STEROIDS

LI. THE TRANSFORMATION OF A SERIES OF 20-OXOSTEROIDS BY A CULTURE OF Bacillus megaterium VNIKhFI-1

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As already reported, the strain of Bacillus megaterium VNIKhFI-1 which is known to possess the capacity for performing the 20α-reduction of 16α, 17α-epoxyprogesterone has also been investigated for its capacity for 20β-reduction in the case of cortisone [1] and for 15β-hydroxylation in the case of progesterone [2].

In a study of the influence of substituents on the course of the reduction of 20-oxosteroids to 20α-hydroxy compounds of a number of cultures [3-5] it was found that the microorganisms studied prefer as substrate steroids of the pregnane series having a hydroxy group in the 17α position or a 16α, 17α epoxide ring.

At the same time, for the majority of microorganisms the reduction of a 20-oxo group to a 20β-hydroxy group requires the presence of a dihydroxyacetone side chain [6-8].

The aim of the present work was to study the influence of substituents in the steroid molecule on the course of the reduction of 20-oxosteroids by a washed culture of Bacillus megaterium.

The nature of the transformation of 18 steroids of the pregnane series has been studied. Such steroids as cortisol, epicortisol, prednisolone, 11α-hydroxy- and 11-oxoprogesterone, 16α-hydroxy- and 16α-methylprogesterone, and also 11α-hydroxy-16α-methylprogesterone, 16α,17α-dimethylprogesterone, and 11α-hydroxy-16α,17α-epoxyprogesterone remained unchanged after treatment for both 10 and 20 h. The formation of new transformation products was observed only for eight of the steroids studied. The transformed steroids could be included in two groups in relation to their structure: 1) steriods having a dihydroxyacetone side chain (cortexolone, Δ^1-dehydrocortexolone, and prednisone), and 2) steroids not having such a side chain (17α-hydroxyprogesterone, DOC,* DOCA, epoxy-DOCA, and 11-oxo-16α,17α-epoxyprogesterone). The products of the transformation of the steroids of the first group were investigated for the presence of the dihydroxyacetone side chain (in view of the possibility of the reduction of a 20-oxo group to a 20-hydroxy group). By means of color reaction, the reduction of the 20-oxo group on transformation by washed cells by Bac. megaterium was shown for cortexolone, Δ^1-dehydrocortexolone, and prednisone. By comparing the R_f values of the reduction production products with those of authentic 20-hydroxy derivatives of the same steroids [in the benzene–isopropanol (7 : 4) and chloroform–acetone (7 : 5) systems], the 20-hydroxy derivatives formed were shown to be 20β-hydroxy compounds.

The transformation of the steroids of the second group took place ambiguously: in addition to an intense new spot on the chromatogram, weak auxiliary spots were found. The main product formed in the transformation of 17α-hydroxyprogesterone was shown not to contain a reduced 20-oxo group by comparing its R_f values with those of authentic samples of 20-dihydro derivatives by chromatography in the benzene–acetone (4 : 1) and chloroform–acetone (7 : 1) systems.

A consideration of the IR, NMR, and mass spectra of the main products of the transformation of DOCA makes it possible to state the following changes took place in the structure of DOCA on its transformation by

*Abbreviations are used for a number of steroids: deoxycorticosterone – DOC; deoxycorticosterone acetate – DOCA; epoxydeoxycorticosterone acetate – epoxy-DOCA.

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Bac. megaterium: the 21-acetoxy group was saponified (Bac. megaterium possesses a strong esterase activity [1]), and a new hydroxy group appeared, as was shown by an increase in molecular weight from 330 to 346. The structure of the steroid skeleton remained basically unchanged, and the 20-oxo and Δ^4-3-oxo groupings were retained. Thus, in the transformation of DOCA there was no reduction of the 20-oxo group and the steroid molecule was hydroxylated, possibly in position 15β, i.e., the reaction that is known for other strains of the species Bac. megaterium [9].

In the transformation of 11-oxo-16α,17α-epoxyprogesterone with Bac. megaterium, the 20-oxo group was reduced. Thus, the IR spectra of the main transformation product showed a strong band of hydroxyl absorption, and the bands in the region of conjugated and nonconjugated carbonyl groupings were retained. The molecular weight increased by two units, being 344. This statement is also supported by the presence in the mass spectrum of peaks with m/e 326, 300, and 299 due to the elimination of H_2O, CH_3CHO, and CH_3CHOH, respectively, and also weak ions with m/e 283, 258, and 240 formed by the stepwise degradation of ring D.

The configuration of the hydroxy group was established by determining the increment in molecular rotation in the acetylation of the transformation product of 11-oxo-16α,17α-epoxyprogesterone (ΔM_D^{OAc-OH} = + 8.53) and the 20β-hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione synthesized (ΔM_D^{OAc-OH} = + 218.6). The 20-hydroxy group of the transformation product was assigned the α configuration [10].

On the basis of the IR spectrum of the transformation product of epoxy-DOCA, it may be assumed that the compound isolated is the 20-hydroxy derivative without the acetyl group in position 21, i.e., in this case, just as in the case of 16α,17α-epoxyprogesterone and 11-oxo-16α,17α-epoxyprogesterone the 20-oxo group underwent reduction and the 21-acetoxy group was deacetylated.

Thus, it has been shown that a culture of Bac. megaterium brings about the reduction of a 20-oxo group to a 20α-hydroxy group only in the case of Δ^4-3-oxosteroids containing a 16α,17α-epoxy ring (16α,17α-epoxyprogesterone, 16α,17α-epoxydeoxycorticosterone, and 11-oxo-16α,17α-epoxyprogesterone). The presence of a 17α-hydroxy group or the absence of an oxygen function in position 17 (DOC, progesterone, 17α-hydroxyprogesterone, etc.) makes the substrate inaccessible for enzymes bringing about the α-reduction of a 20-carbonyl group. The characteristic observed is one more confirmation of the special significance of the 16α, 17α-epoxy ring for the microbiological reduction of 20-oxosteroids to 20α-alcohols [3-5].

The reduction of a 20-carbonyl group to a β-hydroxy group was observed only for cortisone, cortexolone, and their Δ^1-dehydro derivatives and does not take place in the transformation of 17α-hydroxyprogesterone, progesterone, deoxycorticosterone, epoxydeoxycorticosterone, etc.

EXPERIMENTAL

The IR spectra were taken on a UR-10 instrument in the form of suspensions in paraffin oil, and the NMR spectra on a JNM-4H-100/100 MHz instrument (in deuterochloroform, deuteropyridine, or in a mixture of deuterated methanol and carbon tetrachloride). Tetramethylsilane was used as internal standard. The mass spectra were taken on an MKh-1303 instrument.* The molecular weights were determined mass-spectrometrically.

The culture of Bacillus megaterium VNIKhFI-1 was maintained on meat-peptone agar. To a 750-ml flask containing 100 ml of synthetic medium of the composition described [1] was added 1 ml of an aqueous suspension of spores. Growth took place at 28°C on a shaking machine at the rate of 260 rpm for 48 h. The transformation was performed with cells which has been washed free from the medium with distilled water and with phosphate buffer (0.07 M, pH 7). To a 100-ml flask containing 25 ml of a suspension of cells in buffer (27-31 mg/ml as dry biomass) was added 5 mg of a steroid in 0.5 ml of methanol, and the flask was placed in a water thermostat with shaking at 37°C for 20 h. To follow the course of the transformation, the steroid was extracted from 10 ml of the culture liquid twice with 20 ml of chloroform. The extract was concentrated and the steroids in it were separated by thin-layer chromatography on Silufol UV 254 plates or on plates with a fixed layer of KSK silica gel. Color reactions for an α-ketol side chain were used: with triphenyltetrazolium chloride [11], with an aqueous solution of Tetrazolium Blue [12], and with phosphorous pentoxide and sulfuric acid [13] for the plates with a fixed layer of silica gel, and also with ammoniacal solution of silver nitrate [14] for paper chromatograms.

To study the products of the transformation of DOCA, epoxy-DOCA, and 11-oxo-16α,17α-epoxyprogesterone by washed cells of Bac. megaterium, we performed experiments on a larger scale with total amounts

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of DOCA of 400 mg in 40 ml of ethanol, of epoxy-DOCA of 170 mg in 17 ml of ethanol, and of 11-oxo-16α,17α-epoxyprogesterone of 170 mg in 17 ml of ethanol. The steroids were extracted from the culture liquid with methylene chloride three times. The extract was evaporated to dryness and the main transformation product was isolated by thin-layer chromatography on Silufol plates, and the steroids were eluted from the scraped-off spots by boiling them with ethanol in case of the transformation of DOCA, with methylene chloride and methanol for the product of the transformation of epoxy-DOCA, and with ethanol in the case of the transformation of 11-oxo-16α,17α-epoxyprogesterone.

Δ¹-Dehydrocortexolone was obtained by the microbiological dehydrogenation of cortexolone by a culture of Corynebacterium simplex in a medium containing 0.5% of yeast extract and 0.5% of glucose (pH 7) for 12 h. After the end of the dehydrogenation process, the culture liquid was extracted with chloroform, the solvent was distilled off to dryness, and the crude product was recrystallized from isopropanol with carbon, giving Δ¹-dehydrocortexolone with mp 240-242°C, $[\alpha]_D^{20}$ + 71° (c 1.00 in ethanol). Literature data: mp 229-233°C, $[\alpha]_D^{25}$ + 76 ± 3° (c 0.757 in ethanol) [15].

11α-Hydroxy-16α,17α-epoxyprogesterone was synthesized by the microbial hydroxylation of 16α,17α-epoxyprogesterone with Rhizopus nigricans. The 11α-hydroxy-16α,17α-epoxyprogesterone formed was isolated by extracting the culture liquid with methylene chloride and was recrystallized from acetone to give a product with mp 237-238.5°C. Literature data mp 238-238.5°C [16].

11-Oxo-16α,17α-epoxyprogesterone was obtained by the oxidation of 11α-hydroxy-16α,17α-epoxyprogesterone with Kiliani's solution in acetone at 25°C for 30 min. After recrystallization from ethyl acetate, the product had mp 190-191°C. Literature data mp 186-186.5°C [16].

21,15ξ-Dihydroxypregn-4-ene-3,20-dione. The IR spectrum of the main product of the transformation of DOCA was (cm^{-1}): 3450(OH), 1710 (20−CO), 1620, 1650 (Δ⁴−3−CO). NMR spectrum (cm): 0.68(18−CH_3), 1.18(19−CH_3), 4.2(21−CH_3), 5.73(4−H) (in a mixture of deuterated ethanol and CCl_4). M^+ 346.

20α-Hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione. The product of the transformation of 11-oxo-16α,17α-epoxyprogesterone had mp 241.5-242°C; $[\alpha]_D^{20}$ + 188.5°(c 0.885 $CHCl_3$); R_f = 0.45. IR spectrum (cm^{-1}): 3430(OH), 1710(11−CO), 1620, 1670(Δ⁴−3−CO). NMR spectrum (ppm): 0.8(18−CH_3), 1.36(19−CH_3), 3.57(16−H), 4.11(20−H), 5.68(4−H), 2.42(12−H), 1.22(21−CH_3) (in deuterochloroform). Mass spectrum, m/e: 344(M^+), 326(M−H_2O), 300(M−CH_3CHO) 299(M−CH_3CHOH), 283, 258, 243, 326(M−H_2O), 300(M−CH_3CHO) 299(M−CH_3CHOH), 283, 258, 243 (stepwise degradation of ring D).

20ξ,21-Dihydroxy-16α,17α-epoxypregn-4-en-3-one. The IR spectrum of the product of the transformation of 16α,17α-epoxy-DOCA showed an increase in the intensity of the absorption band relating to hydroxy groups (3410-3480 cm^{-1}). The bands at 1620 and 1680 cm^{-1} (Δ⁴-3-CO) remained unchanged.

20β-Hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione was obtained by the reduction of 16α,17α-epoxypregn-4-ene-3,11,20-triol with sodium tetrahydroborate in ethanol at 0-2°C for 45 min followed by the holding of the reaction mixture at 0°C for 1 h. The mixture was neutralized with glacial acetic acid, the ethanol was distilled off in vacuum, and the residue was dissolved in chloroform. The extract was washed, dried, and evaporated to dryness. The residue was purified by chromatography through silica gel. The product of the main fraction was oxidized with manganese dioxide. The reaction was performed at 18-20°C for 4 h, after which the manganese dioxide was filtered off and washed with chloroform, and the filtrate was evaporated to dryness to give a product with mp 212.5-214.5°C (from ethyl acetate), $[\alpha]_D^{20}$ + 197° (c 1.05 $CHCl_3$), R_f = 0.53−0.54 (benzene−acetone (5 :3) system, on Silufol plates). IR spectrum (cm^{-1}): 3510, 3450, 3410(OH), 1705(11−CO), 1620, 1650 (Δ⁴−3−CO); UV spectrum: λ_{max} 239.5 nm; ε = 15600 (in ethanol). NMR spectrum: 0.81 (18−CH_3), 1.36(19−CH_3), 3.33(16−H), 4.28(20−H), 5.67 (4−H), 2.56(12−H), 1.04(21−CH_3) (in deuterochloroform). Mass spectrum (m/e): 344(M^+), 326 (M−H_2O), 299(M−CH_3CHOH), 329(M−CH_3), 316(M−CO), 287, 259, 258 (stepwise degradation of ring D).

20β-Hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione 20-acetate was obtained by the acetylation of 20β-hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione with acetic anhydride in pyridine at room temperature; mp 221-225°C, $[\alpha]_D^{20}$ + 232.2°($CHCl_3$ c 1.258), R_f = 0.83−0.85. IR spectrum (cm^{-1}): 1740(OAc), 1700(11−CO), 1620, 1670(Δ⁴−3−CO). NMR spectrum (in $CDCl_3$; ppm): 0.79 (18−CH_3), 1.33(19−CH_3), 3.29(16−H), 5.46(20−H), 5.66(4−H), 2.55(12−H), 1.02(21−CH_3), 2.03(CH_3 of an acetate group). Mass spectrum (m/e): 386 ($M^{+\cdot}$). The maximum peak is that with m/e 344 (M − $COCH_2$), the further fragmentation of which is analogous the degradation of 20β-hydroxy-16α,17α-epoxypregn-4-ene- 3,11-dione.

20α-Hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione 20-acetate was obtained by the acetylation of 20α-hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione with acetic anhydride in pyridine at room temperature. The

product had: mp 237-240°C $[\alpha]_D^{20}$ + 170° (1.05 $CHCl_3$), R_f = 0.67. IR spectrum (cm^{-1}): 1740(OAc), 1710 (11-CO), 1610, 1665 (Δ^4-3-CO); NMR spectrum ($CDCl_3$; ppm): 0.88 (18-CH_3), 1.35 (19-CH_3), 3.35 (16-H), 5.30 (20-H), 5.68 (4-H), 2.5 (12-H), 1.24 (21-CH_3), 1.98 (CH_3 of an acetate group). Mass spectrum (m/e): 386 ($M^{+\cdot}$). The maximum peak is that with m/e 344 (M - $COCH_2$), the further decomposition of which is similar to the fragmentation of 20α-hydroxy-16α,17α-epoxypregn-4-ene-3,11-dione. The mass spectra of the 20-dihydro compounds of 11-oxo-16α,17α-epoxyprogesterone are practically identical.

SUMMARY

The substrate specificity of an intact culture of Bacillus megaterium in relation to steroids of the pregnane series with a dihydroxyacetone side chain and without it, and also with steroids containing a 16α,17α-epoxy ring, has been studied.

It has been shown that a culture of Bac. megaterium performs the reduction of a 20-oxo to a 20α-hydroxy group only in the case of Δ^4-3-oxo-steroids containing a 16α,17α-epoxy ring.

The necessity of a dihydroxyacetone side chain for the β-reduction of a 20-oxo group has been established.

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