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Preparation and Spatial Structure of Methyl Ether of 7β-Methyl-D-homo-6-oxaestra-1,3,5(10),8(9)-tetraen-17a-one

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Abstract—Procedure of preparation of 7β-methyl-D-homo-6-oxa-1,3,5(10),8,(9)-estratetraen-17a-one methyl ether has been developed; the product conformation in the solution has been studied by NMR spectroscopy. Comparison of the experimental data with simulation of similar compounds docking into the ligand-binding pocket of the estrogens α-receptor has suggested that such steroids are promising for preparation of biologically active substances whose action would not be mediated by the estrogens receptors.

Keywords: conformation analysis, estrogen receptor, antioxidant activity, inhibitor of estrone sulfatase, hormone-dependent oncological disease

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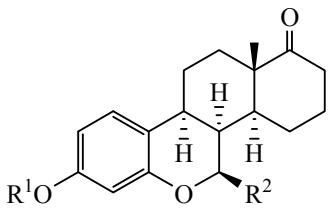
Women breast cancer is considered to be the most widespread oncological disease [1]; it is often estrogen-dependent [2]. In the living organism, the estrogens are formed under the action of aromatase, 17β-hydroxysteroiddehydrogenase, and estrone sulfatase; these enzymes appear suitable targets for the oncologic diseases treatment [3]. A search for new inhibitors of these enzymes should be fruitful among the steroids containing the sulfamate groups, since it is possible to design compounds capable of simultaneous inhibition of estrone sulfatase and aromatase [4, 5] or sulfatase and 17β-hydroxysteroiddehydrogenase [6, 7]. Such inhibitors are promising for the control of the metabolic processes accompanying prostate cancer as well [8, 9].

It was previously shown that 7β-methyl-D-homo-6-oxa-8α-estrone sulfamate **Ia** efficiently inhibited the estrone sulfatase, and the potential metabolite **Ib** of the former not exhibited any uterotropic activity [10]. The decisive part in the drastic decrease of the hormonal action of the estrone **Ia** as compared to that of compound **Ic** was played by the β-methyl group at the C^7 atom; therefore, the preparation and the study of biological activity of other 7β-methyl-D-homo-6-oxasteroids was of definite interest. We chose the steroid **IIa** as the starting model compound, because 8-dehydro analogs of estrogens might possess a higher antioxidant activity [11, 12] (Scheme 1).

The easiest way to prepare compound **IIa** was the selective catalytic hydrogenation of estrapentaene **IIIa**; however, the latter could be obtained via cyclodehydration of compound **IV** by trifluoroacetic acid in the yield as low as 35% [13]. Therefore, we investigated the cyclodehydration reaction under a variety of conditions and performed the catalytic hydrogenation of the **IIIa** + **IIIb** isomers mixture (Scheme 2).

Figure 1 shows the range of ^{1}H NMR spectra of the steroid **IIa** and its isomer **IIb** containing the aliphatic protons signals. The range of 1.55–1.8 ppm was the most challenging for interpretation; it contained the signals of the three axial protons, $H^{12\alpha}$, $H^{15\beta}$, and $H^{16\alpha}$, the latter pair being in the *trans*-diaxial orientation and

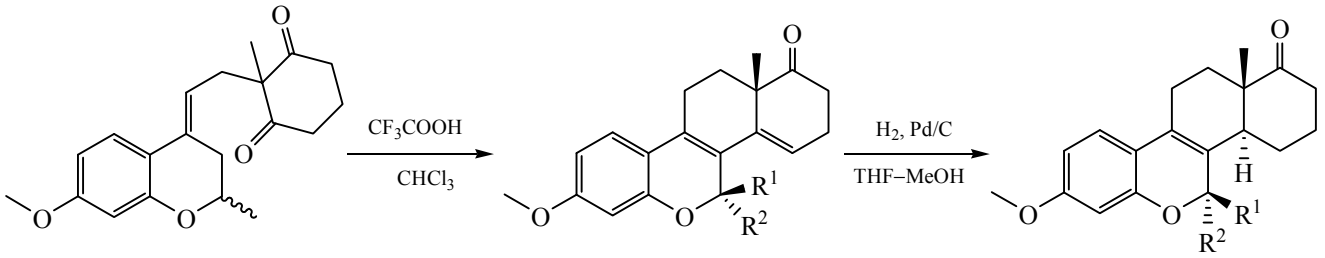
Scheme 1.



I

$R^1 = SO_2NH_2$, $R^2 = CH_3$ (**a**); $R^1 = H$, $R^2 = CH_3$ (**b**); $R^1 = H$, $R^2 = H$ (**c**).

Scheme 2.



IV **III** **II**

$R^1 = CH_3$, $R^2 = H$ (**a**); $R^1 = H$, $R^2 = CH_3$ (**b**).

should have possessed the relatively high (~13 Hz) vicinal constant $^3J_{15\beta-16\alpha}$. Those protons formed the AB spin system, leading to the strong coupling effects for the coupled protons $H^{14\alpha}$, $H^{15\alpha}$, $H^{16\beta}$, $H^{17\alpha}$, and $H^{17\beta}$, each of them representing the X part of the corresponding three-spin ABX system. Consequently, overlapping of the signals of the $H^{15\beta}$ and $H^{16\alpha}$ protons should have resulted in distortion of the multiplet structures of all protons of the D ring. Therefore, in order to interpret the above-mentioned signals and to determine the vicinal constants, we combined the data of various two-dimensional techniques (DQF-COSY, J-COSY, HSQCnd, and NOESY) to simulate the subspectrum of the 7-spin system of the $C^{14}H–C^{15}H_2–C^{16}H_2–C^{17}H_2$ fragment. As a result, we got a set of vicinal constants for the protons of the D ring; their values undoubtedly confirmed the *chair-like* conformation of the ring with axial location of the $H^{14\alpha}$, $H^{15\beta}$, $H^{16\alpha}$, and $H^{17\beta}$ protons. As the $H^{15\beta}$ and $H^{16\alpha}$ interacted, the precise chemical shifts were obtained applying the HSQCnd method diminishing the strong coupling effects (Fig. 2).

The set of the spatial interactions (NOE) of the observed protons in the steroid **IIa** was also important. Not only it independently confirmed the structure of the D ring, but it served as the principal criterion in determining the structure and conformation differences of the estratetraenes **IIa** and **IIb**. The primary question to be so solved was the spatial orientation of the 7-methyl group and the elucidation of its effect on the B and C rings conformation. Figure 3 shows the NOESY spectrum of estratetraene **IIa**; the spatial interactions crucial to confirm its structure are marked in Fig. 4. Among the found interactions that between the C^7H (Me) proton and the D ring protons as well as that between the H^1 aromatic proton and the C $H^{11\alpha}$ and $H^{11\beta}$ ring protons were the most important.

The following spatial interactions were characteristic of the estratetraene **IIa** (Fig. 3): the $H^{7\alpha}$ proton possessed strong interaction with the $H^{15\alpha}$ proton and a relatively weak $H^{7\alpha}/H^{14\alpha}$ cross-peak, whereas the methyl group protons showed the $H^{7\beta}/H^{15\alpha}$ and the $H^{7\beta}/H^{15\beta}$ cross-peaks, the latter being much stronger.

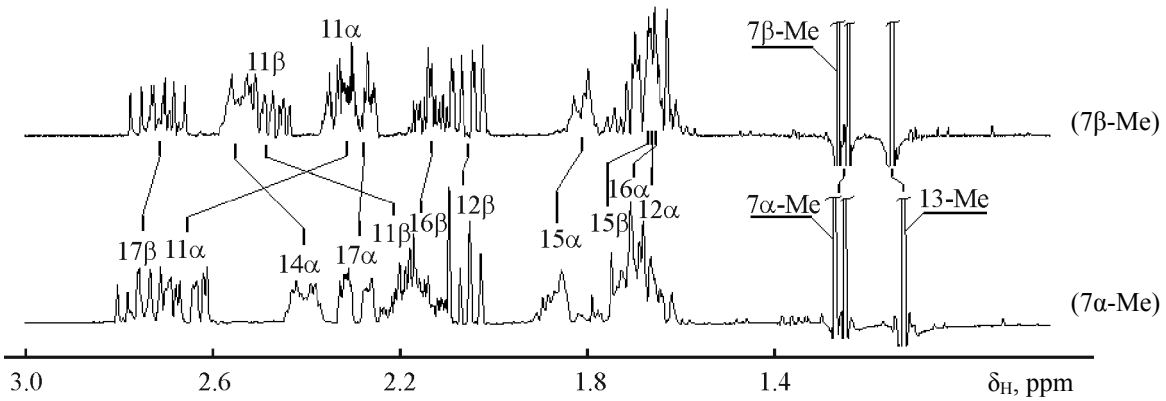


Fig. 1. Parts of ^1H NMR spectra of estratetraenes **IIa** and **IIb** containing the signals of the aliphatic protons.

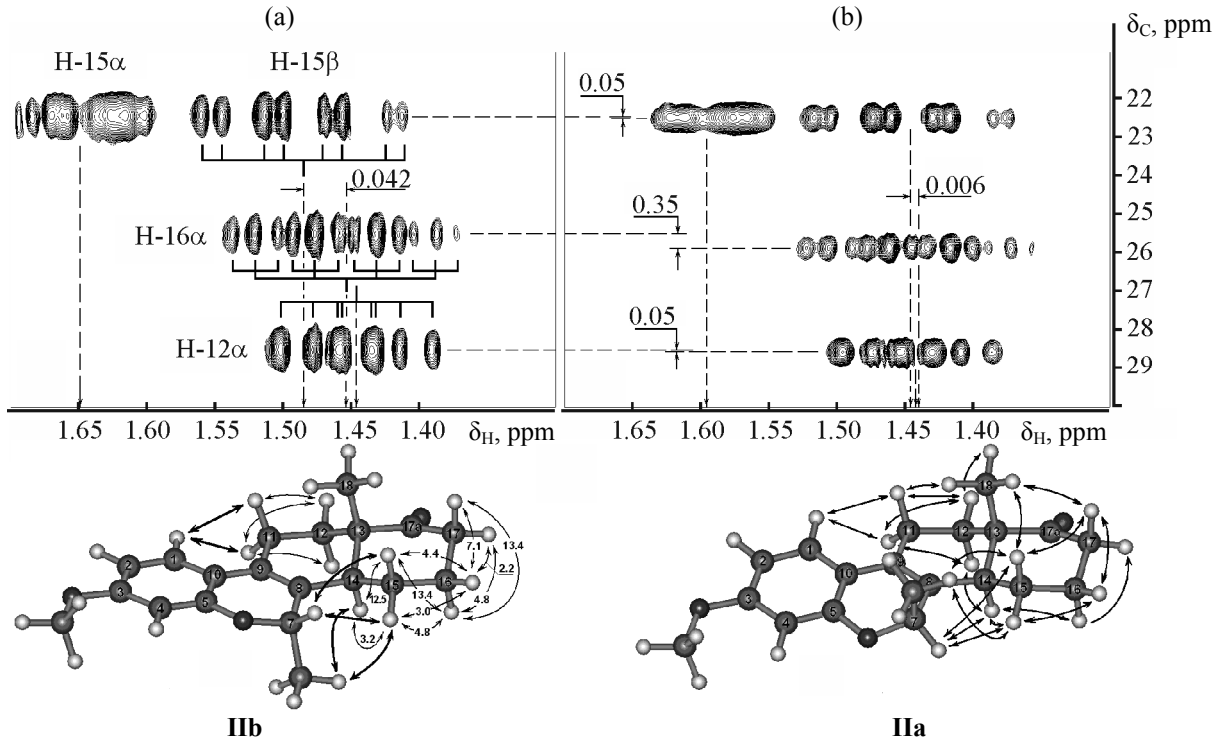


Fig. 2. Parts of HSQCnd spectra of D-homo analogs of estratetraenes (**IIa**, **IIb**) (a, b) and 3D-models of their molecules. (Bold arrows) point at the found direct interactions (NOE), (thin arrows) point at the scalar interactions between the protons of rings C and D (the numbers denote the constants in Hz). Multiplet structures of the $H^{15\beta}$, $H^{16\alpha}$, and $H^{12\alpha}$ proton signals are shown in the spectra; the numbers show the differences between positions of the ^{1}H NMR and ^{13}C NMR signals in the spectra of the estratetraenes (**IIa**, **IIb**), ppm.

Taking into account the relative intensity of the cross-peaks, the spatial orientation (α or β) of the proton and the methyl group in position 7 could be elucidated; furthermore, it was a strong argument for the methyl pseudo-axial orientation in the B ring of the estratetraene **IIa**, as shown by the strong $H^{7\beta}/H^{15\beta}$ cross-peak.

The orientation type (α or β) of the 7-methyl group in the estratetraenes **IIa** and **IIb** resulted in the noticeable conformation changes in the C ring: in the case of **IIb** $r_{1-11\beta} > r_{1-11\alpha}$, whereas in the case of **IIa** $r_{1-11\beta} < r_{1-11\alpha}$. That was a direct experimental confirmation of a slight change of the orientation of the $C^8{=}C^9$ double bond with respect to the A aromatic ring (as compared to the estratetraene **IIb**) in the case of β-orientation of 7-methyl group. In turn, that resulted in the change of the $H^{11\alpha}$ and $H^{11\beta}$ positions with respect to the H^1 proton (and, hence, with respect to the A ring plane) (Fig. 4).

The distances between the H^1 and $H^{11\beta}$ as well as between the H^1 and $H^{11\beta}$ protons as elucidated by the NOESY spectra coincided with those determined via the MM+ simulation. That was a rrason for docking 3-hydroxy analog (**V**) and similar compounds in the ligand-binding part of the estrogens α-receptor, built using the X-ray diffraction data [14] and applied to estimate the uterotropic activity of the structurally related compounds (cf. [15, 16]). In particular, the complex formation of the type **V** steroids with the receptor was unlikely due to the impossibility of the hydrogen bonding between the ligand keto group and the His524 unit. Furthermore, Leu346, Leu349, Ala350, Met388, and Ile424 should have also prevented the efficient complex formation. Hence, such steroids are promising candidates for further development of the substances with the biological action not mediated via the estrogens α-receptors. The preparation of potential inhibitors of estrone sulfatase basing on compound **V** is currently under way and will be reported separately (Scheme 3).

EXPERIMENTAL

All prepared compounds were racemic mixtures. Their purity was checked with TLC (Silufol plates;

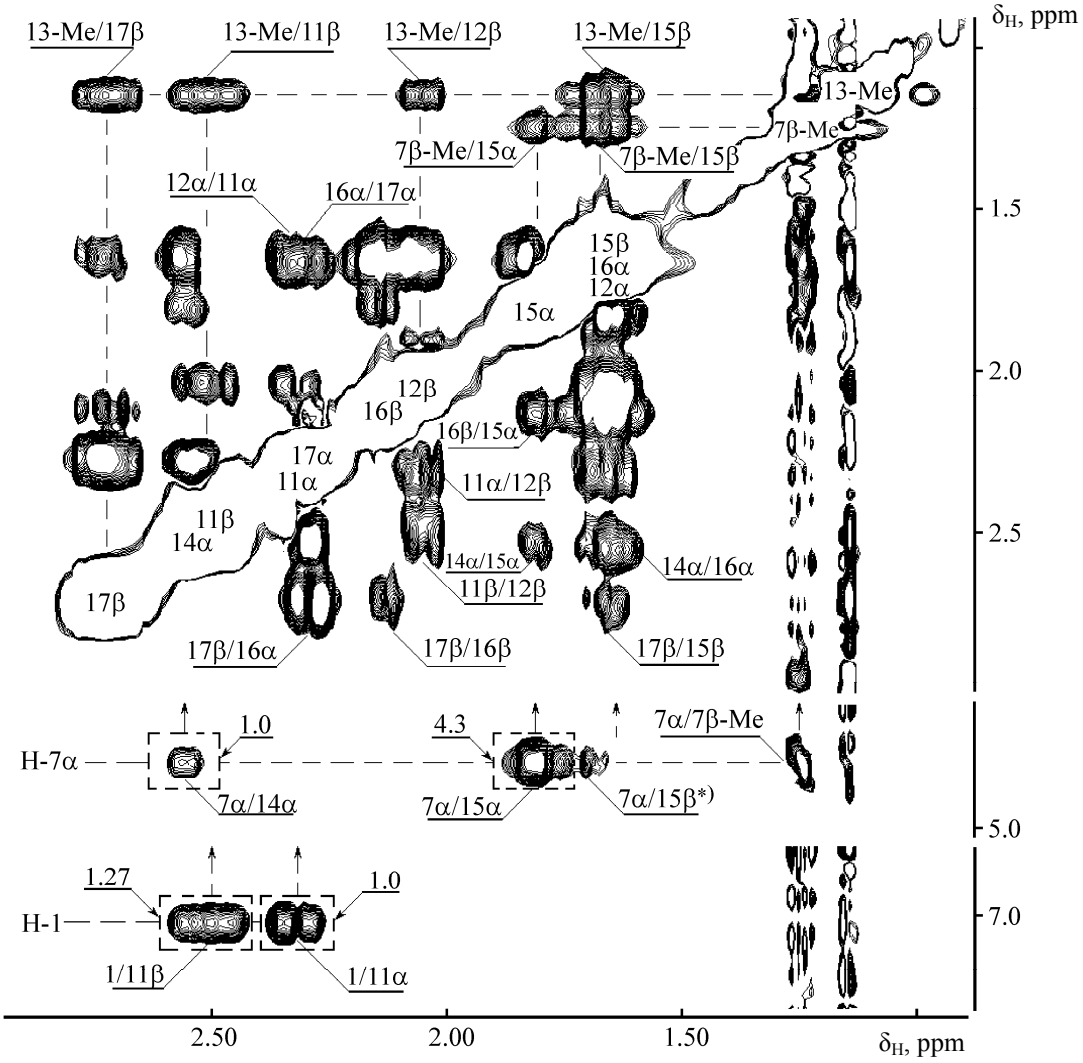


Fig. 3. Parts of NOESY spectrum (τ_m 0.5 s) of estratetraene **IIa**: (asterisk) marks the $H^{7\alpha}/H^{15\beta}$ cross-peak appearing due to the zero-quantum contribution via the $^2J_{15\alpha-15\beta}$ constant; (dashed line marks the $H^{7\alpha}/H^{14\alpha}$, $H^{7\alpha}/H^{15\alpha}$, $H^1/H^{11\alpha}$, and $H^1/H^{11\beta}$ cross-peaks, the numbers denoting the relative volume integrals.

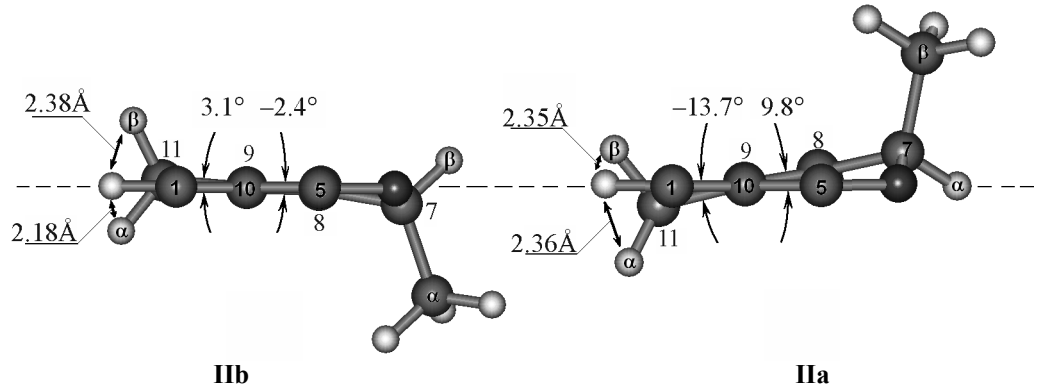
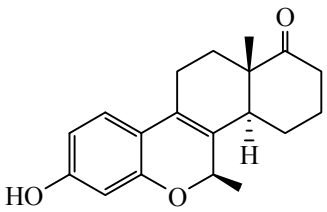


Fig. 4. The Newman projections of estratetraenes (**IIa**, **IIb**) fragments along the C^9–C^{10} bond: (arrows) mark the $C^1C^{10}C^9C^{11}$ and $C^5C^{10}C^9C^8$ torsion angles and the $r_{1-11\alpha}$ and $r_{1-11\beta}$ interprotonic distances; the numbers show their simulated values (the MM^+ geometry optimization).

Scheme 3.



V

eluent petroleum ether – ethyl acetate 6 : 1, 4 : 1, or 3 : 1). NMR spectra were recorded using the DPX-300 Bruker spectrometer at 300.130 MHz (^{1}H) and 75.468 MHz (^{13}C) at 295 K.

7-Methyl-3-methoxy-D-homo-6-oxaestra-1,3,5(10),8,14-pentaen-17a-one (IIIa, IIIb). To a solution of 2.8 g of compound **IV** [13] in 12 mL of $CHCl_3$ was added 1.5 mL of CF_3COOH, and the mixture was stirred at room temperature. The reaction completeness was checked by TLC (petroleum ether–EtOAc, 2 : 1). The resulting mixture was poured into 100 mL of water and extracted with $CHCl_3$ (4 × 50 mL). The organic layer was washed with 100 mL of saturated NaCl solution and with water till neutral reaction of the washings, and dried over Na_2SO_4. The solvent was evaporated using the rotary evaporator, and the residue was crystallized from methanol. After drying in a vacuum, 0.82 g (63%) of the 7 : 1 mixture of **IIIa** and **IIIb** epimers was obtained, mp 145–153°C. The epimers ratio was elucidated from ^{1}H NMR spectrum, complete assignment of the signals was reported elsewhere [13].

The total yields of the epimers **IIIa** and **IIIb** and the corresponding epimers ratios were different in the cases of cyclodehydration by trifluoromethanesulfonic acid in chloroform (37%, 3 : 1), by trifluoroacetic acid in benzene (84%, 3 : 1), and by *p*-toluenesulfonic acid in benzene (64%, 5 : 1).

7β-Methyl-3-methoxy-D-homo-6-oxaestra-1,3,5(10),8(9)-tetraen-17a-one (IIa) and 7α-methyl-3-methoxy-D-homo-6-oxaestra-1,3,5(10),8(9)-tetraen-17a-one (IIb). Hydrogen was bubbled through a solution of 0.4 g of the 7 : 1 mixture of compounds **IIIa** and **IIIb** in 6 mL of the 1 : 1 mixture of THF and MeOH in the presence of 40 mg of 10% Pd/C (atmospheric pressure, room temperature, at stirring) during 5 days. The catalyst was then filtered off, the solvents were removed in a vacuum, and the residue was crystallized from methanol. After drying in a vacuum, 0.25 g (64%) of the estratetraene **IIa** was obtained, mp 179–181°C. ^{1}H NMR spectrum, δ, ppm: 7.02 (C^1H), 6.45 (C^2H), 6.41 (C^4H), 4.79 (C^7H^α), 2.31 ($C^{11}H^\alpha$), 2.48 ($C^{11}H^\beta$), 1.64 ($C^{12}H^\alpha$), 2.05 ($C^{12}H^\beta$), 2.56 ($C^{14}H^\alpha$), 1.80 ($C^{15}H^\alpha$), 1.65 ($C^{15}H^\beta$), 1.64 ($C^{16}H^\alpha$), 2.13 ($C^{16}H^\beta$), 2.28 ($C^{17}H^\alpha$), 2.71 ($C^{17}H^\beta$), 1.14 ($C^{18}H_3$), 3.76 (CH_3O), 1.24 (C^7-βCH_3). ^{13}C NMR spectrum, δ_C, ppm: 123.1 (C^1), 106.6 (C^2), 159.8 (C^3), 102.2 (C^4), 152.1 (C^5), 70.9 (C^7), 128.0 (C^8), 122.6 (C^9), 117.5 (C^{10}), 21.7 (C^{11}), 28.6 (C^{12}), 46.9 (C^{13}), 45.6 (C^{14}), 22.5 (C^{15}), 25.8 (C^{16}), 36.7 (C^{17}), 214.5 (C^{17a}), 16.2 (C^{18}), 55.1 (CH_3O), 19.3 (C^7-βCH_3). Mass spectrum (ESI/MS): *m*/*z* 313.43 (calculated 313.42, $C_{20}H_{22}O_3$).

Chromatographic separation of the residue on the column with 20 g of silica gel yielded 8 mg of compound **IIb**. ^{1}H NMR spectrum, δ, ppm: 7.05 (C^1H), 6.45 (C^2H), 6.39 (C^4H), 4.94 (C^7H^β), 2.65 ($C^{11}H^\alpha$), 2.17 ($C^{11}H^\beta$), 1.64 ($C^{12}H^\alpha$), 2.06 ($C^{12}H^\beta$), 2.40 ($C^{14}H^\alpha$), 1.85 ($C^{15}H^\alpha$), 1.72 ($C^{15}H^\beta$), 1.68 ($C^{16}H^\alpha$), 2.15 ($C^{16}H^\beta$), 2.28 ($C^{17}H^\alpha$), 2.74 ($C^{17}H^\beta$), 1.12 ($C^{18}H_3$), 3.77 (CH_3O), 1.25 (C^7-αCH_3). ^{13}C NMR spectrum, δ_C, ppm: 123.1 (C^1), 106.2 (C^2), 160.1 (C^3), 101.9 (C^4), 152.1 (C^5), 71.2 (C^7), 127.7 (C^8), 122.4 (C^9), 115.7 (C^{10}), 20.8 (C^{11}), 28.5 (C^{12}), 46.5 (C^{13}), 44.3 (C^{14}), 22.4 (C^{15}), 25.5 (C^{16}), 36.6 (C^{17}), 215.0 (C^{17a}), 15.9 (C^{18}), 55.1 (CH_3O), 19.3 (C^7-αCH_3).

The experiments were carried out using the equipment installed in the "Methods of Analysis of the Substances Composition" and "Magnetic Resonance methods of Analysis" Resource Centers of St. Petersburg State University.

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