EtOAc–Et_2O, yield 4.30 g, and combined with 5.40 g from preparation A and recrystallized from EtOAc: yield 7.9 g; mp 188–190 °C; $[\alpha]^{25}D$ −34.8° (1% in 95% EtOH); M^+ 358; UV λ_{max} 238 nm (ϵ 12800); NMR δ 0.88 (3, C-18 CH_3), 1.33 (3 C-19 CH_3), 3.29 (1, ≡CH), 3.60 (2, OCH_2), 4.40 (1, $HOCH_2$), 5.07 (1, 6β-OH), 5.27 (1, 17β-OH), 5.67 (1, C_4=CH). Anal. ($C_{22}H_{30}O_4$) C, H.

Treatment of **8** with Ac_2O–Py afforded the diacetate **9** which was used without purification for NMR (see discourse).

6β,17-Dihydroxy-2-(hydroxymethyl)-17α-pregna-1,4-dien-20-yn-3-one (10). Organism *Melanospora parasitica* (ATCC 11103) (Me_1): five runs of 2.0 g of **7** for 48 h; crude product from EtOAc; yield 4.5 g; mp 226–227 °C; $[\alpha]^{25}D$ −81° (1% in 95% EtOH); M^+ 356; UV λ_{max} 250 nm (ϵ 15800); NMR δ 0.87 (3, C-18 CH_3), 1.41 (3, C-19 CH_3), 3.23 (1, C≡C-H), 4.20 (2, OCH_2), 4.40 (1, CHO), 4.85 (1, CH_2OH), 5.21 (1, CHOH), 5.30 (1, C-17 OH), 6.03 (1, =CH), 7.07 (1, =CH). Anal. ($C_{22}H_{28}O_4$) C, H.

Metabolite Isolation. The following TLC systems were utilized in metabolite studies using silica gel PF_{254} plates: (1) EtOAc; (2) EtOAc–cyclohexane (1:1); (3) CH_2Cl_2–CH_3OH (9:1); (4) EtOAc–cyclohexane–isopropylamine (25:24:1); (5) EtOAc–MeOH (9:1); (6) Et_2O; (7) EtOAc–MeOH (7:3); (8) EtOAc–*n*-hexane (1:1).

Aliquots of crude extracts of urine were applied in narrow bands on TLC plates and developed in one of the above solvent systems. Components were visualized under 253-nm UV light or by spraying sections of the plate with 50% EtOH–H_2SO_4 and heating for color development. Bands were removed and the silica gel was placed in a glass micro column and eluted with MeOH. After evaporation the material was put on another plate which was developed in a second solvent system. Isolated metabolites were compared with reference compounds in several solvent systems.

Monkey Study. A 24-h urine sample was collected from three female Rhesus monkeys medicated orally with danazol in Tween 80 at 50 mg/kg b.i.d. for a total of 664 mg. A 100-ml sample was extracted with *n*-hexane (2 × 200 ml) which was discarded. The sample was then extracted with CH_2Cl_2 (2 × 200 ml) which was dried (Na_2SO_4) and evaporated and the residue purified by TLC with system 1 and then system 8. Several metabolites were observed but only one, ethisterone (**11**), was obtained in sufficient purity to allow identification (Table I).

Human Study. Urine from a female subject administered 800 mg of danazol orally was collected for 24 h. A 1.8-l. aliquot was extracted with CH_2Cl_2 (2 × 4 l.). After being washed with 1 l. of 0.1 N NaOH, 1 l. of 0.1 N HCl, and 1 l. of water, the CH_2Cl_2 solution was dried (Na_2SO_4) and the solvent removed by distillation. The residue was chromatographed using system 3 to give five components which were rechromatographed and the products isolated and identified: metabolite 1, purified in system 2, was identical with ethisterone (**11**); metabolite 2, purified in system 1, was identical with **5**; metabolite 3, purified in system 6, was identical with **7**; metabolite 4, purified in system 5, was identical with **8**; metabolite 5, purified in system 7, was identical with **10**. See Table II.

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Ring D Oxygenated Spirolactones. Characterization of a Human Metabolic Product of Spironolactone

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15α-Hydroxycanrenone (**1b**) was prepared from canrenone (1a) by microbiological oxidation with a *penicillium* species. The product was identical with one obtained from the metabolism of spironolactone (**3**) in human. Oxidation of **1b** with Jones reagent furnished the corresponding 15-oxocanrenone (**1d**) which underwent base-catalyzed β elimination to generate an α,β-unsaturated cyclopentenone system. 15α-Hydroxycanrenone (**1b**) failed to show antimineralocorticoid activity at the screening dose of 2.4 mg while the oxo derivative **1d** exhibited approximately 15% the activity of **3**. Since the activity of canrenone is 38% that of spironolactone, introduction of the carbonyl group at the 15 position of canrenone resulted in a reduction in activity. This effect is opposite to that observed with 6-dehydroprogesterone.

In earlier studies on the antimineralocorticoid activity of various progesterone derivatives, the effect which an oxygen function at the 15 position had on this activity was examined.[1] In extending these studies, we sought to introduce an oxygen function into C-15 of 3-(17β-hydroxy-3-oxoandrosta-4,6-dien-17α-yl)propionic acid γ-lactone (canrenone, **1a**) in order to determine whether it would alter the mineralocorticoid-blocking activity of this

substance.

Using a *penicillium* species (our code number M31-417) which had previously been found to hydroxylate androst-4-ene-3,17-dione at the 15α position in our laboratory (unpublished results), we observed that **1a** was converted into a hydroxylated derivative, presumably 15α-hydroxycanrenone (**1b**). A structure determination of this substance was undertaken because **2a**, the 6,7-dihydro derivative of canrenone, had previously been found to be hydroxylated by a *penicillium* species (M31-399) to afford the 15β-hydroxylated derivative **2b** rather than the epimeric compound **2c**.[2] The latter product was, however, obtained by hydroxylation with a *cephalosporium* species (M60-20).[2] Subsequently **1b** was found to be identical with a substance obtained from the metabolic study of spironolactone **3** in human,[3] and this made an unambiguous assignment of its structure even more crucial.

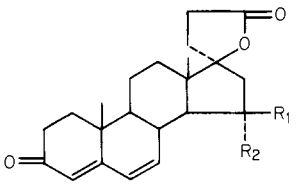
Although **1b** was obtained from the fermentation mixture as an amorphous substance, even after chromatography, it formed a crystalline pyridine complex. Conversion to the acetate **1c** and oxidation to give the ketone **1d** established that the newly introduced hydroxyl group was secondary. The carbonyl stretching frequency of 1745 cm^{-1} revealed that the saturated keto group of **1d** was located in a five-membered ring.[4] Thus, hydroxylation occurred at either C-15 or C-16. Treatment of the ketone **1d** with methanolic sodium methoxide resulted in β elimination to afford the unsaturated keto acid **4a**. The presence of the cyclopentenone chromophore and the 3-keto-$\Delta^{4,6}$ system was demonstrated by ultraviolet maxima at 230–232 and 286–289 nm, respectively. Comparable values were obtained for the corresponding methyl ester **4b**. The results indicated that hydroxylation had occurred at C-15.

Further evidence that C-16 was not the point of attack in the hydroxylation process came from a comparison of the mass spectrum of the ketone **1d** with that of 16-oxocanrenone **5a**. The latter substance was prepared from 3-(3β,16β,17β-trihydroxyandrost-5-en-17α-yl)propionic acid 16,17-acetonide (**9**).[5] Conversion of the 3β-hydroxy-Δ^5 system of **9** into the 3-keto-$\Delta^{4,6}$-diene system of **10** was accomplished by means of a mixture of bromine, lithium bromide, and lithium carbonate in dimethylformamide.[6] Removal of the acetonide-protecting group followed by lactonization afforded **5b**. Oxidation of the 16β-hydroxyl group gave the ketone **5a**. The mass spectra of **5a** and **1d** were found to be dissimilar.

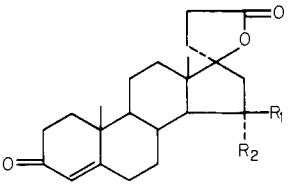
The transformation of canrenone **1a** into the 15-hydroxy derivative **1b** resulted in a dextrorotatory shift of the molecular rotation (ΔM_D = 132). Acetylation or oxidation of the 15-hydroxyl group produced a levorotatory shift (ΔM_D = −20 and −65, respectively). This pattern was observed also in the 6-dehydroprogesterone series in which the hydroxyl group at C-15 is known to be α oriented.[1a] Where the hydroxyl group is 15β, the molecular rotational difference between 6-dehydroprogesterone (**6a**) and the 15-hydroxy derivative **6b** was found to be rather slight (ΔM_D = 6).

The chemical shifts of the 6 and 7 protons are coincident in both canrenone (**1a**) and 6-dehydroprogesterone (**6a**).[1a] This is true also for the corresponding 15α-acetoxy derivatives **1c** and **6d**. However, in the 15α-hydroxy (**1b** and **6c**) and 15-oxo (**1d** and **6e**) derivatives, the chemical shifts of the 6 and 7 protons are different, as they are also in 15β-hydroxy-6-dehydroprogesterone (**6b**).

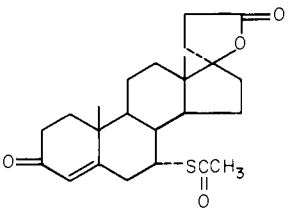
Dehydrogenation of **2d** with dichlorodicyanobenzoquinone in the presence of hydrogen chloride furnished a product which was identical with the $\Delta^{4,6}$ compound **1c**.



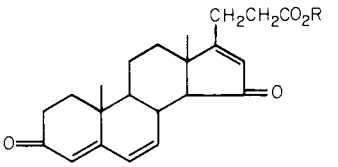
1a, $R_1 = R_2 = H$
b, $R_1 = H$; $R_2 = OH$
c, $R_1 = H$; $R_2 = OAc$
d, $R_1, R_2 = O$
e, $R_1 = H$; $R_2 = OMs$



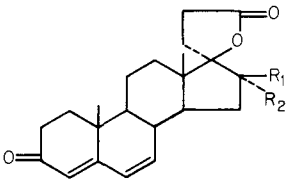
2a, $R_1 = R_2 = H$
b, $R_1 = OH$; $R_2 = H$
c, $R_1 = H$; $R_2 = OH$
d, $R_1 = H$; $R_2 = OAc$
e, $R_1 = H$; $R_2 = OMs$



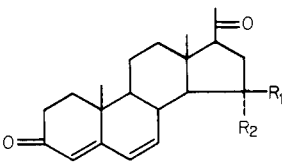
3



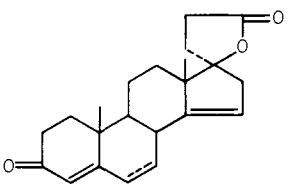
4a, R = H
b, R = CH_3



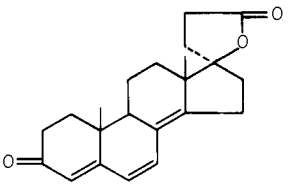
5a, $R_1, R_2 = O$
b, $R_1 = OH$; $R_2 = H$



6a, $R_1 = R_2 = H$
b, $R_1 = OH$; $R_2 = H$
c, $R_1 = H$; $R_2 = OH$
d, $R_1 = H$; $R_2 = OAc$
e, $R_1, R_2 = O$



7a, Δ^6
b, 6,7-dihydro



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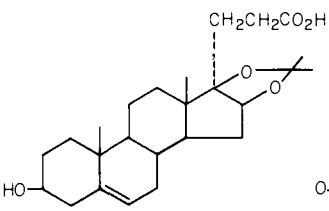
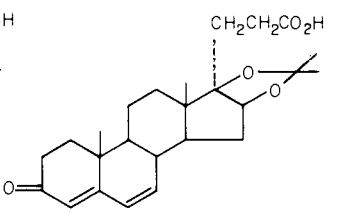


9



10

This provided further evidence that the product obtained from hydroxylation of canrenone (**1a**) with *penicillium* sp. M31-417 was, indeed, 15α-hydroxycanrenone (**1b**).

In both the canrenone and the dihydrocanrenone series, elimination of the 15α-hydroxy group to give the corresponding Δ^{14} derivatives, **7a** and **7b**, was achieved by conversion first to the mesylates **1e** and **2e**, followed by treatment with either potassium or sodium acetate. Dehydrogenation with chloranil converted **7b** into **7a**. Isomerization of the latter substance in an alkaline medium furnished the conjugated trienone **8**.

The antimineralocorticoid effects of the various derivatives of canrenone, in which ring D has been modified, were determined in a standard test employing deoxycorticosterone acetate as the mineralocorticoid. The compounds were administered subcutaneously at a dose of 2.4 mg per rat, each rat weighing between 175 and 200 g. A compound was considered active if the increase in

Table I. Antimineralocorticoid Effects of Ring D Modified Spirolactones

Compd	Urinary log (Na × 10)/K: Treated (T)[a]	Control (C)[b]	T/C[c]
1b	0.57	0.91	0.62
1c	0.77	0.99	0.77
1d	1.04	1.03	1.01
5b	0.97	1.16	0.84
7a	0.55	0.82	0.67
8	0.73	0.89	0.82

[a] Each of the compounds was tested in the adrenalectomized rat at 2.4 mg subcutaneously following the subcutaneous administration of 12 μg of deoxycorticosterone acetate. Eight animals were employed in each group. [b] Spironolactone was given to the adrenalectomized rat at 0.33 mg subcutaneously following the subcutaneous administration of 12 μg of deoxycorticosterone acetate. Groups of eight animals were employed. [c] A compound is considered active when T/C ≥ 1.

the urinary log (Na × 10)/K ratio was equivalent to or greater than that produced by 0.33 mg/rat of spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxyandrost-4-en-17α-yl)propionic acid lactone (3)].[5,7]

With the exception of 15-oxocanrenone (1d), all of the derivatives examined were found inactive in this test at the screening dose (Table I). The activity of 15-oxocanrenone (1d) was approximately 15% that of spironolactone (3). The activity of canrenone (1a), on the other hand, has been reported to be ca. 35% that of spironolactone when administered orally.[8] Given subcutaneously, the activity of 1a is 38% that of 3.[9]

Significantly, introduction of a carbonyl group into the 15 position of canrenone (1a) did not lead to enhancement of antimineralocorticoid activity. This is in contrast to the results obtained with 6-dehydroprogesterone (6a) wherein insertion of the 15-carbonyl group led to increased activity.[1a]

Earlier we had observed that a 6β,7β-methylene group enhanced the antimineralocorticoid effect of a spirolactone derivative while the same group had the opposite effect in the progesterone series.[1b,10] These results indicate that the structural features required for antimineralocorticoid activity differ in the two series of compounds which are known to block the salt-retaining effects of aldosterone.

Experimental Section

Melting points were determined on a Fisher-Johns melting block and are uncorrected. NMR spectra were taken on a Varian A-60 instrument in deuteriochloroform with tetramethylsilane as an internal standard. Optical rotations were determined in $CHCl_3$.

3-(15α,17β-Dihydroxy-3-oxoandrosta-4,6-dien-17α-yl)propionic Acid γ-Lactone (1b). A 12.0-g sample of 3-(17β-hydroxy-3-oxoandrosta-4,6-dien-17α-yl)propionic acid γ-lactone (1a) in 25 l. of cotton seed meal broth was fermented in a 40-l. fermentor for a period of 48 h with *penicillium* sp. M31-417 and worked up in the usual manner.[11] The crude reaction product was chromatographed on 1.2 kg of silica gel. The column was eluted with varying proportions of C_6H_6 and EtOAc. Elution with 50% EtOAc in C_6H_6 gave 2.8 g of 1b as an amorphous substance. A 698-mg sample of 1b was crystallized from pyridine to afford 407 mg of 1b as a pyridine complex: mp 112–117 °C; NMR (Hz) 431–472 (multiplet, C_5H_5N protons), 400 (dd, J = 10, 1.5 Hz, 1 H, C_7-H), 366 (dd, J = 10, 2.5 Hz, 1 H, C_6-H). Anal. ($C_{22}H_{28}O_4 \cdot C_5H_5N$) C, H, N.

A sample of the pyridine complex of 1b was dissolved in EtOAc. The EtOAc solution was evaporated to dryness to afford a viscous oil. The oil was triturated with ether to afford 1b, as an amorphous solid: mp 120–125 °C; $[\alpha]^{25}D$ 60.3° (c 1); λ_{max}^{MeOH} 281–283.5 nm (ε 24 300); ν (KBr) 3695, 3620, 3470, 1770, 1660, 1654, 1621 cm^{-1}; NMR (Hz) 398 (q, J = 10, 2 Hz, 1 H, C_7-H), 367 (q, J = 10, 2 Hz, 1 H, C_6-H). The NMR spectrum showed the absence of pyridine.

3-(15α-Acetoxy-17β-hydroxy-3-oxoandrosta-4,6-dien-17α-yl)propionic Acid γ-Lactone (1c). A mixture of 326 mg of 1b, 1 ml of C_5H_5N, and 1 ml of Ac_2O was heated on the steam bath for 1 h. The cooled reaction mixture was poured into ice water and stirred. The resultant solid was collected, washed with water, and dried. The solid was extracted with boiling ether. The ether extract was concentrated to afford a crystalline product which was recrystallized from ether to furnish 1c: mp 208–212 °C; $[\alpha]^{25}D$ 49° (c 0.1); ν (KBr) 1778, 1747, 1674, 1631, 1595 cm^{-1}; NMR (Hz) 368 (s, 2 H, C_6,C_7-H's), 343 (s, 1 H, C_4-H), 124.5 [s, 3 H, $CH_3C(=O)O$-], 69 (s, C_{10}-CH_3), 65.5 (s, C_{13}-CH_3). Anal. ($C_{24}H_{30}O_5$) C, H.

3-(17β-Hydroxy-3,15-dioxoandrosta-4,6-dien-17α-yl)propionic Acid γ-Lactone (1d). A stirred solution of 350 mg of 1b in 7 ml of acetone was treated portionwise with a solution of 0.35 ml of Jones reagent (8 N CrO_3) in 2 ml of acetone. The reaction mixture was stirred at room temperature for 20 min after which it was diluted with H_2O. The resultant precipitate was collected and dried. Crystallization from acetone afforded 202 mg of 1d: mp 218–219 °C; ν (KBr) 1795, 1745, 1675, 1628, 1593 cm^{-1}; λ_{max}^{MeOH} 280–282 nm (ε 26 000); NMR (Hz) 412 (dd, J = 10, 2 Hz, 1 H, C_7-H), 370 (dd, J = 10, 2.5 Hz, 1 H, C_6-H), 342.5 (1 H, C_4-H), 69.5 (s, 3 H, C_{10}-CH_3), 68.5 (s, 3 H, C_{13}-CH_3). Anal. ($C_{22}H_{26}O_4$) C, H.

3-(15α,17β-Dihydroxy-3-oxoandrosta-4,6-dien-17α-yl)propionic Acid γ-Lactone 15-Methanesulfonate (1e). To a mixture of 100 mg of the pyridine complex of 1b and 0.5 ml of pyridine was added 0.1 ml of methanesulfonyl chloride. The reaction mixture was allowed to stand at room temperature for 1 h with occasional swirling. Then it was allowed to stand at 5 °C for 15 h. The reaction mixture was diluted with H_2O and stirred. The precipitate which resulted was collected, washed with H_2O, and dried. Crystallization from EtOAc–ether afforded 1e, mp 135–140 °C. The presence of a sharp signal at 297.5 Hz (3-H) in the NMR spectrum confirmed the presence of the methanesulfonyl group in 1e.

3-(3,15-Dioxoandrosta-4,6,16-trien-17-yl)propionic Acid (4a). A mixture of 103 mg of the 15-oxospirolactone (1d), 10 ml of MeOH, and 60 mg of NaOMe was stirred at room temperature in an atmosphere of N_2 for 10 min. The reaction mixture was diluted with H_2O and acidified with glacial HOAc. The resultant mixture was concentrated under reduced pressure until a heavy oil appeared. The oil was extracted with EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to afford 118 mg of a yellow foam: λ_{max}^{MeOH} 230–232 nm (ε 12 900), 286–289 (23 100); ν (KBr) 1730, 1708, 1666, 1640, 1618 cm^{-1}. Crystallization of the foam from CH_2Cl_2–C_6H_6 afforded 4a: mp 198–200 °C; NMR (Hz, after D_2O exchange) 389 (dd, J = 10, 1.5 Hz, 1 H, C_6 or C_7-H), 370 (dd, J = 10, 2.5 Hz, 1 H, C_7 or C_6-H), 351 (s, 1 H, C_{16}-H), 342 (s, 1 H, C_4-H).

Methyl 3-(3,15-Dioxoandrosta-4,6,16-trien-17-yl)propionate (4b). A mixture of 75 mg of 4a and 15 ml of an ethereal solution of CH_2N_2 prepared from 700 mg of *N*-nitrosomethylurea was allowed to stand at 5 °C for 66 h. The reaction mixture was evaporated to dryness. The residual oil was chromatographed on 3.0 g of silica gel. The column was eluted with varying proportions of EtOAc in C_6H_6. Elution with 15% EtOAc in C_6H_6 afforded 4b as a yellow viscous oil: λ_{max}^{MeOH} 227.5–231 nm (ε 13 200), 287 (ε 24 800). The appearance of a signal at 222 Hz in the NMR spectrum confirmed the presence of the carbomethoxy group.

3-(15α-Acetoxy-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone (2d). A mixture of 2.0 g of 3-(15α,17β-dihydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone (2c),[2] 10 ml of C_5H_5N, and 6.4 ml of Ac_2O was maintained at room temperature for 20 h after which it was poured into ice H_2O. The resultant precipitate was collected, washed with H_2O, and dried: yield 2.05 g. Crystallization from EtOAc afforded 2d: mp 186–192 °C; NMR (Hz) 345 (s, 1 H, C_4-H), 73 (s, 3 H, C_{10}-CH_3), 63 (s, 3 H, C_{13}-CH_3). Anal. ($C_{24}H_{32}O_5$) C, H.

Dehydrogenation of 3-(15α-Acetoxy-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone (2d). A mixture of 100 mg of 2d, 60 mg of dichlorodicyanobenzoquinone, and 7 ml of 5.9 N HCl in dioxane was stirred and heated under reflux

for 45 min.[12] Then it was allowed to stand at room temperature for 15 h. The reaction mixture was diluted with ether and 5% NaOH. The organic phase was separated, washed successively with 5% NaOH and H_2O, dried (Na_2SO_4), and evaporated to dryness to afford a yellow viscous oil. Crystallization from ether afforded 65 mg of **1c**: mp 208–212 °C; λ_{max}^{MeOH} 279–281 nm (ε 26 200). IR and NMR spectroscopy, as well as GLC, confirmed that the product was identical with that obtained from the acetylation of **1b**.

3-(17β-Hydroxy-3-oxoandrosta-4,6,14-trien-17α-yl)-propionic Acid γ-Lactone (7a). (a) A mixture of 100 mg of methanesulfonate **1e**, 100 mg of fused KOAc, and 8 ml of glacial HOAc was heated under reflux for 1.5 h. Then it was distilled nearly to dryness under reduced pressure. The residue was triturated with H_2O. The resultant solid was collected, washed with H_2O, and dried. Crystallization from EtOAc–ether–hexane afforded 39 mg of **7a**, mp 215–220 °C.

(b) A solution of 5.0 g of 3-(15α,17β-dihydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone (**2c**) in 10 ml of C_5H_5N was cooled in an ice bath and stirred while 3.0 ml of methanesulfonyl chloride was added dropwise over a period of 12 min. Stirring was continued while the reaction mixture was allowed to warm to room temperature over a period of 75 min. The reaction mixture was poured into ice H_2O. The precipitate was collected, washed with H_2O, and dried. The solid was digested with 12 ml of hot EtOH. On cooling, the EtOH solution afforded the mesylate **2e** as a semisolid.

A 3.9-g sample of **2e**, 1.95 g of freshly fused NaOAc, and 12 ml of glacial HOAc was heated under reflux in an atmosphere of N_2 for 25 min. The cooled reaction mixture was diluted with H_2O. The precipitate was collected, washed with H_2O, and dried. Crystallization from MeOH afforded 1.0 g of 3-(17β-hydroxy-3-oxoandrosta-4,14-dien-17α-yl)propionic acid γ-lactone (**7b**): mp 188–191 °C; NMR (Hz) 346 (s, 1 H, C_4-H), 312 (d, J = 2.5 Hz, 1 H, C_{15}-H), 75 (s, 3 H, C_{10}-CH_3), 68 (s, 1 H, C_{13}-CH_3). Anal. ($C_{22}H_{28}O_3$) C, H.

A mixture of 1.0 g of **7b**, 0.8 g of chloranil, and 22 ml of *tert*-amyl alcohol was stirred and heated under reflux for 6.5 h.[12b,13] After the solvent was removed by distillation under reduced pressure, the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed successively with H_2O, 5% NaOH, and H_2O again, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The crystalline residue was crystallized from EtOAc to afford **7a**: mp 220–222 °C; λ_{max}^{MeOH} 280 nm (ε 23 750); NMR (Hz) 377 (d, J = 2.0 Hz, 2 H, C_6,C_7-H's), 344 (s, 1 H, C_4-H), 316 (d, J = 2.5 Hz, 1 H, C_{15}-H), 71 (s, 6 H, C_{10}, C_{13}-CH_3's). Anal. ($C_{22}H_{26}O_3$) C, H. The NMR spectrum was identical with that of the product obtained from **1e**.

3-[17β-Hydroxy-3-oxoandrosta-4,6,8(14)-trien-17α-yl]-propionic Acid γ-Lactone (8). A solution of 450 mg of **7a**, 4.5 ml of 1 N KOH, and 34 ml of MeOH was heated under reflux for 2 h. The cooled reaction mixture was acidified with 6 N HCl and diluted with H_2O. The resultant precipitate was collected, washed with H_2O, and dried. Crystallization from EtOAc afforded **8**: mp 198–201 °C; λ_{max}^{MeOH} 341 nm (ε 22 800); NMR (Hz) 399 (d, J = 10 Hz, 1 H, C_7 or C_6-H), 369 (d, J = 10 Hz, 1 H, C_6 or C_7-H), 348 (s, 1 H, C_4-H), 75 (s, 3 H, C_{13}-CH_3), 63 (s, 3 H, C_{10}-CH_3). Anal. ($C_{22}H_{26}O_3$) C, H.

3-(16β,17β-Dihydroxy-3-oxoandrosta-4,6-dien-17α-yl)-propionic Acid 16,17-Diacetonide (10). A mixture of 614 mg of 3-(3β,16β,17β-trihydroxyandrost-5-en-17α-yl)propionic acid 16,17-acetonide (**9**),[5] 615 mg of LiBr, 615 mg of Li_2CO_3, and 10 ml of DMF was stirred at 75 ± 5 °C. To this warm solution was added dropwise over a period of 25 min a solution of 0.15 ml of Br_2 in 3.5 ml of dioxane. After the addition was complete, the reaction mixture was stirred at 75 ± 2 °C for 2 h. Then it was poured into ice H_2O. The resultant mixture was neutralized with glacial HOAc. The precipitate which formed was collected, washed with H_2O, and dried: yield, 443 mg; mp 124–126 °C; λ_{max}^{MeOH} 282–284 nm (ε 17 500). The crude product, **10**, was not purified further but used directly in the subsequent reaction.

3-(16β,17β-Dihydroxy-3-oxoandrosta-4,6-dien-17α-yl)-propionic Acid γ-Lactone (5b). A mixture of 180 mg of **10**, 15 ml of MeOH, 10 ml of H_2O, and 1 ml of 6 N HCl was allowed to stand at room temperature for 15 h after which it was poured into a dilute solution of $NaHCO_3$. The resultant mixture was extracted with EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to afford a crystalline product. Crystallization from EtOAc–hexane afforded 42 mg of **5b**: mp 235.5–239.5 °C; λ_{max}^{MeOH} 281.5–283.5 nm (ε 21 050); ν (KBr) 3472, 1773, 1653, 1618, 1582 cm^{-1}. Another crystallization from EtOAc raised the mp to 237.5–240 °C. Anal. ($C_{22}H_{28}O_4$) C, H.

3-(17β-Hydroxy-3,16-dioxoandrosta-4,6-dien-17α-yl)-propionic Acid γ-Lactone (5a). To a solution of 0.1 mg of the 16β-hydroxyspirolactone **5b** in 0.1 ml of acetone was added dropwise slightly more than the equivalent amount of Jones reagent. The reaction mixture was allowed to stand at room temperature for 10 min. The excess CrO_3 was destroyed by the dropwise addition of *i*-PrOH. The resultant mixture was diluted to 2 ml with H_2O and extracted with 5 ml of $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to dryness. The residue was subjected to TLC and GC–MS.

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