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SYNTHESIS OF NEW α-CHLOROPYRIDINE DERIVATIVES OF STEROIDAL 3β,5α,6β-TRIOLS AND 3β,5-DIHYDROXY-6-KETONES

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New steroid derivatives containing 6-chloropyridine groups characteristic of the alkaloid epibatidine and neonicotinoid insecticides were synthesized by reacting 3β,5α,6β-trihydroxysteroids or 3β,5-dihydroxy-6-ketosteroids with 2-chloro-5-chloromethylpyridine.

Key words: 3β,5α,6β-trihydroxysteroids, 3β,5-dihydroxy-6-ketosteroids, derivatives, 2-chloro-5-chloromethylpyridine, synthesis.

We have previously reported the synthesis of 6-chloronicotinates of 3β,5α,6β-trihydroxysteroids and 3β,5-dihydroxy-6-ketosteroids containing an α-chloropyridine ring characteristic of the natural analgesic epibatidine isolated from the Ecuadorian frog *Epipedobates tricolor* [2]. Furthermore, this group occurs in modern highly effective neonicotinoid insecticides such as imidacloprid, thiacloprid, nitenpyram, and acetamiprid [3]. In addition, a pyridine ring appears in the phytoecdysteroid diploclidine, which was recently isolated from *Diploclisia glaucescens* (Menispermaceae) [4]. This gives an indication of the importance of this heterocycle for the structures of natural steroids.

Herein we continue the previous studies [1]. The reaction of triol **1** or dihydroxyketones **2** and **3** with 2-chloro-5-chloromethylpyridine produced the corresponding 2-chloropyridine-5-methyl ethers in which the α-chloropyridine ring was bonded to the corresponding steroids not through an ester bond as before [1] but through a metabolically more inert ether bond. 2-Chloro-5-chloromethylpyridine is widely used in the production of neonicotinoids [3], a convenient synthesis of which we recently developed [5].

Reaction of 3β,5α,6β-trihydroxysteroid **1** with 2-chloro-5-chloromethylpyridine in toluene in the presence of aqueous NaOH solution and tetra-*n*-butylammonium bromide formed two monoethers that were isolated in yields of 30 and 5.5%. Their structures as the 3- and 6-regioisomers were established using spectral data. Thus, PMR spectra of both compounds had in the expected ranges proton resonances characteristic of the steroid part and the pyridine ring in a 1:1 ratio. Spectra of the products contained resonances at δ 4.35-4.60 ppm that were characteristic for protons of the benzyl methylene group. The core structure of product **1** with 2-chloro-5-chloromethylpyridine was identified as the 3-monoether **4** based on chemical shifts of H-3α (δ 3.860 ppm) and H-6α (δ 3.55 ppm). Then the minor product was identified as the 6-monoether **5** based on PMR spectra and chemical shifts of protons H-3α (δ 4.08 ppm) and H-6α (δ 3.23 ppm).

Next we studied the reaction of 3β,5α-dihydroxy-6-ketosteroid **2** with 2-chloro-5-chloromethylpyridine under conditions analogous to those described above. As it turned out, the main product was not the 3-monoether **6** but its isomer **7**. The isomerization at C-5 in pyridine under these conditions was not unexpected. It was due to the presence in the reaction mixture of NaOH. It is known in steroid chemistry that 5α-hydroxy-6-ketosteroids under alkaline conditions can easily convert into 5β-hydroxy-6-ketosteroids [1].

Compound **7** was also prepared in >50% yield by direct reaction of 3β,5β-6-ketosteroid **3** with 2-chloro-5-chloromethylpyridine. Its structure was elucidated using spectral data.

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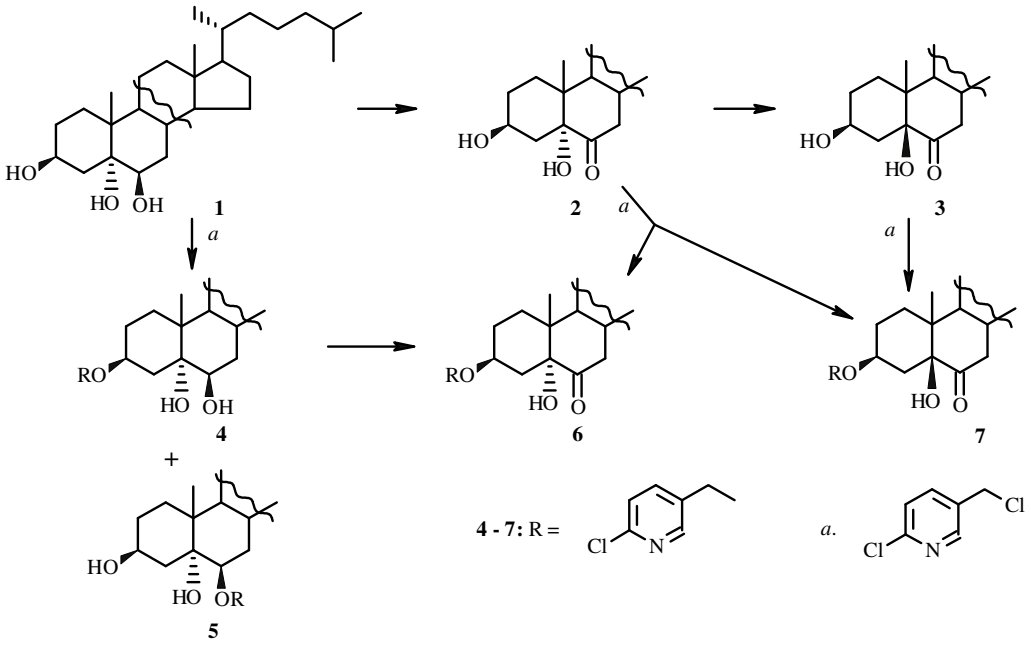

In the final stage, the required 3-monoether of 3β,5α-dihydroxy-6-ketone **6** was synthesized in >60% yield via oxidation under mild conditions of the 6β-hydroxy group in **4** using *N*-bromosuccinimide in aqueous THF. The structure of this compound was found unambiguously from PMR and ^{13}C NMR data.

Thus, we developed methods for synthesizing new derivatives of steroidal 3β,5α,6β-triols and 3β,5-dihydroxy-6-ketones containing also an α-chloropyridine group. Studies of the biological activity of the synthesized compounds will be reported separately.

EXPERIMENTAL

IR spectra in KBr disks were recorded on a UR-20 instrument at 700-3600 cm^{-1}; UV spectra in EtOH, on a Specord M400 spectrophotometer. PMR and ^{13}C NMR spectra in $CDCl_3$ were obtained on a Bruker Avance 500 NMR spectrometer (operating frequency 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C). Chemical shifts are given relative to TMS (internal standard). The course of reactions and the purity of products were monitored using Merck Kieselgel 60F_{254} plates. Melting points were determined on a Kofler block.

Alkylation of 5α-Cholestan-3β,5,6β-triol (1). A mixture of **1** (2.10 g, 5 mmol) (prepared by the literature method [6]), 2-chloro-5-chloromethylpyridine (0.97 g, 6 mmol) (prepared by the literature method [5]), tetra-*n*-butylammonium bromide (0.32 g, 1 mmol), toluene (60 mL), and aqueous NaOH solution (10 mL, 50%) was stirred, heated to 105-110°C for 25 h, treated with additional 2-chloro-5-chloromethylpyridine (0.97 g, 6 mmol), stirred and heated at 105-110°C for 29.5 h, cooled to room temperature, treated with dichloroethane (50 mL) and water (40 mL), and shaken. The resulting precipitate was filtered off, washed on the filter successively with water (10 mL) and dichloroethane (10 mL), and dried in air to afford **1** (0.98 g, 46.7%).

The combined filtrate was transferred to a separatory funnel and shaken. The organic layer was separated. The aqueous layer was extracted with dichloroethane (2 × 20 mL). The combined organic extracts were washed with water (20 mL) and dried over anhydrous Na_2SO_4. Solvent was distilled in a rotary evaporator under reduced pressure (water aspirator). The solid was chromatographed over a column of silica gel with elution by CH_2Cl_2:CH_3OH (300:1) to afford the *bis*-(2-chloropyridine-5-methyl)ether (0.32 g), mp 90-91°C. $C_{12}H_{10}N_2OCl_2$. UV spectrum (λ_{max}, nm, ε): 216 (19,800), 268 (7,500). PMR spectrum (δ, ppm, J/Hz): 4.58 (2H, s, CH_2), 7.35 (1H, d, J = 8.0, H-3), 7.68 (1H, dd, J_1 = 8.0, J_2 = 2.0, H-4), 8.37 (1H, d, J = 2.0, H-6).

Further elution by CH_2Cl_2:CH_3OH (from 80:1 to 50:1) afforded **4** (0.82 g, 30%), mp 183-185°C (dichloroethane). $C_{33}H_{52}ClNO_3$. UV spectrum (λ_{max}, nm, ε): 215 (10,400), 268 (4,300). PMR spectrum (δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.86 (3H, d, J = 6.5, 26-Me), 0.87 (3H, d, J = 6.5, 27-Me), 0.90 (3H, d, J = 6.5, 21-Me), 1.19 (3H, s, 19-Me), 3.55 (1H, br.s, H-6α), 3.860 (1H, m, H-3α), 4.54 (2H, center of AB-system, J_{AB} = 12.5, CH_2O), 7.31 (1H, d, J = 8.0, H-3′), 7.66 (1H, dd, J_1 = 8.0, J_2 = 2.5, H-4′), 8.34 (1H, d, J = 2.5, H-6′).

Further elution by CH_2Cl_2:CH_3OH (20:1) gave **5** (0.15 g, 5.5%), mp 80-82°C (MeOH). PMR spectrum (δ, ppm, J/Hz): 0.66 (3H, s, 18-Me), 0.86 (3H, d, J = 6.5, 26-Me), 0.87 (3H, d, J = 6.5, 27-Me), 0.90 (3H, d, J = 6.5, 21-Me), 1.14 (3H, s, 19-Me), 3.23 (1H, br.s, H-6α), 4.08 (1H, m, H-3α), 4.35 (1H, d, J = 8.0, CH_2O), 4.61 (1H, d, J = 12.5, CH_2O), 7.32 (1H, d, J = 8.0, H-3′), 7.63 (1H, dd, J_1 = 8.0, J_2 = 2.0, H-4′), 8.32 (1H, d, J = 2.0, H-6′).

3β-(2-Chloropyridine-5-methyloxy)-5-hydroxy-5β-cholestan-6-one (7). A. A mixture of **2** (2.09 g, 5 mmol) (prepared from **1** by the literature method [6]), 2-chloro-5-chloromethylpyridine (0.97 g, 6 mmol), tetraethylammonium chloride (0.26 g), toluene (60 mL), and aqueous NaOH solution (5 mL, 50%) was stirred at 105-110°C for 28 h, cooled to room temperature, treated with toluene (50 mL) and water (50 mL), and shaken. The toluene layer was separated. The aqueous layer was extracted with trichloroethylene (3 × 30 mL). The combined organic extracts were washed with water (30 mL) and dried over anhydrous $MgSO_4$. Solvent was distilled in a rotary evaporator at reduced pressure (water aspirator). The solid was chromatographed over a column of silica gel with elution by petroleum ether:EtOAc (5:1) to afford **3** (0.36 g, 17.2%) that was identical by TLC and PMR with an authentic sample.

Further elution isolated a mixture (0.87 g, 32.0%) of C-5 epimers of **7** and **6** in a 5:1 ratio according to PMR spectra. Double recrystallization of this mixture from ethanol produced an analytical sample of **7** (0.36 g), mp 164-165.5°C (EtOH). $C_{33}H_{50}ClNO_3$. IR spectrum (KBr, ν, cm^{-1}): 3470 (OH), 1710 (C=O), 1110 (C–O). UV spectrum (λ_{max}, nm, ε): 212 (1,400), 268 (5,000). PMR spectrum (δ, ppm, J/Hz): 0.66 (3H, s, 18-Me), 0.79 (3H, s, 19-Me), 0.86 (3H, d, J = 6.5, 26-Me), 0.87 (3H, d, J = 6.5, 27-Me), 0.91 (3H, d, J = 6.5, 21-Me), 3.82 (1H, br.s, H-3α), 4.14 (1H, s, 5-OH), 4.47 (1H, d, J = 12.5, CH_2O), 4.58 (1H, d, J = 12.5, CH_2O), 7.31 (1H, d, J = 8.0, H-3′), 7.72 (1H, dd, J_1 = 8.0, J_2 = 2.0, H-4′), 8.33 (1H, d, J = 2.0, H-6′).

B. Compound **3** (2.0 g) (prepared by the literature method [1]) in benzene (30 mL) was treated with 2-chloro-5-chloromethylpyridine (0.90 g), tetraethylammonium iodide (0.1 g), and NaOH solution (14 mL, 50%); stirred at room temperature for 4 h; treated with additional 2-chloro-5-chloromethylpyridine (0.80 g, 1.7 g total) and tetraethylammonium iodide (0.4 g); stirred for an additional 11 h, treated with additional tetraethylammonium iodide (0.5 g); stirred with heating at 40-50°C for 6 h; treated with additional tetraethylammonium iodide (0.5 g, total 1.5 g); stirred with heating for another 2 h; and treated with water (75 mL) and EtOAc (75 mL). The aqueous layer was separated, acidified to pH 4.8 by adding HCl (36%), and extracted with EtOAc (20 mL). The combined extract was washed with water (70, 50, and 50 mL) and evaporated. The solid was chromatographed over a column of silica gel with elution by petroleum ether:EtOAc of increasing polarity (10:1, 8:1, 6:1, 5:1) to isolate starting **3** (0.260 g) and **7** (1.41 g, 54%). The product was recrystallized from petroleum ether (40 mL) to afford **7** (1.32 g), the IR and PMR spectra of which agreed with those of the compound prepared by method **A**.

3β-(2-Chloropyridine-5-methyloxy)-5-hydroxy-5α-cholestan-6-one (6). A solution of **4** (0.55 g, 1 mmol) in THF (9 mL) and water (1 mL) was stirred, treated with *N*-bromosuccinimide (0.2 g, 1.1 mmol), treated after 30 min with *N*-bromosuccinimide (0.02 g, 0.11 mmol), stirred for another 45 min, and transferred to a separatory funnel with aqueous $NaHCO_3$ solution (50 mL, 5%) and CH_2Cl_2 (30 mL). The organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were washed with aqueous Na_2SO_3 solution (5%, 20 mL) and water (2 × 30 mL), and dried over anhydrous $MgSO_4$. The desiccant was filtered off and washed with CH_2Cl_2. The CH_2Cl_2 was evaporated in vacuo. The solid was chromatographed over a column of silica gel with elution first by CH_2Cl_2 (100 mL) and then CH_2Cl_2:CH_3OH (100:1, 2,600 mL; 50:1, 600 mL; 40:1, 400 mL) to afford a product (0.49 g) that was recrystallized successively from benzene and then cyclohexane. Total yield 0.33 g (0.61 mmol, 61%) of **6**, mp 156-160°C. PMR spectrum (δ, ppm, J/Hz): 0.65 (3H, s, 18-Me), 0.82 (3H, s, 19-Me), 0.861 (3H, d, J = 6.5, 26-Me), 0.866 (3H, d, J = 6.5, 27-Me), 0.91 (3H, d, J = 6.5, 21-Me), 2.14 (1H, dd, J_1 = 13.5, J_2 = 4.5, H-4α), 2.20 (1H, s, 5α-OH), 2.71 (1H, t, J = 12.5, H-7α), 3.74 (1H, m, W/2 = 23, H-3α), 4.53 (2H, s, CH_2O–), 7.30 (1H, d, J = 8.0, H-3′), 7.65 (1H, dd, J_1 = 8.0, J_2 = 2.0, H-4′), 8.29 (1H, d, J = 2.0, H-6′). ^{13}C NMR spectrum (δ, ppm): 12.009 (C-18), 14.011 (C-19), 18.613 (C-21), 21.399 (C-11), 22.553 (C-26), 22.817 (C-27), 23.825 (C-23), 23.908 (C-15), 27.100 (C-2), 27.995 (C-25), 28.064 (C-16), 29.553 (C-4), 33.164 (C-1), 35.709 (C-20), 36.076 (C-22), 37.272 (C-8), 39.451 (C-24), 39.542 (C-12), 41.790 (C-7), 42.654 (C-13), 43.902 (C-10), 44.485 (C-9), 56.078 (C-17), 56.312 (C-14), 66.592 (CH_2O), 74.700 (C-3), 80.534 (C-5), 124.153 (C-3′), 133.444 (C-5′), 138.245 (C-4′), 148.439 (C-6′), 150.423 (C-2′), 212.579 (C-6).

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