Aldosterone Antagonists. 4. Synthesis and Activities of Steroidal 6,6-Ethylene-15,16-methylene 17-Spirolactones

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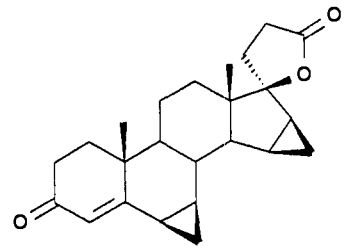
Several steroidal 6,6-ethylene-15,16-methylene 17-spirolactones were synthesized to find new progestogens that exhibit both progestational and antimineralocorticoidal activities. The influence of substituents in the 10- and 13-position of the steroidal framework on both properties was investigated. It was found that only compound 12, carrying methyl groups at the 10- and 13-positions, possesses high progestational and antimineralocorticoidal activity.

The development of steroidal aldosterone antagonists as potassium-retaining diuretics was initiated by the observation that progesterone acts as a competitive antagonist of the renal action of mineralocorticoid hormones.[1,2] Extensive molecular modifications led to the synthesis of the antimineralocorticoid spironolactone, a compound that still exhibits weak progestational activity.[3-6] Our research efforts during the past years have been primarily concerned with the development of new potent antialdosterone derivatives and have been culminated in the synthesis of 6β,7β:15β,16β-dimethylene spirolactones 1 and 2.[6,7]

Both compounds, when tested in rats, exhibited a 7- to 8-fold higher aldosterone antagonistic potency compared to that of spironolactone.[8,9] Compound 1 could not be developed as a diuretic because of its progestational activity as measured in the endometrium transformation test and pregnancy maintenance test.[10,11] Especially in rats 1 showed a progestational potency comparable to cyproterone acetate.[10] These data suggested that aldosterone antagonistic and progestational activity are observed in the same dose range for these compounds.

This combination of activities is only found in naturally occuring progesterone, whereas the synthetic derivatives of 17-hydroxyprogesterone and 19-nortestosterone are only potent progestational agents but do not exhibit antimineralocorticoid activity.[8] The potential advantages of a new progestogen with a profile compared to progesterone have been emphasized[8,11] especially with respect to the development of a new type of progestogen–estrogen combination for fertility control. Upon taking oral contraceptives, some women exhibit an increasing in blood pressure.[12] Hypertension is generally a contraindication for taking the birth control pill. With a progestogen exhibiting antimineralocorticoid activity, the increase in blood pressure should be counteracted, therefore allowing predisposed women to choose a hormonal contraceptive as fertility control method for the first time.

The optimal ratio between aldosterone antagonistic and progestational activity has to be carefully evaluated. Probably the most obvious question is how much antimineralocorticoid activity is necessary at fully active progestational doses. To answer this question, we were looking for derivatives of compound 1 exhibiting comparable aldosterone antagonistic potency but higher progestational potency compared to 1.



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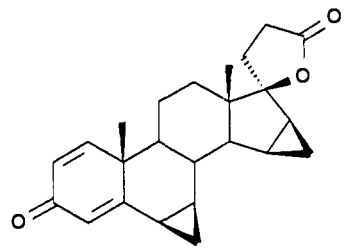

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Chemistry

The structure–activity relationship of spirorenone analogues has been reported.[13] Molecular modification of 1 produced only compounds with reduced aldosterone antagonistic potency and reduced binding to the progesterone receptor. We therefore investigated other basic structures showing both activities. For progestational activity the importance of substituents in the 6-position has been emphasized.[14] Optimal activity is found with rather small and moderate lipophilic substituents, such as methyl or chlorine, but aldosterone antagonists with these substitu-

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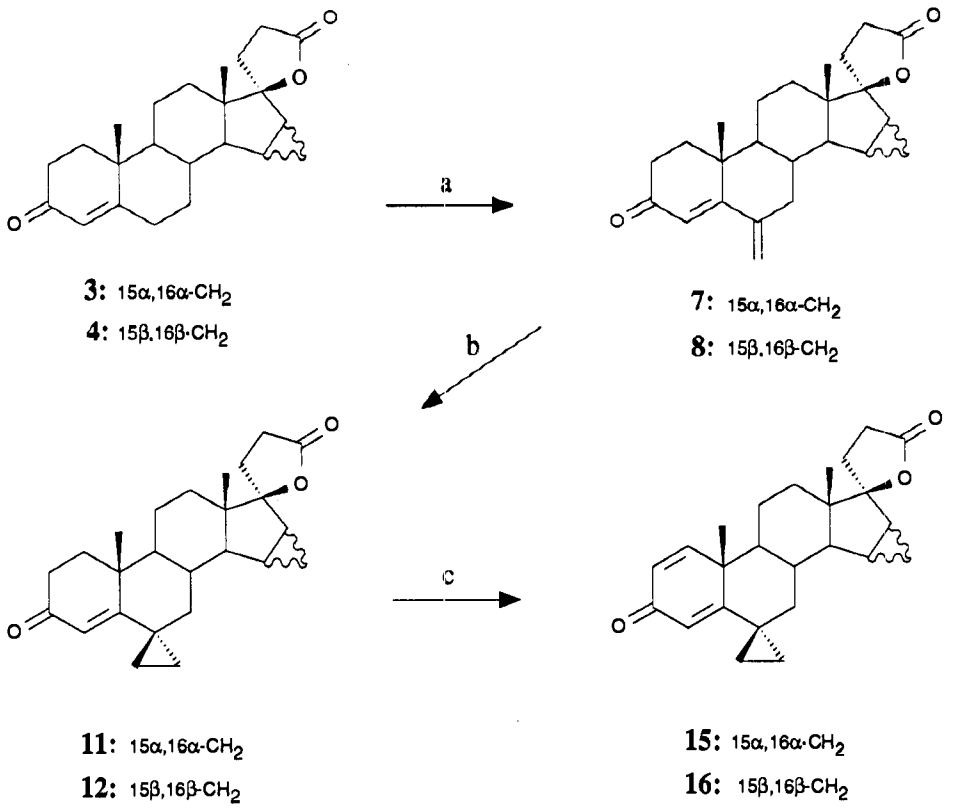
Scheme I[a]



[a] (a) 1. pyrrolidine, MeOH, reflux; 2. CH_2O, EtOH, benzene; 3. HCl, dioxane; (b) $(CH_3)_3S(O)I$, NaH, DMSO; (c) DDQ, toluene, 100 °C.

ents are lacking oral bioavailability. Both requirements are fulfilled by a 6,6-ethylene moiety. Moreover it has been reported that spirolactone derivatives with this moiety exhibit aldosterone antagonistic activity.[15] The progestational activity depends on the nature of substituents at the 17α-position and to some extent at the 10- and 13-positions of the steroid nucleus.[16] Generally speaking 19-nor derivatives are more potent than 10-methyl compounds. The highest progestational potency is found when the 10-position is substtituted by a hydrogen and the 13-position with an ethyl group (e.g., norgestrel).[16] Only a few examples about the systematic comparison of the different series of steroid frameworks on the aldosterone antagonistic activity have been reported.[17] For this reason, we decided to investigate the influence of the substitution pattern in the 10- and 13-position of the steroid framework on the progestational and antimineralocorticoid activity of 6,6-ethylene-15,16-methylene 17-spirolactones.

As starting material for our efforts we chose the 15,16-methylene spirolactones **3–6**. The synthesis of **3** and **4** has been reported.[18] Compounds **5** and **6** were prepared under analogous conditions.[19] The transformation of the 13-methyl derivatives **3** and **4** to the 6,6-ethylene compounds **11** and **12** was accomplished in a four-step sequence (Scheme I). Reaction of the unsaturated ketones with pyrrolidine generated enamines, which were reacted with aqueous formaldehyde to form the hydroxymethyl derivatives. The crude material was transformed to the 6-methylene compounds **7** and **8**. The dienes were converted to the 6,6-ethylene derivatives **11** and **12** by reaction with trimethylsulfoxonium iodide and sodium hydride.

For the synthesis of the 19-nor derivatives **13** and **14**, a slightly different method of synthesis was applied. The starting materials **5** and **6** were transformed via the enamines to the 6-hydroxymethyl derivatives **9** and **10**. The stereochemistry of the 6-substituent was investigated by NMR. The H-4 shows only one allylic coupling of 2 Hz to the H-10, proving that the hydroxymethyl group is in the β-position. All attempts to transform the hydroxymethyl steroids **9** or **10** to the corresponding dienones either under acidic or after transformation to the tosylates under basic conditions were unsuccessful. Only dimeric steroids could be isolated, but their accurate structures were not determined. The desired 6,6-ethylene compounds were finally synthesized by transformation of the hydroxymethyl derivatives **9** and **10** to the tosylates, which were reacted with an excess of trimethylsulfoxonium iodide and sodium hydride in an elimination addition process (Scheme II). The 1,2 double bond was introduced in compounds **11** and **12** by dehydrogenation with DDQ to yield **15** and **16**.

Biological Results and Discussion

The most potent antimineralocorticoid compound of this series is clearly the 15β,16β-methylene derivative **12**, exhibiting a 5-fold higher potency compared to the standard drug spironolactone (oral administration to adrenalectomized rats infused with aldosterone, Table I). The introduction of a 1,2 double bond to yield **16** diminished the aldosterone antagonistic potency. The 19-nor compound **13** and the 13-ethyl-19-nor derivative **14** both showed reduced antimineralocorticoid activity when compared with **12**. The 15α,16α-methylene analogue **11** also

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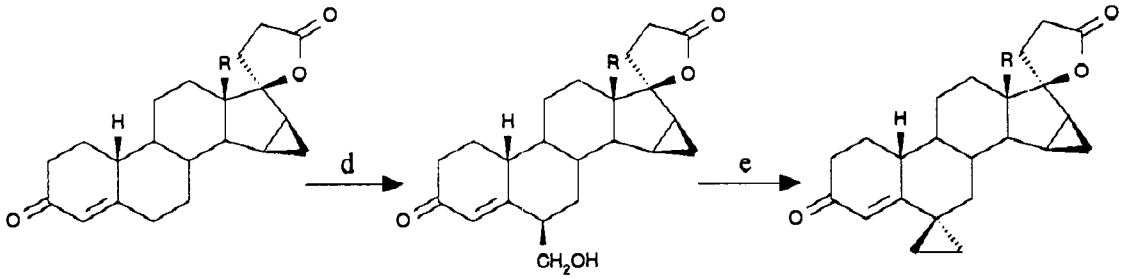
Scheme II[a]



5: R = CH_3 9: R = CH_3 13: R = CH_3

6: R = C_2H_5 10: R = C_2H_5 14: R = C_2H_5

[a] (d) 1. pyrrolidine, MeOH, reflux; 2. CH_2O, EtOH, benzene; (e) 1. *p*-TosCl, pyridine; 2. $(CH_3)_3S(O)I$, NaH, DMSO.

Table I. Biological Activities of Spirolactones

compd	antialdosterone activity maximal relative potency[a] (spironolactone = 100; 95% confidence limits)	progesterone receptor competition factor[c] (progesterone = 1)
11	<100[b]	2.1
12	507 (393–596)	1.1
13	≈100[b]	0.5
14	<100[b]	
15	≈100[b]	4.0
16	≈100[b]	2.3
1	685 (549–1095)	2.7

[a] Relative potency calculated for the hour of maximal activity after oral administration. [b] <, ≈: lower, not significantly different relative potency estimated from single-dose experiments in which the test compound was administered at a 2-fold higher dose (53.6 mg/kg) than the standard spironolactone (26.8 mg/kg). [c] The competition factor is defined as the ratio of concentration of the test compound to progesterone causing the same specific displacement of the radiolabeled standard from the receptor (measured at 50% displacement).

Table II. Progestational Activity: Endometrium Transformation Test in Rabbits after Subcutaneous and Oral Administration and Pregnancy Maintenance Test in Rats after Subcutaneous Treatment

compd	dose, mg	endometrium transformation test McPhail index sc	endometrium transformation test McPhail index po	pregnancy maintenance test % inhibition	
12	3.0			38	
	1.0		2.1	63	
	0.3	2.9	1.6	63	
	0.1	2.6	1.6	58	82
	0.03	1.3	1.5		12
	0.01				0
1	10.0			52	
	3.0		2.4	85	
	1.0	2.7	2.4	74	
	0.3	2.4	1.9	3	
	0.1	1.2	1.6	0	

exhibited decreased antimineralocorticoid activity compared to that of compound 12.

All compounds bind very strongly to the progesterone receptor exhibiting comparable (12) or even higher affinity (13) to the receptor than the naturally occuring progesterone. As it is found with 17α-ethynyl compounds, the 15β,16β-methylene-19-nor derivative 13 exhibited a stronger binding to the progesterone receptor than the 15β,16β-methylene-10-methyl derivative 12.[16] Unfortunately, the 13-ethyl-19-nor derivative 14 could not be tested in vitro because of lack of solubility.

The basic tests clearly showed that only compound 12 exhibits the desired combination of high antimineralocorticoid potency and strong binding to the progestogen receptor. Therefore the progestational activity of the 6,6-ethylene derivative 12 was evaluated in comparison to the 6β,7β-methylene derivative 1 in vivo by using two relevant animal models[10] (endometrium transformation test in estrogen-primed juvenile rabbits and pregnancy maintenance tests in rats, Table II). When tested subcutaneously in rabbits, compound 12 exhibited a progestational activity at least 3-fold higher than that of compound 1, whereas after oral treatment no difference in activity was observed. In the pregnancy maintenance test in rats (sc injection), the progestational activity of the 6,6-ethylene derivative 12 was about 10-fold higher compared to that of compound 1. There is a reasonable correlation between the results of the pregnancy maintenance test and progestational activity in man.[10] Ongoing clinical trials are intended with compound 1 to show if the combination of aldosterone antagonistic with progestational activity is sufficient to overcome typical progesterone side effects. During the course of these studies it will also become evident whether a substance such as 12, which exhibits reduced antimineralocorticoidal activity, would be more suitable for this purpose.

Methods

Antialdosterone Activity in Rats.[20,21] Adrenalectomized male rats (Wistar strain) with a body weight of 140–160 g were treated with glucocorticoids (1 mg of fluocortolone caproate/kg sc on the day of surgery and 10 mg of fluocortolone/kg sc 1 day before the diuresis experiment, which was performed 5 days after adrenalectomy). These glucocorticoid-substituted rats were infused intravenously with a saline–glucose solution (0.05% NaCl, 5% glucose) containing aldosterone (50 μg/L) at a rate of 3 mL/h for 15 h. The aldosterone antagonist was administered 1 h before the start of the aldosterone infusion. Urine excretion was measured in fractions of 1 h. Sodium and potassium concentrations in urine were determined by flame photometry. The antialdosterone activity was assessed by the ability of the compounds to reverse the aldosterone effect on the urinary Na/K ratio. The various antialdosterone derivatives and spironolactone were administered at three oral doses of 6.7, 13.4, and 26.8 mg/kg. The dose–response relationship was tested for each fraction

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Table III. 6,6-Ethylene Carbolactones

compd	prep method	yield, %	mp, °C	formula	Anal.	^{1}H NMR (100 MHz, $CDCl_3$), δ		
						18-CH_3	19-CH_3	other
11	A	21	177	$C_{25}H_{32}O_3$	C, H, O	1.20	1.24	5.61 (s, 1, H-4)
12	A	17	184	$C_{25}H_{32}O_3$	C, H, O	1.01	1.22	5.61 (s, 1, H-4)
13	B	21	214	$C_{24}H_{30}O_3$	C, H, O	1.08		5.7 (d, J = 1.5 Hz, 1, H-4)
14	B	17	165	$C_{25}H_{32}O_3$	C, H, O	0.85 (t)		5.7 (s, 1, H-4)
15	C	87		$C_{25}H_{30}O_3$	C, H, O	1.25	1.35	5.98 (d, J = 2 Hz, 1, H-4) 6.17 (dd, J = 10 + 2 Hz, 1, H-2) 7.0 (d, J = 10 Hz, 1, H-1)
16	C	67	200	$C_{25}H_{30}O_3$	C, H, O	1.07	1.33	5.98 (d, J = 2 Hz, 1, H-4) 6.17 (dd, J = 10 + 2 Hz, 1, H-2) 6.97 (d, J = 10 Hz, 1, H-1)

by regression analysis after logarithmic transformation of the doses. The potency of the standard substance, spironolactone, was allocated the value of 100.

Binding to Progesterone Receptor.[22] For the determination of the affinity of the test substance to the progesterone receptor isolated from the rabbit uterus, tritiated progesterone was used as ligand, and the competition factors are defined as the multiple of the concentration to obtain displacement equivalent to the standard. A high competition factor indicates low binding strength, and a low competition factor indicates high affinity.

Endometrium Transformation Test.[10] Castrated, infantile female rabbits (albino New Zealand) weighing 800–1000 g were administered a daily subcutaneous dose of 0.5 μg of estradiol for 6 days (priming). Thereafter, the animals were treated with varying doses of test compounds (po and sc) for 5 days. On day 12, the animals were sacrificed, and the uteri were excised for histological studies. The rate of the glandular development in endometrium was evaluated by using the McPhail scale (1–4; 1 = inactive, 4 = maximal development of the glands).

Pregnancy Maintenance Test.[10] Pregnant rats were ovarectomized on day of pregnancy. This treatment leads to interruption of pregnancy. The ability of compounds to prevent the loss of pregnancy was used as parameter of gestagenic activity. Daily treatment was performed from day 8 to 21 of pregnancy. At autopsy day 22 the rate of pregnant animals and the rate of surviving fetuses was assessed (10 fetuses/dose = 100 % maintenance). In order to obtain an optimal uterine response to gestagens a dose of 1 μg estrone/day was injected sc in combination with the tested dose of a compound.

Experimental Section

All melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. NMR spectra were taken in $CDCl_3$ on a Bruker MX 90 or a Varian HA-100 spectrometer, using tetramethylsilane as an internal standard unless otherwise stated. Ultraviolet spectra were obtained in methanol on a Cary 14 UV spectrophotometer. Infrared spectra were obtained in KBr tablets on Perkin-Elmer Model 621 and 580 B infrared spectrophotometer. Optical rotations are specific rotations taken in $CHCl_3$ (0.5%).

Method A. 6:15β,16β-Dimethylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (8). A solution of 4.1 g (0.0116 mol) of 4 in 31 mL of methanol was heated under reflux with 2.05 mL of pyrrolidine for 15 min. After cooling in an ice bath, the thus-formed precipitate was suctioned off, washed with a small amount of methanol, and dried. This material was dissolved in 90 mL of ethanol, and 45 mL of benzene and 4.5 mL of a 37% strength formalin solution was added dropwise. After a reaction period of 30 min, the reaction solution was evaporated to dryness under vacuum. The residue was chromatographed on silica gel with hexane/ethyl acetate and the oily residue was agitated for 2 h in 77 mL of dioxane with 9.1 mL of half-concentrated hydrochloric acid. The reaction solution was then combined with an excess of sodium bicarbonate and filtered off from the inorganic salts. The filtrate was diluted with ether and washed with water. After drying and evaporation, the residue was chromatographed on silica gel. Recrystallization from diisopropyl ether/acetone yielded 1.2 g (28%) of 8: mp 173 °C; UV ϵ_{261} = 11 300; IR 1770, 1670, 1620, 1590 cm^{-1}; NMR 1.0 (s, 3, C-18), 1.15 (s, 3, C-19), 5.04 (s (br), 1) and 5.12 (s (br), 1) =CH_2, 5.92 (s, 1, 4-H) ppm. Anal. ($C_{24}H_{30}O_3$) C, H, O.

6:15α,16α-Dimethylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (7). Under the above-described conditions, 5.9 g (0.0166 mol) of 3 was transformed to 2.26 g (37%) of 7: mp 164 °C; UV ϵ_{262} = 11 300; IR 1765, 1675, 1635, 1600 cm^{-1}; NMR 1.15 (s, 3, C-18), 1.23 (s, 3, C-19), 5.02 (s (br), 1) and 5.13 (s (br), 1) =CH_2, 5.94 (s, 1, 4-H) ppm. Anal. ($C_{24}H_{30}O_3$) C, H, O.

6,6-Ethylene-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (12). At room temperature, 1.41 g (0.0064 mol) of trimethylsulfoxonium iodide was agitated for 1.5 h with 239 mg (0.0055 mol) of sodium hydride (55% oil suspension) in 21.5 mL of DMSO. Under argon, 1.14 g (0.0031 mol) of 8 was added to the solution and was stirred for 50 min at room temperature. The reaction mixture was then poured into ice water and was weakly acidified with 2 N sulfuric acid. The thus-formed precipitate was filtered off and taken up in methylene chloride. After drying and evaporation, the residue was chromatographed on silica gel. Recrystallization from diisopropyl ether/acetone yielded 725 mg (61%) of 12: mp 184 °C; UV ϵ_{248} = 14 200.

6,6-Ethylene-15α,16α-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (11). Under the described conditions 1.87 g (0.0051 mol) of 7 was transformed to 1.1 g (57%) of 11: mp 177 °C; UV ϵ_{247} = 13 900.

Method B. 6β-(Hydroxymethyl)-15β,16β-methylene-3-oxo-19-nor-17α-pregn-4-ene-21,17-carbolactone (9). A solution of 4.0 g (0.0117 mol) of 5 in 35 mL of methanol was combined with 2 mL of pyrrolidine and heated for 20 min under reflux. After cooling, the thus-formed precipitate was suctioned off and washed with a small amount of cold methanol and dried. This material was dissolved in 43.6 mL of benzene and 87.2 mL of ethanol, and 4.3 mL of a 37% strength formalin solution was added dropwise. The mixture was stirred for 1 h at room temperature and concentrated under vacuum. The resultant crude mixture was chromatographed on silica gel, thus obtaining after recrystallization from diisopropyl ether 1.9 g (44%) of 9: mp 247 °C; IR 3410, 1765, 1670, 1650, 1615 cm^{-1}; NMR 1.07 (s, 3, C-18), 3.75 (m, 2, CH_2OH), 5.94 (s, 1, H-4) ppm. Anal. ($C_{23}H_{30}O_4$) C, H, O.

6β-(Hydroxymethyl)-18-methyl-15β,16β-methylene-19-nor-17α-pregn-4-ene-21,17-carbolactone (10). Under the above described conditions, 5.0 g (0.0141 mol) of 6 was transformed to 2.33 g (43%) of 10: IR 3450, 1770, 1670, 1610 cm^{-1}; NMR 0.86 (t, J = 7 Hz, 3, CH_2CH_3), 2.71 (dt, J = 5 + 7 Hz, 1, H-6), 3.76 (m, 2, CH_2OH), 5.95 (d, J = 2 Hz, 1, H-4) ppm. Anal. ($C_{24}H_{32}O_4$) C, H, O.

6,6-Ethylene-15β,16β-methylene-3-oxo-19-nor-17α-pregn-4-ene-21,17-carbolactone (13). A solution of 2.06 g (0.0056 mol) of 9 in 20 mL of pyridine was combined with 3.14 g (0.0165 mol) of *p*-toluenesulfonyl chloride. The mixture was stirred for 3 h at room temperature, combined with 0.2 mL of water, and agitated for another hour, and the reaction mixture was precipitated into ice water. The resultant precipitate was filtered off, washed with

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water, and dried. A solution of 3.84 g (0.0174 mol) of trimethylsulfoxonium iodide in 75 mL of DMSO was combined with 566 mg (0.013 mol) of sodium hydride (55% oil suspension) and agitated for 1 h at room temperature. To this solution was added the dried crude tosylation product dissolved in 2 mL of DMSO dropwise. The reaction mixture was stirred for 30 min and precipitated into ice water. The resultant crude product was chromatographed on silica gel, thus producing 985 mg (48%) of 13: mp 214 °C; UV ϵ_{247} = 13500.

6,6-Ethylene-18-methyl-15β,16β-methylene-3-oxo-19-nor-17α-pregn-4-ene-21,17-carbolactone (14). Under the above described conditions 372 mg (0.001 mol) of 10 was transformed to 145 mg (39%) of 14: mp 165 °C; UV ϵ_{247} = 14700.

Method C. 6,6-Ethylene-15β,16β-methylene-3-oxo-17α-pregna-1,4-diene-21,17-carbolactone (16). A solution of 670 mg (0.0018 mol) of 12 and 670 mg (0.003 mol) of DDQ in 13.6 mL of toluene was stirred for 5 h at 100 °C. The reaction solution was then diluted with ether, washed with water sodium bicarbonate solution and water, dried, and evaporated. The residue was then chromatographed on silica gel. After recrystallization from diisopropyl ether/acetone, 445 mg (67%) of 16 was obtained: mp 200 °C; UV ϵ_{243} = 15200.

6,6-Ethylene-15α,16α-methylene-3-oxo-17α-pregna-1,4-diene-21,17-carbolactone (15). Under the above-described conditions, 600 mg (0.0016 mol) of 11 was transformed to 520 mg (87%) of 15 as an oil: UV ϵ_{242} = 14600.

Registry No. 3, 67372-62-7; 4, 67372-68-3; 5, 101765-54-2; 6, 101765-39-3; 7, 101834-16-6; 8, 84529-99-7; 9, 133753-23-8; 10, 133753-24-9; 11, 101834-17-7; 12, 101765-35-9; 13, 101765-58-6; 14, 133753-25-0; 15, 101834-18-8; 16, 101765-36-0; formalin, 50-00-0; aldosterone, 52-39-1.

Synthesis and Evaluation of Antiinflammatory Activities of a Series of Corticosteroid 17α-Esters Containing a Functional Group

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A series of 21-desoxy-21-chlorocorticosteroids that contain a functionalized ester group at 17α has been prepared and examined to separate their systemic activity from topical antiinflammatory activity. Introduction of the functionalized ester group at 17α was carried out by an acid-catalyzed formation of cyclic ortho esters with 17α,21-hydroxyl groups of corticosteroids and subsequent acid-catalyzed hydrolysis. As for the functional group, chloro, methoxy, acetoxy, cyano, cyclopropyl, or alkoxycarbonyl group was introduced at the terminal carbon atom of the 17α-alkanoate group. The topical antiinflammatory activity and systemic activity of these compounds were examined and found to be significantly dependent on the functionalities in the 17α-esters. Among these derivatives, a series of 17α-(alkoxycarbonyl)alkanoates (17α-OCO$(CH_2)_n$COOR) showed an excellent separation of the systemic activity from topical activity. The effects of the number of methylene groups (n) and of the alkyl groups of the ester (R) on either topical or systemic activity of the corticosteroid derivatives were also investigated.

Introduction

Since hydrocortisone was introduced into dermatological use,[1] many structural modifications of the hydrocortisone molecule have been made with the aim at enhancing antiinflammatory potency. Important structural modifications include halogenation,[2] methylation,[3] and introduction of a double bond at C-1 position of the steroidal skeletons.[4]

Also, it is confirmed that esterification of the 17α- and/or 21-hydroxyl groups[5] and replacement of the 21-hydroxyl group with a halogen atom[6] enhance the topical antiinflammatory activity due to improved penetration of the skin and to increased affinity for the glucocorticoid receptor.[7]

As a result of these modifications, 21-chloro-9α-fluoro-11β,17α-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17α-propionate (clobetasol propionate, CP) is found to have the strongest topical antiinflammatory activity in clinical use.[6] On the other hand, CP also shows severe adverse effects such as thymolysis and dermal atrophy.[8]

In the field of glucocorticoids, little success has been achieved in completely separating undesired side effects from topical antiinflammatory activity except for 21-carboxylate[9] and 17β-carboxylate[10] derivatives. However, the antiinflammatory activity of neither derivatives is as strong as that of CP. Teutsch et al.[11] and Kertesz and

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