Mechanisms of Steroid Oxidation by Microorganisms. IV. Seco Intermediates*

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Exposure of androst-4-ene-17βol-3-one to *Nocardia restrictus* gave five new products in addition to the known 3-hydroxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (I) and 3aα-H-4α-[3'-propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II). The structures of 3,9α-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (III), m.p. 195–197°, $[\alpha]_D^{28}$ +48° (EtOH), λ 280 mμ (ϵ 2,450), λ 2.87, 3.04, 5.81, 6.15, 6.28, 6.65, 11.50, and 12.29 μ (Nujol), and 3,9β-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (IV), m.p. 155–157°, $[\alpha]_D^{28}$ +90.3° (EtOH), λ 280 mμ (ϵ 2,400), λ 3.02, 5.75, 6.15, 6.28, 6.63, 11.46, and 12.31 μ (Nujol), was established by conversion to 3-methoxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (VII). The configuration of the 9-hydroxyl group in these steroids was determined from their nuclear magnetic resonance spectra. 3,9β,17β-Trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (IX), m.p. 171.5–172.5°, $[\alpha]_D^{28}$ +19° (EtOH), λ 280 mμ (ϵ 2,200), λ 2.90, 3.03, 6.18, 6.29, 6.68, 11.47, and 12.18 μ was synthesized by the sodium borohydride reduction of 3,9β-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (IV). The structures of 3aα-H-4α-[3'-propionic acid]-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XII), m.p. 124–127°, $[\alpha]_D^{27}$ +23.6° ($CHCl_3$) and 3aα-H-4α-[3'-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII), m.p. 98–104°, were established by oxidation with chromic acid in acetic acid to 3aα-H-4α-[3'-propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II). Hydrogenation of 3aα-H-4α-[3'-propionic acid]-5-hydroxy-7aβ-methyl-3a,4,7,7a-tetrahydro-1-indanone-δ-lactone (XIV) afforded 3aα-H-4α-[3'-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII), m.p. 128.5–130.5°, $[\alpha]_D^{27}$ +52.6° ($CHCl_3$), λ 5.73 μ.

The initial degradative reactions of androst-4-ene-3,17-dione by microorganisms involve a 9α-hydroxylation followed by a 1,2- dehydrogenation (or vice versa) with the formation of 3-hydroxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (I) (Dodson and Muir, 1961). The latter 9,10-secophenol (I) was shown to be oxidized to 3aα-H-4α-[3'-propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II) (Sih and Wang, 1963). Our continued interest in the mechanism of steroid degradation by microorganisms prompted a systematic search for new intermediates in the fermentation broth of *Nocardia restrictus*. Schubert *et al.* (1960) reported the isolation of a phenolic steroid in low yields after exposure of androsterone or dehydroepiandrosterone to *Mycobacterium smegmatis*. On the basis of its infrared and ultraviolet spectra, they partially characterized this compound as 3,9ϵ-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one. 3,17β-Dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-9-one has been previously reported by Dodson and Muir (1961). This paper reports the isolation and characterization of 3,9α-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (III), 3,9β-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (IV), 3,9β,17β-trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (IX), 3aα-H-4α-[3'-propionic acid]-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XII), and 3aα-H-4α-[3'-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII) from the fermentation broth of *Nocardia restrictus*.

Incubation of androst-4-ene-3-one-17βol with *N. restrictus* produced, besides I and II, five additional products (scheme I). The first two products III (m.p. 195–197°) and IV (m.p. 155–157°), were recognized as phenols initially by their ultraviolet spectra $\lambda_{max}^{CH_3OH}$ 280 mμ (ϵ 2,400). The infrared spectrum of III showed bands at 2.87 and 3.04 μ (hydroxyl); 5.81 μ (5-membered ring ketone); and 6.15, 6.28, 6.65, 11.50, and 12.29 μ (aromatic ring and attached hydrogens). The infrared spectrum of IV exhibited bands at 3.02, 5.75, 6.15, 6.28, 6.63, 11.46, and 12.31 μ. Carbon-hydrogen analyses of both III and IV afforded figures in good agreement with $C_{19}H_{26}O_3$. From the foregoing physical constants it was suspected that the 9-keto function in I had been reduced to yield epimeric alcohols III and IV. The following series of reactions was used to establish their structures: Methylation of III and IV with methyl iodide and potassium carbonate in acetone afforded their corresponding methyl ethers, V and VI. Oxidation of V and VI with pyridine–chromic acid afforded 3-methoxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (VII), identical in all respects to a sample obtained by methylation of I. The NMR[1] spectrum of III exhibited bands[2] at 5.90 τ (1 H, doublet, 9-H), 6.68 τ (1 H, doublet J, 2 cps 9-OH), and IV at 6.45 τ (1 H, quadruplet, J's, 3,4,6 cps 9-H), 6.70 τ (1 H, doublet, J 2.5 cps 9-OH). It is well documented that axially oriented protons are generally seen at higher fields than the equatorial protons in rigid systems. Also, J_{aa} (the coupling constant between two axially oriented protons) is in the order of 5–9 cps while $J_{ae} \cong J_{ee} \cong$ 2–3 cps (J_{ae} and J_{ee} denoting the axial-equatorial and the equatorial-equatorial coupling constants, respectively; Jardetzky and Jarketzky, 1962). In addition, on paper chromatograms relative mobility of 0.9 that of III, which is in agreement IV has a with Savard's rule that equatorial hydroxyls are generally more polar than axial ones (Savard, 1953). On the basis of these facts the structure of III was assigned as 3,9α-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one and IV as 3,9β-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one. If the oxidation of V or VI were performed using chromic acid in acetic acid, the major product in the reaction mixture was found to be VIII, which was assigned the structure, 3-methoxy-

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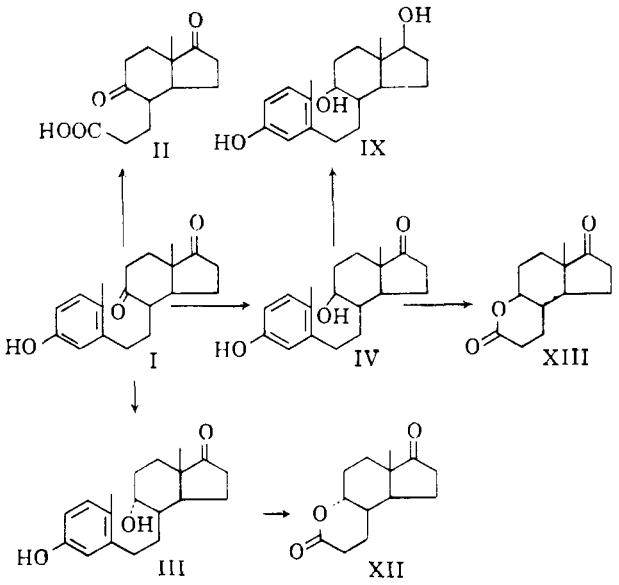
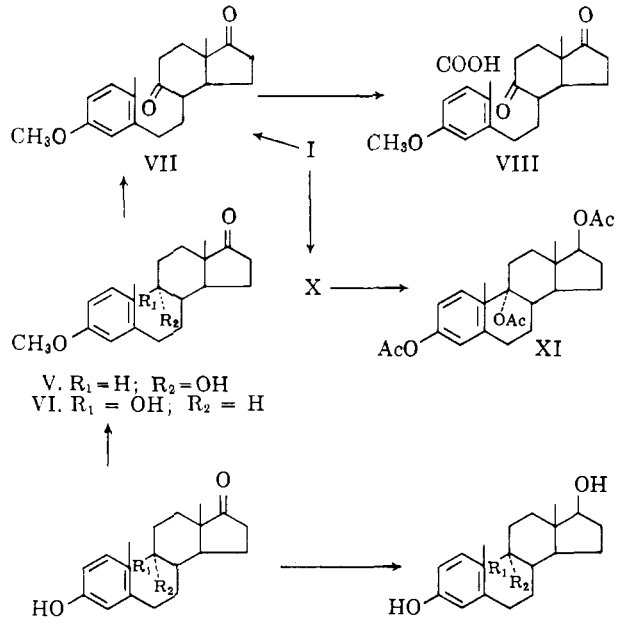
[1] Abbreviation used in this work: NMR, nuclear magnetic resonance.

[2] The fact that the observed resonance does not appear as a clearly defined multiplet is probably due to the fact that the coupling constants with the three adjacent protons are not exactly the same, so that the pattern becomes complicated and badly smeared out. (We thank Dr. D. P. Hollis of Varian Associates for this interpretation).



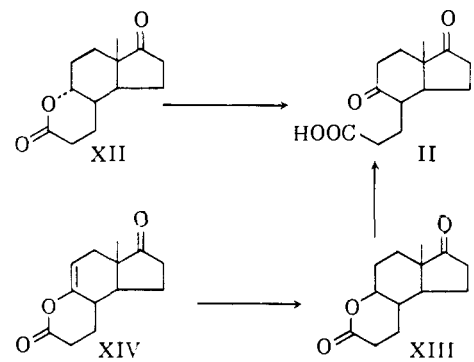
SCHEME I



19 - carboxy - 9,10 - seco - androsta - 1,3,5(10) - triene-9,17-dione (VIII) on the basis of its elemental analysis; ultraviolet spectrum $\lambda_{max}^{CH_3OH}$ 220 mμ (ε 15,400) and 254 mμ (ε 11,800); infrared spectrum (5.74, 5.82, 5.90, 6.20, 6.34, 6.65 μ); its salt in Nujol showed a band at 6.32 μ and its NMR spectrum showed the absence of a band at 7.78τ, characteristic for CH_3 on an aromatic ring.

The third product, IX (mp 171–172°), was assigned the structure, 3,9β,17β-trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (IX) from the following data: Ultraviolet spectrum, $\lambda_{max}^{CH_3OH}$ 280 mμ (ε 2,200) is typical of phenols; its infrared spectrum showed bands at 2.90 and 3.03 μ (hydroxyl), 6.18, 6.29, and 6.68 μ (aromatic ring). Carbon-hydrogen analysis was consistent with $C_{19}H_{28}O_3$. Reduction of IV with $NaBH_4$ gave a product identical in all respects with IX obtained from fermentation. When I was reduced with $NaBH_4$, 3,9α,17β-trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (X) was formed. Its acetate (XI) was identical to a compound obtained by $NaBH_4$ reduction and acetylation of III. It is interesting to note that the reduction of the 9-keto function with $NaBH_4$ gave predominantly the 9α-epimer.

The fourth product, XII (mp 122–124°), was initially recognized as a lactone by virtue of its solubility in NaOH, and the infrared spectrum of its salt showed a band at 6.30 μ (COO^-). Molecular weight analysis gave values of 220 and 216 in two separate determinations, and the carbon-hydrogen analysis was in good agreement with $C_{13}H_{18}O_3$. Oxidation of XII with chromic acid in acetic acid afforded II. The NMR spectrum of XII showed a band at 5.45 τ (1 H, quadruplet, J, 2.0 cps) which indicates that the proton on the same carbon as the oxygen atom bears the equatorial configuration. These results establish the structure of XII as 3aα-H-4α-[3′-propionic acid]-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone. The fifth product, XIII (mp 98–104°), was not obtained in pure form even after repeated chromatography, fractional crystallization, and sublimation. Oxidation of XIII with chromic acid in acetic acid also gave II. The infrared spectrum was almost identical to a sample obtained by hydrogenation of XIV. The NMR spectrum exhibited a band at 6.10 τ (1 H, quadruplet, J's 2.5, 5.0, 5.0 cps) which indicates that the proton on the same carbon atom as the oxygen bears the axial configuration. From these data, XIII probably consisted of a mixture mainly of 3aα,H-4α-[3′-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone and a small amount of XII. Since the mp of XIII, prepared by catalytic reduction (mp 128.5–130.5°) differs widely from the mp of XIII isolated from the fermentation (mp 98–104°) but is close to that of XII isolated from fermentation (mp 124–127°), the infrared spectra of these three compounds are included, as shown in Figure 1a,b, and c, to facilitate the identity of the two compounds with such widely different physical properties.



To ascertain whether these compounds are obligate intermediates in the over-all degradative pathway, I, III, and IV were incubated with *N. restrictus* under identical conditions. Table I shows that the relative rates of disappearance of these three compounds were approximately the same; in fact, I was oxidized slightly faster than III or IV. On paper chromatograms, I was noted to be converted into both III and IV and the reaction is a reversible one; III and IV were further converted into XII and XIII. These results suggest that products III and IV probably are not obligate intermediate in the metabolism of I but rather represent intermediates resulting from side reactions which could also be metabolized in a manner similar to that for I. Apparently the 9-keto function

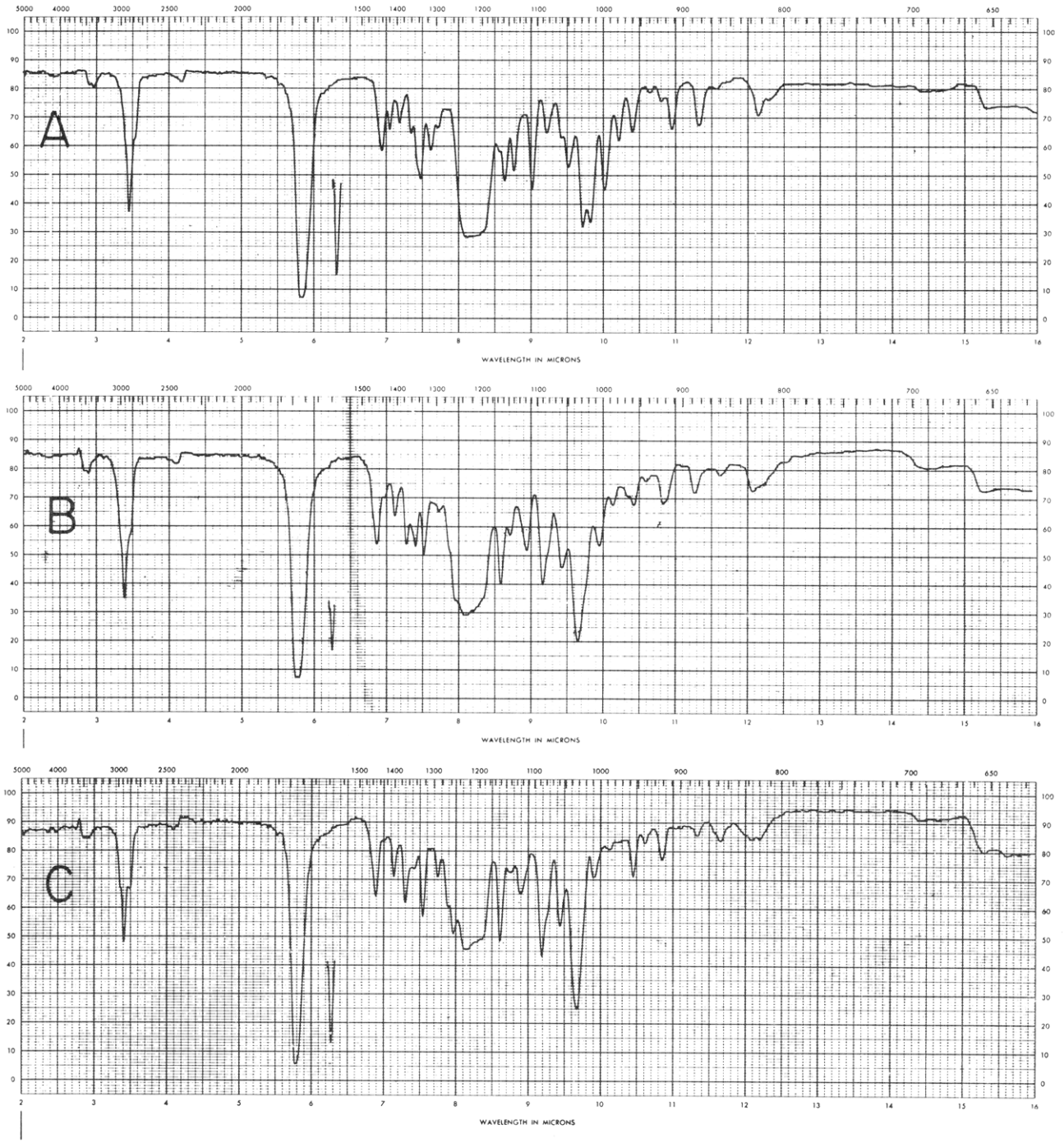


FIG. 1.—(a) The infrared spectrum (in $CHCl_3$) of 3aα-H-4α-[3′-propionic acid]-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XII) (mp 124–127°). (b) The infrared spectrum (in $CHCl_3$) of 3aα-H-4α-[3′-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII) (mp 98–104°) obtained from fermentation. (c) The infrared spectrum (in $CHCl_3$) of 3aα-H-4α-[3′-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII) (mp 128.5–130.5°) obtained by hydrogenation of the enol lactone (XIV).

is unimportant in the oxidative degradation of the aromatic ring.

EXPERIMENTAL

Melting points, determined on a Thomas-Hoover melting point apparatus, are corrected. Ultraviolet absorption spectra were determined on a Cary Model 11 MS recording spectrophotometer and methanol was used as solvent. Infrared spectra were recorded on a Beckman IR 5A double-beam infrared recording spectrophotometer. Microanalyses were carried out by Mr. J. Alicino of Metuchen, N. J. Molecular weights were determined on a Mechro-Lab osmometer. All NMR spectra were determined on a Varian Associates recording spectrometer (A60) at 60 mc in either deuterated chloroform or deuterated acetone. Chemical shifts are reported in τ values (ppm; Tiers, 1958). The paper chromatographic system used throughout this work consisted of toluene–propylene glycol (Zaffaroni *et al.*, 1950). "Petroleum ether" refers to the fraction of bp 60–80°. Silica gel (Mallinckrodt 2847) was washed with acetone-ether (2:1) and dried at 90–100°.

Fermentation of Androst-4-ene-17βol-3-one.—Nocardia restrictus No. 545 (Sih and Wang, 1963) was grown

TABLE I

THE RELATIVE RATE OF METABOLISM OF 9,10-SECOPHENOLS BY *N. Restrictus*[a]

Time (hours)	μMoles of 9,10-Secophenols		
	9-Keto	9α-Hydroxy	9β-Hydroxy
0	42	42	42
2	34	37	39
3	29	32	36
7	16	—	29
9	12	29	24
12	9	15	16

[a] *N. restrictus* was grown in 250-ml Erlenmeyer flasks containing 50 ml of nutrient broth. After 24 hours, 42 μmoles of the respective 9,10-secophenols in 0.2 ml of dimethylformamide was added. At the indicated time intervals, 4-ml samples were withdrawn, acidified with 2 drops of glacial acetic acid, and extracted with 1 ml of chloroform. After thorough mixing and centrifugation, 0.2 ml of the chloroform layer was diluted with 2.8 ml of chloroform and the absorbance at 280 mμ was taken as the measure of the phenol concentration.

in 6.0 liters of Difco nutrient broth (fifteen 2-liter Erlenmeyer flasks) at 25° on a rotary shaker. After 24 hours of incubation, 18 g of androst-4-ene-17β-ol-3-one dissolved in 120 ml of dimethylformamide was distributed equally among the fifteen flasks. After 72 hours the culture broth was acidified with glacial acetic acid to a *p*H of 2.0 and was extracted three times with three 2-liter portions of chloroform. *It is desirable to use acetic acid for pH adjustment because hydrochloric acid will cause the cyclization of the 9,10-secophenol (I) to 1-hydroxy-4-methyl-1,3,5(10),9(11)-estratetraene-17-one.* The combined chloroform extract was dried over sodium sulfate and taken to dryness to give 9.83 g of solids. The residue was taken up in 600 ml of benzene-ether (1:1) and extracted with three 100-ml portions of 6% $NaHCO_3$; the benzene-ether layer was further extracted three times with three 100-ml portions of 5% NaOH. The bicarbonate fraction was acidified with hydrochloric acid and extracted three times with three 100-ml portions of chloroform. The chloroform layer was washed with water, dried over sodium sulfate, and concentrated to dryness to yield 3.1 g of residue. The residue was taken up in ether–petroleum ether and seeded with a few crystals of the acid (II). Two recrystallizations from acetone–petroleum ether afforded 1.95 g of 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II) (mp 109–110°). The sodium hydroxide fraction was acidified with glacial acetic acid and extracted with 150-ml portions of chloroform three times. The chloroform extract was washed with water, dried over sodium sulfate, and taken to dryness to give 2.85 g of residue. The residue was dissolved in 10 ml of chloroform and chromatographed over a silica gel–Celite (90:10) column (2.5 × 45 cm); 10-ml fractions were collected every 10 minutes. Elution with chloroform (fraction 53-74) afforded 802 mg of a mixture, mp 95–125°. Repeated fractional crystallization from acetone–petroleum ether gave 300 mg of 3aα-H-4α-[3′-propionic acid]-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XII), (mp 122–125°); after sublimation, mp 124–127°; $[\alpha]_D^{27}$ +23.6° (*c*, 1.06 in $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.73 μ; its sodium salt in Nujol showed bands at 3.01, 5.73, and 6.30 μ; mw 220 and 216.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 69.93; H, 7.91.

The mother liquor, after repeated crystallization from acetone–petroleum ether, gave 60 mg of 3aα-H-4α-[3′-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII) (mp 98–104°); $\lambda_{max}^{CHCl_3}$ 5.73 μ; its sodium salt in Nujol exhibited bands at 3.02, 5.74, and 6.34 μ.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.59; H, 8.72.

Fraction 83–139 contained the 9,10-secophenol (I). Two recrystallizations from acetone–petroleum ether afforded 80 mg of 3-hydroxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (I) (mp 122–124°). After fraction 120, the mobile phase was changed to a mixture of $CHCl_3$–MeOH (99:1). Fraction 193–208 afforded 80 mg of 3,9α-dihydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (III) after recrystallization from acetone–petroleum ether; mp 195–197°; $[\alpha]_D^{28}$ +48° (*c*, 1.03 in 95% EtOH); λ_{max} 280 mμ (ε 2,450); λ_{max}^{Nujol} 2.87, 3.04, 5.81, 6.15, 6.28, 6.65, 11.50, and 12.29 μ.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.92; H, 8.75.

Further elution of the column (fraction 225–367) gave 1.4 g of 3,9β-dihydroxy-9,10-seco-androsta-1,3,5-(10)-triene-17-one (IV) (mp 155–157°) after two recrystallizations from acetone–petroleum ether; $[\alpha]_D^{28}$ +90.3° (*c*, 0.96 in 95% EtOH); λ_{max} 280 mμ (ε 2,400); λ_{max}^{Nujol} 3.02, 5.75, 6.15, 6.28, 6.63, 11.46, and 12.31 μ.

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.56; H, 8.65.

After fraction 300, the mobile phase was changed to a mixture consisting of $CHCl_3$–MeOH (95:5). Fraction 477–497 contained the dihydroxyphenol (IX). After two recrystallizations from acetone–petroleum ether, 58 mg of 3,9β,17β-trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (IX) (mp 171.5–172.5°) was obtained; $[\alpha]_D^{28}$ +19° (*c*, 0.79 in 95% EtOH); λ_{max} 280 mμ (ε 2,200); λ_{max}^{Nujol} 2.90, 3.03, 6.18, 6.29, 6.68, 11.47, and 12.18 μ.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.97; H, 9.27. Found: C, 74.92; H, 9.27.

3-Methoxy-9α-hydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (*V*).—A solution of 160 mg of III in 7 ml of acetone was treated with 1.6 ml of methyl iodide and 2 g of anhydrous potassium carbonate. The resulting mixture was heated under reflux for 18 hours. The reaction mixture was then evaporated to dryness; the residue was taken up in benzene-ether (1:1) mixture and washed with 5% NaOH, followed by water. The residue was chromatographed over a cellulose powder column (Sih and Bennett, 1960) (1.8 × 30 cm) using propylene glycol as the stationary phase. Elution with cyclohexane afforded 157 mg of V (recrystallized from acetone–petroleum ether; mp 103–105°); $[\alpha]_D^{27}$ +44.8 (*c*, 1.0 in $CHCl_3$); λ_{max} 277 mμ (ε 2,150) and 284 mμ (ε 1,900); $\lambda_{max}^{CHCl_3}$ 2.77, 2.86, 5.75, 6.19, 6.31, and 6.65 μ.

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 76.07; H, 9.14.

3-Methoxy-9β-hydroxy-9,10-seco-androsta-1,3,5(10)-triene-17-one (*VI*).—A solution of 124 mg of IV in 6 ml of acetone was treated with 0.6 ml of methyl iodide and 1.3 g of anhydrous potassium carbonate. After refluxing the reaction mixture for 18 hours, it was evaporated to dryness. The residue was taken up in benzene-ether mixture (1:1) and washed with 5% NaOH and water. The infrared spectrum of the residue in $CHCl_3$ showed bands at 2.78, 2.83, 5.75, 6.18, 6.30, and 6.66 μ. Since many attempts to crystallize this compound have failed, and the product appeared to be homogeneous on paper chromatograms, the residue was used directly for subsequent oxidation.

3-Methoxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (*VII*).—(a) A solution of 1.044 g of the 9,10-secophenol (II) in 50 ml of acetone was treated

with 10 ml of methyl iodide and 10 g of anhydrous potassium carbonate. The resulting suspension was heated under reflux for 18 hours. The reaction mixture was then evaporated to dryness; the residue was taken up in benzene-ether (1:1) mixture, and extracted with 5% NaOH followed by water. The benzene-ether layer was dried over sodium sulfate and evaporated to dryness. Two recrystallizations from acetone–petroleum ether afforded 610 mg of the methyl ether, VII (mp 97–98.5°); $[\alpha]_D^{28}$ +101.6° (*c*, 1.03 in $CHCl_3$); λ_{max} 279 mμ (ε 2,060) and 285 mμ (ε 1,890); $\lambda_{max}^{CHCl_3}$ 5.74, 5.85, 6.20, 6.32, and 6.65 μ.

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.22; H, 8.10.

(b) To 90 mg of V in 25 ml of pyridine was added 80 mg of chromic trioxide and the reaction mixture was left at room temperature for 3 hours. Ethanol was added to destroy the excess chromic acid. The reaction mixture was then diluted with water and extracted with chloroform. The combined chloroform extract was dried over sodium sulfate and taken to dryness. The oily residue was chromatographed over a cellulose-powder column (1.8 × 28 cm) using propylene glycol as the stationary phase; 5-ml fractions were collected every 5 minutes. Elution with cyclohexane (fractions 6–16) afforded 60 mg of VII (mp 96–97.5°) after two crystallizations from acetone–petroleum ether. It was found to be identical in all respects (mmp and infrared spectrum) to that obtained by methylation of I.

(c) When 80 mg of VI was oxidized with chromic trioxide using the same conditions, 36 mg of VII (mp 96–97°) was obtained.

3-Methoxy-19-carboxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (VIII).—To 206 mg of the 9,10-secophenol methyl ether (VII) in 150 ml of 95% acetic acid was added 155 mg of chromic trioxide. The mixture was allowed to stand at room temperature for 3.5 hours. After the excess chromic acid was destroyed with ethanol, the mixture was diluted with 1 liter of water and extracted with chloroform. The combined chloroform extract was dried over sodium sulfate and taken to dryness. The residue was chromatographed over a column (2.5 × 40 cm) consisting of silica gel–Celite (90:10). Chloroform was used as the eluent and 10-ml fractions were collected. Fractions 43–56 consisted of the starting material and fractions 78–105 contained the carboxylic acid, VIII. Two crystallizations from acetone–petroleum ether gave 40 mg of VIII (mp 155.5–156.5°); $[\alpha]_D^{26}$ +96.2° (*c*, 0.86 in chloroform); λ_{max} 220 mμ (ε 15,400) and 254 mμ (ε 11,800); $\lambda_{max}^{CHCl_3}$ 5.74, 5.82, 5.90, 6.20, 6.34, and 6.65 μ.

Anal. Calcd. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.63; H, 6.92.

3,9β,17β-Trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (IX).—Seventy-one mg of IV in 4.5 ml of methanol was treated with 26 mg of $NaBH_4$ in 1.5 ml of methanol. The reaction mixture was left standing for 16 hours at room temperature. The excess $NaBH_4$ was decomposed with acetic acid and the solvent was evaporated to dryness. The residue was taken up in water and extracted with chloroform. The chloroform extract was dried over sodium sulfate and evaporated to dryness. Four crystallizations from acetone–petroleum ether afforded 26 mg of IX (mp 171.5–172.5°), identical in all respects with a sample of IX obtained by fermentation.

3,9α,17β-Trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (X).—(a) To 95 mg of the 9,10-secophenol (I) in 7 ml of methanol was added 50 mg of $NaBH_4$ in 3 ml of methanol and the mixture was allowed to stand for 16 hours at room temperature. The excess $NaBH_4$ was destroyed by the addition of 1 ml of glacial acetic acid and the solvent was removed *in vacuo*. The residue was taken up in 40 ml of water and extracted with chloroform. The chloroform extract was dried over sodium sulfate and evaporated to dryness. Two recrystallizations from acetone–petroleum ether gave 30 mg of X (mp 147–148.5°); $[\alpha]_D^{28}$ −11.1° (*c*, 0.93 in 95% EtOH): λ_{max} 280 mμ (ε 2,310); λ_{max}^{Nujol} 2.91, 3.03, 6.17, 6.28, 6.67, 11.45, and 12.43 μ.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.97; H, 9.27. Found: C, 74.70; H, 9.58.

(b) To 102 mg of III in 5 ml of methanol was added 37 mg of $NaBH_4$ in 2 ml of methanol and the reaction mixture was left standing for 16 hours at room temperature. The excess borohydride was destroyed by the addition of 1 ml of glacial acetic acid and the mixture was evaporated to dryness under nitrogen. The residue was taken up in 30 ml of water, and after acidification the aqueous solution was extracted with three successive 20-ml portions of chloroform. The combined chloroform extract was dried over sodium sulfate and taken to dryness. Two crystallizations from acetone–petroleum ether gave 50 mg of X (mp 192.5–193.5°); λ_{max} 280 mμ (ε 2,325); λ_{max}^{Nujol} 2.90, 3.02, 6.17, 6.28, 6.66, 11.45, and 12.43 μ.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.97; H, 9.27. Found: C, 74.71; H, 9.37.

The infrared spectrum of this material in Nujol differed from that prepared by $NaBH_4$ reduction of I. However, acetates prepared from the two samples were identical. From this we concluded that 3,9α-17-trihydroxy-9,10-seco-androsta-1,3,5(10)-triene (X) exists in two polymorphic forms.

3,9α,17β-Triacetoxy-9,10-seco-androsta-1,3,5(10)-triene (XI).—Nine hundred mg of the 9,10-seco triol (X) was dissolved in 12 ml of pyridine and 12 ml of acetic anhydride. The reaction mixture was left standing at room temperature for 16 hours. After the solvents were removed *in vacuo*, the residue was crystallized from acetone–petroleum ether to yield 850 mg of XI (mp 107.5–109°); $[\alpha]_D^{27}$ +25.4° (*c*, 1.1 in $CHCl_3$); λ_{max} 267 mμ (ε 600) and 273 mμ (ε 600); $\lambda_{max}^{CHCl_3}$ 5.79, 8.10 μ (acetates); 6.21, 6.31, 6.68, 11.05, and 12.22 μ.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.36; H, 7.86.

3aα-H-4α-[3'-Propionic Acid]-7aβ-methylhexahydro-1,5-indanedione (II).—(a) To 90 mg of the lactone, XII, in 5 ml of 95% acetic acid was added 44 mg of chromic trioxide and the reaction mixture was allowed to stand at room temperature for 1 hour. Ethanol was then added to destroy the excess chromic acid and the mixture was extracted with chloroform. The combined chloroform extract was washed with sodium bicarbonate and water, dried over sodium sulfate, and evaporated to dryness. The residue was chromatographed over a silicic acid column (2 × 27.5 cm). Elution with chloroform-methanol (99.5:0.5) afforded 70 mg of the acid, II (mp 109–110.5°), after two crystallizations from ether. The infrared spectrum was superimposable with an authentic sample of II.

(b) When the same chromic trioxide oxidation procedure was repeated using 40 mg of the lactone, XIII, 20 mg of the acid, II (mp 109–110°), was obtained.

3aα-H-4α-[3'-Propionic Acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII).—To 185 mg of the enol lactone (XIV) in 10 ml of 95% ethanol was added 184 mg of 10% palladium-on-carbon and the mixture was stirred under hydrogen at atmospheric pressure and 23°. After 3 hours 1.1 molar equivalents of hydrogen had been absorbed. The suspension was

filtered and the filtrate was evaporated to dryness. The residue was chromatographed over a cellulose-powder column (1.8 × 33 cm) using propylene-glycol as the stationary phase. Elution was effected with cyclohexane-toluene (7:3) and 5-ml fractions were collected. Fractions 88–120 afforded 80 mg of XIII after two crystallizations from acetone–petroleum ether (mp 128.5–130.5°); $[\alpha]_D^{27}$ +52.6° (c, 1.09 in $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 5.73 μ.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 69.90; H, 7.91.

Added in Proof

Since this manuscript was accepted we have isolated pure 3aα-H-4α-[3′-propionic acid]-5β-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone (XIII) from the fermentation broth of *N. restrictus*. It has been shown to be identical in all respects (mmp and infrared spectrum) to a sample of XIII obtained by hydrogenation of the enol lactone (XIV).

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Isolation and Characterization of Human Urinary Metabolites of Aldosterone. IV. The Synthesis and Stereochemistry of Two Bicyclic Acetal Metabolites*

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The synthesis of the urinary metabolite of aldosterone, 3α-hydroxypregnane (11β-18S)(18S-20α) dioxide, M1, reported in this paper, serves to confirm the structure previously proposed on the basis of spectroscopic and degradative evidence, and to establish the configurations at C_{18} and C_{20} as well. The synthesis of a second urinary metabolite of aldosterone, 3α,21α-dihydroxypregnane (11β-18S)(18S-20α) dioxide, M8, also reported here, establishes the configuration at C_{18} but not at C_{20}. However, spectroscopic and chemical evidence support the assignment of the 20α configuration to M8. The synthesis and manipulation of 3α,11β,20α-trihydroxypregnan-18-al (18 → 11β) hemiacetal strongly suggest that the bicyclic acetal metabolites, M1 and M8, are not artifacts of the isolation procedure.

The isolation from human urine and the characterization of two metabolites of aldosterone, 3α-hydroxypregnane-(11β,18)(18,20) dioxide, M1, and 3α,21-dihydroxypregnane-(11β,18)(18,20) dioxide, M8, bearing a bicyclic acetal structure, have been reported (Kelly *et al.*, 1962a). This report concerns the confirmation of these structures by synthesis and the elucidation of the stereochemical configurations at C_{18} and C_{20} in these metabolites.

The synthesis of the metabolites was based on the work of Beal and Pike (1960) and Schmidlin and Wettstein (1962). Recently the latter investigators have described the synthesis of both of the C_{20} epimers of 3-keto-Δ^4-pregnene (11β,18S)(18S,20) dioxide (1α and 1β) (Figure 1). These authors have shown that reduction of (18 → 11) lactone-20-ketosteroids with $LiAlH_4$ leads directly to a mixture of approximately equal amounts of the C_{20} epimers, 1α and 1β. On the other hand, treatment of (18 → 20)-lactone-11-ketosteroids with $LiAlH_4$ gives the (18 → 11β) hemiacetal-20-ol (analogous to (6) in Fig. 2) with retention of configuration at C_{20}. Each of the epimeric hemiacetal-20-ols could then be converted to the corresponding

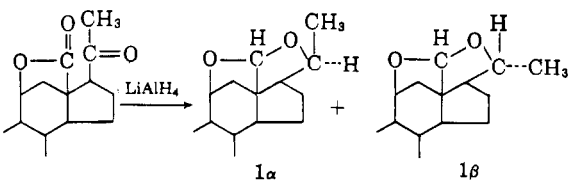

Fig. 1.—Synthesis of bicyclic acetals (Schmidlin and Wettstein, 1962).

bicyclic acetal (1α or 1β), with retention of configuration at C_{20} in each case. Beal and Pike (1960) independently prepared (1β) from 3α-acetoxypregnane-(11β,18S)(18S,20β)-dioxide ((2) in Fig. 2) which in turn was prepared from the known 20β-hydroxy derivative, 3α-acetoxy-20β-hydroxypregnan-11-one. The infrared spectra of (1β) prepared by Beal and Pike (1960) and of (1β) prepared by Schmidlin and Wettstein (1962) were identical (Beal and Pike, personal communication).

Comparison (infrared spectra, nuclear magnetic resonance [NMR][1] spectra, and melting point) of (2) with the acetate of M1 (see Figure 2) established that these two were different compounds. Oxidation of M1 acetate and of (2) with CrO_3 in acetic acid (Kelly *et al.*, 1962a) gave rise in each case to 3α-acetoxy-11β-hydroxy-20-ketopregnane-18-oic (18 → 11β) lactone

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[1] Abbreviation used in this work: NMR, nuclear magnetic resonance.