NOVEL SYNTHESIS OF 2β,3β,20β-TRIACETOXY-5α-PREGNAN-6-ONE

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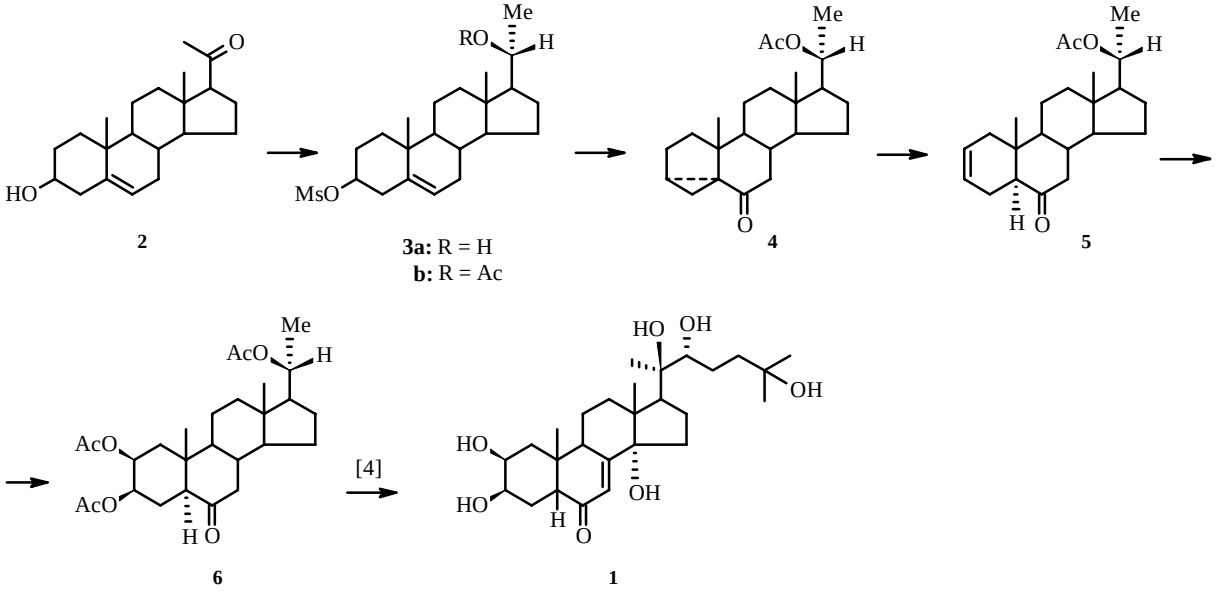
*Pregnenolone (**2**) is used in a novel synthesis of 2β,3β,20β-triacetoxy-6-ketosteroid (**6**), a key intermediate in the preparation of 20-hydroxyecdysone (**1**).*

Key words: pregnenolone, ecdysterone, 2β,3β,20β-triacetoxy-5α-pregnan-6-one.

The ecdysteroid 20-hydroxyecdysone (**1**) is a very important insect molting and metamorphosis hormone [1]. Many synthetic methods have been proposed to prepare this compound [1-3]. However, isolation of it from certain plants, for example, *Rhaponticum carthamoides* roots, is the most economical route. The main reason for the inefficiency of the existing schemes for synthesizing **1** is the structural complexity. However, in our opinion, the syntheses of **1** that were developed previously can be significantly improved by applying recently developed synthetic methods, especially in brassinosteroid chemistry. Therefore, our attention was drawn to a partial synthesis of **1** [4]. One of its characteristics is the use of a 2β,3β,20β-triacetoxy-6-ketosteroid (**6**) as an intermediate. The total synthesis of **6** has also been described [5-7]. It should be mentioned that compounds of similar structure are also used in syntheses of **1** [8] and structural analogs of poststerone [9].

Our goal was to develop a novel synthesis of **6** from pregnenolone **2**, which is a commercially available compound. We used experience accumulated up to now in the preparation of brassinosteroids [10, 11].

In the first step of the synthesis, pregnenolone **2** reacts with methanesulfonyl chloride in pyridine to produce the corresponding mesylate, which is then reduced by $NaBH_4$. The structure of the product **3a** (66% overall yield) was proved by spectral methods. In particular, the β- (or R-) configuration of the 20-hydroxyl was established by comparing chemical shifts of the 18- and 21-methyl protons in the ^1H NMR spectrum of **3a** with the corresponding shifts in spectra of 20α- and 20β-hydroxypregn-4-en-3-ones [12]. Our results for the structure of **3a** agree with those in the literature because it is known [13] that reduction of a 20-ketone in pregnanes by $NaBH_4$ produces 20β-alcohols.



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In the next step, **3a** reacts with acetic anhydride in pyridine to give **3b**, which is solvolyzed without further purification by KOAc in aqueous acetone. The resulting 3α,5-cyclo-6β-alcohol is oxidized without isolating it from the reaction mixture to give 3α,5-cyclo-6-ketosteroid **4** in quantitative yield.

3α,5-Cyclo-6-ketosteroids are currently isomerized into Δ^2-6-ketosteroids by several methods that differ slightly from each other [1-3]. We used an isomerization method [14] that uses *p*-toluenesulfonic acid and LiBr with heating in DMF. This produces compound **5** in 71% yield from **4**. The physicochemical properties agree with those described for **5** in the literature [9-11].

In the final step, the Δ^2-bond in **5** is *cis*-hydroxylated according to Woodward by silver acetate and iodine in aqueous acetic acid. The 2β-acetoxy-3β-hydroxy-6-ketosteroid is acetylated by acetic anhydride without further purification to give the desired 2β,3β,20β-triacetoxy-6-ketosteroid **6** in 46% overall yield.

In conclusion, it should be noted that the scheme used by us to synthesize **6** is simpler and gives a greater yield of the desired compound compared with those described previously [4-7].

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument at 700-3600 cm^{-1} in KBr pellets. ^1H NMR spectra were obtained on a Bruker AC-200 NMR-spectrometer at working frequency 200 MHz. Chemical shifts are given relative to TMS internal standard.

3-Mesylate-pregn-5-en-3β,20β-diol (3a). A solution of **2** (5.0 g) in pyridine (47 mL) was treated with methanesulfonyl chloride (5 mL), held at room temperature for 20 h, and treated with ice. The precipitated product was filtered off, washed thoroughly with water, dried in air, and dissolved in methanol (200 mL) and THF (40 mL). The resulting solution was stirred, cooled on an ice bath, treated with $NaBH_4$ (0.8 g), and stirred with cooling on an ice bath for 1 h 15 min. The excess of reagent was neutralized with acetic acid (1 mL). The mixture was diluted with water. The precipitated product was filtered off and dried in a desiccator. Yield of **3a**, 4.2 g (66%), mp 140-142°C. IR spectrum (ν, cm^{-1}): 3445 (OH), 1640 (C=C). ^1H NMR (δ, ppm): 0.77 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.14 (3H, d, J = 6 Hz, 21-Me), 3.74 (1H, m, W/2 = 20 Hz, H-20), 4.52 (1H, m, W/2 = 23 Hz, H-3α), 5.43 (1H, d, J = 4.5 Hz, H-6).

20β-Acetoxy-3α,5-cyclo-5α-pregnan-6-one (4). Steroid **3a** was dissolved in pyridine (20 mL), treated with acetic anhydride (9 mL), and stored at room temperature for 21 h.

Solvent was removed under vacuum to give **3b**, mp 138-143°C. IR spectrum (ν, cm^{-1}): 1730 (AcO), 1655 (C=C). ^1H NMR (δ, ppm): 0.64 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.16 (3H, d, J = 6 Hz, H-6), 2.02 (3H, s, AcO), 4.52 (1H, m, W/2 = 23 Hz, H-3α), 4.84 (1H, m, W/2 = 20 Hz, H-20), 5.43 (1H, d, J = 4.5 Hz, H-6).

Compound **3b** was dissolved without further purification in acetone (100 mL). The solution was treated with a solution of KOAc (4.0 g) in water (20 mL), refluxed for 8 h, cooled to room temperature, treated with chromic acid (10 mL, 8 N), and stirred at room temperature for 20 min. The excess of oxidant was neutralized with isopropanol (30 mL). The mixture was diluted with water. The oil was separated and chromatographed on a silica-gel column with elution by ether and then THF to give in quantitative yield **4**, mp 85-95°C (petroleum ether), lit. mp 131-132°C [9] and 124-125°C [11]. IR spectrum (ν, cm^{-1}): 1740 (AcO), 1700 (C=C). ^1H NMR (δ, ppm): 0.68 (3H, s, 18-Me), 0.74 (1H, t, J = 5 Hz, H-3β), 1.00 (3H, s, 19-Me), 1.18 (3H, d, J = 6 Hz, 21-Me), 2.02 (3H, s, AcO), 4.86 (1H, m, W/2 = 21 Hz, H-20).

20β-Acetoxy-5α-pregn-2-en-6-one (5). A solution of **4** (4.37 g) in DMF (15 mL) was treated with LiBr (0.609 g) and *p*-toluenesulfonic acid (0.447 g), refluxed for 2 h, cooled to room temperature, treated with ethylacetate (50 mL), washed with water and NaCl solution, dried over $MgSO_4$, and evaporated under vacuum. The solid was recrystallized from a mixture of petroleum ether and ethylacetate. Yield of **5**, 1.268 g. The mother liquor from the recrystallization, which contains a mixture of **4** and **5** (according to TLC) was isomerized again by *p*-toluenesulfonic acid (0.344 g) and LiBr (0.305 g) in DMF (10 mL). The usual treatment gave another 1.836 g of **5** (71% overall yield).

^1H NMR (δ, ppm): 0.64 (3H, s, 18-Me), 0.70 (3H, s, 19-Me), 1.16 (3H, d, J = 6 Hz, 21-Me), 2.02 (3H, s, AcO), 4.84 (1H, m, W/2 = 20 Hz, H-20), 5.55 (1H, br. dd, J_1 = 10 Hz, J_2 = 2 Hz, H-2), 5.69 (1H, br. dd, J_1 = 12 Hz, J_2 = 3 Hz, H-3).

2β,3β,20β-Triacetoxy-5α-pregnan-6-one (6). A solution of **5** (3.104 g) in acetic acid (30 mL) was treated with silver acetate (4.342 g), heated to 60°C, stirred, treated successively with water (2.34 mL) and portions of iodine (3.962 g) in acetic acid (13 mL), stirred at 60°C for 3 h, and cooled to room temperature. The AgI precipitate was filtered off. The solvent was

removed under vacuum. The solid was dissolved in pyridine (20 mL). The solution was treated with acetic anhydride (10 mL) and held at room temperature for 18 h. Most of the solvent was removed under vacuum. The remainder of the pyridine was removed by azeotropic distillation with toluene. The solid was chromatographed on a silica-gel column with elution by petroleum ether:ether (2:1) and then ether. Yield of **6**, 1.834 g (46%), mp 206-217°C. IR spectrum (ν, cm^{-1}): 1745 (AcO). ^1H NMR (δ, ppm): 0.63 (3H, s, 18-Me), 0.92 (3H, s, 19-Me), 1.16 (3H, d, J = 6 Hz, 21-Me), 2.01 (3H, s, AcO), 2.02 (3H, s, AcO), 2.08 (3H, s, AcO), 4.82 (2H, m, W/2 = 25 Hz, H-3α and H-20), 5.30 (1H, br. d, J = 3 Hz, H-2α).

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