PROTON MAGNETIC RESONANCE SPECTRA OF 17ξ-HYDROXY-17ξ-METHYL-5ξ-ANDROSTANE C-3 KETONE AND C-3ξ ALCOHOL ISOMERS IN CHLOROFORM-d AND PYRIDINE-d_5

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Abstract - 17α-Hydroxy-17β-methyl-5β-androstan-3-one, 17β-methyl-5α-androstane-3α,17α-diol, 17β-methyl-5α-androstane-3β,17α-diol, 17α-methyl-5β-androstane-3β,17β-diol, 17β-methyl-5β-androstane-3α,17α-diol and 17β-methyl-5β-androstane-3β,17α-diol were synthesized for the first time. ^{1}H NMR spectra of all four 17ξ-hydroxy/17ξ-methyl C-3 ketones and all eight C-3 alcohols were recorded in chloroform-d and pyridine-d_5. Pyridine-induced chemical shifts are discussed. Thin-layer chromatographic data are given.

INTRODUCTION

Addition of a 17α-methyl group is the synthetic alteration most widely made in a number of anabolic/androgenic drugs derived from testosterone to enhance oral effectiveness (1). The presence of this group has been associated with the development of liver tumors (2). Metabolic epimerization of the 17α-methyl/17β-hydroxy moiety has been reported to occur in humans administered 1-dehydro-17α-methyl-testosterone (methandrostenolone) (3,4).

During studies on the urinary excretion products of 17α-methyl-5α- and 5β-dihydrotestosterone (5,6), 17α-methyltestosterone (7) and 1-dehydro-17α-methyltestosterone (7), the unknown isomers of 17ξ-hydroxy-17ξ-methyl-5ξ-androstan-3-one and 17ξ-methyl-5ξ-androstane-3ξ,-17ξ-diol were synthesized for comparison purposes and the ^{1}H NMR spectra of the four ketone and eight diol isomers were measured in chloroform-d and pyridine-d_5 (see Table I). Other physical properties are reported in the experimental.

EXPERIMENTAL

Infrared (ir) spectra were recorded on a Perkin Elmer Model 367 spectrophotometer. Thin-layer chromatography was carried out on precoated Merck silica gel GF 254 (type 60) plates using 50% v/v ethyl acetate/hexane as the liquid phase. Elemental analyses were performed by Mr. W. Baldeo, School of Pharmacy, University of London, England.

17α-Methyl-5β-dihydrotestosterone (Ib)

17α-Methyltestosterone (25 g) was hydrogenated by the method of Surorov and Yaraslavtseva (8) over 10% $Pd/CaCO_3$ (2.5 g) in dry pyridine (114 ml) to give Ib (21.8 g) MP 78-80° and 117-17.5°C from ether/hexane [lit. (9) 74-6° and 119-21°C]; R_f = 0.40.

3β-Hydroxy-17-methylene-5-androstene

3β-Hydroxy-17-methylene-5-androstene, MP 133-4°C from dichloromethane/ methanol, was prepared by the Wittig method of Macdonald et al. (4) [lit. (10) MP 133-4°C].

17-Methylene-4-androsten-3-one

Oppenauer oxidation (11) of 3β-hydroxy-17-methylene-5-androstene gave 17-methylene-4-androsten-3-one MP 133-4°C [lit. (10) MP 134-5°C].

17-Methylene-5β-androstan-3-one

17-Methylene-4-androsten-3-one (12 g) was selectively hydrogenated (8) over 10% $Pd/CaCO_3$ (1.4 g) in dry pyridine (62 ml) to yield 17-methylene-5β-androstan-3-one (4.8 g), MP 93-5°C from dichloromethane/ methanol, recrystallization gave MP 97-8°C; IR (CCl_4) ν_{max}:1750 (C=O), 1650 ($C=CH_2$) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.81 ($C\text{-}13CH_3$), 1.05 ($C\text{-}10CH_3$), 4.64 W1/2 = 5 Hz, ($C=CH_2$) ppm; MS m/z:286, 271 ($M^+\text{-}CH_3$)

Anal. Found: C, 83.54; H, 10.67. $C_{20}H_{30}O$ requires C, 83.86; H, 10.50.

17α-Hydroxy-17β-methyl-5β-androstan-3-one (IIb)

17-methylene-5β-androstan-3-one (4.5 g) was oxidized with m-chloroperbenzoic acid and the product reduced with lithium aluminum hydride as described by Macdonald et al. (4). The product was oxidized with Jones reagent (12) and chromatographed over alumina to give 17α-hydroxy-17β-methyl-5β-androstan-3-one (IIb) (1.09 g), MP 133-4°C from acetone; R_f = 0.45; IR (CCl_4) ν_{max}: 3620 (OH str.), 1715 (C=O) cm^{-1}; MS m/z:304 (M^+), 289 ($M^+\text{-}CH_3$), 286 ($M^+\text{-}H_2O$).

Anal. Found: C 78.81; H, 10.23, $C_{20}H_{30}O_2$ requires C,78.90; H 10.59. In a separate experiment 17,20-oxido-21-nor-5β-pregnan-3-one was isolated; MP 150-1°C from ethyl acetate; IR (CCl_4) ν_{max}: 1715 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.84 ($C\text{-}13CH_3$), 1.03 ($C\text{-}10CH_3$) 2.72, q, J=4Hz (C-20α-and 20βH) ppm; MS m/z: 304(M^+).

Anal. Found: C,78.89; H, 9.86. $C_{20}H_{32}O_2$ requires C, 79.42; H, 10.00.

3β-Hydroxy-17-methylene-5α-androstane

3β-Hydroxy-5α-androstan-17-one (5 g) was converted to 3β-hydroxy-17-methylene-5α-androstane (4.2g) MP 141-2.5°C from methanol, by the method of Macdonald et al. (3) [lit. (13) MP 144 - 5°C].

3β-Hydroxy-17α,20-oxido-21-nor-5α-pregnane

3β-Hydroxy-17-methylene-5α-androstane (289 mg) was oxidized with m-chloroperbenzoic acid as described by Macdonald et al. (4) to give 3β-hydroxy-17,20-oxido-21-nor-5α-pregnane (223 mg) MP 141-143°C from methanol; ^1H NMR ($CDCl_3$) δ: 0.81 (C-10 and C-13 CH_3), 2.69, q, J=4Hz (C-20α-and 20βH), 3.55, W1/2 = 24Hz (3αH) ppm; MS m/z: 304 (M^+).

Anal.Found:C,77.04;H,10.71.$C_{20}H_{32}O_2.1/2H_2O$ requires C,77.20;H,11.09.

17β-Methyl-5α-androstane-3β,17α-diol (IVb)

To a solution of lithium aluminum hydride (2.15 g) in dry ether (150 ml) was added a solution of 3β-hydroxy-17,20-oxido-21-nor-5α-pregnane (2.10

g) in ether (150 ml) and the mixture heated to reflux for 45 min. Work-up of the product gave the (IVb) (1.4 g), MP 194-5°C from dichloromethane/acetone (see below for the same product from the sodium borohydride reduction of the ketone IIa).

17α-Hydroxy-17β-methyl-5α-androstan-3-one (IIa)

17β-Methyl-5α-androstane-3β,17α-diol (IVb) (1.5 g) in acetone (350 ml) was oxidized with excess Jones reagent (12) to give IIa MP 224-6°C from dichloromethane/ethyl acetate (5); R_f = 0.54.

Sodium borohydride reduction of the C-3 ketones

To the steroid ketone (1 mmole) in 95% ethanol (20 ml) was added sodium borohydride (1.25 mmole) and the mixture stirred at room temperature for 1 hr. The reaction was diluted with water, neutralized with mineral acid and extracted with dichloromethane to give a crude product which was recrystallized from a suitable solvent as follows:

(i)17β-Hydroxy-17α-methyl-5β-androstan-3-one(Ib) (1 g) (R_f = 0.40) gave the 3α-alcohol (Va) (667 mg) MP 164-5°C from dichloromethane [lit. (14) MP 164-6°C]; R_f = 0.26.

(ii)17α-Hydroxy-17β-methyl-5β-androstan-3-one (IIb) (900 mg) gave the 3α-alcohol (VIa) (590 mg) MP 195-6°C from dichloromethane; Rf = 0.18.

Anal. Found: C,76.03; H, 10.65. $C_{20}H_{34}O_2.1/2H_2O$ requires C, 76.14; H, 11.18. Chromatography of the mother liquor over alumina (Brockmann Activity II) in benzene gave on elution with 5-10% v/v ether/benzene the 3β-alcohol (VIb) (90 mg) MP 194-5°C from dichloromethane/methanol; R_f = 0.36.

Anal. Found: C, 78.35; H, 11.28. $C_{20}H_{34}O_2$ requires C, 78.38; H, 11.18.

(iii)17β-Hydroxy-17α-methyl-5α-androstan-3-one (Ia) (912 mg) (R_f = 0.42) gave the 3β-alcohol (IIIb) (50 mg) MP 209-10°C from chloroform [lit. (15) MP 221-12°C]; R_f = 0.30.

(iv)17α-Hydroxy-17β-methyl-5α-androstan-3-one (IIa) (800 mg) gave the 3β-alcohol (IVb) (60 mg) MP 194-5°C from acetone; Rf = 0.33.

Anal. Found: C,77.89; H,11.06. $C_{20}H_{34}O_2$ requires C,78.38; H, 11.18.

Potassium tri-(sec-butyl)-borohydride reduction of the C-3 ketones

To a stirred solution of the steroid ketone (1 mmole) in freshly distilled tetrahydrofuran (20 ml) at -78°C (acetone/dry-ice bath) under nitrogen was added potassium tri-(sec-butyl)-borohydride (1.1 ml of a 1M solution in tetrahydrofuran, K-Selectride, Aldrich Chemical Co., Milwaukee, WI). The reaction was complete by tlc in 1-2 hrs and after coming to room-temperature, 10% aqueous sodium hydroxide (7 ml) and 30% hydrogen peroxide (5 ml) were added and stirred overnight (16)(17). The reaction was extracted with ether and the organic layer washed with water and brine. Evaporation gave a crystalline residue which was recrystallized from a suitable solvent.

(i)17β-Hydroxy-17α-methyl-5β-androstan-3-one (Ib) (304 mg) gave the 3β-alcohol (Vb) (277 mg) MP 178-9°C from ethyl acetate; R_f = 0.35.

Anal.Found:C,75.95; H,11.13. $C_{20}H_{34}O_2.1/2H_2O$ requires C,76.14; H,11.18.

(ii)17β-Hydroxy-17α-methyl-5α-androstan-3-one (Ia) (304 mg) gave the 3α-alcohol (IIIa) (267 mg) MP 181-2°C from acetone/ethyl acetate [lit. (14) MP 188-90°C]; R_f = 0.36.

(iii)17α-Hydroxy-17β-methyl-5α-androstan-3-one (IIa) (421 mg) gave the 3α-alcohol (IVa) (355 mg) MP 188-9°C from acetone; R_f = 0.28.

Anal.Found:C, 74.17; H, 11.00. $C_{20}H_{34}O_2.H_2O$ requires C, 74.02; H, 11.18.

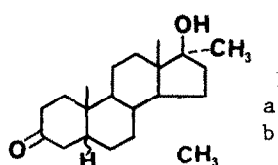
The mass spectra of the isomeric diols (IVa, IVb, Vb, VIa, VIb) showed m/z: 306 (M^+), 291 (M^+-CH_3), 288 (M^+-H_2O), 273 [$M^+-(H_2O+CH_3)$], 270 (M^+-2H_2O), 255 [$M^+-(2H_2O-CH_3)$].

TABLE 1

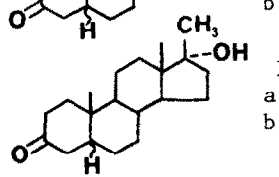
^{1}H NMR SPECTRA OF 17ξ-HYDROXY-17ξ-METHYL-5ξ-ANDROSTAN-3-ONE AND 17ξ-METHYL-5ξ-ANDROSTANE-3ξ,17ξ-DIOL EPIMERS IN CHLOROFORM-d AND PYRIDINE-d_5 WITH SOLVENT DIFFERENCES[a]

	C-3H[b]			C-10CH_3			C-13CH_3			C-17CH_3		
	$CDCl_3$	C_6D_5N	Δ^c	$CDCl_3$	C_6D_5N	Δ^c	$CDCl_3$	C_6D_5N	Δ^c	$CDCl_3$	C_6D_5N	Δ^c
Ia				1.03	0.95	+0.08	0.88	1.12	-0.24	1.22	1.44	-0.22
Ib				1.06	0.95	+0.11	0.89	1.12	-0.23	1.25	1.48	-0.23
IIa				1.03	0.93	+0.10	0.71	0.74	-0.03	1.21	1.39	-0.18
IIb				1.04	0.91	+0.13	0.71	0.70	+0.01	1.21	1.38	-0.17
IIIa	4.02	4.30	-0.27	0.80	0.87	-0.08	0.84	1.13	-0.28	1.21	1.43	-0.22
IIIb	3.60	3.90	-0.30	0.82	0.87	-0.05	0.84	1.11	-0.27	1.19	1.44	-0.25
IVa	4.04	4.28	-0.24	0.80	0.87	-0.07	0.68	0.75	-0.08	1.19	1.38	-0.19
IVb	3.62	3.87	-0.25	0.84	0.85	-0.01	0.68	0.72	-0.04	1.19	1.39	-0.20
Va	3.64	3.92	-0.28	0.95	0.96	-0.01	0.84	1.11	-0.27	1.23	1.45	-0.22
Vb	4.13	4.38	-0.26	0.98	1.09	-0.11	0.84	1.13	-0.29	1.22	1.49	-0.27
VIa	3.62	3.87	-0.25	0.95	0.95	0.00	0.64	0.70	-0.06	1.19	1.37	-0.18
VIb	4.15	4.32	-0.30	0.98	1.05	-0.07	0.69	0.72	-0.03	1.19	1.38	-0.19

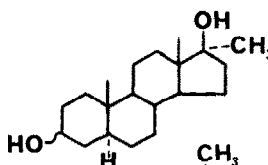
[a]Spectra were recorded on either a Varian 220 mHz, Brucker 90 mHz, NIC 220 mHz or NTC 360 mHz instrument using TMS as internal standard. [b]All axial (IIIb, IVb, Va, VIa) C-3 protons show aa/ae coupling (W1/2≈21 Hz) and equatorial (IIIa, IVa, Vb, VIb) C-3 protons ae/ee coupling (W1/2≈7 Hz). [c]Δ = δ($CHCl_3$)-δ(pyridine-d_5); minus signs indicate a downfield shift.



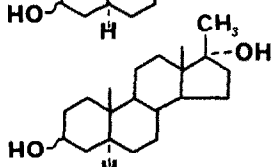
I
a 5α
b 5β



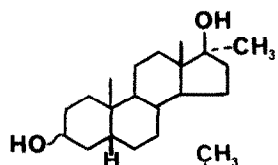
II
a 5α
b 5β



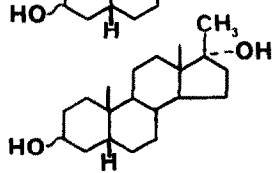
III
a 3α
b 3β



IV
a 3α
b 3β



V
a 3α
b 3β



VI
a 3α
b 3β

RESULTS AND DISCUSSION

The 17α-hydroxy/17β-methyl epimers were synthesized by the method of Sondheimer et al. (13) and Macdonald et al. (4). Generally the C-3 equatorial alcohols were prepared by sodium borohydride reduction of the C-3 ketone, whereas the C-3 axial alcohols were prepared by potassium tri-(sec-butyl)borohydride (K-Selectride, Aldrich) reduction.

The ^{1}H NMR spectra are recorded in chloroform-d and pyridine-d_5 (see Table I). Pyridine-induced chemical shifts in monohydroxylated steroids and other alicyclic compounds have been classified into three types resulting from (a) 1,3-diaxial deshielding, (b) vicinal deshielding and (c) geminal deshielding (18). These effects are rationalized in terms of solute-solvent associations, i.e. hydrogen-bonding appears to be the major effect (19).

The pyridine-induced downfield chemical shift of the C-10 methyl group caused by the presence of a C-3 hydroxyl group is in the range 0 - 0.11 ppm in the 5α-and 5β-series with the effect of the equatorial alcohol (0 - 0.05 ppm) less than that of the axial alcohol (0.07 - 0.11 ppm).

Vicinal deshielding, which is dependent on the dihedral angle, is observed in the C-13 methyl group in the presence of the 17β-hydroxyl group (0.27 - 0.29 ppm) which allows a clear distinction to be made from the epimeric 17α-hydroxyl group (0.03 - 0.07 ppm).

Geminal deshielding observed on the C-17 methyl group is in agreement with generalizations (18) regarding geminal deshielding of protons and methyl groups (0.10 - 0.25 ppm) on a carbon bearing a hydroxy function: the observed shifts are in the 0.16 - 0.30 ppm range. The 17β-hydroxyl/17α-methyl group shows a pyridine-induced shift range of 0.22 -

0.27 ppm compared with a smaller shift of 0.17 - 0.20 ppm for the C-17 epimer again differentiating the C-17 epimers. The C-3 CHOH proton shift is in the 0.24 - 0.30 ppm range and is not significantly different for axial and equatorial alcohols.

Application of the above data to trihydroxylated derivatives is reported in the identification of the neutral urinary excretion metabolites of 17α-methyl-5α-dihydrotestosterone (5), 17α-methyl-5β-dihydrotestosterone (6), 17α-methyltestosterone (7) and 1-dehydro-17α-methyltestosterone (7).

All pairs of epimeric C-3 alcohols can be separated on silica gel thin-layer chomatographic plates in 50% v/v ethyl acetate/hexane. In the 5α-series the 3α-alcohol (axial) is less polar than the 3β-alcohol, whereas for the 5β-series the polarity is less for the 3β-alcohol (also axial). Of the four C-3 ketone compounds the 5α-(Ia,IIa)-and 5β(Ib,IIb)-isomeric pairs do not separate clearly in the solvent system used; nevertheless, the two pairs of C-17 epimers (Ia,Ib and IIa,IIb) can be distinguished.

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GLOSSARY

17α-Methyl-5α-dihydrotestosterone = 17β-hydroxy-17α-methyl-5α-androstan-3-one
17α-Methyl-5β-dihydrotestosterone = 17β-hydroxy-17α-methyl-5β-androstan-3-one
17α-Methyltestosterone = 17β-hydroxy-17α-methyl-4-androsten-3-one
1-Dehydro-17α-methyltestosterone = 17β-hydroxy-17α-methyl-1,4-androstadien-3-one.