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SYNTHESIS OF (22S,23S,24S)-3β,14α,22,23-TETRAHYDROXY--5α-STIGMAST-7-EN-6-0NE, A NEW ECDYSTEROID ANALOG

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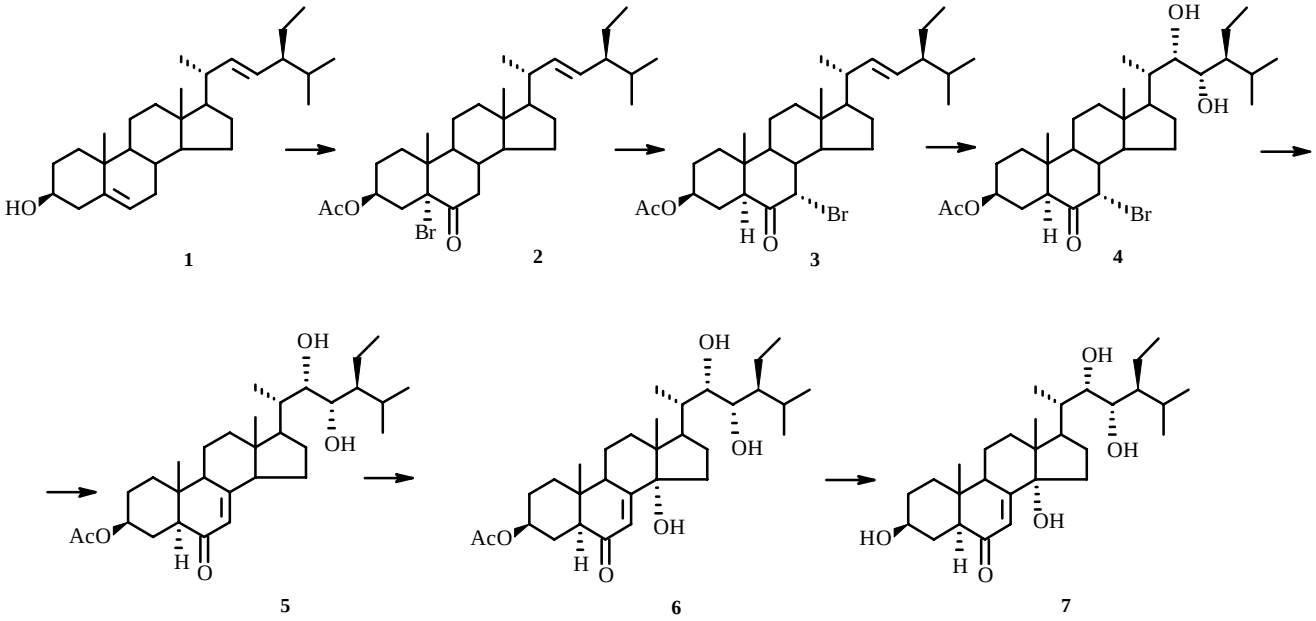
A tetrahydroxyketone that is a structural analog of ecdysteroids is synthesized from stigmasterol.

Key words: synthesis, stigmasterol, (22S,23S,24S)-3β,14α,22,23-tetrahydroxy-5α-stigmast-7-en-6-one.

We previously developed a new synthetic route to insect hormones, ecdysteroids, starting with stigmasterol (**1**) [1]. The key step in this scheme is the rearrangement of a 5α-bromo-6-ketone (**2**) into a 7α-bromo-6-ketone (**3**) by the action of HBr in acetic acid. Because compound **3** contains a Br in the 7-position, the 7(8)-double bond required in ecdysteroids can be simply introduced as necessary in one of the synthetic steps [2]. Compounds with required functional groups in the side chain can be synthesized because a 22(23)-double bond is also present. Such potential was demonstrated [1] using the preparation of ecdysteroid analogs containing a 22(23)-epoxide as an example. In the present article, the possibility of using compound **3** to synthesize ecdysteroid analogs that contain the 22,23-diol moiety is examined. This structural fragment is characteristic of brassinosteroid phytohormones. It should also be noted that the 22,23-diol moiety occurs in the ecdysteroids rapisterone [3], gerardiasterone [4], and 23-hydroxycyasterone [5].

First, the double bond in **3** was *cis*-hydroxylated by OsO_4 according to Criegee to give compound **4**, which has the required 22,23-diol. The structure of steroid **4** as a (22S,23S)-22,23-diol was proved using spectra. In particular, the ^1H NMR of this compound lacks signals for vinylic protons ΔH-22 and H-23. Instead of them, a 2H multiplet for methine protons geminal to 22- and 23-hydroxyls appear at δ 3.60 ppm. The position and shape of these signals are typical of stigmastane (22S,23S)-22,23-diols, which are formed mainly via *cis*-hydroxylation of the Δ^{22}-bond by OsO_4 [6-8]. The presence of a characteristic doublet for methine proton H-7β at δ 4.20 ppm indicates that the Br on C-7 remains in steroid **4**.



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In the next step, dehydrobromination of compound **4** by Li_2CO_3 and LiBr in boiling DMF gave the Δ^7-6-ketone **5**. The presence in the UV spectrum of an absorption band at 244 nm is important for proving the structure of the α,β-unsaturated ketone **5**. The IR spectrum of this compound contains bands for stretches of the ketone and double bond conjugated to it at 1685 and 1635 cm^{-1}, respectively. The appearance in the ^1H NMR spectrum at δ 5.74 ppm of a signal for vinylic proton H-7 also confirms the structure of enone **5**. This signal appears as a triplet with splitting constant J = 2.5 Hz due to allylic coupling with methine protons H-9α and H-14α.

Allylic hydroxylation of steroid **5** by selenium dioxide in dioxane produces the 14α-alcohol **6**. The presence of the 14α-hydroxyl was confirmed by ^1H NMR spectra, in which the signal for H-7 is shifted to weak field at δ 5.90 ppm. Because this proton couples only with H-9α, its signal appears as a doublet with splitting constant J = 2.5 Hz. Furthermore, the signal for H-9α shifts to weak field owing to coupling with the 14α-hydroxyl.

In the final step, the 3β-acetoxy goup in steroid **6** is hydrolyzed by K_2CO_3 in aqueous methanol. This produces the desired 3β,14α,22,23-tetrahydroxy-6-ketone **7** in 84% yield. Its structure follows unambiguously from spectral data. In particular, the ^1H NMR spectrum in deuteropyridine lacks a signal for the acetyl methyl at ~2 ppm. The signal for H-3α is shifted to strong field at δ 3.76 ppm. This shift occurs because this proton in compound **7** is geminal not to an acetoxy but a hydroxy group. The ^1H NMR spectra also show that the remaining functional groups in compound **7** correspond to observed signals.

EXPERIMENTAL

Melting points were measured 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.

(22S,23S,24S)-3β-Acetoxy-22,23-dihydroxy-7α-bromo-5α-stigmastan-6-one (4). A solution of enone **3** (0.393 g, prepared from stigmasterol by the literature method [1]) in pyridine (20 ml) was treated with OsO_4 (0.170 g) in pyridine (2 ml). The reaction mixture was held for 19 h at room temperature and then treated with stirring with sodium bisulfite (8 ml, 40% solution). The mixture was held at 30-40°C for 2 h and then treated with sodium sulfite (0.5 g) and H_2SO_4 (0.15 ml) in water (5 ml). The reaction mixture was stirred at 35-45°C for 1 h, cooled to room temperature, and diluted with water. The reaction product was extracted with $CHCl_3$. The organic layer was washed with water. The solvent was evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by C_6H_6—THF (5:2). Yield of dihydroxyketone **4**, 0.330 g (79%), mp 167-168°C (acetone). IR spectrum (cm^{-1}): 3420 (OH), 1740, 1250 (AcO), 1720 (C=O). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.74 (18-Me, s), 0.78 (19-Me, s), 1.04 (21-Me, d, J = 6.5 Hz), 2.06 (AcO, s), 3.28 (H-5α, dd, J_1 = 13 Hz, J_2 = 2.5 Hz), 3.60 (H-22 and H-23, m, W/2 = 12 Hz), 4.20 (H-7β, d, J = 3.5 Hz), 4.74 (H-3α, m, W/2 = 24 Hz).

(22S,23S,24S)-3β-Acetoxy-22,23-dihydroxy-5α-stigmast-7-en-6-one (5). A solution of 7α-bromoketone **4** (0.320 g) in DMF (8 ml) was treated with Li_2CO_3 (0.315 g) and LiBr (0.160 g). The reaction mixture was boiled for 50 min, cooled to room temperature, and filtered. The filtrate was diluted with water and extracted with ether. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by ether. Yield of amorphous product, 0.255 g. Crystallization from ether gave crystalline enone **5**, 0.120 g (44%), mp 174-176°C (ether). IR spectrum (cm^{-1}): 3470 (OH), 1755, 1255 (AcO), 1685 (C=O), 1635 (C=C). UV spectrum (λ_{max}, nm): 244 (ε = 11,900). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.64 (18-Me, s), 0.88 (19-Me, s), 1.04 (21-Me, d, J = 6.5 Hz), 2.06 (AcO, s), 3.60 (H-22 and H-23, m, W/2 = 12 Hz), 4.74 (H-3α, m, W/2 = 24 Hz), 5.74 (H-7, t, J = 2.5 Hz).

(22S,23S,24S)-3β-Acetoxy-14α,22,23-trihydroxy-5α-stigmast-7-en-6-one (6). A solution of enone **5** (0.230 g) in dioxane (5 ml) was treated with a solution of selenium dioxide (0.230 g) in dioxane (10 ml). The mixture was heated at 80°C under a N_2 atmosphere for 0.5 h, cooled to room temperature, and filtered through a layer of silica gel. The filtrate was diluted with water and extracted with $CHCl_3$. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by ether—hexane (4:1). Yield of 14a-hydroxyderivative **6**, 0.137 g (58%), mp 194-197°C (ether). UV spectrum (λ_{max}, nm): 242 (ε = 10,200). IR spectrum (cm^{-1}): 3470 (OH), 1740, 1250 (AcO), 1675 (C=O), 1635 (C=C). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.72 (18-Me, s), 0.87 (19-Me, s), 1.00 (21-Me, d, J = 6.5 Hz), 2.04 (AcO, s), 2.34 (H-5α, dd, J_1 = 12.5 Hz, J_2 = 3 Hz), 2.73 (H-9α, m, W/2 = 23 Hz), 3.63 (H-22 and H-23, m, W/2 = 12 Hz), 4.73

(H-3α, m, W/2 = 25 Hz), 5.90 (H-7, d, J = 2.5 Hz).

(22S,23S,24S)-3β,14α,22,23-Tetrahydroxy-5α-stigmast-7-en-6-one (7). A solution of acetate **6** (0.125 g) in methanol (8 ml) was treated with water (0.4 ml) and K_2CO_3 (0.070 g). The reaction mixture was refluxed for 25 min, cooled to room temperature, and evaporated under vacuum. The solid was chromatographed on a silca-gel column with elution by ether-THF of increasing polarity from 20:1 to 3:1. Yield of tetraol **7**, 0.097 g (84%), mp 207-209°C (isopropanol). UV spectrum (λ_{max}, nm): 242 (ε = 11,000). ^{1}H NMR spectrum (C_5D_5N, δ, ppm): 0.74 (18-Me, s), 0.92 (19-Me, s), 2.20 (H-5α, dd, J_1 = 12.5 Hz, J_2 = 3 Hz), 2.96 (H-9α, m, W/2 = 23 Hz), 3.76 (H-3α, m, W/2 = 25 Hz), 4.00 (H-22 and H-23, m, W/2 = 9 Hz), 6.15 (H-7, d, J = 2.5 Hz); ($CDCl_3$—CD_3OD, 4:1, δ, ppm): 0.72 (18-Me, s), 0.84 (19-Me, s), 1.02 (21-Me, d, J = 6.5 Hz), 2.32 (H-5α, dd, J_1 = 12.5 Hz, J_2 = 3 Hz), 2.73 (H-9α, m, W/2 = 23 Hz), 3.42-3.80 (H-3α, H-22 and H-23, m), 5.88 (H-7, d, J = 2.5 Hz).

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