SYNTHESIS OF RIA HAPTENS: 3α,11α-DIHYDROXY-5α-PREGNAN-20-ONE 11-HEMISUCCINATE*

Alexander KASAL and Světlana PÁSZTOROVÁ

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6

Received June 16, 1992
Accepted July 16, 1992

5α-Pregnane-3α,11α-diol-20-one (*XVII*) was prepared by hydroxylation od 5α-pregnan-3α-ol-20-one (*XVI*) by *Rhizopus nigricans* and by chemical conversion of 11α-hydroxyprogesterone (*I*). The diol *XVII* was partially acylated with 2-(trimethylsilyl)ethyl hydrogen succinate and the 11α-succinate *XVIII* was converted to the target 11α-hemisuccinate *XXI*.

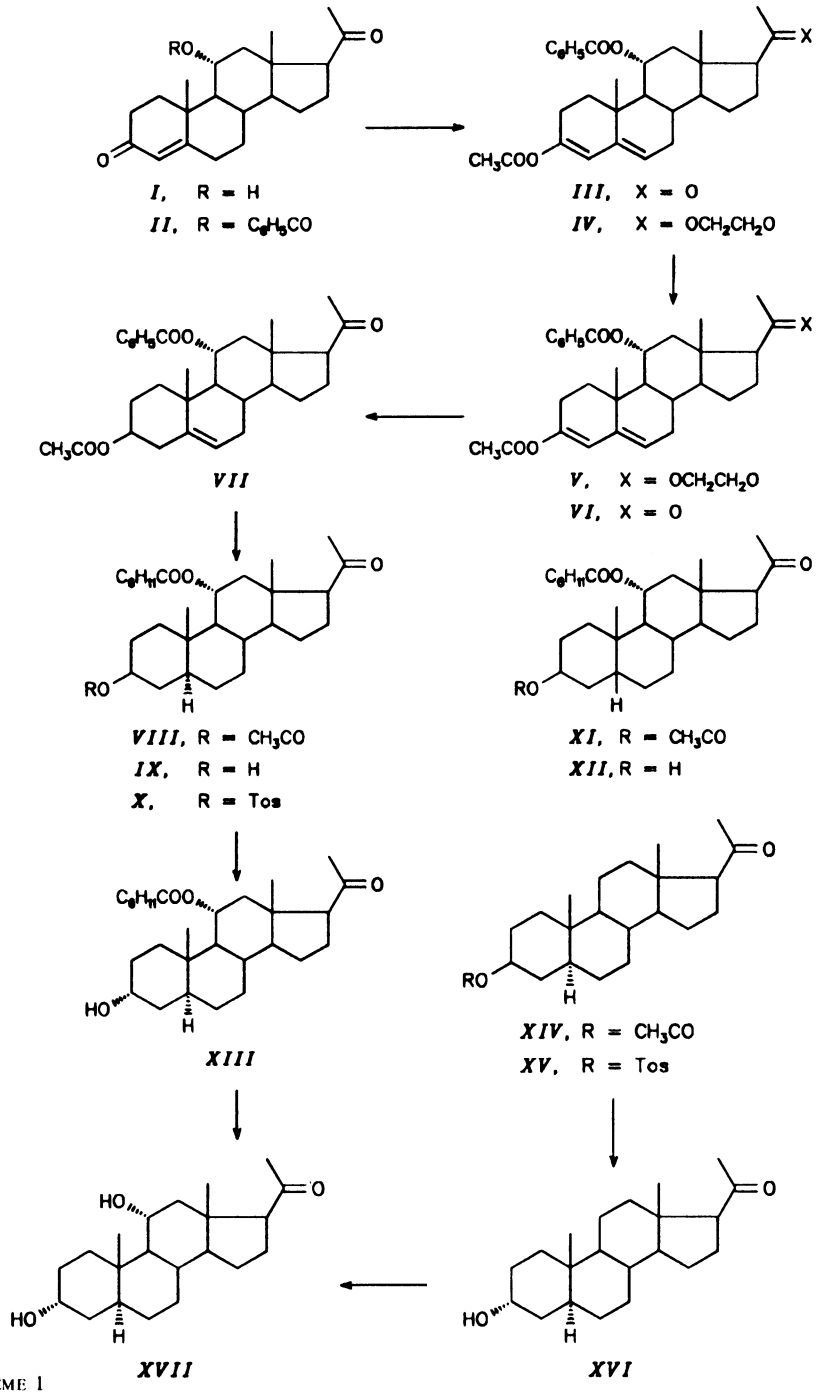
For investigations of the role of 3α-hydroxy-5α-pregnan-20-one (*XVI*) in the organism, as well as for general diagnostic purposes, it was desirable to prepare a suitable hapten on which immunological determination of this compound could be based. Such an antibody has been already prepared by Purdy and coworkers[1] who used as hapten the corresponding 11α-carboxymethyl derivative.

Because of our previous experience with haptens containing the hemisuccinyl group as a spacer[2,3], we decided to use the title compound *XXI* for this purpose. Its preparation is the subject of this paper.

The starting 5α-pregnane-3α,11α-diol-20-one (*XVII*) was prepared by Glaxo researchers in connection with a search for anaesthetics[4,5]. Later on, this compound was prepared in a different way by Ende and Spiteller[6] for a study of mass spectral fragmentation of steroid compounds. We describe now two further syntheses of compound *XVII*; one consisting in chemical transformation of 11α-hydroxyprogesterone (*I*), the other in microbiological hydroxylation of 5α-pregnan-3α-ol-20-one (*XVI*, ref.[7]). Both the starting compounds are commercially available.

In the first reaction pathway (Scheme 1), the Δ^4-3-keto group in 11α-benzoyloxyprogesterone (*II*) was reduced to the Δ^5-3β-hydroxy group; to this end the 3-keto group in the diketone *II* was selectively converted into the enol acetoxy group and then the 20-oxo group in the obtained compound *III* was protected in the form of dioxolane

* Part CCCLXVII in the series On Steroids; Part CCCLXVI: Collect. Czech. Chem. Commun. 58, 600 (1993).


RO
O
O
I, R = H
II, R = C6H5CO
C6H5COO
CH3COO
X
III, X = O
IV, X = OCH2CH2O
V, X = OCH2CH2O
VI, X = O
VII
C6H11COO
VIII, R = CH3CO
IX, R = H
X, R = Tos
XI, R = CH3CO
XII, R = H
HO
XIII
XIV, R = CH3CO
XV, R = Tos
XVI
XVII

SCHEME 1

grouping (compound *IV*). The enol acetate *IV* was reduced with sodium borohydride[8] to give 3β-hydroxy derivative *V*. The product was purified only after deblocking the 20-keto group (compound *VI*). The course of all the reactions was monitored by TLC and structure of the intermediates *III* to *V* was confirmed by IR and ^{1}H NMR spectroscopy (see Experimental and Table I).

As starting compound for the hydrogenation of the Δ^5-double bond we chose 3-acetate *VII*. Its hydrogenation gave the desired 5α-dihydro derivative *VIII* as the main product (as shown by NMR spectrum, the compound has axial 3α-proton), the minor 5β-isomer *XI* (equatorial 3α-proton) being present in the more lipophilic fraction. It proved better

TABLE I
Characteristic parameters of ^{1}H NMR spectra

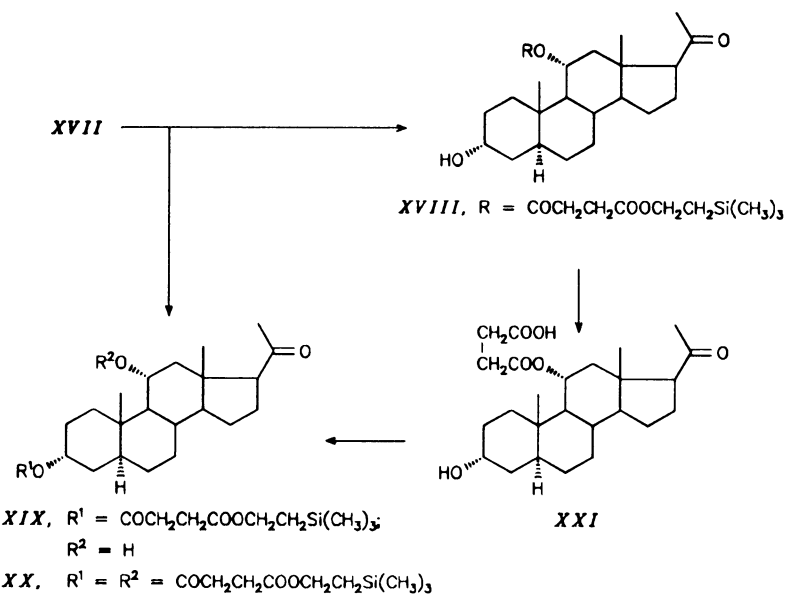
Compound	H-18[a]	H-19[a]	H-21[a]	H-3	H-11[b]	Other signals[c]
I	0.70	1.32	2.14	–	4.04	5.74[d]
II	0.80	1.31	2.09	–	5.55	5.77[d]
III	0.69	1.05	2.10	–	5.45[e]	2.13[f], 5.40[e], 5.69[g]
IV	0.93	1.15	1.25	–	5.45[e]	2.12[f], 3.90[h], 5.40[e], 5.69[g]
V	0.91	1.15	1.25	3.51[i]	5.58	3.92[h], 5.45[j]
VI	0.77	1.14	2.10	3.51[i]	5.58	5.46[j]
VII	0.77	1.15	2.10	4.57[i]	5.56	2.00[f], 5.45[j]
VIII	0.65	0.93	2.09	4.67[k]	5.13	2.01[f]
IX	0.65	0.91	2.09	3.56[k]	5.18	
X	0.63	0.88	2.07	4.43[k]	5.13	2.45[l], 7.34[m], 7.79[m]
XI	0.66	1.07	2.09	5.11[n]	5.11	2.05[f]
XII	0.66	1.06	2.09	4.15[n]	5.13	
XIII	0.65	0.89	2.09	4.00[n]	5.19	
XIV	0.60	0.91	2.10	3.59[k]	–	
XVI	0.61	0.78	2.11	4.04[n]	–	
XVII	0.62	0.93	2.13	4.02[n]	3.94	
XVIII	0.62	0.87	2.08	4.00[n]	5.16	0.02[o], 2.55[p], 4.15[q]
XIX	0.60	0.92	2.12	5.00[n]	3.92	0.02[o], 2.60[p], 4.17[q]
XX	0.65	0.90	2.10	4.99[n]	5.18	2.61[p], 4.19[q], 0.09[o]
XXI	0.65	0.89	1.10	4.04[n]	5.20	2.63[p]

[a] s, 3 H. [b] Unless otherwise stated dt, $J(9\alpha,11\beta) = J(11\beta,12\alpha) = 11$, $J(11\beta,12\beta) = 5.5$. [c] Benzoates exert multiplets of aromatic protons at 7.71 and 8.02. [d] bs, $\sum J = 2$, 1 H (H-4). [e] Overlapping H-4 and H-11. [f] s, 3 H (CH_3COO). [g] d, $J = 2.3$, 1 H (H-6). [h] m, $W = 50$, 2 H (ethylenedioxy group). [i] m, 1 H, $\sum J = 38$. [j] dd, $J = 5.4$, $J' = 0.5$, 1 H (H-6). [k] m, $\sum J = 45$. [l] s, 3 H ($CH_3C_6H_4R$). [m] d, $J = 8.2$, 2 H (aromatic protons). [n] m, 1 H, $\sum J = 18$. [o] s, 9 H ($(CH_3)_3Si$). [p] m, 4 H ($OCOCH_2CH_2COO$). [q] m, $\sum J = 22$, 2 H (OCH_2R).

to hydrolyze the isomeric mixture and only then to separate the corresponding 3β-alcohols *IX* and *XII* by chromatography.

Configurational inversion of the 3β-hydroxy group was realized according to Latrell[9,10], i.e. by solvolysis of the tosylate *X* with sodium nitrite in dimethyl sulfoxide. The principal product *XIII* (61%) exhibited ^{1}H NMR and IR spectral characteristics of an ester with a free axial hydroxy group. Since hydrolysis of the ester group in compound *XIII* was very difficult, we reduced it with lithium aluminium hydride after protection of the 20-oxo group by ketalization; after its liberation we obtained the desired 5α-pregnan-3α,11α-diol-20-one (*XVII*).

The target compound *XVII* was also prepared by microbiological hydroxylation (Scheme 1): 5α-pregnan-3β-ol-20-one (*XIV*, ref.[7]), easily accessible by hydrogenation of the commercial pregnenolone, was converted into the 3α-isomeric alcohol *XVI* by solvolysis of known[11] tosylate *XV*, using the above-described method[9]. The alcohol *XVI* was then hydroxylated by fungi. Whereas *Beauveria bassiana* converted compound *XVII* into a mixture of unidentified polyhydroxy derivatives (R_F of all components was lower than that of diol *XVII*), *Aspergillus ochraceus* afforded a mixture of lipophilic products (R_F higher than that of *XVII*). On the other hand, *Rhizopus nigricans*, in accord with the hypothesis on the directing influences by oxygen groups[12,13], hydroxylated the compound *XVI* in the position 11α as evidenced by ^{1}H NMR spectrum, exhibiting a characteristic signal of the axial 11β-proton (δ 3.94, dt, $J(9\alpha,11) = J(11,12\alpha) = 11$ Hz, $J(11,12\beta) = 5.5$ Hz). The product, obtained in a 97% yield, was in all respects identical with the above-described compound *XVII*.



SCHEME 2

Partial hemisuccinylation of the diol *XVII* was executed in an indirect way[14,15]: treatment of diol *XVII* with 2-(trimethylsilyl)ethyl hydrogen succinate in the presence of N,N'-dicyclohexylcarbodiimide and 4-dimethylaminopyridine as catalyst afforded 11-ester *XVIII* (Scheme 2). Although the direct yield was only 27% (or 44% when taking into account the unreacted *XVII*), both the side-products (the isomeric ester *XIX* and diester *XX*) were hydrolyzed with potassium hydroxide solution to give the starting diol *XVII* which could be again acylated.

The succinate *XVIII* was decomposed with tetrabutylammonium fluoride under formation of the desired hydrogen succinate *XXI* that had the expected physical and chemical properties (see Experimental). Binding of this hapten to BSA, preparation of the antibody, and its properties will be described later.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations and IR spectra (Zeiss UR 20 instrument) were measured in chloroform unless stated otherwise; wavenumbers are given in cm^{-1}. ^1H NMR were taken in deuteriochloroform with tetramethylsilane as internal standard at 23 °C on a Varian XL-200 instrument (200 MHz, FT-mode). Chemical shifts are given in ppm (δ-scale), coupling constants *J* in Hz. All parameters were obtained by first order analysis. Mass spectra were measured on a VG-ZAB-EQ spectrometer (in parentheses relative intensities, referenced to the base peak, and in some cases also assignments). Identity of compounds prepared by different procedures was proved by mixture melting points and comparison of IR spectra. Reaction course and purity of the compounds were followed by thin-layer chromatography (TLC) on silica gel (Woelm DC, detection by spraying with sulfuric acid and heating). Compounds were separated by flash chromatography on a column of silica gel Silpearl (Kavalier, Votice) or on a thin layer of silica gel (PLC, 200 × 200 × 0.7 mm, ICN DC, inspection at 254 nm after spraying with 0.02% solution of morin in methanol).

Cultures of *Rhizopus nigricans*, *Beauveria bassiana* and *Aspergillus ochraceus* were cultivated in a medium consisting of corn-steep (5 g), glucose (11 g), K_2HPO_4 . 3 H_2O (0.52 g) and $MgSO_4$. 7 H_2O (0.4 g) in 1 l of water, in 250 ml flasks under rotatory shaking at 25 °C. After 48 h a solution of the steroid (30 mg of steroid in 0.5 ml of dimethyl sulfoxide into flask containing 100 ml of the mycelium) was added. The mixture was incubated at 25 °C with rotatory shaking for further 48 h.

Steroids used as starting material (11α-hydroxyprogesterone, pregnenolone acetate) were purchased from Steraloids (Wilton, N. H., U.S.A.).

20-Oxo-3,5-pregnadiene-3,11α-diyl 3-Acetate 11-Benzoate (*III*)

A solution of acetic anhydride (9.7 ml, 0.1 mol) in dry ethyl acetate (50 ml) was added at room temperature to a stirred solution of diketone *II* (ref.[16], 2.3 g, 5.29 mmol) in the same solvent. Then a solution of 72% perchloric acid (0.05 ml) in ethyl acetate (20 ml) was added. After 30 min, TLC (20% ether in benzene) showed that all the starting ketone (R_F 0.24) was converted into a nonpolar product (R_F 0.70). The mixture was then stirred with a suspension of potassium hydrogen carbonate (23 g) in water (50 ml) for 30 min. The phases were separated and the aqueous one was washed with ethyl acetate. The combined organic phases were washed with saturated aqueous solution of sodium hydrogen carbonate, dried over sodium sulfate and the solvent was evaporated. The remaining product *III* (2.5 g, 99%) was checked by ^1H NMR spectroscopy (see Table I) and used without purification in the next step.

20,20-Ethylenedioxy-3,5-pregnadiene-3,11α-diyl 3-Acetate 11-Benzoate (*IV*)

A mixture of ketone *III* (6.38 g, 13.4 mmol), ethylene glycol (34 ml, 492 mmol), *p*-toluenesulfonic acid monohydrate (340 mg, 1.79 mmol) and benzene (340 ml) was heated, using the Dean–Stark apparatus. After 6 h, the mixture was cooled and mixed with water. The separated aqueous phase was shaken with benzene, the combined benzene phases were washed with saturated aqueous potassium hydrogen carbonate solution (3 × 50 ml), water, and dried by filtration through a column of magnesium sulfate. The solution was concentrated in vacuo to dryness, leaving 6.4 g (94%) of compound *IV*. The purity of the product was checked by ^{1}H NMR spectroscopy and TLC on silica gel in ether–benzene (1 : 3; R_F 0.70, the plates were pretreated with gaseous ammonia). The product was used in the next step without further purification.

20,20-Ethylenedioxy-3β-hydroxy-5-pregnen-11α-yl Benzoate (*V*)

Sodium borohydride (1.0 g, 26.4 mmol) was added at 0 °C to a stirred solution of enol acetate *IV* (3.8 g, 7.49 mmol) in ethanol (50 ml) during 1 h. After further 2 h, ice was added and the mixture was set aside in a refrigerator for 18 h. The product was collected, dissolved in ether and the solution washed with water and dried. After evaporation of the solvent, the purity of the residue (*V*, 3 g, 83%) was checked by TLC on silica gel in ether–benzene (1 : 5) and by ^{1}H NMR spectroscopy. This product was used in the next experiment without purification.

3β-Hydroxy-20-oxo-5-pregnen-11α-yl Benzoate (*VI*)

A solution of the crude ketal *V* (2 g, 4.16 mmol) and *p*-toluenesulfonic acid monohydrate (0.5 g, 2.63 mmol) in acetone (70 ml) was allowed to stand at room temperature for 24 h. The product was precipitated with saturated sodium chloride solution and taken up in chloroform. The extract was shaken with saturated aqueous solution of potassium hydrogen carbonate and water, concentrated, and the residue was purified by chromatography on a column of silica gel (50 ml, benzene). The main product (874 mg, 48%) had m.p. 203 – 205 °C (acetone–heptane); $[\alpha]_D$ −14° (*c* 1.1). IR spectrum: 3 608 (OH); 1 705, 1 603, 1 585, 1 278 (COO); 1 705, 1 359 (C=O); 1 660 (C=C). For $C_{28}H_{36}O_4$ (436.6) calculated: 77.03% C, 8.31% H; found: 77.15% C, 8.36% H.

20-Oxo-5-pregnene-3β,11α-diyl 3-Acetate 11-Benzoate (*VII*)

A) A solution of 3β-hydroxy derivative *VI* (410 mg, 0.94 mmol) in pyridine (6 ml) and acetic anhydride (4 ml, 42.4 mmol) was set aside at ambient temperature for 18 h. Crushed ice was added to the stirred mixture and after 2 h the precipitated product was filtered and washed with water; yield 420 mg (93%), m.p. 165 – 167 °C, after crystallization from acetone m.p. 167 – 169 °C, $[\alpha]_D$ +4° (*c* 1.2). IR spectrum: 1 707, 1 603, 1 584, 1 277 (C_6H_5COO); 1 728 (sh), 1 257 (CH_3COO); 1 701 (sh, C=O). For $C_{30}H_{38}O_5$ (478.6) calculated: 75.28% C, 8.00% H; found: 75.18% C, 7.99% H.

B) The crude compound *V* (3 g, 6.24 mmol) was acetylated with acetic anhydride (8 ml) and pyridine (10 ml) similarly as described in experiment *A*), and the ethylenedioxy group in the product was cleaved off by treatment with 72% perchloric acid (0.8 ml) in acetone (80 ml). The mixture was worked up as described for the preparation of compound *VI*, and column chromatography afforded 1.47 g (49%) of product whose melting point and IR spectrum agreed with those of compound *VII*.

20-Oxo-5α-pregnane-3β,11α-diyl 3-Acetate 11-Cyclohexanecarboxylate (*VIII*)

Compound *VII* (7.4 g, 15.46 mmol) was hydrogenated on Adams platinum catalyst (1.1 g) in acetic acid (74 ml) under vigorous stirring. After coagulation of the catalyst (5 h), the mixture was filtered and the filtrate concentrated in vacuo to dryness. The residue was dissolved in acetone (70 ml) and oxidized at 0 °C

with Jones reagent. The mixture was poured in a solution of potassium hydrogen carbonate, the precipitated product was extracted with chloroform and the chloroform extract was washed with water and dried over sodium sulfate. After evaporation of the solvent, the residue was crystallized from methanol, m.p. 143 – 145 °C. Yield 2 g (27%); $[\alpha]_D$ +54° (*c* 0.9). IR spectrum: 1 729, 1 170 ($C_6H_{11}COO$); 1 729, 1 245, 1 027 (CH_3COO); 1 709, 1 362 ($COCH_3$). For $C_{30}H_{46}O_5$ (486.7) calculated: 74.04% C, 9.53% H; found: 73.95% C, 9.72% H. The mother liquors (5.2 g, 69%) were hydrolyzed prior to further purification (see preparation of compounds *IX* and *XII*).

3β-Hydroxy-20-oxo-5α-pregnane-11α-yl Cyclohexanecarboxylate (*IX*)

A) A solution of potassium carbonate (1 g, 7.2 mmol) in water (3 ml) was added to a solution of acetate *VIII* (3 g, 6.16 mmol) in methanol (300 ml) and the mixture was set aside for 64 h at 0 °C. The basicity was reduced by addition of acetic acid (5 ml), the mixture was concentrated in vacuo to a quarter of the original volume and mixed with water. The precipitated product (2.6 g, 95%) was crystallized from aqueous acetone; m.p. 127 – 129 °C, $[\alpha]_D$ +44° (*c* 0.9). IR spectrum: 3 620, 1 040 (OH); 1 720, 1 171 (COO); 1 709, 1 358 (C=O). For $C_{28}H_{44}O_4$ (444.7) calculated: 75.63% C, 9.97% H; found: 75.38% C, 9.84% H.

B) The mother liquors after hydrogenation of 7.4 g of benzoate *VII* (5.5 g, 10.7 mmol) were hydrolyzed with potassium carbonate (1.5 g) in methanol (450 ml) and water (5 ml) as described under *A*) and the product was applied onto a column of silica gel (250 ml). Ether–toluene (1 : 8) eluted first the lipophilic minor product *XII* (1.46 g, 21% calculated on the unsaturated benzoate *VII*), m.p. 141 – 143 °C (acetone–ether), $[\alpha]_D$ +13° (*c* 0.9). IR spectrum: 3 626, 1 032 (OH); 1 723, 1 172 (COO); 1 709, 1 356 (C=O). For $C_{28}H_{44}O_4$ (444.7) calculated: 75.63% C, 9.97% H; found: 75.81% C, 9.63% H. The principal component of the mixture (3.54 g, 51% calculated on *VII*) was obtained from subsequent fractions and its m.p. and ^1H NMR spectrum confirmed the identity with compound *IX* prepared under *A*).

20-Oxo-5α-pregnane-3β,11α-diyl 3-Toluenesulfonate 11-Cyclohexanecarboxylate (*X*)

A solution of 3β-hydroxy derivative *IX* (3 g, 6.7 mmol) and *p*-toluenesulfonyl chloride (3 g, 15.7 mmol) in pyridine (15 ml) was allowed to stand at room temperature for 18 h. Crushed ice was added to the mixture and, after 2 h, the precipitated product was collected, washed with water and dissolved in chloroform. The solution was dried over sodium sulfate and the solvent evaporated in vacuo to dryness; yield 3.5 g (87%), m.p. 200 – 205 °C (ether–methanol), $[\alpha]_D$ +25° (*c* 0.8). IR spectrum (CCl_4): 1 721, 1 176 (COO); 1 709 (C=O); 1 366, 1 189, 1 176 (SO_2O). For $C_{35}H_{50}O_6S$ (598.8) calculated: 70.20% C, 8.42% H, 5.35% S; found: 69.91% C, 8.57% H, 4.90% S.

3α-Hydroxy-20-oxo-5α-pregnan-11α-yl 11-Cyclohexanecarboxylate (*XIII*)

A mixture of tosylate *X* (3.5 g, 5.8 mmol), potassium nitrite (7.3 g, 85.8 mmol) and dimethyl sulfoxide (60 ml) was heated at 130 °C under stirring. After 4 h, the mixture was cooled, poured on ice and the precipitate was taken up in chloroform. The extract was washed with saturated ammonium sulfate solution, dried, the solvent evaporated and the residue chromatographed on a column of silica gel (60 g). Benzene eluted the principal product *XIII* (1.8 g, 69%), m.p. 83 – 85 °C, $[\alpha]_D$ +33° (*c* 0.9). IR spectrum (CCl_4): 3 626 (OH); 1 720, 1 172 (COO); 1 708, 1 358 (C=O). For $C_{28}H_{44}O_4$ (444.7) calculated: 75.63% C, 9.97% H; found: 75.97% C, 10.04% H.

3α-Hydroxy-5α-pregnan-20-one (*XVI*)

3β-Hydroxy derivative *XIV* (6 g, 18.9 mmol) in pyridine (20 ml) was converted into tosylate *XV* (ref.[11]) which was solvolyzed with potassium nitrite (21 g, 0.25 mol) in dimethyl sulfoxide (130 °C, 5 h). The mixture was poured in an aqueous solution of ammonium sulfate, the product was extracted with ether, the extract washed with water and dried over sodium sulfate. After evaporation of the solvent, the product was crystallized from toluene to give 1 163 mg of compound *XVI*. Chromatography of the mother liquors on silica gel in benzene, followed by crystallization from toluene, afforded further 1 292 mg of the product (total yield 41% from the starting *XIV*). M.p. 168 – 170 °C, in agreement with the reported[7] value.

3α,11α-Dihydroxy-5α-pregnan-20-one (*XVII*)

A) Under conditions described for the preparation of compound *IV* (using 4 ml of ethylene glycol, 100 ml of benzene and 50 mg of *p*-toluenesulfonic acid monohydrate), ketone *XIII* (1 g, 2.25 mmol) was converted into the corresponding 20,20-ethylenedioxy derivative in which the ester group was then removed by treatment with lithium aluminium hydride (about 100 mg of hydride in 10 ml of tetrahydrofuran, 18 h at room temperature). The protecting ethylenedioxy group in the obtained product (1.1 g, 2.906 mmol) was cleaved by treatment with *p*-toluenesulfonic acid monohydrate (110 mg, 0.58 mmol) in acetone (15 ml). After 24 h, the mixture was concentrated in vacuo, diluted with saturated potassium hydrogen carbonate solution, and the product was extracted with chloroform. The washed extract was dried, concentrated and purified by chromatography on silica gel column (30 g, toluene–ether 10 : 1). Yield 460 mg (61% from *XIII*) of product *XVII*, m.p. 83 – 85 °C (toluene), $[\alpha]_D$ +78° (*c* 1.3) (for a product crystallized from acetone and light petroleum reported[5] m.p. 152 – 154 °C and $[\alpha]_D$ +78°). IR spectrum: 3 613, 3 455, 1 022 (OH); 1 700, 1 358 ($COCH_3$). Mass spectrum, *m/z*: 334 (M^+, 6), 316 (M – H_2O, 100), 301 (316 – CH_3, 32), 298 (316 – H_2O, 60), 283 (316 – H_2O – CH_3, 34), 213 (50), 43 (CH_3CO, 64). For $C_{21}H_{34}O_3$ (334.5) calculated: 75.41% C, 10.25% H; found: 75.38% C, 10.29% H.

B) A solution of hydroxy ketone *XVI* (90 mg, 0.28 mmol) in dimethyl sulfoxide (1.5 ml) was introduced into six 250 ml flasks, containing 2 days-old culture of *Rhizopus nigricans*. The flasks were freely moved on a rotatory shaker at room temperature. After 2 days, their content was extracted with ethyl acetate, dried over sodium sulfate and the solvent was evaporated in vacuo. The residue (300 mg) was subjected to preparative TLC on silica gel (8 plates, 200 × 200 × 0.3 mm) in benzene–ethyl acetate (1 : 1). The lipophilic zone contained residues of the unreacted starting compound *XIV*, the main zone (R_F 0.50) contained compound *XVII* (92 mg, 97%) whose spectra were identical with those of a sample prepared by procedure *A*).

Succinylation of 5α-Pregnane-3α,11α-diol-20-one (*XVII*)

A part of the solvent (3 ml) was distilled from a solution of diol *XVII* (86 mg, 0.26 mmol) in toluene (7 ml). After cooling, 2-(trimethylsilyl)ethyl hydrogen succinate (60 mg, 0.27 mmol), N,N'-dicyclohexylcarbodiimide (50 mg, 0.24 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol) were added. After standing at room temperature for 3 h, the mixture was diluted with methanol (1 ml) and concentrated to dryness in vacuo. The residue was dissolved in chloroform, the solution washed with water, dried over sodium sulfate, and subjected to TLC on silica gel (2 plates) in benzene–ether (1 : 1). The individual zones afforded the following compounds: the starting diol *XVII* (33 mg, 30%); 11α-hydroxy-20-oxo-5α-pregenan-3α-yl 2-(trimethylsilyl)ethyl succinate (*XIX*, 48 mg, 35%), $[\alpha]_D$ +31° (*c* 0.9); 3α-hydroxy-20-oxo-5α-pregnan-11α-yl 2-(trimethylsilyl)ethyl succinate (*XVIII*, 37 mg, 27% or 44% when taking into account recovery of the unreacted compound), $[\alpha]_D$ +27° (*c* 0.9); IR spectrum (CCl_4): 3 626, 1 064 (OH); 1 734, 1 161 (COO), 1 708, 1 353 ($COCH_3$); 20-oxo-5α-pregnen-3α,11α-diyl 2-(trimethylsilyl)ethyl succinate (*XX*, 14 mg, 7%).

3α-Hydroxy-20-oxo-5α-pregnan-11α-yl Hydrogen Succinate (*XXI*)

A solution of tetrabutylammonium fluoride (1 mol l^{-1}, 0.25 ml, 0.25 mmol) was added to a solution of trimethylsilylethyl derivative *XVIII* (57 mg, 0.11 mmol) in tetrahydrofuran (1 ml). After standing at room temperature for 64 h, the solution was diluted with chloroform and concentrated in vacuo to a quarter of the original volume. The solution was washed with dilute hydrochloric acid and water, dried over sodium sulfate, and subjected to preparative TLC in chloroform–acetone–acetic acid (50 : 50 : 1). The zone of R_F 0.65 was eluted with acetone to give 41 mg (88%) of the product, m.p. 148 – 149 °C (ether, −18 °C), $[\alpha]_D$ + 32° (*c* 0.8). IR spectrum: 3 614 (OH), 3 509 (monomeric COOH); 3 086 (associated COOH); 2 677 (dimeric COOH); 1 716 (C=O); 1 716, 1 172, 1 036 (COOR). Mass spectrum, *m/z*: 316 ($M^+ - C_4H_6O_4$, 100), 301 (316 – CH_3, 15), 298 (316 – H_2O, 21), 283 (298 – CH_3, 27), 273 (316 – CH_3CO, 8), 255 (16), 213 (31), 43 (CH_3CO, 86). For $C_{25}H_{38}O_6$ (434.6) calculated: 69.10% C, 8.81% H; found: 68.79% C, 8.95% H. The reaction course was monitored by TLC on silica gel in two solvent systems: (i) chloroform–acetone–acetic acid 50 : 50 : 1 (R_F 0.65) and (ii) chloroform (shaken with ammonia)–acetone 50 : 50 (R_F 0.0).

Hydrolysis of Succinates *XIX* and *XX* to Diol *XVII*

A) A mixture of diester *XX* (125 mg, 0.17 mmol) and 4% methanolic solution of potassium hydroxide (1 ml) was allowed to stand at room temperature for 18 h, diluted with ethyl acetate (30 ml), washed successively with solution of potassium hydrogen carbonate and sodium chloride, and dried over sodium sulfate. The solvents were evaporated in vacuo and the residue purified by TLC on silica gel in benzene–ether (1 : 1). Yield of diol *XVII* was 39 mg (69%).

B) Hydrolysis of the 3α-ester *XIX* (65 mg, 0.12 mmol) was carried out similarly; yield of diol *XVII* was 30 mg (74%).

The authors are indebted to Mrs J. Neumannová for the technical assistance and to Dr J. Smolíková and Mrs K. Matoušková for measurement and interpretation of the IR spectra. For ^{1}H NMR measurements they thank to Mrs J. Jelínková and Mrs M. Snopková, for the elemental analyses to the staff of the Analytical Laboratory (Dr V. Pechanec, Head).

REFERENCES

1. Purdy R. H., Moore P. H., Rao P. N., Hagino N., Yamaguchi T., Schmidt P., Rubinow D. R., Morrow A. L., Paul S. M.: Steroids *55*, 290 (1990).
2. Kasal A., Smolíková J.: Collect. Czech. Chem. Commun. *49*, 2932 (1984).
3. Kasal A., Hampl R., Putz Z., Kohout L., Stárka L.: Collect. Czech. Chem. Commun., in press.
4. Cook M. C., Lawrence R., Phillipps G. H., Hunter A. C., Newall C. E., Stephenson L., Weir N. G. (Glaxo Laboratories, Ltd.): Ger. Offen. 2 162 555; Chem. Abstr. *77*, 102039b (1972).
5. Cook M. C., Lawrence R., Phillipps G. H., Hunter A. C., Newall C. E., Stephenson L., Weir N. G. (Glaxo Laboratories, Ltd.): Brit. 1 380 248; Chem. Abstr. *82*, 171301w (1977).
6. Ende M., Spiteller G.: Tetrahedron *29*, 2457 (1973).
7. Prelog V., Tagmann E., Lieberman S., Ruzicka L.: Helv. Chim. Acta *30*, 1080 (1947).
8. ApSimon J. W., Fenkhoorn J. E.: Can. J. Chem. *52*, 4113 (1974).
9. Lattrell R., Lohaus G.: Justus Liebigs Ann. Chem. *1974*, 901.
10. Radüchel B.: Synthesis *1980*, 292.
11. Ruff A., Reichstein T.: Helv. Chim. Acta *34*, 70 (1951).
12. Jones E. R. H.: Pure Appl. Chem. *33*, 39 (1973).

13. Chambers V. E. M., Denny W. A., Evans J. M., Jones E. R. H., Kasal A., Meakins G. D., Pragnell J.: J. Chem. Soc., Perkin Trans. 1 *1973*, 513.
14. Drašar P., Černý I., Pouzar V., Havel M.: Collect. Czech. Chem. Commun. *49*, 306 (1984).
15. Pouzar V., Drašar P., Černý I., Havel M.: Synth. Commun. *14*, 501 (1984).
16. Peterson D. H., Murray H. C., Eppstein S. H., Reineke L. M., Weintraub A., Meister P. D., Leigh K. M.: J. Am. Chem. Soc. *74*, 5933 (1952).

Translated by M. Tichý.