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Synthesis of Analogs of Ecdysteroids Containing an Isoxaline Ring in the Side Chain

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Abstract—Starting with poststerone a synthesis was performed of ecdysteroid analogs containing an isoxaline ring in the side chain. The key stages of the route to the target products were Normant reaction and the subsequent 1,3-dipolar cycloaddition of nitrile oxides to 20-hydroxy-20-vinylsteroids.

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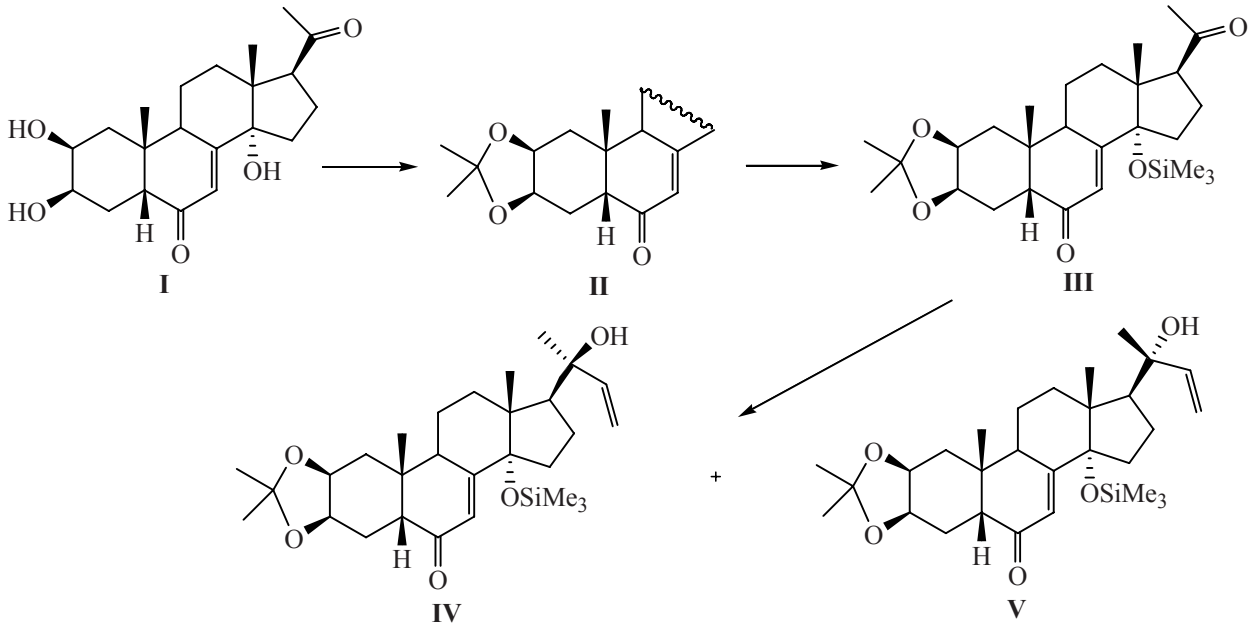
We reported previously on a new method of the side chains formation in natural ecdysteroids, in particular, as rare as ponasterone C and pterosterone, employing as key intermediate compounds steroid isoxalines [1]. In this study we made an attempt to synthesize this type intermediates (20-hydroxy-20-isoxalinylsteroids) with a cyclic part characteristic of ecdysteroids.

We chose for the initial compound poststerone (**I**), naturally occurring ecdysteroid that could also be prepared by a cleavage of 20,22-diol moiety of 20-hydroxyecdysone [2]. We planned to obtain a steroid dipolarophil utilizing the 20-oxo group along the Normant reaction. But the reaction of vinylmagnesium bromide with 20-oxo compound **I** and also with its 2,3-isopropylidenedioxy derivative **II** did not lead to the desired result. In both cases the initial compound was virtually totally recovered. We reached the desired result only after protecting the 14-hydroxy group by preparion of trimethylsilyl ether by a reaction of steroid alcohol **II** with trimethylsilyl triflate in the presence of 2,6-lutidine [3]. Silyloxy derivative **III**

Scheme 1.



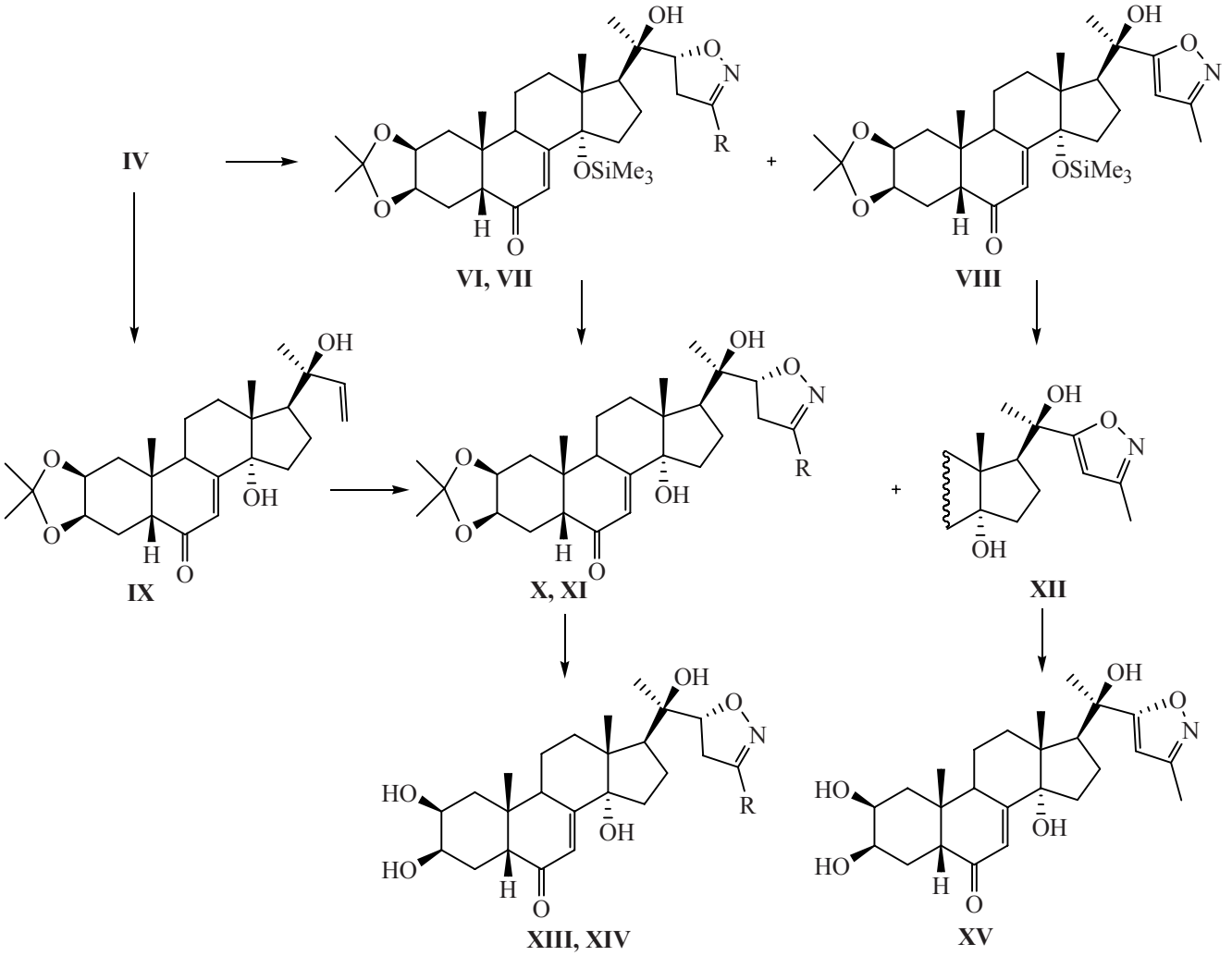
was obtained in 85% yield, and its formation was confirmed by the appearance in the ^{1}H NMR spectrum of a nine-proton singlet at δ 0.11 ppm and by the disappearance in the IR spectrum of the stretching vibrations band of the hydroxy group.

The reaction of 20-oxo compound **III** with vinylmagnesium bromide at boiling in tetrahydrofurane afforded in 65% yield a mixture of allyl alcohols **IV** and **V**. Their structure was confirmed by the presence in their ^{1}H NMR spectrum of signals characteristic of vinyl protons: two doublets of doublets centered at δ 5.00 (for 20*S*-isomer) or 5.01 (20*R*) ppm and 5.16 (for 20*S*-isomer) or 5.24 (20*R*) ppm corresponding to the protons attached to C^{23}, and a doublet of doublets at δ 5.99 (20*S*-isomer) or 6.00 (20*R*) ppm belonging to the proton linked to C^{22}. In the IR spectrum of the obtained allyl alcohols appeared the absorption band of the stretching vibrations of the hydroxy group at 3490 cm.

Compound **IV** was brought into the reaction of 1,3-dipolar cycloaddition with nitryl oxide generated by oxidation of isobutyraldoxime with N-chlorosuccinimide (NCS) by procedure described in [4]. As a result isoxaline **VI** was isolated in 88% yield. Its formation was confirmed by the appearance in the ^{1}H NMR spectrum of the signals from methylene protons as two doublets of doublets (δ 2.77, 2.85 ppm) and one-proton doublet of doublets (δ 4.47 ppm) characteristic of $H^{4'}$ and $H^{5'}$ protons of the isoxaline ring respectively. In the IR spectrum of compound **VI** an absorption band is present in the region 1680 cm^{-1} characteristic of a conjugated C=O bond.

The reaction proceeded with the formation of a single stereoisomer of *R*-configuration of the arising $C^{5'}$-chiral

Scheme 2.



R = $CHMe_2$ (**VI, X, XIII**), Me (**VII, XI, XIV**).

center. A reliable method for establishment of the atom $C^{5'}$ configuration in this type compounds proved to be the method of circular dichroism. An empirical octante rule relates the sign and the value of molecular ellipticity of the band of *n*-π* transition in the azomethine bond in the region 212–220 nm to the conformation of the isoxaline ring and its dissymmetrical surrounding [5]. The positive Cotton effect is characteristic of all 5'*S*-isomers of 20-isoxalinylsteroids, and the negative effect, of 5'*R*-isomers. It our case a negative Cotton effect was observed (–15 deg m^2 mol^{-1}) at 213 nm corresponding to the *R*-configuration of the center $C^{5'}$.

Similarly proceeded the reaction of compound **IV** with a nitrile oxide generated by the oxidation of acetaldoxime resulting in isoxaline **VII** in 68% yield. Same as in event of isobutyraldoxime a single epimer was obtained, 5'*R*-isoxaline, as showed the appearance in the ^{1}H NMR spectrum of methylene protons signals in the form of two doublets of doublets (δ 2.74 and 2.85 ppm) and a one-proton doublet of doublets (δ 4.50 ppm) characteristic of $H^{4'}$ and $H^{5'}$ protons of the isoxaline ring respectively. The IR spectrum of compound **VII** contained an absorp-tion band in the region 1620 cm^{-1} belonging to the stretch-ing vibrations of the C=N bond. In contrast to the rection with the isobutyronitrile oxide alongside 3'-methylisoxaline **VII** in this reaction formed also in 23% yield isoaxazole **VIII** as showed the presence in the ^{1}H NMR spectrum of the singlet belonging to the isoxazole proton (δ 5.90 ppm). The formation of similar compound was observed in the reaction with acetonitrile oxide in the series of pregnane derivatives, but the yield of the isoxazole was insignificant (5–10%) [4].

Similar stereoselectivity with the formation exclusively of 5'*R*-epimer on addition of the Δ^{22}-bond of steroids we had previously observed only twice: at the use of a bulky triazoline protection of the 5,7-diene moiety in the rings A, B [6], and in reaction of nitrile oxide with 1,2-disubstituted Δ^{22}-steroids [7]. In the latter case a low yield of adduct was observed and a low conversion of the initial allyl alcohol.

The stereoselectivity of the discussed cycloaddition may be due to spatial restrictions from the trimethylsilyl group in the position C^{14}. We tested this suggestion by carrying out the 1,3-cycloaddition of allyl alcohol **IX** with a free 14α-hydroxy group. The latter was obtained by deprotection from the silyl group of compound **IV** by treating with tetrabutylammonium fluoride in THF. It turned out that the 1,3-dipolar cycloaddition of isobutyronitrile oxide to compound **IX** with the deprotected 14α-hydroxy group gave in 74% yield also the single 5'*R*-epimer **X** with the center $C^{5'}$. The structure of the obtained isoxaline was proved by the appearance in the ^{1}H NMR spectrum of characteristic methine protons signals at $C^{5'}$ (δ 4.39 ppm), and of signals of methylene protons at $C^{4'}$ in the form of two doublets of doublets at δ 2.77 and 2.85 ppm in keeping with the rules we had previously established [8]. The reaction of compound **IX** with acetaldoxime occurred similarly to the reaction of 14-trimethylsilyloxy derivative and resulted in isoxaline **XI** and isoxazole **XII**.

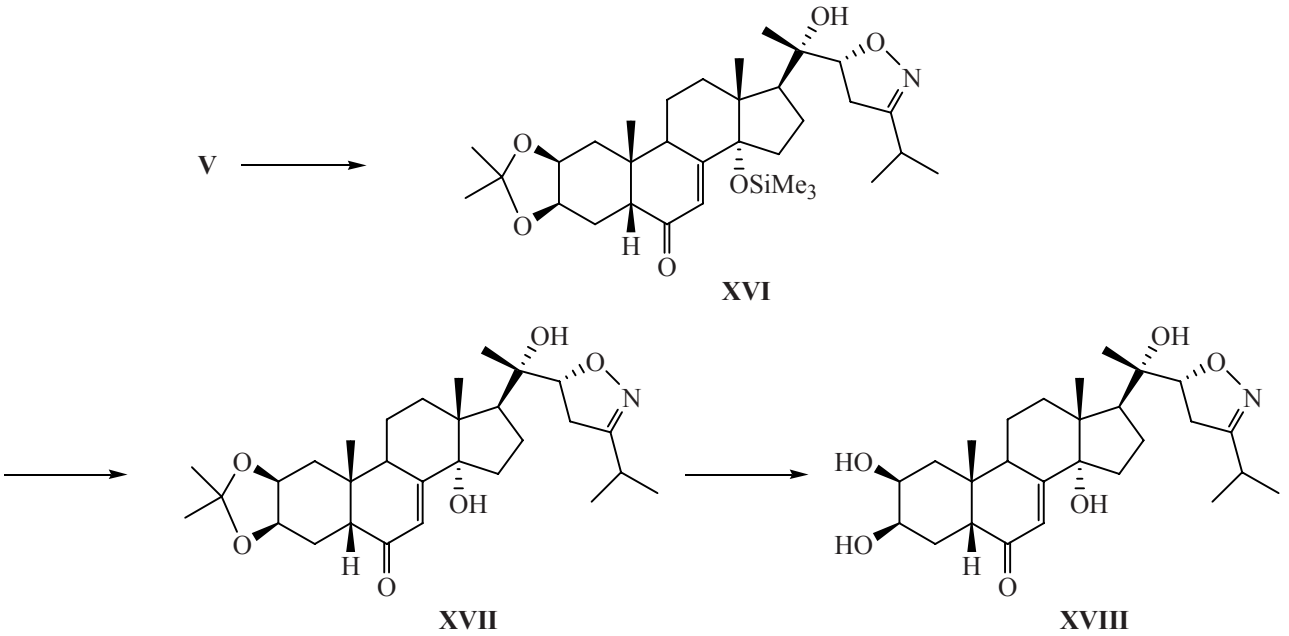
Compounds **X–XII** were also obtained from silyl ethers **VI–VIII** respectively by removing the silyl protection. This way is less favorable for the addition to the olefin with unprotected 14α-hydroxy group **IX** occurred faster and often with a better yield.

The last stage of the synthesis of natural ecdysteroids analogs was the removal of 2,3- isopropylidene protection by treating compounds **X–XII** with a 70% acetic acid solution. The formation of the corresponding tetraols **XIII–XV** was indicated by the appearance in the ^{1}H NMR spectra of the signals of methine protons attached to atoms C^2 and C^3 linked to the hydroxy groups (δ 3.76–3.80 and 3.88–3.92 ppm), by the absence of the protons signals of the methyl groups of the isopropylidene moiety, and also by the considerable complication and intensification of the absorption band of the stretching vibrations of hydroxy groups in the IR spectra.

It was expectable that the changed configuration at C^{20} in the allyl alcohol could essentially affect the stereochemistry of 1,3-dipolar cycloaddition of nitrile oxudes [9]. However the study of 20*R*-isomer **V** as dipolarophile in reaction with isobutyronitrile oxide showed that the cycloaddition also occurred stereoselectively giving exclusively 5'*R*-isoxazoline **XVI**. The most characteristic signals in the ^{1}HNMR spectrum of 20*S*-isomer of isoxaline **XVI** in contrast to its 20*R*-isomer **VI** are the proton signals of 18- and 21-methyl groups (downfield shift by 0.03 and 0.22 ppm respectively).

The successive removal of protective groups of compound **XVI** led to the formation of 20*R*-analogs of ecdysteroids with the isoxaline ring in the side chain **XVII** and **XVIII**. In the circular dichroism spectrum of isoxalinylsteroid **XVII** in the region of the *n*-π* transition of azomethine bond a negative band at 210 nm was observed with the molecular ellipticity –6 deg m^2 mol^{-1} evidencing the *R*-configuration of the formed chiral center $C^{5'}$.

Scheme 3.



The isoxazole derivatives of ecdysteroids obtained for the first time are promising with respect to the biological activity: their brassinosteroid analogs have exhibited high activity in growth regulation and adaptogenic activity [10].

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were registered on a spectrometer Bruker A-500 (at operating frequencies 500 and 125 MHz respectively) in deuterochloroform or deuteromethanol with TMS as internal reference. IR spectra were recorded on a spectrophotometer UR-20. Mass spectra were measured on an instrument Hewlett Packard-5890 under an electron impact (EI) or on an instrument AMD 402 Intectra with electrospray ionization (ESI) under the ionizing radiation energy 70 eV. UV spectra were taken on a spectrophotometer Specord UV VIS from methanol solutions. Circular dichroism spectra (CD) were recorded on a spectropolatimeter JASCO-20 from ethanol solutions. The reaction progress was monitored by TLC on Merck plates (Kieselgel 60 F_{254}). The chromatographic separation of the reaction mixtures was performed on silica gel 40/60 (Kieselgel 60, Merck). The melting points were measured on a Koeffler heating block.

(2β,3β,14α)-14-Hydroxy-2,3-isopropylidenedioxy-5β-pregn-7-ene-6,20-dione (II). A mixture of 545 mg (1.493 mmol) of steroid **I**, 30 ml of acetone, and 21 mg of *p*-toluenesulfonic acid was stirred for 24 h at room temperature, then it was treated with a saturated solution of sodium hydrogen carbonate, the reaction products were extracted into chloroform, the extract was dried with anhydrous sodium sulfate, evaporated, and the residue was subjected to chromatography on silica gel (eluent petroleum ether–ethyl acetate, 2:1). Yield 480 mg (83%), mp 186–187°C (petroleum ether–ethyl acetate). IR spectrum (KBr), ν, cm^{-1}: 3470, 1710, 1680. UV spectrum, λ_{max}, nm (ε): 240 (10 000). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.58 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.34 s (3H, CMe_2), 1.50 s (3H, CMe_2), 2.13 s (3H, 21-Me), 2.37 d.d (1H, C^{5}H, J_1 4.9, J_2 10.7 Hz), 3.15 d.d.d (1H, C^{9}H, J_1 2.2, J_2 6.6, J_3 11.4 Hz), 4.12 d.d (1H, C^{3}H, J_1 5.7, J_2 8.0 Hz), 4.20 d.d (1H, C^{2}H, J_1 4.5, J_2 8.8 Hz), 5.80 d (1H, C^{7}H, J 1.8 Hz).

(2β,3β,14α)-2,3-Isopropylidenedioxy-14-trimethylsilyloxy-5β-pregn-7-ene-6,20-dione (III). In anhydrous THF was dissolved 415 mg (1.041 mmol) of steroid alcohol **II**, to the obtained solution 0.15 ml (1.250 mmol) of 2,6-lutidine was added, the dropwise was added 0.3 ml (1.560 mmol) of trimethylsilyl triflate. The reaction mixture was stirred at room temperature for 30 min, then treated with water, the reaction products were extracted into ethyl ether, the extract was dried with anhydrous sodium sulfate, evaporated, and the residue was subjected to chromatography on silica gel (eluent petroleum ether–ethyl acetate, 3:1). Yield 380 mg (85%), mp 136–137°C (hexane–EtOAc). IR spectrum (KBr), ν, cm^{-1}: 1710, 1670. ^{1}H NMR spectrum ($CDCl_3$),

δ, ppm: 0.11 s (9H, $SiMe_3$), 0.55 s (3H, 18-Me), 1.04 s (3H, 19-Me), 1.33 s (3H, CMe_2), 1.50 s (3H, CMe_2), 2.13 s (3H, 21-Me), 2.37 d.d (1H, C^5H, J_1 4.9, J_2 10.7 Hz), 2.67 d.d.d (1H, C^9H, J_1 2.2, J_2 6.6, J_3 11.4 Hz), 3.13 t (1H, $C^{17}H$, J 8.5 Hz), 4.18 d.d (1H, C^2H, J_1 5.7, J_2 8.0 Hz), 4.26 d.d (1H, C^3H, J_1 4.5, J_2 8.8 Hz), 5.80 d (1H, C^7H, J 1.8 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.95 t.q, 16.65 q, 21.46 t, 21.61 t, 24.20 q, 26.25 t, 26.46 q, 28.66 q, 30.19 t, 30.44 t, 31.54 q, 36.62 d, 37.14 t, 37.49 s, 50.18 d, 50.37 s, 59.03 d, 71.54 d, 72.59 d, 88.03 s, 108.47 s, 122.48 d, 161.93 s, 201.86 s, 209.62 s.

Normant reaction with 20-oxosteroid III. Into a calcined three-neck flask in an argon flow was charged 67 mg (2.660 mmol) of magnesium, several iodine crystals were added, the flask was heated, then 2 ml of THF was poured, and dropwise was added 0.2 ml (2.836 mmol) of vinyl bromide in 3 ml of THF. The stirring was continued till the magnesium completely dissolved, then 320 mg (0.734 mmol) of carbonyl compound **III** in 3 ml of THF was added. The reaction mixture was stirred for 2 h at room temperature, then heated at reflux for 2 h. Afterwards it was treated with a saturated solution of ammonium chloride, the reaction products were extracted into ethyl acetate, and the extract was dried with anhydrous sodium sulfate. On removing the solvent the residue was subjected to chromatography on silica gel (eluent petroleum ether– ethyl acetate, 3:1).

(2β,3β,14α,20*S*)-20-Vinyl-20-hydroxy-2,3-isopropylidenedioxy-14-trimethylsilyloxy-5β-pregn-7-ene-6-one (IV). Yield 210 mg (60%), mp 148–150°C (hexane–EtOAc). UV spectrum, λ_{max}, nm (ε): 245 (11700). IR spectrum (KBr), ν, cm^{-1}: 3490, 1680. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.09 s (9H, $SiMe_3$), 0.74 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.33 s (3H, 21-Me), 1.33 s (3H, CMe_2), 1.50 s (3H, CMe_2), 2.36 d.d (1H, C^5H, J_1 4.8, J_2 11.2 Hz), 2.66 d.d.d (1H, C^9H, J_1 1.9, J_2 6.9, J_3 11.3 Hz), 4.18 t.d (1H, C^2H, J_1 5.7, J_2 8.5 Hz), 4.26 d.d (1H, C^3H, J_1 4.6, J_2 8.1 Hz), 5.00 d.d (1H, $C^{23}H$, J_1 0.9, J_2 10.7 Hz), 5.16 d.d (1H, $C^{23}H$, J_1 0.9, J_2 17.3 Hz), 5.78 d (1H, C^7H, J 2.1 Hz), 5.99 d.d (1H, $C^{22}H$, J_1 10.7, J_2 17.3 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.913 q, 17.29 q, 21.23 t, 22.04 t, 24.08 q, 26.36 t, 26.48 q, 28.65 q, 29.36 q, 29.60 t, 31.45 t, 36.10 d, 37.30 t, 37.56 s, 49.52 s, 50.40 d, 53.50 d, 72.60 d, 71.63 d, 75.47 s, 88.08 s, 108.41 s, 110.83 t, 121.93 d, 145.95 d, 163.22 s, 202.22 s.

(2β,3β,14α,20*R*)-20-Vinyl-20-hydroxy-2,3-isopropylidenedioxy-14-trimethylsilyloxy-5β-pregn-7-ene-6-one (V). Yield 17 mg (5%). UV spectrum, λ_{max}, nm (ε): 245 (10170). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.09 s (9H, $SiMe_3$), 0.67 s (3H, 18-Me), 1.00 s (3H, 19-Me), 1.23 s (3H, 21-Me), 1.32 s (3H, CMe_2), 1.49 s (3H, CMe_2), 2.36 d.d (1H, C^5H, J_1 4.7, J_2 11.2 Hz), 2.66 d.d.d (1H, C^9H, J_1 2.1, J_2 6.9, J_3 9.2 Hz), 4.19 t.d (1H, C^2H, J_1 5.8, J_2 8.6 Hz), 4.26 d.d (1H, C^3H, J_1 4.7, J_2 8.5 Hz), 5.01 d.d (1H, $C^{23}H$, J_1 1.2, J_2 9.6 Hz), 5.21 d.d (1H, $C^{23}H$, J_1 1.2, J 17.3 Hz), 5.79 d (1H, C^7H, J 2.1 Hz), 6.00 d.d (1H, $C^{22}H$, J_1 10.7, J_2 17.3 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.86 t.q, 16.50 q, 21.06 t, 21.16 t, 24.01 q, 26.24 t, 26.40 q, 28.57 q, 29.38 t, 30.54 s, 30.67 q, 36.12 d, 37.15 t, 37.50 s, 49.40 s, 50.28 d, 52.99 d, 71.54 d, 72.52 d, 75.19 s, 88.03 s, 108.41 s, 110.04 t, 121.84 d, 146.10 d, 163.24 s, 202.18 s.

(2β,3β,14α,20*R*,5'*R*)-20-Hydroxy-20-(3'-isopropylisoxalin-5'-yl)-14-trimethylsilyloxy-2,3-isopropylidenedioxy-5β-pregn-7-ene-6-one (VI). To 2 ml of chloroform containing several drops of pyridine 200 mg (1.5 mmol) of NCS was added, and then dropwise was added 130 mg (1.5 mmol) of isobutyraldoxime, the mixture was stirred for 15 min till a transparent solution formed. To the reaction mixture 150 mg (0.299 mmol) of steroid **IV** in 3 ml of chloroform was added, then dropwise within 4 h was added 0.17 g of triethylamine in 3 ml of chloroform. The mixture formed was stirred for 12 h and then treated with water. The reaction products were extracted into ethyl acetate, the extract was washed with a saturated NaCl solution and dried with anhydrous sodium sulfate. On removing the solvent the residue was subjected to chromatography in silica gel (eluent petroleum ether–ethyl acetate, 5:1). Yield 150 mg (88%), mp 154–156°C (hexane–EtOAc). CD spectrum, λ_{max}, nm ([θ], deg m^2 mol^{-1}): 213 (–15). UV spectrum, λ_{max}, nm (ε): 217 (780), 245 (8400). IR spectrum (KBr), ν, cm^{-1}: 3450, 1680, 1620. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.09 s (9H, $SiMe_3$), 0.76 s (3H, 18-Me), 1.03 s (3H, 19-Me), 1.18 d (6H, $CHMe_2$, J 6.9 Hz), 1.33 s (3H, 21-Me), 1.34 s (3H, CMe_2), 1.50 s (3H, CMe_2), 2.36 d.d (1H, C^5H, J_1 4.7, J_2 11.3 Hz), 2.66 m (1H, C^9H), 2.77 d.d (1H, $C^{4'}H$, J_1 8.6, J_2 17.1 Hz), 2.85 d.d (1H, $C^{4'}H$, J_1 10.9, J_2 17.1 Hz), 4.17 t.d (1H, C^2H, J_1 5.7, J_2 8.7 Hz), 4.25 d.d (1H, C^3H, J_1 4.6, J_2 8.2 Hz), 4.47 d.d (1H, $C^{5'}H$, J_1 8.7, J_2 10.8 Hz), 5.78 d (1H, C^7H, J 2.1 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.99 t.q, 16.92 q, 20.17 q, 20.20 q, 20.39 q, 21.15 t, 21.26 t, 24.11 q, 26.36 t, 26.50 q, 28.67 q, 28.07 d, 29.66 t, 31.43 t, 36.15 t, 36.15 br.d, 37.25 t, 37.54 s, 49.56 s, 50.19 d, 50.39 d, 71.62 d, 72.60 d, 75.68 s, 85.51 d, 87.97 s, 108.46 s, 122.07 d, 162.90 s, 163.94 s, 202.25 s.

Likewise from 65 mg (0.248 mmol) of olefin **IV** and acetaldoxime was obtained 50 mg (68%) of isoxaline **VII** and 17 mg (23%) of isoxazole **VIII**.

(2β,3β,14α,20*R*,5'*R*)-20-Hydroxy-2,3-isopropylidenedioxy-20-(3'-methylisoxalin-5'-yl)-14-trimethylsilyloxy-5β-pregn-7-ene-6-one (VII). mp 239–241°C (hexane–ethyl acetate). UV spectrum, λ_{max}, nm (ε): 240 (17290). IR spectrum (KBr), ν, cm^{-1}: 3450, 1680, 1620. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.08 s (9H, $SiMe_3$), 0.77 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.15 s (3H, 21-Me), 1.33 s (3H, CMe_2), 1.50 s (3H, CMe_2), 1.98 s (3H, 3'-Me), 2.74 d.d (1H, $C^{4'}H$, J_1 8,7, J_2 17.3 Hz), 2.85 d.d (1H, $C^{4'}H$, J_1 10.9, J_2 17.2 Hz), 4.17 m (1H, C^3H), 4.25 m (1H, C^2H), 4.50 d.d (1H, $C^{5'}H$, J_1 8.8, J_2 10.9 Hz), 5.79 d (1H, C^7H, J 2.1 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.98 t.q, 13.34 q, 16.87 q, 20.30 q, 21.17 d.t, 24.11 q, 26.36 q, 26.50 t, 28.67 q, 29.66 t, 31.45 t, 36.12 s, 37.35 t, 37.54 s, 39.93 t, 49.64 s, 50.04 d, 50.37 d, 71.61 d, 72.96 d, 75.71 s, 85.85 d, 87.96 s, 108.45 s, 122.08 d, 155.75 s, 162.89 s, 202.24 s.

(2β,3β,14α,20*R*)-20-Hydroxy-2,3-isopropylidenedioxy-20-(3'-methylisoxazol-5'-yl)-14-trimethylsilyloxy-5β-pregn-7-ene-6-one (VIII). mp 141–142°C (hexane–ethyl acetate). UV spectrum, λ_{max}, nm (ε): 240 (10290). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.08 s (9H, $SiMe_3$), 0.65 s (3H, 18-Me), 1.01 s (3H, 19-Me), 1.33 s (3H, CMe_2), 1.49 s (3H, CMe_2), 1.62 s (3H, 21-Me), 2.27 s (3H, 3'-Me), 2.36 d.d (1H, C^5H, J_1 4.7, J_2 11.4 Hz), 2.68 m (1H, C^9H), 4.18 t.d (1H, C^2H, J_1 5.6, J_2 8.7 Hz), 4.26 d.d (1H, C^3H, J_1 4.5, J_2 8.0 Hz), 5.77 d (1H, C^7H, J 2.0 Hz), 5.96 s (1H, $C^{4'}H$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.78 t.q, 11.47 q, 16.28 q, 21.00 t, 21.66 t, 23.93 q, 26.21 t, 26.35 q, 28.52 q, 28.59 q, 29.38 t, 30.97 t, 35.96 br.d, 37.14 t, 37.42 s, 49.09 s, 50.26 d, 53.34 d, 71.50 d, 72.42 d, 74.30 s, 87.66 s, 100.70 d, 108.31 s, 122.00 d, 159.51 s, 162.60 s, 178.03 s, 202.05 s.

(2β,3β,14α,20*S*)-20-Vinyl-14,20-dihydroxy-2,3-isopropylidenedioxy-5β-pregn-7-ene-6-one (IX). In 40 ml of THF was dissolved 100 mg (0.199 mmol) of steroid **IV**, 0.55 ml of 1 M solution of tetrabutylammonium fluoride in THF was added, and the mixture was stirred for 1 h at 15°C. Then a saturated solution of ammonium chloride was added, the reaction products were extracted into ethyl acetate, the extract was dried with anhydrous sodium sulfate, evaporated, and the residue was subjected to chromatography on silica gel (eluent petroleum ether–ethyl acetate, 3:1). Yield 68 mg (79%), mp 217.5–219°C (petroleum ether–ethyl acetate). UV spectrum, λ_{max}, nm (ε): 242 (3780). IR spectrum (film), ν, cm^{-1}: 3450, 1680, 1460. ^1H NMR spectrum, δ, ppm ($CDCl_3$): 0.79 s (3H, 18-Me), 0.97 s (3H, 19-Me), 1.32 s (3H, CMe_2), 1.36 s (3H, 21-Me), 1.48 s (3H, CMe_2), 2.33 d.d (1H, C^5H, J_1 4.3, J_2 9.9 Hz), 2.81 d.d.d (1H, C^9H, J_1 2.0, J_2 7.1, J_3 11.6 Hz), 4.23 m (1H, C^2H), 4.26 m (1H, C^3H), 4.99 d (1H, $C^{23}H$, J 10.9 Hz), 5.16 d (1H, $C^{23}H$, J 17.3 Hz), 5.80 d (1H, C^7H, J 1.9 Hz), 5.99 d.d (1H, $C^{22}H$, J_1 10.8, J_2 17.3 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 17.97 q, 20.62 t, 21.63 t, 23.68 q, 26.58 t, 26.80 q, 28.65 q, 29.18 q, 31.16 t, 31.73 t, 34.52 d, 37.78 t, 37.00 s, 47.37 s, 51.01 d, 53.35 d, 71.75 d, 72.26 d, 75.46 d, 85.12 s, 108.41 s, 110.90 t, 121.54 d, 146.03 d, 163.32 s, 202.87 s.

(2β,3β,14α,20*R*,5'*R*)-14,20-Dihydroxy-2,3-isopropylidenedioxy-20-(3'-isopropylisoxazolin-5'-yl)-5β-pregn-7-ene-6-one (X). *a*. By procedure described for compound **VI** from 88 mg (0.204 mmol) of olefin **IX** was obtained 78 mg (74%) of isoxaline **X**. The product was crystallized from a mixture methanol–water, 1:1, mp 278–280°C (decomp.). CD spectrum, λ_{max}, nm ([θ], deg m^2 mol^{-1}): 213 (–15). IR spectrum (KBr), ν, cm^{-1}: 3460, 1680. ^1H NMR spectrum ($CDCl_3$ + D_3COD), δ, ppm: 0.71 s (3H, 18-Me), 0.88 s (3H, 19-Me), 1.06 s (3H, 21-Me), 1.08 d (6H, $CNMe_2$, J 6.9 Hz), 1.16 s (3H, CMe_2), 1.25 s (3H, CMe_2), 2.19 t (1H, $C^{17}H$, J 8.9 Hz), 2.36 d.d (1H, C^5H, J_1 4.7, J_2 11.3 Hz), 2.77 d.d (1H, $C^{4'}H$, J_1 8.6, J_2 17.1 Hz), 2.85 d.d (1H, $C^{4'}H$, J_1 10.9, J_2 17.1 Hz), 4.13 t.d (1H, C^2H, J_1 5.7, J_2 8.7 Hz), 4.17 d.d (1H, C^3H, J_1 4.6, J_2 8.2 Hz), 4.39 d.d (1H, $C^{5'}H$, J_1 8.7, J_2 10.8 Hz), 5.72 d (1H, C^7H, J 2.1 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 17.97 q, 20.39 2 q, 20.46 q, 21.67 t, 21.60 t, 24.05 q, 27.78 q, 28.87 q, 29.19 q, 30.63 t, 30.70 d, 31.47 t, 32.31 t, 35.68 t, 36.31 s, 38.94 t, 48.27 s, 51.45 d, 52.58 d, 73.20 d, 73.55 d, 76.90 s, 85.29 d, 85.99 s, 109.52 s, 121.91 d, 165.80 s, 167.30 s, 205.73 s.

b. By procedure described for compound **IX** from 25 mg (0.043 mmol) of 14-silyloxy derivative **VI** was obtained 18 mg (79%) of 14-hydroxysteroid **X**.

(2β,3β,14α,20*R*,5'*R*)-14,20-Dihydroxy-2,3-isopropylidenedioxy-20-(3'-methylisoxalin-5'-yl)-5β-pregn-7-ene-6-one (XI). *a*. By procedure described for compound **VI** from 34 mg (0.079 mmol) of olefin **IX** was obtained 30 mg (79%) of isoxaline **XI** and 6 mg (17%) of isoxazole **XII**.

Compound (XI). 269–270°C (hexane–ethyl acetate–MeOH). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.81 s (3H, 18-Me), 0.98 s (3H, 19-Me), 1.16 s (3H, 21-Me), 1.33 s (3H, CMe_2), 1.49 s (3H, CMe_2), 1.97 s (3H, 3'-Me), 2.31 t (1H, $C^{17}H$, J 8.8 Hz), 2.35 d.d (1H, C^5H, J_1 4.6, J_2 12.8 Hz), 2.80 d.d (1H, $C^{4'}H$, J_1 9.2, J_2 17.2 Hz),

2.90 d.d (1H, C$^{4'}$H, J_1 10.6, J_2 17.8 Hz), 4.22 m (1H, C^{3}H), 4.27 m (1H, C^{2}H), 4.50 d.d (1H, C$^{5'}$H, J_1 8.7, J_2 10.9 Hz), 5.83 d (1H, C^{7}H, J 2.4 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.16 q, 17.49 q, 20.20 q, 20.68 t, 20.72 t, 23.55 q, 26.44 q, 26.63 t, 28.53 q, 30.92 t, 31.71 t, 34.31 s, 37.58 t, 37.82 s, 39.94 t, 47.24 s, 49.89 d, 50.81 d, 71.58 d, 72.11 d, 75.50 s, 84.77 d, 85.71 s, 108.28 s, 121.55 d, 155.83 s, 162.81 s, 202.69 s.

(2β,3β,14α,20*R*)-20-Hydroxy-2,3-isopropylidenedioxy-20-(3'-methylisoxazol-5'-yl)-5β-pregn-7-ene-6-one (XII). Oily substance. IR spectrum (film), ν, cm^{-1}: 3450, 1670. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.73 s (3H, 18-Me), 1.00 s (3H, 19-Me), 1.33 s (3H, CMe_2), 1.49 s (3H, CMe_2), 1.64 s (3H, 21-Me), 2.27 s (3H, 3'-Me), 2.36 d.d (1H, C^{5}H, J_1 4.6, J_2 11.3 Hz), 2.64 m (1H, C^{9}H), 4.16 t.d (1H, C^{2}H, J_1 5.8, J_2 8.7 Hz), 4.24 d.d (1H, C^{3}H, J_1 4.6, J_2 8.2 Hz), 5.77 d (1H, C^{7}H, J 2.1 Hz), 5.98 s (1H, C$^{4'}$H).

b. By procedure described for compound **IX** from 22 mg (0.044 mmol) of 14-silyloxy derivative **VII** was obtained 15 mg (73%) of 14-hydroxysteroid **XI**.

(2β,3β,14α,20*R*,5'*R*)-2,3,14,20-Tetrahydroxy-20-(3'-isopropylisoxalin-5'-yl)-5β-pregn-7-ene-6-one (**XIII**). In 1.5 ml 70% acetic acid was dissolved 45 mg (0.09 mmol) of 2,3-isopropylidenedioxy derivative **X**, and the solution was stirred for 7 h at 15°C. The reaction mixture was treated with 50 ml of water, the reaction products were extracted into ethyl acetate, the extract was dried with anhydrous sodium sulfate. On removing the solvent the residue was subjected to chromatography on silica gel (eluent ethyl acetate–methanol, 10:1). Yield 30 mg (71%), 255–257°C (EtOAc–MeOH). CD spectrum, λ_{max}, nm ([θ], deg m^2 mol^{-1}): 213 (–21). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 0.81 s (3H, 18-Me), 0.93 s (3H, 19-Me), 1.14 s (3H, 21-Me), 1.14 d (6H, $CNMe_2$, J 6.9 Hz), 2.35 d.d (1H, C^{5}H, J_1 12.8, J_2 4.6 Hz), 2.41 t (1H, C^{17}H, J 9.0 Hz), 2.65 septet (1H, $CNMe_2$, J 6.9 Hz), 2.84 d.d (1H, C$^{4'}$H, J_1 17.4, J_2 9.2 Hz), 2.97 d.d (1H, C$^{4'}$H, J_1 17.5, J_2 10.9 Hz), 3.80 d.t (1H, C^{2}H, J_1 3.8, J_2 12.1 Hz), 3.92 d (1H, C^{3}H, J 2.0 Hz), 4.46 d.d (1H, C$^{5'}$H, J_1 10.8, J_2 9.4 Hz), 5.78 d (1H, C^{7}H, J 2.3 Hz). ^{13}C NMR spectrum (D_3COD), δ, ppm: 18.16 q, 20.40 2 q, 20.48 q, 21.47 t, 21.76 t, 24.38 q, 29.17 d, 31.70 t, 32.21 t, 32.85 t, 35.07 d, 37.05 t, 37.35 t, 39.25 s, 48.21 s, 51.04 d, 51.78 d, 68.50 d, 68.70 d, 76.86 d, 85.19 s, 87.25 d, 122.21 s, 165.70 s, 167.76 s, 206.44 s. Mass spectrum, *m/z* (I_{rel}, %): 475 [M]$^+$ (2), 457 [M – H_2O]$^+$ (11), 363 [M – isoxaline ring]$^+$ (9), 457 [M – H_2O – isoxaline ring]$^+$ (100), 327, 301, 269, 250. Found: *m/z* 475.29339 [M]$^+$. $C_{27}H_{41}NO_6$. Calculated M 475.29346.

(2β,3β,14α,20*R*,5'*R*)-2,3,14,20-Tetrahydroxy-20-(3'-methylisoxalin-5'-yl)-5β-pregn-7-ene-6-one (XIV). By procedure described for compound **XIII** from 22 mg (0.039 mmol) of 2,3-isopropylidenedioxy derivative **XI** was obtained 9 mg (53%) of 2,3,14,20-tetraol **XIV**, mp 239–241°C (petroleum ether–EtOAc). UV spectrum, λ_{max}, nm (ε): 240 (10530). CD spectrum, λ_{max}, nm ([θ], deg m^2 mol^{-1}): 214 (–19). IR spectrum (KBr), ν, cm^{-1}: 3460, 1660. ^{1}H NMR spectrum (CD_3OD), δ, ppm: 0.78 s (3H, 18-Me), 0.90 s (3H, 19-Me), 1.11 s (3H, 21-Me), 1.89 s (3H, 3'-Me), 2.38 t (1H, C^{17}H, J 9.1 Hz), 2.80 d.d (1H, C$^{4'}$H, J_1 9.2, J_2 17.3 Hz), 2.91 d.d (1H, C$^{4'}$H, J_1 10.9, J_2 17.3 Hz), 3.76 m (1H, C^{3}H), 3.88 m (1H, C^{2}H), 4.45 t (1H, C^{5}H, J 9.7 Hz), 5.75 d (1H, C^{7}H, J 2.0 Hz). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 12.97 q, 18.22 q, 20.44 q, 21.51 t, 21.79 t, 24.42 q, 31.73 t, 32.24 t, 32.91 t, 35.10 t, 37.38 d, 39.29 s, 40.62 t, 48.23 C, 51.10 d, 51.83 d, 68.02 d, 68.74 d, 76.83 s, 85.20 s, 87.61 d, 122.22 d, 157.85 s, 167.82 s, 206.47 s. Mass spectrum, *m/z* (I_{rel}, %): 429 [M – H_2O]$^+$ (8), 363 [M – H_2O – isoxaline ring]$^+$ (14), 345 [M – H_2O – isoxaline ring]$^+$ (100), 327 [M – $2H_2O$ – isoxaline ring]$^+$ (43), 309 (15), 269 (35), 250 (33). Found: *m/z* 447.26315 [M]$^+$. $C_{25}H_{37}NO_6$. Calculated M 447.26209.

(2β,3β,14α,20*R*,5'*R*)-2,3,14,20-Tetrahydroxy-20-(3'-methylisoxalin-5'-yl)-5β-pregn-7-ene-6-one (XV). By procedure described for compound **XIII** from 15 mg (0.027 mmol) 2,3-isopropylidenedioxy derivative **VIII** was obtained 8 mg (68%) of 2,3,14,20-tetraol **XV**. Crystallization from a mixture methanol–EtOAc, mp 254–256°C (decomp.). UV spectrum, λ_{max}, nm (ε): 240 (10530). IR spectrum (KBr), ν, cm^{-1}: 3460, 1670. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.71 s (3H, 18-Me), 0.90 s (3H, 19-Me), 1.55 s (3H, 21-Me), 2.20 s (3H, 3'-Me), 2.32 d.d (1H, C^{5}H, J_1 4.6, J_2 12.6 Hz), 3.10 m (1H, C^{9}H), 3.78 t.d (1H, C^{2}H, J_1 4.1, J_2 12.1 Hz), 4.24 d.d (1H, C^{3}H, J_1 4.6, J_2 8.2 Hz), 5.73 d (1H, C^{7}H, J 2.4 Hz), 6.07 s (1H, C$^{4'}$H). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 11.21 q, 17.65 q, 22.38 q, 24.37 t, 28.06 q, 31.51 t, 31.89 t, 32.86 t, 35.07 d, 37.32 t, 39.25 s, 39.70 s, 48.13 s, 51.80 d, 54.65 d, 60.35 t, 68.49 d, 68.66 d, 80.03 s, 102.19 d, 122.12 d, 161.08 s, 173.50 s, 181.08 s, 207.77 s. Mass spectrum, *m/z* (I_{rel}, %): 445 [M]$^+$ (7), 427 [M – H_2O]$^+$ (15), 409 [M – $2H_2O$]$^+$ (13), 301 [M – side chain – H_2O]$^+$ (21), 250 [M – side chain – $3H_2O$]$^+$ (100). Found: *m/z* 445.2599 [M]$^+$. $C_{25}H_{35}NO_6$. Calculated M 445.24644.

(2β,3β,14α,20*S*,5'*R*)-20-Hydroxy-2,3-isopropylidenedioxy-20-(3'-isopropylisoxazolin-5'-yl)-

14-trimethylsilyloxy-5β-pregn-7-ene-6-one (XVI). By procedure described for compound **VI** from 31 mg (0.061 mmol) of olefin **V** was obtained 29 mg (80%) of oily substance **XVI**. UV spectrum, λ_{max}, nm (ε): 242 (10010). IR spectrum (film), ν, cm^{-1}: 3460, 1680. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.1 s (9H, $SiMe_3$), 0.79 s (3H, 18-Me), 1.01 s (3H, 19-Me), 1.09 s (3H, 21-Me), 1.16 s (3H, $CNMe_2$), 1.18 s (3H, $CNMe_2$), 1.32 s (3H, CMe_2), 1.48 s (3H, CMe_2), 2.35 d.d (1H, C^5H, J_1 4.6, J_2 11.4 Hz), 2.68 m (1H, C^9H), 2.82 d.d (1H, $C^{4'}H$, J_1 10.6, J_2 17.1 Hz), 2.89 d.d (1H, $C^{4'}H$, J_1 9.2, J_2 17.2 Hz), 4.16 t.d (1H, C^2H, J_1 5.8, J_2 8.7 Hz), 4.25 d.d (1H, C^3H, J_1 4.5, J_2 8.1 Hz), 4.49 d.d (1H, $C^{5'}H$, J_1 9.3, J_2 10.5 Hz), 5.78 d (1H, C^7H, J 2.0 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 1.97 t.q, 17.69 q, 20.19 q, 20.21 q, 20.77 q, 21.10 t, 22.35 t, 24.09 q, 26.43 t, 26.53 q, 28.14 d, 28.69 q, 29.58 t, 31.23 t, 35.89 t, 35.98 br.d, 37.36 t, 37.60 s, 49.43 s, 50.14 d, 50.51 d, 71.64 d, 72.59 d, 76.08 s, 85.32 d, 87.71 s, 108.46 s, 122.01 d, 163.17 s, 164.41 s, 202.38 s.

(2β,3β,14α,20*S*,5'*R*)-14,20-Dihydroxy-2,3-isopropylidenedioxy-20-(3'-isopropylisoxalin-5'-yl)-5β-pregn-7-ene-6-one (XVII). By procedure described for compound **IX** from 25 mg (0.043 mmol) of silyloxy derivative **XVI** was obtained 18 mg (79%) of 14-hydroxysteroid **XVII**, mp 220–222°C (hexane–EtOAc). CD spectrum, λ_{max}, nm ([θ], deg m^2 mol^{-1}): 210 (–6). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.86 s (3H, 18-Me), 0.98 s (3H, 19-Me), 1.10 s (3H, 21-Me), 1.16 s (3H, $CNMe_2$), 1.18 s (3H, $CNMe_2$), 1.33 s (3H, CMe_2), 1.49 s (3H, CMe_2), 2.35 d.d (1H, C^5H, J_1 4.6, J_2 12.7 Hz), 2.46 t (1H, $C^{17}H$, J 9.3 Hz), 2.70 septet (1H, $CNMe_2$, J 6.9 Hz), 2.81 m (1H, C^9H), 2.86 m (2H, $C^{4'}H$), 4.16 t.d (1H, C^2H, J_1 6.1, J_2 10.0 Hz), 4.28 m (1H, C^3H), 4.58 t (1H, $C^{5'}H$, J 9.6 Hz), 5.83 d (1H, C^7H, J 2.3 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 18.06 q, 20.19 d.q, 20.55 t, 21.28 q, 21.68 t, 23.69 q, 26.60 q, 26.80 t, 28.08 d, 28.67 q, 29.83 s, 31.02 t, 31.67 t, 34.46 d, 36.14 t, 37.78 t, 38.00 s, 47.29 s, 49.99 d, 51.01 d, 71.75 d, 72.26 d, 85.01 s, 85.15 d, 108.44 s, 121.68 d, 163.03 s, 164.19 s, 202.84 s.

(2β,3β,14α,20*S*,5'*R*)-20-Hydroxy-20-(3'-isopropylisoxalin-5'-yl)-5β-pregn-7-ene-6-one (XVIII). By procedure described for compound **XIII** from 20 mg (0,039 mmol) of 2,3-isopropylidenedioxy derivative **XVII** was obtained 12 mg (71%) of 2,3,14,20-tetraol **XVIII**, mp 155–157°C (hexane–EtOAc). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.86 s (3H, 18-Me), 0.98 s (3H, 19-Me), 1.10 s (3H, 21-Me), 1.16 s (3H, $CNMe_2$), 1.17 s (3H, $CNMe_2$), 2.42 d.d (1H, C^5H, J_1 4.0, J_2 13.6 Hz), 2.52 t (1H, $C^{17}H$, J 9.4 Hz), 2.99 septet (1H, $CNMe_2$, J 7.0 Hz), 2.85 d.d (1H, $C^{4'}H$, J_1 10.8, J_2 17.3 Hz), 2.91 d.d (1H, $C^{4'}H$, J_1 8.7, J_2 17.0 Hz), 2.81 m (1H, C^9H), 3.01 m (2H, $C^{4'}H$), 3.91 m (1H, C^2H), 4.11 m (1H, C^3H), 4.54 t (1H, $C^{5'}H$, J 10.3 Hz), 5.85 d (1H, C^7H, J 1.2 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 18.41 q, 20.20 2 q, 20.96 q, 21.87 t, 22.54 t, 28.07 q, 29.85 t, 30.95 t, 31.59 t, 33.82 br.d, 35.98 t, 36.92 t, 38.46 s, 47.15 s, 49.94 d, 50.26 s, 67.40 d, 67.82 d, 76.17 d, 84.83 d, 85.31 s, 110.18 d, 121.91 d, 163.17 s, 164.08 s, 206.40 s. Mass spectrum, *m/z* (I_{rel}, %): 475 $[M]^+$ (3), 457 $[M - H_2O]^+$ (7), 363 [*M* – isoxaline ring]$^+$ (30), 345 $[M - H_2O$ – isoxaline ring$]^+$ (100), 327 $[M - 2H_2O$ – isoxaline ring$]^+$ (40), 309 $[M - 3H_2O$ – isoxaline ring$]^+$ (20), 285 (30), 269 (25), 250 (20). Found: *m/z* 475.2930 $[M]^+$. $C_{27}H_{41}NO_6$. Calculated *M* 475.29339.

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