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Study by NMR Spectroscopy of Particular Spatial Structure of B,19-Bisnortestosterone 9,10-Isoanalogs

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Abstract—A complete assignment of signals in the ^{1}H and ^{13}C NMR spectra with the help of homonuclear and heteronuclear correlation NMR spectroscopy was carried out for two isoanalogs of B,19-bisnortestosterone. The predominant conformations of the substances in solutions were derived from the values of coupling constants and nuclear Overhauser effect data.

Considerable attention is paid currently to the search for modified steroid hormons with no affinity to nuclear receptors but capable of biological effects mediated by membrane receptors [1–3]. No less interesting is creation of inhibitors for enzymes responsible for hormones metabolism [4–7]. The practical application of new compounds in this case also requires the lack of hormonal effect.

9,10-Isoanalogs of 19-nortestosterone are known to possess some useful biological properties having at the same time strongly depressed hormonal activity [8, 9]. Therefore synthesis of compounds based on these substances and having the above-mentioned qualities seems quite reasonable. Since the synthesis of B,19-bisnor-9,10-isoanalogs of testosterone may be even easier than that of the corresponding compounds with six-membered B ring we carried out at the first stage of the study the synthesis and investigation of particular features of the spatial structure of one representative of this group substances, namely,

16,16-dimethyl-B,19-bisnor-D-homo-9,10-isotestosterone (**I**). The choice of this compound as a model was based on the fact that its analog with similar structure showed only weak uterotropic activity at conserved significant hypocholesterolemia function [8]. The introduction into its structure of two methyl groups in 16 position may further decrease the uterotropic action as it is observed in the series of modified steroid estrogens [10].

The synthesis of target steroid **I** is presented in Scheme 1. The preparation of compound **II** we described in [11]. Acidic hydrolysis of its reduction product obtained under conditions of Birch reaction afforded steroid **I**. A similar scheme was used before in the synthesis of compounds with analogous structure, but the configuration of the C^{10} center was not established [12, 13].

In the present study we reliably proved the configuration of the forming center C^{10} and also established the prevailing conformation of steroid **I** in solution.

The establishing of the spatial structure of 16, 16-dimethyl-B,19-bisnor-D-homo-9,10-isotestosterone **I** requires estimation of the most important for the conformational analysis parameters, namely, of indirect spin-spin coupling constants [14] and the values of nuclear Overhauser effect (NOE) for strongly overlapped multiplet signals of the majority of the aliphatic protons in the steroid molecule located in the upfield region of the ^{1}H NMR spectrum. A preliminary condition for solving this problem is complete and unambiguous assignment of signals in the ^{1}H NMR spectrum, and to this end it is possible to apply various combination of homo-and heteronuclear correlation methods [15, 16]. In this study
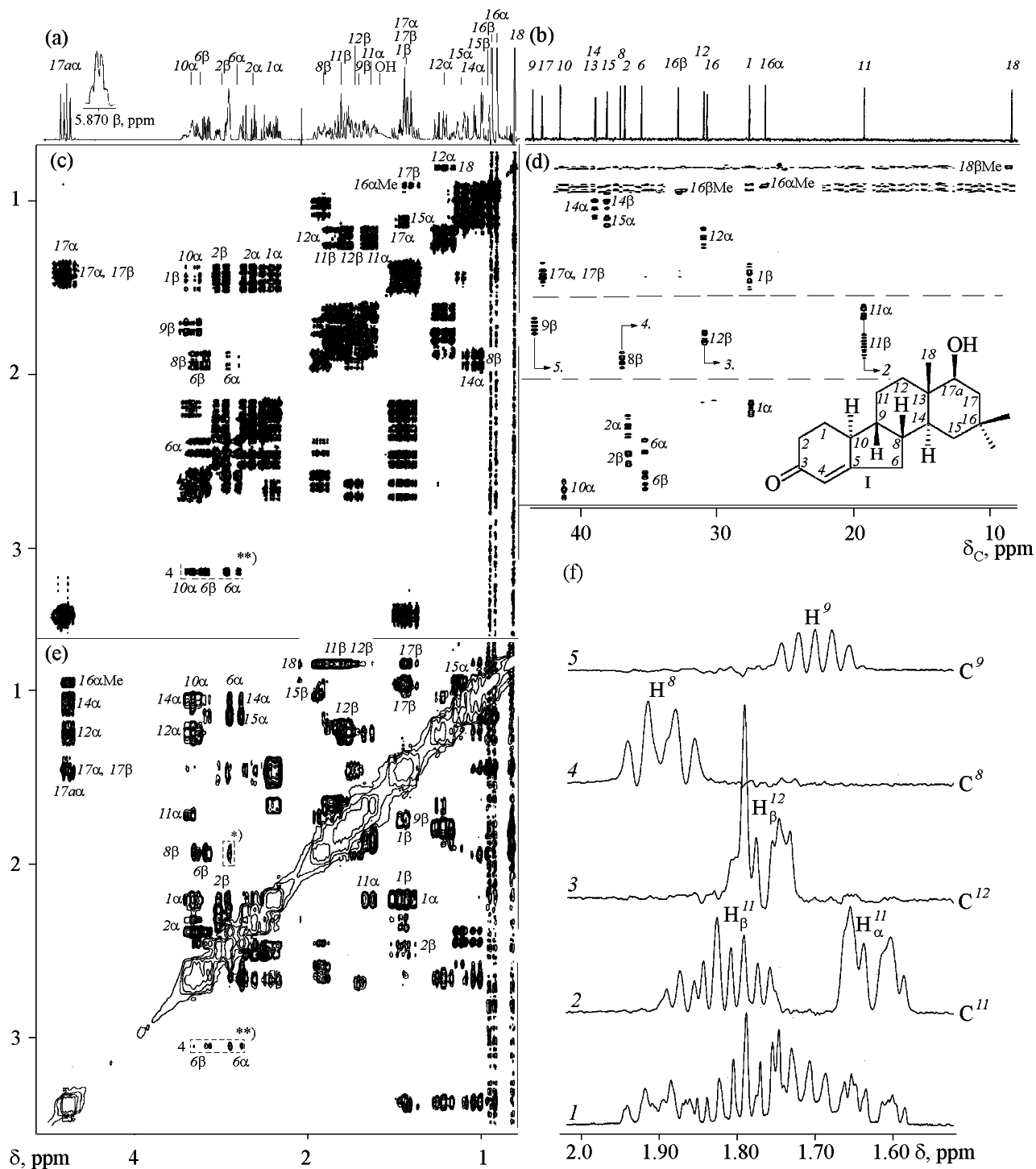
Scheme 1.

(1) Li–NH$_3$/THF
(2) HCl–EtOH–H$_2$O

40%
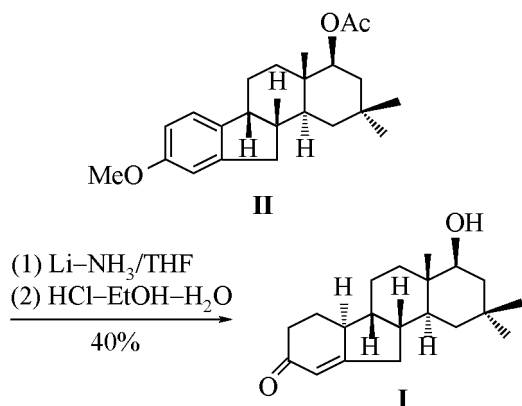
Fig. 1. Upfield regions of the NMR spectra of 17aβ-hydroxy-16,16-dimethyl-3-oxo-D-homo-B,19-bisnor-9,10-isoestr-4-ene (**I**), CDCl₃, 20°C: (a) ^{1}H; (b) ^{13}C; (c) DQF–COSY; (d) HSQC with decoupling from ^{13}C; (e) NOESY at τ_m 0.5 s; (e): (1) a fragment of strongly overlapped region of ^{1}H NMR spectrum (1.5–2 ppm) and F1 profiles in HSQC spectrum (f) at C^{11} 2, C^{12} 3, C^8 4 and C^9 5.

were preferred the methods with the highest resolution for they permit acquiring simultaneously with information on the coupled nuclei also the quantitative estimation of the coupling constants directly from the analysis of the cross-peak multiplicity. Such possibilities are undoubtedly provided for ^1H–^1H correlations by DQF-COSY mode [17], and for ^1H–^{13}C correlations inverse heteronuclear spectroscopy HSQC [18]; the latter is more feasible than HMQC both by resolution and sensitivity [19]. Phase-sensitive NOESY mode [20] was used to determine the spatial orientation of protons.

The ^1H NMR spectrum of compound **I** (Fig. 1a) contains characteristic signals of olefin proton H^4 at 5.86 ppm and of proton H^{17a} at 3.36 ppm, and also three singlets from protons of methyl groups attached to C^{13} and C^{16} in the region 0.8–0.93 ppm. The signals of the rest aliphatic protons are located in the strong field (0.9–2.8 ppm) and appear as four groups of overlapping multiplets. Resonances of protons H^{17a} and H^4 that are present in different parts of the molecule serve as convenient starting point for assignment of the signals in the upfield part of the spectrum. For instance, the signal of methylene protons $C^{17}H_2$ at ~1.4 ppm is easily found from two superimposed cross-peaks in the DQF-COSY spectrum (Fig. 1c) with the signal from proton H^{17a}. The upfield of these cross-peaks is a triplet with a diaxial coupling constant ~13 Hz [for the sake of simplicity here and hereinafter we take into account in defining the multiplicity only large coupling constants (~13 Hz) i.e., geminal and diaxial], and in the NOESY spectrum (Fig. 1e) it has a cross-peak with a methyl group signal at 0.802 ppm that may be identified as $C^{18}H_3$ from its upfield chemical shift at 8.6 ppm in the ^{13}C NMR spectrum (Fig. 1b). Consequently the triplet at 1.391 ppm belongs to the axial proton $H^{17\beta}$, and the resonance of the equatorial proton $H^{17\alpha}$ appears as a multiplet at 1.445 ppm. The signal of the methyl group at C^{16} located in diaxial position with respect to proton $H^{17\beta}$ is present at 0.904 ppm for between them in the DQF-COSY spectrum occurs a cross-peak corresponding to a small coupling constant ^4J [21]. This assignment is confirmed by existence of NOE between the proton H^{17a} and the methyl group at 0.904 ppm (Fig. 1e). Consequently the singlet at 0.935 ppm belongs to the second methyl group at C^{16} that is equatorially oriented. It follows from the DQF-COSY spectrum that the signals of three protons in the region 0.95–1.15 belong to the same spin system, and it is seen in the HSQC spectrum (Fig. 1d) that one among these protons gives a triplet signal and is a methine proton, whereas

the other two, present as a triplet at 0.988 ppm and a doublet at 1.11 ppm, belong to one methylene group. Since the first of the mentioned signals has a cross-peak in the NOESY spectrum with H^{17a}, the second in the same spectrum with $C^{18}H_3$, and for the third in the DQF-COSY is observed a long-range coupling (of *W*-type) with an equatorial proton $H^{17\alpha}$, these signals should belong only to protons $H^{14\alpha}$, axial $H^{15\beta}$, and equatorial $H^{15\alpha}$ respectively.

The results obtained in assignments for the D ring allow identifications to be made in the neighboring B and C rings of steroid **I**. The presence of a cross-peak in the DQF-COSY spectrum between the signal of $H^{14\alpha}$ and that at 1.908 ppm and also the belonging of the corresponding proton to a methine group suggest its assignment to $H^{8\beta}$ proton whose β-orientation is confirmed by the presence in the NOESY spectrum of cross-peaks $18_\beta/8_\beta$ and $15_\beta/8_\beta$. Inasmuch as the next nearest to the H^8 atom are the protons of methylene C^6H_2 and methine C^9H groups their signals at 2.401, 2.597, and 1.711 ppm respectively are easily found by cross-peaks with the H^8 proton in the DQF-COSY spectrum, and their orientation is defined by the presence in the NOESY spectrum of cross-peaks $8_\beta/9_\beta$ and $8_\beta/6_\beta$ (2.597 ppm). It should be noted that although in the NOESY spectrum a sufficiently intense cross-peak $8_\beta/6_\beta$ is observed (marked with an asterisk on Fig. 1, *e*), its occurrence is due to the known specific features of polarization transfer in the strongly coupled systems *ABX* [22], and not to the close position in space of protons $H^{8\beta}$ and $H^{6\alpha}$, as evidenced by characteristic enhanced intensity of the internal component of the doublet corresponding to proton $H^{6\alpha}$. The assignment of the signals from methylene protons $C^{11}H_2$ with the help of cross-peaks with H^9 in the DQF-COSY spectrum is difficult because of their overlapping in the region 1.55–1.95 ppm where are located signals of five protons including those of $H^{9\beta}$, $H^{11\alpha}$ and $H^{11\beta}$ (Fig. 1f, *1*). This follows from the lack of cross-peaks with $H^{9\beta}$ proton in the other regions of DQF-COSY spectrum. Only the signal of H^{10} proton at 2.58 ppm can be distinguished from the corresponding cross-peak. Therefore the position of signals from the protons attached to C^{11} is easier determined from the F1-profile at 1.937 ppm (Fig. 1f, *2*) obtained from the two-dimensional spectrum HSQC (Fig. 1d, profile *2*), for this methylene group is the only one with proton signals in the above mentioned spectral interval. It is seen from this profile that the signal at 1.827 ppm with a triplet-triplet structure belongs to the axial proton $H^{11\beta}$, and the doublet located more

Table 1. Chemical shifts (δ, ppm) in ^{1}H and ^{13}C NMR spectra of 17aβ-hydroxy-16,16-dimethyl-3-oxo-D-homo-B,19-bisnor-9,10-isoestr-4-ene (**I**) and 17β-hydroxy-16,16-dimethyl-3-oxo-B,19-bisnor-9,10-isoestr-4-ene (**III**), CDCl$_3$, 20°C

Atom C no.	Steroid (I)			Steroid (III)		
	δ_C, ppm	^{1}H$_\alpha$, δ, ppm	^{1}H$_\beta$, δ, ppm	δ_C, ppm	^{1}H$_\alpha$, δ, ppm	^{1}H$_\beta$, δ, ppm
1	27.74	2.18	1.434	28.1	2.15	1.43
2	36.86	2.294	2.472	37.14	2.23	2.44
3	199.65	–	–	199.77	–	–
4	122.69	5.869		123.13	5.85	
5	174.51	–	–	175.07	–	–
6	35.63	2.401	2.597	35	2.37	2.59
8	37.19	–	1.906	37.68	–	2.15
9	43.57	–	1.711	43.73	–	1.73
10	41.59	2.647	–	41.99	2.58	–
11	19.37	1.629	1.827	20.72	1.65	1.65
12	31.07	1.202	1.766	27.97	1.01	2.03
13	38.98	–	–	44.08	–	–
14	39.04	1.03	–	45.85	1.06	–
15	38.17	1.11	0.988	23.39	1.58	1.27
16	30.82	–	–	29.69	2.00	1.55
C^{16}-α-CH$_3$	26.58	0.904		–	–	–
C^{16}-β-CH$_3$	32.94	0.935		–	–	–
17	42.9	1.391	1.445	83.43	3.7	–
17a	75.63	3.365	–	–	–	–
18	8.6	–	0.802	16.26	–	1.4
C^{18}–CH$_3$	–	–	–	9.16	1.01	
17β–OH	–	–	–	–	1.62	
17aβ–OH	–	1.58		–	–	–

upfieled at 1.629 ppm corresponds to H$^{11\alpha}$ atom. The orientation of the first proton at C^{11} was established from the NOE with the methyl group C^{18}H$_3$, and the α-orientation was assigned to the H^{10} proton because of two large coupling constants (~13 Hz) corresponding to diaxial coupling with two neighboring protons, one of which was H$^{9\beta}$. Besides in the NOESY spectrum is observed a cross-peak 10$_\alpha$/14$_\alpha$ that supports this conclusion. Now the triplet signal at 1.202 is readily attributed to the axial proton H$^{12\alpha}$ relying on coupling with H$^{11\alpha}$, H$^{11\beta}$ and protons of the methyl group C^{18}H$_3$ (see the corresponding cross-peaks in the spectrum DQF-COSY, Fig. 1c) and on the cross-peaks in NOESY spectrum: 17a$_\alpha$/12$_\alpha$, 14$_\alpha$/12$_\alpha$, 10$_\alpha$/12$_\alpha$ and 11$_\alpha$/12$_\alpha$. Finally, the remaining four multiplets at 2.472, 2294, 2.180, and 1.434 ppm correspond to protons H$^{2e\beta}$, H$^{2a\alpha}$, H$^{1e\alpha}$ and H$^{2a\beta}$, for they all are coupled with each other through the respective cross-peaks in the DQF-COSY spectrum, and the two latter are also coupled with H^{10}. Besides between H$^{2a\alpha}$ and H^{10} is observed NOE in the NOESY spectrum (Fig. 1e), and between H$^{2e\beta}$ and H^4

exists coupling of *W*-type (^4J 0.95 Hz). The two-dimensional spectra DQF-COSY and NOESY in order to enhance the resolution were registered only in the spectral region 0.6–4.5 ppm (see EXPERIMENTAL), therefore the cross-peaks of H^4 proton resonating at 5.86 ppm, i.e. outside this range, appear as reflection, and on Figs. 1c and 1e they are shown by dotted line and marked with two asterisks. These cross-peaks indicate that H^4 proton has a long-range coupling with H$^{6\beta}$ (^4J 2.1 Hz), H$^{6\alpha}$ (^4J 1.8 Hz) and H$^{10\alpha}$ (^4J 1.6 Hz), and also NOE with two former ones. All these facts confirm that the above assignments concerning these protons were correct. Chemical shifts in the ^{1}H and ^{13}C NMR spectra of steroid **I** are listed in Table 1.

The values of coupling constants $^3J_{ij}$ (Table 2) were determined by analysis of the signals multiplicity in ^{1}H NMR spectra, and for overlapping signals from the F1-profiles of the cross-peaks in the DQF-COSY or in HSQC spectrum registered without decoupling from ^{13}C [23]. The calculated by Karplus

Table 2. Experimental and calculated coupling constants $^3J_{ij}$ in steroid (**I**)

i	j	θ, °	$^3J_{ij}$ calc., Hz	$^3J_{ij}$ exp., Hz
1α	2α	53.5	4.9	4.8
1α	2β	62.5	3.7	2.7
1α	10α	57.8	4.3	4.5
1β	2α	170	12.7	13.9
1β	2β	54	4.9	4.4
1β	10α	174.3	12.9	12.6
6α	8β	96.1	2.2	1.8
6β	8β	22	9.7	7.7
8β	9β	41.4	6.9	6.6
8β	14α	173.7	12.9	13
9β	10α	163.6	12.2	12.6
9β	11α	75.9	2.4	1.5
9β	11β	39	7.3	6.6
11α	12α	52.2	5.1	4.8
11α	12β	63.3	3.6	3.2
11β	12α	167.5	12.5	13.4
11β	12β	52	5.2	4.3
14α	15α	63.3	3.6	3.3
14α	15β	179.2	13	12.8
17α	$17a\alpha$	61.5	3.8	5.8
17β	$17a\alpha$	177.8	13	12.8

expression [24] vicinal coupling constants $^3J_{ij}$(calc.) for torsion angles θ_{ij} determined by geometry optimization for steroid **I** molecule along PM3 method in comparison with experimental $^3J_{ij}$ values show good agreement, and thus the spatial structure depicted in Fig.2 is well consistent with the conformation of the molecule in solution.

This conclusion is also supported by estimation of proton-proton distances r_{ij} by measuring nonstationary NOE between the protons where the geometrical factor excludes the contribution from spin diffusion effects [25]. The experimental values r_{ij} (NOE) were obtained by calibration method [26] in isolated spin pair approximation (ISPA) [27] for a model possessing spherical symmetry: $r_{ij} = r_{ref}(\sigma_{ref}/\sigma_{ij})^{1/6}$, where as standard distance r_{ref} was chosen the value 1.767 Å for geminal protons $H^{1\alpha}$ and $H^{1\beta}$. The cross-relaxation rates σ_{ij} and σ_{ref} were determined from a plot of I_{ij}/I_{ii} versus τ_m constructed relying on eight NOESY spectra obtained at mixing times τ_m 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, and 1.2 s. I_{ij} and I_{ii} are volume integrals of the corresponding cross- and diagonal peaks for H^i and H^j protons [28]. Thus obtained values of proton-proton distances r_{ij} (sph) (Table 3) were recalculated for an axially-symmetrical model taking into account the anisotropic diffusion
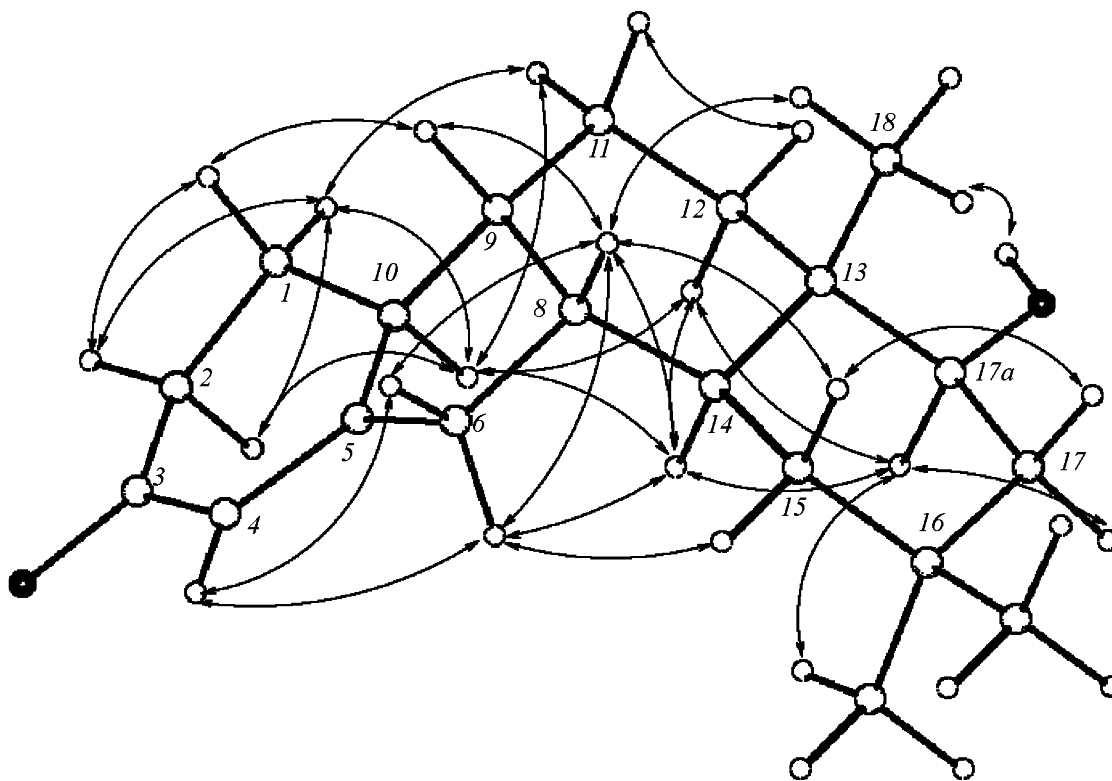

Fig. 2. Spatial structure of conformation prevailing in solution for the molecule of *17*aβ-hydroxy-16,16-dimethyl-3-oxo-D-homo-B,19-bisnor-9,10-isoestr-4-ene (**I**). Arrows indicate NOE the most important for the structure determination.

Table 3. Experimental and calculated values of interatomic distances r_{ij} in steroid **I**

i	j	r_{ij} (exp), Å	r_{ij}, Å[a]	β, deg	$(\tau_c^{ij}/\tau_c^{ref})^{1/6}$	r_{ij}^{β}, Å[b]
1β	1α	1.767	1.767	72	1.000	1.767
11α	1α	2.496	2.248	20	1.083	2.435
12α	12β	1.768	1.787	55	1.016	1.815
12α	11α	2.486	2.460	55	1.016	2.499
1β	9β	2.418	2.248	23	1.078	2.423
12α	$17a\alpha$	2.391	2.190	33	1.058	2.317
14α	$17a\alpha$	2.467	2.274	40	1.043	2.372
8β	6β	2.378	2.200	40	1.043	2.295
11α	10α	2.806	2.710	75	0.999	2.707
1α	10α	2.487	2.400	50	1.024	2.457
12α	10α	2.325	2.150	30	1.064	2.289
14α	10α	2.471	2.311	30	1.064	2.458
1β	2β	2.471	2.470	50	1.024	2.529
14α	6α	2.507	2.366	62	1.007	2.383
15α	6α	1.891	1.805	7	1.098	1.981
15β	8β	2.507	2.285	38	1.048	2.394

[a] For a model with spherical symmetry.   [b] For a model with axial symmetry.

movement of steroid **I** in a liquid [29]. To this end all effective correlation times τ_c^{eff} were calculated from the known Woessner expression [30, 31] for all internuclear vectors r_{ij} and the corresponding polar angles β_{ij} at anisotropy parameter $D_{||}/D_{\perp}$ ~3.2 obtained by calculation of the main inertia moments for molecule **I**: I_a 3352.0, I_b 2884.1 and I_c 969.2 a.u.m.(Å)^2. The polar angles β_{ij} and the corresponding correction factors $(\tau_c^{eff}{}_{ij}/\tau_c^{eff}{}_{ref})^{1/6}$ are given in Table 3.

Correlation between the experimental and calculated values of the proton-proton distances for a spherical (r_{ij}^{sph}) and axially-symmetrical (r_{ij}^{β}) models of data processing for NOE is demonstrated on Figs. 3a and 3b respectively. The comparison of the results evidences that the commonly obtained overestimated values for r_{ij}(NOE) of steroid molecules in the range ~2.0–2.6 Å are not due to intramolecular mobility as is assumed in [32, 33], but primarily due to anisotropic diffusion movement of these molecules in solution and thus to unsuitable model for data processing.

Thus the experimental data on coupling constants and proton-proton distances obtained by NMR measurements in solution are in agreement with the spatial structure of the molecule (Fig. 2) calculated by semiempirical procedure PM3.

It is known that in steroids a conformation transmission is of great importance: as a result modifications in the D ring may lead to changes in conform-

ations of the other rings [34] and to altered reactivity of substituents in the A ring [35]. In this connection it seemed necessary to find out whether the change in the size of ring D would affect the conformation of ring A. As a model compound we selected steroid **III**. The preparation of the compound was reported in [36], and its probable structure was described by analogy with [12] and [13]. We synthesized compound **III** as shown in Scheme 2. However the sample we obtained had mp 190–191°C instead of 155–156°C reported in [36]. Too low melting point and no unambiguous proofs of the structure cast a doubt on preparation in [36] of a good sample of steroid **III**.

In this study we carried out for compound **III** a complete assignment of signals in the ^{1}H and ^{13}C NMR spectra (Table 1) and established the spatial arrangement of its molecules in solution (Fig. 4a). We applied to this problem the methods of correlation NMR spectroscopy similar to those described above for steroid **I**. The key NOE used in determination of compound **III** structure are marked with arrows on Fig. 4a.

As seen from Figs. 2 and 4a, the prevailing conformations of compounds **I** and **III** in solutions are very similar. In the place of *cis*-junction of B and C rings in the molecules of 9,10-isoanalogs of steroid androgens appears a bend that is absent in analogs with the natural junction of the rings. As a result the

Scheme 2.
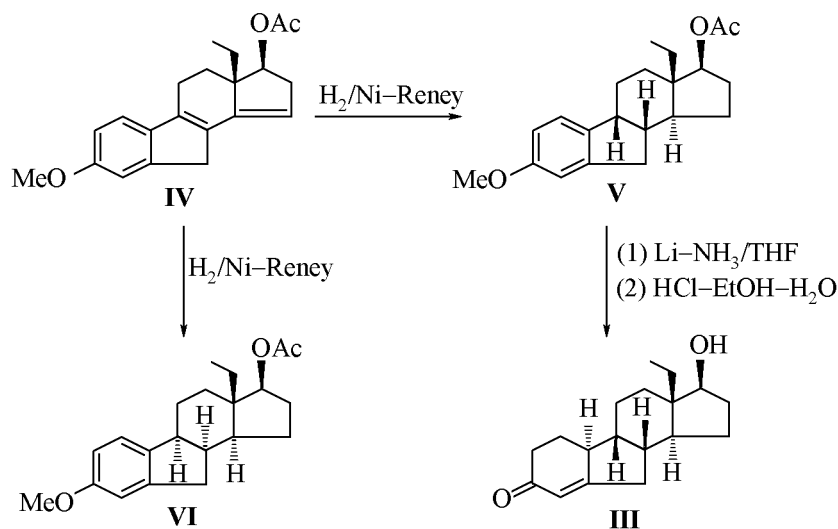
protons $H^{10\alpha}$ and $H^{17a\alpha}$ get near $H^{12\alpha}$ and $H^{14\alpha}$ to ~2.4 A that brings about strong NOE between them.

It should be mentioned that compounds **I** and **III** might exist in more planar conformations, analogous to that shown on Fig.4, *b*. In this conformation atoms $H^{10\alpha}$ and $H^{12\alpha}$, $H^{14\alpha}$ are not drawn together, and the C ring is in the *boat* conformation. Tentative estim-

ations carried out along unrestricted molecular mechanics method MM^+ and semiempirical PM3 procedure suggest that the formation energies of the compared pairs of the alternative conformations of compounds **I** and **III** are similar. Still in the solutions of compounds under investigation were not observed conformations similar to that shown on Fig. 4b.
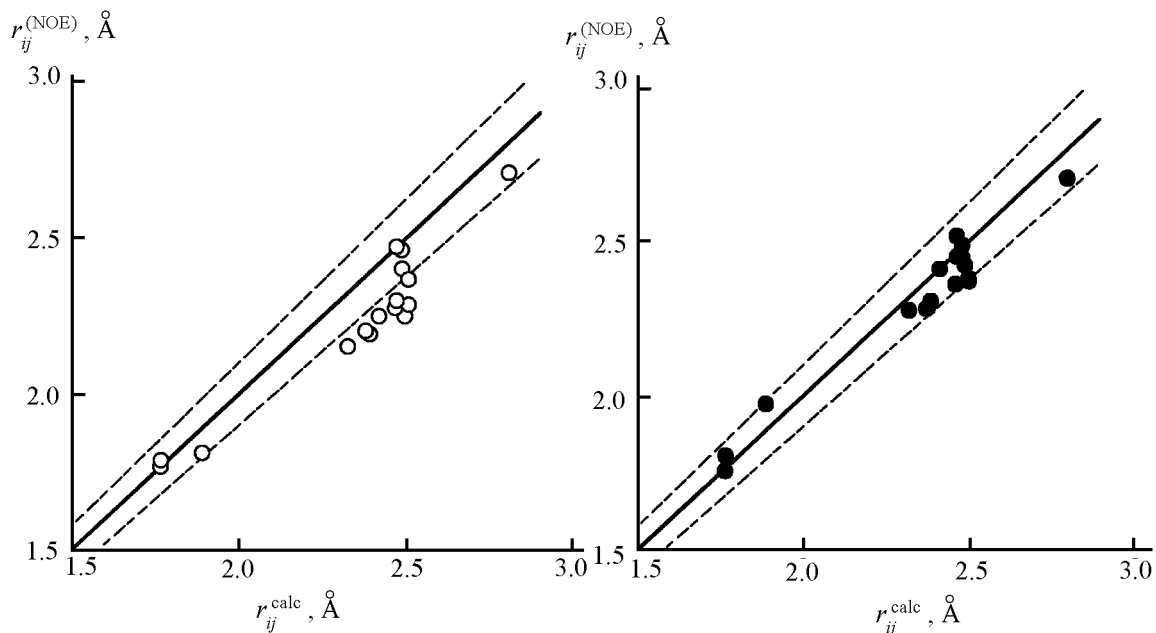

Fig. 3. Correlation of calculated $r_{ij}^{(calc)}$ and experimental proton-proton distances in approximations of spherical-symmetric $r_{ij}^{(NOE)}$ (*a*) and axially-symmetric $r_{ij}^{\beta(NOE)}$ (*b*) models. Dotted lines mark the relative deviation of ±5%.

(a)   (b)
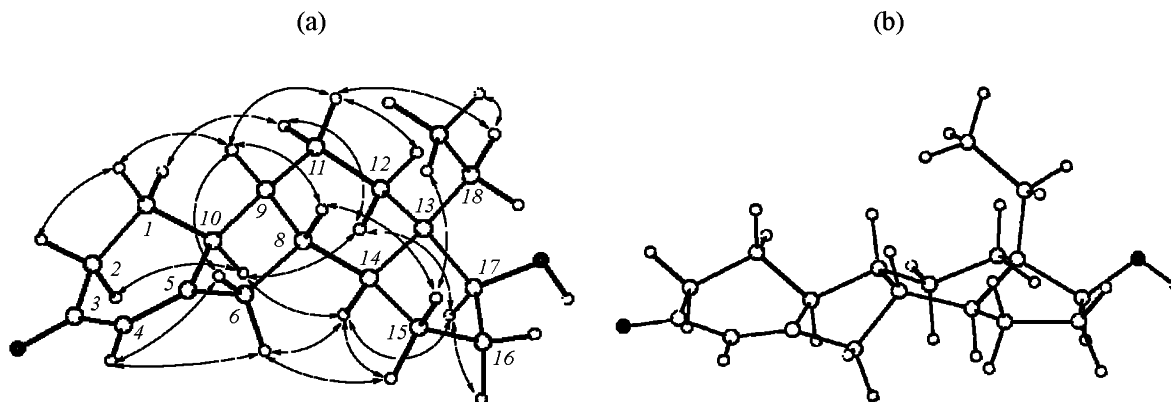

Fig. 4. (a) Spatial structure of conformation prevailing in solution of the molecule of *17*β-hydroxy-16,16-dimethyl-3-oxo-B,19-bisnor-9,10-isoestr-4-ene (**III**). Arrows indicate NOE the most important for the structure determination. (b) Theoretically probable more planar conformation of compound **III** not found in solution.

Finally it may be presumed that the ring A of compounds **I** and **III** exists in the an alternative conformation where protons $H^{1\alpha}$ and $H^{2\beta}$ have pseudoaxial, and protons $H^{1\beta}$ and $H^{2\alpha}$ have pseudoequatorial orientation. However, the results of the present study show that with compounds **I** and **III** this possibility is not actually achieved.

EXPERIMENTAL

Mass spectra were measured on MKh-1321 instrument at ionizing chamber temperature 200–210°C. ^{1}H and ^{13}C NMR spectra were registered at 295K on spectrometer Bruker DPX-300 at operating frequencies 300.130 and 75.468 MHz respectively. The measurements were performed on solutions containing in 0.6 ml of CDCl$_3$ 5–7 mg of compound for ^{1}H NMR spectra and 30–50 mg for ^{13}C NMR spectra.

Chemical shifts were measured relative to TMS; as internal reference served the signals of the solvent (CDCl$_3$ containing 0.1% of CHCl$_3$) that were assigned standard values of chemical shifts for ^{1}H and ^{13}C 7.26 and 76.90 ppm respectively with accuracy no less than ±0.01 ppm. The homonuclear coupling constants were measured with accuracy ±0.02 Hz from ^{1}H NMR spectra obtained after additional processing of the spectra with Lorentz–Gaussian transformation of peaks [37].

All experiments were carried out in a 5-millimeter double-channel probe, magnetic field stabilization was performed on deuterium signal, standard pulse sequence was used in quadrature detection mode. The duration of π/2 pulse at power parameter *PL* –3 dB was for ^{1}H nuclei 7.7 μs, for ^{13} nuclei 7.4 μs.

The registering of ^{13}C NMR spectra with proton decoupling was performed with the use of pulse sequence WALTZ-16 [38], and decoupling from ^{13}C nuclei in the inverse mode HSQC [18] was performed with pulse sequence GARP [39].

In all experiments with two-dimensional spectra before Fourier transform procedures of supplementation with zeros and apodization were carried out. The application of various weight functions depended on the goal of the experiment.

The main parameters of NMR spectra registering and processing were as follows. ^{1}H NMR: number of points for data sampling *TD* 32K; spectral width *SW* 2.4 kHz; number of scans; *NS* 128; relaxation delay *D1* 3 s; parameters of Lorentz–Gaussian trans=formation *LB* –2 Hz, *GM* 0.2; supplementation with zeros: *SI* 64 K or 128 K. ^{13}C NMR: *TD* 32 K; *SI* 64 ¤; *SW* 16.5 kHz; *NS* 512; *D1* 5 s; parameter of exponential weight function *LB* 3 Hz.

COSY-DQF [17]: number of points for data sampling *TD2* 2 K; spectral width $SW1 = SW2$ 1.177 kHz, *NS* 32 for every of 512 t_1-increments; *D1* 1.6 s; dimensions of spectral matrix 2048×1024; apodization function: along t_1 coordinate Lorentz–Gaussian transformation (LB –1, GM 0.1), along t_2 sin(πt/t$_{max}$+ π/3); phase-sensitive detection with TPPI [40].

HSQC [18]: *TD* 1 K; *SW1* 2.858 kHz; *SW2* 0.733 kHz; *NS* 128 for every of 200 t_1-increments; *D1* 1 s; duration of the composite π/2-pulce in GARP sequence 59 μs; apodization function along t_2 coordinate Lorentz–Gaussian transformation (*LB* –2 Hz, *GB* 0.2), along t_1 sin(πt/t$_{max}$+ π/2); dimensions of spectral matrix 4098×1024.

In registering of HSQC spectrum without decoupling from ^{13}C nuclei the last refocusing period was eliminated, and detection started directly after the second INEPT-transfer of polarization; as a result the observed cross-peaks obtained antiphase orientation. *TD2* 1 K; *SW1* 2.858 kHz; *SW2* 0.783 kHz; *NS* 240 for every of 96 t_1-increments. In order to increase the resolution in F1-profiles before the second Fourier transform along the t_2 coordinate was applied an additional procedure of direct linear prediction [41]. The other parameters of registration and processing correspond to those described for HSQC [18].

NOESY [14]: *TD2* 1 K; *SW1* = *SW2* = 1.456 kHz; *NS* 64 for every of 256 t_1-increments. Phase-sensitive detection with TPPI [40]; apodization function along t_1 and t_2 coordinates $\sin(\pi t/t_{max} + \pi/2)$; dimensions of spectral matrix 1024×512. At quantitative estimation of r_{ij} to increase the sensitivity of NOESY spectra registering it was performed with shortened relaxation cycle (fast NOESY) [42]. The repetition period ($AQ + \tau_m + D1$) at spin-lattice relaxation time for protons T_1^i ~0.6–0.9 s was kept constant (2.531 s) in all eight experiments (τ_m 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0 and 1.2 s) by the corresponding variation of the relaxation delay: $D1 = 2.2$ s – τ_m. The contribution of zero quantum coherence into the intensity of cross-peaks was suppressed by random variation of τ_m [43]. The total registration time of each spectrum was ~12 h.

The purity of all compounds was checked by TLC on Silufol plates in solvent systems petroleum ether–ethyl acetate (4:1), (3:1).

17aβ-Hydroxy-16,16-dimethyl-3-oxo-D-homo-B,19-bisnor-9,10-isoestr-4-ene (I). To a solution of steroid **II** (0.7 g, 1.83 mmol) in 80 ml of tetrahydrofuran and 100 ml of liquid ammonia was added at –60°C 1 g (0.143 mol) of finely cut lithium. 5 h later under the same conditions was slowly added 25 ml of anhydrous ethanol. After common workup [44] the reaction product was dissolved in 50 ml of boiling ethanol, to the solution was added 30 ml of 3 M HCl solution, and the mixture was boiled for 2 h. Then it was poured into 100 ml of water, and after common treatment the residue was crystallized from a mixture of petroleum ether and ethyl acetate. The compound remaining in the mother liquor was purified by preparative TLC (on silica gel, 5–40 μm, eluent petroleum ether–ethyl acetate, 6:1, 5:1). We obtained 239 mg (40%) of steroid I as colorless crystals, mp 147–149°C. For ^{1}H and ^{13}C NMR spectra see Table 1. Mass spectrum, *m/z* (I_{rel}, %): 302 (100), 284 (22), 275 (54), 261 (36), 241 (9), 228

(16), 216 (17), 193 (23), 187 (17), 175 (52), 159 (28), 148 (39), 131 (44). Found, %: C 79.75; H 10.24. $C_{21}H_{32}O_2$. Calculated,%: C 79.70; H 10.19.

17β-Hydroxy-18-methyl-3-oxo-B-nor-9,10-iso-estr-4-ene (III) was obtained similarly to compound **I**, mp 190–191°C. For ^{1}H and ^{13}C NMR spectra see Table 1. Mass spectrum, *m/z* (I_{rel}, %): 274 (100), 256 (13), 245 (24), 231 (22), 215 (39), 201 (17), 187 (17), 173 (18), 160 (26), 146 (50), 131 (26), 119 (38). Found, %: C 78.90; H 9.58. $C_{18}H_{26}O_2$. Calculated, %: C 78.79; H 9.55.

17β-Acetoxy-18-methyl-3-methoxy-B-norestra-1,3,5(10),8,14-pentaene (IV) was obtained as described in [45], mp 86–88°C. ^{1}H NMR spectrum (δ, ppm): 0.96 t (3H, C^{18}–CH$_3$), 2.13 s (3H, OCOCH$_3$), 3.83 s (3H, OCH$_3$), 5.14 t (1H, H^{17}, $^3J_{17,16}$ 8.4 Hz), 5.49 s (1H, H^{15}), 6.82 d (1H, H^2, $^3J_{1,2}$ 8.1 Hz), 7.02 s (1H, H^4), 7.15 d (1H, H^1, $^3J_{1,2}$ 8.1 Hz). Found, %: C 77.61; H 7.23. $C_{21}H_{24}O_3$. Calculated, %: C 77.75; H 7.46.

17β-Acetoxy-18-methyl-3-methoxy-B-nor-9-iso-estra-1,3,5(10)-triene (V) was prepared by catalytic hydrogenation of compound **IV** as described in [11], mp 95–97°C (from hexane –ether). ^{13}C NMR spectrum (δ_C, ppm): 8.9, 17.3, 21.0, 21.4, 23.6, 26.7, 28.7, 35.8, 41.7, 42.4, 43.1, 43.9, 55.2, 83.6, 111.2, 123.0, 137.0, 144.6, 158.6, 170.7. Found, %: C 76.56; H 8.68. $C_{21}H_{28}O_3$. Calculated, %: C 76.79; H 8.59. We obtained besides **17β-acetoxy-18-methyl-3-methoxy-B-nor-8-isoestra-1,3,5(10)-triene (VI)**, mp 109.5– 110.5°C (from hexane –ether). ^{13}C NMR spectrum (δ, ppm): 9.2, 18.5, 21.0, 23.5, 27.2, 27.7, 32.2, 33.7, 41.6, 44.1, 45.2, 55.0, 83.6, 110.4, 112.0, 124.3, 140.4, 143.7, 158.6, 170.5. Found, %: C 76.56; H 8.68. $C_{21}H_{28}O_3$. Calculated, %: C 76.65; H 8.61.

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