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Transformation of 20-hydroxyecdysone to inokosterone

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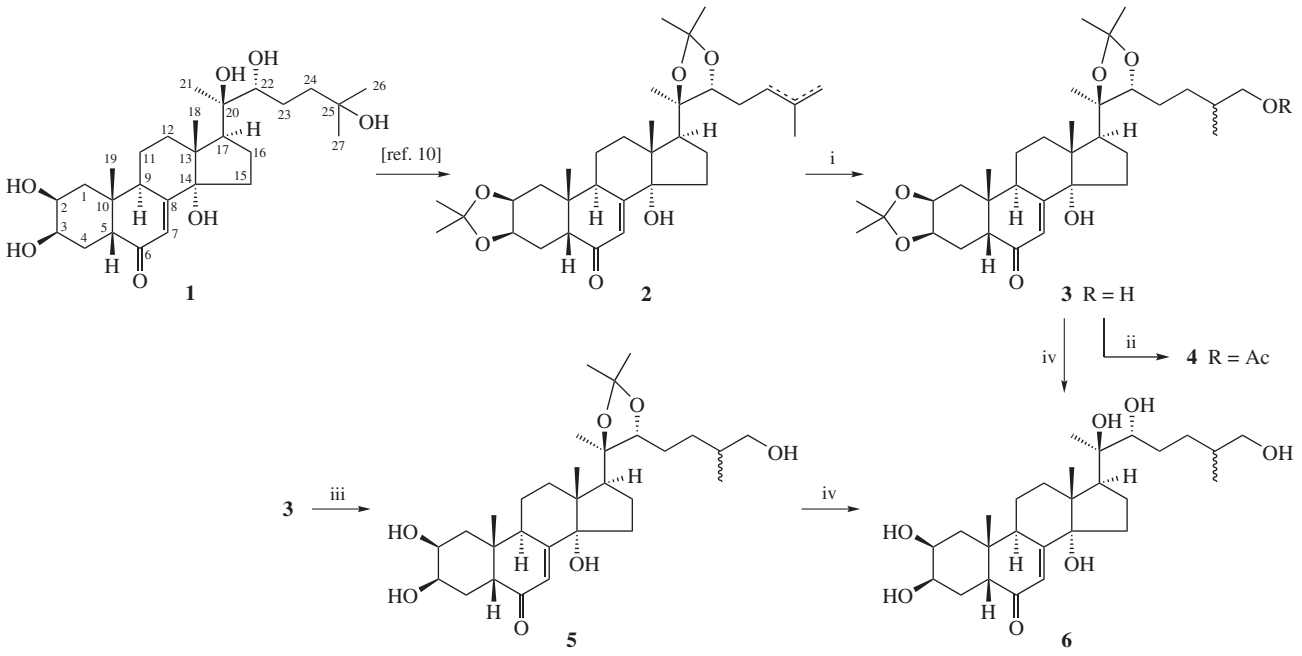
Δ^{24}/Δ^{25}-Derivatives of 20-hydroxyecdysone were hydroborated by sodium borohydride in the presence of CuCl followed by oxidation of organoboron intermediate and deblocking of hydroxyl groups to synthesise the zoo- and phytoecdysteroid inokosterone.

The ecdysteroid inokosterone was originally identified in insect[1] and then isolated from plants.[2,3] Studies of the structure and absolute configuration of inokosterone isolated from roots of a Chinese plant *Achyranthes fauriei*[2] showed the phytoecdysteroid to be a mixture (1:2) of C^{25}(*R*)- and (*S*)-epimers.[4] When isolated from *A. fauriei*[5] inokosterone was used in a synthesis of 26-iodoponasterone.[6] The latter has a very high ecdysone activity, which is 16 times higher than that of 20-hydroxyecdysone, and this permits its use in studies of insect ecdysone receptors.[7]

In order to establish its structure, inokosterone was synthesised from a formylpregnane.[4] A full synthesis of the latter had been carried out previously.[8] The authors have developed a new approach to the synthesis of inokosterone **6**, based on chemical transformations of the more readily available phytoecdysteroid 20-hydroxyecdysone **1**.[9] Thus, a mixture (3:2) of diacetonides of Δ^{24}/Δ^{25}-derivatives of 20-hydroxyecdysones **2**, prepared in two steps[10] from **1**, was subjected to a hydroboration reaction with $NaBH_4$ catalysed by CuCl ($NaBH_4$–CuCl reagent was used previously for α-olefin hydroboration[11]). Oxidation of the organoboron intermediate by the H_2O_2–AcONa reagent led to inokosterone diacetonide **3**.† Along with the required compound an inseparable mixture of more polar compounds and initial mixture of alkenes **2** were isolated (according to ^{1}H NMR spectral data, the Δ^{24}/Δ^{25}-alkene ratio is ≈3:2 as in the initial mixture). The yield of **3** shows that the Δ^{25}-alkene in the initial mixture **2** is commonly transformed to 26-hydroxyderivative, while during the oxidation of the organoboron intermediate, the nonreacted Δ^{24}-alkene probably isomerises to the terminal position and an alkene mixture is formed with the ratio (3:2) Δ^{24}/Δ^{25}-isomers that was stated in dehydration of **1**, respectively.[10]



Scheme 1 *Reagents and conditions*: i, $NaBH_4$/CuCl, THF, 20 °C, H_2O_2/AcONa; ii, Ac_2O/Py/DMAP; iii, AcOH/$ZnCl_2$; iv, 10% $HClO_4$–MeOH, 1:1.

Two single-proton signals at 3.64 and 3.48 ppm in the ^{1}H NMR spectrum and a carbon signal C^{26} at 68.03 ppm in the ^{13}C NMR spectrum reveal the appearance of hydroxyl at C^{26} in compound **3**. The presence of two doublets (J 6.5 Hz) H_3C^{27} of equal intensities at 0.93 and 0.94 ppm in the ^{1}H NMR spectrum shows that compound **3** is an equimolar mixture of 25*R*- and 25*S*-epimers. A similar picture is observed in this part of ^{1}H NMR spectra for diacetonide 25-acetate **4**.‡ In its ^{13}C NMR spectra two signals for the carbonyls of the acetate groups were observed. An acetylation of hydroxyl group at C^{26} leads to a shift of C^{27} signal to a low field, and C^{25} signal – to a high one and a doubling of their signals in the ^{13}C NMR spectrum of compound **4** (a result of the presence of epimers at 25-tertiary centre).

Under the normal conditions of the removal of an acetonide protection in ecdysteroids (AcOH/$ZnCl_2$)[12] the hydrolysis of **3** leads only to 20,22-acetonide of inokosterone **5**.‡ The treatment of acetonide **5** or diacetonide **3** by a different method[6] gives inokosterone **6**.‡

† *Hydroboration–oxidation of* **2**. To a solution of **2** (a mixture Δ^{24}/Δ^{25}-alkenes, 3:2, prepared by method[10]) (0.3 g, 0.55 mmol) in dry THF (20 ml) was added CuCl (0.056 g, 0.56 mmol) with mixing, the mixture was cooled to 0 °C and $NaBH_4$ (0.021 g, 0.55 mmol) was added in one portion. After 20 min temperature was raised to ~20 °C for 8 h. Then at 0 °C H_2O (1 ml), H_2O_2 (0.2 ml, 20%) and solution of NaOAc (0.082 g, 1 mmol) in H_2O (1 ml) were added to the reaction mixture. The organic layer was separated from water, extracted by EtOAc. The common organic layers were washed by NaCl, dried by $MgSO_4$ and evaporated under vacuum. A residue was chromatographed (SiO_2) eluating by chloroform to give an initial mixture of alkenes **2** (0.08 g, R_f 0.64, Silufol, $CHCl_3$–MeOH, 10:1), **3** (0.12 g, 38.5% or 97% taking into account Δ^{25}-alkene in **2**, R_f 0.48) and a mixture of polar compounds (0.1 g, R_f 0.25–0.36).

For **3**: mp 130–132 °C, $[\alpha]_D^{27}$ +23.2° (*c* 5.82, $CHCl_3$). IR (KBr, ν/cm^{-1}): 1680, 3450. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ: 0.79 (s, 3H, H_3C^{18}), 0.93 and 0.94 (2d, 3H, H_3C^{27}, J 6.5 Hz), 0.98 (s, 3H, H_3C^{19}), 1.14 (s, 3H, H_3C^{21}), 1.32 and 1.41 (2s, 6H, 20,22-Me_2C), 1.33 and 1.49 (2s, 6H, 2,3-Me_2C), 1.50–2.26 (m, 18H, HC, H_2C), 2.34 (dd, 1H, HC^5, J 12.5 and 4.4 Hz), 2.81 (m, 1H, HC^9, $w_{1/2}$ 22.0 Hz), 3.48 (m, 2H, HC^{22}, H_aC^{26}, $w_{1/2}$ 10.0 Hz), 3.64 (dd, 1H, H_bC^{26}, J 10.4 and 5.5 Hz), 4.23 (m, 2H, HC^2, HC^3, $w_{1/2}$ 10.0 Hz), 5.82 (br. s, 1H, HC^7, $w_{1/2}$ 6.0 Hz). ^{13}C NMR [75.47 MHz, $CDCl_3$, the signals were assigned using a pulse sequence of J-modulated spin echo (JMOD)] δ: 16.58 and 16.64 (C^{27}), 17.03 (C^{18}), 20.54 (C^{16}), 21.19 (C^{11}), 21.89 (C^{21}), 23.60 (C^{19}), 26.42 and 28.50 (2,3-Me_2C), 26.87 and 29.00 (20,22-Me_2C), 26.67 (C^{23}), 30.65 (C^{15}), 30.87 (C^{12}), 31.03 (C^{24}), 31.61 (C^4), 34.58 (C^9), 35.77 and 35.81 (C^{25}), 37.59 (C^1), 37.75 (C^{10}), 47.48 (C^{13}), 49.08 and 49.14 (C^{17}), 50.78 (C^5), 68.03 (C^{26}), 71.62 (C^3), 72.16 (C^2), 81.55 and 81.64 (C^{22}), 84.12 and 84.16 (C^{14}), 84.95 and 85.00 (C^{20}), 106.84 and 106.88 (20,22-Me_2C), 108.27 (2,3-Me_2C), 121.34 (C^7), 163.23 and 163.29 (C^8), 202.80 (C^6). Found (%): C, 70.68; H, 9.35. Calc. for $C_{33}H_{52}O_7$ (%): C, 71.07; H, 9.21.

‡ For **4**: R_f 0.68 (Silufol, $CHCl_3$–MeOH, 10:1), mp 96–98 °C, $[\alpha]_D^{18}$ +25.5° (*c* 2.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 0.77 (s, 3H, H_3C^{18}), 0.90 and 0.94 (2d, 3H, H_3C^{27}, J 6.7 Hz), 0.95 (s, 3H, H_3C^{19}), 1.11 (s, 3H, H_3C^{21}), 1.30 and 1.38 (2s, 6H, 20,22-Me_2C), 1.30 and 1.47 (2s, 6H, 2,3-Me_2C), 2.03 (s, 3H, MeCOO), 1.50–2.26 (m, 18H, CH, CH_2), 2.31 (dd, 1H, HC^5, J 12.4 and 4.1 Hz), 2.80 (m, 1H, HC^9, $w_{1/2}$ 25 Hz), 3.60 (m, 1H, HC^{22}, $w_{1/2}$ 15 Hz), 3.73–4.05 (m, 2H, HC^{26}), 4.23 (m, 2H, HC^2, HC^3, $w_{1/2}$ 15 Hz), 5.79 (br. s, 1H, HC^7, $w_{1/2}$ 5 Hz). ^{13}C NMR, δ: 17.03 (C^{18}), 17.28 and 18.39 (C^{27}), 20.51 (C^{16}), 20.90 (*Me*COO), 21.15 (C^{11}), 21.83 (C^{21}), 23.53 (C^{19}), 26.37 and 28.45 (2,3-Me_2C), 26.62 (C^{23}), 26.81 and 28.93 (20,22-Me_2C), 29.58 (C^{15}), 30.69 (C^{12}), 30.99 (C^{24}), 31.50 (C^4), 32.48 and 32.69 (C^{25}), 34.52 and 34.57 (C^9), 37.55 (C^1), 37.69 (C^{10}), 47.44 and 47.49 (C^{13}), 48.80 and 49.06 (C^{17}), 50.74 (C^5), 69.20 (C^{26}), 71.58 (C^3), 72.11 (C^2), 81.35 and 81.43 (C^{22}), 83.80 and 84.08 (C^{14}), 84.80 (C^{20}), 106.83 and 106.91 (20,22-Me_2C), 108.18 (2,3-Me_2C), 121.26 (C^7), 163.35 (C^8), 170.47 and 171.20 (Me*C*OO), 202.76 (C^6). Found (%): C, 69.74; H, 9.03. Calc. for $C_{35}H_{54}O_8$ (%): C, 69.78; H, 9.00.

For **5**: R_f 0.67 (Silufol, $CHCl_3$–MeOH, 5:1), mp 118–120 °C, $[\alpha]_D^{17}$ +47.4° (*c* 2.2, $CHCl_3$). IR (KBr, ν/cm^{-1}): 1640, 3280. UV [$CHCl_3$, λ_{max}/nm (ε)]: 242 (12000). ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, 3H, H_3C^{18}), 0.96 (m, 3H, H_3C^{27}, $w_{1/2}$ 7 Hz), 0.97 (s, 3H, H_3C^{19}), 1.15 (s, 3H, H_3C^{21}), 1.33 and 1.41 (2s, 6H, 20,22-Me_2C), 1.50–2.50 (m, 19H, HC, CH_2), 3.03 (m, 1H, HC^9, $w_{1/2}$ 19.6 Hz), 3.47 (m, 1H, HC^{22}, $w_{1/2}$ 12 Hz), 3.65 (m, 2H, HC^{26}, $w_{1/2}$ 20.0 Hz), 3.85 (m, 1H, HC^2, $w_{1/2}$ 17.4 Hz), 3.99 (m, 1H, HC^3, $w_{1/2}$ 12 Hz), 5.84 (br. s, 1H, HC^7, $w_{1/2}$ 6.0 Hz). ^{13}C NMR, δ: 16.62 and 16.69 (C^{27}), 17.07 (C^{18}), 20.50 (C^{16}), 21.20 (C^{11}), 21.92 (C^{21}), 23.90 (C^{19}), 26.50 (C^{23}), 26.90 and 29.01 (20,22-Me_2C), 30.10 (C^{15}), 31.02 (C^{12}), 31.50 (C^4), 31.35 (C^{24}), 35.58 and 35.91 (C^{25}), 34.20 (C^9), 36.62 (C^1), 38.18 (C^{10}), 47.27 (C^{13}), 49.11 and 49.17 (C^{17}), 50.06 (C^5), 67.39 (C^3), 67.73 (C^2), 67.85 (C^{26}), 81.20 and 81.30 (C^{22}), 84.19 (C^{14}), 84.86 (C^{20}), 106.87 (20,22-Me_2C), 121.42 (C^7), 165.39 (C^8), 204.47 (C^6). Found (%): C, 69.20; H, 9.29. Calc. for $C_{30}H_{48}O_7$ (%): C, 69.27; H, 9.21.

For **6**: R_f 0.24 (Silufol, $CHCl_3$–MeOH, 5:1), mp 176–178 °C, $[\alpha]_D^{22}$ +48.7° (*c* 0.8, MeOH) [*cf.* for mixture (1:2) 25*R*/*S*-epimers: mp 255 °C,[4] $[\alpha]_D^{27}$ +59.4° (*c* 0.78, MeOH)[13]]. IR (KBr, ν/cm^{-1}): 1660, 3240. ^{1}H NMR (C_5D_5N) δ: 1.04 (d, 3H, H_3C^{27}, J 6.9 Hz), 1.06 (s, 3H, H_3C^{19}), 1.22 (s, 3H, H_3C^{18}), 1.57 and 1.59 (2s, 3H, H_3C^{21}), 1.70–2.70 (m, 17H, HC, H_2C), 2.95–3.05 (m, 2H, HC^5, HC^{17}), 3.60 (m, 1H, HC^9, $w_{1/2}$ 22 Hz), 3.65–3.82 (m, 2H, H_2C^{26}), 3.86 (dd, 1H, HC^{22}, J 10.1 and 1.4 Hz), 4.21 (m, 1H, HC^2, $w_{1/2}$ 22 Hz), 4.28 (m, 1H, HC^3, $w_{1/2}$ 8 Hz), 6.26 (d, 1H, HC^7, J 2.0 Hz) (*cf.* refs. 3, 4 and 13). ^{13}C NMR, δ: 17.06 (C^{27}), 17.64 (C^{18}), 20.82 (C^{16}), 21.40 (C^{11}), 23.07 (C^{21}), 24.07 (C^{19}), 29.21 (C^4), 31.42 (C^{23}), 31.80 (C^{12}, C^{15}, C^{24}), 34.13 (C^9), 36.48 (C^{25}), 37.68 (C^1), 38.41 (C^{10}), 47.59 (C^{13}), 49.78 (C^{17}), 50.96 (C^5), 67.11 (C^{26}), 67.78 (C^3), 68.00 (C^2), 73.27 (C^{22}), 76.65 (C^{20}), 83.92 (C^{14}), 121.38 (C^7), 166.16 (C^8), 203.10 (C^6) (*cf.* ref. 13).

Thus, the four-step transformation of the readily available ecdysteroid 20-hydroxyecdysone to 25*R*/*S*-inokosterone was developed, a key step in which is hydroboration.

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