The Effect of Certain Steroids Upon the Growth of *Trichophyton rubrum*

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SUMMARY

The effect of thirty-seven different steroids on the growth of *Trichophyton rubrum* is reported. The effects varied from complete inhibition of growth to stimulation. Highly inhibitory compounds included androstan-3,17-dione, testosterone, 11-deoxycorticosterone, progesterone and 7-dehydrocholesterol. 17 α -Hydroxypregnenolone was stimulatory but all other compounds with the 17 α -hydroxyl group were inactive. Structural requirements for activity are discussed.

INTRODUCTION

Steroid derivatives have been reported to influence micro-organisms in a variety of ways—being essential growth factors for certain species (e.g. Edward & Fitzgerald, 1951; Butler & Knight, 1960; Van Wagtendonk, Conner, Miller & Rao, 1953); stimulators of growth for others (Buetow & Levedahl, 1957; Jefferson & Sisco, 1959); but most frequently growth inhibitors (e.g. Lester, Stone & Hechter, 1958). Included among the organisms for which inhibitions by steroids have been reported are several dermatophytes (Reiss, 1949; Tarbet, Oura & Sternberg, 1953; Casas-Campillo, Balandrano & Galarza, 1960). The dermatophytes are of particular interest since changes in the incidence of fungal infections have been noted at times of variation in the content of steroid hormones in the tissue. Microsporium infections usually cure spontaneously at puberty and dissemination of *Trichophyton* infections has been noted in association with Cushing's syndrome (Nelson & McNiece, 1959) and during cortisone therapy (Cremer, 1955). Thus steroids may influence either directly or indirectly the development of these infections and it seemed of interest to study in more detail the nature of the effect of these substances upon the fungi themselves. A preliminary report (Chattaway, Townsley & Barlow, 1959) showed the effect of a small number of steroids upon several dermatophytes. In the present paper this has been extended to a study of the effect of 37 steroids upon *Trichophyton rubrum*.

METHODS

Organism. The strain used was the OS strain of *Trichophyton rubrum* isolated from a foot infection in 1958.

Medium and inoculum. The growth medium contained (per 9.5 ml.): L-glutamic acid, 21 mg.; KH₂PO₄, 18 mg.; MgSO₄.7H₂O, 1 mg.; D-glucose, 100 mg.; D-biotin,

20 $\mu\text{g.}$; thiamine hydrochloride, 200 $\mu\text{g.}$; i-inositol, 200 $\mu\text{g.}$ Aqueous solutions adjusted to pH 6.8 containing glutamic acid, vitamins and phosphate were autoclaved (121° for 10 min.) separately from those containing glucose and magnesium sulphate. Medium (9.5 ml.) was placed aseptically in 100 ml. conical flasks and its volume made 10 ml. by addition of the inoculum, which consisted of homogenized washed mycelium suspended in sterile water, having been grown initially on Sabouraud's maltose broth.

Steroids were added in chloroform ethanol (1 + 1, v/v) solution to the sterile 100 ml. conical flasks and the solvent removed by evaporation for 18 hr. at 33° before addition of medium.

Measurement of growth. This was done by determination of mycelial dry weight by filtering on to dried and weighed Whatman No. 1 filter paper (3.0 cm. diam.), washing three times with 50 ml. distilled water and drying at 105° for 18 hr. All such determinations were made in triplicate and after incubation at 33° for 7 days.

RESULTS

The earlier survey showed that 11-deoxycorticosterone was highly inhibitory and the effect of different concentrations showed that 50 % inhibition of growth occurred at 17 $\mu\text{g./ml.}$ and maximal inhibition at 75 $\mu\text{g./ml.}$

For routine testing steroids were examined at 250 $\mu\text{g./ml.}$ medium; the results are shown in Table 1. All compounds were tested on at least three separate occasions. Results are expressed as percentage inhibition or stimulation of growth in the absence of steroid. The normal growth was determined at the same time. Some of the compounds previously tested were included for completeness. While no close correlation of structure with activity can be discerned, some structural requirements can be seen. A considerable number of the compounds with high inhibitory activity are C_{17} or C_{19} steroids and all such compounds have a C_3 carbonyl group; reduction of this to a 3 α - or 3 β -hydroxyl group leads to considerable decrease or complete loss of inhibitory effect. Among inhibitory compounds the C_3 carbonyl group is often accompanied by unsaturation between C_4 and C_5 , but this is not essential for activity since androstan-3,17-dione and 5 β -androsten-3-one-17 β -ol displayed high activity. The activity of the latter compound also suggests that the planarity of the steroid nucleus is not an essential feature. The nature of substituents at C_{17} afford some interesting comparisons. Thus high activity is seen with a C_{17} carbonyl, β -hydroxyl, β -acetyl and β -acetoxy groups and with an 8-carbon side-chain, but the presence of a 17 α -hydroxyl group leads to complete loss of activity. Substitution at other sites (e.g. C_9 or C_{11}) also leads to reduction or loss of activity.

The series of sterols is of some interest since activity is seen with 7-dehydrocholesterol, lumisterol and ergosterol all of which have a $\Delta^{5,7}$ -diene structure while the six compounds with the 3 β -hydroxyl and Δ^5 double bond all show no inhibition or slight stimulation. Combination of this structure with a 17-hydroxyl group as in 17 α -hydroxypregnenolone causes appreciable stimulation of growth.

There appears to be no parallel between inhibitory power and known function in mammals; thus 11-deoxycorticosterone displays high activity while 2 α -methyl-9 α -fluoro-hydrocortisone, the most active mineralocorticoid known, was without inhibitory effect. Similarly corticosterone displayed some activity while the much

more powerful glucocorticoid 16 α -methyl-9 α -fluoro-prednisolone was without activity. Also compounds such as androstan-3,17-dione showed high activity while possessing no great hormonal activity in the mammal.

Table 1. *Effect of steroids on growth of Trichophyton rubrum, strain OS*

Results are expressed as % inhibition (+ve) or stimulation (–ve) of growth in the presence of 250 μ g. steroid/ml. medium as compared with growth obtained in the absence of added steroid. The steroids are listed in order of decreasing inhibitory effects.

Compound	No. of expts.	Mean % inhibition or stimulation
Androstan-3,17-dione	4	96
Δ^1 -androsten-3,17-dione	4	96
Nortestosterone	3	94
11-deoxycorticosterone*	4	91
Δ^4 -androsten-3,17-dione	4	89
5 β -androstan-3-one-17 β -ol	3	88
Calciferol*	4	79
Testosterone*	4	77
7-Dehydrocholesterol*	4	77
$\Delta^1,^4$ -androstadiene-3-one-17 β -ol	3	72
Progesterone*	4	70
5 α -androstan-3-one-17 β -ol	3	62
11 α -hydroxyprogesterone*	4	59
Androsterone	3	57
Corticosterone	4	44
Lumisterol	4	42
Oestradiol-17 β *	4	39
Oestradiol-17 α *	4	39
Ergosterol	3	28
Epitestosterone	3	20
Oestrone	3	11
Δ^5 -androsten-3 β ,17 β -diol	3	10
17 α -hydroxyprogesterone	3	10
Prednisolone	3	7
Androstan-3 α ,17 β -diol	3	4
Aldosterone†	1	0
Pregnenolone	3	0
Hydrocortisone*	4	–1
Cortisone*	4	–3
17 α -hydroxy-11-deoxycorticosterone	4	–4
Lanosterol*	4	–5
2 α -methyl-9 α -fluorohydrocortisone	3	–9
9 α -fluorohydrocortisone	3	–12
Stigmasterol	3	–14
Cholesterol*	4	–18
Sitosterol	3	–18
17 α -hydroxypregnenolone	3	–37

* Previously reported (Chattaway *et al.* 1959).

† 50 μ g./ml. medium.

DISCUSSION

There has been some emphasis in the literature on the marked ability of 11-deoxycorticosterone to inhibit fungal growth; thus Lester *et al.* (1958) reported it as the most active steroid in a group of 33 which inhibited the growth of *Neurospora crassa*,

Tarbet *et al.* (1953) found a similar result with *Microsporium gypseum* and Casas-Campillo *et al.* (1960) reported that it, together with 19-norprogesterone, was the most effective inhibitor of *Trichophyton mentagrophytes*. The present study also shows this compound to have high activity against *T. rubrum*, but nor-testosterone and androstane-3,17-dione were equally potent inhibitors and compounds with such widely different biological activity as calciferol and progesterone showed activity little below that of 11-deoxycorticosterone. Thus little support is given by the present work to the concept that steroids in micro-organisms may control processes analogous to those under hormonal control in mammals. Evidence in support of this concept was advanced by Lester & Hechter (1959) in the effect of 1-deoxycorticosterone on sodium and potassium concentration in *N. crassa* and by the same authors (1961) in the antagonistic effects displayed by oestradiol-17 β and 11-deoxycorticosterone upon the growth of *Penicillium puberulum*. The inhibitory activity of testosterone on *T. rubrum* is comparable with that reported by Reiss (1949) for dermatophytes, Lester *et al.* (1958) for *N. crassa* and Maxwell, McGuire & Tomkins (1960) for *Saccharomyces fragilis*. Tarbet *et al.* (1953), however, found it to be slightly stimulatory for *M. gypseum* when assayed by its effect on the germination of macroconidia. Similarly androstan-3,17-dione and Δ^1 -androstene-3,17-dione were reported to have fungistatic effects (Maxwell *et al.* 1960) and the inactivity of 17 α -hydroxy steroids has been reported by a number of workers. Thus in general it appears that growth of *T. rubrum* was optimally inhibited by C₁₇ or C₁₉ steroids with a C₃-carbonyl substituent associated with a variety of C₁₇ substituents of the β -configuration and that such activity was decreased by modifications at other sites in the molecule and abolished by the presence of a 17 α -hydroxyl group.

Of the sterols tested ergosterol has been reported as inhibitory for *Penicillium chrysogenum*; stimulatory for the growth of other moulds (Matkovics, 1957, Matkovics & Sipos, 1959) and without effect upon *Neurospora crassa* (Lester *et al.* 1958). The low inhibitory activity of ergosterol for *Trichophyton rubrum* is of interest since this compound has been found in the normal mycelium (unpublished results). The considerable inhibitory power of 7-dehydrocholesterol, calciferol and lumisterol does not appear to have been recorded previously. The low stimulatory power of cholesterol, stigmasterol and sitosterol together with the marked stimulation by 17 α -hydroxypregnenolone suggests that structures may be found which have even greater powers of stimulation.

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