Synthesis of pregnane ketols as metabolic precurs ors of analogues of plant growth regulators

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The synthesis of seven new D-ring functionalised 5α-hydroxylated biosynthetic precursors of brassinosteroid analogues is described.

Keywords: steroids, 5-hydroxy-pregnan-6-ones, hydroxypregnanes

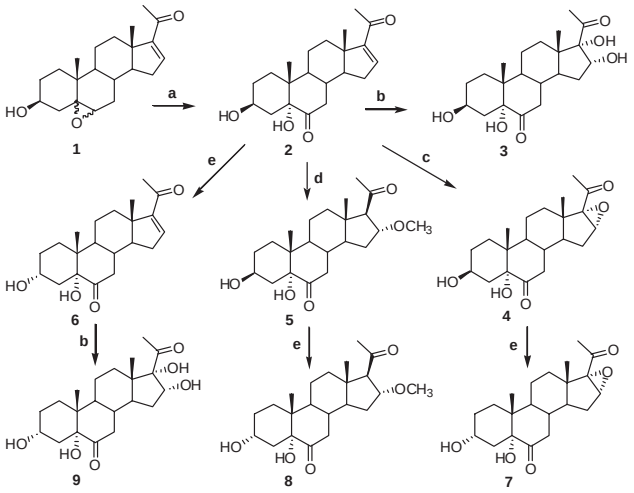
The brassinosteroids comprise a family of phytohormones that display high growth-promoting activity and anti-stress properties.[1] The possible replacement of these naturally occurring hormones with synthetic analogues in agricultural applications has been widely discussed over the last few years.[2-5] The excellent results which have been obtained for some synthetic compounds, have been the focus of considerable attention in both chemical and biological communities. The emphasis has been on more synthetically accessible analogues, such as those bearing the 20-oxo, (20)-five-carbon-atom ester and (20)-four-carbon-atom ester functionalities,[6-8] or molecules having chemical functions which are considered to be biosynthetic precursors of the brassinosteroids.[2-5]

With a view toward applying this concept, 5α-hydroxylated analogues of the naturally occurring biosynthetic precursors tifasterol and teasterone, have been described.[5] The 3α-OH and 3β-OH functions, even in the presence of the 5α-OH group, can be biosynthetically transformed to the 2α,3α-diol one and thereby further recognised by the brassinosteroid receptor.

As part of our program involving the synthesis of novel brassinosteroid-like compounds, we synthesised seven new pregnane ketols which might act as precursors of pregnane analogues of brassinosteroids. Our goal took advantage of the straightforward synthesis of the key intermediate **2** from the epoxide mixture **1**, via a simple acid-catalysed epoxide opening and subsequent selective oxidation of the axial 6β-OH with N-bromosuccinimide (NBS). The epoxide mixture **1** was prepared from the readily available 16-dehydropregnenolone using a previously reported method.[9]

The known epoxidation of the Δ^{16} double bond to furnish the final epoxyketol **4** was performed using cold hydrogen peroxide in alkaline methanol. On the other hand, several equivalents of N-methylmorpholine N-oxide (NMO), non-catalytic amounts of OsO_4 as well as long reaction times were required to afford the ketol **3** in good yields. The Michael type nucleophilic addition of a methoxy group to afford ketol **5** was accomplished in 68% yield by treatment with sodium in anhydrous methanol at room temperature. Heating was avoided because of the possible inversion of the C-5 configuration which has been reported for 5α-hydroxy-6-oxo functions under drastic conditions.[10]

Additionally, epimerisation of C-3 using the successful Mitsunobu reaction provided the desired set of D-ring functionalised 3α-ketols. Thus, treatment of the 3β-OH derivatives **2**, **4** and **5** with diethylazodicarboxylate (DEAD)/Ph_3P/HCOOH in THF and subsequent cleavage of the 3α-formyloxy group with $NaHCO_3$/MeOH-H_2O afforded the corresponding C-3 epimers **6**, **7** and **8**, respectively. Final dihydroxylation of compound **8** led to the tetraol **9** in 54% yield from intermediate **2**.



Scheme 1 Reagents and conditions: (a) i, $HClO_4/H_2O$–CH_3COCH_3; ii, NBS/AcOH–CH_3COCH_3; (b) OsO_4/NMO/THF–BuOH–H_2O; (c) H_2O_2/NaOH/MeOH/0°C; (d) MeONa/MeOH; (e) i, HCOOH/Ph_3P/DEAD/THF; ii, $NaHCO_3$/MeOH–H_2O.

1D and 2D NMR experiments were performed on compounds **3**–**9** allowing the unequivocal assignment of the functionalised carbons and confirming the structure and stereochemistry of the ketols. The C-16 configuration in compounds **3**, **4** and **5** was demonstrated using NOE difference experiments, in which an enhancement of H-16 upon irradiation of H-18 confirmed the assigned 16α stereochemistry of the oxygenated functions at C-16.

Our approach allowed the introduction of a variety of oxygen functions (hydrogen bond acceptors and/or donors) on ring D, which along with the 3β-and 3α,5α-hydroxy-6-oxo functions represent a unique structural feature not found in any known brassinosteroid and thereby not biologically tested.

Experimental

Melting points were determined on a Stuart Scientific Apparatus and are uncorrected. IR spectra were obtained on a Nicolet 205 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker ACF-250. Elemental Analyses were performed in a Leco CHNS-932 instrument. "Usual work-up" refers to extracting with an organic solvent, washing the extract, drying over anhydrous $MgSO_4$ and concentrating under reduced pressure.

3β,5-Dihydroxy-5α-pregn-16-ene-6,20-dione (**2**): A suspension of the epoxide mixture **1** (7.6 g, 23.0 mmol) in 200 ml of acetone was treated with water (10 ml) and 70% $HClO_4$ (2.5 ml) and stirred at room temperature for 4 h. Usual work-up (EtOAc) yielded a white solid (pure by TLC) which was consecutively dissolved in the mixture acetone (150 ml)/water (15 ml), treated with NBS (2.5 g, 14.0 mmol) and AcOH (2 ml) and stirred at room temperature for 2 h. Usual work-up yielded a crude product, which was purified by flash column chromatography (hexane/EtOAc 3:1) to give the ketol **2**

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Table 1 ^{13}C NMR spectral data of final compounds

Compound	3	4	5	7	8	9
C-1	29.7	29.9	29.7	28.3	28.3	28.3
C-2	29.7	29.7	29.7	25.4	25.4	25.4
C-3	66.6	67.0	66.9	67.4	67.5	67.5
C-4	35.2	35.6	35.6	31.4	31.4	31.4
C-5	79.9	80.1	80.0	80.3	80.3	80.2
C-6	213.8	213.5	213.5	211.9	211.8	212.0
C-7	41.4	41.4	41.4	41.7	41.7	41.7
C-8	37.1	36.7	36.9	37.5	37.5	37.5
C-9	44.0	44.0	44.0	44.8	44.8	44.8
C-10	42.2	42.3	42.2	43.4	43.4	43.4
C-11	19.5	21.0	21.1	21.2	21.1	21.2
C-12	34.5	38.6	38.6	38.6	38.6	34.5
C-13	47.0	41.6	44.9	41.6	44.9	47.0
C-14	48.9	44.8	53.9	44.8	53.9	48.9
C-15	27.9	27.3	31.5	27.3	31.5	27.9
C-16	74.0	60.4	81.2	60.3	81.2	74.0
C-17	77.2	70.8	71.1	70.8	71.1	77.2
C-18	16.0	15.3	14.4	15.3	14.4	16.0
C-19	14.5	14.0	13.8	14.0	13.7	13.9
C-20	216.5	205.1	207.9	205.0	207.9	216.5
C-21	29.7	25.9	31.5	25.9	31.5	29.7

(6.1 g, 76%). m.p. (EtOH): 193–195°C. IR (KBr, cm^1): 3443, 3335 (O–H); 1705 (C=O); 1661 (C=O); 1685 (C=C); 1086, 1048 (C–O). ^1H NMR ($CDCl_3$): δ_H 0.81 (3H, s, 18-H); 0.80 (3H, s, 19-H); 2.23 (3H, s, 21-H); 3.87 (1H, m, 3α-H); 6.66 (1H, d, *J*=2.0 Hz, 16-H).

General dihydroxylation procedure: NMO (600 mg, 5 mmol) and a solution of OsO_4 in *t*-BuOH (5 ml, 12.5 mg/ml) were added to a solution of the corresponding 16-ene-20-one (600 mg, 1.73 mmol) in the solvent mixture THF/t-BuOH/H_2O (10:10:1) (80 ml). The reaction was stirred under nitrogen atmosphere at room temperature for 72 h, then treated with a saturated solution of Na_2SO_3 and stirred for an additional hour. Usual work-up (AcOEt) yielded a crude product, which was purified by flash column chromatography (CH_2Cl_2/EtOAc 5:1) to give the 16α,17α-diol.

3β,516α,17α-Tetrahydroxy-5α-pregnane-6,20-dione (**3**)*:* 390 mg (65%). m.p. (EtOAc): 219–221°C. IR (KBr, cm^{-1}): 3337, 3414, 3439 (O-H); 1697 (C=O); 1186, 1048 (C–O). ^1H NMR ($CDCl_3$+CD_3OD 95:5): δ = 0.70 (3H, s, 18-H); 0.73 (3H, s, 19-H); 2.07 (3H, s, 21-H); 3.84 (1H, m, 3α-H); 3.97 (1H, d, *J*=7.5 Hz, 16β-H). For $C_{21}H_{32}O_6$; Calc: C, 66.3; H, 8.5; Found: C 66.35; H, 8.7%

16a,17a-Epoxy-3b,5-dihydroxy-5a-pregnane-6,20-dione (**4**): A solution of the enone **2** (1.0 g, 2.87 mmol) in 80 ml of MeOH was cooled to 0°C and treated successively with $H_2O_2$30% (6 ml) and cold NaOH 4 N (2 ml). The reaction mixture was stirred and kept at 0–5°C for 24 h. Usual work-up (Et_2O) yielded an oil, which was purified by flash column chromatography (hexane/EtOAc 3:1) to give the epoxyketol **4** (780 mg, 77%). m.p. (acetone): 186–189°C. IR (KBr, cm^{-1}): 3540, 3330 (O-H); 1705 (C=O); 1700 (C=O); 1092, 1043 (C–O). ^1H NMR ($CDCl_3$): δ = 0.95 (3H, s, 18-H); 0.73 (3H, s, 19-H); 1.97 (3H, s, 21-H); 3.63 (1H, d, *J*=2.5 Hz, 16β-H); 3.84 (1H, m, 3α-H). For $C_{21}H_{30}O_5$; Calc: C, 69.6; H, 8.3; Found: C 69.4; H, 8.25%

3β,5-Dihydroxy-16α-methoxy-5α-pregnane-6,20-dione (**5**): A freshly prepared solution of sodium (1.1 g, 51.5 mmol) in 30 ml of dry MeOH was added to a solution of **5** (2 g, 5.15 mmol) in 100 ml of MeOH. The reaction mixture was stirred for 8 h at room temperature and then concentrated. Usual work-up (EtOAc) yielded a white solid, which was purified by flash column chromatography (hexane/EtOAc 2:1) to obtain the methoxyketol **5** (1.4 g, 72%). m.p. (acetone): 193–194°C. IR (KBr, cm^{-1}): 3495, 3463 (O-H); 1700 (C=O); 1702 (C=O); 1380, 1090 (C–O). ^1H NMR ($CDCl_3$): δ = 0.57 (3H, s, 18-H); 0.72 (3H, s, 19-H); 2.15 (3H, s, 21-H); 2.54 (1H, d, *J*=6.3 Hz, 17α-H); 3.16 (3H, s, OCH_3); 3.94 (1H, br m, 3α-H); 4.29 (1H, t, *J*=6.5 Hz, 16β-H). For $C_{22}H_{34}O_5$; Calc: C, 69.8; H, 9.05; Found: C, 69.75; H, 9.1%

General Mitsunobu reaction: To a stirred solution of the 3β-ketol (1.6 mmol), formic acid (0.20 ml) and triphenylphosphine (635 mg, 2.49 mmol) in THF (50 ml) was added dropwise a solution of DEAD (510 mg, 2.94 mmol) in 5 ml of THF. The mixture was stirred at room temperature for 24 h, and then the solvent was evaporated under reduced pressure. The resultant crude product was dissolved in 80 ml of a saturated solution of $NaHCO_3$ in MeOH-H_2O (4:1) and stirred for 1 h at room temperature. Usual work-up (EtOAc) yielded a crude, which was purified by flash column chromatography (Hexane/EtOAc 4:1) to afford the corresponding 3α-ketol.

16α,17α-Epoxy-3α,5-dihydroxy-5α-pregnane-6,20-dione (**7**): 356 mg (56%). m.p. (acetone): 188–190°C. IR (KBr, cm^{-1}): 3445, 3368 (O-H); 1705 (C=O); 1699 (C=O); 1105, 1092 (C–O).^1H NMR ($CDCl_3$): δ = 0.95 (3H, s, 18-H); 0.75 (3H, s, 19-H); 1.97 (3H, s, 21-H); 4.25 (1H, m, 3β-H); 3.63 (1H, d, *J*=2.5 Hz, 16β-H). For $C_{21}H_{30}O_5$; Calc: C, 69.6; H, 8.3; Found: C, 69.5; H, 8.45%

3α,5-Dihydroxy-16α-methoxy-5α-pregnane-6,20-dione (**8**): 387 mg (64%). m.p. (EtOH): 196–198°C. IR (KBr, cm^{-1}): 3398, 3325 (O-H); 1700 (C=O); 1702 (C=O); 1368, 1092 (C-O). ^1H NMR ($CDCl_3$): δ = 0.58 (3H, s, 18-H); 0.75 (3H, s, 19-H); 2.15 (3H, s, 21-H); 2.54 (1H, d, *J*=6.3 Hz, 17α-H); 3.16 (3H, s, OCH_3); 4.23 (1H, m, 3β-H); 4.29 (1H, t, *J*=6.5 Hz, 16β-H). For $C_{22}H_{34}O_5$; Calc: C, 69.8; H, 9.05; Found: C, 69.65; H, 9.0%

3α,5-Dihydroxy-5α-pregn-16-ene-6,20-dione (**6**): 382 mg (69%). m.p. (MeOH): 191–193°C. IR (KBr, cm^{-1}): 3396, 3327 (O-H); 1702 (C=O); 1661 (C=O); 1682 (C=C); 1105, 1048 (C-O). ^1H NMR ($CDCl_3$): δ = 0.81 (3H, s, 18-H); 0.77 (3H, s, 19-H); 2.23 (3H, s, 21-H); 4.25 (1H, m, 3β-H); 6.65 (1H, d, *J*=2.0 Hz, 16-H). For $C_{21}H_{30}O_4$; Calc: C, 72.8; H, 8.7; Found: C, 72.9; H, 9.0.

3α,5,16α,17α-Tetrahydroxy-5a-pregnane-6,20-dione (**9**): Obtained from **6** through the general dihydroxylation procedure. 318 mg (76 %). m.p. (AcOEt): 217–220°C. IR (KBr, cm^1): 3456, 3398, 3325 (O-H); 1700 (C=O); 1698 (C=O); 1280, 1105, 1060 (C-O). ^1H NMR ($CDCl_3$+CD_3OD 95:5): δ = 0.70 (3H, s, 18-H); 0.75 (3H, s, 19-H); 4.25 (1H, m, 3α-H); 3.97 (1H, d, *J*=7.5 Hz, 16β-H). For $C_{21}H_{32}O_6$; Calc: C, 66.3; H, 8.5; Found: C, 66.25; H, 8.6.

We are grateful to Prof. Dr. Carlos. S. Pérez from University of Havana, Chemical Physics Department, for the spectroscopic measures.

Received 2 February 2004; accepted 6 March 2004
Paper 04/2334

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