SYNTHESIS OF 19-HYDROXYSTEROIDS

I. NEW SYNTHESIS OF 19-HYDROXYTESTOSTERONE

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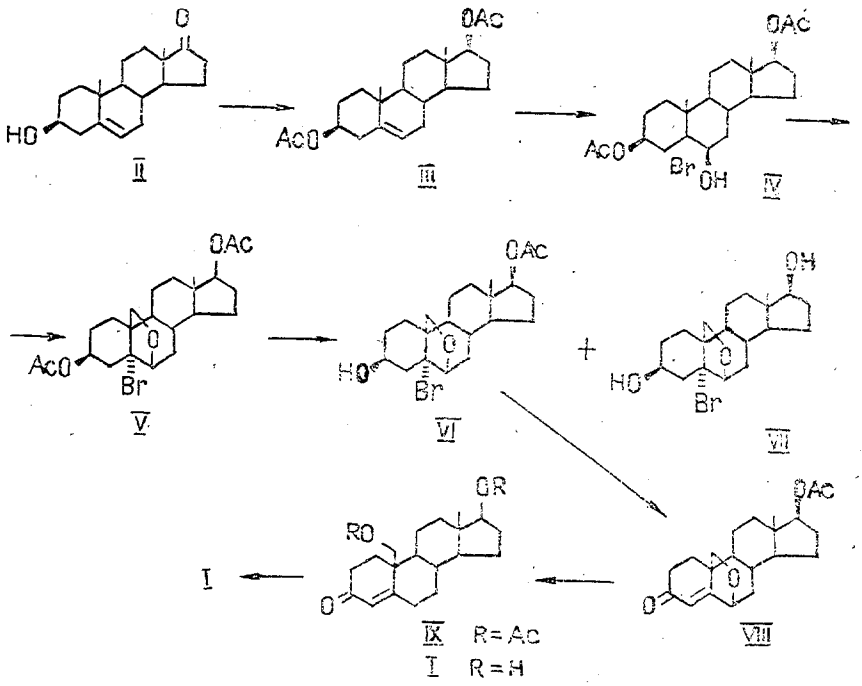
UDC 547.92

For obtaining 19-hydroxytestosterone from dehydroepiandrosterone a new scheme of synthesis has been developed the key stages of which are the reduction of the 17-keto group to a 17-alcohol, the functionalization of the 19-methyl group via the bromohydrin with the formation of a 6β,19-epoxide, the selective hydrolysis of the free β-acetoxy group, the conversion of the 3β-hydroxy-5α-bromo derivative into the Δ^4-3-ketone, and the reductive cleavage of the 6β,19-epoxide ring.

In the radioimmunological analysis of the male sex hormone testosterone, the use of its derivative, 19-hydroxytestosterone (I), appears extremely promising [1]. This substance was first obtained as the result of a complex and multistage synthesis from the cardiotonic steroid strophanthidin [2]. Subsequently, more practical approaches for the preparation of steroid (I) were proposed which were based on dehydroepiandrosterone (II) [3, 4]. In the process of developing a new method for the radioimmunological analysis of testosterone in the Institute of Bioorganic Chemistry of the Belorus Academy of Sciences, we came up against the necessity for obtaining 19-epitestosterone in large amounts. To fulfil this task, we have developed a new scheme for synthesizing steroid (I) from dehydroepiandrosterone (II) which is distinguished by a smaller number of stages than in the method known previously and a high overall yield.



In the first stage of the synthesis, the 17-keto group in dehydroepiandrosterone (II) was reduced with sodium tetrahydroborate in methanol and then the resulting 3,17-diol was converted into the diacetate (III) with an overall yield of 97%. As a result of the addition

Institute of Bioorganic Chemistry, Belorussian Academy of Sciences, Minsk. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 672-678, November-December, 1992. Original article submitted April 24, 1992.

 0009-3130/92/2806-0584$12.50

of the elements of hypobromous acid to the 5(6)-double bond in compound (III), the bromohydrin (IV) was obtained with a yield of 64%. Its structure followed unambiguously from its spectra. Thus, in the PMR spectrum of steroid (IV), the signals of the characteristic vinyl proton at C_6 (δ 5.38 ppm) in the spectra of the initial substance (III) had disappeared.

The presence of a 6-hydroxy group in compound (IV) was shown by the signal in the PMR spectra of the proton geminal to it at 4.19 ppm. Since this signal had the form of a doublet (J = 4.0 Hz) it was possible to conclude that the 6-hydroxy group had the β-configuration. Attention is attracted by the downfield shift to 1.33 ppm of the signal of the 19-methyl group. This was due to the action of the 6β-hydroxy group in the 1,3-diaxial position with respect to it. An analogous downfield shift to 5.19 ppm of the signal of the C_3–H_α proton showed the presence of a bromine atom at C_5 in the 1,3-diaxial position with respect to it. It must be mentioned that the addition of the elements of hydrobromous acid in compounds of the 17-oxoandrosterand series take place fairly ambiguously [5]. Together with the 5a-bromo-6β-hydroxy derivatives considerable amounts of the 6β-bromo-5α-hydroxy isomers are formed.

Our investigations have shown that the preliminary reduction of the 17-ketone to a 17β-alcohol substantially changes the ratio of the isomers in the direction of the predominance of the required bromohydrin (IV).

This influence of the fairly remote C_{17}-substituent on a reaction of the 5(6)-double bond can be explained by conformational transmission.

The cyclization of the bromohydrin (IV) under the action of lead tetraacetate and iodine in boiling benzene under illumination gave a 91% yield of the 5α-bromo-6β,19-epoxysteroid (V). Its structure could be judged from the disappearance from the PMR spectrum of the signal of the angular 16-methyl group and the appearance at 3.74 and 3.94 ppm of two doublets of the AB system of a 19-methylene group to which an oxygen atom was attached.

In steroid (V), the acetoxy groups differ considerably with respect to their steric accessibility. Therefore, in 3β,17β-diacetoxyandrostanes the selective hydrolysis of the 3β-acetoxy group with the aim of the subsequent conversion of the 3β-hydroxy group into a Δ^4-3-ketone grouping is possible [4]. In actual fact, we have established that the action of potassium carbonate on the diacetate (V) in methanol at room temperature for 2.5 h take place mainly with the formation of the 17β-acetoxy-3β-hydroxysteroid (VI), which is was possible to isolate from the reaction mixture with a yield of 77%. The second product of this reaction, which we obtained with a yield of 21%, was the 3β,17β-diol (VII). An upfield shift in the PMR spectrum of the C_3-Hα signal to 4.13 ppm in comparison with the position of this signal in the spectrum of the initial diacetate (V) (δ 5.19 ppm) was important for demonstrating the structure of the hydroxyacetate (VI). At the same time, the chemical shifts of the C_{17}–H_α proton in the spectra of compounds (V) and (VI) practically coincided, which indicated the retention of the 17-acetoxy group in the steroid (VI). In the IR spectrum of compound (VII), the minor product of the hydrolysis of the diacetate (V), bands of the stretching vibrations of the acetoxy groups were absent, and in the PMR spectrum the signals of the protons of the acetoxy groups were absent. The characteristic upfield shift of the C_3–H_α and C_{17}–H_α signals (δ 4.07 and 3.63 ppm, respectively) observed in addition to this unambiguously showed the structure of the compound under discussion as a 3,17-diol.

The acetylation of steroid (VII) gave a 92% yield of the diacetate (V), which may also be used for the synthesis of the hydroxyacetate (VI).

By the Jones oxidation of the 3β-hydroxy group in compound (VI) with chromium trioxide in acetone and dehydrobromination of the unstable 5α-bromo-6-ketone formed, we obtained the Δ^4-3-ketone. In this way, the use of the method of dehydrobrominating bromoketosteroids by the action of lithium carbonate and bromide in dimethylformide at the boil which is widely used in the synthesis of ecdysteroids [6] enabled us to obtain the enone (VIII) from the alcohol (VI) with an overall yield of 91%. The UV spectrum of the steroid (VIII) had the absorption band at 239 nm that is characteristic for α,β-unsaturated ketones. The presence in the IR spectrum of the bands of the stretching vibrations of a keto group at 1680 cm^{-1} also demonstrated the structure of the enone (VIII). A confirmation of this was the presence in the PMR spectrum of the enone (VIII) of, in addition to other characteristic signals, a singlet of the C_4–H vinyl proton at 5.82 ppm.

It must be mentioned that an attempt to split out the bromine atom from the molecule of steroid (V) by the action of lithium carbonate and bromide in dimethyl formamide at the boil was unsuccessful. Only the initial substance was recovered, in high yield.

We carried out the reductive cleavage of the 6β,19-oxide ring under the action of zinc dust in acetic acid. Under these conditions, partial acetylation of the 19-hydroxy group took place. To avoid the procedure of separating the 19-alcohol and its acetate, the reaction products were subjected to acetylation. As a result, the 17,19-diacetate (IX) was obtained from the 6β,19-epoxide (VIII) with an overall yield of 57%. Judging from its IR, UV, and PMR spectra, compound (IX) had retained the Δ^4-3-keto grouping. Characteristic for the PMR spectrum of the initial steroid (VIII) was the presence of the signal of the C_6–H_α methine proton (δ 4.71 ppm) in the form of a doublet with a constant of 5.0 Hz, which showed the attachment of the oxygen of the epoxy ring to the C_6 atom. The absence of this signal from the PMR spectrum of compound (IX) therefore showed the opening of the oxide ring. In addition, the spectrum contained two doublets of the AB system of a 19-methylene group geminal to an acetoxy group.

In the concluding stage, the required 19-hydroxytestosterone was obtained with a yield of 84% by the hydrolysis of the diacetate (IX) with potassium carbonate in methanol in room temperature. Its structure followed unambiguously from its IR and PMR spectra. The overall yield of compound (I) from dihydroepiandrosterone (II) by the scheme developed amounted to 20%.

EXPERIMENTAL

Melting points were determined on Kofler block. IR spectra were obtained on a UR-20 instrument. UV spectra were recorded on a Specord UV-Vis spectrophotometer. 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.

Androst-5-ene-3β,17β-diol Diacetate (III). A suspension of 5.0 g of dehydroepiendrosterone (II) and 0.6 g of technical sodium tetrahydroborato in 100 ml of anhydrous methanol were stirred at room temperature for 1 h. Then 1.5 ml of glacial acetic acid was added to the mixture and it was evaporated to dryness, and the residue was treated with 100 ml of water. The precipitate that deposited was filtered off, washed on the filter with water, and dried in a vacuum desiccator over phosphorus pentoxide. The filtrate was extracted with chloroform (5 × 30 ml), the organic extracts were dried with magnesium sulfate, and the solvent was evaporated off in vacuum.

The combined reduction products were kept at room temperature in a mixture of 40 ml of pyridine and 20 ml of acetic anhydride for 72 h. Then the reaction mixture was evaporated in vacuum and the residue was chromatographed on a column of silica gel with elution by hexane-ether (7:3). This gave 6.3 g of the diacetate (III). Yield 97%, mp 163-165°C (ether).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1730 (C=O), 1630 (C=C), 1250 (C–O). PMR spectrum (δ, ppm): 0.80 (s, 18-Me), 1.03 (s, 19-Me), 2.04 (s, OAc), 2.05 (s, OAc), 4.60 (2H, m, W/2 = 18.0 Hz, C_3–H_α and C_{17}–H_α), 5.38 (1H, br, d, W/2 = 9.0 Hz, C_6–H).

5-Bromo-5α-androstane-3β,6β,17β-triol 3,17-Diacetate (IV). With stirring in the dark, 1.55 g of N-bromoacetamide was added in portions to a solution of 2.1 g of the diacetate (III) in 150 ml of dioxane containing 13 ml of water and 3.2 ml of 70% perchloric acid solution. After the end of the addition, the mixture was stirred at room temperature for 30 min, and then a solution of 0.6 g of sodium thiosulfate in 150 ml of water was added. After 10 min, an additional 0.6 g of thiosulfate dissolved in 100 ml of water was added. The mixture was extracted with chloroform (4 × 50 ml), the chloroform extracts were dried with magnesium sulfate, and the solvent was evaporated off in vacuum. The residue was dissolved in ether, the solution was filtered through a small layer of silica gel, and the residue was crystallized from ether–hexane. This gave 1.7 g of the bromohydrin (IV). The yield was 64%, mp 169-171°C, lit. mp 159°C [7], 174-175°C [8].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3480 (OH), 1740 (C=O), 1710 (C=O), 1270 (C–O), 1250 (C–O). PMR spectrum (δ, ppm): 0.80 (3H, s, 18-Me), 1.33 (3H, s, 19-Me), 2.04 (6H, s, 17β-OAc and 3β-OAc), 4.19 (1H, m, W/2 = 9.5 Hz C_6–H_α), 4.61 (1H, dd, J = 9.0 Hz, J = 8.0 Hz, C_{17}–H_α) 5.47 (1H, m, W/2 = 18 Hz, C_3–H_α).

5-Bromo-6β-19-epoxy-5α-androstane-3β,17β-diol 3,17-Diacetate (V). A mixture of 1.1 g of the bromohydrin (IV), 1.6 g of lead tetraacetate, and 0.25 g of iodine in 30 ml of benzene was boiled under reflux with stirring and illumination by a 60 W lamp for 50 min. After

cooling to room temperature, the precipitate was filtered off, and was washed on the filter with benzene. The benzene solution was washed with 5% sodium thiosulfate solution (2 × 50 ml) and with water and was dried with magnesium sulfate. After the benzne had been evaporated off, the residue was chromatographed on a column of silica gel with elution by hexane–ether (3:1). This gave 1.0 g of product (V). Yield 91% mp 180-183°C (ether–hexane), lit. mp 136-137°C [8], 178-180°C [4].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1740 (C=O), 1260 (C–O). PMR spectrum (δ, ppm): 0.80 (s, 18-Me), 2.04 (s, 17β-OAc, 3β-OAc), 3.74 (1H, d, J_{AB} = 8.5 Hz, C_{19}–H), 3.94 (1H, d, C_{19}–H), 4.07 (1H, d, J = 4.0 Hz, C_6–H_α), 4.62 (1H, dd, J = 9.0 Hz, J = 7.0 Hz, C_{17}–H_α), 5.19 (1H, m, W/2 = 22.0 Hz, C_3–H_α).

Methanolysis of the Diacetate (V). A mixture of 3.4 g of the diacetate (V), 0.8 g of potassium carbonate, and 150 ml of dry methanol was stirred at room temperature for 2.5 h, and then 1.0 ml of glacial acetic acid was added and it was evaporated in vacuum. The residue was chromatographed on a column of silica gel with eluation by hexane–ethyl acetate (1:1). This gave 2.4 g of 5-bromo-6β,19-epoxy-5α-androstane-3β,17β-diol 17-acetate (VI). Yield 77%, mp 220-221°C (ether–ethyl acetate).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3460 (OH), 1720 (C=O), 1250 (C–O). PMR spectrum (δ, ppm): 0.83 (s, 18-Me), 2.04 (s, 17β-OAc), 3.72 (1H, d, J_{AB} = 8.5 Hz, C^{19}–H), 3.93 (1H, d, C_{19}–H), 4.08 (1H, d, J = 4.0 Hz, C_6–H_α), 4.13 (1H, m, W/2 = 24 Hz, C_3–H_α), 4.63 (1H, dd, J = 9.0 Hz, J = 7.0 Hz, C_{17}–H_α).

Further eluation with hexane–ethyl acetate (1:2) gave 0.6 g of 5-bromo-6β,19-epoxy-5α-androstane-3β,17β-diol (VII). Yield 21%, mp 234-237°C (ethyl acetate), lit.: 184-186°C [8], 233-234°C [9].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3400 (OH). PMR spectrum (δ, ppm): 0.76 (s, 18-Me), 3.63 (1H, t, J = 8.0 Hz, C_{17}–H_α), 3.74 (1H, d, J_{AB} = 8.5 Hz, C_{19}–H), 3.94 (1H, m, C_{19}–H), 4.07 (2H, m, W/2 = 8.0 Hz, C_6–H_α and C_3–H_α).

Acetylation of the Diol (VII). A solution of 0.40 g of the diol (VII) in a mixture of 10 ml of pyridine and 10 ml of acetic anhydride was left at room temperature for 72 h. Then it was evaporated in vacuum, the residue was dissolved in ether, and the solution was filtered through a small layer of silica gel and was evaporated. The residue was crystallized from a mixture of ether and hexane, giving 0.45 g of the diacetate (V), yield 92%, mp 180-182°C. The substance obtained gave no depression of the melting point in a mixture with an authentic sample.

17β-Acetoxy-6β,19-epoxyandrost-4-en-3-one (VIII). A solution of 0.8 g of the alcohol (VI) in 50 ml of acetone was treated with 1.5 ml of an 8 N solution of chromic acid. After 15 min, the excess of oxidant was eliminated by the addition of 5 ml of isopropanol, and the reaction mixture was evaporated in vacuum. The residue was treated with 50 ml of water and was extracted with chloroform (4 × 20 ml). The chloroform extracts were dried with magnesium sulfate and evaporated in vacuum. The residue was boiled under reflux with 25 ml of dimethylformamide in the presence of 0.8 g of lithium carbonate and 0.3 g of lithium bromide for 30 min. After cooling, the residue was filtered off, and the filtrate was diluted with 100 ml of water and was extracted with ether (4 × 50 ml). The ethereal extracts were dried with magnesium sulfate, and evaporated in vacuum, and the residue was chromatographed on a column of silica gel with elution by hexane–ether (2:3). This gave 0.58 g of the enone (VIII), yield 91%, mp 156-159°C (ether–hexane); lit.: mp 149-152°C [3], 157-159°C [4].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1740 (C=O), 1680 (C=O), 1640 (C=C), 1250 (C–O). UV spectrum (λ_{max}^{EtOH} (ε), nm): 239 (14400). PMR spectrum (δ, ppm): 0.89 (s, 18-Me), 2.05 (s, 17β-OAc), 3.52 (1H, d, J_{AB} = 8.5 Hz, C_{19}–H), 4.22 (1H, d, C_{19}–H), 4.62 (1H, dd, J = 9.0 Hz, J = 7.0 Hz, C_{17}–H_α), 4.71 (1H, d, J = 5.0 Hz, C_6–H_α), 5.82 (1H, s, C_4–H).

19-Hydroxytestosterone Diacetate (IX). A solution of 1.6 g of the epoxyenone (VIII) in 70 ml of glacial acetic acid was boiled under reflux for 1.5 h with stirring in the presence of 2.0 g of zinc dust. Then another 1.0 g of zinc dust was added to the mixture and boiling was continued for 1 h. After cooling, the solid matter was filtered off and was washed on the filter with ethyl acetate. The filtrate was evaporated in vacuum and the residue was dissolved in a mixture of 30 ml of pyridine and 30 ml of acetic anhydride. After 20 h, the reaction 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 1.03 g of the diacetate (IX). Yield 57%, mp 122-124°C (ether–pentane); lit.: mp 128.5-130°C [2].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1740 (C=O), 1680 (C=O), 1640 (C=C), 1250 (C–O). UV spectrum (λ_{max}^{EtOH} (ε), nm): 240 (16300). PMR spectrum (δ, ppm): 0.83 (s, 18-Me), 2.01 (s, OAc), 2.05 (s, OAc), 4.16 (1H, d, J_{AB} = 11.5 Hz, C_{19}–H), 4.59 (1H, dd, J = 9.0 Hz, J = 7.5 Hz, C_{17}–H_α), 4.66 (1H, d, C_{19}–H), 5.92 (1H, c, C_4–H).

19-Hydroxytestosterone (I). A solution of 0.19 g of the diacetate (IX) in 15 ml of methanol was treated with 0.20 g of potassium carbonate. The mixture was kept at room temperature for 25 h, and then the excess of potassium carbonate was neutralized by the addition of 0.5 ml of acetic acid. The solvent was evaporated off in vacuum and the residue was chromatographed on a column of silica gel with elution by hexane–ethyl acetate (1:1). This gave 0.12 g of 19-hydroxytestosterone. Yield 84%, mp 204-205.5°C (methanol–ether); lit.: mp 201-205°C [2], 197°C [3].

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3310 (OH), 3370 (OH), 1650 (C=O), 1620 (C=C). PMR spectrum (δ, ppm): 0.79 (s, 18-Me), 3.65 (1H, m, W/2 = 20.5 Hz, C_{17}–H_α), 3.92 (1H, dd, J = 11 Hz, J = 6.5 Hz, C_{19}–H), 4.06 (1H. br.d, C_{19}–H), 5.96 (1H, s, C_4–H).

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