Synthesis of Four New Brassinosteroids Analogeues 11-Oxo-Functionalized on C Ring, with 24-Nor Side Chain and Containing 5β-Cholanic Acid Skeleton

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Abstract
In this work, I report the synthesis of four new brassinosteroids analogues with 24-nor side chain and 11-oxo functionalized on C ring, containing 5β-cholanic acid skeleton: 3α, 12β-diacetoxy-22(S), 23-dihydroxy-24-nor-5β-cholan-11-one (20); 3a, 12β, 22(S), 23-tetrahydroxy-24-nor-5β-cholan-11-one (21); 3α, 12β, 22(S), 23-tetraacetoxy-24-nor-5β-cholan-11-one (22) and 3a, 12β-diacetoxy-[2,2-dimethyl-22(S)], 23-dioxolane]-24-nor-5β-cholan-11-one (23) derivatives from commercial deoxycholic acid.

Keywords: Brassinosteroids; C-functionalized; 24-nor side chain

Introduction
Brassinosteroids (Brs) are a naturally occurring steroidal plant hormones group that regulates plant growth and development by producing an array of physiological changes. Brs occur at low concentrations throughout the plant kingdom. They have been detected in all plant organs (pollen, anthers, seeds, leaves, stems, roots, flowers, and grains) and also in the insect and crown galls [1,2]. Further work has demonstrated that Brs not only induce stem elongation but also increase biomass and total crop yield. Moreover, Brs are recognized to have an ameliorative role in plants subjected to various biotic and abiotic stresses, such as high temperature [3], heavy metals excess [4,5], salinity [6], water stress [7,8] and extreme temperatures [9]. Several authors and mainly Hayat et al. have reported structure-activity relationships (SAR) of brassinosteroid [10]. These SAR are based on the functions contained in the A, B ring, A/B ring fusion and in the side chain. However, in recent decades, efforts have been focused on the synthesis of new brassinosteroid analogues, keeping common patterns of organic functions in the A/B rings and cis-trans fusion between these, as in some natural brassinosteroids, but with dramatic structural changes in the side chain (shorter side chains, different oxygenated functions, spinosteranic, aromatic and cyclic substituents, esters, carboxylic acids, etc.) and oxygenated functions in C ring. Surprisingly these analogs have presented very important biological activities. On the other hand, the isolation of natural brassinosteroids with oxygenated function in ring C has not been reported. However, the synthesis of this type of analogs is very important for SAR studies of this kind of phytohormone. In this direction, hecogenin (I) is an abundantly available steroidal sapogenin, used as raw material in the production of a large number of Brs spinosteranic analogs with oxygenated function in ring C [11-21]. Examples of brassinosteroid analogues o xo-functionalized in C ring are shown in Figure 1. Others active Brs C- oxo and oxa functionalized analogs (6-8) bearing a cholic acid skeleton were derived from cholic acids [22]. Nevertheless, C-functionalized analogs 9-16 (Figure 2) were obtained from deoxycholic acid [23-25]. The plant growth-promoting activity of compounds 9 and 13 was tested in the hypocotyls elongation and cotyledons expansion of radish bioassay, where the compound 9 showed growth promoting activity at 10-3 mg/mL concentration in both bioassays, whereas compound 13 showed inhibiter effect in the cotyledons expansion test at the same concentration [24]. Compound 14 showed an increase of 38.6% by weight at 10-3 mg/mL concentration in cotyledons expansion of radish bioassay [25]. In this work is reported the synthesis and structural determination of four new Brs analogues, obtained from deoxycholic acid, with a cis-A/B ring junction, 24-nor side chain and 11-oxo-12β-hydroxy/acetate function on C ring containing with 5β-cholanic acid skeleton.

Materials and Methods

General
All reagents were purchased from commercial suppliers and used without further purification (Merck, Darmstadt, Germany or Aldrich, St. Louis, MO, USA). Melting points were measured on a Stuart-Scientific SMP3 apparatus (Staffordshire, ST15 OSA, UK) and are uncorrected. Optical rotations were obtained for CHCl3 or CH3OH, solutions on a Perkin-Elmer 241 polarimeter (Wellesley, Massachusetts, USA) and their concentrations are expressed in g/100 mL. NMR spectra were recorded on a Bruker AM-200 (Bruker, Rheinstetten, Germany) spectrometer operating at 200.1 MHz for 1H and 50.3 MHz for 13C. Chemical shifts are expressed in ppm downfield relative to TMS (6 scale) in CDCl3 solutions and coupling constants (J) are given in hertz. Carbon multiplicity were established by a DEPT pulse sequence. IR spectra were recorder as KBr disks in a Bruker FT-IR Vector-22 (Bruker, Germany) and frequencies are in cm-1. Elemental analyses were obtained on a Fisons-Carlo-Erba EA-1108 Automatic microanalyzer (Fisons Instruments/Carlo-Erba Instruments, Milano, Italy) For analytical TLC, Merck silica gel 60 in 0.25 mm layer was used and TLC spots were detected by heating after spraying with 25% H2SO4 in H2O. Chromatographic separations were carried out by conventional column on Merck silica gel 60 (230-400 Mesh) using hexane-EtOAc gradients of increasing polarity. All organic extracts were dried over anhydrous magnesium sulfate and evaporated under reduced pressure, below 40°C.

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Synthesis

24-oic 3α, 12β-diacetoxy-11-oxo-5β-cholan acid (17)

A solution of 16 (3.2 g, 7.61 mmol) and K₂CO₃ (0.8 g, 7.55 mmol) in MeOH (150 mL) was stirred at room temperature for 1.5 h. The end of the reaction was verified by TLC. Then the solvent was removed (until a 40 mL approximate volume) and the residue acidified with 2% HCl (15 mL) and extracted with EtOAc (3 × 20 mL). The organic layer was washed with 5% NaHCO₃ (30 mL) and water (2 × 15 mL), dried over Na₂SO₄, and filtered. The solvent was evaporated under reduced pressure. The crude was re-dissolved in CH₂Cl₂ (5 mL) and chromatographed on silica gel with petroleum ether/EtOAc mixtures of increasing polarity (19.8:0.2→10.2:9.8). Compound 17 (3.17 g, 85% yield) was as a colorless solid: m.p. 85.5-91.7°C (MeOH/Et₂O); [α]₂⁰+46.2° (c=0.405, CHCl₃); IR: 3432-2516; 1735; 1243; 1028. ¹H NMR: 0.69 (s, 3H, H-18); 0.93 (d, J=6.3 Hz, 3H, H-21); 1.16 (s, 3H, H-19);
To a solution of 18 (1.58 g, 3.55 mmol) in MeOH (60 mL) was added K₂CO₃ (0.79 g, 7.54 mmol), then the suspension was stirred at room temperature for 6 h. The end of the reaction was verified by TLC. Then the solvent was removed (until a 10 mL approximate volume) and the residue acidified with 5% HCl (2 mL) and extracted with EtOAc (2 x 10 mL). The organic layer was washed with 5% NaHCO₃ (10 mL) and water (2 x 10 mL), dried over Na₂SO₄ and filtered. The solvent was evaporated under reduced pressure.

To a solution of 17 (3.42 g, 7.69 mmol) in anhydrous benzene (300 mL) were added Cu(OAc)₂ (0.25 g, 1.38 mmol) and pyridine (1.0 mL). Then refluxed and Pb(OAc)₄ (8.34 g, 18.81 mmol) was added. The reaction was continued for 1 h. The end of reaction was verified by TLC, and then the mixture was filtered and the solvent was evaporated under reduced pressure. The crude was re-dissolved in CH₂Cl₂ (5 mL) and chromatographed on silica gel with petroleum ether/EtOAc mixtures of increasing polarity (19.8:0.2 → 14.2:5.8), to yield pure compound 18 (0.0485 g, 84.1% yield) which it was identified as a colorless solid: m.p. 152.8-154°C (MeOH/Et₂O); [α]D+99.5° (c=0.20, MeOH); IR: 3428; 1700; 1066; 1022. Elemental analysis: found C, 67.75%; H, 8.85%; C₂₃H₂₄O₃ requires C, 67.75%; H, 8.85%.

3a, 12β-diacetoxy-24-nor-5β-cholan-22-one-11-one (21)

To a solution of 20 (0.07 g, 0.146 mmol) in MeOH (50 mL) was added K₂CO₃ (0.035 g, 0.215 mmol), then the suspension was stirred at room temperature for 6 h. The end of the reaction was verified by TLC. Then the solvent was removed (until a 10 mL approximate volume) and the residue acidified with 2% HCl (2 mL) and extracted with EtOAc (2 x 10 mL). The organic layer was washed with 5% NaHCO₃ (10 mL) and water (2 x 10 mL), dried over Na₂SO₄ and filtered. The solvent was evaporated under reduced pressure. Subsequent recrystallization (MeOH/Et₂O) gave compound 21 (0.0485 g, 84.1% yield) which it was identified as a colorless solid: m.p. 152.8-154°C (MeOH/Et₂O); [α]D+99.5° (c=0.20, MeOH); IR: 3428; 1700; 1066; 1022. Elemental analysis: found C, 69.71%; H, 9.61%; C₂₃H₂₄O₃ requires C, 70.02%; H, 9.71%.

3a, 12β, 22(S), 23-tetrahydroxy-24-nor-5β-cholan-11-one (22)

Compound 20 (0.09 g, 0.188 mmol) was dissolved in CHCl₃ (30 mL) and pyridine (3.0 mL). Later 4-N,N-dimethylaminopyridine (DMAP, 5 mg) and Ac₂O (30 mL) were added to the solution. The end of the reaction was verified by TLC (2 h), and then the solvent was reduced to a volume about 5 mL, extracted with EtOAc (2 x 10 mL). The organic layer was washed with 5% KHSO₃ (2 x 5 mL) and water (2 x 10 mL), dried over Na₂SO₄ and filtered. The solvent was evaporated under reduced pressure. The crude was redissolved in CHCl₃ (3 mL) and chromatographed on silica gel with petroleum ether/EtOAc mixtures of increasing polarity (19.8:0.2 → 14.2:5.8), to yield pure 21 (0.09 g, 85.1% yield) as a colorless solid: m.p. 78.1-79.5°C (hexane/EtOAc); [α]D+6.9 ° (c=0.44, CHCl₃).

References
Results and Discussion

Previously we have reported the synthesis, and structural determination, of compounds 15 and 16 from deoxylchoholic acid [25]. Selective saponification of 16 in K$_2$CO$_3$/MeOH at room temperature afforded acid 17 with 85% yield (Scheme 1). The presence of the carboxylic function was confirmed by the signal at $\delta_{H}$=179.27 ppm in $^{13}$C NMR spectrum (see Table 1). The olefinic intermediate 18 (Scheme 1) was obtained with 72.3% yield by decarboxylation reaction of 17 in Pb(OAc)$_4$/Cu(AcO)$_2$ system, as reported for the preparation of other derivatives [24,26-28]. The presence of terminal exocyclic double bond with catalytic OsO$_4$ in NMO and purification of reaction crude by C.C. (Scheme 1) was obtained with 72.3% yield by decarboxylation reaction of 17, whereas standard acetylation (Ac$_2$O/DMAP/CH$_2$Cl$_2$) of compound 20 produces the tetra-acetylated derivative 22 with 85.1% yield. $^1$H NMR spectroscopic evidence indicates the presence of four singlets signals at $\delta_{H}$=2.01, 2.04, 2.07 and 2.14 ppm (3H, CH$_3$CO each), while six signals at $\delta_{H}$=20.86, 20.90, 21.25, 21.40, 169.94, 2 x 170.46 and 170.94, ppm were observed in $^{13}$C NMR (Table 1), so confirming the presence of four acetate groups in the molecule. Finally, ketalization reaction of 20 with (CH$_3$)$_2$CO/CuSO$_4$ anhydrous system, the ketol derivative 19 with 98% yield (Scheme 1) (1H NMR spectrum at room temperature for six hours) produces the ketol 19 with 98% yield (Scheme 1) (1H NMR spectrum at room temperature for six hours) produces the ketol 19 with 98% yield (Scheme 1) The presence of two signals at $\delta_{H}$=3.57 ppm (m, 1H) and $\delta_{H}$=3.84 ppm (s, 1H) in the $^1$H NMR spectrum confirms the removal of acetate groups. These carbinolic hydrogens were assigned as H-C3 and H-C12, respectively, according to their observed hydrogen multiplicities. The next step was the dihydroxylation reaction of olefin 18, however, some authors have reported that the electrophilic reactions at the steroidal C-22 double bond with OsO$_4$, RuCl$_3$/NaIO$_4$, Prevost-Woodward reaction with I$_2$/AgOAc produces predominantly (S) configuration at C-22 [29-31]. Then treatment of the alkene 18, with catalytic OsO$_4$ in NMO and purification of reaction crude by C.C. and subsequent crystallization, afforded compound 20 with 68% yield (Scheme 1). The presence of three carbinolic proton signals at $\delta_{H}$=3.47 ppm (m, 1H); 3.63 ppm (m, 1H) and 3.72 ppm (m, 1H) in the $^1$H NMR spectrum confirms the presence of four acetate groups in the molecule. Finally, ketalization reaction of 20 with (CH$_3$)$_2$CO/CuSO$_4$ anhydrous system, the ketol derivative 19 was obtained with 99.2% yield, according to the methodology previously described. In the $^1$H NMR spectrum of compound 21 two singlets signals at $\delta_{H}$=1.29 and 1.36 ppm were observed, these were assigned to the methyl of acetonide group [O$_2$C(CH$_3$)$_2$]. While in the $^{13}$C NMR spectrum, the signals observed at $\delta_{C}$=25.33 (CC H$_3$), 26.39 (CCH$_3$) and 108.30 ppm [O$_2$C(CH$_3$)$_2$], confirming the presence of acetonide group.

Conclusions

We have designed a synthetic sequence, which allows the obtention of three new synthetic derivatives (compounds 17-19) from commercial deoxychoholic acid and four new brassinosteroids analogues (compounds 20-23) with 24-nor side chain, oxygenated function at C-22 (S) and C-23 and 11-oxo functionalized on C ring, containing 5β-cholanic acid skeleton. Bioassays in Rice Lamina Inclination Test (RLIT) and growth in Arabidopsis thaliana, to detect possible biological activity of compounds 20-23, are under being developed and these results will be reported later.

Scheme 1: Synthetic route developed for intermediates 17-19 and Brs analog 20. Conditions and reagents: a. K$_2$CO$_3$/MeOH, r.t. 1.5 h, 85%. b. Pb(OAc)$_4$/Cu(OAc)$_2$/C$_6$H$_5$, reflux, 2.5 h, 72.3%. c. K$_2$CO$_3$/MeOH, r.t. 6 h, 98%. d. OsO$_4$, 4%, (CH$_3$)$_2$CO/NMO, r.t. 12 h, 68%. 

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Scheme 2: Synthetic route developed for Brs analog 21-23. Conditions and reagents: a. K₂CO₃/MeOH, r.t. 6 h, 84.1%. b. Ac₂O/DMAP/py/CH₂Cl₂, 2 h, 85.1%. c. CuSO₄/(CH₃)₂CO, r.t. 5 days, 69.2%.

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Table 1: δ ¹³C NMR (CDCl₃, 50.3 MHz) for compounds 17-20 and 22-23.

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References


