SYNTHESIS OF (19*E*)-3,20-DIOXOPREGN-4-EN-19-AL 19-(O-CARBOXYMETHYL)OXIME*

Jan FAJKOŠ[a], Vladimír POUZAR[a] and Karel VEREŠ[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

[b] *Institute of Nuclear Biology and Radiochemistry, Czechoslovak Academy of Sciences, 142 20 Prague 4*

Received September 6, 1990
Accepted October 2, 1990

Dedicated to the memory of Professor František Šorm.

Hydride reduction of the ketone *I* gave the (20*R*)-hydroxy derivative *II*. Cleavage of the diacetate *III* with zinc dust, followed by mild Jones' oxidation led to the aldehyde *V*. Oximation with (O-carboxymethyl)hydroxylamine hemihydrochloride and methylation with diazomethane afforded the ester *VI*. Alkaline hydrolysis to the acid *VII*, followed by methylation yielded the diol methylester *VIII* which on Jones' oxidation and acid catalyzed rearrangement afforded the dione *IX*. Mild hydrolysis gave the desired hapten *X* derived from progesterone.

In our recent paper[1] we described the synthetic approach to a new type of immunogens carrying the O-(carboxymethyl)oxime (CMO) grouping at C(19) and reported on the synthesis of the C(19)-CMO derivative of testosterone. The desired low crossreactivity with 5α-dihydrotestosterone which this immunogen exhibited[2] prompted us to extend our interest to other derivatives of this group. In this paper we present the synthesis of such hapten *X* derived from progesterone.

The synthesis started with the known[3] ketone *I*. Reduction of the 20-oxo group with lithium tri-tert-butoxyaluminium hydride in tetrahydrofuran afforded the (20*R*)-hydroxy derivative *II* which was transformed to the diacetate *III*. Cleavage of the 6β,19-epoxide ring was performed with zinc dust in a tert-butanol–water mixture (3 : 2) to yield the 19-hydroxy derivative *IV*. Synthesis of this compound by different route was described in the literature[4].

The title compound *X* was synthesized by the reaction sequence *IV* → *V* → *VI* → → *VII* → *VIII* → *IX* → *X* which is essentially analogous to the described synthesis of the C(19)-CMO derivative of testosterone[1]. Oxidation of alcohol *IV* with Jones' reagent at +5°C gave the aldehyde *V* which on reaction with (O-carboxymethyl)-

* Part CCCLVI in the series On Steroids; Part CCCLV: Collect. Czech. Chem. Commun. *56*, 1070 (1991).

hydroxylamine hemihydrochloride in pyridine, followed by methylation with diazomethane afforded the oxime ester *VI*. ^{1}H NMR spectrum confirms this structure without any doubt: the corresponding signals prove safely the presence of the CH= =N—OCH_2COOCH_3 grouping at C(10) (see Table I), and the *E* configuration of the oxime moiety follows from the chemical shift of H-19 (cf. ref.[5]). Alkaline hydrolysis of compound *VI* followed by esterification with diazomethane gave the diol *VIII* which on Jones' oxidation and acid catalyzed rearrangement yielded the dione *IX*. Its structure follows from the ^{1}H NMR spectrum (see Table I) as well as from the IR spectrum: bands at 1 681 and 1 623 cm^{-1} are characteristic for the *s-trans* conjugated system C=C—C=O. Additional confirmation for this structure was provided by the mass spectrum: the loss of the OCH_2COOCH_3 fragment (*m*/*z* 89) from the molecular ion (compounds *VI* a *IX*), or from the molecular ion formed after loss of water molecule (compound *VIII*), this being typical for compounds containing the CH=N—OCH_2COOCH_3 grouping[1]. Mild alkaline hydrolysis then led to the desired C(19)-CMO derivative of progesterone *X*. ^{1}H NMR

TABLE I

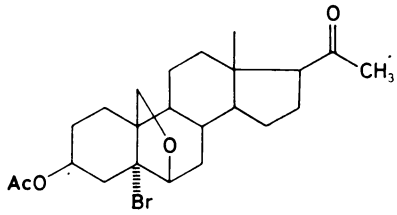
^{1}H NMR spectral parameters of 19-substituted pregnane derivatives[a]

Compound	H-18 (3 H)	H-19 (1 H)	H-3α (1 H)	H-6 (1 H)	H-20 (1 H)	H-21 (3 H)	OCH_2CO (2 H)	$COOCH_3$ (3 H)
II[b]	0·79 s	3·90[c]	5·20 m[d]	4·06 d[e]	[f]	1·14 d[g]	—	—
III[h]	0·67 s	3·83[i]	5·20 m[d]	4·06 d[e]	4·84 dq[j]	1·15 d[k]	—	—
IV[h]	0·70 s	3·71 m	4·65 m[d]	5·76 bd[e]	4·84 dq[j]	1·15 d[k]	—	—
V[l]	0·59 s	9·66 d[m]	4·60 m[d]	5·88 bd[n]	4·82 dq[j]	1·15 d[k]	—	—
VI[o]	0·62 s	7·38 s	[p]	5·62 bd[q]	4·82 dq[j]	1·15 d[k]	4·63 s	3·75 s
VII[r]	0·64 s	7·35 s	[s]	5·48 bd	[s]	0·99 d[t]	4·48 s	—
VIII	0·74 s	7·40 s	[u]	5·60 bd	[u]	1·14 d[g]	4·63 s	3·76 s
IX[v]	0·66 s	7·65 s	—	[s]	—	2·12 s	4·60 s	3·73 s
X[r,x]	0·54 s	7·77 s	—	[s]	—	2·06 s	4·51 s	—

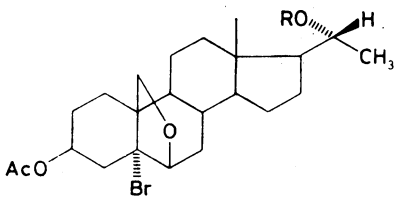
[a] Measured on Tesla BS-476 instrument (100 MHz, FT mode) in deuterochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling contants (*J*) and width (*W*) in Hz. All values are obtained by first order analysis. [b] Other signal: 2·03 s, 3 H (CH_3COO). [c] AB system, 2 H, Δ(A, B) = 17·8, *J* = 8·5. [d] *W* ≈ 32. [e] *J* = 4. [f] Overlapped with signal of H-19. [g] *J*(20, 21) = 6·1. [h] Other signals: 2·01 s and 2·03 s, 2 × 3 H (2 × CH_3COO). [i] AB system, 2 H, Δ(A, B) = 19·5, *J* = 8·5. [j] *J*(17, 20) = 9·9; *J*(20, 21) = 6·2. [k] *J*(20, 21) = 6·2. [l] Other signal: 2·00 s, 6 H (2 × CH_3COO). [m] *J* = 1·4. [n] *J* = 5·5. [o] Other signals: 2·00 s and 2·01 s, 2 × 3 H (2 × CH_3COO). [p] Overlapped with signal of OCH_2COO group. [q] *J* = 4·5. [r] Measured in CD_3SOCD_3 with tetramethylsilane as internal standard. [s] Undeterminable value. [t] *J*(20, 21) = = 6·0. [u] Overlapped with signal of $COOCH_3$ group. [v] Other signal 5·86 bs, 1 H (H-4). [x] Other signal 5·80 bs, 1 H (H-4).

data (see Table I) as well as the IR spectrum (cf. Experimental) prove safely this structure.

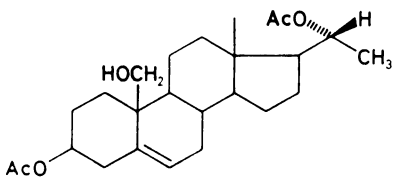
The antigenic properties of antiboies of this new hapten will be reported elsewhere.



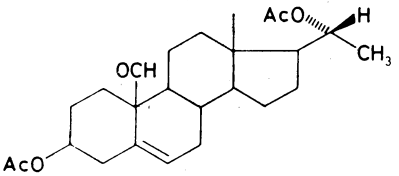
I



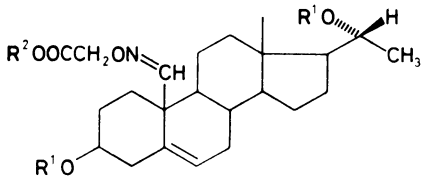
II, R = H
III, R = Ac



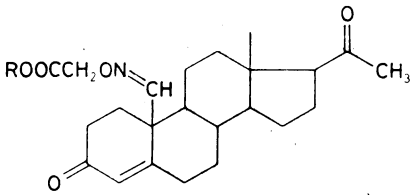
IV



V



VI, R^1 = Ac ; R^2 = CH_3
VII, R^1 = R^2 = H
VIII, R^1 = H ; R^2 = CH_3



IX, R = CH_3
X, R = H

In formulae *III*-*VIII*: Ac = $OCCH_3$

EXPERIMENTAL

Melting points were determined on a Koffer block. Optical rotations were carried out in chloroform with an accuracy of $\pm 2°$ at 23°C. The infrared spectra were recorded on the Zeiss UR 20 spectrometer in chloroform; wavenumbers are given in cm^{-1}. ^1H NMR spectra were measured on a Tesla BS-476 instrument (100 MHz, FT mode) in deuterochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and width

(W) in Hz. All values were obtained by first order analysis. Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer (energy of ionizing electrons 70 eV, ion source temperature 170–200°C). The identity of samples prepared by different procedures was checked by thin-layer chromatography (TLC, silica gel G Woelm, detection with sulfuric acid) and by infrared spectra. The working up of a reaction mixture in the usual way means extraction of the product with an organic solvent and washing the extract with 5% aqueous hydrochloric acid, water, 5% aqueous sodium hydrogen carbonate, water, drying over magnesium sulfate, and evaporation of the solvent in vacuo at 50°C.

(20*R*)-5-Bromo-6β,19-epoxy-20-hydroxy-5α-pregnan-3β-yl Acetate (*II*)

The ketone[3] *I* (10·0 g, 22·1 mmol) was dissolved in tetrahydrofuran (150 ml), treated at 0°C with solid lithium tri-tert-butoxyaluminium hydride (20·0 g, 78·7 mmol) and allowed to stand at 5°C for 30 min. The reaction mixture was decomposed with ice and conc. hydrochloric acid (50 ml) and the product was extracted with ethyl acetate. The extract was worked up in the usual way and the solvent was removed in vacuo. The residue which was slightly contaminated with the polar (20*S*)-isomer was chromatographed over silica gel (320 g) in benzene–ether (9 : 1). Fractions with the lipophilic (20*R*)-hydroxy derivative *II* were combined, solvents removed and the product was crystallized from methanol to afford 8·20 g (82%) of the alcohol *II*, m.p. 167–168°C, $[\alpha]_D$ −8° (*c* 1·9). IR spectrum (chloroform): 3 610, 3 455 (O—H); 1 730 (C=O); 1 250, 1 039 (C=O). Mass spectrum, *m/z*: 438/436 (M − H_2O). For $C_{23}H_{35}BrO_4$ (455·4) calculated: 60·66% C, 7·75% H, 17·55% Br; found: 60·42% C, 7·68% H, 17·38% Br.

(20*R*)-5-Bromo-6β,19-epoxy-5α-pregnane-3β,20-diyl Diacetate (*III*)

The alcohol *II* (4·50 g, 9·88 mmol) was acetylated with acetic anhydride (30 ml) in pyridine (50 ml). After 18 h at room temperature the reaction mixture was decomposed with ice and the product was taken into ethyl acetate. Usual working up and crystallization from chloroform–light petroleum yielded 3·8 g (77%) of the diacetate *III*, m.p. 171–173°C, $[\alpha]_D$ +19° (*c* 2·6). Literature[6] gives m.p. 169·5–171·5°C, $[\alpha]_D$ +20·8°. IR spectrum (tetrachloromethane): 1 735 (C=O); 1 242, 1 040 (C—O). Mass spectrum, *m/z*: 438/436 (M − CH_3COOH), 357 (438/436 − Br), 297 (357 − CH_3COOH). For $C_{25}H_{37}BrO_5$ (497·5) calculated: 60·36% C, 7·50% H, 16·06% Br; found: 60·21% C, 7·39% H; 15·86% Br.

(20*R*)-19-Hydroxypregn-5-ene-3β,20-diyl Diacetate (*IV*)

The epoxide *III* (5·20 g, 10·5 mmol) in 2-methyl-2-propanol (140 ml) and water (100 ml) was treated with zinc dust (40·0 g, 0·62 mol) and the reaction mixture was refluxed under efficient stirring for 1 h. The metal was removed by suction, washed well with ethanol and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate, the solution was washed with 5% hydrochloric acid and worked up. The product after evaporation of the solvent was crystallized from methanol to afford 4·10 g (94%) of the alcohol *IV*, m.p. 132–134°C, $[\alpha]_D$ −27° (*c* 2·8). Literature[4] gives m.p. 132–133°C, $[\alpha]_D$ −27°. IR spectrum (chloroform): 3 623, 3 570, 3 440 (O—H); 1 723 (C=O); 1 255, 1 032 (C—O). Mass spectrum, *m/z*: 358 (M − CH_3·COOH). For $C_{25}H_{38}O_5$ (418·6) calculated: 71·74% C, 9·15% H; found 71·53% C, 9·04% H.

(20*R*)-19-Oxopregn-5-ene-3β,20-diyl Diacetate (*V*)

The alcohol *IV* (1·50 g, 3·58 mmol) in acetone (25 ml) was 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 and the product was taken into ethyl acetate. The organic layer was worked up and the residue was chromatographed on a silica gel column (50 g) in benezene–ether (98 : 2). Fractions with the desired compound were combined, solvents were distilled off in vacuo and the product was crystallized from methanol to afford 1·05 g (70%) of the aldehyde *V*, m.p. 155–157°C, $[\alpha]_D$ −197° (*c* 2·2). IR spectrum (tetrachloromethane): 2 810, 2 702 (CHO); 1 730 (C=O); 1 665 (C=C); 1 245, 1 035 (C–O). Mass spectrum, *m/z*: 356 (M − CH_3COOH). For $C_{25}H_{36}O_5$ (416·5) calculated: 72·08% C, 8·71% H; found: 72·00% C, 8·65% H.

(19*E*,20*R*)-19-Oxopregn-5-ene-3β,20-diyl Diacetate
19-(O-Carboxymethyl)oxime Methylester (*VI*)

The aldehyde *V* (4·20 g, 10·1 mmol) in pyridine (40 ml) was treated with (O-carboxymethyl) hydroxylamine hemihydrochloride (3·00 g, 20·1 mmol) and allowed to stand at room temperature for 20 h. The reaction mixture was decomposed with ice and conc. hydrochloric acid (60 ml) and the product was extracted with ethyl acetate. After thorough washing with water the solvent was distilled off in vacuo, the residue was dissolved in methanol (5 ml) and ether (60 ml) and treated with ethereal solution of diazomethane. The excess diazomethane was removed with acetic acid and after working up and evaporation of the solvents the crude product was crystallized from methanol to give 3·75 g (74%) of the oxime *VI*, m.p. 144–145°C, $[\alpha]_D$ −112° (*c* 1·9). IR spectrum (tetrachloromethane): 1 763 (C=O of $COOCH_3$); 1 735 (C=O of acetate); 1 248, 1 035 (C–O). Mass spectrum, *m/z*: 503 (M^+), 443 (M − CH_3COOH), 414 (M − $OCH_2COO.CH_3$), 354 (M − CH_3COOH − OCH_2COOCH_3). For $C_{28}H_{41}NO_7$ (503·6) calculated: 66·78% C, 8·21% H, 2·78% N; found: 66·61% C, 8·17% H, 2·64% N.

(19*E*,20*R*)-3β,20-Dihydroxypregn-5-en-19-al 19-(O-Carboxymethyl)oxime (*VII*)

The ester *VI* (1·20 g, 2·38 mmol) in methanol (60 ml) was treated with a solution of potassium hydroxide (1·0 g, 17·8 mmol) in water (10 ml) and refluxed for 4 h. The excess alkali was removed with acetic acid and methanol was distilled off under reduced pressure. The product was dissolved in ethyl acetate and the solution was worked up. The residue after evaporation of the solvent was crystallized from ethyl acetate–ether to yield 720 mg (75%) of the oxime *VII*, m.p. 228–230°C (recrystallization at 132–136°C), $[\alpha]_D$ −135° (*c* 1·6, dioxane). IR spectrum (KBr): 3 395 (O–H); 1 740, 1 705 (C=O); 1 097 (C–O). For $C_{23}H_{35}NO_5$ (405·5) calculated: 68·12% C, 8·70% H, 3·45% N; found: 67·95% C, 8·62% H, 3·42% N.

(19*E*,20*R*)-3β,20-Dihydroxypregn-5-en-19-al 19-(O-Carboxymethyl)oxime Methylester (*VIII*)

The acid *VII* (560 mg, 1·38 mmol) was dissolved in tetrahydrofuran (8 ml), ether (40 ml) and methanol (1 ml) were added and the solution was treated with diazomethane in ether. The excess diazomethane was destroyed with acetic acid and the solution was washed with 5% potassium hydrogen carbonate. After working up the residue was crystallized from chloroform–ether–light petroleum to yield 480 mg (83%) of the ester *VIII*, m.p. 190–192°C, $[\alpha]_D$ −150° (*c* 2·4). IR spectrum (chloroform): 3 610, 3 485 (O–H); 1 753, 1 735 shoulder (C=O). Mass spectrum, *m/z*: 419 (M^+), 401 (M − H_2O), 312 (M − H_2O − OCH_2COOCH_3). For $C_{24}H_{37}NO_5$ (419·6) calculated: 68·71% C, 8·89% H, 3·34% N; found: 68·58% C, 8·71% H, 3·27% N.

(19*E*)-3,20-Dioxypregn-4-en-19-al 19-(O-Carboxymethyl)oxime Methylester (*IX*)

The solution of the diol *VIII* (3·60 g, 8·58 mmol) in acetone (120 ml) was treated with Jones' reagent and allowed to stand at room temperature for 15 min. Methanol was added to remove

the excess reagent, the reaction mixture was diluted with water and acetone was distilled off in vacuo. The oily product was extracted with ethyl acetate and the extract was worked up in the usual way. The residue was dissolved in methanol (80 ml) and treated with conc. hydrochloric acid (1 ml) to accomplish the rearrangement of the 5,6-double bond to the 4,5-position. After 30 min at room temperature the reaction mixture was neutralized with 5% sodium hydrogen carbonate and solvents were removed under reduced pressure. The product was taken into ethyl acetate, the extract was worked up and the solvent was removed. The residue was chromatographed over silica gel (120 g) in benzene–ether (95 : 5) to yield after working up of the corresponding fractions 2·60 g (73%) of the dione *IX* which resisted all attempts at crystallization. $[\alpha]_D$ +161° (*c* 2·4). IR spectrum (tetrachloromethane): 1 764, 1 745 (COOR); 1 706 ($COCH_3$); 1 681, 1 623 (C=C—C=O). Mass spectrum, *m/z*: 415 (M^+), 326 ($M - OCH_2COOCH_3$). For $C_{24}H_{33}NO_5$ (415·5) calculated: 69·37% C, 8·00% H, 3·37% N; found: 69·28% C, 7·83% H, 3·19% N.

(19*E*)-3,20-Dioxopregn-4-en-19-al 19-(O-Carboxymethyl)oxime (*X*)

The methylester *IX* (2·80 g, 6·74 mmol) in methanol (100 ml) was treated dropwise under stirring with a solution of potassium hydroxide (500 mg, 8·91 mmol) in water (50 ml) at room temperature. The reaction mixture was stirred for additional 1 h, the excess alkali was neutralized with conc. hydrochloric acid and methanol was distilled off in vacuo. The residue was acified with hydrochloric acid and the product was extracted with ethyl acetate. Working up and crystallization from tetrahydrofuran–ether afforded 2·10 g (78%) of the oxime *X*, m.p. 230—232°C, $[\alpha]_D$ +169° (*c* 1·2, dioxane). IR spectrum (KBr): 3 500—2 500, 1 760, 1 745 shoulder (COOH); 1 695 shoulder (C=O of $COCH_3$); 1 674, 1 620 (C=C—C=O). For $C_{23}H_{31}NO_5$ (401·5) calculated: 68·80% C, 7·78% H, 3·49% N; found: 68·67% C, 7·75% H, 3·24% N.

The authors are indebted to Mrs I. Jurinová for technical assistance. Our thanks are due to Dr S. Vašičková for taking and interpretation of IR *spectra and to Mrs J. Jelínková and Mrs M. Snopková for measurements of* ^{1}H NMR *spectra. We are also indebted to the staff of the Laboratory of Mass Spectrometry (Dr K. Ubik, Head) for measurements of mass spectra and to the staff of Analytical Laboratory (Dr V. Pechanec, Head) for carrying out the elemental analyses.*

REFERENCES

1. Fajkoš J., Pouzar V., Vereš K.: Collect. Czech. Chém. Commun. *55*, 2086 (1990).
2. Vereš K.: Unpublished results.
3. Bowers A., Villotti R., Edwards J. A., Denot E., Halpern O.: J. Am. Chem. Soc. *84*, 3204 (1962); Santaniello E., Hadd H. E., Caspi E.: J. Steroid Biochem. *6*, 1505 (1975).
4. Kalvoda J., Heusler K., Ueberwasser H., Anner G., Wettstein A.: Helv. Chim. Acta *46*, 1361 (1963).
5. Martin G. L., Martin M. L.: Progr. Nucl. Magn. Reson. Spectrosc. *8*, 195 and 243 (1972).
6. Barton D. H. R., Hesse R. H., O'Brien R. E., Pechet M. M.: J. Org. Chem. *33*, 1562 (1968).

Translated by the author (J.F.).