SYNTHESIS OF 19-HYDROXYSTEROIDS. III. AN INVESTIGATION OF APPROACHES TO THE SYNTHESIS OF 19-HYDROXYCORTISOL FROM CORTISOL AND CORTISONE

N. V. Kovzanko, Zh. N. Kashkan, and Yu. G. Chernov

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Methods have been developed for the chemical transformation of cortisol and cortisone in derivatives of 19-hydroxycortisol.

Various methods have been proposed for the determination in biological media of the most important hormone of the adrenal cortex, cortisol (I), of which the most widely used in recent years has been radioimmunological analysis [1]. Progress in the creation of a more effective method for the radioimmunological determination of cortisol as compared with the existing procedure is being largely determined by the use for the preparation of antisera of new steroid—protein complexes in which the protein is attached through a bridge molecule to a carbon atom of the steroid having no primary functional group. In view of this, undoubted interest is presented by the preparation of steroid—protein complexes in which cortisol is bound covalently with the protein through the C-19, which presupposes in the first place the synthesis of 19-functional derivatives of it — for example, 19-hydroxycortisol (II).

The present investigation was devoted to a search for routes to the synthesis of 19-hydroxycortisol from the initial commercially available substances cortisol (I) and cortisone (III). In the solution of this problem we used experience that we have accumulated previously in the development of new methods for the synthesis of 19-hydroxytestosterone [2] and 19-hydroxyprogesterone [3]. It must be mentioned that to functionalize the C-19 atom in corticoids it is possible to use a method the key stage of which is the photolysis of the corresponding 11β-nitrites [14]. However, the formation in this procedure of a mixture of 18- and 19-functional derivatives with low yields makes it unsuitable in practice for obtaining the final product in large amounts. We therefore planned to introduce 19-oxygen-containing functions into the molecules of the initial substances (I) and (III) with the aid of the cyclization of the corresponding 5α-bromo-6β-hydroxyderivatives under the action of lead tetraacetate and iodine with the formation of 6β,19-epoxides, an expedient which is widely used in steroid chemistry. We have previously considered in detail the corresponding methods of synthesis in the androstane and pregnane series.

The side-chain of the molecule of each of the initial steroids (I) and (III) contains a labile 17α,21-dihydroxy-20-keto grouping. In the first stage of the synthesis, therefore, cortisol and cortisone were transformed by reaction with paraformaldehyde in an acid medium [5] into the corresponding 17α,20:20,21-bismethylenedioxy derivatives (IV) and (V) with yields of 86 and 82%. In accordance with the selected scheme of synthesis, it was then necessary to convert the Δ^4-3-ketones (IV) and (V) into 3β-hydroxy-Δ^5-steroids. We planned to perform this procedure as the result of the initial formation from the Δ^4-3-ketones of the corresponding enol acetates and their subsequent reduction with sodium tetrahydroborate. Since in this procedure the formation of 3β-hydroxy-Δ^4-steroids is also possible, the determination of the structures of the reaction products from their spectra is a fairly complex problem. In order to simplify it, we first performed the reduction of the Δ^4-3-ketone (IV) with sodium tetrahydroborate, which took place with the formation of the 3-hydroxy-Δ^4-steroids. After acetylation of the reaction products with acetic anhydride in pyridine in the presence of N,N-dimethylaminopyridine, the 3,11-diacetate (VIa) and the 3-monoacetate (VIb) were obtained with yields of 22 and 77%. In the PMR spectra of steroids (VIa) and (VIb) signals in the form of broadened singlets with δ 5.27 and 5.19 ppm, respectively, corresponded to the C_4—H vinyl proton. Since, judging from the PMR spectra, in steroids (VIa) and (VIb) the C_3—H methine proton had the quasi-axial orientation, the 3-acetoxy group had to be assigned the quasi-equatorial, i.e., β-, orientation.

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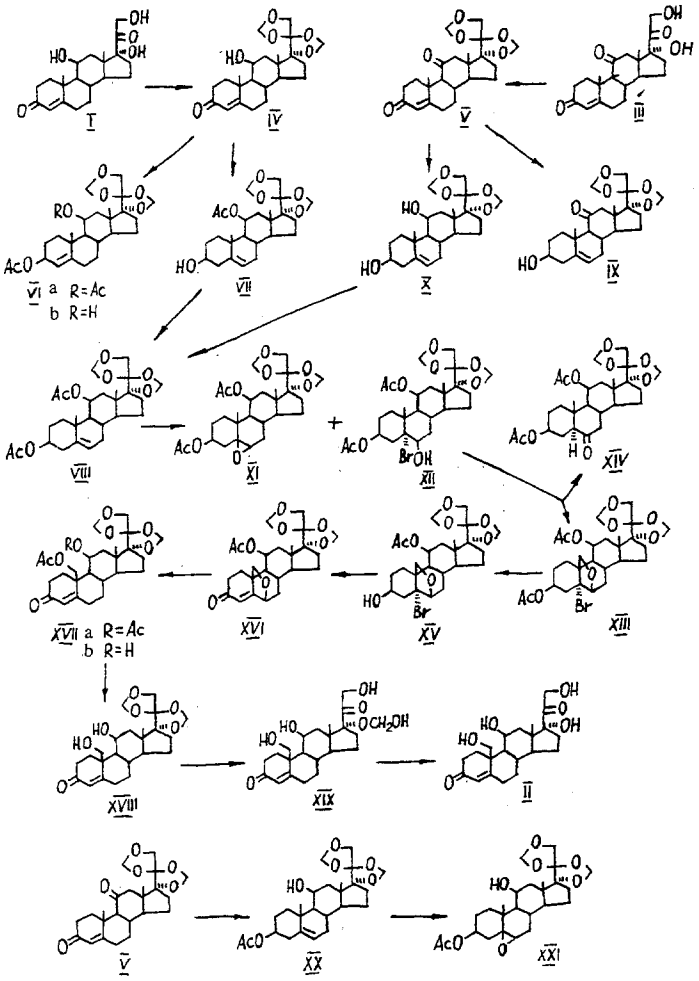

On the interaction of the Δ^4-3-ketone (IV) with acetic anhydride, tert-butyldimethylchlorosilane, and sodium iodide, according to [6], the corresponding enol acetate was obtained, and without additional purification this was subjected to reduction with sodium tetrahydroborate ethanol, with the formation in 84% yield of the 11-monoacetate (VII) of the 3β,11β-dihydroxy-Δ^5-steroid (VII). The further acetylation of compound (VII) formed the 3,11-diacetate (VIII). The structures of compounds (VII) and (VIII) were shown by comparing their PMR spectra with the spectra of the Δ^4-steroids (VIa) and (VIb). In particular, the presence in the PMR spectrum of each of steroids (VII) and (VIII) of the signal of the C_6—H vinyl proton in the form of a doublet of doublets at 5.51 ppm is important for establishing the position of the double bond. Attention is attracted by the fact that in the process of obtaining the enol acetate under the action of acetic anhydride, tert-butyldimethylchlorosilane and sodium iodide, the ready acetylation of the sterically hindered 11β-hydroxy group also takes place. We made use of this observation subsequently.

Steroid (VIII) is the key compound in the synthesis of 9-hydroxycortisol. For this reason, we undertook an alternative synthesis of it from cortisone (III). As a result of the action on the Δ^4-3,11-diketone (V) of acetic anhydride, trimethylchlorosilane, and sodium iodide and reduction of the resulting enol formed with sodium tetrahydroborate in ethanol at room temperature for 3.5 h, the 3β-hydroxy-Δ^5-11-ketosteroid (IX) was obtained with a yield of 67%. A second substance isolated in this reaction with a yield of 18% was the 3,11-diol (X). It was possible to raise the yield of compound (X) to 68% by the use in the reduction stage of additional amounts of sodium tetrahydroborate and by boiling the reaction mixture. The acetylation of the diol (X) with acetic anhydride in the presence of trimethylchlorosilane and sodium iodide gave a 71% yield of the 3,11-diacetate (VIII), identical with that synthesized previously from cortisol.

The addition of the elements of hypobromous acid to the 5,6-double bond of compound (VIII) did not take place as smoothly as in the case of other steroids. Although in this case the reaction product, isolated with a yield of 54%, was the required bromohydrin (XII), nevertheless formation of fairly large amounts of the product of its further transformation having the structure of the 5β,6β-epoxide (X) was observed.

When the bromohydrin (XII) was cyclized under the action of lead tetraacetate and iodine in benzene, a mixture of at least four substances was formed. By means of column chromatography on silica gel we succeeded in isolating the desired 5-bromo-6β,19-epoxide (XIII) from this mixture with a yield of 13%. Other reliably identified products of this reaction were the 5β,6β-epoxide (XI) and the 6-ketone (XIV). The formation of the 6-ketosteroid (XIV) under the given conditions can probably be explained by a rearrangement of the 5,6-epoxide (XI).

The subsequent transformation of the 3β,11β-diacetoxy-5α-bromosteroid (XIII) included the selective hydrolysis of the spatially more accessible 3β-acetoxy group under the action of potassium carbonate in methanol at room temperature, with the formation of the 11β-acetoxy-3β-hydroxy derivative (XV) with a yield of 85%. The Δ^4-3-ketone (XVI) was obtained with an overall yield of 92% by the oxidation of the free hydroxy group in compound (XV) with chromic acid in acetone by Jones' method and dehydrobromination of the resulting unstable 5-bromo-3-ketone with lithium carbonate and lithium bromide in dimethylformamide. The reduction of the 6β,19-oxide ring in compound (XVI) with zinc dust in isopropanol was accompanied by the partial hydrolysis of the 11β-acetoxy group. After acetylation of the mixture of reaction products with acetic anhydride, the 11β,19-diacetate (XVIIa) and the 19-acetate (XVIIb) were obtained in approximately equal amounts. The hydrolysis of both steroids with potassium carbonate in methanol at room temperature formed high yields of the 11β,19-diol (XVIII). Such ready hydrolysis of the spatially hindered 11-acetoxy group in undoubtedly explained by the presence of the 19-hydroxy group.

An attempt to remove the protective grouping in the side-chain of steroid (XVIII) under the action of a mixture of trifluoroacetic acid and hydrochloric acids [5] led to the formation of the 17-hemiacetal (XIX) with a yield of 73%. We showed the structure of (XIX) as the result of an analysis of IR, PMR, and mass spectra. In particular, according to the IR spectrum, steroid (XIX) contained a Δ^4-3-keto grouping and a 20-keto group to which vibration bands at 1650, 1620, and 1700 cm^{-1} corresponded. The presence in compound (XIX) of a Δ^4-3-keto grouping was confirmed by the presence in its PMR spectrum of the signal of the C_4—H vinyl proton (δ 5.84 ppm). The spectrum also contained the signals of the C_{11}—H methine proton (δ 4.16 ppm) and of the protons of a 19-methylene group (δ 3.55 and 3.97 ppm), which reliably showed the presence of 11β- and 19-hydroxy groups in steroid (XIX). It was possible to decide that compound (XIX) contained a free 21-hydroxy group from the form and magnitudes of the chemical shifts of the signals of the protons of the 21-methylene group (δ 4.28 and 4.68 ppm), which agreed well with the positions of the analogous signals in the PMR spectrum of cortisol. The signals of these protons appeared in the spectrum in the form of well-resolved doublets of doublets with splitting constants 20.0 and 4.0 Hz. The large constant here is a geminal constant, and the additional splitting is due to interaction with the hydroxylic proton. This interaction disappeared after deuteroexchange. In addition to the signals mentioned, the PMR spectrum of the compound under discussion contained two doublets (δ 4.67 and 4.73 ppm) corresponding to the protons of an isolated methylene group to which at least one oxygen atom was attached. As a result of the consideration of several variants of the structure, we came to the conclusion that these signals corresponded to the protons of a 17-(hydroxymethoxy) group. This conclusion was confirmed by the mass spectrum. It was possible to obtain the desired 19-hydroxycortisol (II) by the subsequent hydrolysis of steroid (XIX).

We also investigated the possibility of obtaining 19-hydroxycortisol from cortisone without the preliminary protection of the 11-hydroxy group. With this aim, the 3-monoacetate (XX) was synthesized with an overall yield of 73% by the conversion of the 3-keto group of the bismethylenedioxy derivative of cortisone (V) into an enol acetate group and reduction with sodium tetrahydroborate, followed by selective acetylation of the more accessible 3-hydroxy group. However, attempts to obtain the corresponding 5β-bromo-6β-hydroxy derivative from the Δ^5-steroid (XX) by the addition of the elements of hypobromous acid were unsuccessful. In this process it was not the bromohydrin but the product of its dehydrobromination, the 5α,6β-epoxide (XXI) that was formed.

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 M-400 spectrophotometer. CD spectra were obtained on a Jasco J-20 spectropolarimeter. PMR spectra were recorded 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. The solvent, unless specially stated otherwise, was deuterochloroform. Mass-spectra were obtained on a Varian MAT 311 instrument with an energy of the ionizing radiation of 70 eV.

Reduction of 11β-Hydroxy-17α,20:20,21-bismethylenedioxypregn-4-en-3-one (IV). A solution of 0.60 g of the unsaturated ketone (IV) (obtained with a yield of 86% from cortisol (I) by the procedure given in [3], mp 228-229°C

(ether—hexane); literature [7]: mp 217-222°C) in 120 ml of ethanol was treated with 0.55 g of sodium tetrahydroborate and the mixture was stirred at room temperature for 2 h. Then 12 ml of 2 N hydrochloric acid was added to it and stirring was continued for another 40 min. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with saturated sodium bicarbonate solution and with water and was evaporated in vacuum. The residue was dissolved in 10 ml of pyridine, and then 0.033 g of N,N-dimethylaminopyridine and 15 ml of acetic anhydride were added. After 66 h, the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with 16% hydrochloric acid and with water and was dried with anhydrous magnesium sulfate. The solvent was driven off in vacuum. The residue was chromatographed on a column of silica gel with the aid of elution by hexane—ethyl acetate (10:1). This gave 0.134 g of 17α,20:20,21-bismethylenedioxypregn-4-ene-3β,11β-diol diacetate (VIa). Yield 22%, amorphous.

PMR spectrum (δ, ppm): 1.06 (3H, s, 18-Me), 1.14 (3H, s, 19-Me), 2.02 (6H, s, AcO), 3.96 (2H, br.s, C_{21}—H_2), 4.98 (2H, d, J 1.5 Hz), 5.01 (1H, s), 5.18 (1H, s) (O—CH_2—O)$_2$, 5.27 (1H, br.s, C_4—H), 5.40 (2H, m, C_3—H_α and C_{11}—H_α).

Further elution gave 0.515 g of 17α,20:20,21-bismethylenedioxypregn-4-ene-3β,11β-diol 3-acetate (VIb). Yield 77%, amorphous.

IR spectrum (ν_{max}^{film}, cm^{-1}): 3540 (OH), 1730, 1245 (AcO), 1665 (C=C). PMR spectrum (δ, ppm): 1.10 (3H, s, 18-Me), 1.34 (3H, s, 19-Me), 2.06 (3H, s, AcO), 4.00 (2H, d, J 1.5 Hz, C_{21}—H_2), 4.38 (1H, m, W/2 8 Hz, C_{11}—H_α), 5.04 (2H, d), 5.06 (1H, s), 5.24 (1H, s) (O—CH_2—O)$_2$, 5.19 (1H, br.s, C_4—H), 5.20 (1H, m, C_3—H_α); (Py-d_5): 1.45 (3H, s, 18-Me), 1.61 (3H, s, 19-Me), 2.08 (3H, s, AcO), 4.16 (2H, dd, J_1 9.0 Hz, J_2 16.5 Hz, C_{21}—H_2), 4.59 (1H, m, W/2 8.5 Hz, C_{11}—H_α), 5.50 (1H, m, W/2 18.0 Hz, C_3—H_α).

17α,20:20,21-Bismethylenedioxy-5-ene-3β,11β-diol 11-Acetate (VII). A suspension of 0.150 g of steroid (IV) and 2 ml of acetic anhydride cooled in an ice bath was treated with 0.228 g of tert-butyldimethylchlorosilane in 5 ml of acetic anhydride. After this, with cooling and stirring by a magnetic stirrer, 0.287 g of sodium iodide was added. Stirring was continued in the dark for 1 h, and then the reaction mixture was poured with stirring into a mixture of 50 g of ice, 100 mg of saturated sodium bicarbonate solution, and 50 ml of chloroform. Then 20 ml of 10% sodium thiosulfate solution was added to this mixture and it was stirred for 10 min. The chloroform layer was separated off and the aqueous layer was extracted with chloroform. The combined chloroform extract was washed with sodium bicarbonate solution and with water, the bulk of the solvent was evaporated off in vacuum, and, after the addition of 25 ml of methanol, the residue was evaporated in vacuum to dryness.

The final residue was dissolved in 40 ml of ethanol, and this solution was treated with 0.250 g of sodium tetrahydroborate and the mixture was stirred with a magnetic stirrer at room temperature for 3 h 15 min. Then 5 ml of 2 N hydrochloric acid was added to the reaction mixture and stirring was continued for 30 min, after which it was diluted with water and extracted with chloroform. The chloroform extract was washed with saturated sodium bicarbonate solution and was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane—ethyl acetate (2:1). This gave 0.140 g of the amorphous acetate (VII). Yield 84%.

PMR spectrum (δ, ppm): 0.97 (3H, s, 18-Me), 1.08 (3H, s, 19-Me), 2.03 (3H, s, AcO), 3.52 (1H, m, W/2 19.0 Hz, C_3—H_α), 3.98 (2H, s, C_{21}—H_2), 5.00 (2H, d, J 3.0 Hz), 5.02 (1H, d, J 1.0 Hz), 5.18 (1H, s) (O—CH_2—O)$_2$, 5.27 (1H, m, W/2 10.0 Hz, C_{11}—H_α), 5.51 (1H, dd, J_1 8.1 Hz, J_2 3.0 Hz, C_6—H).

3β-Hydroxy-17α,20:20,21-bismethylenedioxypregn-5-en-11-one (IX). A suspension of 0.49 g of the bismethylenedioxy derivative of cortisone (V) (obtained with a yield of 82% from cortisone (III) by the procedure of [3], mp 235-237°C (methanol); lit. mp 258-261°C [7], 249-253°C [8]) in 10 ml of acetic anhydride cooled in an ice bath was treated with 0.7 ml of trimethylchlorosilane, 5 ml of acetic anhydride, and 1.00 g of sodium iodide. The mixture was stirred in the dark with cooling for 2 h and was then poured with stirring into a mixture of 60 g of ice, 30 ml of saturated sodium bicarbonate solution, and 50 ml of chloroform. The resulting emulsion was treated with 20 ml of a 10% solution of sodium thiosulfate, the mixture was stirred for 15 min, the chloroform layer was separated off, and the aqueous layer was extracted with chloroform. The combined chloroform extracts were washed with saturated sodium bicarbonate solution and with water and were dried with magnesium sulfate, and the solvent was driven off in vacuum. The residue was dried in vacuum and was then dissolved in 100 ml of ethanol, and 1.00 g of sodium tetrahydroborate was added.

The mixture was stirred at room temperature for 3.5 h and the excess of sodium tetrahydroborate was eliminated by the addition of 20 ml of 2 N hydrochloric acid with stirring for 20 min, and then the bulk of the solvent was evaporated off in vacuum and the residue was dissolved in 100 ml of water and 50 ml of chloroform. The chloroform layer was separated off and the aqueous layer was extracted with chloroform. The combined chloroform extracts were dried with magnesium

sulfate, the solvent was evaporated off in vacuum, and the residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (1:3). This gave 0.33 g of the hydroxyketone (IX). Yield 67%, mp 155-157°C (ethyl acetate—hexane). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3450 (OH), 1705 (C=O), 1635 (C=C). PMR spectrum (δ, ppm): 0.81 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 2.62 (1H, d, J_{AB} 14.4 Hz, C_{12}—H_β), 2.81 (1H, d, C_{12}—H_α), 3.51 (1H, m, W/2 19 Hz, C_3—H_α), 3.96 (2H, center of an AB system, J_{AB} 9.6 Hz, C_{21}—H_2), 5.01 (1H, s), 5.06 (1H, s), 5.07 (1H, s), 5.20 (1H, s) (O—CH_2—O)$_2$, 5.35 (1H, br.d, J 5.5 Hz, C_5—H).

Further elution with the same solvent system gave 0.09 g of the 3,11-diol (X), identical with an authentic sample. Yield 18%.

17α,20:20,21-Bismethylenedioxypregn-5-ene-3β,11β-diol (X). A suspension of 0.51 g of the 3,11-diketone (V) in 5 ml of acetic anhydride coooled in an ice bath was treated with 0.7 ml of trimethylchlorosilane, 2 ml of acetic anhydride, and 1.00 g of sodium iodide. The mixture was stirred in the dark with cooling for 2 h. The subsequent working up procedure was similar to that described in the preparation of (IX). This gave 0.03 g of the hydroxyketone (IX). Yield 6%.

Further elution led to the isolation of 0.35 g of the diol (X). Yield 68%, mp 237-239°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3510 (OH), 1630 (C=C). PMR spectrum (δ, ppm): 1.10 (3H, s, 18-Me), 1.29 (3H, s, 19-Me), 3.53 (1H, m, W/2 24.0 Hz, C_3—H_α), 4.00 (2H, center of an AB system, J_{AB} 9.0 Hz, C_{21}—H_2), 4.46 (1H, m, W/2 8.0 Hz, C_{11}—H_α), 5.03 (1H, s), 5.05 (1H, s), 5.06 (1H, s), 5.23 (1H, s) (O—CH_2—O)$_2$, 5.26 (1H, br.d, J 3.0 Hz, C_6—H).

17α,20:20,21-Bismethylenedioxypregn-5-ene-3β,11β-diol Diacetate (VIII). A. A suspension of 0.42 g of the diol (X) in 5 ml of acetic anhydride cooled in an ice bath was treated with 1.4 ml of trimethylchlorosilane, 2.00 g of sodium iodide, and 2 ml of acetic anhydride. The mixture was stirred in the dark with cooling with 2.5 h. The subsequent procedure was similar to that described in the preparation of (IX).

The residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (1:7). This gave 0.36 g of the diacetate (VIII). Yield 71%, mp 183-185°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1735, 1250 (AcO). PMR spectrum (δ, ppm): 0.98 (3H, s, 18-Me), 1.08 (3H, s, 19-Me), 2.03 (3H, s, AcO), 2.05 (3H, s, AcO), 3.98 (2H, center of an AB system, J_{AB} 9.0 Hz, C_{21}—H_2), 4.59 (1H, m, W/2 28.0 Hz, C_3—H_α), 5.00 (1H, s), 5.02 (1H, d, J 1.0 Hz), 5.03 (1H, d, J 1.0 Hz), 5.18 (1H, s) (O—CH_2—O)$_2$, 5.29 (1H, m, W/2 8.5 Hz, C_{11}—H_α), 5.51 (1H, dd, J_1 7.0 Hz, J_2 3.0 Hz, C_6—H).

B. A solution of 0.104 g of the alcohol (VII) in 15 ml of pyridine was treated with 1 ml of acetic anhydride. The reaction mixture was stirred at room temperature for 18 h and was then poured into water. After extraction with chloroform, the chloroform extract was washed with a solution of 15 ml of hydrochloric acid in 50 ml of water and then with saturated sodium carbonate solution. The solvent was driven off in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane—ethyl acetate (2:1). This gave 0.116 g of the acetate (VIII). The yield was quantitative. The sample had a PMR spectrum completely identical with that of the substance obtained by the acetylation of the diol (X).

5-Bromo-17α,20:20,21-bismethylenedioxy-5α-pregnane-3β,6β,11β-triol 3β,11β-diacetate (XII). With stirring in the dark, 0.20 g of N-bromoacetamide was added to a solution of 0.29 g of the diacetate (XIII) in 17 ml of dioxane containing 1.5 ml of water and 0.35 g of 70% perchloric acid. The mixture was stirred at room temperature for 1 h, and then 20 ml of 10% sodium sulfite solution was added and stirring was continued for 30 min. After extraction with chloroform, the extracts were dried with magnesium sulfate, the solvent was driven off in vacuum, and the residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (1:4). This gave 0.08 g of the amorphous 5,6β-epoxy-17α,20:20,21-bismethylenedioxy-5β-pregnane-3β,11β-diol diacetate (XI). Yield 27%.

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1735, 1250 (AcO). PMR spectrum (δ, ppm): 0.94 (3H, s, 18-Me), 1.04 (3H, s, 19-Me), 3.13 (1H, d, J 3.0 Hz, C_6—H_α), 3.97 (2H, center of an AB system, J_{AB} 9.5 Hz, C_{21}—H_2), 4.76 (1H, m, W/2 28.0 Hz, C_3—H_α), 4.98 (1H, s), 5.00 (1H, d, J 1.0 Hz), 5.02 (1H, d, J 1.0 Hz), 5.17 (1H, s) (O—CH_2—O)$_2$, 5.24 (1H, m, W/2 6.0 Hz, C_{11}—H_α).

Further elution gave 0.19 g of the bromohydrin (XII). Yield 54%, mp 155-158°C (ether).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3440 (OH), 1740, 1710, 1270-1240 (AcO). PMR spectrum (δ, ppm): 0.97 (3H, s, 18-Me), 1.36 (3H, s, 19-Me), 2.01 (3H, s, AcO), 2.03 (3H, s, AcO), 3.98 (2H, center of an AB system, J_{AB} 9.5 Hz, C_{21}—H_2), 4.18 (1H, m, W/2 7.0 Hz, C_6—H_α), 4.99 (1H, s), 5.02 (1H, s), 5.04 (1H, s), 5.18 (1H, s) (O—CH_2—O)$_2$, 5.36 (1H, m, C_{11}—H_α), 5.44 (1H, m, C_3—H_α).

5-Bromo-6β,19-epoxy-17α,20:20,21-bismethylenedioxy-5α-pregnane-3β,11β-diol Diacetate (XIII). A mixture of 1.16 g of the bromohydrin (XII), 1.40 g of lead tetraacetate, 0.21 g of iodine, and 25 ml of benzene was boiled under reflux with stirring and illumination with 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 filtrate was washed with 10% sodium thiosulfate solution and with water and was dried with magnesium sulfate. The solvent was eliminated in vacuum, and the residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (1:4). This gave 0.25 of a mixture of the epoxide (XI) with another compound. This mixture was not investigated. Further elution led to 0.44 g of the pure desired product (XIII). Yield 38%, mp 170-173°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1740, 1250 (AcO). PMR spectrum (δ, ppm): 0.97 (3H, s, 18-Me), 2.03 (6H, s, AcO), 3.93 (1H, d, J_{AB} 8.5 Hz, C_{19}—H), 3.97 (2H, s, C_{21}—H_2), 4.08 (1H, d, J 4.5 Hz, C_6—H_α), 4.23 (1H, d, C_{19}—H), 4.98 (1H, s), 5.02 (1H, d, J 1.0 Hz), 5.04 (1H, d, J 1.0 Hz), 5.19 (1H, s) (O—CH_2—O)$_2$, 5.13 (1H, m, C_3—H_α), 5.20 (1H, m, C_{11}—H_α).

Further elution led to the isolation of 3β,11β-diacetoxy-17α,20:20,21-bismethylenedioxy-5α-pregnan-6-one (XIV). Yield 19%, mp 184-186°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1710 (C=O), 1735, 1250 (AcO). PMR spectrum (δ, ppm): 0.87 (3H, s, 18-Me), 0.97 (3H, s, 19-Me), 2.04 (6H, s, AcO), 3.99 (2H, s, C_{21}—H_2), 4.66 (1H, m, W/2 23.0 Hz, C_3—H_α), 5.00 (1H, s), 5.04 (1H, s), 5.05 (1H, s), 5.19 (1H, s) (O—CH_2—O)$_2$, 5.51 (1H, m, W/2 5.0 Hz, C_{11}—H_α).

5-Bromo-6β,19-epoxy-17α,20:20,21-bismethylenedioxy-5α-pregnane-3β,11β-diol 11β-Acetate (XV). A solution of 0.42 g of the diacetate (XIII) in 50 ml of dry ethanol containing 0.10 g of potassium carbonate was stirred at room temperature for 4 h, the excess of potassium carbonate was neutralized by the addition of 0.1 ml of glacial acetic acid, and the solution was evaporated in vacuum to dryness. The residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (1:1). This gave 0.33 of steroid (XV). Yield 85%, mp 198-200°C (ether—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3460 (OH), 1740, 1240 (AcO). PMR spectrum (δ, ppm): 0.97 (3H, s, 18-Me), 2.04 (3H, s, AcO), 3.93 (1H, d, J_{AB} 8.5 Hz, C_{19}—H), 3.97 (2H, center of an AB system, J_{AB} 9.5 Hz, C_{21}—H_2), 4.09 (1H, d, J 4.0 Hz, C_6—H_α), 4.16 (1H, m, C_2—H_α), 4.22 (1H, d, C_{19}—H), 4.99 (1H, s), 5.03 (1H, s), 5.04 (1H, d, J 1.0 Hz), 5.19 (1H, s), (O—CH_2—O)$_2$, 5.20 (1H, m, C_{11}—H_α).

11β-Acetoxy-6β,19-epoxy-17α,20:20,21-bismethylenedioxypregn-4-en-3-one (XVI). A solution of 0.30 g of the alcohol (XV) in 15 ml of acetone was treated with 0.45 ml of 8 N chromic acid. The mixture was stirred at room temperature for 15 min, and then the excess of oxidant was eliminated by the addition of 1.5 ml of isopropanol, and the solvent was evaporated off in vacuum. The residue was treated with 20 ml of water and 20 ml of chloroform, the chloroform layer was separated off, and the aqueous layer was extracted with chloroform. The combined chloroform extracts were dried with magnesium sulfate and were then evaporated in vacuum. The residue was dissolved in 10 ml of dimethylformamide, and the solution was treated with 0.24 g of lithium carbonate and 0.11 g of lithium bromide. The mixture was boiled under reflux for 30 min. After cooling, the solid matter was filtered off, and the filtrate was diluted with 30 ml of water and was extracted with ether. The ethereal extract was dried with magnesium sulfate, and then the solvent was driven off in vacuum. The residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (2:3). This gave 0.23 g of the enone (XVI). Yield 92%, mp 252-254°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1735, 1250 (AcO), 1680 (C=O), 1640 (C=C). UV spectrum [λ_{max}^{EtOH} (ε), nm]: 238 (12000). PMR spectrum (δ, ppm): 1.02 (3H, s, 18-Me), 2.08 (3H, s, AcO), 3.50 (1H, d, J_{AB} 8.5 Hz, C_{19}—H), 3.98 (2H, center of an AB system, J_{AB} 9.0 Hz, C_{21}—H_2), 4.62 (1H, d, C_{19}—H), 4.71 (1H, d, J 5.0 Hz, C_6—H_α), 4.98 (1H, s), 5.01 (1H, s), 5.02 (1H, s), 5.20 (1H, s) (O—CH_2—O)$_2$, 5.35 (1H, m, W/2 8.0 Hz, C_{11}—H_α), 5.83 (1H, s, C_4—H).

Reduction of the Epoxide (XVI). A solution of 0.21 g of steroid (XVI) in 10 ml of isopropanol containing 0.05 ml of acetic acid was treated with 1.40 g of zinc dust. The mixture was boiled under reflux with stirring for 45 h. After cooling, the solid matter was filtered off and washed with ethyl acetate, the filtrate was evaporated to dryness, and the residue was dissolved in 50 ml of chloroform. The chloroform solution was washed with 25 ml of 2% hydrochloric acid and then with water and was dried with magnesium sulfate. The solvent was evaporated off in vacuum and the residue was dissolved in a mixture of 10 ml of pyridine and 10 ml of acetic anhydride. The reaction mixture was kept at room temperature for 23 h and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane (1:3). This gave 0.07 g of 11β,19-diacetoxy-17α,20:20,21-bismethylenedioxypregn-4-en-3-one (XVIIa). Yield 30%, mp 209-211°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1735, 1720, 1250 (AcO), 1660 (C=O), 1620 (C=C). UV spectrum [λ_{max}^{EtOH} (ε), nm]: 240 (14300). CD spectrum [λ_{max}^{EtOH} ($\Delta\varepsilon$), nm): 236 (+14). PMR spectrum (δ, ppm): 0.98 (3H, s, 18-Me), 1.99 (3H, s, 19-OAc), 2.12 (3H, s, 11β-OAc), 3.98 (2H, s, C_{21}—H_2), 4.05 (1H, d, J_{AB} 11.5 Hz, C_{19}—H), 4.74 (1H, d, C_{19}—H), 4.99 (1H, s), 5.00 (1H, s), 5.02 (1H, s), 5.19 (1H, s) (O—CH_2—O)$_2$, 5.45 (1H, m, W/2 6.0 Hz, C_{11}—H_α), 5.92 (1H, s, C_4—H).

Further elution led to the isolation of 0.06 g of 11β-hydroxy-19-acetoxy-17α,20:20,21-bismethylenedioxy-pregn-4-en-3-one (XVIIb). Yield 29%, mp 228-231°C (ethyl acetate—hexane).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3500 (OH), 1720, 1240 (AcO), 1670 (C=O), 1630 (C=C). UV spectrum [λ_{max}^{EtOH} (ε), nm]: 244 (13400). CD spectrum [λ_{max}^{EtOH} (Δε), nm]: 222 (+10). PMR spectrum (δ, ppm): 1.11 (3H, s, 18-Me), 1.98 (3H, s, 19-OAc), 3.99 (2H, center of an AB system, J_{AB} 9.5 Hz, C_{21}—H_2), 4.42 (1H, m, W/2 8.0 Hz, C_{11}—H_α), 4.62 (1H, d, J_{AB} 12.0 Hz, C_{19}—H), 4.79 (1H, d, C_{19}—H), 5.04 (2H, s), 5.08 (1H, s), 5.22 (1H, s) (O—CH_2—O)$_2$, 5.89 (1H, s, C_4—H).

11β,19-Dihydroxy-17α,20:20,21-bismethylenedioxypregn-4-en-3-one (XVIII). A. A solution of 0.05 g of the monoacetate (XVIIb) in 15 ml of anhydrous methanol containing 0.05 g of potassium carbonate was stirred at room temperature for 2 h. The excess of potassium carbonate was neutralized by the addition of 0.05 ml of acetic acid, and the solvent was evaporated off in vacuum. The residue was chromatographed on a column of silica gel, and elution by ethyl acetate gave 0.04 g of steroid (XVIII). Yield 89%, mp 265-267°C (ethyl acetate).

UV spectrum [λ_{max}^{EtOH} (ε), nm]: 246 (12500). CD spectrum [λ_{max}^{EtOH} (Δ′ε), nm]: 243 (+8). PMR spectrum (δ, ppm): 1.14 (3H, s, 18-Me), 3.19 (2H, m, 19-OH, 11β—OH), 3.55 (1H, s, J_{AB} 12.0 Hz, C_{19}—H), 4.00 (2H, center of an AB system, J_{AB} 9.0 Hz, C_{21}—H_2), 4.12 (1H, d, C_{19}—H), 4.41 (1H, m, W/2 8.0 Hz, C_{11}—H_α), 5.03 (3H, s), 5.22 (1H, s) (O—CH_2—O)$_2$, 5.76 (1H, s, C_4—H).

B. Using method A and increasing the reaction time to 6 h, 0.05 g of the diol (XVIII), identical with that prepared above, was obtained from a solution of 0.06 g of the diacetate (XVIIa). The yield was quantitative.

11β,19,21-Trihydroxy-17α-(hydroxymethoxy)pregn-4-ene-3,20-dione (XIX). A solution of 0.090 g of the diol (XVIII) in a mixture of trifluoroacetic acid and 8 ml of 2 N hydrochloric acid solution was stirred at room temperature for 4 h and was then diluted with 40 ml of chloroform and 20 ml of water. The chloroform layer was separated off and the aqueous layer was extracted with chloroform (3 × 10 ml). The combined chloroform extracts were washed successively with water, saturated sodium bicarbonate solution, and water again 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 ethyl acetate—hexane (1:1). This gave 0.064 g of steroid (XIX). Yield 73%, mp 235-237°C (ethyl acetate).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3480 (OH), 1700 (C=O), 1650 (C=O), 1620 (C=C). PMR spectrum (δ, ppm): 0.93 (3H, s, 18-Me), 3.55 (1H, d, J_{AB} 12.0 Hz, C_{19}—H), 3.97 (1H, d, C_{19}—H), 4.16 (1H, m, W/2 8.0 Hz, C_{11}—H_α), 4.28 (1H, dd, J_{AB} 20.0 Hz, J_{OH} 4.5 Hz, C_{21}—H), 4.68 (1H, dd, C_{21}—H), 4.67 (1H, d, J_{AB} 4.0 Hz), 4.73 (1H, d) (O—CH_2—O), 5.84 (1H, s, C_4—H). Mass spectrum, *m/z*: 390 ($M^+ - H_2O$), 372 ($M^+ - 2H_2O$), 360 ($M^+ - H_2O$—CH_2O).

17α,20:20,21-Bismethylenedioxypregn-5-ene-3β,11β-diol 3-Acetate (XX). A suspension of 3.70 g of the diketone (V) in 40 ml of acetic anhydride, cooled in an ice bath, was treated with 5.6 ml of trimethylchlorosilane and 7.85 g of sodium iodide. After the mixture had been stirred for 3 h, another 1.4 ml of trimethylchlorosilane and 2.00 g of sodium iodide were added. Stirring was continued for 40 min. Then the reaction mixture was poured into a mixture of 400 g of ice, 150 ml of saturated sodium bicarbonate solution, and 300 ml of chloroform; 150 ml of 20% sodium thiosulfate solution was added, and the mixture was stirred for 0.5 h. The chloroform was separated off, and was washed with water and with saturated sodium bicarbonate solution and was dried with magnesium sulfate. The solvent was evaporated off in vacuum and the residue was dissolved in 150 ml of ethanol, and this solution was evaporated in vacuum. The final residue was dissolved in 400 ml of ethanol and 8.5 g of sodium tetrahydroborate was added, after which the reaction mixture was boiled for 2.5 h. Then it was cooled to room temperature, 200 ml of 2 N hydrochloric acid was added, and the mixture was stirred at room temperature for 25 min, after which it was diluted with water and extracted with chloroform.

The chloroform extract was washed with saturated sodium bicarbonate solution and with water and was evaporated in vacuum. The residue was dissolved in 40 ml of pyridine, and then 10 ml of acetic anhydride was added and the mixture was kept at —12°C for 2.5 days. After this, it was diluted with water and was extracted with chloroform. The chloroform extract was washed with 10% hydrochloric acid and then with saturated sodium bicarbonate solution and was dried with magnesium sulfate. The solvent was evaporated off in vacuum and the residue was chromatographed on a column of silica gel with elution by ethyl acetate—hexane—chloroform (1:1:0.065). This gave 3.0 g of the monoacetate (XX). Yield 72%, mp 187-189°C (ethyl acetate).

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3540 (OH), 1730, 1250 (AcO), 1635 (C=C). PMR spectrum (δ, ppm): 1.11 (3H, s, 18-Me), 1.30 (3H, s, 19-Me), 2.06 (3H, s, AcO), 4.00 (2H, d, J 1.5 Hz, C_{21}—H_2), 4.47 (1H, m, W/2 10.0 Hz, C_{11}—H_α), 4.62 (1H, s, W/2 24.0 Hz, C_3—H_α), 5.04 (1H, s), 5.07 (2H, s), 5.24 (1H, s), (O—CH_2—O)$_2$, 5.28 (1H, m, C_6—H).

5,6β-Epoxy-17β,20:20,21-bismethylenedioxy-5β-pregnane-3β,11β-diol 3-acetate (XXI). At room temperature, with stirring, 14.2 ml of water and 3.3 ml of 70% perchloric acid were added to a solution of 2.9 g of steroid (XX) in 170 ml of freshly distilled dioxane, and then 1.9 g of N-bromoacetamide was added in portions. The reaction mixture was stirred in the dark for 1 h, and then a solution of 2.5 g of sodium sulfite in 30 ml of water was added and stirring was continued for 25 min. 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 dried with anhydrous magnesium sulfate. The solvent was driven off in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane—ethyl acetate—chloroform (5:1:0.3). This gave 1.05 g of the epoxide (XXI). Yield 35%.

PMR spectrum (δ, ppm): 0.77 (3H, s, 18-Me), 1.25 (3H, s, 19-Me), 2.05 (3H, s, AcO), 3.15 (1H, d, J 2.5 Hz, C_6—H_α), 3.96 (3H, m, C_{11}—H_α, C_{21}—H_2), 4.73 (1H, m, W/2 24.0 Hz, C_3—H_α), 5.01 (1H, s), 5.06 (2H, s), 5.20 (1H, s) $(O—CH_2-O)_2$.

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