SYNTHESIS OF (15*E*)-17β-HYDROXY-5α-ANDROSTANE-3,15-DIONE 15-[*O*-(CARBOXYMETHYL)]OXIME, NEW HAPTEN FOR DIHYDROTESTOSTERONE (17β-HYDROXY-5α-ANDROSTAN-3-ONE)*

Ivan CERNY[1], †Tereza SLAVIKOVA and Vladimir POUZAR[2]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] cerny@uochb.cas.cz, [2]pouzar@uochb.cas.cz

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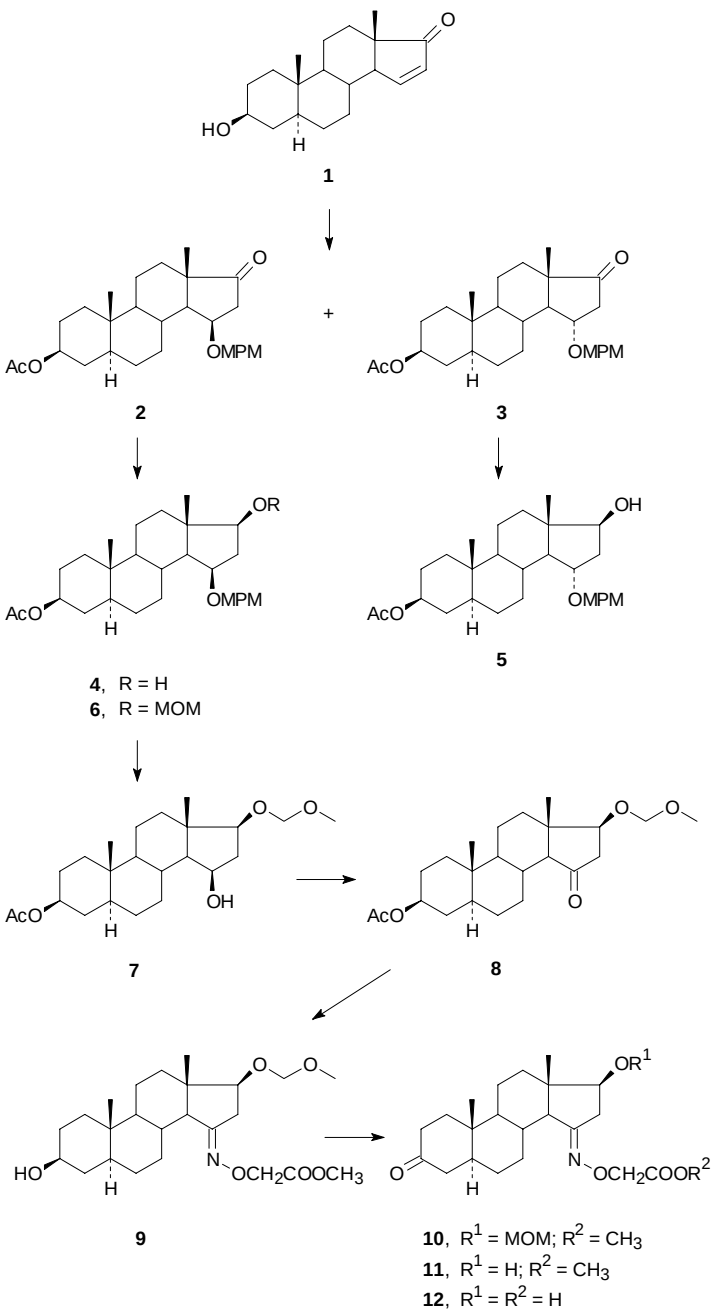
Addition of 4-methoxybenzyl alcohol to 3β-hydroxy-5α-androst-15-en-17-one gave the mixture of isomeric 15-(4-methoxyphenyl)methoxy derivatives from which, after acetylation and chromatography, the major 15β isomer was separated. Borohydride reduction gave 17β-hydroxy derivative which was protected as methoxymethyl ether. Oxidative cleavage of protecting group at position 15 and the subsequent Jones oxidation afforded corresponding 15-ketone. Its oximation with *O*-(carboxymethyl)hydroxylamine, deacetylation and methylation with diazomethane gave protected *O*-(carboxymethyl)oxime derivative with free hydroxy group at position 3. Its oxidation afforded dihydrotestosterone derivative and successive deprotection of position 17 and of carboxy group led to final (15*E*)-17β-hydroxy-5α-androstane-3,15-dione 15-[*O*-(carboxymethyl)]oxime. The title compound was designed as dihydrotestosterone hapten for heterologous radioimmunoassays.
Key words: Steroids; DHT; CMO; Protecting groups; MPM; Hapten.

Continuing our work on 15β-hydroxy derivatives in androstane series[2], we have prepared some haptens with a connecting bridge in position 15 derived from testosterone[3]. We further modified this method for preparation of derivatives with amino group in this position and we prepared[4] 15β-succinamido derivative of dihydrotestosterone (DHT). Preliminary immunological studies revealed good sensitivity of antibodies generated using this hapten but the bridge effect causing irreversible binding was observed. For heterologous immunoassays, we needed other haptens: preparation of one of them is the subject of our contribution.

The synthesis is based on addition of 4-methoxybenzyl alcohol to 3β-hydroxy-5α-androst-15-en-17-one[5] (**1**) (Scheme 1). A mixture of resulting 15-(4-methoxyphenyl)-methoxy (MPM) derivatives was acetylated and the obtained 15β and 15α isomers, **2** and **3**; the former prevailing, was partially separated by column chromatography.

* Part CCCXCI in the series On Steroids; Part CCCXC see ref.[1].


HO
O
H
1
AcO
OMPM
O
H
2
3
OR
OH
4, R = H
6, R = MOM
5
O
OH
7
8
HO
N
OCH2COOCH3
9
OR1
OCH2COOR2
10, R1 = MOM; R2 = CH3
11, R1 = H; R2 = CH3
12, R1 = R2 = H

SCHEME 1

Reduction with sodium borohydride gave in both cases corresponding 17β-hydroxy derivatives, **4** and **5**, differing in configuration of the protected hydroxy group in position 15. The major derivative **4** was converted to 17-methoxymethoxy (MOM) derivative **6**. The free hydroxy group in position 15 was revealed by mild deprotection with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and Jones oxidation of the resulting derivative **7** gave finally the key intermediate, 17β-(methoxymethoxy)-15-oxo-5α-androstan-3β-yl acetate (**8**).

The successive oximation with *O*-(carboxymethyl)hydroxylamine, deacetylation and methylation with diazomethane gave protected oxime **9** with free 3-hydroxy group. The variation of this procedure using deacetylation step before oximation was not successful, compound **8** was unstable toward alkali and partial elimination to the corresponding 16-en-15-one occurred.

Oxidation of compound **9** by Jones reagent led to the dihydrotestosterone series giving ketone **10**. Acid cleavage of MOM group at position 17 afforded methyl ester **11** from which the (15*E*)-17β-hydroxy-5α-androstane-3,15-dione 15-[*O*-(carboxymethyl)]oxime (**12**, 15-CMO DHT) was prepared by hydrolysis with 2 M methanolic sodium hydroxide.

The structure of oxime **12** was confirmed by spectroscopic methods. In its IR spectrum bands corresponding to the presence of carboxylic acid at 3 496 and 1 768 cm^{-1} (monomer), 2 665, 2 565, and 1 732 cm^{-1} (dimer), of ketone at 1 707 cm^{-1}, and of hydroxy group at 3 612 cm^{-1}, were present. Its ^1H NMR spectrum contained characteristic signals of H-17α (δ 3.81 t, J = 8.4 Hz) and of 15-CMO group (δ 4.60 AB-system, J = 19.7). The ^{13}C NMR spectrum, when compared with the spectrum of 17β-hydroxy-5α-androstan-3-one[6], exhibited reasonable substituent shifts (in ppm) corresponding to the presence of 15-CMO substitution: 141.6 at C-15; 4.7 and 3.9 at C-14 and C-16; –4.1 and –2.3 at C-17 and C-8; 1.4 at C-13; other differences are less than 1 ppm. The assignment was made tentatively by the comparison with known data and with the aid of attached proton test experiment. The immunological properties of this new hapten will be reported separately.

EXPERIMENTAL

Melting points were determined on a Boetius micro melting point apparatus (Dresden, Germany). Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter; $[\alpha]_D$ values are given in ° [10^{-1} deg cm^2 g^{-1}]. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer in chloroform. ^1H NMR spectra were taken on a Varian UNITY-200 (200 MHz) spectrometer with tetramethylsilane as an internal standard and ^{13}C NMR spectrum on a Varian UNITY-500 (125.7 MHz) beeing referenced on CDCl3 (77.00); both at 23 °C in deuteriochloroform. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and width of multiplets (W) in Hz. Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biochemicals) and was followed by spraying with concentrated sulfuric acid and heating. Column chromatography was performed on silica gel (60–120 μm, Service Laboratory of the Institute). Prior to evaporation on a

rotary evaporator *in vacuo* (bath temperature 50 °C), solutions in organic solvents were dried over magnesium sulfate. Analytical samples were dried over phosphorus pentoxide at 40 °C/26 Pa for 12 h.

15β-[(4-Methoxyphenyl)methoxy]-17-oxo-5α-androstan-3β-yl Acetate (**2**)

To a suspension of compound **1** (2 g, 6.9 mmol) in 4-methoxybenzyl alcohol (4 ml, 32 mmol) under argon was added 50% oil suspension of sodium hydride (40 mg, 0.8 mmol). The mixture was stirred for 5 h and then left stand overnight. Pyridine (5 ml) and acetic anhydride (20 ml) were added and stirring was continued for 4 h. The mixture was poured on ice and extracted with ethyl acetate (250 ml) in three portions. Combined organic phases were washed with saturated aqueous NaCl and after drying and evaporation, the product was chromatographed on silica gel column (80 g) in petroleum ether–benzene mixture (1 : 1) with 2% of acetone. After elution of 4-methoxybenzyl acetate, the oily main product **2** (1.5 g, 46%) was eluted. The analytical sample was purified by additional chromatography in the same solvent system. MPM derivative **2**, foam, $[\alpha]_D$ –2° (*c* 1.0, chloroform). IR spectrum (chloroform): 1 729 (C=O, acetate and ketone); 1 613, 1 587, 1 515, 1 443, 1 252, 1 168, 827 (arom.); 1 466, 1 302, 1 049 (CH_3O, MPM); 1 367 (CH_3, acetate); 1 264, 1 025 (C–O, acetate); 1 076 (C–O). ^{1}H NMR spectrum: 7.22 m, 2 H, *W* = 17, (H-2′ and H-6′); 6.88 m, 2 H, *W* = 17 (H-3′ and H-5′); 4.69 m, 1 H, *W* = 36, (H-3α); 4.48 and 4.29 AB system, 2 H, *J*(AB) = 11.4 (OCH_2, MPM); 4.12 t, 1 H, *J* = 5.4 (H-15α); 3.81 s, 3 H (OCH_3); 2.64 d, 1 H, *J* = 19.5 (H-16β); 2.29 dd, 1 H, *J* = 19.7, 6.0 (H-16α); 2.03 s, 3 H, (CH_3COO); 1.14 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19). For $C_{29}H_{40}O_5$ (468.7) calculated: 74.33% C, 8.60% H; found: 74.55% C, 8.71% H.

15α-[(4-Methoxyphenyl)methoxy]-17-oxo-5α-androstan-3β-yl Acetate (**3**)

Continuing the chromatography from the above experiment gave 460 mg of a mixture of **2** and more polar isomer **3**. This mixture was rechromatographed in the same solvent system and from more polar fractions, 110 mg (3%) of isomer **3** were isolated as an oil, $[\alpha]_D$ +62° (*c* 1.2, chloroform). IR spectrum (chloroform): 1 730 (C=O, acetate and C=O, ketone); 1 612, 1 587, 1 514, 1 443, 1 253, 1 173, 832 (arom.); 1 465, 1 303 (CH_3O, MPM); 1 367 (CH_3, acetate); 1 264, 1 030 (C–O, acetate); 1 072 (C–O). ^{1}H NMR spectrum: 7.22 m, 2 H, *W* = 17, (H-2′ and H-6′); 6.88 m, 2 H, *W* = 17 (H-3′ and H-5′); 4.68 m, 1 H, *W* = 36, (H-3a); 4.41 AB system, 2 H, *J*(AB) = 11.4 (OCH_2, MPM); 3.99 ddd, 1 H, *J* = 9.8, 7.6, 6.1 (H-15β); 3.80 s, 3 H (OCH_3); 2.89 dd, 1 H, *J* = 19.1, 7.5 (H-16β); 2.09 dd, 1 H, *J* = 19.2, 6.2 (H-16α); 2.01 s, 3 H, (CH_3COO); 0.87 s, 3 H (3 × H-18); 0.85 s, 3 H (3 × H-19). For $C_{29}H_{40}O_5$ (468.7) calculated: 74.33% C, 8.60% H; found: 74.51% C, 8.83% H.

17β-Hydroxy-15β-[(4-methoxyphenyl)methoxy]-5α-androstan-3β-yl Acetate (**4**)

To a solution of **2** (1.0 g, 2.13 mmol) in a mixture of ethyl acetate (5 ml) and methanol (10 ml), stirred and cooled to 10 °C, was added gradually in 5 min sodium borohydride (120 mg, 3.2 mmol). After additional 5 min of stirring at the same temperature, the excess hydride was destroyed by acetic acid (0.3 ml) and water (0.3 ml). The solution was concentrated under reduced pressure, diluted with ethyl acetate, and washed successively with saturated aqueous NaCl, 10% HCl, water, saturated aqueous $KHCO_3$, and water. After drying and evaporation, the product was recrystallized from a minimum amount of ethanol. The yield of **4** was 580 mg (58%), m.p. 169–170 °C, $[\alpha]_D$ –66° (*c* 1.0, chloroform). IR spectrum (chloroform): 3 611, 3 491 (O–H); 1 722 (C=O); 1 613, 1 587, 1 514, 1 442, 1 251, 1 173, 827 (arom.); 1 466, 1 302, 1 046 (OCH_3); 1 367 (CH_3, acetate); 1 263, 1 027 (C–O, acetate); 1 081 (C–O). ^{1}H NMR spectrum: 7.22 m, 2 H, *W* = 17 (H-2′ and H-6′); 6.87 m, 2 H, *W* = 17 (H-3′ and H-5′); 4.68 m, 1 H, *W* = 36 (H-3α); 4.42 and 4.18 AB system, 2 H, *J*(AB) = 11.4 (OCH_2, MPM); 3.80 s, 3 H (OCH_3); 3.76 m, 1 H, *W* = 19 (H-15α); 3.58 m, 1 H, *W* = 25 (H-17α);

2.40 ddd, 1 H, *J* = 14.2, 8.7, 7.0 (H-16α); 2.02 s, 3 H (CH_3COO); 0.98 s, 3 H (3 × H-18); 0.85 s, 3 H (3 × H-19). For $C_{29}H_{42}O_5$ (470.7) calculated: 74.01% C, 8.99% H; found: 73.89% C, 9.01% H.

17β-Hydroxy-15α-[(4-methoxyphenyl)methoxy]-5α-androstan-3β-yl Acetate (**5**)

The mixture after chromatography of **3** (300 mg; see above) was reduced by borohydride in the same manner as for **4**. The resulting mixture of isomers **4** and **5** was chromatographed in a benzene–ether (100 : 1) mixture giving 120 mg of foamy compound **5**, $[\alpha]_D$ +32° (*c* 1.0, chloroform). IR spectrum (chloroform): 3 613, 3 488 (O–H); 1 722 (C=O); 1 612, 1 587, 1 514, 1 443, 1 252, 1 173, 830 (arom.); 1 466, 1 303 (OCH_3); 1 367 (CH_3, acetate); 1 263, 1 030 (C–O, acetate); 1 082 (C–O). ^{1}H NMR spectrum: 7.24 m, 2 H, *W* = 17 (H-2′ and H-6′); 6.85 m, 2 H, *W* = 17 (H-3′ and H-5′); 4.68 m, 1 H, *W* = 36 (H-3α); 4.39 and 4.29 AB system, 2 H, *J*(AB) = 11.1 (OCH_2, MPM); 3.86 dd, 1 H, *J* = 9.2, 6.3 (H-15β); 3.80 s, 3 H (OCH_3); 3.69 dt, 1 H, *J* = 3.3, 8.8 (H-17α); 2.02 s, 3 H (CH_3COO); 0.83 s, 3 H (3 × H-19); 0.78 s, 3 H (3 × H-18). For $C_{29}H_{42}O_5$ (470.7) calculated: 74.01% C, 8.99% H; found: 73.95% C, 9.03% H.

17β-(Methoxymethoxy)-15β-[(4-methoxyphenyl)methoxy]-5α-androstan-3β-yl Acetate (**6**)

Hydroxy derivative **4** (950 mg, 2.0 mmol) was dissolved in dichloromethane (10 ml) and *N*,*N*-diisopropylethylamine (1.5 ml, 10.7 mmol). Chloromethyl methyl ether (0.4 ml, 5.3 mmol) was added dropwise to a stirred solution and the stirring continued at room temperature for 4 h. Then the reaction mixture was poured on ice with saturated aqueous NaCl and extracted with ether (50 ml). The ethereal layer was washed twice with 10% HCl, saturated aqueous $KHCO_3$ and saturated aqueous NaCl. After drying, the solution was filtered with short column of aluminium oxide and concentrated giving 800 mg (77%) of oily **6**. The analytical sample was purified by column chromatography on silica gel in a mixture of petroleum ether–benzene (1 : 1) with 1% of acetone. Oily **6**, $[\alpha]_D$ –50° (*c* 1.5, chloroform). IR spectrum (chloroform): 1 723 (C=O); 1 612, 1 587, 1 514, 1 442, 1 252, 1 173, 830 (arom.); 1 466, 1 302 (CH_3O, MPM); 1 367 (CH_3, acetate); 1 263, 1 029 (C–O, acetate); 1 150, 1 102, 1 045 (C–O, MOM); 1 081 (C–O); 913 (CH_2, MOM). ^{1}H NMR spectrum: 7.22 m, 2 H, *W* = 17 (H-2′ and H-6′); 6.85 m, 2 H, *W* = 17 (H-3′ and H-5′); 4.68 m, 1 H, *W* = 36 (H-3α); 4.64 s, 2 H (OCH_2O); 4.44 and 4.17 AB system, 2 H, *J*(AB) = 11.4 (OCH_2, MPM); 3.80 s, 3 H (OCH_3); 3.76 m, 1 H, *W* = 22 (H-15α); 3.46 t, 1 H, *J* = 8.5 (H-17α); 3.36 s, 3 H (C**H**$_3$OCH$_2$); 2.38 ddd, 1 H, *J* = 13.8, 8.8, 7.2 (H-16α); 2.02 s, 3 H (CH_3COO); 1.02 s, 3 H (3 × H-18); 0.84 s, 3 H (3 × H-19). For $C_{31}H_{46}O_6$ (514.7) calculated: 72.34% C, 9.01% H; found: 72.45% C, 8.97% H.

15β-Hydroxy-17β-(methoxymethoxy)-5α-androstan-3β-yl Acetate (**7**)

MPM derivative **6** (700 mg, 1.36 mmol) was dissolved in dichloromethane (20 ml) and water (6 drops) was added. Then 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (350 mg, 1.54 mmol) was added under stirring. After 20 min the reaction mixture was diluted with chloroform, washed with saturated aqueous $KHCO_3$ (three times) and water, dried, and the solid residue was chromatographed on a silica gel column (100 g) in a benzene–petroleum ether (1 : 1) mixture with 5% of acetone. The yield of hydroxy derivative **7** was 470 mg (88%), m.p. 123–125 °C, $[\alpha]_D$ –31° (*c* 1.1, chloroform). IR spectrum (chloroform): 3 619, 3 500 (O–H); 1 723 (C=O); 1 367 (CH_3, acetate); 1 261, 1 255, 1 029 (C–O, acetate); 1 150, 1 102, 1 046 (C–O, MOM); 1 079 (C–O); 913 (CH_2, MOM). ^{1}H NMR spectrum: 4.69 m, 1 H, *W* = 36 (H-3α); 4.63 s, 2 H (OCH_2O); 4.19 m, 1 H, W = 22 (H-15α); 3.43 t, 1 H, *J* = 8.5 (H-17α); 3.36 s, 3 H (C**H**$_3$OCH$_2$); 2.57 dt, 1 H, *J* = 14.7, 8.3 (H-16α); 2.02 s, 3 H (CH_3COO); 1.04 s, 3 H (3 × H-18); 0.87 s, 3 H (3 × H-19). For $C_{23}H_{38}O_5$ (394.6) calculated: 70.02% C, 9.71% H; found: 70.25% C, 9.80% H.

17β-(Methoxymethoxy)-15-oxo-5α-androstan-3β-yl Acetate (**8**)

A solution of hydroxy derivative **7** (400 mg, 1.0 mmol) in acetone (8 ml) was treated with excess Jones reagent. After 5 min at room temperature, the excess reagent was destroyed with methanol, saturated aqueous $KHCO_3$ was added (10 ml), and acetone was removed under reduced pressure. The mixture was diluted with saturated aqueous $KHCO_3$ and extracted with ethyl acetate. The extract was washed with saturated aqueous $KHCO_3$ (two times) and with water, dried, and evaporated. The residue was crystallized from methanol to yield 329 mg (83%) of ketone **8**, m.p. 145–146 °C, $[\alpha]_D$ +30° (*c* 1.0, chloroform). IR spectrum (chloroform): 1 735 (C=O, ketone); 1 727 (C=O, acetate); 1 367 (CH_3, acetate); 1 261, 1 256, 1 029 (C–O, acetate); 1 151, 1 110, 1 043 (C–O, MOM); 915 (CH_2, MOM). ^{1}H NMR spectrum: 4.68 m, 1 H, *W* = 36 (H-3α); 4.66 s, 2 H (OCH_2O); 3.87 t, 1 H, *J* = 8.1 (H-17α); 3.37 s, 3 H (CH_3OCH_2); 2.62 m, 2 H, *W* = 28 (H-16α and H-7β); 2.10 dd, 1 H, *J* = 18.9, 8.0 (H-16β); 2.02 s, 3 H, (CH_3COO); 0.84 s, 6 H (3 × H-18 and 3 × H-19). For $C_{23}H_{36}O_5$ (392.5) calculated: 70.38% C, 9.24% H; found: 70.32% C, 9.13% H.

(15*E*)-3β-Hydroxy-17β-(methoxymethoxy)-5α-androstan-15-one *O*-[(Methoxycarbonyl)-methyl]oxime (**9**)

Ketone **8** (250 mg, 0.64 mmol) in pyridine (3 ml) was treated with *O*-(carboxymethyl)hydroxylamine hemihydrochloride (177 mg, 1.9 mmol) and the mixture was stirred for 52 h at room temperature. After pouring on ice with 10% hydrochloric acid (30 ml), the product was extracted with ethyl acetate. The extract was washed with saturated aqueous NaCl (three times), dried, and the solvent was evaporated. The product was deacetylated by stirring with tetrahydrofuran (5 ml), methanol (5 ml), and 10% aqueous KOH (3 ml). After 2.5 h, a small piece of solid carbon dioxide was added and the mixture was evaporated to dryness. Saturated aqueous NaCl was added and the mixture was made acidic with 10% H_2SO_4. The product was extracted with ethyl acetate (50 ml in three portions), the extract was dried and evaporated. The residue was dissolved in methanol (1 ml), ether (4 ml) was added, and the mixture was treated with excess diazomethane in ethereal solution (*ca* 6 ml) at 0 °C. After 5 min, the excess reagent was removed with acetic acid and the reaction mixture was evaporated to dryness. The oily residue crystallized after addition of ether, yielding 235 mg (84%) of oxime **9**, m.p. 121–122 °C, $[\alpha]_D$ +9° (*c* 0.9, chloroform). IR spectrum (chloroform): 3 610, 3 487 (O–H); 1 757, 1 741 (C=O, CMO); 1 653 (C=N); 1 439 ($COOCH_3$); 1 147, 1 106, 1 044 (C–O, MOM); 914 (CH_2, MOM); 844, 836 (N–O). ^{1}H NMR spectrum: 4.64 and 4.62 AB system, 2 H, *J* = 6.7 (OCH_2COO); 4.57 s, 2 H (OCH_2O); 3.74 s, 3 H ($COOCH_3$); 3.62 t, 1 H, *J* = 8.4 (H-17α); 3.60 m, 1 H, *W* = 36 (H-3α); 3.35 s, 3 H (CH_3OCH_2); 2.80 dd, 1 H, *J* = 18.6, 8.5 (H-16α); 2.57 dq, *J* = 12.8, 3.1 (H-7β); 2.50 dd, 1 H, *J* = 18.6, 8.5 (H-16β); 0.82 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19). For $C_{24}H_{39}NO_6$ (437.6) calculated: 65.88% C, 8.98% H, 3.20% N; found: 66.01% C, 9.03% H, 3.15% N.

(15*E*)-17β-(Methoxymethoxy)-5α-androstane-3,15-dione 15-{*O*-[(Methoxycarbonyl)methyl]}oxime (**10**)

Jones reagent (*ca* 10 drops) was added to a solution of hydroxy derivative **9** (120 mg, 0.27 mmol) in acetone (1.5 ml). After stirring for 10 min at room temperature, the excess reagent was decomposed with methanol. The mixture was diluted with saturated aqueous $KHCO_3$ (3 ml) and extracted with ethyl acetate (3 × 1 ml). Combined organic phases were washed with saturated aqueous $KHCO_3$ (2 × 1 ml) and water, dried and evaporated, giving 116 mg (97%) of **10**, which was used in the next step without purification. The analytical sample was crystallized from methanol, m.p. 146–147 °C, $[\alpha]_D$ +11° (*c* 1.0, chloroform). IR spectrum (chloroform): 1 757, 1 739 (C=O, CMO); 1 707 (C=O, ketone); 1 659 (C=N); 1 439 ($COOCH_3$); 1 151, 1 103, 1 045 (C–O, MOM); 914 (CH_2, MOM); 849, 840 (N–O). ^{1}H NMR spectrum: 4.64 and 4.62 AB system, 2 H, *J* = 6.7 (OCH_2COO); 4.57 s, 2 H

(OCH_2O); 3.74 s, 3 H ($COOCH_3$); 3.62 t, 1 H, *J* = 8.4 (H-17α); 3.35 s, 3 H (CH_3OCH_2); 2.80 dd, 1 H, *J* = 18.6, 8.5 (H-16α); 2.62 dq, J = 13.1, 3.1 (H-7β); 2.52 dd, 1 H, *J* = 18.5, 7.8 (H-16β); 1.03 s, 3 H (3 × H-19); 0.83 s, 3 H (3 × H-18). For $C_{24}H_{37}NO_6$ (435.6) calculated: 66.18% C, 8.56% H, 3.22% N; found: 66.20% C, 8.64% H, 3.17% N.

(15*E*)-17β-Hydroxy-5α-androstane-3,15-dione 15-{*O*-[(Methoxycarbonyl)methyl]}oxime (**11**)

To a solution of MOM derivative **10** (100 mg, 0.23 mmol) in a mixture of benzene (4 ml) and methanol (6 ml) was added 35% $HClO_4$ (0.2 ml). The mixture was stirred at 50° C for 16 h, then poured into ice cool saturated aqueous $NaHCO_3$ and the product was extracted with ethyl acetate (30 ml) in three portions. The organic layer was washed with saturated aqueous $NaHCO_3$ and water, dried and solvents were evaporated. Column chromatography on silica gel (25 g) in a benzene–acetone (20 : 1) mixture gave 45 mg (50%) of foamy **11**, $[\alpha]_D$ –6° (*c* 1.4, chloroform). IR spectrum (chloroform): 3 613, 3 492 (O–H); 1 756, 1 738 (C=O, CMO); 1 706 (C=O, ketone); 1 662 (C=N); 1 439 ($COOCH_3$); 1 099, 1 059 (C–O); 850, 840 (N–O). ^{1}H NMR spectrum: 4.57 AB system, 2 H, *J* = 16.7 (OCH_2COO); 3.79 dt, 1 H, *J* = 5.1, *J* = 8.9 (H-17α); 3.75 s, 3 H ($COOCH_3$); 2.82 dd, 1 H, *J* = 18.6, 8.5 (H-16α); 2.62 dq, *J* = 13.1, 3.1 (H-7β); 2.46 dd, 1 H, *J* = 18.8, 8.5 (H-16β); 1.04 s, 3 H (3 × H-19); 0.80 s, 3 H (3 × H-19). For $C_{22}H_{33}NO_5$ (391.5) calculated: 67.49% C, 8.50% H, 3.58% N; found: 67.51% C, 8.62% H, 3.48% N.

(15*E*)-17β-Hydroxy-5α-androstane-3,15-dione 15-[*O*-(Carboxymethyl)]oxime (**12**)

Methyl ester **11** (40 mg, 0.10 mmol) was dissolved in 2 M methanolic sodium hydroxide (25 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified with ice-cold 10% H_2SO_4 (2 ml) and the product was extracted with ethyl acetate (2 ml) in three portions. The extract was washed with water (four times) and the solvent was evaporated. The chromatography of the residue on silica gel column (10 ml) in a mixture of chloroform–methanol (20 : 1) with 1% of acetic acid gave 25 mg (65%) of compound **12**, m.p. 155–156 °C (ethyl acetate–ether–hexane), $[\alpha]_D$ –8° (*c* 2.2, chloroform). IR spectrum (chloroform): 3 612 (O–H); 3 496 (COOH, monomer); 2 665, 2 565 (COOH, dimer); 1 768, 1 732 (C=O, CMO); 1 707 (C=O, ketone); 1 662 (C=N); 1 097, 1 058 (C–O); 839 (N–O). ^{1}H NMR spectrum: 4.60 AB system, 2 H, *J* = 19.7 (OCH_2COO); 3.81 t, 1 H, *J* = 8.4 (H-17α); 2.82 dd, 1 H, *J* = 18.8, 8.4 (H-16α); 2.58 dq, *J* = 12.8, 3.4 (H-7β); 1.04 s, 3 H (3 × H-19); 0.80 s, 3 H (3 × H-19). ^{13}C NMR spectrum: 212.1 (C-3), 174.0 (COOH), 165.0 (C-15), 77.6 (C-17), 70.0 (–OCH_2–), 55.6 (C-14), 53.5 (C-9), 46.6 (C-5), 44.5 (C-4), 44.4 (C-13), 38.6 (C-1), 38.1 (C-2), 35.9 (C-10), 35.8 (C-12), 34.4 (C-16), 33.2 (C-8), 31.5 (C-7), 28.6 (C-6), 20.5 (C-11), 11.5 (C-18), 11.4 (C-19). For $C_{21}H_{31}NO_5$ (377.5) calculated: 66.82% C, 8.28% H, 3.71% N; found: 66.76% C, 8.34% H, 3.68% N.

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