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NEW SYNTHESIS OF 2β-HYDROXY-19-OXOANDROST-4-ENE-3,17-DIONE AND ITS 2β-^{18}O ANALOG

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Summary—Treatment of 19-[oxygenated]-androst-4-ene-3,17-dione with $Mn(AcO)_3$ and $ClCH_2COOH$ in benzene gave epimeric mixtures of the corresponding 2ξ-chloroacetates and 2ξ-acetates. The products were processed to give the title compound. For the synthesis of the 2-^{18}O analog, $ClCH_2C^{18}OOH$ was used, which was prepared from $ClCH_2COCl$.

INTRODUCTION

2β-Hydroxy-19-oxoandrost-4-ene-3,17-dione [compound (**6a**)] was proposed as an intermediate in the biosynthetic elaboration of estrone from androgens [1]. The reported syntheses of compound (**6a**) were unsatisfactory [1–4] and proceeded in very poor yield (0.09%). Our subsequent effort to improve the synthesis, which also involved recyclization of certain intermediates, was only marginally successful [4]. The synthetic difficulties and scarcity of the compound, and particularly of its isotopically labeled analog, hindered a thorough evaluation of its role in the biosynthesis. There was an obvious need for a better synthesis, which is described herein.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian EM-390 or 500 MHz spectrometer for solutions in C^2HCl_3 and are reported in δ values relative to internal tetramethylsilane or chloroform. An HP-5970 instrument equipped with a mass selective detector and a 15 m DB1 column (J&W Scientific, Folsom, CA) was used. In general, the samples were analyzed by E.I. mass spectrometry and/or E.I.GC-MS of their MO-TMS derivatives. The samples were introduced by splitless injection at 50°C and, following a 3-min hold, the oven was taken to its starting temperature of 210°C at the rate of 27°C/min. The temperature was then increased linearly by 3°C/min to a final temperature of 320°C. Mass spectra were acquired by continuously scanning over the range 100–600 a.m.u. Compounds were analyzed by GC-MS of oxime-silyl ethers (MO-TMS). Several products were analyzed by desorption chemical ionization (DCI) using ammonia as the reagent gas.

High performance liquid chromatography (HPLC) was carried out on an instrument equipped with: (1) Waters Co., Model 510, twin pumps and an automatic gradient controller; (2) Micromeritics Co., Model 788, Dual Variable Detector; and (3) Houston Instrument Co., Omni Scribe Series 5000 recorder. In all instances, an Alltech Nucleosil 50 column (silica 5μ: 4mm/25 cm) was used. The column was eluted with the indicated solvent systems at a flow rate of 1 ml min^{-1}; and detector setting at 240 and 280 nm.

Thin layer (TLC) and preparative (PLC) chromatography was carried out using precoated plates [silica gel 60 (HF 254–366)] (Analtech, Inc., Newark, DE).

Water labeled with ^{18}O was purchased from MSD isotopes (Montreal, Canada). Melting points (m.p.) were taken on a Kofler hot-stage apparatus and are reported as read.

*2ξ-Chloroacetoxy-17-testosterone acetate (**1c**)*

A mixture of $Mn(AcO)_3$ dihydrate (402 mg, 1.5 mmol) and chloroacetic acid (284 mg, 3 mmol) in dry benzene (50 ml) was refluxed for 45 min under a Dean-Stark separator and

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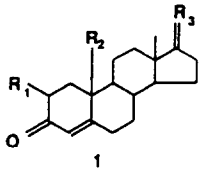


1

a) R_1 = -H; R_2 = -CH_2OAc; R_3 = O

b) R_1 = -H; R_2 = -CH_3; R_3 = (β)-OAc

c) R_1 = -$OCOCH_2Cl$; R_2 = -CH_3; R_3 = (β)-OAc

d) R_1 = -OAc; R_2 = -CH_3; R_3 = (β)-OAc;

e) R_1 = -H; R_2 = -CH_3; R_3 = β-$OCOCH_2Cl$

f) R_1 = -$OCOCH_2Cl$; R_2 = -CH_3; R_3 = (β)-$OCOCH_2Cl$

g) R_1 = -OH; R_2 = -CH_3; R_3 = (β)-OAc

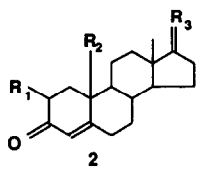
h) R_1 = -OH; R_2 = -CH_3; R_3 = (β)-OH

i) R_1 = -OH; R_2 = -CH_2OAc; R_3 = O

k) R_1 = -H; R_2 = -CH_2OAc; R_3 = (β)-OAc

Scheme 1

cooled to room temperature (RT). Then testosterone acetate (**1b**) (83 mg, 0.25 mmol) was added and the reaction mixture was refluxed for 20 h. Following cooling to RT, ethyl acetate (100 ml) was added and the solution was washed successively with 1M hydrochloric acid, saturated aq. sodium bicarbonate, saturated aq. NaCl and dried (Na_2SO_4). Removal of solvent gave 120 mg of a crude product. An aliquot of the crude product (60 mg) was fractionated by PLC (cyclohexane–EtOAc, 1:1, v/v) to give 2ξ-chloroacetate-testosterone acetate (**1c**) 28 mg (53%) and 2ξ-hydroxy-testosterone diacetate (**1d**) 10 mg (21%). For (**1c**) NMR δH ($CDCl_3$), 0.84 (3H,s,13-CH_3), 1.22 (3H,s,10-CH_3, 2.0 (3H,s,-$OCOCH_3$), 4.14 (2H,s,-$OCOCH_2$-Cl), 4.64 (1H,t,17-H), 5.38 (1H,dd,J_1 = 5.5 Hz, J_2 = 11.9 Hz, 2-H), 5.38 (1H,s,4-H).



2

a) R_1 = -$OCOCH_2Cl$; R_2 = -CH_2OAc: R_3 = O

b) R_1 = -OAc; R_2 = -CH_2OAc; R_3 = O

c) R_1 = -$^{18}OCOCH_2Cl$; R_2 = -CH_2OAc; R_3 = O

d) R_1 = -$OCOCH_2Cl$; R_2 = -CH_2OAc; R_3 = (β) - OAc

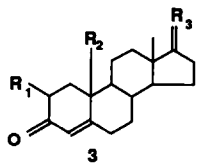
e) R_1 = -OAc; R_2 = -CH_2OAc; R_3 = (β)-OAc

f) R_1 = -OH; R_2 = -CH_2OH; R_3 = (β)-OAc

g) R_1 = -OH; R_2 = -CH_2OAc; R_3 = -(β)-OAc

h) R_1 = H; R_2 = -CH_2OH; R_3 = O

Scheme 2



3

a) R_1 = (β)-OH; R_2 = -CH_2OH; R_3 = O

b) R_1 = (α)-OH; R_2 = -CH_2OH; R_3 = O

c) R_1 = (β)-^{18}OH; R_2 = -CH_2OH; R_3 = O

d) R_1 = (α)-^{18}OH; R_2 = -CH_2OH; R_3 = O

e) R_1 = (β)-$OCOCH_2Cl$; R_2 = -CH_2OAc; R_3 = (β)-OAC

f) R_1 = (α)-$OCOCH_2Cl$; R_2 = -CH_2OAc; R_3 = (β)-OAc

g) R_1 = (β)-OAc; R_2 = -CH_2OAc; R_3 = (β)-OAc

h) R_1 = (α)-OAc; R_2 = -CH_2OAc; R_3 = (β)-OAc

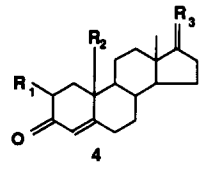
i) R_1 = (β)-OH; R_2 = -CH_2OH; R_3 = (β)-OAc

k) R_1 = (α)-OH; R_2 = -CH_2OH; R_3 = (β)-OAc

l) R_1 = (β)-OH; R_2 = -CH_2OAc; R_3 - (β)-OAc

m) R_1 = (α)-OH; R_2 = -CH_2OAc; R_3 - (β)-OAc

Scheme 3



4

a) R_1 = β-tBDMSO; R_2 = -CH_2OH; R_3 = O

b) R_1 = β-tBDMSO; R_2 = $CH_2OSMDBt$ R_3 = O

c) R_1 = β-tBDMS^{18}O; R_2 = -CH_2OH; R_3 = O

d) R_1 = -tBDMSO; R_2 = -CH_2OAc; R_3 = (β)-OAc

e) R_1 = (β)-tBDMSO; R_2 = -CH_2OAc; R_3 = (β)-OAc

f) R_1 = (α)-tBDMSO; R_2 = -CH_2OAc; R_3 = (β)-OAc

g) R_1 = -tBDMSO; R_2 = -CH_2OH; R_3 = (β)-OH

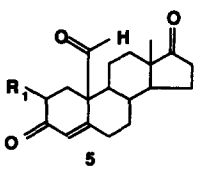
h) R_1 = (β)-tBDMSO; R_2 = -CH_2OH; R_3 = (β)-OH

i) R_1 = (α)-tBDMSO; R_2 = -CH_2OH; R_3 = (β)-OH

k) R_1 = (β)-tBDMSO; R_2 = -CH_2OH; R_3 = (β)-OAc

l) R_1 = (α)-tBDMSO; R_2 = -CH_2OH; R_3 = (β)-OAc

Scheme 4



5

a) R_1 = (β)-tBDMSO

b) R_1 = (β)-tBDMS^{18}O

c) R_1 = (α)-tBDMSO

d) R_1 = (β)-OH

e) R_1 = (β)-^{18}OH

f) R_1 = (α)-OH

g) R_1 = -H

h) R_1 = $-OCOCH_2Cl$

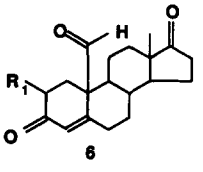
Scheme 5

*2ξ-Hydroxy-testosterone diacetate (**1d**)*

NMR δH ($CDCl_3$) 0.83 (3H,s,13-CH_3), 1.19 (3H,s,10-CH_3), 2.0 (3H,s,17-OCOCH_3), 2.11 (3H,s,2-OCOCH_3), 4.64 (1H,t,17-H), 5.15 (1H,dd, $J_1 = 5.4$ Hz $J_2 = 12$ Hz, 2-H), 5.14 (1H,s,4-H).

*2ξ-Hydroxy-testosterone 17-acetate (**1g**)*

The remainder of the crude product from the above reaction (60 mg) was dissolved in methanol (10 ml) and a solution of K_2CO_3 (25 mg) in water (0.5 ml) was added. The mixture was stirred at room temperature (30 min), diluted with water (5 ml) and the products were recovered (dichloromethane) and processed in the usual way to give a residue (**1g**) (41 mg). ^{1}H NMR δH ($CDCl_3$) 0.88 (3H,s,13-CH_3), 1.2 (3H,s,10-CH_3), 2.07 (3H,s,17-OCOCH_3), 3.5 (1H,br.s,2-OH), 4.2 (1H, dd, $J_1 = 5.5$ Hz, $J_2 = 13$ Hz, 2-H), 4.64 (1H,s,17-H), 5.84 (1H,s,4-H).



6

a) R_1 = (β)-OH

b) R_1 = (β)-^{18}OH

c) R_1 = (α)-OH

Scheme 6

*2ξ-Hydroxy-testosterone dichloroacetate (**1f**)*

A mixture of $Mn(AcO)_3$ (402 mg, 1.5 mmol) and chloroacetic acid (284 mg, 3 mmol) in dry benzene (50 ml) was dried as above then testosterone chloroacetate (**1e**) (92 mg, 0.25 mmol) was added. The reaction mixture was refluxed for 18 h. The cooled reaction mixture was processed in the described manner and concentrated to yield a crude residue (105 mg). PLC fractionation (EtOAc–cyclohexane, 1:1, v/v) gave (**1f**) 95 mg (82%). ^{1}H NMR δH ($CDCl_3$) 0.9 (3H,s,13-CH_3), 1.25 (3H,s,10-CH_3), 4.07 (2H,s,17-OCOCH_2-Cl), 4.18 (2H,s,2-OCO-CH_2-Cl), 4.74 (1H,m,17-H), 5.4 (1H,dd,$J_1 = 6$ Hz, $J_2 = 12$ Hz, 2-H), 5.84 (1H,s,4-H).

*2ξ-Hydroxy-testosterone (**1h**)*

To a solution of dichloroacetate (**1f**) (50 mg, 0.11 mmol) in methanol (7 ml) potassium carbonate (20 mg) in water (0.5 ml) was added. The reaction mixture was stirred at RT (45 min), then diluted with water (10 ml), and the recovered dichloromethane products were processed to give a residue (34 mg). Following PLC (EtOAc–cyclohexane, 1:1, v/v) 2-hydroxy-testosterone was obtained (28 mg; 84%). ^{1}H NMR δH ($CDCl_3$) 0.8 (3H,s,13-CH_3), 1.2 (3H,s,10-CH_3), 3.66 (br.m, 17-H and -OH), 4.19 (1H,m,2-H), 5.84 (1H,s,4-H).

*Treatment of 19-hydroxyandrost-4-ene-3,17-dione acetate (**1a**) with $Mn(AcO)_3$/$ClCH_2COOH$*

$Mn(AcO)_3$ dihydrate (2.0 g, 7.46 mmol) and chloroacetic acid (1.17 g, 12.43 mmol) in dry benzene (100 ml) was dried (reflux, 45 min D-S trap). To the cooled mixture (RT) (**1a**) (611 mg, 1.776 mmol) was added and the reaction mixture was refluxed for 20 h. Ethyl acetate (100 ml) was added (at RT) and the solution was processed and concentrated to give a crude residue (882 mg). HPLC indicated the presence of four major components.

*2β,19-dihydroxyandrost-4-ene-3,17-dione (**3a**)*

A solution of the above crude product (441 mg) in methanol (25 ml) was stirred under argon at RT, then aq. 1M KOH (1.5 ml) was added. After 15 min, the reaction was terminated and neutralized with 1N acetic acid. Most of the MeOH was removed under reduced pressure (water bath temp. 30°C), and the remaining liquid was extracted several times with $CHCl_3$ (100 ml). The extract was processed in the usual manner and concentrated to a residue (200 mg). The residue was fractionated by PLC

($MeOH–CHCl_3$, 1:9, v/v) to give (**3a**) (63 mg; 25%) and (**3b**) (45 mg; 18%). For (**3a**) m.p. 140–42°C (reported [4] 140–142°C); NMR; δH ($CDCl_3$) 0.9 (3H,s,13-$C\underline{H}_3$), 3.64 (1H,d,J = 10.5 Hz, 1H of 19-$C\underline{H}_2$), 4.1 (2H,m,2α-$\underline{H}$; 1H of 19-$C\underline{H}_2$-OH), 4.6 (1H, br.m.; D_2O exchangeable-O$\underline{H}$), 5.96 (1H,s,4-$\underline{H}$).

*2α,19-Dihydroxyandrost-4-ene-3,17-dione (**3b**)*

The (**3b**) showed m.p. 202–204°C (reported [4] 202–204°C); NMR: δH 0.9 (3H,s,13-$C\underline{H}_3$), 3.64 (1H,m; D_2O exchangeable, -O$\underline{H}$), 4.07 (2H,d,J = 2.5 Hz, 19-$C\underline{H}_2$), 4.70 (1H,dd,J_1 = 6 Hz, J_2 = 13 Hz, 2β-$\underline{H}$), 6.0 (1H,s,4-$\underline{H}$).

*2β,19-Dihydroxyandrost-4-ene-3,17-dione 2-t-BDMS (**4a**)*

A mixture of 2β,19-dihydroxyandrost-4-ene-3,17-dione (38 mg, 0.12 mmol), *t*-butyldimethylchlorosilane (*t*-BDNSCl) (60 mg, 0.4 mmol) imidazol (95 mg; 1.4 mmol) in dry DMF (5 ml) was stirred at RT under argon for 1 h. Then diethylether (50 ml) was added, the solution was washed with water, dried, and the solvent removed. The recovered product was resolved by PLC (cyclohexane–EtOAc, 1:3, v/v) to give (**4a**) (25 mg; 48%) and (**4b**) (20 mg; 30%).

For (**4a**) m.p. 131–132°C; NMR: δH ($CDCl_3$), 0.14 (3H,s,Si-$C\underline{H}_3$) 0.22 (3H,s,Si-$C\underline{H}_3$), 0.91 [9H,s,C($C\underline{H}_3$)$_3$], 0.95 (3H,s,13-$C\underline{H}_3$), 3.61–4.24 (3H,2α-$\underline{H}$ and 19-$C\underline{H}_2$), 5.98 (1H,s,4-$\underline{H}$).

*2β,19-Dihydroxyandrost-4-ene-3,17-dione bis-t-BDMS (**4b**)*

Semi-solid, NMR; δH ($CDCl_3$), 0.00 (3H,s,Si-$C\underline{H}_3$), 0.05 [6H,s,Si($C\underline{H}_3$)$_2$], 0.08 (3H,s,Si-$C\underline{H}_3$), 0.81 (3H,s,13-$C\underline{H}_3$), 0.84 [18H,s,SiC($C\underline{H}_3$)$_3$ × 2], 3.84 (2H,d,J = 3 Hz, 19-$C\underline{H}_2$), 4.1 (1H,m,2α-$\underline{H}$), 5.77 (1H,s,4-$\underline{H}$).

*2β-Hydroxy-19-oxoandrost-4-ene-3,17-dione 2-t-BDMS (**5a**)*

(a) A mixture of 2β,19-dihydroxyandrost-4-ene-3,17-dione 2-*t*-BDMS (**4a**) (17 mg, 0.04 mmol), pyridinium chlorochromate (pcc) (excess) and dichloromethane (10 ml) was stirred at RT for 2 h. Then anhydrous diethylether (50 ml) was added and the solids were removed by filtration through a celite column. The filtrate and column washings were combined and concentrated. The product was purified (PLC) (cyclohexane–EtOAc, 1:3, v/v) to give (**5a**) (12 mg; 70%); m.p. ($MeOH/CHCl_3$) 186–188°C (reported [4] 186–188°C); NMR; δH ($CDCl_3$), 0.01 (3H,s,Si-$C\underline{H}_3$), 0.11 (3H,s,Si-$C\underline{H}_3$), 0.83 (9H,s,-C($C\underline{H}_3$)$_3$), 0.86 (3H,s,13-$C\underline{H}_3$), 4.07 (1H,m,2α-$\underline{H}$), 5.94 (1H,s,4-$\underline{H}$), 9.94 (1H,s,10-C$\underline{H}$O). Mass spectrum (C.I.; $N\underline{H}_3$) *m/z* 448 [$(M^+ + NH_4)^+$ 100%], 431[$(M + H)^+$; 100%], 420 {[$(M + NH_4) - CO)]^+$; 15%} 373 [$(M - C_4H_9)^+$; 8%], 316 [$(M - SiC_5H_{15})^+$; 15%].

(b) A mixture of 2β-*t*-BDMS,17,19-diol (**4h**) (21 mg, 0.048 mmol), dichloromethane (5 ml), and pcc (excess) was stirred at RT for 2 h. Then anhydrous diethylether (25 ml) was added and the organic extract was filtered through a column of celite. Evaporation of the filtrate yielded a crude product. PLC of the residue (EtOAc–cyclohexane, 1:3, v/v) gave (**5a**) (14 mg, 67%). m.p. (MeOH; white needles) 179–183°C; NMR. Identical to (**5a**) from (a); mass spectrum (MO-*t*-BDMS) *m/z* 517 (M, 2%), 502 (2%), 486 (7%), 460 (100%), 385 (80%), 354 (12%).

*2β-Hydroxy-19-oxoandrost-4-ene-3,17-dione (**6a**)*

To a solution of 2-*t*-BDMS (**5a**) (11.5 mg, 0.027 mmol) in acetonitrile (2 ml) aq. (48%) HF (0.8 ml) was added and the reaction was stirred 1 h at RT. The reaction was terminated, $CHCl_3$ (25 ml) was added, and the organic layer was washed with water, dried and evaporated to give the crude product (9.5 mg). Following HPLC (isopropanol–isooctane, 3:17, v/v), (**6a**) [retention time (rt) 11 min] (5 mg; 60%) was obtained; m.p. 166–168°C (reported [4] 164–168°C); NMR: δH ($CDCl_3$), 0.96 (3H,s,13-$C\underline{H}_3$), 3.29 [1,H,s (D_2O-exchangeable 2-O$\underline{H}$)], 4.18 (1H,dd,J_1 = 6 Hz, J_2 = 10 Hz, 2α-$\underline{H}$), 6.06 (1H,s,4-$\underline{H}$), 9.62 (1H,s,19-C$\underline{H}$O). Mass spectrum (C.I.; NH_3) *m/z* 334[$(M + NH_4)^+$ 100%], 307 [$(M + NH_4)$-28) 25%].

*2ξ-Chloroacetoxy-17β,19-dihydroxyandrost-4-en-3-one diacetate (**2d**)*

A mixture of $Mn(AcO)_3$ (1.9 g, 7.09 mmol) and chloroacetic acid (1.349 g, 14.3 mmol) in dry benzene (100 ml) was refluxed for 45 min under a Dean-Stark trap. The mixture was cooled, then 17,19-diacetoxy (**1k**) (475 mg, 1.224 mmol) was added and the reaction was refluxed (22 h). The products were recovered (ethyl acetate; 100 ml) and processed as described above to yield a residue (585 mg). PLC

fractionation (cyclohexane–EtOAc, 1:1, v/v) gave (**2d**) (315 mg; 55.5%) and (**2e**) (126 mg; 24%).

For (**2d**); NMR: δH ($CDCl_3$) 0.84 (3H,s,13-CH_3), 2.0 (s,$OCOCH_3$), 2.03 (s,-$COCH_3$), 4.04 (1H, B—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.17 (2H,s,2-$OCOCH_2$-Cl), 4.58 (1H,m,17-H), 4.77 (1H, A—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 5.44 (1H,dd,J_1 = 7.5 Hz, J_2 = 12 Hz; 2α-H), 5.90 (1H,dd,J_1 = 6 Hz, J_2 = 14 Hz; 2β-H), 5.92 (1H,s,4H).

HPLC analysis of (**2d**) (isopropanol–isooctane, 1:9, v/v) and quantitation of products (Hewlett Packard 3390A integrator) gave (**3e**) (rt 5.8 min) and (**3f**) (rt 6.2 min) in nearly 1:1 ratio.

The triacetate (**2e**) was analyzed in the same manner and showed that (**3g**) (rt 7.8 min) and (**3h**) (rt 8.1 min) are present in nearly 1:1 ratio.

*2ξ,17β,19-Trihydroxyandrost-4-en-3-one 17-acetate (**3i**)*

(a) To a solution of the crude residue from the above experiment (50 mg) in MeOH (10 ml), K_2CO_3 (12.5 mg) in water (0.5 ml) was added and the mixture was stirred (30 min) at RT. Water was then added (5 ml) and the products were recovered (methylene chloride) and processed to yield 2ξ,19-diol-17-acetate (**2f**), and 2ξ-hydroxy-17,19-diacetate (**2g**).

(b) To a solution of (**2d**) (72 mg, 0.15 mmol) in methanol (12 ml) a solution of potassium carbonate (30 mg) in water 0.5 ml was added. The mixture was stirred at RT (30 min), diluted with water (5 ml) and the products were recovered (dichloromethane) and processed in the usual way to give the diol (**2f**) (37 mg). Following PLC (EtOAc–benzene, 3:1, v/v × 3) (**3i**) (18 mg; 33%), and (**3k**) (13 mg; 24%) were obtained.

For (**3i**); m.p. (MeOH) 166–169°C; NMR: δH ($CDCl_3$) 0.84 (3H,s,13-CH_3), 2.0 (1H,s,17-$OCOCH_3$), 3.58 (1H, B—part of an AB system, J = 12 Hz 1H of 19-CH_2), 4.08 (1H, A—part of an AB system, J = 12 Hz 1H of 19-CH_2-), 4.1 (1H,m,2α-H), 4.62 (1H,m,17-H), 5.97 (1H,s,4-H); mass spectrum (MO-TMS), *m/z* 535 (M^+, 2%), 520 (12%), 504 (2%), 445 (100%), 342 (25%), 283 (37%), 251 (21%).

*2α,17β,19-Trihydroxyandrost-4-en-3-one 17-acetate (**3k**)*

The product showed: m.p. (MeOH) 144–147°C; NMR δH ($CDCl_3$) 0.84 (1H,s,13-CH_3), 2.0 (3H,s,17-$OCOCH_3$), 3.9 (1H, B—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.1 (1H, A—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.62 (1H,m,17-H), 4.74 (1H,m,2β-H), 5.98 (1H,s,4-H); mass spectrum (MO-TMS) *m/z* 535 (M^+; 10%), 504 (43%), 445 (25%), 432 (18%), 414 (100%), 342 (40%), 282 (40%), 251 (33%).

*2β,17β,19-Trihydroxyandrost-4-en-3-one triacetate (**3g**)*

Acetylation of 2β,19-diol (**3i**) (15 mg, 0.04 mmol) [pyridine (2 ml); acetic anhydride (1 ml)] gave triacetate (**3g**) (15 mg; 85%); m.p. (MeOH) 138–142°C; NMR δH ($CDCl_3$), 0.84 (3H,s,13-CH_3), 2.0 (6H,s,-$OCOCH_3$ × 2), 2.14 (3H,s,2-$OCOCH_3$), 4.14 (1H, B—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.64 (1H, A—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.65 (1H,m,17-H), 5.38 (1H,dd,J_1 = 9 Hz, J_2 = 11.5 Hz, 2α-H), 5.9 (1H,s,4-H); mass spectrum (MO) *m/z* 475 (M^+, 13%), 415 (60%), 373 (54%), 342 (100%), 324 (44%), 282 (35%).

*2α,17,19-Trihydroxyandrost-4-en-3-one triacetate (**3h**)*

Acetylation of the 2α,19-diol (**3k**) (13 mg, 0.036 mmol) [pyridine (2 ml); acetic anhydride (1 ml)] gave triacetate (**3h**) (12.5 mg; 78%); m.p. (MeOH) 149–152°C; NMR δH ($CDCl_3$), 0.92 (3H,s,13-CH_3), 2.0 (3H,s,-$OCOCH_3$), 2.04 (3H,s,-$OCOCH_3$), 2.12 (3H,s,2-$OCOCH_3$), 4.24 (1H, B—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.62 (1H,m,17-H), 4.75 (1H, A—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 5.8 (1H,dd,J_1 = 5.4 Hz, J_2 = 14.2 Hz, 2β-H), 5.94 (1H,s,4-H); mass spectrum (MO) *m/z* 475 (M^+, 6%), 444 (3%), 415 (41%), 373 (41%), 355 (22%), 342 (100%), 324 (26%), 282 (41%).

*2ξ,17β,19-Trihydroxyandrost-4-en-3-one 17,19-diacetate (**2g**)*

To a solution of 2ξ-chloroacetoxy-17,19-diacetate (**2d**) (255 mg, 0.53 mmol) in MeOH (20 ml), 5% aq. potassium bicarbonate (2 ml) was added and the mixture was stirred (30 min) at RT. Then water (10 ml) was added and the product was recovered (methylene chloride) and processed in the usual manner (230 mg). PLC fractionation (EtOAc–cyclohexane, 2:1, v/v) gave (**2g**) (142 mg; 66%), (**3i**) (21 mg; 11%) and (**3k**) (10 mg; 5%).

For (**2g**): NMR δH ($CDCl_3$), 0.87 (3H,s,13-CH_3), 2.04 (6H,s,$OCOCH_3$ × 2), 3.06 (1H, br.s, 2-OH), 4.1 (1H,m,2α-H), 4.26 (B—part of an

AB system, J = 12 Hz, 1H of 19-CH_2), 4.6 (2H,br,m,2β-H and 17-H), 4.8 (1H, A—part of an AB system, J = 12, 1H of 19-CH_2), 6.0 (1H,s,4-H).

HPLC of (**2g**) (isopropanol–iso-octane, 1:5, v/v) gave pure (**31**) (rt 6.1 min) and (**3m**) (rt 6.8 min) at about 1:1 ratio (HP. integrator).

2ξ,17,19-Trihydroxyandrost-4-en-3-one 2-t-BDMS 17,19-diacetate (4d)

A mixture of 2-hydroxy-17,19-diacetate (**2g**) (142 mg; 0.35 mmol), *t*-BDMSCl (240 mg, 1.6 mmol), imidazole (120 mg, 1.76 mmol) in dry DMF (5 ml) was stirred (20 h) at RT. Then, water was added and the recovered product ($CHCl_3$) was fractionated (PLC) (EtOAc–cyclohexane, 1:2, v/v) to yield (**4d**) (182 mg, 100%); NMR δH ($CDCl_3$), 0.84 (3H,s,13-CH_3), 0.92 [9H,s,C(CH_3)$_3$], 2.0 (6H,s,-OCO-CH_3 × 2), 4.14 (2H, br.m, 2ξ-H and 1H of 19-CH_2), 4.67 (3H, br.m., 2ξ-H,17-H and one of 19-CH_2), 5.84 (1H,s,4-H).

An aliquot of (**4d**) was purified by HPLC (isopropanol–isooctane, 1:19, v/v) to give 2β-*t*-BDMS (**4e**) (rt 4.5 min) and 2α-*t*-BDMS (**4f**) (rt 5.0 min). For (**4e**) mass spectrum (MO) *m/z* 547 (M^+ not detected), 532 (7%), 516 (8%), 490 (100%), 390 (32%).

For (**4f**) mass spectrum (MO) *m/z* 547 (M^+; not detected), 532 (4%), 490 (69%), 430 (100%), 356 (61%), 296 (36%).

2α,17β,19-Trihydroxyandrost-4-en-3-one 2-t-BDMS (4g)

A solution of (**4d**) (182 mg, 0.35 mmol) in MeOH (18 ml) was purged with argon, then 5% aqueous sodium hydroxide (0.78 ml) was added, and the mixture was stirred (2 h) at RT. Water was added and the product was recovered (chloroform) and processed in the conventional manner to give a residue (120 mg). PLC fractionation of the crude product (methanol–chloroform, 1:19, v/v) gave (**4h**) (21 mg, 14%); (**4i**) (39 mg, 26%); (**4k**) (34 mg, 22%); and (**4l**) (16 mg, 10%).

2β,17β,19-Trihydroxyandrost-4-en-3-one 2-t-BDMS (4h)

The obtained (**4h**) (21 mg) (see above) showed: m.p. (methanol, white prisms) 180–184°C; NMR δH ($CDCl_3$), 0.10 (3H,s,Si-CH_3), 0.2 (3H,s,Si-CH_3), 0.78 (3H,s,13-CH_3), 0.90 [9H,s,C(CH_3)$_3$], 3.67 (1H, B—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 3.7 (2H, Br.s, -OH × 2), 4.04 (2H,m,2α-H and 17-H), 4.22 (1H, A—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 5.94 (1H,s,4-H); mass spectrums (MO-TMS) *m/z* 607 (M^+ not detected), 592 (7%), 550 (86%), 475 (100%).

2α,17β,19-Trihydroxyandrost-4-en-3-one 2-t-BDMS (4i)

Treatment of (**4l**) (30 mg, 0.063 mmol) in MeOH (5 ml) with 5% aq. NaOH (0.6 ml) at 40°C for 1 h was followed by the usual workup and chromatography to give (**4i**) (18 mg; 65%); m.p. (MeOH) 110–111°C; NMR, δH ($CDCl_3$), 0.10 (3H,s,Si-CH_3), 0.18 (3H,s,Si-CH_3), 0.79 (3H,s,13-CH_3), 0.94 [9H,s,C(CH_3)$_3$], 3.65 (1H,m,17-H), 3.91 (1H, B—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.13 (1H, A—part of an AB system, J = 12 Hz, 1H of 19-CH_2), 4.78 (1H,dd,J_1 = 8 Hz, J_2 = 12.9 Hz, 2β-H), 5.85 (1H,s,4-H); mass spectrum (MO-TMS) *m/z* 607 (M^+, 2%), 592 (20%), 578 (10%), 550 (100%), 428 (42%).

2β,17β,19-Dihydroxyandrost-4-en-3-one 2-t-BDMS 17-acetate (4k)

The isolated (**4k**) (34 mg) (see above) showed: m.p. (MeOH) 153–156°C; NMR δH ($CDCl_3$), 0.17 (3H,s,Si-CH_3), 0.24 (3H,s,Si-CH_3), 0.87 (3H,s,13-CH_3), 0.94 [9H,s,C(CH_3)$_3$], 2.05 (3H,s,OCOCH_3), 3.57–4.41 (3H, 2α-H and 19-CH_2), 4.60 (1H,m,17-H), 5.96 (1H,s,4-H); mass spectrum (TMS) *m/z* 548 (M^+; not detected), 533 (3%), 491 (100%), 401 (48%), 341 (29%), 329 (7%).

2α,17β,19-Trihydroxyandrost-4-en-3-one 2-t-BDMS 17-acetate (4l)

The recovered (**4l**) (see above) showed: NMR δH ($CDCl_3$) 0.11 (3H,d,J = 2 Hz, Si-CH_3), 0.19 (3H,s,Si-CH_3), 0.84 (3H,s,13-CH_3), 0.99 [9H,s,C(CH_3)$_3$], 2.04 (3H,s,-OCO-CH_3), 3.89 (1H, B part of an AB system, J = 10.8 Hz, 1H of 19-CH_2), 4.12 (1H, A part of an AB system, J = 10.8 Hz, 1H of 19-CH_2), 4.57 (1H,m,17-H), 4.79 (1H,dd,J_1 = 6 Hz, J_2 = 12.6 Hz, 2β-H), 0.86 (1H,s,4-H); mass spectrum (TMS) *m/z* 548 (M^+; 2%), 533 (4%), 491 (100%), 401 (76%), 341 (25%), 329 (8%).

2α-Hydroxy-19-oxoandrost-4-ene-3,17-dione 2-t-BDMS (5c)

A mixture of 2α-*t*-BDMS-17,19-dihydroxy (**4:1**) (27 mg, 0.062 mmol), dichloromethane

(5 ml) and pcc (excess) was stirred at RT for 2 h and processed as described for (**5a**). Following PLC (cyclohexane–EtOAc, 2:1, v/v) (**5c**) (25 mg, 93%) was obtained; m.p. (MeOH; white needles) 179–183°C; NMR δH ($CDCl_3$), 0.14 (3H,s,-$SiCH_3$), 0.24 (3H,s,$SiCH_3$), 0.95 (3H,s,13-CH_3), 1.00 [9H,s,$C(CH_3)_3$], 4.27 (1H,dd,$J_1 = 6$ Hz, $J_2 = 12$ Hz, 2β-H), 5.94 (1H,s,4-H), 10.07 (1H,s,10-CHO); mass spectrum (MO-TMS) *m/z* 517 (M^+ not detected), 502 (2%), 486 (6%), 460 (100%), 428 (7%), 371 (23%).

2α-Hydroxy-19-oxoandrost-4-ene-3,17-dione (6c)

To a solution of 2α-hydroxy-19-oxoandrost-4-ene-3,17-dione 2-t-BDMS (**5c**) (16 mg, 0.037 mmol) in acetonitrile (1.5 ml) aq. HF (48%) (0.6 ml) was added and the reaction mixture was stirred (1 h) at RT. $CHCl_3$ (10 ml) and water (2 ml) were added and the organic phase was washed with water (×2), dried (Na_2SO_4) and evaporated to a residue. PLC (EtOAc–cyclohexane, 1:2, v/v) gave (**6c**) (11 mg, 93%); m.p. (MeOH) 205–208°C; (lit [4] 205–208°C); NMR δH ($CDCl_3$), 0.9 (3H,s,13-CH_3), 3.57 [1H,s (D_2O exchangeable), 2-OH], 4.17 (1H,dd,$J_1 = 5.5$ Hz, $J_2 = 13.5$ Hz, 2β-H), 6.04 (1H,s,4-H), 10.07 (1H,s,10-CHO); mass spectrum (MO-TMS) *m/z* 475 (M^+, 2%), 460 (16%), 444 (100%), 429 (46%), 385 (22%).

2ξ-Hydroxy-19-oxoandrost-4-ene-3,17-dione-2-chloroacetate (5h)

The conventional $Mn(AcO)_3$/chloroacetic/benzene oxidation (44 h) of 19-oxoandrost-4-ene-3,17-dione (**5g**) (210 mg) gave after workup (**5h**) (291 mg); NMR δH ($CDCl_3$) 0.9 (3H,s,13-CH_3), 4.02 (2H,s,$OCOCH_2Cl$), 5.4 (1H,br.m,2-H), 6.08 (1H,s,4-H), 9.87 (1H,s,10-CHO).

Attempted saponification of (**5h**) under a variety of conditions failed and only estrone was obtained.

[^{18}O]Chloroacetic acid

A mixture of chloroacetylchloride (5.04 g, 45 mmol) and $H_2{}^{18}O$ (99.8% ^{18}O) (1 g, 50 mmol) was stirred at RT for 1 h under argon. The obtained solid was dried under reduced pressure. The resulting crystalline [^{18}O]chloroacetic acid 4.3 g (100%) showed: m.p. 62–64°C; NMR δH ($CDCl_3$) 4.1 (s,Cl-CH_2); mass spectrum (TMS ester) *m/z* 155 (33%), 153 [(M + 2) − 15; 40%], 96 [(M + 2) − TMS; 24%], 94 (M − TMS; 29%), 73 (TMS + 1; 100%); ^{18}O-content 38–40%.

[2-^{18}O]2ξ,19-dihydroxyandrost-4-ene-3,17-dione 2-chloroacetate 19-acetate (2c)

A mixture of $Mn(AcO)_3$ dihydrate (2.0 g, 7.46 mmol), [^{18}O]chloroacetic acid (1.17 g, 12.23 mmol) in dry benzene (100 ml) was dried in the manner described above and cooled to RT. Then 19-hydroxyandrost-4-ene-3,17-dione acetate (**1a**) (611 mg, 1.776 mmol) was added and the mixture was refluxed for 20 h. The reaction was cooled (RT), ethyl acetate (100 ml) was added and the solution was processed to give a crude product (896 mg). HPLC (isopropanol–isooctane, 1:9, v/v) showed the presence of four major compounds.

The crude product was fractionated by PLC (diethyl ether–dichloromethane, 1:1, v/v) to give an isomeric mixtures of [2-^{18}O]2ξ,19-dihydroxy 2-chloroacetates 19-acetate (**2c**) (389 mg) and 2ξ,19-dihyroxy diacetates (**2b**) (138 mg).

For (**2c**) NMR, δH ($CDCl_3$) 0.89 (s,13-CH_3), 2.04 (s,O-CO-CH_3), 2.09 (s,OCO-CH_3), 3.98-4.9 (19-CH_2), 4.13 (s,-$OCOCH_2Cl$), 4.21 (s,$OCOCH_2Cl$), 5.41 (m,2α-H), 5.9 (m, 2β-H), 5.91 (s,4-H).

[2-^{18}O]2β,19-dihydroxyandrost-4-ene-3,17-dione (3c)

To a solution of the (**2c**) (389 mg) in methanol (25 ml) under argon, 1N KOH (1.5 ml) was added and the mixture was stirred 15 min at RT. The reaction was neutralized (1N acetic acid) and most of the MeOH was removed under reduced pressure (water bath temp. 30°C). The remaining liquid was extracted several times with $CHCl_3$ (100 ml) and the extract was processed and concentrated to a residue (216 mg). The crude product was fractionated by PLC (MeOH–$CHCl_3$, 1:9, v/v) to give (**3c**) (63 mg; 11%) and (**3d**) (62 mg; 11%).

For (**3c**): m.p. 140–142°C; NMR; δH ($CDCl_3$) 0.9 (3H,s,13-CH_3), 3.64 (1H,d,J = 10.5 Hz, 1H of the 19-CH_2), 4.1 (2H,m,2α-H and 1H of 19-CH_2), 5.96 (1H,s,4-H); mass spectrum (TMS) *m/z* 464 [(M + 2)$^+$; 7%], 462 (M^+; 12%), 449 {[(M + 2) − 15]$^+$; 15%}, 447 [(M − 15)$^+$; 29%], 434 {[(M + 2) − (2 × 15)]$^+$; 47%}, 432 [(M − 2 × 15)$^+$; 100%]; 419 {[(M + 2) − 1]$^+$; 10%}, 417 [M^+; 15%], 344 {[(M + 2) − (91 + 29)]; 48%}, 342 {[M − (89 + 29)]; 74%}.

*[2-^{18}O]2α,19-dihydroxyandrost-4-ene-3,17-dione (**3d**)*

The (**3d**) showed: m.p. 202–204°C, NMR: δH ($CDCl_3$) 0.9 (3H,s,13-CH_3), 3.64 [1H,m(D_2O exchangeable), -OH], 4.07 (2H,d,J = 2.5 Hz,19-CH_2-OH), 4.70 (1H,dd,J_1 = 6 Hz, J_2 = 13 Hz, 2β-H), 6.0 (1H,s,4-H); mass spectrum (TMS) *m/z* 464 [(M + 2)$^+$; 25%], 462 (M$^+$; 48%), 449 {[(M + 2) − 15]$^+$; 13%}, 447 [(M − 15)$^+$; 23%], 434 {[(M + 2) − 2 × 15]$^+$; 6%}, 432 [(M − 2 × 15)$^+$; 12%], 419 (5%), 417 (7%), 361 (36%), 359 (100%).

*[2-^{18}O]2β,19-dihydroxyandrost-4-ene-3,17-dione 2-t-BDMS (**4c**)*

A mixture of [2-^{18}O]2β,19-dihydroxy (**3c**) (50 mg, 0.16 mmol), *t*-BDMSCl (83 mg, 0.55 mmol) imidazole (132 mg) in dry DMF (5 ml) was stirred at RT under argon for 1 h. Then the reaction mixture was diluted with diethylether (50 ml), and processed in the conventional manner. The recovered product was purified by PLC (cyclohexane–EtOAc, 1:3, v/v) to give 32 mg (47%) of (**4c**). It showed: m.p. 130–133°C, NMR: δH ($CDCl_3$), 0.14 (3H,s,SiCH_3), 0.22 (3H,s, SiCH_3), 0.91 [9H,s,C(CH_3)$_3$], 0.90 (3H,s,13-CH_3), 3.61–4.24 (3H, 2α-H and 19-CH_2), 5.98 (1H,s,4-H); mass spectrum (TMS) *m/z* 504 [(M + 2)$^+$; not detected], 502 (M$^+$; not detected), 449 {[(M + 2) − 57]$^+$; 23%}, 447 [(M − 57)$^+$; 46%], 401 {[(M + 2) − 101]$^+$; 15%}, 359 {[(M + 2) − (57 + 90)]$^+$; 38%}, 357 {[M − (57 + 90)]$^+$; 100%}.

*[2-^{18}O]2β-hydroxy-19-oxoandrost-4-ene-3,17-dione t-BDMS (**5b**)*

A mixture of [2-^{18}O]2β,19-dihydroxyandrost-4-ene-3,17-dione 2-*t*-BDMS (**4c**) (18 mg, 0.042 mmol), pcc (excess) and dichloromethane (10 ml) was stirred 2 h at RT. Then anhydrous diethylether (50 ml) was added and the solids were removed by filtration through a celite column. The eluate and washings were combined concentrated and the recovered crude product was purified by PLC (cyclohexane–EtoAc, 1:3, v/v) to give (**5b**) (13 mg; 71%), m.p. 186–188°C. NMR: δH ($CDCl_3$), 0.01 (3H,s,Si-CH_3), 0.11 (3H,s,Si-CH_3), 0.83 [9H,s,-C(CH_3)$_3$], 0.86 (3H,s,13-CH_3), 4.07 (1H,m,2α-H), 5.94 (1H,s,4-H), 9.94 (1H,s,10-CHO). Mass spectrum (C.I.; NH_3) *m/z* 450 {[(M + 2) + NH_4]$^+$, 50%}, 448[(M + NH_4)$^+$ 100%], 433 {[(M + 2) + H]$^+$ 50%}, 431 [(M + H)$^+$ 100%], 375 {[(M + 2) − C_4H_9]$^+$; 4%}, 373 [(M − C_4H_9) 8%], 318 (11%), 316 (22%); ^{18}O-content 33–34%.

*[2-^{18}O]2β-hydroxy-19-oxoandrost-4-ene-3,17-dione (**6b**)*

To a solution of [2-^{18}O]2β-hydroxy-19-oxoandrost-4-ene-3,17-dione *t*-BDMS (**5b**) (13 mg, 0.03 mmol) in acetonitrile (2.5 ml) aq. (48%) HF (0.8 ml) was added and the reaction mixture was stirred 1 h at RT. Then $CHCl_3$ (25 ml) was added, and following a conventional workup and solvent removal, a residue (12 mg) was obtained. HPLC purification (isopropanol–isooctane, 3:17, v/v); [retention time 11 min] gave homogeneous (**6b**) (6 mg; 63%); m.p. 166–168°C; NMR: δH ($CDCl_3$) 0.96 (3H,s,13-CH_3), 3.29 [1H,s,(D_2O-exchangeable), 2-OH], 4.18 (1H,dd,J_1 = 6 Hz, J_2 = 10 Hz, 2α-H), 6.06 (1H,s,4-H), 9.62 (1H,s,10-CHO); mass spectrum (C.I.; NH_3) *m/z* 336 {[(M + 2) + NH_4]$^+$; 50%}, 334 [(M + NH_4)$^+$; 100%]; ^{18}O-content 33–34%.

DISCUSSION AND RESULTS

Anhydrous manganese tri-acetate Mn(AcO)$_3$ has been successfully used for α-acetoxylation of (conjugated) enones (R″-C = C-CO-CH-R′) [5–7]. We were intrigued by the report that when the reaction was carried out in the presence of benzoic acid or chloroacetic acid, the products were isomeric mixtures of benzoyloxy and chloroacetoxy enones, respectively [7]. We anticipated that this procedure may allow differential protection and eventually selective deprotection of hydroxyls in the projected synthesis of (**6a**).

To evaluate the system, testosterone acetate (**1b**) was added to a dry mixture of Mn(AcO)$_3$, $ClCH_2COOH$ in benzene and the reaction was refluxed for 22 h under a Dean Stark trap. The recovered products were resolved by PLC to give epimeric mixtures of 2ξ-chloroacetate-17β-acetate (**1c**) (53%) and 2ξ,17β-diacetates (**1d**) (23%). When the same reaction was repeated with testosterone chloroacetate (**1e**), only 2ξ; 17(β)-chloroacetates (**1f**) was obtained in nearly quantitative yield. The major products of the reactions were the 2β-epimers.

Exposure of 2ξ-chloroacetate-17-acetate (**1c**) to aqueous methanolic potassium bicarbonate gave epimeric 2ξ-hydroxy-testosterone 17-acetate (**1g**) in excellent yield.

Based on these observations, several approaches to the synthesis of (**6a**) were explored.

Treatment of 19-hydroxyandrost-4-ene-3,17-dione acetate (**1a**) with $Mn(AcO)_3$ and chloroacetic acid in benzene as above gave epimeric mixtures of 2ξ-chloroacetate-19-acetate (**2a**) (60%) and 2ξ; 19-diacetate (**2b**) (20%). Selective saponification of (**2a**) with aq. methanolic potassium bicarbonate gave a mixture of 2ξ-hydroxy-19-acetates (**1i**). Unfortunately, the epimeric 2ξ-hydroxy-19-acetates could not be satisfactorily resolved and this approach was not pursued further.

Exploratory investigation revealed that the corresponding 2ξ,19-diols could be separated. Consequently, the crude reaction products were saponified (1M KOH/MeOH, RT; 15 min) and the resulting mixture of C-2 epimeric diols was resolved (PLC) into 2α,19-dihydroxy (**3b**) and 2β,19-dihydroxy (**3a**). The diol (**3a**) was treated with *t*-BDMSCl in DMF/Imidazole and the resulting 2β-*t*-BDMS-19-hydroxy (**4a**) (46%) was oxidized (PCC/CH_2Cl_2) to yield 19-oxo (**5a**) (70%). Exposure of (**5a**) to aq. HF in acetonitrile gave after HPLC fractionation the required (**6a**) (60%). The overall yield of (**6a**) starting from 19-OAc (**1a**) was 5–6%.

We have also explored the route starting with 17β,19-dihydroxyandrost-4-en-3-one diacetate (**1k**) previously prepared in our laboratory [8]. The conventional $Mn(AcO)_3/ClCH_2COOH$ oxidation of (**1k**) gave after workup and PLC fractionation 2ξ-chloroacetate-17,19-diacetates (**2d**) (55.5%) and 2ξ,17,19-triacetates (**2e**) (24%). HPLC analysis indicated that (**2d**) and (**2e**) are 1:1 mixtures of the respective 2β and 2α epimers. Treatment of the C-2 epimeric chloroacetates (**2d**) with aq. methanolic $KHCO_3$ gave 2ξ-hydroxy-17,19-diacetates (**2g**) (66%) together with smaller amounts of 2β- and 2α-19-diol-17-acetates (**3i**) and (**3k**), respectively. A similar mixture of products was obtained when the crude $Mn(AcO)_3$ oxidation residue was exposed to aq. methanolic $KHCO_3$.

Alternatively, saponification of (**2d**) with aq.methanolic K_2CO_3 resulted in 2ξ,17,19-trihydroxy 17-acetates (**2f**) which were resolved by PLC to give 2β,17,19-trihydroxy 17-acetate (**3i**) and 2α,17,19-trihydroxy 17-acetate (**3k**). Acetylation of diols (**3i**) and (**3k**) gave triacetates (**3g**) and (**3h**), respectively.

The epimeric alcohols (**2g**) were converted into 2ξ-*t*-BDMS-17,19-diacetates (**4d**), saponified and the resulting product was resolved (PLC) to yield (**4h**; 14%); (**4i**; 26%), (**4k**; 22%), and (**4l**; 10%).

The 2β-*t*-BDMS-diol (**4h**) were oxidized and the obtained 2β-*t*-BDMS-19-oxo-3,17-dione (**5a**) was hydrolyzed with aq. HF in acetonitrile to give the required 2β-hydroxy-19-oxo-(**6a**). Similar treatment of 2α-*t*-BDMS-diol (**4i**) gave 2α-hydroxy-19-oxo (**6c**).

It is of interest that analogous treatment of 19-oxoandrost-4-ene-3,17-dione (**5g**) [$Mn(AcO)_3$/chloroacetic acid/benzene] gave 2ξ-chloroacetoxy (**5h**) in very good yield. Unfortunately, attempts to saponify the chloroac tate failed and estrone was obtained. Similar oxidation of 19-hydroxy (**2h**) resulted in a complex mixture of esters which was not investigated further.

It is apparent that C-2 oxygenation of conjugated 3-keto-4-ene steroids in the system $Mn(AcO)_3/ClCH_2COOH$ proceeds via two different routes and is not stereospecific. The major products were epimeric 2-chloroacetates which were accompanied by lesser amounts of epimeric 2-acetates. Also of importance was the observation that the epimeric 2-chloroacetates and 2-acetates could be resolved and processed differentially. These observations provided the basis for the preparation 2-^{18}O-labeled (**6b**), which was the primary synthetic objective of the investigation. Our synthetic strategy was to introduce isotopic oxygen with the use of [$^{18}O_1$]chloroacetic acid. Separate the resulting 2ξ-[^{18}O]chloroacetates from the accompanying unlabeled 2ξ-acetates and process only the 2ξ-[^{18}O]chloroacetates.

First we concentrated on the synthesis of the required [$^{18}O_1$]chloroacetic acid. After several false starts we discovered that stirring of chloroacetyl chloride ($ClCH_2COCl$; liquid) with a 5%-molar excess of ([^{18}O]H_2-99.8%) (at ambient temperature) gave crystalline $ClCH_2C^{18}OOH$ in quantitative yield (100%). The product was dried under reduced pressure (RT) and the GC-mass spectrum of its TMS-ester showed that 38–40% of the molecules were $^{18}O^1$-labeled. In different preparations the incorporation of ^{18}O varied in the range of 30–48%.

For the synthesis of [2-^{18}O](**6b**), the 19-acetate (**1a**) was treated with a mixture of $Mn(AcO)_3$, [^{18}O]chloroacetic acid in benzene in the usual manner. Following workup and PLC fractionation [2-^{18}O]2ξ-chloroacetate-19-acetate (**2c**) and 2ξ,19-diacetate (**2b**) were obtained. Considering that the diacetate could not be labeled with isotopic oxygen it was set aside.

Saponification of (**2c**) (1M KOH/MeOH, RT; 15 min) gave [2-^{18}O]2ξ,19-dihydroxyandrost-4-ene-3,17-dione, which was resolved (PLC) to yield [2-^{18}O]2β,19-dihydroxy (**3c**) (11%) and [2-^{18}O]2α,19-dihydroxy (**3d**) (11%). The [^{18}O]diol (**3c**) was selectively silylated and the resulting 2-*t*-BDMS (**4c**) (47%) was oxidized to yield 19-oxo (**5b**) (70%). Removal of *t*-BDMS moiety provided the required [2-^{18}O]2β-hydroxy-19-oxoandrost-4-ene-3,17-dione (**6b**; 60%). The overall yield of [2-^{18}O](**6b**) starting from 19-OAc (**1a**) was 3%.

It should be stressed that no attempts were made to recycle intermediates and/or optimize yields.

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REFERENCES

1. Hosoda H. and Fishman J.: Unusually facile aromatization of 2β-hydroxy-19-oxo-4-androstene-3,17-dione to estrone. Implications in estrogen biosynthesis. *J. Am. Chem. Soc.* **96** (1974) 7325.
2. Caspi E., Wicha J., Arunachalam T., Nelson P. and Spiteller G.: Aspects of estrogen biosynthesis: Concerning the pathway via 2β-hydroxy-10β-formyl-androst-4-ene-3,17-dione. In *Mechanisms of Enzymatic Reactions: Stereochemistry* (Edited by P. A. Frey). Elsevier, New York (1986) pp. 281–292.
3. Caspi E., Wicha J., Arunachalam T., Nelson P. and Spiteller G.: Estrogen biosynthesis. Concerning the obligatory intermediacy of 2β-hydroxy-10β-formylandrost-4-ene-3,17-dione. *J. Am. Chem. Soc.* **106** (1984) 7282–7283.
4. Njar V. C. O., Spiteller G., Wicha J. and Caspi E.: Observations on the preparation of 2β-hydroxy-19-oxoandrost-4-ene-3,17-dione: Synthesis of 2β,19-oxaandrost-4-ene-3,17-dione. *Heterocycles* **28** (1989) 1051–1060.
5. Williams G. J. and Hunter N. R.: Situselective α-acetoxylation of some α,β-enones by manganic acetate oxidation. *Can. J. Chem.* **54** (1976) 3830.
6. Dunlap N. K., Sabol M. R. and Watt D. S.: Oxidation of enones to α-acetoxyenones using manganese triacetate. *Tetrahedron Lett.* **25** (1984) 5839.
7. Demir A. S., Jeganathan A. and Watt D. S.: Synthesis of α-acyloxy enones from enones using manganese (III) acetate in combination with manganese (II) carboxylates or carboxylic acids. *J. Org. Chem.* **54** (1989) 4020.
8. Njar V. C. O., Dharmaratne H. R. W. and Caspi E.: Synthesis of [16,16,19-^{2}H$_3$; 19-^{3}H] 19-oxoandrost-4-ene-3,17-dione. *J. Chromat.* **440** (1988) 415–420.