

SYNTHESIS OF ANTHERIDIOL-(22,23-³H)

Trevor C. McMorris and Thangavel Arunachalam
University of California, San Diego, La Jolla, California, U.S.A.
Received on March 10, 1975.
Revised on August 20, 1975.

SUMMARY

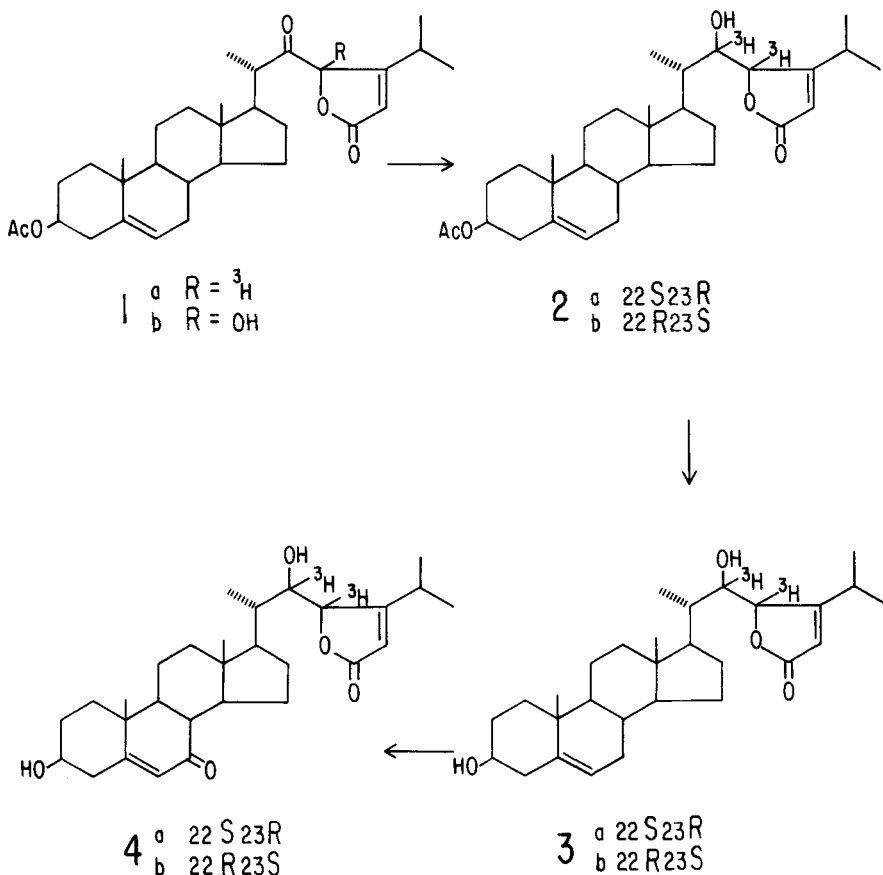
Antheridiol-(22,23-³H) has been prepared by reduction of 3-acetoxy-23,23-dihydroxy-22-oxo-5, 24(28)-ergostadiene-28-carboxylic acid gamma-lactone with tritiated sodium borohydride followed by hydrolysis, photo-oxygenation and cupric chloride catalysed rearrangement.

The sequence of events leading to sexual reproduction in the water mould, *Achlya*, begins with secretion of the steroidal hormone, antheridiol (4a), by female strains. This hormone causes the formation of antheridial branches, or male sex organs, in male strains and also stimulates the male to secrete a second hormone (hormone B), which induces the female to produce oogonial branches, or female sex organs.⁽¹⁾ Antheridiol is secreted in minute amounts but it can be detected by biological assay in as low a concentration as 6×10^{-12} g/ml of solution. In order to study the metabolic fate of antheridiol, we required labelled hormone of high specific activity. Our recent synthesis of antheridiol⁽²⁾ provided a straightforward method for introducing tritium at C-22 and C-23 of the steroidal side chain.

The keto-lactol intermediate (1b) was synthesized in good yield by condensation of 3-acetoxy-22,23-bisnorchol-5-en-24-al with the anion of 3-isopropylbutenolide followed by Jones oxidation and autoxidation, as reported previously.⁽²⁾ Reduction of 1b with tritiated sodium borohydride in tetrahydrofuran-methanol (2:1) gave the tritiated hydroxy butenolide, 2a,b (mixture of erythro isomers). The isomer with the natural stereochemistry (2a) was isolated by preparative thin layer chromatography. It was diluted ten times with unlabelled compound and recrystallized to constant activity. Hydrolysis with dilute sulfuric acid in dioxane gave 3a, which was

converted to 22,23-³H antheridiol (4a) by photooxygenation and cupric chloride catalyzed rearrangement. The pure product 4a had mp 233-241° (MeOH), specific activity 0.76 mCi/mmol.

In an attempt to produce material with higher activity the reduction was repeated using tritiated sodium borohydride which had an activity 60% greater than that used previously. The solvent for the reduction was dioxane-tritiated water. Tritiated water was used to ensure that if any exchange of hydrogen attached to boron were to occur, it would not lead to a decrease in the amount of tritium incorporated into 2a,b.⁽³⁾ The activity of the antheridiol obtained by this method was 97% higher than that of the previous experiment.



The proportion of 22S 23R isomer to 22R 23S isomer was lower (1:4.5) in the second reduction than in the first (1:2.5). This difference may be a result of steric factors. The 22-keto group and to a lesser extent the (potential) 23-keto group in 1b are hindered and are situated in an asymmetric environment. The proportions of the alcohols formed by reduction will depend on the size of the attacking species. This in turn depends on the type of solvent used in the reaction.⁽⁴⁾

Removal of the tritium atoms in 2a (specific activity 0.78 mCi/mmol) by sequential oxidation showed that the activity was almost evenly distributed between the C-22 and C-23 positions (0.38 and 0.32 mCi/mmol respectively). The residual activity in the oxidation product 1b may be due to tritiation of the hydroxyl group.

EXPERIMENTAL⁽⁵⁾

Reduction of 1b with tritiated sodium borohydride

The keto-lactol 1b⁽²⁾ (40 mg) in tetrahydrofuran-methanol, 2:1 (5 ml), was treated with tritiated sodium borohydride (40 mg, 87 mCi/mmol) and the mixture kept at room temperature for sixteen hours. The solvent was removed in a stream of N₂ and dilute HCl was added to the residue. The white insoluble solid was collected, washed (H₂O), dried, and chromatographed by preparative thin layer chromatography on silica gel with hexane-ethyl acetate (3:1) to give 2a, 6 mg, 10.5 mCi/mmol, 2b, 14 mg, 17.8 mCi/mmol and a mixture of the corresponding deacetylated compounds (11mg). Both 2a and 2b were diluted ten times with the corresponding unlabelled compounds and recrystallized to constant activity to give 2a, 38 mg, mp 158-160° (ethyl acetate-hexane), 0.78 mCi/mmol; and 2b, 92 mg, mp 204-208°, 1.17 mCi/mmol.

Repetition of the reduction with 120 mg of 1b and tritiated sodium borohydride (27 mg, 138.9 mCi/mmol) in dioxane-tritiated water, 18:1 (9ml) for twenty hours at 5°C gave 2a, 18 mg, 23.5 mCi/mmol, and 2b, 83 mg, 32.7 mCi/mmol.

Conversion of 2a and 2b to antheridiol-(22,23-³H), 4a and its isomer, 4b

A solution of the acetate 2a (35mg) in dioxane (15 ml) was refluxed with 5% aqueous H₂SO₄ (7ml) for one hour. The solvent was removed under reduced pressure

and the residue diluted with water. The insoluble diol 3a was collected and dried 30 mg, mp 228-232°.

The diol 3a (28 mg) and hematoporphyrin (3 mg) were dissolved in pyridine (5ml) in a pyrex tube and irradiated for twenty-four hours with two 15-W fluorescent lamps placed close to the tube while oxygen was passed through the solution. The dark brown solution was diluted with ether (20 ml), stirred with activated charcoal and then filtered through Celite. Removal of the solvent under reduced pressure gave the hydroperoxide which was dissolved in pyridine (3 ml) and, after adding cupric chloride (1.5 mg), was allowed to stand at room temperature for three days. The pyridine was removed under reduced pressure and the residue taken up in ethyl acetate and filtered through a short column of silica gel. Purification was effected by preparative thin layer chromatography on silica gel with chloroform-methanol (15:1) which gave 4a, 13 mg, mp 238-241° (MeOH), 0.76 mCi/mmol. A sample of 2a (10 mg) obtained by the second reduction, yielded 4a, 4.5 mg, 15 mCi/mmol. Similarly, 2b (42 mg from first reduction) was converted to 4b, 18 mg, mp 255-259° (MeOH), 1.1 mCi/mmol.

Oxidation of 2a

The acetate 2a (10 mg, from first reduction) dissolved in acetone was treated with Jones reagent (0.1 ml). The mixture was stirred at 0°C for one hour then a few drops of methanol were added to destroy excess reagent. Most of the solvent was removed under reduced pressure and the residue diluted with water to give the crystalline ketone 1a (23R), 8 mg, mp 150-155°, 0.4 mCi/mml. Thus 49% of the activity was lost.

A solution of 1a (6 mg) in 3 ml tetrahydrofuran-methanol (2:1) was stirred with silica gel (0.3 g) for twenty-four hours. The mixture was filtered and the solvent removed from the filtrate leaving a white solid which on purification by preparative thin layer chromatography on silica gel with hexane-ethyl acetate (2:1), afforded 1b, 4 mg, with an activity of less than 0.08 mCi/mmol. Therefore, more than 90% of the radioactivity was lost in the two step oxidation sequence.

ACKNOWLEDGMENT

This work was supported by grant GM 21350 from the National Institutes of Health.

REFERENCES AND NOTES

1. Gooday G.W. - Ann. Rev. of Biochem. 43: 35 (1974).
2. McMorris T.C., Seshadri R. and Arunachalam T. - J. Org. Chem. 39: 669 (1974).
3. Cornforth has found that decomposition of lithium borohydride in tetrahydrofuran by water, alcohols or acetic acid is accompanied by extensive exchange of hydrogen attached to boron. The same effect was obtained with sodium borohydride in excess of ethanol but only to a very small extent with sodium borohydride and water: Cornforth R.H. - Tetrahedron 26: 4635 (1970).
4. cf Morrison J.D. and Mosher H.S. - "Asymmetric Organic Reactions", Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1971, p. 95.
5. NaB³H₄ and ³H₂O were purchased from New England Nuclear, Boston, Mass. All samples were counted by liquid scintillation (Nuclear Chicago Model 6810).