CHEMISTRY

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1965

SovietRxiv

Abstract

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A NEW STEROID HYDROXYLATION REACTION FOR ACTINOMYCETES

(Presented by Academician M. M. Shemyakin, 28 VIII 1964)

The introduction of a hydroxyl group into position 17α of pregnane steroids is of considerable interest, since the presence of this group determines the physiological activity of many hormonal preparations. In view of the prospects for sterins as sources of raw material for the production of steroid hormones, microbiological 17α-hydroxylation is of special interest, since it makes it possible to introduce a 17α-oxy group into the progesterone molecule ([1]).

It is known that some microorganisms are capable of introducing a 17α-hydroxyl group into pregnane steroids. 17α-hydroxylating microorganisms have been described that belong to *Dendroides* ([1]), *Trichothecium* ([2,3]), *Cephalothecium* ([4]), *Trichoderma* ([5]), *Sepedonium* ([6]), *Kabatiella* ([7]), *Gliocladium* ([8]), *Leptosphaeria*, *Cucurbita*, *Lophotrichus*, *Melanospora* and *Thielaria* ([9]).

Despite intensive investigations, carried out over almost two decades, of the ability of actinomycetes to transform steroids, for actinomycetes (microorganisms widely distributed in nature) the ability to hydroxylate steroids at position 17α remained unknown.

In studying the ability of actinomycetes of various species to transform steroids, we found that some actinomycetes hydroxylate pregnane steroids at position 17α. During fermentation with progesterone (I) of a culture of *Actinomyces spheroides* LNGI-56, we isolated from the culture fluid, along with unchanged starting diketone (I), products of its hydroxylation—17α-oxyprogesterone (II) and Δ^4-pregnendiol-17α, 20β-one-3 (III):

[reaction scheme: progesterone (I) $\rightarrow$ 17α-oxyprogesterone (II) + Δ^4-pregnendiol-17α,20β-one-3 (III)]

For fermentation, a nutrient medium containing 1% starch, 0.2% $(NH_4)_2SO_4$, 0.1% $MgSO_4$, 0.1% NaCl, 0.3% $CaCO_3$, and 0.1% K_2HPO_4 in tap water (pH 7.0) was inoculated with a culture of *Act. spheroides* LNGI-56 grown for 48 h on a nutrient medium containing 1% corn extract, 2% glucose, 0.5% NaCl, and 0.5% $CaCO_3$ in tap water (pH 7.0), then aerated by shaking on a shaker (220 rpm) at 28° for 24 h; a solution of 100 mg of progesterone (I) in 5 ml of acetone was added, and fermentation was carried out under the same conditions

reaction scheme: IV → V, VI, VII

Figure 1: reaction scheme: IV → V, VI, VII

reaction scheme: VIII → IX

Figure 2: reaction scheme: VIII → IX

for 70 h. By extraction of the culture fluid with methylene chloride, followed by the usual work-up and preparative thin-layer chromatography on aluminum oxide (activity III–IV, layer thickness 1 mm, ethyl acetate), progesterone (I), m.p. 125—126.5°, 17α-oxyprogeste-

rone (II), m.p. 215–218°, IR spectrum (in a paste with vaseline oil): 3400, 1707, 1670, and 1618 cm^{-1}, and Δ^4-pregnenediol-17α,20β-ol-3 (III), m.p. 199–202°, IR spectrum (in a paste with vaseline oil): 3435, 1652, and 1615 cm^{-1}.

In the next series of experiments we carried out chromatographic identification of the reaction products.

During fermentation with 17α-hydroxyprogesterone (II), the culture of *Act. spheroides* also forms diolone III, as shown by chromatography of extracts of samples of the culture fluid on paper in the ethylene glycol/benzene system. During fermentation with Δ^4-pregnenol-20β-ol-3, hydroxylation in the 17α position does not occur. Thus, it may be concluded that during fermentation with progesterone (I) the first stage for cultures of *Act. spheroides* LNGI-56 is hydroxylation at the 17α position, and then reduction of the 20-keto group of 17α-hydroxyprogesterone (II) occurs.

For the reduction of the 20-keto group to take place, the presence of a hydroxy group specifically in the 17α position is not a necessary condition. Thus, during fermentation with 11α-hydroxyprogesterone (IV), cultures of *Act. spheroides* LNGI-56 also undergo hydroxylation at the 17α position and reduction of the 20-keto group, with formation of 11α,17α-dihydroxyprogesterone (V) and Δ^4-pregnenetriol-11α,17α,20β-ol-3 (VI). However, in this case a substance obtained by reduction of the starting steroid—Δ^4-pregnenediol-11α,20β-ol-3 (VII)—was found among the reaction products:

(Chromatography on paper in the ethylene glycol/toluene—dioxane = 6 : 4 system.)

Upon the action of the culture *Act. spheroides* LNGI-56 on corticosterone (VIII), we were able to detect in the fermentation mass (chromatography on paper in the ethylene glycol/toluene—dioxane 6 : 4 system), in addition to the starting steroid, only Δ^4-pregnenetetraol-11β,17α,20β,21-ol-3 (IX):

During fermentation with Reichstein's substance S and 16,17α-epoxyprogesterone, cultures of *Act. spheroides* LNGI-56, along with the starting substances, revealed the corresponding products of reduction of the 20-keto group.

reaction scheme: X XI

Figure 3: reaction scheme: X XI

Using fermentation with testosterone (X) and androstenedione (XI) as examples, we showed that the culture *Act. spheroides* LNGI-56 effects interconversion of the 17β-hydroxy and 17-keto groups of C_{19}-steroids:

(Paper chromatography in the ethylene glycol/cyclohexane–benzene system = 3:1.)

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Received
27 VIII 1964

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Note: Figure translations are in progress. See original paper for figures.