Notes

Synthesis of New 11β-Substituted Spirolactone Derivatives. Relationship with Affinity for Mineralocorticoid and Glucocorticoid Receptors

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Received March 30, 1993

Various steroidal 17-spirolactones substituted in the 11β-position were synthesized to study the relationship between the nature of the 11β-arm and their affinity for cytosolic mineralocorticoid (MR) and glucocorticoid (GR) receptors prepared from adrenalectomized rabbit kidney or liver. One of them, the 11β-allenyl-3-oxo-19-nor-17-pregna-4,9-diene-21,17-carbolactone derivative, exhibited the same affinity for MR as aldosterone and a 5-fold higher affinity than mespirenone. Its affinity for GR was found to be relatively low. As suggested by molecular modeling, the marked differences in mineralocorticoid receptor binding affinity could be related to the structural features induced by this 11β-allenic substituent.

During the past decade, the synthesis of new steroidal aldosterone antagonists has been developed[1-6] in an attempt to obtain derivatives with higher antagonistic activity than spironolactone and devoid of the side effects exhibited by this compound, i.e. gynecomastia and impotence in men and menstrual irregularities in women, which are due to its antiandrogenic and progestative properties.[7]

Slight modifications of the spirolactone skeleton have been shown to induce important variations in the affinity and specificity for the mineralocorticoid (MR). Several A or D ring substituted steroidal 7α-alkoxycarbonyl spirolactones have recently been synthesized leading to a reduction in the affinity for the androgen (AR) and progesterone (PR) receptors, whereas the mineralocorticoid activity was maintained at a high level.[2] The binding characteristics of these molecules for the MR were not described. On the other hand, some 11,12-dehydropregnane derivatives were found to exhibit high affinities for the MR,[6] but displayed agonistic rather than antagonistic activities. Introduction of bulky unsaturated chains (vinylic or aromatic) on the 11β-position of 17α-ethynyl or propynyl 19-norsteroids led to enhancement of the affinities for GR, PR, or estrogen (ER) receptors.[8] The nature of the substituents involved in these studies led to the hypothesis that a large "hydrophobic pocket" could be located in the region of the GR and PR corresponding to the 11β-position of the steroid.[9] Comparison of the amino acid sequences demonstrated a high degree of homology between DNA binding domains (~90%) but also steroid binding domains (>50%) of the ER, GR, PR, and MR.[10] The hypothetical hydrophobic pocket in GR and PR could thus be postulated for MR as well. In order to investigate this hypothesis, we synthesized various 11β-substituted 19-norsteroids bearing a 17γ-lactone moiety and we determined their relative binding affinities (RBA) for cytosolic MR and GR prepared from adrenalectomized rabbit kidney or liver. This animal model is more appropriate than rat since aldosterone binds to a single class of sites in rabbit kidney cytosol, whereas two types of sites are detected in the cytosolic fraction of rat kidney.[11] The results of these experiments are listed in Table I. Aldosterone was taken as the reference steroid for MR and dexamethasone for GR (RBA = 100). Spironolactone, canrenone, and mespirenone, a new antialdosterone compound synthesized by Schering laboratories,[2] were also tested. In our experimental conditions, spironolactone and canrenone exhibited RBA values which are in agreement with that recently published by a Japanese group in a similar study.[6]

Chemistry

11β-Allenyl-3-oxo-19-nor-17α-pregna-4,9-diene-21,17-carbolactone (**9**) was synthesized starting from 3,3-[(2,2-dimethyltrimethylene)dioxy]-5α,10α-epoxyestr-9(11)-en-17β-ol by a method similar to that recently employed in our laboratory (**1**, **3**, **4**, and **5**)[12] or by other authors (**2** and **6**)[13] (Scheme I). Fixation of the 11β-allenyl chain was achieved using the Grignard reagent prepared from propargyl bromide and magnesium. Selective blockade of the 3-enone by ethanedithiol (compound **7**) regardless of the diketal moiety was necessary before formation of the 17γ-lactone as described in a recent paper.[14] 11-Ethylidene-3-oxo-19-nor-17α-pregna-4,9-diene-21,17-spirolactone (**10**) and 11-(3-propenylidene)-3-oxo-19-nor-17α-pregna-4,9-diene-21,17-spirolactone (**11**) were synthesized from either 11β-vinyl and 11β-allenyl moieties **6** or **9** after acidic rearrangement of these unsaturated functions, according to a procedure already described in our laboratory for the steroidal precursors 3,17-diones.[15] The stereochemistry of the ethylidene chain of compound **10** and the 11-diene system of compound **11** was resolved, as described in a recent article by using NOESYs experiments.[15] Nuclear Overhauser effects observed between 11^1-H and 1α,β-H suggested a trans conformation for compound **10**. A nuclear Overhauser effect observed between 11^2-H and 12β-H and between 11^1-H and 1α,β-H

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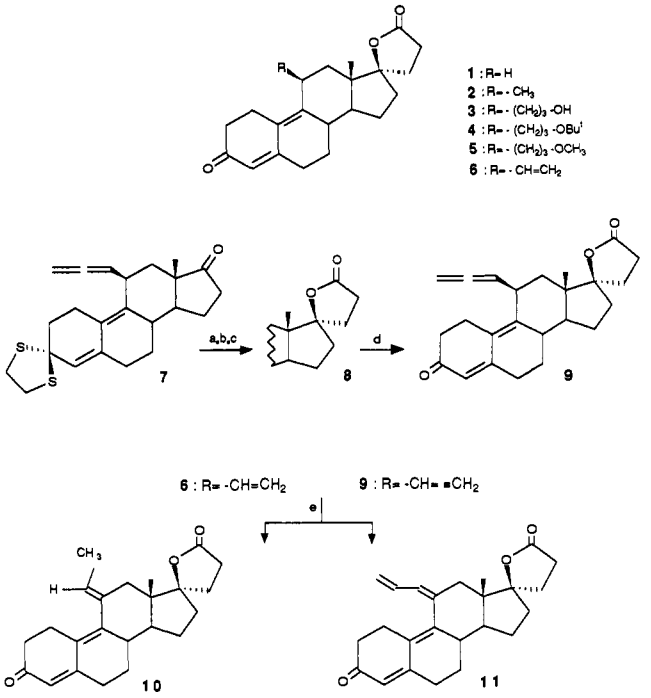
0022-2623/93/1836-2404$04.00/0

Table I. Relative Binding Affinity (RBA) for Mineralocorticoid (MR) and Glucocorticoid Receptors (GR) of 11β-Steroidal Spirolactones

compound	RBA %[a]	
	MR (Ald = 100%)	GR (Dex = 100%)
aldosterone	100	10
dexamethasone	20	100
spironolactone	4	<0.1
canrenone	1	<0.1
mespirenone	21	<0.1
1	13	1
2	6	4
3	<0.1	<0.1
4	<0.1	2
5	0.5	0.5
6	14	4
9	110	5
10	7	5
11	2	2

[a] Aliquots (0.1 mL) of the cytosolic fraction were incubated either with 2 mM [^{3}H]1,2-aldosterone (40–60 Ci/mol) or 20 nM) [^{3}H]1,2,4-dexamethasone (40–50 Ci/mol) in the absence or presence of increasing concentrations of the various spirolactone derivatives to be tested (1–500-fold excess). Unlabeled aldosterone and dexamethasone were used as reference compound for MR and GR, respectively. After 20 h at 4 °C (corresponding to the steady-state), bound and free steroids were separated by the charcoal–dextran method previously described,[11] and the bound fraction counted for radioactivity. Competition curves were established for each competitor or reference compound, and the relative binding affinity was determined from the concentrations needed to obtain a 50% displacement of [^{3}H]aldosterone or [^{3}H]dexamethasone from their binding sites. The RBA values of the reference compounds was taken as 100. Values given in Table I represent the mean of three separate experiments.

Scheme I[a]



[a] Reagents: (a) NaH, DMSO 75 °C; (b) $(CH_3)_3Si$, THF −5 °C; LDA, CH_3CN, THF −40 °C; (c) KOH, CH_3OH; HCl, CH_3OH, reflux; (d) NCS, $AgNO_3$, CH_3CN, H_2O; Na_2SO_3, $NaHCO_3$, CH_2Cl_2; (e) HCl, MeOH reflux 1 h.

suggested the same trans conformation for compound 11 (Scheme I).

Methods

As already described,[11] New Zealand rabbits (~1 kg) were adrenalectomized under pentobarbital anesthesia and maintained on 0.9% normal saline for 72 h. They were then sacrificed and their kidneys were perfused with 30 mL of 0.9% normal saline and 30 mL of homogenization buffer (HB: 20 mM Tris-HCl, 1 mM EDTA, 20 mM sodium tungstate, and 10% glycerol v/v, pH 7.4). The kidneys were then cut into pieces and immediately frozen in liquid nitrogen. The frozen kidneys were ground in a mortar under liquid nitrogen and homogenization buffer (2 mL/g) was added to the resulting buffer. Immediately after thawing, the mixture was vortexed at 0 °C and homogenized in a Teflon-glass Potter. The homogenate was centrifuged at 105 000g for 60 min at 4 °C. The supernatant was then filtered through a 0.45-μm porous membrane and frozen in liquid nitrogen until further use.

Results

The RBA for MR and GR exhibited by the differently 11β-substituted spirolactones are summarized in Table I. The 19-nor compound 1, unsubstituted at 11β, demonstrated a higher RBA for MR than canrenone and spironolactone but also a low but significant affinity for GR. Introduction of a methyl group in the C-11 position (compound 2) decreased the RBA for MR, whereas RBA for GR was enhanced. Compound 4, with a larger hydrophobic substituent than the methyl group, displayed a lower RBA for GR and no affinity for MR. Compounds 3 and 5 with bulky hydrophilic substituents had almost no affinity for MR and GR. The C-11 substitution with unsaturated groups yielded derivatives showing wide variations of RBA for MR, depending on the nature of the unsaturated moieties. Introduction of a vinylic substituent (compound 6) led to a RBA for MR identical to that observed for the compound 1 but there was a higher RBA for GR (4 vs 1). The 11β-allenyl group (compound 9) dramatically enhanced the RBA for MR leading to a derivative which displayed the same affinity as aldosterone and 5–6-fold higher than mespirenone. The RBA for GR of this compound was also enhanced as compared to the unsubstituted derivative 1 but to a lower degree. Contrary to what was expected, the acidic rearrangement of the vinylic and allenic moieties yielding 11-ethylidene and 11-(3-propenylidene) spirolactones respectively (compounds 10 and 11), substantially decreased the RBA for MR and either did not modify (10) or only moderately lowered (11) the RBA for GR.

Discussion

The 11β-substituted spirolactones that we synthesized showed a wide range of RBA for MR and as expected for spirolactone derivatives a low affinity for GR. The hypothetical "hydrophobic pocket" located in the MR region corresponding to the 11β position, was confirmed by our results. This conformation probably differs from that suggested for the GR and PR in the 17α–propynyl (or ethynyl) series.[8] The 11β-allenic derivative 9 was the most interesting compound in this series. Simple addition of another double bond to the vinylic compound 6, strongly increased the RBA for MR (14–110%) with almost no modification of the RBA for GR (4–5%). Such enhancement could be attributed to the π potential of the 11β-allenic chain with π bonds orthogonal to each other.[16] These results clearly indicate that there must be a specific bonding interaction of the 11β-allenic moiety with part of the MR. When the orientation of the 11-unsaturated group is different, as was the case for the propenylidene moiety

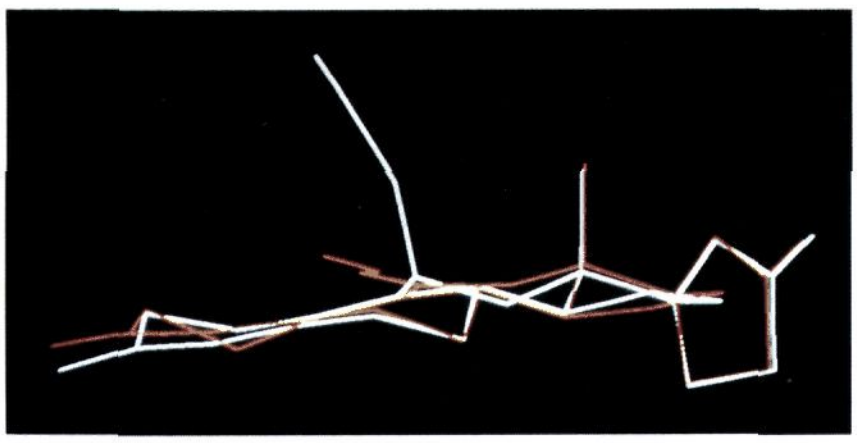


Figure 1. Relaxed stereoview of the superimposition of **9** (white) and **11** (orange). For the sake of clarity, hydrogen atoms have been removed.

(compound **11**) resulting from an acidic rearrangement of **9**, the RBA for MR dramatically decreased (from 110% to 2%) and that for GR (from 5% to 2%) to a lesser extent. The wide difference in the RBA results could be explained by this different steric orientation, as illustrated by the superimposition of the atomic skeleton of compounds **9** and **11** simultaneously fitted and energy minimized (Figure 1). Precise and accurate X-ray determination of the crystal and molecular structures of these two compounds detailed in another paper[17] could provide a base upon which to specify orientation of these side chains and to correlate conformational and geometric variations with affinities. Further improvement of the affinity and specificity could probably be obtained with steroidal spirolactones bearing an 11β side chain derived from this new type of 11β-allenic substituent. The preparation of new 11β-allenic derivatives is currently underway in our laboratory, and the synthesis as well as RBA data for MR, GR, and PR will be reported in due course.

Experimental Section

Starting material, 3,3-(2,2-dimethyltrimethylenedioxy)-5(10),9(11)-estradien-17β-ol (ZK 37875) was obtained from Professor Wiechert of Schering. Starting reactions were carried out under an argon atmosphere with dry solvents used under anhydrous conditions. Melting points (mp) were determined on a Bioblock melting point apparatus and were uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 983 spectrophotometer by means of potassium bromide disks unless otherwise stated. Optical rotations on chloroform solutions were measured with a Roussel-Jouan micropolarimeter. Proton magnetic resonance (^{1}H NMR) spectra were recorded at 32 °C on a Bruker WM 360 WB spectrometer. Compounds were dissolved in $CDCl_3$ from which the residual signal was taken as reference at 7.24 ppm for ^{1}H. The ^{1}H–^{1}H COSY were recorded in 512 experiments under standard conditions.[18] Two-dimensional correlated ^{13}C–^{1}H were performed in the inverse detection mode, according to Bax's sequence.[19] Column chromatography was carried out with silica gel Merck 60 A CC. Analytical high-pressure liquid chromatography (HPLC) was carried out on a 25-cm × 4.6-mm i.d. stainless steel column packed with 5-μm Hypersil. The mobile phase of methanol/water (7:3) was used and detection measured with the variable wavelength Kontron detector model. Analytical thin-layer chromatography (TLC) was performed using 0.25-mm silica gel plates with F-254 indicator. Analytical results indicated by elemental symbols were determined for C, H, and O and were within 0.4% of the theoretical values. MAD [20] was used to predict and display the structural and molecular characteristics of these compounds. Molecular mechanics techniques (MM_2)[21] were performed to determine preferred conformations *in vacuo* on the basis of previous X-ray structure determinations.[22] All simulations were computed on an IBM 3090 V 600 and all molecular graphics were displayed on an IBM 5080/5082 workstation.

11β-Allenyl-3-oxo-19-nor-17α-pregna-4,9-diene-21,17-carbolactone (9). The procedure we followed for the formation of the 17γ-lactone has been published elsewhere for other compounds.[12] It consisted of a methyl transfer reaction to the 17-keto function of 410 mg (0.93 mmol) of compound **7** with dimethyl sulfonium methylide, producing 17-spirooxirane. Condensation of acetonitrile on the 17-spirooxirane according to Creger's method[23] enabled us to convert the 17α-hydroxycyanohydrin after alkaline hydrolysis and acidic treatment to 300 mg (64%) of the 17γ-lactone **8**. Dethioketalization was performed on 140 mg (0.34 mmol) of **8** in acetonitrile (15 mL) and H_2O (3.6 mL). After rapid stirring, 99 mg (0.74 mmol) of *N*-chlorosuccinimide (NCS) and 130 mg (0.77 mmol) of silver nitrate ($AgNO_3$) where then added and the whole yellow mixture was stirred for 30 min. Next, saturated aqueous solutions (1 mL) of sodium sulfite (Na_2SO_3), $NaHCO_3$ and sodium chloride (NaCl) were successively added to the reaction mixture. All material was dissolved in 25 mL of CH_2Cl_2, washed with water and saturated aqueous NaCl solution, dried, and evaporated. The product was purified by column silica gel chromatography using hexane/AcOEt (5:5) as an eluent. Recrystallization from diisopropyl ether gave 60 mg (52%) of **9**: mp 202–204 °C $[\alpha]^{25}_D$ 126° (*c* 0.5); IR 1951, 1768, 1659 cm^{-1}; NMR ($CDCl_3$) 1.14 (s, 3, C-18), 3.86 (m, 1, 11α-H), 4.67 (m, 2, 11^3-H), 5.15 (m, 1, 11^1-H), 5.70 (s, 1, H-4) ppm. Anal. ($C_{24}H_{28}O_3$) C, H, O.

11-Ethylidene-3-oxo-19-nor-17α-pregna-4,9-diene-21,17-carbolactone (10). A solution of 300 mg (0.85 mmol) of 11β-vinyl-3-oxo-19-nor-17α-pregna-4,9-diene- 21,17-carbolactone (**6**)[12,13] in 10 mL of methanol and 74 μL concentrated HCl was heated to reflux for 1 h. The solution was poured into cold, saturated $NaHCO_3$ and extracted with EtOAc. After drying and evaporation, the residue was chromatographed on silica gel with dichloromethane/ethyl acetate (9:1), thus obtaining, after recrystallization from diisopropyl ether 240 mg (80%) of **10**: mp 148–150 °C; $[\alpha]^{25}_D$ −484° (*c* 0.5); IR 1772, 1661 cm^{-1}; NMR ($CDCl_3$) 0.97 (s, 3, C-18), 1.65 (m, 1, 12α-H), 1.70 (d, 3, 11^2-H), 2.85 (m, 1, 12β-H), 5.54 (q, 1, 11^1-H), 5.70 (s, 1, 4-H) ppm. Anal. ($C_{23}H_{28}O_3$) C, H, O.

11-(3-Propenylidene)-3-oxo-19-nor-17α-pregna-4,9-diene-21,17-carbolactone (11). Under the above-described conditions, 100 mg (0.27 mmol) of **6** was transformed to 84 mg (84%) of **11** after recrystallization in diethyl ether: mp 183–185 °C; $[\alpha]^{25}_D$ −146° (*c* 0.5); IR 1769, 1663, 1625 cm^{-1}; NMR ($CDCl_3$) 0.95 (s, 3, C-18), 1.90 (m, 1, 12α-H), 3.00 (m, 1, 12β-H), 5.2–5.3 (m, 2, 11^3-H), 5.70 (s, 1, 4-H), 6.02 (m, 1, 11^1-H), 6.60 (m, 1, 11^2-H) ppm. Anal. ($C_{24}H_{28}O_3$) C, H, O.

Acknowledgment. We would like to express our gratitude to Professor R. Wiechert of Schering Laboratories for the generous supply of the starting steroid ZK 37875.

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