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A Short Way to Invert Configuration of the 2,3-Hydroxy Groups in Ecdysteroids

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Abstract—3-*epi*-2-Dehydro-20-hydroxyecdysone and its 20,22-acetonide were reduced with lithium tris(*sec*-butyl)hydridoborate selectively at the 2-oxo group with formation of 2β,3β-dihydroxy derivatives and the corresponding 2α,3α epimers which were separated by chromatography.

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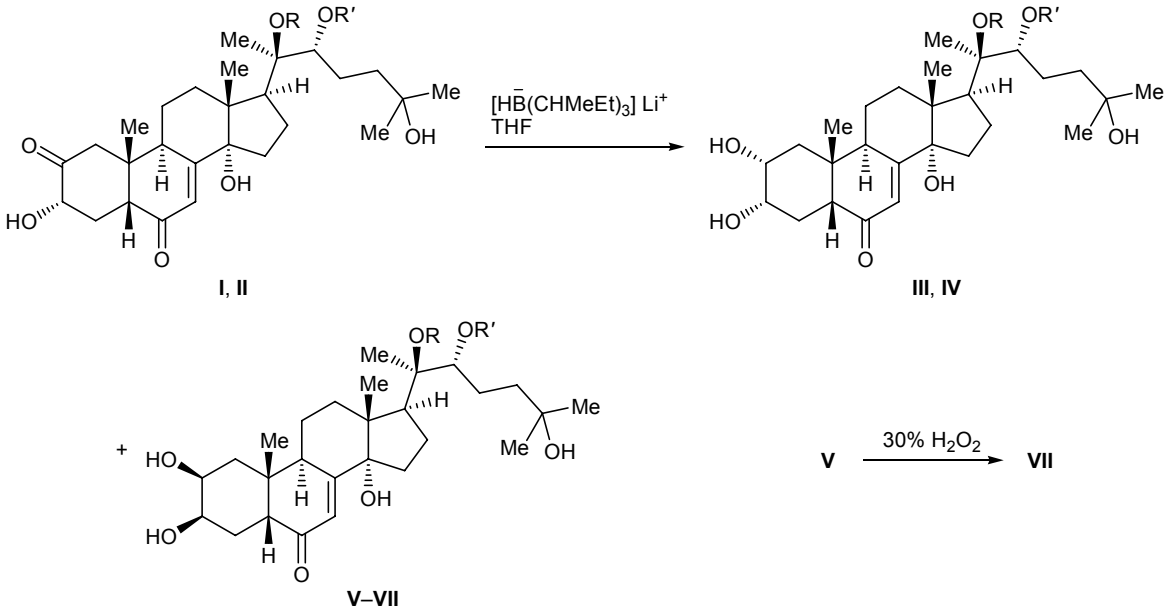
Ecdysteroids are hormones responsible for moulting, metamorphosis, and reproduction of insects and other arthropods. They are also produced by plants even at much higher concentrations (up to 2–2.5% of the air-dry weight of some plant species) [1–4]. The hydroxy groups in the A ring of ecdysteroids are usually 2β,3β-configured; however, some plant species contain minor ecdysteroid components with α-oriented hydroxy groups in the A ring [5–8]. The most promising way of synthesis of such minor ecdysteroids and their analogs is 2,3-epimerization of one of the most widely known ecdysteroid, 20-hydroxyecdysone, and its derivatives. However, the known methods for epimerization of ecdysteroids include many steps [9, 10].

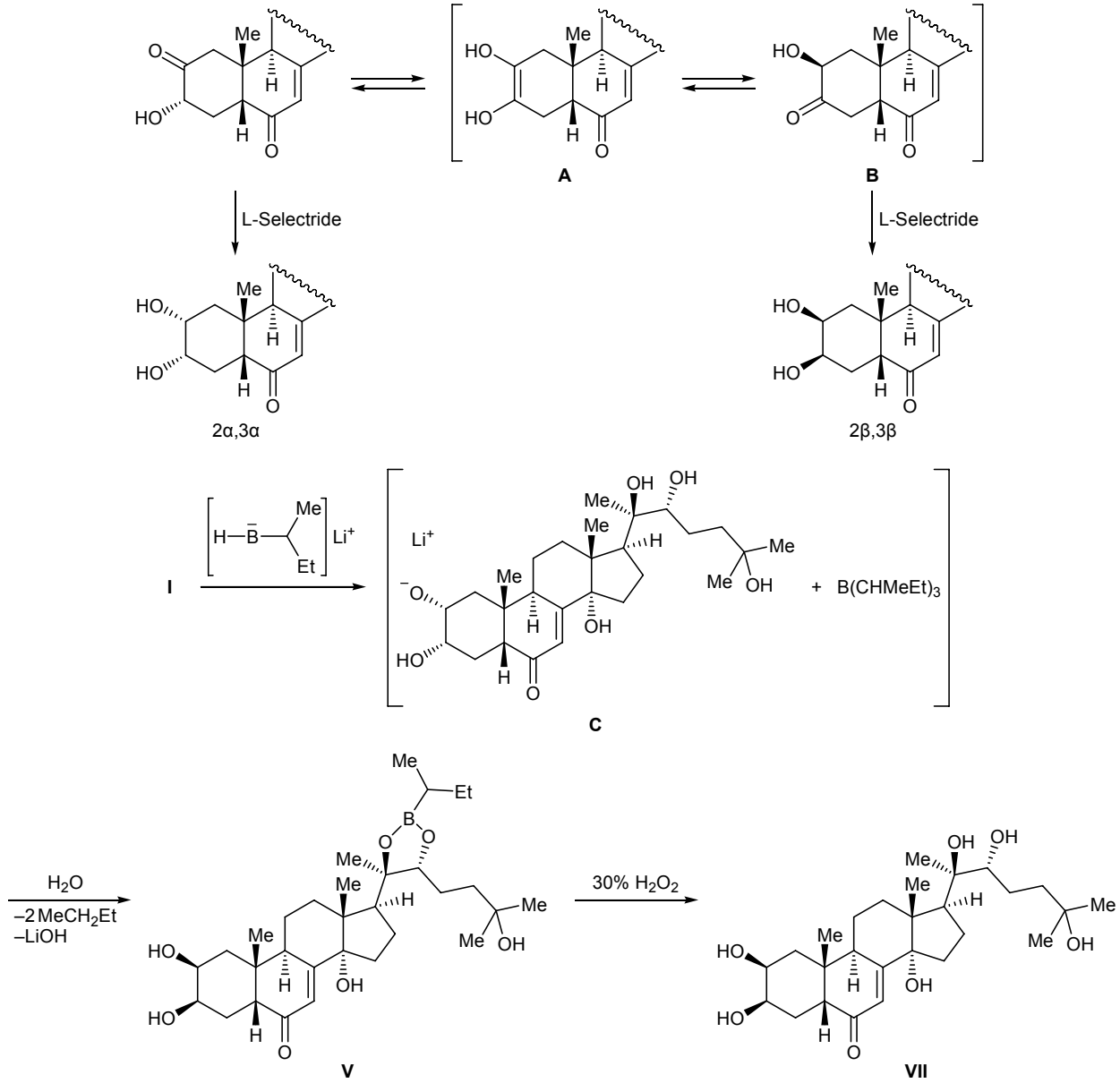
We previously reported on a one-step synthesis of 3-*epi*-2-dehydro-20-hydroxyecdysone (**I**) and its 20,22-acetonide (**II**) via ozonation in pyridine [11]. Selective reduction of the 2-oxo group in compounds **I**

Scheme 1.



I, **III**, **VII**, R = R′ = H; **II**, **IV**, **VI**, RR′ = Me_2C; **V**, RR′ = MeEtCHB.

Scheme 2.



and **II** could provide the shortest way to 2,3-di-*epi*-20-hydroxyecdysone (**III**) and its 20,22-acetonide **IV**.

The reduction of diketones **I** and **II** with alkali metal hydride complexes ($LiAlH_4$, $NaBH_4$, $NaBH_4$–$CeCl_3$) involved both oxo groups in positions *2* and *6*. Treatment of **I** and **II** with 9-bora[3.3.1]bicyclononane ensured selective reduction of the 2-oxo group, but the products were 20-hydroxyecdysone and its 20,22-acetonide, respectively.

2,3-Di-*epi*-ecdysteroids **III** and **IV** were obtained by reaction of diketones **I** and **II** with lithium tris(*sec*-butyl)hydridoborate (L-Selectride) in THF at –10°C. However, apart from 2α,3α-epimers **III** and **IV**, we isolated from the reaction mixtures by column chromatography on silica gel 20-hydroxyecdysone 20,22-(*sec*-butyl)boronate (**V**) and 20-hydroxyecdysone 20,22-acetonide (**VI**), respectively. Removal of the boronate group from **V** by treatment with 30% hydrogen peroxide afforded 20-hydroxyecdysone (**VII**) (Scheme 1).

Thus the final products of the hydride reduction of 3-*epi*-2-dehydroecdysteroids **I** and **II** with L-Selectride are the corresponding 2α,3α- and 2β,3β-dihydroxy derivatives **III**, **IV** and **VI**, **VII**, respectively, at a ratio of ~30:70. With account taken of the number of steps in the synthesis of 3-*epi*-2-dehydro-20-hydroxyecdy-

sone [11], we have proposed a two-step procedure for the inversion of configuration of the 2,3-hydroxy groups in 20-hydroxyecdysone; the known synthesis of 2,3-di-*epi*-20-hydroxyecdysone includes six steps [10].

Presumably, the formation of 2β,3β epimers in the examined reactions is the result of keto–enol tautomerism known for α-hydroxy ketones. Intermediate α,β-enediol **A** is likely to occur in equilibrium with initial 2-oxo-3α-hydroxy derivative **I** and its 3-oxo-2β-hydroxy isomer **B**, and their reduction with L-Selectride yields 2α,3α and 2β,3β epimers, respectively (Scheme 2). Boronic ester **V** is formed due to the known ability of trialkylboranes to react with hydroxy compounds; this reaction is used to protect hydroxy groups [12], e.g., in the synthesis of shidasterone [13] and viticosterone E [14]. In keeping with the generally accepted mechanism of the reduction of ketones with L-Selectride [15], addition of hydride ion to the carbonyl group releases tris(*sec*-butyl)borane which reacts with vicinal hydroxy groups to produce 20-hydroxyecdysone 20,22-(*sec*-butyl)boronate (**V**) (Scheme 2). The corresponding boronic acid ester derived from 2,3-di-*epi*-20-hydroxyecdysone is likely to be less stable, so that it undergoes hydrolysis during chromatography on silica gel to give 2,3-di-*epi*-20-hydroxyecdysone (**III**).

The ^{1}H and ^{13}C NMR spectra of 2α,3α epimers **III** and **IV**, as well as of ecdysteroids **VI** and **VII**, were identical to those reported previously [10, 16, 17]. Signals in the ^{1}H and ^{13}C NMR spectra of **III** and **IV** were assigned with the aid of homo- and heteronuclear shift correlation techniques (HSQC, HMBC, COSY, ROESY).

The configuration of the 2,3-dihydroxy fragment in **III** was confirmed by the coupling constants observed for 2-H and 3-H and the neighboring protons (1-H and 4-H) in the ^{1}H NMR spectrum recorded at 323 K. Small coupling constants between 2-H (δ 4.33 ppm) and the axial and equatorial protons on C^1 (δ 1.22 and 2.42 ppm, $^3J_{2,1\text{-}ax} = {}^3J_{2,1\text{-}eq} = 2.4$ Hz) indicated equatorial orientation (β-configuration) of 2-H. Correspondingly, the 2-hydroxy group in **III** is axial (α-configuration). The coupling constant of 3-H (δ 3.90 ppm) with the axial proton on C^4 (δ 1.85 ppm) is equal to 10.6 Hz, indicating its axial orientation (β-configuration). This means that the hydroxy group on C^3 occupies equatorial position (α-configuration).

The structure of boronic ester **V** was determined on the basis of spectral data. Signals in the ^{1}H and ^{13}C NMR spectra of **V** were assigned using JMOD, HSQC, HMBC, COSY, and ROESY techniques. The spectral parameters of **V** are fairly similar to those observed for 20-hydroxyecdysone 20,22-acetonide (**VI**) [10, 16, 17]. Some differences are determined by the presence of 20,22-*O*-(*sec*-butyl)boronate moiety in molecule **V** instead of 20,22-*O*-isopropylidene group in **VI**. The result is that there is no acetal carbon signal (δ_C 106 ppm) in the ^{13}C NMR spectrum of **V**. The ^{1}H NMR spectrum of **V** contained a triplet at δ 1.03 ppm (J = 8 Hz) and a doublet at δ 1.05 ppm (J = 10 Hz) from the methyl protons in the $MeCHCH_2Me$ fragment instead of two Me_2C singlets (δ 1.26 and 1.33 ppm) present in the spectrum of **VI**. Compound **V** displayed ion peaks with m/z 569 $[M + Na]^+$ and 585 $[M + K]^+$ in the MALDI-TOF mass spectrum.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded from solutions in pyridine-d_5. The homo- and heteronuclear DEPT-135°, COSY, HSQC, and HMBC spectra were measured on Bruker Avance-400 (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) and Bruker Avance II 600 (600.13 MHz for ^{1}H and 150.76 MHz for ^{13}C) instruments equipped with an inverse broad-band probe. The chemical shifts were determined relative to tetramethylsilane as internal reference. The melting points were measured using a Boetius micro-hot stage. The optical rotations were measured on a Perkin Elmer-141 polarimeter. Analytical TLC was performed on Silufol plates; spots were developed with a solution of vanillin in ethanol acidified with sulfuric acid.

2,3-Di-*epi*-20-hydroxyecdysone (III) and (20*R*,22*R*)-2β,3β,14α,25-tetrahydroxy-20,22-(butan-2-yl)boranediyldioxy-5β-cholest-7-en-6-one [V, 20-hydroxyecdysone 20,22-(*sec*-butyl)boronate]. A solution of 0.15 g (0.31 mmol) of compound **I** {mp 136–138°C, $[\alpha]_D^{20}$ = +13.05° (c = 2.18, $CHCl_3$) [11]} in 10 ml of anhydrous THF was cooled to –10°C, 0.56 ml (0.47 mmol) of a 1 M solution of L-Selectride in THF was added under argon, and the mixture was stirred until the initial compound disappeared (2 h, TLC). The mixture was evaporated, and the residue was subjected to chromatography on 4.5 g of silica gel (gradient elution with chloroform to chloroform–methanol, 10:1) to isolate 0.025 g (20%) of compound **III**, R_f 0.46 ($CHCl_3$–MeOH, 3:1), whose ^{1}H and ^{13}C NMR spectra were identical to those reported in [10], and 0.07 g (60%) of boronate **V**, R_f 0.45 ($CHCl_3$–MeOH, 5:1), mp 136–138°C, $[\alpha]_D^{20}$ = +63.8° (c = 0.58, MeOH). ^{1}H NMR spectrum, δ, ppm: 0.99 s (3H, $C^{18}H_3$), 1.03 t (3H, CH_3CH_2CHB, J = 8.0 Hz), 1.05 d

(3H, C**H**$_3$CHB, J = 10.0 Hz), 1.12 s (3H, C^{19}H$_3$), 1.38 s (9H, C^{21}H$_3$, C^{26}H$_3$, C^{27}H$_3$), 1.66 m and 2.09 m (2H, 1-H), 2.72 m (1H, 17-H), 3.00 br.d (1H, 5-H, J = 3.9 Hz), 3.52 br.s (1H, 9-H, $w_{1/2}$ = 7.2 Hz), 4.12 m (1H, 2-H), 4.18 m (1H, 22-H), 4.21 m (1H, 3-H), 6.20 s (1H, 7-H). ^{13}C NMR spectrum, δ_C, ppm: 13.15 (**C**H$_3$CH$_2$CHB), 14.66 (C**H**$_3$CHB), 18.17 (C^{18}), 19.90 (C^{11}), 20.86 (C^{16}), 21.74 (C^{21}), 23.37 (C^{19}), 25.65 (C^{23}), 29.53 and 29.64 (C^{26}, C^{27}), 30.46 (C^{12}, C^{15}), 31.38 (C^4), 33.26 (C^9), 36.89 (C^1), 37.65 (C^{24}), 40.87 (C^{10}), 46.47 (C^{13}), 50.33 (C^5), 53.78 (C^{17}), 64.32 (C^8), 67.68 (C^2, C^3), 68.24 (C^{25}), 83.08 (C^{14}), 83.86 (C^{22}), 85.00 (C^{20}), 120.85 (C^7), 202.38 (C^6). Mass spectrum: m/z 569 $[M + Na]^+$ and 585 $[M + K]^+$. Found, %: C 68.12; H 9.41. $C_{31}H_{51}BO_7$. Calculated, %: C 68.38; H 9.04.

Deprotection of 20-hydroxyecdysone 20,22-(*sec*-butyl)boronate (V). Compound **V**, 0.07 g (0.12 mmol), was dissolved in 5 ml of aqueous THF (THF–H_2O, 9:1), 0.003 ml (0.10 mmol) of 30% hydrogen peroxide was added, and the mixture was stirred until the initial compound disappeared (10 min, TLC). The mixture was treated with 0.015 ml of $Na_2S_2O_3$ and extracted with butan-1-ol (3×5 ml), the combined extracts were evaporated, and the residue was subjected to chromatography on 3 g of silica gel (gradient elution with chloroform to chloroform–methanol, 10:1) to isolate 0.034 g (58%) of compound **VII**, R_f 0.45 ($CHCl_3$–MeOH, 3:1), whose ^{1}H and ^{13}C NMR spectra were identical to those reported previously [5].

2,3-Di-*epi*-20-hydroxyecdysone 20,22-acetonide (IV) and 20-hydroxyecdysone 20,22-acetonide (VI). A solution of 0.12 g (0.23 mmol) of compound **II** {mp 100–102°C, $[\alpha]_D^{20}$ = +24.2° (c = 3.8, $CHCl_3$) [11]} in 10 ml of anhydrous THF was cooled to –10°C, 0.42 ml (0.35 mmol) of a 1 M solution of L-Selectride in THF was added under argon, and the mixture was stirred until the initial compound disappeared (2 h, TLC). The mixture was evaporated, and the residue was subjected to chromatography on 4 g of silica gel (gradient elution with chloroform to chloroform–methanol, 20:1) to isolate 0.03 g (30%) of compound **IV**, R_f 0.46 ($CHCl_3$–MeOH, 5:1), and 0.06 g (60%) of **VI**, R_f 0.47 ($CHCl_3$–MeOH, 5:1). The ^{1}H and ^{13}C NMR spectra of compounds **IV** and **VI** were identical to those reported previously [10].

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