Synthesis of 17ß-Hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one

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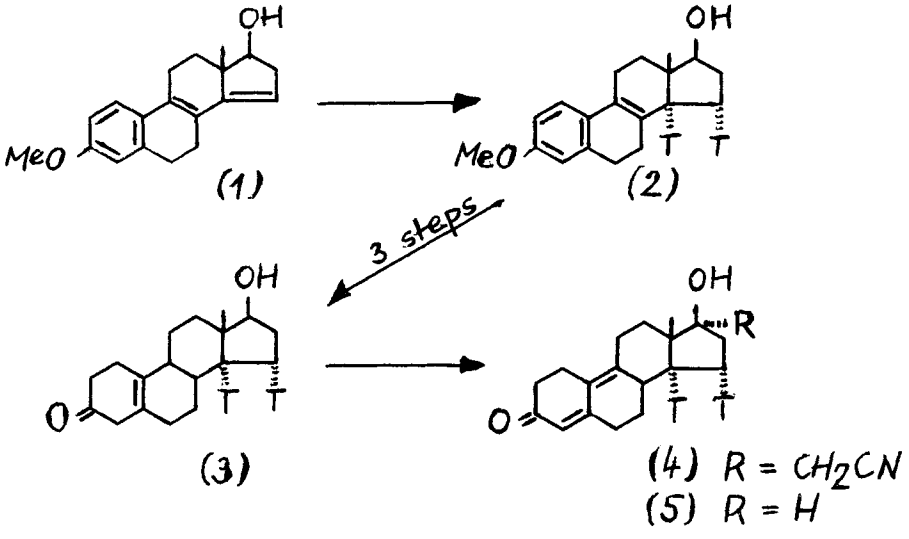
SUMMARY: Starting with catalytic tritiation of 3-methoxy-estra-1,3,5(10),8,14-pentaen-17ß-ol (1) the preparation of 17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one (5) is described. The dienone (5) was obtained with a specific activity of 400 GBq/mmol and a radiochemical purity better than 96 %. The intermediate unconjugated ketone (3) and also the dienone (5) were found to be very sensitive to self-radiolysis.

KEY WORDS: tritium labelled steroids, estrogens, syntnesis, purification, self-radiolysis

In a previous publication we described the ring D labelling of steroids and synthesized 17α-cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one (4) from 3-methoxy-estra-1,3,5(10),8,14-pentaen-17ß-ol (1) [1,2]. Now the synthesis of the tritium labelled form (5) of the pharmacologically also important 17ß-hydroxy-estra-4,9-dien-3-one [3,4,5] is described.



0362-4803/82/070855-05$01.00
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Received September 11, 1981
Revised March 15, 1982

RESULTS and DISCUSSION

The reactions (1)→(2)→(3) were carried out as previously reported [2]. We synthesized (2) with a specific activity of 1300 GBq/mmol and diluted with its inactive form to 430 GBq/mmol. 17β-Hydroxy-[14α,15α-^{3}H]estra-5(10)-en-3-one (3) was obtained with a radiochemical purity of 93 %.

The steroid (3) represents a sensitive one because the unconjugated ketone is converted quickly into the more stable conjugated 3-keto-Δ^4-compound (6) [2]. Therefore on preparation of (3) we continued the synthesis immediately. The bromination-dehydrobromination step (3)→(5) was successfully followed by radio-thin-layer chromatography (radio-t.l.c.). Within a few minutes of the addition of bromine 38 % of the dienone (5) was present, which increased to 80 % on warming the solution 10 minutes to 60 °C. By extraction with ethanol-free chloroform we obtained the dienone (5) in high yield. The product was purified by washing with cold acetone. In this way crystalline material was isolated with 96 % radiochemical purity. However, high loss of substance occurred by such treatment.

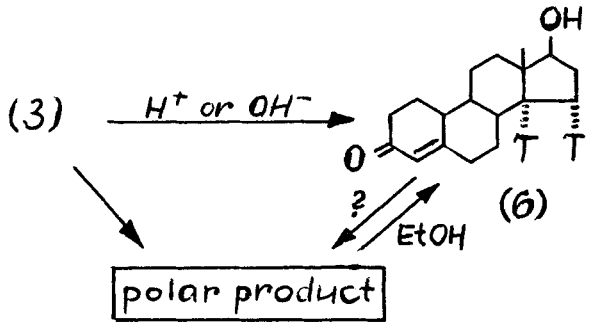
In order to investigate the degree of any self-decomposition an aliquote of (3) and the final product (5) were stored at 6 °C as solutions with a radioactive concentration of about 8 GBq/ml. The labelled ketone (3) was found to be the most sensitive steroid. Even after storing the ketone (3) a few days a polar product was formed whereas (3) was decreased in about the same amount. The distribution of activity measured by radio-t.l.c. is shown in table 1.

Table 1: Radio-t.l.c. of a benzene solution of the unconjugated ketone (3) stored at 6 °C; c≈ 8 GBq (3)/ml

storing time	distribution of activity (%)	
[days]	polar product	unconjugated ketone (3)
1	1	94
2	3	92
5	17	78
7	28	68
9	32	62
13	53	44

The radio-chromatograms of the stored ketone (3) developed in benzene/ethyl acetate/chloroform (60 : 30 : 10) showed that $R_f(3) = 0.2$ with the polar product remaining at the start. It was thus possible to separate the polar product by a simple column chromatography procedure. Washing the ketone (3) with cold acetone also removed the polar product.

The structure of the polar product was not established, but indications suggested that it was a mixture of polymers. For instance, an uncharacteristic i.r. spectrum was obtained except the well defined C=O absorption at 1720 cm^{-1}. This value belongs to a saturated 3-ketone [6]. The u.v. light absorption spectrum in ethanolic solution of the polar product absorbed at 241 nm corresponding to that of a 3-keto-Δ^4-steroid [7]. We were able to show that the conjugated ketone (6) was present on all radio-t.l.c. plates developed in solvents containing ethanol. However, (6) was absent after developing the radio-t.l.c. plates in ethanol free solvents. The polymers apparently decomposed into monomers by ethanol in which the favoured conjugated ketone (6) is formed.



The pure dienone (5) we stored at a concentration of 0.02 mmol/ml in benzene/methanol (9 : 1) at 6 °C. The radiochemical purity of (5) which was determined from time to time steadily decreased with time. The measured values are shown in table 2. The radiograms showed that similarly as in case of the unconjugated ketone (3) a polar product ($R_f \approx 0$) had been formed. Moreover three further polar decomposition products occurred R_f values of which were lower than R_f (5). The chromatographic purification of the solution containing decomposition products yielded pure (5) again, but with lower specific activity.

Table 2: Radio-t.l.c. of a solution of the dienone (5) stored at 6 °C in benzene/methanol (9 : 1); c≈8 GBq (5)/ml

storing time [weeks]	radiochemical purity of (5) (%)
0	96
1	95
4	90
8	82
14	64

EXPERIMENTAL

17β-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one (5)

The unconjugated ketone (3) (273 mg = 1 mmol) was dissolved in pyridine (5 ml). Under stirring at -10 °C bromine was added dropwise until a yellow colour persisted when the addition of bromine was stopped. The solution was warmed 10 minutes to 60 °C and allowed to cool at room temperature. The solution was adjusted to pH 1.0 and extracted with chloroform (3 x 3 ml) thoroughly. The combined extracts were washed and the chloroform was removed in vacuo. The residue was treated with benzene/methanol (9 : 1) (10 ml) and lyophilized. The residue was purified by washing with acetone (0.5 ml, 20 min at 55 °C). After cooling the solvent was decanted cautiously from the grey crystals which were again washed with cold acetone (0.5 ml) and finally dried to give the dienone (5) (116.5 mg = 0.43 mmol = 43 %). Radiochemical purity: 96 %; specific activity: 400 GBq/mmol.

Chromatographic purification of (5)

Dienone (5) (110 mg) which was stored three months in benzene/MeOH (9 : 1) at 6 °C was purified on a preparative plate three times in succession in benzene/ethyl acetate/acetone (6 : 3 : 1). The u.v. visible area containing the dienone (5) was extracted thoroughly with benzene/MeOH (1 : 1) (5 x 10 ml). Pure dienone (5) (49 mg) was obtained. Chemical yield: 45 %; radiochemical purity: 95 %; specific activity: 266 GBq/mmol.

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