SYNTHESIS OF (15*E*)-3β-HYDROXYANDROST-5-ENE-15,17-DIONE 15-(*O*-CARBOXYMETHYL)OXIME; THE NEW HAPTEN FOR 3β-HYDROXYANDROST-5-EN-17-ONE (DEHYDROEPIANDROSTERONE, DHEA)*

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From 17β-hydroxy-15β-[(4-methoxyphenyl)methoxy]androst-5-en-3β-yl acetate (**1**) by acetylation, removal of (4-methoxyphenyl)methyl group, and oxidation with Jones reagent, 15-oxoandrost-5-ene-3β,17β-diyl 3,17-diacetate (**4**) was prepared. (*O*-Carboxymethyl)hydroxylamine treatment and subsequent diazomethane methylation gave methyl ester of corresponding 15-(*O*-carboxymethyl)oxime derivative. Partial acid hydrolysis gave 3-acetate as a minor product, therefore the major 17-acetate was transformed in two steps into the 3-benzoate. Oxidation at position 17 and subsequent deprotection gave for both products final (15*E*)-3β-hydroxyandrost-5-ene-15,17-dione 15-(*O*-carboxymethyl)oxime (**14**), but for 3-acetyl derivative the whole synthesis is shorter and gave higher yield.
Key words: Dehydroepiandrosterone; Hapten; Oxime.

Recently, we described the synthesis[2] of 15-(*O*-carboxymethyl)oxime (CMO) derivative of testosterone, designed as hapten for testosterone immunoassays. Continuing this study, we present now a synthesis of analogous 15-CMO derivative of dehydroepiandrosterone (DHEA, 3β-hydroxyandrost-5-en-17-one). DHEA plays role in androgen hormones metabolism and in last years its connection with various living processes in human organism (obesity, aging, Alzheimer disease, cancer, AIDS, etc.) has been intensively studied[3].

As a starting compound we used 17β-hydroxy-15β-[(4-methoxyphenyl)methoxy]-androst-5-en-3β-yl acetate (**1**), prepared previously[4]. Acetylation and subsequent selective removal[5] of (4-methoxyphenyl)methyl (MPM) group gave derivative **3** with free hydroxyl group at position 15, which was then oxidized into ketone giving 15-oxo derivative **4**. The key intermediate, oxime **5**, was then prepared by the reaction of **4** with (*O*-carboxymethyl)hydroxylamine and by subsequent methylation with diazomethane.

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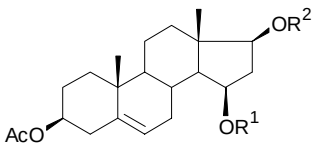
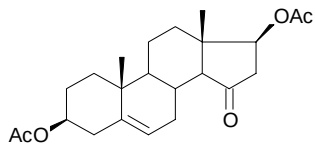
The structure of 15-CMO derivative **5** was documented by the ^{1}H NMR spectrum, which besides two singlets of acetyl groups (δ 2.03 and 2.06) and signals of protected CMO group (δ 3.74 s, and δ 4.55 and 4.58, AB system) contains resolved signals of three C-ring protons. Two doublets of doublets at δ 2.95 (H-16α) and δ 2.53 (H-16β were very similar, with geminal coupling constant amounting 18.9 Hz and about the same coupling constants with H-17α (8.6 and 8.2 Hz), consequently H-17α was observed as a triplet at δ 4.73. The configuration on the double bond C=N of CMO group was assigned to 15*E* due to steric reasons.



	R^1	R^2
1	MPM	H
2	MPM	Ac
3	H	Ac



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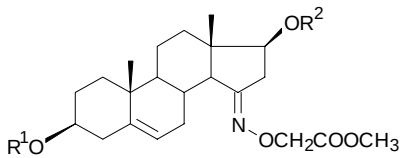
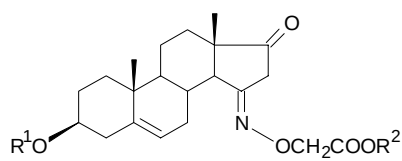


	R^1	R^2
5	Ac	Ac
6	H	Ac
7	Ac	H
8	H	H
9	Bz	Ac
10	Bz	H



	R^1	R^2
11	Ac	CH_3
12	Bz	CH_3
13	H	CH_3
14	H	H

MPM = (4-Methoxyphenyl)methyl; Ac = Acetyl; Bz = Benzoyl

For the further synthetic steps the derivative with protected hydroxyl group at position 3 and with free one at position 17 was needed. Partial hydrolysis of diacetate **5** by hydrochloric acid in methanol gave 33% of 17-acetate **6** and only 19% of desired 3-acetate **7**, besides 16% of diol **8** and 12% of unreacted starting diacetate **5**. The structure of reaction products was confirmed by ^{1}H NMR spectroscopy. For distinguishing among variously acylated compounds, protons H-3α and H-17α were suitable, resonating at δ 3.52 and 4.73 for compound **6**, at δ 4.60 and 3.81 for **7**, and at δ 3.52 and 3.80 for diol **8**, respectively.

The major monoacetylated product **6** was transformed into derivative suitable for further synthesis by benzoylation at position **3** and subsequently by deacetylation of resulted acetate benzoate **9**. The deacetylation[6] was carried out by hydrochloric acid in methanol and gave 3-benzoate **10** in a 72% yield. Its ^{1}H NMR spectrum contains multiplet of H-3α at δ 4.86 and triplet H-17α at δ 3.82.

Oxidation of 17β-hydroxyl group in derivatives **7** and **10** by Jones reagent afforded ketones **11** and **12**, respectively. In the case of acetyl derivative **11**, the acetyl group was hydrolyzed together with methyl ester by sodium carbonate in aqueous methanol to give final CMO derivative **14**. For the removal of benzoate protecting group in **12**, potassium hydroxide in aqueous methanol is necessary and methyl ester is split simultaneously as well. However, in this case the purification was more complicated due to presence of benzoic acid. The raw product was firstly methylated by diazomethane and then the resulting methyl ester **13** was purified by column chromatography. Mild hydrolysis with potassium hydrogen carbonate in aqueous methanol gave CMO derivative **14**.

The overall yields of oxime **14** were 2.2% in the case of the synthesis via acetate **11** (7 steps) and 1.7% via benzoate **12** (10 steps). The structure of oxime **14** was confirmed by IR and ^{1}H NMR spectra. In the IR spectrum bands at 3 500–2 500 cm^{-1} (carboxylic acid), 1 749 cm^{-1} (carboxylic acid, monomer), 1 725 cm^{-1} (ketone and carboxylic acid, dimer), and 3 606 cm^{-1} (hydroxyl), were present. The ^{1}H NMR spectrum displayed characteristic signals of H-6 (δ 5.36 bd, J = 4.5 Hz), H-3α (δ 3.56 m, W = 32 Hz), and of 15-CMO group (δ 4.65 and 4.62, AB-system). The immunological properties of this new hapten will be reported separately.

EXPERIMENTAL

Melting points were determined on a Boetius micro melting point apparatus (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. ^{1}H NMR spectra were taken on a Varian UNITY-200 (200 MHz) spectrometer at 23 °C in deuteriochloroform with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and width of multiplets (W) in Hz. The purity of the products and reaction course were checked by thin-layer chromatography (TLC) performed on silica gel G (ICN Biochemicals) developed in benzene–ether mixture (9 : 1 to 1 : 1) followed by spraying with concentrated sulfuric acid and heating. Column chromatography was performed on silica gel (60–120 μm, Service Laboratories, this 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]androst-5-ene-3β,17β-diyl 3,17-Diacetate (**2**)

The hydroxy derivative[4] **1** (5.0 g, 10.7 mmol) in pyridine (50 ml) was treated with acetic anhydride (40 ml) and then allowed to stand for 18 h at room temperature. The excess reagent was decomposed with ice and water and the product was extracted with ethyl acetate. The extract was sequentially washed with 5% hydrochloric acid, water, 5% sodium hydrogen carbonate solution, dried and the

solvent was removed in vacuo. The oily product was purified by column chromatography over silica gel (300 g) in benzene–ether 3 : 1 to yield 4.6 g (84%) of the oily diacetate **2**, $[\alpha]_D$ –67° (*c* 1.7, chloroform). IR spectrum: 1 727 (C=O); 1 252 (C–O, acetate); 1 033 (C–O). ^{1}H NMR spectrum: 7.20 bd, 2 H, $J \approx 8$ (H-2 and H-6 of 4-$CH_3OC_6H_4CH_2O$); 6.86 bd, 2 H, $J \approx 8$ (H-3 and H-5 of 4-$CH_3OC_6H_4CH_2O$); 5.36 bd, 1 H, $J \approx 4.5$ (H-6); 4.59 m, 1 H, $W = 32$ (H-3α); 4.56 t, 1 H, $J = 8.5$ (H-17α); 4.44 and 4.18 AB-system, 2 H, J(AB) = 11.4 (OCH_2); 4.12 t, 1 H, $J = 5.2$ (H-15α); 3.80 s, 3 H (CH_3O); 2.53, 1 H, $W = 31$ (H-16β); 2.06 s, 3 H (CH_3COO); 2.04 s, 3 H (CH_3COO); 1.04 s, 6 H (3 × H-19 and 3 × H-18). For $C_{31}H_{42}O_6$ (510.7) calculated: 72.91% C, 8.29% H; found: 72.86% C, 8.16% H.

15β-Hydroxyandrost-5-ene-3β,17β-diyl 3,17-Diacetate (**3**)

The derivative **2** (4.5 g, 8.8 mmol) was dissolved in dichloromethane (350 ml) and water (3 ml) was added. Then 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (11.0 g, 58 mmol) was added in one portion under vigorous stirring. After 20 min the reaction mixture was diluted with chloroform, washed with 5% solution of potassium hydrogen carbonate, water, dried, and the solid residue was chromatographed on a silica gel column (280 g) in benzene–ether (20 : 1). Crystallization from methanol afforded 2.76 g (80%) of the hydroxy derivative **3**, m.p. 229–230 °C, $[\alpha]_D$ –82° (*c* 1.9, chloroform). IR spectrum: 3 619, 3 500 (O–H); 1727 (C=O); 1 254 (C–O, acetate); 1 034 (C–O). ^{1}H NMR spectrum: 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 4.60 m, 1 H, $W = 32$ (H-3α); 4.53 t, 1 H, $J = 8.7$ (H-17α); 4.27 m, 1 H, $W = 19$ (H-15α); 2.69, 1 H, $W = 31$ (H-16β); 2.06 s, 3 H (CH_3COO); 2.03 s, 3 H (CH_3COO); 1.06 s, 6 H (3 × H-19 and 3 × H-18). For $C_{23}H_{34}O_5$ (390.5) calculated: 70.74% C, 8.78% H; found: 70.62% C, 8.63% H.

15-Oxoandrost-5-ene-3β,17β-diyl 3,17-Diacetate (**4**)

A solution of hydroxy derivative **3** (1.2 g, 3.1 mmol) in acetone (120 ml) was treated with excess Jones reagent. After 5 min at room temperature the excess reagent was destroyed with methanol, water was added (50 ml), and acetone was distilled off under reduced pressure. The crystalline product was collected by suction, dissolved in ethyl acetate, and the solution was washed with water, dried, and the solvent was distilled off in vacuo. The residue was crystallized from methanol to yield 970 mg (81%) of the ketone **4**, m.p. 184–186 °C, $[\alpha]_D$ –36° (2.6, chloroform). IR spectrum: 1 734 (C=O); 1 251 (C–O, acetate). ^{1}H NMR spectrum: 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 4.96 t, 1 H, $J = 8.4$ (H-17α); 4.60 m, 1 H, $W = 32$ (H-3α); 2.09 s, 3 H (CH_3COO); 2.03 s, 3 H (CH_3COO); 1.03 s, 3 H (3 × H-19); 0.89 s, 3 H (3 × H-18). For $C_{23}H_{32}O_5$ (388.5) calculated: 71.11% C, 8.30% H; found: 70.92% C, 8.19% H.

(15*E*)-15-Oxoandrost-5-ene-3β,17β-diyl 3,17-Diacetate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**5**)

The ketone **4** (8.5 g, 21.8 mmol) in pyridine (80 ml) was treated with (*O*-carboxymethyl)hydroxylamine hemihydrochloride (6.0 g, 65.2 mmol) and the mixture was allowed to stand for 18 h at room temperature. After pouring on ice containing concentrated hydrochloric acid (115 ml), the product was extracted with ethyl acetate. The extract was washed with a saturated sodium chloride solution to neutrality, dried, and the solvent was removed in vacuo. The residue was dissolved in methanol (50 ml) and ether (200 ml) and treated with excess diazomethane in ethereal solution. The excess diazomethane was removed with acetic acid, the reaction mixture was diluted with ethyl acetate, and the solution was washed with 5% sodium hydrogen carbonate. The oily residue after evaporation of the solvents was purified by column chromatography over silica gel in benzene–ether (5 : 1) to yield 7.2 g (69%) of the oily oxime **5**, $[\alpha]_D$ –44° (*c* 1.6, chloroform). IR spectrum: 1 754 (C=O,

$COOCH_3$); 1 730 (C=O, acetate); 1 252 (C–O, acetate); 1 068, 1 047, 1 034 (C–O). ^{1}H NMR spectrum: 5.37 bd, 1 H, $J \approx 4.5$ (H-6); 4.73 t, 1 H, $J = 8.4$ (H-17α); 4.60 m, 1 H, $W = 32$ (H-3α); 4.58 and 4.55 AB-system, 2 H, J(AB) = 16.3 (OCH_2COO); 3.74 s, 3 H ($COOCH_3$); 2.95 dd, 1 H, $J = 18.9$, $J' = 8.6$ (H-16α); 2.53 dd, 1 H, $J = 18.9$, $J' = 8.2$ (H-16β); 2.06 s, 3 H (CH_3COO); 2.03 s, 3 H (CH_3COO); 1.04 s, 3 H (3 × H-19); 0.84 s, 3 H (3 × H-18). For $C_{26}H_{37}NO_7$ (475.6) calculated: 65.66% C, 7.84% H, 2.95% N; found: 65.49% C, 7.76% H, 2.92% N.

(15*E*)-3β-Hydroxy-15-oxoandrost-5-en-17β-yl 17-Acetate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**6**)

The diacetate **5** (10.5 g, 22 mmol) in methanol (200 ml) was treated with 1% methanolic hydrochloric acid (400 ml) and the mixture was allowed to stand at 18 °C for 3 h. After neutralization with 5% sodium hydrogen carbonate solution, methanol was distilled off under reduced pressure. The product was taken into ethyl acetate, the solution was washed with water, dried and the solvent was removed in vacuo. The residue consisted of four products: The lipophilic starting diacetate **5**, the 3-hydroxy derivative **6**, the 17-hydroxy derivative **7** and the diol **8**. It was chromatographed on silica gel (500 g) in benzene–ether (19 : 1). Fractions with the most lipophilic component afforded 1.3 g (12%) of the starting diacetate **5**. Further elution with the same solvent mixture yielded fractions with the monoacetate **6**. Crystallization from methanol gave 3.1 g (33%) of **6**, m.p. 64 °C, $[\alpha]_D$ –33° (*c* 1.7, chloroform). IR spectrum: 3 609 (O–H); 1 754 (C=O, $COOCH_3$); 1 733 (C=O, acetate); 1 254 (C–O, acetate); 1 095, 1 062 (C–O). ^{1}H NMR spectrum: 5.34 bd, 1 H, $J \approx 4.5$ (H-6); 4.73 t, 1 H, $J = 8.5$ (H-17α); 4.58 and 4.54 AB-system, 2 H, J(AB) = 16.4 (OCH_2COO); 3.74 s, 3 H ($COOCH_3$); 3.52 m, 1 H, $W = 32$ (H-3α); 2.94 dd, 1 H, $J = 18.9$, $J' = 8.5$ (H-16α); 2.53 dd, 1 H, $J = 18.9$, $J' = 8.2$ (H-16β); 2.06 s, 3 H (CH_3COO); 1.03 s, 3 H (3 × H-19); 0.84 s, 3 H (3 × H-18). For $C_{24}H_{35}NO_6$ (433.5) calculated: 66.49% C, 8.14% H, 3.23% N; found: 66.38% C, 8.19% H, 3.14% N.

(15*E*)-17β-Hydroxy-15-oxoandrost-5-en-3β-yl 3-Acetate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**7**)

Continuing the chromatography from the foregoing experiment by the elution with the same solvent mixture yielded fractions containing the hydroxy derivative **7**. Evaporation left 1.8 g (19%) of an oil, $[\alpha]_D$ –68° (*c* 1.6, chloroform). IR spectrum: 3 612, 3 501 (O–H); 1 758 (C=O, $COOCH_3$); 1 727 (C=O, acetate); 1 255 (C–O, acetate); 1 031 (O–H). ^{1}H NMR spectrum: 5.37 bd, 1 H, $J \approx 4.5$ (H-6); 4.60 m, 1 H, $W = 32$ (H-3α); 4.59 s, 2 H (OCH_2COO); 3.81 t, 1 H, $J = 8.7$ (H-17α); 3.74 s, 3 H ($COOCH_3$); 2.83 dd, 1 H, $J = 18.9$, $J' = 8.6$ (H-16α); 2.46 dd, 1 H, $J = 18.9$, $J' = 8.6$ (H-16β); 2.03 s, 3 H (CH_3COO); 1.05 s, 3 H (3 × H-19); 0.80 s, 3 H (3 × H-18). For $C_{24}H_{35}NO_6$ (433.5) calculated: 66.49% C, 8.14% H, 3.23% N; found: 66.27% C, 8.10% H, 3.05% N.

(15*E*)-3β,17β-Dihydroxyandrost-5-en-15-one 15-(*O*-Carboxymethyl)oxime Methyl Ester (**8**)

Further elution (see above) with benzene–ether (3 : 1) yielded fractions with the diol **8**. Crystallization from methanol–water (7 : 3) gave 1.4 g (16%) of **8**, m.p. 98–99 °C, $[\alpha]_D$ –59° (*c* 1.3, chloroform). IR spectrum: 3 610, 3 466 (C–O); 1 756 (C=O); 1 092, 1 069 (C–O). ^{1}H NMR spectrum: 5.34 bd, 1 H, $J \approx 4.5$ (H-6); 4.56 s, 2 H (OCH_2COO); 3.80 bt, 1 H, $J \approx 8.5$ (H-17α); 3.74 s, 3 H ($COOCH_3$); 3.52 m, 1 H, $W = 32$ (H-3α); 2.82 dd, 1 H, $J = 18.9$, $J' = 8.5$ (H-16α); 2.46 dd, 1 H, $J = 18.9$, $J' = 8.7$ (H-16β); 1.03 s, 3 H (3 × H-19); 0.80 s, 3 H (3 × H-18). For $C_{22}H_{33}NO_5$ (391.5) calculated: 67.49% C, 8.50% H, 3.58% N; found: 67.31% C, 8.29% H, 3.57% N.

(15*E*)-15-Oxoandrost-5-ene-3β,17β-diyl 17-Acetate 3-Benzoate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**9**)

The hydroxy derivative **6** (1.15 g, 2.67 mmol) was dissolved in pyridine (10 ml) and treated with benzoyl chloride (1.5 ml, 12.9 mmol). After 18 h at room temperature, the excess reagent was decomposed with ice and water, and the crystalline product was collected by suction. The product was dissolved in ethyl acetate, the solution was washed with 5% hydrochloric acid, water, 5% sodium hydrogen carbonate, dried, and the solvent was removed in vacuo. Crystallization from a chloroform–methanol mixture afforded 1.15 g (71%) of the benzoate **9**, m.p. 175–176 °C $[\alpha]_D$ –16° (*c* 1.9, chloroform). IR spectrum: 1 756 (C=O, $COOCH_3$); 1 733 (C=O, acetate); 1 713 (C=O, benzoate); 1 276 (C–O, benzoate); 1 250 (C–O, acetate), 1 034 (C–O). ^{1}H NMR spectrum: 8.04 m, 2 H (H-2 and H-6 of C_6H_5COO); 7.50 m, 3 H (H-3, H-4 and H-5 of C_6H_5COO); 5.42 bd, 1 H, $J \approx 4.5$ (H-6); 4.86 m, 1 H, $W = 32$ (H-3α); 4.74 t, 1 H, $J = 8.4$ (H-17α); 4.59 and 4.54 AB-system, 2 H, J(AB) = 16.5 (OCH_2COO); 3.75 s, 3 H ($COOCH_3$); 2.95 dd, 1 H, $J = 18.8$, $J' = 8.7$ (H-16α); 2.55 dd, 1 H, $J = 18.8$, $J' = 7.8$ (H-16β); 2.07 s, 3 H (CH_3COO); 1.09 s, 3 H (3 × H-19); 0.86 s, 3 H (3 × H-18). For $C_{31}H_{39}NO_7$ (537.7) calculated: 69.25% C, 7.31% H, 2.61% N; found: 69.17% C, 7.14% H, 2.56% N.

(15*E*)-17β-Hydroxy-15-oxoandrost-5-en-3β-yl 3-Benzoate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**10**)

The acetate **9** (1.3 g, 2.4 mmol) was dissolved in chloroform (10 ml) and treated with 1% methanolic hydrochloric acid (10 ml). After 18 h at 45 °C, the reaction mixture was neutralized with 5% sodium hydrogen carbonate solution, solvents were distilled off under reduced pressure, and the product was extracted with ethyl acetate. The solution was washed with water, dried, and the solvent was removed. The residue gave on crystallization from ethanol 850 mg (72%) of the monoester **10**, m.p. 164 °C, $[\alpha]_D$ –35° (*c* 1.4, chloroform). IR spectrum: 3 612 (O–H); 1 756 (C=O, $COOCH_3$); 1 711 (C=O, benzoate); 1 277 (C–O, benzoate); 1 257 (C–O, $COOCH_3$); 1 095, 1 025 (C–O). ^{1}H NMR spectrum: 8.03 m, 2 H (H-2 and H-6 of C_6H_5COO); 7.49 m, 3 H (H-3, H-4 and H-5 of C_6H_5COO); 5.42 bd, 1 H, $J \approx 4.5$ (H-6); 4.86 m, 1 H, $W = 32$ (H-3α); 4.56 s, 2 H (OCH_2COO); 3.82 bt, 1 H, $J \approx 9$ (H-17α); 3.74 s, 3 H ($COOCH_3$); 2.84 dd, 1 H, $J = 18.6$, $J' = 8.6$ (H-16α); 2.48 dd, 1 H, $J = 18.6$, $J' = 9.5$ (H-16β); 1.09 s, 3 H (3 × H-19); 0.81 s, 3 H (3 × H-18). For $C_{29}H_{37}NO_6$ (495.6) calculated: 70.28% C, 7.52% H, 2.83% N; found: 70.16% C, 7.44% H, 2.76% N.

(15*E*)-15,17-Dioxoandrost-5-en-3β-yl 3-Acetate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**11**)

The hydroxy derivative **7** (1.85 g, 4.3 mmol) in acetone (40 ml) was treated with excess Jones reagent. After 10 min at room temperature, methanol (3 ml) was added to destroy the oxidizing agent, the reaction mixture was diluted with water (15 ml), and the solvents were distilled off under reduced pressure. The crystalline product was collected by suction, dissolved in ethyl acetate, and the solution was washed with water, dried, and the solvent was distilled off. Crystallization from methanol gave 1.42 g (77%) of the ketone **11**, m.p. 169–170 °C, $[\alpha]_D$ –53° (*c* 2.1, chloroform). IR spectrum: 1 751 (C=O, $COOCH_3$, ketone); 1 728 (C=O, acetate); 1 254 (C–O, acetate); 1 102, 1 031 (C–O). ^{1}H NMR spectrum: 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 4.64 and 4.58 AB-system, 2 H, J(AB) = 16.2 (OCH_2COO); 4.60 m, 1 H, $W = 32$ (H-3α); 3.75 s, 3 H ($COOCH_3$); 3.59 d, 1 H, $J = 22.3$ (H-16α); 2.78 d, 1 H, $J = 22.3$ (H-16β); 2.03 s, 3 H (CH_3COO); 1.06 s, 3 H (3 × H-19); 0.98 s, 3 H (3 × H-18). For $C_{24}H_{33}NO_6$ (431.5) calculated: 66.80% C, 7.71% H, 3.25% N; found: 66.63% C, 7.56% H, 3.17% N.

(15*E*)-15,17-Dioxoandrost-5-en-3β-yl 3-Benzoate 15-(*O*-Carboxymethyl)oxime Methyl Ester (**12**)

The hydroxy derivative **10** (1.5 g, 3 mmol) was oxidized with Jones reagent in acetone (40 ml) as described in the previous experiment. Similar work-up and crystallization from ethanol afforded 1.03 g (68%) of the ketone **12**, m.p. 187–188 °C, $[\alpha]_D$ –17° (*c* 1.9, chloroform). IR spectrum: 1 752 (C=O, $COOCH_3$); 1 711 (C=O, benzoate and ketone); 1 277 (C–O, benzoate); 1 099, 1 026 (C–O). ^{1}H NMR spectrum: 8.04 m, 2 H (H-2 and H-6 of C_6H_5COO); 7.49 m, 3 H (H-3, H-4 and H-5 of C_6H_5COO); 5.43 bd, 1 H, $J \approx 4.5$ (H-6); 4.86 m, 1 H, $W = 32$ (H-3α); 4.65 and 4.59 AB-system, 2 H, J(AB) = 16.5 (OCH_2COO); 3.75 s, 3 H ($COOCH_3$); 3.61 d, 1 H, $J = 22.3$ (H-16α); 2.80 d, 1 H, $J = 22.3$ (H-16β); 1.11 s, 3 H (3 × H-19); 1.00 s, 3 H (3 × H-18). For $C_{29}H_{35}NO_6$ (493.6) calculated: 70.57% C, 7.15% H, 2.84% N; found: 70.44% C, 7.02% H, 2.80% N.

(15*E*)-3β-Hydroxyandrost-5-ene-15,17-dione 15-(*O*-Carboxymethyl)oxime Methyl Ester (**13**)

The benzoate **12** (2.3 g, 4.7 mmol) in methanol (40 ml) was treated with 5% potassium hydroxide solution in 80% methanol (20 ml) and set aside for 18 h. The alkali was removed with 2% hydrochloric acid and methanol was distilled off in vacuo. The residue was extracted with ethyl acetate, the extract was washed with cold saturated sodium chloride solution to neutrality, dried, and the solvent was distilled off. To remove the benzoic acid the residue was dissolved in methanol (10 ml) and treated with excess diazomethane in ether. After 10 min the excess diazomethane was destroyed with acetic acid, the reaction mixture was diluted with ether, washed with 5% sodium hydrogen carbonate solution, and solvents were removed. The residue was chromatographed over silica gel (100 g) in benzene. After elution of methyl benzoate the product was eluted with benzene–ether (20 : 1). The yield of the oily ester **13** was 1.28 g (70%), $[\alpha]_D$ –39° (*c* 1.2, chloroform). IR spectrum: 3 608, 3 489 (O–H); 1 752 (C=O, $COOCH_3$); 1 706 (C=O, ketone); 1 104, 1 045 (C–O). ^{1}H NMR spectrum: 5.37 bd, 1 H, $J \approx 4.5$ (H-6); 4.64 and 4.58 AB-system, 2 H, J(AB) = 16.4 (OCH_2COO); 3.75 s, 3 H ($COOCH_3$); 3.60 d, 1 H, $J = 22.4$ (H-16α); 3.54 m, 1 H, $W = 32$ (H-3α); 2.79 d, 1 H, $J = 22.4$ (H-16β); 1.05 s, 3 H (3 × H-19); 0.99 s, 3 H (3 × H-18). For $C_{22}H_{31}NO_5$ (389.5) calculated: 67.84% C, 8.02% H, 3.60% N; found: 67.74% C, 7.88% H, 3.55% N.

(15*E*)-3β-Hydroxyandrost-5-ene-15,17-dione 15-(*O*-Carboxymethyl)oxime (**14**)

A) The acetate methyl ester **11** (1.2 g, 2.7 mmol) in methanol (70 ml) was treated with a solution of sodium carbonate (350 mg, 3.3 mmol) in water (15 ml) and allowed to stand under nitrogen for 12 h at room temperature. Methanol was distilled off under reduced pressure and the residue was acidified with 2% hydrochloric acid. The product was extracted with ethyl acetate, the extract was washed with a cold saturated sodium chloride solution to neutrality, dried, and the solvent was removed in vacuo. The yield of the oily oxime **14** was 420 mg (40%), $[\alpha]_D$ –36° (*c* 1.0, chloroform). IR spectrum: 3 606 (O–H); 3 500–2 500 (O–H, acid); 1 749 (C=O, acid monomer); 1 725 (C=O, ketone and acid dimer); 1 045 (C–O). ^{1}H NMR spectrum: 5.36 bd, 1 H, $J \approx 4.5$ (H-6); 4.65 and 4.62 AB-system, 2 H, J(AB) = 17.1 (OCH_2COO); 3.58 d, 1 H, $J = 22.3$ (H-16α); 3.56 m, 1 H, $W = 32$ (H-3α); 2.80 d, 1 H, $J = 22.3$ (H-16β); 1.05 s, 3 H (3 × H-19); 0.98 s, 3 H (3 × H-18). For $C_{21}H_{29}NO_5$ (375.5) calculated: 67.18% C, 7.79% H, 3.73% N; found: 67.06% C, 7.61% H, 3.65% N.

B) A solution of the methyl ester **13** (420 mg, 1.1 mmol) in methanol (40 ml) was treated with a solution of potassium hydrogen carbonate (500 mg) in water (10 ml) and allowed to stand for 7 days at 50 °C. Methanol was removed in vacuo, the residue was acidified with 2% hydrochloric acid, and the product was extracted with ethyl acetate. The extract was washed with a cold saturated sodium chloride solution to neutrality, dried, and the solvent was removed. The yield of the oily oxime **14** was 230 mg (57%) with properties identical in all respects with the product prepared by procedure *A*).

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