Synthesis of Analogues of Brassinosteroids from Chenodeoxycholic Acid

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The synthesis and spectroscopic characterization of two new bioactive analogues of brassinosteroids with a 24-hydroxylated cholanic side chain, an A/B ring *cis*-junction and oxygenated functions in C-7 is described.

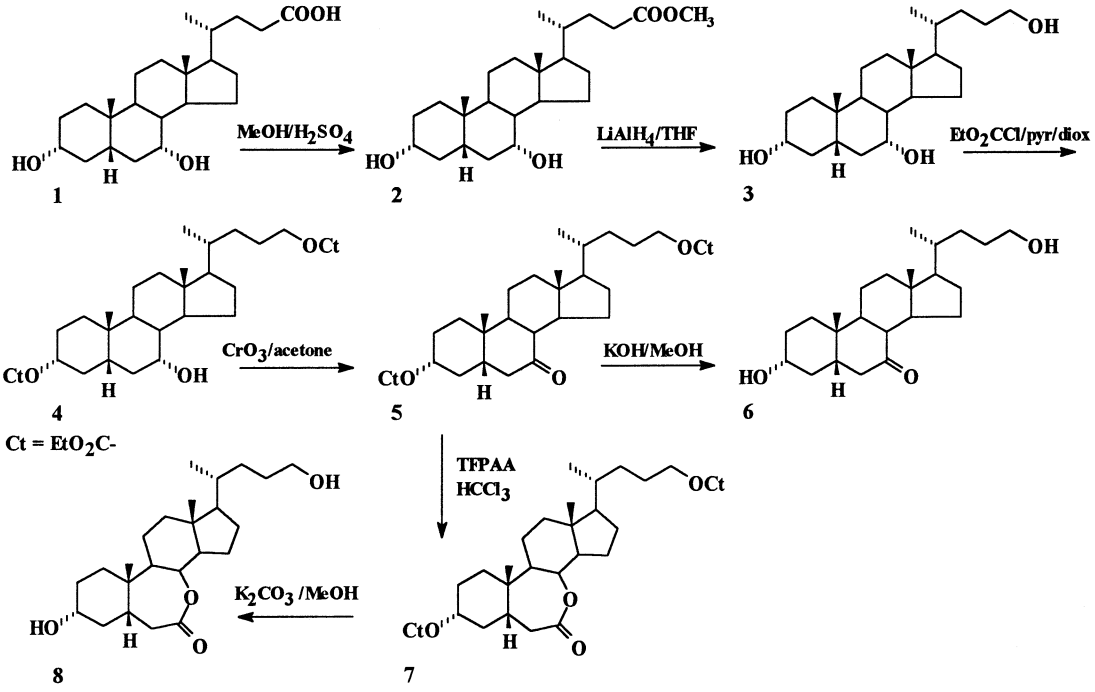
Introduction

Brassinosteroids are plant hormones[1] with remarkable biological activity that have stimulated many laboratories to undertake their synthesis[2] and the study of the relationship between molecular structure and biological activity.[3] Although it is generally accepted that a high brassinosteroid activity requires (a) a 2α,3α-diol in the A ring, (b) a 6-one or 6-oxo-7-oxalactone moiety in the B ring, (**c**) an A/B *trans*-fused ring junction, (d) a (22*R*,23*R*)-diol and (e) a 24-methyl or -ethyl substituent,[1] a great variety of bioactive analogues with modifications in the steroidal nucleus and side chain have been synthesized.[4] Recently, we have reported on the synthesis of some bioactive spirostanic[5] and furostanic[6] analogues of brassinosteroids starting from steroidal sapogenins. We now wish to describe the synthesis of two new bioactive analogues of brassinosteroids with a 24-hydroxylated cholanic side chain, an A/B ring *cis* junction and oxygenated functions in C-7.

Results and Discussion

Methyl chenodeoxycholate (**2**) was obtained by a procedure already described.[7] $LiAlH_4$ reduction of **2** in tetrahydrofuran (THF) led to the triol **3**[8] which was selectively cathylated to afford the 7α-hydroxy-5β-cholan-3α,24-diyl dicathylate (**4**). Oxidation of **4**, followed by hydrolysis with 5% methanolic KOH afforded 3α,24-dihydroxy-5β-cholan-

Scheme 1. Synthetic sequence



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7-one (**6**) in 79% overall yield. Baeyer-Villiger reaction of **5** with trifluoroperoxyacetic acid (TFPAA) obtained "in situ" by the trifluoroacetic anhydride (TFAA)/hydrogen peroxide procedure,[8] led to pure lactone **7**. Selective hydrolysis of **7** with 1% methanolic K_2CO_3 during 15 hours afforded the desired compound 3α,24-dihydroxy-B-homo-8-oxa-5β-cholan-7-one (**8**) in 66% overall yield (Scheme 1).

The biological activity of the brassinosteroid analogues **6** and **8** was examined by the Radish test.[9] Compound **6** produced, at the concentration of 10^{-6} μg/ml, an increase of 36% (referred to an untreated control) in the weight of cotyledons and at 10^{-4} and 10^{-6} μg/ml an increase of 16 and 17% respectively, in the length of hypocothyls. Compound **8** produced at the concentration of 10^{-4} μg/ml just a small increase of 9% in the weight of cotyledons.

Conclusions

Two new analogues of brassinosteroids **6** and **8** with an A/B ring *cis* junction, oxygenated functions in C-7 and a 24-hydroxylated cholanic side chain were synthesized from chenodeoxycholic acid (**1**). In contrast with the expected results, the compounds which bear drastic structural modifications, showed biological activity. The biological activity of the 7-oxo compound **6** proved to be higher than that of the lactone **8**.

Experimental Section

General: IR spectra were recorded from KBr cells with a Phillips Analytical PU9800 FT-IR spectrometer. − NMR: Bruker 250 ACF (250 MHz and 62.9 MHz, for ^1H and ^{13}C, respectively). For ^1H NMR, $CDCl_3$ or CD_3OD (solvents) and TMS (internal standard); for ^{13}C NMR, $CDCl_3$ or CD_3OD (δ_C = 77.0 and 49.0) were used. Temperature of the samples was 300 K and concentrations were 30 mg/ml for ^{13}C spectra and 10 mg/ml for ^1H spectra. − MS: JEOL JMS-DX 300 (70 eV). − Melting points were measured with Electrothermal 9100 equipment and are uncorrected.

5β-Cholane-3α,7α,24-triol (**3**): To a suspension of 500 mg of $LiAlH_4$ in 15 ml of THF, a solution of 2.54 g ($6.72\cdot10^{-3}$ mol) of methyl chenodeoxycholanate (**2**) in 15 ml of THF was added dropwise, and the mixture was stirred at reflux temp. for 4 h, poured into cool water and 10% H_2SO_4 (10 ml) was added. The product was extracted with ethyl acetate (3 × 30 ml) and the combined organic extracts were washed with brine (2 × 20 ml) and water (2 × 20 ml), dried with $MgSO_4$ and concentrated to give 2.25 g of **3** (95%), colorless crystals, m.p. 104.8−105.8°C. − IR (KBr): $\tilde{\nu}$ = 3404 cm^{-1} (OH). − ^1H NMR (CD_3OD): δ = 3.51 (m, 1 H, 3-H_{ax}), 3.79 (m, 1 H, 7-H_{eq}), 0.70 (s, 3 H, 18-H), 0.93 (s, 3 H, 19-H), 0.97 (d, *J* = 6.21 Hz, 3 H, 21-H), 3.40 (t, 2 H, 24-H). − ^{13}C NMR (CD_3OD): δ = 36.9 (C-1), 33.6 (C-2), 73.2 (C-3), 41.5 (C-4), 43.6 (C-5), 36.3 (C-6), 69.4 (C-7), 41.2 (C-8), 34.4 (C-9), 36.6 (C-10), 22.2 (C-11), 40.8 (C-12), 44.0 (C-13), 51.9 (C-14), 25.1 (C-15), 29.8 (C-16), 57.9 (C-17), 12.7 (C-18), 23.9 (C-19), 37.5 (C-20), 19.7 (C-21), 31.8 (C-22), 30.7 (C-23), 63.9 (C-24). − MS (70 eV); *m/z* (%): 378 (4.9) [M^+], 360 (33.6) [$M^+ - H_2O$], 342 (71.3) [$M^+ - 2H_2O$], 283 (2.8) [$M^+ - 2\,H_2O - C_3H_7O$], 255 (28.7) [$M^+ - 2\,H_2O - C_5H_{11}O$], 41 (100) [C=C−$C^+$]. − $C_{24}H_{42}O_3$ (378.59): calcd. C 76.14, H 11.18; found C 76.10, H 11.24.

7α-Hydroxy-5β-cholane-3α,24-diyl Dicathylate (**4**): To a solution of 1.65 g ($4.36\cdot10^{-3}$ mol) of **3** in 15 ml of dioxane and 8 ml of pyridine at 0°C, 10 ml of ethyl chloroformate was added dropwise, and the mixture was stirred at room temp. for 2 h. 8 ml of 32% HCl and 50 ml of water were added and the product was extracted with dichloromethane. The organic phase was washed with water, dried with $MgSO_4$ and the solvent removed under reduced pressure to give 2.21 g of **4** (97%), white crystalline solid, m.p. 107.4−109.4°C. − IR (KBr): $\tilde{\nu}$ = 3536 cm^{-1} (OH), 1740 (C=O). − ^1H NMR ($CDCl_3$): δ = 4.44 (m, 1 H, 3-H_{ax}), 3.84 (m, 1 H, 7-H_{eq}), 0.66 (s, 3 H, 18-H), 0.92 (s, 3 H, 19-H), 0.93 (d, *J* = 7.0 Hz, 3 H, 21-H), 4.09 (t, 2 H, 24-H), 1.29−1.31 (t, 6 H, *J* = 7.1 Hz, C*H*$_3$CH$_2$OCO$_2$), 4.16−4.19 (q, *J* = 7.2 Hz and *J* = 7.1 Hz, 4 H, CH$_3$C*H*$_2$OCO$_2$). − ^{13}C NMR ($CDCl_3$): δ = 34.9 (C-1), 26.6 (C-2), 78.1 (C-3), 35.1 (C-4), 41.1 (C-5), 34.3 (C-6), 68.3 (C-7), 39.4 (C-8), 32.7 (C-9), 35.0 (C-10), 20.5 (C-11), 39.6 (C-12), 42.6 (C-13), 50.3 (C-14), 23.6 (C-15), 28.2 (C-16), 55.8 (C-17), 11.7 (C-18), 22.6 (C-19), 35.4 (C-20), 18.4 (C-21), 31.7 (C-22), 25.2 (C-23), 68.4 (C-24), 14.3 double intensity (*C*H$_3$−CH$_2$−O), 63.5/63.8 (CH$_3$−*C*H$_2$−O), 155.3/154.7 (O$_2$*C*=O).

3α,24-Dicathyloxy-5β-cholan-7-one (**5**): To a solution of 2 g of **4** ($3.85\cdot10^{-3}$ mol) in 15 ml of acetone at 0°C, 1.5 ml of Jones reagent was added dropwise and the mixture was stirred for 10 min. 0.5 ml of 2-propanol and enough water to produce a precipitate was added. The precipitate was collected by filtration, washed with abundant water and dried at 60°C to give 1.85 g of **5** (98%), syrup. − IR (KBr): $\tilde{\nu}$ = 1744, 1715 cm^{-1} (C=O). − ^1H NMR ($CDCl_3$): δ = 4.54 (m, 1 H, 3-H_{ax}), 0.65 (s, 3 H, 18-H), 1.21 (s, 3 H, 19-H), 0.93 (d, *J* = 6.4 Hz, 3 H, 21-H), 4.09 (t, 2 H, 24-H) 1.29−1.31 (t, *J* = 7.1 Hz, 6 H, C*H*$_3$CH$_2$OCO$_2$), 4.16−4.19 (q, *J* = 7.1 Hz, 4 H, CH$_3$C*H*$_2$OCO$_2$). − ^{13}C NMR ($CDCl_3$): δ = 33.7 (C-1), 26.0 (C-2), 76.5 (C-3), 33.0 (C-4), 42.6 (C-5), 45.2 (C-6), 211.5 (C-7), 48.9 (C-8), 45.7 (C-9), 35.1 (C-10), 21.7 (C-11), 38.9 (C-12), 42.6 (C-13), 49.5 (C-14), 25.4 (C-15), 28.3 (C-16), 54.8 (C-17), 12.0 (C-18), 22.9 (C-19), 35.3 (C-20), 18.6 (C-21), 31.7 (C-22), 24.8 (C-23), 68.4 (C-24), 14.2/14.3 (*C*H$_3$−CH$_2$−O), 63.7/63.8 (CH$_3$−*C*H$_2$−O), 155.3/154.5 (O$_2$*C*=O).

3α,24-Dihydroxy-5β-cholan-7-one (**6**): A mixture of 600 mg of **5** ($1.59\cdot10^{-3}$ mol) and 10 ml of 5% methanolic KOH was stirred at room temp. for 15 min and then poured into water. The solid was collected by filtration, washed with abundant water and dried at 60°C. Chromatographic separation on silica gel with hexane/ethyl acetate (3:2) as solvent system gave 391 mg of **6** (90%), white crystalline solid, m.p. 148−149°C. − IR (KBr): $\tilde{\nu}$ = 3496, 3350 cm^{-1} (OH), 1713 (C=O). − ^1H NMR ($CDCl_3$): δ = 3.58 (m, 1 H, 3-H_{ax}), 0.66 (s, 3 H, 18-H), 1.20 (s, 3 H, 19-H), 0.94 (d, *J* = 6.5 Hz, 3 H, 21-H), 3.58 (t, 2 H, 24-H). − ^{13}C NMR ($CDCl_3$): δ = 34.1 (C-1), 29.6 (C-2), 70.5 (C-3), 37.1 (C-4), 42.8 (C-5), 45.4 (C-6), 213.0 (C-7), 49.5 (C-8), 46.1 (C-9), 35.1 (C-10), 21.6 (C-11), 38.9 (C-12), 42.5 (C-13), 48.8 (C-14), 24.7 (C-15), 28.3 (C-16), 54.9 (C-17), 11.9 (C-18), 23.0 (C-19), 35.4 (C-20), 18.6 (C-21), 31.8 (C-22), 29.2 (C-23), 63.1 (C-24). − MS (70 eV); *m/z* (%): 376 (9.2) [M^+], 358 (13.7) [$M^+ - H_2O$], 340 (7.8) [$M^+ - 2H_2O$], 299 (3.9) [$M^+ - H_2O - C_3H_7O$], 271 (7.8) [$M^+ - H_2O - C_5H_{11}O$], 41 (100) [C=C−C^+]. − $C_{24}H_{40}O_3$ (376.58): calcd. C 76.55, H 10.71; found C 76.49, H 10.70.

3α,24-Dicathyloxy-B-homo-8-oxa-5β-cholan-7-one (**7**): To a solution of trifluorperoxyacetic acid [prepared from 30% aqueous H_2O_2 (0.7 ml, 5.85 mmol) and TFAA (3.7 ml)] in 5 ml of chloroform, a solution of 216 mg of **6** ($0.42\cdot10^{-3}$ mol) in 15 ml of chloroform was added dropwise at 0°C and the mixture was stirred for 5 min and diluted with 10 ml of chloroform. The resulting solution was washed with water (1 × 3 ml), aqueous Na_2CO_3 (1 × 3 ml), aqueous $NaHSO_3$ (1 × 3 ml), brine (1 × 3 ml) and water (1 × 3 ml),

and dried with $MgSO_4$. The solvent was removed by evaporation to give 209 mg of **5** (94%), white crystalline solid, m.p. 92.4−94.4°C. − IR (KBr): $\tilde{\nu}$ = 1743 cm^{-1} (C=O). − ^1H NMR ($CDCl_3$): δ = 4.59 (m, 1 H, 3-H_{ax}), 4.18 (t, 1 H, 8-H_{ax}), 0.66 (s, 3 H, 18-H), 1.08 (s, 3 H, 19-H), 0.92 (d, *J* = 6.5 Hz, 3 H, 21-H), 4.09 (t, 2 H, 24-H), 1.29−1.31 (t, *J* = 7.1 Hz, 6 H, $CH_3CH_2OCO_2$), 4.18 (q, *J* = 7.1 Hz, 4 H, $CH_3CH_2OCO_2$). − ^{13}C NMR ($CDCl_3$): δ = 35.3 (C-1), 26.4 (C-2), 76.3 (C-3), 31.6 (C-4), 41.5 (C-5); 35.5 (C-6), 173.9 (C-7), 79.6 (C-8), 39.9 (C-9), 37.5 (C-10), 22.5 (C-11), 38.4 (C-12), 43.2 (C-13), 54.4 (C-14), 25.1 (C-15), 27.8 (C-16), 55.8 (C-17), 11.5 (C-18), 23.4 (C-19), 35.1 (C-20), 18.4 (C-21), 31.7 (C-22), 25.3 (C-23), 68.3 (C-24), 14.2/14.3 (CH_3−CH_2−O), 63.8 (CH_3−CH_2−O), 155.3/154.5 (O_2C=O).

3α,24-Dihydroxy-B-homo-8-oxa-5β-cholan-7-one (**8**): A mixture of 200 mg of **7** ($0.51 \cdot 10^{-3}$ mol) and 10 ml of 1% methanolic K_2CO_3 was stirred at room temp. for 15 h and then poured into water. The solid was collected by filtration, washed with abundant water and dried at 60°C. Chromatographic separation on silica gel with hexane/ethyl acetate (3:2) as solvent system gave 130 mg of **8** (89%), white crystalline solid, m.p. 119.0−119.4°C. − IR (KBr): $\tilde{\nu}$ = 3342 cm^{-1} (OH), 1743 (C=O). − ^1H NMR ($CDCl_3$): δ = 3.58 (m, 1 H, 3-H_{ax}), 4.21 (t, 1 H, 8-H_{ax}), 0.67 (s, 3 H, 18-H), 1.07 (s, 3 H, 19-H), 0.93 (d, *J* = 6.4 Hz, 3 H, 21-H), 3.58 (t, 2 H, 24-H). − ^{13}C NMR ($CDCl_3$): δ = 35.6 (C-1), 29.9 (C-2), 70.4 (C-3), 35.7 (C-4), 41.6 (C-5) 35.6 (C-6) 174.9 (C-7), 80.0 (C-8), 39.8 (C-9), 37.4 (C-10), 22.4 (C-11), 38.4 (C-12), 43.1 (C-13), 54.3 (C-14), 25.0 (C-15), 27.8 (C-16), 55.9 (C-17) 11.4 (C-18), 23.4 (C-19), 35.2 (C-20), 18.5 (C-21), 31.7 (C-22), 29.3 (C-23), 63.1 (C-24). − MS (70 eV); *m/z* (%): 392 (2.9) [M^+], 374 (8) [$M^+ - H_2O$], 356 (7.2) [$M^+ - 2H_2O$], 315 (1.5) [$M^+ - H_2O - C_3H_7O$], 305 (4.3) [$M^+ - C_5H_{11}O$], 287 (4.3) [$M^+ - H_2O - C_5H_{11}O$], 41 (100) [C=C−C^+]. − $C_{24}H_{40}O_4$ (392.58): calcd. C 73.43, H 10.27; found C 73,49, H 10.35.

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