3β,21β-Diaetoxyserrfat-14-ene (IVa). The mixture containing compound (IV) (0.028 g) was acetylated, and, after chromatography of the reaction mixture, 0.06 g of compound (IVa) was isolated with mp 221-225°C (acetone), $[\alpha]_D^{23}$ − 11° (c 0.91), lit.: mp 225-229°, $[\alpha]_D$ − 29° (c 2.06) [12]. PMR spectrum (AC-200): 0.68, 0.85, 0.92 (each 3H), 0.82 (12H, s, tertiary methyl groups), 2.02, 2.06, (s, each 3H—$OCOCH_3$), 4.44 (dd, J = 6.0 and 10.0 Hz, 1H-3), 4.66 (t, J = 2.0 Hz, 1H-21), 5.31 (m, 1H-14). Its ^{13}C NMR spectrum is given in Table 1.

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SYNTHESIS OF 3α-HYDROXY-6-KETOBRASSINOSTEROIDS

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The synthesis has been effected of the new brassinosteroids (22S,23S)-28-homotyphasterol, 24-epityphasterol, and (22S,23S)-24-epityphasterol, which belong to the 3α-hydroxy-6-oxosteroids. For obtaining (22S,23S)-28-homotyphasterol from stigmasterol, a new scheme of synthesis has been developed the key stages of which are the reduction of a 2α,3α-epoxy-6-ketone with lithium tetrahydroaluminate and the selective oxidation of the resulting 3α,6β-diol to the 3α-hydroxy-6-ketone.

The brassinosteroids include phytohormones with a polyhydroxysteroid structure that have been detected in plants in recent years and which possess high plant-growth stimulating activity and increase the resistance of agricultural crops to unfavorable conditions [1]. One of the brassinosteroids is typhasterol, which was isolated in 1983 from the pollen of the cattail Typha latifolia [2] and of the pine Pinus thunbergii [3]. Continuing an investigation on the synthesis of brassinosteroids and compounds related to them from accessible steroid raw material, we have obtained a number of new brassinosteroid belonging, like typhasterol, to the 3α-hydroxy-6-ketones and being close structural analogs of it. (Formula, top, following page.)

As a result of the solvolysis of the tosylates of the initial sterols β-sitosterol (IIa) and stigmasterol (IIb) and the Jones oxidation of the resulting 6β-hydroxy-3α,5-cyclosteroids we obtained the 6-oxo-3α,5-cyclosteroids (IIIa, b). The opening of the three-membered rings in compounds (IIIa, b) with hydrobromic acid formed the 3β-bromo-6-ketones (IVa, b), the dehydrobrimlination of which with lithium carbonate and bromide in dimethyl formamide led to the corresponding Δ^2-6-ketones (Va, b). The epoxidation of the Δ^2-bond in compound (Va) with m-chloroperbenzoic acid gave a 65% yield of the 2α,3α-epoxy-6-ketone (VIa). Its structure followed from its IR and PMR spectra. In the PMR spectrum of the epoxyketone (VIa) the signals of the vinyl protons at 5.58 and 5.69 ppm characteristic for the spectrum of the initial com-

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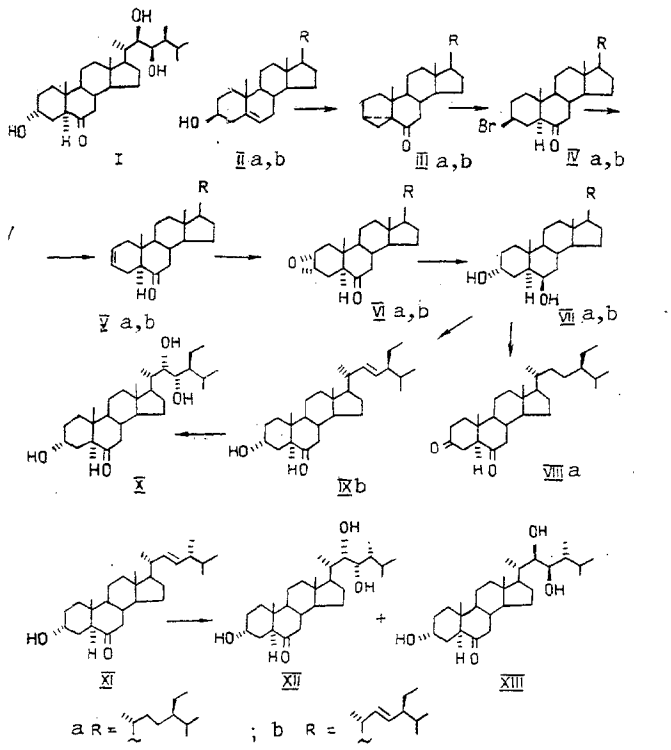
pound (Va) had disappeared, and the signals of the C_2–H and C_3–H methine protons geminal to the epoxy ring appeared at 3.14 and 3.28 ppm, respectively.



The conclusion of the α-configuration of the epoxide ring in compound (VIa) that we drew on the basis of the greater accessibility of a Δ^2-bond for attack by a reagent from the α-side of the steroid molecule was completely confirmed by the subsequent course of the synthesis. The analogous monoepoxidation of the 2,22-dien-6-one (Vb) with the calculated amount of the m-perbenzoic acid led to the 2α-3α-epoxide (VIb) with a yield of 54%. The PMR spectra of compound (VIb) contained the signals of vinyl protons at C_{22} and C_{23} with chemical shifts of 5.02 and 5.14 ppm, which showed the presence of a Δ^{22}-bond. At the same time, the signals of the vinyl protons at C_2 and C_3 characteristic for the spectrum of the initial steroid (Vb) were absent from the PMR spectrum of compound (VIb).

The presence of the signals of methine protons in the spectrum at 3.13 and 3.27 ppm the positions and forms of which completely agreed with the analogous signals in the spectrum of steroid (VIa) enabled us to show unambiguously the structure of the product the epoxidation of the 2,22-diene-6-ketone (Vb) as a 2α,3α-epoxy-Δ^{22}-6-ketone. In this case, the selective epoxidation of the Δ^2 bond is explained by its greater steric accessibility. On the interaction of the 2α,3α-epoxy-6-oxosteroids (VIa, b) with lithium tetrahydroaluminate, both the 2α, 3α-epoxide ring and the 6-keto group were reduced. Then the main reaction product, isolated with yields of 36 and 59%, respectively, were the 3,6-diols (VIIa, b).

The PMR spectra of compounds (VIIa, b) contained the signals of two methine protons geminal to hydroxy groups (δ 3.76 and 4.18-4.20 ppm). It followed from the half-width of these signals (8 Hz) that in compounds (VIIa and b) both hydroxy groups had an axial orientation. In the light of this, it is possible to ascribe only the β-configuration to the 6-hydroxy group. At the same time, depending on the configuration of the 2,3-epoxy ring, on its reduction with lithium tetrahydroaluminate the formation of an axial 2β-alcohol (for the 2β,3β-epoxide) or a 3α-alcohol (for the 2α,3α-epoxide) is possible in principle.

A convincing proof of the structures of the products of the reduction of the 2,3-epoxy-6-ketones (VIa, b) as 3α,6β-diols was the oxidation of compound (VIIa) with an excess of chromium trioxide in acetone by the Jones method to the known 3,6-ketone (VIIIa) identical with that which we had obtained previously by an alternative method [4]. As a result of the selective Jones oxidation of one of the hydroxy groups in steroid (VIIa), the 3α-hydroxy-6-ketone (IXb) was obtained with a yield of 52%. Its structure followed from a comparison of its PMR spectrum with the spectrum of the 3α-hydroxy-6-ketone (XI) that we had synthesized previously

from ergosterol [5]. The cis-hydroxylation of the Δ^{22}-steroid (IXa) with osmium tetroxide gave (22S,23S)-28-homotyphasterol with a yield of 97%. The analogous transformation of the Δ^{22}-steroid (XI) led to the formation of (22S,23S)-24-epityphasterol (XII) and 24-epityphasterol (XIII), which were isolated with yields of 49 and 29%, respectively. The structures of the brassinosteroids (XI-XIII) were shown unambiguously by a comparison of their PMR spectra with that of typhasterol (I) given in [2].

EXPERIMENTAL

Melting points were determined on a Kofler block. PMR spectra were recorded in deuterochloroform on Bruker AC-200 and Bruker WM-360 NMR spectrometers with working frequencies of 200 and 360 MHz, respectively. Chemical shifts are given relative to TMS as internal standard. IR spectra were obtained on a UR-20 instrument in KBr tablets. Mass-spectrometric characteristics were obtained on a Varian MAT-311 instrument at an energy of the ionizing electrons of 70 eV.

(24R)-5α-Stigmast-2-en-6-one (Va). A solution of 1.5 g of (24R)-3β-bromo-5α-stigmastan-6-one (IVa) (obtained by the procedure of [6]) in 50 ml of dimethylformamide was treated with 1.5 g of lithium carbonate and 0.5 g of lithium bromide. The reaction mixture was boiled under reflux for 3 h. After cooling to room temperature, it was diluted with water and extracted with hexane. The hexane extract was washed with water and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (5:1). This gave 1.02 g of the enone (Va). Yield 85%. IR spectrum ν_{max}^{KBr}, cm^{-1}: 1705 (C=O), 1660 (C=C). PMR spectrum (δ, ppm): 0.66 (s, 18-Me), 0.70 (s, 19-Me), 0.80 (d, J = 7.2 Hz, 26-Me), 0.84 (t, J = 7.2 Hz, 29-Me), 0.86 (d, J = 7.2 Hz, 27-Me), 0.92 (d, J = 6.0 Hz, 21-Me), 5.58 (m, W/2 = 18.0 Hz, C_2–H), 5.69 (m, W/2 = 20.4 Hz, C_3–H).

(24R)-2α-3α-Epoxy-5α-stiamastan-6-one (VIa). A solution of 0.80 g of the enone (Va) in 50 ml of chloroform was treated with 0.44 g of m-chloroperbenzoic acid (containing 85% of the main substances) and 0.20 g of sodium bicarbonate. The reaction mixture was kept at room temperature for 19 h, and it was then washed with saturated sodium bicarbonate solution and with water. The organic layer was separated from the aqueous layer and was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (5:1). This gave 0.548 g of the epoxide (VIa). Yield 65%, mp 159-162°C (hexane–ether). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1705 (C=O). PMR spectrum (δ, ppm): 0.64 (s, 18-Me), 0.70 (s, 19-Me), 0.80 (d, J = 7.0 Hz, 26-Me), 0.83 (d, J = 7.0 Hz, 27-Me), 0.84 (t, J = 7.5 Hz, 29-Me), 0.92 (d, J = 6.0 Hz, 21-Me), 3.14 (dd, J_1 = 5 Hz, J_2 = 7 Hz, C_2–H_β), 3.28 (t, J = 2 Hz, C_3–H_β).

(24S)-2α,3α-Epoxy-5α-stigmast-22-en-6-one (VIb). A solution of 1.85 g of the dienone (Vb) (obtained by the procedure of [7]) in 25 ml of chloroform was treated with 0.896 g of m-chloroperbenzoic acid (containing 85% of the main substance), and the mixture was kept at room temperature for 66.5 h. Then it was treated with 30 ml of saturated sodium bicarbonate solution and stirred at room temperature for 15 min. The organic layer was separated from the aqueous layer, washed with water, and evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (10:1). This gave 1.015 g of the epoxide (VIb). Yield 54%, mp 159-165°C (hexane). Found, %: C 81.71; H 10.88. Calculated for $C_{29}H_{46}O_2$ %: C 81.63, H 10.86.

IR spectrum, (ν_{max}^{KBr}, cm^{-1}): 1710 (C=O), 1630 (C=C). PMR spectrum (δ, ppm): 0.69 (s, 18-Me), 0.73 (s, 19-Me), 0.78 (d, J = 7.2 Hz, 26-Me), 0.80 (t, J = 7.2 Hz, 29-Me), 0.84 (d, J = 6.6 Hz, 27-Me), 1.01 (d, J = 7.2 Hz, 21-Me), 2.34 (t, J = 3.6 Hz, C_7–H_α), 3.13 (dd, J_1 = 36. Hz, J_2 = 6.0 Hz, C_2 – H_β), 3.27 (t, J = 2.4 Hz, C_3 – H_β), 5.02 (dd, J_1 = 8.4 Hz, J_2 = 15.6 Hz, C_{22}–H), 5.14 (dd, J_1 = 8.4 Hz, J_2 = 14.4 Hz, C_{23}–H). Mass-spectrum, m/z: 426 (M^+).

(24R)-5α-Stigmastane-3α-6β-diol (VIIa). With continuous stirring at room temperature, 0.2 g of lithium tetrahydroaluminate was added to a solution of 0.50 g of the epoxyketone (VIa) in 60 ml of anhydrous ether. The reaction mixture was stirred for 1 h and was then treated with a solution of ethyl acetate in ether. The solvent was evaporated off in vacuum and the residue was chromatographed on a column of silica gel with elution by chloroform–methanol (10:1). This gave 0.179 g of the diol (VIIa). Yield 36%, mp 174-177°C. IR spectrum (ν_{max}, cm^{-1}): 3400 (OH). PMR spectrum (δ, ppm): 0.67 (s, 18-Me), 0.92 (s, 19-Me), 0.78 (d, J = 50 Hz, 26-Me), 0.88 (d, J = 4.0 Hz, 21-Me), 0.81 (d, J = 5.0 Hz, 27-Me), 0.82 (t, J = 7.0 Hz, 29-Me), 3.76 (m, W/2 = 9.5 Hz, C_6 – H_α) 4.18 (m, W/2 = 8.0 Hz, C_3 – H_β).

(22S)-5α-Stigmast-22-ene-3α,6β-diol (VIIb). With continuous stirring at room temperature, 0.876 g of lithium tetrahydroaluminate was added to a solution of 1.23 g of the epoxy derivative (VIb) in 86.5 ml of anhydrous ether. The reaction mixture was stirred at room temperature for 1 h 35 min, and was then treated with a solution of ethyl acetate in ether. The solvent was evaporated off in vacuum, and the residue was chromatographed on a column of silica gel with elution by chloroform–methanol (30:1). This gave 0.73 g of the diol (VIIb). Yield 59%, mp 207-210°C (methanol).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3360 (OH). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.78 (d, J = 6.0 Hz, 26-Me), 0.85 (d, J = 6.0 Hz, 27-Me), 0.80 (t, J = 7.0 Hz, 29-Me), 1.00 (s, 19-Me), 1.02 (d, J = 6.0 Hz, 21-Me), 3.76 (m, W/2 = 8.0 Hz, $C_6 - H_\alpha$), 4.20 (t, J = 3.0 Hz, $C_3 - H_\beta$), 5.00 (dd, J_1 = 8.0 Hz, J_2 = 15.0 Hz, C_{22}–H), 5.16 (dd, J_1 = 8.0 Hz, J_2 = 15.0 Hz, $C_{23} - H$).

(24R)-5α-Stigmastane-3,6-dione (VIIIa). A solution of 0.18 g of the diol (VIIa) in 70 ml of acetone was treated with 0.35 ml of 8 N chromic acid. The reaction mixture was kept at room temperature for 20 min. Then the excess of oxidant was eliminated by the addion of 1 ml of isopropanol. The mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and the solvent was evaporated off in vacuum. The residue was chromatographed on a column of silica gel with elution by chloroform–methanol (50:1). This gave 0.15 g of the diketone (VIIIa), identical with that synthesized by the procedure of [4]. Yield 82%, mp 203-205°C (hexane–methanol); lit [4]: mp 205-206.5°C.

(24S)-3α-Hydroxy-5α-stimast-22-en-6-one (IXb). With cooling in an ice bath, 0.41 ml of 8 N chromic acid was added to a solution of 0.717 g of the diol (VIIIb) in 150 ml of acetone. The mixture was kept with cooling for 10 min and was then diluted with water. The reaction product was extracted with ether, and the ethereal extract was washed with water and evaporated in vacuum. The residue was chromatographed on a column os silica gel with elution by chloroform–ethyl acetate (50:1). This gave 0.375 g of the hydroxyketone (IX). Yield 52%, mp 190-194°C (methanol).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3430 (OH), 1712 (C=O). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.74 (s, 19-Me), 0.80 (d, J = 6.0 Hz, 26-Me), 0.86 (d, J = 6.0 Hz, 27-Me), 0.82 (t, J = 3.0 Hz, 29-Me), 1.02 (d, J = 7.0 Hz, 21-Me), 2.31 (dd, J_1 = 4.0 Hz, J_2 = 8.0 Hz, $C_7 - H_\beta$), 2.73 (quintet, J = 8.0 Hz, $C_5 - H_\alpha$), 4.18 (m, W/2 = 7.0 Hz, $C_3 - H_\beta$), 5.02 (dd, J_1 = 8.0 Hz, J_2 = 15.0 Hz, $C_{22} - H$), 5.16 (dd, J_1 = 8.0 Hz, J_2 = 15.0 Hz, C_{23}–H).

(22S,23S,24S)-3α,22,23-Trihydroxy-5α-stigmastan-6-one ((22S,23S)-28-Homotyphasterol) (X). A solution of 0.160 g of the hydroxyenone (Ix) in 7.5 ml of pyridine was treated with 0.095 g of osmium tetroxide, and the reaction mixture was kept at room temperature for 22.5 h. Then, with stirring, a solution of 0.3 g of sodium sulfate and 0.075 ml of sulfuric acid in 5 ml of water was added to it over 40 min. The reaction product was extracted with chloroform. The organic layer was washed with water and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (1:1). This gave 0.168 g of the brassinosteroid (X). Yield 97%, mp 170-172°C (methanol).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3420 (OH), 1710 (C=O). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.73 (s, 19-Me), 0.88 (d, J = 7.0 Hz, 26-Me), 0.96 (t, J = 7.0 Hz, 29-Me), 1.00 (d, J = 4.0 Hz, 27-Me), 1.03 (d, J = 7.0 Hz, 21-Me), 2.72 (quintet, J = 8.0 Hz, $C_5 - H_\alpha$), 3.44-3.68 (m, C_{22}– and C_{23}–H), 4.18 (m, W/2 = 8.0 Hz, C_3–H_β).

Hydroxylation of (24R)3α-Hydroxy-5α-ergost-22-en-6-one (XI).. A solution of 0.150 g of the enone (XI) (obtained by the procedure of [5]) in 2 ml of pyridine was treated with a solution of 0.100 g of osmium tetraoxide in 2 ml of pyridine. The reaction mixture was kept at room temperature for 22.5 h. Then, with stirring, a solution of 0.300 g of sodium sulfate and 0.075 ml of sulfuric acid in 5 ml of water was added. The resulting mixture was stirred with heating to 40°C for 1 h and was then 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 ether.

This gave 0.146 g of (22R,23R,24R)-3α,22,23-Trihydroxy-5α-ergostan-6-one (24-epityphasterol) (XIII). Yield 29%, mp 233-235°C (hexane–ether). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3430 (OH), 1705 (C=O). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.75 (s, 19-Me), 1.00 (d, J = 6.0 Hz, 21-Me), 0.87 (d, J = 7.2 Hz, 26-Me), 0.89 (d, J = 7.2 Hz, 27-Me), 0.93 (d, J =- 7.2 Hz, 28-Me), 2.75 (quintet, J = 8.4 Hz, $C_5 - H_\alpha$), 3.42 (m, W/2 = 13.2 Hz, C_{22}–H), 3.70 (m, W/2 = 10.8 Hz, C_{23}–H).

Further elution with ether yielded 0.7059 g of (22S,23S,24R)-3α,22,23-Trihydroxy-5α-ergostan-6-one ((22S,23S)-24-epityphasterol) (XII). Yield 49%, mp 230-235°C (methanol), IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3435 (OH), 1710 (C=O). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.75 (s, 19-Me), 0.88 (d, J = 7 Hz, 26-Me), 0.90 (d, J = 7 Hz, 27-Me), 0.95 (d, J = 7 Hz, 28-Me), 1.02 (d, J = 7.5 Hz, 21-Me), 2.31 (dd, J_1 = 6.0 Hz, J_2 = 12.0 Hz, C_7–H_β), 2.72 (quintet, J = 8.0 Hz, C_5–H_α), 3.61 (m, W/2 = 12 Hz, C_{22}–H), 3.73 (m, W/2 = 11 Hz, C_{23}–H), 4.18 (m, W/2 = 8.0 Hz, C_3 – H_β).

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