MICROBIOLOGICAL TRANSFORMATIONS OF PROGESTERONE

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Transformation of progesterone by various microorganisms such as actinomycetes, yeast and mould fungi of different 53 strains had been studied. 19 different strains was able to oxygenate progesterone at C₁₁. The transformation products, other than 11 α -hydroxyprogesterone, which were formed by these active strains had been identified. These studies proved that the active organisms were capable of introduction of oxygen function at different positions other than position 11 of progesterone *e.g.* positions 17 and 21. Side chain degradation reaction with and without lactone formation of ring D of progesterone had been also found to be performed by some of these organisms.

Of the many chemical changes brought about in steroids by microorganisms the one at C₁₁ is by far the most important. The microbial-11- α hydroxylations of progesterone, PETERSON and MURRAY (1), especially with *Rhizopus arrhizus* served as the initial impetus in the study of microbiological transformation from the practical and technical view. Hydroxylation of steroids in that strategic position is performed by many fungi other than *Rhizopi*. Several stimulatory reviews on this subject have been appeared during the last ten years *e.g.* VISCHER and WETTSTEIN (2), PETERSON (3), VAN DYKE (4) and CAPEK *et al.* (5)

The present work aims to study the ability of different representatives of locally isolated microorganisms to introduce 11- α -hydroxyl group into progesterone. Identification of 11 α -hydroxyprogesterone as well as other transformation products formed by the active strains were further carried out in a trial to throw some light on the possible utilization of these reactions in the preparation of some cortical hormones.

EXPERIMENTALS

Organisms. The different organisms used in this work were collected from the centre of cultures of the Microbiological Chemistry Lab., N.R.C.,

Cairo. These strains were isolated from local habitats and identified through the American Type Culture Collection, Washington; the Centraalbureau Voor Schimmelcultures, Baarn (Holland) and the B.I.R.F. (England).

Cultivation. The medium formulated by CAPEK *et al.* (6) was used as a nutritive solution. This medium has the following composition (g/liter): glucose, 40.0; peptone, 1.0; KH_2PO_4 , 0.74; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0; Yeast extract, 1.0 and asparagine, 0.7. The pH was adjusted to 5.6. The cultivation was performed in 250-ml Erlenmeyer flasks each containing 50 ml medium. The flasks were sterilized at 1 atmosphere for 30 min, and inoculated with 2 ml inoculum of a 48 hr cultures of the pure organism. The culture flasks were agitated on a reciprocal shaker (110 strokes/min, amplitude 7 cm) at $30^\circ \pm 2$ for 48 hr. Thereafter, 20 mg of progesterone, dissolved in 1 ml 96% ethyl alcohol, was added to each flask and fermentation was continued for another 72 hr.

Extraction. At the end of the fermentation period, the contents of each flask (medium+mycelium) were homogenized in a blender (8,000 rpm) with two volumes of chloroform (100 ml). The extraction was repeated three times in order to ensure that all the transformation products were extracted. The combined chloroform extracts were washed with half its volume 5% sodium bicarbonate solution; followed by an equal volume distilled water, dried over anhydrous sodium sulphate, filtered, then distilled.

Analysis of the transformation mixtures. The residues obtained after evaporation of the chloroform extracts were dissolved separately in 5 ml chloroform-methanol (1: 1). Analysis was carried out by the thin-layer chromatographic technique on silica gel G plates as described by the authors in the next article SALLAM *et al.* (7). The following two solvents: cyclohexane: chloroform: iso-propanol (100: 50: 20) and cyclohexane: acetone: chloroform (75: 25: 20) proved to be satisfactory for the resolution of all the transformation products encountered during this work. The developed plates were sprayed with I_2/KI solution. The fact that 11α -hydroxyprogesterone gave a characteristic blue colour with this reagent was used to select the organisms capable of oxygenation of progesterone at C_{11} position. The transformation products other than 11α -hydroxyprogesterone were further identified by studying the thin-layer chromatographic picture of each product compared with the authentic steroids upon using different solvent systems and colour reagents (SALLAM) (7) *et al.*

RESULTS AND DISCUSSION

Oxygenation of progesterone at C_{11}

The data presented in Table 1 showed the ability of the different experimental organisms to transform progesterone to 11α -hydroxyprogesterone. The presence of 11α -hydroxylation was indicated by the sign (+). The different strains of *Rhizopus nigricans* tested as well as most of the *Aspergilli*, except

Table 1. The ability of different microorganisms to oxygenate the C₁₁ of progesterone.

Organism	11 α -OH Prog.
<i>Streptomyces</i> sp. A1	---
<i>Streptomyces</i> sp. A2	---
<i>Streptomyces</i> sp. A3	---
<i>Streptomyces</i> sp. A4	---
<i>Streptomyces</i> sp. A5	---
<i>Saccharomyces cerevisiae</i>	---
<i>Saccharomyces carlsbergensis</i>	---
<i>Saccharomyces delbruecki</i>	---
<i>Saccharomyces fermentati</i>	---
<i>Rhodotorula minuta</i>	---
<i>Torulopsis famata</i>	---
<i>Cryptococcus neoformans</i>	---
<i>Geotrichum candidum</i> (Link)	---
<i>Mucor fragilis</i> (Bain)	+
<i>Cunninghamella echinulata</i> (Curie & Thom)	---
<i>Verticillium dahliae</i> (Klebahn-F. Zonatum V. Beyma)	---
<i>Penicillium notatum</i> (Westl)	---
<i>Penicillium oxalicum</i> (Curie & Thom)	---
<i>Penicillium requeforti</i> (P-21-1)	---
<i>Penicillium chrysogenum</i> (Dierckx)	---
<i>Penicillium brevicompactum</i>	+
<i>Cladosporium cladosporioides</i> (Fres) de Vries powdery type	+
<i>Cladosporium herbarum</i> (Link)	---
<i>Helminthosporium speciferum</i> (Bain)	--
<i>Aspergillus niger</i> (AMN)	---
<i>Aspergillus niger</i> (74)	---
<i>Aspergillus niger</i> (3 N)	+
<i>Aspergillus niger</i> (58F)	+
<i>Aspergillus niger</i> (73)	+
<i>Aspergillus niger</i> (100)	+
<i>Aspergillus niger</i> (200)	+
<i>Aspergillus niger</i> (567)	+
<i>Aspergillus niger</i> (1 R)	+
<i>Aspergillus niger</i> (108)	+
<i>Aspergillus niger</i> (37)	+
<i>Aspergillus fischeri</i> (Wehmer)	+
<i>Aspergillus terreus</i>	---
<i>Aspergillus flavus</i>	---
<i>Aspergillus fumigatus</i> (Fres)	---
<i>Aspergillus ochraceus</i> (Wilhelm)	+
<i>Aspergillus uncinatus</i> (518) ^a	+
<i>Rhizopus nigricans</i> (LTF) ^a	+
<i>Rhizopus nigricans</i> (YSF) ^a	+
<i>Rhizopus nigricans</i> (REF) ^a	+
<i>Fusarium oxysporium</i>	---
<i>Fusarium equiseti</i> (Forma)	---
<i>Fusarium semitectum</i>	---
<i>Fusarium sambucinum</i> (Forma)	---
<i>Alternaria tenuis</i> (Nees)	---
<i>Alternaria tenuis</i> (Auct)	---
<i>Gibberella fujikuroi</i> (117)	---
<i>Epicoccum purpurascens</i>	+

^a These organisms are isolated and identified by the authors.

Aspergillus niger AMN and 74; *Asp. terreus*; *Asp. flavus* and *Asp. fumigatus*, showed rather extensive ability to perform 11α -hydroxylation. On the other hand, the strains of *Fusarium* spp., *Alternaria* spp., *Streptomyces* spp. and Yeast spp. fail to produce 11α -hydroxylations. Moreover, the *Penicillium* spp. investigated except *Pen. brevicompactum* were unable to carry out this reaction. These results are in close agreement with the findings of many authors e.g. DULANEY *et al.* (8), CAPEK and HANC (9, 10).

Transformation products other than 11α -hydroxyprogesterone

It was rather of interest to identify the transformation products, other than 11α -hydroxyprogesterone, produced by the active strains. The results tabulated in Table 2 showed that almost every strain investigated seemed to possess a certain type remarkable activity in its steroid transforming reaction. That is, although all the *Aspergilli* gave 11α -hydroxy and 6β , 11α -dihydroxyprogesterone derivatives, other products were detected with the various strains tested. For instance, with *Asp. fischeri* and *Asp. suniculosus*, 11α -hydroxyallopregnane was formed. The latter compound was also detected as a transformation product with *Rhiz. nigricans* LTF 127 and REF 129 respectively. *Asp. niger* 100 and 567, *Asp. fischeri*, *Rhiz. nigricans* REF 129 and *Epicoccum purpurascens* were able to introduce hydroxyl group at C_{21} of progesterone. Furthermore, 11α , 21-dihydroxyprogesterone was detected with *Asp. niger* 3N, 200, 567 and 1R, respectively. ZAFFARONI *et al.* (11) confirmed the microbiological 21-hydroxylation of several steroids by the use of *Asp. niger*. Furthermore, WEISZ *et al.* (12) and WIX *et al.* (13) found that a strain of *Asp. niger* was able to introduce both 11α and 21 hydroxyl groups simultaneously into progesterone, while RUBIN *et al.* (14) showed that *Curvularia lunata* affected 11β , 21 dihydroxylations of progesterone. Reactions of this type are of considerable importance since these two hydroxyl groups are constituents of most of the antinflammatory steroids, PETERSON (3).

Carbon 17 is another site of hydroxyl group representing an important constituent of the biologically significant adrenal cortical hormones and derivatives. Oxygenation at that position was successfully achieved by *Cladosporium cladosporioides*, *Asp. niger* 37 and 100, and *Rhiz. nigricans* REF 129. The microbiological introduction of the tertiary 17α -hydroxyl group along with simultaneous introduction of the biologically important oxygen at C_{11} was also observed with the local strains, *Clad. cladosporioides*, *Asp. niger* 100 and 37, *Asp. fischeri* and *Rhiz. nigricans* REF 129. Progesterone was recorded to be transformed to 11α , 17α -dihydroxyprogesterone derivative by *Cephalothecium roseum*, MEISTER *et al.* (15); *Dactylium dendroides*; *Scopulariopsis brevicaulis* and *Tricostema roseum*; TIMOFEEVA *et al.* (16) and DULANEY and MCALEER (17).

An interesting reaction is that recorded with *Clad. cladosporioides*, *Asp. niger* 100 and *Rhiz. nigricans* REF 129 whereby progesterone was hydroxylated at C_{11} , C_{17} and C_{21} positions to give 11α , 17α , 21-trihydroxyprogesterone

Table 2. Transformation of progesterone by the 11 α -hydroxylator *microorganisms*.

Organism	Transformation		Products ^a	
	Mono-hydroxy	Dihydroxy	Trihydroxy	Other products
<i>Mucor fragilis</i> (Bain)	11 α ; 15 α	6 β , 11 α	----	----
<i>Penicillium brevi-compactum</i>	11 α ; 15 α	----	----	testololactone testosterone androstendione
<i>Cladosporium cladosporioides</i> (Fres, de Vries powdery type)	11 α ; 11 β ; 17 α	6 β , 11 α ; 11 α , 17 α	11 α , 17 α , 21 11 β , 17 α , 21	----
<i>Aspergillus niger</i> (3 N)	11 α	6 β , 11 α ; 11 α , 21	----	----
<i>Aspergillus niger</i> (58 F)	11 α	6 β , 11 α	----	----
<i>Aspergillus niger</i> (73)	11 α	6 β , 11 α	----	----
<i>Aspergillus niger</i> (100)	11 α ; 17 α ; 21	6 β , 11 α ; 11 α , 17 α	11 α , 17 α , 21	----
<i>Aspergillus niger</i> (200)	11 α	6 β , 11 α ; 11 α , 21	----	----
<i>Aspergillus niger</i> (567)	11 α ; 21	6 β , 11 α ; 11 α , 21	----	----
<i>Aspergillus niger</i> (1R)	11 α	6 β , 11 α ; 11 α , 21	----	----
<i>Aspergillus niger</i> (37)	11 α ; 17 α	11 α , 15 β ; 11 α , 17 α	----	----
<i>Aspergillus fischeri</i> (Wehmer)	11 α ; 11- hydroxy allopreg- nane; 21	11 α , 17 α	----	testololactone
<i>Aspergillus ochraceus</i> (Wilhelm)	11 α	6 β , 11 α	----	----
<i>Aspergillus suniculis</i> (518)	11 α ; 11- hydroxy allopreg- nane	6 β , 11 α	----	testololactone
<i>Rhizopus nigricans</i> (YSF 128)	11 α	6 β , 11 α	----	----
<i>Rhizopus nigricans</i> (REF 129)	11 α ; 11 α - hydroxy allopregn- ane; 17 α	6 β , 11 α 11 α , 17 α	11 α , 17 α , 21	----
<i>Epicoccum purpurascens</i>	11 α ; 11 β ; 21	6 β , 11 α ; 11 α , 21	----	----

^a Mono, di and trihydroxy=monohydroxylation, dihydroxylation and trihydroxylation of progesterone at the different recorded positions.

(epicortisole). Furthermore, cortisole (11β , 17α , 21-trihydroxyprogesterone) was also produced with *Clad. cladosporioides*. According to the available literatures, the only report on the microbiological hydroxylation of progesterone at these three carbon atoms with *Rhiz. nigricans* and *Cunninghamella blakesleeana* was given by CIBA (18).

Other interesting reaction is the detection of the epimers 11α ; and 11β hydroxylations with *Clad. cladosporioides* and *Epic. purpurascens*. Both of these bio-oxidations, viz., 11α -hydroxylation of progesterone and 11β -hydroxylation of compound S by *Curvularia lunata* have been scaled up in large production plants as a mean of making the cortical hormones and their derivatives, SHULL and KITA (19). SHIRASAKA *et al.* (20) also reported the transformation of progesterone to 11α and 11β hydroxyderivatives by *Botrytis fabae*. Moreover, CAPEK and HANK (10) showed that the proportion of 11α , and 11β epimers depended on steroid substrate. In progesterone, the best ratio obtained with *Absidia orchidis* was $11\alpha: 11\beta=7: 1$.

The microbiological side chain degradation with and without lactonization of ring D of progesterone was observed with *Asp. fischeri*, *Asp. suniculosis* and *Pen. brevi-compactum*. These findings are in harmony with PETERSON *et al.* (21) and FRIED *et al.* (22) who showed that C_{21} -steroids such as progesterone, desoxycorticosterone and compound S could be converted to J^4 -androstene 3, 17-dione, as well hydroxylated steroids and testololactone, using *Gliocladium* spp., *Penicillia* and *Aspergilli*. SEBEK *et al.* (23) also noted that *Pen. lilacinum* metabolized progesterone successively to 20β -hydroxy-4-prognen-3-one, testosterone, androstenedione and testololactone, of which testosterone and androstenedione were readily interconvertible.

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