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Synthesis and biological activity of ring-A difluorinated brassinosteroids

Sofía L. Acebedo, Fernando Alonso, Lydia R. Galagovsky, Javier A. Ramírez*

Departamento de Química Orgánica and UMYMFOR (CONICET – Facultad de Ciencias Exactas y Naturales), Universidad de Buenos Aires, Pabellón 2, Piso 3, Ciudad Universitaria, C1428EGA Buenos Aires, Argentina

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ABSTRACT

In this paper we report the synthesis of four ring-A difluorinated analogs of brassinosteroids. The bioactivity of these new compounds was evaluated using the rice lamina inclination test. The results show that one of these analogs elicits a bioactivity comparable to that of 28-homocastasterone, a highly active natural brassinosteroid. This finding suggests that both hydroxyls at C-2 and C-3 in active brassinosteroids are involved as hydrogen bond acceptors in their interactions with the cellular receptor.

1. Introduction

In 1979, Groove et al. showed that the unique growth-promoting activity of *Brassica* pollen extracts was conferred by brassinolide (**1**, Fig. 1), a steroid with an unusual lactone B-ring structure [1]. Since then, brassinolide and more than 60 related compounds – known as brassinosteroids (BRs) – have been isolated from a wide variety of plant species [2], and their biological activity studied in great detail [3,4]. Their exogenous application leads to a spectrum of growth responses, such as stem elongation, inhibition of root growth, leaf epinasty and xylem differentiation, which are partly brought about by changes in enzyme activity and gene expression. BRs are able to increase yield and stress resistance of a number of commercially important crops, particularly when grown under conditions of biotic or abiotic stress [5].

In the past few years, important progress has been made in understanding how BRs are perceived and how the information is transduced to promote genomic responses [6]. In contrast to animal steroid signals, BRs are perceived by a plasma membrane-localized receptor kinase. This kinase is encoded by the BRI1 gene, which is part of a large, plant-specific family of leucine-rich repeat receptor-like kinases (LLR-LRK) [7]. Although binding assays have allowed the identification of a 90-amino acid region of the extracellular domain of BRI1 as the BR-binding domain, the nature of the interaction between this receptor and its ligands remains obscure.

It is known that fluorinated analogs of biologically active molecules are useful tools for physiological studies [8,9]. If the fact that BRs are polyhydroxylated steroids and the fact that fluorine can sometimes resemble the presence of an atom of oxygen are taken together, the design and synthesis of fluorinated BRs is an interesting task both from the chemical and biological points of view.

In previous works, our group has reported the synthesis of several BR analogs, fluorinated at C-2, C-3, C-5 and C-6, and described the effects of this substitution on their biological activity [10–13]. In order to extend these studies, here we present the synthesis of four new difluorinated BRs (compounds **2**–**5**, Fig. 1). The bioactivity of these analogs on the rice lamina inclination test was evaluated and analyzed, giving some insights into the binding features of BRs to their receptor.

2. Experimental procedures

2.1. General

All the reagents were purchased from Sigma–Aldrich Chemical Co. EI-MS were measured either in a VG Trio-2 or in a Shimadzu QP-5000 mass spectrometer at 70 eV by direct inlet. Melting points were determined on a Fisher Johns apparatus and are uncorrected. All NMR spectra were recorded on a Bruker AM-500 (500 MHz for ^{1}H and 125.1 MHz for ^{13}C). Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constant (J) values are in Hz. All solvents and reagents were of analytical grade.

2.2. Synthesis of compounds

2.2.1. (22E)-2α,3α-difluoro-5α-stigmast-22-en-6-one (7)

Compound **6** [13] (97 mg, 0.22 mmol) was dissolved in 5 mL dry dichloromethane at −70 °C under argon. Diethylaminosulfur trifluoride (85 μL, 0.65 mmol) was added dropwise and the reaction

* Corresponding author. Tel.: +54 01145763346; fax: +54 01145763385.
E-mail address: jar@qo.fcen.uba.ar (J.A. Ramírez).

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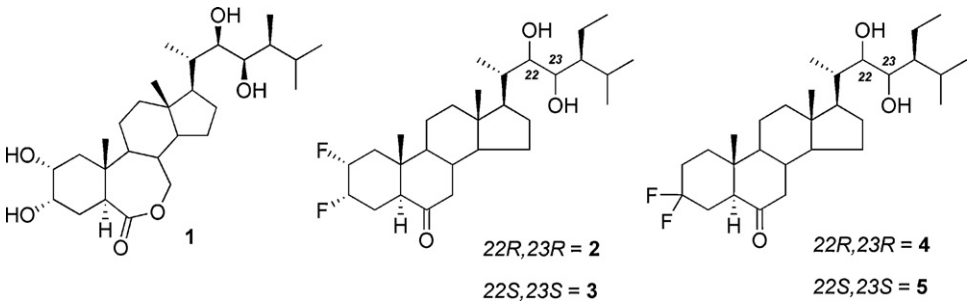


Fig. 1. Structure of brassinolide (**1**) and synthesized difluorinated analogs.

was allowed to reach ambient temperature. The organic phase was washed with 5 mL of water, diluted $NaHCO_3$ and water, and dried over Na_2SO_4. Once the solvent was evaporated at reduced pressure, the crude extract was purified by silica gel column chromatography (hexane/EtOAc 97:3). Compound **7** was obtained in a 61% yield (59 mg).

M.p: 183–184 °C. ^{1}H NMR: 5.15 (H-22, 1H, *dd*, *J* = 8.7 and 15.3), 5.03 (H-23, 1H, *dd*, *J* = 8.7 and 15.3), 5.01 (H-3β, 1H, *dm*, $^2J_{HF}$ = 51.8), 4.59 (H-2β, 1H, *dm*, $^3J_{HF}$ = 28.3 and $^2J_{HF}$ = 44.8), 2.65 (H-5α, 1H, *dd*, *J* = 3.2 and 12.7), 2.32 (H-7α, 1H, *dd*, *J* = 4.7 and 13.3), 1.03 (H-21, 3H, *d*, *J* = 6.7), 0.84 (H-27, 3H, *d*, *J* = 6.8), 0.81 (H-29, 3H, *t*, *J* = 7.3), 0.79 (H-26, 3H, *d*, *J* = 6.6), 0.77 (H-19, 3H, *s*), 0.69 (H-18, 3H, *s*). ^{13}C NMR: 210.5 (C-6), 137.9 (C-22), 129.6 (C-23), 88.5 (C-2, *dd*, $^2J_{CF}$ = 18.6 and $^1J_{CF}$ = 180.7), 87.9 (C-3, *dd*, $^2J_{CF}$ = 16.5 and $^1J_{CF}$ = 176.2), 56.6 (C-14), 55.8 (C-17), 53.6 (C-9), 51.2 (C-24), 51.0 (C-5), 46.5 (C-7), 42.8 (C-13), 42.6 (C-10, *d*, $^2J_{CF}$ = 10.9), 40.4 (C-20), 39.1 (C-12), 37.5 (C-8), 37.5 (C-1, *dd*, $^3J_{CF}$ = 1.7 and $^2J_{CF}$ = 18.1), 31.8 (C-25), 28.7 (C-16), 25.4 (C-28), 25.2 (C-4, *dd*, $^3J_{CF}$ = 6.4 and $^2J_{CF}$ = 19.2), 24.0 (C-15), 21.2 (C-11), 21.1 and 21.1 (C-21 and C-27), 18.9 (C-26), 13.5 (C-19), 12.2 and 12.2 (C-18 and C-29). Anal. calculated for $C_{29}H_{46}F_2O$: C, 77.63; H, 10.33. Found: C, 77.59; H, 10.49.

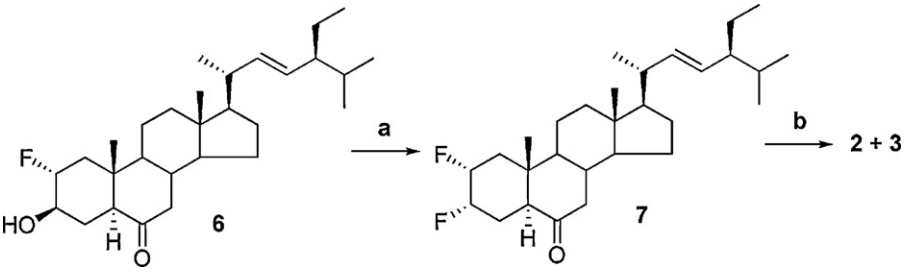
*2.2.2. (22R,23R)-2α,3α-difluoro-22,23-dihydroxy-5α-stigmastan-6-one (**2**) and (22S,23S)-2α,3α-difluoro-22,23-dihydroxy-5α-stigmastan-6-one (**3**)*

A mixture of **7** (43 mg, 95.8 μmol), *t*-butanol/water (1:1, 1 ml), (DHQD)2-Phal (30 mg, 38.5 μmol), methansulfonamide (18 mg, 0.19 mmol), potassium ferricyanide (151 mg, 0.44 mmol), potassium carbonate (63 mg, 0.44 μmol), and potassium osmate dihydrate (3.5 mg, 9.5 μmol) was stirred at room temperature for 7 days. An excess of $NaHSO_3$ was added until no evolution of bubbles was observed. Layers were separated and the aqueous phase was thoroughly extracted with CH_2Cl_2/MeOH (95:5). Combined organic layers were washed with 0.25 M H_2SO_4 and 2% NaOH. Purification by column chromatography (CH_2Cl_2/acetonitrile gradient) yielded 6 mg of compound **3** (12% yield). M.p: 146–147 °C. ^{1}H NMR: 5.02 (H-3β, 1H, *dm*, $^2J_{HF}$ 52.4), 4.59 (H-2β, 1H, *m*), 3.62 (H-22 and H-23, 2H, *m*), 2.64 (H-5α, 1H, *dd*, *J* = 3.3 and 12.8), 2.33 (H-7α, 1H, *dd*, *J* = 4.5 and 13.3), 1.04 (H-21, 3H, *d*, *J* = 6.9), 0.97 (H-29, 3H, *t*, *J* = 7.4), 0.95 (H-27, 3H, *d*, *J* = 6.9), 0.88 (H-26, 3H, *d*, *J* = 6.7), 0.78 (H-19, 3H, *s*), 0.71 (H-18, 3H, *s*). ^{13}C NMR: 210.3 (C-6), 88.2 (C-2, *dd*, $^2J_{CF}$ = 18.3 and $^1J_{CF}$ = 179.8), 87.9 (C-3, *dd*, $^2J_{CF}$ = 16.5 and $^1J_{CF}$ = 176.2), 72.1 (C-22), 70.6 (C-23), 56.1 (C-14), 53.5 (C-9), 52.5 (C-17), 51.0 (C-5), 49.6 (C-24), 46.5 (C-7), 43.5 (C-13), 42.6 (C-10, *d*, $^2J_{CF}$ = 11.2), 42.2 (C-20), 39.2 (C-12), 37.5 (C-1, *d*, $^3J_{CF}$ = 18.3), 37.5 (C-8), 27.8 (C-16), 26.9 (C-25), 25.2 (C-4, *dd*, $^3J_{CF}$ = 6.3 and $^2J_{CF}$ = 19.6), 24.2 (C-15), 21.7 (C-27), 21.2 (C-11), 18.5 (C-28), 17.7 (C-26), 14.5 (C-29), 14.1 (C-21), 13.5 (C-19), 11.9 (C-18). Anal. calculated for $C_{29}H_{48}F_2O_3$: C, 72.16; H, 10.02. Found: C, 72.02; H, 10.31.

Further elution gave 36 mg of (22*R*,23*R*)-2α,3α-difluoro-22,23-dihydroxy-5α-stigmastan-6-one (**2**, 75% yield). M.p: 213–214 °C. ^{1}H NMR: 5.02 (H-3β, 1H, *dm*, $^2J_{HF}$ = 52.0), 4.59 (H-2β, 1H, *dm*, $^3J_{HF}$ = 28.4 and $^2J_{HF}$ = 44.9), 3.72 (H-23, 1H, *d*, *J* = 8.6), 3.59 (H-22, 1H, *d*, *J* = 8.6), 2.66 (H-5α, 1H, *dd*, *J* = 3.0 and 12.5), 2.33 (H-7α, 1H, *dd*, *J* = 4.3 and 13.2), 0.97 (H-27, 3H, *d*, *J* = 6.7), 0.96 (H-26, 3H, *d*, *J* = 6.7), 0.95 (H-29, 3H, t, *J* = 7.5), 0.92 (H-21, 3H, *d*, *J* = 6.7), 0.78 (H-19, 3H, *s*), 0.69 (H-18, 3H, *s*). ^{13}C NMR: 210.4 (C-6), 88.4 (C-2, *dd*, $^2J_{CF}$ = 18.7 and $^1J_{CF}$ = 180.1), 87.9 (C-3, *dd*, $^2J_{CF}$ = 16.5 and $^1J_{CF}$ = 176.5), 74.5 (C-22), 72.7 (C-23), 56.4 (C-14), 53.5 (C-9), 52.4 (C-17), 50.9 (C-5), 46.5 (C-7), 46.3 (C-24), 42.8 (C-13), 42.6 (C-10, *d*, $^2J_{CF}$ = 10.6), 39.2 (C-12), 37.6 (C-8), 37.5 (C-1, *dd*, $^3J_{CF}$ = 1.5 and $^2J_{CF}$ = 18.1), 36.9 (C-20), 28.8 (C-25), 27.6 (C-16), 25.1 (C-4, *dd*, $^3J_{CF}$ = 6.4 and $^2J_{CF}$ = 19.8), 23.8 (C-15), 21.2 (C-11), 21.2 (C-27), 19.4 (C-26), 18.8 (C-28), 14.2 (C-29), 13.4 (C-19), 11.9 and 11.9 (C-18 and C-21). Anal. calculated for $C_{29}H_{48}F_2O_3$: C, 72.16; H, 10.02. Found: C, 72.21; H, 9.97.

*2.2.3. (22E)-3,3-difluoro- 5α-stigmast-22-en-6-one (**9**)*

The diketosteroid **8** [13] (100 mg, 0.23 mmol) was dissolved in 5 mL of dry toluene and 50 μL of DAST were added dropwise. The reaction was kept at room temperature for 1 h and poured into an ice/water mixture. The organic layer was washed with 10 mL of diluted $NaHCO_3$ with water, and dried over Na_2SO_4. The solvent was evaporated at reduced pressure to give a crude extract that was purified by silica gel column chromatography (hexane/EtOAc 98:2) yielding 62 mg (59%) of compound **9**. M.p. 136–137 °C. ^{1}H NMR: 5.14 (H-22, 1H, *dd*, *J* = 8.7 and 15.2), 5.03 (H-23, 1H, *dd*, *J* = 8.7



Scheme 1. Synthesis of difluorinated brassinosteroids **2** and **3**. *Reagents and conditions*: (a) DAST/CH_2Cl_2/-70 °C. (b) K_2OsO_4/$K_3Fe(CN)_6$/$(DHQD)_2$Phal/K_2CO_3/t-BuOH/H_2O/$CH_3SO_3NH_2$, r.t.