# MICROBIOLOGICAL REDUCTION OF CORTISOL AND PREDNISOLONE BY A CULTURE OF Act. roseoviridis AND THE STUDY OF THE STRUCTURE OF THE ACETONIDES OF 11β,17α,20β,21-TETRAHYDROXYPREGN-4-EN-3-ONE

E. G. Balashova, K. N. Gabinskaya,
L. M. Alekseeva, V. F. Shner,
O. V. Messinova, and N. P. Suvorov

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The enzymatic reduction of the keto group at C-20 is one of the main routes for the metabolism and inactivation of corticosteroids in mammals [1]; a similar process is widely known also for various microorganisms, including actinomycetes [2].

We have studied the reduction of cortisol (I) and prednisolone (II) by a number of actinomycetes. Of the strains tested Act. viridochromogenes 3356 ATCC, Act. aureofaciens 10762 VNIIA, Act. variabilis 526/56, Act. viridochromogene 9343εTH, Act. lavendulae 9911εTH, and Act. lavendulae 8664 ATCC do not transform substrates (I) and (II); when Act. roseoviridis 13040 INA was used, the corresponding 20β-hydroxy compounds (III) and (IV) were formed smoothly. The structure of the compounds obtained and of their 20,21 diacetates was confirmed by comparison with literature data.

In a chromatographic study of the composition of the culture liquids it was found that the tetrols (III) and (IV) are the main products, and other steroid compounds were present in only trace amounts. Thus, in contrast to many other known cultures which perform this reduction, Act. roseoviridis 13034 INA is characterized by a high specificity of reduction in the course of which the other groupings in the steroid molecule are not affected.

In connection with the considerable biological role of the reduction of the carbonyl and C-20, the development of a method for determining 20-hydroxy steroids in the organism is extremely urgent. It must be observed that 17α,20,21-triols containing a glycerol side chain are distinguished by a low stability, and therefore they were analyzed in the form of derivatives. Thus, such compounds and, in particular, Reichstein's substance E (III) were converted into their acetonides and were then determined by GLC and mass spectrometry [3], the authors ascribing structure (V) to the acetonide. The opinion has been expressed recently [4] that in the reaction of compound (III) with acetone in the presence of p-toluenesulfonic acid the acetonide (V) is formed, while in the presence of perchloric acid a mixture of the acetonides (V) and (VI) is formed.

We have studied this reaction with the aid of the TLC method, compound (V) and compound (VI) being separated by preparative chromatography. In contrast to literature information, it was found that when both TSOH and $HClO_4$ were used as catalysts and also when the methods described in the literature [3, 4] were repeated, a mixture of the acetonides (V) and (VI), readily separable by chromatography, was always formed. Compound (VI), in which the least sterically hindered 21-hydroxy group is free, possesses a lower chromatographic mobility than its isomer (V). The structures of these isomers follow from the ease of acetylation of the acetonide (VI) with the formation of the acetate (VII) and the resistance of the acetonide (V) to acetylation under mild conditions.

It is also interesting to note that we have observed an interconversion of the acetonides: When a solution of an individual compound (V) or (VI) in acetone was treated with acid catalysts, after only a short time

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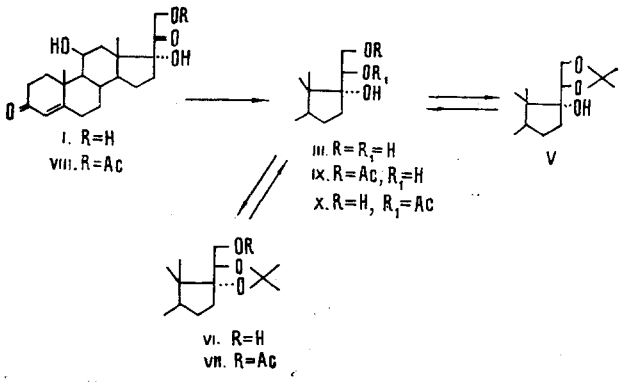
both isomers were detected by the TLC method. Thus, bearing in mind the reversibility of the reaction for the preparation of the acetonides in an acid medium, on the basis of the results obtained it may be considered that the process takes place under thermodynamic control, the ratio of the isomeric compounds (V) and (VI) being determined by the relative stabilities of the dioxolane rings formed with the participation of carbon atoms 17 and 20 or 20 and 21.




In the PMR spectra of the acetonides (V)-(VII) there are the following singlet signals: the protons of four methyl groups (C-18, C-19, and the acetonide grouping) in the 1-1.5 ppm region and of the vinyl protons at C-4 in the 5.7 ppm region. In addition, a complex system of signals can be seen in the spectrum with an intensity of four proton units in the 3.5-4.5 ppm region from the protons at C-11, C-20, and C-21, while acetylation of a hydroxy group leads to a normal downfield shift of the signal from the C-21 protons. In contrast to acetylation, the inclusion of the hydroxy group in a dioxolane ring leads to practically no change in the chemical shift of the geminal proton at C-21. The two acetonides (V) and (VI) have practically identical mass spectra.

Since the conversion of the tetrol (III) into an acetonide leads to the formation of a mixture of the isomers (V) and (VI), the analysis of such compounds by the method described by Bailey [3] is insufficiently reliable. It seems to us desirable for analysis to stabilize them in the form of diacetates or, for example, trimethylsilyl ethers.

It is known that reduction by complex metal hydrides of the keto group at $C_{20}$ in cortisol acetate (VIII) takes place stereospecifically but is accompanied by side reactions. In the reduction of the acetate (VIII) by $NaBH_4$ [5] we have observed the formation of a mixture of the monoacetates (IX) and (X) and also of the tetrol (III). It was found that the yield of compounds (III) can be substantially increased if the mixture of acetates (IX) and (X) is hydrolyzed without purification.

## EXPERIMENTAL METHOD

The IR spectra were obtained on a Perkin-Elmer 457 spectrometer in paraffin oil, the UV spectra on an EPF-3 (Hitachi) spectrometer in ethanol, and the PMR spectra on a JNM-4N-100 instrument in deuterochloroform (chemical shifts given in the $\delta$ scale from the signal of TMS taken as 0), and the specific rotations were measured for 1% solutions on an LPU-01 polarimeter. Thin-layer chromatography was performed on "Silufol" plates (Kavalier, Czechoslovakia) and preparative separation of columns of type KSK silica gel. The analyses of all the compounds obtained corresponded to the calculated C and H contents.

The steroid alcohols were acetylated with acetic anhydride in pyridine (4 h at ~20° C), and the acetates were isolated after the reaction mixtures had been poured into dilute hydrochloric acid at 0° C.

11$\beta$, 17$\alpha$, 20$\beta$, 21-Tetrahydroxypregn-4-en-3-one (III). Method A [Microbiological Reduction of (I)]. The cultures, which were obtained from the collection of Kazan' State University, were maintained on a medium containing (%): starch – 2; potassium nitrate – 0.1; dipotassium phosphate – 0.05; magnesium sulfate – 0.05; sodium chloride – 0.05; and agar – 2; pH 7.2. The culture was grown on a medium (%): maize extract – 0.5 (as dry weight); starch – 3; ammonium nitrate – 0.5; potassium carbonate – 0.5; sodium chloride – 0.4; pH 6.4. An aqueous suspension of actinomycetes spores was added in 2-ml portions to 250-ml conical flasks containing 100 ml of the medium; the mycelium was grown on a shaking machine at 28°C; after 24 h, a solution of 50 mg of the steroid in 1 ml of ethanol was added. After the completion of the transformation (48 h, checked by the TLC method), the culture liquid [3.5 g of the initial (I)] was filtered, and the filtrate was saturated with 1850 g of NaCl and extracted four times with ethyl acetate (3.5 liters + 7 liters + 7 liters + 3.5 liters). On evaporation in vacuum to ~25 ml, the combined extracts deposited

2.46 g (yield 70%) of (III); mp 120°C (from ethyl acetate), $[\alpha]_D^{20}$ + 88° (0.5% dioxane); it was identical in properties with the compound obtained by method B. According to the literature [6, 7] – mp 120-126°C (from ethyl acetate).

The 20,21-diacetate had mp 228-230° C (from acetone) $[\alpha]_D^{20}$ +162° (acetone) and was identical in its properties with the diacetate of the compound obtained by method B. According to the literature – mp 228-229° C (from acetone); $[\alpha]_D^{20}$ +165° (dioxane) [6]; mp 229-231° C, $[\alpha]_D^{20}$ +161.3° (acetone) [8].

Method B (reduction with $NaBH_4$). A solution of 4.4 g of $NaBH_4$ in 100 ml of water was added over 4 h (~ 20° C) to a solution of 20 g of (VIII) in 800 ml of dimethylformamide, and then the mixture was cooled to 0° C and 35.7 ml of 30 % acetic acid was added in drops. In vacuum, 700 ml of dimethylformamide was evaporated off at a bath temperature of 50° C, and the residue was treated with 700 ml of water, and was left at ~0° C for 2 h. The resulting mixture of (IX) and (X) (0.56 g) was filtered off. The aqueous filtrate was extracted with ethyl acetate (5 × 200 ml), and the extract was washed with water (2 × 30 ml) and dried with $Na_2SO_4$. Fractional evaporation of the extract yielded portions of a crystalline mixture of (IX) and (X) (total 7.71 g) and then 4.49 g of (III). The aqueous layer was additionally extracted with ethyl acetate (5 × 500 ml), and evaporation of the extract gave 17.51 g of residue [a mixture of (IX) and (X)]. To a solution of 7.71 g of the crystalline mixture of (IX) and (X) in 386 ml of methanol was added a solution of 3.93 g of potassium carbonate in 3.93 ml of water, and after 2 h (~ 20° C) 88 ml of water was added and the mixture was acidified with 30 % acetic acid. After 30 min, the methanol was evaporated off in vacuum, the residue was extracted with ethyl acetate (5 × 300 ml), and the extract was washed with water, dried, and evaporated. The residue was recrystallized from methanol–acetone (1 : 1), giving 4.57 g of (III). In a similar manner, 17.51 g of a mixture of (IX) and (X) was hydrolyzed, giving 6.49 g of (III), the total amount of (III) therefore being 15.55 g, the yield being 86.4% calculated on the (VIII).

11β, 17α, 20β, 21-Tetrahydroxypregna-1,4-dien-3-one (IV). As in the preceding case (method A), after the transformation of 0.95 g of (II), 0.95 g of unpurified tetrol (IV) was obtained.

20,21-Diacetate, mp 241.5-243° C (from isopropanol), $[\alpha]_D^{20}$ + 109.5° (dioxane). According to the literature – mp 244-247° C (from acetone), $[\alpha]_D^{20}$ +110° (dioxane) [9]; mp 234.5-237.5° C (from a mixture of acetone and petroleum ether), $[\alpha]_D^{20}$ +114° (dioxane) [6].

The 20,21-Acetonide (V) and the 17,20-Acetonide (VI) of 11β, 17α, 20β, 21-Tetrahydroxypregn-4-en-3-one. A solution of 8.33 g of (III) and 3 ml of 43.4 % $HClO_4$ in 200 ml of acetone was left at ~20° C for 12 h and was then poured into 800 ml of water and extracted with chloroform; the extract was washed successively with water, 5 % $NaHCO_3$, and water again, and was dried and evaporated in vacuum. The residue (11.2 g) was transferred to a column of silica gel and eluted with chloroform and then with a mixture of chloroform and acetone (20 : 1). From the corresponding portions of the eluate were isolated successively 2.81 g (yield 30.4 %) of (V), mp 205-206° C (from a mixture of ethyl acetate and hexane), $\lambda_{max}$ 243 nm (ε 15,800), $\nu_{max}$ 1610 and 1660 $cm^{-1}$ ($\Delta^4$-3-ketone) and 3460, 3550 $cm^{-1}$ (OH), $[\alpha]_D^{20}$ + 100.0° (methanol), and 2.26 g (yield 24.5 %) of (VI), mp 251.5-253°C (from a mixture of ethyl acetate and hexane), $\lambda_{max}$ 243 nm (ε 16,000) $\lambda_{max}$ 1610 and 1670 $cm^{-1}$ ($\Delta^4$-3-ketone) and 3480, 3560 $cm^{-1}$ (OH), $[\alpha]_D^{20}$ + 78.0° (methanol). According to the literature [4]: (V) has mp 205-207.5° C, $[\alpha]_D^{20}$ + 99.4° (methanol), and (VI) has mp 257.5-259.5° C, $[\alpha]_D^{20}$ + 83.7° (methanol).

21-Acetate of 11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one. mp 188-190° C (from a mixture of ethyl acetate and hexane), $\lambda_{max}$ 243 nm (ε 16,000), $\lambda_{max}$ 1615 and 1655 $cm^{-1}$ ($\Delta^4$-3-ketone), 1735 $cm^{-1}$ (ester CO), and 3415 $cm^{-1}$ (OH). According to the literature [4]: mp 196-197° C.

## SUMMARY

1. It has been established that the reduction of cortisol and of pregnisolone with a culture of Act. roseoviridis forms the corresponding 20β-hydroxy compounds.

2. In a study of the preparation of acetonides from Reichstein's substance E the formation of a mixture of isomers has been shown.

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