Summary

Ethyl 2-methoxymethylene-3-ethoxypropionate (Ⅲ) undergoes condensation with urea, and N-substituted urea in ethanol solution in the presence of hydrochloric acid. With urea, Ⅲ gave ethyl 2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (V), with N-methylurea a mixture of ethyl 2-oxo-3-methyl-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (XⅢ) and its isomeric 1-methyl compound (XIV), which was separated into each isomer, and with N-phenylurea the 1-phenyl compound (XV) was exclusively obtained. Conversion into pyrimidines was achieved by dehydrogenation and subsequent chlorination of V.

(Received April 11, 1964)

Chem. Pharm. Bull. 12 (7) 808 ~ 812

UDC 547.92.02 : 577.176.3

115. Terumi Aoki, Hiroko Yamamura, Kyoko Takei,[*1] and Hiromu Mori[*2] : Synthesis of 16-Oxygenated Androst-5-en-3β-ol Derivatives.

(*School of Medicine, Gunma University,*[*1] *and Research Laboratory, Teikoku Hormone Mfg. Co., Ltd.*[*2])

Androst-5-ene-3β, 16α, 17β-triol,[1~3] androst-5-ene-3β, 16β, 17β-triol,[4] and 3β, 16α-dihydroxyandrost-5-en-17-one[5] are all steroid metabolites isolated from human urine. In our course of study on steroid metabolism, these compounds became necessary as standard samples. Androst-5-ene 3β,16α,17β-triol was first prepared by Huffman and Lott,[6] from 3β-hydroxyandrost-5-en-17-one through nine steps, but much more convenient method seems not to be reported. This paper describes much more convenient method of synthesis of androst-5-ene-3β,16α,17β-triol and related compounds.

In Huffman's method of synthesis, key steps for introduction of oxygen at C-16 contain three reactions (I→Ⅱ→Ⅲ→Ⅳ); the condensation of 17-oxo steroid (I) with isoamyl nitrite to 16-oximino-17-oxo compound (Ⅱ), reductive hydrolysis of Ⅱ with zinc dust in aqueous acetic acid to 17β-hydroxy-16-oxo compound (Ⅲ) and reduction of Ⅲ with sodium amalgam to 16α,17β-glycol (Ⅳ). The last step (Ⅲ→Ⅳ) is not stereospecific reaction, so that considerable amount of 16β,17β-glycol is also produced[7]. On the other hand, Gallagher and his coworkers[8] developed another method of synthesis of 16α,17β-glycol from 17-oxo steroid which is more stereospecific; I is transformed into 17-enol acetate (V), which on oxidation with peracid gave the oxide (Ⅵ), followed by reduction with lithium aluminum hydride to 16α,17β-glycol (Ⅳ). This elegant method could be applied for synthesis of androst-5-ene-3β,16α,17β-triol.

[*1] 280 Iwakami-cho, Maebashi-shi (青木照美, 山村弘子, 武井京子).

[*2] 1640 Shimosakunobe, Kawasaki-shi (森 弘).

1) H. Hirschman : J. Biol. Chem., **150**, 363 (1943).

2) G.F. Marrian : Nature, **154**, 19 (1944); G.F. Marrian, G.C. Butler : Biochem J., **38**, 322 (1944).

3) H.L. Mason, E.J. Kepler : J. Biol. Chem, **161**, 235 (1945).

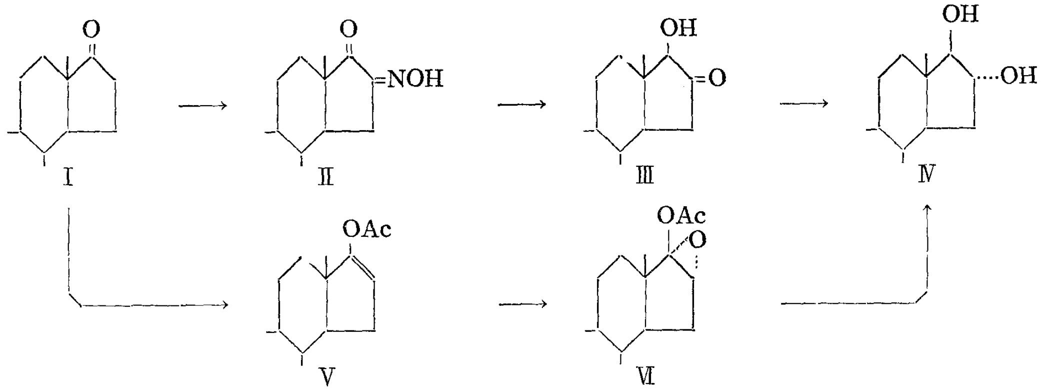
4) K. Fotherby : Biochem. J., **67**, 259 (1957).

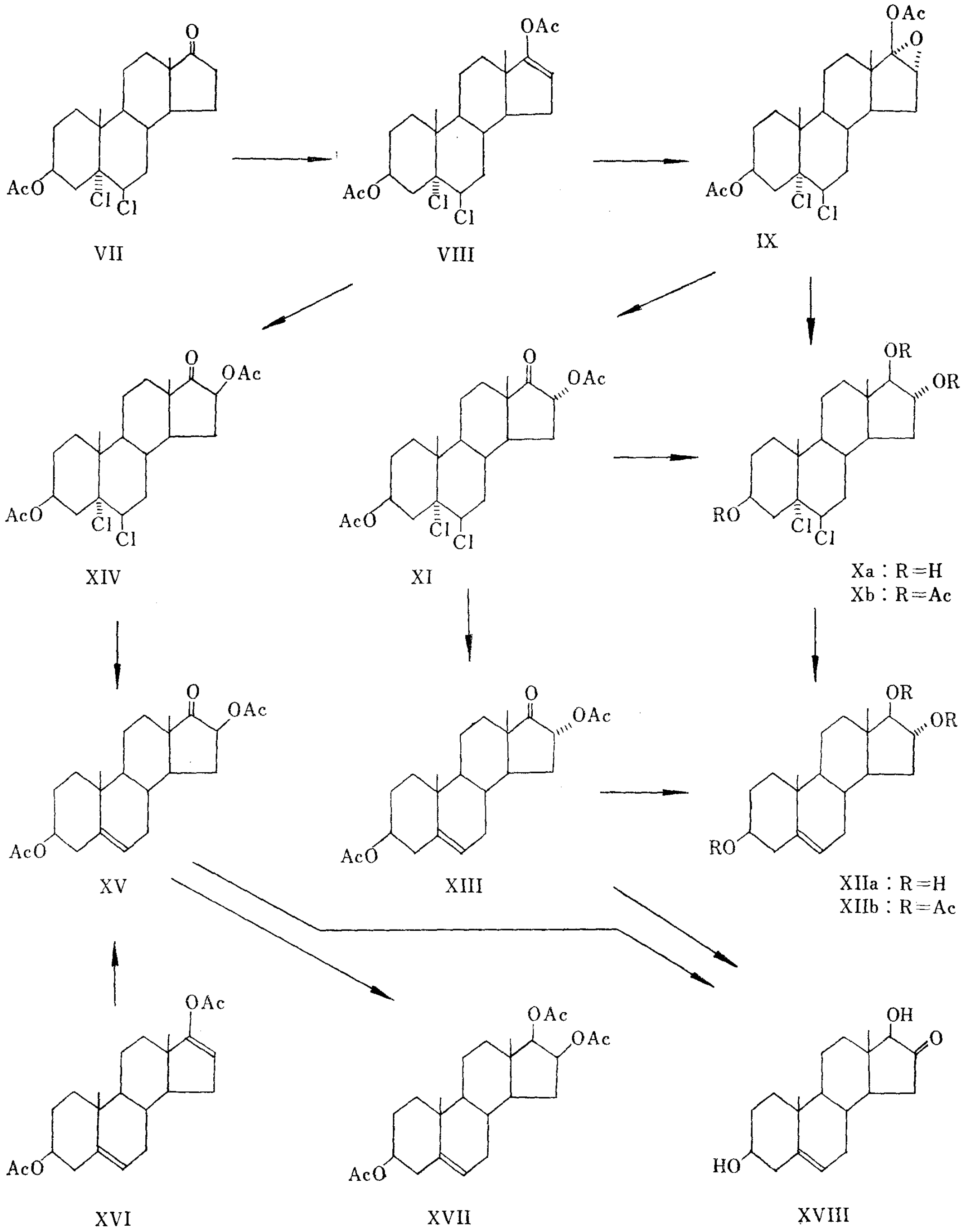
5) K. Fotherby, A. Colas, S.M. Atherden, G.F. Marrian : *Ibid.*, **66**, 664 (1957).

6) M.N. Huffman, M.H. Lott : J. Biol. Chem., **172**, 789 (1948).

7) *Idem* : J. Am. Chem. Soc., **71**, 719 (1949).

8) N.S. Leeds, D.K. Fukushima, T.F. Gallagher : *Ibid.*, **76**, 2943 (1954).


O
O
=NOH
OH
=O
OH
....OH
I
II
III
IV
OAc
OAc
O
V
VI


O
AcO
Cl
Cl
VII
OAc
AcO
Cl
Cl
VIII
OAc
O
AcO
Cl
Cl
IX
O
OAc
AcO
Cl
Cl
XIV
O
OAc
AcO
Cl
Cl
XI
OR
OR
RO
Cl
Cl
Xa : R=H
Xb : R=Ac
O
OAc
AcO
XV
O
OAc
AcO
XIII
OR
OR
RO
XIIa : R=H
XIIb : R=Ac
OAc
AcO
XVI
OAc
OAc
AcO
XVII
OH
O
HO
XVIII

3β-Acetoxy-5α,6β-dichloroandrostan-17-one (VII)[9] was used as a starting material, and was converted to enol acetate (VIII) without any trouble by refluxing with isopropenyl acetate in the presence of catalytic amount of sulfuric acid. When enol acetate (VIII) was oxidized with perphthalic acid, oxide (IX) was given, but recrystallization was not effective for purification. The crude oxide (IX) was reduced with sodium borohydride[*3] and then hydrolized by alkali to give 5α,6β-dichloroandrostane-3β,16α,17β-triol (Xa), the structure of which was proved by introduction of Xa to androst-5-ene-3β,16α,17β-triol triacetate (XIIb). Treatment of Xa with zinc dust in acetic acid afforded halogen free triol (XIIa), which on acetylation with acetic anhydride in pyridine gave triacetate (XIIb). The infrared spectrum of XIIb was identical with that for an authentic sample.[10]

When the oxide (IX) was treated with perchloric acid, a rearrangement product, 3β, 16α-diacetoxy-5α, 6β-dichloroandrostan-17-one (XI) was obtained.[8] Reduction of X with sodium borohydride and acetylation of the reduction product gave triacetate (Xb), which was identical with acetylation product of Xa. Dechlorination of XI with zinc dust gave 3β,16α-diacetoxyandrost-5-ene-17-one (XIII), which could be transformed into triacetate (XIIb) by reduction and acetylation.

Johnson and his coworkers[11] have reported that oxidation of 5α-androst-16-ene-3β,17-diol diacetate with lead tetraacetate affords 3β,16β-diacetoxy-5α-androstan-17-one, and this reaction was extended to estrogen series by Biggerstaff and Gallagher.[12] The oxidation of enol acetate (VIII) with lead tetraacetate gave the expected product, 3β,16β-diacetoxy-5α,6β-dichloroandrostan-17-one (XIV). Dechlorination of XIV with zinc dust afforded 3β,16β-diacetoxyandrost-5-en-17-one (XV), which on reduction with sodium borohydride followed by acetylation gave androst-5-ene-3β, 16β, 17β-triol triacetate (XVII) identical with an authentic sample. XV was also obtained from enol acetate (XVI) by lead tetraacetate oxidation. Both XV and XIII were transformed into 3β,17β-dihydroxyandrost-5-en-16-one (XVIII) on treatment with alkali. It is generally accepted that the most stable 16,17-ketol is 17β-hydroxy-16-oxo compound in C/D trans steroid.[11,13]

Experimental[*4]

3β-Acetoxy-5α,6β-dichloroandrostan-17-one 17-Enol Acetate (VIII)——A solution of 3β-acetoxy-5α, 6β-dichloroandrostan-17-one (VII, 4.0 g.) ih isopropenyl acetate (23 ml.) containing a catalyst solution (2.5 ml.)[*5] was stirred at 90~95° for 2 hr., and then distilled slowly until 7 ml. of isopropenyl acetate was removed. Isopropenyl acetate (16 ml.) and catalyst solution (0.8 ml.) were added and the resulting solution was refluxed for 1 hr. After 20 ml. of isopropenyl acetate were removed by distillation, Et_2O was added and Et_2O solution was washed well with H_2O and dried over Na_2SO_4. Et_2O solution was passed through alumina (100 g.) column, and the column was eluated with another portion of Et_2O. The combined Et_2O solution was condensed to dryness, and the residue was recrystallized from Me_2CO-hexane to give enol acetate (VIII, 2.9 g.) m.p. 140~146°. Further recrystallization from the same solvent afforded an analytical sample as colorless cubes, m.p. 146~150°, $[\alpha]_D^{27}$ $-22°$ (c=1.05), IR ν_{max}^{KBr} cm^{-1}: 1757 (C=O at 17-OAc), 1734 (C=O at 3-OAc). *Anal.* Calcd. for $C_{23}H_{32}O_4Cl_2$: C, 62.44; H, 7.29. Found : C, 62.51; H, 7.37.

5α,6β-Dichloro-16α,17α-epoxyandrostane-3β,17β-diol Diacetate (IX)——A solution of perphthalic acid in Et_2O (2 g. in 32 ml.) was added dropwise to an ice cold solution of the enol acetate (VIII, 2.0 g.) in $CHCl_3$

[*3] Gallagher, *et al.* used $LiAlH_4$ for this type of reaction, but $NaBH_4$ was also a satisfactory reagent for reduction.

[*4] All melting points are uncorrected and optical rotations are measured in $CHCl_3$ solution unless otherwise stated.

[*5] Catalyst solution was a mixture of isopropenyl acetate (5 ml.) and H_2SO_4 (0.1 ml.).

9) H. Mori, J. Yamada : This Bulletin, **11**, 1418 (1963).

10) K. Dobriner, E.R. Katzenellenbogen, R.N. Jones : "Infrared Absorption Spectra of Steroids," 82 (1953), Interscience Publishers, Inc., New York.

11) W.S. Johnson, B. Gastambide, R. Pappo : J. Am. Chem. Soc., **79**, 1991 (1957).

12) W.R. Biggerstaff, T.F. Gallagher : J. Org. Chem., **22**, 1220 (1957).

13) J. Fisman : J. Am. Chem. Soc., 82, 6143 (1960).

(10 ml.). The resulting solution was stored overnight in an ice box. Et_2O (150 ml.) was added and the Et_2O solution was washed with 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. Solvent was evaporated *in vacuo* to give white solid (2.0 g.), m.p. 158~161°, which was used for further transformation without purification.

5α,6β-Dichloroandrostane-3β,16α,17β-triol (Xa)——A solution of the crude epoxide obtained above (IX, 2.0 g.) and $NaBH_4$ (2.0 g.) in MeOH (100 ml.) was refluxed for 1 hr., and 4% NaOH (4 ml.) was added. The solution was refluxed for additional half an hour and poured into 10% H_2SO_4 (600 ml.). Precipitates were collected by filtration and washed well with H_2O. Recrystllization from Me_2CO-hexane gave 5α, 6β-dichloroandrostane-3β,16α,17β-triol (Xa, 970 mg.), m.p. 170~175°. Further recrystallization from MeOH gave an analytical sample as colorless plates, m.p. 170~172°, $[\alpha]^{27}_{D}$ −65° (c=1.06, dioxane), IR: ν^{KBr}_{max} 3300~3480 cm^{-1} (-OH). *Anal.* Calcd. for $C_{19}H_{30}O_3Cl_2 \cdot H_2O$: C, 57.72; H, 8.14. Found: C, 57.99; H, 8.24.

3β,16α-Diacetoxy-5α,6β-dichloroandrostan-17-one (XI)——The crude epoxide obtained above (IX, 2.0 g.) was dissolved in AcOH (120 ml.). After addition of 70% $HClO_4$ (0.4 ml.), the solution was stored in a dark place for 1.5 hr. Et_2O (500 ml.) was added and Et_2O solution was washed with 5% $NaHCO_3$ and 4% NaCl, and dried over Na_2SO_4. After evaporation of the solvent, the residue was dissolved in dry pyridine (40 ml.) and Ac_2O (40 ml.) and the solution was allowed to stand overnight and poured into H_2O. The product was extracted with Et_2O, and the ethereal solution was washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. Evaporation of the solvent gave colorless solid, which on recrystallization from MeOH afforded 3β,16α-diacetoxy-5α,6β-dichloroandrostan-17-one (XI, 1.57 g.), m.p. 155~156°. Further recrystallization from MeOH gave an analytical sample as colorless cubes, m.p. 155~156°, $[\alpha]^{27}_{D}$ +4° (c=1.02), IR $\nu^{CS_2}_{max}$ cm^{-1}: 1765, 1750. *Anal.* Calcd. for $C_{23}H_{32}O_5Cl_2$: C, 60.13; H, 7.02. Found: C, 60.34; H, 7.11.

5α,6β-Dichloroandrostane-3β,16α,17α-triol Triacetate (Xb). a) From 5α,6β-Dichloroandrostane-3β,16α, 17β-triol (Xa)——A solution of 5α,6β-dichloroandrostane-3β,16α,17β-triol (Xa, 500 mg.) in dry pyridine (10 ml.) and Ac_2O (10 ml.) was allowed to stand overnight at room temperature, and poured into H_2O. The product was extracted with Et_2O, and the Et_2O solution was washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. The solvent was removed by distillation, and the residue was recrystallized from MeOH to give 5α,6β-dichloroandrostane-3β,16α,17β-triol triacetate (Xb, 350mg.), m.p. 202~204°. $[\alpha]^{27}_{D}$ −91° (c=0.98), IR: ν^{KBr}_{max} 1730 cm^{-1} (-OAc). *Anal.* Calcd. for $C_{25}H_{36}O_6Cl_2$: C, 59.64; H, 7.21. Found: C, 59.84; H, 7.38.

b) From 3β,16α-Diacetoxy-5α,6β-dichloroandrostan-17-one (XI)——A solution of 3β,16α-diacetoxy-5α,6β-dichloroandrostan-17-one (XI, 100 mg.) and $NaBH_4$ (50 mg.) in MeOH (10 ml.) was stirred at room temperature for 3 hr. After decomposition of excess $NaBH_4$ by one drop of AcOH, the solution was poured into H_2O, and the product was extracted with Et_2O. The Et_2O solution was washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the residue was dissolved in dry pyridine(5 ml.) and Ac_2O (5 ml.) and the solution was stored at room temperature overnight, and poured into H_2O. The product was extracted with Et_2O, and the Et_2O solution washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from MeOH to give triacetate (Xb, 70 mg.). Further recrystallization from MeOH afforded an analytical sample as colorless needless, m.p. 200~202°, which was identical with the sample obtained above.

Androst-5-ene-3β,16α,17β-triol (XIIa)——Zn dust (300 mg.) was added portionwise to a solution of 5α,6β-dichloroandrost-5-ene-3β,16α,17β-triol (Xa, 290 mg.) in AcOH (12 ml.) and the resulting suspension was stirred at room temperature for 2 hr. Zn cake was removed by filtration and washed with MeOH. The combined solution was poured into H_2O, and precipitates were collected by filtration and washed with H_2O. Recrystallization from MeOH afforded androst-5-ene-3β,16α,17β-triol (XIIa, 80 mg.), m.p. 257~258°. An analytical sample was obtained by further recrystallization from MeOH as colorless plates, m.p. 263~265°, $[\alpha]^{27}_{D}$ −67° (c=0.6, AcOH). (reported, m.p. 267~270°, $[\alpha]^{28}_{D}$ −75.7° (90% AcOH)). IR: ν^{KBr}_{max} 3260~3320 cm^{-1} (-OH).

3β,16α-Diacetoxyandrost-5-en-17-one (XIII)——Zn dust (1.6 g.) was added portionwise to a solution of 3β,16α-diacetoxy-5α,6β-dichloroandrostan-17-one (XI, 1.57 g.) in AcOH (64 ml.) and the resulting suspension was stirred for 2 hr. at room temperature. Zn cake was removed by filtraton and washed with MeOH. The combined filtrate was poured into H_2O and precipitates were collected by filtration and washed with H_2O. Recrystallization from MeOH afforded 3β,16α-diacetoxyandrost-5-en-17-one (XIII, 1.0 g.) m.p. 153~165°. Further recrystallization from MeOH gave an analytical sample as colorless needles, m.p. 166~168°, $[\alpha]^{27}_{D}$ −15° (c=1.00), IR $\nu^{CS_2}_{max}$ cm^{-1}: 1763, 1748 (-OAc, C=O). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.01; H, 8.30. Found: C, 71.02; H, 8.32.

Androst-5-ene-3β,16α,17β-triol Triacetate (XIIb). a) From Androst-5-ene-3β,16α,17β-triol (XIIa)——A solution of androst-5-ene-3β,16α,17β-triol (XIIa, 80 mg.) in pyridine (8 ml.) and Ac_2O (8 ml.) was allowed to stand overnight at room temperature, and poured into H_2O. The product was extracted with Et_2O, and the Et_2O solution was washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4.

After evaporation of the solvent, the residue was recrystallized from MeOH to give androst-5-ene-3β,16α,17β-triol triacetate (XIIb). An analytical sample was obtained by further recrystallization from MeOH as colorless plates, m.p. 186~187°, $[\alpha]_D^{27}$ −91° (c=1.05). IR: $\nu_{max}^{CS_2}$ 1742 cm^{-1} (-OAc). (reported, m.p. 184~185°, $[\alpha]_D^{28}$ −102° (95% EtOH)). The IR spectrum of XIIb was the same as that for an authentic sample.

b) From 3β,16α-Diacetoxyandrost-5-en-17-one (XIII)——A solution of 3β,16α-diacetoxyandrost-5-en-17-one (XIII, 300 mg.) and $NaBH_4$ (150 mg.) in MeOH (20 ml.) was stirred at room temperature for 3 hr. After decomposition of excess $NaBH_4$ by one drop of AcOH, the solution was poured into H_2O and the product was extracted with Et_2O. The ethereal solution was washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the residue was dissolved in dry pyridine (10 ml.) and Ac_2O (10 ml.). The solution was stored at room temperature overnight, and poured into H_2O. The product was extracted with Et_2O and the Et_2O solution was washed with 10% H_2SO_4, 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized twice from MeOH to give pure XIIb as colorless plates, which was identical with the sample obtained above in all respects.

3β,16β-Diacetoxy-5α,6β-dichloroandrostan-17-one (XIV)——The enol acetate (XIII, 2.0 g.) was dissolved in AcOH (32 ml.) and Ac_2O (3.2 ml.), and $Pb(OAc)_4$ (2.4 g.) was added to the solution. The solution was allowed to stand at room temperature for 18 hr. The solvent was distilled *in vacuo* and the product was dissolved in Et_2O. The Et_2O solution was washed with 5% $NaHCO_3$, and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from Me_2CO-hexane to give 3β,16β-diacetoxy-5α,6β-dichloroandrostan-17-one (XIV, 1.7 g.), m.p. 191~193°. An analytical sample was obtained by further recrystallization from the same solvent, m.p. 195~196°, $[\alpha]_D^{27}$ 0° (c=1.02). IR ν_{max}^{KBr} cm^{-1}: 1730, 1745 (C=O, -OAc). *Anal.* Calcd. for $C_{23}H_{32}O_5Cl_2$: C, 60.13; H, 7.02. Found: C, 59.95; H, 7.20.

3β,16β-Diacetoxyandrost-5-en-17-one (XV). a) From 3β,16β-Diacetoxy-6β-dichloroandrostan-17-one (XIV)——Zn dust (500 mg.) was added in portions to a solution of 3β,16β-diacetoxy-5α,6β-dichloroandrostan-17-one (XIV, 500 mg.) in AcOH (20 ml.) and the resulting suspension was stirred at room temperature for 2 hr. After removal of Zn cake by filtration, the filtrate was poured into H_2O. The product was extracted with Et_2O, and the Et_2O solution was washed with 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. The solvent was evaporated to dryness and the residue was recrystallized from Me_2CO-hexane to give 3β,16β-diacetoxyandrost-5-en-17-one (XV, 210 mg.), m.p. 168~170°. Further recrystallization from the same solvent gave an analytical sample as colorless plates, m.p. 172~173°, $[\alpha]_D^{27}$ +9° (c=1.00), IR ν_{max}^{KBr} cm^{-1}: 1730, 1745 (-OAc, C=O). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.19; H, 8.39.

b) From 3β-Acetoxyandrost-5-en-17-one 17-Enol Acetate (XVI)——The enol acetate (XVI, 1 g.) was treated with $Pb(OAc)_4$ (1.2 g.), AcOH (16 ml.), and Ac_2O (1.6 ml.) as described above. Recrystallization from MeOH gave 3β,16β-diacetoxyandrost-5-en-17-one (XV, 620 mg.), m.p. 164~168°, the IR spectrum of which was identical with the product obtained above.

Androst-5-ene-3β,16β,17β-triol Triacetate (XVII)——3β,16β-Diacetoxyandrost-5-en-17-one (XV, 100 mg.) was treated with $NaBH_4$ (50 mg.) and the reduction product was acetylated with Ac_2O in pyridine as described above. Recrystallization from Me_2CO-hexane gave androst-5-ene-3β,16β,17β-triol triacetate (XVII, 50 mg.) as colorless plates, m.p. 212~214°, $[\alpha]_D^{27}$ −30° (c=1.02), the IR spectrum of which was identical with that for an authentic sample.

3β,17β-Dihydroxyandrost-5-en-16-one (XVIII). a) From 3β,16β-Diacetoxyandrost-5-en-17-one (XV)——A solution of 3β,16β-diacetoxyandrost-5-en-17-one (XV, 100 mg.) and KOH (100 mg.) in MeOH (10 ml.) was refluxed for 2 hr., and poured into H_2O. The product was extracted with $CHCl_3$-MeOH (50:4) and organic layer was washed with 10% HCl, 5% Na_2CO_3 and H_2O and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from MeOH to give 3β,17β-dihydroxyandrost-5-en-16-one (XVIII, 50 mg.) as colorless plates, m.p. 207~211°, the IR spectrum of which was identical with that for an authentic sample.

b) From 3β,16α-Diacetoxyandrost-5-en-17-one (XIII)——3β,16α-Diacetoxyandrost-5-en-17-one (XIII, 100 mg.) was treated as the same way described above and the same product (XVIII) was obtained.

The authors are very grateful to Prof. K. Shida, Gunma University and Dr. I. Chuman, the Director of Research Laboratory, Teikoku Hormone Mfg. Co., Ltd. for their encouragements throughout this work, and also to Dr. S. Wada, Research Laboratory, Teikoku Hormone Mfg. Co., Ltd. for his kind advices.

Summary

Some 16-oxygenated androst-5-ene steroids, which are all steroid metabolites, were prepared from 3β-acetoxy-5α,6β-dichloroandrostan-17-one.

(Received February 22, 1964)