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Metabolism of Progesterone by *Streptomyces griseus*

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The microbial side chain degradation of progesterone, leading to testosterone, Δ^4-androstene-3,17-dione, and testolo-lactone or their 1-dehydroderivatives, is well known.[1-5] The cleavage of the side chain is suggested to proceed in a way, similar to the non-enzymatic action of peracids on ketones, [5] as shown in Fig. 1. Formation of the intermediate testosterone acetate from progesterone has only been demonstrated in *Cladosporium resinae* (Fonken *et al.*).[6] In some other micro-organisms high esterase activity is believed to prevent accumulation of this compound [6,7] and therefore only the later products in this reaction sequence have been detected.

As one part of a study, regarding the progesterone metabolism of some different microorganisms, the fungus *Streptomyces griseus* was investigated. Upon incubation of progesterone with this fungus three products were demonstrated: testosterone acetate (main product), testosterone and Δ^4-androstene-3,17-dione.

Experimental. A spore culture of *Streptomyces griseus* was obtained as a gift from AB KABI, Stockholm. The fungus was grown up on beer-wort-distilled water 1:1 in cotton-plugged Erlenmeyer flasks on a shaking table at 25–27°C. After completed fermentation the mycelia were harvested, washed with distilled water and resuspended to original density in 0.1 M Na_2HPO_4 (pH 8.3). The suspension was distributed in 50 ml portions into 200 ml Erlenmeyer flasks and the steroid substrate (29.0 mg of progesterone, dissolved in 1.0 ml of methanol) was pipetted into each flask. The flasks were stopped with cotton plugs and the incubation took place on a shaking table at 25–27°C.

At definite time intervals duplicate flasks were withdrawn and the steroids were extracted with two 50 ml portions of chloroform. By evaporation at 50°C the extracts were concentrated to 50 ml volume and 0.5 ml aliquots of these solutions were analyzed by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

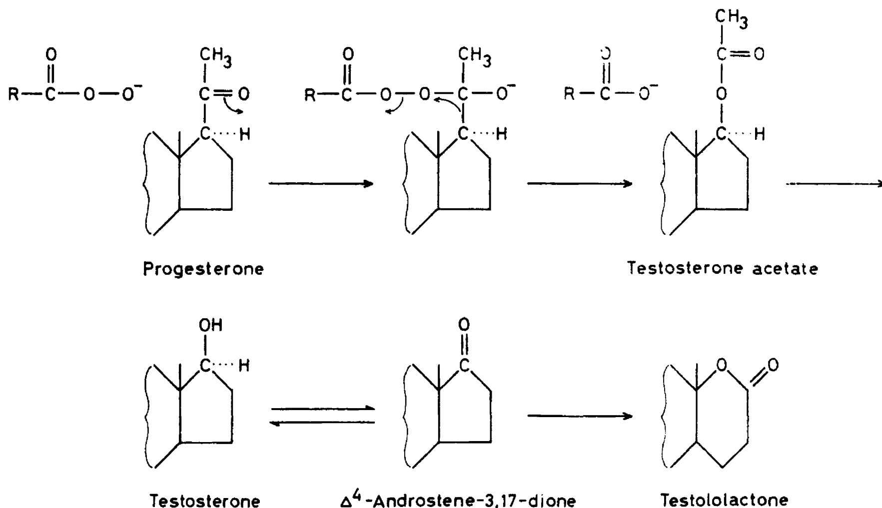

Fig. 1. Pathway of the microbial side chain degradation of progesterone.

The TLC was performed on Silica Gel G plates (200 × 100 mm) using ethyl acetate-benzene-hexane 1:1:1 as solvent. The plate was divided into three strips, the first twice as wide as two others. On the wide strip the 0.5 ml aliquot from the chloroform extract was placed, on the second strip a 0.2 ml aliquot from the same extract and on the third strip a mixture of progesterone, testosterone acetate, testosterone, and Δ^4-androstene-3,17-dione dissolved in chloroform. After running the chromatogram the wide strip was protected with an aluminium sheet. The two narrow strips were sprayed with 10 % $SbCl_5$ in chloroform and quickly heated in a Bunsen flame. R_F-values in this system were: testosterone acetate 0.47, progesterone 0.38, Δ^4-androstene-3,17-dione 0.29, and testosterone 0.20. The zones on the wide strip, corresponding to the standards, were cut out and each eluted with three 5 ml portions of ethanol. After centrifugation 16.6 μg of cholestane, dissolved in benzene, was added as an internal standard. The solvents were evaporated under nitrogen at 50°C. The dry residues were dissolved in 50 μl of chloroform and subjected to GLC.

The GLC was carried out in a Perkin-Elmer F-11 Gas Chromatograph fitted with a flame ionization detector. The column was a stainless steel 2 m × 1/8″ packed with 1 % Nitrile Silicone XE-60 on HMDS-treated Chromosorb W, 80–100 mesh. Column temperature was 230°C, injection temperature 300°C, and carrier gas flow (nitrogen) 50 ml/min. The retention times were: cholestane 39 sec, Δ^4-androstene-3,17-dione 138 sec, testosterone 144 sec, testosterone acetate 162 sec, and progesterone 174 sec.

For further identification of the steroids additional TLC and GLC systems were used. This TLC was carried out on Al_2O_3 G plates with 0.5 % ethanol in benzene as solvent. R_F-values in this system were: testosterone acetate 0.50, progesterone 0.48, Δ^4-androstene-3,17-dione 0.26, and testosterone 0.10. The additional GLC was performed on 3 % Silicone Rubber SE-30 + 0.1 % Versamid 900 on AW-HMDS-Chromosorb W 80–100 mesh. The column temperature was 240°C, injection temperature 300°C, and nitrogen flow 50 ml/min. The retention times were: Δ^4-androstene-3,17-dione 258 sec, testosterone 264 sec, testosterone acetate 408 sec, and progesterone 432 sec.

For the IR-spectroscopic study testosterone acetate was isolated from the fermentation extracts by using column chromatography on neutral Al_2O_3. The extracts were evaporated to dryness and the residues were dissolved in benzene-hexane 1:1. The column was prepared with the same solvent. Elution took place with benzene-hexane 1:1, benzene, and finally benzene with increasing amounts of ethyl acetate. The testosterone acetate appeared in the ethyl acetate-benzene 1:10 fraction. After evaporation of the solvent it was recrystallized from hexane. The IR-spectra were recorded on a Perkin-Elmer 421 IR-spectrometer.

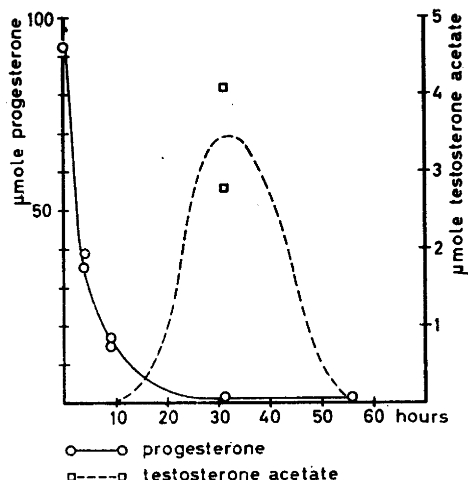

Fig. 2. Composition of the steroid mixture during a progesterone fermentation with *Streptomyces griseus*. Testosterone and Δ^4-androstene-3,17-dione were not detectable until the appearance of the testosterone acetate and were only present in trace amounts.

Results and discussion. The composition of the steroid mixture during the fermentation is given in Fig. 2. No testololactone could be demonstrated. Before the appearance of the testosterone acetate only progesterone was detected. After its appearance traces of testosterone and Δ^4-androstene-3,17-dione were demonstrated. Only traces of the four steroids were detectable after 56 h of fermentation.

The compound, tentatively identified as testosterone acetate, had the same mobility as the reference standard in the two TLC systems. The GLC retention times on the two different liquid phases corresponded to those of the authentic testosterone acetate. After heating the compound with 1 M KOH in methanol at 60°C for 30 min, followed by isolation of the hydrolysis product, it showed the

same behaviour in the two TLC and GLC systems as testosterone. IR-spectrum of the fermentation compound was idential to that of authentic testosterone acetate.

From these results it can be concluded that progesterone is metabolized by *Streptomyces griseus* in the same way as by *Cladosporium resinae*.[6] This finding supports the hypothesis that this way might be the general route in which the side chain of progesterone is cleaved by microorganisms.[6-8]

The esterase activity in *Streptomyces griseus* seems to be rather low, even at high pH (8.3) at which the esterases generally are stable. The optimum pH for a steroid esterase, prepared from *Nocardia restrictus*, is 8.0.[9]

The rapid disappearance of the progesterone without formation of corresponding amounts of other steroids indicates complete degradation of the steroid moiety to low-molecular compounds. This has been reported for other microorganisms by some authors [10-12] and has also been observed in this laboratory after progesterone fermentations with *Penicillium lilacinum* and *Aspergillus flavus*.

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A Novel Route to 1,3-Dioxoles *via* a General Acid-Catalyzed Isomerization Reaction

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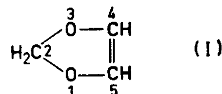
The first successful synthesis of 1,3-dioxole (I) and one of its alkyl derivatives was reported a few years ago by Field.[1] The reaction scheme consisted of a Diels-Alder addition of vinylene



carbonate to anthracene, hydrolysis of the adduct to a 1,2-diol, acetalization of the diol to give a 1,3-dioxolane derivative, followed then by thermal cracking of the latter to yield the 1,3-dioxole desired. Some 2,4-diaryl-substituted 1,3-dioxoles have been prepared recently by Ried and Omran [2] from diazoketones and aromatic aldehydes.

For kinetic studies of the hydrolysis of 5-membered cyclic vinyl ethers an easy route to a number of alkyl-substituted 1,3-dioxoles was desired. In a recent paper [3] it had been shown that the hydrolysis of vinyl ethers exhibits general acid catalysis involving a rate-determining proton transfer step which yields a mesomeric oxonium-carbonium ion as the first reaction intermediate. This reaction mechanism suggested that the intermediates generated by the hydrolysis of 4-alkyl-1,3-dioxoles (*e.g.*, eqn. (II)) should be just the same as those formed from the respective 4-alkylidene-1,3-dioxolanes (*e.g.*, eqn. (III)). It was therefore inferred that, under conditions where the hydrolytic cleavage of the intermediates could not occur, 1,3-dioxoles and the alkylidene dioxolanes would be readily interconvertible by general acid catalysis. This was verified by the actual experiments. Because alkylidene dioxolanes can be easily obtained from 4-α-chloroalkyldioxolanes, the con-