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Synthesis of Hoodigogenin A, aglycone of natural appetite suppressant glycosteroids extracted from *Hoodia gordonii*

Philippe Geoffroy[a], Blandine Ressault[a], Eric Marchioni[b], Michel Miesch[a,*]

[a] Université de Strasbourg-Institut de Chimie, UMR 7177-Laboratoire de Chimie Organique Synthétique-1, rue Blaise Pascal, BP 296/R8, 67008 Strasbourg-Cedex, France
[b] Université de Strasbourg, Faculté de Pharmacie, IPHC, UMR 7178, Laboratoire de Chimie Analytique et de Science de l'Aliment, 74 route du Rhin, 67640 Illkirch Graffenstaden, France

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ABSTRACT

14β-hydroxy pregnane glycosides extracted from *Hoodia gordonii*, a succulent plant isolated from Apocynaceae are suggested to have appetite suppressant properties in animals and humans. However, limited reports on biological studies concerning the appetite suppressant properties are available in the open literature. One reason for that is the poor availability of these glycosteroids because *H. gordonii* is a protected plant and the yield of extraction lies between 0.003% and 0.02%. Starting from 3α,12α-diacetoxy-pregnanone **1**, we disclose in this report the synthesis of Hoodigogenin A, the aglycone of the natural 14β-hydroxy pregnane glycosides extracted from *H. Gordonii*.

1. Introduction

Obesity is one of the major health concerns in the 21st century. Worldwide, more than 300 million people are obese and one billion are overweight [1]. The increase of childhood obesity is a worrisome problem [2,3]. Obesity is also associated with several health problems such as diabetes, heart diseases and dyslipidemia, and glucose intolerance. Obesity is a disease that requires careful attention and pragmatic treatment. Lifestyle changes, exercising, dieting and weight loss are needed for a complete treatment and prevention of relapse. However, diet pills can help in fighting obesity. In this context, there is a growing interest for *Hoodia gordonii*, a succulent plant isolated from Apocynaceae (previously Asclepiadaceae) which grows in South Africa and Namibia [4]. It is claimed that this plant presents appetite suppressant properties. However, only limited reports on biological studies concerning the appetite suppressant properties are available in the open literature [5–7]. The extraction of *H. gordonii* provided numerous oxypregnane glycosides, for example P57AS3, characterized by a common aglycone called Hoodigogenin A (12-O-β-tigloyl-3β, 14β-dihydroxy-pregn-5-ene-20-one) (Fig. 1) [8–10].

In the frame of a collaborative study concerning the synthesis, the extraction and the biological evaluation of Hoodigogenin A, we developed an original synthesis of Hoodigogenin A starting from commercially available reagents. The main reason for our interest in the synthesis of the Hoodigogenin A is because there is limited access to the active compounds which are a priori responsable for the appetite suppressant properties. According to the literature, the yield of extraction of the oxypregnane glycosides from *H. gordonii* lies between 0.003% and 0.02% [7–10]. In addition, *H. gordonii* is a protected plant and therefore of limited access. Furthermore, to the best of our knowledge, no synthesis of Hoodigogenin A has been reported in the open literature [11]. We report herein the synthesis of Hoodigogenin A, the key step being a Norrish type I – Prins reaction.

2. Experimental

2.1. General

Melting points were measured on a Stuart Scientific melting point apparatus (SMP 3) and are uncorrected. Reactions were carried out under argon with magnetic stirring and degassed solvents. Et_2O and THF were distilled from Na/benzophenone. Thin layer chromatography (TLC) was carried out on silica gel plates (Merck $60F_{254}$) and the spots were visualized under UV lamp (254 or 365 nm) and sprayed with phosphomolybdic acid solution (25 g phosphomolybdic acid, 10 g cerium sulfate, 60 mL H_2SO_4, 940 mL H_2O) followed by heating on a hot plate. For column chromatography, silica gel (Merck Si 60 40–60 μm) was used. IR spectra were

* Corresponding author. Tel.: +33 0 368851752; fax: +33 0 68851754.
E-mail address: m.miesch@unistra.fr (M. Miesch).

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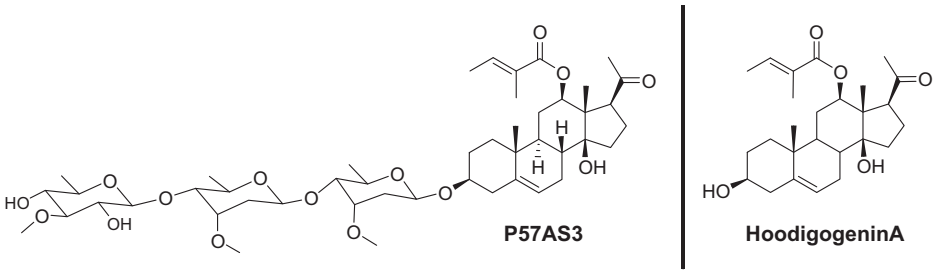

Fig. 1.

recorded on Bruker Alpha (ATR) spectrophotomer. ^{1}H NMR spectra were recorded at 300 MHz (Bruker AC-300) and ^{13}C NMR spectra at 75 MHz (Bruker AC-300) using the signal of the residual non-deuterated solvent as internal reference. Significant ^{1}H NMR data are tabulated in the following order: chemical shift (δ) expressed in ppm, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants in hertz, number of protons. High-resolution mass spectra (HRMS) were performed on a Agilent 6520 Accurate Mass Q-TOF.

2.2. 3α-hydroxy,12α-acetoxy-5Hβ-pregnan-20-one (**1a**)

To a solution of compound **1** (3.37 g, 8.05 mmol) in MeOH (80 mL) was added K_2CO_3 (1.00 g, 7.24 mmol). The reaction mixture was stirred for 1 h at r.t. and treated with water (50 mL). The aqueous layer was extracted with Et_2O (3 × 60 mL). The combined organic layers were washed with brine (1 × 50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The white residue was purified by column chromatography (30 g silica gel, petroleum ether–ethyl acetate, 3:7), to afford compound **1a** as a white solid (2.48 g, 83%, mp 201.5–203.8 °C). IR (ATR, cm^{-1}): 3472, 1714, 1696. ^{1}H NMR ($CDCl_3$) δ: 5.12 (t, 1H, J = 2.7 Hz, H-12β); 3.63 (tt, 1H, J = 10.9–4.6 Hz, H-3β); 2.95 (t, 1H, J = 9.3 Hz, H-17α); 2.20–2.05 (1H, m); 2.14 (s, 3H, H-21); 2.01 (s, 3H, CH_3, Ac); 1.95–0.85 (m, 20H); 0.89 (s, 3H, CH_3); 0.67 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$) δ: 208.9 (C=O, C-20); 170.5 (C=O, OAc); 74.5 (CH, C-3); 71.6 (CH, C-12); 55.6 (CH); 49.6 (CH); 46.7 (C); 41.9 (CH); 36.2 (CH_2); 35.6 (CH); 35.0 (CH_2); 34.5 (CH); 34.1 (C); 31.1 (CH_3); 30.4 (CH_2); 27.0 (CH_2); 26.0 (CH_2); 25.6 (CH_2); 23.7 (CH_2); 23.0 (CH_3); 22.3 (CH_2); 21.4 (CH_3); 13.8 (CH_3). HRMS (ESI) m/z: $C_{23}H_{36}NaO_4$ [M+Na]$^+$ calcd. 399.2506, found 399.2518; $[\alpha]^{20}_D$: +154 (c 0.01, $CHCl_3$).

2.3. 12α-acetoxy-5Hβ-pregnan-3,20-dione (**2**)

A solution of compound **1a** (1.00 g, 2.66 mmol) in acetone (60 mL) at 0 °C was treated dropwise with Jones reagent (2.7 mL). The orange solution was stirred for 1 h at r.t. and treated with water (50 mL). The aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with sat. $NaHCO_3$ (1 × 50 mL) and then with brine (1 × 50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford compound **2** as a white solid (1.034 g, quant., mp 124–125.7 °C) which was used directly for the next step. IR (ATR, cm^{-1}): 1714–1699. ^{1}H NMR ($CDCl_3$) δ: 5.16 (t, 1H, J = 2.7 Hz, H-12β); 2.93 (t, 1H, J = 9 Hz, H-17α); 2.63 (dd, 1H, J = 13.5–15.3 Hz); 2.30–1.05 (m, 19H); 2.11 (s, 3H, CH_3, H-21); 2.00 (s, 3H, CH_3, Ac); 0.98 (3H, s, CH_3); 0.70 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$) δ: 212.6 (C=O, C-3); 208.6 (C=O, C-20); 170.3 (C=O, OAc); 74.3 (CH, C-12); 55.5 (CH); 49.5 (CH); 46.7 (C); 43.8 (CH); 42.1 (CH_2); 36.8 (CH_2); 36.5 (CH_2); 35.3 (CH); 34.7 (CH); 34.3 (C); 31.1 (CH_3); 26.3 (CH_2); 25.9 (CH_2); 25.7 (CH_2); 23.6 (CH_2); 22.3 (CH_2); 22.3 (CH_3); 21.3 (CH_3); 13.9 (CH_3). HRMS (ESI) m/z: $C_{23}H_{34}NaO_4$ [M+Na]$^+$ calcd. 397.2349, found 397.2357. $[\alpha]^{20}_D$: +146 (c 0.01, $CHCl_3$).

2.4. 4β-bromo,12α-acetoxy-5Hβ-pregnan-3,20-dione (**3**)

To a solution of compound **2** (2.14 g, 5.71 mmol) in AcOH (60 mL), was added dropwise over 45 min a solution of Br_2 (0.29 mL, 5.65 mmol) in AcOH (25 mL). The solution was stirred for an additional 30 min and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water (3 × 30 mL). The combined aqueous layers were extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with sat. $NaHCO_3$ (3 × 30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield compound **3** as a white solid (2.89 g, quant., mp 167.4–169.9 °C) which was used directly for the next step. IR (ATR, cm^{-1}): 1726, 1698. ^{1}H NMR ($CDCl_3$) δ: 5.16 (t, 1H, J = 2.4 Hz, H-12β); 4.92 (d, 1H, J = 11.7 Hz, H-4α); 2.92 (t, 1H, J = 9.3 Hz, H-17α); 2.47 (dt, 1H, J = 3.9–14.4 Hz); 2.35–0.60 (17H, m); 2.11 (s, 3H, CH_3, H-21); 2.00 (s, 3H, CH_3, Ac); 0.88 (s, 3H, CH_3); 0.71 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 208.5 (C=O); 201.8 (C=O); 170.2 (C=O, OAc); 74.1 (CH, C-12); 59.5 (CH); 55.4 (CH); 53.9 (CH); 49.4 (CH); 46.6 (C); 37.6 (C); 36.3 (CH_2); 36.2 (CH_2); 35.8 (CH); 35.4 (CH); 31.1(CH); 26.0 (CH_2); 25.4 (CH_2); 24.7 (CH_2); 23.6 (CH_2); 2.9 (CH_3); 22.4 (CH_2); 21.3 (CH_3); 13.9 (CH_3). HRMS (ESI) m/z: $C_{23}H_{33}BrNaO_4$ [M+Na]$^+$ calcd. 477.1437, found 477.1445. $[\alpha]^{20}_D$: +151 (c 0.01, $CHCl_3$).

2.5. 12α-acetoxy-pregn-4-ene-3,20-dione (**4**)

To a solution of the crude compound **3** (2.60 g, 5.73 mmol) in DMF (65 mL), was added LiCl (1.20 g, 28.24 mmol). The reaction mixture was refluxed for 1 h and the medium was concentrated under reduced pressure. The orange residue was dissolved in AcOEt (50 mL) and washed with water (3 × 30 mL). The combined aqueous layers were extracted with AcOEt (3 × 30 mL). The combined organic layers were washed with brine (1 × 30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography (60 g silica gel, petroleum ether–ethyl acetate, 8:2) to afford compound **4** as a slightly yellow solid (1.43 g, 67%, mp 147.4–151 °C). IR (ATR, cm^{-1}): 1729, 1700, 1665, 1618. ^{1}H NMR ($CDCl_3$) δ: 5.71 (s, 1H, H-4); 5.15 (t, 1H, J = 2.7 Hz, H-12β); 2.91 (t, 1H, J = 9 Hz, H-17α); 2.40–0.60 (m, 17H); 2.16 (s, 3H, CH_3, H-21); 2.00 (s, 3H, CH_3, Ac); 1.14 (s, 3H, CH_3); 0.72 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 208.5 (C=O, C-20); 199.1 (C=O, C-3); 170.2 (C=C, C-5); 170.0 (C=O, OAc); 124.1 (CH, C-4); 74.3 (CH, C-12); 55.3 (CH, C-17); 48.7 (CH);47.9 (CH); 46.4 (C); 38.0 (C); 35.7 (CH); 35.6 (CH_2); 33.8 (CH_2); 32.6 (CH_2); 31.6 (CH_2); 31.1 (CH_3); 25.6 (CH_2); 23.6 (CH_2); 22.2 (CH_2); 21.2 (CH_3); 17.1 (CH_3); 13.7 (CH_3). HRMS (ESI) m/z: $C_{23}H_{32}NaO_4$ [M+Na]$^+$ calcd. 395.2193, found 395.2202. $[\alpha]^{20}_D$: +217 (c 0.01, $CHCl_3$).

2.6. 3,12α-diacetoxy-pregna-3,5-diene-20-one (**5**)

A solution of compound **4** (2.80 g, 3.73 mmol) in Ac_2O (18 mL) was treated with AcCl (30 mL) and heated under reflux for 1 h. After cooling to r.t., the reaction mixture was concentrated under