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Synthesis and Transformations of 20-Isoxazolylsteroids with Modified D Ring: I. Synthesis of 16α,17α-Epoxyderivatives*

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Abstract—Synthesis of 16α,17α-epoxy-20-isoxazolylsteroids was carried out starting with dehydropregnenolone acetate. Transformation procedures for preparation therefrom of open-chain compounds were considered. Physico-chemical characteristics of compounds synthesized were investigated.

A selective introduction into a steroid molecule with natural structure of an additional functional group that would not significantly affect the main biological characteristics (that would not hamper binding with a presumed receptor) would have permitted performing further modification and conjugation of the steroid to an appropriate protein molecule and thus to prepare desired antigenes and antibodies to the steroid compounds under investigation. This problem is urgent for various steroid groups.

Achievement of this target with regard to a new phytohormone class, brassinosteroids, should transfer the studies thereof to a new qualitative level. Thus would arise possibilities to investigate the reception of brassinosteroids, to develop highly sensitive microanalytical reagents necessary for the study of brasinosteroids spreading in plants, their biosynthesis, metabolism etc. It is presumable that the preparation of synthetic analogs would furnish new bioactive substances.

The target of the present study was a development of procedures for regio and stereoselective chemical modification of brassinosteroids and preparation of their analogs with an additional functional moiety attached to one of the carbon atoms (preferably to C^{16} or C^{17}).

The traditional ways of brassinosteroid synthesis based on transformation of ergosterol or stigmasterol and involving Δ^{22} bond for subsequent hydroxylation or cleavage into C^{22}-aldehyde [1–3] cannot be applied to the target problem since the method of substituents introduction into the D ring of these compounds is lacking. Therefore we chose as the starting compound dehydropregnenolone acetate (**I**), and the synthetic approach was based on the isoxazole procedure for building up the carbon backbone of the brassinosteroid side chain [1, 4].

The first step in modification of the D ring in the steroid molecule was the synthesis of 16α,17α-epoxide **II** by selective epoxidation of the conjugated double bond in dehydropregnenolone acetate (**I**) effected by hydrogen peroxide in the alkaline medium [5]. With the synthesized epoxide **II** was carried out a reaction with Grignard reagent, acetylenemagnesium bromide. We established that the arising acetylene alcohol **III** contained two epimers at C^{20} atom which were virtually indistinguishable both by chromatography and ^{1}H NMR spectra. The formation of two epimers was detected only with the use of ^{13}C NMR spectroscopy (the ^{13}C NMR spectrum contained a double set of signals from the atoms C^{12}, C^{13}, C^{14}, C^{16}, C^{20} and C^{23} indicating the presence of two epimers at the C^{20} atom).

The building up of the side chain for C^{27}-steroids was further performed along the nitriloxide procedure [4, 6] that we had tested before for unsubstituted in the D ring pregnane compounds. The addition to acetylene alcohol **III** of isobutyronitriloxide generated by treating the isobutyraldoxime with *N*-chlorosuccinimide in the presence of triethylamine resulted in regioselective formation of 20-hydroxy-20-isoxazolylsteroid (**IV**). The addition occurred at the triple bond and did not affect the $\Delta^{5(6)}$ bond.

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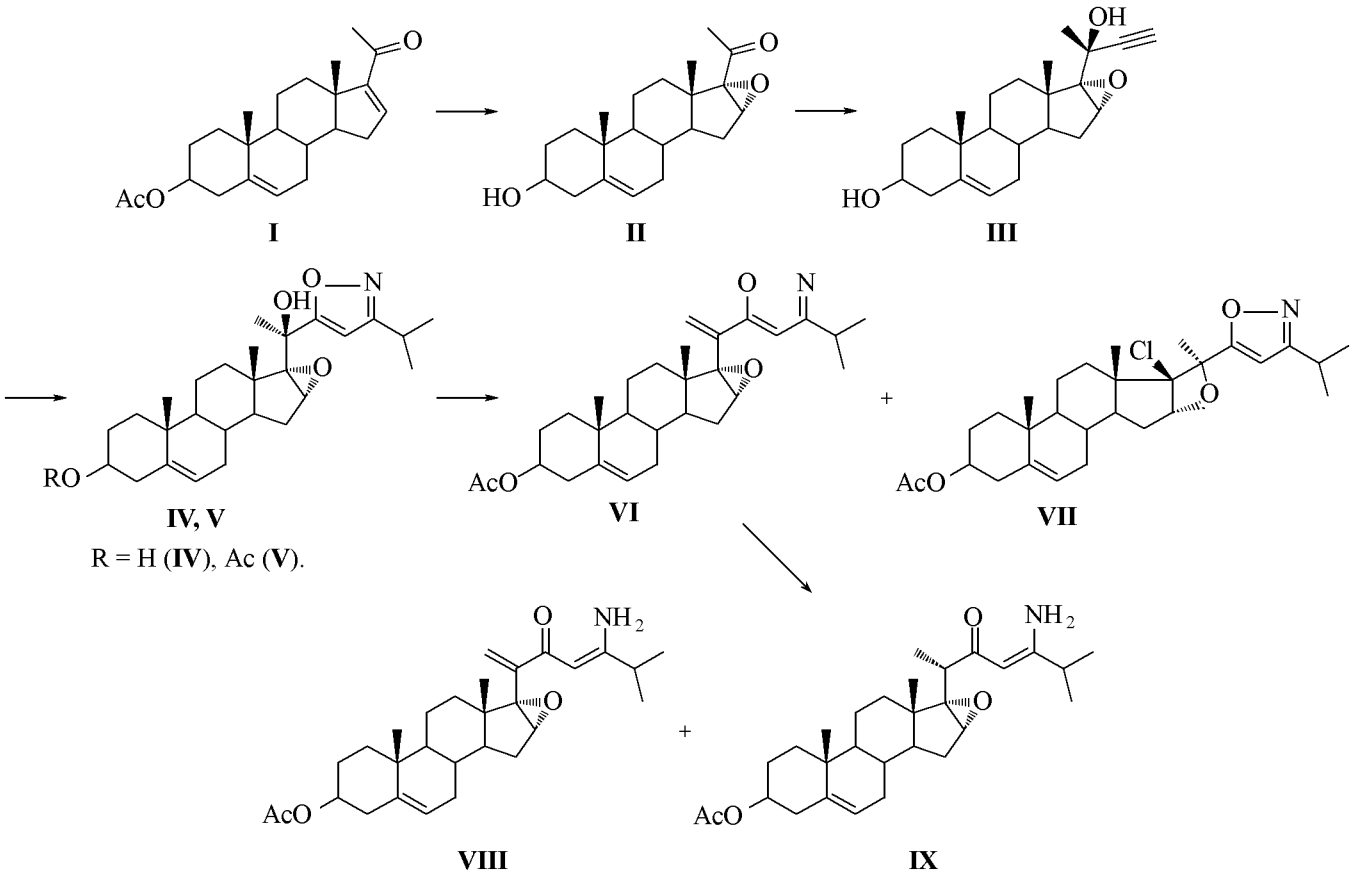
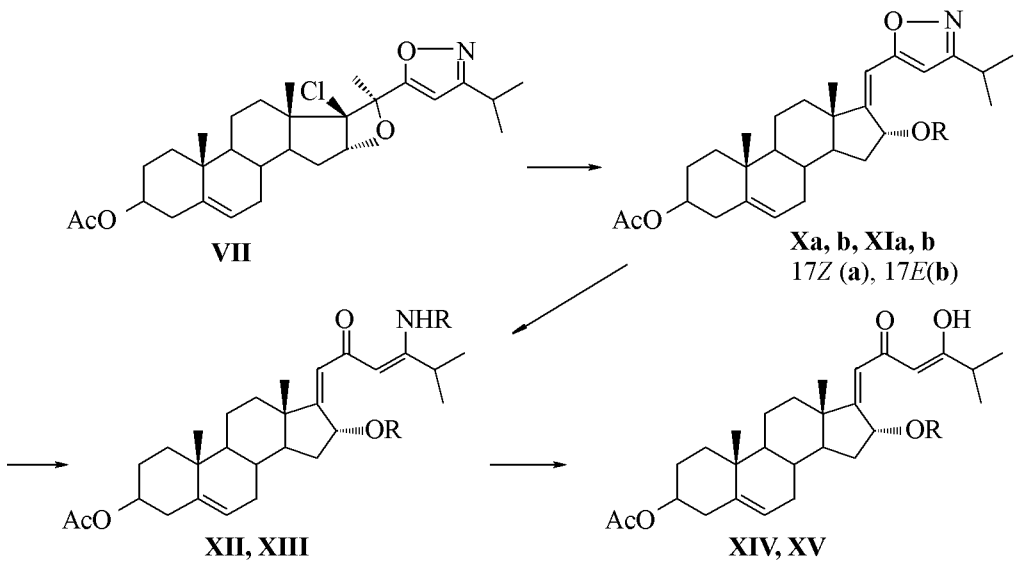


The obtained 20-hydroxy-20-isoxazolylsteroid (**IV**) after protection of the hydroxy group was subjected to treatment with thionyl chloride in tetrahydrofuran in the presence of pyridine to perform dehydration [7]. The use of freshly distilled thionyl chloride provided in good yield $\Delta^{20(21)}$-steroid (**VI**) whose structure was confirmed by its physico-chemical characteristics: the lack of absorption band from stretching vibrations of hydroxy group in the IR spectrum, and the presence in the ^{1}H NMR spectrum of two one-proton singlets from vinyl protons attached to C^{21} (δ 5.48 and 6.10 ppm). At use in the above reaction of thionyl chloride with hydrogen chloride traces alongside dehydration product **VI** was also obtaine 17β-chloroderivative **VII** originating from cleavage of the epoxy ring followed by cyclization of the arising 16,20-diol into the corresponding oxetane. The structure and composition of the compound were determined from ^{1}H, ^{13}C NMR, IR, and mass spectra, and also from elemental analysis with chlorine trapping by silver powder [8].

The transition from the isoxazoles obtained to the steroids with an open functionalized cholesterol chain was done by reduction on Raney nickel in ethanol. After this procedure was effected with $\Delta^{20(21)}$-en-20-isoxazolylsteroid (**VI**) were separated two products: 24-amino-*20*(*21*),23-diene-22-one (**VIII**) produced by opening of the isoxazole ring, and 24-amino-23-en-22-one (**IX**) originating from heterocycle opening and hydrogenation of the $\Delta^{20(21)}$-bond. The hydrogenation time virtually did not affect the ratio of the enamines formed. Note that the separated $\Delta^{20(21)}$-steroid (**VIII**) under the indicated conditions can be further transformed into compound **IX** with the use of catalytical amounts of Raney nickel. Probably the complete hydrogenation of the $\Delta^{20(21)}$-bond requires the presence of fresh catalyst. It should be noted that the reduction of the double bond yielded a single stereoisomer.

The structure of compound **VIII** is confirmed by the presence in its ^{1}H NMR spectrum of two signals from vinyl protons attached to atom C^{21} (δ 5.48 and 6.12 ppm), two broadened one-proton singlets (δ 5.22 and 10.05 ppm) characteristic of amino group, and a singlet from vinyl proton linked to C^{23} atom (δ 5.56 ppm). A characteristic signal in the ^{1}H NMR



R = H (**X, XII, XIV**); Ac (**XI, XIII, XV**).

spectrum of compound **IX** is the three-proton doublet at 1.18 ppm inherent to the protons of 21-methyl group, and no vinyl protons at C^{21} atom.

The reductive cleavage of 16,20-oxy-20-isoxazolylsteroid (**VII**) on Raney nickel proceeded in several directions affording a product mixture therefrom we succeeded to isolate only Δ^{17}-20-isoxazolylsteroid (**X**) formed by cleavage of the oxetane ring and dehydrochlorination, and 24-amino-23-en-22-one (**XII**), resulting from the cleavage of both oxetane and isoxazole rings followed by dehydrochlorination. Δ^{17}-20-Isoxazolylsteroid (**X**) formed as a mixture of two geometrical isomers **Xa, b** with respect to the bond $\Delta^{17(20)}$ as follows from the ^{1}H NMR spectrum. In the latter appear two singlets belonging to the protons of methyl groups attached to the double bond (δ 2.12 and 2.14 ppm), and a doublet from the proton at C^{16} atom (δ 4.82 ppm). The presence of a hydroxy group is confirmed by the absorption band of stretching vibrations at 3410 cm^{-1} in the IR spectrum, and also by appearance in the ^{1}H NMR spectrum of an additional three-proton singlet of acetate methyl after acetylation of compound **X** into acetate **XI**. In the ^{1}H NMR spectrum of the latter the signal of the proton at C^{16} atom is sifted downfield (δ 5.80 ppm). Acetylation also removes the absorption band of the hydroxy group stretching vibrations from the IR spectrum. We succeded to isolate the individual isomers by chromatography. The assignement of **Xa** to E-isomer and **Xb** to *Z*-isomer was done basing on ^{1}H NMR spectra and literature data [9] for the signal of 21-methyl group protons in the *Z*-isomer was located in the stronger field (see Scheme).

Isoxazole **X** was cleaved on Raney nickel freshly saturated with hydrogen to afford 24-amino-23-en-22-one (**XII**) in 50% yield. The structure of the latter was confirmed by ^{13}C NMR spectrum and appearance of characteristic signals in the ^{1}H NMR spectrum (three-proton singlet of the methyl group attached to the double bond at δ 2.03 ppm, one-proton doublet of the 16β-proton at δ 4.42 ppm, one-proton singlet of the vinyl proton at C^{23} atom, δ 5.18 ppm, and two broadened singlets of the protons of the amino group at δ 5.24 and 10.08 ppm). In the IR spectrum of compound **XII** is observed an absorption band of stretching vibrations of hydroxy group (3420 cm^{-1}). The acetylation of the secondary alcohol **XII** with acetic anhydride in pyridine at room temperature results in 3,16-diacetoxy-24-acetamido derivative **XIII** as revealed by ^{1}H NMR spectrum: downfield shift of the resonance from the proton attached to C^{16} (δ 5.86), three three-proton singlets belonging to acetate groups (δ 1.97, 2.03, and 2.16 ppm), and only one singlet from the amino group proton (δ 12.54 ppm).

Thus we developed a synthesis of 16α,17α-epoxy-20-isoxazolylsteroids proceeding from dehydropregnenolone. The reductive cleavage of the compounds obtained on Raney nickel permits transformation thereof into open-chain compounds, in particular, into 24-amino-23-en-22-ones. The transition from the latter to the known key intermediates leading to brassinisteroids we already described before [10].

EXPERIMENTAL

^{1}H NMR spectra were registered on spectrometer Bruker A-200 (200 MHz) in deuterochloroform, internal reference TMS. IR spectra were recorded on UR-20 spectrophotometer (from thin film, solution in CCl_4, or KBr pellets). Mass spectra were measured on Hewlett-Packard 5890 instrument with the linear temperature programming from 40 to 280°C at the rate 10 deg min^{-1}, ionizing electrone energy 70 eV. UV spectra were obtained on Specord M-400 spectrophotometer from methanol or ethanol solutions. The melting points were determined on Koeffler heating block.

The reactions were monitored by TLC on Silufol-UV-254 and Kieselgel 60 F_{254} (Merck) plates. Chromatographic separation was carried out on silica gel 40/60 μ (Kieselgel 60, Merck).

Epoxidation of 16-dehydropregnenolone acetate (I). To a solution of 1 g (3 mmol) of dehydropregnenolone acetate (**I**) in 10 ml of ethanol at 30–40°C was added 6 ml of 30% hydrogen peroxide and 3.6 ml of 4 N NaOH. The reaction mixture was stirred for 2 h, then 300 ml of cold water was added. The precipitate was filtered off, the solution was dried with sodium sulfate. The solvent was evaporated, the residue was crystallized from methanol–dichloromethane mixture. The yield of epoxide **II** 0.84 g (91%).

3β-Hydroxy-16α,17α-epoxypregn-5-ene (II). mp 179–180°C (methanol). ^{1}H NMR spectrum (δ, ppm): 1.04 s (3H, 18-Me), 1.08 s (3H, 19-Me), 2.05 s (3H, 21-Me), 3.58 m (1H, H^3), 3.70 br.s (1H, H^{15}), 5.35 m (1H, H^6). ^{13}C NMR spectrum (δ_C, ppm): 15.5 q, 19.3 q, 20.4 t, 25.9 q, 27.5 t, 29.7 d, 31.3 t, 31.4 t, 31.5 t, 36.7 c, 37.0 t, 41.4 s, 42.1 t, 45.5 d, 50.3 d, 60.5 d, 71.0 s, 71.5 d, 121.0 d, 141.1 s, 205.0 s. IR spectrum (cm^{-1}): 3450, 2950, 2915, 2870, 1705, 1645, 1450, 1380, 1310. Mass spectrum (*m/z*): 331, 312, 296, 294, 262, 253.

3β,20S-Dihydroxy-16α,17α-epoxy-24-norchol-5-en-22-yne (III). Into Grignard reagent prepared from 0.36 g (15 mmol) of magnesium and 1.4 ml (16 mmol) of ethyl bromide in 40 ml of anhydrous tetrahydrofuran 10 min after complete dissolution of magnesium was passed acetylene for 30 min (the reaction mixture self-heated to 40°C). After cooling the reaction mixture to room temperature a solution of 0.98 g (3 mml) of epoxyketone **II** in 20 ml of tetrahydrofuran was added, and the mixture was stirred for 2 h at room temperature. Then the reaction mixture was treated with saturated ammonium chloride solution, the reaction product was extracted with ethyl acetate, the extract was dried with sodium sulfate, and the solvent was evaporated. The residue was charged into a column packed with the silica gel, elution was performed with a mixture toluene–ethyl acetate (4 : 1). We obtained 0.96 g (92%) of the acetylene alcohol**III**. mp 192–193°C (methanol). ^{1}H NMR spectrum (δ, ppm): 1.03 s (3H, 18-Me), 1.05 s (3H, 19-Me), 1.63 s (3H, 21-Me), 2.55 s (1H, H^{23}), 3.09 s (OH), 3.47 s (1H, H^{16}), 3.55 m (1H, H^3), 5.35 m (1H, H^6). ^{13}C NMR spectrum (δ_C, ppm): 16.6 q, 19.3 q, 20.6 t, 26.9 t, 28.6 and 28.8 q, 30.0 d, 31.2 t, 31.6 t, 33.2 two t, 36.7 s, 37.2 t, 41.9 t, 42.9 two s, 47.0 d, 50.5 d, 59.4 and 59.6 d, 66.6 and 67.5 s, 71.3 d, 72.5 and 73.4 s, 72.6 and 72.7 s, 86.3 and 86.8 d, 121.2 d, 141.3 s. IR spectrum (KBr, cm^{-1}): 3450, 3300, 2950, 2920, 2875, 1650, 1480, 1450, 1390, 1360. Mass spectrum (*m/z*): 356, 323, 305, 270.

3β,20S-Dihydroxy-20-(isopropylisoxazol-5-yl)-16α,17α-epoxypregn-5-ene (IV). To a suspension of 0.8 g (6 mmol) of *N*-chlorosuccinimide in 5 ml of chloroform was added several drops of pyridine and then a solution of 0.45 g (6 mmol) of isobutyraldoxime in 2 ml of chloroform. The mixture was stirred for 15 min, and thereto was added 0.68 g (2 mmol) of acetylene alcohol **III** in 2 ml of chloroform. In 10 min was started addition of 0.85 ml (6 mmol) of triethylamine in chloroform that was performed dropwise and proceeded for 4 h. Then the reaction mixture was left overnight. The reaction mixture was washed with water, dried with sodium sulfate, the solvent was evaporated, and the residue was subjected to chromatography on a column packed with silica gel, eluent toluene–ethyl acetate, 1 : 1. We obtained 0.77 g (91%) of isoxazole **IV** as oily substance. ^{1}H NMR spectrum (δ, ppm): 0.90 s (3H, 18-Me), 0.97 s (3H, 19-Me), 1.24 d (6H, CH$\underline{Me}_2$, *J* 7 Hz), 1.66 s (3H, 21-Me), 3.04 m (1H, C$\underline{H}$Me$_2$), 3.48 m (1H, H^3), 3.52 s (1H, H^{16}), 5.30 m (1H, H^6), 6.14 s (1H, H$^{4'}$).

3β-Acetoxy-20-(isopropylisoxazol-5-yl)-16α,17α-epoxypregna-5,20(21)-diene (VI). To a stirred solution of 0.480 g (1 mmol) of steroid alcohol **V** in 20 ml of tetrahydrofurane was added 0.3 ml of pyridine. The mixture was cooled to –50°C, and 0.33 ml (1.84 mmol) of freshly distilled thionyl chloride in 5 ml of tetrahydrofuran was added thereto. Within 1 h the reaction mixture was warmed to room temperature, and then 100 ml of 5% solution of sodium hydrogen carbonate was added to the reaction mixture. The reaction product was exreacted into

ethyl acetate, the extract was washed with the solution of sodium hydrogen carbonate, dried with anhydrous sodium sulfate, and evaporated. The residue was subjected to chromatography on a column packed with silica gel, eluent hexane–ethyl acetate, 3:1. We obtained 0.35 g (80%) of compound **VI** as oily substance. ^{1}H NMR spectrum (δ, ppm): 0.93 s (3H, 18-Me), 1.05 s (3H, 19-Me), 1.30 d (6H, CHMe$_2$, J 7 Hz), 2.03 s (3H, OAc), 3.07 s (1H, CHMe$_2$), 3.54 s (1H, H^{16}), 4.60 m (1H, H^3), 5.38 m (1H, H^6), 5.48 s and 6.10 s (2H, H^{22}), 6.27 s (1H, H$^{4'}$). ^{13}C NMR spectrum (δ_C, ppm): 16.3 q, 19.9 q, 21.3 t, 22.0 q, 22.4 q, 27.1 q, 28.0 t, 28.3 t, 31.3 t, 32.2 t, 33.2 t, 37.4 s, 37.5 t, 38.7 t, 43.1 s, 46.2 d, 50.9 d, 61.6 d, 71.4 s, 74.4 d, 101.5 d, 121.3 t, 122.7 d, 130.9 s, 140.6 s, 167.9 s, 170.2 s, 171.1 s. IR spectrum (film, cm^{-1}): 2980, 2950, 2870, 1745, 1590, 1480, 1460, 1390, 1380, 1260. UV spectrum [EtOH, λ8_max, nm (χ)]: 250 (6500).

Along the same procedure of application of the thionyl chloride with traces of hydrogen chloride was obtained from 0.42 g of stroid alcohol **V** 0.18 g (40%) of diene **VI** and 0.23 g (54%) of 17-chloro-derivative **VII**.

3β-Acetoxy-20-(3-isopropylisoxazol-5-yl)-16α,20-oxy-17β-chloropregna-5-ene (VII). mp 137–138°C (methanol). ^{1}H NMR spectrum (δ, ppm): 0.55 s (3H, 18-Me), 1.00 s (3H, 19-Me), 1.30 d (6H, CHMe$_2$, *J* 7 Hz), 1.80 s (3H, 21-Me), 2.03 s (3H, OAc), 3.04 m (1H, H^{25}), 3.70 br.s (1H, H^{16}), 4.60 s (1H, H^3), 5.36 m (1H, H^6), 6.36 s (1H, H$^{4'}$). ^{13}C NMR spectrum (δ_C, ppm): 14.4 q, 19.1 q, 20.5 t, 21.4 q, 21.6 q, 21.7 q, 26.6 q, 27.0 t, 27.7 t, 29.6 d, 29.9 d, 31.4 t, 33.6 t, 36.7 s, 36.8 t, 38.0 t, 44.6 s, 46.6 d, 50.0 d, 61.0 d, 67.3 s, 71.1 s, 73.8 d, 102.0 d, 122.1 d, 139.9 s , 169.3 s, 170.4 s, 173.6 s. IR spectrum (cm^{-1}): 2980, 2960, 2880, 1745, 1610, 1475, 1460, 1380, 1250. Mass spectrum (*m/z*): 387, 361, 324, 309, 279, 253. Found, %: C 68.88, 68.57; H 7.93, 8.14; Cl 6.94, 6.58; N 2.79. $C_{29}H_{39}ClNO_4$. Calculated, %: C 69.39; H 7.97; Cl 7.07; N 2.68.

Cleavage of 20-isoxazolylsteroids. Raney nickel of W-2 grade (100 mg) was saturated with hydrogen at stirring in 10 ml of ethanol for 2 h. Then 0.1 mmol of 20-isoxazolylsteroid in 10 ml of ethanol was added. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 3 h. At the end of the reaction the catalyst was filtered off, and the solvent was evaporated. The residue was purified by chromatography on silica gel (eluent toluene–ethyl acetate, 3:1).

By this procedure from 0.15 g isoxazolylsteroid **VI** was obtained 0.02 g (14%) of 20(21),23-dien-22-one **VIII** and 0.015 g (10%) of enaminoketone **IX**.

24-Amino-3β-acetoxy-16α,17α-epoxycholesta-5,20(21),23-trien-22-one (VIII) was isolated as oily substance. ^{1}H NMR spectrum (δ, ppm): 0.88 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.20 d (6H, CHMe$_2$, *J* 7 Hz), 2.04 s (3H, OAc), 2.40 m (1H, CHMe$_2$), 3.52 s (1H, H^{16}), 4.60 m (1H, H^3), 5.22 br.s (1H, NH), 5.38 m (1H, H^6), 5.48 d and 6.12 d (2H, H^{21}, *J* 1.5 Hz), 5.56 s (1H, H^{23}), 10.05 br.s (1H, NH). IR spectrum (film, cm^{-1}): 2980, 2960, 2870, 1745, 1625, 1540, 1470, 1455, 1370, 1255.

24-Amino-3β-acetoxy-16α,17α-epoxycholesta-5,23-dien-22-one (IX) was isolated as oily substance. ^{1}H NMR spectrum (δ, ppm): 0.92 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.18 d (9H, 21-Me and CHMe$_2$, *J* 7 Hz,), 2.04 s (3H, OAc), 3.52 s (1H, H^{16}), 4.60 m (1H, H^3), 5.04 br.s (1H, NH), 5.38 m (1H, H^6), 5.58 s (1H, H^{23}), 9.80 br.s (1H, NH). IR spectrum (film, cm^{-1}): 2980, 2960, 2870, 1735, 1620, 1535, 1470, 1455, 1370, 1255.

By the above procedure from 0.195 g (0.4 mmol) of compound **VII** was obtained 0.151 g (83%) of isomer mixture of compound **X** and 0.012 g (6%) of compound **XII**.

(17*E*,*Z*)-3β-Acetoxy-16α-hydroxy-20-(3-isopropylisoxazol-5-yl)pregna-5,17-diene (X) was isolated as oily substance. ^{1}H NMR spectrum (δ, ppm): 0.98 s and 1.05 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.30 d (6H, CHMe$_2$, *J* 7 Hz), 2.02 s and 2.04 s (3H, OAc), 2.12 s and 2.14 s (3H, 21-Me), 3.04 (1H, CHMe$_2$), 4.60 m (1H, H^3), 4.82 d (1H, H^{16}, *J* 5 Hz), 5.38 m (1H, H^6), 5.97 s and 6.20 s (1H, H$^{4'}$). IR spectrum (film cm^{-1}): 3440, 2975, 2960, 2915, 2880, 1745, 1680, 1590, 1470, 1460, 1425, 1380, 1245. UV spectrum [EtOH, λ_{max}, nm (ε)]: 256 (13470).

The isomers of compound **X** obtained were separated on a column packed with silica gel, eluent toluene–ethyl acetate, 5:1. The more polar isomer **Xa** is of *E*-confuguration, the less polar one, **Xb**, possesses *Z*-configuration.

(17*E*)-3β-Acetoxy-16α-hydroxy-20-(3-isopropylisoxazol-5-yl)pregna-5,17-diene (Xa) was isolated as oily substance. ^{1}H NMR spectrum (δ, ppm): 1.05 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.30 d (6H, CHMe$_2$, *J* 7 Hz), 2.04 s (3H, OAc), 2.14 s (3H, 21-Me), 3.05 (1H, CHMe$_2$), 4.60 m (1H, H^3), 4.82 d (1H, H^{16}), *J* 5 Hz), 5.38 m (1H, H^6), 6.20 s (1H, H$^{4'}$). IR spectrum (cm^{-1}): 3440, 2980, 2960,

2915, 2880, 1745, 1680, 1590, 1470, 1460, 1425, 1380, 1240.

(17*Z*)-3β-Acetoxy-16α-hydroxy-20-(3-isopropylisoxazol-5-yl)pregna-5,17-diene. mp 58–61°C (crystals obtained by grinding with hexane). ^{1}H NMR spectrum (δ, ppm): 0.98 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.30 d (6H, CHMe$_2$, *J* 7 Hz), 2.04 s (3H, OAc), 2.12 s (3H, 21-Me), 3.05 (1H, CHMe$_2$), 4.60 m (1H, C3H^3), 4.82 d (1H, 6H^{16}, *J* 5 Hz), 5.38 m (1H, C6H^6), 5.97 s (1H, H$^{4'}$). IR spectrum (cm^{-1}): 3440, 2980, 2960, 2915, 2880, 1745, 1680, 1590, 1470, 1460, 1425, 1380, 1240.

By the above procedure from 0.07 g of isoxazole **Xb** was obtained 0.035 g (50%) of compound **XII**. **(17*Z*)-24-Amino-3β-acetoxy-16α-hydroxycholesta-5,17,23-trien-22-one (XII).** mp 165–167°C (methanol). ^{1}H NMR spectrum (δ, ppm): 0.92 s (3H, 18-Me), 1.04 s (3H, 19-Me), 1.20 d (6H, CHMe$_2$, *J* 7 Hz), 2.03 s (3H, 21-Me), 2.04 s (3H, OAc), 4.42 d (1H, H^{16}, *J* 5 Hz), 4.62 m (1H, H^3), 5.18 s (1H, H^{23}), 5.38 m (1H, H^6), 5.24 br. s (1H, H^{16}), 10.08 s (1H, NH). IR spectrum (cm^{-1}): 3420, 3200, 2980, 2960, 2920, 2880, 1740, 1620, 1530, 1480, 1450, 1380, 1260. UV spectrum [EtOH, λ_{max}, nm (ε)]: 318 (6700).

Acetylation of secondary steroid alcohols. In 1 ml of pyridine was dissolved 0.16 mmol of steroid alcohol and thereto was added dropwise 0.5 ml of acetic anhydride. The reaction mixture was left standing for 18–20 h, then was treated with water, and the reaction products were extracted into ether. The extract was washed with 0.5% solution of hydrochloric acid till neutral, dried with anhydrous sodiumsulfate, and the solvent was evaporated. The residue was dissolved in a little of chloroform and purified by filtration through a silica gel bed. Yields of acetates 95–97%.

(20S)-3β-Acetoxy-20-hydroxy-20-(3-isopropylisoxazol-5-yl)-16α,17α-epoxypregna-5-ene (V) was prepared from alcohol **IV**. mp 185–186°C (methanol). ^{1}H NMR spectrum (δ, ppm): 0.90 s (3H, 18-Me), 0.98 s (3H, 19-Me), 1.28 d (6H, CHMe$_2$, *J* 7 Hz), 1.68 s (3H, 21-Me), 2.02 s (3H, OAc), 3.06 m (1H, CHMe$_2$), 3.56 s (1H, H^{16}), 4.58 m (1H, H^3), 5.36 m (1H, H^6), 6.14 s (1H, H$^{4'}$). IR spectrum (KBr, cm^{-1}): 3460, 2980, 2950, 2870, 1745, 1475, 1460, 1380, 1260. Mass spectrum (*m/z*): 331, 310, 279, 270.

(17*E,Z*)-3β,16α-Diacetoxy-20-(3-isopropylisoxazol-5-yl)pregna-5,17-diene (XI) was prepared from 16-hydroxysteroid **X** as a mixture of two isomers. mp 197–199°C (methanol). ^{1}H NMR spectrum (δ, ppm): 1.00 s and 1.04 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.27 d and 1.30 d (6H, CHMe$_2$, *J* 7 Hz), 1.87 s and 1.90 s (3H, OAc), 2.04 s (3H, OAc), 2.12 and 2.15 s (3H, 21-Me), 3.04 (1H, CHMe$_2$), 4.60 m (1H, H^3), 5.38 m (1H, H^6), 5.80 d (1H, H^{16}, *J* 5 Hz), 5.98 s and 6.02 s (1H, H$^{4'}$). IR spectrum (film, cm^{-1}): 2975, 2960, 2915, 2880, 1745, 1595, 1580, 1470, 1460, 1425, 1380, 1250.

24-*N*-acetamido-3β,16α-diacetoxycholesta-5,17,23-trien-22-one (XIII) was obtained from the mixture of the two isomers of 17,23-dien-24-amino-16-ol **XII** as a mixture of $\Delta^{17(20)}$-isomers. Oily substance ^{1}H NMR spectrum (δ, ppm): 0.97 s and 1.05 s (3H, 18-Me), 1.02 s (3H, 19-Me), 1.13 d (6H, CHMe$_2$, *J* 7 Hz), 1.89 s and 1.97 s (3H, 21-Me), 2.05 s (3H, OAc), 2.16 s (3H, NAc), 3.92 m (1H, H^{25}), 4.62 m (1H, H^3), 5.38 m (1H, H^6), 5.50 s and 5.52 s (1H, H^{23}), 5.72 and 5.86 br.s (1H, H^{16}), 12.54 s (1H, NH). IR spectrum (film cm^{-1}): 2975, 2950, 2870, 1745, 1620, 1600, 1530, 1470, 1450, 1380, 1260.

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