SYNTHESIS OF 5α-ANDROSTAN-3β,17β-DIOL FROM TIGOGENIN

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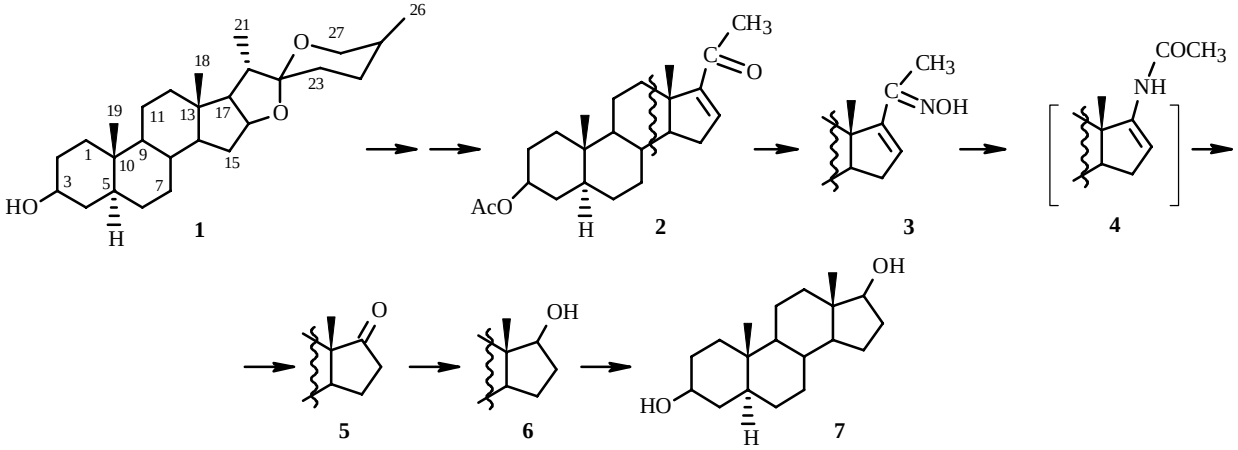
5α-Androstan-3β,17β-diol (3β-adiol), a known inhibitor of prostate cancer cell growth, was synthesized from tigogenin. Its structure was confirmed by NMR and IR spectroscopy and mass spectroscopy.

Key words: 3β-adiol, 5α-steroids, tigogenin, synthesis.

Despite the early diagnosis and treatment of prostate cancer, it remains one of the principal male diseases. The risk factor increases with age, as does the level of estrogens in plasma (17-estradiol and estrone) and the ratio of estrogens to androgens [1-3].

Lately studies of the effect of 5α-androstan-3β,17β-diol (3β-adiol) on cancer of the prostate gland have been published [4-8]. It has been shown that development of prostate cancer depends in the early stages on androgens and is usually modulated pharmacologically by their blockade [9]. However, such therapy may induce androgen-independent prostate cancer, which is often more aggressive. It was found that a 5α-reduced testosterone derivative, dihydrotestosterone (DHT), inhibits migration of cancer cells through an androgen-receptor-independent mechanism. A metabolite of DHT, 3β-adiol, does not bind to androgen receptors although it effectively binds to estrogen receptor β (ERβ) and inhibits powerfully the spread of prostate cancer cells by ERβ activation. Moreover, 3β-adiol induces through ERβ the appearance of the protein E-cadgerin, a known metastasis inhibitor for breast and prostate cancer [6].



As reported previously, the Institute of Pharmaceutical Chemistry of the Academy of Sciences of Georgia proposed the steroidal sapogenin tigogenin as starting material for synthesizing hormonal preparations of the 5α-series. Tigogenin is isolated from the plant *Yucca gloriosa*, which is cultivated in Georgia [10]. We developed a synthetic scheme for 3β-adiol based on tigogenin (**1**) that involves conversion of **1** to pregnenolone acetate (**2**), of **2** to epiandrosterone acetate, then reduction to 5α-androstan-3β,17β-diol. For conversion of **1** to **2**, we chose oxidative dehydration using $TiCl_4$ as catalyst. This avoided an autoclave step in the oxidative dehydration of the spiroketal part of tigogenin and enabled **2** to be synthesized in one step. The yield of **2** from **1** was 69.5% [11]. Compound **2** was converted to epiandrosterone acetate using the Schmidt-Thome method [12], according to which pregnenolone acetate oxime (**3**) underwent Beckmann rearrangement by $POCl_3$ in pyridine. Acid hydrolysis of intermediate 17-acetylamino derivative **4** gave epiandrosterone acetate (**5**) in 65% yield [13]. Reduction of **5** using

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$NaBH_4$ in methanol produced 3β-acetoxy-5α-androstan-17β-ol (**6**), base hydrolysis of which in methanol gave 5α-androstan-3β,17β-diol (**7**) in 95% yield.

Thus, the yield of **7** starting from tigogenin was 40.8%.

EXPERIMENTAL

Melting points were determined on a Gallenkamp block. IR spectra in KBr disks were recorded on a Magna-IR Spectrometer 550 instrument. Mass spectra were measured on a Finnigan AQA Navigator instrument (EI, 70 eV). NMR spectra were obtained on a Bruker AM 300 instrument. Proton chemical shifts are given on the δ-scale (ppm) with TMS internal standard and DMSO-d_6 solvent. Elemental analyses were performed on a Perkin—Elmer CHN 2004 instrument and agreed for all compounds with those calculated. The course of reactions and purity of products were monitored by TLC on Silufol 254 plates (Kavalier, Czech Rep.) using benzene:acetone (3:1). Spots were developed by spraying with phosphomolybdic acid (10%) in ethanol with subsequent heating.

3β-Acetoxy-5α-pregn-16-en-20-one (2). A mixture of **1** (50 g, 120.0 mmol), $(CH_3CO)_2O$ (150 mL), and C_5H_5N (10 mL) was boiled for 1 h, cooled to 100°C, stirred, treated with $TiCl_4$ (2.5 g, 13.16 mmol) in $(CH_3CO)_2O$ (2.5 mL), boiled an additional 2 h, cooled to 40°C, treated gradually with CH_3COONa (10 g) dissolved in water (25 mL), stirred 20 min, cooled to room temperature, poured into CH_3COCH_3 (220 mL) and CH_3COOH (220 mL), oxidized by addition of CrO_3 (15 g) in water (7.5 mL) at 15-18°C, stirred an additional hour, treated with isopropanol (7.5 mL), gradually heated to distill off acetone and reach a temperature of 115-117°C, boiled for 1.5 h, cooled to room temperature, and treated with water (425 mL). The resulting precipitate was filtered off, washed with water, and recrystallized from methanol:acetone (3:1) to afford **2** (29 g, 69.5%), mp 158-162°C, lit. mp 158-162°C [11].

5α-Pregn-16-en-3β-ol-20-one Acetate Oxime (3). A mixture of **2** (2.5 g, 6.97 mmol), $NH_2OH \cdot HCl$ (0.55 g, 7.91 mmol), and dry C_5H_5N (12 mL) was heated at 65-68°C for 2 h, cooled to room temperature, treated with water (45 mL), and stirred for 30 min. The resulting precipitate was filtered off and washed with water to afford **3** (2.5 g, 98.07%), mp 196-198°C, lit. mp 195.5-198.5°C [13].

3β-Acetoxy-5α-androstan-17-one (5). A mixture of **3** (1 g, 2.67 mmol), dry C_5H_5N (3.2 mL), and dry CH_3COCH_3 (3.2 mL) at 18-20°C was treated with $POCl_3$ (1.2 mL), stirred for 30 min, cooled to -5°C, treated with dilute HCl (1:1 with water, 28 mL), stirred for 30 min, and treated with water until neutral. The resulting precipitate was filtered off and washed with water to afford crude product (0.83 g) that was chromatographed over a column of silica gel (L 100-160) with elution by low-boiling petroleum ether:ether (20:1) to afford **5** (0.58 g, 65%), mp 111-113°C, lit. mp 111-113°C [13].

3β-Acetoxy-5α-androstan-17β-ol (6). A solution of **5** (4.7 g, 14.13 mmol) in methanol (10 mL) at 0°C was treated in portions with $NaBH_4$ (1 g, 26.31 mmol), stirred at 20°C for an hour, acidified with acetic acid until weakly acidic, and poured into water (200 mL). The precipitate was filtered off, washed with water, and recrystallized from methanol to afford **6**, (0.97 g, 97%), mp 106-107°C, lit. mp 106.5°C [14]. IR spectrum (ν, cm^{-1}): 3455 (OH).

5α-Androstan-3β,17β-diol (7). A solution of **6** (4.46 g, 13.33 mmol) in methanol (100 mL) was treated with NaOH (0.53 g), boiled for 10 min, cooled, evaporated to 40 mL, and poured into water. The resulting precipitate was filtered off, washed with water until the rinsings were neutral, and recrystallized from benzene:hexane (10:1) to afford **7**, (4.2 g, 95%), mp 163-165°C, lit. mp 161°C [15]. IR spectrum (ν, cm^{-1}): 3504 (OH). PMR spectrum (ppm): 0.61 (3H, s, CH_3-18), 0.72 (3H, s, CH_3-19), 3.18 (1H, br.s, OH-C-3), 3.4 (1H, br.s, OH-C-17), 4.3 (1H, s, C-3), 4.3 (1H, s, C-17), ^{13}C NMR spectrum (δ, ppm): 11.37 (C-18), 12.22 (C-19), 60.39 (C-3), 80.13 (C-17). Mass spectrum (*m*/*z*, I_{rel}, %): 292 (7) $[M]^+$, 257 (12), 208 (15).

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