β-HSD type 1 have already been described [9]; however, no inhibitor of 17β-HSD type 2 was known. Herein we report a synthesis of a spiro-γ-lactone derivative of estradiol and its potent inhibitory effect on 17β-HSD type 2.

Chemistry (scheme 1):

The synthesis of spiro-γ-lactone **6** was performed following a sequence of five steps starting with commercially available estrone (**1**). The first step was to protect the phenolic group by formation of a *tert*-butyldimethylsilyl (TBDMS) ether **2**. By a Grignard reaction, an allyl group was stereoselectively introduced in the 17α-position of TBDMS-estrone (**2**) to obtain the corresponding 17α-allyl derivative **3**. Oxidative hydroboration of olefin **3** by known methodology (BH_3, NaOH, H_2O_2) afforded the desired diol **4**. Further oxidation of the primary alcohol **4** with Jones' reagent (CrO_3, H_2SO_4, acetone) led to carboxylic, acid which undergoes a cyclization to form the more stable five-member ring spirolactone **5**. After removal of TBDMS with tetrabutylamonium fluoride, the spiro-γ-lactone **6** was obtained. All intermediates of synthesis (compounds **2-5**) were fully characterized by IR, NMR, and MS spectral data (data not shown), and data for the spiro-γ-lactone **6** were reported in note 10.



Scheme 1. Synthesis of spiro-γ-lactone **6** from commercially available estrone (**1**). Reagents and conditions are: a) TBDMS-Cl, imidazole, DMF, rt, 17 h (69%); b) CH_2=$CHCH_2MgCl$, THF, 0°C to rt, 5 h (88%); c) 1. BH_3, THF, rt, 3 h; 2. NaOH (3N), H_2O_2 (30%, w/v), rt, 1 h (69%); d) Jones' reagent, acetone, 0°C, 1 h; e) Bu_4NF, THF, rt, 0.5 h (50%, two steps).

Inhibition of 17β-HSD type 2:

The 17β-HSD activity present in microsomes of human placenta (17β-HSD type 2) was used to evaluate the inhibitory effect of spiro-γ-lactone **6**. In the enzymatic assay (note 11), [^{3}H] Δ4-dione was transformed to the more active androgen [^{3}H] T by partially purified 17β-HSD type 2 and the percent of transformation was calculated. The interfering aromatase activity responsible for the transformation of [^{3}H] Δ4-dione to [^{3}H] estrone was selectively blocked with an aromatase inhibitor [12]. Competition of labelled Δ4-dione (the substrate) by increasing the concentration of inhibitors (spiro-γ-lactone **6** or unlabelled Δ4-dione) gave the inhibition curves shown in Fig. 1A. From these curves, the concentration of inhibitor that causes a 50% inhibition was determined. Both compounds inhibit 17β-HSD type 2, but spiro-γ-lactone **6** (IC_{50} = 0.27 μM) is a better inhibitor than Δ4-dione itself (IC_{50} = 1.41 μM). When compound **6**, at a concentration of 1 μM, was incubated with 17β-HSD type 2 and cofactor, no inactivation of enzyme was observed according to time (Fig. 1B). In fact, enzymatic activity is entirely restored after removal of inhibitor, suggesting that spiro-γ-lactone **6** acts as a reversible inhibitor. Using the Cheng-Prusoff equation [13] to calculate the inhibition constant (Ki) from the IC_{50} value, a Ki value of 0.25 μM was found for spiro-γ-lactone **6** inhibition. Since 17β-HSD type 1 catalyse specifically the interconversion of estrone and estradiol [14] and could not transform Δ4-dione, the present result obtained with Δ4-dione substrate is thus selectif for 17β-HSD type 2. To determine the ability of spiro-γ-lactone **6** to inhibit 17β-HSD type 1, a 100000 g supernatant soluble fraction obtained from human placental fractionation which did not possess the ability to transform Δ4-dione, was used with estrone as substrate, a Ki value greater

than 40 μM was obtained (data not shown). The results thus indicate that spiro-γ-lactone **6** inhibits more potently type 2 (>160 fold) than 17β-HSD type 1. The exact mechanism by which spiro-γ-lactone **6** inhibits the 17β-HSD type 2 is unknown however, and is related not only to the presence of a γ-lactone group, as introduction of a similar group on androgenic nucleus (C-19 steroid) does not provoke an inhibition of 17β-HSD type 2 (data not shown). Thus, further studies will be necessary to better understand the mechanism of action and to optimize this first inhibitor of 17β-HSD type 2.

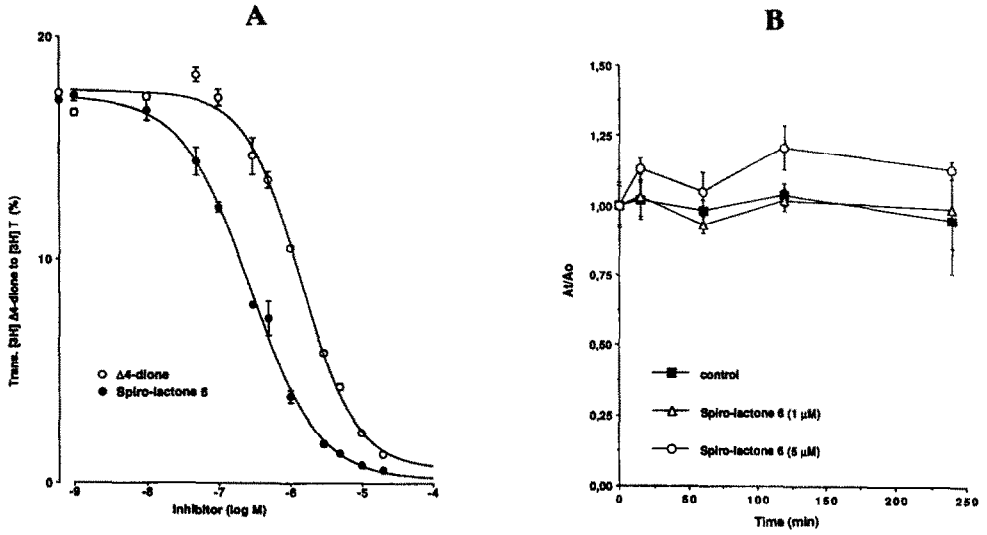


Figure 1. A. Inhibition of 17β-HSD type 2 by increasing concentration of spiro-γ-lactone **6** (IC_{50} = 0.27 μM) and Δ4-dione (IC_{50} = 1.41 μM). B. Inactivation of 17β-HSD type 2 by spiro-γ-lactone **6**. At: enzymatic activity at time t, and Ao: initial activity.

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10. 3-Hydroxy-19-nor-17α-pregna-1,3,5(10)-triene-21,17β-carbolactone (spiro-γ-lactone **6**): Colorless needles, mp 244-246°C (acetone/hexane); IR ν (KBr): 3350 (OH, phenol), 1750 (C=O, lactone); ^{1}H NMR δ (acetone-d_6): 0.95 (s, 3H, CH_3-18), 2.52 (m, 2H, CH_2C=O), 2.77 (m, 2H, CH_2-6), 6.53 (d, J = 2.6 Hz, 1H, CH-4), 6.60 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2), 7.10 (d, J = 8.5 Hz, 1H, CH-1), 7.97 (s, 1H, OH phenol); ^{13}C NMR δ (acetone-d_6):15.00 (C-18), 23.24 (C-15), 27.00 (C-11), 28.04 (C-7), ~ 29 (C-6 and C-21 masked in solvent peaks), 31.74 (C-12), 32.61 (C-20), 36.16 (C-16), 40.26 (C-8), 44.34 (C-9), 46.73 (C-13), 49.30 (C-14), 96.00 (C-17), 113.70 (C-2), 116.00 (C-4), 127.09 (C-1), 131.61 (C-10), 138.40 (C-5), 156.06 (C-3), 176.62 (C=O of lactone); MS m/e (rel. intensity): 326 M^+, 100), 253(13), 226 (14), 213 (59), 160 (62); HRMS calcd. for $C_{21}H_{26}O_3$ (M^+): 326. 1882, found 326. 1877; Anal. Calcd. for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03, found: C, 77.26; H, 8.31.
11. Enzymatic assay (briefly): **(A)** To 90 µl of human placental microsome preparation containing 17β-HSD type 2 activity was added 10 µl of a solution of aromatase inhibitor (EM-330, 10^{-3} M) [12], NADH (1 mM), [^{3}H] Δ4-dione (3.2 nM) and 10 µl of an ethanolic solution of spiro-γ-lactone **6** or unlabelled Δ4-dione. The volume was completed until 1 ml with a phosphate-based buffer (glycerol 20%, KH_2PO_4 50 mM, EDTA 1mM at pH 7.4). The mixture was incubated for 1 h at 37°C, and the reaction was stopped by adding a solution of unlabelled Δ4-dione and T before extraction with diethyl ether and evaporation of organic solvent. The residue was dissolved with CH_2Cl_2 in order to be spotted on a silica gel plate (TLC, 20 X 20 cm X 0.2 mm Kieselgel 60 F_{254}) and eluted with CH_2Cl_2/ethyl acetate (80:20). Δ4-dione (less polar) and T (more polar) are identified on TLC as two rows of visible spots under UV light. Each spot on the plate is cut, stored in a vial with 1 ml of ethanol and 10 ml of scintillating solution and radioactivity measured to obtain the % of transformation. **(B)** For inactivation assay, 100 µl of buffer solution containing 17β-HSD type 2, NADH, and appropriate concentrations of spiro-γ-lactone **6** was incubated at 37°C. At specified intervals, the samples (triplicate) were diluted 20-fold with buffer solution of [^{3}H] Δ4-dione and enzymatic assay performed as above. The data were plotted according to Kitz and Wilson [15].
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