SYNTHESIS OF C7-$^2H_2$ STEROIDS FOR HUMAN METABOLISM STUDIES

Ian A. Blair*, George Phillipou and Christopher Seaborn,
Department of Obstetrics and Gynaecology, University of Adelaide,
The Queen Elizabeth Hospital, Woodville, South Australia 5011.
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SUMMARY

Seven deuterium labelled steroids were prepared in good yield. The synthetic strategy embodies C7 functionalisation by Cr VI allylic oxidation followed by deoxygenation of the resulting ketone with aluminium chlorodeuteride. Subsequent elaboration of the di-deuterated compounds using standard procedures allowed the preparation of biologically important steroids containing 96% deuterium at C7.

Key Words: Isotopic labelling, deuterated steroids, aluminium chlorodeuteride, GC-MS

INTRODUCTION

As part of a continuing programme (1) designed to study human steroid metabolism both *in vitro* and *in vivo*, we required a series of deuterium-labelled steroid hormones. Such compounds allow three important measurements in biomedical research: steroid production rates (2), absolute levels using isotope dilution (3) and intermediate steroid metabolism without recourse to radioactive materials.

Previous methods for the preparation of biologically important deuterated steroids (1, 4-8) either did not satisfy certain general requirements for GC-MS analysis (9) or were not generally applicable. Our unsuccessful attempts (10) to synthesize C18-$^2H_3$ steroids led us to investigate the possibility of labelling at C7. Budzikiewicz (11) has reported the preparation of the simple steroid 7-$^2H_2$-5-androstene-3β-ol in poor yield (14%) but we envisaged that this could provide the basis for a general route to C7-$^2H_2$ steroid hormones.

DISCUSSION

The sequence used for the preparation of the deuterated steroids is shown in Scheme I. 5-Pregnene-3β,20β-diol diacetate (1), 5-androstene-3β,17β-diol diacetate (2) and 5-cholesten-3β-yl acetate (3) were all oxidised in good yield by the method of Dauben (12) to the corresponding ketones (4), (5) and

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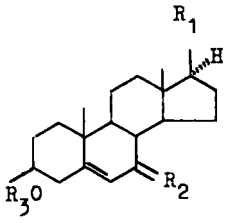
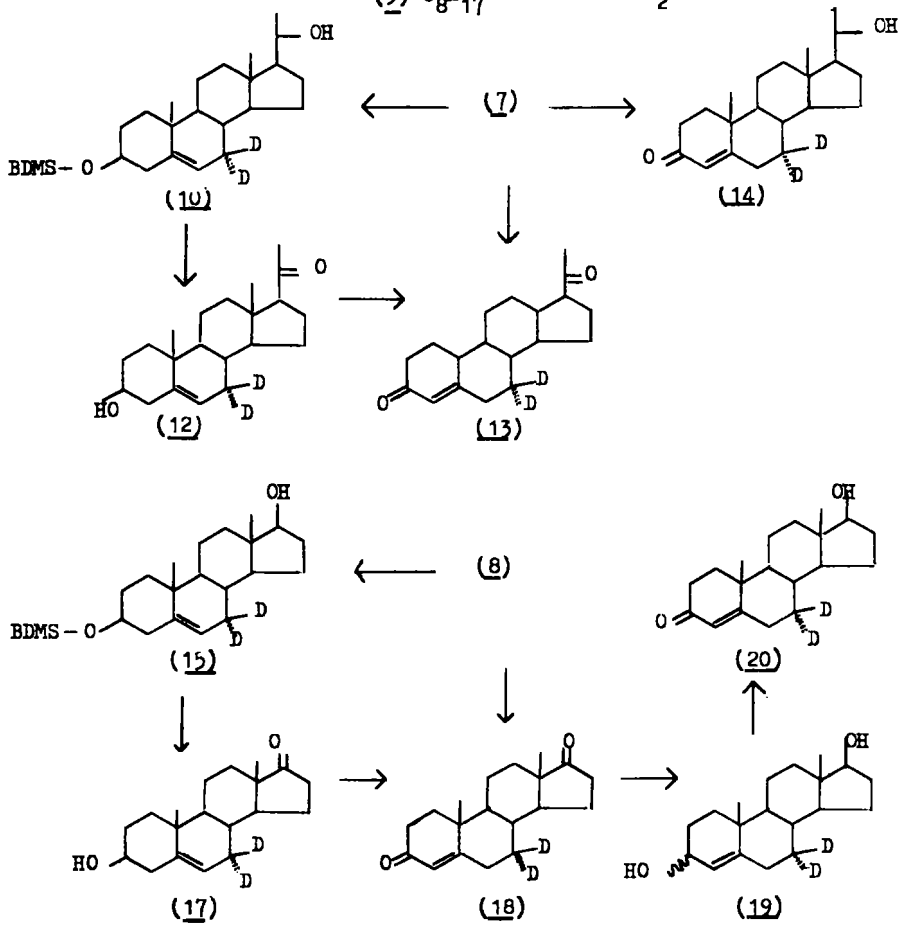
SCHEME I



	R_1	R_2	R_3
(1)	CH_3CH-β OAc	$^1H_2$	Ac
(2)	OAc	$^1H_2$	Ac
(3)	C_8H_{17}	$^1H_2$	Ac
(4)	CH_3CH-β OAc	O	Ac
(5)	OAc	O	Ac
(6)	C_8H_{17}	O	Ac
(7)	CH_3CH-β OAc	$^2H_2$	^1H
(8)	OH	$^2H_2$	^1H
(9)	C_8H_{17}	$^2H_2$	^1H



(6) respectively. A detailed investigation of the reduction step showed that simple 1,2 reduction was the major process using the Budzikiewicz (11), Brown (13) procedures. Under suitable conditions (as detailed in the experimental section) deoxygenation could, however, become the dominant reaction. Reduction of the ketones (4), (5) and (6) with Li $Al^2H_4/AlCl_3$ in ether/THF gave the corresponding C7-$^2H_2$ alcohols (7), (8) and (9) in yields of 70%, 65% and 75% respectively.

The diols (7) and (8) were smoothly converted (14) to their mono t-butyldimethylsilyl ethers, oxidised with chromium trioxide in pyridine, followed by acid hydrolysis to give 7-$^2H_2$-3β-hydroxy-5-pregnene-20-one (12) and 7-$^2H_2$-3β-hydroxy-5-androsten-17-one (17) in overall yields of 61% and 59% respectively. Oppenauer oxidation of these compounds provided 7-$^2H_2$-4-pregnene-3-20-dione (13) and 7-$^2H_2$-4-androstene-3-17-dione (18) in 81% and 78% yields respectively. A more direct route to the diones (13) and (18) was available by Oppenauer oxidation of the diols (7) and (8). Use of the modified Oppenauer conditions (15) (where N-methyl piperidone functions as the hydride acceptor) on the diol (7), resulted in selective oxidation at the less hindered 3-position to give 7-$^2H_2$-20β-hydroxy-4-pregnen-3-one (14).

7-$^2H_2$-4-androstene-3-17-dione (18) was reduced with lithium aluminium hydride to the diol (19), subsequent oxidation with DDQ in benzene gave 7-$^2H_2$-17β-hydroxy-4-androsten-3-one (20) in 81% overall yield. The deuterium contents of the compounds prepared in this study are collated in Table 1. Their use for the evaluation of clinical conditions is currently under investigation and preliminary results are due to appear elsewhere shortly.

TABLE 1

	$^2H_0$	$^2H_1$	$^2H_2$	$^2H_3$
5-cholesten-3β-ol (9)	1.7	0.9	96.5	0.9
3β-hydroxy-5-pregnene-20-one (12)	2.1	0.7	96.1	1.1
4-pregnene-3, 20-dione (13)	2.2	0.9	95.9	1.0
20β-hydroxy-4-pregnen-3-one (14)	1.6	0.7	96.6	1.1
3β-hydroxy-5-androsten-17-one (17)	1.7	0.9	96.5	0.9
4-androstene-3,17-dione (18)	1.4	0.4	97.4	0.8
17β-hydroxy-4-androsten-3-one (20)	1.4	0.7	96.8	1.1

EXPERIMENTAL

Melting points are uncorrected. Mass spectral data was obtained using a double beam AEI MS-30 mass spectrometer equipped with a DS-50 data system and interfaced to a Pye 104 GC (column 2m x 2mm I.D. 1% OV 101, 260°) via a single stage glass jet separator (S.G.E., Melb., Aust.).

Rotations were run on a Perkin Elmer 141 instrument operating at the sodium D line. Infra-red data was obtained on a Pye Unicam SP 1025 infrared spectrophotometer. All compounds were shown to be homogeneous by t.l.c. and g.l.c. analysis.

3β, 20β-Diacetoxy-5-pregnen-7-one (4) Chromium trioxide (7.8g, 78 mmol) dried over P_2O_5, was added to a magnetically stirred solution of pyridine (12.50 g, 12.74 ml, 156 mmol) in methylene chloride (200 ml). After stirring for 15 minutes at room temperature under anhydrous conditions 5-pregnene-3β, 20β-diol diacetate (1) (1.9g, 4.7 mmol) in methylene chloride (20 ml) was added and the mixture stirred over 48 hours.

The mixture was decanted and the black residue washed with ether (200 ml). The combined ether and methylene chloride was extracted with aqueous sodium bicarbonate (5 x 50 ml) and the washings extracted once with ether (40 ml). The combined organic extracts were washed with 5% hydrochloric acid (4 x 40 ml), 5% sodium bicarbonate, brine (1 x 40 ml) and dried ($MgSO_4$) evaporated to give a pale yellow solid which was recrystallised from methanol to give white plates of the ketone (4) (1.5 g, 79%). m.p. 197-198° ($C_{25}H_{36}O_5$ requires C,72.07; H,8.71. Found: C,72.13; H,8.83%)

3β, 17β-Diacetoxy-5-androsten-7-one (5) Prepared from 5-androstene-3β, 17β-diol diacetate (2) by the method Dauben (12)in 76% yield m.p. 220-221° lit (12) m.p. 218-221°.

3β-Acetoxy-5-cholesten-7-one (6) Prepared from 5-cholesten-3β-yl acetate (3) by the method of Dauben (12) in 74% yield m.p. 160-161° lit (12) m.p. 161-163°.

7-$^2H_2$-5-Pregnene-3β, 20β-diol (7) Lithium aluminium deuteride (1.5g, 36 mmol) in dry ether (60 ml) under nitrogen was cautiously treated with aluminium chloride (15g, 112 mmol) in dry ether (100 ml) with cooling.

The mixture was stirred for 0.5 h under reflux, cooled to room temperature

and 3β, 20β-diacetoxy-5-pregnen-7-one (4) (2g, 5 mmol) in THF (30 ml) added so as to maintain a gentle reflux. After refluxing a further 1 h, the reaction mixture was cooled and water (20 ml) carefully added. The aqueous layer was separated and the organic phase washed with water (30 ml) dried($MgSO_4$) and evaporated to give the crude diol (1.7 g). Chromatography on Grade III alumina eluting with chloroform/hexane afforded the pure 7-$^2H_2$-5-pregnene-3β, 20βdiol (1.1g, 70%) m.p. 202-203° $(\alpha)_D^{22}$ - 66.1° lit (16) for C7-$^1H_2$ compound m.p. 201.5-203.5° $(\alpha)_D$ - 68.5°. A portion sublimed in vacuum had m.p. 206-208°.

7-$^2H_2$-5-Androstene-3β, 17β-diol (8) Lithium aluminium deuteride (0.2g, 4.8 mmol) in ether (5 ml) was treated with aluminium chloride (2g, 15 mmol) in ether (10 ml). The mixture was treated with 3β, 17β-diacetoxy-5-androsten-7-one (5) (0.2g, 0.48 mmol) in THF (3 ml) and the reaction carried out as for (7). The 7-$^2H_2$-5-androsten-3β, 17β-diol (8) was obtained in 65% yield after chromatography and recrystallisation from methanol. m.p. 182-183, $(\alpha)_D^{23}$-52° (EtOH). The C7-$^1H_2$ compound has (17) m.p. of 178°, $(\alpha)_D$ - 55.5° (EtOH).

7-$^2H_2$-5-Cholesten-3β-ol (9) Lithium aluminium deuteride (1 g, 24 mmol) in ether (30 ml) was treated with aluminium chloride (10g, 72 mmol) in ether (50 ml). The mixture was treated with 3β-acetoxy-5-cholesten-7-one (6) (1g, 2.3 mmol) in THF (10 ml) and the reaction carried out as for (7). The deuterated 5-cholesten-3β-ol (9) was obtained in 75% yield after chromatography and recrystallisation from ethanol/water. m.p. 146.5-147° undepressed an admixture with an undeuterated authentic sample, $(\alpha)_D^{23}$ - 57° ($CHCl_3$). The C-$^1H_2$ compound has (18) m.p. 148.5 $(\alpha)_D$ - 39° ($CHCl_3$).

7-$^2H_2$-3β-t-Butyldimethylsiloxy-5-pregnen-20-ol (10) To a solution of 5-pregnene-3β, 20β-diol (7) (1.7 g, 5.3 mmol) in dry pyridine (20 ml) was added t-butyldimethylsilyl chloride (2.4 g, 15.9 mmol) with stirring. The reaction was followed by t.l.c. which indicated that mono silylation was complete after 4 h. The reaction mixture was diluted with ether (80 ml) and water (20 ml), the aqueous phase separated and extracted with a further portion of ether. The ether extracts were washed with water (3 x 50 ml) dried ($MgSO_4$) and evaporated to give the crude ether (10). Chromatography on silica eluting with

ether/petrol followed by recrystallisation from ethyl acetate afforded the pure t-butyldimethylsilyl ether (10) (2.1g, 91%) m.p. 135-136°. ν max (nujol) 3500 cm^{-1}. (Calculated for C7-$^1H_2$ compound $C_{27}H_{48}O_2$ Si C, 74.94; H, 11.18 Found C, 74.87; H 11.24%).

7-$^2H_2$-3β-t-butyldimethylsiloxy-5-pregnen-20-one (11) Chromium trioxide (3.9g, 39 mmol) was added under nitrogen to pyridine (70 ml) at 0° with stirring. The mixture was allowed to stir until a red colour developed (ca 45 mins) then a solution of t-butyldimethylsilyl ether (10) (1.7g, 3.9 mmol) added dropwise at 0° under nitrogen. The reaction was allowed to warm to room temperature, left stirring overnight, then decanted into ether (150 ml). The chromium salts were filtered off through celite and the pale yellow ethereal solution washed with saturated aqueous sodium chloride (3 x 50 ml), 5% hydrochloric acid (3 x 50 ml), water (1 x 50 ml), dried ($MgSO_4$) and evaporated to leave a white solid which was recrystallised from ethyl acetate to give the pure pregnen-20-one (11) (3.7 g, 94%) m.p. 166-167° ν max (nujol) 1720 cm^{-1} (Calculated for C7-$^1H_2$ compound $C_{27}H_{46}O_2$ Si C, 75.29; H, 10.76 Found C, 75.26; H, 10.76%.)

7-$^2H_2$-3β-Hydroxy-5-pregnen-20-one (12) The ketone (11) (1.1 g, 2.7 mmol) in acetone (60 ml) was treated with 6N hydrochloric acid (1 ml) and stirred for 1 h. The reaction mixture was diluted with ethyl acetate (60 ml) and washed with 10% aqueous sodium bicarbonate (2 x 20 ml), brine (1 x 20 ml), dried ($MgSO_4$) and evaporated. Chromatography on grade III alumina eluting with ether-petrol followed by recrystallisation from ethyl acetate afforded pure 7-$^2H_2$-3β-hydroxy-5-pregnen-20-one (12) (0.7 g, 78%) m.p. 189-190° undepressed an admixture with authentic undeuterated compound, $(\alpha)_D^{24}$ + 31° (EtOH). The C7-$^1H_2$ compound has (19) m.p. 189-190° $(\alpha)_D$ + 28° (EtOH).

7-$^2H_2$-4-Pregnene-3,20-dione (13).

Method A. 7-$^2H_2$-3β-Hydroxy-5-pregnen-20-one (12) (0.3 g, 1mmol) in dry toluene (30 ml) was treated with redistilled cyclohexanone (1.5 ml, 15 mmol) and toluene (5 ml) distilled off. Aluminium isopropoxide in toluene (1 ml of a solution containing 0.12 g/ml), 0.12 g, 1 mmol) was added and the solution refluxed for 1 h. A further addition of cyclohexanone (0.5 ml, 5 mmol) and

aluminium isopropoxide (1 ml of solution containing 0.12 g/ml) followed by refluxing a further hour pushed the reaction to completion. The reaction mixture was steam distilled, the aqueous phase extracted with chloroform (3 x 50 ml), dried ($MgSO_4$) and evaporated. Chromatography on silica GF_{254} p.l.c.* plates developing with chloroform followed by recrystallisation from ethanol gave the pure 7-$^2H_2$-4-pregnene-3, 20-dione (13) (0.24 g, 81%) m.p. 124-125° undepressed on admixture with authentic undeuterated material, $(\alpha)_D^{23}$ + 188° ($CHCl_3$) the C7-$^1H_2$ compound has (20) m.p. 128.5°, $(\alpha)_D$ + 192° ($CHCl_3$).

Method B. 7-$^2H_2$-5-Pregnene-3 β, 20 β-diol (7) (0.1 g, 0.3 mmol) in dry toluene (25 ml) was treated with cyclohexanone (0.5 ml, 5 mmol) and toluene (4 ml) distilled off. Aluminium isopropoxide in toluene (0.4 ml of a solution containing 0.12 g/ml, 0.50 g, 0.2 mmol) was diluted with toluene (4 ml) and added dropwise as toluene was distilled off at a slightly faster rate under nitrogen. After the addition was complete further portionsof cyclohexanone (0.5 ml) and aluminium isopropoxide (0.5 ml, 0.12 g/ml) were added. The reaction mixture was distilled until a further 5 ml of toluene had distilled over. The residual solution was steam distilled and the aqueous phase extracted with chloroform (3 x 40 ml) dried ($MgSO_4$) and evaporated to give the crude dione (13). Chromatography on a silica GF_{254} p.l.c. plate developing with chloroform followed by recrystallisation from ethanol gave pure 7-$^2H_2$-4-pregnene-3, 20-dione (0.07 g, 71%) m.p. 124-125° identical in all respects with the compound obtained by method A.

7-$^2H_2$-20β-Hydroxy-4-pregnen-3-one (14). 7-$^2H_2$-Pregnene-3β, 20β-diol (7) (0.1 g, 0.3 mmol) in toluene (20 ml) was treated (15) with N-methyl piperidone (0.7 g, 0.6 mmol) and 5 ml of toluene was distilled off (19). Aluminium isopropoxide (0.4 g, 1.9 mmol) in toluene (5 ml) was added and the solution refluxed for a further 2 h. The reaction mixture was diluted with ether (50 ml) and washed with 5% sulphuric acid (2 x 20 ml), the acid washings re-extracted with ether (2 x 50 ml), the combined ether extracts were washed with water (2 x 30 ml) dried and evaporated to give the crude ketone (14). Chromatography by p.l.c. on silica GF_{254} eluting with 10% acetone/chloroform followed by recrystallisation from di-isopropyl ether/petrol gave 7-$^2H_2$-20β-hydroxy-4-pregnen-

*p.l.c.= preparative layer chromatography

3-one (14) undepressed an admixture with authentic undeuterated compound (0.60g, 59%) m.p. 170-171° $(\alpha)_D^{23}$ + 82° ($CHCl_3$). The C7-$^1H_2$ compound has (21) m.p. 171-172° $(\alpha)_D^{24}$ + 84° ($CHCl_3$).

7-$^2H_2$-3β-t-Butyldimethylsiloxy-5-androsten-17β-ol (15). To a solution of 5-androstene-3β, 17β-diol (8) (0.23 g, 0.8 mmol) in pyridine (5 ml) was added t-butyldimethylsilyl chloride (0.36 g, 2.4 mmol) and the reaction carried out as for (13). Chromatography on Grade III alumina eluting with ether/petrol followed by recrystallisation from aqueous methanol gave pure t-butyldimethylsilyl ether (15) in 89% yield m.p. 173-174°C ν_{max} (nujol) 3,500 cm^{-1}. (Calculated for undeuterated $C_{25}H_{44}O_2Si$ C, 74.19; H, 10.96 Found C, 74.02; H, 11.13%. The lit. (14) m.p. 171-172 (for $C_{25}H_{44}O_2Si. \frac{1}{2} H_2O$).

7-$^2H_2$-3β-t-Butyldimethylsiloxy-5-androsten-17-one (16). Chromium trioxide (0.25 g, 2.5 mmol) was added under nitrogen to pyridine (6 ml) at 0° with stirring. The mixture was allowed to stir for 45 mins and then a solution of the alcohol (15) (0.1 g, 0.25 mmol) in pyridine (6 ml) added dropwise at 0°. Work-up as for ketone (11) afforded the pure C7-$^2H_2$-3β-t-butyldimethylsiloxy-5-androsten-17-one (16) as a white solid (0.09 g, 90%). A sample recrystallised from aqueous methanol had a melting point of 149-150°C. lit. (14) for undeuterated compound 146-147°.

7-$^2H_2$-3β-hydroxy-5-androsten-17-one (17) 6N Hydrochloric acid (0.2 ml) was added to a solution of 7-$^2H_2$-3β-t-butyldimethylsiloxy-5-androsten-17-one (16) (0.07 g, 0.2 mmol) in acetone (5 ml) and the reaction stirred under nitrogen for 2 h. Ethyl acetate (20 ml) was added and the organic phase washed with saturated sodium bicarbonate (2 x 20 ml) water (20 ml) dried ($MgSO_4$) and evaporated to give pure 7-$^2H_2$-3β-hydroxy-5-androsten-17-one (17) (0.05 g, 100%). Recrystallisation from n.hexane/ethyl acetate gave an analytically pure sample m.p. 149-151° undepressed on admixture with an undeuterated authentic sample. $(\alpha)_D^{23}$ + 11° The 7-$^1H_2$ compound has (22) m.p. 147-148° $(\alpha)_D$ + 13.5°.

7-$^2H_2$-4-Androstene-3, 17-dione (18)

Method A. 7-$^2H_2$-3β-hydroxy-5-androsten-17-one (17) (0.1 g, 0.3 mmol) in toluene (15 ml) was treated with cyclohexanone (0.33 g, 3.4 mmol) then refluxed, and toluene (5 ml) allowed to distill off. Aluminium isopropoxide (0.3 ml of

0.12 g/ml in toluene, 0.035 g, 0.17 mmol) was added and the solution stirred under reflux for 2 h. The reaction mixture was worked up as for dione (13). Chromatography by p.l.c. on silica GF_{254} developing with 5% acetone in chloroform followed by recrystallisation from hexane gave pure 7-$^2H_2$-4-androsten-3, 17-dione (0.08 g, 78%) m.p. 170-172° undepressed on admixture with an authentic sample of undeutrated material $(\alpha)_D^{21}$ + 184° (EtOH). The C7-$^1H_2$ compound has (23) m.p. 173-4° $(\alpha)_D^{18}$ (24) + 185° (EtOH).

Method B. 7-$^2H_2$-5-androstene-3β, 17β-diol (8) (0.1 g, 0.34 mmol) in toluene (15 ml) was treated with cyclohexanone(0.23 g, 3.4 mmol) then refluxed and toluene (5 ml) allowed to distill off. Aluminium isopropoxide (0.5 ml of 0.12 g/ml in toluene, 0.035 g, 0.17 mmol) was added and the solution stirred under reflux for 3 h. Work-up and chromatography as for method A gave the pure 7-$^2H_2$-4-androsten-3, 17-dione (0.065 g, 66% m.p. 170-172° identical in all respects with the compound obtained by method A.

7-$^2H_2$-4-Androstene diol (19). To a suspension of lithium aluminium hydride (0.13 g, 3.5 mmol) in THF was added dropwise a solution of 7-$^2H_2$-4-androstene-3, 17-dione (0.065 g, 0.23 mmol) in THF (4 ml). The reaction mixture was refluxed for 1 h then diluted with ether (40 ml) and water (10 drops) added. The organic layer was washed with brine (15 ml) dried ($MgSO_4$) and evaporated to leave a mixture of 3β, 17βdiol and the 3α, 17βdiol (0.060 g, 96%) (c.f. 25) which was oxidised without purification.

7-$^2H_2$-17β-Hydroxy-4-androsten-3-one (20). A solution of 7-$^2H_2$-4-androstene-diol (0.06 g, 0.2 mmol) in benzene (3 ml) was treated with DDQ (0.10 g, 0.42 mmol) and was allowed to stir overnight at room temperature in the dark. The reaction mixture was diluted with ether (30 ml) and then washed with 10% sodium hydroxide (4 x 15 ml), saturated brine (15 ml) dried and evaporated to give an off white solid which was chromatographed by p.l.c. on silica GF_{254} eluting with 5% acetone in chloroform. Recrystallisation from acetone gave pure 7-$^2H_2$-17β-hydroxy-4-androsten-3-one (20) (0.055 g, 86%) m.p. 153-154° undepressed on admixture with authentic undeuterated material. $(\alpha)_D^{24}$ + 107° (EtOH). The C7-$^1H_2$ compound has (23) m.p. 154-154.5° $(\alpha)_D$ + 109° (EtOH).

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