PREPARATION OF 7-[*O*-(CARBOXYMETHYL)OXIME] DERIVATIVES OF DEHYDROEPIANDROSTERONE AND PREGNENOLONE*

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Novel simple syntheses of 7-(*O*-carboxymethyloxime) derivatives of 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone) and of 3β-hydroxypregn-5-en-20-one (pregnenolone) are reported. In the first one, 17-oxoandrost-5-en-3β-yl acetate was oxidized to give the 7,17-dione which was then selectively reduced in the position 17. Oximation, reoxidation, and deacetylation yielded the desired oxime. The second synthesis started with (20*R*)-pregn-5-ene-3β,20-diol 3-acetate which was converted to the 20-nitrate, oxidized to the C-7 ketone, oximated, partially deprotected in position 20, reoxidized, and deacetylated. Both the 7-(*O*-carboxymethyloxime) derivatives have been devised as immunoassay components.

Key words: Steroid haptens; NMR spectra; Synthesis.

The cross-reactivity of a particular steroid immunoassay depends on the hapten used either in the antibody generation or as a tracer component. One of the important factors is the position where a spacer, attaching the carrying protein or label, is bound to the steroid molecule. The best results have been achieved with connecting bridge in positions other than those in which characteristic functional groups are located[2]. Thus, for haptens of androgens, gestagens, and their metabolites the positions 7, 11, 15 and 19 of the steroid skeleton are preferred.

One of the commonly used type of connecting bridge has been a substituted oxime derivative, *O*-(carboxymethyl)oxime (CMO), which is formed by reaction of a steroid carbonyl group with *O*-(carboxymethyl)hydroxylamine[3]. However, steroid hormones and their metabolites contain frequently carbonyl group as an hormone binding site and it is necessary not only to introduce another carbonyl group into the steroid molecule but also to protect the original ketone against the reaction with *O*-(carboxymethyl)-hydroxylamine. This goal can be achieved in two ways: (i) by a suitable protection of the original carbonyl group (in the form of ketal) or (ii) by generation of the carbonyl

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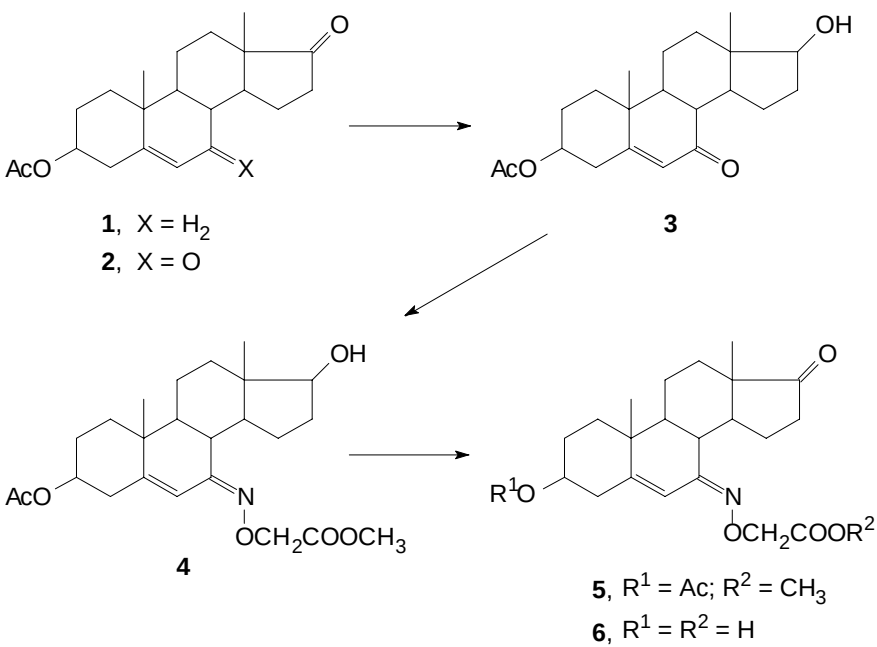
group from a suitable precursor (oxidation of a hydroxyl group) after the attachment of the connecting bridge.

Both these concepts were used in the synthesis of 19-CMO derivatives of dehydroepiandrosterone[4], testosterone[5], progesterone[6] and 17α-hydroxypregnenolone[7]. Concerning the 7-CMO derivatives of steroids, syntheses of dehydroepiandrosterone[8,9] and 16α-hydroxypregnenolone[10] derivatives have already been described. However, for the synthesis of 7-CMO dehydroepiandrosterone **6** only limited experimental data have been published[8,9].

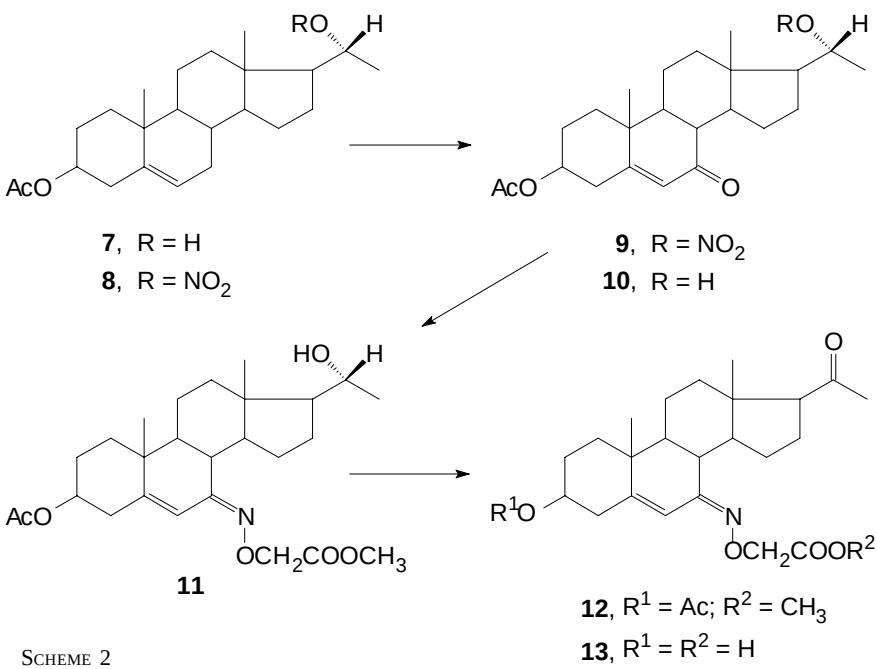
As a part of our program dealing with the preparation of steroid CMO derivatives, we decided to synthesize 7-CMO dehydroepiandrosterone **6** using a new approach starting from 17-oxoandrost-5-en-3β-yl acetate (**1**) (see Scheme 1). This derivative was transformed into 7-keto derivative **2** by oxidation with complex of chromium(VI) oxide and 3,5-dimethylpyrazole in dichloromethane. Different reactivity of carbonyl groups in positions 7 and 17 allowed selective reduction of the 17-keto group with sodium borohydride[11] which gave 17-hydroxy derivative **3**. The proposed structure was confirmed by IR spectrum which contained characteristic bands of hydroxy group (3 615 and 3 486 cm^{-1}) and conjugated ketone (1 668 and 1 633 cm^{-1}). ^1H NMR spectrum exhibited characteristic signals of H-17α (doublet of doublets at δ 3.65; J = 7.6 + 8.8 Hz), of H-6 (a doublet at δ 5.70; J = 1.5 Hz), and of H-3α (δ 4.71; W = 32 Hz).



SCHEME 1

Reaction of ketone **3** with *O*-(carboxymethyl)hydroxylamine and subsequent methylation with diazomethane gave the 7-CMO derivative **4**. The ^{1}H NMR spectrum of **4** contained characteristic signals of the C=N–OCH_2COOCH_3 moiety, a singlet of methyl group at δ 3.75, and an AB system of methylene group at δ 4.56. For steric reasons, only one of the two possible geometrical isomers at the C=N bond, the 7*Z*-isomer, was formed. The *syn*-arrangement of the oxygen of the oxime group and of the H-6 proton was confirmed by the value of the corresponding chemical shift (δ 6.54) which was in accord with data published for the corresponding oxime derivatives[12].

Oxidation of the 17-hydroxyl group in derivative **4** with Jones reagent and subsequent hydrolysis of the protecting groups gave the desired 7-CMO derivative of dehydroepiandrosterone (**6**) in 39% overall yield. Of the two known papers, dealing with preparation of **6**, one gave only the IR spectrum and elemental analysis[8]; the other[9] gave also the ^{1}H NMR data, but the published[9] melting point and optical rotation disagree with the values found by us (cf. Experimental). Our product was characterized by spectral methods (IR, NMR and MS) and elemental analysis, the structure **6** being unequivocally confirmed. Most convincing in this sense was the ^{13}C NMR spectrum of the corresponding methyl ester **5**, which could be compared with that[13] of the parent compound **1**, differing in the neighbourhood of the attached oxime. The corresponding substituent shifts of the carbons C-5 to C-9 are +17.0, –7.9, +121.9, +5.5, and –4.0, respectively, in accord with the substitution on C-7.



SCHEME 2

For the preparation of 7-CMO pregnenolone **13** the trivial selective reduction of 7,20-dioxopregn-5-en-3β-yl acetate with sodium borohydride was not feasible because, according to the ^{1}H NMR spectra, this reaction gave mainly products with reduced carbonyl group in position 7. We succeeded with another procedure, starting with transformation of the 20-hydroxy derivative **7** into the nitrate **8** (see Scheme 2). The subsequent allylic oxidation was again performed with chromium(VI) oxide complex with 3,5-dimethylpyrazole in dichloromethane and the nitrate protecting group was split off with zinc in acetic acid under formation of compound **10**. Its IR spectrum contained characteristic bands of hydroxyl group (3 608 and 3 500 cm^{-1}) and conjugated carbonyl group (1 668 and 1 634 cm^{-1}). ^{1}H NMR spectrum displayed signals of H-20 (doublet of quartets at δ 3.73; *J*(17,20) = 9.5 Hz, *J*(20,21) = 6.1 Hz), H-6 (doublet at δ 5.71; *J* = 1.5 Hz), and H-3α (multiplet at δ 4.72; *W* = 32 Hz).

The obtained 20-hydroxy-7-oxo derivative **10** was transformed into the 7-CMO derivative **11** by reaction with *O*-(carboxymethyl)hydroxylamine and subsequent methylation with diazomethane. As in the case of the androstane derivative **4**, only one isomer (7*Z*) was formed. This was confirmed by the ^{1}H NMR spectrum: a substantial shift of the H-6 proton in *syn*-arrangement with the oxime oxygen atom was observed, compared with the H-6 proton in the 7-oxo derivative (δ 6.54 vs δ 5.72).

Oxidation of compound **11** with Jones reagent and subsequent hydrolysis of the protecting groups gave 7-CMO derivative of pregnenolone **13** in the overall yield of 18% (from derivative **7**).

Both the 7-CMO derivatives **6** and **13** were prepared in satisfactory overall yields and will be used in components for immunoassay kits for dehydroepiandrosterone (DHA) and for pregnenolone. The importance of DHA assays is recently increasing due to the discovery of immunomodulating effects of this compound[14,15]. Pregnenolone assay is necessary for differential diagnosis of congenital adrenal hyperplasia[16].

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. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer in chloroform solution, unless stated otherwise. NMR spectra were taken on UNITY-200 (^{1}H, 200 MHz, FT mode) and UNITY-500 (^{13}C, 125.7 MHz, FT mode) Varian spectrometers at 23 °C in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (*W*) in Hz. For ^{13}C NMR spectrum the number of directly bonded hydrogen atoms was determined from the proton decoupled "attached proton test". Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer (energy of ionizing electrons 70 eV; ion source temperature 170–200 °C). Thin-layer chromatography was performed on silica gel G (ICN Biochemicals), detection by spraying with concentrated sulfuric acid followed by heating. For column chromatography silica gel 60–120 μm was used. Prior to evaporation on a rotary evaporator in vacuo (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

7,17-Dioxoandrost-5-en-3β-yl Acetate (**2**)

To a suspension of chromium(VI) oxide (10.0 g, 100 mmol) in dichloromethane (65 ml) was added at –25 °C 3,5-dimethylpyrazole (10.0 g, 104 mmol). The mixture was stirred at the same temperature for 15 min, then a solution of dehydroepiandrosterone acetate **1** (1.98 g, 6 mmol) in dichloromethane (10 ml) was added dropwise. The reaction mixture was stirred at –20 °C for 4 h, diluted with benzene–ethyl acetate (60 ml, 7 : 3) and filtered through a short column of silica gel (25 g) layered with Celite. The column was washed with the same solvent mixture, and the solvents were evaporated in vacuo. The residue was chromatographed on a column of silica gel (100 g) in benzene–ethyl acetate (95 : 5) yielding 1.33 g (64%) of ketone **2**, m.p. 183–185 °C (methanol), $[\alpha]_D$ –81° (*c* 1.2, chloroform). Literature[17] reports m.p. 185–186 °C, $[\alpha]_D$ –74° (*c* 2.3, ethanol). ^{1}H NMR spectrum: 5.75 d, 1 H, J = 1.5 (H-6); 4.72 m, 1 H, W = 32 (H-3α); 2.04 s, 3 H (CH_3COO); 1.22 s, 3 H (3 × H-19); 0.89 s, 3 H (3 × H-18).

17β-Hydroxy-7-oxoandrost-5-en-3β-yl Acetate (**3**)

Sodium borohydride (80 mg, 2.1 mmol) was added at 0 °C in four portions to a solution of ketone **2** (690 mg, 2.0 mmol) in methanol (6 ml) and ethyl acetate (6 ml). After stirring at 0 °C for 20 min, acetic acid (0.25 ml) was added dropwise and the mixture was diluted with ethyl acetate (200 ml). The solution was washed with water, 5% hydrochloric acid, water, saturated aqueous potassium hydrogen carbonate solution and water. The solvent was evaporated in vacuo and the residue was chromatographed on a column of silica gel (30 g) in benzene–ethyl acetate (9 : 1). Yield of hydroxy derivative **3** was 570 mg (82%), m.p. 167–169 °C (acetone–water), $[\alpha]_D$ –122° (*c* 2.3, chloroform). IR spectrum: 3 615, 3 486 (OH); 1 729 (C=O, acetate); 1 668 (C=O, ketone); 1 633 (C=C); 1 249 (C-O, acetate); 1 036 (C–O). ^{1}H NMR spectrum: 5.70 d, 1 H, J = 1.5 (H-6); 4.71 m, 1 H, W = 32 (H-3α); 3.65 dd, 1 H, J = 7.6, J' = 8.8 (H-17α); 2.04 s, 3 H (CH_3COO); 1.22 s, 3 H (3 × H-19); 0.76 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 286 (100, M – CH_3COOH), 271 (13, M – CH_3COOH – CH_3), 253 (10, M – CH_3COOH – CH_3 – H_2O), 187 (16), 174 (23), 161 (32). For $C_{21}H_{30}O_4$ (346.5) calculated: 72.80% C, 8.93% H; found: 72.65% C, 8.73% H.

(7*Z*)-17β-Hydroxy-7-oxoandrost-5-en-3β-yl Acetate 7-(*O*-Carboxymethyl)oxime Methyl Ester (**4**)

A mixture of ketone **3** (2.00 g, 5.8 mmol), (*O*-carboxymethyl)hydroxylamine hemihydrochloride (1.27 g, 11.8 mmol) and pyridine (17 ml) was heated at 60 °C under stirring for 5 h. Toluene (20 ml) was added and the solvents were evaporated in vacuo. The residue was dissolved in ethyl acetate (150 ml) and 5% hydrochloric acid, the aqueous phase was extracted with ethyl acetate, the combined organic phases were washed with 5% hydrochloric acid, water, and the solvent was evaporated in vacuo. The residue was dissolved in ether (100 ml) and methanol (25 ml) and treated with an ethereal solution of diazomethane at 0 °C for 5 min. The excess diazomethane and the solvents were evaporated in vacuo and the residue was chromatographed on a column of silica gel (200 g) in a benzene–ether mixture (95 : 5). Yield of compound **4** was 2.27 g (91%), m.p. 89–92 °C, $[\alpha]_D$ –185° (*c* 1.7, chloroform). IR spectrum: 3 619, 3 500 (OH); 1 751 (C=O, ester); 1 729 (C=O, acetate); 1 633 (C=C); 1 250 (C–O, acetate); 1 033 (C–O). ^{1}H NMR spectrum: 6.54 bd, 1 H, $J \approx 1.5$ (H-6); 4.67 m, 1 H, W = 32 (H-3α); 4.58 and 4.54, AB system, 2 H, J(A,B) = 16.4 (OCH_2COO); 3.75 s, 3 H ($COOCH_3$); 3.64 dd, 1 H, J = 7.5, J' = 8.5 (H-17α); 2.04 s, 3 H (CH_3COO); 1.13 s, 3 H (3 × H-19); 0.76 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 373 (100, M – CH_3COOH), 284 (66, M – CH_3COOH – OCH_2COOCH_3), 266 (66, M – CH_3COOH – OCH_2COOCH_3 – H_2O). For $C_{24}H_{35}NO_6$ (433.5) calculated: 66.49% C, 8.14% H, 3.23% N; found: 66.72% C, 8.37% H, 3.04% N.

(7*Z*)-7,17-Dioxoandrost-5-en-3β-yl Acetate 7-(*O*-Carboxymethyl)oxime Methyl Ester (**5**)

Jones reagent (2.0 ml) was added to a solution of hydroxy derivative **4** (2.13 g, 4.9 mmol) in acetone (100 ml). After stirring at room temperature for 10 min, the excess reagent was decomposed by methanol (10 ml). The solvents were evaporated in vacuo and the residue was partitioned between ether and water. The aqueous layer was extracted with ether and the combined ethereal phases were washed with water, saturated aqueous potassium carbonate solution and water. The solvent was evaporated and the residue was crystallized from light petroleum–ether. Yield of ketone **5** was 2.08 g (98%), m.p. 113–115 °C, $[\alpha]_D$ –146° (*c* 1.6, chloroform). IR spectrum: 1 751 (C=O, ester and ketone); 1 732 (C=O, acetate); 1 640 (C=C); 1 251 (C–O, acetate). ^{1}H NMR spectrum: 6.59 bd, 1 H, $J \approx 1.5$ (H-6); 4.68 m, 1 H, $W = 32$ (H-3α); 4.60 s, 2 H (OCH_2COO); 3.77 s, 3 H ($COOCH_3$); 2.05 s, 3 H (CH_3COO); 1.14 s, 3 H (3 × H-19); 0.90 s, 3 H (3 × H-18). ^{13}C NMR spectrum: 220.66 (C-17); 170.68 (C=O, acetate); 170.25 (C=0, methyl ester); 156.86 (C-5); 153.39 (C-7); 113.94 (C-6); 72.60 (C-3); 70.55 (OCH_2CO); 51.78 ($COOCH_3$); 49.75 (C-14); 47.84 (C-13); 46.09 (C-9); 38.43 (C-10); 38.02 (C-1); 37.02 (C-8); 36.16 (C-4); 35.47 (C-16); 30.62 (C-12); 27.35 (C-2); 24.88 (C-15); 21.23 (CH_3, acetate); 20.08 (C-11); 17.91 (C-19); 13.90 (C-18). Mass spectrum, *m/z* (%): 371 (100, M – CH_3COOH), 343 (18), 328 (15), 282 (26, M – CH_3COOH – OCH_2COOCH_3), 261 (11), 254 (71), 239 (29). For $C_{24}H_{33}NO_6$ (431.5) calculated: 66.80% C, 7.71% H, 3.25% N; found: 66.48% C, 7.53% H, 2.98% N.

(7*Z*)-3β-Hydroxyandrost-5-ene-7,17-dione 7-(*O*-Carboxymethyl)oxime (**6**)

Methyl ester **5** (1.00 g, 2.3 mmol) was dissolved in a mixture of tetrahydrofuran (23 ml) and methanol (4 ml). After addition of 0.4 M aqueous sodium hydroxide (15 ml) the mixture was stirred at 42 °C for 3 h. The excess alkali was neutralized with 5% hydrochloric acid and the solvents were evaporated in vacuo. The residue was acidified with 5% hydrochloric acid and the product was extracted with dichloromethane (250 ml). The extract was washed with water (3 times) and the solvent was evaporated in vacuo. The residue was crystallized from methanol–water, yielding 729 mg (84%) of acid **6**, m.p. 135–137 °C (decomposition), $[\alpha]_D$ –136° (*c* 0.7, dioxane). Literature[9] gives m.p. 220 °C, $[\alpha]_D$ –286° (*c* 0.7, dioxane). IR spectrum (KBr pellet): 3 376 (OH); 1 741, 1 728 (C=O); 1 660 (C=C); 1 292, 1 088, 1 033 (C–O). ^{1}H NMR spectrum: 6.51 bd, 1 H, $J \approx 1.2$ (H-6); 4.53 s, 2 H (OCH_2COO); 3.53 m, 1 H, $W \approx 32$ (H-3α); 1.08 s, 3 H (3 × H-19); 0.85 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 375 (42, M^+), 347 (31), 332 (13), 300 (39, M – OCH_2COOH), 283 (15), 282 (15, M – OCH_2COOH – H_2O), 272 (94), 258 (55), 255 (47), 240 (76), 191 (35), 187 (29), 178 (31), 174 (45), 160 (100). For $C_{21}H_{39}NO_5$ (375.5) calculated: 67.18% C, 7.79% H, 3.73% N; found: 67.45% C, 7.84% H, 3.98% N.

(20*R*)-Pregn-5-ene-3β,20-diyl 3-Acetate 20-Nitrate (**8**)

Nitric acid (65%, 5.9 ml, 85 mmol) was added dropwise at –25 °C to acetic anhydride (29 ml, 0.32 mol). After stirring at this temperature for 10 min, a solution of hydroxy derivative **7** (4.5 g, 12.5 mmol) in dichloromethane (78 ml) was added during 20 min. The mixture was stirred at –25 °C for 1.5 h and then poured on a mixture of ice (500 g) and concentrated aqueous ammonia (110 ml). The product was taken up in ether, the extract was washed with saturated solution of potassium hydrogen carbonate, water, and the solvent was evaporated in vacuo. The residue was chromatographed on a column of silica gel (98 g) in light petroleum–benzene (1 : 1) yielding 4.12 g (81%) of nitrate **8**, m.p. 178–179 °C (ether–light petroleum), $[\alpha]_D$ –56° (*c* 1.1, chloroform). IR spectrum: 1 726 (C=O, acetate); 1 652 (C=C); 1 621, 1 274 (NO_2); 1 255 (C–O). ^{1}H NMR spectrum: 5.37 d, 1 H, $J = 5.2$ (H-6); 5.04 dq, 1 H, $J(17,20) = 10.6$, $J(20,21) = 6.0$ (H-20); 4.60 m, 1 H, $W = 32$ (H-3α); 2.03 s, 3 H (CH_3COO);

1.32 d, 3 H, *J*(20,21) = 6.1 (3 × H-21); 1.02 s, 3 H (3 × H-19); 0.71 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 345 (100, M – CH_3COOH), 330 (6, M – CH_3COOH – CH_3), 284 (10, M – CH_3COOH – 61), 255 (13, M – CH_3COOH – 90), 159 (13), 145 (16), 133 (15). For $C_{23}H_{35}NO_5$ (405.5) calculated: 68.12% C, 8.70% H, 3.45% N; found: 68.38% C, 8.82% H, 3.42% N.

(20*R*)-7-Oxopregn-5-ene-3β,20-diyl 3-Acetate 20-Nitrate (**9**)

To a suspension of chromium(VI) oxide (10 g, 100 mmol) in dichloromethane (70 ml), 3,5-dimethylpyrazole (10 g, 104 mmol) was added at –25 °C. The mixture was stirred at the same temperature for 20 min, then a solution of nitrate **8** (2.43 g, 6 mmol) in dichloromethane (10 ml) was added dropwise. The reaction mixture was stirred at –20 °C for 4 h, diluted with a benzene–ethyl acetate mixture (60 ml, 7 : 3) and filtered through a short column of silica gel (25 g) layered with Celite. The column was washed with the same solvent mixture and the solvents were evaporated in vacuo. The residue was chromatographed on a column of silica gel (110 g) in benzene–light petroleum–ether (49 : 49 : 2) yielding 1.37 g (54%) of ketone **9**, m.p. 208–210 °C (ether–light petroleum), $[\alpha]_D$ –119° (*c* 1.2, chloroform). IR spectrum: 1 731 (C=O, acetate); 1 669 (C=O, ketone); 1 630 (C=C); 1 621, 1 281 (NO_2); 1 249, 1 036 (C–O). ^{1}H NMR spectrum: 5.72 d, 1 H, *J* = 1.5 (H-6); 5.04 dq, 1 H, *J*(17,20) = 10.4, *J*(20,21) = 6.1 (H-20); 4.72 m, 1 H, *W* = 32 (H-3α); 2.03 s, 3 H (CH_3COO); 1.33 d, 3 H, *J*(20,21) = 5.8 (3 × H-21); 1.21 s, 3 H (3 × H-19); 0.71 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 359 (87, M – CH_3COOH), 298 (16, M – CH_3COOH – 61), 269 (100, M – CH_3COOH – 90), 254 (16). For $C_{23}H_{33}NO_6$ (419.5) calculated: 65.85% C, 7.93% H, 3.34% N; found: 65.64% C, 8.O4% H, 3.47% N.

(20*R*)-20-Hydroxy-7-oxopregn-5-en-3β-yl Acetate (**10**)

Zinc powder (2.75 g, 42 mmol) was added during 30 min to a stirred mixture of nitrate **9** (1.47 g, 3.5 mmol), tetrahydrofuran (84 ml), acetic acid (21 ml) and water (4.2 ml). After further stirring for 1 h, the solid material was removed by filtration through Celite, which was then washed with chloroform. The solution was washed with water, saturated potassium hydrogen carbonate solution, water, and the solvents were evaporated in vacuo. The residue was chromatographed on a column of silica gel (80 g) in benzene–ether (95 : 5) yielding 1.28 g (98%) of ketone **10**, m.p. 156–158 °C (ether–light petroleum), $[\alpha]_D$ –140° (*c* 1.3, chloroform). IR spectrum: 3 608, 3 500 (O–H); 1 730 (C=O, acetate); 1 668 (C=O, ketone); 1 634 (C=C); 1 250, 1 036 (C–O). ^{1}H NMR spectrum: 5.71 d, 1 H, *J* = 1.5 (H-6); 4.72 m, 1 H, *W* = 32 (H-3α); 3.73 dq, 1 H, *J*(17,20) = 9.5, *J*(20,21) = 6.1 (H-20); 2.03 s, 3 H (CH_3COO); 1.23 s, 3 H (3 × H-19); 1.16 d, 3 H, *J*(20,21) = 6.0 (3 × H-21); 0.77 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 374 (8, M^+), 314 (100, M – CH_3COOH), 299 (5, M – CH_3COOH – CH_3), 296 (5, M – CH_3COOH – H_2O), 270 (6), 227 (5), 187 (16), 174 (32), 161 (20). For $C_{23}H_{34}O_4$ (374.5) calculated: 73.76% C, 9.15% H; found: 73.76% C, 9.32% H.

(7*Z*,20*R*)-20-Hydroxy-7-oxopregn-5-en-3β-yl Acetate 7-(*O*-Carboxymethyl)oxime Methyl Ester (**11**)

A mixture of ketone **10** (1.05 g, 2.8 mmol), (*O*-carboxymethyl)hydroxylamine hemihydrochloride (0.61 g, 5.7 mmol) and pyridine (20 ml) was heated under stirring at 60 °C for 5 h. Then (*O*-carboxymethyl)hydroxylamine hemihydrochloride (0.30 g, 2.8 mmol) was added, and the reaction mixture was heated for additional 1 h. Toluene (20 ml) was added and the solvents were evaporated in vacuo. The residue was dissolved in ether and water, the aqueous phase was extracted with ether, the combined organic phases were washed with 5% hydrochloric acid, water, and the solvent was evaporated in vacuo. The residue was dissolved in ether (40 ml) and methanol (20 ml) and treated with ethereal solution of diazomethane for 5 min at 0 °C. The excess diazomethane and the solvents were evaporated in vacuo and the residue was chromatographed on a column of silica gel (50 g) in toluene–acetone

(97 : 3). Yield of methyl ester **11** was 1.23 g (95%), m.p. 159–161 °C (ether), $[\alpha]_D$ –195° (*c* 1.0, chloroform). IR spectrum: 3 608 (O–H); 1 752 (C=O, ester); 1 730 (C=O, acetate); 1 641 (C=N); 1 590 (C=C); 1 252, 1 034 (C–O). ^{1}H NMR spectrum: 6.54 d, 1 H, *J* = 1.2 (H-6); 4.67 m, 1 H, *W* = 32 (H-3α); 4.60 and 4.56, AB system, 2 H, *J*(A,B) = 16.2 (OCH_2COO); 3.76 s, 3 H (CH_3O); 3.73 m, 1 H, *W* ≈ 29 (H-20); 2.04 s, 3 H (CH_3COO); 1.15 d, 3 H, *J*(20,21) ≈ 7 (3 × H-21); 1.13 s, 3 H (3 × H-19); 0.77 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 401 (100, M – CH_3COOH), 356 (2), 312 (33, M – CH_3COOH – OCH_2COOCH_3), 294 (8). For $C_{26}H_{39}NO_6$ (461.6) calculated: 67.65% C, 8.52% H, 3.03% N; found: 67.90% C, 8.69% H, 2.99% N.

(7*Z*)-7,20-Dioxopregn-5-en-3β-yl Acetate 7-(*O*-Carboxymethyl)oxime Methyl Ester (**12**)

Jones reagent (1 ml) was added to a solution of hydroxy derivative **11** (1.02 g, 2.2 mmol) in acetone (50 ml). After stirring at room temperature for 10 min, the excess reagent was decomposed with methanol (5 ml). The solvents were evaporated in vacuo and the residue was partitioned between ether and water. The aqueous phase was extracted with ether, the combined organic phases were washed with water, saturated potassium hydrogen carbonate solution, water, and the solvent was evaporated in vacuo. The residue was crystallized from light petroleum–ether yielding 950 mg (94%) of ketone **12**, m.p. 150–151 °C, $[\alpha]_D$ –164° (*c* 1.2, chloroform). IR spectrum: 1 754 (C=O, ester); 1 731 (C=O, acetate); 1 699 (C=O, ketone); 1 641 (C=N); 1 593 (C=C); 1 252, 1 034 (C–O). ^{1}H NMR spectrum: 6.55 d, 1 H, *J* = 1.2 (H-6); 4.67 m, 1 H, *W* = 32 (H-3α); 4.58 s, 2 H (OCH_2COO); 3.76 s, 3 H ($COOCH_3$); 2.13 s, 3 H (3 × H-21); 2.04 s, 3 H (CH_3COO); 1.12 s, 3 H (3 × H-19); 0.66 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 399 (100, M – CH_3COOH), 370 (4), 356 (5, M – CH_3COOH – $COCH_3$), 342 (6), 310 (84, M – CH_3COOH – OCH_2COOCH_3), 274 (20). For $C_{26}H_{37}NO_6$ (459.6) calculated: 67.95% C, 8.11% H, 3.05% N; found: 67.98% C, 8.28% H, 2.98% N.

(7*Z*)-3β-Hydroxypregn-5-ene-7,20-dione 7-(*O*-Carboxymethyl)oxime (**13**)

Methyl ester **12** (690 mg, 1.5 mmol) was dissolved in a mixture of tetrahydrofuran (15 ml) and methanol (2.5 ml). After addition of 0.4 M aqueous sodium hydroxide (9.2 ml), the mixture was stirred at 42 °C for 3 h. The excess alkali was neutralized with 5% hydrochloric acid and the solvents were evaporated in vacuo. The residue was acidified with 5% hydrochloric acid and the product was extracted with dichloromethane (3 × 300 ml). The collected extracts were washed with water and the solvent was evaporated in vacuo. The residue was crystallized from dichloromethane–methanol–ether yielding 270 mg (47%) of acid **13**, m.p. 201–209 °C (decomposition), $[\alpha]_D$ –177° (*c* 1.1, dioxane). IR spectrum (KBr pellet): 3 368 (O–H); 2 653, 2 631, 2 561, 2 520 (O–H, dimer-COOH); 1 708 (C=O, dimer-COOH); 1 680 (C=O, ketone). ^{1}H NMR spectrum: 6.47 d, 1 H, *J* = 1.2 (H-6); 4.52 s, 2 H (OCH_2COO); 3.53 m, 1 H, *W* = 32 (H-3α); 2.09 s, 3 H (3 × H-21); 1.05 s, 3 H (3 × H-19); 0.60 s, 3 H (3 × H-18). Mass spectrum, *m/z* (%): 403 (38, M^+), 388 (16, M – CH_3), 360 (4, M – $COCH_3$), 347 (5), 328 (59, M – OCH_2COOH), 311 (52), 296 (18), 268 (24), 186 (59), 160 (70), 43 (100). For $C_{23}H_{33}NO_5$ (403.5) calculated: 68.46% C, 8.24% H, 3.47% N; found: 68.32% C, 8.34% H, 3.29% N.

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