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SYNTHESIS OF HIGHLY TRITIATED 7-DEOXY-7 DIHYDROANTHERIDIOL AND ANTHERIDIOL*

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SUMMARY

The synthesis of tritiated male-activating steroids of the aquatic fungus *Achlya*, 7-deoxy-7-dihydroantheridiol (7-DA) and antheridiol, has been achieved by aldol condensation of 3β-acetoxy-23,24-dinorchola-1,5-dien-22-al and the carbanion of 3-isopropyl-2-butenolide. The product with the desired stereochemistry (22S,23R) was isolated and reduced with $^3H_2$ in the presence of tris(triphenylphosphine)rhodium chloride. Acid hydrolysis of the acetate gave 1,2[^3H]–7*DA* which had a specific activity of 40 Ci/mmol. 1,2[^3H]–7*DA* was converted to 1,2[^3H]-antheridiol by protecting the hydroxyl groups as the disilyl ethers, oxidation to the 7-ketone with chromium trioxide-dimethylpyrazole and removal of the protecting groups by gentle acid treatment. 1,2[^3H]–7*DA* has been used to detect a protein receptor in the cytosol of *Achlya*.

Key Words: fungal sex steroids, cytosol receptors.

INTRODUCTION

Although there is now a fairly well accepted scheme for the early events of steroid hormone action, the biochemical mechanism of action for all steroid hormones remains unknown.

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There is little information on the effects of steroid binding on the receptor molecule, how the receptor complex moves to nuclear sites, or what the receptor complex does at nuclear sites to alter gene expression.

An attractive organism for studying such questions is the primitive eukaryote *Achlya (1)*. Female strains of this fungus, eg *A. ambisexualis* 734, secrete the steroid antheridiol (1) which acts on male strains, eg *A. ambisexualis* E87, inducing differentiation of sexual hyphae (antheridial branches). Antheridiol also induces the production of further sex steroids, oogoniol (2) and dehydrooogoniol (3) by the male. The latter steroids cause sexual differentiation in the female leading to formation of the oogonium *(2)*.

The hyphae of *A. ambisexualis* E87 are extremely sensitive to antheridiol and under suitable conditions will respond to concentrations of antheridiol as low as 6 picograms/mL ($10^{-11}M$) within two hours.

Developmental responses include an early stimulation of RNA and protein synthesis within the first few hours. Inhibitor studies show that the morphological changes depend upon RNA and protein synthesis *(3,4)*. These results are not unlike those obtained for steroid hormone action in higher organisms. Therefore, although *Achlya* cells differ greatly from cells in the rat uterus or chick oviduct, it is tempting to speculate that the basic mechanism of steroid hormone action in these target cells may be quite similar.

In this paper we report on our efforts to synthesize tritium-labelled antheridiol with a specific activity sufficient to initiate studies on the binding and fate of the hormone in target cells.

Several approaches were studied and are summarized as follows:

A. The most expedient method for obtaining a tritiated compound was to synthesize antheridiol-3-acetate using [^3H]-acetic anhydride.[4] This was prepared with a specific activity of about 3 Ci/mmol. Antheridiol acetate is biologically active, but it is not known whether the acetate remains intact within the organism. Initial attempts to observe specific binding of this compound to *Achlya* were unsuccessful.

B. We investigated ways of introducing the label into ring C of the steroid. Specifically, a derivative containing an 11,12 double bond was prepared with the hope of introducing tritium. However, selective reduction of this double bond was not possible. In another approach the 12-keto derivative of 7-deoxy-7-dihydroantheridiol (7-DA) was prepared. However, attempts to introduce

deuterium were not very efficient and resulted in a compound with rather low incorporation of isotope.

C. A tritium-labelled steroid was prepared by reduction of the 3-keto derivative of 7-DA with NaB^3H_4 (10-15 Ci/mmol). Initial attempts to measure specific binding with this compound were not promising. In retrospect, this was due to the low specific activity (approximately 5 Ci/mmol).

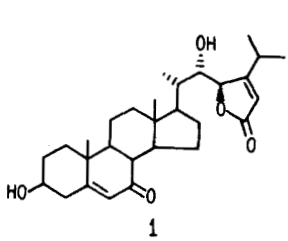
D. The most successful approach has been the synthesis and tritiation of the acetate of 1,2-dehydro-7-DA (**7**). Thus 1,2-[^3H]-7-*DA* (**8**) has been prepared with a specific activity of 40 Ci/mmol. Our synthetic plan was similar to that which had been employed for the synthesis of 7-DA and antheridiol *(5)*. This required a dinor-cholenaldehyde derivative to which the sidechain containing the lactone ring could be attached by means of an aldol reaction.

RESULTS AND DISCUSSION

Commercially available 20-(hydroxymethyl)pregna-1,4-dien-3-one (**4**) proved to be a most suitable starting material. The hydroxyl group in this compound was protected by forming the tetrahydropyranyl (THP) ether. A solution of the derivative in dimethyl sulfoxide-diethyl ether was treated with potassium *t*-butoxide and the resulting dienolate anion solution was poured into ice-water thus generating the 1,5-dien-3-one derivative. Reduction of the C-3 ketone with $Ca(BH_4)_2$ furnished the corresponding C-3β alcohol (**5**) which was converted to the acetate with acetic anhydride and pyridine. The tetrahydropyranyl protecting group was then removed by mild acid treatment and the resulting alcohol was oxidised to the aldehyde (**6**) with pyridinium chlorochromate. The overall yield of aldehyde (**6**) from the starting compound (**4**) was 60%.

In order to construct the side chain of antheridiol, an aldol reaction was carried out with the aldehyde (**6**) and the anion of 3-isopropyl-2-butenolide which was prepared as follows: bromination of 3-methyl-2-butanone under kinetic conditions afforded the 1-bromo derivative. The bromo group was displaced by acetate on refluxing a solution of the derivative in acetone with anhydrous KOAc. Reaction of the resulting keto-acetate with the anion from triethylphosphonoacetate afforded the desired butenolide in 55% yield.

A solution of the butenolide in freshly distilled tetrahydrofuran was added to a cold (-40°C) solution of lithium triphenylmethide, generated from triphenylmethane and n-butyllithium. The resulting solution of butenolide anion was cooled to -72°C and a solution of the aldehyde (**6**) was added gradually. The reaction mixture was allowed to warm to 0°C and then quenched with NH_4Cl solution.



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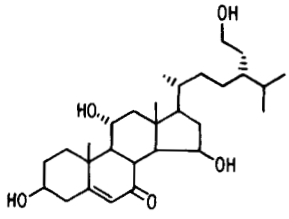

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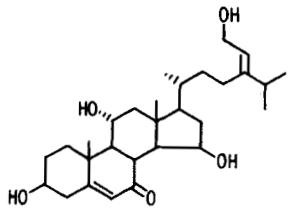

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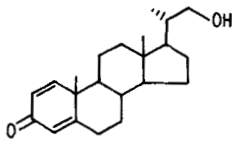

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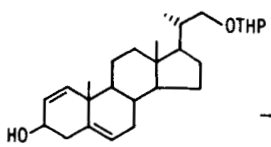

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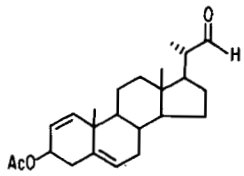

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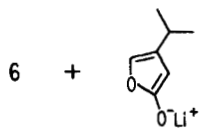

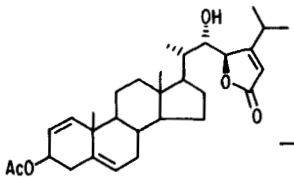

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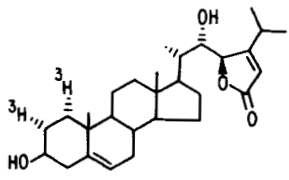

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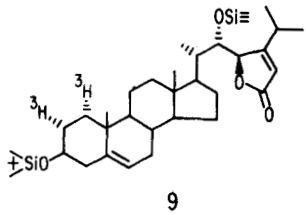

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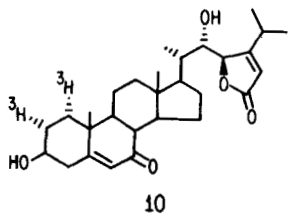

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Chromatography of the crude product gave the desired isomer (7,22S,23R) in low yield (3%). The major product was the 22R,23S isomer (29%), and smaller amounts of the 22R,23R (10%) and 22S,23S (3%) isomers were obtained as well. The preferred stereochemistry at C-22 in the aldol reaction is predicted by the Cram or Felkin-Anh models for the transition state *(6)*. The stereochemistry at C-23 can be rationalized in terms of steric factors as was indicated in the recently reported synthesis of brassinolide by a similar aldol reaction *(7)*.

The intermediate (7) possesses two trisubstituted double bonds and one disubstituted double bond. The latter would be expected to be more reactive than the former to catalytic hydrogenation and it has been reported that the Δ^1 double bond in steroids can be selectively hydrogenated in the presence of a Δ^5 double bond by employing tris-(triphenylphosphine)rhodium chloride as catalyst *(8)*. When a solution of intermediate (7) and this catalyst were shaken in an atmosphere of deuterium, at 20 psi for 24 hours, a high yield of the desired 1,2-dideutero compound was obtained, and the other double bonds were unaffected.

A sample of compound 7 was therefore submitted to Amersham Laboratories, Arlington Heights, Illinois, for reduction with carrier free tritium gas. The crude product (50 mCi) was returned and was analyzed by thin layer chromatography (TLC) with solvent system, chloroform-methanol 98:2. There was one major radioactive spot which corresponded in Rf to authentic 7-deoxy-7-dihydroantheridiol-3-acetate (Rf 0.46), and one unidentified spot (Rf 0.93).

The radioactive acetate was converted to 7-DA by heating the solution in dioxane with 5% aqueous H_2SO_4. Analysis of the hydrolysis product (chloroform-methanol, 95:5) showed one major spot (Rf 0.40) which corresponded to authentic (7-DA), another spot (Rf 0.76) for the acetate of 7-DA, and an unidentified spot (Rf 0.96). The 7-DA was purified by TLC to give a single radioactive spot. The yield was 24.8 mCi at 97% radiochemical purity.

In order to determine the specific activity of the radioactive 7-DA, samples of unlabelled 7-DA of known weight were converted to the silyl derivatives with N,0-bis(trimethylsilyl)acetamide. The derivatives were injected into a gas chromatography system and the areas of the resulting peaks were measured. A standard curve based on triplicate injections of each concentration was plotted of area versus sample weight. Samples of the purified radioactive 7-DA (approximately 0.5 mCi each) were derivatized and injected into the chromatography system as above. From the observed area of the peak, the specific activity of 1,2-[^3H]-7*DA* was determined to be approximately 40 Ci/mmol.

7-DA can be converted to antheridiol by photooxygenation followed by rearrangement but the yield is rather low (< 50%). We investigated an alternative route which involved protecting the hydroxyl groups in 7-DA as the silyl ethers, oxidation with CrO_3-dimethylpyrazole complex to introduce the ketone, and deprotection of the hydroxyls with mild acid treatment. This method gave reproducible yields (54%) of antheridiol. 1,2-[^3H]-*7-DA* was therefore treated with *t*-butyldimethylsilylchloride in the presence of dimethylaminopyridine and triethylamine to form the 3-silylether. Trimethylsilylchloride and more triethylamine were added to silylate the C-22 hydroxyl. The disilylether was purified by thin layer chromatography and then oxidised with CrO_3-dimethylpyrazole complex in methylene chloride. The crude reaction product was treated with dilute acetic acid and the resulting 1,2-[^3H]-*antheridiol* was then isolated by chromatography.

Since 7-DA has good biological activity (about 10% that of antheridiol), as soon as 1,2-[^3H]-*7DA* was prepared it was tested and was found to bind with high affinity to a macromolecular component of the cytosol of male cells. This receptor is specific, saturable and of low capacity and partial characterization has shown it to be remarkably similar to steroid receptors in higher organisms *(9,10)*. Further work is now in progress to isolate the receptor.

EXPERIMENTAL METHODS

General. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. NMR spectra were taken in $CDCl_3$ with Me_4Si as internal standard on Varian EM 390 (80 MHz) and IBM NR 80 (90 MHz) spectrometers. Mass spectra were determined with a Kratos MS 30 spectrometer. Column chromatography was carried out with silica gel (230-400 mesh) and thin layer chromatography with silica gel 60F-245 plates (E. Merck Laboratories). Dry tetrahydrofuran (THF) was prepared by heating the reagent grade liquid at reflux over potassium in a circulating still. In the work-up of reactions, the solutions containing the products were washed with aqueous Na_2SO_4 solution (10%), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure.

23,24-Dinorchola-1,4-dien-3-one-22-ol-THP ether. 20-(Hydroxymethyl)pregna-1,4-dien-3-one, 4,(3.28 g, 10.0 mmol) was dissolved in 100mL methylene chloride. Dihydropyran (0.925 g, 11.0 mmol) and pyridinium 4-toluenesulfonate (0.100 g, 0.40 mmol) were added and the solution was stirred at 25°C for 18 h. The solution was poured into 200 mL of 5% $NaHCO_3$ solution, the layers were separated and the aqueous layer extracted with an additional 100 mL methylene chloride. On work-up the combined organic extracts yielded 3.92 g (95%) of a colorless oil which crystallized on standing.

An analytical sample was recrystallized from hexane: mp 127-9° C; NMR δ 0.75 (s, 18H), 1.05 (d, J=6 Hz, 21*H*), 1.24 (s, 19*H*), 6.08 (d, J=2 Hz, 4H), 6.23 (dd, J=2 Hz, 10 Hz, 2H), 7.05(d, J=10 Hz, 1H). IR (KBr) 1665, 1628, 1602 cm^{-1}. Anal. Calcd for $C_{27}H_{40}O_3$: C, 78.60; H, 9.77. Found: C, 78.89; H, 10.16.

23,24-Dinorchola-1,5-dien-3β,22-diol-22-THPether (**5**). The dienone (3.30 g, 8.00 mmol) was dissolved in 90 mL DMSO and 30 mL diethyl ether under a nitrogen atmosphere and then cooled to 10° C. Potassium *t*-butoxide (4.50 g, 40.2 mmol) was added and the solution was stirred at 10-15° C for 1 h. The solution was then slowly poured into 500 mL of rapidly stirred ice-water. The aqueous mixture was extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were washed with 10% Na_2SO_4 solution (3 x 150 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent removed at reduced pressure.

$NaBH_4$ (1.50 g, 39.5 mmol) in 120 mL 95% ethanol was added to a solution of $CaCl_2$ (3.0 g, 27 mmol) in 75 mL methanol at -10° C. After 15 min at -10° C, a solution of the crude reaction product from above in 50 mL THF was added during 5 min to the $Ca(BH_4)_2$ solution. The mixture was stirred at -10°C for 30 min, and then poured into 500 mL 10% NaCl solution. The resulting mixture was extracted with ethyl acetate (2 x 150 mL). Work up of the combined extracts gave a product which was triturated with hexane and filtered to yield 2.65 g (80%) of **5** as white crystals, mp 116-119°C. An analytical sample was recrystallized from toluene-hexane, mp 120-121°C. NMR δ 0.72 (s, 3, 18H), 1.07 (d, J=6 Hz, 21*H*), 1.11 (s, 19*H*), 4.25 (m, 3H), 4.54 (m, THP methine-H), 5.40 (m, 6H), 5.54 (d, J= 10 Hz, 1H) 5.78 (dd, J=10, 1.6 Hz, 2H)., IR (KBr) 3400 cm^{-1}. Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.10; H, 10.18.

23,24-Dinorchola-1,5-dien-3β,22-diol-3-acetate-22-THPether. The alcohol **5**(2.49 g, 6.00 mmol) was dissolved in pyridine (25 mL) and acetic anhydride (2.5 mL, 24.0 mmol). The reaction mixture was stirred at 25°C for 16 h, and then added to 200 mL 5% $NaHCO_3$ solution. The resulting mixture was extracted with ether (2 x 100 mL) and the combined organic extracts were washed with 5% $NaHCO_3$ solution (100 mL) and worked up to yield 2.65g (97%) of a colorless oil, homogeneous by TLC.

23,24-Dinorchola-1,5-dien-3β,22-diol-3-acetate. The acetate from above (2.28g, 5.00 mmol) was dissolved in 100 mL THF. Concentrated HCl (1.0 mL) was added, and the resulting solution was stirred at 25°C. for 2 h. The solution was added to 5% $NaHCO_3$ solution (150 mL) and the whole

extracted with ethyl acetate (2 x 100 mL). The combined organic extracts on work-up gave the crude product which was triturated with hexane and filtered to yield 1.67 g (91%) of a white crystalline solid, mp 105-107°C. An analytical sample was recrystallized from hexane, mp 107-108°C; NMR δ 0.73 (s, 18*H*), 1.05 (d, J=7Hz, 21*H*), 1.10 (s, 19H), 2.06 (s, acetate), 5.17 (m, 3H), 5.37 (m, 1H 6H), 5.80 (dd J=10, 2Hz, 2H); IR (KBr) 3350, 1740 cm^{-1}. Anal. Calcd for $C_{24}H_{36}O_3$: C, 77.38; H, 9.74. Found: C, 77.44 H, 9.86.

3β-Acetoxy-23,24-dinorchola-1,5-dien-22-al (**6**). The alcohol (1.48 g, 4.0 mmol) was dissolved in methylene chloride (100 mL). Pyridinium chlorochromate (1.72 g, 8.0 mmol) was added and the reaction mixture was stirred at 25°C for 18 h. Ether (100 mL) was added and the mixture was filtered through a short pad of TLC grade silica, and the pad washed with an additional 200 mL of 1:1 diethyl ether-methylene chloride. The combined filtrate was evaporated to dryness to yield the aldehyde **6** as light yellow crystals (1.30 g, 88%), mp 123-126°C. The product was recrystallized from hexane to yield 1.15 g (78%) of **6**: mp 127-9°C; NMR δ 0.75 (s, 18H), 1.11 (s, 19H), 1.12 (d, J=6 Hz 21H), 2.06 (s, acetate), 5.17 (m, 3H), 5.40 (m, 1H 6H), 5.80 (dd J=10, 2Hz, 2H), 9.58 (d, J=3 Hz, 22H), IR (KBr) 2730, 1740, 1730 cm^{-1}. Anal. Calcd for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found: C, 77.65, H, 9.32.

3-Isopropyl-2-butenolide. A solution of 3-methyl-2-butanone (43.0 g, 0.50 mol) in methanol (500 mL) was cooled to -15°C. Bromine (80.0 g, 0.50 mol) was added rapidly in one portion. The cooling bath was removed and the reaction was allowed to warm. After approximately 25 min, the solution became colorless and was immediately added to ice cold 5% $NaHCO_3$ solution (1L). The mixture was extracted with petroleum ether (3 x 150 mL). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated to a volume of 75 mL. This crude lachrymatory product was then added to a refluxing solution of anhydrous KOAc (80 g, 0.82 mol) in acetone (1L). After 18 h, the mixture was poured into water (2 L) and the whole extracted with diethyl ether (3 x 300 mL). The combined ether extracts were washed with 5% aqueous $NaHCO_3$ solution, dried ($MgSO_4$), filtered, concentrated and then distilled (110-115°C at 3mm) to yield 54.0 g(75%) of the ketoacetate: NMR δ 1.15 (d, J=7 Hz, isopropyl CH_3), 2.2 (acetate), 2.75 (septet, J=7 Hz, methine H), 4.77 (s, methylene H).

Triethylphosphonoacetate (84.0 g, 0.375 mol) was added over 30 min to a cooled suspension of 50% NaH (9.0 g, 0.375 mol) in THF (350 mL), while maintaining the temperature below 30°C. The resulting solution was cooled to 15°C and the ketoacetate (54.0 g, 0.375 mol) was added over 5 min. After 30 min at 25°C, the solution was poured into 10% aqueous Na_2SO_4 solution (1L), which was

then extracted with diethyl ether (3 x 200 mL). The combined ether extracts were washed (10% Na_2SO_4), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting yellow oil was distilled (80-90°C at 0.3 mm) to yield 42.9 g of a mixture of the butenolide and the diester. The mixture was dissolved in methanol (200 mL) and 6N aqueous NaOH (200 mL). After standing 24 h at 25°C, the reaction mixture was acidified to pH 1 with concentrated HCl. After 1 h, the solution was poured into 10% aqueous Na_2SO_4 solution (1 L) and the whole extracted with diethyl ether (3 x 200 mL). The ether extracts were dried ($MgSO_4$) and distilled (80-85°C at 0.3 mm) to yield 34.5 g (73% from the ketoacetate) of the butenolide: NMR δ 1.22 (d, J=7 Hz, isopropyl CH_3), 2.75 (m, methine H), 4.78 (d, J=2 Hz, methylene H), 5.79 (q, vinyl H), IR (NaCl) 1785, 1750, 1635 cm^{-1}.

Condensation of **6** *with 3-isopropylbutenolide.* Triphenylmethane (0.50 g, 2.0 mmol) was dissolved in anhydrous THF (5 mL) and the solution was cooled to -40°C under N_2 atmosphere. *n*-Butyllithium (1.0 mmol, 0.105 mL of 9.5 M solution in hydrocarbon solvent) was added; the stirred mixture was allowed to warm to 25°C, and then maintained at 25°C for 1 h. The mixture was cooled to -40°C, and a solution of β-isopropylbutenolide (0.126 g, 1.0 mmol) in THF (2 mL) was added dropwise during 5 min. The pale yellow solution was cooled to -72°C. A solution of **6** (0.370 g, 1.0 mmol) in THF (3 mL) was added dropwise during 10 min. The mixture was kept at -72°C, for 45 min then warmed to 0°C. After an additional 1.5 h, the reaction mixture was added to saturated NH_4Cl solution (50 mL) and the whole extracted with diethyl ether (2 x 50 mL). The crude product from work up was purified by silica gel column chromatography (hexane-ethyl acetate). After an initial forerun of 120 mL, 20 mL fractions were collected. Unreacted aldehyde **6** (0.100 g) was eluted in fractions 3-7. Fractions 38-41 contained nearly pure **7** (0.011 g) which was re-chromatographed and recrystallized from ether-hexane to yield pure **7**; mp 155-156°C; NMR δ 0.72 (18H), 1.06 (d, J=7 Hz, 21H), 1.10 (19H), 1.17 and 1.22 (pair of d, J=7 Hz, 26H and 27H), 2.06 (acetate), 3.6 (m, 22H), 4.9 (d, J=9 Hz, 23H), 5.17 (m,3H), 5.43 (m, 1H and 6H), 5.8 (t, J=1 Hz, 28H), 5.9 (dd, J=10, 2Hz, 2H), IR (KBr) 1760, 1740, 1625 cm^{-1}; mass spectrum m/z 436 (M^+-60), 418.

Fractions 42-49 contained the 22R,23S, isomer of **7** (0.104g). The product was recrystallized from ethyl acetate-hexane; mp 209-210°C; NMR δ 0.73 (18H), 1.04 (d, J=6.5 Hz, 21H), 1.10 (19H), 1.18 and 1.23 (pair of d, J=7 Hz, 26H and 27H), 2.06 (acetate), 3.6 (m, 22H), 4.9 (d, J=9 Hz, 23H), 5.23 (m, 3H), 5.47 (m, 1H and 6H), 5.8 (t, J=1 Hz, 28H), 5.9 (dd, J=2, 10 Hz, 2H); IR (KBr) 1820, 1768, 1742, 1718, 1630 cm^{-1}. Mass spectrum m/z 436, 418.

Fractions 55-57 contained the 22R,23R isomer of 7 (0.038 g). The product was recrystallized from ethyl acetate-hexane; mp 214-216°C; NMR δ 0.75 (18H), 1.09 (d, J=7 Hz, 21H), 1.10 (19H), 1.17 and 1.23 (pair of d, J=7 Hz, 26H and 27H), 2.03 (acetate), 3.9 (broad d, J=5 Hz, 22H), 4.9 (broad s, 23H), 5.20 (m, 3H), 5.43 (m, 1H and 6H), 5.73 (t, J=1 Hz, 28H), 5.87 (d, J=11 Hz, 2H); IR(KBr) 1760, 1740, 1640 cm^{-1}; mass spectrum m/z 436, 418.

Fractions 60-61 contained the 22S,23S isomer of 7 (0.012 g). The product was recrystallized from ethyl acetate-hexane; mp 216-218°C; NMR δ 0.77 (18H), 1.12 (d, J=7 Hz, 21H), 1.12 (19H), 1.20 and 1.23 (pair of d, J=8 Hz, 26H and 27H), 2.15 (acetate), 3.9 (broad s, 22H), 5.03 (broad s, 23H), 5.20 (m, 3H), 5.43 (m, 1H and 6H), 5.77 (t, J=1 Hz, 28H), 5.87 (d, J=11 Hz, 2H); IR (KBr) 1765 (sh), 1735, 1635 cm^{-1}; mass spectrum m/z 436, 418.

1,2[^2H]-7-deoxy-7-dihydroantheridiol-3-acetate. The acetate 7 (4.90 mg, 0.01 mmol) was dissolved in anhydrous benzene (10 mL). To the solution was added $(PPh_3)_3RhCl$ (5.0 mg, 0.006 mmol). The reaction flask was evacuated and deuterium was introduced. A pressure of 20 psi was established and the reaction flask was shaken vigorously for 24 h at 25°C. The reaction mixture was then filtered through a short pad of TLC grade silica. The silica was washed with 50 mL of a 70:30 mixture of benzene-ethyl acetate. Evaporation of the solvent yielded the 1,2 dideutero compound (4.0 mg): NMR δ 0.72 (18H), 1.03 (19H), 1.05 (d, J=7 Hz, 21H), 1.18 and 1.23 (pair of d, J=7 Hz, 26H and 27H), 2.03 (acetate), 3.6 (d, J=8.5 Hz, 22H), 4.91 (d, J=8.5 Hz, 23H), 5.4 (m, 6H), 5.77 (t, J=1 Hz, 28H); mass spectrum m/z 440, 422, 314.

1,2[^3H]7-deoxy-7-dihydroantheridiol **(8).** The acetate 7 (0.490 mg, 1.00 μmol) was submitted to Amersham Laboratories for reduction with carrier free tritium gas. The procedure described for deuterium reduction was followed on 1/10 scale. The crude product (50 mCi) was returned in toluene solution (50 mL). The product was analyzed by TLC followed by zonal scraping of the 5 x 20 cm glass TLC plate (silica, solvent system: 98:2 chloroform-methanol). The silica was mechanically removed in 0.4 cm segments and transferred to forty scintillation vials. After solubilization of the product from the silica by the addition of ethanolic acetic acid (5 mL) to each vial, scintillation fluid (15 mL) was added and the vials were counted. The crude product showed one major radioactive peak at *Rf* 0.46 (68.7%) which corresponded in *Rf* to authentic 7-DA-3-acetate, and one unidentified peak (20.9%) at *Rf* 0.93.

One half of the crude product (25 mL toluene solution) was transferred to a 30 mL conical glass vial and the toluene was removed in a stream of N_2. The residue was dissolved in a solution of dioxane (4 mL) and 5% aqueous H_2SO_4 (1 mL). The solution was heated at 100°C for 1.5 h, cooled to 25°C, and an aqueous solution of 10% Na_2SO_4 (5 mL) was added. The reaction mixture was extracted with chloroform (5 x 4 mL), and the combined extracts were dried (Na_2SO_4) and filtered. Analysis of the crude hydrolysis product by TLC with zonal scraping (solvent system: 95:5 chloroform-methanol) showed one major peak at *Rf* 0.40 (52.4%) which corresponded to authentic 7-DA, one peak at *Rf* 0.76 (18.1%) which corresponded to 7-DA-3-acetate, and one unidentified peak at *Rf* 0.96 (19.1%). The chloroform solution was reduced in volume under a stream of N_2 to ca. 100 μL. The solution was applied to a 5 x 20 cm^2 silica gel TLC plate (0.5 mm thickness), and the plate was developed in a 95:5 chloroform-methanol solvent system. After the plate was dried, a zone corresponding to *Rf* 0.30 to 0.55 was scraped from the plate. The silica was extracted with absolute ethanol (5 x 2 mL) and the mixture was filtered through a plug of glass wool. The purified product was analyzed by zonal scraping (TLC solvent system: 95:5 chloroform-methanol), and showed a single radioactive peak at *Rf* 0.44 (97%). Yield: 12.4 mCi at 97% radiochemical purity.

Determination of Specific Activity. Five standard solutions of authentic 7-DA at 10, 20, 30, 40, and 50 ng/μL were prepared. Aliquots (100 μL) of each were taken to dryness and treated with pyridine (25 μL) and N,O-bis(trimethylsilyl)acetamide for 1 h at 60°C. The samples were taken to dryness and redissolved in chloroform (100 μL). Samples (1 μL) of each standard concentration were injected into a Carlo Erba gas chromatography system (260°C, Hewlett-Packard OV-1 capillary column, 30 m x 0.32 mm, helium carrier gas). The column peaks were detected by flame ionization and were recorded on a Hewlett-Packard integrator model 3388. The derivatized standards migrated as a single peak with a retention time of 5.7 min. A standard curve of area vs. sample weight was plotted based on triplicate injections of each concentration.

Two samples of the purified radioactive steroid **8** (approximately 0.5 mCi each) were treated as described for the unlabelled standards. The mass of each sample was determined by plotting GC area vs. the standard curve. Triplicate injections of each sample were made. Specific activity was then calculated by determining the radioactivity present in aliquots (1 μL) of each sample. Samples 1 and 2 were found to be 42.6 and 39.3 Ci/mmol respectively.

1,2-[^3H]-7-deoxy-7-dihydroantheridiol-3-t-butyldimethylsilyl-22-trimethylsilylether (**9**). Another

sample of acetate 7 (5 mg, 10 μmol) was tritiated as above and the crude product (198 mCi) was returned as a solution in benzene-ethyl acetate (7:3). One third of the product was converted to 1,2-[^3H]-7-DA which was silylated with *t*-butyldimethylsilylchloride (1 mg, 6.6 μmol), 4-dimethylaminopyridine (1 mg, 8.2 μmol), triethylamine (0.05 mL, 0.36 mmol) in methylene chloride (5 mL) at room temperature under argon for 15 h. Chlorotrimethylsilane (0.5 mL, 3.9 mmol) and triethylamine (0.5 mL, 3.6 mmol) were then added and the solution stirred for 24 h more. The solution was filtered through a short pad of silica which was then eluted with 10% ethyl acetate in hexane. TLC of the eluate showed one major peak of radioactivity which corresponded with authentic disilylether of 7. The disilylation reaction of 7-DA had previously been carried out as above and found to give a 97% yield of product.

1,2-[^3H]-antheridiol (10). Dimethylpyrazole (5 mg, 50 μmol) was added to a stirred mixture of CrO_3(*5 mg*, *50 μmol*) and methylene chloride (2 mL) under argon at -20°C. After 15 min. a solution of crude disilyl ether (9) in methylene chloride (2 mL) was added and the stirred mixture was kept at -10° to -20°C for 7 hr. Aqueous NaOH (5N, 2mL) was next added and the stirring continued at 0°C for 1 hr more. The mixture was extracted with chloroform (3 x 5 mL), and the combined extract washed with 10% HCl, brine and dried (Na_2SO_4). The crude product was dissolved in acetic acid (3 mL)-THF (1 mL) - water (1 mL) and the solution was stirred at room temperature for 3 days. It was taken up in chloroform and the organic layer was washed with water then dried (Na_2SO_4). Analysis of the product by TLC (98:2 $CHCl_3$–CH_3OH) with zonal scraping showed one major peak corresponding to antheridiol (Rf 0.25) and two others, at *Rf* 0.6 (7-DA) and Rf 0.9 (unidentified). The crude 1,2-[^3H]-antheridiol solution was concentrated then applied to a 20 x 20 cm^2 TLC plate (0.25 mm thickness) and the plate was developed in a 98:2 chloroform-methanol solvent system. The zone corresponding to *Rf* 0.1 to 0.3 was scraped from the plate and extracted with absolute ethanol. The purified product showed a single radioactive peak which corresponded to that of authentic antheridiol.

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REFERENCES

1. O'Day, D.H., Horgen, P.A.: Sexual Interactions in Eukaryotic Microbes, Academic Press, New York, 1981.
2. McMorris, T.C., Le, P.H., Preus, M.W., Schow, S.R., Weihe, G. R.: J. Org. Chem. 48, 3370 (1983).
3. Timberlake, W.E.: Dev. Biol. 51, 202 (1976).
4. Horgen, P.A., Iwanochko, M., Bettiol, M.F.: Arch. Microb. 134, 314 (1983).
5. McMorris, T.C., Seshadri, R., Arunachalam, T.: J. Org. Chem. 39, 669 (1974).
6. Anh, N.T., Eisenstein, O.: Nouv J. Chim. 1, 61 (1977).
7. Donaubauer, J.R., Greaves, A.M., McMorris, T.C.: J. Org. Chem. 49, 2833 (1984).
8. Djerassi, C., Gutzwiller, J.: J. Am. Chem. Soc. 88, 4537 (1966).
9. Riehl, R.M., Toft, D.O. Meyer, M.D., Carlson, G.L., McMorris, T.C.: Exp. Cell Res. 153, 544 (1984).
10. Riehl, R.M., Toft, D.O.: J. Biol. Chem. 259, 15324 (1984).