Response of *Achlya ambisexualis* E87 to the Hormone Antheridiol and Certain Other Steroids

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SUMMARY

Of 53 steroids tested for their ability to induce the formation of antheridial branches in the water mould *Achlya ambisexualis* Raper, strain E87, 34 were inactive, including 18 steroids having hormonal activity in mammals and six related to antheridiol, a C-29 steroid controlling the formation of antheridia in *Achlya*. The C-22, C-23 stereoisomers of antheridiol and two intermediates in its synthesis had about 10^{-3} to 10^{-6} the activity of antheridiol. Certain modifications in the side chain of antheridiol resulted in a loss of activity.

INTRODUCTION

In *Achlya*, a filamentous water mould, the male sex organ (antheridium) is formed at the tip of a lateral branch produced in response to antheridiol, a C-29 steroid (Barksdale, 1969; Raper, 1942). This compound has been isolated from the culture liquid of *Achlya bisexualis* Coker & Couch (strains T5 and 369) and *Achlya ambisexualis* Raper (strain 734) (McMorris & Barksdale, 1967; Green, Edwards, Barksdale & McMorris, 1971). The methods used at The New York Botanical Garden and at Syntex Research for the synthesis of antheridiol involve the steroid 3- β -hydroxy-22,23-bisnor- Δ^6 -cholenaldehyde on which the antheridiol side chain is constructed. Four stereoisomers are obtained in this synthesis, as well as several intermediates, which differ from antheridiol mainly in the structure of the side chain (Edwards, Mills, Sundeen & Fried, 1969; Edwards *et al.* 1972; McMorris, Arunachalam & Seshadri, 1972 and unpublished results; and McMorris & Seshadri, 1971). These compounds and a number of unrelated steroids have been tested for the ability to evoke a response from *Achlya ambisexualis* E87, a strain which reacts to extremely low concentrations of antheridiol (Barksdale, 1963, 1969). The results of these tests are discussed in this paper.

METHODS

Source of steroids (Tables 1 and 2). Compounds (18), (19) and (20) were synthesized at Syntex Research. The last one (20) is the C-22 epimer of 23-deoxy-antheridiol, a steroid which is present with antheridiol in culture liquids of *Achlya*. The other compounds were prepared in the laboratory of The New York Botanical Garden.

Steroids having hormonal activity in mammals were obtained from Calbiochem. Ecdysterone, which is a moulting hormone isolated from a crustacean, was purchased from

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Mann Research Laboratories, New York. Strophanthidin was kindly supplied by Dr A. Yoda of the University of Wisconsin. The remaining steroids were either obtained commercially or prepared by standard methods and are of interest because of their relationship to the steroids in Table 1 or else important to the synthesis of these compounds.

Preparation of solutions. Each of the compounds in Table 1 was dissolved in methanol. The solutions of antheridiol isomers (Table 2) were diluted further with methanol to give a concentration of 10 or 20 $\mu\text{g/ml}$. Serial aqueous dilutions (1:100, 1:1000, etc.) were then made from the methanolic solutions.

The steroids inactive at 5.0 $\mu\text{g/ml}$ were dissolved in ethanol to give a concentration of 1 mg/ml and 1 ml of each solution was diluted with an equal part of water. The steroids inactive at 0.5 to 1.0 $\mu\text{g/ml}$ were dissolved in absolute methanol from which aqueous dilutions of 1:100 and 1:1000 were made.

Bioassay. One ml of each aqueous dilution of compounds (1) to (25) (Table 1) and other compounds in methanol was added to 10 ml of culture fluid in a Petri dish containing a mycelial mat of *Achlya ambisexualis*, strain E87 (diameter of mat approximately 60 mm; dry wt 150 to 160 mg), which had been grown from zoospores for 72 h at 25 °C. Only 0.1 ml of the solution in 50 % ethanol was added to the mat. The composition of the culture medium was as follows (per litre of distilled water): Edamin (hydrolysed lactalbumin from Sheffield Chemical of Norwich, Connecticut), 400 mg; glucose, 2400 mg; calcium glycerophosphate, 80 mg; 2 M-KCl, 0.5 ml; 0.5 M-MgSO₄·7H₂O, 1.0 ml; Metal Mix No. 4 solution, 0.5 ml (see Barksdale, 1963, for formula); tris (hydroxy methyl)amino-methane, 100 mg; pH adjusted to 6.8 with HCl.

The assay dishes were incubated at 25 °C for 2 h and then examined for the presence of antheridial branches. Strain E87 will produce antheridial branches in response to as little antheridiol as 5 to 10 picograms/ml. The concentrations given in Tables 1 and 2 are either the minimal concentration evoking a response or the highest concentration that failed to evoke a response.

RESULTS AND DISCUSSION

The most significant conclusion to be drawn from the assays is the importance of the stereochemistry at C-22 and C-23 in antheridiol (Tables 1 and 2). The *erythro* isomer (4) (22R, 23S) and the *threo* isomer (10) (22S, 23S) were less than 1/1000 as active as antheridiol (1) (22S, 23R). The *threo* isomer (7) (22R, 23R) appeared to be even less active. This great difference in activity raises the question of whether the weak activity of the isomers may have been the result of contamination with antheridiol. The four compounds were prepared from the acetates of 7-deoxy-7-dihydroantheridiol and its C-22, C-23 isomers, i.e. (3), (6), (9) and (12). The acetates were, in turn, obtained from a condensation which created two new asymmetric centres leading to four stereoisomers. Although (3) was a minor product (13 %) compared with (6), the major product (67 %), and although the isomers could be cleanly separated by chromatography, it is possible that contamination of (6), (9) and (12) by (3), or its hydrolysed derivative (2), would explain their activity. On the other hand, the activity observed with (18) suggests that the hydroxy butenolide system of antheridiol is not a necessary condition for some biological activity. Compounds (4) to (12) (Table 1) may therefore possess inherent activity.

The 7-keto group appears to be relatively unimportant, since antheridiol (1) was only 20 times as active as its 7-deoxy analog (2). Compound (2) and its acetate (3) had the same activity, which suggests that a free hydroxyl at C-3 is not essential for full biological activity.

Table 1. Structure and activity of antheridiol and related steroids: minimal concentration inducing branching or highest concentration not inducing branching in *A. ambisexualis* E87

[See Table 2 for activity of compounds (1-12)]

Table 2. *Minimal concentration of stereoisomers of antheridiol inducing branching in Achlya ambisexualis E87*

Stereoisomer	Erythroisomer		Threoisomer	
	22S 23R (ng/ml)	22R 23S (ng/ml)	22R 23R (ng/ml)	22S 23S (ng/ml)
Antheridiol	(1) 0.006	(4) 20	(7) 400	(10) 20
7-deoxy-7-dihydro-antheridiol	(2) 0.110	(5) 40	(8) 400	(11) 40
7-deoxy-7-dihydro-antheridiol 3-acetate	(3) 0.110	(6) 40	(9) > 200*	(12) 40

* Highest concentration tested.

Numbers in brackets refer to compounds in Table 1.

Oxidation of the 22-hydroxyl causes a sharp fall in activity in the case of compound (3). The product from (3) and (9) is (14), while that from (6) and (12) is (13). It is noteworthy that (14), which has the same configuration as antheridiol at C-23, was considerably more active than (13). [The sample of (14) was prepared from (9), so the difference in activity cannot be attributed to contamination.] The activity was completely destroyed on oxidation to (15).

The furan (16) was tested as a mixture with its inactive isomer (22) in a proportion of two parts of (22) to one part of (16), so (16) itself would be expected to be three times more active than shown. The activity of (16) is interesting because the furan and the butenolide are closely related. Formally, the furan can be converted to the butenolide by hydroxylation at C-28 in (16), followed by rearrangement. Furans and related butenolides have been isolated from the same source, and it has been proposed that the former are precursors of the latter (Novotný, Herout & Šorm, 1964).

Except for (23), the compounds (21) to (25) were all found to be inactive. This is not surprising since they all differ appreciably in structure from antheridiol. The activity of (23) is almost certainly the result of contamination with (3). It was obtained from the same reaction which produces (3).

If compound (24) (McMorris, 1970) was added with antheridiol in a ratio of 50 nanograms (24):0.25 nanogram (1), it completely inhibited the formation of antheridia on antheridial branches in strain E87.

The mammalian hormones and related compounds inactive at 5.0 µg/ml were: 21-hydroxy-4-pregnene-3,20-dione (desoxy-corticosterone); estrone; 5β-pregnane-3α,20α-diol diacetate; cortisone; 5α-pregnane-3β,20β-diol; 3β-hydroxy-5α-androstan-17-one; 3α-hydroxy-5α-androstan-17-one; progesterone; 21-hydroxy-4-pregnene-3,11-,20-trione (11-dehydrocorticosterone); 3α-hydroxy-5α-androstan-17-one; 17α,21-dihydroxy-4-pregnene-3,20-dione (Reichstein's substances); 5β-pregnane-3α,20α-diol; 3β-hydroxy-5-androsten-17-one; 3α-hydroxy-5β-androstan-17-one; hydrocortisone; 4-androstene-3,11,17-trione; estradiol; and estriol. Ecdysterone and strophanthidin were also inactive at 5.0 µg/ml.

The steroids inactive at 0.5 to 1.0 µg/ml were: stigmasterol; 7-keto-stigmasterol; 7ξ-hydroxystigmasterol; 7-ketostigmasteryl acetate; cholesterol; 3β-hydroxy-5-cholenic acid; 3β-acetoxy-7-keto-22,23-bisnor-5-cholenic acid; and 3β-hydroxy-7-keto-22,23-bisnor-5-cholenic acid.

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