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Synthesis of 16-Substituted 23,24-Dinorcholane Derivatives

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Abstract—16-Substituted 22,24-dinorcholanes with variously modified A and B rings were synthesized starting from 17-oxo steroids through 17-ethylidene derivatives via ene reaction and subsequent transformations at the Δ^{16}-bond.

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Recent discoveries of new steroids possessing a hydroxy group (or its derivatives) at C^{16}, primarily of steroids isolated from marine living species [1, 2], require development of procedures for their synthesis. We focused our efforts on the development of methods for preparation of steroid compounds modified via introduction of oxygen-containing functional groups into the 16-position.

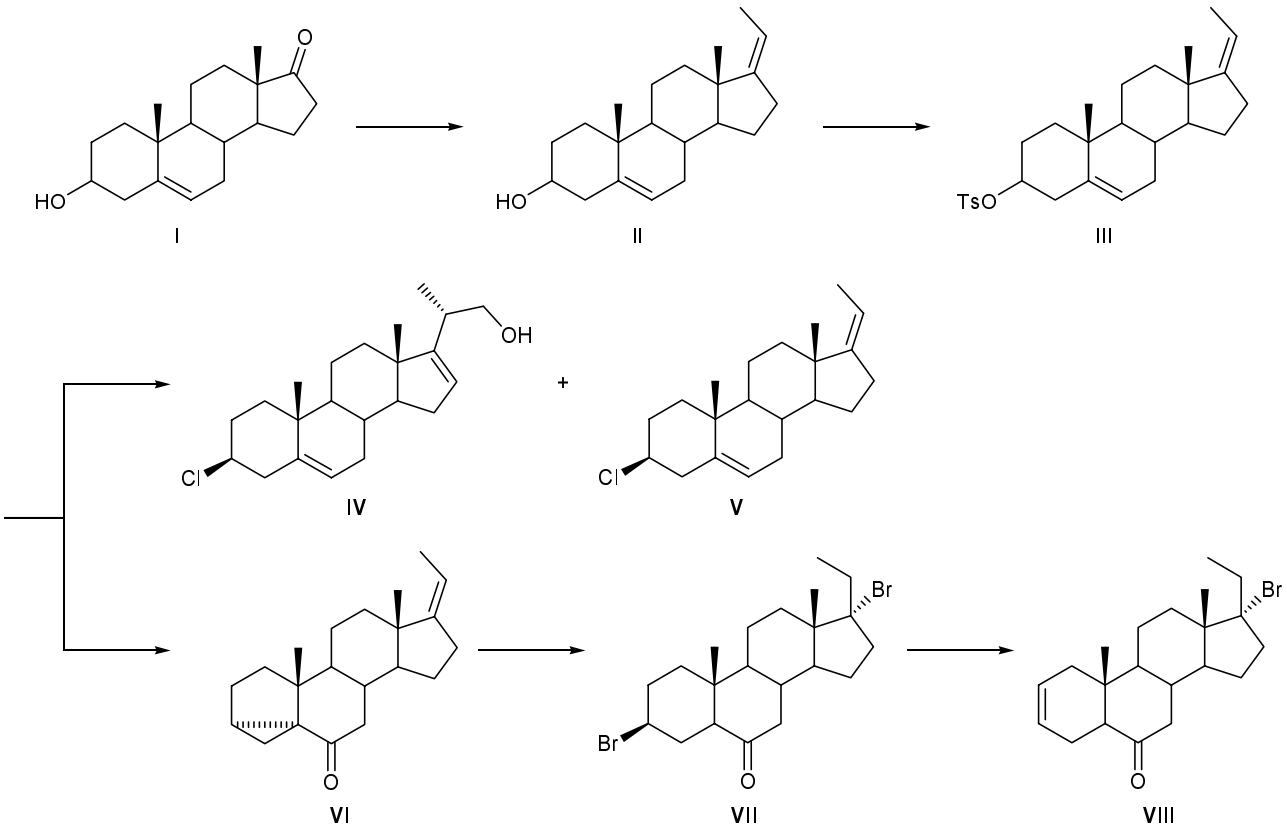
In the present article we describe the synthesis of 16-substituted steroid C^{22}-aldehydes and C^{22}-alcohols with variously modified A and B rings, which are convenient precursors of many natural polyhydroxy steroids and their analogs [3, 4]. As starting compound we selected an accessible 17-keto steroid, androstenolone, and the key stage in the synthesis of 23,24-dinorcholane derivatives was ene reaction involving 17-ethylidene steroids [5, 6].

17-Ethylidene derivative **II** was obtained in 93% yield by the Wittig reaction using potassium bis(trimethylsilyl)amide to generate the corresponding ylide from ethyltriphenylphosphonium bromide. Analysis of the ^{1}H NMR spectra and subsequent transformations of olefin **II** allowed us to refine previous assignment [7] of signals from protons on C^{20} (δ 5.14 ppm) and C^{21} (δ 1.64 ppm), thus bringing them into consistency with the data for 17-ethylidene steroids [6, 8]. The hydroxy group in **II** was protected by treatment with *p*-toluenesulfonyl chloride in pyridine with a view to use the resulting tosyloxy derivative in isosteroid rearrangement. Compound **III** was subjected to reaction with paraformaldehyde in methylene chloride in the presence of diethylaluminum chloride as catalyst. The product was Δ^{16}-22-hydroxy derivative **IV** (yield 74%). In addition, 3β-chloro-17-ethylidene steroid (**V**) was isolated as by-product; the latter was formed as a result of partial transformation only at the C^3 center (Scheme 1).

The structure of **IV** is confirmed by the IR spectrum containing an absorption band at 3450 cm^{-1} due to stretching vibrations of the hydroxy group. In the ^{1}H NMR spectrum of **IV** we observed a one-proton signal at δ 5.44 ppm from the vinyl proton on C^{16} and a two-proton multiplet at δ 3.58 ppm from the $C^{22}H_2$ group. The assumed structure of **IV** and **V** is also supported by comparing the shape and position of signals from the α-protons on C^3 with the corresponding signals of 3β-chloro derivatives which were prepared by us previously via opening of cyclopropane ring [9]. Our results suggest initial formation of 3β-chloro-17-ethylidene steroid **V** which is then involved in the ene reaction.

Further transformation of 3β-chloro derivatives into Δ^2-6-keto steroids with a view to subsequently introduce a brassino- or ecdysteroid functionality into the A and B rings is usually characterized by a poor yield. Therefore, we tried to effect such functionalization prior to ene reaction. For this purpose, *p*-toluenesulfonate **III** was heated with potassium acetate in boiling acetone, and the alcohol thus formed was oxidized *in situ* with chromic acid to obtain 3α,5-cyclo-6-oxo steroid **VI** (Scheme 1). Following the traditional procedure for opening of the three-membered ring by the action of acetic acid in the presence of hydrobromic acid [10], the formation of 3β-bromo derivative was accompanied by addition of hydrogen bromide at the C^{17}=C^{20} bond, while the subsequent dehydrobromina-

Scheme 1.



tion of **VII** by treatment with lithium carbonate in boiling dimethylformamide was not accompanied by bromine elimination from position *17*. As a result, compound **VIII** was isolated.

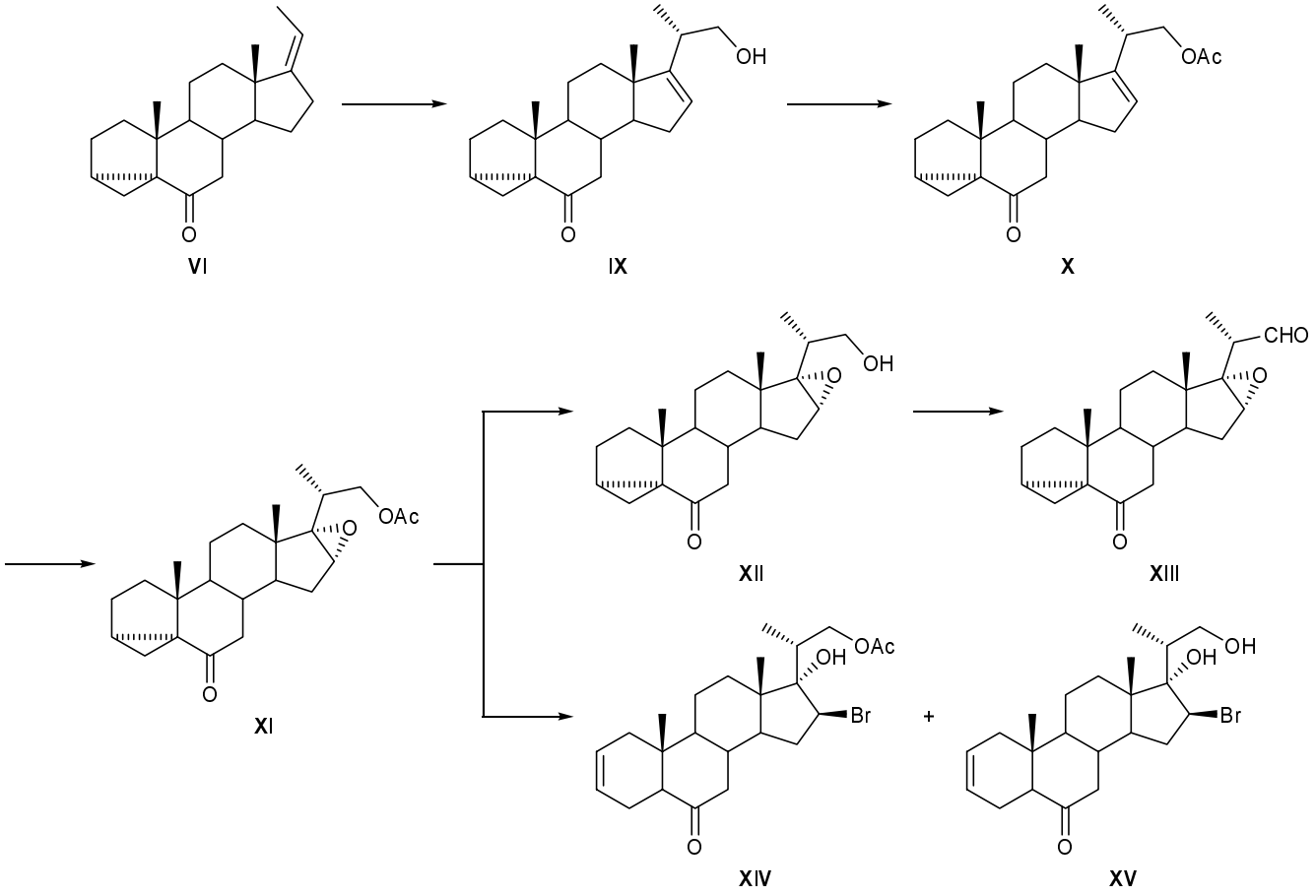
The ene reaction of olefin **VI** with paraformaldehyde in the presence of diethylaluminum chloride in methylene chloride afforded 68% of 22-hydroxy-Δ^{16} steroid **IX** (Scheme 2). The product characteristically showed in the IR spectrum an absorption band due to stretching vibrations of the hydroxy group. Its ^{1}H NMR spectrum contained a signal at δ 5.44 ppm from the olefinic proton on C^{16} and a two-proton multiplet from the $C^{22}H_2$ group at δ 3.60 ppm. The position and multiplicity of the 16-H and 22-H signals fully coincided with those observed for compound **IV**. Acetylation of **IX** with acetic anhydride in pyridine gave acetoxy derivative **X**. The IR spectrum of **X** lacked hydroxy group absorption, but absorption bands typical of ester moiety appeared. In addition, the multiplet signal from protons on C^{22} was displaced downfield (δ 3.90– 4.20 ppm).

The presence of double C^{16}=C^{17} bond in molecules **IX** and **X** provides the possibility for introducing substituents into the D ring. Oxidation of **X** with *m*-chloroperoxybenzoic acid (2 h at room temperature) led to formation of 16α,17α-epoxy derivative **XI** in 90% yield. In the ^{1}H NMR spectrum of **XI**, we observed no olefinic proton signal, and the 16-H signal appeared as a singlet at δ 3.37 ppm. The ^{13}C NMR spectrum of **XI** was also consistent with the assumed structure. The mass spectrum of **XI** contained a weak peak from the molecular ion, *m*/*z* 386 $[M]^+$, while the most abundant was fragment ion with *m*/*z* 326, which corresponds to elimination of acetic acid from $[M]^+$.

Hydrolysis of epoxy derivative **XI** with potassium hydroxide in methanol gave alcohol **XII** which characteristically showed an absorption band at 3420 cm^{-1} (OH) in the IR spectrum. By oxidation of alcohol **XII** with pyridinium chlorochromate in methylene chloride in the presence of potassium acetate we obtained 59% of aldehyde **XIII**. The aldehyde proton in **XIII** gave a doublet at δ 9.52 ppm (J = 3 Hz) in the ^{1}H NMR spectrum, and the IR spectrum of this compound contained absorption bands at 1735 and 1700 cm^{-1}, belonging to the aldehyde carbonyl group and ketone carbonyl at the 6-position.

Compound **XI** was subjected to isomerization involving the transformation of the 3α,5-cyclo-6-oxo

Scheme 2.

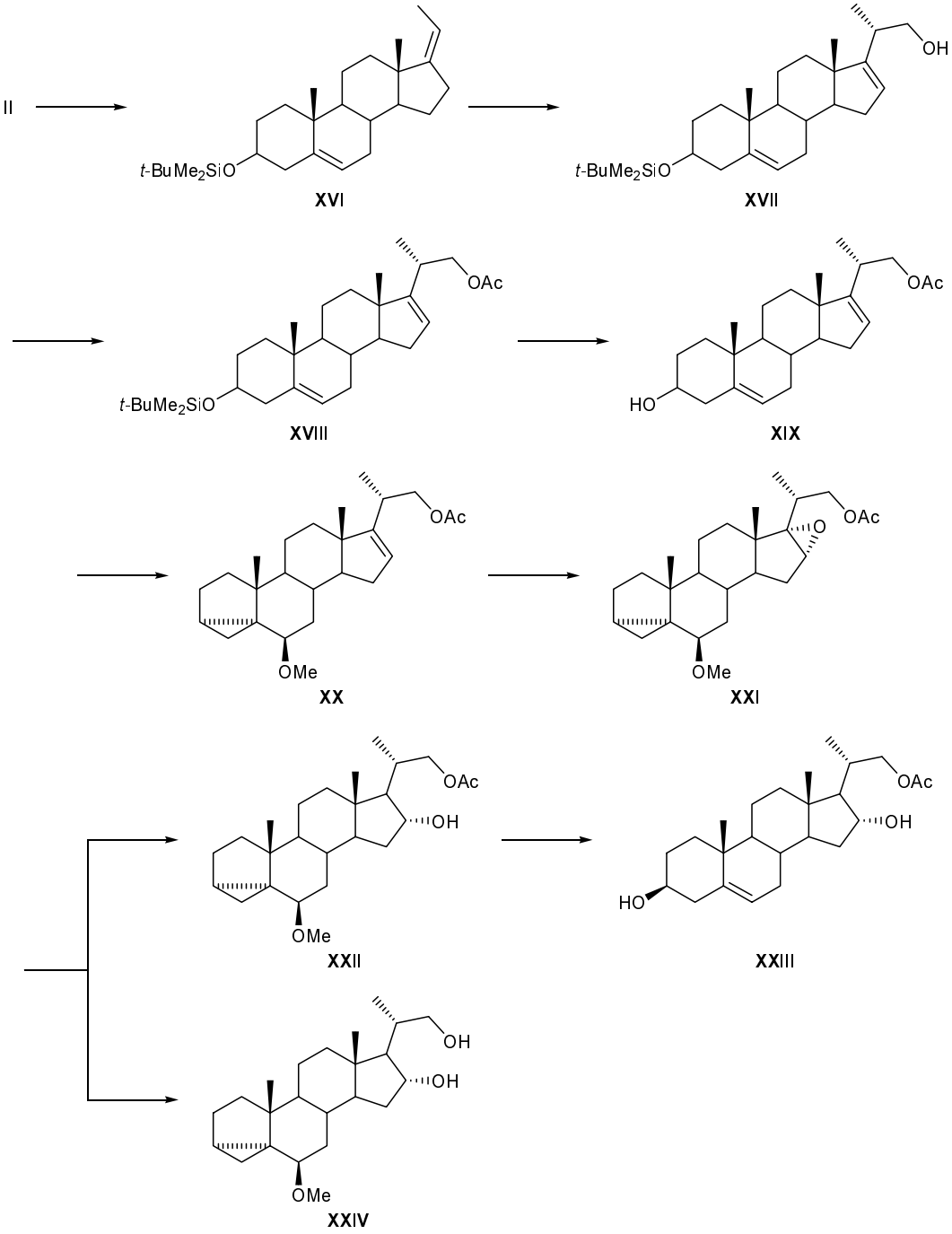


fragment into Δ^2-6-oxo moiety. For this purpose, steroid **XI** was heated with pyridine hydrobromide in boiling dimethylformamide [11]. The isomerization was accompanied by opening of the oxirane ring to afford Δ^2-6-oxo derivative **XIV** in 53% yield. In addition, 12% of diol **XV** was formed as by-product via hydrolysis of the 22-acetoxy group. The formation of double $C^2=C^3$ bond follows from the presence in the ^{1}H NMR spectra of **XIV** and **XV** of signals from 2-H and 3-H as two one-proton multiplets at δ 5.60 and 5.72 ppm and the absence of signals assignable to cyclopropane ring protons. The 16-H signal appeared as a one-proton triplet, while singlet from proton in epoxy ring was lacking, indicating opening of the oxirane ring with formation a bromohydrin fragment. This is consistent with published data on the synthesis of 17α-hydroxy-16β-bromo derivatives [12]. The most abundant ions detected in the mass spectrum of **XIV** were those corresponding to elimination of acetic acid and hydrogen bromide from the molecular ion (or of water and hydrogen bromide in the spectrum of **XV**). Compound **XIV** showed in the IR spectrum absorption bands due to acetate moiety and hydroxy group, and the IR spectrum of diol **XV** contained only a strong absorption band belonging to OH stretching vibrations.

We also tried to effect an analogous reaction sequence with various protected 3-hydroxy steroids. As an example, we examined reactions with *tert*-butyldimethylsilyl ether **XVI** which was synthesized by treatment of alcohol **II** with *tert*-butyl(chloro)dimethylsilane in the presence of imidazole. The IR spectrum of **XVI** contained no OH stretching vibration band, while a nine-proton singlet from the *tert*-butyl group (δ 0.92 ppm) and a six-proton singlet from the methyl groups on the silicon atom (δ 0.10 ppm) were present in the ^{1}H NMR spectrum.

The ene reaction of 17-ethylidene derivative **XVI** with paraformaldehyde in methylene chloride in the presence of boron trifluoride–ether complex as catalyst gave 77% of Δ^{16}-22-hydroxy steroid **XVII** which was treated with acetic anhydride in pyridine to obtain acetoxy derivative **XVIII** (Scheme 3). The structure of 22-hydroxy and 22-acetoxy-Δ^{16} steroids **XVII** and

Scheme 3.



XVIII was confirmed by the ^{1}H NMR spectra which contained a signal from olefinic proton on C^{16} at δ 5.46 ppm and a two-proton multiplet from the methylene protons on C^{22}. Compound **XVII** displayed in the IR spectrum an absorption band at 3430 cm^{-1} due to OH stretching vibrations, and the acetoxy group in **XVIII** gave rise to IR absorption bands at 1745 and 1240 cm^{-1}.

Compound **XVIII** possesses two differently protected hydroxy groups; therefore, selective deprotection of these groups may be possible. On the one hand, hydrolysis of the silyloxy group in the presence of a catalytic amount of *p*-toluenesulfonic acid gave steroid **XIX** having a 3-hydroxy-Δ^5 fragment which is convenient for further functionalization of the A and B rings in many polyhydroxy steroids. On the other

hand, protection of the Δ^5-3-hydroxy moiety makes it possible to modify the D ring and release the 22-hydroxy group for further transformations. This possibility was demonstrated using compound **XIX** as an example. It was subjected to isosteroid rearrangement in methanol, and 6β-methoxy-3α,5-cyclo derivative **XX** thus obtained was converted into 16α,17α-epoxy compound **XXI** (as described above for steroid **X**). Compound **XXI** was identified by the presence in its ^{1}H NMR spectrum of a signal at δ 3.33 ppm due to 16-H instead of olefinic proton signal. It was found that purification of **XXI** by chromatography on silica gel with an eluent containing ~1% of acetic acid is accompanied by opening of the oxirane ring with formation of 16-hydroxy-22-acetoxy derivative **XXII**.

The IR spectrum of **XXII** confirmed the presence of a hydroxy group in its molecule (ν_{OH} 3450 cm^{-1}), and the ^{1}H NMR spectrum of **XXII** contained a doublet of doublets typical of 16α-H in 16β-hydroxy steroids, while signal from proton in the epoxy ring (s, 1H) was absent. The observed reaction pattern indicates the possibility for modification at C^{16}, as well as of the A and B rings, specifically to restore the Δ^5-3β-hydroxy fragment, as in the synthesis of **XXIII**. The Δ^5-3β-hydroxy fragment in **XXIII** gives rise to signals at δ 5.36 (6-H) and 3.46 ppm (3-H) in the ^{1}H NMR spectrum.

Treatment of 16α,17α-epoxy derivative **XXI** with diborane (prepared by reaction of boron trifluoride with sodium tetrahydridoborate in tetrahydrofuran [13]) gave 97% of 16,22-diol **XXIV** as a result of oxirane ring opening contrary to the Krasuskii rule and simultaneous removal of the acetate protection. The ^{1}H NMR spectrum of **XXIV** contained a two-proton multiplet in the region δ 3.44–3.70 ppm, which may be assigned to the methylene protons on C^{22}. In addition, a doublet of doublets centered at δ 4.20 ppm was present (16α-H). The IR spectrum of this compound lacked ester absorption, but a strong band at 3450 cm^{-1} appeared due to OH stretching vibrations.

Thus the ene reaction of 17-ethylidene steroids obtained from the corresponding 17-oxo compound afforded a series of 23,24-dinorcholan-16-ene derivatives. Transformations of the latter and 16α,17α-epoxy steroids derived therefrom led to compounds having various substituents on C^{16}.

EXPERIMENTAL

The melting points were determined on a Kofler apparatus. The IR spectra were recorded on a UR-20 spectrometer in the range from 700 to 3600 cm^{-1}; samples were examined as neat substances (film) or pellets pressed with KBr. The UV spectra were measured on a Specord M-400 spectrophotometer from solutions in methanol. The mass spectra (electron impact, 70 eV) were run on a Micromass MasSpec instrument. The NMR spectra were obtained on a Bruker AC-E 200 spectrometer (200 MHz for ^{1}H) using chloroform-*d* as solvent and TMS as internal reference. The progress of reactions was monitored by TLC on Silufol UV-254 and Kieselgel 60 F_{254} (Merck) plates. Kieselgel 60 silica gel (40–60 μm, Merck) was used for preparative chromatography.

(17*Z*)-3β-Hydroxypregna-5,17(20)-diene (II). To a suspension of 5.12 g (12.3 mmol) of triphenylphosphonium iodide in 10 ml of tetrahydrofuran we added 20 ml (12 mmol) of a 0.5 M solution of potassium bis(trimethylsilyl)amide in 10 ml of toluene under stirring at room temperature. The mixture was stirred for 5 min and cooled to –78°C, and a solution of 1.2 g (4.1 mmol) of androstenolone (**I**) in 25 ml of anhydrous tetrahydrofuran was added. The cooling bath was removed, and the mixture was stirred for 1 h at room temperature, heated for 3 h under reflux in an argon atmosphere, and left overnight at room temperature. The mixture was diluted with 50 ml of ethyl acetate, washed with water and a saturated solution of sodium chloride, and dried over anhydrous sodium sulfate. The solvent was distilled off, and the residue was chromatographed on a column charged with silica gel (eluent petroleum ether–ethyl acetate, 3:1). Yield 1.17 g (93%), mp 118–120°C (from hexane–petroleum ether). IR spectrum (KBr), ν, cm^{-1}: 3450, 1450, 1380. ^{1}H NMR spectrum, δ, ppm: 0.91 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.64 d (3H, 21-Me, J = 8 Hz), 3.56 m (1H, 3-H), 5.15 m (1H, 20-H), 5.38 m (1H, 6-H). ^{13}C NMR spectrum, δ_C, ppm: 3.14 q, 16.64 q, 19.38 q, 21.26 t, 24.50 t, 31.46 d, 31.66 t, 31.74 t, 35.98 s, 36.60 t, 36.68 t, 37.00 t, 42.30 t, 44.05 s, 50.18 d, 56.55 d, 71.78 d, 113.50 d, 121.58 d, 140.81 s, 150.24 s. Mass spectrum, *m*/*z* (I_{rel}, %): 300 $[M]^+$ (10), 282 $[M - H_2O]^+$ (100), 267 $[M - H_2O - Me]^+$ (36).

(17*Z*)-3β-*p*-Tolylsulfonyloxypregna-5,17(20)-diene (III). Compound **II**, 0.53 g (1.77 mmol), was dissolved in 5 ml of pyridine, 1 g (5.3 mmol) of *p*-toluenesulfonyl chloride was added, and the mixture was left to stand for 24 h at room temperature. The mixture was then poured into 100 ml of water, and the precipitate was filtered off, dried under reduced pressure (water-jet pump), and recrystallized from methanol. Yield 0.548 g (71%), mp 137–138°C (from methanol). IR spectrum (KBr), ν, cm^{-1}: 3450, 1370.

^{1}H NMR spectrum, δ, ppm: 0.88 s (3H, 18-Me), 1.00 s (3H, 19-Me), 1.64 d (3H, 21-Me, J = 7 Hz), 4.32 m (1H, 3-H), 5.15 m (1H, 20-H), 5.38 m (1H, 6-H).

Ene reaction of compound III with paraformaldehyde in the presence of diethylaluminum chloride. To a suspension of 0.065 g (2.1 mmol) of paraformaldehyde in 3 ml of methylene chloride, stirred at –78°C under argon, we added 3.5 ml of a 1 M solution of diethylaluminum chloride in hexane. After 15 min, a solution of 0.24 g (0.55 mmol) of steroid **III** in 3 ml of methylene chloride was added under stirring, and the mixture was stirred for 2 h at –78°C, allowed to warm up to room temperature, and treated with 15 ml of a saturated solution of sodium hydrogen carbonate. The products were extracted into ethyl acetate, and the extract was washed with water and a saturated solution of sodium chloride, and dried over anhydrous sodium sulfate. The solvent was distilled off, and the residue was subjected to column chromatography on silica gel using toluene–ethyl acetate (10:1) as eluent to isolate 0.14 g (74%) of compound **IV** and 30 mg of **V**.

3β-Chloro-22-hydroxy-23,24-dinorchola-5,16-diene (IV). mp 105–107°C (from hexane). IR spectrum (KBr), ν, cm^{-1}: 3450, 1470, 1380, 1275, 1100, 1030, 810. ^{1}H NMR spectrum, δ, ppm: 0.82 s (3H, 18-Me), 0.98 d (3H, 21-Me, J = 7 Hz), 1.02 s (3H, 19-Me), 3.58 m (2H, 22-H), 3.78 m (1H, 3-H), 5.40 m (1H, 6-H), 5.44 br.s (1H, 16-H).

3β-Chloropregna-5,17(20)-diene (V). mp 103–104°C (from hexane). IR spectrum (KBr), ν, cm^{-1}: 1470, 1380, 1275, 1110, 1040. ^{1}H NMR spectrum, δ, ppm: 0.90 s (3H, 18-Me), 1.04 s (3H, 19-Me), 1.56 s (3H, 21-Me), 3.78 m (1H, 3-H), 5.14 m (1H, 20-H), 5.40 m (1H, 6-H).

(17*Z*)-3α,5-Cyclo-5α-pregn-17(20)-en-6-one (VI). Compound **III**, 0.6 g (1.37 mmol), was dissolved in 150 ml of acetone, and a solution of 0.6 g of potassium acetate in 5 ml of water was added. The mixture was heated for 7 h under reflux and cooled, 1.5 ml of 8 N chromic acid was added, and the mixture was kept for 20 min and diluted with 2 ml of isopropyl alcohol. The precipitate was filtered off and washed with 30 ml of acetone, the solvent was distilled off from the filtrate, and the residue was subjected to chromatography on silica gel using cyclohexane–ethyl acetate (9:1) as eluent to isolate 0.37 g (88%) of compound **VI** as an oily substance. IR spectrum (film), ν, cm^{-1}: 1700, 1470, 1380, 1300, 1170. ^{1}H NMR spectrum, δ, ppm: 0.74 t (1H, 3-H, J = 4 Hz), 0.94 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.66 d (3H, 21-Me, J = 8 Hz), 5.14 m (1H, 20-H). Mass spectrum, m/z (I_{rel}, %): 298 $[M]^+$ (100), 283 $[M - Me]^+$ (70), 267 $[M - H_2O - Me]^+$ (60).

3β,17α-Dibromopregnan-6-one (VII). Compound **VI**, 0.3 g (1 mmol), was dissolved in 8 ml of glacial acetic acid, and 0.8 ml of hydrobromic acid was added. The mixture was stirred for 1 h at room temperature, treated with 20 ml of water, and extracted with chloroform. The extract was washed with a saturated solution of sodium hydrogen carbonate and water and dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was chromatographed on a column charged with silica gel (eluent petroleum ether–ethyl acetate, 5:1). Yield 0.42 g (91%), oily substance. IR spectrum (film), ν, cm^{-1}: 1710, 1470, 1380. ^{1}H NMR spectrum, δ, ppm: 0.80 s and d (6H, 18-Me, 21-Me), 0.98 s (3H, 19-Me), 3.96 m (1H, 3-H).

17α-Bromo-5α-pregn-2-en-6-one (VIII). A mixture of 0.5 g (1.1 mmol) of compound **VII** and 0.8 g of lithium carbonate in 10 ml of dimethylformamide was heated for 3 h under reflux, cooled, and poured into 100 ml of water. The precipitate was filtered off, washed with water, and dissolved in chloroform, the solution was dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was chromatographed on a column charged with silica gel (eluent hexane–ethyl acetate, 5:1). Yield 0.32 g (78%), oily substance. IR spectrum (film), ν, cm^{-1}: 1720, 1470. ^{1}H NMR spectrum, δ, ppm: 0.72 s (3H, 18-Me), 0.98 s and d (6H, 19-Me, 21-Me), 5.60 m and 5.72 m (2H, 2-H, 3-H).

22-Hydroxy-3α,5-cyclo-23,24-dinorchol-16-en-6-one (IX). A solution of 0.5 g (1.67 mmol) of compound **VI** in 2 ml of methylene chloride was added to a suspension obtained from 0.13 g (4.2 mmol) of paraformaldehyde in methylene chloride and 7 ml of a 1 M solution of diethylalyuminum chloride in hexane, cooled to –78°C. The mixture was stirred for 3 h on cooling and for 12 h at room temperature, treated with 15 ml of a saturated solution of sodium hydrogen carbonate, and extracted with ethyl acetate. The extract was washed with a solution of sodium chloride and dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was subjected to column chromatography on silica gel using toluene–ethyl acetate (4:1) as eluent. Yield 0.38 g (68%), mp 142–143°C (from hexane–ethyl acetate). IR spectrum (film), ν, cm^{-1}: 3450, 1700, 1470, 1380, 1300, 1170. ^{1}H NMR spectrum, δ, ppm: 0.76 t (1H, 3-H, J = 4 Hz), 0.86 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.04 d (3H,

21-Me, J = 7 Hz), 3.60 m (2H, 22-H), 5.44 br.s (1H, 16-H).

22-Acetoxy-3α,5-cyclo-23,24-dinorchol-16-en-6-one (X). 22-Hydroxy derivative **IX**, 0.2 g (0.61 mmol), was dissolved in 1 ml of pyridine, 0.3 ml (3 mmol) of acetic anhydride was added, and the mixture was kept for 20 h at room temperature, treated with 10 ml of a saturated solution of ammonium chloride, and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid and dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was subjected to chromatography on silica gel using toluene–ethyl acetate (8:1) as eluent to isolate 0.21 g (95%) of compound **X** as an oily substance. IR spectrum (film), ν, cm^{-1}: 1745, 1700, 1470, 1380, 1240. ^{1}H NMR spectrum, δ, ppm: 0.75 t (1H, 3-H, J = 4 Hz), 0.84 s (3H, 18-Me), 1.05 s (3H, 19-Me), 1.08 d (3H, 21-Me, J = 7 Hz), 2.04 s (3H, OAc), 3.90–4.20 m (2H, 22-H), 5.44 br.s (1H, 16-H). Mass spectrum, m/z (I_{rel}, %): 310 $[M - AcOH]^+$ (100), 285 $[M - AcOH - Me]^+$ (95), 269 $[M - AcOH - H_2O - Me]^+$ (16).

22-Acetoxy-3α,5-cyclo-16α,17α-epoxy-23,24-dinorcholan-6-one (XI). Compound **X**, 0.12 g (0.33 mmol), was dissolved in 5 ml of methylene chloride, 0.06 g of *m*-chloroperoxybenzoic acid was added, and the mixture was stirred for 2 h at room temperature, treated with 10 ml of a saturated solution of sodium hydrogen carbonate, and extracted with chloroform. The extract was washed with a saturated solution of sodium chloride and dried over anhydrous sodium sulfate. The solvent was distilled off, and the residue was chromatographed on a column charged with silica gel using toluene–ethyl acetate (7:1) as eluent. Yield 0.112 g (90%), mp 158–160°C (from hexane–ethyl acetate). IR spectrum (KBr), ν, cm^{-1}: 1750, 1700, 1470, 1380, 1245. ^{1}H NMR spectrum, δ, ppm: 0.74 t (1H, 3-H, J = 4 Hz), 0.87 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.04 d (3H, 21-Me, J = 7 Hz), 2.05 s (3H, OAc), 2.50 m (1H, 20-H), 3.37 s (1H, 16-H), 3.69 d.d (1H, 22-H, J_1 = 9, J_2 = 11 Hz), 3.84 d.d (1H, 22-H, J_1 = 6, J_2 = 11 Hz). ^{13}C NMR spectrum, δ_C, ppm: 11.6 t, 13.6 q, 15.9 q, 19.7 q, 20.9 q, 22.5 t, 25.80 t, 27.4 t, 29.2 d, 32.8 t, 33.2 d, 33.4 t, 35.3 d, 42.7 s, 44.4 t, 45.5 d, 46.4 s, 46.4 d, 46.8 s, 59.9 d, 65.7 t, 70.8 s, 170.8 s, 208.9 s. Mass spectrum, m/z (I_{rel}, %): 386 $[M]^+$ (5), 326 $[M - AcOH]^+$ (100), 328 $[M - AcOH - H_2O]^+$ (35), 285 (16).

3α,5-Cyclo-16α,17α-epoxy-22-hydroxy-23,24-dinorcholan-6-one (XII). Compound **XI**, 0.22 g (0.57 mmol), was dissolved in 5 ml of a 4% solution of potassium hydroxide in methanol, and the solution was stirred for 2 h at room temperature, diluted with 20 ml of water, and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid and with water and dried over anhydrous sodium sulfate. The solvent was distilled off, and the residue was purified by recrystallization. Yield 0.19 g (97%), mp 138–140°C (from hexane–ethyl acetate). IR spectrum (KBr), ν, cm^{-1}: 3420, 1690, 1470, 1380. ^{1}H NMR spectrum, δ, ppm: 0.74 t (1H, 3-H, J = 4 Hz), 0.89 s (3H, 18-Me), 1.00 s (3H, 19-Me), 1.10 d (3H, 21-Me, J = 7 Hz), 3.48 d.d (1H, 22-H, J_1 = 6, J_2 = 11 Hz), 3.57 d.d (1H, 22-H, J_1 = 4, J_2 = 11 Hz), 3.55 s (1H, 16-H).

(20*S*)-3α,5-Cyclo-16α,17α-epoxy-6-oxo-5α-pregnane-20-carbaldehyde (XIII). A solution of 0.22 g (0.64 mmol) of compound **XII** in 2 ml of methylene chloride was added dropwise under stirring to a solution of 0.04 g of potassium acetate and 0.03 g of pyridinium chlorochromate in 3 ml of anhydrous methylene chloride. The mixture was stirred for 1 h at room temperature, diluted with 10 ml of ethyl acetate, and subjected to flash chromatography on silica gel to isolate 0.129 g (59%) of compound **XIII** as an oily substance. IR spectrum (film), ν, cm^{-1}: 2860, 1735, 1700, 1470, 1380, 1300. ^{1}H NMR spectrum, δ, ppm: 0.90 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.28 d (3H, 21-Me, J = 7 Hz), 2.96 m (1H, 20-H), 3.48 s (1H, 16-H), 9.52 d (1H, CHO, J = 3 Hz).

Reaction of 22-acetoxy-3α,5-cyclo-16α,17α-epoxy-23,24-dinorcholan-6-one (XI) with pyridine hydrobromide. Pyridine hydrobromide, 0.1 g, was added to a solution of 0.11 g (0.29 mmol) of compound **XI** in 3 ml of dimethylformamide. The mixture was heated for 1.5 h at the boiling point, poured into 20 ml of water, and extracted with ethyl acetate. The extract was washed with a saturated solution of sodium chloride and dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was subjected to chromatography on silica gel using petroleum ether–ethyl acetate (4:1) as eluent to isolate 0.063 g (53%) of compound **XIV** and 0.014 g (12%) of **XV**.

22-Acetoxy-16β-bromo-17α-hydroxy-23,24-dinorchol-2-en-6-one (XIV). Oily substance. IR spectrum (film), ν, cm^{-1}: 3460, 1740, 1720, 1250. ^{1}H NMR spectrum, δ, ppm: 0.70 s (3H, 18-Me), 1.06 s and d (6H, 19-Me, 21-Me, J = 7 Hz), 2.06 s (3H, OAc), 3.84 d.d (1H, 22-H, J_1 = 8, J_2 = 11 Hz), 4.12 t (1H, 16-H, J = 7 Hz), 4.36 d.d (1H, 22-H, J_1 = 3, J_2 = 11 Hz), 5.60 m and 5.72 m (2H, 2-H, 3-H). Mass spectrum, m/z (I_{rel}, %): 326 $[M - HBr - AcOH]^+$ (100), 311

$[M - HBr - AcOH - Me]^+$ (6), 308 $[M - HBr - AcOH - H_2O]^+$ (10), 284 $[M - HBr - 17\text{-}R]^+$ (32) (cleavage of the C^{17}–C^{20} bond), 269 $[M - HBr - 17\text{-}R - Me]^+$ (10).

16β-Bromo-17α,22-dihydroxy-23,24-dinorchol-2-en-6-one (XV). Oily substance. ^{1}H NMR spectrum, δ, ppm: 0.70 s (3H, 18-Me), 0.98 d (3H, 21-Me, J = 7 Hz), 1.18 s (3H, 19-Me), 3.35 t (1H, 16-H, J = 7 Hz), 3.90 d.d (1H, 22-H, J_1 = 6, J_2 = 8 Hz), 4.26 d.d (1H, 22-H, J_1 = 2, J_2 = 6 Hz), 5.60 m and 5.72 m (2H, 2-H, 3-H). Mass spectrum, m/z (I_{rel}, %): 326 $[M - HBr - H_2O]^+$ (100), 308 $[M - HBr - 2H_2O]^+$ (10), 284 $[M - HBr - 17\text{-}R]^+$ (34), 269 $[M - HBr - 17\text{-}R - Me]^+$ (22).

(17*Z*)-3β-(*tert*-Butyldimethylsiloxy)pregna-5,17(20)-diene (XVI). Compound **II**, 4 g (13 mmol), was dissolved in 50 ml of dimethyl sulfoxide, 2.6 g (39 mmol) of imidazole and 4 g (22 mmol) of *tert*-butyl(chloro)dimethylsilane were added, and the mixture was stirred for 3 h at room temperature, treated with 100 ml of water, and extracted with ethyl acetate. The extract was washed with a saturated solution of sodium chloride and dried over anhydrous sodium sulfate, and the solvent was distilled off to leave 5.5 g (96%) of oily compound **XVI** which was used in further syntheses without additional purification. ^{1}H NMR spectrum, δ, ppm: 0.10 s (6H, $SiMe_2$), 0.92 s (12H, 18-Me, *t*-Bu), 1.06 s (3H, 19-Me), 1.62 d (3H, 21-Me, J = 8 Hz), 3.52 m (1H, 3-H), 5.18 m (1H, 20-H), 5.38 m (1H, 6-H).

3β-*tert*-Butyldimethylsiloxy-22-hydroxy-23,24-dinorchola-5,16-diene (XVII). Boron trifluoride–ether complex, 0.015 ml, was added under argon to a suspension consisting of 0.4 g (1 mmol) of compound **XVI**, 0.2 g (5 mmol) of paraformaldehyde, and 5 ml of methylene chloride. The mixture was kept for 10 min at room temperature, treated with 50 ml of water, and extracted with methylene chloride. The extract was washed with a saturated solution of sodium hydrogen carbonate and dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was subjected to column chromatography on silica gel using toluene–ethyl acetate (4:1) as eluent to isolate 0.33 g (77%) of compound **XVII** as an oily substance. IR spectrum (film), ν, cm^{-1}: 3430, 1470, 1380, 1260, 1100. ^{1}H NMR spectrum, δ, ppm: 0.10 s (6H, $SiMe_2$), 0.83 s (3H, 18-Me), 0.92 s (9H, *t*-Bu), 1.05 d (3H, 21-Me, J = 7 Hz), 1.06 s (3H, 19-Me), 3.40–3.70 m (3H, 3-H, 22-H), 5.36 m (1H, 6-H), 5.46 br.s (1H, 16-H).

22-Acetoxy-3β-*tert*-butyldimethylsiloxy-23,24-dinorchola-5,16-diene (XVIII) was synthesized by the procedure described above for compound **X** from 0.07 g (0.15 mmol) of alcohol **XVII**. Yield 0.072 g (95%), oily substance. IR spectrum (film), ν, cm^{-1}: 1745, 1470, 1375, 1240. ^{1}H NMR spectrum, δ, ppm: 0.80 s (3H, 18-Me), 1.04 s (3H, 19-Me), 1.08 d (3H, 21-Me, J = 7 Hz), 2.04 s (3H, OAc), 3.54 m (1H, 3-H), 3.94–4.12 m (2H, 22-H), 5.40 m (2H, 6-H, 16-H).

22-Acetoxy-3β-hydroxy-23,24-dinorchola-5,16-diene (XIX). Compound **XVIII**, 0.052 g (0.11 mmol), was dissolved in 3 ml of acetone, a catalytic amount of *p*-toluenesulfonic acid was added, and the mixture was stirred for 3 h at room temperature and poured into 50 ml of water. The precipitate was filtered off and dried in air. Yield 0.034 g (86%), mp 119–120°C (from hexane–ethyl acetate). IR spectrum (KBr), ν, cm^{-1}: 3450, 3260, 1745, 1250. ^{1}H NMR spectrum, δ, ppm: 0.80 s (3H, 18-Me), 1.04 s (3H, 19-Me), 1.08 d (3H, 21-Me, J = 7 Hz), 2.04 s (3H, OAc), 3.54 m (1H, 3-H), 3.94–4.12 m (2H, 22-H), 5.40 m (2H, 6-H, 16-H).

22-Acetoxy-3α,5-cyclo-6β-methoxy-23,24-dinor-5α-chol-16-ene (XX). Compound **XIX**, 1.3 g (3.6 mmol), was dissolved in 20 ml of pyridine, 1.3 g (6.8 mmol) of *p*-toluenesulfonyl chloride was added, and the mixture was kept for 12 h at room temperature and was then carefully diluted with 100 ml of ice water. The precipitate was filtered off, dried in air, and dissolved in 6 ml of pyridine, 50 ml of methanol was added, and the mixture was heated for 3 h under reflux. The solvent was distilled off, and the residue was subjected to column chromatography using petroleum ether–ethyl acetate (8:1) as eluent to isolate 0.9 g (69%) of compound **XX** as an oily substance. ^{1}H NMR spectrum, δ, ppm: 0.44 m and 0.66 m (2H, 3-H, 4-H), 0.84 s (3H, 18-Me), 1.01 d (3H, 21-Me, J = 7 Hz), 1.02 s (3H, 19-Me), 2.03 s (3H, OAc), 2.79 t (1H, 6-H, J = 2 Hz), 3.34 s (3H, OMe), 3.92–4.12 m (2H, 22-H), 5.42 br.s (1H, 16-H).

22-Acetoxy-3α,5-cyclo-16α,17α-epoxy-6β-methoxy-23,24-dinor-5α-cholane (XXI). Compound **XX**, 1.3 g (3.3 mmol), was dissolved in 20 ml of anhydrous methylene chloride, 0.7 g (4 mmol) of *m*-chloroperoxybenzoic acid was added, and the mixture was stirred for 2 h at room temperature, washed with 10 ml of a saturated solution of sodium hydrogen carbonate, and dried over anhydrous sodium sulfate. The solvent was distilled off, and the residue was chromatographed on a column charged with silica gel using petroleum ether–ethyl acetate (4:1) as eluent. Yield 1.01 g (75%), oily substance. IR spectrum (film), ν, cm^{-1}: 1750, 1460, 1380, 1240, 1100. ^{1}H NMR spectrum, δ, ppm: 0.45 m and 0.68 m (2H, 3-H, 4-H), 0.88 s (3H,

18-Me), 1.03 d (3H, 21-Me, J = 8 Hz), 1.05 s (3H, 19-Me), 2.04 s (3H, OAc), 2.52 m (1H, 20-H), 2.79 t (1H, 6-H, J = 2 Hz), 3.34 s (4H, 16-H, OMe), 3.64–3.90 m (2H, 22-H).

22-Acetoxy-3α,5-cyclo-16α-hydroxy-6β-methoxy-23,24-dinor-5α-cholane (XXII). Column chromatography of 1 g (2.49 mmol) of compound **XXI** on silica gel using toluene–ethyl acetate (8:1) containing 1% of acetic acid as eluent gave 0.105 g (10%) of initial compound **XXI** and 0.81 g (81%) of compound **XXII** as an oily substance. IR spectrum (film), ν, cm^{-1}: 3460, 1740, 1465, 1380, 1250. ^{1}H NMR spectrum, δ, ppm: 0.45 m and 0.62 m (2H, 3-H, 4-H), 0.92 s (3H, 18-Me), 1.00 d (3H, 21-Me, J = 7 Hz), 1.02 s (2H, 19-Me), 2.00 s (3H, OAc), 2.76 t (1H, 6-H, J = 2 Hz), 3.27 s (3H, OMe), 3.70 d.d (1H, 22-H, J_1 = 9, J_2 = 11 Hz), 4.05 t.t (1H, 16-H, J = 8 Hz), 4.27 d.d (1H, 22-H, J_1 = 3, J_2 = 11 Hz).

22-Acetoxy-3β,16α-dihydroxy-23,24-dinorchol-5-ene (XXIII). Compound **XXII**, 0.02 g (0.05 mmol), was dissolved in 2 ml of a 3:1 dioxane–water mixture, the solution was heated to 60°C, the heating bath was removed, and the mixture was kept for 1 h at room temperature. It was then diluted with 5 ml of water and extracted with ethyl acetate, the extract was dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (4:1) as eluent. Yield 0.015 g (79%). IR spectrum (film), ν, cm^{-1}: 3430, 1730, 1470, 1380, 1255. ^{1}H NMR spectrum, δ, ppm: 0.90 s (3H, 18-Me), 0.98 d (3H, 21-Me, J = 7 Hz), 1.02 s (3H, 19-Me), 2.01 s (3H, OAc), 3.48 m (1H, 3-H), 3.65 d.d (1H, 22-H, J_1 = 9, J_2 = 11 Hz), 4.05 t (1H, 16-H, J = 7 Hz), 4.26 d.d (1H, 22-H, J_1 = 3, J_2 = 11 Hz), 5.36 m (1H, 6-H).

3α,5-Cyclo-16α,22-dihydroxy-6β-methoxy-23,24-dinor-5α-cholane (XXIV). Sodium tetrahydridoborate, 0.038 g, was added to a mixture of 0.08 g (0.24 mmol) of compound **XXI** and 0.5 ml of a 1 M solution of BH_3 in tetrahydrofuran, cooled to 0°C. The mixture was kept for 16 h in a refrigerator, 10 ml of ethyl acetate and 10 ml of water were added, and the mixture was extracted with ethyl acetate. The extract was washed with a saturated solution of sodium chloride and dried over anhydrous sodium sulfate, the solvent was distilled off, and the residue was subjected to column chromatography on silica gel using toluene–ethyl acetate (4:1) as eluent. Yield 0.07 g (97%), oily substance. IR spectrum (film), ν, cm^{-1}: 3450, 1640, 1430, 1380, 1100. ^{1}H NMR spectrum, δ, ppm: 0.45 m and 0.67 m (2H, 3-H, 4-H), 0.83 s (3H, 18-Me), 1.04 s and d (6H, 19-Me, 21-Me, J = 8 Hz), 2.80 t (1H, 6-H, J = 2 Hz), 3.34 s (3H, OMe), 3.44–3.70 m (2H, 22-H), 4.20 d.d (1H, 16-H, J_1 = 2, J_2 = 11 Hz).

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