metabolic rate and other evidence that intestinal absorption of many substances is impaired in myxedema.

Acknowledgments

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Synthesis and Reactions of 3,11β-Dihydroxy-Δ^4-androsten-17-one*

David K. Fukushima, Shirley Dobriner, and H. Leon Bradlow

ABSTRACT: 3α,11β-Dihydroxy-Δ^4-androsten-17-one and its 3β-epimer have been synthesized from cortisol by reduction with lithium aluminum hydride followed by periodate oxidation in dioxane. The interconversion of the epimers was studied at various pH values and time periods. The equilibrium mixture of 1:1 could be achieved at pH 4 or 5 without dehydration to the $\Delta^{3,5}$-diene.

The four isomeric 4,5-oxides were prepared and characterized. 3β-Methoxy-11β-hydroxy-Δ^4-androsten-17-one was obtained when cortisol was reduced with sodium borohydride and oxidized with periodate in methanol solution. The interconversion of the 3-methoxy-Δ^4- and 3-hydroxy-Δ^4-steroids has been studied.

In the preceding paper the isolation and characterization of 3α,11β-dihydroxy-Δ^4-androsten-17-one (Ia) and its 3β-epimer IIa as metabolites of 11β-hydroxy-Δ^4-androstene-3,17-dione (III) in man were reported (Fukushima *et al.*, 1966). The present study describes the partial synthesis of these two allylic alcohols and some of their derivatives.

Cortisol (IV) was chosen as the starting material since it has the required 11β-hydroxyl group, the Δ^4-3-keto group which can be readily reduced to the Δ^4-3-hydroxy epimers, and a side chain which can easily be converted to the 17-ketone. Reduction with lithium aluminum hydride yielded the unsaturated pentol which, without isolation, was oxidized with periodate buffered at pH 6.5. The oxidized product contained a mixture of I, II, and 11β-hydroxy-Δ^4-androstene-3,17-dione (III). 3β,11β-Dihydroxy-Δ^4-androsten-17-one (II) was readily separated by Celite chromatography whereas more difficulty was encountered in the purification of the 3α-epimer I. Losses were incurred by the ready dehydration, allylic rearrangement, and apparent oxidation to the ketone of the 3α-hydroxyl group (Ward *et al.*, 1965). Most of the Δ^4-3-keto steroid III was formed because of incomplete reduction of the unsaturated ketone of hydrocortisone under the present condition.

The orientation of the C-3 hydroxyl group of I and II was assigned on the basis of molecular rotatory difference between the Δ^4-3-keto and the 3-hydroxy-Δ^4-steroids and by nuclear magnetic resonance spectrometry. The transformation to the 3β-epimer has been reported to be accompanied by a large negative molecular rotation change, *ca.* -170 for the 3β-OH and -295 for the 3β-acetate (Nes and Kim, 1963; Table I). Therefore, the allylic alcohol with molecular rotatory differences of -250 and -384, respectively, has been assigned as the 3β-epimer II and its acetate IIb (Table I). The nuclear magnetic spectra of IIa and its acetate IIb confirm the β orientation of the C-3 substituent of II. The alcohol IIa in pyridine exhibited a doublet at δ = 5.27 ppm (J = 4 cps) for the vinyl

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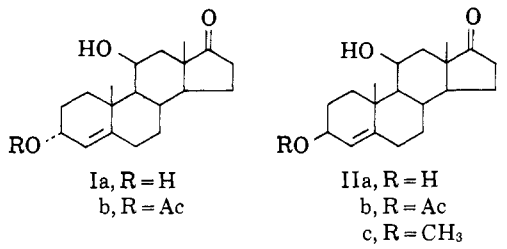
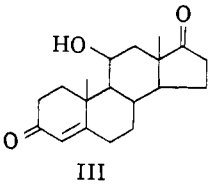


Ia, R = H
b, R = Ac

IIa, R = H
b, R = Ac
c, R = CH_3



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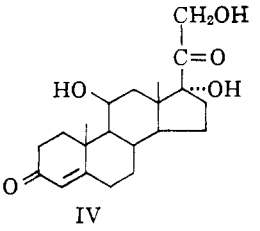

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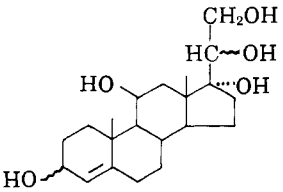

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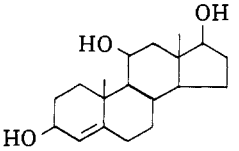

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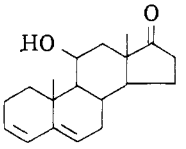


VII

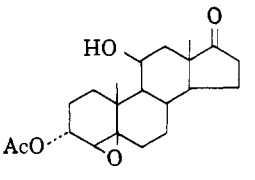
4-hydrogen, whereas the 3-acetate IIb in deuteriochloroform exhibited a singlet at δ = 5.23 ppm; Ward *et al.* (1965) have ascribed the peak at δ = 5.32 (J = 2 cps) for the vinyl 4-hydrogen of 3β-hydroxy-Δ^4-androsten-17-one in deuteriochloroform. Burstein and Ringold (1964) have also found a broad singlet at 5.26–5.28 ppm for the vinyl hydrogen of a number of Δ^4-3β-hydroxysteroids in deuteriochloroform. These authors have examined the molecular model of these allylic alcohols and have predicted from the Karplus equation that there would be no coupling of the C-4 proton with the 3α proton. The splitting of the signal due to the 4-H in IIa can probably be ascribed to pyridine, the solvent in which the spectrum was examined.

The 3,11β-dihydroxy-Δ^4-androsten-17-one epimer which had positive molecular rotatory change in the transformation of the Δ^4-3-ketone to the allylic alcohol (+6 for Ia and +316 for the acetate Ib) is assigned the 3α-epimer structure I (Nes and Kim, 1963; Table I). The nuclear magnetic resonance[1] spectra of Ia in pyridine and the acetate Ib in deuteriochloroform both exhibit a doublet (J = 4 cps) at δ = 5.47 and 5.37 ppm, respectively, for the vinyl 4-hydrogen. Burstein and Ringold (1964) have found that 3α-hydroxy-Δ^4-steroids exhibited a doublet (J = 5 cps) centered at 5.46–5.47 ppm as predicted.

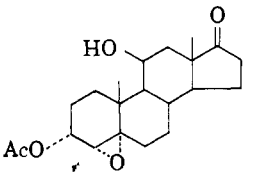
In another preparation of the allylic alcohol I and II, the intermediate unsaturated pentol V was oxidized with periodic acid in methyl alcohol solution. In this case, the principal product isolated was the methyl ether, 3β-methoxy-11β-hydroxy-Δ^4-androsten-17-one (IIc), formed under acidic conditions from the Δ^4-3-hydroxysteroid (Nes and Kim, 1963). The 3β orientation was assigned from molecular rotation difference and from the broad singlet at δ = 5.30 ppm in deuteriochloroform observed for the vinylic 4-hydrogen in the nmr spectrum (Nes and Kim, 1963). Attempted synthesis of I and II by selective borohydride reduction of the 3-keto group in 11β-hydroxy-Δ^4-androstene-3,17-dione (III) resulted primarily in the initial reduction of the 17-ketone. Complete reduction of III afforded Δ^4-androstene-3β,11β,17β-triol (VI) as the principal product as shown earlier (Slaunwhite *et al.*, 1964).

3-Hydroxy-Δ^4-steroids are readily dehydrated under acidic conditions to $\Delta^{3,5}$-dienes (Butenandt and Heusner, 1938). We have found that II and its derivatives afforded 11β-hydroxy-$\Delta^{3,5}$-androstadien-17-one (VII) with minimum formation of by-products when they are heated in 70% aqueous acetic acid for a short time on a steam bath. Similarly triol VI was dehydrated to the corresponding $\Delta^{3,5}$-dienediol.

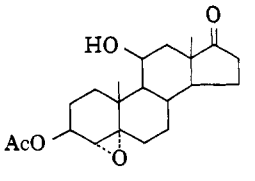
The isolation of 3α,11β-dihydroxy-Δ^4-androsten-17-one from urine extract was not readily accomplished due to mixture with the saturated analog, 3α,11β-dihydroxy-5β-androstan-17-one (Fukushima *et al.*, 1966). Separation was achieved by the formation of 4,5-oxides of the Δ^4-steroid. In order to characterize the oxides formed, the four oxides derived from the two 3-hydroxy-Δ^4-epimers I and II have been prepared. Oxidation of 3α-acetoxy-11β-hydroxy-Δ^4-androsten-17-one (Ib) with *m*-chloroperbenzoic acid afforded two products. The β conformation was assigned to the major product, 3α-acetoxy-11β-hydroxy-4β,5β-oxidoandrostan-17-one (VIII), since it has been demonstrated by Henbest and Wilson (1957) that cyclic allyl acetates



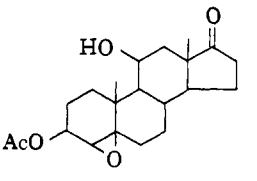
VIII



IX



X



XI

preferentially yield the oxide *trans* to the acetoxyl group. The minor product of the epoxidation of Ib, 3α-acetoxy-11β-hydroxy-4α,5α-oxidoandrostan-17-one

[1] Abbreviations used: nmr, nuclear magnetic resonance; tlc, thin layer chromatography.

TABLE I: Molecular Rotatory Differences between Δ^4-3-Keto and Δ^4-3-OR Steroids.[a]

		ΔMD (C=O → COR)						
		3β			3α			
	MD	R = H	R = CH_3	R = Ac	R = H	R = CH_3	R = Ac	Ref
Δ^4-Androstene-3,17-dione	+566							*b*
3β-Hydroxy-Δ^4-androsten-17-one	+326	−240						*c*
3β-Methoxy-Δ^4-androsten-17-one	+330		−236					*c*
3α-Methoxy-Δ^4-androsten-17-one	+668					+102		*c*
3α-Hydroxy-Δ^4-androsten-17-one	+650				+84			*c*
17β-Hydroxy-Δ^4-androsten-3-one	+314[d]							*b*
Δ^4-Androstene-3β,17β-diol	+137[d]	−177						*f*
Δ^4-Androstene-3α,17β-diol	+426[d]				+112			*f*
17β-Acetoxy-Δ^4-androsten-3-one	+300							*c*
Δ^4-Androstene-3β,17β-diol diacetate	+4			−296				*c*
Δ^4-Androstene-3α,17β-diol diacetate	+545						+245	*c*
Δ^4-Cholesten-3-one	+340							*b*
Δ^4-Cholesten-3β-ol	+186	−154						*b*
Δ^4-Cholesten-3β-ol acetate	+39			−301				*b*
Δ^4-Cholesten-3β-ol methyl ether	+154		−186					*b*
Δ^4-Cholesten-3α-ol	+445				+105			*b*
Δ^4-Cholesten-3α-ol acetate	+755						+415	*b*
Δ^4-Cholesten-3α-ol methyl ether	+280					−60[e]		*b*
11β-Hydroxy-Δ^4-androstene-3,17-dione	+664							*b*
3β,11β-Dihydroxy-Δ^4-androsten-17-one	+414	−250						*c*
3β-Acetoxy-11β-hydroxy-Δ^4-androsten-17-one	+280			−384				*c*
3β-Methoxy-11β-hydroxy-Δ^4-androsten-17-one	+426		−241					*c*
3α,11β-Dihydroxy-Δ^4-androsten-17-one	+670				+6			*c*
3α-Acetoxy-11β-hydroxy-Δ^4-androsten-17-one	+980						+316	*c*
11β,17β-Dihydroxy-Δ^4-androsten-3-one	+472							*b*
Δ^4-Androstene-3β,11β,17β-triol	+244	−228						*c*
11β-Hydroxy-17β-acetoxy-Δ^4-androsten-3-one	+426							*b*
Δ^4-Androstene-3β,11β,17β-triol 3,17-diacetate	+53			−373				*c*

[a] The molecular rotation differences were calculated from specific rotation values obtained in chloroform unless otherwise noted. [b] Jacques *et al.*, 1965. [c] This laboratory. [d] In ethyl alcohol. [e] This value suggests that the product was contaminated by the 3β-methoxy epimer. Shoppee *et al.*, 1957. [f] Ungar *et al.*, 1957.

(IX), was more polar on thin layer chromatography than the 4β,5β-oxide. Additional evidence for the preferential *trans* epoxidation of steroidal allyl acetates was obtained for the 3β-acetoxy derivatives by nmr spectrometry. The major product of epoxidation of IIb was 3β-acetoxy-11β-hydroxy-4α,5α-oxidoandrostan-17-one (X), which exhibited chemical shifts at δ = 2.92 ppm for the 4β proton and 4.98 ppm for the 3α proton. The minor product and more polar epimer by tlc, 3β-acetoxy-11β-hydroxy-4β,5β-oxidoandrostan-17-one (XI), exhibited chemical shifts for the 4α and 3α protons at 3.22 (J = 4 cps) and 5.17 ppm, respectively. These values are consistent with those reported for the corresponding cholestane analogs. Collins and his coworkers (1963) have found that the C-4 hydrogen in *cis*-4,5-epoxy-3-alcohols appears as a doublet while the 4-hydrogen appears as a singlet in the *trans*-4,5-epoxy-3-alcohols in accord with calculation from the Karplus equation. Furthermore in the 3β-acetoxycholestane derivatives, the chemical shift of the 4-hydrogen of the β-oxide appears further downfield than that of the α-oxide.

The solvolysis of allylic alcohols and their derivatives is well known. Thus solvolysis of 3β-chloro-Δ^4-cholestene in aqueous acetone at 45–55° in presence of bicarbonate or in moist ether in the presence of silver hydroxide at 20° has been shown by Young and coworkers (1959) to yield an equilibrium mixture of 3α-hydroxy-Δ^4-cholestene and its β-epimer in equal proportions. Furthermore both epimers of 3,11β-dihydroxy-Δ^4-androsten-17-one were obtained from the urine of these subjects following administration of 11β-hydroxy-Δ^4-androstene-3,17-dione in different proportions depending on the length of incubation of the

urine at pH 5.0 (Fukushima *et al.*, 1966). The interconversion of 3α- and 3β,11β-dihydroxy-Δ⁴-androsten-17-one (Ia and IIa) was, therefore, studied at various pH values and time periods. It was found that an equilibrium mixture of equal amounts of the two epimers was achieved from either isomer. At pH 5.0 and 38° the 3α-hydroxy-Δ⁴-steroid equilibrium was achieved within 6 days, whereas the equilibrium mixture was not obtained until the 10th day with the 3β-hydroxy epimer. There was little or no dehydration to 11β-hydroxy-Δ³,⁵-androstadien-17-one (VII) under these conditions. There was no interconversion of the compounds at pH 6.0 during 6 days at 38°. More rapid interconversions were observed on lowering the pH accompanied by greater formation of the dehydration product. Addition of Tween 80 or β-glucuronidase (Ketodase) did not alter the rate of solvolysis at pH 5.0.

3β-Methoxy-Δ⁴-steroid IIc was slowly transformed to 3α- and 3β,11β-dihydroxy-Δ⁴-androsten-17-one (Ia and IIa) without significant dehydration upon treatment in aqueous solution at pH 5.0 and 38° (Table II). There was unchanged methyl ether remaining after 7 days with complete transformation by 10 days. The equilibrium mixture of the 3-hydroxy epimers was achieved by the 14th day. The reverse reaction, the methanolysis of the 3β-hydroxy-Δ⁴-steroid (IIa), can be readily achieved in methanol under acidic conditions; both epimeric methyl ethers were obtained. Similar transformation has been observed on the treatment of 17,17-ethylenedioxy-Δ⁴-androsten-3-ol with methyl alcohol and acetic acid (Nes and Kim, 1963).

TABLE II: Interconversion of 3α- and 3β,11β-Dihydroxy-Δ⁴-androsten-17-one at 38°.

	Time (days)	Ratio of Products[a] 3α-OH	3β-OH	Δ³,⁵
3α,11β-Dihydroxy-Δ⁴-androsten-17-one				
pH 5.0	2	3	1	...
	6	1	1	Trace
pH 6.0	2	Unchanged		
	6	Unchanged		
3β,11β-Dihydroxy-Δ⁴-androsten-17-one				
pH 3.0	2	1	1	1.5
	6	0	0	All
pH 4.0	2	1	1.5	
	6	1	1	1.5
pH 5.0	2	Trace	Mostly	...
	6	1	2	
	8	1	1.5	...
	10	1	1	...
	14	1	1	...
3β-Methoxy-11β-hydroxy-Δ⁴-androsten-17-one				3β-OCH_3
pH 5.0	2	1	2	2.5
	5	1	2	1
	7	1	1.5	0.5
	10	1	1.3	0
	14	1	1	0

[a] 3α-OH, 3α,11β-dihydroxy-Δ⁴-androsten-17-one (Ia). 3β-OH, 3β,11β-dihydroxy-Δ⁴-androsten-17-one (IIa). Δ³,⁵, 11β-hydroxy-Δ³,⁵-androstadien-17-one (VII). 3β-OCH_3, unreacted starting material, 3β-methoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIc); only trace amount of Δ³,⁵-diene was observed.

Experimental Section

Melting points were taken on a micro hot stage and are corrected. Optical rotations were determined in chloroform unless specified. Infrared spectra were determined on a Beckman IR-9 spectrophotometer; br = broad, sh = shoulder. Nuclear magnetic resonance spectra (nmr) were determined on a Varian A-60 spectrometer in the solvent specified; the chemical shifts are reported in δ relative to tetramethylsilane as internal standard; tlc on silica gel GF; R_F values reported are for those in system ethyl acetate–cyclohexane (1:1) unless otherwise stated.

3α,11β-Dihydroxy-Δ⁴-androsten-17-one (Ia) and Its 3β-Epimer (IIa). A solution of 10 g of cortisol (IV) in freshly distilled tetrahydrofuran was added dropwise to a suspension of 4 g of lithium aluminum hydride in 1000 ml of anhydrous ether. The reaction mixture was refluxed for 2 hr and allowed to stand overnight at room temperature. Saturated sodium sulfate solution was added and the mixture was extracted with large volumes of ethyl acetate. The organic layer was washed with water and dried, and the solvent was evaporated to give 8.4 g of unsaturated pentol (V). This was dissolved in 400 ml of dioxane and 1400 ml of water and added to a solution of 12.0 g of sodium metaperiodate in 400 ml of water and 200 ml of phosphate buffer at pH 6.5. The mixture was kept in the dark for 20 hr at room temperature, extracted with ethyl acetate, and washed with water, sodium carbonate solution, and brine. The extract was dried over sodium sulfate and the solvent was evaporated to give 5.1 g of product. This material was chromatographed on 350 g of Celite 545 in the system 2,2,4-trimethylpentane–*t*-butyl alcohol–methyl alcohol–water (5:2:2:2). The Celite was impregnated with the lower phase (2:1, w/v). The chromatogram was developed with the upper phase and fractions collected at a rate of 40 ml/30 min. Fractions 3–35 (412 mg) contained traces of 3α,11β-dihydroxy-Δ⁴-androsten-17-one; the principal component

was a steroid with R_F 0.40 on tlc with ethyl acetate–cyclohexane (1:1). Fractions 36–107 contained 2.88 g of mixtures of varying composition of 11β-hydroxy-Δ⁴-androstene-3,17-dione (III) and the epimeric 3β- and 3α,11β-dihydroxy-Δ⁴-androsten-17-ones, as judged by tlc with ethyl acetate–cyclohexane (1:1). Fractions 108–170 (510 mg) contained principally 3β,11β-dihydroxy-Δ⁴-androsten-17-one.

A portion (2.1 g) of the combined fractions (36–107) was rechromatographed on 100 g of Celite 545 in the same system as above. Selected fractions were rechromatographed on Celite in the system 2,2,4,-trimethylpentane–toluene–methyl alcohol–water (3:5–4:1) and on thin layer silica gel G impregnated with ethylene glycol in the system benzene–chloroform–cyclohexane (1:1:1). Following repeated chromatography, 99 mg of reasonably pure 3α,11β-dihydroxy-Δ⁴-androsten-17-one (Ia) was obtained. Recrystallizations from acetone–petroleum ether (bp 60°) yielded 55 mg of Ia, mp 169–170°; $[\alpha]_D^{23}$ +220°; ν_{max}^{KBr} 3515, 3485, 3440, 3000, 1735, 1661, 1406, 1101, 1074, 1056, 1045, 1025, 1001, 873 cm⁻¹; nmr in pyridine 1.30 (s, 18-CH_3), 1.50 (s, 19-CH_3), 4.37 (m, 11α-H), 4.55 (m, 3β-H), and 5.47 ppm (doublet, J = 4 cps, 4-H); tlc, R_F 0.13. Acetylation of Ia and recrystallization from ethyl acetate–cyclohexane and acetone gave 3α-acetoxy-11β-hydroxy-Δ⁴-androsten-17-one (Ib), mp 182, 185–188°; $[\alpha]_D^{23}$ +284°; ν_{max}^{KBr} 3609, 3580, 3010, 1734, 1726, 1706, 1662, 1407, 1258, 1239, 1018, 954, 891, 873, 658 cm⁻¹; nmr in $CDCl_3$ 1.28 (s, 18-CH_3), 1.13 (s, 19-CH_3), 2.04 (s, acetate-CH_3), 4.45 (m, 11α-H), 5.13, (m, half-height width 8 cps, 3β-H), and 5.37 (d, J = 4 cps; 4-H); tlc, R_F 0.41.

The chromatographic fractions containing principally 3β,11β-dihydroxy-Δ⁴-androsten-17-one (IIa) were combined (773 mg) and recrystallized from acetone, acetone–petroleum ether, and ether to give 202 mg of IIa, mp 177–180°; $[\alpha]_D^{26}$ +142°, +136° (ethyl alcohol); ν_{max}^{KBr} 3475, 3430, 3383, 3010, 1729, 1666, 1403, 1084, 1051, 1029, 855 cm⁻¹; nmr in pyridine 1.50 (s, 18-CH_3), 1.27 (s, 19-CH_3), 4.30 (m, 11α-H), 4.53 (m, 3α-H), and 5.27 ppm (d, J = cps, 4-H); tlc, R_F 0.16.

Anal. Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.85; H, 9.50.

Acetylation with acetic anhydride and pyridine at room temperature afforded the monoacetate IIb. Recrystallization from ethyl acetate–cyclohexane yielded IIb which melted at 164–169, 171°; $[\alpha]_D^{23}$ +80.9°; ν_{max}^{KBr} 3450 (br), 3001, 1730, 1659, 1405, 1252, 1243, 1090, 1026, 1017, 655 cm⁻¹; nmr in $CDCl_3$ 1.00 (s, 18-CH_3), 1.33 (s, 19-CH_3), 2.05 (s, acetate-CH_3), 4.43 (m, 11α-H), and 5.23 ppm (s, 4-H; m, half-height width 18 cps, 3α-H); tlc, R_F 0.41.

11β-Hydroxy-Δ³,⁵-androstadien-17-one (VII). A solution of 55 mg of 3β,11β-dihydroxy-Δ⁴-androsten-17-one (IIa) in 2.5 ml of 70% aqueous acetic acid was heated on a steam bath for 30 min. Thin layer chromatography on the reaction product in ethyl acetate–cyclohexane (1:1) showed one spot of R_F 0.59 which absorbed in ultraviolet light and gave an immediate pink color with 77% sulfuric acid. The solvent was removed *in vacuo* and the residue was recrystallized from ether to give 28 mg of 11β-hydroxy-Δ³,⁵-androstadien-17-one (VII), mp 126.5–127.5°, 135–136°; $[\alpha]_D^{26}$ +54.9°; $\lambda_{max}^{ethanol}$ 228 mμ (ε 18,700), 234 mμ (ε 20,300), and 243 mμ (ε 13,800); ν_{max}^{KBr} 3532, 3451, 3009, 1733, 1652, 1583, 1407, 1094, 1054, 1019, 810, 796, 621 cm⁻¹; Rothman and Wall (1960) reported mp 161.5–165° and 178–179°; $[\alpha]_D^{25}$ +52.1°; λ_{max} 237 mμ (ε 21,900) with other maxima at 230 and 245 mμ; $\nu_{max}^{CS_2}$ 3600, 1743, 821, 811, and 865 cm⁻¹. Neeman *et al.* (1960) reported mp 148°; $\lambda_{max}^{ethyl\ alcohol}$ 228 mμ (log ε 4.27), 235 mμ (log ε 4.31), and 243 mμ (log ε 4.14).

Δ⁴-Androstene-3β,11β,17β-triol (VI). A solution of 1.0 g of 11β-hydroxy-Δ⁴-androstene-3,17-dione and 300 mg of sodium borohydride in 25 ml of methyl alcohol was allowed to stand at room temperature for 90 min. The product (1.1 g) was a mixture of two compounds as judged by chromatography on thin layer silica gel G with 5% methyl alcohol in ethyl acetate, R_F 0.47 and 0.41. Recrystallization from methyl alcohol afforded 346 mg of Δ⁴-androstene-3β,11β,17β-triol (VI), mp 210–212°. The analytical sample melted at 216–219°; $[\alpha]_D^{25}$ +80° (ethyl alcohol); ν_{max}^{KBr} 3460–3390 (br), 3005, 1662–1650 (br), 1052, 1025, 812 (sh), and 807 cm⁻¹; tlc, R_F 0.47 on silica gel G with 5% methyl alcohol in ethyl acetate; R_F 0.31 in ethyl acetate. The reported melting point of this triol was 214–216° (Slaunwhite *et al.*, 1964). The diacetate prepared with acetic anhydride and pyridine at room temperature was recrystallized from methyl alcohol, mp 129.5–131°; $[\alpha]_D^{25}$ +13.6° (ethyl alcohol); reported mp 145–146° (Slaunwhite *et al.*, 1964).

Treatment of Δ⁴-androstene-3β,11β,17β-triol with 70% acetic acid on a steam bath for 30 min yielded Δ³,⁵-androstadiene-11β,17β-diol, mp 151°; $\lambda_{max}^{ethyl\ alcohol}$ 228 mμ (ε 17,600), 234 mμ (ε 19,400), 243 mμ (ε 13,200); reported mp 150–151° and λ_{max} 228 mμ (ε 16,800), 236 mμ (ε 18,800), 243 mμ (ε 12,800) (Slaunwhite *et al.*, 1964).

3α-Acetoxy-4,5-oxido-11β-hydroxyandrostan-17-one (VIII and IX). A solution of 12 mg of 3α-acetoxy-11β-hydroxy-Δ⁴-androsten-17-one (Ib) and 11 mg of *m*-chloroperbenzoic acid in 1.1 ml of chloroform was stored at room temperature overnight. The mixture was extracted with ether, washed with dilute alkali and water, and dried over sodium sulfate. The solvent was evaporated to give 10 mg of product which was chromatographed on paper in the system 2,2,4-trimethylpentane–methyl alcohol–water (10:8:2) to yield 6 mg of 3α-acetoxy-4β,5β-oxido-11β-hydroxyandrostan-17-one (VIII). Recrystallization from ether gave 3 mg of VIII, mp 175–177°; $[\alpha]_D^{23}$ +168°; ν_{max}^{KBr} 3500, 3465 (sh), 1735 (br), 1410, 1250 (br), and 1028 cm⁻¹; tlc, R_F 0.37.

The mother liquor from the recrystallization of the β-oxide was chromatographed on thin layer silica gel GF in the system ethyl acetate–cyclohexane (1:1). An additional 1 mg of the β-oxide was obtained. Elution of the oxide with R_F 0.41 gave 1 mg of 3α-acetoxy-4α,5α-oxido-11β-hydroxyandrostan-17-one (IX). Recrystallization from ether gave needles of IX, mp 150°, 215–217°; ν_{max}^{KBr} 3540, 3460, 1739, 1722, 1416, 1405, 1243, and 1030 cm⁻¹.

3β-Acetoxy-4,5-oxido-11β-hydroxyandrostan-17-one (X and XI). A solution of 70 mg of 3β-acetoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIb) and 108 mg of *m*-chloroperbenzoic acid in 3 ml of chloroform was stored at room temperature overnight. The mixture was worked up as above for the 3α-acetate. Preparative thin layer chromatography on silica gel GF in ethyl acetate–cyclohexane (1:1) gave 43 mg of 3β-acetoxy-4α,5α-oxido-11β-hydroxyandrostan-17-one (X), R_F 0.45. Recrystallization from ether yielded 29 mg of α-oxide X, mp 176, 185–187°; $[\alpha]_D^{23}$ +118°; ν_{max}^{KBr} 3478, 1735 (br), 1404, 1237, 1226 (sh), 1070, 1054, 1031, and 1018 cm^{-1}; nmr in $CDCl_3$ 1.13 (s, 18-CH_3), 1.33 (s, 19-CH_3), 2.08 (s, acetate-CH_3), 2.92 (s, 4β-H), 4.33 (m, 11α-H), and 4.97 ppm (t, 3α-H).

The more polar (R_F 0.33) and minor product of oxidation was eluted to give 20 mg of 3β-acetoxy-4β,5β-oxido-11β-hydroxyandrostan-17-one (XI). Recrystallization from ether gave fine needles of β-oxide XI, mp wide range with many transformations 120, 130, 160, 168°; $[\alpha]_D^{23}$ +27.2°; ν_{max}^{KBr} 3450 (br), 1738 (br), 1407, 1250 (sh), 1240, and 1030 cm^{-1}; nmr in $CDCl_3$ 1.17 (s, 18-CH_3), 1.32 (s, 19-CH_3), 2.13 (s, acetate-CH_3), 3.22 (d, J = 4 cps, 4α-H), 4.30 (m, 11α-H), and 5.17 ppm (m, 3α-H).

3β-Methoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIc). Sodium borohydride (1.5 g) was added in portions to a solution of 6.0 g of cortisol (IV) in 120 ml of methyl alcohol and 2 ml of 5% sodium bicarbonate solution at room temperature. The mixture was allowed to stand at room temperature overnight and was diluted with 150 ml of water. The solution was then acidified to pH 6.5 and 50 ml of phosphate buffer was added. Sodium metaperiodate (10 g) was added and the reaction mixture stored overnight at room temperature and extracted with ethyl acetate. The extract was washed with sodium sulfite and brine and dried. Upon concentration 2.48 g of crystals separated and were collected by filtration and recrystallized from acetone to yield 3β-methoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIc), mp 153–156°; $[\alpha]_D^{26}$ +133°; ν_{max}^{KBr} 3450, 1741, 1723, 1667 (br), 1075 cm^{-1}; nmr in $CDCl_3$ 1.10 (s, 18-CH_3), 1.30 (s, 19-CH_3), 3.34 (s, OCH_3), 3.75 (m, 11α-H), 4.41 (m, 3α-H), and 5.30 (s, 4-H); tlc, R_F 0.40.

Anal. Calcd for $C_{20}H_{30}O_3$: C, 75.44; H, 9.50. Found: C, 75.11; H, 9.36.

Interconversion of 3α,11β-Dihydroxy-Δ⁴-androsten-17-one (Ia) and Its 3β-Epimer (IIa). A solution of 1 mg of steroid in 1 ml of ethyl alcohol was added to 90 ml of water and 10 ml of the appropriate buffer solution. Citrate phosphate buffer was used for solutions at pH 3.0, 4.0, and 6.0 while acetate buffer was employed at pH 5.0. The mixture was incubated at 38° and portions were removed at several time intervals. The portions were extracted with ethyl acetate, washed with 5% sodium hydroxide solution and brine, and dried, and the solvent was evaporated. A sample of the residue was chromatographed on thin layer silica gel GF with ethyl acetate. The chromatogram was sprayed with 77% sulfuric acid and the relative amounts of the steroids were estimated by visual comparison of the pink color formed with known quantities of pure compounds chromatographed simultaneously. The spots were identified by the mobilities of the steroids in this system, 3α,11β-dihydroxy-Δ⁴-androsten-17-one (Ia) R_F 0.42; 3β,11β-dihydroxy-Δ⁴-androsten-17-one (IIa) R_F 0.46; 3β-methoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIc) R_F 0.58; and 11β-hydroxy-Δ³,⁵-androstadien-17-one (VII) R_F 0.70. The results are recorded in Table II.

The following modifications of the acidic medium containing 3β,11β-dihydroxy-Δ⁴-androsten-17-one at pH 5.0 and 38° were made: (1) addition of 300 units of Ketodase/ml of solution; (2) no ethyl alcohol, the steroid was homogenized in 10 ml of acetate buffer and diluted to 100 ml; (3) no ethyl alcohol and no buffer, the steroid was homogenized in 10 ml of water at pH 5.0 containing Tween 80 and diluted to 100 ml. The results were essentially identical with that reported for this compound under the general conditions described above.

Interconversion of 3-Methoxy-11β-hydroxy-Δ⁴-androsten-17-one and 3,11β-Dihydroxy-Δ⁴-androsten-17-one. A solution of 1 mg of 3β-methoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIc) in 1 ml of ethyl alcohol was added to 90 ml of water and 10 ml of acetate buffer at pH 5.0. The mixture was incubated at 38° and portions treated as above for the 3α–3β-hydroxy interconversion study. The results are recorded in Table II.

A solution of 1 mg of 3β,11β-dihydroxy-Δ⁴-androsten-17-one (IIa) in 1.9 ml of methyl alcohol, 0.4 ml of water, and 0.4 ml acetic acid was refluxed for 3 hr (Nes and Kim, 1963). The mixture was extracted with ethyl acetate and washed with dilute base and brine. The extract was dried and the solvent was evaporated. The residue was chromatographed on silica gel GF with ethyl acetate and sprayed with 77% sulfuric acid. There were two main products with R_F 0.58 and 0.55; based on the intensity of the pink color formed these products were present in the ratio of 3:1, respectively. The less polar compound, R_F 0.58, had the mobility of 3β-methoxy-11β-hydroxy-Δ⁴-androsten-17-one (IIc). Based on the studies of Nes and Kim (1963), the more polar product should be the 3α-methoxy epimer. There were only traces of compounds present in the reaction mixture with the mobility of the starting material and the dehydrated product, diene VII.

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Pathway and Stereochemistry of the Formation of Estriols in Man*

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ABSTRACT: Following the administration of estradiol-16β-^{3}H and -16α-^{3}H to human subjects the tritium content of the urinary estriol and estra-1,3,5(10)-triene-3,16β,17β-triol (16-epiestriol) was determined. From the values it is evident that the hydroxylation at C-16 whether α or β proceeds by replacement of hydrogen and that no common enol form is involved in the reaction. Further, 16-keto compounds cannot be considered as intermediates in the biosynthesis of estriol or 16-epiestriol. The difference in the recovery of tritium in urine and body water in the 16β-^{3}H and 16α-^{3}H studies suggests that the substantial portion of the administered dose which is never recovered in the urine is altered by 16α and not 16β substitution.

The metabolism of the female sex hormone in man is distinguished by competitive hydroxylation at C-2 and C-16 (Fishman *et al.*, 1965). The latter course, formation of estriol and its 16β-epimer, is sometimes quantitatively the more important and the metabolic sequence leading to these products is, therefore, of considerable interest. It has been shown (Fishman *et al.*, 1960) that oxidation of estradiol to estrone is a requisite stage of the *in vivo* formation of estriol and 16-epiestriol[1] and that 16-hydroxylation of estradiol, as such, does not occur to any significant extent (Fishman *et al.*, 1961). The subsequent steps by which estriol and 16-epiestriol are derived have been the subject of considerable speculation, in particular with respect to the 16-keto compounds, 16-ketoestradiol (Layne and Marrian, 1958) and 16-ketoestrone (Serchi, 1953; Slaunwhite and Sandberg, 1956). It has been suggested that these compounds are either precursors for estriol and 16-epiestriol or oxidation products of the estriols in the human (Breuer, 1960; Dorfman and Ungar, 1965) and both *in vivo* (Levitz *et al.*, 1956, 1958, 1960; Stimmel, 1958; Nocke *et al.*, 1961) and *in vitro* (Breuer and Knuppen, 1958; Breuer *et al.*, 1958, 1959); evidence has been offered for either point of view.

The stereochemical nature of C-16 hydroxylation leading to estriol and 16-epiestriol is of particular interest. This question arises because estrone is the substrate for C-16 hydroxylation, and therefore an enolizable hydrogen α to a ketone is involved. It has been demonstrated (Bergstrom *et al.*, 1958; Corey *et al.*, 1958; Hayano *et al.*, 1958) that enzymatic hydroxylation at other unactivated (in the chemical sense) positions in the steroid molecule proceeds with replacement rather than displacement of the hydrogen involved. This stereochemistry need not apply to hy-

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[1] Abbreviations used: 16-epiestriol, estra-1,3,5(10)-triene-3,16β,17β-triol; 16α-hydroxyestrone, 3,16α-dihydroxyestra-1,3,-5(10)-trien-17-one; 16β-hydroxyestrone, 3,16β-dihydroxyestra-1,3,5(10)-trien-17-one; epiestriol acetonide, 16β,17β-isopropylenedioxyestra-1,3,5(10)-trien-3-ol; 16-ketoestradiol, 3,17β-dihydroxyestra-1,3,5(10)-trien-16-one; 16-ketoestrone, 3-hydroxyestra-1,3,5(10)-triene-16,17-dione; 2-methoxyestrone, 2-methoxy-3-hydroxyestra-1,3,5(10)-trien-17-one.