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Synthesis of 3-methyl-3-hydroxy-6-oxo-androstane derivatives☆

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Abstract

3α,17β-Dihydroxy-3β-methyl-5α-androstan-6-one (**1**) and 3β,17β-dihydroxy-3α-methyl-5α-androstan-6-one (**13**) were prepared by the reaction of methylmagnesium bromide with the 3-ketosteroids. Structures and configurations in position 3 were determined by NMR spectra. Substitution in the position 6 influences the ratio of the products.

Keywords: Biologically active steroids; 3-Methyl-3-hydroxy-steroids

1. Introduction

Biologically active steroid compounds with 3α-hydroxy group can be easily found in nature [1–3]. Their physiological properties present a great potential to pharmacological industry and agriculture. A significant drawback for their real life applications is often caused by their low stability in living organisms; their fast metabolic deactivation, presumably by conjugation of the 3-hydroxy group or its oxidation to the corresponding ketone. The problem can be solved by protection of 3-hydroxy group by introduction of geminal methyl group in position 3. This can prevent the mentioned deactivation [4].

The published data [5] dealing with the reaction of an organometallic reagent with 3-oxo-5α-steroid show, that in the resulting mixture 3β-hydroxyderivative prevails with the ratio being (3:2). The opposite tendency can be observed by using a more complex reaction sequence utilizing methylsulfoxonium reagent. This reaction gives an oxirane intermediate ((3*R*)-*spiro*[oxiran-2′,5α-steroid]). The cleavage of this oxirane with sodium iodide and acetic acid followed with hydrogenolysis of 3α-hydroxy-3β-iodomethyl derivative give the target 3α-hydroxy-3β-methyl compound. In this paper, we dealt with reaction of organometallic reagent with 3-oxo-5α-steroid containing a bulky substituent in position 6α. We assumed the reversion of the product ratio formed under these conditions.

2. Experimental

2.1. General methods and equipment

Melting points were determined on a Koefler melting point micro apparatus Boetius (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured in chloroform using an Autopol IV (Rudolf Research Analytical, Flanders, USA, $[\alpha]_D$ values are given in 10^{-1} degree cm^2 g^{-1}). IR spectra were recorded on a Bruker IFS 88 spectrometer in chloroform solutions, wavenumbers are given in cm^{-1}. Detailed NMR study of C(3)-epimeric pairs (**1**, **13**), (**8**, **9**), and (**11**, **12**) was done on Varian UNITY-500 and Buker AVANCE-500 instruments (^{1}H at 500 MHz; ^{13}C at 125.7 MHz). Proton NMR spectra of other compounds were measured on spectrometers Varian UNITY-200 (at 200 MHz) and/or Bruker AVANCE-400 (at 400 MHz) in $CDCl_3$ with tetramethylsilane as internal reference. TAI stands for trichloroacetyl isocyanate. TAC stands for trichloroacetyl carbamate. Chemical shifts are given in ppm (δ-scale), coupling constants and widths of multiplets in Hz. Unless otherwise stated, the data were interpreted as the first-order spectra. Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals). Preparative TLC (PLC) was carried out on 200 mm × 200 mm plates coated with a 0.7-mm thick layer of the same material. For

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column chromatography, 60–120 μm silica gel was used. Methylmagnesium bromide, 3 M solution in diethyl ether was purchased from Sigma-Aldrich (Prague, Czech Republic). Jones reagent was prepared by the dissolving of CrO_3 (5.2 g) in concentrated H_2SO_4(4.6 ml) and water (4 ml) and subsequently diluting with water (20 ml). Whenever aqueous solutions of hydrochloric acid was used, concentration was 5%. Solvents were evaporated on a rotatory evaporator in vacuo (0.25 kPa, bath temperature 40 °C).

2.2. 3β-(2-Tetrahydropyranyloxy)-androst-5-en-17β-yl pivalate (3)

To a stirring solution of the alcohol **2** [6] (4.5 g, 12.0 mmol) in pyridine (10 ml) at 0 °C was added pivaloyl chloride (1.2 eq., 1.7 ml, 14.4 mmol). The mixture was warmed to 30 °C and let stand overnight. Water was added and the mixture was extracted with ethyl acetate (3 × 30 ml) and the combined extracts were washed with saturated aqueous sodium hydrogen carbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure (4.9 g). Chromatography on the column of silica gel (150 g, elution with light petroleum/acetone, 19:1) and crystallization (ethyl acetate/light petroleum) provided 3.3 g (60%) of protected derivative **3**, mp 173–174 °C, $[\alpha]_D$ +51.7 (*c* 0.51). IR: 1717 (C=O); 1673 (C=C); 1173, 1160 (C–O); 1077. ^{1}H NMR: 5.30 m, 1 H (H-6); 4.62 dd, 1 H, $J_1 = 3$, $J_2 = 7$ (H-2′ (THP)); 4.56 dd, 1 H, $J = 9.1$ and 7.7 (H-17); 3.89 m, 1 H, $W = 23$ (H-6′$_b$ (THP)); 3.57 m, 1 H, $W = 32$ (H-3); 3.44 m, 1 H (H-6′$_a$ (THP)); 2.22 m, 1 H (H-5); 1.19 s, 9 H ($(CH_3)_3$ (Piv)); 1.02 s, 3 H (19-H_3); 0.82 s, 3 H (18-H_3). Analysis calculated for $C_{29}H_{46}O_4$ (458.7): 75.94% C, 10.11% H; found: 75.97% C, 10.28% H.

2.3. 6α-Hydroxy-3β-(2-tetrahydropyranyloxy)-5α-androstan-17β-yl pivalate (4)

To a suspension of sodium borohydride (0.5 g, 13.2 mmol) in THF (20 ml) a solution of boron trifluoride etherate (2 ml, 12.5 mmol) in THF (10 ml) was added via syringe through septum under argon at 0 °C. The resultant mixture was stirred for 30 min. Then the solution of olefin **3** (4.0 g, 8.7 mmol) in THF (30 ml) was added and the mixture was stirred at 0 °C for 9 h. The mixture was treated with aqueous solution of potassium hydroxide (0.5 g in 5 ml of water) and hydrogen peroxide (30%, 3 ml) and stirred again for 80 min at 0 °C. The product was extracted with ether (300 ml), and the ethereal phase was washed with water (1 l in four portions) and dried over anhydrous sodium sulfate. After evaporation of the solvent, the crude product was purified by column chromatography (silica gel, 80 g). Elution with the mixture of light petroleum/ether (95:5) afforded 3.07 g (74%) of **4**, mp 205–207 °C (ethanol), $[\alpha]_D$ +25.8 (*c* 0.68). IR: 1717 (C=O); 1174, 1294, 1026 (C–O); 1077. ^{1}H NMR: 4.62 dd, 1 H, $J_1 = 3$, $J_2 = 7$ (H-2′ (THP)); 4.56 dd, 1 H, $J_1 = 9.1$, $J_2 = 7.7$ (H-17); 3.89 m, 1 H, $W = 23$ (H-6′$_b$ (THP)); 3.57 m, 1 H, $W = 32$ (H-3); 3.44 m, 2 H (H-6 and H-6′$_a$ (THP)); 2.22 m, 1 H (H-5); 1.19 s, 9 H ($(CH_3)_3$ (Piv)); 0.82 s, 3 H (19-H_3); 0.79 s, 3 H (18-H_3). Analysis calculated for $C_{29}H_{48}O_5$ (476.7): 73.07% C, 10.15% H; found: 73.15% C, 10.28% H.

2.4. 6α-(tert-Butyldimethylsilyloxy)-3β-(2-tetrahydropyranyloxy)-5α-androstan-17β-yl pivalate (5)

tert-Butyldimethylsilyl chloride (1.56 g, 10.4 mmol) was added at 0 °C to a solution of hydroxyderivative **4** (2.25 g, 4.72 mmol) and imidazole (1.26 g, 18.5 mmol) in *N*,*N*-dimethylformamide (50 ml). The reaction mixture was allowed to stand at room temperature overnight, diluted with ether (200 ml), and washed successively with 5% aqueous citric acid (three times), water (three times), saturated aqueous sodium hydrogen carbonate (three times) and water (twice). Combined organic extracts were dried over the anhydrous sodium sulfate. The solvent was evaporated, and the residue was chromatographed on a column of silica gel (80 g) with a mixture of light petroleum/ether (98:2 to 90:10). The yield of **5** was 2.56 g (92%), mp 140–142 °C (ether), $[\alpha]_D$ +61.8 (*c* 0.27). IR: 1717 (C=O); 1174, 1160 (C–O); 1077 (C–OSi). ^{1}H NMR: 4.73 dd, 1H, $J_1 = 3.1$, $J_2 = 7$ (H-2′ (THP)); 4.56 dd, 1H, $J_1 = 7.7$, $J_2 = 8.9$ (H-17); 3.89 m, 1 H, $W = 25.3$ (H-6′$_b$ (THP)); 3.46 m, 2 H (H-3 and H-6′$_a$ (THP)); 3.38 dt, 1 H, $J_1 = 4.5$, $J_2 = 9.8$ (H-6); 1.19 s, 9 H ($(CH_3)_3$ (Piv)); 0.88 s, 9 H ($(CH_3)_3CSi$); 0.82 s, 3 H (19-H_3); 0.79 s, 3 H (18-H_3); 0.05 s, 3 H (CH_3Si); 0.03 s, 3 H (CH_3Si). Analysis calculated for $C_{35}H_{62}O_5Si$ (591.0): 71.14% C, 10.57% H; found: 70.81% C, 10.61% H.

2.5. 6α-(tert-Butyldimethylsilyloxy)-3β-hydroxy-5α-androstan-17β-yl pivalate (6)

Tetrahydropyranyl derivative **5** (1 g, 1.69 mmol) was added at 0 °C to a solution of magnesium bromide [7,8] (1.3 g, 7.0 mmol) in the mixture of absolute ether (5.4 ml) and absolute benzene (0.6 ml). The reaction mixture was allowed to stand at room temperature overnight, diluted with ether (20 ml), and saturated aqueous ammonium chloride solution (20 ml). Ethereal layer was separated and water phase extracted with ether (three times). Combined organic extracts were washed with saturated aqueous ammonium chloride solution (three times), water (twice) and dried over the anhydrous sodium sulfate. Evaporation of the solvent and crystallization of the residue from light petroleum afforded 790 mg (94%) of **6**, mp 170–172 °C (light petroleum), $[\alpha]_D$ +37.3 (*c* 0.21). IR: 1717 (C=O); 1294, 1286, 1172 (C–O); 1077 (C–OSi). ^{1}H NMR: 4.58 dd, 1H, $J_1 = 9.1$, $J_2 = 7.7$ (H-17); 3.58 m, 1 H, $W = 32$ (H-3); 3.38 m, $W = 32$, 1 H (H-6); 1.19 s, 9 H ($(CH_3)_3$ (Piv)); 0.88 s, 9 H ($(CH_3)_3CSi$); 0.82 s, 3 H (19-H_3); 0.79 s, 3 H (18-H_3); 0.40 s, 3 H (CH_3Si); 0.34 s, 3 H (CH_3Si). Analysis calculated for $C_{30}H_{54}O_4Si$ (506.8): 71.09% C, 10.74% H; found: 71.50% C, 10.85% H.

2.6. 6α-(tert-Butyldimethylsilyloxy)-3-oxo-5α-androstan-17β-yl pivalate (7)

To a stirring solution of hydroxyderivative **6** (400 mg, 0.79 mmol) in the absolute dichloromethane (10 ml) was added pyridinium chlorochromate (100 mg, 0.46 mmol) and the mixture was stirred for 1 h and then left at room temperature over night. The mixture was filtered over a small column of silica gel. Crystallization from the mixture of acetone/heptane gave 384 mg (96%) of the ketone **7**, mp 158–159 °C, $[\alpha]_D$ +47.5 (*c* 1.9). IR: 1710 (C=O); 1294, 1171, 1155 (C–O); 1092, 1078 (C–O–Si); 858, 838 ($Si(CH_3)_2$). ^{1}H NMR: 4.58 dd, 1H, $J_1 = 8.0$, $J_2 = 9.0$ (H-17); 3.45 dt, 1 H, $J_{5,6} = J_{6,7}\alpha = 10.4$, $J_{6,7}\beta = 4.5$ (H-6); 1.19 s, 9 H ($(CH_3)_3$ (Piv)); 1.02 s, 3 H (19-H_3); 0.87 s, 9 H ($(CH_3)_3CSi$); 0.82 s, 3 H (18-H_3); 0.27 s, 6 H (2 × CH_3Si). Analysis calculated for $C_{30}H_{52}O_4Si$ (504.8): 71.38% C, 10.38% H; found: 71.21% C, 10.54% H.

2.7. 6α-(tert-Butyldimethylsilyloxy)-3α-hydroxy-3β-methyl-5α-androstan-17β-yl pivalate (8) and 6α-(tert-butyldimethylsilyloxy)-3β-hydroxy-3α-methyl-5α-androstan-17β-yl pivalate (9)

Solution of methylmagnesium bromide in diethyl ether (3 M, 0.5 ml) was added over 20 min dropwise to the stirring solution of ketone **7** (360 mg, 0.71 mmol) in THF (23 ml) cooled to −78 °C, under argon atmosphere. The reaction mixture was stirred at −50 °C for 6 h and then allowed to warm to room temperature. Reaction mixture was diluted with a saturated aqueous ammonium chloride solution (5 ml), concentrated in vacuo on rotatory evaporator and filtered under suction. Chromatography of obtained crude product (380 mg) on a silica gel column (20 g) in light petroleum (gradient to 2% of acetone) afforded isomers **8** and **9**.

The yield of compound **9** was 38 mg (10%): mp 157–159 °C (ether), $[\alpha]_D$ +49.9 (*c* 0.43). IR: 3603 (OH); 1717 (C=O); 1294, 1171 (C–O); 1092 (C–OSi); 860, 838 ($Si(CH_3)_2$); ^{1}H and ^{13}C NMR data (see Tables 1 and 2).

The yield of compound **8** was 270 mg (73%). ^{1}H and ^{13}C NMR data (see Tables 1 and 2). This crude oily product was used directly for the next step.

2.8. 3α,6α-Dihydroxy-3β-methyl-5α-androstan-17β-yl pivalate (10)

To a solution of silyl derivative **8** (200 mg, 0.39 mmol) in acetone (10 ml), three drops of concentrated hydrochloric acid were added. After 30 min standing at room temperature reaction mixture was diluted with water (30 ml) and neutralized with aqueous ammonia solution (25%, 0.5 ml). Crystalline product **10** (136 mg, 87%) was collected by filtration and crystallized from a mixture of acetone/heptane: mp 226–228 °C, $[\alpha]_D$ +32.0 (*c* 0.39). IR: 1710 (C=O); 1294, 1171, 1155 (C–O). ^{1}H NMR: 4.57 dd, 1 H, $J_1 = 7.8$, $J_2 = 9.1$ (H-17); 3.38 dt, 1 H, $J_1 = 4.5$, $J_2 = 9.8$ (H-6); 2.16 m, 1 H, $W = 45$ (H–16); 1.25 s, 1 H (C(3)-CH_3); 0.80 s, 3 H (19-H_3); 0.77 s, 3 H (18-H_3). Analysis calculated for $C_{25}H_{42}O_4$ (406.6): 73.85% C, 10.41% H; found: 73.61% C, 10.51% H.

Table 1
Proton NMR data of compounds **1**, **8**, **9**, **11**, **13**, and **12** in $CDCl_3$

Proton	**1**	**8**	**11**	**13**	**9**	**12**
H-1α	1.5–1.6	∼1.34	∼1.50	1.30	1.06	1.31
H-1β	1.5–1.6	∼1.50	∼1.50	1.74	1.62	1.73
H-2α	1.51	∼1.56	1.5–1.6	∼1.60	∼1.53	∼1.58
H-2β	1.51	∼1.50	1.5–1.6	∼1.60	∼1.60	∼1.58
Me-3	1.267 s	1.225 s	1.268 s	1.210 s	1.229 s	1.213 s
H-4α	∼1.58	1.84	∼1.58	1.6–1.7	1.90 ddd	1.6–1.7
H-4β	∼1.58	∼1.18	∼1.58	1.6–1.7	∼1.26	1.6–1.7
H-5	2.68 td	1.43 dd	2.69 ddd	2.24 bdd	1.11	2.25 ddd
H-6	–	3.36 dt	–	–	3.37 dt	–
H-7α	1.98 dt	0.97 dt	2.01 dt	1.95 dt	0.93 dd	1.98 dt
H-7β	2.31 dd	1.86 dt	2.31 dd	2.32 dd	1.86 dt	2.32 dd
H-8	1.86 m	∼1.47	1.86 m	1.85 m	1.47	1.87 m
H-9	1.21	0.78	1.31	1.21	0.71	1.30
H-11α	1.74	∼1.56	∼1.70	1.72	∼1.54	1.67
H-11β	1.36	1.21	1.31	1.38	∼1.24	1.34
H-12α	1.15	∼1.15	1.26	1.16	1.15	1.26
H-12β	1.87	∼1.71	1.77	1.88	1.71	1.78
H-14	1.36	1.10	1.39	1.27	1.08	1.30
H-15α	1.56	∼1.66	1.61	1.56	∼1.66	1.61
H-15β	1.27	∼1.32	1.32	1.28	∼1.31	1.32
H-16α	2.08	2.17 ddt	2.17	2.09	2.17 dddd	2.17
H-16β	1.45	∼1.46	1.48	1.46	∼1.46	1.49
H-17	3.68 bt	4.57 dd	4.62 dd	3.68 bt	4.57 dd	4.62 dd
3H-18	0.748 s	0.793 s	0.806 s	0.751 s	0.793 s	0.808 s
3H-19	0.720 s	0.757 s	0.727 s	0.776 s	0.822 s	0.772 s
TBDMS	–	0.023 s	–	–	0.025 s	–
		0.026 s			0.031 s	
		0.879 s			0.879 s	
Piv	–	1.190 s	1.196 s	–	1.191 s	1.196 s

2.9. 3α-Hydroxy-3β-methyl-6-oxo-5α-androstan-17β-yl pivalate (11)

To a solution of hydroxyderivative **10** (120 mg, 0.3 mmol) in chloroform (10 ml) at 0 °C Jones reagent (10 drops) were added. After 30 min the reaction mixture was filtered through the column of silica gel (5 g) in a mixture of chloroform/ether (5:1). Solvents were evaporated and crystallization of the residue from acetone/heptane afforded 110 mg (92%) of ketone **11**, mp 205–206 °C, $[\alpha]_D$ −7.5 (*c* 0.25). IR: 1715, 1703 (C=O); 1293, 1287, 1172, 1155 (C–O). ^{1}H and ^{13}C NMR data (see Tables 1 and 2). Analysis calculated for $C_{25}H_{40}O_4$ (404.6): 74.22% C, 9.97% H; found: 74.10% C, 10.05% H.

2.10. 3α,17β-Dihydroxy-3β-methyl-5α-androstan-6-one (1)

To a solution of the pivaloyl derivative **11** (60 mg, 0.16 mmol) in methanol potassium hydroxide (0.1 g,

Table 2
Carbon-13 chemical shifts of compounds **1**, **8**, **9**, **11**, **13**, and **12** in $CDCl_3$

Carbon	**1**	**8**	**11**	**13**	**9**	**12**
C-1	33.55	34.41	33.59	36.19	37.00	36.17
C-2	33.63	34.25	33.52	35.57	36.03	35.54
C-3	69.21	69.23	69.17	71.02	71.52	71.01
C-4	34.24	36.87	34.21	35.06	38.06	35.04
C-5	53.70	48.05	53.65	56.46	51.21	56.42
C-6	212.21	70.34	212.14	210.59	70.13	210.47
C-7	46.38	41.48	46.33	46.24	41.50	46.20
C-8	38.08	34.13	37.78	37.96	34.13	37.69
C-9	51.52	53.66	51.26	51.47	53.89	51.25
C-10	41.02	36.37	40.99	41.29	36.82	41.26
C-11	20.97	20.40	20.83	21.17	20.63	21.06
C-12	36.38	36.91	36.65	36.33	36.91	36.62
C-13	43.46	42.86	43.26	43.46	42.88	43.29
C-14	53.93	50.58	53.74	54.26	50.60	54.09
C-15	23.20	23.53	23.35	23.19	23.54	23.35
C-16	30.43	27.50	27.39	30.41	27.50	27.38
C-17	81.60	82.32	81.92	81.57	82.28	81.87
C-18	11.09	12.14	12.11	11.10	12.14	12.11
C-19	12.36	12.44	12.34	12.84	13.07	12.83
Me-3	31.84	31.85	31.82	25.90	26.62	25.93
TBDMS	–	−4.00	–	–	−3.95	–
		−4.60			−4.61	
		18.15			18.13	
	–	25.96			25.95	
Piv	–	178.48	178.14	–	178.51	178.52
		38.86	38.88		–[a]	–[a]
		27.22	27.22		27.23	27.22

[a] The signal was not detected.

1.8 mmol) was added and the mixture was heated to 60 °C for 4 h under argon atmosphere. Then the reaction mixture was poured on ice. Precipitate (45 mg) was separated by filtration and dried. Crystallization from ether afforded methyl derivative **1** (38 mg, 80%), mp 224–225 °C, $[\alpha]_D$ −8.5 (*c* 0.21). IR: 3607, 3457 (OH); 1700 (C=O); 1168, 1155, 1050 (C–O). ^{1}H and ^{13}C NMR data (see Tables 1 and 2). Analysis calculated for $C_{20}H_{32}O_3$ (320.5): 74.96% C, 10.06% H; found: 74.70% C, 10.05% H.

2.11. 3β-Hydroxy-3α-methyl-6-oxo-5α-androstan-17β-yl pivalate (12)

To a solution of silyl derivative **9** (30 mg, 0.06 mmol) in acetone (5 ml) 1 drop of concentrated hydrochloric acid was added. After 30 min standing at room temperature the reaction mixture was diluted with water (5 ml) and neutralized with aqueous ammonia solution (25%, 0.2 ml). Crystalline product was separated by filtration and dried. Then it was dissolved in chloroform (2 ml) and the Jones reagent (five drops) was added at 0 °C. After 30 min the reaction mixture was filtered through the column of silica gel in a mixture of chloroform/ether (5:1). Evaporation of solvents afforded solid compound **12** (22 mg, 95%). ^{1}H and ^{13}C NMR data (see Tables 1 and 2).

2.12. 3β,17β-Dihydroxy-3α-methyl-5α-androstan-6-one (13)

Ketone **12** (20 mg, 0.05 mmol) was dissolved in methanol (4 ml). Potassium hydroxide (0.02 g, 0.36 mmol) was added and the mixture was heated to 60 °C for 4 h under argon atmosphere and then poured on ice. Precipitate (15 mg) was separated by filtration and dried. Crystallization from ether afforded methyl derivative **13** (8 mg, 51%), mp 213–215 °C, $[\alpha]_D$ −4.2 (*c* 0.17). IR: 3434 (OH); 1701 (C=O); 1125, 1051 (C–O). ^{1}H and ^{13}C NMR data (see Tables 1 and 2). Analysis calculated for $C_{20}H_{32}O_3$ (320.5): 74.96% C, 10.06% H; found: 74.82% C, 10.15% H.

2.13. 3,6-Dioxoandrost-4-en-17β-yl benzoate (15)

To a solution of hydroxyderivative **14** [9,10] (500 mg, 1.27 mmol) in acetone (35 ml) Jones reagent (1 ml) was added. The mixture was vigorously stirred in the open flask on the sun light at room temperature for 2 h and then poured into the water (500 ml). Solid material was separated by filtration and dried on air. This crude product was dissolved in chloroform (20 ml), filtrated through alumina column and the solvent was evaporated. Crystallization of the residue from methanol gave 410 mg (80%) of diketone **15**, mp 182–185 °C (decomposition), $[\alpha]_D$ +20.2 (*c* 0.21). IR: 1713, 1693 (C=O); 1634 (C=C); 1603, 1585 (arom); 1279 (C–O). ^{1}H NMR: 8.04 m, 2 H, (H-2 and H-6 (OBz)); 7.57 m, 1 H (H-4 (OBz)); 7.45 m, 2 H (H-3 and H-5 (OBz)); 6.20 s, 1 H (H-4); 4.91 dd, 1 H, $J_1 = 7.6$, $J_2 = 9.3$ (H-17); 1.20 s, 3 H (19-H_3); 1.00 s, 3 H (18-H_3). Analysis calculated for $C_{26}H_{30}O_4$ (406.5): 76.82% C, 7.44% H; found: 74.70% C, 10.05% H.

2.14. 3,6-Dioxo-5α-androstan-17β-yl benzoate (16)

To a solution of diketone **15** (300 mg, 0.74 mmol) in ethyl acetate (4 ml) and ethanol (1 ml) palladium on carbon (5%, 30 mg) was added and the mixture was vigorously stirred in the slight overpressure of hydrogen at room temperature for 6 h. Then the catalyst was filtered off and solvents were evaporated. Crystallization of the residue from methanol gave compound **16** (180 mg, 60%), mp 213–216 °C (decomposition), $[\alpha]_D$ +28.8 (*c* 0.49). IR: 1712 (C=O); 1634 (C=C); 1603, 1585 (arom); 1280 (C–O). ^{1}H NMR: 8.03 m, 2 H (H-2 and H-6 (OBz)); 7.56 m, 1 H (H-4 (OBz)); 7.44 m, 2 H (H-3 and H-5 (OBz)); 4. 91 dd, 1 H, $J_1 = 7.7$, $J_2 = 9.1$ (H-17); 0.99 s, 3 H (19-H_3); 0.97 s, 3 H (18-H_3).

2.15. 3α,17β-Dihydroxy-3β-methyl-5α-androstan-6-one (1) and 3β,17β-dihydroxy-3α-methyl-5α-androstan-6-one (13) from 3,6-diketone 16

A solution of methylmagnesium bromide in diethyl ether (3 M, 0.5 ml) was added through septum over 20 min dropwise to the stirring solution of diketone **16** (400 mg,

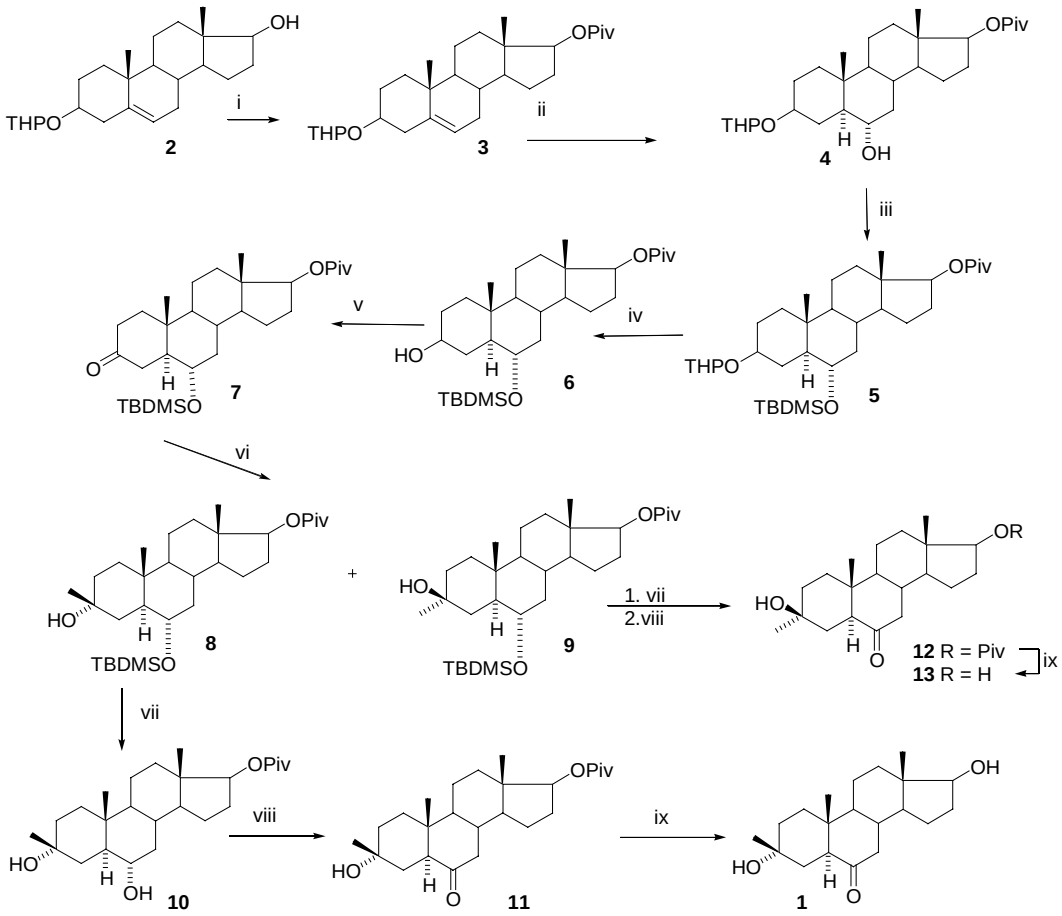
0.98 mmol) in THF (20 ml) cooled to −78 °C, under argon atmosphere. The reaction mixture was stirred at −50 °C for 1 h and then diluted with a saturated aqueous ammonium chloride solution (5 ml), concentrated in vacuo and filtered under suction. Crude product (220 mg) column chromatography (silica gel, elution with a gradient from 100% light petroleum to 2% light petroleum/acetone) afforded 38 mg (10%) of starting material and 350 mg (84%) of the mixture of 3-methyl derivatives **17** and **18** with small amounts of other products. This mixture was dissolved in methanol (20 ml). Potassium hydroxide (0.30 g, 5.34 mmol) was added and the mixture was heated to 60 °C for 4 h under argon atmosphere. Then the reaction mixture was poured on ice. Precipitate (310 mg) was separated by filtration and dried. Preparative TLC in a mixture of light petroleum/acetone (4:1) afforded **1** (96 mg, 32%) and **13** (136 mg, 45%) according to mp, IR, and NMR spectra identical with samples mentioned above.

3. Results and discussion

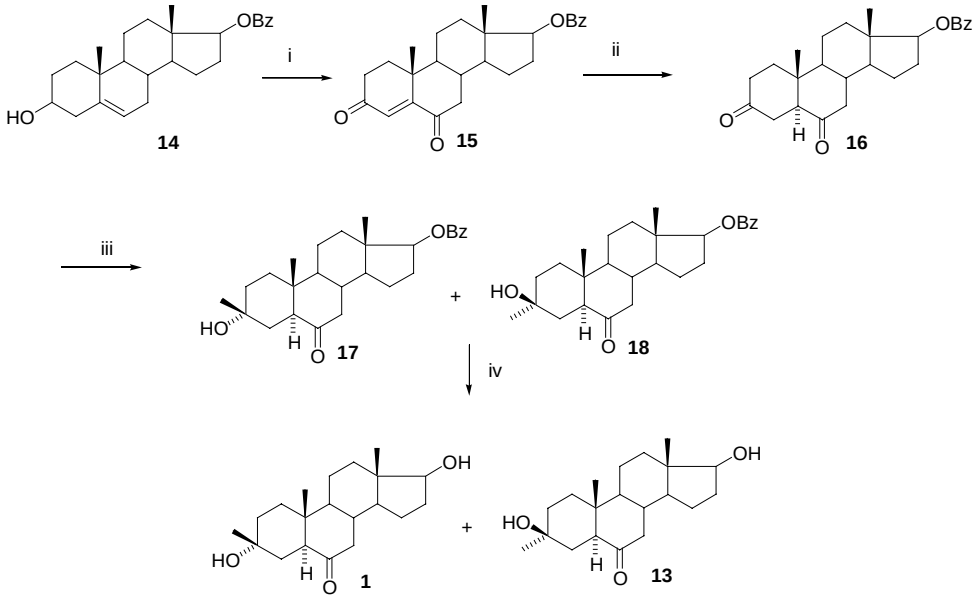
Selectively protected androstenediol **2** was used for the synthesis of the 3α-methylcarbinol **1** (Scheme 1). The free hydroxyl group in compound **2** was further on protected with dihydropyrane giving compound **3**. 6α-Hydroxy group was introduced by hydroboration reaction of the double bond 5,6 and new hydroxyl group was subsequently protected by *tert*-butyldimethylsilyl group. The individual protecting groups were chosen in a way to take advantage of their different reactivity for the following selective deprotection reactions. Tetrahydropyranyl was cleaved off with magnesium bromide [7,8]. The 3-hydroxy derivative **6** yielded by this reaction was oxidized with pyridinium chlorochromate to give ketone **7**.

The reaction of 3-oxo derivative **7** with methylmagnesium bromide yielded a mixture of methylcarbinols **8** and **9** which were separated on a silica gel column. The amount of the less polar crystalline product **9** was 10%. Conditions to crystallize the more abundant (73%) product **8** were not found either by choosing different solvents, or by lowering the temperature, or prolonging the time of crystallization. The analysis of NMR experiments, which will be described in a latter paragraph, proved the product **8** to have the α-configuration of hydroxyl group.

The product ratio **8**:**9** could be explained by the presence of the bulky substituent (6α-(*tert*-butyldimethylsilyloxy)



Scheme 1. (i) Pivaloyl chloride, pyridine; (ii) $NaBH_4$, BF_3-etherate, H_2O_2, KOH, THF; (iii) TBDMSCl, Im, DMF; (iv) $MgBr_2$, ether, benzene; (v) PCC, CH_2Cl_2; (vi) MeMgBr, −50 °C, THF; (vii) HCl, acetone; (viii) Jones reagent, $CHCl_3$; (ix) KOH, MeOH.



Scheme 2. (i) Jones reagent, acetone, air, sunlight; (ii) H_2, Pd/C, EtOH, EtOAc; (iii) MeMgBr, −50 °C, THF; (iv) KOH, MeOH.

group) on the α-side of the molecule. In the case of selective reaction of 3,6-diketone **16** (lacking a bulky substituent on α-side) under similar conditions the 3α-methyl derivative prevails, see below. The product ratio changed with the reaction temperature. At −10 °C the desired product **8** dominated only slightly in the reaction mixture, the ratio α:β being 3:2. This ratio increased significantly in favor of the desired product **8** when the reaction temperature was lowered to −50 °C. The reaction was quantitative, no side products were isolated.

The reaction sequence was terminated by the deprotection of the hydroxy group in position 6. This group was oxidized with the Jones reagent, as there were no acid labile protecting groups in the molecule any more, to afford ketone **11**. The last step was basic hydrolysis of the pivaloyl ester group in compound **11**. This reaction needs to be carefully controlled, as the isomerization in position 5 could take place and would lead to the change of annulation of rings A and B. From the reaction mixture only product **1** was isolated having the 5α-configuration.

The selective reaction of 3,6-diketone with methylmagnesium bromide presents a shorter way to 3-methylcarbinol **1**. It starts with 3β-hydroxyandrost-5-en-17β-yl benzoate **14** [9,10]. This compound was oxidized with Jones reagent [11] to give the 3,6-diketone **15** under conditions when the oxidizing agent itself was Cr(IV) formed in situ. Hydrogenation of this unsaturated diketone **15** in ethyl acetate/ethanol catalysed by paladium on carbon gives a mixture of 5β- and 5α-dihydroderivatives where the 5α one predominates. Diketone **16** on reaction with methylmagnesium bromide at −50 °C yielded quickly a reaction mixture that contained a small amount of the starting compound and chromatographically unseparable mixture of 3α-hydroxy-3β-methyl- and 3β-hydroxy-3α-methyl-derivatives (identified with NMR) accompanied by a small amounts of other products. The selectivity of the reaction in respect to the two keto groups in the molecule is caused by different steric hindrance.

The isomeric 3-methylderivatives were successfully separated after basic hydrolysis of the benzoyl protecting group at C-17 to give compounds **1** and **13**, identical with the derivatives prepared via the reaction sequence mentioned above (Scheme 1).

The reaction sequence in Scheme 1 has nine steps starting with the 17-protected derivative **2** and the over all yield of the desired product **1** being 17%. The shorter alternative showed in Scheme 2 has four steps, starting also with the 17-protected derivative **14** and the yield of the isomer **1** was only 13%, while the yield of the unwanted isomer **13** was 21%.

Although the reaction sequence 1 is more time and material consuming, it was necessary to take this path. The orthogonal protection of individual functional groups allowed us to unambiguously identify and characterize the obtained products, and to prepare a sample of the desired 3α-hydroxyderivative. We found out the best reaction conditions (low temperature, relatively short time, and excess of reagent and solvent) for the key step—the reaction of 3-oxo group with the Grignard reagent. It is important to realize, that the reaction of the diketone **16** with methylmagnesium bromide can theoretically yield two isomeric methyl derivatives for each position 3 and 6, as well as various combinations of 3,6-dimethyl derivatives. More complications arise from the possibility of isomerization in position 5. The conditions typical for Grignard reaction could even potentially cause partial cleavage of the protecting group in position 17. Searching for the desired products in this complex mixture of compounds with similar R_f without any standards was quite difficult.

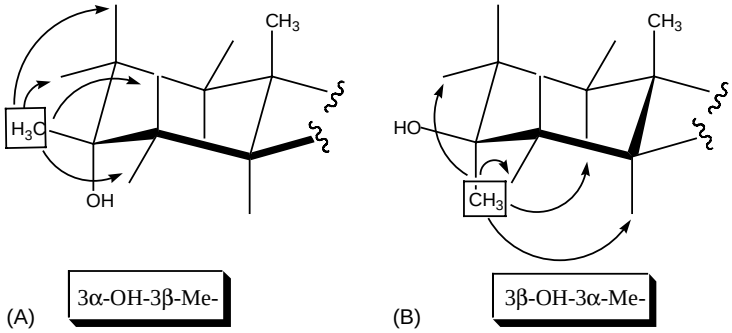


Fig. 1. The NOE-contacts of C(3)-methyl group in 3α-OH-3β-Me- (A) and 3β-OH-3α-Me-derivatives (B).

3.1. Configuration of C(3)-methyl group in the epimeric carbinols

Determination of the configuration at position 3 in epimeric pairs of carbinols (**1**, **13**), (**8**, **9**), and (**11**, **12**) from NMR spectra is not straightforward due to the absence of any proper interproton coupling. To solve this problem we have combined the proton 2D-NOE spectra with in situ TAI-acylation shifts observed in both ^{1}H and ^{13}C NMR spectra. The first necessary step was the structural assignment of proton and carbon signals. It has been achieved using 2D NMR methods—mainly H,H-COSY and H,C-HSQC spectra—together with signal multiplicity and chemical shift arguments. Geminal CH_2 protons at α- and β-side of steroid skeleton were distinguished by NOE contacts with protons of known stereochemistry (mainly 18- and 19-Me-groups). The additional NOE contacts observed between C(3)-methyl protons and axial hydrogen H(1α) and H(5α) in compound **9** indicate an axial α-orientation of C(3)-methyl group (see Fig. 1B). On the other hand C(3)-methyl group in the epimer **8** showed the NOE contacts only to the hydrogens in vicinal positions 2 and 4 in agreement with the situation expected from models (see Fig. 1A). The chemical relation between compounds **8**, **11**, and **1** similarly as between compounds **9**, **12**, and **13** allowed to assign the configuration of C(3)-methyl groups for all studied carbinols. ^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2.

The in situ TAI-acylation [12,13] was used to prepare 3-OTAC derivatives of compounds **8**, **9**, **11**, **12** and 3,17-diOTAC derivatives of diols **1**, **13**. All proton and carbon signals were structurally assigned in their NMR spectra similarly as described above. The observed TAI-acylation shifts of selected protons and carbons in the vicinity of reaction site (at C(3)) are summarized in Table 3. In ^{1}H NMR spectra the TAI-acylation shift of C(3)-Me group is roughly same for whole series but the characteristic differences are observed for geminal protons in position 2 and 4. Compounds **1**, **8**, and **11** show large positive shifts for protons H(2α) ∼0.85 and H(4α) ∼0.65 while their β-oriented partners H(2β) and H(4β) show very small negative shifts (around −0.05). On the other hand in compounds **13**, **9**, and **12** the induced shifts for all protons in vicinal position to OTAC group are approximately the same (+0.40 to +0.50 ppm). These observations are in a good agreement with stereochemical conclusions made from NOEs since for 3α-OH derivatives **1**, **8**, and **11** we should expect larger induced acylation shifts for *gauche*-oriented hydrogens H(2α) and H(4α) than for *trans*-oriented H(2β) and H(4β) (see Fig. 2A). On the contrary for 3β-OH derivatives **13**, **9** and **12** all vicinal protons adopt *gauche*-orientation to OTAC group and therefore show similar induced acylation shifts (see Fig. 2B). In case of diols **1** and **13** the additional TAI-acylation of 17-OH group results in the induced acylation shifts observed for protons on ring D and Me-18 (the largest 1.08 ppm at H(17)). In ^{13}C NMR spectra the largest positive TAI-acylation shifts (around +17 ppm) were observed in whole series at C(3), while for carbons in β-positions (C(2), C(4), and C(3)-Me) significant negative induced shifts (−2.4 to −5.7 ppm) were found. Much

Table 3
TAI-acylation shifts (in ppm) of selected proton and carbon atoms in carbinols **1**, **8**, **9**, **11**–**13**

	3α-OH-3β-Me-derivatives			3β-OH-3α-Me-derivatives		
	1	8	11	13	9	12
Proton						
H-1α	∼0	−0.08	−0.02	0.05	0.04	0.05
H-1β	∼0	∼0	0.11	0.07	0.06	0.05
H-2α	0.91	0.83	0.87	0.40	0.50	0.47
H-2β	−0.03	−0.04	−0.07	0.40	0.43	0.47
3-Me	0.368	0.375	0.362	0.379	0.345	0.374
H-4α	0.65	0.66	0.64	0.40	0.40	0.40
H-4β	0.02	−0.04	0.01	0.40	0.43	0.40
H-5	−0.03	−0.05	−0.06	0.06	0.05	0.05
3H-19	0.044	0.044	0.030	0.054	0.052	0.049
Carbon						
C-1	−0.17	−0.19	−0.18	0.13	−0.62	0.34
C-2	−2.97	−2.81	−2.88	−4.10	−4.65	−4.03
C-3	16.84	16.76	16.88	16.11	16.99	16.23
Me-3	−5.72	−5.39	−5.70	−3.48	−3.54	−3.50
C-4	−2.49	−2.48	−2.42	−3.81	−4.03	−3.73
C-5	−0.50	−0.20	−0.45	−1.00	−0.57	−0.95
C-10	−0.40	−0.34	−0.33	−0.15	−0.07	−0.06
C-19	0.31	0.44	0.31	0.01	0.04	0.03

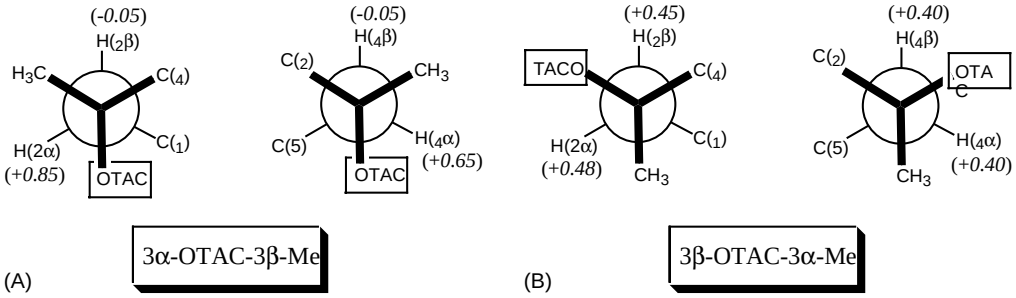


Fig. 2. The Newman projection along C(3)–C(2) and C(3)–C(4) bond in: (A) 3α-OTAC-3β-Me-, and (B) 3β-OTAC-3α-Me-derivatives. The characteristic observed TAI-acylation shifts of hydrogens at positions 2 and 4 are given in parentheses.

smaller induced shifts are observed in γ- and δ-positions. Although some characteristic difference of acylation shifts between both series are obvious (see Table 3) their interpretation in the sense of configuration at C(3) is not straightforward. Diols **1** and **13** show the additional remarkable TAI-acylation shifts for carbons of ring D and 3H(18) (ca +4.25 ppm at C(17), −3.23 ppm at C(16), −0.17 ppm at C(13), and +1.05 ppm at C(18)).

The key step of the reaction sequence is the methylation of 3-ketone with the Grignard reagent. The reaction of Grignard reagents with 3-oxo-5α-steroids yields dominantly 3α-methyl-3β-hydroxy derivatives [1]. A similar analogue 3α-hydroxy-3β-methyl derivative **17** was unfortunately the minor product of the shorter reaction sequence, where a reaction of 3,6-diketone **16** with methylmagnesium bromide yields more of the undesired 3β-hydroxy-3α-methyl derivative **18**.

The synthetic methods described above can be used for the synthesis of 3α-hydroxy-3β-methyl derivative **1** in relatively simple way and reasonable yield. The longer reaction sequence using orthogonal protection of the functional groups in the intermediates, therefore leading to the most straightforward course of all the reaction steps involved in the synthesis, along with NMR spectroscopy, allowed unambiguous identification and structure as well as configuration determination of products formed. The second approach offers a faster solution to synthesize the desired products.

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