Aldosterone Antagonists. 1. Synthesis and Biological Activities of 11β,18-Epoxypregnane Derivatives

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Several steroid derivatives having the 11β,18-epoxypregnane skeleton, **7**, **8**, **19**, **20**, **21**, and **31**, were synthesized to evaluate their antialdosterone activity. Among them, 3-(9α-fluoro-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone (**31**) possessed fairly strong binding affinity for the cytoplasmic mineralocorticoid receptor of rat kidney and exhibited good aldosterone antagonist activity in an in vivo assay. However, its agonistic nature cannot be ignored. The properties of **31** as an aldosterone antagonist were enhanced by its very low to negligible binding affinity for the androgen, progestin, estrogen, and glucocorticoid receptors.

Aldosterone is a very potent mineralocorticoid[1] which regulates the electrolyte balance of body fluids by promoting excretion of potassium and retention of sodium ions.[2] Antimineralocorticoid therapy has been considered to be effective for treating edematous diseases and essential hypertension, as inappropriate overproduction of this hormone is observed with these conditions.[3]

The clinical usefulness of spironolactone[4] and potassium canrenoate,[5] the well-established aldosterone antagonists, is often limited because of adverse side effects attributed to their antiandrogenic and progestational properties.[6] Therefore, increasing interest is being focused on the search for new aldosterone antagonists which will not produce such hormonal side effects.

18-Deoxyaldosterone (21-hydroxy-11β,18-epoxypregn-4-ene-3,20-dione, **7**), an analogue of aldosterone in which the aldehyde hemiacetal structure is replaced by a stable 11β,18-epoxy ring, was first synthesized by Schmidlin and Wettstein in 1961[7] and then by Kondo et al. in 1965[8] as an intermediate in the microbiological synthesis of aldosterone from corticosterone.

This compound had been shown by Ulick et al. to possess high binding affinity for the cytoplasmic mineralocorticoid receptor of rat kidney (about one-third of the binding affinity of aldosterone) and very low affinity[9] for the androgen receptor of rat prostate. They also showed that it exhibited an approximate 2:1 antagonist to agonist ratio in both toad bladder and adrenalectomized rat bioassay systems.[9]

Bioassay of 18-deoxyaldosterone in our own systems, both in vitro and in vivo, gave almost the same results. These interesting findings prompted us to carry out structural modification of 18-deoxyaldosterone by 9α-fluorination to increase its activity and by replacing the 17β-hydroxyacetyl side chain with 17β-hydroxy-17-propionic acid γ-lactone to improve its antagonistic nature.

Chemistry. 18-Deoxy-9α-fluoroaldosterone (**8**) was synthesized by a method similar to that employed for the synthesis of 18-deoxyaldosterone (**7**).[8] 9α-Fluorocorticosterone (**2**)[10] was oxidized with the resting mycelium of *Corynespora cassiicola* (IMI 56007) to afford 9α-fluoro-18-hydroxycorticosterone 18,20-acetal (**4**) or its dimer **6** along with several byproducts. The crude oxidation products were treated with hot aqueous acetic acid and subsequently separated by column silica gel chromatography to furnish about a 17% yield of 9α-fluoro-18-deoxyaldosterone (**8**) and a 3% yield of its 21-acetate (Scheme I).

3-(17β-Hydroxy-3-oxo-11β,18-epoxyandrost-4-en-17α-yl)propionic acid γ-lactone derivatives were synthesized according to the procedure described in Scheme II. 11β,18-Dihydroxy-3-oxoandrost-4-ene-17β-carboxylic acid 18,20-lactone (**9**), obtained by sodium bismutate oxidation of the microbiological oxidation products of corticosterone, **3**, or **5**,[8] was treated with methanol and hydrochloric acid to yield 17β-carbomethoxy-11β,18-epoxyandrost-4-en-3-one (**10**). The corresponding 9α-fluoro derivative **22** was prepared by α-ketol cleavage of 9α-fluoro-18-deoxyaldosterone (**8**) with periodic acid and subsequent esterification with diazomethane. Ketalization of **10** and **22** with ethylene glycol gave **11** and **23**, respectively, which were reduced with diisobutylaluminum hydride in toluene at −78 °C to give the corresponding aldehydes **12** and **24**. The accompanying overreduction byproducts **13** and **25** could be transformed into **12** and **24**, respectively, by Collins oxidation. These aldehydes were first converted into the corresponding morpholine enamines which were then subjected to sodium perchromate oxidation to afford the corresponding 17-keto derivatives **14** and **26**, respectively. 17α-Ethynyl-17β-hydroxy derivatives **15** and **27** derived from **14** and **17**, respectively, by ethynylation with lithium acetylide in tetrahydrofuran–ether, was treated with *n*-butyllithium in tetrahydrofuran followed by carboxylation with gaseous carbon dioxide to yield **16** and **28**, respectively. Stepwise hydrogenation of the triple bond in **16** and **28** was effected first with deactivated Lindlar catalyst in dioxane and then with palladium-on-carbon catalyst in dioxane–ethanol to yield upon hydrolysis of the ketal function 3-(17β-hydroxy-3-oxo-11β,18-epoxyandrost-4-en-17α-yl)propionic acid γ-lactone (**19**) and its 9α-fluoro derivative **31**, respectively. Chloranil oxidation of **31** or DDQ oxidation of the ethyl dienol ether derivative of **31** failed, probably because of the effect of the 9α-fluoro substituent. However, chloranil oxidation of **19** smoothly gave the corresponding 3-oxo 4,6-diene derivative **20**, which was

(1) Gomez-Sanchez, C.; Holland, O. B.; Higgins, J. R.; Mathieu, R.; Gruber, G. M.; Milewich, L.; Kaplan, N. M. *J. Lab. Clin.* **1976**, *88*, 571.
(2) Luetscher, J. A., Jr.; Johnson, B. B. *J. Clin. Invest.* **1954**, *23*, 1441.
(3) Ochs, H.; Greenblatt, D. J.; Bodam, G.; Smith, T. W. *Am. Heart J.* **1978**, *96*, 389.
(4) (a) Cella, J. A.; Kagawa, C. M. *J. Am. Chem. Soc.* **1957**, *79*, 4808. (b) Kagawa, C. M. *Endocrinology* **1960**, *67*, 125.
(5) Kagawa, C. M.; Bouska, D. J.; Anderson, M. L.; Krol, W. F. *Arch. Int. Pharmacodyn. Ther.* **1964**, *149*, 18.
(6) Loriaux, D. L. *Ann. Int. Med.* **1976**, *85*, 630.
(7) Schmidlin, J.; Wettstein, A. *Helv. Chim. Acta* **1961**, *44*, 1596.
(8) Kondo, E.; Mitsugi, T.; Tori, K. *J. Am. Chem. Soc.* **1965**, *87*, 4655.
(9) Ulick, S.; Marver, D.; Adam, W.; Funder, J. W. *Endocrinology* **1979**, *104*, 1352.
(10) Fried, J.; Herz, J. E.; Sabo, E. F.; Borman, A.; Singer, F. M.; Numerlf, P. *J. Am. Chem. Soc.* **1955**, *77*, 1068.

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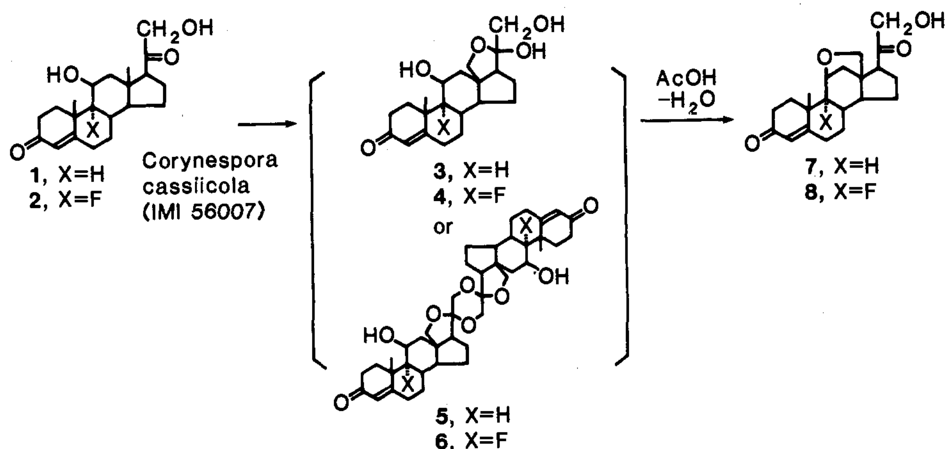
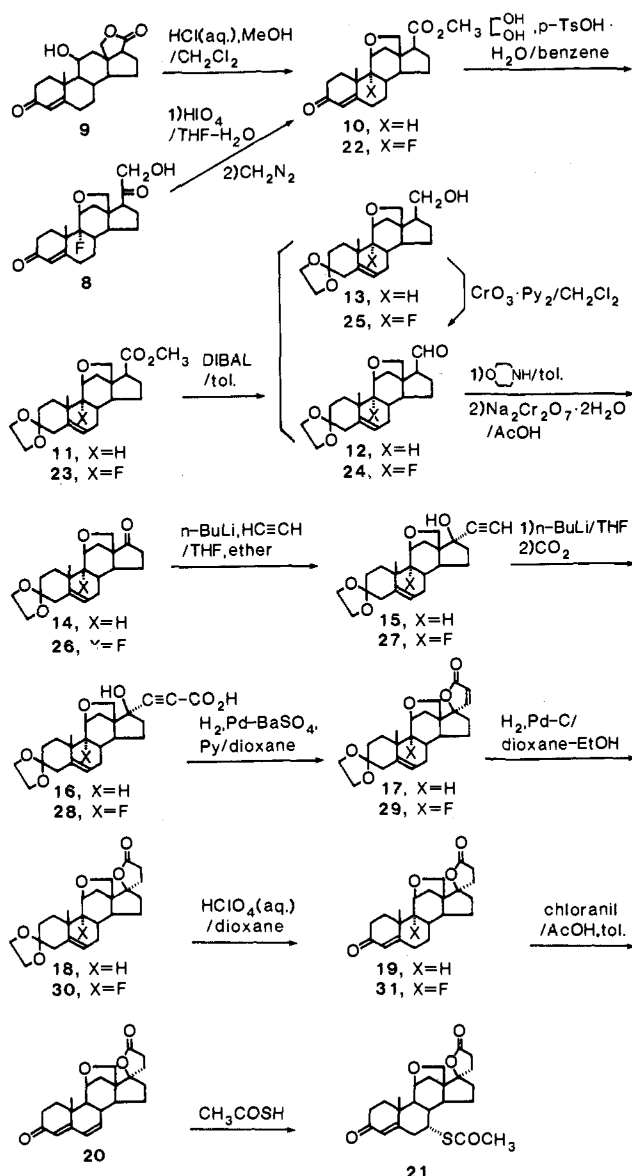
Scheme I



Scheme II



further converted into the corresponding 7α-acetylthio derivative **21**.

Receptor Binding Studies

Method. [^{3}H]Aldosterone (^{3}H-Ald, 44 Ci/mmol etc.), [^{3}H]dexamethasone (^{3}H-DM, 42 Ci/mmol), and [^{3}H]estradiol (^{3}H-E2, 60 Ci/mmol) were obtained from Amersham International. [^{3}H]Dihydrotestosterone (^{3}H-DHT, 50.6 Ci/mmol etc.), [^{3}H]methyltrienolone (^{3}H-MT 87 Ci/mmol), and [^{3}H]promegestone (^{3}H-R5020, 87 Ci/mmol etc.) were purchased from New England Nuclear (NEN). ^{3}H-DM (47.5 Ci/mmol) from NEN was also used. Unlabeled steroids and spironolactone (SP) were obtained from Sigma. Phosphate buffer (P-buffer, pH 7.5, 0.02 M KH_2PO_4–Na_2HPO_4 containing 0.5 mM dithiothreitol and 0.25 M sucrose) was used throughout. Steroids were first dissolved in ethanol then diluted with P′-buffer (P-buffer without sucrose) to the required concentrations. In some cases, ethylene glycol was added to the P′-buffer to dissolve the steroids. The final concentrations of ethanol and ethylene glycol at incubation were 1 and 5 vol %, respectively. To stabilize the steroid receptors, sodium molybdate (10 mM final concentration) was added to the homogenization buffer and/or ^{3}H-ligand solutions.[11-13] The mineralocorticoid receptor (MR) assay used kidneys from male Sprague–Dawley rats (4–8 weeks old) which had been adrenalectomized bilaterally 1 or 2 days earlier, and the androgen receptor (AR) source was ventral prostates from the rats (4–5 weeks old) which had been castrated 1 day earlier. The tissues were homogenized in five volumes (10 or 20 volumes for prostates) of P-buffer and the homogenate was centrifuged for an hour (2 h for prostates) at 200000*g*. In competitive binding assays, 100 μL of cytosol thus obtained was incubated with 50 μL of various concentrations of steroids (0.4 nM–40 μM) of P′-buffer (for control) and 50 μL of about 4 nM of ^{3}H-ligand. For Scatchard plot analysis,[14] cytosols were incubated with P′-buffer and various concentrations (1.2–40 nM) of ^{3}H-ligands. Nonspecific bindings were determined in parallel incubations with 100- and 1000-fold excess of unlabeled steroids and subtracted from the total bindings. After incubation for 42–48 h at 0 °C or 100–120 min at either 25 or 26 °C for MR and for 16–20 h at 0 °C or 2 h at 15 °C for AR, bound and free steroids were separated by the Dextran-coated charcoal (DCC) method.[15,16] Standard competition curves for unlabeled Ald or DHT were prepared in the respective assays and the relative binding affinity (RBA) was determined from: RBA = 100 × ${}^{50}_{0}C/{}^{50}C$ (%), ${}^{50}_{0}C$ = concentration of the standard at 50%

(11) Nielsen, C. J.; Sando, J. J.; Vogel, W. M.; Pratt, W. B. *J. Biol. Chem.* **1977**, *252*, 7568.
(12) Grekins, R. J.; Sider, R. S. *J. Steroid Biochem.* **1980**, *13*, 835.
(13) Noma, K.; Nakao, K.; Sato, B.; Nishizawa, Y.; Matsumoto, K.; Yamamura, Y. *Endocrinology* **1980**, *107*, 1205.
(14) Scatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660.
(15) Korenman, S. G. *J. Clin. Endocrinol.* **1968**, *28*, 127.
(16) DeHertogh, R.; Van der Heyden, I.; Ekka, E. *J. Steroid Biochem.* **1975**, *6*, 1333.

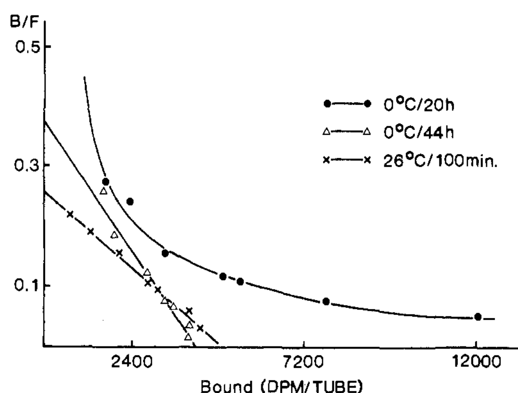


Figure 1. Schatchard plots of [^{3}H]aldosterone binding to rat renal cytosol. Renal cytosol was prepared with P-buffer. Portions of cytosol were incubated with various concentrations of [^{3}H]aldosterone containing 10 mM Na_2MoO_4 for 20 and 44 h at 0 °C or for 100 min at 26 °C. Bound and free forms were separated by the DCC method.

inhibition of ^{3}H-ligand bindings of control, and ^{50}C = concentration of the competitor at 50% inhibition of ^{3}H-ligand bindings of the control. Radioactivities were counted with Tricarb 460C or Aloka LSC-673 (counting efficiencies were 40–50%). As progestin receptor (PR), estrogen receptor (ER), and glucocorticoid receptor (GR) sources, uteri from rats primed with estradiol, uteri from immature rats, and livers from adrenalectomized male rats were used, respectively. Competitive binding assays were carried out with use of ^{3}H-R5020, ^{3}H-E2, and ^{3}H-DM as ligands.

Results. Scatchard plots of ^{3}H-Ald bindings are presented in Figure 1. Association constants of ^{3}H-Ald and MR were 2.3×10^9 M^{-1} (0 °C, 44 h) and 1.0×10^9 M^{-1} (26 °C, 100 min). Shorter incubation, for example, 20 h at 0 °C or 30 min at 26 °C, gave a hyperbolic curve (Figure 1) which could be analyzed into two binding components by the method of Feldman (data not shown).[17] Funder et al.[18] reported that kidney cytoplasm contained type I and type II binding components for ^{3}H-Ald and postulated that type I binding component, which had the higher affinity, is the true MR and type II is the GR. We used low concentration of ^{3}H-Ald (about 1 nM) in which the contribution of GR for Ald binding is very small. We also found that longer incubation time (about 2 days at 0 °C or 100–120 min at either 25 or 26 °C) gave a straight line which had a higher association constant as described above. Funder et al.[19] reported a value similar to ours for the ^{3}H-Ald-MR complex at 25 °C. Under our incubation conditions, GR was more labile than MR (to be published elsewhere). These incubation conditions were adequate and convenient for MR binding assay and therefore were adopted. Relative binding affinities for MR and AR are shown in Table I. Compound 7 had RBA of about one-third to one-half of that of Ald for MR. Fluorine-containing compounds 8 and 31 had relatively higher RBA when compared with their corresponding defluoro compounds 7 and 19. Both 19 and 21 had weak affinities. SP, as a reference compound, showed moderate affinity for MR at 25–26 °C, but it had a smaller RBA at 0 °C. Compounds 7, 8, and 31 had almost no affinity for AR, but SP had 4.7% and 5.6% RBA at 0 and 15 °C, respectively. Binding affinity of 31 for PR was weak, and the RBA of 31 was smaller than that of SP (1.5% for 31 and 2.8% for SP at 0 °C with progesterone = 100%). Binding affinities of 31 and SP were also weak for GR and were negligible for ER (data not shown).

Table I. Relative Binding Affinity for Mineralocorticoid and Androgen Receptors

	RBA, %			
	MR		AR	
compd	0 °C	25 °C (26 °C)	0 °C	15 °C
Ald	100	100		
DHT			100	100
7	36–63 (3)[a]	32 (1)	N (1)[b,c]	
8	320 (1)	270, 300 (2)	N (1)[c]	N (1)[c]
19	1.8, 5.5 (2)	1.1, 2.3 (2)		
21	0.1, 0.2 (2)	0.4, 0.5 (2)		
31	44–58 (4)	23–37 (4)	N (1)[c] N (4)	N (1)[c]
SP	2.3–8.0 (5)	11–24 (4)	3.3 (1)[c] 2.7–7.9 (5)	4.7 (1)[c] 6.5 (1)

[a] Values in parentheses are the numbers of measurements. [b] N: negative, RBA < 0.1%. Rat renal (or prostatic) cytosol was incubated with various concentrations of the compounds and about 1 nM ^{3}H-Ald (or ^{3}H-DHT or [^{3}H]methyltrienolone) for 42–48 h at 0 °C or for 100–120 min at 25 or 26 °C (or for 16–20 h at 0 °C or 2 h at 15 °C). Bound and free steroids were separated by the DCC method. RBA was calculated as described in the Methods section. [c] Experiments with ^{3}H-MT as the ligand. Most of AR experiments for 31 and SP were performed with ^{3}H-DHT, but in experiments for 7 and 8 and in one set of experiment for 31 and SP, ^{3}H-MT was used instead. Although Raynaud et al.[21a] pointed that DHT is metabolized and MT is not in prostatic cytosol at 0 °C, our results with ^{3}H-DHT gave almost the similar RBA values as those with ^{3}H-MT.

Table II. Aldosterone Antagonist and Agonist Activity: Mean ± SE for Urinary Na/K

		aldosterone, μg/kg	
compd	dose, μg/kg	0	5
7	0	3.5 ± 0.7 (8)[c]	0.8 ± 0.2 (8)[c]
	250	1.8 ± 0.6 (8)	1.7 ± 0.3 (8)
8	0	4.8 ± 1.5 (6)	1.0 ± 0.3 (7)
	2.5	2.8 ± 0.8 (6)	0.7 ± 0.2 (7)
	25	0.3 ± 0.1[b] (6)	0.1 ± 0.5 (5)
19	0	7.7 ± 1.2 (6)	1.1 ± 0.2 (5)
	2500	8.8 ± 1.0 (6)	2.4 ± 0.3 (6)
	0	3.8 ± 0.6 (9)	0.7 ± 0.1 (10)
	25000	3.8 ± 0.6 (10)	2.2 ± 0.1[a] (12)
21	0	7.7 ± 1.2 (6)	1.1 ± 0.2 (5)
	2500	6.4 ± 0.7 (6)	1.6 ± 0.4 (6)
31	0	5.1 ± 0.3 (6)	0.4 ± 0.1 (7)
	2500	3.5 ± 0.6[b] (6)	2.5 ± 0.4[a] (8)

[a] Statistically significant at $p < 0.05$ against aldosterone. [b] Against control. [c] Values in parentheses are the numbers of rats.

In Vivo Mineralo- and Antimineralocorticoid Studies

Method. Antimineralocorticoid activity was determined in vivo by a modification of the method of Kagawa.[4b] Male Slc:Wistar rats (7 weeks old) were bilaterally adrenalectomized 2 days before the assay and maintained on 0.45% NaCl and ordinary diet (Jcl CA-1). The next evening sugar cubes were substituted for the diet, and 6 mL of 0.45% NaCl/100 g of body weight was injected intraperitoneally. On the morning of the assay (the third day), an additional 6 mL of 0.45% NaCl/100 g of body weight was administered intraperitoneally. Aldosterone (5 μg in 1 mL of sesame oil/kg of body weight) was injected subcutaneously, and immediately thereafter the compound to be assayed was administered subcutaneously. For mineralocorticoid assay, the rats received the compound alone. Animals were then placed individually in metabolism cages, and their urine was collected for the 4-h period between 2 and 6 h

(17) Feldman, H. A. *Anal. Biochem.* 1972, *48*, 317.
(18) Funder, J. W.; Feldman, D.; Edelman, I. S. *Endocrinology* 1973, *92*, 994.
(19) Funder, J. W.; Feldman, D.; Highland, E.; Eldman, I. S. *Biochem. Pharmacol.* 1974, *23*, 1493.

Table III. Antagonist Activity of Compound 31 and Spironolactone: Mean ± SE for Urinary Na/K

dose, μg/kg			
compd	aldosterone	31	spironolactone
250	5	1.33 ± 0.22[a] (10)[b]	0.93 ± 0.23 (10)[b]
2500	5	2.08 ± 0.30[a] (9)	1.47 ± 0.20[a] (9)
25000	5	2.13 ± 0.38[a] (9)	2.83 ± 0.61[a] (10)
0	5	0.75 ± 0.14 (10)	
0	0	4.07 ± 0.62 (9)	

[a] Statistically significant at $p < 0.05$ against aldosterone. [b] Values in parentheses are the numbers of rats.

Table IV. Agonist Activity of Compound 31: Mean ± SE for Urinary Na/K

compd	dose, μg/kg	Na/K
control		6.02 ± 0.50 (8)[b]
31	250	3.42 ± 0.35[a] (7)
	2500	2.70 ± 0.19[a] (8)
	25000	1.95 ± 0.25[a] (8)
aldosterone	1	1.48 ± 0.16[a] (8)

[a] Statistically significant against control at $p < 0.01$. [b] Values in parentheses are the numbers of rats.

after steroid administration. Sodium and potassium in the urine samples were measured with an atomic absorption spectrophotometer (Toshiba-Beckman). The potency was evaluated from the urinary Na/K ratio of the sample to the control.

Results. The in vivo effects of **7**, **8**, **19**, **21**, and **31** on renal electrolyte excretion are shown in Table II. Compound **7** showed both an appreciable antialdosterone and a mineralocorticoid activity.

Compound **8** seemed to have no antialdosterone activity but a significant sodium-retaining activity. It was approximately as potent as aldosterone. In contrast, compound **19** showed no appreciable agonistic activity, but it exhibited slight antialdosterone activity at very high doses. Compound **21** seemed to have neither antialdosterone nor aldosterone activity.

Compound **31**, when compared with **19**, showed remarkably stronger antialdosterone activity (roughly 10 times), but it also has slight agonistic activity. In view of its strong antialdosterone activity, a comparative study with spironolactone was carried out (Table III), and it was found to have substantially stronger antialdosterone activity at low doses but weaker activity at high doses. Because of the absence of dose dependency on antialdosterone activity, the agonistic activity of **31** was examined (Table IV), and **31** was found to possess weak but significant activity.

Discussion

As expected, the binding affinity of 9α-fluoro-18-deoxyaldosterone (**8**) to the mineralocorticoid receptor was greater than that of 18-deoxyaldosterone (**7**). However, **8** was found to be an aldosterone agonist. Compounds 19 and 31, obtained by replacing the 17β-hydroxyacetyl function of **7** and **8**, respectively, by the spiro-γ-lactone function, showed reduced binding affinity to the mineralocorticoid receptor and reduced mineralo- and/or antimineralocorticoid activity. However, there was an increase in the antagonist to agonist ratio, as expected.

As for the derivatives of 3-(17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone,[20] the values of the relative binding affinity to the mineralocorticoid receptor decreased upon introduction of the 11β,18-epoxy ring, as in **19**, but further substitution of the 9α-fluoro function led to a recovery of its affinity to a value greater than that of the parent compound, for example, **31**. This shift tendency is also seen in a series of compounds consisting of deoxycorticosterone, corticosterone (**1**), and 9α-fluorocorticosterone (**2**).[21]

The antialdosterone activity of **31** is interesting, for it appears intrinsically to be a stronger antagonist against aldosterone than spironolactone, as evidenced by its higher potency at lower doses. However, its lower efficacy at higher doses cannot be ignored. This phenomenon may be due to its weak but significant agonistic nature. In contrast, a straight dose–response curve is obtained with spironolactone, which was found to have no agonist activity. A favorable property of **31** was its lack of or very weak binding affinity for the androgen, progestin, estrogen, and glucocorticoid receptors. Binding to these receptors can give rise to adverse reactions in the clinic.

Experimental Section

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere with use of dry solvents under anhydrous conditions and with anhydrous $MgSO_4$ used as a drying agent for extracts, and organic solvents were removed by evaporation under reduced pressure with a rotary evaporator. Medium-pressure column chromatographies on Merck "Lobar" prepacked columns packed with LiChroprep Si 60 [size A (240–10 mm, 40–69 μm), size B (310–25 mm, 40–63 μm), and size C (440–37 mm, 63–125 μm)] were carried out for separation and purification of the products. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were determined with a Hitachi Model 260-10 spectrophotometer, and NMR spectra were determined on a Varian EM-390 spectrometer or a Varian T-60A spectrometer. Analytical results indicated by elemental symbols were within 0.4% of the theoretical values.

9α-Fluoro-21-hydroxy-11β-,18-epoxypregn-4-ene-3,20-dione (8). Three-hundred 500-mL Sakaguchi flasks, each containing 100 mL of sterilized medium composed of 3.5% glucose, 2.0% polypeptone, and 0.3% corn steep liquor (pH 6.8–7.0), were inoculated with *Corynespora cassiicola* (IMI 56007). After incubation for 4 days at 28 °C on a reciprocal shaker, mycelia were harvested by filtration and washed with deionized water. The harvested wet mycelia (3 kg) were equally divided into 300 shaking flasks, each containing 100 mL of deionized water and 70 mg of 9α-fluoro-11β,20-dihydroxypregn-4-ene-3,20-dione (**2**)[10] [total amount was 21 g (57.6 mmol)], and then the flasks were shaken for 2 days at 28 °C. Whole reaction mixtures were collected and the products were isolated by AcOEt extraction. The AcOEt layer was washed with water, dried, and evaporated, giving 22.5 g of a mixture of products. The crude mixture of 9α-fluoro-11β,18,21-trihydroxypregn-4-ene-3,20-dione 18,20-acetal (**4**) and its dimer (**6**) was obtained by crystallization of the residue from acetone–AcOEt. The crystals were suspended in a mixture of 700 mL of AcOH and 300 mL of water, and the mixture was stirred under reflux for 1.5 h. The solvents were evaporated, and acetone was added to isolate the desired product. The acetone-soluble materials were separated by column silica gel chromatography using an AcOEt–benzene (1:2) mixture as an eluent. From the less polar fraction, 647 mg (2.8%) of 21-acetoxy-9α-fluoro-11β,18-epoxypregn-4-ene-3,20-dione, which was recrystallized from CH_2Cl_2–ether, mp 173–175 °C, was obtained. From the polar fraction, 3.52 g (17.0%) of **8** was obtained, and a portion was recrystallized from CH_2Cl_2–ether: mp 180–181 °C; IR ($CHCl_3$) 1618, 1655 (sh), 1665, 1710, 3490 cm^{-1}; NMR ($CDCl_3$) δ 1.35 (3 H, s), 3.03 (1 H, d, J = 8 Hz), 3.35 (1 H, d, J = 8 Hz), 3.38 (1 H, d, J = 8 Hz), 3.73 (1 H, d, J = 8 Hz), 4.23 (2 H, s), 4.45 (1 H, m),

(20) Chinn, L. J.; Salamon, K. W.; Desai, B. N. *J. Med. Chem.* **1981**, *24*, 1103. Binding affinity for MR of 3-(17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone was described in this paper.

(21) (a) Raynaud, J. P.; Ojasoo, T.; Burton, M. M.; Philibert, D. "Drug Design"; Academic Press: New York, 1979; Vol. VIII, p 169 and references therein. (b) The values of RBA to MR of these compounds were determined independently by our assay method; unpublished results.

5.78 (1 H, br s). Anal. ($C_{21}H_{27}O_4F$) C, H.

3,3-(Ethylenedioxy)-17β-carbomethoxy-11β,18-epoxyandrost-5-ene (11). Four drops of concentrated H_2SO_4 was added to a solution of 4.0 g (12.1 mmol) of 11β,18-dihydroxy-3-oxoandrost-4-ene-17β-carboxylic acid 18,20-lactone (9)[8] in 200 mL of MeOH and 40 mL of CH_2Cl_2. The mixture was stirred under reflux in a flask equipped with a Dean–Stark water separator containing 4A molecular sieve for 15 h. After the mixture cooled, the product was isolated by AcOEt extraction. The AcOEt layer was washed with water, dried, and evaporated. Part of the residue was purified by recrystallization from CH_2Cl_2–ether to obtain analytically pure 17β-carbomethoxy-11β,18-epoxyandrost-4-en-3-one (10): mp 193–194 °C; IR ($CHCl_3$) 1608, 1660, 1722 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (3 H, s), 3.30 (1 H, d, J = 8 Hz), 3.43 (1 H, d, J = 8 Hz), 4.43 (1 H, d, J = 6 Hz), 5.73 (1 H, br s). Anal. ($C_{21}H_{28}O_4$) C, H. The residual crude reaction product (10), 2 g of ethylene glycol (32.2 mmol), and 60 mg (0.3 mmol) of *p*-toluenesulfonic acid monohydrate were dissolved in 30 mL of benzene. The solution was stirred under reflux for 4 h in a flask equipped with a Dean–Stark water separator containing 4A molecular sieve. After the mixture cooled, the product was isolated by benzene extraction. The benzene layer was washed with aqueous 2 N Na_2CO_3 and water then dried, and evaporated. Recrystallization of the residue from CH_2Cl_2–MeOH yielded 1.64 g (95.3%) of 11: mp 171–175 °C; IR ($CHCl_3$) 1725 cm^{-1}; NMR ($CDCl_3$) δ 1.12 (3 H, s), 3.25 (1 H, d, J = 8 Hz), 3.72 (1 H, d, J = 8 Hz), 3.68 (3 H, s), 3.79 (4 H, s), 4.42 (1 H, d, J = 6 Hz), 5.38 (1 H, m). Anal. ($C_{23}H_{32}O_5$) C, H.

3,3-(Ethylenedioxy)-17β-formyl-11β,18-epoxyandrost-4-ene (12). A solution of 2.87 g (7.38 mmol) of 11 in 180 mL of toluene was cooled to −78 °C, and 5.05 mL (7.38 mmol) of a 1.46 M solution of diisobutylaluminum hydride in toluene was added dropwise. The mixture was stirred at −78 °C for 45 min. Saturated aqueous NH_4Cl was added to the reaction mixture, and the product was isolated by AcOEt extraction. The AcOEt layer was washed with aqueous NH_4SO_4 and water, dried, and evaporated. The products were separated by column silica gel chromatography using AcOEt–benzene (1:4–1:1) as an eluent. From the fraction eluted with AcOEt–benzene (1:4), 1.53 g (53.3%) of 12 was obtained. An analytical sample was obtained by recrystallization from CH_2Cl_2–ether: mp 142–144 °C; IR ($CHCl_3$) 1720, 2720, 2830 cm^{-1}; NMR ($CDCl_3$) δ 1.13 (3 H, s), 3.48 (1 H, d, J = 8 Hz), 3.75 (1 H, d, J = 8 Hz), 3.95 (1 H, s), 4.43 (1 H, d, J = 6 Hz), 5.35 (1 H, m), 9.73 (1 H, d, J = 1 Hz). Anal. ($C_{22}H_{30}O_4$) C, H. From the fraction eluted with AcOEt–benzene (1:1), 0.22 g (8.1%) of 3-(ethylenedioxy)-17β-(hydroxymethyl)-11β,18-epoxyandrost-4-ene (13) was obtained. An analytically pure sample was obtained by recrystallization from CH_2Cl_2–MeOH: mp 242–243 °C; NMR ($CDCl_3$) δ 1.13 (3 H, s), 3.60 (4 H, br s), 3.95 (4 H, s), 4.40 (1 H, d, J = 6 Hz), 5.37 (1 H, m). Anal. ($C_{22}H_{32}O_4$) C, H.

3,3-(Ethylenedioxy)-11β,18-epoxyandrost-5-en-17-one (14). A solution of 2.11 g (5.87 mmol) of 12 and 0.56 mL (6.5 mmol) of morpholine in 42 mL of toluene was stirred under reflux for 3.5 h. After cooling, the solvent and excess morpholine were evaporated under reduced pressure. To a cooled solution of the residue in 30 mL of benzene at 0 °C was added a cooled solution of 3.50 g (11.74 mmol) of $Na_2Cr_2O_7{\cdot}2H_2O$ in 17.5 mL of anhydrous AcOH and, the mixture was stirred at 0 °C for 2 h. Next, 5.8 mL of MeOH was added, and the mixture was stirred for another 0.5 h. The product was isolated by AcOEt extraction. The AcOEt layer was washed with 7% aqueous NaOH and saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:2) as an eluent. Recrystallization from ether gave 1.55 g (77.0%) of 14: mp 222–224 °C; IR ($CHCl_3$) 1730 cm^{-1}; NMR ($CDCl_3$) δ 1.14 (3 H, s), 3.40 (1 H, d, J = 8 Hz), 4.01 (1 H, d, J = 8 Hz), 3.93 (4 H, s), 4.58 (1 H, d, J = 6 Hz), 5.37 (1 H, m). Anal. ($C_{21}H_{28}O_4$) C, H.

3,3-(Ethylenedioxy)-17α-ethynyl-17β-hydroxy-11β,18-epoxyandrost-5-ene (15). A mixture of 20 mL of THF and 10 mL of ether was saturated with acetylene at −40 °C. To the solution was added 3.53 mL (5.3 mmol) of a solution of 1.5 M *n*-butyllithium in *n*-hexane in 10 min and then a solution of 183 mg (0.53 mmol) of 14 in 8 mL of THF was added dropwise. The mixture was stirred at −40 °C for 15 min and at 0 °C for 30 min, and then saturated aqueous NH_4Cl was added. The product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:2) as an eluent, and 158 mg (81%) of 15 was obtained on recrystallization from CH_2Cl_2–ether: mp 220–224 °C; IR ($CHCl_3$) 3590, 3295 cm^{-1}; NMR ($CDCl_3$) δ 1.14 (3 H, s), 2.53 (1 H, s), 3.68 (1 H, d, J = 8 Hz), 3.88 (1 H, d, J = 8 Hz), 3.92 (4 H, s), 4.45 (1 H, d, J = 6 Hz), 5.32 (1 H, m). Anal. ($C_{23}H_{30}O_4$) C, H.

3-[3,3-(Ethylenedioxy)-17β-hydroxy-11β,18-epoxyandrost-5-en-17α-yl]propiolic Acid (16). A solution of 200 mg (0.54 mmol) of 15 in 5 mL of THF was cooled to −78 °C and a solution of 1.5 M *n*-butyllithium in *n*-hexane was added dropwise. After the reaction had proceeded for 30 min at −78 °C, dry ice was added at once, the temperature of the reaction mixture was allowed to rise to room temperature, and saturated aqueous NH_4Cl was added. The product was isolated by AcOEt extraction. The AcOEt layer was washed with water, dried, and evaporated. Recrystallization of the residue from AcOEt gave 164 mg (72%) of 16: mp 188–191 °C; IR (Nujol) 1719, 2215, 2500–3500 cm^{-1}; NMR ($CDCl_3$) δ 1.13 (3 H, s), 3.71 (1 H, d, J = 8 Hz), 3.90 (1 H, d, J = 8 Hz), 3.96 (4 H, s), 4.50 (1 H, d, J = 6 Hz), 5.33 (1 H, m). Anal. ($C_{24}H_{30}O_6$) C, H.

3-[3,3-(Ethylenedioxy)-17β-hydroxy-11β,18-epoxyandrost-5-en-17α-yl]propenoic Acid γ-Lactone (17). A solution of 160 mg (0.39 mmol) of 16 and 0.16 mL of pyridine in 1.6 mL of dioxane containing 50 mg of 5% Pd–$BaSO_4$ was hydrogenated under hydrogen atmosphere for 1 h. The catalyst was separated by filtration and washed with dioxane. The solvent was evaporated, the residue was purified by column silica gel chromatography using AcOEt–benzene (2:1) as an eluent, and 91 mg (59.1%) of 17 was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 288–291 °C; IR ($CHCl_3$) 1760 cm^{-1}; NMR ($CDCl_3$) δ 1.13 (3 H, s), 3.93 (6 H, s) 4.45 (1 H, d, J = 6 Hz), 5.33 (1 H, m), 5.98 (1 H d, J = 5 Hz), 7.48 (1 H, d, J = 5 Hz). Anal. ($C_{24}H_{30}O_5$) C, H.

3-(17β-Hydroxy-11β,18-epoxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone (19). A solution of 300 mg (0.75 mmol) of 17 containing 60 mg of 5% Pd–C in a mixture of 20 mL of dioxane and 4 mL of EtOH was hydrogenated under hydrogen atmosphere for 20 min. The catalyst was separated by filtration and washed with dioxane. The filtrate was evaporated, and recrystallization of part of the residue from CH_2Cl_2–ether gave 3-[3-(ethylenedioxy)-17β-hydroxy-11β,18-epoxyandrost-4-en-17α-yl]propionic acid γ-lactone (18): mp 285–286 °C; IR ($CHCl_3$) 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.13 (3 H, s), 3.79 (2 H, s), 3.93 (4 H, s), 4.47 (1 H, d, J = 6 Hz), 5.33 (1 H, m). Anal. ($C_{24}H_{32}O_5$) C, H. The crude reaction product 18 was dissolved in a mixture of 1.12 mL of 60% aqueous $HClO_4$, 35 mL of dioxane, and 12.5 mL of water and the mixture was allowed to react at room temperature for 15 h. The product was isolated by AcOEt extraction. The AcOEt layer was washed with water, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent, and 233 mg (73.3%) of 19 was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 186–187 °C; IR ($CHCl_3$) 1605, 1660, 1762 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (3 H, s), 3.83 (2 H, s), 4.47 (1 H, d, J = 6 Hz), 5.70 (1 H, br s). Anal. ($C_{22}H_{28}O_4$) C, H.

3-(17β-Hydroxy-11β,18-epoxy-3-oxoandrosta-4,6-dien-17α-yl)propionic Acid γ-Lactone (20). A solution of 310 mg (0.87 mmol) of 19 and 227 mg (0.91 mmol) of chloranil in a mixture of 8 mL of acetic acid and 2 mL of toluene was stirred under reflux for 1.5 h. After cooling, the product was isolated by $CHCl_3$ extraction. The $CHCl_3$ layer was washed with 10% aqueous NaOH and saturated aqueous NaCl, dried, and evaporated. Recrystallization of the residue from CH_2Cl_2–ether gave 204 mg (66%) of 20: mp 205–208 °C; IR ($CHCl_3$) 1610, 1650 (sh), 1660, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 3.88 (2 H, s), 4.52 (1 H, d, J = 6 Hz), 5.63 (1 H, s), 6.11 (2 H, s). Anal. ($C_{22}H_{26}O_4$) C, H.

3-[7α-(Acetylthio)-17β-hydroxy-11β,18-epoxy-3-oxoandrost-4-en-17α-yl]propionic Acid γ-Lactone (21). A solution of 250 mg (0.71 mmol) of 19 in 1 mL (14 mmol) of thioacetic *S*-acid was stirred under reflux for 1 h. Excess thioacetic *S*-acid was evaporated under reduced pressure, and the product was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent. One hundred and eighty milligrams (59%) of 21 was obtained. A portion of the product was recrystallized from ether: mp 259–262 °C; IR ($CHCl_3$) 1615, 1675, 1693, 1775 cm^{-1};

NMR ($CDCl_3$) δ 1.32 (3 H, s), 2.33 (3 H, s), 3.85 (2 H, s), 4.10 (1 H, m), 4.45 (1 H, d, *J* = 6 Hz), 5.68 (1 H, br s). Anal. ($C_{24}H_{30}O_5S$) C, H.

9α-Fluoro-17β-carbomethoxy-11β,18-epoxyandrost-4-en-3-one (22). To a solution of 1.1 g (3 mmol) of **8** in 12 mL of THF was added a solution of 1.4 g (6.2 mmol) of $HIO_4 \cdot 2H_2O$ in 3 mL of water, and the mixture was stirred at room temperature for 15 h. The product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and filtered. To esterify the acid product in the filtrate, a solution of CH_2N_2 in ether was added, and then the solvents were evaporated. Recrystallization of the residue from CH_2Cl_2–ether gave 996 mg (90.8%) of **22**: mp 194–196 °C; IR ($CHCl_3$) 1627, 1675, 1735 cm^{-1}; NMR ($CDCl_3$) δ 1.38 (3 H, s), 3.71 (3 H, s), 3.32 (1 H, dd, *J* = 8 and 2 Hz), 3.75 (1 H, d, *J* = 8 Hz), 4.43 (1 H, m), 5.80 (1 H, br s). Anal. ($C_{21}H_{27}O_4F$) C, H.

3,3-(Ethylenedioxy)-9α-fluoro-17β-carbomethoxy-11β,18-epoxyandrost-5-ene (23). A 965-mg (2.66 mmol) sample of **22** was ketalized by the same procedure as described for the preparation of 11. The product was purified by column silica gel chromatography using AcOEt–benzene (1:1) as an eluent, and 958 mg (88.5%) of **23** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 151–153 °C; IR ($CHCl_3$) 1722 cm^{-1}; NMR ($CDCl_3$) δ 1.22 (3 H, s), 3.67 (3 H, s), 3.92 (4 H, s), 4.43 (1 H, m), 5.37 (1 H, m). Anal. ($C_{23}H_{31}OF$) C, H.

3,3-(Ethylenedioxy)-9α-fluoro-17β-formyl-11β,18-epoxyandrost-5-ene (24). A 951-mg (2.34 mmol) sample of **22** was reduced with diisobutylaluminum hydride by the same procedure as described for the preparation of **12**. The products were separated by column silica gel chromatography using AcOEt–benzene (1:4–1:1) as an eluent. From the fraction eluted with AcOEt–benzene (1:4), 619 mg (70.3%) of **24** was obtained. An analytical sample was obtained by recrystallization from CH_2Cl_2–ether: mp 155–159 °C; IR ($CHCl_3$) 1718, 2700, 2825, cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 3.48 (1 H, dd, *J* = 8 and 2 Hz), 3.72 (1 H, d, *J* = 8 Hz), 3.93 (4 H, s), 4.48 (1 H, m), 5.38 (1 H, m), 9.78 (1 H, d, *J* = 2 Hz). Anal. ($C_{22}H_{29}O_4F$) C, H. From the fraction eluted with AcOEt–benzene (1:1), 229 mg of **25** (25.9%) was obtained. An analytical sample was obtained by recrystallization from CH_2Cl_2–MeOH: mp 223–226 °C; IR ($CHCl_3$) 3610, 3300–3600 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 3.60 (4 H, br s), 3.92 (4 H, s), 4.40 (1 H, m), 5.35 (1 H, br s). Anal. ($C_{22}H_{31}O_4F$) C, H.

3,3-(Ethylenedioxy)-9α-fluoro-11β,18-epoxyandrost-5-en-17-one (26). The morpholine enamine derived from 619 mg (1.64 mmol) of **24** was oxidized with $Na_2Cr_2O_7 \cdot 2H_2O$, and the product was purified by the same procedures as described for the preparation of 14. Recrystallization of the product from CH_2Cl_2–ether gave 315 mg (52.9%) of **26**: mp 229–231 °C; IR ($CHCl_3$) 1738 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 4.25 (4 H, s), 4.62 (1 H, m), 5.37 (1 H, m). Anal. ($C_{21}H_{27}O_4F$) C, H.

3,3-(Ethylenedioxy)-17α-ethynyl-9α-fluoro-17β-hydroxy-11β,18-epoxyandrost-5-ene (27). A 450-mg (1.24 mmol) sample of **26** was ethynylated, and the product was purified by the same procedures as described for the preparation of **15**. Three hundred and sixty-two milligrams (75.1%) of **27** was obtained on recrystallization from CH_2Cl_2–ether: mp 239–240 °C; IR ($CHCl_3$) 3300, 3580 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 2.33 (1 H, s), 3.68 (1 H, d, *J* = 8 Hz), 3.90 (1 H, d, *J* = 8 Hz), 3.93 (4 H, s), 4.52 (1 H, m), 5.35 (1 H, m). Anal. ($C_{23}H_{29}O_4F$) C, H.

3-[3,3-(Ethylenedioxy)-9α-fluoro-17β-hydroxy-11β,18-epoxyandrost-5-en-17α-yl]propiolic Acid (28). A 416-mg (1.07 mmol) sample of **27** was carboxylated by the same procedure as described for the preparation of **16**. Four hundred and eighty milligrams of crude **28** thus obtained was submitted to the next reaction without further purification.

3-[3,3-(Ethylenedioxy)-9α-fluoro-17β-hydroxy-11β,18-epoxyandrost-5-en-17α-yl]propenoic Acid γ-Lactone (29). A 480-mg (ca. 1.1 mmol) sample of crude **28** was hydrogenated with 5% Pd–$BaSO_4$ by the same procedure as described for the preparation of **17**. This gave 378 mg of crude product **29**: IR ($CHCl_3$) 1760 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 3.92 (6 H, s), 4.49 (1 H, m), 5.37 (1 H, m), 5.98 (1 H, d, *J* = 6 Hz), 7.50 (1 H, d, *J* = 6 Hz). This was submitted to the next reaction without further purification.

3-[3,3-(Ethylenedioxy)-9α-fluoro-17β-hydroxy-11β,18-epoxyandrost-4-en-17α-yl]propionic Acid γ-Lactone (30). A 378-mg (ca. 0.9 mmol) sample of **29** was hydrogenated further with 5% Pd–C by the same procedure as described for the preparation of **18**. Recrystallization of the product from CH_2Cl_2–ether gave 339 mg (75.7% from **27**) of **30**: mp 239–241 °C; IR ($CHCl_3$) 1767 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3 H, s), 3.84 (2 H, s), 3.93 (4 H, s), 4.55 (1 H, m), 5.38 (1 H, m). Anal. ($C_{24}H_{31}O_5F$) C, H.

3-(9α-Fluoro-17β-hydroxy-11β,18-epoxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone (31). The ketal function of compound **30** (254 mg, 0.65 mmol) was cleaved by the same procedure as described for the preparation of **19**. The product was purified by column silica gel chromatography using AcOEt–benzene (1:1) as an eluent and 198 mg (87.1%) of **31** was obtained. It was recrystallized from CH_2Cl_2–ether: mp 252–253 °C; IR ($CHCl_3$) 1620, 1670, 1767 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (3 H, s), 3.87 (2 H, s), 4.51 (1 H, m), 5.78 (1 H, br s). Anal. ($C_{22}H_{27}O_4F$) C, H, F.

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