CONSTRUCTION OF THE SIDE-CHAIN IN 14β-ANDROST-5-ENE DERIVATIVES. PREPARATION OF 14β-PREGNENOLONE*

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Stepwise side-chain construction schemes leading to pregnane derivatives were tested in the 14β-androst-5-ene series. 6β-Methoxy-3α,5-cyclo-5α,14β-androstan-17-one (*I*) gave after methylenation and hydroboration the 17α-hydroxymethyl derivative *III*. Subsequent oxidation to the aldehyde *VI*, Grignard reaction with methylmagnesium iodide, and reoxidation led to the ketone *VII* and to the isomeric 17β-derivative *VIII* as a minor product. Final i-steroid cleavage of *VII* furnished the known 14β,17α-pregnenolone *IX*; the minor product *VIII* gave the 17β-isomer *X*. Alternatively, the ketone *X* was prepared from 6β-methoxy-3α,5-cyclo-5α,14β-androstan-17α-ol *p*-toluenesulfonate (*XI*) by cyanide substitution, diisobutylaluminum hydride reduction to the aldehyde *XIV*, and further as described for *IX*. The mass and NMR spectra of the four pregnenolone derivatives *IX*, *X*, *XVIII*, and *XIX*, isomeric in positions 14 and 17, were studied.

Construction of the side chain of steroids represents a frequent task in syntheses leading to less accessible steroids as, e.g., in the case of unusual fusion of rings. Recently[1], we developed a simplified approach to 14β-androst-5-ene derivatives and in this work we are continuing the study in checking the stereoselectivity of several side-chain building schemes, leading to 14β-pregn-5-ene derivatives of 17α- or 17β-configuration. 14β-Pregnenolone is the only one of the four possible configuration isomers at the important ring D centers (14α,17α; 14α,17β; 14β,17α; 14β,17β), that has not been synthesized so far, and is interesting in connection with the slight cardiotonic activity found for 14β-hydroxy or 14β-amino pregnane derivatives[2] with *trans*-junction of rings A and B.

We tested stepwise syntheses of pregnenolones[3,4], exploring 17-carbaldehydes as intermediates which may extend the approach to other side-chain derivatives. As starting compound we used 6β-methoxy-3α,5-cyclo-5α,14β-androstan-17-one[1] (*I*) with the parent 3-hydroxy-5-ene arrangement protected in the i-steroid form. We investigated first the

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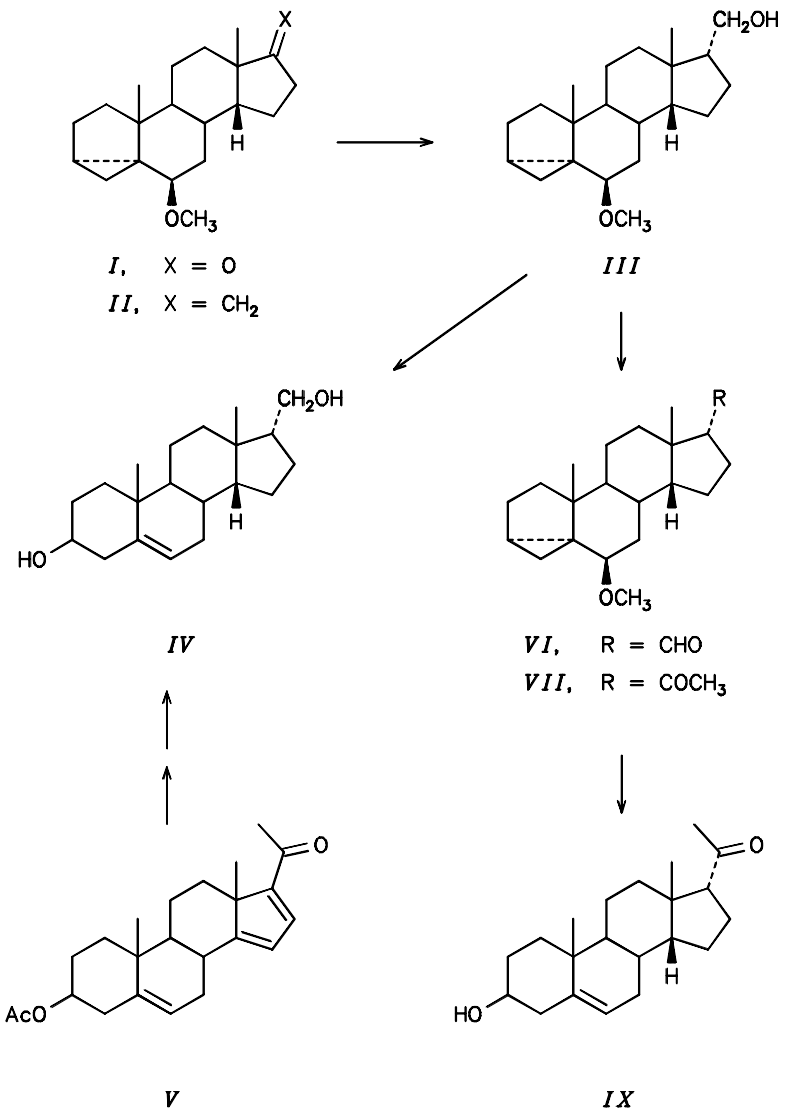
methylenation of *I* with triphenylphosphonium methylide and hydroboration of the resulting methylene derivative *II* which led to the 17α-hydroxymethyl derivative *III* (Scheme 1).



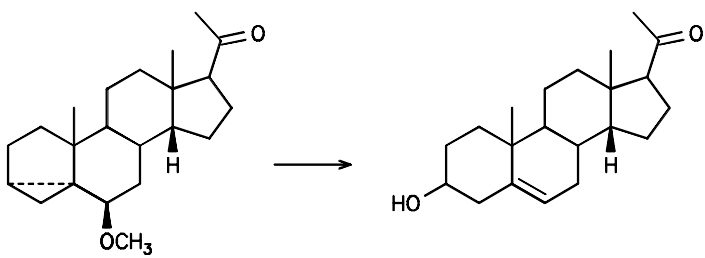
SCHEME 1

Originally we supposed that this derivative had 17β-configuration on the basis of a NOE ^{1}H NMR experiment (see Experimental) and in analogy with the stereospecificity of this reaction sequence in the case of 8α,9α,14β-1,3,5(10)-estratriene skeleton[5], also with C,D-*cis*-arrangement. However, a detailed analysis of ^{1}H and ^{13}C NMR spectra and transformation into the diol *IV*, which was compared with the authentic sample, proved definitely the configuration 17α. An independent synthesis of 21-nor-14β,17α-pregn-5-ene-3β,20-diol (*IV*) was accomplished[6] from 3β-acetoxypregna-5,14,16-trien-20-one[7] (*V*).

The compound *III* was converted into aldehyde *VI* by oxidation with chromium(VI) oxide–pyridine complex and the chain was elongated by addition of methylmagnesium iodide and subsequent reoxidation. The obtained 3α,5α-cyclo ketone *VII* was deprotected by treatment with perchloric acid to give the known[8] 14β,17α-pregnenolone *IX*. We isolated the isomeric 17β-ketone *VIII* (Scheme 2) present in crude *VII* as a minor side-product; due to similar chromatographic behavior several HPLC runs were necessary. Acid splitting of the i-steroid arrangement in ketone *VIII* gave 14β-pregnenolone *X*, physical constants and spectral data of which could be distinguished from those of the isomer *IX*. This comparison further supported the structural assignments.

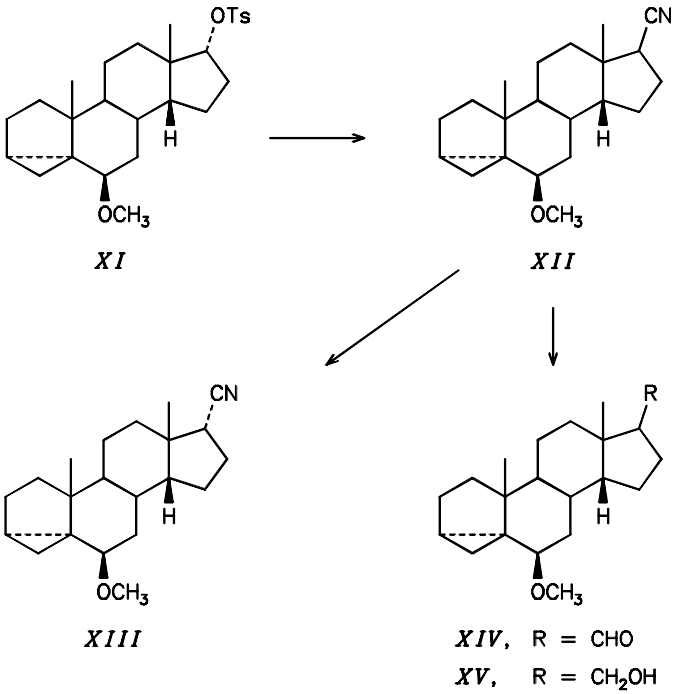
Variations of the side-chain elongation, consisting either in epoxidation of the methylene derivative *II* with 3-chloroperoxybenzoic acid or in methylenation of the ketone *I* with dimethyloxosulfonium methylide, subsequent diborane reduction of the resulting epoxides, and deprotection led in both cases to mixtures of the isomeric diols, in which the 17α-derivative *IV* prevailed.

In an alternative successful approach to the ketone *X* (Scheme 3) we subjected the tosylate *XI* to a nucleophilic reaction with sodium cyanide in hexamethylphosphoric triamide. The resulting nitrile *XII* was reduced with diisobutylaluminum hydride (DIBAH) to give the 17β-aldehyde *XIV*. Side-chain elongation was performed as described above, the obtained 17β-derivative *X* being identical with the minor product of the previous synthesis.



VIII *X*

SCHEME 2

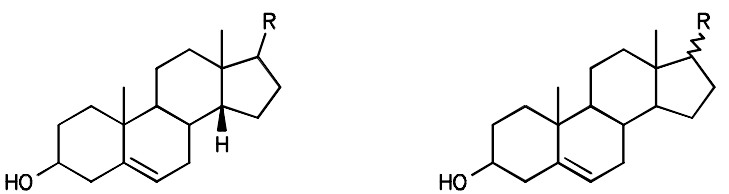


XI *XII*

XIII

XIV, R = CHO
XV, R = CH_2OH

SCHEME 3



XVI, R = CH_2OH
XVII, R = H

XVIII, R = α-$COCH_3$
XIX, R = β-$COCH_3$
XX, R = H

During the synthesis we carefully followed the epimeric purity of products. The cyanide substitution is highly stereospecific; we did not find any detectable amount (^{1}H NMR) of the isomeric 17α-derivative *XIII*. The authentic *XIII* was obtained by epimerization of *XII* with potassium *tert*-butoxide in boiling *tert*-butyl alcohol for 5 h. Neither DIBAH reduction nor Grignard reaction or chromium(VI) oxide oxidation in the presence of pyridine caused any isomerizations. Surprisingly, an attempt to transform directly nitrile *XII* into ketone *X* by reaction with methyllithium in benzene resulted (after deprotection) in a 1 : 1 mixture of 17α- and 17β- derivatives *IX* and *X*. The same procedure with 14β-hydroxy-3β-(tetrahydropyranyloxy)-5β-androst-9(11)-en-17β-yl cyanide was reported[4] to give pure 17β-isomer.

DISCUSSION

Mass Spectra

We studied mass spectra of isomers *IX* and *X* in comparison with the corresponding derivatives[8,9] *XVIII* and *XIX* of the 14α-series. EI mass spectra (Table I) of all the pregnenolone derivatives are very similar. In all cases, the base peak corresponds to the molecular ion, in the high mass region of the spectrum fragments arising by loss[10] of methyl group (301, M – 15), water (298, M – 18), or both (283, M – 33) are discernible. At lower masses, the spectra exhibit minor ions corresponding to rupture of the C-17/C-20 bond (273, M – 43), but their dehydrated equivalents (255, M – 61) are more abundant. The ions of *m*/*z* 265 probably suggest possible loss of methyl group and two molecules of water (M – 51). The ion of *m*/*z* 231 (M – 85) and its dehydrated equivalent of *m*/*z* 213 (M – 103) correspond to loss of a fragment containing atoms C-15, C-16, C-17, C-20, and C-21. In the lower mass region (see Experimental), an ion of *m*/*z* 71, arising from C-16, C-17, C-20 and C-21 of this fragment can be noted; in

TABLE I
Mass spectra (*m*/*z*) of isomeric pregnenolone derivatives *IX*, *X*, *XVIII*, and *XIX*; relative abundancies in %. For conditions and lower masses see Experimental

Compound	316 (M^+)	301 (M – 15)	298 (M – 18)	283 (M – 33)	273 (M – 43)	265 (M – 51)	255 (M – 61)	231 (M – 85)	213	205
IX	100	10	41	29	2	16	5	16	38	11
X	100	8	18	33	5	10	15	21	33	7
XVIII	100	3	10	26	0	9	6	17	45	6
XIX	100	14	48	38	5	9	13	41	25	21

both series this ion is more abundant for the 17α- isomer (cf. ref.[11]). However, the differences in abundance of particular ions are small and irregular to be of any diagnostic use; in general, the spectra of the 17α- and 17β-isomers are more similar in the 14β- than in the 14α-series.

NMR Spectra

The ^{13}C and ^{1}H NMR spectra of the same set of compounds as above (*IX*, *X*, *XVIII*, and *XIX*), together with androst-5-ene derivatives *XVII* and *XX*, were studied to get information about substituent effects of the 17α- and 17β-acetyl groups in the 14α- and 14β-androst-5-ene series and about possible changes in skeleton conformation. Full structural assignment of carbon atoms and protons, especially in the case of unusual 14β-derivatives, will represent a base for studies on more complex derivatives.

In the ^{13}C NMR spectral analysis we distinguished the type of carbon atoms (CH_3, CH_2, CH or C) by the "attached proton test" (APT) experiment, and by comparison of the data with those published on related compounds[12,13]. For all the four pregnenolone derivatives (*IX*, *X*, *XVIII*, and *XIX*) the assignment of C-15 and C-16 was confirmed by a $^{1}H,^{13}C$-heteronuclear multiple quantum coherence (2D-HMQC) experiment.

The carbon chemical shifts and substituent effects are summarized in Table II. The α-effects (21.01 to 23.44 ppm) and β-effects (2.32 to 4.89 ppm) are of limited diagnostic value. On the other hand, the γ-effects in positions 12, 14, and 18 reflect significantly the configuration at C-17 in both the 14α- and 14β-series. High negative values of substituent effects are caused by γ-*gauche* arrangement as in the case of C-12 in the 17α-derivatives *IX* and *XVII* (–4.37 and –3.63 ppm), C-18 in the 17β-derivatives *X* and *XIX* (–3.99 and –4.03 ppm), and C-14 in the 14β,17β-derivative *X* (–2.15 ppm) and in the 14α,17β-derivative *XVIII* (–5.37 ppm).

For ^{1}H NMR spectra of steroids the full analysis even on a high-field NMR spectrometer represents a complex task due to the huge amount of spin–spin interactions and overlapping multiplets. Strongly coupled systems may give higher-order splitting patterns. The problem was solved by the simultaneous use of several 1D- and 2D-NMR methods. In the 1D-NMR spectra of compounds *IX* – *XX* we identified the signals of methyl protons H-18, H-19, and H-20, olefinic proton H-6, allylic H-4, and the heteroatom-influenced protons H-3 and H-17 in the pregnenolone derivatives. Starting from these basic value sets, homonuclear correlation experiments (2D-COSY) enabled us to assign nearly all the remaining protons. Additional information was obtained from the 2D-J-resolved spectra. Structural resolution of protons, especially in the methylene groups, was achieved by 2D-HMQC, making use of ^{13}C NMR data obtained previously. Finally, difference 1D-NOE spectra, taken under irradiation of methyl protons H-18 and H-19, contributed to the stereochemical assignment of many β-protons in the influenced methylenes. This combination resulted in determination of all proton chemical shifts for the whole series under study (Table III).

TABLE II

Carbon-13 chemical shifts of isomeric pregnenolone derivatives *IX*, *X*, *XVIII*, *XIX* and androstane derivatives *XVII*, *XX* used as reference compounds for estimation of substituent effects of $COCH_3$ group

Carbon	Chemical shifts (in $CDCl_3$)						Substituent effect of $COCH_3$			
	14β-skeleton			14α-skeleton			14β-skeleton		14α-skeleton	
	IX	*X*	*XVII*	*XVIII*	*XIX*	*XX*	17α	17β	17α	17β
C-1	37.00	36.99	37.07	37.20	37.24	37.31	–0.07	–0.08	–0.11	–0.07
C-2	31.53	31.50	31.59	31.55	31.60	31.67	–0.06	–0.09	–0.12	–0.07
C-3	71.69	71.71	71.79	71.61	71.69	71.79	–0.10	–0.08	–0.18	–0.10
C-4	42.21	42.19	42.26	42.18	42.23	42.31	–0.05	–0.07	–0.13	–0.08
C-5	140.00	139.95	139.92	140.39	140.75	140.74	0.08	0.03	–0.35	0.01
C-6	121.81	122.10	122.20	121.62	121.38	121.73	–0.39	–0.10	–0.11	–0.35
C-7	30.27	30.78	30.80	31.98	31.76	32.18	–0.53	–0.02	–0.20	–0.42
C-8	29.64	29.32	29.69	32.01	31.84	32.14	–0.05	–0.37	–0.13	–0.30
C-9	42.71	42.71	42.93	50.51	49.97	50.42	–0.22	–0.22	0.09	–0.45
C-10	36.40	36.61	36.54	36.44	36.51	36.63	–0.14	0.07	–0.19	–0.12
C-11	21.01	20.98	21.50	21.00	21.08	21.12	–0.49	–0.52	–0.12	–0.04
C-12	27.52	34.81	31.89	35.06	38.83	38.69	–4.37	2.92	–3.62	0.14
C-13	43.89	44.66	40.25	45.46	43.99	40.57	3.64	4.41	4.89	3.42
C-14	50.93	47.12	49.27	49.47	56.90	54.84	1.66	–2.15	–5.37	2.06
C-15	23.50	25.36	25.40	26.00	24.47	25.61	–1.90	–0.04	0.60	–1.14
C-16	22.96	24.74	20.02	24.32	22.81	20.49	2.94	4.72	4.30	2.32
C-17	64.65	62.80	41.95	61.27	63.70	40.26	22.70	20.85	21.01	23.44
C-18	23.90	20.15	24.14	20.61	13.21	17.24	–0.24	–3.99	3.37	–4.03
C-19	19.52	19.43	19.52	19.34	19.37	19.43	0.00	–0.09	–0.09	–0.06
C-20	210.21	211.17	–	212.79	209.57	–	–	–	–	–
C-21	31.88	32.76	–	32.80	31.53	–	–	–	–	–

TABLE III

Proton chemical shifts of isomeric pregnenolone derivatives *IX*, *X*, *XVIII*, *XIX* and androstane derivatives *XVII*, *XX* used as reference compounds for estimation of substituent effects of $COCH_3$ group

Carbon	Chemical shifts (in $CDCl_3$)						Substituent effect of $COCH_3$			
	14β-skeleton			14α-skeleton			14β-skeleton		14α-skeleton	
	IX	*X*	*XVII*	*XVIII*	*XIX*	*XX*	17α	17β	17α	17β
H-1α	1.07	1.07	1.08	1.05	1.10	1.09	–0.01	–0.01	–0.04	0.01
H-1β	1.86	1.85[a]	1.88	1.82[a]	1.86	1.86	–0.02	–0.03	–0.04	0.00
H-2α	1.83[a]	1.83[a]	1.84[a]	1.80[a]	1.85	1.84	–0.01	–0.01	–0.04	0.01
H-2β	1.49	1.49[a]	1.50[a]	1.49	1.50[a]	1.50[a]	–0.01	–0.01	–0.01	0.00
H-3α	3.53	3.52	3.53	3.52	3.53	3.53	0.00	–0.01	–0.01	0.00
H-4α	2.30	2.30	2.30	2.29	2.31	2.30	0.00	0.00	–0.01	0.01
H-4β	2.24	2.23	2.24	2.22	2.24	2.24	0.00	–0.01	–0.02	0.00
H-6	5.37	5.37	5.37	5.34	5.36	5.36	0.00	0.00	–0.02	0.00
H-7α	1.80[a]	1.84[a]	1.83[a]	1.63	1.57	1.56[a]	–0.03	0.01	0.07	0.01
H-7β	1.83[a]	1.84[a]	1.83[a]	2.00	2.00	2.01	0.00	0.01	–0.01	–0.01
H-8β	1.84[a]	1.75[a]	1.81[a]	1.44	1.51[a]	1.41	0.03	–0.06	0.03	0.10
H-9α	1.17	1.20	1.16	0.92	0.99	0.96	0.01	0.04	–0.04	0.03
H-11α	1.51	1.50[a]	1.51[a]	1.58	1.64[a]	1.58[a]	0.00	–0.01	0.00	0.06
H-11β	1.28	1.40	1.36[a]	1.49	1.46[a]	1.46	–0.08	0.04	0.03	0.00
H-12α	1.37	1.50[a]	1.38[a]	1.17	1.44	1.15[a]	–0.01	0.12	0.02	0.29
H-12β	1.05	1.31	1.13	1.77	2.05	1.75	–0.08	0.18	0.02	0.30
H-14α	–	–	–	1.25[a]	1.15	0.90	–	–	0.35	0.25
H-14β	1.73	1.92	1.60[a]	–	–	–	0.13	0.32	–	–
H-15α	1.62[a]	1.83[a]	1.62[a]	1.80[a]	1.69	1.66[a]	0.00	0.21	0.14	0.03
H-15β	1.62[a]	1.61	1.56[a]	1.25[a]	1.24	1.18[a]	0.06	0.05	0.07	0.06
H-16α	2.10	1.78[a]	1.56[a]	1.92	1.64[a]	1.58[a]	0.54	0.22	0.34	0.06
H-16β	1.68	1.78[a]	1.56[a]	1.73	2.18	1.66[a]	0.12	0.22	0.07	0.52
H-17α	–	2.74	1.45[a]	–	2.54	1.44	–	1.29	–	1.10
H-17β	2.74	–	1.45[a]	2.81	–	1.16[a]	1.29	–	1.37	–
Me-18	1.26	1.01	1.02	0.93	0.63	0.72	0.24	–0.01	0.21	–0.09
Me-19	0.98	0.98	0.99	1.00	1.01	1.02	–0.01	–0.01	–0.02	–0.01
Ac-17	2.15	2.15	–	2.13	2.13	–	–	–	–	–

[a] Signal position was determined from 2D-spectra (COSY, J-resolved and/or HMQC).

Nevertheless, an attempt to obtain the complete set of coupling constants failed due to the presence of strongly coupled systems in nearly all the compounds and conformation analysis of ring D employing vicinal couplings was thus not feasible. The 14β-derivatives generally give less analyzable spectra with complex multiplets of the H-7α, H-7β, and H-8 and other protons on ring C and/or ring D. Couplings available for rings A, B, and C confirm the usual geometry of the steroid skeleton. Long range couplings over four single bonds were detected between equatorial protons 2α and 4 (ca 2.4 Hz), methyl protons H-19 and H-18, and axial H-1α and H-12α protons (0.5 – 0.8 Hz), and for the 14β-derivatives *IX*, *X*, and *XVII* also between the equatorial protons H-12β and H-14β (about 1.7 Hz). For the whole set of compounds, the allylic and homoallylic interactions ($J(4\beta,6) \approx 2$ and $J(4\beta,7\alpha) \approx 3$ Hz) are characteristic.

Concerning the substituent effects for the 17α- and 17β-acetyl groups (Table III) the α-effect in 14β-series is identical for the 17α- and 17β-substituents (1.29 ppm) whereas in the 14α-series the value is higher for the 17α-isomer (1.37 vs 1.10 ppm). The β-effects in position 16 are significant; the proton *cis*-oriented toward the 17-acetyl group shows mostly higher chemical shift (except in 14β-derivative *X* with equal substituent effects). Concerning the γ-effects, the only important ones are those for the methyl protons H-18 (>0.2 for 17α- and <0.1 for 17β-acetyl group in both series). The effects in more distant positions are generally lower than 0.08 ppm. The substituent effects, found for the 17β-acetyl group in the 14α-series, are in good agreement with the data published[14] for the androst-4-en-3-one skeleton.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany). Optical rotations were measured on a Perkin–Elmer 141 MC polarimeter at 25 °C. IR spectra were taken on a Perkin–Elmer PE 580 spectrometer (wavenumbers in cm^{-1}). Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer (energy of ionizing electrons 70 eV, ion source temperature 170 – 200 °C).

NMR spectra of compounds *II – IV*, *XII – XVI* were obtained with a Varian XL-200 spectrometer (^1H at 200 MHz, for compound *XV* also ^{13}C at 50.3 MHz) at room temperature in deuteriochloroform with tetramethylsilane as internal reference, unless stated otherwise. NMR spectra of compounds *IX*, *X*, *XVII – XX* were measured on a Varian UNITY-500 spectrometer (^1H at 500 MHz, ^{13}C at 125.7 MHz) under the same conditions. Chemical shifts (in ppm, δ-scale) and coupling constants (in Hz) were obtained by the first-order analysis. For the complete structural assignment of proton and carbon signals of compounds *IX – XX* the following additional NMR experiments were done: (i) Proton difference 1D-NOE spectra – from the first spectrum obtained with a selective irradiation of methyl protons H-18 (and/or H-19) during 6 s interval before the acquisition (acquisition time 4 s, 64 scans accumulated) a second spectrum obtained under the identical conditions but the off-resonance irradiation frequency was subtracted. (ii) Homonuclear 2D-COSY spectra[15] – sequence 90° – t_1 – 45°, typical parameters: spectral width 3 500 Hz, acquisition time 0.15 s, 4 transients for each of ca 300 increments, relaxation delay 1 s, data matrix zero filled to 1 024 × 1 024 data points, sinebell weighing function used for resolution enhancement in both dimensions. (iii) Homonuclear 2D-J-resolved spectra[16] – sequence 90° – $t_1/2$ – 180° – $t_1/2$, typical parameters: spectral width ca 3 500 Hz in F_2 (δ(H))

and 50 Hz in F_1 (J(H,H)) dimension, acquisition time 0.17 s, relaxation delay 1 s, 16 transients for each of 64 increments, data matrix zero filled to 2 048 × 256 data points, sinebell weighing function used for resolution enhancement in both dimensions. (iv) APT spectra[17] – used for distinguishing carbon signals according to the number of directly bonded protons. (v) 2D-HMQC spectra[18] were measured for the correlation between proton and protonated carbon signals under the following experimental conditions: indirect probe (5 mm) with decoupler channel tuned for ^{13}C (125.7 MHz) and equipped with 135 MHz band pass and 77 MHz reject filters, high pass filter (375 MHz) used in observe ^{1}H channel, the BIRD null delay optimized to 0.5 s, relaxation delay 1.6 s, proton pulse length 19 μs at spectral width 3 500 Hz, decoupler field strength γB_2 = 6 000 Hz used for decoupling during acquisition while γB_2 = 10 000 Hz was used for ^{13}C pulses (24 μs = 90°), ^{13}C spectral width 20 000 Hz, hypercomplex method[19] used for obtaining phase-sensitive spectra, 2 × 360 increments (32 scans for each) collected, data matrix zero filled to 2 048 × 1 024 data points, sinebell and shifted sinebell weighing functions used for resolution enhancement in F_2 and F_1 dimension, respectively.

Column chromatography was performed on silica gel (60 – 120 μm) or on neutral alumina (Reanal, activity II), thin-layer chromatography on silica gel G according to Stahl (ICN Biochemicals). Solutions in organic solvents were dried over anhydrous sodium sulfate and the solvents were evaporated in vacuo (about 2 kPa) on a rotary evaporator. Analytical samples were dried over phosphorus pentoxide at 40 °C/26 Pa for 12 h. The identity of samples prepared by different routes was checked by comparison of their IR and ^{1}H NMR spectra, thin-layer chromatography and mixture melting point determination.

6β-Methoxy-17-methylene-3α,5-cyclo-5α,14β-androstane (*II*)

To sodium hydride (50% suspension in mineral oil, 0.8 g, 16.7 mmol), prewashed under argon with light petroleum (3 × 5 ml), was added dimethyl sulfoxide (20 ml) and the mixture was stirred and heated at 65 °C for 1 h. After cooling to room temperature, triphenylmethylphosphonium iodide (6.68 g, 16.5 mmol) was added and the stirring was continued for further 30 min. Then a solution of ketone *I* (1.0 g, 3.31 mmol) in dimethyl sulfoxide (10 ml) was added and the mixture was stirred at 65 °C for 24 h. A part of dimethyl sulfoxide (ca 20 ml) was then distilled off under diminished pressure (oil pump), water was added (100 ml) and the product was extracted with ether (3 × 50 ml). After concentration in vacuo, the raw product was dried in vacuo at 50 °C and then chromatographed on an alumina column (180 g) in light petroleum–ether (20 : 1) mixture yielding 860 mg (86%) of oily methylene derivative *II*. ^{1}H NMR spectrum (200 MHz, tetrachloromethane, external lock): 4.60 bt, 2 H, J = 2.5 (H-20, H-20′); 3.25 s, 3 H (OCH_3); 2.67 bt, 1 H, J = 3 (H-6α); 1.13 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19). For $C_{21}H_{32}O$ (300.5) calculated: 83.94% C, 10.73% H; found: 84.07% C, 10.79% H.

6β-Methoxy-3α,5-cyclo-21-nor-5α,14β,17α-pregnan-20-ol (*III*)

A solution of methylene derivative *II* (0.82 g, 2.73 mmol) in tetrahydrofuran (30 ml) was cooled to –5 °C and under stirring sodium borohydride (516 mg, 13.6 mmol) was added. Boron trifluoride etherate (0.67 ml, 5.45 mmol) was added dropwise under argon at –5 °C and stirring was continued for 2 h at –5 to 0 °C and then for 30 min at room temperature. The mixture was cooled with ice and water (1 ml), 25% potassium hydroxide solution (25 ml), and 30% hydrogen peroxide (15 ml) were added dropwise in succession. The reaction was completed by stirring at 0 °C for 30 min and at room temperature for 20 min. The mixture was poured into saturated aqueous potassium hydrogen carbonate solution and the product was taken up in ether. After drying, the solvent was evaporated, the residue coevaporated with benzene and the product was chromatographed on a column of alumina

(150 g) in ether affording 745 mg (86%) of oily hydroxy derivative *III*. IR spectrum (tetrachloromethane): 3 635 (OH); 3 065, 3 000 (cyclopropane ring); 2 825 (OCH_3); 1 106, 1 092, 1 030, 1 023 (C–O). ^{1}H NMR spectrum (200 MHz): 3.75 dd, 1 H, J = 5.1, J'= 10.5 (H-20); 3.48 dd, 1 H, J = 8.0, J'= 10.5 (H-20′); 3.33 s, 3 H (OCH_3); 2.79 t, 1 H, J = 2.9 (H-6α); 1.10 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 0.64 dd, 1 H, J = 3.8, J'= 5.0 (H-4); 0.44 dd, 1 H, J = 8.0, J'= 5.0 (H-4′). NOE: irradiation at δ 3.48 induced 5% at δ 1.10, irradiation at δ 1.10 induced 5% at δ 3.48 and 3.5% at δ 3.75. For $C_{21}H_{34}O_2$ (318.5) calculated: 79.19% C, 10.76% H; found: 79.31% C, 10.70% H.

21-Nor-14β,17α-pregn-5-ene-3β,20-diol (*IV*)

The hydroxy derivative *III* (60 mg, 0.19 mmol) was stirred in acetone (5 ml) with a perchloric acid–water mixture (0.5 ml, 1: 1) at room temperature for 1 h. Then the mixture was diluted with chloroform and washed with aqueous sodium hydrogen carbonate. Organic phases were dried, evaporated, and the residue was crystallized from methanol. Diol *IV* (37 mg, 65%) had m.p. 171 – 173 °C; $[\alpha]_D$ −33° (*c* 0.2, chloroform). A sample prepared by an independent procedure from derivative *V* (cf. ref.[6]) had m.p. 173 – 175 °C, $[\alpha]_D$ −28° (*c* 0.2, chloroform). ^{1}H NMR spectrum (200 MHz): 5.36 m, 1 H, W = 12 (H-6α); 3.75 dd, 1 H, J = 5.0, J'= 10.4 (H-20); 3.49 m, 1 H, W = 32 (H-3); 3.47 dd, 1 H, J = 8.3, J'= 10.4 (H-20′); 1.08 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19). For $C_{20}H_{32}O_2$ (304.5) calculated: 78.90% C, 10.59% H; found: 79.03% C, 10.58% H.

6β-Methoxy-3α,5-cyclo-21-nor-5α,14β,17α-pregnan-20-al (*VI*)

Pyridine (1.9 ml, 23.5 mmol) was added dropwise under argon to a stirred suspension of chromium(VI) oxide (1.14 g, 11.4 mmol) in dichloromethane (70 ml). After stirring for 5 min, a solution of hydroxy derivative *III* (0.5 g, 1.57 mmol) in dichloromethane (5 ml) was introduced, the mixture was stirred for 15 min and then diluted with ether (50 ml). The insoluble parts were filtered off on a celite column, which was then washed with ether. The ethereal solution was washed with ice-cold 5% hydrochloric acid, water, and brine. After drying and evaporation, the product was subjected to chromatography on alumina (5 g) in ether. Yield 470 mg (95%) of crude aldehyde *VI*, used further without purification.

3β-Hydroxy-14β,17α-pregn-5-en-20-one (*IX*)

A solution of crude aldehyde *VI* (250 mg, 0.79 mmol) in ether (3 ml) was added dropwise to a solution of methylmagnesium iodide (prepared from Mg (125 mg, 5.1 mmol) and methyl iodide (0.35 ml, 5.6 mmol) in ether (5 ml)) and stirred at room temperature for 2 h under argon. Saturated aqueous ammonium chloride was added, the mixture was extracted with ether, and washed successively with 5% aqueous citric acid, saturated aqueous potassium hydrogen carbonate, and water. After drying and evaporation, the resulting mixture of 20-hydroxy derivatives (860 mg) in dichloromethane was added under stirring to complex of chromium(VI) oxide (1.9 g, 19 mmol) and pyridine (3.1 ml, 38.3 mmol) in dichloromethane (100 ml). Stirring was continued for 30 min at room temperature and the solids were filtered off on alumina layered with celite which was then washed with ether. The combined filtrates were washed with 5% aqueous citric acid, saturated aqueous potassium hydrogen carbonate, and water, dried and evaporated. The obtained mixture of ketones *VII* and *VIII* (230 mg, 88%) was separated by repeated HPLC on Lichrosorb Si column (2.5 × 30 cm) in toluene. A sample of ketone *VII* (80 mg, 0.24 mmol) was cleaved by stirring in acetone (5 ml) with a perchloric acid–water mixture (0.5 ml, 1 : 1) at room temperature for 30 min. Then the mixture was diluted with chloroform and washed with aqueous sodium hydrogen carbonate solution. Organic phase was dried, the solvent evaporated, and the residue crystallized from methanol. Ketone *IX* (50 mg, 65%)

had m.p. 206 – 208 °C (from 200 °C subl.); $[\alpha]_D$ –32° (*c* 0.8, chloroform). Mass spectrum, *m*/*z* (%): 187 (8), 176 (20), 171 (13), 161 (8), 159 (26), 158 (42), 157 (17), 147 (10), 146 (9), 145 (37), 143 (22), 133 (15), 131 (19), 121 (9), 120 (16), 119 (19), 117 (10), 107 (26), 105 (32), 95 (15), 93 (18), 91 (28), 85 (24), 81 (21), 79 (23), 77 (12), 71 (17), 67 (14), 55 (15), for the higher mass ions see Table I. A sample prepared by hydrogenation of 3β-acetoxypregna-5,14,16-trien-20-one (*V*) over 10% palladium on activated carbon and subsequent deacetylation had m.p. 208 – 209 °C (from 200 °C subl.); $[\alpha]_D$ –29° (*c* 1.0, chloroform), ref.[8] gives m.p. 204 – 205 °C; $[\alpha]_D$ –13.3° (*c* 0.7, chloroform).

3β-Hydroxy-14β-pregn-5-en-20-one (*X*)

A. A sample of ketone *VIII* (20 mg) from the foregoing experiment was deprotected by aqueous perchloric acid in acetone as mentioned above. The ketone *X* obtained was identical with the product prepared according to procedure *B* (NMR).

B. Crude aldehyde *XIV* (400 mg, 1.26 mmol) was treated as described for the preparation of the isomeric derivative *IX*, final chromatography afforded 320 mg (77%) of ketone *VIII*. The ketone *VIII* (140 mg) was then converted by the above-mentioned acid hydrolysis and chromatography on silica gel (30 ml) in benzene–acetone (20 : 1) into 101 mg (75%) of the title ketone *X*, m.p. 202 – 203 °C (acetone); $[\alpha]_D$ +50° (*c* 1.0, chloroform). Mass spectrum, *m*/*z* (%): 187 (9), 176 (6), 173 (12), 171 (8), 161 (9), 159 (16), 158 (11), 157 (9), 147 (10), 146 (6), 145 (24), 143 (12), 133 (13), 131 (13), 121 (8), 120 (11), 119 (14), 117 (8), 107 (26), 105 (26), 95 (12), 93 (15), 91 (22), 85 (12), 81 (15), 79 (17), 77 (9), 71 (8), 67 (9), 55 (11), for higher mass ions see Table I. For $C_{21}H_{32}O_2$ (316.5) calculated: 79.70% C, 10.19% H; found: 79.81% C, 10.17% H.

6β-Methoxy-3α,5-cyclo-5α,14β-androstane-17β-carbonitrile (*XII*)

Tosylate[1] *XI* (1.0 g, 2.18 mmol) was heated with sodium cyanide (2.0 g, 40.8 mmol) in hexamethylphosphoric triamide (10 ml) to 80 °C for 12 h. The reaction mixture was diluted with ethyl acetate, washed with brine, 10% aqueous iron(II) sulfate, and brine again, dried and evaporated. Chromatography of the product on alumina (30 g) in benzene–light petroleum–ether (10 : 10 : 1) gave 231 mg (34%) of nitrile *XII*, m.p. 115 – 116 °C; $[\alpha]_D$ –36° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 060 (cyclopropane ring); 2 820 (OCH_3); 2 235 (C≡N); 1 086, 1 102 (C–O). ^{1}H NMR spectrum (200 MHz): 3.34 s, 3 H (OCH_3); 2.80 t, 1 H, *J* = 2.9 (H-6α); 2.56 dd, *J* = 3.2, *J*′= 8.8 (H-17); 1.31 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-19). Mass spectrum, *m*/*z* (%): 313 (23, M^+); 298 (46, M – 15); 281 (52, M – 32); 258 (100, M – 55). For $C_{21}H_{31}NO$ (313.5) calculated: 80.46% C, 9.97% H, 4.47% N; found: 80.17% C, 9.99% H, 4.34% N.

Isomerization of Nitrile *XII*

Nitrile *XII* (25 mg, 0.08 mmol) in *tert*-butyl alcohol (1 ml) was refluxed with potassium *tert*-butoxide (ca 20 mg, 0.18 mmol) for 5 h. The solution was concentrated, the product partitioned between water and benzene, the organic layer was dried and the solvent was evaporated. From the spectrum of the raw product (ca 10% *XII*) the following data for *XIII* were obtained: ^{1}H NMR spectrum (200 MHz): 3.34 s, 3 H (OCH_3); 2.80 t, 1 H, *J* = 2.8 (H-6α); 2.52 t, 1 H, *J* = 9.9 (H-17); 1.19 s, 3 H (3 × H-18); 1.02 s, 3 H (3 × H-19).

6β-Methoxy-3α,5-cyclo-21-nor-5α,14β-pregnan-20-al (*XIV*)

A solution of 1.5 M disobutylaluminum hydride in toluene (2.5 ml, 3.75 mmol) was added at –76 °C to a solution of nitrile *XII* (513 mg, 1.64 mmol) in toluene (8 ml), and the mixture was stirred for

1 h at this temperature. Methanol (2 ml) was added, followed by water (1 ml), and stirring was continued at room temperature for 2 h. After dilution with ether, the solution was washed with 5% aqueous citric acid, saturated aqueous potassium hydrogen carbonate solution, and water, dried and evaporated. After coevaporation with benzene and drying in vacuo, 527 mg of oily aldehyde *XIV* was obtained and used in the next step without purification. ^{1}H NMR spectrum (200 MHz): 9.68 d, 1 H, J = 4 (H-20); 3.33 s, 3 H (OCH_3); 2.80 t, 1 H, $J \approx 4$ (H-6α); 1.16 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-19); 0.64 dd, 1 H, J = 3.8, J'= 5 (H-4); 0.45 dd, 1 H, J = 8, J'= 5 (H-4′).

6β-Methoxy-3α,5-cyclo-21-nor-5α,14β-pregnan-20-ol (*XV*)

The crude aldehyde *XIV* (prepared from 100 mg, 0.32 mmol of nitrile *XII*) was reduced in ethyl acetate (2 ml) with sodium borohydride (50 mg, 1.3 mmol). After 10 min at room temperature, the excess hydride was decomposed by several drops of acetic acid and the mixture was worked up as in the preparation of aldehyde *XIV*. Chromatography on silica gel (5 g) in benzene–acetone (100 : 1) yielded 81 mg (80%) of hydroxy derivative *XV*, m.p. 100 – 101 °C (acetone), $[\alpha]_D$ +82° (*c* 0.8, chloroform). IR spectrum (tetrachloromethane): 3 638 (OH); 3 061, 3 000 (cyclopropane ring); 2 820 (OCH_3); 1 102, 1 090, 1 027, 1 020 (C–O). ^{1}H NMR spectrum (200 MHz): 3.73 dd, 1 H, J = 5.6, J'= 10.4 (H-20); 3.39 dd, 1 H, J = 8.0, J'= 10.4 (H-20′); 3.33 s, 3 H (OCH_3); 2.79 t, 1 H, J = 2.8 (H-6α); 1.04 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 0.64 dd, 1 H, J = 3.8, J'= 5.0 (H-4); 0.44 dd, 1 H, J = 8.0, J'= 5.0 (H-4′). ^{13}C NMR spectrum: 33.54, 25.04, 21.49, 13.21, 43.42, 82.77, 35.82, 28.08, 39.96, 35.18, 22.60, 35.89, 42.22, 49.54, 24.15, 25.51, 52.32, 19.76, 19.14 (C-1 to C-19); 65.17 (CH_2OH); 56.60 (CH_3O). For $C_{21}H_{34}O_2$ (318.5) calculated: 79.19% C, 10.76% H; found: 79.31% C, 10.70% H.

21-Nor-14β-pregn-5-ene-3β,20-diol (*XVI*)

Hydroxy derivative *XV* (15 mg, 0.05 mmol) in acetone (1 ml) was treated with a perchloric acid–water mixture (0.1 ml, 1 : 1) for 1 h. Saturated aqueous potassium hydrogen carbonate (0.15 ml) was added and acetone was removed in a stream of nitrogen. After addition of water (1 ml), the product was taken up in chloroform, the solution was dried and concentrated to yield 10 mg (70%) of diol *XVI*, m.p. 175 – 178 °C (from 120 °C subl.), $[\alpha]_D$ +21° (*c* 0.2, chloroform). ^{1}H NMR spectrum (200 MHz): 5.38 m, 1 H, W = 12 (H-6); 3.73 dd, 1 H, J = 5.6, J'= 10.5 (H-20); 3.53 m, 1 H, W = 32 (H-3); 3.41 dd, 1 H, J = 7.9, J'= 10.5 (H-20′); 1.01 s, 3 H (3 × H-18); 0.99 s, 3 H (3 × H-19). For $C_{20}H_{32}O_2$ (304.5) calculated: 78.90% C, 10.59% H; found: 79.17% C, 10.61% H.

3β-Hydroxy-17α-pregn-5-en-20-one (refs[8,9], *XVIII*)

Mass spectrum, *m*/*z* (%): 187 (9), 176 (4), 173 (13), 171 (10), 161 (6), 159 (13), 158 (11), 157 (9), 147 (10), 146 (4), 145 (16), 143 (18), 133 (11), 131 (11), 121 (7), 120 (8), 119 (13), 117 (6), 107 (14), 105 (19), 95 (12), 93 (11), 91 (20), 85 (18), 81 (12), 79 (14), 77 (8), 71 (16), 67 (9), 55 (13), for higher mass fragments see Table I.

3β-Hydroxypregn-5-en-20-one (*XIX*)

Mass spectrum, *m*/*z* (%): 187 (17), 176 (3), 173 (12), 171 (9), 161 (21), 159 (22), 158 (8), 157 (9), 147 (18), 146 (7), 145 (27), 143 (13), 133 (16), 131 (14), 121 (12), 120 (15), 119 (16), 117 (10), 107 (26), 105 (29), 95 (16), 93 (19), 91 (26), 85 (21), 81 (16), 79 (20), 77 (10), 71 (8), 67 (12), 55 (37), for higher mass fragments see Table I.

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