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SYNTHESIS OF (24R)-3β-HYDROXYSTIGMAST-5-EN-7-ONE

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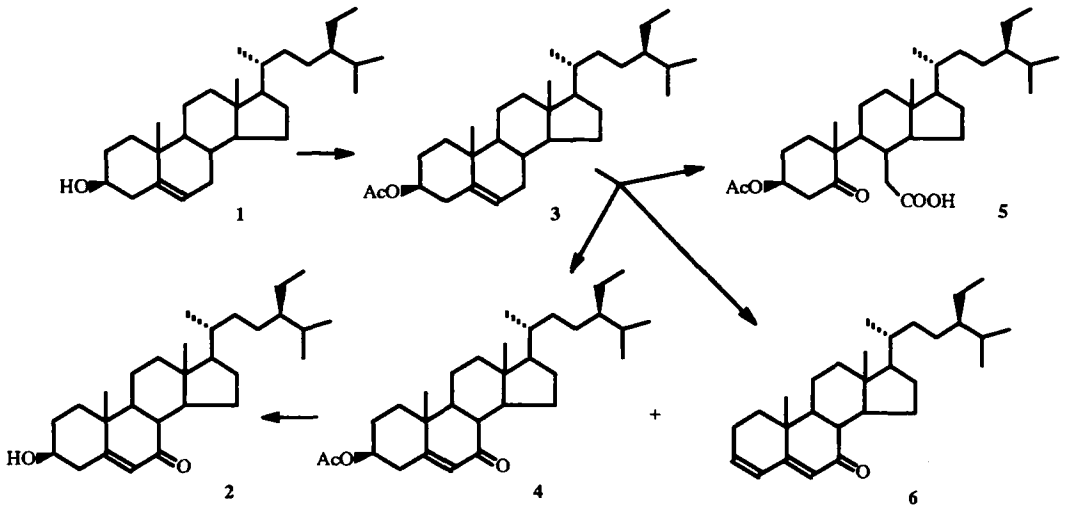
The natural compound (24R)-3β-hydroxystigmast-5-en-7-one has been synthesized from β-sitosterol.

The unsaturated compound (24R)-3β-hydroxystigmast-5-en-7-one (**2**) is one of the oxidized derivatives of the extremely important phytosterol β-sitosterol (**1**). Sterol (**2**) is a natural compound; it has been isolated from plants repeatedly [1—7]. Nothing is known about the reasons for the presence of the hydroxyketone (**2**) in plants and its biological activity. It is assumed only that steroid (**2**) may be responsible for the biological action of the plant *Euphorbia fischeriana*, which is used in Siberian and Mongolian medicine as an antitumoral agent [2].



In continuation of investigations on the production of various oxidized derivatives of sterols [8], we have synthesized the hydroxyketone (**2**) from β-sitosterol (**1**). In the first stage, the acetate (**3**) was obtained in a yield of 88% by the interaction of β-sitosterol with acetic anhydride in pyridine. The following stage of the synthesis had to consist in the allyl oxidation of compound (**3**) with the formation of the Δ^5-7-ketone (**4**). It had been established previously [9] that compound (**4**) is formed on the oxidation of compound (**3**) with chromic acid in acetic acid by a procedure described in the cholestane series [10]. Unfortunately, no experimental details whatever permitting the method of obtaining the acetoxyenone (**3**) to be reproduced are given in [9].

We have made an attempt to reproduce the procedure of [10] in application to compound (**3**). It was established that a complex mixture of substances was formed during the reaction, but the required acetoxyketone (**4**) was isolated from this with a yield of only 6%. The main oxidation product was the seco-acid (**5**), isolated with a yield of 38%. Another product, which we succeeded in isolating with a yield of 4%, had the structure of a 3,5-dien-7-one.

The structures of steroids (**3**—**5**) followed unambiguously from their spectra. Thus, in the mass spectrum of compound (**3**) there was the peak of the molecular ion with *m/z* 470. It structure as an α,β-unsaturated ketone was confirmed by the presence in its IR spectrum of the bands of a keto group at 1685 cm^{-1} and of a double bond conjugated with it at 1640 cm^{-1}. Characteristic for the ^1H NMR spectrum of the substance under discussion was the presence of the signal of a vinyl proton at

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5.78 ppm. This value of the chemical shift corresponds to its α-position with respect to the keto group. The retention of the acetoxy group in the structure of compound (**4**) was shown, in the first place, by the presence of a three-proton singlet at 2.08 ppm, corresponding to its methyl fragment, and, in the second place, by the chemical shift and form of the signal of the methine proton geminal to it, H-3α, having the form of a broadened multiplet at 4.78 ppm.

Furthermore, according to the result of chemical analysis, the molecule of steroid (**5**) contained five oxygen atoms. Characteristic for its mass spectrum was a peak with the maximum mass at *m/z* 444, resulting from the splitting out of acetic acid from the molecular ion. The presence of acetoxy, carboxy, and keto groups can be well seen from the IR spectrum, in which there are two intense absorption bands at 1745 and 1715 cm^{-1}. Again, the ^1H NMR spectrum of compound (**5**) lacks signals of any vinyl protons whatever. It must be mentioned that the formation of a seco-acid of analogous structure but belonging to the 5,6-secocholestane series has been observed previously under the same experimental conditions on the oxidation of cholesterol acetate with chromic acid [10]. This substance is probably produced as a result of the epoxidation of the 5(6)-double bond in the molecule of β-sitosterol acetate (**3**), with the subsequent opening of the epoxide, oxidation of the resulting 5α,6β-diol to form a 5α-hydroxy-6-ketone, and subsequent cleavage of the 5(6) bond.

Important for proving the structure of compound (**6**) was the presence in its UV spectrum of a very intense band at 281 nm, which is characteristic for steroidal 3,5-dien-7-ones [11]. This was confirmed by an analysis of the IR spectrum, which contained the absorption band of a keto group at 1660 cm^{-1} and bands of double bonds conjugated with it at 1630 and 1600 cm^{-1}. The ^1H NMR spectrum of the dienone contained signals of the vinyl protons H-3 (δ 6.24 ppm), H-4 (δ 6.17 ppm), and H-6 (δ 5.67 ppm). Steroid (**6**) was most probably formed as a result of the splitting out of acetic acid from the molecule of the acetoxyenone (**4**). It must be mentioned that the dienone (**6**) is a natural compound that has been detected in plants [12, 13].

Thus, the formation in the preceding reaction of the seco-acid (**5**) and the dienone (**6**) permitted the conclusion that the experimental conditions used were too severe. When the oxidation of β-sitosterol acetate was performed subsequently under milder conditions — namely, at room temperature with a lower concentration of chromic acid — it was possible to raise the yield of the acetoxyenone (**4**) to 26%. Hydrolysis of the acetoxyenone (**4**) under the action of potassium carbonate in methanol then gave the desired phytosterol (**2**) with a yield of 82%.

Judging from its IR spectrum, compound (**2**) had retained the α,β-unsaturated keto grouping. At the same time, its IR spectrum lacked the absorption band of an acetoxy group and had a band of the stretching vibrations of a hydroxy group at 3450 cm^{-1}. The ^1H NMR spectrum of steroid (**2**) included the signal of the H-6 vinyl proton and lacked signals of the protons of an acetoxy group. Since the signal of the H-3α methine proton had a chemical shift, δ, of 3.72 ppm, it is possible to draw the conclusion of its geminal position in relation to a hydroxy group. We may note that the characteristics of the spectra of steroid (**2**) that we had obtained agree well with those given in the literature for the natural compound [5].

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EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument in the 700—3600 cm^{-1} range in KBr tablets. ^1H NMR spectra in deuterochloroform were obtained on a Bruker WM-360 NMR spectrometer with a working frequency of 360 MHz. Chemical shifts are given relative to TMS as internal standard. Electron-impact mass spectra were obtained on a Varian MAT-311 instrument at an energy of the ionizing electrons of 70 eV. UV spectra were taken on a Specord UV-Vis spectrophotometer. For column chromatography we used type L 40/100 silica gel from Lachema (Czech Republic). The course of the reaction and the purity of the compounds obtained were monitored with the aid of Silufol UV-254 plates from Kavalier (Czech Republic).

β-Sitosterol Acetate (3). A solution of 20.0 g of technical β-sitosterol (**1**) (content of the main substance 60%) in 200 ml of pyridine was treated with 40 ml of acetic anhydride. After 19 h, the solvent was evaporated off in vacuum, and the residue was dissolved in chloroform. The chloroform solution was washed with water and was then evaporated in vacuum, and the residue was dissolved in hexane. The hexane solution was filtered through a layer of alumina and was then evaporated in vacuum. The residue was recrystallized from hexane, giving 11.6 g of the acetate (**3**). The yield was 88%, calculated on pure β-sitosterol. mp 120—122°C hexane), lit. [14] mp 118—119°C. IR spectrum (cm^{-1}): 1730 (AcO), 1630 (C=C). ^1H NMR spectrum (δ, ppm): 0.68 (3H, s, 18-Me), 0.82 (3H, d, J = 7 Hz, 26-Me), 0.84 (3H, d, J = 8 Hz, 27-Me), 0.86 (3H, t, J = 8 Hz,

29-Me), 0.93 (3H, d, J = 7 Hz, 21-Me), 1.03 (3H, s, 19-Me), 2.05 (3H, s, AcO), 4.66 (1H, m, W/2 = 25 Hz, H-3α), 5.44 (1H, m, H-6). Mass spectrum (*m/z*) 456 (M^+).

Oxidation of β-Sitosterol Acetate (3) with Chromic Acid. A. With stirring, a solution of 7.0 g of chromium trioxide in a mixture of 10 ml of acetic acid and 10 ml of water was added to a solution of 7.0 g of the acetate (**3**) in 300 ml of acetic acid and 15 ml of chloroform at 50°C. The reaction mixture was stirred at 50°C for 30 min and was then cooled to room temperature and, after the addition of 15 ml of methanol, it was diluted with water and extracted with hexane. The hexane extract was washed with water and evaporated in vacuum. The residue was chromatographed on a column of silica gel, with elution by hexane—ether (1:2). Four fractions were obtained.

Fraction 1 — 0.75 g of the initial β-sitosterol acetate (**3**), identical with an authentic specimen. Yield 11%.

Fraction 2 — 0.25 g of (24R)-stigmasta-3,5-dien-7-one (**6**). Yield 4%. mp 109.5—111°C (hexane), lit mp 105°C [15]. IR spectrum (cm^{-1}): 1660 (C=O), 1630, 1600 (C=C). UV spectrum (nm): λ_{max} 281 (ε 28,000) (ethanol). ^1H NMR spectrum (δ, ppm): 0.72 (3H, s, 18-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), 0.95 (3H, d, J = 6 Hz, 21-Me), 1.13 (3H, s, 19-Me), 5.67 (1H, s, H-6), 6.17 (1H, br. d, J = 9.6 Hz, H-4), 6.24 (1H, m, W/2 = 19 Hz, H-3). Mass spectrum (*m/z*): 410 (M^+).

Fraction 3 — 0.4 g of (24R)-3β-acetoxystigmast-5-en-7-one (**4**). Yield 6%. mp 166—167.5°C (hexane), lit. mp 169—170°C [5], 170°C [9]. IR spectrum (cm^{-1}): 1735 (AcO), 1685 (C=O), 1640 (C=C). ^1H NMR spectrum (δ, ppm): 0.69 (3H, s, 18-Me), 0.82 (3H, d, J = 7 Hz, 26-Me), 0.84 (3H, d, J = 8 Hz, 27-Me), 0.85 (3H, t, J = 8 Hz, 29-Me), 0.94 (3H, d, J = 6 Hz, s, 21-Me), 1.22 (3H, s, 19-Me), 2.08 (3H, s, AcO), 4.78 (1H, m, W/2 = 24 Hz, H-3α), 5.78 (1H, br. s, H-6). Mass spectrum (*m/z*): 470 (M^+).

Fraction 4 — 2.95 g of (24R)-3β-acetoxy-5,6-secostigmast-5-on-6-oic acid. Yield 38%. mp 146—148°C (hexane). IR spectrum (cm^{-1}): 1745, 1715 (COOH, AcO, C=O). ^1H NMR spectrum (δ, ppm): 0.70 (3H, s, 18-Me), 0.81 (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 = 7 Hz, 21-Me), 1.06 (3H, s, 19-Me), 2.04 (3H, s, AcO), 5.47 (1H, m, W/2 = 10 Hz, H-3α). Mass spectrum (*m/z*): 470 (M^+ - AcOH).

B. With stirring, at room temperature, a solution of chromic acid obtained from 5.0 g of chromium trioxide, 7 ml of acetic acid and 7 ml of water was added to a solution of 10.0 g of β-sitosterol acetate in 300 ml of acetic acid and 70 ml of chloroform. The reaction mixture was stirred at room temperature for 5.5 h, and then 10 ml of ethanol was added. The mixture was diluted with water and was extracted first with hexane and then with ether, and the combined extract was evaporated in vacuum. The residue was chromatographed on a column of silica gel, with elution by hexane—ether (5:1). Two fractions were obtained.

Fraction 1 — 2.55 g of the initial β-sitosterol acetate (**3**), identical with an authentic specimen.

Fraction 2 — 2.0 g of the acetoxyenone (**4**), identical with an authentic specimen. Yield 26%, calculated on the β-sitosterol acetate that had reacted.

(24R)-3β-Hydroxystigmast-5-en-7-one (2). A mixture of 2.0 g of the acetate (**4**), 2.0 g of potassium carbonate, and 100 ml of methanol was boiled under reflux for 1.5 h, and then the solvent was evaporated off in vacuum. The residue was chromatographed on a column of silica gel, with elution by methanol—chloroform (1:10). This gave 1.5 g of the hydroxyketone (**2**). Yield 82%. mp 130—131°C (hexane), lit. mp 132—133°C [5]. IR spectrum (cm^{-1}): 3450 (OH), 1680 (C=O), 1660—1640 (C=C). ^1H NMR spectrum (δ,ppm): 0.69 (3H, s, 18-Me), 0.82 (3H, d, J = 7 Hz, 26-Me), 0.84 (3H, d, J = 8 Hz, 27-Me), 0.85 (3H, t, J = 8 Hz, 29-Me), 0.94 (3H, d, J = 6 Hz, 21-Me), 1.22 (3H, s, 19-Me), 3.72 (1H, m, W/2 = 20 Hz, H-3α), 5.76 (1H, d, J = 2 Hz, H-6). Mass spectrum (*m/z*): 428 (M^+).

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