Synthesis of New Steroidal 11β-Substituted Spirolactones

Hassane Faraj,[a] Michel Claire,[a] Anne Rondot,[a] André Aumelas[b] and Gilles Auzou*[a]
[a] *U 300 INSERM, Faculte de Pharmacie, 15 Avenue Charles Flahault, 34060, Montpellier Cedex 1, France*
[b] *U 264 CNRS-INSERM, Rue de la Cardonille, BP 5055, 34094, Montpellier Cedex*

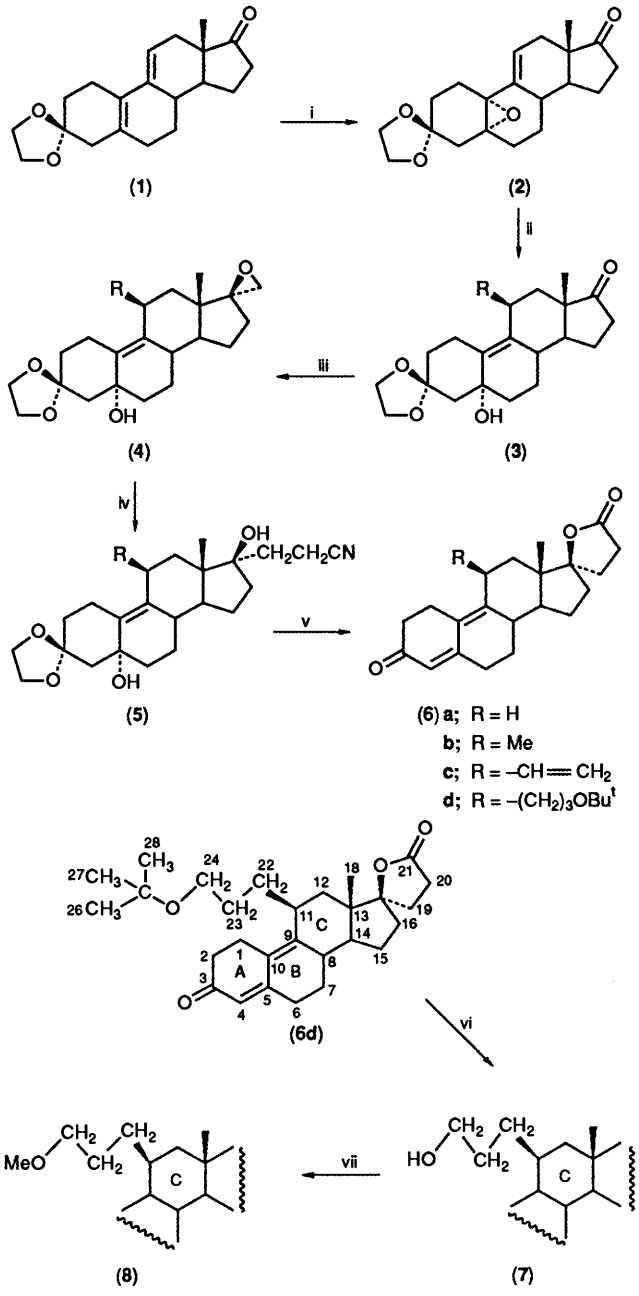
A stereoselective synthesis of new 3-oxo-11β-(3-alkoxypropyl)-19-nor-17α-pregna-4,9-diene-21,17-carbolactones has been achieved. The purpose of this study was to synthesize new 11β-substituted spirolactones by a new approach. A variety of NMR techniques were utilized to make complete assignments of this type of steroids in solution.

Steroid receptors are closely related structurally and in their mechanisms of action. Nevertheless, they exhibit large differences in their specificity for the various steroid hormones, and this has led to the identification of receptor superfamily and subfamilies.[1] Steroidal receptor ligands for each of these subfamilies present typical binding areas such as the 4-ene-3-one A-ring, also present specific planarity of the A-ring for androgen receptor (AR), progestin receptor (PR), glucocorticoid receptor (GR), and mineralocorticoid receptor (MR).[2] Slight modifications of the steroidal skeleton have been found to induce important affinity and specificity variations for the corresponding receptors. Several A- and D-ring-substituted steroidal 7α-alkoxycarbonyl spirolactones have recently been synthesized with the purpose of increasing the aldosterone antagonistic potency relative to the standard drug spironolactone.[3] Various 11β-substitutions of 17α-ethynyl or -propynyl 19-norsteroids with PR and GR specificities could drastically modify their affinity for the receptors.[4] The introduction of bulky unsaturated chains (vinylic, aromatic) in the C-11 position was found to enhance affinities for PR, GR,[4] or estrogen receptor (ER).[5] These observations led to the hypothesis that, in the region corresponding to the 11β-position of the steroids, a pocket exists on the GR, PR, and ER receptors, where hydrophobic interactions are probably involved. On the other hand, it has also been observed[6] that electronic interactions could be involved in the same area since 11β-halogen or 11β-bulky substituents bearing a heteroatom can also enhance PR- or GR-binding activity.[4] Putative pocket characteristics have never been explored for MR. Specific 11β-substituted MR ligands, agonists, or antagonists are necessary to perform this study. In the present paper, we describe the synthesis of new 11β-substituted 19-norsteroids bearing a 17γ-lactone moiety, this latter is typical of the MR steroidal antagonist. An 11β-hydroxypropyl chain was chosen first because it enables us to (1) condense various groups on the hydroxy group (2) make a further study of the influence of length, size, and electronic, geometric, and stereochemical interactions of these new 11β-arms with a binding affinity for MR. This hydroxypropyl chain could also be easily linked to fluorescent molecules, enabling further hormone receptor interaction studies to be undertaken. Fluorescent label derivatives have been prepared previously with steroidal mineralocorticoids or antimineralocorticoids substituted in the 7α-position.[7] Only one paper[8] describes the synthesis of a few spirolactones bearing 11β-alkyl, -alkenyl or -cycloalkyl substituents. The authors used different methods, but these methods required several steps. The starting material for the preparation of our new 17-spirolactone derivatives was 3,3-(ethylenedioxy)estra-5(10),9(11)-dien-17-one (**1**) (Scheme 1). Access to final derivatives was carried out by, first, introduction of the 11β-function, then the 17γ-lactone. In so doing, we took advantage (1) of avoiding the 3-deacetalization during hydrolysis of the intermediate 17-hydroxy-21-carbonitrile moiety (**5**) and (2) of having ease of access to dienone (**6**) in a one step from compound (**5**).

Results and Discussion

The key intermediates leading to these compounds are 5α,10α-epoxyestr-9(10)-enes (**2**). Selectivity in the α-epoxidation was improved by using hydrogen peroxide in the presence of hexafluoroacetone sesquihydrate.[9] With these reagents, epoxidation affords predominantly the 5α,10α-epoxide, with only 10–20% of the 5β,10β-conjugated epoxide, easily separated from the parent compound. When epoxide (**2**) was treated with the corresponding organomagnesium halide in the presence of a catalytic amount of copper(I) chloride,[9] the required product was obtained. The reaction is regio- and stereo-specific and only affords the 11β-substituted product (**3**).

The most obvious approaches to synthesis of the desired γ-lactones involve either propargyl alcohol fixation on the 17-keto derivative followed by hydrogenation and Oppenauer oxidation,[10] ethynylation of the same function with lithium acetylide followed by carboxylation and hydrogenation,[11] or the Sturtz reaction.[12] In our case, these methods gave poor yields and often mixtures of undesired products. Steric hindrance, caused by the 11β-hydroxypropyl and 18-methyl groups, could be a possible explanation for these results. The procedure we followed for this reaction consisted of methyl transfer to the 17-keto function by reaction with dimethylsulphonium methylide,[13] and condensation of acetonitrile on the thus produced spiro-oxirane (**4**) according to Creger's method.[14] This method enabled us, by a one-step procedure, to convert the 17α-cyanoethyl-17β-hydroxy steroid (**5**), after alkaline hydrolysis and acidic treatment into the 17γ-spirolactone derivative, which was then deacetylized and concomitantly dehydrated to the dienones (**6a–d**). Compounds (**6b**) and (**6c**) had already been synthesized by another method.[8] Further hydrolysis of the t-butoxypropyl group of compound (**6d**) by conc. HCl in dioxane produced compound (**7**). Etherification of the hydroxypropyl group by dimethyl sulphate and triethylbenzylammonium chloride as a phase-transfer catalyst (ptc) involving 50% aq. sodium hydroxide gave the methyl ether (**8**) in higher yields than usually obtained by other methods. In this case, purification involved preparative HPLC prior to chromatography on a silica gel column. The structure assignments were firmly established by a variety of NMR techniques. Generally, all compounds were characterized by COSY and ^{13}C spectra. Using the DEPT sequence, multiplicities of different carbon signals were defined. Where necessary, ^{1}H–^{1}H COSY and heterocorrelated ^{13}C–^{1}H experiments were performed on compound (**6d**). Results are reported in



Scheme 1. *Reagents and conditions:* i, H_2O_2–CF_3COCF_3, $6H_2O$, CH_2Cl_2–C_5H_5N, 99:1, 0 °C; ii, $Bu^tO(CH_2)_3MgCl$, THF, CuCl, −30 °C; iii, NaH–DMSO, 75 °C; Me_3SI, −5 °C, THF; iv, LDAMeCN, THF, −40 °C; v, KOH–MeOH, HCl–MeOH, reflux; vi, HCl-Diox, room temp; vii, TEBA–Cl, NaOH, CH_2Cl_2, room temp.

Table 1. The molecules synthesized in this study are now being tested for MR and GR affinities and activities. Further details of the relative affinity of these derivatives for the MR receptor and structure–reactivity studies will be published elsewhere.

Experimental

M.p.s were determined on a Bioblock melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 983 spectrophotometer for potassium bromide discs unless otherwise stated. NMR data were recorded on a WM 360 WB spectrometer (Brucker) at 360 MHz for ^1H, and 90 MHz for the ^{13}C nuclei. Compounds were dissolved in $CDCl_3$ from which the residual signal was taken as reference at δ_H 7.24 for ^1H and δ_C 77.0 for ^{13}C. In two-dimensional experiments, the ^1H–^1H COSY spectra was recorded in 512 experiments under standard conditions,[15] and the ^1H–^{13}C spectra were obtained in 160 experiments according to Bax's sequence.[16] Optical rotations on chloroform solutions were measured with a Roussel-Jouan micropolarimeter. All reactions were carried out under argon with dry solvents being used under anhydrous conditions. Column chromatography was carried out with silica gel (Merck 60 A CC). Analytical and small-scale preparative HPLC were carried out on a 25 cm × 4.6 mm i.d. stainless steel column packed with 5μ Hypersil (Beckman). Preparative HPLC purification of compound (7) was carried out on a 25 cm × 22.5 mm i.d. stainless steel column packed with 5μ Hypersil (S.F.C.C.). A mobile phase of methanol–water (7:3) was used and detection was at 254 nm on a Gilson Model 111 LC detector. Analytical results were determined for C and H and were within 0.3% of the calculated values. All new compounds described in this Experimental section were homogeneous on TLC. Epoxidation of estra-5(10),9(11)-diene (1) was carried out by a standard method,[9] and yielded mainly the 5α,10α-epoxide (2). Light petroleum refers to the fraction boiling in the range 148–150 °C.

Table 1. The ^{13}C and ^1H chemical-shift assignments for the steroid (**6d**) ($CDCl_3$; 307 K).

Atom	δ ^{13}C	δ ^1H
1	37.18	2.42
2	26.35	2.83 and 2.60
3	199.35	
4	122.48	5.67
5	157.15	
6	30.48	2.38
7	29.30	2.50
8	36.33	2.44
9	126.73	
10	149.05	
11	36.91	3.07
12	34.34	1.75 and 1.58
13	45.76	
14	50.48	1.29
15	22.86	1.68 and 1.47
16	35.72	2.29 and 1.83
17	96.24	
18	16.89	1.14
19	26.89	1.94 and 1.29
20	31.70	2.35 and 1.91
21	176.39	
22	33.19	1.59
23	29.48	1.57 and 1.44
24	61.03	3.28
25	72.05	
26, 27, 28	27.51	1.14

11β-(3-*t-Butoxypropyl*)-3-*oxo*-19-*nor*-17α-*pregna*-4,9-*diene*-21,17-*carbolactone* (**6d**).—A 1.4M-solution of (3-t-butoxypropyl)magnesium chloride (22 ml, 31 mmol) was cooled to −30 °C and a solution of copper(I) chloride (480 mg, 4.9 mmol) in tetrahydrofuran (THF) (20 ml) was added. The reaction mixture was stirred for 0.5 h and then a solution of the pure epoxide (2) (2.9 g, 8.8 mmol) in THF (30 ml) was added dropwise, and the mixture was stirred for 3 h while the temperature rose from −30 to −10 °C. The reaction mixture was poured into cold, saturated aq. ammonium chloride and extracted with AcOEt. The extract was washed successively with saturated brine and water, dried over sodium sulphate, and

evaporated to dryness. Chromatography on silica gel with light petroleum–ethyl acetate (7:3) as eluant yielded 11β-(3-*t-butoxypropyl*)-3,3-*ethylenedioxy*-5-*hydroxy*-19-*nor*-5α-*estr*-9-*ene*-17-*one* (**3**) (2.9 g, 74%) as a white powder from hexane, m.p. 78–80 °C (Found: C, 72.6; H, 9.55. $C_{27}H_{42}O_5$ requires C, 72.6; H, 9.5%); $[\alpha]_D$ −214.5° (*c* 0.5); ν_{max} 3504 (OH), 1743 (17γ-ketone) and 1606 cm^{-1} (C=C); δ_H 1.00 (3 H, s, 18-H_3), 3.25 (2 H, m, OCH_2), 3.95 (4 H, m, OCH_2CH_2O) and 4.25 (1 H, s, OH).

A suspension of 60% sodium hydride (0.3 g, 7.5 mmol) in mineral oil in dimethyl sulphoxide (DMSO) (14 ml) was stirred and heated at 70 °C for 1 h. The cooled mixture was diluted with THF (20 ml) and further cooled to −5 °C. Next, a solution of trimethylsulphonium iodide (1.4 g, 6.9 mmol) in DMSO (10 ml) was added and the reaction mixture was stirred for 35 min at below 0 °C, followed by treatment with a solution of compound (**3**) (880 mg, 2 mmol) in THF (8 ml). The mixture was then stirred at 0 °C for 2 h, and then at room temperature for 20 h. The reaction mixture was poured into cold aq. ammonium chloride and extracted with AcOEt. The extract was washed with water, dried over sodium sulphate, and evaporated to dryness. The product, purified by column silica gel chromatography with cyclohexane–AcOEt (4:1) as eluant, yielded the 17-oxirane (**4**) (550 mg, 60%). This amorphous residue was characterized by IR spectroscopy (absence of 17-C=O at 1742 cm^{-1}) and was then used immediately for the next step without further purification.

A standard solution of butyl-lithium in hexane (1.6M; 1.7 ml, 2.6 mmol) was added to a solution of diisopropylamine (0.27 g, 2.6 mmol) in anhydrous THF (10 ml) at −30 °C and the mixture was stirred for 35 min. Next a solution of acetonitrile (0.15 ml, 2.6 mmol) in THF (3 ml) was added and the mixture was stirred at −30 to −20 °C for 1 h. A solution of the 17-oxirane (**4**) (0.4 g, 0.87 mmol) in THF (6 ml) was added dropwise during 10 min and the reaction mixture was stirred at ambient temperature for 20 h, poured into aq. ammonium chloride, and extracted with AcOEt. The extract was washed with water, dried over sodium sulphate and evaporated to dryness. The crude product (0.7 g) was purified by column silica gel chromatography with methylene dichloride–AcOEt (7:3) as eluant to yield 11β-(3-t-butoxypropyl)-3,3-ethylenedioxy-5,17β-dihydroxy-5α-estr-9-ene-21-carbonitrile (**5**) (350 mg, 80.5%), ν_{max} 3457 (OH) and 2249 cm^{-1} (CN).

A mixture of compound (**5**) (300 mg, 0.6 mmol), methanol (0.4 ml) and 6M-KOH (0.35 ml) was heated to reflux for 4 h. The cooled mixture was acidified with conc. HCl (0.4 ml) and refluxed for 1.5 h. The solution was diluted with water and extracted with AcOEt. The extract was washed with water, dried over sodium sulphate, and evaporated to dryness to give a crude product (0.26 g). Chromatography on silica gel with CH_2Cl_2–AcOEt (4:1) as eluant yielded 11β-(3-*t-butoxypropyl*)-3-*oxo*-19-*nor*-17α-*pregna*-4,9-*diene*-21,17-*carbolactone* (**6d**) (130 mg, 50%) (Found: C, 76.2; H, 9.5. $C_{28}H_{40}O_2$ requires C, 76.3; H, 9.15%); $[\alpha]_D$ −36.1° (*c* 0.5) as a white powder from diethyl ether–pentane, m.p. 122–123 °C; ν_{max} 1772 (γ-lactone), 1662 (α,β-unsaturated ketone) and 1610 cm^{-1} (C=C); δ_H 1.14 (12 H, m, Bu^t, 18-H), 3.07 (1 H, m, 11-H) and 5.67 (1 H, s, 4-H).

11β-(3-*Hydroxypropyl*)-3-*oxo*-19-*nor*-17α-*pregna*-4,9-*diene*-21,17-*carbolactone* (**7**).—Conc. HCl (2.3 ml) was added to a solution of compound (**6d**) (900 mg, 1.6 mmol) in 1,4-dioxane (6 ml). The reaction mixture was stirred for 1.5 h, then poured onto cold water and extracted with AcOEt. The extract was washed successively with saturated aq. sodium carbonate and water, dried over sodium sulphate, and evaporated to dryness. The crude product was purified by preparative reversed-phase HPLC with a mobile phase of methanol–water (7:3) to yield *compound* (**7**) (650 mg, 83%) as a major component, separated as a white powder from light petroleum, m.p. 157–159 °C (Found: C, 74.65; H, 8.55. $C_{24}H_{32}O_4$ requires C, 74.95; H, 8.4%); $[\alpha]_D$ −78.5° (*c* 0.3); ν_{max} 3437 (OH), 1771 (γ-lactone) and 1656 cm^{-1} (α,β-unsaturated ketone); δ_H 1.16 (3 H, s, 18-H_3), 3.09 (1 H, m, 11-H), 3.63 (2 H, m, CH_2O) and 5.7 (1 H, s, 4-H).

11β-(3-*Methoxypropyl*)-3-*oxo*-19-*nor*-17α-*pregna*-4,9-*diene*-21,17-*carbolactone* (**8**).—Triethylbenzylammonium chloride (3 mg, 0.01 mmol) and 50% aq. NaOH (30 mg, 0.75 mmol) was added to a solution of compound (**7**) (40 mg, 0.1 mmol) in methylene dichloride (1.2 ml). Dimethyl sulphate (30 mg, 0.23 mmol) is then added dropwise, with cooling, for 1 h at a rate such that the temperature did not exceed 35 °C. The whole mixture was then stirred for 24 h with frequent TLC controls. After addition of methylene dichloride (24 ml) the mixture was finally poured in aq. NH_4Cl and the organic phase was separated, washed with water, and dried over Na_2SO_4. Elution with CH_2Cl_2–AcOEt (9:1) gave compound (**8**) with some impurities. By reversed-phase HPLC with a mobile phase of methanol–water (7:3), *compound* (**8**) (22.5 mg, 54%) was separated as a white powder from pentane–diethyl ether (8:2), m.p. 119–121 °C (Found: C, 75.5; H, 8.45. $C_{25}H_{34}O_4$ requires C, 75.3; H, 8.6%); $[\alpha]_D$ −20° (*c* 0.5); ν_{max} 1772 (γ-lactone) and 1665 cm^{-1} (α,β-unsaturated ketone); δ_H 1.16 (3 H, s, 18-H_3), 3.30 (3 H, s, OMe), 3.33 (2 H, m, OCH_2) and 5.70 (1 H, s, 4-H).

3-*Oxo*-19-*nor*-17α-*pregna*-4,9-*diene*-21,17-*carbolactone* (**6a**).—Similar treatment of the ketone (**1**) as described for the reaction (**3**) ⟶ (**4**) yielded the 17-oxirane (81%) (absence of 17-C=O at 1740 cm^{-1}). When treated as for reaction (**4**) ⟶ (**5**), the 17-oxirane steroid afforded the 17α-cyanoethyl-17β-hydroxy compound (74%) purified by chromatography on silica gel with CH_2Cl_2–AcOEt (9:1) as eluant; ν_{max} 2245 cm^{-1}. Similar treatment as described for reaction (**5**) ⟶ (**6d**) afforded compound (**6a**) as needles after recrystallization from diethyl ether, m.p. 172–175 °C (Found: C, 77.1; H, 8.0. $C_{21}H_{26}O_3$ requires C, 77.3; H, 8.0%); $[\alpha]_D$ −214.5° (*c* 0.55); ν_{max} 1765 (γ-lactone) and 1655 cm^{-1} (α,β-unsaturated ketone); δ_H 1.02 (3 H, s, 18-H_3) and 5.7 (1 H, s, 4-H).

11β-*Methyl*-3-*oxo*-19-*norpregna*-4,9-*diene*-21,17-*carbolactone* (**6b**).—Similar treatment of the epoxide (**2**) as described for reaction (**2**) ⟶ (**3**), but using methylmagnesium bromide, afforded the 11β-methyl intermediate with 10% of the disubstituted 11β,17α-dimethyl moiety easily separated by chromatography on silica gel with hexane–AcOEt (6:4) as eluant. The 17-oxirane (75%) was obtained as for compound (**4**) (absence of 17-C=O at 1740 cm^{-1}) and treated as for reaction (**4**) ⟶ (**5**) to yield the 17α-cyanoethyl-17β-hydroxy steroid (72%), purified by chromatography on silica gel with CH_2Cl_2–AcOEt (8:2) as eluant; ν_{max}(KBr) 2250 cm^{-1} (CN).

Similar treatment as described for (**6d**) afforded compound (**6b**) as needles after recrystallization from diethyl ether, m.p. 208–210 °C (lit.,[8] 198–201 °C) (Found: C, 77.6; H, 8.3. Calc. for $C_{22}H_{28}O_3$: C, 77.1; H, 8.00%); $[\alpha]_D$ −91.8° (*c* 0.5); ν_{max} 1722 (γ-lactone), 1656 (α,β-unsaturated ketone) and 1607 cm^{-1} (C=C); δ_H($CDCl_3$) 1.13 (3 H, s, 18-H_3), 1.17 (3 H, d, 11-Me), 3.23 (1 H, m, 11-H) and 5.63 (1 H, s, 4-H).

11β-*Vinyl*-3-*oxo*-19-*norpregna*-4,9-*diene*-21,17-*carbolactone* (**6c**).—Similar treatment of the epoxide (**2**) with vinylmagnesium bromide afforded the 11β-vinyl intermediate (75%), with 15% of the 11β,17α-divinyl moiety, easily separated by chromatography on silica gel with hexane–AcOEt (6:4) as eluant. The 17-oxirane (73%) was obtained as for compound (**4**) (absence of 17-C=O at 1740 cm^{-1}) and treated in the usual way to yield the 17α-cyanoethyl-17β-hydroxy steroid (85%), purified by chromatography on silica gel with hexane–AcOEt (7:3) as eluant; ν_{max} 2247 cm^{-1} (CN).

Similar treatment as described for (**6d**) afforded compound (**6c**) as a white powder after recrystallization from diethyl ether, m.p. 143–144 °C (lit.,[8] 133–135 °C) (Found: C, 77.95; H, 7.9. Calc. for $C_{23}H_{28}O_3$: C, 78.36; H, 8.02%); $[\alpha]_D$ −30° (*c* 0.5); ν_{max} 1770 (γ-lactone), 1664 (α,β-unsaturated ketone), 1630 (C=C 11β-vinyl) and 1608 cm^{-1} (C=C); δ_H 1.04 (3 H, s, 18-H_3), 3.77 (1 H, m, 11-H), 4.75 (1 H, m, *J* 17.2 Hz, 22-H), 4.95 (1 H, m, *J* 10.2 Hz, 23-H), 5.70 (1 H, s, 4-H) and 5.82 (1 H, m, *J* 10.2 and 17.2 Hz, 23-H).

Acknowledgements

We are very grateful to Mr. A. Ramet of Roussel Uclaf for his generous gift of 3,3-(ethylenedioxy)-estra-5(10),9(11)-dien-17-one.

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Paper 0/02301I
Received 23*rd May* 1990
Accepted 13*th July* 1990