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A NEW ROUTE TO [5α,6α-^{3}H]ANDROST-16-EN-3-ONE

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SUMMARY: A new simple synthesis of the boar pheromone [5α,6α-^{3}H]-androst-16-en-3-one with a specific activity of about 50 Ci/mmol and a radiochemical purity >99% is described.

KEY WORDS: Tritium labelled steroids, synthesis, labelled androst-16-enes, boar pheromone.

5α-Androst-16-en-3-one, the so-called boar pheromone, is found in low concentrations in plasma and adipose tissue of the sexually mature boar [1]. Its determination requires a sensitive analytical procedure and a radioimmunoassay (RIA) is most suitable for this purpose.

Previously, we had sucessfully synthesized [5α,6α-^{3}H]androst-16-en-3-one (3) from 3β-acetoxy-androst-5-en-17-one (1) in five steps [2]. After catalytic hydrogenation of the 5-double bond of (1) with T_2 the corresponding 17-tosylhydrazone had to be prepared and treated with methyl lithium under precise exclusion of air and moisture [3]. [5α,6α-^{3}H]Androst-16-en-3β-ol (2) was formed. Jones oxidation [4] then gave the desired RIA tracer (3) with specific activities >20 Ci/mmol.

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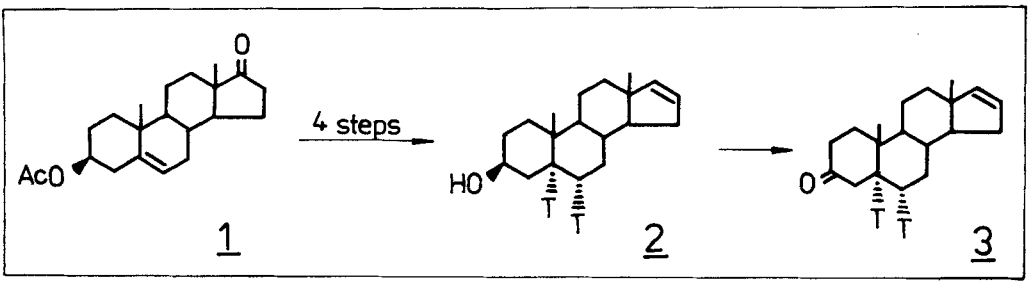


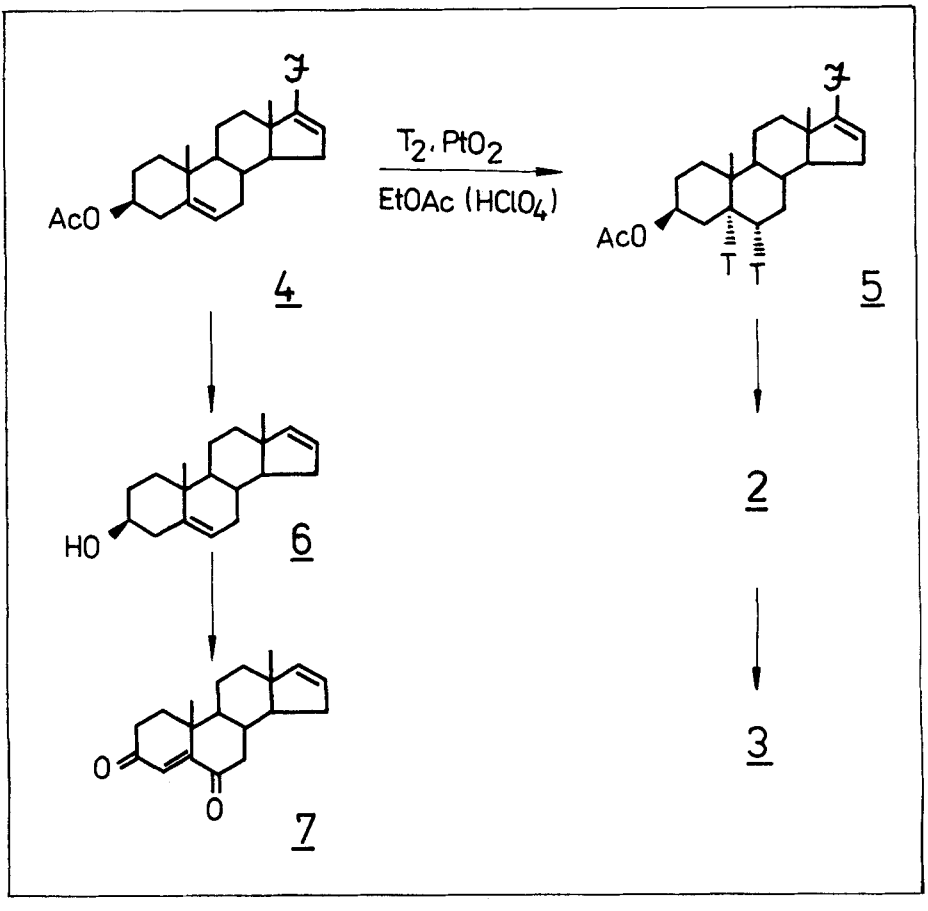
This route had some disadvantages. It was time consuming and failed when small substrate amounts were to be converted. Therefore, we looked for a simpler procedure which would yield multimillicuries of (3).

The development of the new procedure was based on the use of an androsta-5,16-diene as substrate for hydrogenation in order to avoid problems with introducing the 16-double bond at a later stage in the synthesis. 3β-Acetoxy-androsta-5,16-dien-17-iodide (4) with an iodine atom in the 17-position to protect the 16-double bond from hydrogenation was a suitable starting material. It was obtained by a 3 steps procedure as described by Barton [5].

RESULTS AND DISCUSSION

Non-radioactive catalytic hydrogenation experiments demonstrated that the 16-double bond of (4) was protected but unexpectedly the double bond in the 5-position also resisted catalytic hydrogenation. Even in the presence of PtO_2 with ethyl acetate containing perchloric acid the 5-double bond was not hydrogenated, although these conditions had been successfully used for catalytic hydrogenation of cholesterol [6]. When using tritium however, a partial conversion of (4) into 3β-acetoxy-[5α,6α-^{3}H]androst-16-en-17-iodide (5) occurred under the aforementioned drastic conditions. The 17-iodine atom and the 16-double bond were not attacked under these conditions. Using about 50 μmol (4) in 2.5 ml ethyl acetate and a ratio of (4):(PtO_2) of 3:5 by weight were found to be favourable reaction conditions.

After catalytic hydrogenation because it was not possible to separate (5) from the unconverted (4) by chromatography, the reaction mixture was hydrolyzed and deiodinated according to Barton's procedure [5]. The resulting mixture of (2) and androsta-5,16-dien-3β-ol (6) also could not be separated by chromatography and were converted to (3) and androsta-4,16-diene-3,6-dione (7) by Jones oxidation [4]. Compound (7) is a new member among the androst-16-enes having a characteristic odour [7]. Because of their different polarity (3) and (7) were readily separated by chromatography over silica gel (plates).



The new route allowed the preparation of multimillicuries of (3) with a radiochemical purity >99% and with a specific activity of about 50 Ci/mmol. The synthesis is quick and without significant difficulties. Because in the first step the reaction mixture had been stirred some hours in a tritium atmosphere and tritiated water of high specific activity had been formed by PtO_2 reduction, the 3β-acetoxy steroids (4) and (5) were additionally labelled by catalytic exchange. The exchange labelling of (4) resulted in a specific activity contribution of about 4 Ci/mmol to the final product (7).

The results which we obtained in thin layer chromatography (TLC) of the inactive steroids are noted in Table 1. They show the favourable conditions (R_f value, u.v. activity) for separating the final mixture of (3) and (7).

Table 1: Results of TLC of the inactive steroids on silica gel plates

Compound	R_f value[a)]	u.v. activity	Colour[b)] hot	cold
4	0.70	-	blueblack	blueblack
5	0.70	-	blueblack	blueblack
2	0.23	-	purple	bluegrey
6	0.23	-	darkpurple	blueblack
3	0.60	-	purple	brown
7	0.46	+	pink	green

a) benzene/acetone (9:1) as solvent

b) after spraying with vanillin in sulphuric acid and heating

EXPERIMENTAL

Materials and methods: The catalytic hydrogenation with T_2 (product of USSR) was carried out in the Institute of Nuclear Research Rossendorf [8]. Specific activities were determined using a LS-233 spectrometer

(Beckman, USA). The activity on radio-TLCs was detected by a scanner LB 2723 (Berthold-Friesecke, FRG). Preparative radio-TLC was carried out on silica gel plates (2mm, Merck, FRG). Analytical radio-TLC on silufol plates (Kavalier, CSSR) was used to determine the activity distribution and the radiochemical purity of purified products. The eluting solvent in all cases was benzene/acetone (9:1).

[5α,6α-^{3}H]Androst-16-en-3-one - (4) (25mg = 57 µmol) was dissolved in ethyl acetate (2,5 ml) containing 1 drop $HClO_4$ (conc.). After adding PtO_2 (40 mg) the reaction mixture was stirred for 5 h under tritium gas atmosphere. The excess of T_2 was reabsorbed, the reaction mixture filtered and after removing solvent and labile tritium the residue (4 and 5) was dissolved in ethanol (99.9%, 10 ml). Excess metallic sodium (ca. 200mg in small pieces) was added. After heating under reflux for 1 h the solution was poured into HNO_3 (1:10, 10 ml) and the resulting steroids were extracted with ether (2 x 20 ml). After removal of ether in vacuo the residue (6 and 2) was dissolved in acetone (5ml) and cooled to O°C. Jones reagent (26.72 g CrO_3 + 23 ml H_2SO_4 + H_2O to 100 ml) was added until a visible yellow colour persisted. Excess reagent was reduced with isopropanol after 1 h. The mixture was poured in water (80 ml), and extracted with benzene (2 x 15 ml). Removal of solvent gave the final mixture (7 and 3) which was separated on a silica gel plate. Benzene/ether (1:1) was used to extract the zone of (3) (R_f = 0.60) and (7) (R_f = 0.46). Removal of the solvent gave (3) (1.2 mg, 205 mCi; 47 Ci/mmol) and (7) (6.6 mg, 106 mCi; 4.6 Ci/mmol).

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