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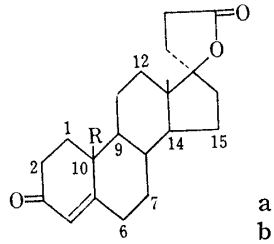
121. Robert C. Tweit, Edward A. Brown, Stephen Kraychy, Seth Mizuba, R. D. Muir, and Robert T. Nicholson : Steroidal Aldosterone Blockers. VIII.*1 Hydroxylated Steroidal 17-Spirolactones.

(*Divisions of Chemical and Biological Research, G. D. Searle & Co.**2)

Previous work has demonstrated that some steroidal spirolactones bearing hydroxyl groups[1] or thioesters[2] exhibit an enhanced ability to block the mineralocorticoid activity of deoxycorticosterone acetate, compared to the parent spirolactones. In this paper we

TABLE I. Positions hydroxylated by Various Organisms



a : R=CH_3
b : R=H

Compd.	Position of hydroxyl	Organisms
Ia	none	
IIa	1β	*Mucor* sp. M 10-77[a]
IIIa	2β	*Trichoderma* sp. M 1254
IVa	6β	*Gelasinospora* sp. M 40-10; *Gelasinospora tetraspora* M 1114 (A. T. C. C. 14512)[b]
Va	7α	*Mucor* sp. M 10-77; *Gelasinospora* sp. M 40-10; *Gelasinospora tetraspora* M 1114 (A. T. C. C. 14512)
VIa	7β	*Gliocladium* sp. M 30-128 (A. T. C. C. 14513)
VIIa	9α	*Nocardia* sp. A-20-13 (A. T. C. C. 13934)
VIIIb	10β	*Gliocladium* sp. M 30-128 (A. T. C. C. 14513)
IXa	12β	*Gliocladium* spp. M 30-128 (A. T. C. C. 14513), M 30-142, M 1125, *Phoma* sp. M 65-4
Xa	14α	*Phoma* sp. M 1210
XIa	15α	*Cephalosporium* sp. M 60-20
XIIa	15β	*Penicillium* sp. M 31-399
XIIb	15β	*Fusarium* sp. M 1632

a) The M-numbers are our designations for these molds.
b) American Type Culture Collection number.

*1 Part VII. E. A. Brown, R. R. Burtner : J. Med. Chem., **6**, 732 (1963).
*2 P. O. Box 5110, Chicago, Illinois 60680, U. S. A.
1) E. A. Brown, R. D. Muir, J. A. Cella : J. Org. Chem., **25**, 96 (1960).
2) J. A. Cella, R. C. Tweit : *Ibid.*, **24**, 1109 (1959).

wish to report on the preparation by microbiological means of some additional hydroxylated compounds as well as on their anti-mineralocorticoid activity.

Table I lists the new substances which were prepared and the organisms which produced them.

The 1β-hydroxy compound (IIa) was isolated as a minor product from a fermentation using a *Mucor* sp. The maximum in the ultraviolet spectrum of IIa in basic solution shifted from 240 to 245 mμ, typical of the dehydration of a 1-hydroxy group to give a $\Delta^{1,4}$-3-one system,[3] and oxidation produced a material, XX, which appeared from the infrared and ultraviolet spectra to exist as an equilibrium mixture of diketone and $\Delta^{1,4}$-1-hydroxy-3-oxo-compound, typical of β-diketones. The molecular rotatory contribution of the hydroxy group in IIa is $-135°$; this value is in agreement with the range of $-116°$ to $-218°$ reported for 1β-hydroxy compounds[4] and does not agree with the values of $-14°$ and $-26°$ reported for 1α-hydroxy-Δ^4-3-oxosteroids.[3]

The main point of oxidation with this organism was at the 7α-position to give Va, also obtained from two *Gelasinospora* species. The epimeric 7β-compound (VIa) was isolated from the fermentation of Ia with a *Gliocladium* sp. M 30-128, and these two hydroxyls were identified with the 7-position by the shift in their ultraviolet spectra in base from 241 to 284.5 mμ, typical of the dehydration to a $\Delta^{4,6}$-3-one-system.[5] The shifts in the 18- and 19-methyl resonance positions in the nuclear magnetic resonance spectra for each epimer agreed with the values reported in the literature[6] and the changes in molecular rotation for the corresponding acetates: 7α, XIII; 7β, XIV; and the positions of the 7-hydrogens in the nuclear magnetic resonance spectra of XIII and XIV also agree with literature values[7] (see Table II).

Table II. Changes in Molecular Rotation and Nuclear Magnetic Resonance Peaks produced by 7-Substituents

Compd.	ΔM_D	NMR		7-H position[b]
		18-CH_3 shift[a]	19-CH_3 shift[a]	
Va	$-61°$	1.0	1.0	238 (broad singlet)
7α-OH	$-56°$ to $-136°$[c]	0.3[d]	-0.6[d]	
XIII	$-262°$	1.5	2.5	302 (〃)
7α-OAc	$-250°$ to $-433°$[c]	1.5[d]	1.9[d]	307 (〃)[c]
VIa	$-26°$	2	1.5	240～265 (multiplet)
7β-OH	$-4°$ to $-169°$[c]	2.4[d]	1.5[d]	
XIV	$-15°$	3	3	275～290 (〃)
7β-OAc	$-52°$ to $+53°$[c]	2.5[d]	2.6[d]	280 (center of multiplet)[c]

a) Shift in c.p.s. (positive shift is downfield from tetramethylsilane resonance).
b) Position in c.p.s. at 60 Mc./sec.
c) Ref. 7
d) Ref. 6

Two other products were isolated from the *Gliocladium* M 30-128 fermentation. One was identified by comparison of infrared spectra as 3-oxo-11β,17β-dihydroxyandrost-4-ene-17α-propionic acid γ-lactone,[1] the other was shown to be the 12β-hydroxy compound (IXa) which was also isolated from oxidations with two other *Gliocladium* and one

3) R. M. Dodson, A. H. Goldkamp, R. D. Muir: J. Am. Chem. Soc., **82**, 4026 (1960).
4) R. M. Dodson, S. Kraychy, R. T. Nicholson, S. Mizuba: J. Org. Chem., **27**, 3159 (1962).
5) C. W. Greenhalgh, H. B. Henbest, E. R. H. Jones: J. Chem. Soc., **1952**, 2375.
6) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, K. Tsuda: This Bulletin, **10**, 338 (1962).
7) R. C. Tweit, A. H. Goldkamp, R. M. Dodson: J. Org. Chem., **26**, 2856 (1961).

Phoma species. The position of the hydroxyl was established by its oxidation to a six-membered ring ketone, XV, (IR : λ_{max} 5.88 μ) whose nuclear magnetic resonance spectra had one peak for both 18- and 19-methyls at 75 c.p.s.; only a 12-oxo-function can account for this shift of 3 c.p.s. for the 19-methyl and 17 c.p.s. for the 18-methyl. The configuration at 12 was suggested by examination of the nuclear magnetic resonance spectra of the 12-acetate (XIX) in which a quartet appeared at 288, 293, 297, and 302 c.p.s., consistent with an axial hydrogen attached to the same carbon atom as an equatorial acetoxy group.*3, 7)

The same micro-organism species, *Gliocladium* sp. M 30-128, converted the 19-nor compound (Ib) to the 10β-hydroxy derivative (Ⅷb) in low yield. This was indicated by the ultraviolet maximum at 234.5 mμ, which shifted in acid to low peaks at 278 and 287 mμ.[8] In addition, in the nuclear magnetic resonance spectrum there was no absorption in the 175~340 c.p.s. region, where the resonance for a hydrogen attached to a carbon carrying a hydroxyl group would be expected to appear.[9] The 10β-hydroxyl shifts the 18-CH_3 signal by 4 c.p.s. to lower frequency and moves the 4-H peak to 349 c.p.s. The main product was a mixture of monohydroxy derivatives of Ib which could not be separated by chromatography.

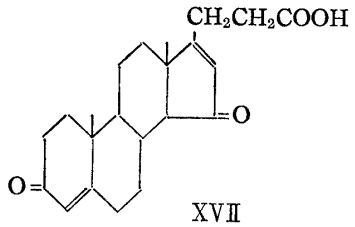
Two tertiary hydroxy derivatives of Ia were formed; the 9α-compound (Ⅶa) and the 14α-substance (Xa). No peaks were present in the 165~350 c.p.s. region of their nuclear magnetic resonance spectra and the changes in the methyl peaks are consistent with

Table Ⅲ. Shifts in the Main Peaks in the Nuclear Magnetic Resonance Spectra of Various Hydroxy Compounds

Compd.	Position of hydroxy group	18-CH_3[a]	19-CH_3[a]	Compd.	Position of hydroxy group	18-CH_3[a]	19-CH_3[a]
Ⅶa		1	7	Ⅺa		1	1
	9α	0~1[b]	8~9[b]		15α	1~3[c]	0~1[c]
Xa		7.5	1	Ⅻa		17	3
	14α	7~8[b,c]	0~3[b,c]		15β	16[c]	2[c]
Ⅳa		2.5	11.5				
	6β	2~3[b,c]	11~13[b,c]				

a) Shift in c.p.s. at 60 Mc./sec. (positive shift is downfield from tetramethylsilane).
b) K. Tori, E. Kondo : Tetrahedron Letters, No. 10, 645 (1963).
c) Ref. 6

the literature values as seen in Table Ⅲ. The 4,5α-dihydro derivative (XVⅢ) of Xa was also isolated from the *Phoma* sp. M 1210 fermentation. A 6β-hydroxylated compound, Ⅳa, was identified by its ultraviolet absorption peak at 235.5 mμ,[10] its strongly shifted 19-methyl resonance in the nuclear magnetic resonance (Table Ⅲ), and a peak at 260 c.p.s. Two other secondary hydroxy derivatives, Ⅺa and Ⅻa, can be oxidized to the same ketone



XVⅡ

*3 These data are in good agreement with those of the corresponding progesterone derivatives. 12α-Acetoxyprogesterone (prepared by the method of G. Just, Ch. R. Engel : J. Org. Chem., 23, 12 (1958)) exhibited a broad singlet at 312 c.p.s., whereas 12β-acetoxyprogesterone (unpublished results from this laboratory) showed a quartet at 278, 283, 288, and 293 c.p.s. Further, the shifts produced by the introduction of a 12-ketone into progesterone are 21.5 c.p.s. for the 18-methyl and 7 c.p.s. for the 19-methyl group.

8) R. L. Pederson, J. A. Campbell, J. C. Babcock, S. H. Eppstein, H. C. Murray, A. Weintraub, R. C. Meeks, P. D. Meister, L. M. Reineke, D. H. Peterson : J. Am. Chem. Soc., 78, 1512 (1956); J. Perez Ruelas, J. Iriarte, F. Kincl, C. Djerassi : J. Org. Chem., 23, 1744 (1958); F. Alvarez : Steroids, 3, 13 (1964).

9) Y. Kawazoe, Y. Sato, T. Okamoto, K. Tsuda : This Bulletin, 11, 328 (1963).

10) A. S. Meyer : J. Org. Chem., 20, 1240 (1955).

(XVI) which exhibits a peak at 5.7 μ in the infrared spectrum and which can be dehydrated with base to an acid (XVII) containing two α,β-unsaturated ketone systems and two vinyl hydrogens. These changes suggest the presence of 15-hydroxy groups in XIa and XIIa and the nuclear magnetic resonance (Table III) shows XIa to be α and XIIa to be β. The 15β-hydroxy derivative of Ib, XIIb, was isolated from a *Fusarium* sp., M 1632, and shown to be the same as material isolated by Dr. J. A. Cella from an *Aspergillus* sp., M 78. Finally the 2β-hydroxy compound (IIIa) was identified by the characteristic change of its ultraviolet spectrum in base[10] and the strongly negative molecular rotatory contribution of the hydroxy group.

The anti-mineralcorticoid activities of these compounds are listed in Table IV. Compounds (VIa), (VIIIb), and (IXa), the most interesting biologically, did not have oral activities comparable to their sub-cutaneous activities.

Table IV. Deoxycorticosterone Acetate Blocking Potencies[a]

Compd.	MED[b]	Compd.	MED[b]	Compd.	MED[b]
IIa	>2.4	IXa	0.28	XV	>0.6
IIIa	>2.4	Xa	>2.4	XVI	1.6
IVa	>2.4	XIa	>2.4	XVII	>2.4
Va	>0.5	XIIa	1.7	XVIII	>2.4
VIa	0.69	XIIb	>0.6	XIX	>0.6
VIIa	>2.4	XIII	1.0	Spironolactone[c]	0.33
VIIIb	0.26	XIV	>2.4		

a) We are indebted to Dr. C.M. Kagawa and Mr. Robert Jacobs for these results.
b) MED is the minimal effective dose (mg./rat) which, when used with 12 μg. of DOCA produces the same urinary Na/K ratio as that which results from the use of 6 μg. of DOCA alone. All of these results are for s.c. administration.
c) Aldactone®.

Experimental*4

Fermentations——In the method used for most of the compounds, the organism was grown for 40~48 hr. in a stainless steel fermentor containing cotton seed flour medium*5 or nutrient broth*6 (in the case of *Nocardia* sp.) which had been sterilized by direct steam. The culture was agitated by a paddle-type stirrer operating at 200 revolutions per minute and was aerated with 10 L. per minute of sterile air which entered through a sparger located below the agitator. The temperature was maintained at 25°. The steroid, dissolved in 125~250 ml. of Me_2CO, was added and incubation was continued for 7.5 to 48 hr. as determined by paper chromatography of previous shaken flask runs and by IR spectra run during the actual fermentation. The mixture was extracted with methylene chloride.

3-(3-Oxo-9α,17β-dihydroxy-4-androsten-17α-yl)propanoic Acid Lactone (VIIa)——The starting material, Ia, 10 g., was incubated for 7.5 hr. with *Nocardia* sp. A 20-13 using the above method. The extracts were concentrated to dryness and the residue was dissolved in benzene and chromatographed on 1 kg. of silica gel. From the 25% AcOEt-benzene eluates, 6.05 g. of Ia, m.p. 168~169.5°, was recovered. Further development of the column gave 1.08 g. of the 9α-hydroxy compound (VIIa) m.p. 226~229°, from the 80% eluates. Two crystallizations from Me_2CO-hexane raised the melting point to 229~232°. $[\alpha]_D$ +76.1°. IR $\lambda_{max}^{CHCl_3}$ μ : 2.76, 5.65, 5.98, 6.18. UV : λ_{max}^{MeOH} 241 mμ (ε 15,740). *Anal.* Calcd. for $C_{22}H_{30}O_4$ (VIIa) : C, 73.71; H, 8.44. Found : C, 73.62; H, 8.47.

Fermentation of Ia with a *Gliocladium* sp.——The starting material (Ia), 5.5 g., was incubated for 18 hr. with a *Gliocladium* sp. M 30-128 (A. T. C. C. 14513) by the method described above. The extracts were concentrated and the residue was rinsed with ligroin and Et_2O and then dissolved in 10% AcOEt

*4 The rotations were taken in $CHCl_3$ at 25±2°, unless otherwise noted. The NMR spectra were run in $CDCl_3$ on a Varian A-60 using tetramethylsilane as a reference standard. The melting points are corrected.
*5 Cotton seed flour, 150 g., 1 kg. of commercial dextrose; 90 ml. of corn steep liquor; 5 g. of silicone antifoam and 40 L. of hot tap water.
*6 Dehydrated nutrient broth, 200 g.; 5 g. of silicone antifoam and 40 L. of hot tap water.

in benzene and chromatographed on 400 g. of silica gel. The first 6 L. of 30% AcOEt-benzene eluate contained material which gave 0.18 g. of solid, m.p. 206~207.5°, after two crystallizations from Me_2CO-ligroin. This compound was identified as 3-(3-oxo-11β,17β-dihydroxy-4-androsten-17α-yl)propanoic acid lactone by comparison of its IR spectrum with that of an authentic sample.[1]

From the 40% AcOEt in benzene eluate, 1.43 g. of the 7β-hydroxy compound (Va) was obtained by crystallization of the residue from Me_2CO, m.p. 174~177°. $[\alpha]_D$ +66°. UV $\lambda_{max}^{0.1N\ KOH\ in\ MeOH}$ mμ (ε): 241.5 (14,900), shifts to 283 (20,200) in 24 hr. IR λ_{max}^{KBr} μ: 2.97, 5.63, 6.03. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (Va): C, 73.71; H, 8.44. Found: C, 73.63; H, 8.53.

The mother liquors of Va were concentrated and crystallized from Me_2CO-Et_2O to yield 0.33 g. of the 12β-hydroxy compound (IXa), m.p. 225~226°, which was identified by comparison of IR spectra with that of material obtained fermentations with *Phoma* sp. M 65-4 and *Gliocladium* spp. M 30-142 and M 1125.

3-(3-Oxo-12β,17β-dihydroxy-4-androsten-17α-yl) propanoic Acid Lactone (IXa)——This compound was prepared by a variation of the general method using a cotton seed flour medium*[7] in a stainless steel fermentor with a capacity of 400 L. The medium was aerated at 30 L. per minute and 117 g. of Ia was incubated for 20.5 hr. with *Gliocladium* sp. M 30-142. The extracts were concentrated and the residue was washed with Et_2O and crystallized from MeOH to yield 44.4 g. of IXa, m.p. 204~208°. Two crystallizations from AcOEt raised the melting point to 227~229°. $[\alpha]_D$ +72°. UV: λ_{max}^{MeOH} 240 mμ (ε 16,600). IR $\lambda_{max}^{CHCl_3}$ μ: 2.73, 2.84, 5.62, 5.96, 6.17. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (IXa): C, 73.71; H, 8.44. Found: C, 73.80; H, 8.17.

3-(3-Oxo-15β,17β-dihydroxy-4-androsten-17α-yl)propanoic Acid Lactone (XIIa)——Ten grams of Ia was fermented with a *Penicillium* sp. M 31-399 for 30.5 hr. using the general method above. The residue obtained by evaporation of the extracts was chromatographed on 1200 g. of silica gel. The desired product was eluted with 65% AcOEt in benzene and was crystallized twice from AcOEt to yield 0.46 g. of XIIa, m.p. 193~195°. UV: λ_{max}^{MeOH} 240 mμ (ε 16,000). IR $\lambda_{max}^{CHCl_3}$ μ: 2.73, 2.84, 5.63, 5.98, 6.18. $[\alpha]_D$ +39° (MeOH). *Anal.* Calcd. for $C_{22}H_{30}O_4$ (XIIa): C, 73.71; H, 8.44. Found: C, 73.62; H, 8.59.

3-(3-Oxo-15α,17β-dihydroxy-4-androsten-17α-yl) propanoic Acid Lactone (XIa)——Compound (Ia), 15 g. was fermented with a *Cephalosporium* sp. M 60-20 under the general conditions described above for 28 hr. The extracts were concentrated and the residue was chromatographed on 1300 g. of silica gel. The material (about 2 g.) eluted with 70% AcOEt-benzene could not be crystallized, but was identified as XIa by the analysis, rotation, and spectral data. IR $\lambda_{max}^{CHCl_3}$ μ: 2.75, 2.85, 5.63, 5.98, 6.18. $[\alpha]_D$ +97°. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (XIa): C, 73.71; H, 8.44. Found: C, 73.42; H, 8.25.

Fermentation with a *Mucor* sp.——The starting material (Ia), 10 g., was fermented with a *Mucor* sp. M 10-77 for 23 hr. using the general method. When the extracts were concentrated, a solid formed. This was crystallized from MeOH to 1.4 g. of crude 7α-compound (Va) which was crystallized two times more to m.p. 285~288° (gas evolution). The remaining material was chromatographed on 700 g. of silica gel. The 50% AcOEt-benzene eluates gave 0.30 g. of 3-(3-oxo-1β,17β-dihydroxy-4-androsten-17α-yl)propanoic acid lactone (IIa), m.p. 222~226°, after crystallization from AcOEt. $[\alpha]_D$ +35.5°. UV λ_{max}^{MeOH} mμ (ε): 240 (14,600), shifts to 245 in 3 hr. in *N*KOH. IR λ_{max}^{KBr} μ: 2.89, 5.69, 5.96, 6.21. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (IIa): C, 73.71; H, 8.44. Found: C, 73.68; H, 8.30.

From the 60% eluates another 0.96 g. of Va was obtained. This compound was also obtained by fermentation of Ia with two *Gelasinospora* spp.

Fermentation with *Gelasinospora tetraspora*——The starting material (Ia), 10 g., was fermented with *Gelasinospora tetraspora* M 1114 (A. T. C. C. 14512) for 7.5 hr. by the general method. The residue from the extracts was washed with Et_2O and then crystallized three times from AcOEt-Et_2O to yield 90 mg. of 7α-hydroxy material (Va), m.p. 268~270° (gas evolution). $[\alpha]_D$ +56°. UV λ_{max}^{MeOH} mμ (ε): 241 (15,000), shifts in 24 hr. in *N*KOH in MeOH to 284.5 (21,300). IR λ_{max}^{KBr} μ: 2.91, 5.63, 6.03, 6.18. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (Va): C, 73.71; H, 8.44. Found: C, 73.84; H, 8.45.

The mother liquors were concentrated and chromatographed on 750 g. of silica gel. The 25% AcOEt-benzene eluates removed 1.76 g. of recovered Ia and the 35 and 40% eluates gave 0.43 g. of material which was crystallized from AcOEt to yield 3-(3-oxo-11β,17β-dihydroxy-4-androsten-17α-yl) propanoic acid lactone, m.p. 210~212.5°, identified by comparison of the IR with that of an authentic sample.[1] The 50% AcOEt eluates were concentrated and the residue, 0.44 g., was crystallized from Me_2CO-ligroin and then AcOEt to give the 6β-hydroxy compound (IVa), m.p. 216~217.5°. $[\alpha]_D$ +19°. UV λ_{max}^{MeOH} mμ (ε): 235.5 (13,900), shifts in 24 hr. in *N*KOH in MeOH to 257 (8450). IR λ_{max}^{KBr} μ: 2.85, 5.6, 5.94, 6.2. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (IVa): C, 73.71; H, 8.44. Found: C, 73.37; H, 8.56.

The 60% fractions were concentrated to 1.87 g. of residue which was crystallized from Me_2CO-ligroin to pure 3-(3-oxo-11α,17β-dihydroxy-4-androsten-17α-yl) propanoic acid lactone[1] whose identity

*[7] Cotton seed flour, 1 kg.; 5 kg. of commercial dextrose; 600 ml. of corn steep liquor; 15 g. of silicone antifoam; 50 ml. of conc. HCl and 300 L. of tap water.

was proven by comparison of IR spectra and a mixture melting point (no depression). Elution with more polar solvent mixtures yielded an additional 0.25 g. of Va.

3-(3-Oxo-14α,17β-dihydroxy-4-androsten-17α-yl)propanoic Acid Lactone (Xa)——Ten grams of Ia was fermented with *Phoma* sp. M 1210 for 48 hr. by the general method and the residue from the extraction was chromatographed on 1700 g. of silica gel. The early 30% AcOEt eluates afforded 0.37 g. of impure 3-(3-oxo-17β-hydroxy-5α-androstan-17α-yl)propanoic acid lactone which was crystallized from Me_2CO-ligroin to m.p. 170.5~171.5°, $[\alpha]_D$ +12.5°, IR identical with that of an authentic sample.[11] The later 30% fractions gave 4.53 g. of Ia, while the 50 and 60% fractions yielded 0.26 g. of the 5α-dihydro-14α-hydroxy derivative (XVIII), m.p. 217~221° (gas evolution). $[\alpha]_D$ +33°. IR λ_{max}^{KBr} μ: 2.9, 5.66, 5.83. *Anal.* Calcd. for $C_{22}H_{32}O_4$ (XVIII): C, 73.30; H, 8.95. Found: C, 73.24; H, 8.75.

Attempted oxidation with chromic acid*8 was unsuccessful. The 70% AcOEt eluates were concentrated to 0.46 g. of residue which was crystallized to give Xa, m.p. 205.5~208.5°. $[\alpha]_D$ +95°. UV λ_{max}^{MeOH}mμ (ε): 240.5 (15,500). IR λ_{max}^{KBr} μ: 2.79, 2.85, 5.65, 5.97, 6.18. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (Xa): C, 73.71; H, 8.44. Found: C, 73.31; H, 8.47.

3-(3-Oxo-2β,17β-dihydroxy-4-androsten-17α-yl)propanoic Acid Lactone (IIIa)——The starting material, Ia, 15 g., was incubated in a cotton seed flour medium*5 with *Tricoderma* sp., M 1254, by the general method described earlier for 31.5 hr. The extracts were concentrated and the residue was dissolved in benzene and chromatographed on 1 kg. of silica gel. From the 20% AcOEt-benzene eluates 6.05 g. of Ia was recovered and from the 35 and 50% eluates 1 g. of IIIa was obtained as a glass. $[\alpha]_D$ −26.5°. UV $\lambda_{max}^{0.1N\ KOH\ in\ MeOH}$ mμ (ε): 242 (12,900) shifts to 230 (14,300) in 24 hr. IR $\lambda_{max}^{CHCl_3}$ μ: 2.85, 5.65, 5.95, 6.2. *Anal.* Calcd. for $C_{22}H_{30}O_4$ (IIIa): C, 73.71; H, 8.44. Found: C, 73.96; H, 8.70.

3-(3,12-Dioxo-17β-hydroxy-4-androsten-17α-yl) propanoic Acid Lactone (XV)——The 12β-hydroxy compound (IXa), 0.52 g., was dissolved in 20 ml. of Me_2CO and to this solution was added dropwise with stirring 0.68 ml. of chromic acid solution.*8 The stirring was continued for 5 min. and then 5 drops of iso-PrOH was added, followed by 50 ml. of H_2O. This solution was concentrated under N_2 to remove Me_2CO. The precipitate which formed was separated by filtration and washed with H_2O to yield 320 mg. of crude XV. Crystallization from AcOEt produced pure material, m.p. 206~210°. $[\alpha]_D$ +112.5°. UV λ_{max}^{MeOH} mμ (ε): 238.5 (15,900). IR λ_{max}^{KBr} μ: 5.62, 5.85, 6.00, 6.20. *Anal.* Calcd. for $C_{22}H_{28}O_4$ (XV): C, 74.13; H, 7.92. Found: C, 73.78; H, 8.25.

3-(3,15-Dioxo-17β-hydroxy-4-androsten-17α-yl) propanoic Acid Lactone (XVI)——The15α-hydroxy compound (XIa), 130 mg., was oxidized with 0.17 ml. of the chromic acid reagent*8 as described above. The precipitate was crystallized first from AcOEt-hexane and then from MeOH to yield XVI, m.p. 198~201°. $[\alpha]_D$ +114°. UV λ_{max}^{MeOH} mμ (ε): 238 mμ (15,900). IR $\lambda_{max}^{CHCl_3}$ μ: 5.59, 5.70, 5.94, 6.17. *Anal.* Calcd. for $C_{22}H_{28}O_4$ (XVI): C, 74.13; H, 7.92. Found: C, 73.91; H, 7.90.

Similar oxidation of 140 mg. of the 15β-hydroxy compound (XIIa) with 0.17 ml. of the reagent*8 gave 25 mg. of XVI after two crystallizations from AcOEt-hexane, m.p. 196~201°, undepressed when mixed with material obtained from XIa.

3-(3,15-Dioxo-4,16-androstadien-17α-yl) propanoic Acid (XVII)——To a solution of 0.54 g. of XVI in 45 ml. of MeOH was added 5.4 ml. of *N* methanolic sodium methoxide. This solution was maintain undered N_2 for 10 min., diluted with 50 ml. of H_2O and then acidified with glacial AcOH. Next the solution was concentrated *in vacuo* until a solid formed. The mixture was cooled to 5° for 18 hr. and then filtered to yield 0.41 g. of crude XVII. Two crystallizations from MeOH gave material of m.p. 232~236° (decomp.). $[\alpha]_D$ +265° (MeOH). UV: λ_{max}^{MeOH} 236.5 mμ (ε 26,100). IR λ_{max}^{KBr} μ: 5.73, 5.88, 6.07, 6.20. *Anal.* Calcd. for $C_{22}H_{28}O_4$ (XVII): C, 74.13; H, 7.97. Found: C, 73.97; H, 7.86.

3-(3-Oxo-7β-acetoxy-17β-hydroxy-4-androsten-17α-yl)propanoic Acid Lactone (XIV)——The 7β-hydroxy compound (VIa), 0.7 g., was dissolved in 3 ml. of pyridine and 2 ml. of Ac_2O. The next day the solution was poured onto ice and a solid formed which was separated by filtration. The solid was recrystallized from Me_2CO-Et_2O to yield 0.6 g. of ester (XIV), m.p. 217~218°. $[\alpha]_D$ +69°. UV λ_{max}^{MeOH} mμ (ε): 238 (16,200). *Anal.* Calcd. for $C_{24}H_{32}O_5$ (XIV): C, 71.97; H, 8.05. Found: C, 72.03; H, 8.40.

3-(3-Oxo-7α-acetoxy-17β-hydroxy-4-androsten-17α-yl)propanoic Acid Lactone (XIII)——The hydroxy compound (Va) was acetylated to give (XIII), m.p. 152~156°, 214~216°. $[\alpha]_D$ 0. UV: λ_{max}^{MeOH} mμ (ε): 238 (16,550). IR λ_{max}^{KBr} μ: (high melting material) 5.62, 5.75, 6.0, 6.17, 7.97. *Anal.* Calcd. for $C_{24}H_{32}O_5$ (XIII): C, 71.97; H, 8.05. Found: C, 72.00; H, 7.87.

3-(3-Oxo-6β-acetoxy-17β-hydroxy-4-androsten-17α-yl)propanoic Acid Lactone——This compound was prepared by acetylation of IVa and had a m.p. 237.5~240.5°. $[\alpha]_D$ +12°. UV λ_{max}^{MeOH} mμ(ε): 235.5 (13,300). IR λ_{max}^{KBr} μ: 5.62, 5.72, 5.94, 6.13, 8.06. *Anal.* Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.69; H, 8.17.

3-(3-Oxo-12β-acetoxy-17β-hydroxy-4-androsten-17α-yl) propanoic Acid Lactone (XIX)——A solution of 350 mg. of Xa in 3.5 ml. of pyridine and 2.1 ml. of Ac_2O was heated on the steam bath for 1½ hr.,

*8 100 g. of CrO_3 per 500 ml. of 6*N* H_2SO_4.

11) J. A. Cella, E. A. Brown, R. R. Burtner: J. Org. Chem., **24**, 743 (1959).

and then poured into 80 ml. of ice and H_2O. The resulting precipitate was separated by filtration, washed with H_2O, dried and crystallized twice from AcOEt to yield 51 mg. of XIX, m.p. 234~236°. IR λ_{max}^{KBr} μ : 5.65, 5.76, 5.98, 6.19. *Anal.* Calcd. for $C_{24}H_{32}O_5$ (XIX) : C, 71.97; H, 8.05. Found : C, 71.90; H, 8.14.

3-(1,3-Dioxo-17β-hydroxy-4-androsten-17α-yl)propanoic Acid Lactone (XX)——To a solution of 100 mg. of 1β-hydroxy compound (IIa) in 5 ml. of Me_2CO was added 0.14 ml. of chromic acid reagent.*[8] Stirring was continued for 2 min. more and then 20 ml. of H_2O was added. The precipitate was separated by filtration to yield 40 mg. of XX which was crystallized from AcOEt to yield the hemihydrate (after drying 2 hr. *in vacuo* at 153°), m.p. 143°. UV λ_{max}^{MeOH} mμ (ε) : 225, 243 (10,950), 289. IR $\lambda_{max}^{CHCl_3}$ μ : 5.65, 5.80, 5.95, 6.18. λ_{max}^{KBr} μ : 3.08, 3.76, 3.90, 5.65, 6.05, 6.39. *Anal.* Calcd. for $C_{22}H_{28}O_4 \cdot \frac{1}{2}H_2O$ (XX) : C, 72.29; H, 7.99. Found : C, 71.96; H, 7.95.

3-(3-Oxo-10β,17β-dihydroxy-4-estren-17α-yl)propanoic Acid Lactone (VIIIa)——The 19-nor compound (Ib) was fermented by another method. *Gliocladium* sp. M 30-128 was grown for 48 hr. in a 20 L. bottle in hydrolyzed casein medium*[9] which had been sterilized at 120° for 1/2 hr. Sterile air was bubbled through the medium to provide agitation and aeration with no attempt made to measure the amount of air. The steroid (Ib), 5 g., in 100 ml. of Me_2CO was added to the medium and incubation was continued for 47 hr. at room temperature. The mixture was extracted with 5 L. of methylene chloride which was concentrated. The residue was dissolved in benzene and chromatographed on 450 g. of silica gel. Recovered Ib, about 1 g., was eluted with 20% AcOEt-benzene. From the early 35% eluates 0.08 g. of crystalline (VIIIb) was obtained by crystallization from Me_2CO-Et_2O-pentane, m.p. 208~211°. UV λ_{max}^{MeOH} mμ(ε): 234.5 (15,850), shifts with acid to 278, 287 (1600). IR λ_{max}^{KBr} μ : 2.9, 5.6, 5.98. *Anal.* Calcd. for $C_{21}H_{28}O_4$ (VIIIb) : C, 73.22; H, 8.19. Found : C, 73.39; H, 8.30.

The major product was eluted with 60% AcOEt-benzene and appeared to be a mixture of two monohydroxy derivatives of Ib which could not be induced to crystallize nor separated by repeated chromatography.

3-(3-Oxo-15β,17β-dihydroxy-4-estren-17α-yl)propanoic Acid Lactone (XIIb)——*Fusarium* sp. M 1632 was grown for 16 hr. in 30 L. of hydrolyzed casein medium (same composition as described earlier*[9]) in a stainless steel fermentor

The substrate was 10 g. of Ib and it was incubated for 40.5 hr. The mixture was extracted with 20 L. of methylene chloride and the extracts were concentrated. The residue was washed twice with ligroin, dissolved in benzene and chromatographed on 700 g. of silica gel. From the 20% AcOEt-benzene eluates, 2.5 g. of Ib was recovered. When the 60% fractions were concentrated, two crops of XIIb were obtained : 0.55 g., m.p 228~231, and 0.2 g., m.p. 224~229°. The higher-melting meterial was analyzed. $[\alpha]_D$ −4°. UV λ_{max}^{MeOH} mμ(ε): 239 (16,700). IR λ_{max}^{KBr} μ : 2.88, 5.67, 5.97, 6.17. *Anal.* Calcd. for $C_{21}H_{28}O_4$ (XIIb) : C, 73.22; H, 8.19. Found : C, 73.14; H, 8.24.

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Summary

The preparation, by microbiological methods, of some derivatives of 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propanoic acid lactone, hydroxylated at 1β, 2β, 6β, 7α, 7β, 9α, 11α, 11β, 12β, 14α, 15α, or 15β, is described as well as of two 19-nor compounds. Some of these compounds block the effects of deoxycorticosterone acetate on the rat kidney.

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*[9] Hydrolyzed casein, 65 g., 162 g. of commercial dextrose, 10 ml. of corn steep liquor, 3.9 ml. of conc. HCl, 5 g. of silicone antifoam and 13 L. of tap water.