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AN INVESTIGATION OF PATHWAYS TO THE SYNTHESIS OF 2β,3β,5β-TRI-HYDROXY-6-KETOSTEROIDS

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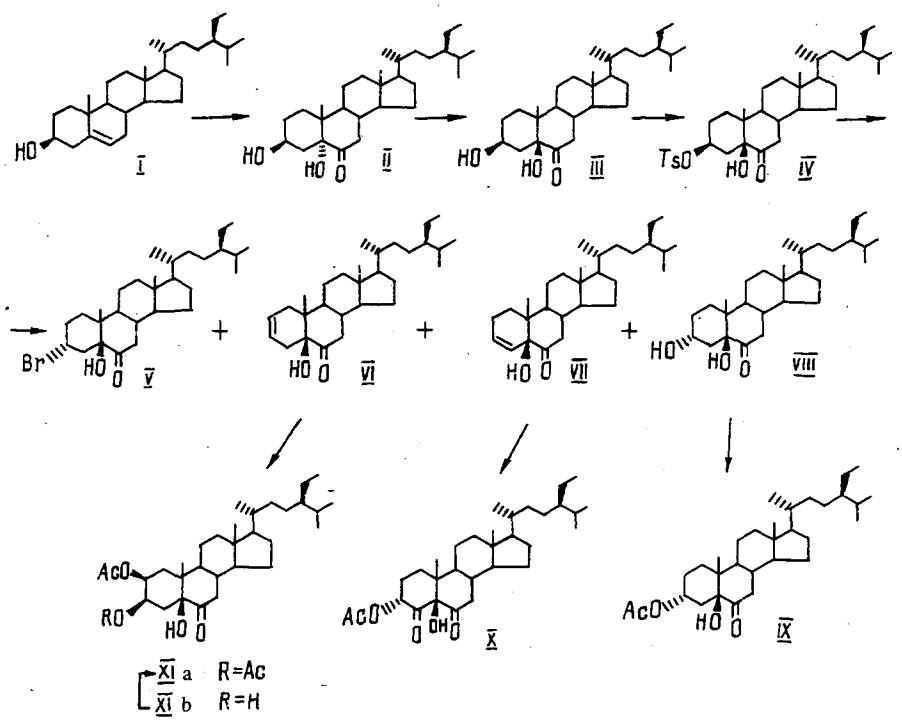
Using β-sitosterol as an example, an investigation has been mde of the introduction into 3β-hydroxy-Δ^5-sterols of the 2β,3β,5β-trihydroxy-6-keto grouping that is characteristic for some phytoecdysteroids.

One of the unsolved problems of the chemistry of the insect hormones ecdysteroids is the development of methods of obtaining derivatives containing in rings A and B a 2β,3β,5β-trihydroxy-Δ^7-6-keto grouping. This structural fragment is present in the molecules of such phytoecdysteroids as polypodine B, ponasterone C and sengosterone, which possess a high activity as insect hormones [1]. It must be mentioned that the number of plant sources of these natural substances is extremely limited. The absence of methods for their chemical synthesis therefore considerably hinders the all-sided study of their biological activity and the possibility of their practical use.

The present work was devoted to a study of the possibilities of the introduction of a 2β,3β,5β-trihydroxy-6-keto grouping into the 3β-hydroxy-Δ^5-sterols that are widely used for the synthesis of various steroids. As the initial compound we chose β-sitosterol (I), which is isolated from the wastes of the pulp and paper industry and, for this reason, is one of the cheapest steroidal raw materials [2].

In the first stage, using a method that we had developed earlier [3], by the trans-hydroxylation of β-sitosterol with hydrogen peroxide in formic acid and subsequent selective Jones oxidation of the 3β,5α,6β-triol formed with chromium trioxide in acetone we synthesized the 3β,5α-dihydroxy-6-ketone (II) with an overall yield of 55%.



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The reversal of the configuration of the 5α-hydroxy group in compound (II) was achieved by reaction with potassium hydroxide in methanol at the boil [4]. This gave a 61% yield of the 3β,5β-dihydroxy-6-ketone (III). The subsequent reaction of the diol (III) with p-toluenesulfonyl chloride in pyridine led with quantitative yield to the formation of the 3-monotosylate (IV). The structures of compounds (III) and (IV) followed unambiguously from their IR, PMR, and mass spectra, which agreed well with those described in the literature [4] for the corresponding derivatives of the cholestane series.

It is knowm [5, 6] that the elimination of a 3-substituent (for example, a benzoyl group) from derivatives of 2β,3β-dihydroxy-6-ketosteroids leads mainly to the formation of Δ^3-5β-hydroxy-6-ketones. However, the simultaneous formation of the Δ^2-5β-hydroxyketones as by-products is observed, and we planned to use these subsequently in the synthesis of the 2β,3β,5β-trihydroxy-6-ketosteroids. With this aim, we studied the reaction of the tosylate (IV) with lithium carbonate and lithium bromide in dimethylformamide at the boil. It was found that this reaction formed compounds (V-VIII). By column chromatography on silica gel we succeeded in obtaining in the individual state the products of nucleophilic substitution with configuration reversal — the 3α-bromo-5β-hydroxy-6-ketone (V) and the 3α,5β-dihydroxy-6-ketone (VIII). At the same time, the Δ^2-5β-hydroxy-6-ketone (VI) and the Δ^3-5β-hydroxy-6-ketone (VII) formed in the elimination reaction have very close chromatographic characteristics in various eluent systems. For this reason, it was very difficult to separate a mixture of them. The use of alumina activated with silver nitrate as adsorbent enabled us to isolate only a small amount of compound (VII), sufficient for its total identification.

The structures of steroids (V-VIII) were established by an analysis of their spectra. Thus, the presence of bromine at C_3 in compound (V) followed from its mass spectrum and was also confirmed by a positive Beilstein test. Its equatorial, 3α, orientation followed from the half-width of the signal of the $C_3-H\beta$ methine proton geminal to it (δ 4.35 ppm, W/2 24 Hz). Analogously, in the PMR spectra of the 3α-alcohol (VIII) and of the 3-monoacetate (IX) formed on its acetylation the signals of the $C_3-H\beta$ protons (δ 4.03 ppm, W/2 26 Hz, and δ 5.08 ppm, W/2 23 Hz, respectively) unambiguously showed the the α-orientation of the hydroxy and acetoxy groups. The PMR spectrum of the Δ^3-steroid contained the signals of the C_3-H and C_4-H vinyl protons at 6.01 and 5.42 ppm in the form of a doublet of triplet and of a doublet, respectively. The same signals were present in the PMR spectrum of a mixture of steroids (V) and (VII). The presence in this spectrum of the signals of vinyl protons, as well, at 5.61 and 5.72 ppm in form of complex multiplets proved the structure of the second substance as a Δ^2-steroid.

In the Δ^2-5β-hydroxy-6-ketosteroid (VI), because of the cis-A/B linkage, the β-side is more accessible for the attack of reagents. To introduce a 2β,3β-diol grouping, therefore, we used the Criegee cis-hydroxylation of the double bond in steroid (VI) under the action of a catalytic amount of osmium tetroxide and N-methylmorpholine N-oxide. As a result of subsequent acetylation and the separation of the mixture of products by column chromatography, we succeeded in obtaining — together with considerable amounts of the unchanged Δ^3-steroid (VII) and the 3α-acetoxy-5β-hydroxy-4,6-diketone (X) — the 2β,3β,5β-trihydroxy-6-ketone in the form of the 2,3-diacetate (XIa) and the 2-monoacetate (XIb).

The structures of compounds (X) and (XIa, b) followed unambiguously from their spectra. Thus, in the IR spectrum of steroid (X) bands of the stretching vibrations of a hydroxy group, of an acetoxy group, and of two keto groups were observed. The PMR spectrum of the hydroxyacetate had at 4.28 ppm a signal in the form of a singlet corresponding to the 5-hydroxy proton, which disappeared on deuterium exchange. It was possible to conclude from the magnitude of the chemical shift and the form of the signal of the methine proton geminal to the 3-acetoxy group that the second keto group was located at C^4. It must be mentioned that the further oxidation of the alcohols formed in the Criegee reaction to ketones is a disdavantage of the use of the variant with catalytic amounts of osmium tetroxide.

The PMR spectrum of the monoacetate (XIb) contained signals of the 3- and 5-hydroxy protons at 4.38 and 4.54 ppm in the form of a doublet and a singlet, respectively. To the acetoxy group and the secondary hydroxy group corresponded the signals of the methine protons geminal to them, at 4.92 and 4.13 ppm, respectively. The use of the double resonance procedure enabled it to be shown unambiguously that these groups were vicinal. It followed from the form of the signal at 4.92 ppm that the acetoxy group geminal to it was equatorial. It was found that when a sample was irradiated with the frequency corresponding to the resonance absorption of the hydroxy proton at 4.38 ppm the signal of the proton geminal to the hydroxy group broadened into a multiplet with a half-width W/2 of 8 Hz. It followed from this that the secondary hydroxy group in steroid (XIb) was axial. Of the two structures possible in principle (i.e., 3α-acetoxy-2α-hydroxy- and 2β-acetoxy-3β-hydroxy), we made our choice in favor of the latter on the basis of the mechanism of the Criegee reaction.

The structure of the diacetate (XIa) was proved by a similar analysis of its PMR spectrum and was confirmed by its formation on the acetylation of the monoacetate (XIb).

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were obtained on a UR-10 instrument in KBr tablets. PMR spectra of solutions in $CDCl_3$ were recored on Bruker WM-360 and Bruker AC-200 NMR spectrometers with working frequencies of 360 and 200 MHz, respectively. Chemical shifts are given relative to TMS as internal standard. Mass-spectrometric characteristics were obtained on a Varian MAT instrument at an energy of the ionizing electrons of 70 eV.

(24R)-3β,5-Dihydroxy-5β-stigmastan-6-one (III). A mixture of 2.5 mg of the 3β,5α-dihydroxy-6-ketone (II) (obtained as described in [3]) and 90 ml of a 10% solution of potassium hydroxide in methanol was boiled under reflux for 12.5 h. After the addition of a further 110 ml of 10% methanolic potassium hydroxide to the reaction mixture, boiling was continued for another 11.5 h. The reaction mixture was cooled to room temperature and was diluted with water and extracted with ether. The ethereal extract was washed with water, and then the solvent was eliminated in vacuum. The residue was chromatographed on a column of silica gel with elution by chloroform–methanol (100:1). This gave 1.53 g of the 3β,5β-dihydroxy-6-ketone (III). Yield 61%, mp 108-110°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3500, 3420 (OH), 1710 (C=O). PMR spectrum (δ, ppm): 0.66 (3H, s, 18-Me), 0.75 (3H, s, 19-Me), 0.75 (3H, s, 19-Me), 0.80 (3H, d, J 5 Hz, 26-Me), 0.84 (3H, d, J 5 Hz, 27-Me), 0.85 (3H, t, J 7 Hz, 29-Me), 0.92 (3H, d, J 6 Hz, 21-Me), 4.06 (1H, m, W/2 16 Hz, C_3–Hα), 4.35 (1H, d, J 10 Hz, C_3–OH), 4.42 (1H, s, C_5–OH). By further elution, 0.40 g of the initial 3β,5β-dihydroxy-6-ketone (II) was isolated. Yield 17%.

(24R)-3β,5-Dihydroxy-5β-stigmastan-6-one 3-Tosylate (IV). A solution of 1.45 g of the 3β,5β-dihydroxy-6-ketone (III) in 5 ml of pyridine was treated with 2.9 g of p-toluenesulfonyl chloride. The reaction mixture was kept at room temperature for 7 days. Then it was diluted with water and extracted with ether. The ethereal extract was washed with saturated sodium bicarbonate solution and then with water, and it was dried with anhydrous magnesium sulfate. After the solvent had been eliminatd in vacuum, the residue was chromatographed on a column of silica gel, with elution by chloroform. This gave 1.9 g of the tosylate (IV). Yield 97%, mp 159-161°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3480 (OH), 1705 (C=O), 1600 $(C=C)_{arom}$. PMR spectrum (δ, ppm): 0.63 (3H, s, 18-Me), 0.73 (3H, s, 19-Me), 0.80 (3H, d, J 5 Hz, 26-Me), 0.83 (3H, d, J 5 Hz, 27-Me), 0.835 (3H, t, J 7 Hz, 29-Me), 0.90 (3H, d, J 6 Hz, 21-Me), 2.43 (3H, s, Me–Ar), 3.84 (1H, s, C_5–OH), 4.92 (1H, m, W/2 8 Hz, C_3–Hα), 7.30, 7.82 (4H, Ar–H).

Interaction of the Tosylate (IV) with Lithium Carbonate and Bromide in Dimethylformamide. A. A solution of 0.9 g of the tosylate (IV) in 35 ml of dimethylformamide was treated with 0.9 g of lithium carbonate and 0.3 g of lithium bromide, and the reaction mixture was boiled for 30 min. After cooling to room temperature, it was diluted with water and extracted with hexane. The extract was washed with water and evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by ethyl acetate–hexane (1:1). This gave 0.40 g of a mixture of steroids (V-VII). Further elution with the given solvent system gave 0.20 g of (24R)-3α,5-dihydroxy-5β-stigmastan-6-one (VIII). Yield 30%, mp 151-153°C (ethyl acetate–hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3450 (OH), 1710 (C=O). PMR spectrum (δ, ppm): 0.66 (3H, s, 18-Me), 0.71 (3H, s, 19-Me), 0.81 (3H, d, J 6 Hz, 26-Me), 0.84 (3H, d, J 6 Hz, 27-Me), 0.86 (3H, t, J 7 Hz, 29-Me), 3.99 (1H, s, C_5–OH), 4.03 (1H, m, W/2 26 Hz, C_3–Hβ). Mass spectrum, m/z: 446 (M^+), 428 ($M^+ - H_2O$), 410 ($M^+ - 2H_2O$).

The rechromatography of 0.3 g of the mixture of steroids (V-VII) on a column of silica gel, with elution by hexane–ether (5:1) gave 0.10 g of (24R)-3α-bromo-5-hydroxy-5β-stigmastan-6-one (V). Yield 17%, mp 110-113°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3485 (OH), 1690 (C=O). PMR spectrum (δ, ppm): 0.65 (3H, s, 18-Me), 0.69 (3H, s, 19-Me), 0.82 (3H, d, J 8 Hz, 26-Me), 0.84 (3H, d, J 8 Hz, 27-Me), 0.85 (3H, t, J 8 Hz, 29-Me), 0.93 (3H, d, J 6 Hz, 21-Me), 3.95 (1H, s, C_5–OH), 4.35 (1H, m, W/2 24 Hz, C_3–Hβ). Mass spectrum, m/z: 508, 510 (M^+), 490, 492 ($M^+ - H_2O$), 428 (M^+ — HBr), 410 (M^+ — HBr — H_2O). On subsequent elution, 0.190 g of a mixture of (24R)-5-hydroxy-5β-stigmast-3-en-6-one (VII) and (24R)-5-hydroxy-5β-stigmast-2-en-6-one (VIII) was obtained. The combined yield from (IV) was 39%).

PMR spectrum (δ, ppm): 0.66, 0.67 (s, 18-Me), 0.77, 0.78 (s, 19-Me), 0.81 (d, J 7 Hz, 26-Me), 0.84 (d, J 7 Hz, 27-Me), 0.85 (t, J 7 Hz, 29-Me), 0.92 (d, J 6 Hz, 21-Me), 4.06, 4.18 (s, OH), 5.41 (d, J 9 Hz, C_4–H), 5.61, 5.72 (m, C_2–H and C_3–H), 5.97 (dt, J_1 9 Hz, J_2 4 Hz, C_3–H).

B. A mixture of 2.6 g of the 3β,5α-dihydroxy-6-ketone (II) in 300 ml of a 10% solution of potassium hydroxide in methanol was boiled under reflux for 25 h. Then the reaction mixtrue was evaporated to a volum eof 100 ml and was diluted with water and extracted with ether. The ethereal extract was washed with water and evaporated in vacuum. The residue was

dissolved in 15 ml of puridine and treated with 4.0 g of p-toluenesulfonyl chloride. After 5 days, the reaction mixture was diluted with water and extracted with ether. The ethereal extract was washed with saturated sodium bicarbonate solution and then with water, and was dried with anhydrous magnesium sulfate. The solvent was eliminated in vacuum, and the residue was dissolved in 60 ml of dimethylformamide; 2.1 g of lithium carbonate and 0.7 g of lithium bromide were added, and the reaction mixture was boiled for 30 min. Then it was cooled to room temperature, diluted with water, and extracted with hexane. The hexane extract was washed with water, dried with magnesium sulfate, and evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (30:1). This gave 0.20 g of the 3α-bromo-5β-hydroxy-6-ketone (V) (yield 7%), 0.48 g of a mixture of Δ^2- and Δ^3-5β-hydroxy-6-ketones (VI) and (VII) (yield 19%), and 0.34 g of the 3α,5β-dihydroxy-6-ketone (VIII) (yield 13%). On rechromatography of the mixture of olefins (VI) and (VII), on alumina activated with silver nitrate by the procdure of [7], we succeeded in isolating, together with 0.310 g of the initial mixture, 0.045 g of pure (24R)-5-hydroxy-5β-stigmast-3-en-6-one (VII). mp 125-128°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3455 (OH), 1710 (C=O), 1640 (C=C). PMR spectrum (δ, ppm): 0.63 (3H, s, 18-Me), 0.76 (3H, s, 18-Me), 0.76 (3H, s, 19-Me), 0.80 (3H, d, J 8 Hz, 26-Me), 0.82 (3H, d, J 8 Hz, 27-Me), 0.83 (3H, t, J 7 Hz, 29-Me), 0.91 (3H, d, J 6 Hz, 21-Me), 4.19 (1H, s, C_5–OH), 5.42 (1H, d, J 10 Hz, C_4–H), 6.01 (1H, dt, J_1 10 Hz, J_2 4 Hz, C_3–H). Mass spectrum, m/z: 428 (M^+), 410 ($M^+ - H_2O$).

(24R)-3α,5-Dihydroxy-5β-stigmastan-6-one (IX). A solution of 0.200 g of the 3α,5β-dihydroxy-6-ketone (VII) in 6 ml of pyridine was treated with 3 ml of acetic anhydride. The reaction mixture was kept at room temperature for 23 h and was then diluted with water and extracted with ether. The etheral extract was washed successively with water, with 15% hydrochloric acid, and with saturated sodium bicarbonate solution. The solvent was eliminated in vacuum, and the residue was chromatographed on a column of alumina with elution by ether–hexane (1:2). Thus gave 0.214 g of the monoacetate (IX). Yiled 98%, mp 113-114°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3480 (OH), 1740, 1235 (AcO), 1705 (C=O). PMR spectrum (δ, ppm): 0.65 (3H, s, 18-Me), 0.72 (3H, s, 19-Me), 0.81 (3H, d, J 5 Hz, 26-Me), 0.84 (3H, d, J 5 Hz, 27-Me), 0.86 (3H, t, J 6 Hz, 29-Me), 0.92 (3H, d, J 6 Hz, 21-Me), 2.03 (3H, s, AcO), 3.98 (1H, s, C_5–OH), 5.08 (1H, m, W/2 23 Hz, C_3–Hβ).

Criegee hydroxylation of the Mixture of the Δ^2- and Δ^3-Steroids (VI) and (VII). With magnetic stirring at room temperature, 0.260 g of N-methylmorpholine N-oxide monohydrate and 0.020 g of osmium tetroxide were added to a solution of 0.285 g of the mixture of Δ^2- and Δ^3- derivatives (VI) and (VII) in 100 ml of a mixture of tetrahydrofuran, tert.-butanol, and water (10:10:1). The reaction mixture was stirred at room temperature for 1 day and was then treated with a solution of 1.5 g of sodium sulfite and 0.49 ml of 96% sulfuric acid in 14 ml of water. Stirring was stopped after 30 min, and the mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with saturated sodium bicarbonate solution and then with water and was evaporated in vacuum. The residue was dissolved in 7 ml of pyridine, and 4 ml of acetic anhydride was added. The reaction mixture was kept at room temperature for 19 h and was then diluted with water and extracted with hexane. The hexane extract was washed successively with 15% hydrochloric acid, saturated sodium bicarbonate solution, and, twice, with water, and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by ether–hexane (1:2). This gave 0.092 g of the unchanged Δ^3-steroid (VII). Yield 32%. Further elution led to the isolation of 0.073 g of the 3-acetate of (24R)-3α,5-dihydroxy-5β-stigmastane-4,6-dione. (X). Yield 22%, mp 206-209°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3460 (OH), 1760, 1745, 1235 (AcO), 1725, 1710 (C=O). PMR spectrum (δ, ppm): 0.63 (3H, s, 18-Me), 0.80 (3H, d, J 7 Hz, 26-Me), 0.81 (3H, d, J 7 Hz, 27-Me), 0.835 (3H, t, J 7 Hz, 29-Me), 0.85 (3H, s, 19-Me), 0.895 (3H, d, J 7 Hz, 21-Me), 2.15 (3H, s, AcO), 4.28 (1H, s, C_5–OH), 5.54 (1H, dd, J_1 12 Hz, J_2 7 Hz, C_3–Hβ). Mass spectrum, m/z: 502 (M^+), 484 ($M^+ - H_2O$), 442 (M^+ — AcOH), 424 (M^+ — AcOH — H_2O). On subsequent elution, 0.035 g of the 2,3-diacetate of (24R)-2β,3β,5-trihydroxy-5β-stigmastan-6-one (XIa) was obtained. Yield 10%, mp 153-156°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3460 (OH), 1740, 1250 (AcO), 1705 (C=O). PMR spectrum (δ, ppm): 0.63 (3H, s, 18-Me), 0.80 (3H, s, 19-Me), 0.81 (3H, d, J 8 Hz, 26-Me), 0.83 (3H, d, J 8 Hz, 27-Me), 0.84 (3H, t, J 7 Hz, 29-Me), 0.91 (3H, d, J 7 Hz, 21-Me), 2.03 (3H, s, AcO), 2.10 (3H, s, AcO), 3.96 (1H, s, C_5–OH), 5.07 (1H, dt, J_1 13 Hz, J_2 4 Hz, C_2–Hα), 5.26 (1H, m, W/2 8 Hz, C_3–Hα). Mass spectrum, m/z: 486 (M^+ — AcOH). Further elution gave 0.055 g of the 2-acetate of (24R)-2β,3β,5-trihydroxy-5β-stigmastan-6-one (XIb). Yield 16%, mp 123-125°C (hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3430 (OH), 1725, 1260 (AcO), 1705 (C=O). PMR spectrum (δ, ppm): 0.63 (3H, s, 18-Me), 0.796 (3H, s, 19-Me), 0.80 (3H, d, J 8 Hz, 26-Me), 0.82 (3H, d, J 8 Hz, 27-Me), 0.83 (3H, t, J 8 Hz, 29-Me), 0.91

(3H, d, J 7 Hz, 21-Me), 2.11 (3H, s, AcO), 4.13 (1H, m, W/2 17 Hz, $C_3-H\alpha$), 4.38 (1H, d, J 9 Hz, C_3-OH), 4.54 (1H, s, C_5-OH), 4.92 (1H, dt, J_1 13 Hz, J_2 3 Hz, $C_2-H\alpha$). Mass spectrum, m/z: 504 (M^+), 486 ($M^+ - H_2O$).

Acetylation of the 2β-Acetoxy-3β,5β-dihydroxy-6-ketone (XIb). A solution of 0.045 g of steroid (XIb) in 3 ml of pyridine was treated with 1 ml of acetic anhydride, and the reaction mixture was kept at room temperature for 22 h and was then diluted with water and extracted with ether. The ethereal extract was washed successively with water, 15% hydrochloric acid, water, saturated sodium bicarbonate solution, and water, and was evaporated in vacuum. Since, according to TLC, the reaction mixture contained a considerable amount of the initial alcohol (XIb), acetylation was repeated. For this purpose, the mixture was dissolved in 2 ml of pyridine and 0.5 ml of acetic anhydride and the solution was kept at room temperature for 4 days. The residue obtained after a similar working up of the reaction mixture was chromatographed on a column of silica gel. Elution with hexane–ether (2:1) led to the isolation of 0.023 g of the diacetate (XIa), identical with an authentic sample, to judge from its PMR spectrum. Yield 47%.

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