Synthesis of (19*E*)-3β,17-dihydroxy-20-oxopregn-5-en-19-al 19-(*O*-carboxymethyl)oxime, new steroidal hapten for 17-hydroxypregnenolone

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*A synthesis of (19E)-3β,17-dihydroxy-20-oxopregn-5-en-19-al 19-(O-carboxymethyl)oxime (**15**), is reported. Hydride reduction of ketone **1** gave the (20R)-hydroxy derivative **2** as the main product. Formylation of **2** followed by cleavage of the epoxide ring and mild Jones oxidation afforded aldehyde **6**. Oximation with (O-carboxymethyl)hydroxylamine and subsequent methylation yielded methyl ester **8** which was selectively hydrolyzed to alcohol **9** and oxidized to ketone **10**. Enolacetylation, epoxidation, and hydrolysis led to the desired 19-(O-carboxymethyl)oxime derivative of 17-hydroxypregnenolone **15**.* (*Steroids* **59**:696–701, 1994)

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Introduction

In our previous studies[1–3] we synthesized a series of steroid haptens of a novel type containing an *O*-(carboxymethyl)oxime (CMO) group in position 19. Placement of this functional group into position 19 leaves free the active groups of the steroid molecule. This fact, together with the relatively good accessibility, makes these compounds very suitable haptens.

In the present communication we describe the preparation of 19-CMO derivative of 17-hydroxypregnenolone **15**. The synthesis of this compound was prompted by the fact that 17-hydroxypregnenolone is an important marker for differential diagnosis[4] of congenital adrenal hyperplasia (CAH) that enables disclosure of a more rare case of CAH due to Δ^5-3β-hydroxysteroid dehydrogenase-isomerase deficiency.

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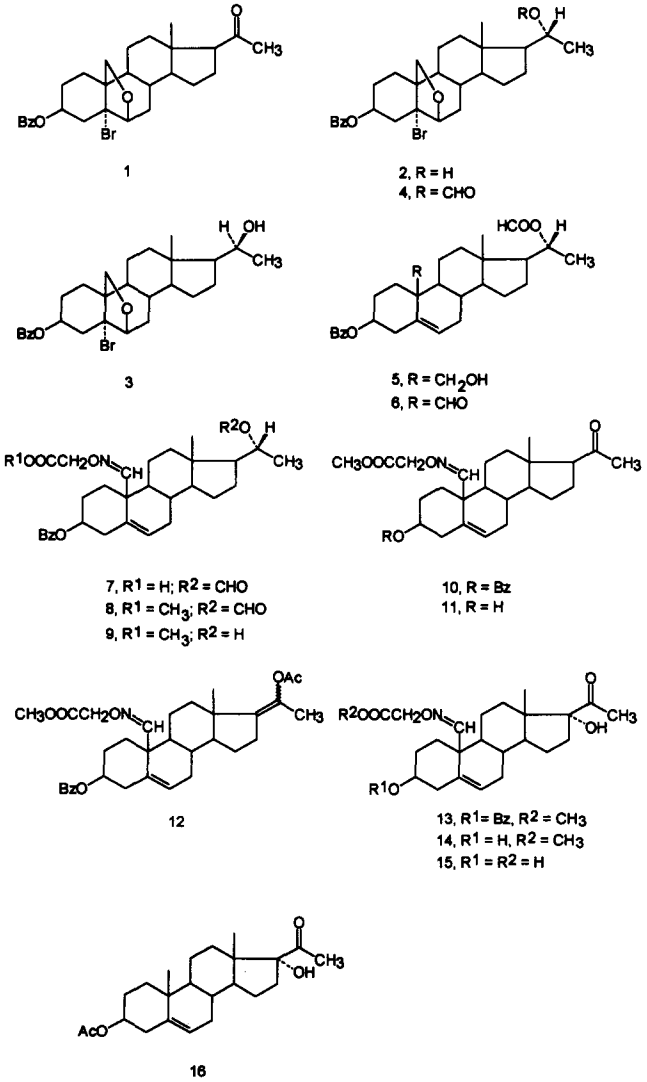
Experimental

Melting points (m.p.) were determined on a micro melting point apparatus Boetius (Germany). Optical rotations were measured at 25°C on a Perkin-Elmer 141 MC polarimeter in chloroform unless stated otherwise. Infrared spectra (wavenumbers in cm^{-1}) were recorded on Bruker IFS 88 spectrometer in chloroform, unless stated otherwise. ^1H NMR spectra were taken on a Varian UNITY-200 (200 MHz) and ^{13}C NMR spectra on a Varian UNITY-500 (125.7 MHz) spectrometers at 23°C in deuteriochloroform with tetramethylsilane as an internal standard unless stated otherwise. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (W) in Hz. The number of directly bonded hydrogen atoms in ^{13}C NMR spectra was determined from the proton decoupled "attached proton test" spectra.[5,6] The purity of the products and reaction course were checked by thin-layer chromatography (TLC) performed on silica gel G (ICN Bioehmicals) 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 lab., Institute of Organic Chemistry & Biochemistry). Tetrahydrofuran was distilled from sodium and stored under argon. Prior to evaporation in vacuo (bath temperature 50°C), solutions in organic solvents were dried over magnesium sulfate.

(20R)-5-Bromo-6β, 19-epoxy-20-hydroxy-5α-pregnan-3β-yl benzoate (2)

The ketone **1** (8.3 g, 16 mmol, Scheme 1)[7] was dissolved in tetrahydrofuran (150 mL) by moderate heating and after cooling to 0°C solid lithium tri-*tert*-butoxyaluminum hydride (15 g, 59 mmol) was added. After 30 min at 5°C the reaction mixture was treated with ice and the excess hydride was decomposed with hydrochloric acid. The steroid was isolated with ethyl acetate. The extract was washed with 5% aqueous hydrochloric acid, water, 5% aqueous sodium hydrogen carbonate, water, solvents were removed under reduced pressure. According to the TLC the residue contained about 15% of a more polar (20*S*)-isomer **3**. It was chromatographed on a silica gel column (280 g) in benzene-ether (20:1). Fractions containing the less polar (20*R*)-isomer **2** yielded after working up and crystallization from methanol 6.2 g (74%) of the alcohol **2**, m.p. 208–209°C, $[\alpha]_D$ −3° (c = 0.8). IR: 3,608 (O—H), 1,714 (C=O), 1,603, 1,586 (Ar), 1,278 (C—O, benzoate). ^{1}H NMR: 8.03 (2H, m, 2-H and 6-H of C_6H_5COO), 7.47 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 5.47 (1H, m, W = 31, 3α-H), 4.10 (1H, d, *J* = 4.3, 6α-H), 4.01 and 3.80 (2H, AB system, *J*(A,B) = 8.2, 19-H_2), 3.73 (1H, m, 20-H), 1.15 (3H, d, *J*(21,20) = 6.2, 21-H_3), 0.80 (3H, s, 18-H_3). Anlaysis calculated for $C_{28}H_{37}BrO_4$ (517.5): C, 64.99; H, 7.21; Br, 15.44. Found: C, 65.23; H, 7.04; Br, 15.13.



Scheme 1

(20S)-5-Bromo-6β, 19-epoxy-20-hydroxy-5α-pregnan-3β-yl benzoate (3)

Further elution with the same solvent mixture gave 0.96 g of a more polar isomer **3** purified by additional recrystallization from methanol. Pure product, 0.76 g (9%) showed m.p. 207–208°C, $[\alpha]_D$ +5° (c = 1.8). IR: 3,616 (O—H), 1,714 (C=O), 1,603, 1,586 (Ar), 1,278 (C—O, benzoate). ^{1}H NMR: 8.03 (2H, m, 2-H and 6-H of C_6H_5COO), 7.49 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 5.47 (1H, m, W = 31, 3α-H), 4.11 (1H, d, *J* = 4.0, 6α-H), 4.01 and 3.78 (2H, AB system, *J*(A,B) = 8.4, 19-H_2), 3.71 (1H, m, 20-H), 1.23 (3H, d, *J*(21,20) = 6.4, 21-H_3), 0.71 (3H, s, 18-H_3). Analysis calculated for $C_{28}H_{37}BrO_4$ (517.5): C, 64.99; H, 7.21; Br, 15.44. Found: C, 65.25; H, 7.28; Br, 15.56.

(20R)-5-Bromo-6β, 19-epoxy-5α-pregnane-3β,20-diyl 3-benzoate 20-formate (4)

The alcohol **2** (27.0 g, 49.5 mmol) in 98% formic acid (250 mL) was treated with *p*-toluenesulfonic acid monohydrate (2.5 g, 13.1 mmol) and allowed to stand at 30°C for 18 h. The reaction mixture was diluted with water (1.5 L) and the product which separated was collected by suction filtration, washed well with water, and dried at 50°C. It was crystallized from chloroform-methanol to yield 20.5 g (72%) of the diester **4**, m.p. 196–198°C, $[\alpha]_D$ +32° (c = 0.9). IR: 1,714 (C=O), 1,603, 1,585 (Ar), 1,278 (C—O, benzoate), 1,195 (C—O, formate). ^{1}H NMR: 8.02 (3H, m, overlapped HCOO and 2-H and 6-H of C_6H_5COO), 7.50 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 5.47 (1H, m, W = 31, 3α-H), 4.92 (1H, dq, *J*(20,17) = 9.6, *J*(20,21) = 6.0, 20-H), 4.10 (1H, d, *J* = 4.3, 6α-H), 4.00 and 3.77 (2H, AB system, *J*(A,B) = 8.5, 19-H_2), 1.21 (3H, d, *J*(21,20) = 6.0, 21-H_3), 0.69 (3H, s, 18-H_3). Analysis calculated for $C_{29}H_{37}BrO_5$ (545.5): C, 63.85; H, 6.84; Br, 14.65. Found: C, 64.04; H, 7.02; Br, 14.35.

(20R)-19-Hydroxypregn-5-ene-3β,20-diyl 3-benzoate 20-formate (5)

The epoxide **4** (20 g, 42.9 mmol) in tert-butanol (500 mL) and water (160 mL) was treated with zinc dust (140 g, 2.14 mol) and the reaction mixture was refluxed under vigorous stirring for 1 h. Zinc was filtered off by suction, washed well with hot ethyl acetate and the filtrate was evaporated to dryness. Crystallization from chloroform-ligroin afforded 10.8 g (63%) of the 5-pregnene derivative **5**. The mother liquors (5.2 g) were purified by column chromatography over silica gel (150 g) in benzene-ether (9:1) to give after crystallization, the additioal 3.2 g (19%) of **5** thus increasing the total yield to 14.0 g (82%), m.p. 233–236°C (chloroform-ligroin), $[\alpha]_D$ +15° (c = 0.9). IR: 3,623 (O—H), 1,714 (C=O, ester), 1,603, 1,585 (Ar), 1,277 (C—O, benzoate), 1,196 (C—O, formate), 1043 (C—O, alcohol). ^{1}H NMR: 8.02 (3H, m, overlapped HCOO and 2-H and 6-H of C_6H_5COO), 7.48 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 5.82 (1H, bd, *J* = 4, 6-H), 4.95 (2H, m, overlapped 3α-H and 20-H), 3.90 and 3.66 (2H, AB system, *J*(A,B) = 11.5, 19-H_2), 1.21 (3H, d, *J*(21,20) = 6.1, 21-H_3), 0.73 (3H, s, 18-H_3). Analysis calculated for $C_{29}H_{38}O_5$ (466.6): C, 74.65; H, 8.21% H. Found: C, 73.68; H, 8.27% H.

(20R)-3β,20-Dihydroxypregn-5-en-19-al 3-benzoate 20-formate (6)

The alcohol **5** (12.5 g, 26.9 mmol) was dissolved in acetone (3 L), cooled to 0°C and treated with excess Jones reagent. After 5 min at +5°C the excess reagent was removed with methanol. Water was added (600 mL) and acetone was distilled off in vacuo. The product was taken into chloroform, the extract was washed with water, dried, and chloroform was distilled off. The residue was treated with benzene and the crystals were collected by suction filtration. The benzene mother liquors were evaporated and the residue was crystallized from chloroform-methanol the overall yield of the 19-aldehyde **6** was 10.7 g (86%); m.p. 175–180°C, $[\alpha]_D$ −147° (c = 1.4). IR: 2,815, 2,711 (C—H, aldehyde), 1,715 (C=O), 1,603, 1,585 (Ar), 1,278 (C—O, benzoate), 1,196 (C—O, formate). ^{1}H NMR: 9.70 s, 1 H (19-H), 8.00 (3H, m, overlapped HCOO and 2-H and 6-H of C_6H_5COO), 7.48 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 5.92 (1H, bd, J = 4, 6-H), 4.92 (2H, m, overlapped 3α-H and 20-H), 1.21 (3H, d, J(21,20) = 6.0, 21-H_3), 0.62 (3H, s, 18-H_3). Analysis calculated for $C_{29}H_{36}O_5$ (464.6): C, 74.97; H, 7.81% H. Found: C, 75.25; H, 8.13% H.

(19E,20R)-3β,20-Dihydroxypregn-5-en-19-al 3-benzoate 20-formate 19-(O-carboxymethyl) oxime (7)

The aldehyde **6** (10.0 g, 18.6 mmol) in pyridine (100 mL) was treated with (*O*-carboxymethyl)hydroxylamine hemihydrochloride (7.0 g, 65.4 mmol) and allowed to stand at room temperature for 20 h. The reaction mixture was poured on ice (400 g) containing concentrated hydrochloric acid (140 mL) and the product was extracted with ethyl acetate. The extract was washed with water, dried, and the solvent was distilled off under reduced pressure. Yield 9.2 g (79%) of acid **7**, m.p. 235–238°C, $[\alpha]_D$ −72° (c = 11.1). IR (KBr pellet): 3,500–2,500 (O—H, COOH), 1,772 (C=O, COOH monomer), 1,716 (C=O, benzoate, formate, COOH dimer), 1,602, 1,585 (Ar), 1,273 (C—O, benzoate), 1,187 (C—O, formate), 1,101, 1,092 (C—O). ^{1}H NMR: 8.02 (3H, m, overlapped HCOO and 2-H and 6-H of C_6H_5COO), 7.48 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.42 (1H, s, 19-H), 5.67 (1H, bd, J = 5, 6-H), 4.97 (1H, dq, J(20,17) = 9.0, J(20,21) = 6.2, 20-H), 4.88 (1H, m, W = 31, 3α-H), 4.64 (2H, s, OCH_2COO), 1.21 (3H, d, J(21,20) = 6.2, 21-H_3), 0.63 (3H, s, 18-H_3). Analysis calculated for $C_{31}H_{39}NO_7$ (537.7): C, 69.25; H, 7.31; N, 2.61. Found: C, 69.20; H, 7.29; N, 2.75.

(19E,20R)-3β,20-Dihydroxypregn-5-en-19-al 3-benzoate 20-formate 19-(O-carboxymethyl)oxime methylester (8)

The acid **7** (12.0 g, 21.8 mmol) was dissolved in methanol (40 mL), diluted with ether (100 mL), and treated with ethereal solution of diazomethane. The excess diazomethane was removed with acetic acid, the solution was washed with a 5% solution of potassium hydrogen carbonate, dried, and solvents were distilled off. The residue was crystallized from chloroform-methanol to give 8.8 g (71%) of the methylester **8**, m.p. 156–159°C, $[\alpha]_D$ −75° (c = 1.4). IR: 1,756 (C=O, $COOCH_3$), 1,714 (C=O, benzoate and formate), 1,603, 1,585 (Ar), 1,278 (C—O, benzoate), 1,258 shoulder, 1,229 (C—O, $COOCH_3$), 1,197 (C—O, formate). ^{1}H NMR: 8.02 (3H, m, overlapped HCOO and 2-H and 6-H of C_6H_5COO), 7.47 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.43 (1H, s, 19-H), 5.67 (1H, bd, J = 5, 6-H), 4.98 (1H, dq, J(20,17) = 9.0, J(20,21) = 6.0, 20-H), 4.89 (1H, m, W = 31, 3α-H), 4.65 (2H, s, OCH_2COO), 3.76 (3H, s, $COOCH_3$), 1.21 (3H, d, J(21,20) = 6.0, 21-H_3), 0.65 (3H, s, 18-H_3). Analysis calculated for $C_{32}H_{41}NO_7$ (551.7): C, 69.67; H, 7.49; N, 2.54. Found: C, 69.52; H, 7.45; N, 2.64.

(19E,20R)-3β,20-Dihydroxypregn-5-en-19-al 3-benzoate 19-(O-carboxymethyl)oxime methylester (9)

The formate **8** (11.0 g, 21 mmol) was dissolved in chloroform (100 mL), treated with a solution of concentrated hydrochloric acid (7.5 mL) in methanol (250 mL), and allowed to stand for 18 h at 35°C. The desired hydrolyzed alcohol **9** was the sole product according to the TLC. The reaction mixture was diluted with ethyl acetate, the solution was washed with water and 5% potassium hydrogen carbonate, dried, and solvent was distilled off in vacuo. Crystallization from ethanol afforded 7.6 g (73%) of the alcohol **9**, m.p. 176–179°C, $[\alpha]_D$ −104° (c = 1.1). IR: 3,609 (O—H), 1,754, 1,740 (C=O, $COOCH_3$), 1,714 (C=O, benzoate), 1,603, 1,585 (Ar), 1,279 (C—O, benzoate), 1,260 shoulder, 1,229 (C—O, $COOCH_3$). ^{1}H NMR: 8.02 (2H, m, 2-H and 6-H of C_6H_5COO), 7.46 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.45 (1H, s, 19-H), 5.68 (1H, bd, J = 5, 6-H), 4.89 (1H, m, W = 32, 3α-H), 4.62 (2H, s, OCH_2COO), 3.77 (3H, s, $COOCH_3$), 3.73 (1H, m, 20-H), 1.14 (3H, d, J(21,20) = 6.0, 21-H_3), 0.76 (3H, s, 18-H_3). Analysis calculated for $C_{31}H_{41}NO_6$ (523.7): C, 71.10; H, 7.89; N, 2.67. Found: C, 71.21; H, 7.91; N, 2.66.

(19E)-3β-Hydroxy-20-oxopregn-5-en-19-al 3-benzoate 19-(O-Carboxymethyl)oxime methylester (10)

A solution of the alcohol **9** (9.0 g, 17.3 mmol) in acetone (500 mL) was treated with excess Jones reagent. After 20 minutes at room temperature the excess oxidizing agent was removed with methanol, water was added (100 mL) and acetone was distilled off under reduced pressure. After cooling the crystals were collected by suction filtration, dried, and recrystallized from chloroform-methanol to give 7.8 g (87%) of the ketone **10**, m.p. 141–142°C, $[\alpha]_D$ −46° (c = 1.5). IR: 1,756, 1,741 (C=O, $COOCH_3$), 1,704 (C=O, benzoate and ketone), 1,603, 1,585 (Ar), 1,278 (C—O, benzoate). ^{1}H NMR: 8.03 (2H, m, 2-H and 6-H of C_6H_5COO), 7.47 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.43 (1H, s, 19-H), 5.68 (1H, bd, J = 5, 6-H), 4.89 (1H, m, W = 32, 3α-H), 4.65 (2H, s, OCH_2COO), 3.75 (3H, s, $COOCH_3$), 2.12 (3H, s, 21-H_3); 0.62 (3H, s, 18-H_3). Analysis calculated for $C_{31}H_{39}NO_6$ (521.7): C, 71.38; H, 7.54; N, 2.69. Found: C, 71.46; H, 7.48; N, 2.47.

(19E)-3β-Hydroxy-20-oxopregn-5-en-19-al 19-(O-carboxymethyl)oxime methylester (11)

Benzoate **10** (52 mg, 0.1 mmol) was dissolved in tetrahydrofuran (1 mL) and methanol (0.1 mL). After addition of 0.4N sodium hydroxide (0.75 mL) the mixture was stirred at 65°C for 6 h. The excess alkali was neutralized with dilute hydrochloric acid (1:4) and the solvents were evaporated in vacuo. The residue was acidified with dilute hydrochloric acid and the product was extracted with ether. The extract was washed with water (3 times) and the solvent was evaporated in vacuo. The residue was dissolved in ether (2 mL) and methanol (3 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 preparative silica gel plate (200 × 200 × 0.3 mm) in benzene-ether (1:1). Yield 33 mg (79%) of hydroxy derivative **11**, m.p. 116–119°C (hexane-ether), $[\alpha]_D$ −78° (c = 1.4). ^{1}H NMR: 7.37 (1H, s, 19-H), 5.59 (1H, bd, J = 4, 6-H), 4.61 (2H, s, OCH_2COO), 3.74 (3H, s, $COOCH_3$), 3.54 (1H, m, W = 31, 3α-H), 2.11 (3H, s, 21-H_3), 0.60 (3H, s, 18-H_3). ^{1}H NMR ($CDCl_3$, after addition of trichloroacetyl isocyanate): 8.36 (1H, s, $CCl_3CONHCOO$), 7.39 (1H, s, 19-H), 5.66 (1H, bd, J = 4, 6-H), 4.73 (1H, m, W = 31, 3α-H), 4.63 (2H, s, OCH_2COO), 3.75 (3H, s, $COOCH_3$), 2.12 (3H, s, 21-H_3), 0.61 (3H, s, 18-H_3). Anlaysis calculated for $C_{24}H_{35}NO_5$ (417.6): C, 69.04; H, 8.45; N, 3.35. Found: C, 69.23; H, 8.35; N, 3.07.

(19E)-3β,17-Dihydroxy-20-oxopregn-5-en-19-al 3-benzoate 19-(O-carboxymethyl)oxime methylester (13)

To a suspension of ketone **10** (1.5 g, 2.87 mmol) in acetic anhydride (30 mL) *p*-toluenesulfonic acid monohydrate (450 mg, 2.37 mmol) was added. During 3 h 20 mL of distillate was collected, the residue was cooled and poured on ice (200 g) and pyridine (1 mL). The separated product was extracted with ether, and the extract was washed with 5% aqueous sodium hydrogen carbonate solution (5 times) and water. Evaporation of solvent afforded crude enol acetate **12** (1.5 g) which was used directly in the next step. ^{1}H NMR: 8.03 (2H, m, 2-H and 6-H of C_6H_5COO), 7.47 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.36 (1H, s, 19-H), 5.69 (1H, bd, J = 5, 6-H), 4.89 (1H, m, W = 31, 3α-H), 4.66 (2H, s, OCH_2COO), 3.76 (3H, s, $COOCH_3$), 2.11 (1.9H, s, CH_3COO), 2.10 (1.1H, s, CH_3COO), 1.89 (1.1H, s, 21-H_3), 1.79 (1.9H, s, 21-H_3), 0.90 (1.1H, s, 18-H_3), 0.84 (1.9H, s, 18-H_3). Enol acetate **12** (1.5 g, 2.7 mmol) in benzene (70 mL) was treated with 3-chloroperoxybenzoic acid (70%, 708 mg, 2.87 mmol). After 1 h stirring at room temperature, the reaction mixture was diluted with ether (200 mL) and washed with aqueous sodium hydrogen carbonate solution (5 times) and water. The solvents were evaporated in vacuo and the residue was dissolved in benzene (30 mL) and methanol (40 mL). After addition of 0.4N aqueous sodium hydroxide (4 mL) the mixture was stirred at room temperature for 2 h. The excess alkali was neutralized with dilute hydrochloric acid (1:4) and the solvents were evaporated in vacuo. The residue was acidified with dilute hydrochloric acid and the product was extracted with ether. The extract was washed with water (3 times) and the solvent was evaporated in vacuo. The residue was dissolved in ether (30 mL) and methanol (5 mL) and treated with ethereal solution of diazomethane for 10 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 (100 g) with benzene-ether (95:5). Yield 450 mg (29%) of hydroxy ketone **13**, m.p. 145–148°C, (dichloromethane-ether), $[\alpha]_D$ −113°C (c = 1.9). IR: 3,606, 3,507 broad (O—H), 1,756, 1,741 shoulder (C=O, $COOCH_3$), 1,709 (C=O, benzoate and ketone), 1,603, 1,585 (Ar), 1,278 (C—O, benzoate). ^{1}H NMR: 8.02 (2H, m, 2-H and 6-H of C_6H_5COO), 7.47 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.44 (1H, s, 19-H), 5.68 bd (1H, J = 5, 6-H), 4.89 (1H, m, W = 31, 3α-H), 4.65 (2H, s, OCH_2COO), 3.76 (3H, s, $COOCH_3$), 2.28 (3H, s, 21-H_3), 0.73 (3H, s, 18-H_3). ^{1}H NMR ($CDCl_3$, after addition of trichloroacetyl isocyanate): 8.49 (1H, s, $CCl_3CONHCOO$), 8.02 (2H, m, 2-H and 6-H of C_6H_5COO), 7.48 (3H, m, 3-H, 4-H, and 5-H of C_6H_5COO), 7.44 (1H, s, 19-H), 5.68 (1H, bd, J = 5, 6-H), 4.89 (1H, m, W = 31, 3α-H), 4.65 (2H, s, OCH_2COO), 3.75 (3H, s, $COOCH_3$), 2.16 (3H, s, 21-H_3), 0.68 (3H, s, 18-H_3). Analysis calculated for $C_{31}H_{39}NO_7$ (537.7): C, 69.25; H, 7.31; N, 2.61. Found: C, 69.13; H, 7.44; N, 2.76.

(19E)-3β,17-Dihydroxy-20-oxopregn-5-en-19-al 19-(O-carboxymethyl)oxime methylester (14)

Benzoate **13** (295 mg, 0.55 mmol) was dissolved in tetrahydrofuran (6 mL) and methanol (0.6 mL). After addition of 0.4N aqueous sodium hydroxide (4.1 mL) the mixture was stirred at 65°C for 6 h. The excess alkali was neutralized with dilute hydrochloric acid (1:4) and the solvents were evaporated in vacuo. The residue was acidified with dilute hydrochloric acid and the product was extracted with ether. The extract was washed with water (3 times) and the solvent was evaporated in vacuo. The residue was dissolved in ether (12 mL) and methanol (18 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 (15 g) in benzene-acetone (9:1). Yield 176 mg (74%) of hydroxy derivative **14**, m.p. 143–145°C (dichloromethane-hexane), $[\alpha]_D$ −153° (c = 1.8). IR: 3,609, 3,502 broad (O—H), 1,754 (C=O, $COOCH_3$), 1,705, 1,692 (C=O, ketone), 1,106, 1,088, 1,050, 1,023 (C—O). ^{1}H NMR: 7.38 (1H, s, 19-H), 5.59 (1H, bd, J = 4, 6-H), 4.61 (2H, s, OCH_2COO), 3.72 (3H, s, $COOCH_3$), 3.54 (1H, m, W = 32, 3α-H), 2.26 (3H, s, 21-H_3), 0.71 (3H, s, 18-H_3). ^{1}H NMR ($CDCl_3$, after addition of trichloroacetyl isocyanate): 8.45 (1H, s, $CCl_3CONHCOO$), 8.39 (1H, s, $CCl_3CONHCOO$), 7.88 (1H, s, 19-H), 5.66 (1H, bd, J = 4, 6-H), 4.71 (1H, m, W = 32, 3α-H), 4.61 (2H, s, OCH_2COO), 3.74 (3H, s, $COOCH_3$), 2.14 (3H, s, 21-H_3), 0.66 (3H, s, 18-H_3). Analysis calculated for $C_{24}H_{35}NO_6$ (433.5): C, 66.49; H, 8.14; N, 3.23. Found: C, 66.31; H, 8.23; N, 3.16.

(19E)-3β,17-Dihydroxy-20-oxopregn-5-en-19-al 19-(O-carboxymethyl)oxime (15)

Methyl ester **14** (125 mg, 0.29 mmol) was dissolved in tetrahydrofuran (3 mL) and methanol (0.3 mL). After addition of 0.4N aqueous sodium hydroxide (1.5 mL) the mixture was stirred at 65°C for 6 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 ethyl acetate. The extract was washed with water (3 times) and the solvent was evaporated in vacuo. The residue was recrystallized from ethyl acetate-hexane mixture. Yield 75 mg (62%) of acid **15**, m.p. 200–203°C, $[\alpha]_D$ −119° (c = 1.2, methanol). IR (KBr pellet): 3,500–2,500 broad (O—H, acid), 3,392 (O—H, hydroxyl), 1,764 shoulder (C=O, COOH monomer), 1,707, 1,697 (C=O, COOH dimmer and ketone). ^{1}H NMR (CD_3SOCD_3): 7.36 (1H, s, 19-H), 5.49 (1H, bd, J = 4, 6-H), 4.60 (2H, s, OCH_2COO), 5.20 (1H, s, OH), 4.59 (1H, bs, OH), 2.08 (3H, s, 21-H_3), 0.44 (3H, s, 18-H_3). Analysis calculated for $C_{23}H_{33}NO_6$ (419.5): C, 65.85; H, 7.93; N, 3.34. Found: C, 65.67; H, 8.08; N, 3.45.

17-Hydroxy-20-oxopregn-5-en-3β-yl Acetate (16)

^{1}H NMR: 5.38 (1H, bd, J = 4, 6-H), 4.60 (1H, m, W = 32, 3α-H), 2.28 (3H, s, 21-H_3), 2.03 (3H, s, CH_3COO), 1.03 (3H, s, 19-H_3), 0.73 (3H, s, 18-H_3). ^{1}H NMR ($CDCl_3$, after addition of trichloroacetyl isocyanate): 8.48 (1H, s, $CCl_3CONHCOO$), 5.37 (1H, bd, J = 4, 6-H), 4.58 (1H, m, W = 31, 3α-H), 2.15 (3H, s, 21-H_3), 2.02 (3H, s, CH_3COO), 1.02 (3H, s, 19-H_3), 0.68 (3H, s, 18-H_3).

Results and discussion

In the synthesis of the title hapten **15** we have applied our experience with the synthesis of 19-CMO progester-

one.[2] The presence of hydroxy group in position 17α represents a complication, and therefore a suitable combination of protecting groups and a proper sequence of steps in building the structural elements of the molecule play a crucial role for the whole synthesis. On the basis of preliminary experiments, bromo epoxide **1**, protected with benzoate group in position 3, was chosen as the starting compound. Reduction of the keto group in position 20 afforded both the isomeric 20-hydroxy derivatives **2** and **3** in a ratio of about 8:1. By way of analogy,[2] we can expect that the main reduction product has the 20*R* configuration. This assumption was confirmed by comparison of chemical shifts of the 18-H_3 and 21-H_3 signals in the ^{1}H NMR spectra. According to the known rules,[8] the configuration of the principal product **2** is 20*R* whereas that of the minor product **3** is 20*S*. Alcohol **2** was converted into 20-formate **4** whose bromo expoxide grouping was reduced with zinc in aqueous 2-methyl-2-propanol to give 19-hydroxy derivative **5**. The reaction sequence **2** → **4** → **5** → **6** → **7** → **8** was analogous to that already employed[2] in the synthesis of 19-CMO derivative of progesterone.

The formate protecting group in position 20 of compound **8** was selectively removed by treatment with hydrochloric acid in a mixture of chloroform and methanol. The obtained 20-hydroxy derivative **9** was oxidized with Jones reagent to give 20-keto derivative **10**. Ketone **10** was also converted into ketone **11**. The hydroxy group was introduced into the position 17α using a method[9] consisting of epoxidation of an enol acetate and subsequent opening of the epoxide ring. Ketone **11** was treated with acetic anhydride and p-toluenesulfonic acid to give enol acetate **12** which, according to the ^{1}H NMR spectrum, was a mixture (about 1.9:1.1) of two isomers differing in configuration at the 17,20-double bond. Epoxidation of this mixture of enol acetates with one equivalent of 3-chloroperoxybenzoic acid afforded a complex mixture of products which was immediately hydrolyzed with 0.4NNaOH in a mixture of methanol and benzene. The obtained product mixture was methylated with diazomethane and the desired 17α-hydroxy derivative **13** was isolated as the only pure product in 29% yield.

The structure of compound **13** was confirmed by spectral methods. Its IR spectrum exhibited hydroxyl bands, in the ^{1}H NMR spectrum we found signals characteristic of a benzoate group, a 19-CMO derivative of configuration 19E,[1] a 5,6-double bond, and a 20-keto group. Upon addition of trichloroacetyl isocyanate (TAI), the spectrum of the obtained trichloroacetyl carbamate (TAC) displayed a singlet at 8.49 ppm, characteristic of a $Cl_3CONHCOO$ grouping.[10] The differences between chemical shifts in the spectra of hydroxy compound **13** and its TAC derivative (for 18-H_3: −0.05, for 21-H_3: −0.12) correspond to those found for 17-hydroxy-20-oxopregn-5-en-3β-yl acetate (**16**) as a model compound. The structure of compound **13** has also been confirmed by its ^{13}C NMR spectrum and by spectrum of its TAC derivative, shown in Table 1. Table 1 also contains spectra of the model compound **16** and its TAC derivative. Characteristic for both compounds is a strong acylation shift of the C-17 signal and an identical effect on the chemical shifts of carbon atoms in the vicinity of position 17.

Table 1 ^{13}C NMR spectra of 17α-hydroxy compounds **13**, **14**, and **16**.

Position	13[a]	13 + TAI[b]	dif.[c]	14[d]	14 + TAI[e]	dif.	16[f]	16 + TAI[g]	dif.
1	31.55	31.38	−0.17	33.51[h]	30.46[i]	−3.05	36.97[i]	36.85	−0.12
2	28.37	28.35	−0.03	29.92	28.09	−1.83	27.71	27.67	−0.04
3	73.87	73.81	−0.06	71.19	76.96	5.77	73.82	73.75	−0.08
4	38.65	38.63	−0.03	42.70	38.30	−4.40	38.06[j]	38.00	−0.06
5	134.68	134.81	0.13	135.73	134.04	−1.69	139.63	139.71	0.09
6	125.58	125.28	−0.31	124.42	125.98	1.55	122.32	122.05	−0.27
7	33.17[k]	33.11	−0.05	32.16	31.33	−0.83	31.87	31.70	−0.17
8	32.10	32.27	0.17	32.11	32.21	0.09	31.79	31.98	0.19
9	50.31	51.22	0.91	50.32	51.15	0.83	50.70[l]	51.59[m]	0.88
10	43.67	43.61	−0.06	43.51	43.40	−0.11	36.59	36.56	−0.03
11	21.39	21.50	0.11	21.41	21.47	0.07	20.45	20.57	0.12
12	29.93	30.51[n]	0.58	31.51	32.81	1.30	30.05	30.54	0.49
13	48.18	47.45	−0.73	48.21	47.41	−0.79	48.21	47.53	−0.68
14	49.33	48.81	−0.52	49.34	48.71	−0.63	49.57[l]	49.02[m]	−0.55
15	23.98	23.90	−0.08	24.00	23.87	−0.13	24.13	24.07	−0.06
16	33.53[k]	30.82[n]	−2.71	33.44[h]	30.76[l]	−2.68	33.58	30.90	−2.69
17	89.84	100.38	10.54	89.82	100.35	10.53	90.05	100.54	10.49
18	15.31	14.29	−1.03	15.33	14.26	−1.07	15.31	14.33	−0.98
19	155.43	155.26	−0.16	155.54	154.80	−0.75	19.30	19.23	−0.07
20	211.64	202.67	−8.97	211.72	202.62	−9.09	211.67	202.77	−8.90
21	27.91	26.74	−1.17	27.93	26.71	−1.22	27.87	26.70	−1.17

[a] Other signals: 51.82, 70.41, 170.46 (OCH_2COOCH_3); 128.25, 129.50, 130.65, 132.77, 165.95 (C_6H_5COO). [b] +TAI = spectrum after addition of trichloroacetyl isocyanate (e.g. corresponding trichloroacetylcarbamate (TAC)). [b] Other signals: 51.77, 70.36, 170.38 (OCH_2COOCH_3); 92.27, 150.14 and 157.46 ($CCl_3CONHCOO$); 128.24, 129.48, 130.61, 132.77, 165.89 (C_6H_5COO). [c] dif. = differentiation of chemical shifts of TAC derivative and parent compound. [d] Other signals: 51.85, 70.30, 170.52 (OCH_2COOCH_3). [e] Other signals: 51.80, 70.26, 170.35 (OCH_2COOCH_3); 91.73, 92.27, 148.98, 150.21, 157.45, 157.59 (2 × CCl_3 CONHCOO). [f] Other signals: 21.40, 170.52 (CH_3COO). [g] Other signals 21.32, 170.43 (CH_3COO); 92.27, 150.08, 157.42 ($CCl_3CONHCOO$). [h-n] Signals with the same symbols can be mutually interchanged. For other conditions see Experimental.

Compound **13** was converted into dihydroxy derivative **14** by alkaline hydrolysis and subsequent methylation with diazomethane. The structure of compound **14** was again confirmed by its ^{1}H and ^{13}C NMR spectra (Table 1). The desired hapten **15**, i.e., 19-CMO derivative of 17-hydroxypregnenolone, was obtained by alkaline hydrolysis of compound **14**.

The antigenic properties of antibodies of this new hapten will be reported elsewhere.

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