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NOVEL SYNTHESIS OF (24R,6E)-24-ETHYLCHOLEST-6-HYDROXYIMINO-4-EN-3-ONE, A STEROIDAL OXIME FROM *Cinachyrella* spp. SPONGES

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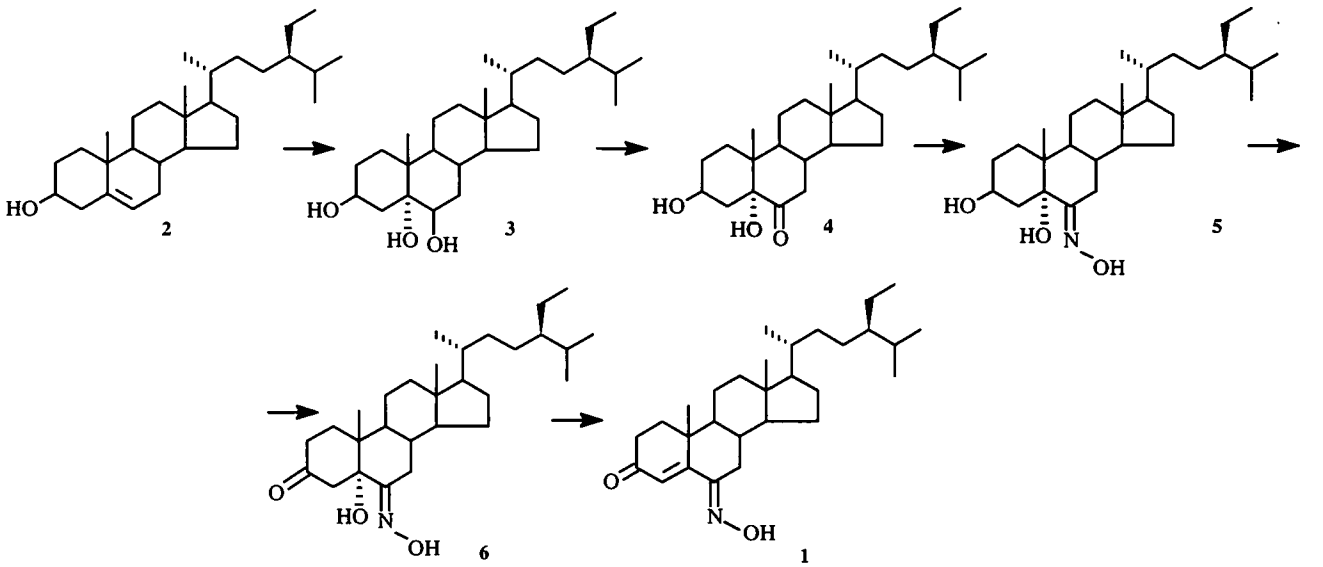
(24R,6E)-24-ethylcholest-6-hydroxyimino-4-en-3-one (1), which was isolated previously from Cinachyrella *spp. sponges, is synthesized in five steps from β-sitosterol in 30% overall yield.*

Key words: β-sitosterol, (24R,6E)-24-ethylcholest-6-hydroxyimino-4-en-3-one, synthesis.

Marine sponges are a rich source of large quantities of steroids with varied structures [1-3]. The variety of structures observed in sponges frequently have no analogs among other natural compounds. This makes sponge steroids attractive as starting materials for new medicinal preparations. It was recently reported [4] that two steroidal oximes were isolated from *Cinachyrella alloclada* and *C. apion*. One of these has the structure (24R,6E)-24-ethylcholest-6-hydroxyimino-4-en-3-one (**1**). The second is its cholestane derivative. It is noteworthy that oximes of analogous structure that were specially synthesized were active inhibitors of aromatase [5]. The enzyme aromatase is a key intermediate in the biosynthesis of estrogens. Its inhibitors are very interesting as potential antitumor agents [6].



In addition to the isolation of steroid **1** [4], its synthesis from β-sitosterol (**2**) in seven steps has also been reported [5]. However, experimental details were not revealed. The goal of the present investigation was to develop a new synthesis that would yield the desired oxime **1** by a shorter route in rather high overall yield.

In the first synthetic step, starting β-sitosterol undergoes *trans*-diaxial hydroxylation by H_2O_2 in formic acid according to the literature [7]. This produces 3β,5α,6β-triol **3** in quantitative yield. Then triol **3** is converted by oxidation with N-bromosuccinimide in aqueous dioxane in 78% yield to 3β,5α-dihydroxy-6-ketone **4**. The properties of compound **4** that is synthesized this way agree completely with those of the authentic compound that was synthesized previously [7] by selective oxidation of triol **3** using the Jones reaction.

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Next, oxime **5** is prepared by reaction of dihydroxysteroid **4** with hydroxylamine in ethanol in the presence of NaOAc in >80% yield. The structure of compound **5** was proved by us using spectral data. The lack in the IR spectrum of a band for the ketone stretching is interesting. This band is characteristically present in the spectrum of **4**. However, the IR spectrum of steroid **5** contains a band at 1660 cm^{-1} that is characteristic of the C=N oxime bond. It is important that the ^1H NMR in a mixture of $CDCl_3$ and CD_3OD contains a broad multiplet at 3.99 ppm for the axial proton H-3α, which is geminal to the 3β-hydroxy group. It is also noteworthy that the spectrum of steroid **5** contains a doublet of doublets at 3.10 ppm. Use of double resonance demonstrated that the splitting of this signal is not due to interaction with methine proton H-3α. This indicates that the signal at 3.10 ppm cannot be assigned to H-4α or H-4β. Therefore, the presence of this signal in the spectrum is due to resonance of H-7β. Judging from the literature [4, 5], the magnitude of the chemical shift for H-7β unambiguously indicates that the oxime has (E)-geometry in **5**. Additional signals of the oxime proton (δ 12.51 ppm), two hydroxyl protons (δ 4.86 and 6.64 ppm), two methylene protons H-4α and H-4β (δ 2.91 and 2.60 ppm, respectively), and methylene proton H-7α (δ 2.36 ppm) could be observed in the ^1H NMR spectrum of **5** in C_5D_5N. These were assigned using double resonance. Thus, saturation of H-3α converts the signals of H-4α and H-4β from quartets to doublets with splitting constant J = 14 Hz. Saturation of these signals produces a slight simplification of the H-3α multiplet. Saturation of H-7β changes the triplet with δ 2.36 ppm to a doublet with J = 14 Hz. Therefore, the signal at δ 2.36 ppm is assumed to correspond to H-7α.

The ^{13}C NMR spectrum of oxime **5** provides useful information for proving its structure. We compared these spectra with those of the starting 6-ketosteroid **4** [8] in order to assign the signals. Chemical shifts of corresponding C atoms in the ^{13}C NMR spectra of steroid **5** clearly demonstrate the presence of 3β- and 5α-hydroxy groups and a 6-ketoxime in **5**.

Oxidation of compound **5** by chromic acid in THF gives in 55% yield 3-ketosteroid **6**. The IR spectrum of **6** contains stretching vibrations for OH at 3350 cm^{-1} and C=N at 1660 cm^{-1} in addition to a band for ketone stretching at 1710 cm^{-1}. The ^1H NMR spectrum of steroid **6** in C_5D_5N lacks a signal for the methine proton H-3α, the presence of which is characteristic of the starting 3,5-dihydroxy-6-ketoxime **5**. Signals of the oxime and hydroxyl protons can also be identified as singlets at δ 12.80 and 7.52 ppm, respectively. The presence of a signal for H-7β at 3.78 ppm indicates that the oxime has (E)-geometry. It is also noteworthy that the signals of the methylene protons H-4 shift to weak field (δ 3.14 and 3.36 ppm) with the 3-ketone in the structure of compound **6**.

In the final step, steroid **6** was dehydrated by basic aluminum oxide in boiling dioxane. This produced oxime **1** in 83% yield. The structure of compound **1** follows unambiguously from the spectral data. Thus, the UV spectrum exhibits a strong absorption band with a maximum at 271 nm. This agrees with the literature data [4]. However, use of the fourth derivative showed that the exact position of this maximum is 279 nm. Furthermore, another band with maximum at 234 nm is observed as a shoulder in the UV spectrum. The ^1H NMR spectra of compound **1** synthesized by us agree well with those in the literature [4]. There are slight differences in the chemical shifts of the oxime and H-4β protons. Apparently this is due to the different conditions under which the spectra were recorded. In particular, we found (see Experimental) that these chemical shifts change noticeably depending on the concentration.

Thus, the synthesis developed by us includes five steps and enables the target steroid **1** to be prepared from β-sitosterol in 30% overall yield.

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. UV spectra of ethanol solutions were recorded on a Specord M-400 instrument. The exact positions of absorption maxima were determined using the fourth derivative. ^1H and ^{13}C NMR spectra were obtained on a Bruker AC-200 spectrometer at working frequencies 200 and 50 MHz, respectively. Chemical shifts are reported relative to TMS as an internal standard.

(24R,6E)-24-Ethyl-5α-cholestan-6-hydroximino-3β,5-diol (5). A boiling solution of dihydroxyketone **4** (1.12 g) in ethanol (40 ml, 95%) was treated dropwise with hydroxylamine hydrochloride (0.70 g) and NaOAc trihydrate (1.40 g) in ethanol (50%, 20 ml). The mixture was boiled for 2 h, diluted with water (40 ml), and left overnight in a refrigerator. The precipitate was filtered off, washed on the filter with ethanol (50%), and dried in air. Recrystallization from ethanol gave oxime **5** (0.96 g), yield 83%, mp 254-256°C (ethanol). IR spectrum (ν, cm^{-1}): 3400 (OH), 1660 (C=N). ^1H NMR spectrum ($CDCl_3$—CD_3OD, 4:1, δ, ppm): 0.66 (18-Me, s), 0.83 (19-Me, s), 0.83 (26-Me, d, J = 6.0 Hz), 0.84 (27-Me, d, J = 6.0 Hz), 0.85 (29-Me, t, J =

6.0 Hz), 0.92 (21-Me, d, J = 6.0 Hz), 3.10 (H-7β, dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz), 3.99 (H-3α, m, W/2 = 18 Hz); (C_5D_5N, δ, ppm): 0.67 (18-Me, s), 0.87 (26-Me, d, J = 6.0 Hz), 0.89 (27-Me, d, J = 6.0 Hz), 0.90 (29-Me, d, J = 6.0 Hz), 0.99 (21-Me, d, J = 6.0 Hz), 1.11 (19-Me, s), 2.36 (H-7α, dd, J = 14.0 Hz), 2.60 (H-4β, dd, J_1 = 14.0 Hz, J_2 = 11.0 Hz), 2.91 (H-4α, dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz), 3.77 (H-7β, dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz), 4.86 (H-3α, 3β-OH, m). ^{13}C NMR spectrum ($CDCl_3$—CD_3OD, 4:1, δ, ppm): 12.0 (C-18), 12.2 (C-29), 14.4 (C-19), 18.9 (C-21), 19.1 (C-26), 19.9 (C-27), 21.6 (C-11), 23.2 (C-28), 24.2 (C-15), 25.1 (C-4), 26.3 (C-23), 28.4 (C-16), 29.3 (C-25), 30.1 (C-1), 30.1 (C-2), 34.1 (C-22), 35.0 (C-8), 36.3 (C-20), 37.9 (C-7), 40.0 (C-12), 41.1 (C-10), 43.2 (C-13), 44.9 (C-9), 46.0 (C-24), 56.3 (C-17), 56.5 (C-14), 67.2 (C-3), 77.0 (C-5), 162.6 (C-6).

(24R,6E)-5-Hydroxy-24-ethyl-5α-cholestan-6-hydroximino-3-one (6). A solution of **5** (0.92 g) in THF (50 ml) was treated dropwise with stirring with chromic acid (1 ml, 8 N). Additional chromic acid (0.2 ml, 8 N) was added after 20 min. The excess of oxidant was removed after 5 min by adding *iso*-propanol (2 ml). The mixture was filtered through a thin layer of aluminum oxide. The solvent was removed under vacuum. The solid was crystallized from a mixture of dioxane and ethanol. Yield 0.51 g of compound **6** (55%), mp 267-269°C. Found, %: C 75.89, H 10.81, N 3.26. Calc. for $C_{29}H_{49}NO_3$, %: C 75.77, H 10.74, N 3.05. IR spectrum (ν, cm^{-1}): 3350 (OH), 1710 (C=O), 1660 (C=N). ^1H NMR spectrum (C_5D_5N, δ, ppm): 0.68 (18-Me, s), 0.88 (26-Me, d, J = 6.0 Hz), 0.90 (27-Me, d, J = 6.0 Hz), 0.91 (29-Me, t, J = 6.0 Hz), 1.00 (21-Me, d, J = 6.0 Hz), 1.17 (19-Me, s), 3.14 (H-4, d, J = 15.0 Hz), 3.36 (H-4, d, J = 15.0 Hz), 3.78 (H-7β, dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz), 7.52 (5α-OH, s), 12.80 (N–OH, s).

(24R,6E)-24-Ethylcholest-6-hydroximino-4-en-3-one (1). A solution of steroid **6** (0.15 g) in dioxane (20 ml) was boiled for 1 h with basic aluminum oxide (0.30 g). After cooling the precipitate was filtered off. The solvent was removed under vacuum. The solid was crystallized from methanol. Yield 0.12 g of **1** (83%), mp 198-200°C. UV spectrum (λ_{max} nm): 234 (ε 6900), 279 (ε 10400). IR spectrum (ν, cm^{-1}): 1670 (C=N, C=C–C=O). ^1H NMR spectrum (C_5D_5N, δ, ppm): 0.67 (18-Me, s), 0.89 (26-Me, d, J = 6.0 Hz), 0.91 (27-Me, d, J = 6.0 Hz), 0.92 (29-Me, t, J = 6.0 Hz), 1.01 (21-Me, d, J = 6.0 Hz), 1.07 (19-Me, s), 3.86 (H-7β, d, J = 12.0 Hz), 6.66 (H-4, s), 13.94 (N–OH, s); (19 mg/0.5 ml $CDCl_3$, δ, ppm): 0.70 (18-Me, s), 0.82 (26-Me, d, J = 6.0 Hz), 0.84 (27-Me, d, J = 6.0 Hz), 0.86 (29-Me, t, J = 6.0 Hz), 0.93 (21-Me, d, J = 6.0 Hz), 1.14 (19-Me, s), 3.42 (H-7β, dd, J_1 = 16.0 Hz, J_2 = 4.0 Hz), 6.55 (H-4, s), 10.77 (N–OH, br. s); (8 mg/0.5 ml $CDCl_3$, δ, ppm): 0.71 (18-Me, s), 0.82 (26-Me, d, J = 6.0 Hz), 0.84 (27-Me, d, J = 6.0 Hz), 0.86 (29-Me, t, J = 6.0 Hz), 0.93 (21-Me, d, J = 6.0 Hz), 1.13 (19-Me, s), 3.43 (H-7β, dd, J_1 = 16.0 Hz, J_2 = 4.0 Hz), 6.50 (H-4, s), 10.26 (N–OH, br. s).

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