Synthesis of spirostanic analogues of brassinosteroids via homogeneous permanganate dihydroxylation

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Homogeneous non-aqueous permanganate dihydroxylation is used to synthesize spirostanic analogues of brassinosteroids bearing a variety of oxygen functions on ring B.

Keywords: steroids, brassinosteroid analogues, homogeneous permanganate oxidation

Brassinosteroids are considered as a new class of steroidal phytohormones due to their high plant growth-promoting activity and anti-stress effect.[1] The 2α,3α-dihydroxy function is one of the structural requirements for high bioactivity.[2] Most synthetic strategies are directed toward the introduction of both 2α,3α and 22R,23R-diol groups, for which the most straightforward method is the osmium-catalysed asymmetric dihydroxylation.[3]

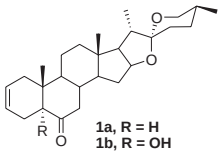
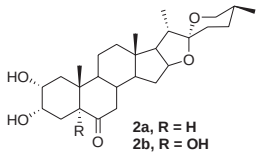
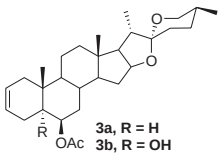
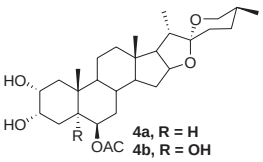
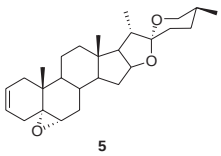
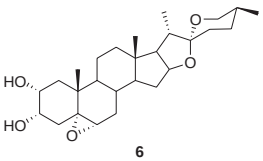
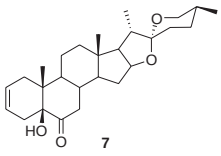
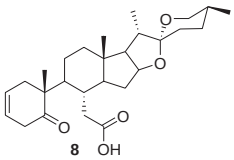
We have reported the synthesis of spirostanic analogues of brassinosteroids that showed high plant growth-promoting activity in both bioassays and field trials.[4-8] Because our compounds do not require transformations in the spiroketal side chain, and with the aim of establishing an alternative way for the preparation of 2α,3α-diols that avoids the use of the expensive and toxic OsO_4 (osmium tetroxide), we studied the scope of the homogeneous permanganate dihydroxylation and its effect on several oxygen functions usually found on ring B of natural brassinosteroids or their synthetic analogues.

The Δ^2-spirostenes (Table 1) bearing ketone, α-ketol, epoxy, hydroxy and acetoxy functions were submitted to homogeneous non-aqueous permanganate oxidation upon phase transfer catalysis[9]. The importance must be noted of utilising 1.5 eq. of $KMnO_4$, is an anhydrous freshly prepared organic solution and a rigorous control of the temperature and speed of dropwise addition to achieve the stability of the organomanganese intermediates before being quenched by basic aqueous solutions. For entries 1, 2 and 3 we obtained the desire 2α,3α-diols in good yields besides of a small amount of the 2,3-seco-spirostan-2,3-dioic acids, without affecting the key oxygen functions of ring B. The oxidation of olefin **7** resulted in opening of ring B to afford the 5,6-seco-steroid without previous hydroxylation.

These results may suggest that the configuration of C-5 decides the possibility of the 5-hydroxy-6-oxo function of being re-oxidised by MnO_4^-. Thus, the equatorial disposition of the 5β-hydroxyl allows the α-ketol to adopt a conformation in which the hydroxyl group is almost eclipsed with respect to the oxygen of the carbonyl group. This allows the permanganate complex to form with the subsequent cleavage of the function. In the case of compound **1b**, it seems that the axial disposition of the hydroxyl, anticlinal to the oxygen of the carbonyl group, makes impossible the complex formation. Compounds **2a** and **4a** were compared with those previously obtained by the osmium tetroxide dihydroxylation procedure[4,6], the ^{1}H NMR data as well as NOESY experiments confirmed the 2α,3α stereochemistry of the diol in compounds **2b**, **4b** and **6**.

Our results reveal a set of B-ring oxygen functions found in brassinosteroids or analogues, which are not affected by the homogeneous non-aqueous permanganate dihydroxylation. Standardization of this method on different analogues could lead to the establishment of an alternative A-ring functionalising procedure.

Table 1 Homogeneous permanganate oxidation of B-ring functionalised Δ^2-spirotanic olefins.

Entry	Substrate	Product[a]	Yield/%[b]
1	 **1a, R = H** **1b, R = OH**	 **2a, R = H** **2b, R = OH**	2a, 68 2b, 67
2	 **3a, R = H** **3b, R = OH**	 **4a, R = H** **4b, R = OH**	3a, 69 3b, 71
3	 **5**	 **6**	72
4	 **7**	 **8**	68

[a]For entries 1,2 and 3; the corresponding (25R)-2,3-seco-5α-spirostan-2,3-dioic acids were obtained in 10–15 %.
[b]Yield of isolated pure product.

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 spectrometer at 250.13 MHz and 62.9 MHz for ^{1}H and ^{13}C, respectively, using TMS as internal standard. The high resolution ESI mass spectra were obtained from a Bruker Appex 70e Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer.

General procedure: The Δ^2-spirostenes (4.0 mmol) were dissolved in CH_2Cl_2 (60 ml) and cooled to 0°C. A freshly prepared solution of $KMnO_4$ (6.0 mmol) and triethylbenzylammonium chloride (6.0 mmol) in CH_2Cl_2 (60 ml) was slowly added dropwise, being the temperature kept at 0–3 °C. When addition was completed (30 min),

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the reaction was allowed to reach room temperature and stirred an additional hour until permanganate ion was completely consumed. The brown mixture was then treated with NaOH 3 % (50 ml) and stirred under nitrogen atmosphere for 15 h. The organic layer was separated, washed with saturated solution of oxalic acid, dried over $MgSO_4$ and concentrated at reduced pressure. The obtained crude in each case was purified by flash column chromatography (hexane/EtOAc 5:1) to furnish the products shown in Table 1.

(25R)-2α,3α-Dihydroxy-5α-spirostan-6-one (**2a**): m.p. (heptane/AcOEt): 224.2–225.5°C (lit[4]: 224–225°C). IR (KBr, cm^{-1}): 3475, 3387, 3335 (νO-H); 1721 (νC=O); 1110, 1050 (νC–O), 980, 920, 860 (ν spiroketal system). ^1H NMR ($CDCl_3$): δ = 0.71 (3 H, s, H-19); 0.75 (3 H, s, H-18); 3.73 (^1H, br m, H-2β); 4.02 (1 H, m, H-3β). ^{13}C NMR ($CDCl_3$): δ = 68.2 (C-2); 68.3 (C-3); 50.8 (C-5); 212.0 (C-6); 16.3 (C-18); 16.6 (C-19).

(25R)-2α,3α,5-Trihydroxy-5α-spirostan-6-one (**2b**): m.p. (heptane/AcOEt): 278.7–279.1°C. IR (KBr, cm^{-1}): 3477, 3420, 3335 (νO-H); 1697 (νC=O); 1121, 1080, 1034 (νC-O); 978, 920, 864 (ν spiroketal system). ^1H NMR ($CDCl_3$): δ = 0.79 (3 H, s, H-19); 0.77 (3 H, s, H-18); 3.79 (1 H, br m, H-2β); 4.10 (1 H, m, H-3β). ^{13}C NMR ($CDCl_3$): δ = 67.2 (C-2); 69.3 (C-3); 79.3 (C-5); 211.7 (C-6); 16.1 (C-18); 14.4 (C-l9). HRMS (ESI-FT-ICR) *m/z*: 485.28790 $[M+Na]^+$ (Calculated for $C_{27}H_{42}O_6Na$: 485.28791).

(25R)-5α-Spirostane-2α,3α,6β-triol 6-acetate (**4a**): m.p. (heptane/AcOEt): 227.5–229.0°C (lit[6]: 227.0–228.8°C). IR (KBr, cm^{-1}): 3470, 3382 (νO-H); 1736 (νC=O, Ac); 1244 (νC-O, Ac); 1086, 1048, 1018 (νC-O). 989, 920, 860 (ν spiroketal system).^1H NMR ($CDCl_3$): δ = 1.08 (3 H, s, H-19); 0.77 (3 H, s, H-18); 2.02 (3 H, s, CH_3-Ac); 3.77 (1 H, br m, H-2β); 4.03 (1 H, m, H-3β), 4.90 (1H, m, H-6β). ^{13}C NMR ($CDCl_3$): δ = 68.4 (C-2); 69.0 (C-3); 41.2 (C-5); 72.3 (C-6); 16.1 (C-18); 15.5 (C-19), 170.0 (CO-Ac).

(25R)-5α-Spirostane-2α,3α,5,6β-tetraol 6-acetate (**4b**): m.p. (heptane/AcOEt): 275.0–276.2°C. IR (KBr, cm^{-1}): 3513, 3468, 3399 (νO-H); 1732 (νC=O, Ac); 1240, 1050 (νC-O, Ac); 1115, 1060, 1020 (νC-O). 980, 920, 862 (ν spiroketal system). ^1H NMR ($CDCl_3$): δ = 1.10 (3 H, s, H-19); 0.78 (3H,s,H-18); 2.04 (3H,s,CH_3-Ac); 3.90 (1H,brm,H-2β); 4.11 (1H,m,H-3β); 4.81 (1H,m,H-6β). ^{13}C NMR ($CDCl_3$): δ = 68.0 (C-2); 70.5 (C-3); 73.6 (C-5); 75.0 (C-6); 16.6 (C-18); 17.0 (C-19); 170.1 (CO-Ac). HRMS (ESI-FT-ICR) *m/z*: 529.31412 $[M+Na]^+$ (Calculated for $C_{29}H_{46}O_7Na$: 529.3 1412).

(25R)-5,6α-Epoxy-5α-spirostane-2α,3α-diol (**6**): m.p. (MeOH): 226.3–227.5°C. IR (KBr, cm^{-1}): 3498, 3412 (νO-H); 1078, 1012 (νC-O). 978, 918, 859 (ν spiroketal system). ^1H NMR ($CDCl_3$): δ = 0.73 (3H,s,H-19); 0.77 (3H,s,H-18); 3.84 (1H,brm,H-2β); 4.07 (1H,m,H-3β); 2.88 (1H,d, *J*=4.3 Hz, H-6β). ^{13}C NMR ($CDCl^3$): δ = 67.5 (C-2); 69.1 (C-3); 65.2 (C-5); 59.0 (C-6); 16.2 (C-18); 14.8 (C-19). HRMS (ESI-FT-ICR) *m/z*: 469.29298 $[M+Na]^+$ (Calculated for $C_{27}H_{42}0_5Na$: 469.29299).

(25R)-5,6-Seco-5-oxo-spirost-2-en-6-oic acid (**8**): m.p. (MeOH): 175.6–176.3°C. IR (KBr, cm^{-1}): 3025 (νO-H); 1708, 1700 (νC=O); 977, 920, 900 (ν spiroketal system). ^1H NMR ($CDCl_3$): δ = 1.07 (3 H, s, H-19); 0.82 (3 H, s, H-18); 3.42 (1 H, dd, *J*=10.5/3.0 Hz, H-4); 5.54 (2 H, m, H-2 + H-3). ^{13}C NMR ($CDCl_3$): δ = 125.6 + 125.7 (C-2 + C-3); 216.1 (C-5); 177.3 (C-6); 16.1 (C-18); 17.5 (C-19). HRMS (ESI-FT-ICR) *m/z*: 467.27738 $[M+Na]^+$ (Calculated for $C_{27}H_{40}O_5Na$: 467.27734).

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