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Hydroxylation and epimerization of ecdysteroids in alkaline media: Stereoselective synthesis of 9α-hydroxy-5α-ecdysteroids

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ABSTRACT

Autoxidation of diacetonides of 20-hydroxyecdysone and ponasterone A under treatment with excess of NaOH in methanol leads to the formation of 9α-hydroxy-5α-ecdysteroids previously not described. Their structures have been determined by detailed NMR analysis. Catalytic hydrogenation (Pd-C, MeOH–MeONa) of hydroxylated ecdysteroids affords the 7,8α-dihydro-9α-hydroxy-5α-ecdysteroids.

1. Introduction

Ecdysteroids are polyhydroxysterols with a *cis*-fused A/B ring junction and 14α-hydroxy-7-en-6-one system. These compound exhibit physiological activities in insects and has been shown in both invertebrates and plant species [1]. Originally, these hormones were identified from insects, but they were later isolated from plants, where their concentrations reach 2–3% of the dry weight in some species, making it possible to study the physiological properties of the ecdysteroids and their synthetic transformations [2,3]. A variety of chemical transformations of an available phytoecdysteroid 20-hydroxyecdysone leading to rare ecdysteroids and their analogues with new properties have been carried out [4–9]. A *cis*-fused A/B ring of the ecdysteroids structure requires the β-configuration of the hydrogen atom at C-5-position. Nearly all the naturally occurring ecdysteroids have the 5β-hydrogen atom. However, the isolation of 5α-20-hydroxyecdysone from plants *Achyranthes fauriei* and Leuzea carthamoides, as well as 22-*O*-benzoyl-5α-20-hydroxyecdysone from the plant Silene scabrifolia has been also demonstrated [10–13]. Partial inversion of the C-5 chiral atom of 20-hydroxyecdysone in the presence of a base is well known [13,14].

It has been found that the unsubstituted 9-position is additional essential feature for molting hormonal activity [14]. The number, location, and stereochemistry of hydroxyl groups in the molecule are also responsible for high activity of ecdysteroids. In most cases, their synthesis implies introduction of new hydroxyl groups into a starting molecule of ecdysteroids. That is rather problematic, especially for the C-9 position of steroid skeleton. The first representatives of 9-hydroxylated ecdysteroids, a namely, 9α,20-dihydroxy- [15] and 9β,20-dihydroxyecdysone [16] have been isolated from *Silene italica* ssp. *nemoralis*. These and other 9-hydroxylated ecdysteroids are interesting as new low molecular weight bioregulators.

The base-catalyzed (2% aqueous methanolic solution) autoxidation of 20-hydroxyecdysone have been shown [17] for the synthesis of 9α,20-dixydroxyecdysone (29%) along with calonysterone (35%) but the configuration of C5-hydrogen atom was not established in this report. On the other hand, the synthesis of 9α,20-dihydroxyecdysone diacetonide via the Si–O bond cleavage of 14α-trimethylsilyl precursor under the action of the 6-fold excess of tetrabutylammonium fluoride was reported [18]. In such a case, the inversion of the C(5)-hydrogen atom was improbable and the synthesized 9α,20-dihydroxyecdysone was 5β-oriented. The alpha (α) or beta (β) configuration of the hydrogen atom at C-5-position agrees with a A/B-ring (cis or trans) junction, respectively and it is a very important aspect for the ecdysonic activity [2].

In this paper we report the stereoselective synthesis of 9α-hydroxy-5α-ecdysteroids via autoxidation of 5β-ecdysteroids in alkaline media (10% NaOH-MeOH).

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2. Experimental

One-dimensional (^{1}H and ^{13}C) and two-dimensional (COSY, NOESY, HSQC, and HMBC) NMR spectra of compounds were recorded on Bruker Avance 400 spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C), equipped with broadband observer probe; 9α,20-dihydroxy-5α-ponasterone A diacetonide (**4**), 7,8α-dihydro-9α,20-dihydroxy-5α-ponasterone A diacetonide (**11**) were recorded on Bruker Avance III 500 spectrometer (500.17 MHz for ^{1}H and 125.78 MHz for ^{13}C), and NMR spectra of 9α,20-dihydroxy-5α-ecdysone (**5**) were recorded on Bruker Avance II 600 spectrometer (600.13 MHz for ^{1}H and 150.76 MHz for ^{13}C), equipped with an inverse broadband probe. All the experiments were set up with standard Bruker methods. Chemical shifts are given in ppm using TMS as the internal standard. Mass-spectra were measured by MALDI TOF methods on Bruker Autoflex III spectrometer with registration of positive ions; 2,5-dihydroxybenzoic and α-cyano-4-hydroxycinnamic acids were used as matrix. Specific rotations were measured on Perkin–Elmer-341 polarimeter. Melting points were determined on Boetius hot stage. Column chromatography and TLC were performed using silica gel (<0.06 mm) and pre-coated silica gel (Silufol plates), respectively; spots were processed by treatment with a solution of 4-hydroxy-3-methoxybenzaldehyde in ethanol, acidified with sulfuric acid.

The stock compounds were synthesized from 20-hydroxyecdysone [(mp 239–240 °C, $[\alpha]_D^{20} + 54.3^\circ$ (c 1.45, MeOH); literature: mp 241–242.5 °C, $[\alpha]_D^{20} + 61.8^\circ$ (MeOH) [3]; mp 246 °C (EtOAc–MeOH, 9:1), ,$[\alpha]_D^{20} + 65.3^\circ$ (c 1.0, MeOH) [19]]. 20-Hydroxyecdysone was isolated from the juice of *Serratula coronata* L. [19]. 20-Hydroxyecdysone diacetonide (**1**) [mp 233–234 °C, $[\alpha]_D^{20} + 26.8^\circ$ (c 2.54, $CHCl_3$); literature: mp 234–235 °C, $[\alpha]_D^{20} + 39.4^\circ$ (c 1.1, $CHCl_3$) [20]], ponasterone A diacetonide (**2**) [mp 110 °C, $[\alpha]_D^{20} + 25.4^\circ$ (c 1.75, $CHCl_3$); literature: mp 100–102 °C, $[\alpha]_D^{20} + 31.8^\circ$ (c 3.07, $CHCl_3$) [21]]. ^{1}H and ^{13}C NMR data for 20-hydroxyecdysone, diacetonide (**1**) and (**2**) were identical with NMR data in Refs. [19-21] accordingly.

*2.1. (20R,22R)-9α,14α,25-Trihydroxy-2β,3β:20,22-diisopropylidenedioxy-5α-cholest-7-en-6-one (**3**)*

Compound **1** (0.20 g, 0.36 mmol) was added under stirring to 10 mL of 10% solution of NaOH in MeOH. The mixture was stirred for 3 h at room temperature without exclusion of O_2, and evaporated under reduced pressure. The residue was chromatographed on SiO_2 (9 g) ($CHCl_3$ was used as the eluent) to yield **3** (0.14 g, 68%). Mp 143–145 °C, $[\alpha]_D^{20} + 11.2^\circ$ (*c* 1.63, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H, 18-Me), 1.11 (s, 3H, 19-Me), 1.19 (s, 3H, 21-Me), 1.24 (s, 3H, 26-Me), 1.25 (s, 3H, 27-Me), 1.33 (s, 6H, 2,3-CMe_2), 1.42 and 1.51 (both s, 6H, 20,22-CMe_2), 1.92 and 2.46 (both m, 2H, 1-H), 2.28 (m, 1H, 17-H), 3.01 (dd, *J* = 2.0 Hz, *J* = 7.0 Hz, 1H, 5-H), 3.67 (m, 1H, 22-H), 4.11 (m, 1H, 3-H), 4.30 (m, 1H, 2-H), 5.90 (s, 1H, 7-H); ^{13}C NMR ($CDCl_3$) δ 17.16 (C18), 18.19 (C19), 20.90 (C11), 21.90 (C21), 23.53 (C16), 24.39 (C15), 26.25 and 26.86 (20,22-$\underline{Me_2}$C), 27.96 (C4, C12), 28.68 and 28.93 (2,3-$\underline{Me_2}$C), 29.33 (C26), 29.41 (C27), 30.54 (C1), 31.23 (C23), 41.33 (C24), 41.74 (C10), 45.07 (C5), 46.99 (C13), 48.83 (C17), 70.53 (C25), 73.46 (C2), 73.94 (C3), 74.00 (C9), 82.02 (C22), 84.34 (C20), 86.14 (C14), 107.02 (20,22-$Me_2\underline{C}$), 108.17 (2,3-$Me_2\underline{C}$), 124.01 (C7), 158.08 (C8), 200.85 (C6). MALDI TOF *m/z* 599.35 $[M+Na]^+$, calcd for $C_{33}H_{52}O_8Na$ 599.76; *m/z* 615.34 $[M+K]^+$ calcd for $C_{33}H_{52}O_8K$ 615.76.

*2.2. (20R,22R)-9α,14α-Dihydroxy-2β,3β:20,22-diisopropylidenedioxy-5α-cholest-7-en-6-one (**4**)*

Compound **4** (0.19 g, 70%) was obtained from **2** (0.27 g, 0.5 mmol) in a similar manner to that used to synthesize **3**. Mp 108–110 °C, ,$[\alpha]_D^{20} + 18.7^\circ$ (*c* 0.43, $CHCl_3$); ^{1}H NMR (500.17 MHz, $CDCl_3$) δ 0.82 (s, 3H, 18-Me), 0.92 (d, *J* = 6.5 Hz, 3H, 27-Me), 0.93 (d, *J* = 6.5 Hz, 3H, 26-Me), 1.10 (s, 3H, 19-Me), 1.17 (s, 3H, 21-Me), 1.33 (s, 6H, 2,3-CMe_2), 1.42 and 1.50 (both s, 6H, 20,22-CMe_2), 2.29 (m, 1H, 17-H), 2.98 (dd, *J* = 3.0 Hz, *J* = 13.0 Hz, 1H, 5-H), 3.62 (m, 1H, 22-H), 4.07 (m, 1H, 3-H), 4.27 (m, 1H, 2-H), 5.81 (s, 1H, 7-H); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 17.23 (C18), 18.15 (C19), 20.91 (C11), 21.94 (C16, C21), 22.52 (C27), 22.61 (C26), 24.46 (C15), 26.26 (C12), 26.89 and 27.93 (20,22-$\underline{Me_2}$C), 28.27 and 29.02 (2,3-$\underline{Me_2}$C), 26.78 (C23), 28.70 (C25), 30.49 (C1), 31.39 (C4), 36.46 (C24), 41.70 (C10), 45.11 (C13), 46.93 (C5), 48.87 (C17), 73.47 (C2), 73.75 (C3), 73.98 (C9), 81.75 (C22), 84.09 (C20), 86.19 (C14), 106.78 (20,22-$Me_2\underline{C}$), 108.12 (2,3-$Me_2\underline{C}$), 123.95 (C7), 158.67 (C8), 201.35 (C6).

*2.3. (20R,22R)-2β,3β,9α,14α,20,22,25-Heptahydroxy-5α-cholest-7-en-6-one (**5**) and (20R,22R)-2β,3β,9α,14α,25-pentahydroxy-20,22-isopropylidenedioxy-5α-cholest-7-en-6-one (**6**)*

Compound **3** (0.17 g, 0.29 mmol) was dissolved in MeOH (5 mL), and 3.4 mL of 10% $HClO_4$ was added. The mixture was stirred at ambient temperature for 2 h. A saturated solution of $NaHCO_3$ (3.4 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was chromatographed on SiO_2 (5 g, eluent – $CHCl_3$) to yield compound **6** (0.08 g, 51%), and compound **5** (0.06 g, 42%) ($CHCl_3$–MeOH (10:1) was used as the eluent).

Compound **5**. Mp 193–195 °C, $[\alpha]_D^{20} + 16.4^\circ$ (*c* 0.92, CH_3OH); ^{1}H NMR (600.13 MHz, C_5D_5N) δ 1.22 (s, 3H, 18-Me), 1.41 (s, 6H, 27-Me, 26-Me), 1.57 (s, 3H, 19-Me), 1.60 (s, 3H, 21-Me), 1.96 (m, 1H, 11α-H), 2.10 and 2.70 (both m, 2H, 1-H), 2.26 (m, 1H, 11β-H), 2.44 (m, 1H, 4α-H), 2.52 (m, 1H, 4β-H), 2.97 (m, 1H, 17-H), 3.63 (m, 1H, 5-H), 3.89 (m, 1H, 22-H), 4.02 (m, 1H, 3-H), 4.52 (m, 1H, 2-H), 6.19 (s, 1H, 7-H); ^{13}C NMR (150.76 MHz, C_5D_5N) δ 18.20 (C18), 19.96 (C19), 21.29 (C16), 21.82 (C21), 25.47 (C4), 27.61 (C23), 28.90 (C11), 29.44 (C12), 30.19 (C27), 30.48 (C26), 31.32 (C15), 36.06 (C1), 42.86 (C24, C10), 48.23 (C13), 48.40 (C5), 50.10 (C17), 69.74 (C25), 70.63 (C2), 72.08 (C3), 75.26 (C9), 76.99 (C20), 77.78 (C22), 86.05 (C14), 124.23 (C7), 159.33 (C8), 200.91 (C6). MALDI TOF *m/z* 519.33 $[M+Na]^+$, calcd for $C_{27}H_{44}O_8Na$ 519.39.

Compound **6**. Mp 157–159 °C, $[\alpha]_D^{20} + 24.1^\circ$ (*c* 1.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H, 18-Me), 1.12 (s, 3H, 19-Me), 1.19 (s, 3H, 21-Me), 1.25 (s, 3H, 26-Me), 1.27 (s, 3H, 27-Me), 1.34 and 1.43 (both s, 6H, 20,22-CMe_2), 2.27 (m, 1H, 17-H), 3.11 (m, 1H, 5-H), 3.66 (m, 1H, 22-H), 4.08 (m, 1H, 3-H), 4.14 (m, 1H, 2-H), 5.87 (s, 1H,7-H); ^{13}C NMR ($CDCl_3$) δ 17.17 (C18), 18.89 (C11), 19.04 (C19), 20.92 (C16), 21.95 (C21), 23.48 (C15), 23.78 (C23), 26.88 and 29.04 (20,22-$\underline{Me_2}$C), 27.93 (C12), 28.95 (C26), 29.71 (C27), 31.12 (C4), 34.34 (C1), 41.36 (C24), 42.17 (C10), 47.15 (C5, C13), 48.87 (C17), 69.46 (C2), 70.69 (C25), 71.22 (C3), 74.64 (C9), 82.03 (C22), 84.30 (C20), 86.25 (C14), 107.03 (20,22-$Me_2\underline{C}$), 124.04 (C7), 157.73 (C8), 200.96 (C6). MALDI-TOF *m/z* 559.34 $[M+Na]^+$, calcd for $C_{30}H_{48}O_8Na$ 559.30.

*2.4. (20R,22R)-2β,3β,9α,14α,20,22-Hexahydroxy-5α-cholest-7-en-6-one (**7**) and (20R,22R)-2β,3β,9α,14α-tetrahydroxy-20,22-isopropylidenedioxy-5α-cholest-7-en-6-one (**8**)*

Compound **7** (0.08 g, 44%) and compound **8** (0.10 g, 51%) were obtained from **4** (0.21 g, 0.37 mmol) in a similar manner to that used to synthesize **5** and **6**.

Compound **7**. Mp 181–183 °C, $[\alpha]_D^{20} + 21.8^\circ$ (*c* 0.28, CH_3OH); ^{1}H NMR (C_5D_5N) δ 0.79 (s, 3H, 18-Me), 0.81 (d, *J* = 6.5 Hz, 3H, 27-Me), 0.86 (d, *J* = 6.5 Hz, 3H, 26-Me), 1.11 (s, 3H, 19-Me), 1.19 (s, 3H, 21-Me), 2.12 (m, 1H, 17-H), 3.22 (m, 1H, 5-H), 3.99 (m, 1H, 22-H), 3.81 (m, 1H, 3-H), 4.52 (m, 1H, 2-H), 6.23 (s, 1H, 7-H); ^{13}C NMR (C_5D_5N)