SYNTHESIS OF NATURAL PHYTOSTEROIDS OF THE 6-KETOSTIGMASTANE SERIES AND COMPOUNDS RELATED TO THEM

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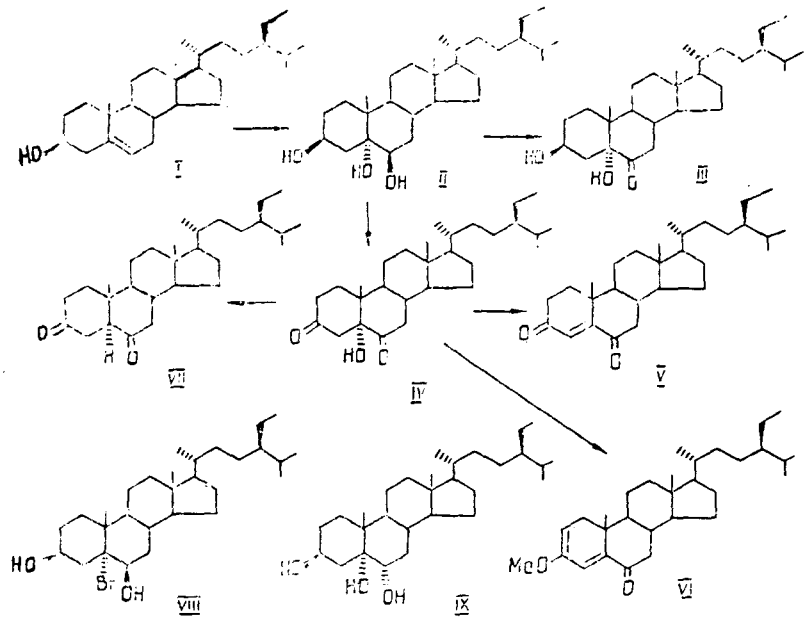
Preparative methods of synthesizing a number of natural 6-ketosteroids and 6-alcohols related to them from β-sitosterol have been developed.

In recent years, a number of compounds have been detected in various plants that may be considered as phytosteroid derivatives and, in the first place, β-sitosterol [I] oxidized in rings A and B at at C_3, C_5, and C_6. Such compounds include 5α-stigmastane-3β, 5, 6β-triol (II) [1, 2], 3β,5-dihydroxy-5α stigmastan-6-one (III) [2, 3],stigmast-4-ene-3,6-dione (V) [4-6], and 5α-stigmastane-3,6-dione (VII) [6-11]. We may note that the reasons for the presence in the plants of the compounds that have been detected have not been explained. This is connected primarily with the absence of methods of obtaining them in amounts sufficient for biological investigations. the isolation of the 6-ketostigmastanes from natural sources is difficult because of their low concentration and the complexity of their separation from impurities. Sporadic attempts at the synthesis of steroids (II), (V), and (VII) that have been reported in the literature [2, 3, 8, 12] cannot be regarded as satisfactory, since they were mainly intended for the comparison of synthetic and natural samples in order to demonstrate the structures of the latter, and they are distinguished by a large number of stages and by low overall yields.



Within the framework of the realization of a program on the synthesis and study of C_{27}–C_{29} polyhydroxysteroids, we have developed preparative methods for the synthesis of natural 6-ketones from β-sitosterol (I) – a promising raw material for the production of other steroids [13, 14]. In our investigations we used a technical product consisting of a fairly complex mixture containing 60% of the main substance. On the trans- hydroxylation of β-sitosterol (I) by the method of Fieser and Rajagopalan [15] under the action of 30% hydrogen

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peroxide in formic acid, which is widely used in the synthesis of ecdysteroids [16], with subsequent purification by crystallization, the 3β,5α-6β-triol (II) was obtained with a yield of 62%. The recrystallization of the mixture of products of trans-hydroxylation of technical β-sitosterol as a method of isolating the trol (II) is not completely convenient, since it is accompaned by considerable losses of substance. Therefore, in the subsequent transformations, the triol (II) was not isolated but was subjected directly to the necessary reactions. On the interaction of compound (II) with aqueous chromic acid in acetone by Jones' method for 10 min the selective oxidation of the axial 6β-hydroxy group took place. This led with an overall yield of 93%, calculated on the β-sitosterol, to the 3,5-dihydroxy-6-ketone (III). The IR spectrum of the steroid (III) had a band of the stretching vibrations of the keto group at 1715 cm^{-1}. The fact that it was just the 6β-hydroxy group, and not the 3β-hydroxy group, that was oxidized during reaction was shown by the presence in the PMR spectrum of the signal of the C_3–H_α proton (δ 4.00 ppm) and the absence of the signal of the proton at C_6.

The more prolonged Jones oxidation of the triol (II), for 1-2 days permitted the production with an overall yield of 81% of the 5-hydroxy-3,6-dione (IV). The mass spectrum of this compound was characterized by the presence of the peak of the molecular ion, and in the IR spectrum there were the absorption bands of a hydroxy group (3130 cm^{-1}) and of keto groups (1715 cm^{-1}). The PMR spectrum of the steroid (IV) under discussion lacked the signals of methine protons at C_3 and C_6 the presence of which was characteristic for the spectrum of the initial triol (II).

The hydroxydiketone (IV) is a convenient intermediate for obtaining the natural diketones (V) and (VII). Thus, the dehydration of compound IV) with hydrochloric acid in chloroform form the enedione (V). The yield of steroid (V) from β-sitosterol was more than 30%. The presence in the UV spectrum of intense absorption at 252 nm, which is characteristic for a conjugated Δ^4-3,6-diketo grouping was extremely important for proving the structure of steroid (V). In the IR spectrum of this compound absorption bands at 1690 and 1600 cm^{-1}, characteristic of conjugated keto groups and of a double bond, respectively, corresponded to this grouping. In the PMT spectrum of steroid (V) there was the singlet of the C_4–H vinyl proton (δ 6.25 ppm).

We established that when the dehydration of the hydroxydiketone (IV) was carried out with hydrochloric acid in a mixture of methanol and chloroform the yield of the unsaturated diketone (V) decreased sharply. The main product of the reaction in this case, isolated with a yield of more than 40%, was the methyl ether (VI). The presence in compound (VI) of a characteristic 2,4-dien-6-one grouping followed from the presence of absorption at 308 nm in the UV spectrum and of the absorption bands of a 6-keto group and of double bonds conjugated with it at 1660, 1620, and 1590 cm^{-1} in the IR spectrum. The presence in the PMR spectrum of the signal of a methoxy group (δ 3.62 ppm) and of vinyl protons at C_2 (δ 5.19 ppm) and C_4 (δ 6.40 ppm) also confirmed the structure of steroid (VI).

We may note that the 5α-hydroxy-3,6-dione (IV) is a very unstable compound and gradually decomposes on storage with the formation of the enedione (V). The reduction of both the hydroxydiketone (IV) and the unsaturated ketone (V) with zinc dust in a mixture of acetic acid and chloroform formed the 3,6-diketone (VII) in high yield. Therefore, in the approach to the synthesis to steroid (VII) that we have developed we used the reduction of a mixture of steroids (IV) and (V) with zinc dust. The structure of the compound obtained followed unambiguously from its spectroscopic characteristics.

Thus, as the result of the investigations that we have performed, preparative methods have been developed for the synthesis of the natural 6-ketosteroids (III), (V), and (VII) with high overall yield. These compounds are convenient starting materials for the synthesis of various C_{29} polyhydroxy steroids, as we have demonstrated for the case of the synthesis of 24R-ethyl-5α-cholest-7-ene-3β,5α,6β-triol, which has been isolated from the marine sponge Spongionella gracilis [17].

By the addition to the 5(6)-double bond of β-sitosterol of hypobromous acid we obtained the bromohydrin (VIII). In the PMR spectrum of this steroid the characteristic signals of the C_3–H_α and C_6–H_α protons (δ 4.55 and 4.18 ppm, respectively) were observed, among others. Attempts to oxidize the bromohydrin (VIII) by Jones' method and subsequent transformations of the 5α-bromo-3,6-dione formed did not lead to the development of a convenient methods of synthesizing steroids (V) and (VII). This was due mainly to the extreme instability of the 5α-bromo-3,6-diketone because of the ready elimination of hydrogen bromide. We also synthesized the 3β,5α,6β-triol (IX), which is the isomer at C_6 of the natural triol (II), as the

result of the cis-hydroxylation of β-sitosterol with osmium tetroxide. Characteristic for the PMR spectrum of this compound was the presence of the broad signal of the C_6–H_β axial proton (δ 4.04 ppm, W/2 = 24 Hz), which unambiguously showed the orientation of the 6-hydroxy group. The half-width of the signal of the C_3–H_α methine proton (δ 4.76 ppm, W/2 = 28 Hz) showed trans-A/B linkage and, therefore, the α-orientation of the 5-hydroxy group in steroid (IX).

The study of the biological activities of the compounds synthesized is in its initial stage, and its results will be published later.

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EXPERIMENTAL

Melting points were determined on a Kofler stage. IR spectra were obtained on a UR-20 instrument. PMR spectra were recorded on a Bruker WM-360 NMR spectrometer with a working frequency of 360 MHz. Chemical shifts are given relative to TMS as internal standard. Mass spectrometric characteristics were obtained on a Varian MAT-311 instrument at an energy of the ionizing electrons of 70 eV. UV spectra were recorded on a Specord UV-Vis instrument.

5α-Stigmastane-3β,5,6β-triol (II). A suspension of 5.0 g of technical β-sitosterol (I) (content of the main substance 60%) in 50 ml of 90% of formic acid was stirred at 70-80°C for 5 min. After the reaction mixture had cooled to room temperature, 5.0 ml of 30% hydrogen peroxide was added and the resulting mixture was slowly heated to 37°C and was slowly cooled. After 18 h, the solvent was evaporated in vacuum in the form of an azerotropic mixture with toluene. The residue was dissolved in 250 ml of methanol, 5.0 g of potassium carbonate was added, and the mixture was boiled under reflux for 2 h. After evaporation of the methanol in vacuum, the residue was recrystallized from chloroform. This gave 2.0 g of the triol (II). Yield 62%, mp 241-244°C ($CHCl_3$-C_2H_5OH); according to the literature, mp 250-252°C [1], 242-245°C [2].

IR spectrum; ν_{max}^{KBr}, cm^{-1}: 3610, 3440 (OH). PMR spectrum (C_5D_5N, δ, ppm.): 0.76 (3H, s, 18-Me), 0.86 (3H, d, J = 7.2 Ha, 26-Me), 0.87 (3H, s, 19-Me), 0.885 (3H, d, J = 7.2 Ha 27-Me), 0.89 (3H, t, J = 7.2 Hz, 29-Me), 1.01 (3H, d, J = 7.3 Hz, 21-Me), 4.17 (1H, m, W/2 = 10 Hz, C_6–H_α), 4.91 (2H, m, W/2 = 41.4 Hz, C_2–H_α and OH). Mass spectrum, m/z 448 (M^+).

3β,5-Dihydroxy-5α-stigmastan-6-one (III) and 5-Hydroxy-5α-stigmastane-3,6-dione (IV). A suspension of 10.0 g of technical β-sitosterol (I) in 100 ml of 90% formic acid was stirred at 70-80°C for 10 min. After cooling to room temperature, 10 ml of 30% hydrogen peroxide was added. Then the mixture was slowly heated to 38°C and was slowly cooled. After 21 h, the formic acid was evaporated off in the form of an azeotropic mixture with toluene. The residue was dissolved in 500 ml of methanol, 10.0 g of potassium carbonate was added, and the mixture was boiled under reflux for 2 h. The methanol was driven off in vacuum, and the residue was dissolved in mixture of 250 ml of acetone and 250 ml of chloroform and was subjected to Jones oxidation by two different methods:

Method A. The solution was treated with 9.0 ml of 8 N chromic acid. After 10 min, the excess of oxidant was eliminated by the addition of 15 ml of isopropanol, and the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by chloroform–methanol (30:1). This gave 6.0 g of the dihydroxyketone (III). Yield 93%. mp 255-258°C ($CHCl_3$–MeOH); according to the literature, mp: 246-248°C [3], 242-244°C [2].

IR spectrum: ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1715 (C=O). PMR spectrum, ($CDCl_3$, δ, ppm): 0.65 (3H, s, 18-Me), 0.83 (3H, d, J = 7.2 Hz, 26-Me), 0.835 (3H, s, 19-Me), 0.85 (3H, d, J = 7.2, Hz, 27-Me), 0.86 (3H, t, J = 7.2 Hz, 29-Me), 0.93 (3H, d, J = 6 Hz, 21-Me), 4.00 (1H, m, W/2 = 24 Hz, C_3–H_α). Mass spectrum: m/z 446 (N^+).

Method B. The solution was treated with 70 ml of 8 N chromic acid. After one day, the excess of oxidant was eliminated with 90 ml of isopropanol. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by chloroform–methanol (30:1). This gave 5.2 g of the hydroxy-diketone (IV). Yield 81%, mp 222-225°C ($CHCl_3$–ether); according to the literature, mp 242-241°C [2].

IR spectrum: ν_{max}^{KBr}, cm^{-1}: 3430 (OH), 1715 (C=O). PMR spectrum, ($CDCl_3$, δ, ppm): 0.68 (3H, s, 18-Me), 0.83 (3H, d, J = 7.2 Hz, 26-Me), 0.85 (3H, d, J = 7.2 Hz, 27-Me), 0.86 (3H, t, J = 7.2 Hz, 29-Me), 0.93 (3H, d, J = 7.3 Hz, 21-Me), 1.01 (3H, s, 19-Me). Mass spectrum, m/z: 444 (M^+).

Stigmast-4-ene-6-dione (V). A suspension of 10.0 g of technical β-sitosterol in 100 ml of 90% formic acid was stirred at 70-80°C for 12 min. After the mixture had cooled to room temperature, 10 ml of 30% hydrogen peroxide was added and it was then slowly heated to 38°C and slowly cooled. After 18 h, the formic acid was evaporated off in the form of an azeotropic mixture with toluene. The residue was dissolved in 500 ml of methanol, 10 g of potassium carbonate was added and the mixture was boiled for 2 h. After the methanol had been driven off in vacuum, the residue was dissolved in a mixture of 250 ml of acetone and 250 ml of chloroform, and 90 ml of 8 N chromic acid was added. The mixture was left for 2 days and, after the excess of oxidant had been eliminated with 15 ml of isopropanol, it was diluted with water and was extracted with chloroform. The chloroform extract was washed with water and was evaporated in vacuum. The residue was dissolved in 300 ml of chloroform and, after the addition of 5 ml of hydrochloric acid, the solution was kept at room temperature for 1 h 40 min. The acid was neutralized with sodium bicarbonate, the solution was filtered, and the filtrate was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (10:1). This gave 2.0 g of the enedione (V). Yield 32%, mp 183-185°C (hexane).

IR spectrum: ν_{max}^{KBr}, cm^{-1}: 1690 (C=O), 1600 (C=C). UV spectrum, ν_{max}^{MeOH}, nm: 252 (lg ε 4.15). PMR spectum, ($CDCl_3$, δ, ppm): 0.73 (3H, s, 18-Me), 0.82 (3H, d, J = 7.2 Hz, 26-Me), 0.83 (3H, d, J = 7.2 Hz, 27-Me), 0.85 (3H, t, J = 7.2 Hz, 29-Me), 0.95 (3H, d, J = 7.3 Hz, 21-Me), 1.18 (3H, s, 19-Me), 6.25 (1H, s, C_4-H). Mass spectrum: m/z (M^+).

Dehydration of the Hydroxydiketone (IV). A solution of 3.5 g of the hydroxydiketone (IV) in 350 ml of methanol and 30 ml of chloroform was treated with 5 ml of concentrated hydrochloric acid. The reaction mixture was boiled for 1.5 h and was then filtered through a layer of alumina. The filtrate was evaporated in vacuum, and the residue was chromatographed on a column of silica gel with elution by hexane–ether (20:1). This gave 1.46 g of 3-methoxystigmasta-2,4-dien-6-one (VI). Yield, 42%, mp 112-114°C (hexane).

IR spectrum: ν_{max}^{KBr}, cm^{-1}: 1660 (C=O), 1620, 1590 (C=C). UV spectrum: ν_{max}^{MeOH}, nm: 308 (lg ε 3.93). PMR spectrum, ($CDCl_3$, δ, ppm): 0.78 (3H, s, 18-Me), 0.83 (3H, d, J = 7.5 Hz, 26-Me), 0.85 (3H, d, J = 7.5 Hz, 27-Me), 0.86 (3H, t, J = 7.5 Hz, 29-Me), 0.94 (3H, d, J = 6 Hz, 21-Me), 1.13 (3H, s, 19-Me), 3.62 (3H, s, MeO), 5.19 (1H, m, C_2–H), 6.30 (1H, s, C_4–H). Mass spectrum: m/z: 438 (N^+).

Further elution led to 0.18 g of the enedione (V), identical with an authentic sample. Yield 18%.

5α-Stigmastane-3,6-dione (VII). A solution of a mixture of 2.7 g of the hydroxydiketone (IV) and 1.0 g of the enedione (V) in 300 ml of acetic acid and 170 ml of chloroform was boiled under reflux with 19.0 g of zinc dust for 5 h. After the usual working up, 2.9 g of the dione (VII) was obtained. Yield 80%, mp 205-206.5°C (hexane); according to the literature, mp 196-199°C [13], 198-199°C [10], 200-202°C [8].

IR spectrum: ν_{max}^{KBr}, cm^{-1}: 1720, 1710 (C=O). PMR spectrum ($CDCl_3$, δ, ppm): 0.70 (3H, s, 18-Me), 0.82 (3H, d, J = 7.2 Hz, 26-Me), 0.85 (3H, d, J = 7.2 Hz, 27-Me), 0.86 (3H, t, J = 7.2 Hz, 29-Me), 0.94 (3H, d, J = 6.6 Hz, 21-Me), 0.97 (3H, s, 19-Me). Mass spectrum: m/z: (M^+).

5-Bromo-5α-stigmastane-3β,6β-diol (VIII). With stirring 40 ml of water and 8 ml of 70% perchloric acid, followed by 6.6 g of N-bromoacetamide in portions were added to a solution of 10.0 g of technical β-sitosterol in 640 ml of dioxane. After 30 min, the reaction mixture was diluted with 300 ml of water, 2 g of sodium sulfite were added, and extraction was carried out with chloroform. The chloroform extract was washed with water and was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–chloroform (3:1). This gave 1.5 g of the bromohydrin (VIII). Yield 20%, mp 144-148°C (hexane). Found %: C 68.13; H 9.96; Br 15.45. Calculated for $C_{29}H_{51}BrO_2$ %: C 68.08; H 10.05; Br 15.62.

IR spectrum: ν_{max}^{KBr}, cm^{-1}: 3450 (OH). PMR spectrum ($CDCl_3$, δ, ppm): 0.65 (3H, s, 18-Me), 0.81-Me), 0.81 (3H, d, J = 7.2 Hz, 26-Me), 0.83 (3H, d, J = 7.2 Hz, 27-Me), 0.84 (3H, t,

J = 7.2 Hz, 29-Me), 0.90 (3H, d, J = 7.2 Hz, 21-Me), 0.99 (3H, s, 19-Me), 3.11 (1H, d, J = 6.7 Hz, C_4–H_α), 4.18 (1H, m, W/2 = 12 Hz, C_6–H_α), 4.55 (1H, dd, J_1 = 12 Hz, J_2 = 4.8 Hz, C_3–H_α). Mass spectrum: m/z: 415 (M^+–HBr–Me).

5α-Stigmastane-3β,5,6α-triol (IX). A solution of 1.63 g of technical β-sitosterol in 20 ml of pyridine was treated with 1.0 g of osmium tetroxide. The mixture was kept at room temperature for one day and was then treated with a solution of 2.83 g of sodium sulfite and 0.93 ml of sulfuric acid in 25 ml of water and 10 ml of chloroform. After being stirred at room temperature for 30 min, the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by chloroform–ethanol (20:1). This gave 0.6 g of the triol (IX). Yield 57%, mp 230-232°C (MeOH).

IR spectrum: ν_{max}^{KBr}, cm^{-1}: 3450 (OH). PMR spectrum C_5D_5N, δ, ppm): 0.71 (3H, s, 18-Me), 0.88 (3H, d, J = 7.3 Hz, 26-Me), 0.90 (3H, d, J = 7.3 Hz, 27-Me), 0.92 (3H, t, J = 7.3 Hz, 29-Me), 1.00 (3H, d, J = 6.0 Hz, 21-Me), 1.10 (3H, s, 19-Me), 4.04 (1H, m, W/2 = 24 Hz, C_6–H_β), 4.76 (1H, m, W/2 = 28 Hz, C_3–H_α). Mass spectrum, m/z: 430 (M^+–H_2O).

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