THE SYNTHESIS OF 3β,11β-DIHYDROXY-5-ANDROSTEN-17-ONE, 5-PREGNENE-3β,11β,17α,20α-TETROL AND 5-PREGNENE-3β,11β, 17α,20β-TETROL TRITIATED AT C-1

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SUMMARY

The synthesis of the following C-1-tritiated-11-oxygenated-3β-hydroxy-steroids, is described: 3β,11β-dihydroxy-5-androsten-17-one, 5-pregnene-3β,11β,17α,20α-tetrol and 5-pregnene-3β,11β, 17α,20β-tetrol.

Key Words: 11-oxygenated-3β-hydroxysteroids, C-11-tritiated-steroids.

INTRODUCTION AND DISCUSSION

The direct 11β-hydroxylation of 3β-hydroxy-5-ene-steroids in the C_{19} and C_{21}-series was demonstrated in men (1-6). Compounds 4,5 and 8 tritiated at C-1 were synthesized in order to facilitate studies on the biosynthesis and metabolism of the 11-oxygenated-3β-hydroxy-5-ene-steroids in biological systems.

The synthesis is based on a previously published method (7,8), using the commercially available C-1,2-tritiated 21-deoxy-cortisone (1) or cortisone (2) as starting materials. After dienolacetylation to 3 the material was reduced under alkaline conditions to a mixture of 4 and 5 or 6 and 7. At this stage the tritium at C-2 (i.e., at the α-position to the enol-acetylated keto group) was lost, reducing the specific activity by about 50%; this yielded C-1-tritiated products of sa. of 15-20 Ci/mmol.

The epimers 4,5 or 6,7 could be separated from each other by chromatographic systems containing boric acid (8-10); they could also be cleaved directly to 3β,11β-dihydroxy-5-androsten-17-one (8). Compound 8 prepared from cortisone in gram amounts was crystallized directly (35-40% yield) without chromatographic purification. The physical constants were consistent with those in the literature (11,2). Similar yields were obtained when microgram amounts of tritiated 1 or 2 were used as starting materials.

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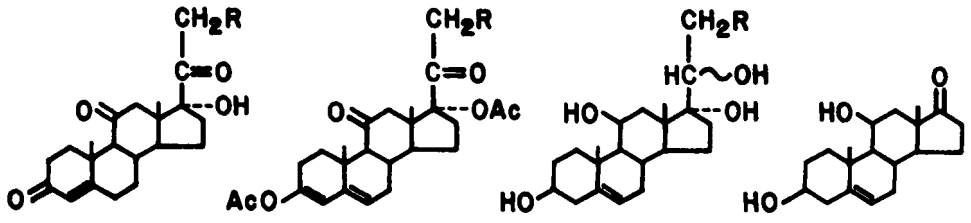

1 R=H
2 R=OH

3
R=H or OAc

4 R=H, 20∝OH
5 R=H, 20βOH
6 R=OH, 20∝OH
7 R=OH, 20βOH

8

EXPERIMENTAL

3β,11β-Dihydroxyandrosten-17-one-1-^{3}H (8). 250 μC of cortisone-1,2-$^{3}H_2$ (2) (sa. 40 Ci/mmol) purchased from New England Nuclear Corporation was dissolved in 40 ml of acetic anhydride-acetyl chloride (1:1 v/v). The mixture was refluxed under a stream of nitrogen for 90 minutes (7). The solvents were evaporated under reduced pressure in a water bath. The residue (200 μCi) was dissolved in benzene (50 ml) and washed with a saturated solution of bicarbonate and then with H_2O. The resulting enolacetate (190 μCi) was dissolved in ice cold ethanol (40 ml) to which 2 grams of sodium borohydride was added. The solution was kept at 4°C overnight and then 4 ml of 1 N NaOH was added. The mixture was kept at room temperature for 48 h and then was extracted with ethyl acetate (50 ml). The organic phase was re-extracted with ethyl acetate (25 ml). The combined organic phases contained 80 μCi while 100 μCi were found in the water phase. The ethyl acetate was evaporated under reduced pressure and the following were added to the residue: ethanol (10 ml), pyridine (1 ml) and 50% HIO_4 $2H_2O$ w/w (1 ml). The mixture was kept at room temperature for 60 minutes and then 80 ml of benzene was added. After washing with bicarbonate and H_2O, the organic layer contained 64 μCi. The resulting 3β,11β-dihydroxy-5-androsten-17-one-1-^{3}H (8) was purified on paper in the Bush type system petroleum ether:benzene:methanol 80% (5:3:8); approximately 90% of the radioactivity was recovered from the region corresponding to 8 (19-21 cm from the origin). The radiochemical purity was confirmed as follows: an aliquot of the eluate (32,000 dpm) was mixed with 20 mg of crystalline 3β,11β-dihydroxy-5-androsten-17-one m.p. 190-192°C;

ir(KBr) 3395,3350,1740,1100 cm^{-1}; nmr($CDCl_3$): δ 1.16(s,3H,18-CH_3), 1.30(s,3H,19-CH_3), 4.50(m,1H,11α-H), 5.29(m,1H,6C-H) ppm; mass spectrum of the 3β-TMSi 376(M^+), M-18, M-90, 129(base peak). The mixture was successively crystallized from ethyl acetate, ethyl acetate-petroleum ether and methanol yielding crystals with specific activities of 1580, 1620 and 1580 dpm/mg respectively. The identity of the tritiated compound was further verified by demonstrating its enzymic conversion to 11β-hydroxy-4-androstene-3,17-dione in 90% yield (6).

5-Pregnene-3β,11β,17α,20α-tetrol-1-^3H (4) and 5-Pregnene-3β,11β,17α,20β-tetrol-1-^3H (5), 11.3 mCi of 21-deoxycortisone-1,2-$^3H_2$ (1) (sa. 34 Ci/nmol, purchased from Nuclear Research Center, Beer-Sheba, Israel) was subject to enol-acetylation and reduction-hydrolysis as described above. The ethyl acetate extract (5 mCi) was chromatographed on paper-boric acid (9) in the system toluene/75% methanol (1:1) for 24 hr. Two radioactive zones were found: one (0.56 mCi) was located 12.5-15.0 cm from the origin and the second (3.7 mCi) was located 21-24 cm from the origin, which corresponded to compounds 4 and 5 respectively. The identity of the compound having the same mobility as 4 was confirmed by mixing 22,000 dpm of the eluate with 21 mg of crystalline 4 and successively crystallizing it from ethyl acetate, methanol and acetone; the specific activities of the crystals were: 1100, 1100 and 1050 dpm/mg respectively. Similarly, the material having the same chromatographic mobility as 5 (45,000 dpm) was added to 22 mg of crystalline 20β-tetrol and was crystallized as above. It yielded crystals with specific activities of 2100, 2000 and 2100 dpm/mg. The identity of the tetrols was further confirmed by degradation to 8 (2). Aliguots of the tetrols were converted to the corresponding C-1-tritiated 11-keto-derivatives of 4 and 5 (8) in nearly quantitative yields.

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