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Mimicking natural phytohormones. 26-Hydroxycholestan-22-one derivatives as plant growth promoters

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ABSTRACT

26-Hydroxycholestan-22-one derivatives with oxygenated functions in the rings A and/or B were successfully synthesized from diosgenin. After the modifications of rings A and B, the spiroketal side chain was selectively opened through a Lewis acid mediated acetolysis to afford the cholestane derivatives. These compounds incorporate pharmacophores, which mimic the activity of natural phytohormones and show high growth promoting activity in Mexican rice cultivars using the rice lamina inclination test.

1. Introduction

Plant growth promoters are a class of phytohormones defined as chemical substances able to regulate physiological growth processes [1–3]. Such substances are synthesized in tissues or organs by the plant itself and then transported to other sites where they produce specific effects on its development [1]. Phytohormones are biosynthesized in tiny amounts becoming precious targets in the organic synthesis and in the subsequent biological processes. The most sophisticated phytohormones known to date are brassinosteroids (BSs). BSs are considered the sixth class of phytohormones and the most potent among them. The first BS identified, a campestane derivative, was isolated in 1979 from bee-collected pollen of *Brassica napus* L. and named brassinolide (Fig. 1) [4]. Three years later, castasterone was isolated [5]. These two compounds are structurally very close, brassinolide contains a campestano-6,7-lactone skeleton and castasterone a campestan-6-one structure. Later, stigmastane and cholestane BSs as homobrassinolide and norbrassinolide were found. Nowadays, more than 70 compounds are recognized as BSs.

Most BSs presents a variety of functional groups in rings A and B and in the side chain, usually oxygenated functional groups. The modification of such pharmacophores can modulate (increase or decrease) the activity of a synthetic phytohormone [1]. The reader interested in more extended information of BSs and analogs is referred to the excellent reviews reported in references [1–3] and [6,7]. All BSs contain an oxygenated functional group at C-3 with or without extra hydroxyl groups in the ring A. The most potent BSs known contain a 2α,3α-diol, but compounds with other stereochemistry at C-2 and C-3 are also active [1,2,8,9]. Many analogs have been synthesized functionalizing and/or modifying the ring B e.g. lactams [10,11], 6-oxalactones [12], and 5α-hydroxy-6-oxo compounds [13–15], among others. Different steroidal side chains have been reported [16–20] and analogs with an atypical stereochemistry of the vicinal diol present at C-22/C-23 have also been reported and all of them promote plant growth activity [1,21].

Some of us reported the first synthesis of the 22-oxocholestane side chain in a one-pot process starting from spirostanes [22–27] and recently, we have stated their first role as plant growth promoters [28]. In an attempt to mimic natural phytohormones, herein, we report the synthesis of 22-oxocholestanes starting from diosgenin (**1**), bearing a hydroxyl group at C-26, an acetate group at C-16, and additional oxygenated functions in the rings A and B. We found that the extra acetate

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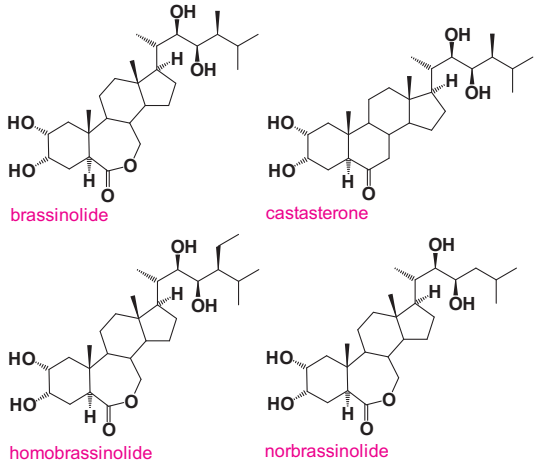


Fig. 1. Structure of some naturally occurring brassinosteroids.

group inserted at C-16 increases the bioactivity, mimicking the oxygenated function present in natural BSs containing a vicinal 22*R*,23*R*-diol. This bioactivity was evaluated via the rice lamina inclination test (RLIT) employing Mexican rice cultivars.

2. Experimental

2.1. General remarks

Optical rotations were measured at 24 °C in an Anton Paar MCP500 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$ on a Varian Mercury NMR instrument. The ^{1}H NMR spectra were referenced to residual protonated solvent (δ 7.26 ppm) and the ^{13}C NMR spectra to the middle signal of $CDCl_3$ (δ 77 ppm). Coupling constants are expressed in hertz (Hz). All assignments were confirmed with the aid of two-dimensional experiments (COSY, HSQC, and HMBC). Processing of the spectra was performed using MestReNova software (http://mestrelab.com). High-resolution mass spectra were obtained by the fast atom bombardment (FAB) technique, using a JEOL The MStation spectrometer. IR spectra were recorded on an Agilent Cary 630 FTIR spectrometer (range: 4000–600 cm^{-1}). Column chromatography was performed using a Teledyne Isco *Combiflash* apparatus and analytical thin-layer chromatography (TLC) on aluminum plates precoated with Silica Gel 60F-254. Two Mexican rice (*Oryza sativa*) cultivars, Morelos A06 and Morelos A08 were used in this study and donated by INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campus Morelos). Homobrassinolide (HB) was purchased from Sigma-Aldrich and used as authentic standard. The RLIT were performed in the Jardín Botánico BUAP.

2.2. Chemical synthesis

*Diosgenyl tosylate (**2**)*. Pyridine (5.8 mL, 72.4 mmol) was added dropwise to a solution of **1**, (3.0 g, 7.2 mmol), and *p*-TsCl (13.8 g, 72.4 mmol) in CH_2Cl_2 (30.0 mL). The reaction mixture was maintained in the darkness under stirring for a period of 3 h. The organic phase was washed with an ice cooled solution of 5% HCl (5 × 50.0 mL), distilled water (2 × 50.0 mL), extracted with CH_2Cl_2 (3 × 50.0 mL), washed with a saturated solution of $NaHCO_3$ (3 × 50.0 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give 4.1 g of **2**. This compound was immediately used in the next step without further purification [29].

2.2.1. Compounds

3–5, (25*R*)-6-oxo-5α-spirostan-3β-yl tosylate, (25*R*)-3α,5-cyclo-5α-spirostan-6-one, and (25*R*)-3β-hydroxy-5α-spirostan-6-one (laxogenin), respectively, were synthesized starting per the procedures described in reference [30].

2.2.2. (25R)-26-Hydroxy-22-oxospirost-5-en-3β,6,16β-triyl triacetate (6)

Compound **4** (1.0 g, 2.5 mmol) was dissolved in CH_2Cl_2 (20.0 mL) and added dropwise to a previously cooled (0 °C) mixture of Ac_2O (3.4 mL) and $BF_3{\cdot}OEt_2$ (4.3 mL). The mixture was stirred for 30 min and the resulting syrup poured into ice-water (50.0 mL). The organic phase was washed with a saturated solution of $NaHCO_3$ (4 × 50.0 mL), distilled water (1 × 50.0 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by chromatography on a *Combiflash* apparatus with a gradient of hexane/ethyl acetate 10:0 → 7:3 for 15 min to yield 1.2 g (85%) of **6**; white powder; mp 201–202 °C; $[\alpha]_D$ = -12.0 (*c* 1.0, $CHCl_3$). IR: 2923 (CH, aliphatic), 1728 (C═O, ketone), 1664 (C═O, acetate). ^{1}H NMR (δ): 4.97 (1H, ddd, $J_{16,17}$ = 8.0 Hz, $J_{16,15}$ = 4.3 and 6.1 Hz, H-16), 4.53 (1H, m, H-3), 3.42 (2H, d, $J_{26,25}$ = 5.8 Hz, CH_2-26), 2.95 (1H, dq, $J_{20,21}$ = 7.1 Hz, $J_{20,17}$ = 14.2 Hz, H-20), 2.70 (1H, m, H-4*eq*), 2.63 (1H, m, H-23a), 2.40 (1H, m, H-23b), 2.37 (1H, m, H-15a), 2.12 (3H, s, $C\underline{H_3}CO_2$-6), 2.03 (3H, s, $C\underline{H_3}CO_2$-3), 1.96 (3H, s, $C\underline{H_3}CO_2$-16), 1.14 (3H, d, $J_{21,20}$ = 7.1 Hz, CH_3-21), 1.07 (3H, s, CH_3-19), 0.91 (3H, d, $J_{27,25}$ = 6.8 Hz, CH_3-27), 0.87 (3H, s, CH_3-18). ^{13}C NMR (δ): 36.8 (C-1), 27.3 (C-2), 72.8 (C-3), 28.2 (C-4), 126.3 (C-5), 141.6 (C-6), 33.2 (C-7), 35.4 (C-8), 49.2 (C-9), 36.7 (C-10), 20.7 (C-11), 39.4 (C-12), 41.8 (C-13), 53.5 (C-14), 34.7 (C-15), 75.5 (C-16), 54.9 (C-17), 13.1 (C-18), 19.4 (C-19), 43.4 (C-20), 16.8 (C-21), 213.6 (C-22), 38.5 (C-23), 26.1 (C-24), 31.3 (C-25), 67.4 (C-26), 16.6 (C-27), 170.4 ($CH_3\underline{C}O_2$-3), 169.7 ($CH_3\underline{C}O_2$-16), 169.0 ($CH_3\underline{C}O_2$-6), 21.4 ($\underline{C}H_3CO_2$-3), 21.1 ($\underline{C}H_3CO_2$-16), 20.8 ($\underline{C}H_3CO_2$-6). HRMS (FAB) *m/z* for $C_{33}H_{50}O_8$ Calcd: 574.3506. Found: 575.3554 $[M + H]^+$.

2.2.3. (25R)-26-hydroxy-6,22-dioxo-5α-spirostan-3β,16β-diyl diacetate (7)

Compound **7** was synthesized per the procedure described for **6**. The crude product was purified by chromatography on a *Combiflash* apparatus with a gradient of hexane/ethyl acetate 10:0 → 1:1 for 10 min to yield 1.0 g, 83%; mp 199–201 °C; $[\alpha]_D$ = + 2.0 (*c* 1.0, $CHCl_3$). IR: 3518 (OH), 2950 (CH, aliphatic), 1735 (C═O, ketone), 1674 (C═O, acetate). ^{1}H NMR (δ): 4.99 (1H, ddd, $J_{16,17}$ = 8.2 Hz, $J_{16,15}$ = 4.3 and 6.0 Hz, H-16), 4.66 (1H, m, H-3), 3.42 (2H, d, $J_{26,25}$ = 5.9 Hz, CH_2-26), 2.96 (1H, dq, $J_{20,21}$ = 7.1 Hz, $J_{20,17}$ = 11.0 Hz, H-20), 2.65 (1H, ddd, $J_{23a,24}$ = 6.5 y 8.7 Hz, J_{gem} = 18.0 Hz, H-23a), 2.41 (1H, m, H-23b), 2.35 (1H, m, H-15a), 2.29 (1H, m, H-17), 2.28 (1H, m, H-7*eq*), 2.03 (3H, s, $C\underline{H_3}CO_2$-3), 1.97 (3H, s, $C\underline{H_3}CO_2$-16), 1.14 (3H, d, $J_{21,20}$ = 7.1 Hz, CH_3-21), 0.91 (3H, d, $J_{27,25}$ = 6.8 Hz, CH_3-27), 0.86 (3H, s, CH_3-19), 0.77 (3H, s, CH_3-18). ^{13}C NMR (δ): 38.5 (C-1), 26.1 (C-2), 72.6 (C-3), 26.6 (C-4), 53.4 (C-5), 209.6 (C-6), 46.1 (C-7), 36.1 (C-8), 53.7 (C-9), 38.5 (C-10), 21.1 (C-11), 40.7 (C-12), 42.4 (C-13), 56.3 (C-14), 34.3 (C-15), 75.0 (C-16), 54.9 (C-17), 12.9 (C-18), 13.3 (C-19), 43.2 (C-20), 16.7 (C-21), 213.4 (C-22), 37.0 (C-23), 25.9 (C-24), 35.2 (C-25), 67.3 (C-26), 16.5 (C-27), 170.5 ($CH_3\underline{C}O_2$-3), 169.6 ($CH_3\underline{C}O_2$-16), 21.2 ($\underline{C}H_3CO_2$-3), 21.0 ($\underline{C}H_3CO_2$-16). HRMS (FAB) *m/z* for $C_{31}H_{48}O_7$ Calcd: 532.3400. Found: 533.3461 $[M + H]^+$.

2.2.4. (25R)-5α-spirost-2-en-6-one (8)

Compound **3** (1.8 g, 3.0 mmol) was dissolved in 37.5 ml of DMF, then 3.0 g (34.3 mmol) of LiBr and 2.5 g (33.8 mmol) of Li_2CO_3 were added. The reaction mixture was refluxed for 1 h, left to cool down to rt and poured into a 10% HCl solution. The precipitate formed was filtered and washed with distilled water (6 × 50.0 ml), and purified by chromatography on a *Combiflash* apparatus with a gradient of hexane/ethyl acetate 10:0 → 7:3 for 10 min to yield a white powder (0.85 g, 70%); white needles; mp 160–162 °C; $[\alpha]_D$ = + 18.0 (*c* 0.5, $CHCl_3$) [31]. IR: 2954 (CH, aliphatic), 1736 (C═O, ketone), 1618 (C═C), 982, 860 (O—C–O spiroketal). ^{1}H NMR (δ): 5.58 (1H, m, H-2), 5.48 (1H, m,

H-3), 4.31 (1H, ddd, $J_{16,17}$ = 7.3 Hz, $J_{16,15}$ = 4.0 and 6.1 Hz, H-16), 3.36 (1H, dd, $J_{26eq,25}$ = 4.0 Hz, J_{gem} = 10.8 Hz, H-26*eq*), 3.26 (1H, dd, $J_{26ax,25}$ = 11.4 Hz, J_{gem} = 10.8 Hz, H-26*ax*), 2.27 (1H, m, H-7*eq*), 2.26 (1H, m, H-5), 2.16 (1H, m, H-4*eq*), 0.88 (3H, d, $J_{21,20}$ = 6.9 Hz, CH_3-21), 0.72 (3H, d, $J_{27,25}$ = 6.4 Hz, CH_3-27), 0.72 (3H, s, CH_3-19), 0.66 (3H, s, CH_3-18). ^{13}C NMR (δ): 39.0 (C-1), 124.6 (C-2), 124.1 (C-3), 20.6 (C-4), 53.0 (C-5), 210.7 (C-6), 46.6 (C-7), 36.8 (C-8), 53.4 (C-9), 39.6 (C-10), 21.4 (C-11), 39.2 (C-12), 40.4 (C-13), 56.2 (C-14), 31.2 (C-15), 80.0 (C-16), 61.8 (C-17), 13.2 (C-18), 16.0 (C-19), 41.3 (C-20), 14.2 (C-21), 108.8 (C-22), 31.0 (C-23), 28.4 (C-24), 29.9 (C-25), 66.4 (C-26), 16.8 (C-27). HRMS (FAB) *m/z* for $C_{27}H_{40}O_3$ Calcd: 412.2977. Found: 413.2981 $[M + H]^+$.

2.2.5. (25R)-2α,3α-dihydroxy-5α-spirostan-6-one (9)

To a solution of **8** (2.1 g, 5.0 mmol) in 5 ml of *t*-BuOH, freshly prepared CTAP (2.0 g, 5.0 mmol) in 20 ml of *t*-BuOH/water (8:2) was added dropwise. The reaction mixture was stirred at rt for 24 h and then diluted in CH_2Cl_2 (50.0 mL). After that, 15.0 mL of 5% NaOH were added and the stirring was maintained for 2 h. The crude product was extracted with CH_2Cl_2 (2 × 30.0 ml) and the combination of the extracts was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting solid was purified by chromatography on a *Combiflash* apparatus with a gradient of hexane/ethyl acetate 10:0 → 2:3 for 15 min to yield a white solid (1.5 g, 66%) colorless needles; mp 224–226 °C [31]. $[\alpha]_D$ = + 3.5 (*c* 0.5, $CHCl_3$). IR: 3475, 3387 (OH), 2936 (CH, aliphatic), 1721 (C═O, ketone), 980, 920, 860 (O—C–O spiroketal). ^{1}H NMR (δ): 4.41 (1H, ddd, $J_{16,17}$ = 8.4 Hz, $J_{16,15}$ = 4.2 and 6.0 Hz, H-16), 4.04 (1H, br.s, H-3), 3.75 (1H, m, H-2), 3.47 (1H, ddd, $J_{26eq,25}$ = 4.4 Hz, J_{gem} = 10.8 Hz, $J_{26eq,24eq}$ = 1.2 Hz, H-26*eq*), 3.36 (1H, dd, $J_{26ax,25}$ = J_{gem} = 10.8 Hz, H-26*ax*), 2.68 (1H, dd, $J_{5,4eq}$ = 3.2 Hz, $J_{5,4ax}$ = 12.6 Hz, H-5), 2.31 (1H, m, H-7*eq*), 0.98 (3H, d, $J_{21,20}$ = 6.8 Hz, CH_3-21), 0.79 (3H, d, $J_{27,25}$ = 6.4 Hz, CH_3-27), 0.77 (3H, s, CH_3-19), 0.77 (3H, s, CH_3-18). ^{13}C NMR (δ): 40.1 (C-1), 68.2 (C-2), 68.3 (C-3), 26.3 (C-4), 50.7 (C-5), 211.8 (C-6), 46.8 (C-7), 37.1 (C-8), 53.7 (C-9), 40.9 (C-10), 21.0 (C-11), 39.4 (C-12), 42.5 (C-13), 56.4 (C-14), 31.5 (C-15), 80.4 (C-16), 61.9 (C-17), 13.6 (C-18), 16.4 (C-19), 41.6 (C-20), 14.5 (C-21), 109.3 (C-22), 31.3 (C-23), 28.7 (C-24), 30.2 (C-25), 66.8 (C-26), 17.1 (C-27). HRMS (FAB) *m/z* for $C_{27}H_{42}O_5$ Calcd: 446.3032. Found: 447.3045 $[M + H]^+$.

2.2.6. (25R)-26-hydroxy-6,22-dioxo-5α-cholestan-2α,3α,16β-triyl triacetate (10)

Compound **10** was synthesized from **9**, per the procedure described for **6**. The crude product was purified by chromatography on a *Combiflash* apparatus with a gradient of hexane/ethyl acetate 10:0 → 6:4 for 10 min to yield 1.1 g (83%) of a pale cream powder, mp 260–262 °C. $[\alpha]_D$ = -27.0 (*c* 0.5, $CHCl_3$). IR: 3536 (OH), 2952 (CH, aliphatic), 1732 (C═O, ketone), 1670 (C═O, acetate). ^{1}H NMR (δ): 5.37 (1H, br-s, H-3), 4.97 (1H, m, H-16), 4.97 (1H, m, H-2), 3.42 (2H, d, $J_{26,25}$ = 5.91 Hz, H-26), 2.98 (1H, m, H-20), 2.66 (1H, m, H-23a), 2.61 (1H, dd, $J_{5,4eq}$ = 4.8 Hz, $J_{5,4ax}$ = 11.9 Hz, H-5), 2.41 (1H, m, H-23b), 2.37 (1H, m, H-15a), 2.29 (1H, dd, J_{gem} = 13.2 Hz, $J_{7eq,8}$ = 4.5 Hz, H-7*eq*), 2.10 (3H, s, C$\underline{H_3}CO_2$-3), 2.06 (1H, m, H-7*ax*), 2.00 (3H, s, C$\underline{H_3}CO_2$-2), 1.98 (3H, s, C$\underline{H_3}CO_2$-16), 1.15 (3H, d, $J_{21,20}$ = 7.12 Hz, CH_3-21), 1.05 (1H, m, H-15b), 0.91 (3H, d, $J_{27,25}$ = 6.7 Hz, CH_3-27), 0.86 (3H, s, CH_3-18), 0.83 (3H, s, CH_3-19). ^{13}C NMR (δ): 37.2 (C-1), 68.9 (C-2), 67.8 (C-3), 24.6 (C-4), 51.6 (C-5), 210.0 (C-6), 46.0 (C-7), 36.6 (C-8), 53.6 (C-9), 42.2 (C-10), 20.8 (C-11), 39.0 (C-12), 42.3 (C-13), 53.2 (C-14), 34.2 (C-15), 74.9 (C-16), 54.8 (C-17), 13.4 (C-18), 13.2 (C-19), 43.1 (C-20), 16.6 (C-21), 213.5 (C-22), 38.5 (C-23), 26.1 (C-24), 35.2 (C-25), 67.2 (C-26), 16.4 (C-27), 170.2 ($CH_3\underline{C}O_2$-2), 169.9 ($CH_3\underline{C}O_2$-3), 169.7 ($CH_3\underline{C}O_2$-16), 20.9 (C$\underline{H_3}CO_2$-2), 21.0 (C$\underline{H_3}CO_2$-3), 20.9 (C$\underline{H_3}CO_2$-16). HRMS (FAB) *m/z* for $C_{33}H_{50}O_9$ Calcd: 590.3455. Found: 591.3529 $[M + H]^+$.

2.3. Biological activity

2.3.1. The rice lamina inclination test

Seeds of two rice (*Oryza sativa*) varieties (Morelos A06 and Morelos A08) were germinated in the darkness for 7 days, and uniform seedlings were selected. Then, leaf segments, each consisting of the second lamina (length 1 cm), a lamina joint, and sheath (length 1 cm), were excised and grown in distilled water at 30 °C [32]. They were then incubated in 1.0 mL of 2.5 mM potassium maleate solution containing a finite amount of a test sample for 48 h at 30 °C under the dark conditions, using a dim red light bath. The induced angles between laminae and sheaths were measured after the incubation period. Untreated leaves and a commercial sample of homobrassinolide (HB) were used as controls.

3. Results and discussion

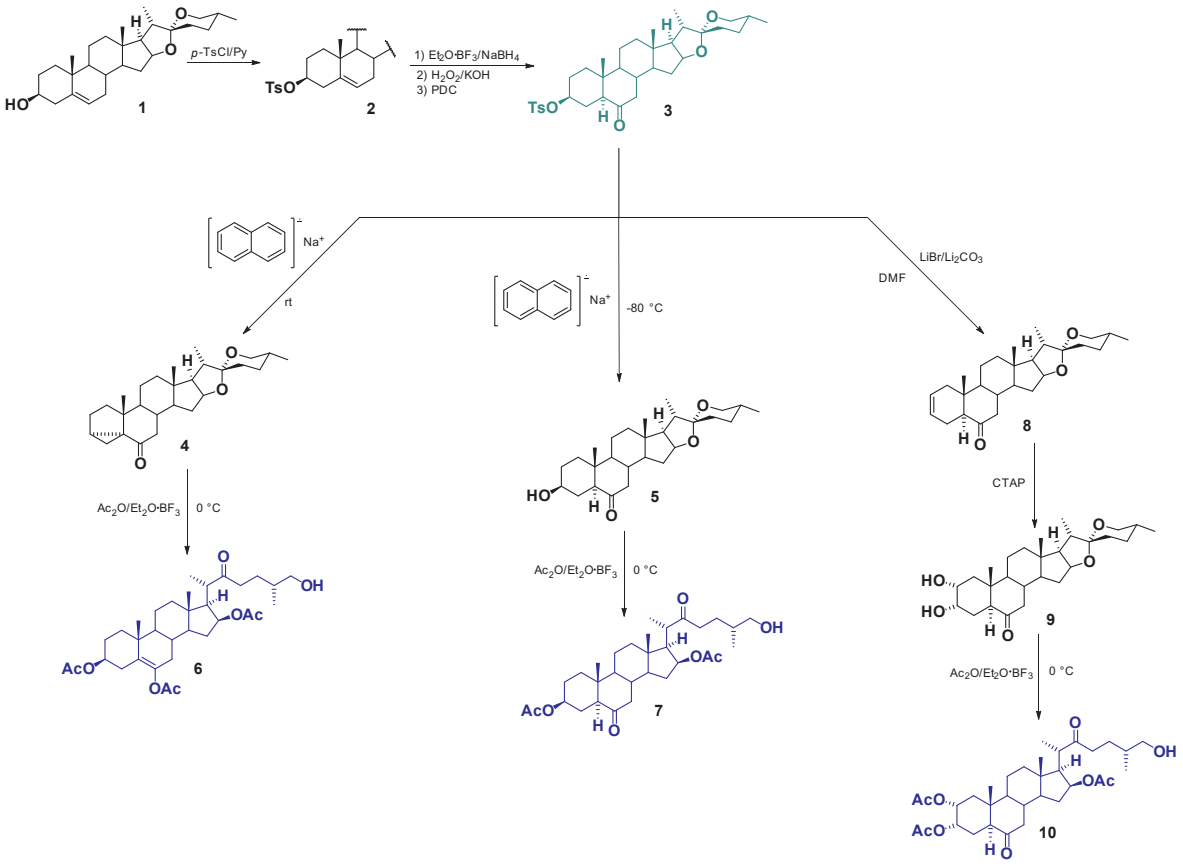
Recently, we reported that cholestan-22-one steroids promote plant growth [28], showing that such a side chain could be considered as a pharmacophore. Herein we report the synthesis of 26-hydroxycholestan-22-one derivatives bearing a hydroxyl function at C-16 in the acetate form, and introducing oxygenated functional groups in rings A and B.

The synthetic pathway is described in Scheme 1. As starting material diosgenin (**1**) was chosen, since it is one of the most versatile and commercially available steroidal sapogenins. **1** was tosylated under standard conditions to obtain quantitatively compound **2**. The latter was immediately used in the next step without further purification. Compound **2** was functionalized under the hydroboration-oxidation protocol previously reported, followed by a subsequent oxidation with PDC to obtain compound **3** [30]. The tosyl group remained unchanged under these reductive and alkaline conditions, for which strong evidence is the formation of the 6α-hydroxy derivative which is the major compound obtained after the oxidation of the mixture of alkylboranes [33]. The overall yield for this step is 92%. For the synthesis of the title compounds, **3** was selected as starting point.

To synthesize **6** and **7**, compound **3** reacted with the radical anion sodium naphthalenide (SN) to reduce the tosyl group under two reaction conditions, at room temperature and at -80 °C [30]. The resulting 3α,5-cyclo-5α-steroid **4** (reaction at rt) was obtained in 91% [34]. The cleavage of the tosylate by SN produced a radical at C-3 and the 3α,5-cyclo group was generated by the cleavage of the C—H bond at C-5. When the reaction with SN was performed at -80 °C, the C—O bond of the tosylate could be selectively cleaved and laxogenin (**5**) was obtained in 89% [18,30,35]. The spiroketal side chains of **4** and **5** were opened after a Lewis acid mediated acetolysis by means of $Ac_2O/BF_3{\cdot}OEt_2$ at 0 °C (see Scheme 1). The *i*-steroid **4** provided the enol ester **6** in 85%. To explain the formation of **6,** we must consider the activation of the oxygen atom of the carbonyl at C-6 by the Lewis acid, which reacted with acetic anhydride to trigger the nucleophilic attack of an acetate ion at C-3β, pushing the bond between C-3 and C-5 to C-5 – C-6. Laxogenin (**5**) furnished compound **7** in 83% under similar reaction conditions, providing a different route to obtain such compound [36].

To obtain **10**, the first step was the elimination of the tosylate group of compound **3**, by means of $LiBr/Li_2CO_3$ in DMF producing **8** in 70%. An α-dihydroxylation of the double bond in **8** was performed employing cetyltrimethylammonium permanganate (CTAP) as the oxidizing agent [37]. Usually this step is carried out employing OsO_4, but it is costly and extremely toxic, so CTAP represents an excellent alternative. Treatment with a solution of CTAP in *t*-BuOH/H_2O (8:2), followed by an alkaline work-up provided after 24 h the 2α,3α-diol (compound **9**) in 66% yield. Next, to settle down the 26-hydroxycholesta-22-one side chain, the Lewis acid mediated acetolysis referred above produced compound **10** in 88% yield.

The three final 26-hydroxycholestan-22-one derivatives are quite stable. A combination of COSY, HSQC, and HMBC experiments helped



Scheme 1. Synthesis of 26-hydroxycholestan-22-one derivatives.

to complete the ^{1}H and ^{13}C NMR assignment of **6**, **7**, and **10**. Selected ^{1}H NMR shifts are shown in Fig. 2. The mass spectra of the target compounds yield a characteristic molecular ion pattern. For compound **6**, the presence of the enol ester can be confirmed with the shift of H-4*eq*, which is observed at 2.70 ppm and the presence of the acetates at C-3 and C-6, where H-3 displays a characteristic shift of proton base of acetate at 4.53 ppm. The methyl groups of the acetates appear at 2.03, 2.12, and 1.96 ppm for positions 3, 6, and 16, respectively. The ^{13}C shifts of C-5 (126.3 ppm) and C-6 (141.6 ppm) confirm the structure of the enol ester (see Table 1). For compound **7**, the signal of H-3 at 4.66 ppm corroborates the presence of the acetate. The chemical shifts of H-5 (2.35 ppm) and H-7*eq* (2.28 ppm) are characteristic of acid carbonyl-alpha hydrogens. The molecule displays two methyl groups of acetate at 2.03 and 1.97 ppm for positions 3 and 16, respectively. The ^{13}C shift of C-6 (209.6 ppm) supports the presence of the carbonyl group in the ring B. For compound **10**, H-2 and H-3 are observed at 4.96 and 5.37 ppm, respectively, characteristic chemical shifts for protons base of acetates. In a similar way to compound **7**, the chemical shifts of H-5 (2.61 ppm) and H-7*eq* (2.29 ppm) are characteristic of acid carbonyl-alpha hydrogens and the ^{13}C shift of C-6 (210.0 ppm) reinforces the proposed structure. Three methyl groups for acetates are observed at 2.00, 2.10, and 1.98 ppm, which belong to the positions 2, 3, and 16, respectively. For the side chain, similar chemical shifts are observed for compounds **6**, **7** and **10**. For example, for compound **6**, H-20 (2.95 ppm) and the two H-23 (2.63 and 2.40 ppm) evidence the presence of a carbonyl group at C-22 (observed in the ^{13}C experiment at 213.6 ppm). The two H-26 are found at 3.42 ppm and their corresponding carbon at 67.4 ppm endorse the primary hydroxyl group.

Since 22-oxocholestanes (which bear the side chain of the potent anticancer agent OSW-1) have shown significant plant growth promoting activity [28], our strategy was to mimic some of the oxygenated functions that are shown in natural brassinosteroids. Thus, the carbonyl at C-22 and the acetate at C-16 mimic the vicinal 22*R*,23*R*-diol and the different pharmacophores inserted in the A/B ring provide the required electronic density in such zone of the molecule. Compounds **6**, **7**, and **10** were evaluated using the RLIT that represents an extremely sensitive and specific bioassay for phytohormones.

Although the pharmacophores in natural BSs responsible for plant growth promoting activity are well identified, several synthetic compounds with few or many structural differences have shown activity. Two Mexican rice cultivars were tested: Morelos A06 and Morelos A08 (Figs. 3 and 4, respectively). The plant growth promoting activity of **6**, **7**, and **10** was compared with the commercially available brassinosteroid homobrassinolide (HB). The purity of **6**, **7**, and **10** was assured by recrystallizing the pure samples obtained from chromatography until constant melting point. With respect to the rice cultivars, Morelos A06 is the least sensitive cultivar, giving angles between 26° and 79°. With Morelos 08, angles close to 180° can be obtained.

Regarding Morelos A06, compound **6** reaches the highest angle of 70° at 0.05 mg/L, but its activity is not higher than the HB with an angle of 79°. While compound **7** gives 76° at 0.0005 mg/L, HB reaches 49° at the same concentration, this makes **7** a better plant growth promoter than HB. At 0.00005 mg/L, compound **10** gives 63° and compound **7** gets 65°. In comparison with 43° for HB, **7** is the most active in this experiment. Compound **6** does not show a consistent behavior compared with **7** and **10**. Interestingly, all the 22-oxocholestanes surpass the activity of HB at the lowest concentration tested, 0.00005 mg/L.

Concerning Morelos A08, which is the most sensitive cultivar, compound **6** reaches the highest angle of 171° at 0.005 mg/L, which is higher than HB with 164°. At 0.05 mg/L, compound **7** gives 176° and HB 170°. Finally, compound **10** gets 170° at 0.05 mg/L, showing this behavior since 0.0005 mg/L. This variety is too sensitive but the trend is similar to Morelos A06 where all the compounds are more active than HB.

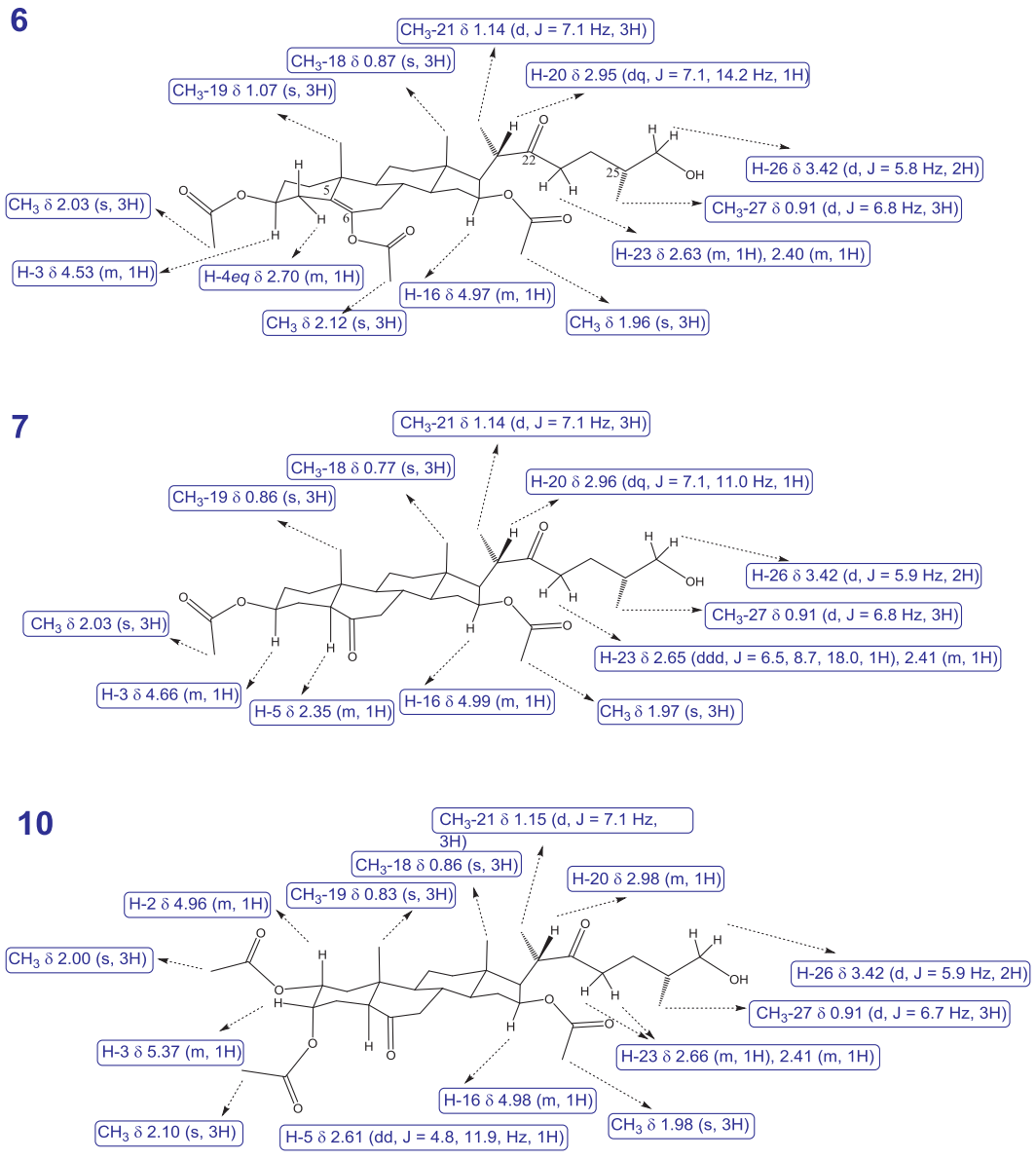


Fig. 2. ^{1}H NMR chart of **6**, **7** and **10**.

Table 1
Selected ^{13}C NMR chemical shifts δ (ppm) of **6**, **7**, and **10** in $CDCl_3$.

	6	7	10
C-2	27.3	26.1	68.9
C-3	72.8	72.6	67.8
C-5	126.3	53.4	51.6
C-6	141.6	209.6	210.0
C-16	75.5	75.0	74.9
C-22	213.6	213.4	213.5
C-26	67.4	67.3	67.2

For both cultivars, the activity decreases at concentrations higher than 0.05 mg/L, hence the lowest concentrations are preferred. At concentrations above 0.05 mg/L, the cultivars drop sensitivity and the assay loses practical applicability. Data sets are shown in Figs. 3 and 4 using the same scale. More specific graphics are included in the Supplementary Data file.

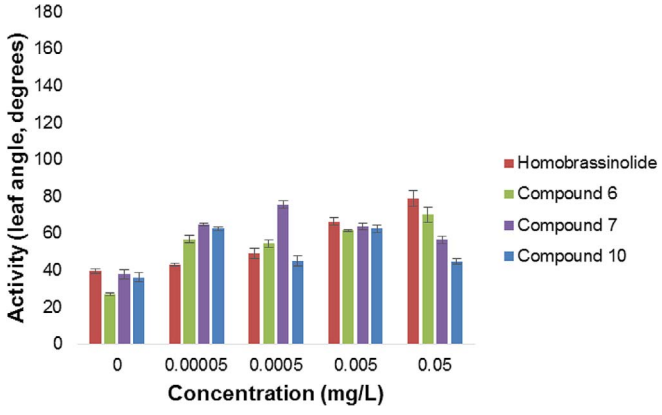


Fig. 3. Effect of compounds **6**, **7**, and **10** on the inclination activity measured by the rice lamina inclination test using the rice cultivar Morelos A06. Untreated segments and segments treated with HB were used as control. Each data point represents the mean of 10 replicates ± S.E.

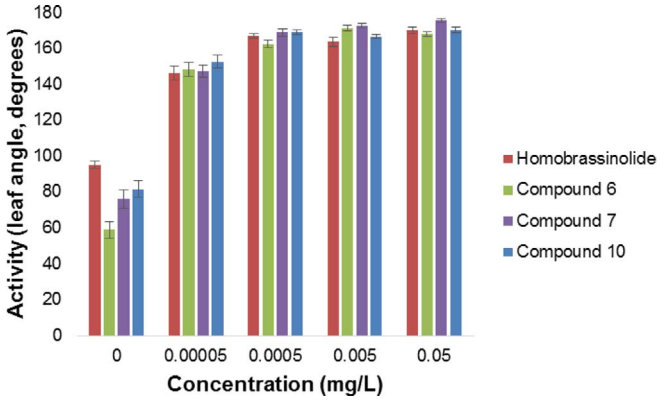


Fig. 4. Effect of compounds **6**, **7**, and **10** on the inclination activity measured by the rice lamina inclination test using the rice cultivar Morelos A08. Untreated segments and segments treated with HB were used as control. Each data point represents the mean of 10 replicates ± S.E.

4. Conclusions

In summary, we report the synthesis and characterization of 26-hydroxycholestan-22-one derivatives starting from the commercially available spirostan diosgenin. The title compounds bear oxygenated functions in the A/B rings and in the side chain trying to mimic the ones found in natural brassinosteroids. The syntheses were mostly carried out in good to excellent yields. An enol ester is found in **6** and the presence of the double bond at C-5 did not decrease the activity compared to the other analogs. The *cis*-diol of **10** was inserted in good yield employing CTAP as an alternative oxidizing agent, instead of using the expensive and more toxic OsO_4. The plant growth promoting activity of the title compounds was tested through the RLIT in two Mexican rice cultivars showing a potent activity compared with HB, one of the most active brassinosteroids. Surprisingly, for Morelos A06, compounds **7** and **10** showed the highest activity. This result is expected, being **10** the compound that most resembles natural brassinosteroids but **7** does not contain the vicinal 2α,3α-diol, so, this reinforces our hypothesis about the contribution for the activity given by the 22-oxocholestane side chain. For Morelos A08, the three compounds showed a trend like HB but with higher angles of the leaf bending. These results prove that the 26-hydroxycholestan-22-one side chain is a functionality that exerts significant plant growth promoting activity, so we can consider the title compounds as potential phytohormones (not necessarily BSs until the disclosure of the specific signaling pathways). We believe, therefore, that these compounds serve as promising lead candidates for further optimization provided that every cultivar reacts specifically. We succeeded in mimicking the activity found in natural BSs by inserting different oxygenated functional groups.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2017.06.004.

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