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NEW SYNTHESIS OF ECDYSTEROIDS BASED ON STIGMASTEROL

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A new synthesis of ecdysteroids from stigmasterol has been developed.

Key words: synthesis, stigmasterol, ecdysteroids.

Various steroids are used as starting materials in the chemical synthesis of ecdysteroids [1-2]. The overall yield of the total synthesis often dictates the choice of starting material. Stigmasterol (**1**) is one of the compounds that has been used in this area. Stigmasterol contains a 3β-hydroxyl and 5(6)- and 22(23)-double bonds that provide a basis for many synthetic means of introducing a wide array of functional groups into the cyclic part and the side chain.

Our goal was to develop a new approach to synthesizing ecdysteroids from stigmasterol through several intermediates that have a significant number of the necessary functional groups in the cyclic part and the untransformed side chain. The availability of such compounds would enable a wide range of final products to be prepared via various reactions of the 22(23)-double bond, which have been well studied in recent years in the chemistry of brassinosteroids. It is also important that the configuration of chiral C-20 and C-24 remains unchanged by these reactions. This would significantly simplify the total synthesis.

The approach that we developed is based on previous experience [3] in the synthesis of physanol A and B analogs from β-sitosterol. In the first step, **1** is reacted with acetic anhydride by the literature method [4] to give the acetate **2** in 90% yield. Then, selective addition of HBrO, obtained from the calculated amount of N-bromoacetamide, at the sterically more accessible 5(6)-double bond and subsequent Jones oxidation of the bromohydrin by chromic acid gave 5α-bromo-6-ketone **3** in 40% overall yield. The IR spectrum of **3** contains a band at 1720 cm^{-1} that corresponds to stretching vibrations of the 6-ketone. The signal for the methine proton H-3α in the ^1H NMR spectrum undergoes a significant weak-field shift to δ 5.32 ppm compared to its position in the spectrum of the starting acetate (δ 4.62 ppm). Such a shift is undoubtedly due to the 5α-bromine, which is 1,3-diaxial to H-3α. Furthermore, signals for two vinyl protons H-22 and H-23 (δ 5.02 and 5.16 ppm, respectively) are observed in the ^1H NMR of **3**. This indicates that the 22(23)-double bond persists.

Reaction of **3** with aqueous HBr in acetic acid by the literature method [3] produced 7α-bromo-6-ketone **4** in 76.5% yield. The ^1H NMR spectrum of **4** contains a doublet at δ 4.20 ppm with splitting constant J = 3.5 Hz that is characteristic of methine proton H-7β geminal to the Br. Furthermore, the spectrum also contains two signals for the vinyl protons H-22 and H-23 (δ 5.02 and 5.16 ppm, respectively). This unambiguously indicates that the 22(23)-double bond persists. It is noteworthy that reductive debromination—bromination has been proposed for the rearrangement of 5α-bromo-6-ketosteroids into 7α-bromo-6-ketosteroids by HBr [5]. Therefore it is supposed that the 5α-H-6-ketone and molecular bromine are formed first via the reaction of HBr with the 5α-bromo-6-ketone. These then react to form the desired 7α-bromo-6-ketone. However, in our instance, if the reaction were to occur by such a mechanism, the products would necessarily contain 22,23-dibromides. Therefore, it can be proposed that the conversion of **3** into **4** occurs most probably via an S_N2' substitution mechanism. For example, such a mechanism was proposed for an analogous acetolysis of 3β-acetoxy-5-hydroxy-5α-cholestan-6-one [6].

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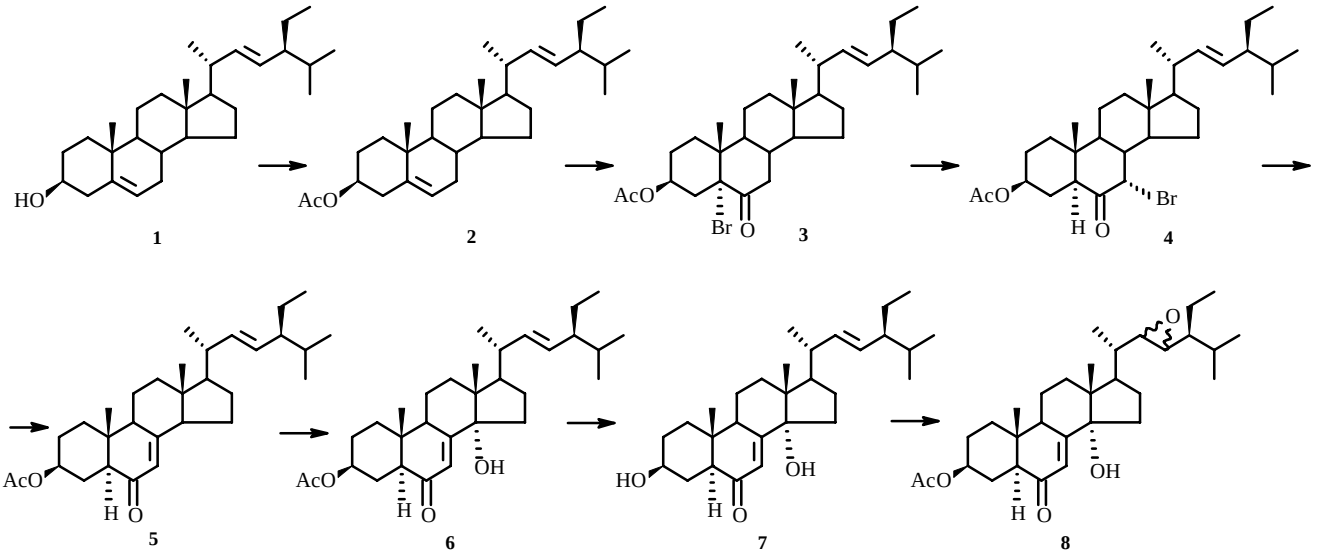

Dehydrobromination of **4** by lithium carbonate and bromide in boiling DMF gives 7,22-dien-6-one **5** in 60% yield. The UV spectrum of **5** has a strong band at 245 nm that is consistent with an α,β-unsaturated ketone. The IR spectrum suggests this same conclusion because it has absorption bands of a 6-ketone and a 7(8)-double bond conjugated to it at 1685 and 1635 cm^{-1}, respectively. The ^1H NMR spectrum of **5** exhibits a signal for the vinyl proton H-7 with δ 5.74 ppm. This signal appears as a poorly resolved triplet with J = 2.5 Hz that is due to allylic coupling with methine protons H-9α and H-14α.

In the next step **5** underwent allylic oxidation by selenium dioxide in dioxane to introduce the 14α-hydroxyl. This produced 14α-hydroxy-7,22-dien-6-one **6** in 77% yield. The presence of the additional 14α-hydroxyl in **6** can easily be proved by the changes that are observed in the ^1H NMR spectrum. First, methine proton H-9α, which occupies a 1,3-diaxial position relative to the 14α-hydroxyl, is significantly shifted to weak field compared to its position in the spectrum of **5**. Second, the signal of vinyl proton H-7 in the spectrum of **6** is a doublet. This is consistent with its allylic coupling to only one H-9α.

Subsequent hydrolysis of the acetoxy group in **6** by K_2CO_3 in methanol produced alcohol **7** in quantitative yield. Its structure is demonstrated unambiguously from spectral data. In particular, the ^1H NMR spectrum lacks a signal for the acetoxy methyl at ~2 ppm. The signal for H-3α is shifted to 3.58 ppm (i.e., by -1.15 ppm) relative to its position in the spectrum of **6**. This shift is due to the fact that this proton in **7** is geminal to a hydroxyl and not an acetoxy. The cyclic part of **7** has the minimal set of functional groups that are characteristic of ecdysteroids. Nevertheless, the presence of the 22(23)-double bond enables the necessary functional groups to be introduced into the side chain. As an example, we demonstrated this by conversion of **7** into the 22,23-epoxide **8**. Several reports on the isolation of ecdysteroids with a 22,23-epoxy group have recently appeared. These compounds include polyporusterones C and E [7], atrosterone B, and 25-hydroxyatrosterone B [8]. Furthermore, it is known from the chemistry of brassinosteroids that 22,23-epoxysteroids are convenient starting materials for the synthesis of *cis*-22,23-dihydroxysteroids.

We synthesized **8** in 85% yield by the usual reaction of Δ^{22}-steroid **7** with *m*-chloroperbenzoic acid. The products are a mixture of the (22R,23R)- and (22S,23S)-isomers with the first predominating. The structure of **8** was proved by the absence in the ^1H NMR spectrum of signals for the vinyl protons of the side chain. Instead of them the spectrum contains signals for methine protons H-22 and H-23 with δ 2.60 and 2.84 ppm that are geminal to the epoxide. The ^1H NMR data also are consistent with the presence of the remaining functional groups in **8**.

The possibility of using the synthesis developed by us to prepare other ecdysteroids is being investigated. These results will be reported later.

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. ^1H NMR spectra were obtained on a Bruker AC-200 NMR spectrometer at working frequency 200 MHz. Chemical shifts are given relative to

TMS as an internal standard.

(22E,24S)-3β-Acetoxy-5-bromo-5α-stigmast-22-en-6-one (3). A solution of stigmasterol acetate (**2**, 1.00 g, obtained by acetylation of **1** according to the literature method [4]) in dioxane (65 ml) at room temperature was treated with water (2.5 ml), perchloric acid (0.5 ml, 32%), and N-bromoacetamide (0.40 g). The mixture was stirred for 17 min, treated with chromic acid (1.5 ml, 8 N), and stirred for another 50 min. The excess of oxidant was decomposed with isopropanol (10 ml). The solution was filtered through a layer of aluminum oxide and diluted with water (50 ml). The product was extracted by a mixture of hexane and ether. The organic layer was washed with 5% $NaHCO_3$ and water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane:THF (25:1). Yield 0.46 g (40%) of 5α-bromoketone **3**, mp 179-181°C (isopropanol). IR spectrum (cm^{-1}): 1745, 1735, 1260, 1240 (AcO), 1720 (C=O). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.68 (18-Me, s), 1.00 (19-Me, s), 1.04 (21-Me, d, J = 7 Hz), 2.04 (AcO, s), 5.02 (H-22, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.16 (H-23, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.32 (H-3α, m, W/2 = 24 Hz).

(22E,24S)-3β-Acetoxy-7α-bromo-5α-stigmast-22-en-6-one (4). A solution of **3** (0.33 g) in acetic acid (30 ml) was heated to 60°C and treated with stirring with HBr (1 ml, 40%). The mixture was stirred at 60-63°C for 2 h, cooled to room temperature, and diluted with water. The product was extracted with toluene. The organic layer was washed with water, 5% $NaHCO_3$, and again with water. The solvent was evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by toluene. Yield 0.25 g (76.5%) of 7α-bromoketone **4**, mp 183-186°C (hexane). IR spectrum (cm^{-1}): 1745, 1250 (AcO), 1720 (C=O). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.72 (18-Me, s), 0.80 (19-Me, s), 1.04 (21-Me, d, J = 7 Hz), 2.05 (AcO, s), 4.20 (H-7β, d, J = 3.5 Hz), 4.74 (H-3α, m, W/2 = 24 Hz), 5.02 (H-22, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.16 (H-23, dd, J_1 = 15 Hz, J_2 = 8 Hz).

(22E,24S)-3β-Acetoxy-5α-stigmast-7,22-dien-6-one (5). A solution of **4** (0.24 g) in DMF (10 ml) was treated with Li_2CO_3 (0.24 g) and LiBr (0.12 g). The mixture was boiled for 1 h, cooled to room temperature, and filtered. The filtrate was diluted with water and extracted with toluene. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel plate with elution by cyclohexane:ether (4:1). Yield 0.12 g (60%) of dienone **5**, mp 158-163°C. IR spectrum (cm^{-1}): 1750, 1250 (AcO), 1685 (C=O), 1635 (C=C). UV spectrum (λ_{max}, nm): 245 (ε = 15,500). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.62 (18-Me, s), 0.88 (19-Me, s), 1.04 (21-Me, d, J = 7 Hz), 2.04 (AcO, s), 4.72 (H-3α, m, W/2 = 25 Hz), 5.04 (H-22, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.18 (H-23, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.74 (H-7, t, J = 2.5 Hz).

(22E,24S)-3β-Acetoxy-14α-hydroxy-5α-stigmast-7,22-dien-6-one (6). A solution of **5** (0.100 g) in dioxane (4 ml) heated to 80°C was treated with selenium dioxide (0.100 g) in dioxane (6 ml). The mixture was heated at 81-83°C under N_2 for 30 min, cooled to room temperature, and filtered through a layer of silica gel. The filtrate was diluted with water and extracted with toluene. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel plate with elution by cyclohexane:THF (4:1). Yield 0.081 g (77%) of the 14α-hydroxy derivative **6**, mp 203-206°C (hexane—ether). UV spectrum (λ_{max}, nm): 241 (ε = 14,700). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.70 (18-Me, s), 0.88 (19-Me, s), 1.04 (21-Me, d, J = 7 Hz), 2.05 (AcO, s), 2.34 (H-5α, dd, J_1 = 12 Hz, J_2 = 3.5 Hz), 2.73 (H-9α, m, W/2 = 23 Hz), 4.73 (H-3α, m, W/2 = 26 Hz), 5.06 (H-22, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.20 (H-23, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.90 (H-7, d, J = 2.5 Hz).

(22E,24S)-3β,14α-Dihydroxy-5α-stigmast-7,22-dien-6-one (7). A solution of **6** (0.080 g) in methanol (5 ml) was treated with water (0.2 ml) and K_2CO_3 (0.046 g). The reaction mixture was refluxed for 25 min, cooled to room temperature, and evaporated under vacuum. The residual solvent was azeotroped off with toluene. The solid was chromatographed on a silica-gel plate with elution by cyclohexane:THF (2:3). Yield 0.071 g (97%) of diol **7**, mp 191-193°C (ether). UV spectrum (λ_{max}, nm): 241 (ε = 15,500). ^1H NMR spectrum ($CDCl_3$—CD_3OD, 9:1, δ, ppm): 0.70 (18-Me, s), 0.88 (19-Me, s), 1.04 (21-Me, d, J = 7 Hz), 2.33 (H-5α, dd, J_1 = 12 Hz, J_2 = 3.5 Hz), 2.72 (H-9α, m, W/2 = 22 Hz), 3.58 (H-3α, m, W/2 = 33 Hz), 5.06 (H-22, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.20 (H-23, dd, J_1 = 15 Hz, J_2 = 8 Hz), 5.88 (H-7, d, J = 2.5 Hz).

(22RS,23RS,24S)-3β,14α-Dihydroxy-22,23-epoxy-5α-stigmasta-7,22-dien-6-one (8). A solution of **7** (0.060 g) in methylenechloride (5 ml) was treated with stirring with $NaHCO_3$ (0.050 g) and *m*-chloroperbenzoic acid (85%, 0.043 g). The reaction mixture was stirred for 19 h at room temperature. The solvent was removed under vacuum. The solid was chromatographed on a silica-gel plate with elution by toluene:THF (3:2). Yield 0.053 g (85%) of epoxysteroid **8**, mp 155-165°C (isopropanol). ^1H NMR spectrum ($CDCl_3$—CD_3OD, 9:1, δ, ppm): 0.68, 0.70 (18-Me, s), 0.88 (19-Me, s), 1.06, 1.20 (21-Me, d, J = 6.5 Hz), 2.32 (H-5α, dd, J_1 = 12 Hz, J_2 = 3.5 Hz), 2.60 (m, W/2 = 12 Hz) and 2.84 (dd, J_1 = 7.5 Hz, J_2 = 2.5 Hz) (total 2H, H-22 and H-23), 2.74 (H-9α, m, W/2 = 22 Hz), 3.58 (H-3α, m, W/2 = 33 Hz), 5.88 (H-7, d, J = 2.5 Hz).

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