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SYNTHESIS OF OXIMES OF STIGMASTANE 5β-HYDROXY-6-KETOSTEROIDS

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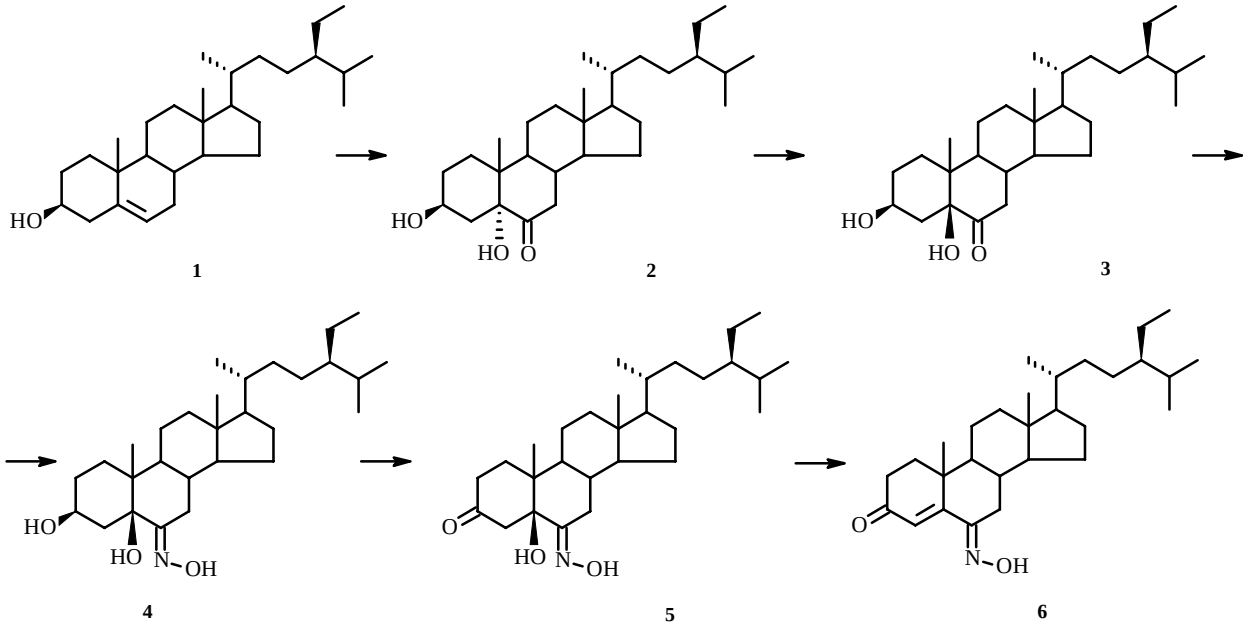
β-*Sitosterol **1** and the intermediate 5-hydroxy-6-ketosteroids **2** and **3** were used to synthesize 5*β*-hydroxy-6-ketosteroid oximes **4** and **5**. Dehydration of **5** from* Cinachyrella *sponges forms (24R,6E)-24-ethylcholest-6-hydroximino-4-en-3-one **6**, a steroidal oxime.*

Key words: 6-ketosteroids, oximes.

We previously synthesized from β-sitosterol (**1**) the unsaturated steroid oxime **6** [1], which has been isolated from *Cinachyrella* sponges [2]. Oximes of the corresponding 5α-hydroxy-6-ketosteroids were used as intermediates. In our opinion, these steroids are interesting as potential biologically active compounds. For example, active progestins [3] and aromatase inhibitors [4] are known among steroid oximes. These observations prompted us to investigate further the synthesis of 6-ketosteroid oximes.

The present article reports the preparation of new steroid 6-ketoximes, the structures of which also contain 5β-hydroxyls.

The starting material was β-sitosterol (**1**), which is transformed in the first step via *trans*-hydroxylation with hydrogen peroxide in formic acid by the literature method [5] in quantitative yield into the corresponding 3β,5α,6β-triol. Selective oxidation of this triol by N-bromosuccinimide in aqueous dioxane gave 3β,5α-dihydroxy-6-ketone **2** in 80% yield. Then, isomerization of **2** by KOH in ethanol gave 3β,5β-dihydroxy-6-ketone **3** in 70% yield. The structure of **3** was proved by comparing IR and ^{1}H NMR spectra with those of the authentic compound that was isolated previously [6].



1 **2** **3** **4** **5** **6**

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Subsequent reaction of **3** with hydroxylamine in ethanol in the presence of NaOAc gave oxime **4** in quantitative yield. The structure of **4** follows unambiguously from spectral data. Thus, the IR spectrum lacks a stretching band of the ketone, the presence of which is characteristic of the spectrum of starting **3**. The IR spectrum of **4** contains a band for the C=N oxime bond at 1650 cm^{-1}. The presence in the ^1H NMR spectrum of **4** in a mixture of $CDCl_3$ and CD_3OD of a broad doublet for methylene proton H-7β, which is located next to the oxime, is important for confirming the structure. Judging from published data [1-3], such a chemical shift for H-7β is consistent with (E)-geometry of the oxime in **4**. It is noteworthy that the signal for methine proton H-3α is not resolved in the ^1H NMR spectrum of **4** because of the strong solvent peak. However, this signal is well resolved at 4.28 ppm in the spectrum recorded in Py-d_5. Furthermore, signals for oxime protons (δ 13.17 ppm) and hydroxyl protons on C-3 (δ 5.44 ppm) and C-5 (δ 6.06 ppm) are also observed in the ^1H NMR spectrum of **4** in Py-d_5.

In the next step, **4** was oxidized by chromic acid in THF to transform the 3β-hydroxyl into a carbonyl. Unfortunately, this reaction does not occur smoothly like the oxidation of the analogous 3β,5α-dihydroxy-6-ketoxime [1]. We isolated only a mixture of the expected 5β-hydroxy-3-ketosteroid **5** and unsaturated steroid **6** by chromatography of the oxidation products. Pure **5** could be obtained from the mixture by crystallization. Its structure was determined from spectral data. Thus, the IR spectrum of **5** contains stretching bands of the 3-ketone at 1720 cm^{-1} in addition to stretching bands of hydroxyl at 3450 cm^{-1} and C=N at 1650 cm^{-1}. The ^1H NMR spectrum of **5** in $CDCl_3$ lacks a signal for methine proton H-3α, the presence of which is characteristic of the spectrum of the starting 3,5-dihydroxy-6-ketoxime **4**. Furthermore, the spectrum exhibits signals of oxime and hydroxyl protons as singlets at δ 9.32 and 5.18 ppm, respectively. The presence of a signal for H-7β at δ 3.36 ppm indicates that the oxime has (E)-geometry.

The final step involves dehydration of the mixture of **5** and **6** prepared in the preceding step by $Al(OH)_3$ in boiling dioxane to give in excellent yield pure unsaturated oxime **6**. The structure of **6** was proved by direct comparison with an authentic sample that we synthesized earlier [1].

EXPERIMENTAL

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

(24R)-24-Ethyl-5β-cholestan-3β,5-diol-6-one (3). Dihyhdroxyketosteroid **2** (6.00 g, prepared from β-sitosterol by the literature method [1, 5]) and KOH (60 g) in ethanol (550 mL, 95%) was boiled for 6.5 h under Ar, cooled to 40°C, treated with HCl (110 mL), evaporated in vacua to half the volume, diluted with water (300 mL), and extracted with $CHCl_3$ (3×100 mL). The $CHCl_3$ extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness. The solid was chromatographed over a silica-gel column with elution by a mixture of cyclohexane and ethylacetate (5:1). Yield of **3**, 4.2 g (70%), mp 109-110°C (hexane), lit. [6] mp 108-110°C. IR and ^1H NMR spectra of **3** agree with those reported previously [6].

(24R,6E)-24-Ethyl-5β-cholestan-6-hydroximino-3β,5-diol (4). A solution of **3** (3.65 g), hydroxylamine hydrochloride (2.24 g), and NaOAc trihydrate (4.40 g) in methanol (150 mL, 75%) was boiled for 1 h, evaporated in vacua to half the volume, and diluted with water (100 mL). The resulting precipitate was filtered off, washed on the filter with ethanol (50%), and dried in air.

Recrystallization from ethanol gave **4**, 3.71 g (98%), mp 133-135°C (ethanol). IR spectrum (ν, cm^{-1}): 3430 (OH), 1650 (C=N). ^1H NMR spectrum ($CDCl_3$—CD_3OD, δ, ppm, J/Hz): 0.66 (3H, s, 18-Me), 0.80 (3H, s, 19-Me), 0.82 (3H, d, J = 6.0, 26-Me), 0.83 (3H, d, J = 6.0, 27-Me), 0.86 (3H, t, J = 6.0, 29-Me), 0.92 (3H, d, J = 6.0, 21-Me), 2.25 (1H, dd, J_1 = 15.0, J_2 = 3.0, H-4β), 3.25 (1H, br.d, J = 14, H-7β); (C_5D_5N, δ, ppm): 0.64 (3H, s, 18-Me), 0.89 (3H, d, J = 6.0, 26-Me), 0.90 (3H, d, J = 6.0, 27-Me), 0.92 (3H, t, J = 6.0, 29-Me), 1.02 (3H, d, J = 6.0, 21-Me), 1.02 (3H, s, 19-Me), 2.45 (1H, dd, J_1 = 15.0, J_2 = 3.0, H-4β), 3.74 (1H, br.d, J = 10.0, H-7β), 4.28 (1H, m, W/2 = 13.0, H-3α), 5.44 (1H, d, J = 8.0, 3β-OH), 6.06 (1H, s, 5β-OH), 13.17 (1H, br.s, N–OH).

(24R,6E)-5-Hydroxy-24-ethyl-5β-cholestan-6-hydroximino-3-one (5). A stirred solution of **4** (2.12 g) in THF (50 mL) was treated dropwise with chromic acid (2.5 mL, 8 N) and with an additional portion (0.5 mL, 8 N) after 15 min. The excess of oxidant was destroyed after 10 min by adding isopropanol (3 mL). The mixture was filtered through a thin layer of aluminum oxide. The solvent was evaporated in vacua. The solid was chromatographed over a silica-gel column with elution by a mixture of dichloroethane and ethanol (20:1) to give a mixture of **5** and **6** (3:2 ratio according to the ^1H NMR spectrum)

(0.55 g). Crystallization three times from a mixture of ether and hexane gave pure **5**, 0.024 g, mp 218-220°C. IR spectrum (ν, cm^{-1}): 3450 (OH), 1720 (C=O), 1650 (C=N). ^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69 (3H, s, 18-Me), 0.81 (3H, d, J = 7.0, 26-Me), 0.84 (3H, d, J = 7.0, 27-Me), 0.85 (3H, t, J = 6.0, 29-Me), 0.90 (3H, s, 19-Me), 0.93 (3H, d, J = 6.0, 21-Me), 3.13 (1H, d, J = 14.0, H-4), 3.36 (1H, dd, J_1 = 15.0, J_2 = 4.0, H-7β), 5.18 (1H, s, 5β-OH), 9.32 (1H, br.s, N-OH).

(24R,6E)-24-Ethylcholest-6-hydroximino-4-en-3-one (6). A solution of a mixture of **5** and **6** (0.11 g) in dioxane (15 mL) was boiled for 1 h with basic aluminum oxide (0.20 g) and cooled to room temperature. The precipitate was filtered off. The solvent was evaporated in vacua. The solid was crystallized from methanol. Yield of **6**, 0.080 g, identical in all properties with an authentic sample [1]. Mp 197-200°C, lit. [1] mp 198-200°C.

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