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METABOLISM OF PROGESTERONE BY *FUSARIUM OXYSPORUM*

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SUMMARY

Progesterone was found to be transformed by *F. oxysporum* first to 1:2-dehydroprogesterone, then to 1:2-dehydrotestosterone, further to 1,4-androstadiene-3,17-dione and finally to 1:2-dehydrotestololactone (13α-hydroxy-3-oxo 13,17-seco-androst-1,4-dien 17-oic-lactone) as revealed by a thin-layer chromatographic method. These metabolites were isolated and identified.

INTRODUCTION

THE ABILITY to hydroxylate the steroid molecule may be considered as a generic characteristic of the genus *Fusarium*. However, certain strains of these fungi are also capable of splitting off the side chain of progesterone and introducing the 1:2-double bond thus giving rise to 1,4-androstadiene-3,17-dione. Of all hitherto tested *Fusaria* only three species, *F. solani*, *F. caucasicum* and *F. lateritium*[1], can produce 1,4-androstadiene-3,17-dione from progesterone, while *F. javanicum var. ensiforme*[2, 3] is known to effect, in addition, concomitant 11α-hydroxylation. Investigating the effect of various factors on the transformation of progesterone to 1,4-androstadiene-3,17-dione by *Fusarium spp.*, we found that this transformation is effected not only by the already known 1,4-androstadiene-3,17-dione producing *Fusaria*, but also by *F. oxysporum*[4]. It seemed, therefore, worthwhile to elucidate the pathway of this transformation.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Rotations were taken in $CHCl_3$ in a 1 dm cell at 20°. Infrared spectra were recorded with a Perkin–Elmer Model 521 spectrophotometer. Thin-layer chromatography was done either on 0·25 or 1·5 mm thick silica gel *G* chromatoplates using cyclohexane-ethyl acetate (1:2) as the developing phase and 50 per cent sulphuric acid for detection as previously described[5].

The reference compounds used were: progesterone (Merck) 1,4-androstadiene-3,17-dione (Koch-Light), 1:2-dehydro-testololactone (Dr. O. Hanč, Prague). 1:2-dehydrotestosterone was prepared from testosterone and 1:2-dehydroprogesterone from progesterone by the reaction with 2,3-dichloro-5,6-dicyanobenzoquinone according to Burn and Petrow[6].

F. oxysporum used in this study was obtained from the Phytopathological Institute at the University of Zagreb, Yugoslavia, and its identity confirmed by the Department of Microbiology, Chemical Institute "Boris Kidrič".

Incubation

F. oxysporum spores were maintained at 28° on potato agar slants. The cultures were grown in 500 ml flasks containing 100 ml of the medium composed of 0·005 per cent glucose, 0·6 per cent corn steep liquor (50 per cent dry weight) and 1·5 per cent peptone. Before sterilisation, the pH was adjusted to 6·5 with diluted alkali. After 48 hr of incubation at 28° on a reciprocal shaker, 20 mg of progesterone was added to each flask and the incubation continued for 24 hr.

Isolation and identification of metabolites

At definite time intervals (2,4,6,8,10,12 hr) the pooled broths obtained by the incubation of 1·2 g of progesterone, were extracted with methylene dichloride. The combined extracts were dried over anhydrous sodium sulphate and evaporated under reduced pressure. The oily residue (880 mg) was dissolved in chloroform–methanol (2:1) and applied to preparative thin-layer chromatoplates. Steroidal products were eluted with methanol and purified by repeated chromatography and recrystallisation.

RESULTS

When the transformation of progesterone by *F. oxysporum* was followed by the thin-layer chromatographic method of Sočič and Belič[5], the presence of five compounds was revealed. By comparing their R_f value in cyclohexane: ethyl acetate system, colour reaction and u.v. fluorescence with authentic samples they were tentatively identified as progesterone (R_f:0·65), 1:2-dehydroprogesterone (R_f:0·53), 1,4-androstadiene-3,17-dione (R_f:0·45), 1:2-dehydrotestosterone (R_f:0·35) and 1:2-dehydrotestololactone (R_f:0·20).

After 2 hr of incubation, three main spots corresponding to progesterone, 1:2-dehydroprogesterone and 1,4-androstadiene-3,17-dione, respectively, and a small fourth spot, corresponding to 1:2-dehydrotestosterone, were visible. After 6 hr of incubation, the same four compounds were present but the relative amount of progesterone had decreased, whereas that of 1,4-androstadiene-3,17-dione had greatly increased and 1:2-dehydroprogesterone and 1:2-dehydrotestosterone were present in small amounts. In addition, the over-oxidation product, 1:2-dehydrotestololactone, began to appear after 3 hr of incubation and increased with the time. Complete transformation of progesterone was achieved after 12 hr of incubation. The production of the principal metabolite, 1,4-androstadiene-3,17-dione, reached a maximum of 69±7 per cent in 6–8 hr as determined by the Kober–Haenni reaction[6].

The resulting metabolites isolated by preparative thin-layer chromatography in crystalline form, were additionally characterized on the basis of the following properties:

1:2-dehydroprogesterone (cryst. from aqueous methanol) m.p. 151–153°C; $[\alpha]_D$ + 124°; $\lambda_{max.}^{ETOH}$: 244 mμ; $\lambda_{max.}^{Nujol}$: 5·87, 6·0, 6·12, 6·22 μ.
Literature[8] requires: m.p. 151°; $[\alpha]_D$ + 122°; $\lambda_{max.}^{ETOH}$: 244 mμ.
1:2-dehydrotestosterone (cryst. from aqueous methanol) m.p. 168°–172°C; $\lambda_{max.}^{ETOH}$: 243 mμ; $\lambda_{max.}^{Nujol}$: 5·95, 6·0, 6·15, 6·25 μ.
Literature[8, 9] requires: m.p. 167°–168°C; λ_{max}^{ETOH}: 243 mμ.
1,4-androstadiene-3,17-dione (cryst. from ether) m.p. 141°–144°C; $[\alpha]_D$ + 108°; $\lambda_{max.}^{ETOH}$: 243 mμ; $\lambda_{max.}^{Nujol}$: 5·72, 6·0, 6·15, 6·22 μ.
Literature[8–10] requires: m.p. 140°–144°C; 138°–139·5°C; 145°–146°C; $[\alpha]_D$ + 115°; 110°; $\lambda_{max.}^{ETOH}$: 243 mμ.
1:2-dehydrotestololactone (cryst. from aqueous methanol) m.p. 217°–219°C; $[\alpha]_D$ − 46°; $\lambda_{max.}^{ETOH}$: 242 mμ; $\lambda_{max.}^{Nujol}$: 5·82, 6·0, 6·12, 6·23 μ.
Literature[8, 9] requires: m.p. 214°–215°C; 218°–219°C; $[\alpha]_D$ − 43·7°; − 44°; $\lambda_{max.}^{ETOH}$; 242 mμ.

In all the above cases mixed melting points showed no depression and infrared spectra were superimposable on those of authentic samples. The infrared spectra of the different steroids isolated are indicated in Figs. 1(a–d).

Infrared spectra of the different steroids isolated

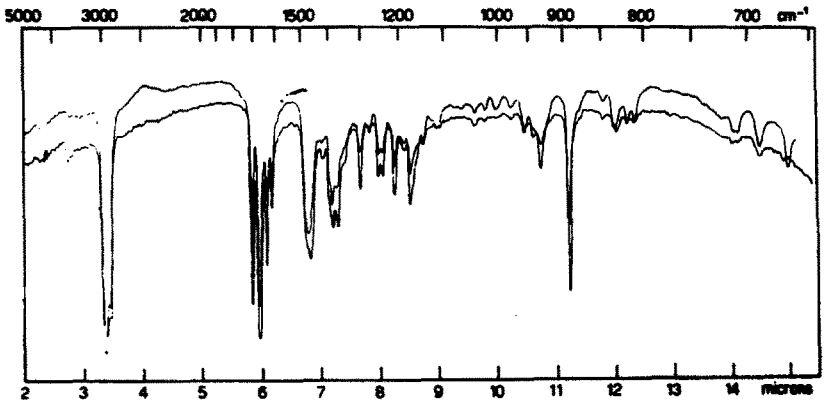

Fig. 1(a). Infrared spectra of 1:2-dehydroprogesterone (*above*; synthetic steroid: *below*; isolated steroid).

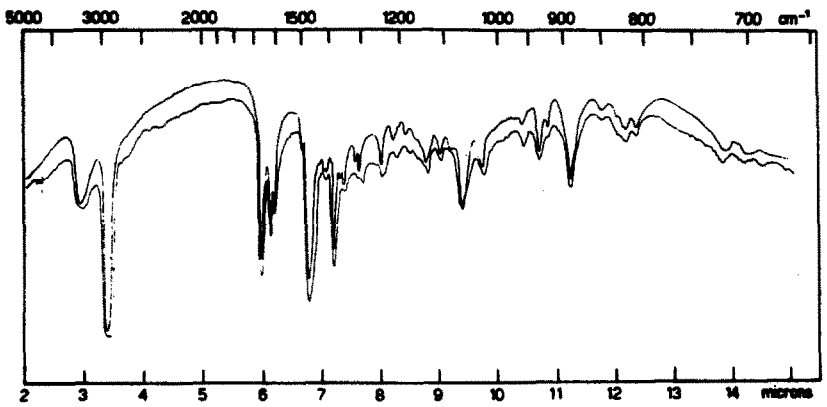

Fig. 1(b). Infrared spectra of 1:2-dehydroprogesterone (*above*; synthetic steroid: *below*; isolated steroid).

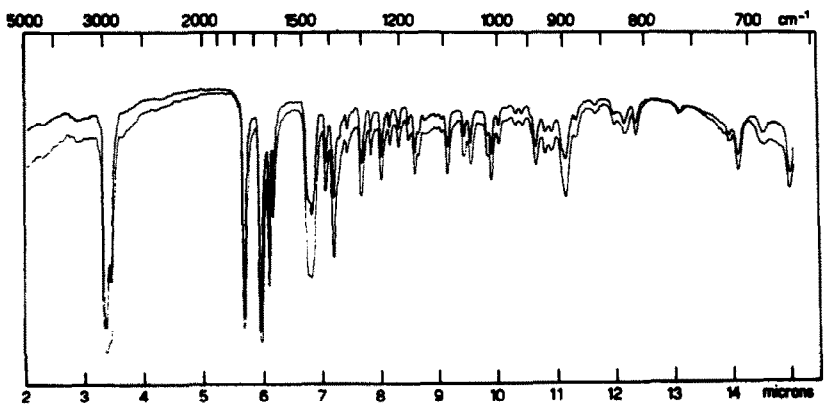

Fig. 1(c). Infrared spectra of 1,4-androstadiene-3,17-dione (*above*; synthetic steroid: *below*; isolated steroid).

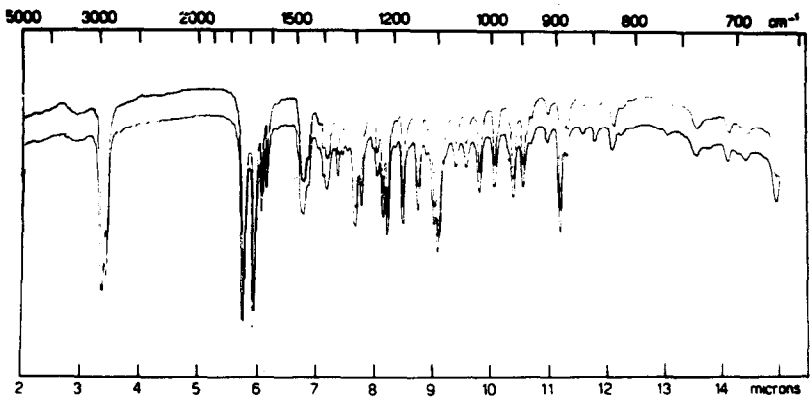

Fig. 1(d). Infrared spectra of 1,4-androstadiene-3,17-dione (*above*; synthetic steroid: *below*; isolated steroid).

DISCUSSION

Since Wisher and Wettstein's[10] discovery of the ability of *F. solani* and *F. caucasicum* to produce 1,4-androstadiene-3,17-dione from progesterone, several attempts have been made to determine the metabolic pathway of this transformation. Nishikawa, Noguchi, Hasegawa and Bano[8] found that with *F. solani* the first step in this transformation is the introduction of the 1:2 double bond resulting in the formation of 1:2-dehydroprogesterone which is further transformed to 1:2-dehydrotestosterone and then to 1,4-androstadiene-3,17-dione. On the other hand, Szpilvogel, de Winter and Alsche[11] found for *F. solani var. eumarthii* and Wix and Albrecht[12] for *F. caucasicum*, that the first intermediate is 4-androstene-3,17-dione, which in turn is transformed to 1,4-androstadiene-3,17-dione.

Later, this problem was re-examined more extensively by Čapek and Hanč [13], who studied the pathway of several 1,4-androstadiene-3,17-dione producing *Fusaria*. In the first place they found no difference in the metabolic pathway of progesterone in all the tested *Fusaria* and in the second, that the side chain of progesterone was first split off, as a consequence of which 4-androstene-3,17-dione and testosterone were simultaneously formed. It should be noted, however, that only Nishikawa *et al.* proved the presence of the described metabolites by actual isolation and identification, the other authors limiting themselves to paper chromatographic methods only.

Our findings that *F. oxysporum* produces 1,4-androstadiene-3,17-dione differ from those of Čapek and Hanč, according to which *F. oxysporum* belongs to the predominant group of hydroxylating *Fusaria*. Such discrepancies are not uncommon and the fact, pointed out by Fried, Thoma, Perlman, Herz and Borman [14], that different compounds are produced by the same species must be ascribed to differences in the strains employed. Our results indicate that *F. oxysporum* transforms progesterone first to 1:2-dehydroprogesterone, which in turn is metabolized to 1,4-androstadiene-3,17-dione; 1:2-dehydrotestosterone is most probably an intermediate in the latter step. Finally, 1,4-androstadiene-3,17-dione is over-oxidized to 1:2-dehydrotestololactone, which is the end product common to both pathways of the progesterone transformation by *Fusaria*.

The transformation of progesterone by *Fusarium oxyporom* is indicated in Fig. 2.

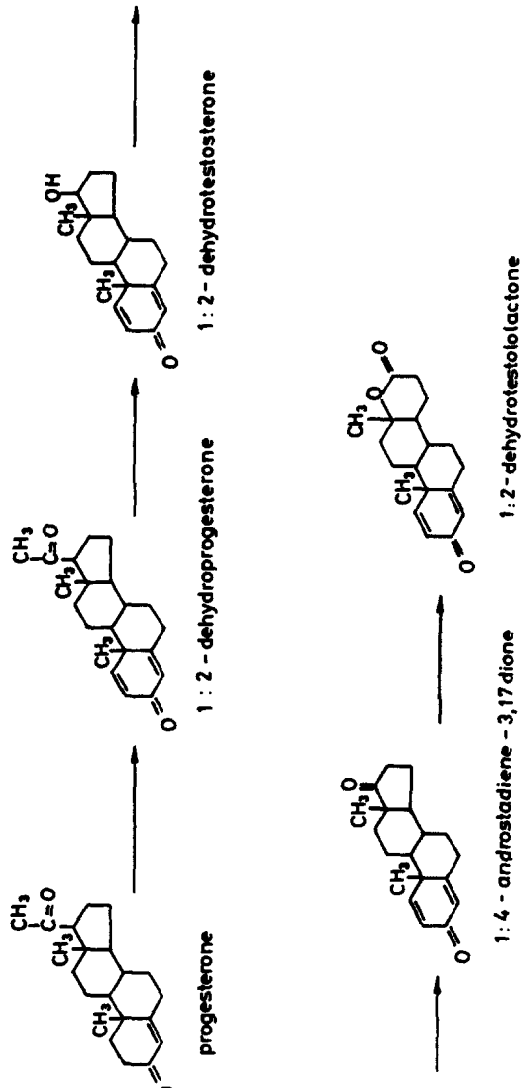

Fig. 2. Transformation of progesterone by *Fusarium oxysporum*.

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