SYNTHESIS OF 19-HYDROXYSTEROIDS

II. NEW SYNTHESIS OF 19-HYDROXYPROGESTERONE

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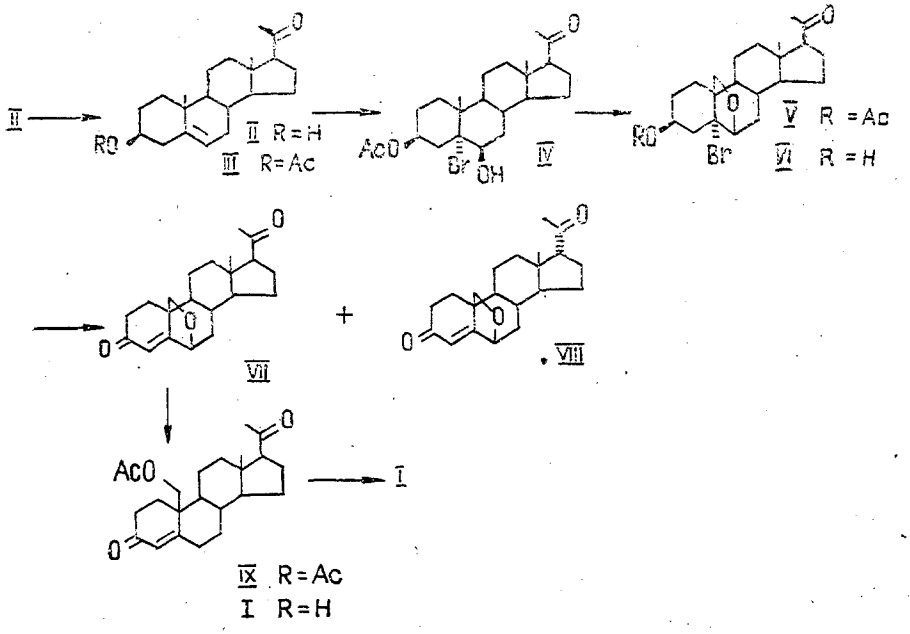
UDC 547.92

19-Hydroxyprogesterone has been synthesized in seven stages from pregnenolone with an overall yield of 11%.

During the creation in the Institute of Bioorganic chemistry of the Belorus Academy of Sciences of new methods for the radioimmunological analysis of the pregnancy hormone progesterone (I) in large amounts. This substance was first synthesized by a fairly complex method with a low overall yield from the difficultly accessible cardiotonic steroid strophanthidin [1]. Several methods were subsequently developed for its production, using more accessible steroids of the pregnane series as the starting material [2-7].

On analyzing the approaches to the synthesis of 19-hydroxy progesterone known from the literature, we settled our choice on a scheme proposed as the best with respect to the number of stages and the overall yield of the desired product [2, 7]. Unfortunately, the relevant publications [2, 7] have the nature of brief communications without a detailed description of the experimental work. This considerably hindered their reproduction and required the special development of the most practical procedures. In this, we made use of the experience accumulated in the process of developing the synthesis of 19-hydroxytestosterone. The present communication is devoted to the results obtained from the investigations.



By the acetylation of pregenolone (II) with acetic anhydride in pyridine we obtained the acetate (III) in quantitative yield. The addition of the elements of hypobromous acid obtained directly in the reaction mixture by the decomposition of N-bromoacetate under acid conditions to the 5(6)-double bond of this compound took place with the formation mainly of the bromohydrin (IV). We isolated compound (IV) with a yield of 75%. The structure was

Institute of Bioorganic Chemistry, Belorussian Academy of Sciences, Minsk. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 678-682, November-December, 1992. Original article submitted January 12, 1992. Original article submitted April 24, 1992.

0009-3130/92/2806-0589$12.50

determined as the result of spectral analysis. In this, the PMR spectrum was of the greatest importance, containing, together with others, the signal of the C_6—H_α proton (δ 4.20 ppm) geminal to a hydroxy group. It was possible to deduce the β-orientation of the 6-hydroxy group on the basis of the half-width of this signal (W/2 8 Hz) and also the downfield shift to 1.33 ppm of the signal of the 19-methyl group in the 1,3-diaxial position with respect to it. The analogous shift on the signal of the C_3—h_α methine proton (δ 5.48 ppm) acted as proof of the presence of the bromine atom in the 1,3-diaxial position to it.

Cyclization of the bromohydrin (IV) under the action of lead tetraacetate and iodine in boiling benzene under illumination enabled us to obtain the 5α-bromo-6β,19-epoxide (V) with a yield of 81%. The PMR spectrum of this steroid lacked the signal of the angular methyl group and contained at 3.73 and 3.94 ppm the signals of the protons of the 19-methylene group geminal to the oxygen atom of a 6β-19-epoxide ring. The realization of the scheme that we selected [3, 7] presupposed passage from the 3β-acetoxy-5α-bromo derivative (V) to the Δ^4-3-ketone. With this aim, in the following stage we hydrolyzed the 3β-acetoxy group in compound (V) under the action of potassium carbonate in methanol. The structure of the alcohol (VI) so obtained with a yield of 77% was shown with the aid of IR and PMR spectra. For example, the IR spectrum of steroid (VI) lacked bands of the stretching vibrations of an acetoxy group at 1740 and 1250 cm^{-1}. Similarly, the PMR spectrum also lacked the signals of the protons of an acetoxy group. An upfield shift to 4.16 ppm of the signal of the C_3—H_α methine proton was proof of the fact that this proton was geminal not to the acetoxy group but to the hydroxy group. When alcohol (VI) was subjected to Jones oxidation with chromium trioxide in acetone and the resulting unstable 5α-bromo-3-ketone was subjected to dehydrobromination under the action of lithium carbonate and bromide in dimethylformamide at the boil, we obtained two substances. The structure of the main product, isolated with a yield of 57%, was established as the result of an analysis of its spectroscopic characteristics as the Δ^4-3-ketone (VII). For example, the presence of an α,β-unsaturated keto group in compound (VII) follows from a characteristic absorption band at 242 nm in its UV spectrum. A similar conclusion was permitted by the IR spectrum, in which, together with the band of the stretching vibrations of the 20-keto group at 1705 cm^{-1}, there were the bands of a 3-keto group at 1680 cm^{-1} and of a 4(5)-double bond conjugated with it at 1640 cm^{-1}. In the PMR spectrum, together with the signals of the protons corresponding to the main structural fragments, was a singlet of the C_4—H vinyl proton (δ 5.84 ppm). In its turn, in the molecule of the minor reaction product (VIII), judging from its IR, UV, and PMR spectrum, there were also a 20-keto group, a Δ^4-3-keto grouping and a 6β-19-epoxide grouping. From this it was possible to conclude that steroid (VII) and (VIII) were isomers at C_{17}.

For the accurate determination of the structure of compound (VIII), the presence in its PMR spectrum of the signal of the C_{17}—H_β methine proton at 2.95 ppm is of great importance. Such a chemical shift is characteristic for 17α-pregnan-20-one derivatives [8]. At the same time, in a number of pregnane derivatives with the natural side-chain the C_{17}—H_α signals appear at smaller values of the chemical shift (usually 2.45-2.60 ppm) [8]. In spectra recorded on instruments with low working frequencies, therefore, they fall into the region of the methylene hump which also took place in our case for compound (VII). The circular dichroism spectra also permitted similar conclusions concerning the structures of steroids (VII) and (VIII).

By the reductive cleavage of the 6β,19-oxide ring in steroid (VII) under the action of zinc dust in acetic acid followed by acetylation of the resulting mixture of 19-hydroxy and 19-acetoxy derivatives, we obtained 19-acetoxyprogesterone (IX) with a yield of 61%. The structure of this compound was determined from its spectra, in which it was possible reliably to identify all its elements. When the acetate (IX) was hydrolyzed with potassium carbonate in methanol, 19-hydroxyprogesterone (I) was synthesized with a yield of 67%. The overall yield of steroid (I) from pregnenolone (II) was 11%. The spectroscopic characteristics of the substance that we had synthesized corresponded completely to the structure ascribed to it and agreed well with those known from the literature [2-8].

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were obtained on a UR-20 instrument. UV spectra were recorded on a Specord UV-VIS spectrophotometer and circular dichroism spectra on a JASCO J-20 spectropolarimeter. PMR spectra were recorded in deuterochloroform on a Bruker AC-200 NMR spectrometer with a working frequency of 200 MHz. Chemical shifts are given relative to TMS as internal standard.

3β-Acetoxypregn-5-3n-20-one (III). A solution of 5.0 g of pregnenolone (II) in a mixture of 40 ml of pyridine and 20 ml of acetic anhydride was kept at room temperature for 60 h and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution with hexane–ether (4:1). This gave 5.6 g of the acetate (III). Yield 99%, m 145-148°C (ether–hexane).

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 1730 (C=O), 1705 (C=O), 1240 (C–O). PMR spectrum (δ, ppm): 0.63 (s, 18-Me), 1.02 (s, 19-Me), 2.04 (s, 3β-OAc), 2.13 (s, 21-Me), 4.61 (1H, m, W/2 20 Hz, C_3–H_α), 5.40 (1H, bnd, J = 5.0 Hz, C_6–H).

3β-Acetoxy-5-bromo-6β-hydroxy-5α-pregnan-20-one (IV). With stirring in the dark, 4.4g of N-bromoacetamide was added in portions to a solution of 5.6 g of the acetate (III) in 420 ml of freshly distilled dioxane containing 36 ml of water and 9 ml of 70% perchloric acid. After the end of addition, stirring was continued at room temperature for 30 min, and then 3.0 g of sodium thiosulfate in 40 ml of water was added to the mixture and it was stirred for another 10 min. After this it was extracted with chloroform (5 × 100 ml), the chloroform extracts were dried with magnesium sulfate, and the solvent was distilled off in vacuum. Crystallization of the residue from 50 ml of ethyl acetate yielded 2.4 g of the bromohydrin (IV). The mother solution was evaporated and the residue was chromatographed on a column of silica gel with elution by hexane–ethyl acetate (2:1). This gave another 2.9 g of the bromohydrin. The total yield was 5.3 g (75%, mp 163-165° (ethyl acetate); lit.: mp 165-167°C [2], 166-168°C [7], 155-160°C [9].

IR spectrum; ν_{max}^{KBr}, cm^{-1}: 3440 (OH), 1740 (C=O), 1705 (C=O), 1250 (C–O). PMR spectrum (δ, ppm): 0.64 (s, 18-Me), 1.32 (s, 19-Me), 2.04 (s, 3β-OAc), 2.13 (s, 21-Me), 4.20 (1H, m, W/2 = 8 Hz, C_6–H_α) 5.48 (1H, m, W/2 = 21 Hz, C_3–H_α).

3β-Acetoxy-5-bromo-6β,19-epoxy-5α-pregnan-20-one (V). A mixture of 5.3 g of the bromohydrin (IV), 150 ml of benzene, 8.1 g of lead tetraacetate, and 1.3 g of iodine was boiled under reflux with stirring and illumination by a 60 W lamp for 1 h. After cooling, the precipitate was filtered off and was washed on the filtrate with benzene; the benzene solutions were washed with 5% sodium thiosulfate solution (100 ml) and with water (75 ml) and were dried with magnesium sulfate. After the solvent had been distilled off, the residue was chromatographed on a column of silica gel with elution by hexane–ether (3:1). This gave 4.3 g of the epoxysteroid (V). Yield 81%, mp 134-136°C (ether–pentane); lit.: mp 152-154°C [2], 154-157°C [5], 148-151°C [7].

IR spectrum; ν_{max}^{KBr}, cm^{-1}: 1740 (C=O), 1700 (C=O), 1240 (C–O). PMR spectrum (δ, ppm): 0.65 (s, 18-Me), 2.05 (s, 3β-OAc), 2.12 (s, 21-Me), 3.73 (1H, d, J_{AB} = 8.5 Hz. C_{19}–H), 3.94 (1H, d, C_{19}–H), 4.08 (1H, d, J = 4.0 Hz, C_6–H_α), 5.20 (1H, m, W/2 = 22 Hz, C_3–H_α).

3β-Hydroxy-5-bromo-6β,19-epoxy-5α-pregnan-20-one (VI). A solution of 4.3 g of the acetate (V) in 200 ml of methanol containing 1.0 g of potassium carbonate was stirred at room temperature for 2.5 h. Then the excess of potassium carbonate was eliminated by the addition of 1 ml of glacial acetic acid, and the mixture was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ethyl acetate (1:1). This gave 3.0 g of the alcohol (VI). Yield 77%, mp 184-186°C (hexane–ethyl acetate); lit.: mp 179-180°C [2], 186-187°C [5], 180-181°C [7].

IR spectrum; ν_{max}^{KBr}, cm^{-1}: 3420 (OH), 1700 (C=O). PMR spectrum (δ, ppm): 0.66 (s, 18-Me), 2.12 (s, 21-Me), 3.72 (1H, d, J_{AB} = 8.5 Hz, C_{19}–H), 3.94 (1H, d, C_{19}–H), 4.10 (1H, d, J = 4.0 Hz, C_6–H_α), 4.16 (1H, m, W/2 = 31 Hz, c_3–H_α).

6β,19-Epoxypregn-4-ene-3,20-dione (VII). A solution of 2.9 g of compound (VI) in 200 ml of acetone was treated with 6.0 ml of an 8 N solution of chromic acid. The mixture was stirred at room temperature for 20 min, and then the excess of oxidant was eliminated by the addition of 20 ml of isopropanol, and the solvent was distilled off in vacuum. The residue was treated with 200 ml of water and extracted with chloroform. The chloroform extracts were dried with magnesium sulfate, and the solvent was distilled off in vacuum. The residue was boiled under reflux in 100 ml of dimethylformamide in the presence of 3.1 g of lithium carbonate and 1.2 g of dimethylformamide in the presence of 3.1 g of lithium carbonate and 1.2 g of lithium bromide for 30 min. After cooling, the precipitate was filtered off, and the filtrate was diluted with 300 ml of water and extracted with ether (5 × 50 ml). The ethereal extracts were dried with magnesium sulfate, the solvent was distilled off in vacuum, and the residue was chromatographed on a column of silica gel with elution by hexane–ethyl acetate (2:1). This gave 0.40 g of 6β,19-epoxy-17α-pregn-4-ene-3,20-dione (VIII). Yield, 17%, mp 151-153°C (ether–hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1705 (C=O), 1670 (C=O), 1640 (C=C). UV spectrum, λ_{max}^{EtOH}, (ε), nm: 241 (11800). CD spectrum (λ_{max}^{EtOH} ($\Delta\varepsilon$), nm)): 230 (-20.32), 323 (-3.64). PMR spectrum (δ, ppm): 1.00 (s, 18-Me), 2.14 (s, 21-Me), 2.95 (1H, dd, J = 3.0 Hz, C_{17}—H_β), 3.50 (1H, d, J_{AB} = 8.0 Hz, C_{19}—H), 4.19 (1H, d, C_{19}—H), 4.69 (1H, d, J = 5.0 Hz C_6—H_α 5.80 (1H, s, C_4—H).

Further elution led to 1.3 g of the main product (VII). Yield 57%, mp 136-138°C (ether—hexane); lit. mp 143-145°C [3], 142-145°C [5].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1705 (C=O), 1680 (C=O), 1640 (C=C). UV spectrum (λ_{max}^{EtOH} (ε), nm): 242 (11700). CD spectrum (λ_{max}^{EtOH} ($\Delta\varepsilon$), nm): 230 (-24.02), 286 (+4.63), 328 (-2.61). PMR spectrum (δ, ppm): 0.72 (s, 18-Me), 2.14 (s, 21-Me), 3.52 (1H, d, J_{AB} = 8.0 Hz, C_{19}—H), 4.22 (1H, d, C_{19}—H), 4.72 (1H, d, J = 5.0 Hz, C_6—H_α), 5.84 (1H, s, C_4—H).

19-Hydroxyprogesterone Acetate (IX). A solution of 1.2 g of the epoxyenedione (VIII) in 70 ml of glacial acetic acid in the presence of 4.0 g of zinc dust was boiled under reflux with stirring for 2.5 h. After cooling, the solid matter was filtered off and was washed on the filter with ethyl acetate, and the filtrates were evaporated to dryness. The residue was kept at room temperature in a mixture of 25 ml of pyridine and 25 ml of acetic anhydride for 20 h. Then the mixture was evaporated in vacuum and the residue was chromatographed on a column of silica gel with elution by hexane—ether (2:3). This gave 0.81 g of 19-acetoxyprogesterone (IX). Yield 61%, mp 125-126°C (hexane); lit. [1]: double mp 89-95°C/125-126°C.

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1740 (C=O), 1705 (C=O), 1680 (C=O), 1620 (C=C). UV spectrum (λ_{max}^{EtOH} (ε), nm): 242 (13600). CD spectrum (λ_{max}^{EtOH} ($\Delta\varepsilon$), nm): 235 (+11.92), 2.85 (+4.60), 325 (—1.35). PMR spectrum (δ, ppm): 0.67 (s, 18-Me), 2.02 (s, 19-OAc), 2.13 (s, 21-Me), 4.18 (1H, d, J_{AB} = 11.0 Hz, C_{19}—H), 4.68 (1H, d, C_{19}—H), 5.94 (1H, s, C_4—H).

19-Hydroxyprogesterone (I). A solution 0.80 g of 19-acetoxyprogesterone (IX) in 60 ml of dry methanol containing 0.4 g of potassium carbonate was stirred at room temperature for 5 h. Then the excess of potassium carbonate was eliminated by the addition of 0.5 ml of glacial acetic acid, and the mixture was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane—ethyl acetate (1:1). This gave 0.49 g of 19-hydroxyprogesterone (I). Yield 67%, mp 160-162°C (hexane—ethyl acetate); lit.: mp 171-172° [1], 169-171°C [2], 169-170°C [3], 168-170°C [4], 165-168°C [5], 166-168°C [6], 166-169°C [7].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3340 (OH), 1710 (C=O), 1660 (C=O), 1620 (C=C). PMR spectrum (δ, ppm): 0.66 (s, 18-Me), 2.12 (s, 21-Me), 3.90 (1H, dd, J_{AB} = 10.0 Hz, J = 7.0 Hz, C_{19}—H), 4.07 (1H, dd, J = 3.0 Hz, C_{19}—H), 5.95 (1H, s, C_4—H).

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