CHEMISTRY OF 3α-HYDROXY-Δ^5-ANDROSTENE-17-ONE[1]

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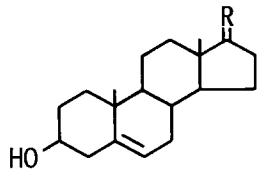
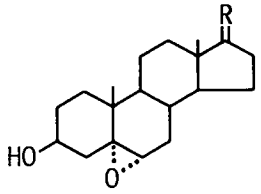
The synthesis and reactions of 3α-hydroxy-Δ^5-androstene-17-one are described. The preparation of 3β-hydroxy-Δ^5-androstene-17-one-3α-H^3 and its epimer, 3α-hydroxy-Δ^5-androstene-17-one-3β-H^3, is also reported.

Recently, the <u>in vivo</u> conversion of Δ^5-androstene-3,17-dione to 3α-hydroxy-Δ^5-androstene-17-one (Ec) was reported[3]. In order to study the mechanism of this and related biological transformations, a supply of 3α-hydroxy-Δ^5-androstene-17-one and the isotopically labeled isomers of 3-hydroxy-Δ^5-androstene-17-one-3-H^3 were required. A convenient preparation of these compounds is described.

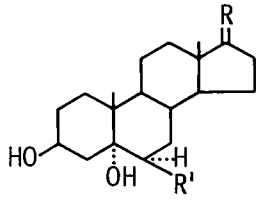
Ruzicka and Goldberg[4] prepared 3α-hydroxy-Δ^5-androstene-17-one (Ec) by hydrogenation of Δ^5-androstene-3,17-dione with Raney nickel. The predominant product of the reduction was the 3β-hydroxy isomer Aa which was removed by digitonin separation. This separation proved tedious and the reaction itself would have been difficult to adapt to the radioactive preparations. Therefore the techniques by which Plattner et al[5] converted cholesterol to epicholesterol were utilized in



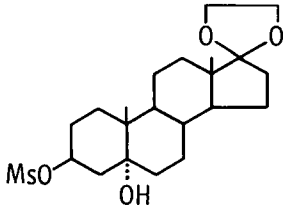
A a) R=O
b) R= 



B a) R= 
b) R=O



C a) R=  R'=H
b) R=O, R'=H
c) R=O, R'=OH



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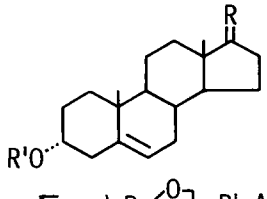
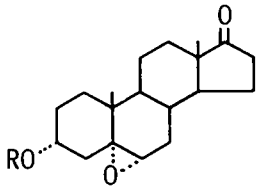
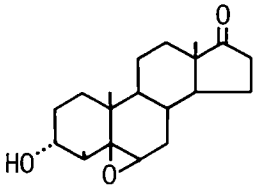


E a) R=  R'=Ac
b) R=  R'=H
c) R=O, R'=H
d) R=O, R'=Ac



F a) R=H
b) R=Ac



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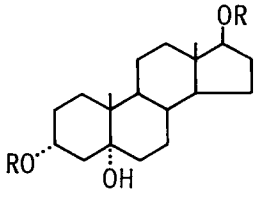
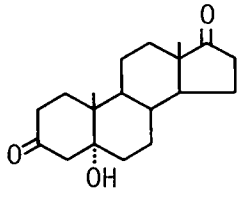


H a) R=H
b) R=Ac



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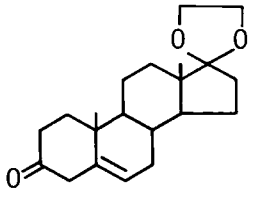

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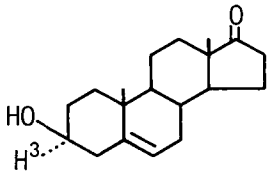
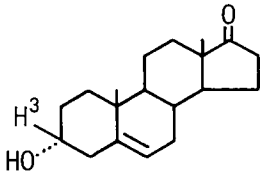


K



L

the present study. Monoperphthalic acid oxidation of the ethylene ketal of 3β-hydroxy-Δ^5-androstene-17-one (Ab) gave 5,6α-oxide (Ba) as the principal product. Hydrolysis of the crude oxide with acid gave the known 3β-hydroxy-5,6α-oxidoandrostane-17-one[4] (Bb) accompanied by 3β,5,6β-trihydroxy-androstane-17-one[6] (Cc) resulting from hydrolytic opening of the oxide ring. Reduction of the crude oxide with lithium aluminum hydride in tetrahydrofuran gave a mixture of products from which 3β,5-dihydroxyandrostane-17-one ethylene ketal (Ca) was readily isolated by crystallization from acetone. Hydrolysis of Ca with acid gave the known 3β,5-dihydroxyandrostane-17-one[7] (Cb). Treatment of ketal Ca with methanesulfonyl chloride in pyridine gave 3β-methanesulfonoxy-5-hydroxyandrostane-17-one ethylene ketal (D) in nearly quantitative yield. The mesylate D was refluxed with acetyl chloride and diethylaniline in chloroform followed by saponification of epimerized product Ea to give the 3α hydroxy derivative Eb. Without isolation Eb was treated with acid to remove the protective ketal group to yield the desired 3α-hydroxy-Δ^5-androstene-17-one in 79% overall yield from mesylate D. The overall yield from dehydroisoandrosterone (Aa) was 40%.

Derivatives which would be useful in the biochemical studies of this compound were prepared from 3α-hydroxy-Δ^5-androstene-17-one. Oxidation of Ec with monoperphthalic acid gave 3α-hydroxy-5,6α-oxidoandrostane-17-one (Fa), $[\alpha]_D^{28} = -26.8°$ as the principal product and a small amount

of the 5,6β-oxide G, $[\alpha]_D^{22} = +46.5°$. Catalytic reduction of the principal product, oxide Fa, to androstane-3α,5,17β-triol (Ha) and oxidation with chromic acid in acetone yielded the known 5-hydroxyandrostane-3,17-dione (I). The hydroxydione I was also prepared from 3β,5-dihydroxyandrostane-17-one (Cb) by the same reagent. Dehydration of the 5α-hydroxy group in I was readily accomplished with base to yield Δ^4-androstene-3,17-dione confirming the point of attachment of the hydroxyl group at C-5. Thus the assignment of the 5α,6α-oxide to the principal product Fa from epoxidation of 3α-hydroxy-Δ^5-androstene-17-one (Ec) is in agreement with earlier findings that epoxidation of the 5,6-double bond gives primarily the 5α,6α-oxide and that this isomer has a more negative specific rotation than the corresponding 5β,6β-oxide[8]. The mobilities of the oxides Fa and G on paper in system 2,2,4-trimethylpentan:methanol:water (10:8:2) and on thin layer of Silica Gel G in ethyl acetate:cyclohexane (1:1) are shown in Table I. Their mobilities were much lower than that of 3α-hydroxy-Δ^5-androstene-17-one (Ec) which has a mobility close to that of its metabolites, androsterone (3α-hydroxyandrostane-17-one) and etiocholanolone (3α-hydroxyetiocholane-17-one).

Radioactive dehydroisoandrosterone labeled at C-3, 3β-hydroxy-Δ^5-androstene-17-one-3α-H^3(K), 4.75×10^6 cpm/mg, was prepared by the reduction of Δ^5-androstene-3,17-dione

TABLE I

CHROMATOGRAPHIC MOBILITY OF 3α-HYDROXY-17-KETOSTEROIDS

Steroid	Paper* cm from origin	TLC** R_f
3α-Hydroxyandrostane-17-one	66 - 71	0.34
3α-Hydroxy-Δ^5-androstene-17-one	55 - 60	0.28
3α-Hydroxyetiocholane-17-one	48 - 51	0.25
3α-Hydroxy-5,6α-oxidoandrostane-17-one	28 - 34	0.14
3α-Hydroxy-5,6β-oxidoetiocholane-17-one	5 - 7	0.07

* Whatman #1 paper (18 x 118 cm), 40 hours at 25° in 2,2,4-trimethylpentane:methanol:water (10:8:2).

** Silica Gel G (250μ) with cyclohexane-ethyl acetate (1:1); solvent front 15 cm.

17-ethylene ketal (J) with $LiAlH_4^3$ followed by acid hydrolysis. The unsaturated ketone J was synthesized by the oxidation of 3β-hydroxy-Δ^5-androstene-17-one ethylene ketal (Ab) with Jones' reagent and contained about 5-10% of the conjugated Δ^4-3-ketone. Radioactive purity of 3β-hydroxy-Δ^5-androstene-17-one-3α-H^3 (K) was demonstrated and 99.5% of the isotope was removed on oxidation to Δ^4-androstene-3,17-dione by the Oppenauer method.

The epimerically labeled steroid, 3α-hydroxy-Δ^5-androstene-17-one-3β-H^3 (L) was prepared from radioactive dehydroisoandrosterone (K) through the reaction sequence,

Aa to Ec, reported above for the non-isotopic synthesis without purification of the intermediates. There was no change of specific activity during the synthesis and therefore the epimerization at C-3 during the reaction of the 3β-mesylate D to the 3α-acetoxy group with acetyl chloride and diethylaniline proceeded without loss of tritium.

EXPERIMENTAL[9]

3β-Hydroxy-5,6α-oxidoandrostane-17-one Ethylene Ketal (Ba).

To a cooled (10°) solution of 5 g of 3β-hydroxy-Δ^5-androstene-17-one ethylene ketal[10], m.p. 159.5-162° (Ab) was added 150 ml of 0.28 M monoperphthalic acid in ether. The mixture was stored at 5° overnight, 200 ml of 10% sodium hydroxide was added and then extracted with ethyl acetate. The organic solution was washed with water, 2 ml of pyridine added, and the solution dried and evaporated to dryness. The partially crystalline residue was recrystallized from cyclohexane containing a trace of pyridine followed by 4 recrystallizations from acetone-ligroin, containing a trace of pyridine, to give 313 mg of 3β-hydroxy-5,6α-oxidoandrostane-17-one ethylene ketal (Ba), m.p. 166-167°; $[\alpha]_D^{26} = -98.8°$; $\nu_{max}^{CS_2}$ 3600, 1311, 1170, 1110-1102, 1040, 997, 951, 907 cm^{-1}.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26

Found: C, 72,26; H, 9.59.

Acid Hydrolysis of 3β-hydroxy-5,6-oxidoandrostane-17-one Ethylene Ketal.

A mixture of 159 mg of crude 3β-hydroxy-5,6-oxidoandrostane-17-one ethylene ketal, 10 ml of ethanol, 5 ml of water and 5 drops of conc. hydrochloric acid was allowed to stand at room temperature for 2½ hrs. The acid was neutralized with 5% sodium bicarbonate solution and the mixture was extracted with ethyl acetate to give 132 mg of crystalline material. Chromatography on acid washed alumina gave (10% ethyl acetate in benzene) 83 mg of 3β-hydroxy-5,6α-oxidoandrostane-17-one (Bb) which melted at 227-229° after recrystallization from cyclohexane-acetone; reported[6] m.p. 229-230°. The infrared spectrum of the material was identical with that of an authentic sample. Further elution of the alumina column with 10% ethanol in ethyl acetate gave 33 mg of 3β,5,6β-trihydroxyandrostane-17-one (Cc) which melted at 304-307° after recrystallization from aqueous methanol; reported[11] m.p. 299-302°. The infrared spectrum of the material was identical with that of an authentic sample.

3β,5-Dihydroxyandrostane-17-one Ethylene Ketal (Ca).

One and one-half grams of lithium aluminum hydride (LAH) was added to a solution of 4.0 g of crude 3β-hydroxy-5,6-oxidoandrostane-17-one ethylene ketal in 100 ml of tetrahydrofuran (freshly distilled from LAH) and the mixture was refluxed for ½ hr. Excess LAH was decomposed by the cautious addition of ethyl acetate, followed by 10%

sodium hydroxide to give a thick white sludge. The liquid was decanted and evaporated to dryness. The residue was combined with the original thick white sludge and extracted thoroughly with ethyl acetate. The ethyl acetate solution was washed with water, dried and evaporated to dryness. The residue was recrystallized from acetone several times to give 2.62 g of 3β,5-dihydroxyandrostane-17-one ethylene ketal (Ca) with m.p. 220-221°. The analytical sample melted at 224-225°; $[\alpha]_D^{27}$ = - 18.1°; ν_{max}^{KBr} 3495, 1316, 1176, 1129, 1108-1093, 1037, 994, 915 cm^{-1}.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78
Found: C, 71.63; H, 9.77.

3β,5-Dihydroxyandrostane-17-one (Cb).

A solution of 88 mg of 3β,5-dihydroxyandrostane-17-one ethylene ketal (Ca) in 10 ml of ethanol, 5 ml of water and 5 drops of conc. hydrochloric acid was allowed to stand at room temperature for 2 hrs. The acid was neutralized with sodium bicarbonate solution, the solution was concentrated and diluted with water to give 60 mg of 3β,5-dihydroxyandrostane-17-one (Cb) which melted at 277.5-278° after recrystallization from methanol; $[\alpha]_D^{22}$ = + 83.6°, reported[12] m.p. 281-282°; $[\alpha]_D^{23}$ = + 88°.

3α-Hydroxy-Δ^5-androstene-17-one (Ec).

To a cooled solution of 1.12 g of 3β,5-dihydroxy androstane-17-one ethylene ketal (Ca) in 20 ml of pyridine was added 1 ml of methanesulfonyl chloride. The mixture was allowed to stand at room temperature for

two hours and then was stirred into a mixture of ice and water. The resultant mixture was extracted with ethyl acetate, the organic extract was washed with cold 5% sulfuric acid followed by cold 5% sodium hydroxide, then by water till neutral. The organic solution was dried and evaporated and the residue was triturated with a small volume of ether to give 1.28 g of crystalline 3β-methanesulfonoxy-5-hydroxyandrostane-17-one ethylene ketal (D).

A mixture of 1.16 g of mesylate D, 10 ml of chloroform, 10 ml of acetyl chloride and 10 ml of diethylaniline was refluxed for 5 hrs. The solution was concentrated under reduced pressure and extracted with ethyl acetate. The organic solution was washed with cold 10% sulfuric acid, cold 10% sodium hydroxide and with water, then was dried and evaporated. The oily residue was passed through a short alumina column with petroleum ether and benzene to remove polar impurities. The resultant oil was dissolved in 20 ml of methanol and 10 ml of 10% aqueous potassium hydroxide and the solution was refluxed for 15 minutes. The methanol was removed and the residue was extracted with ethyl acetate in the usual manner. The oily product Eb from the saponification was dissolved in 20 ml of ethanol, 10 ml of water and 1 ml of conc. hydrochloric acid. The mixture was refluxed for 15 minutes; then extracted with ethyl acetate. The crystalline residue after solvent removal was triturated with benzene and

recrystallized from acetone to give 361 mg of 3α-hydroxy-Δ^5-androstene-17-one (Ec), m.p. 224-225°; $[\alpha]_D^{22} = -7.6°$; reported[4] m.p. 221°; $[\alpha]_D = 0°$. The benzene solution from the trituration and the mother liquor from the recrystallization were combined and evaporated. The residue was chromatographed on acid washed alumina to give (with 5% ethyl acetate in benzene) an additional 258 mg of Ec. The overall yield from D was 79%.

Acetylation with acetic anhydride in pyridine gave 3α-acetoxy-Δ^5-androstene-17-one (Ed) which melted at 169.5-171.5° after recrystallization from methanol; reported[4] m.p. 173.5-174.5°.

3α-Hydroxy-5,6α-oxidoandrostane-17-one (Fa).

A solution of 500 mg of 3α-hydroxy-Δ^5-androstene-17-one (Ec) in 50 ml of chloroform and 50 ml of 0.3 N monoperphthalic acid in ether was stored at 5° for 5 hrs. Ethyl acetate was added and the extract washed with dilute base and water and dried. Evaporation of the solvent afforded 548 mg of product which was chromatographed on 50 g of acid washed alumina. Elution with 3% ethyl acetate-benzene gave 293 mg of 3α-hydroxy-5,6α-oxido-androstane-17-one (Fa) which on recrystallization from acetone-petroleum ether yielded 233 mg, m.p. 164.5-165°; $[\alpha]_D^{28} = -26.8°$; ν_{max}^{KBr} 3555, 1745, 1733 (sh), 1215, 1155, 1056, 1007, 829, 795 cm^{-1}.

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

Found: C, 74.72; H, 9.66.

Acetylation with acetic anhydride and pyridine afforded acetate Fb, m.p. 199-199.5°; $[\alpha]_D^{21}$ = + 2.5°; ν_{max}^{KBr} 1740, 1727, 1262, 1243, 1155, 1111, 1017, 841, 798 cm^{-1}.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73
Found: C, 72.42; H, 8.65

Elution with 5% ethyl acetate-benzene gave 25 mg of 3α-hydroxy-5,6β-oxidoetiocholane-17-one which on recrystallization from methanol melted at 201-203°; $[\alpha]_D^{22}$ = + 46.5°; ν_{max}^{KBr} 3475, 1738(sh), 1729, 1262, 1150, 1086, 1070, 1031, 846, 775 cm^{-1}.

Androstane-3α,5,17β-triol (Ha).

A solution of 19 mg of 3α-hydroxy-5,6α-oxidoandrostane-17-one (Fa) in 20 ml of tetrahydrofuran was added to 100 mg of lithium aluminum hydride. The reduction product was chromatographed on acid washed alumina and elution with 1% ethanol-ethyl acetate afforded 12 mg of androstane-3α,5,17β-triol (Ha). Recrystallization from acetone-petroleum ether gave Ha, m.p. 194.5-196°; $[\alpha]_D^{28}$ = + 1° (ethanol); ν_{max}^{KBr} 3485, 3370(sh), 3300, 3180(sh), 1678[13], 1117, 1072, 1056, 1006, 915-911, 860, 822 cm^{-1}.

Anal. Calcd. for $C_{19}H_{32}O_3 \cdot H_2O$: C, 69.90; H, 10.50
Found: C, 70.29; H, 10.38.

Acetylation with acetic anhydride and pyridine afforded 3,17-diacetate Hb, m.p. 198.5-199°; $[\alpha]_D^{25}$ = + 1.2°; $\nu_{max}^{CS_2,CCl_4}$ 3595, 1747, 1737(sh), 1246, 1228(sh), 1222, 1195, 1116, 1043, 1028, 1016, 987, 918, 820 cm^{-1}.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.25
Found: C, 70.09; H, 9.39.

5-Hydroxyandrostane-3,17-dione (I).

A solution of 20 mg of 3β,5-dihydroxyandrostane-17-one (Cb) in 20 ml of acetone and 0.025 ml of chromic acid solution[14] (26.72 g of chromic acid in 23 ml of conc. sulfuric acid diluted to 100 ml with water) was allowed to stand at room temperature for 10 minutes. The solution was poured into water, extracted with ethyl acetate and worked up in the usual manner. Recrystallization from petroleum ether-acetone yielded 15 mg of 5-hydroxyandrostane-3,17-dione (I), m.p. 213-214.5°, reported[15] m.p. 212-213°.

A solution of 6 mg of androstane-3α,5,17β-triol (Ha) in 3 ml of acetone was treated with 0.02 ml of chromic acid reagent as above. Recrystallization from acetone-petroleum ether gave 5-hydroxyandrostane-3,17-dione (I), m.p. 211-214°. The infrared spectrum was identical with the product obtained from 3β,5-dihydroxyandrostane-17-one.

Hydroxydiketone I in 10 ml of methanol and 300 mg of potassium hydroxide was heated on a steam bath for 3 minutes. The product obtained had the same mobility on thin layer of Silica Gel G in ethyl acetate:cyclohexane 1:1 as Δ^4-androstene-3,17-dione and the identity was confirmed by infrared spectrometry.

Δ^5-Androstene-3,17-dione 17-ethylene ketal (J).

A solution of 1.5 g of 3β-hydroxy-Δ^5-androstene-17-one ethylene ketal in 200 ml of acetone and 1.2 ml of 7.64 N chromic acid-sulfuric acid solution was allowed to stand at 15° for 4 minutes under nitrogen. The solution was poured

into ice and water, extracted with ethyl acetate and worked up in the usual manner. The crude product, 1.1 g, showed no hydroxy or 17-ketone absorption bands in the infrared region and had $\lambda_{max}^{240m\mu}$ $E_{1cm}^{1\%}$ = 30 or about 6% of Δ^4-3-ketone. Addition of a drop of 5% potassium hydroxide gave $E_{1cm}^{1\%}$ = 450, equivalent to the presence of approximately 90% of the Δ^4-3-ketone. Recrystallization of a small portion of the oxidation product from ethanol gave Δ^5-androstene-3,17-dione 17-ethylene ketal (J), m.p. 141-146°; $[\alpha]_D^{26}$ = - 44.1°. ν_{max}^{KBr} 1712, 1667, 1274, 1204, 1145, 1115, 1063, 1052, 1000, 981, 957, 901, 852 cm^{-1}.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.36; H, 9.09
Found: C, 75.77; H, 9.15

3β-Hydroxy-Δ^5-androstene-17-one-3α-H^3 (K).

A solution of 1.0 g of Δ^5-androstene-3,17-dione 17-ethylene ketal (J) in 25 ml of ether was added during 30 minutes to a stirred solution of 125 mg of $LiAlH_4^3$ (25 mc). The reaction mixture was stirred for an additional 30 minutes and worked up as usual for a LAH reduction. The reduction production was refluxed in 100 ml of ethanol containing 10 drops of conc. hydrochloric acid for 3 hours. The solution was diluted with water, extracted with ether and worked up in the usual manner. The residue was chromatographed on acid washed alumina. Elutions with ether-benzene gave two fractions of dehydroisoandrosterone, a) 8.65×10^8 cpm and b) 4.76×10^8 cpm. Approximately 2×10^5 cpm from each fraction was mixed with 200 μg of carrier dehydroisoandro-

sterone and chromatographed on Whatman #1 paper (18 x 57 cm) in the system 2,2,4-trimethylpentane:toluene (3:1) methanol: water (3:1) for 4 hours. Fraction a contained mainly radioactive dehydroisoandrosterone with a radioactive contaminant which had a slightly greater mobility suggestive of the 3α-hydroxy epimer. Fraction b showed only one radioactive peak which coincided exactly with the position of the carrier dehydroisoandrosterone.

A mixture of 1.90×10^6 cpm of fraction b (3β-hydroxy-Δ^5-androstene-17-one-3α-H^3, 4.75×10^6 cpm/mg) and 1.15 g of non-isotopic 3β-hydroxy-Δ^5-androstene-17-one was counted, 1620 cpm/mg. Approximately 60 mg of the mixture was acetylated with acetic anhydride and pyridine. Recrystallization of dehydroisoandrosterone acetate from methanol gave m.p. 168°, 1660 cpm/mg corrected to the unacetylated compound.

The remainder of the diluted mixture was oxidized by the Oppenauer method with acetone and aluminum tert-butylate in benzene for 10 hours. Chromatography of the product on alumina gave Δ^4-androstene-3,17-dione which on recrystallization from acetone-petroleum ether, melted at 145°, 5 cpm/mg. Further purification by chromatography and recrystallization gave Δ^4-androstene-3,17-dione, m.p. 169-170°, 3 cpm/mg.

3α-Hydroxy-Δ^5-androstene-17-one-3β-H^3 (L).

The sequence of reactions described above for 3β-hydroxy-Δ^5-androstene-17-one (Ec) was carried out on 50 mg (238×10^6 cpm) of 3β-hydroxy-Δ^5-androstene-17-one-

3α-H^3 (K) without purification of any intermediates until the final stage. Mechanical loss at one point led to a much lower yield than anticipated.

The crude 3α-hydroxy-Δ^5-androstene-17-one-3β-H^3 was chromatographed on 8 g of acid washed alumina to give about 2 mg of crystalline material. This material was streaked on two 18 x 118 cm Whatman #1 papers and run for 30 hours in the system isooctane:methanol:water 5:4:1. The radioactivity was located with a Vanguard Automatic Chromatogram Scanner and was extracted from the paper with methanol. The residue from the methanol was dissolved in 50 ml of hot benzene. The benzene solution contained 9.1×10^6 cpm of tritium.

An aliquot containing 2.55×10^4 cpm was added to 51.8 mg of non-isotopic 3α-hydroxy-Δ^5-androstene-17-one (Ec) and the mixture was recrystallized twice from acetone to give 460 cpm/mg and 500 cpm/mg respectively; calculated 492 cpm/mg. The residues from the recrystallizations were acylated with acetic anhydride in pyridine in the usual manner and the acetate was recrystallized twice from methanol to give specific activities corrected to the "free" steroid of 472 and 462 cpm/mg respectively. The results indicated a purity of 96% for 3α-hydroxy-Δ^5-androstene-17-one-3β-H^3 (L). A qualitative paper chromatogram of L showed radioactivity only in the region expected for 3α-hydroxy-Δ^5-androstene-17-one.

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REFERENCES

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