Synthesis of 26-Iodoponasterone, a New and very Active Ecdysteroid

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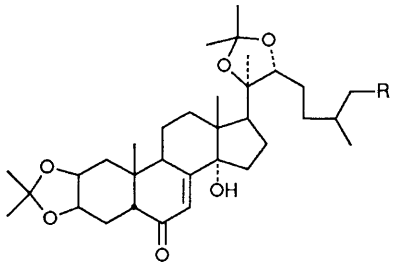
26-Iodoponasterone A, prepared from inokosterone in four steps, is 160 times as active as the parent compound in an ecdysone assay on Drosophila Kc cells, and is one of the most active ecdysones known; the ^{125}I is being used for the detection of ecdysone receptors.

20-Hydroxyecdysone, the moulting hormone of arthropods, like other steroids, is a regulator of gene expression, and the molecular biology of its actions is being studied in numerous laboratories.[1,2,3] Work on ecdysone receptors requires the use of hormone analogues more active than the natural hormone 20-hydroxyecdysone,[2,4,5] and the development of potent ecdysteroids suitable for radiolabelling has been critical to progress in this field. Here, we report the synthesis of a new ecdysone analogue, 26-iodoponasterone, starting from the phytoecdysone inokosterone **1**. 26-Iodoponasterone proves to be a very active ecdysone suitable for use in receptor studies and for radiolabelling.[6]

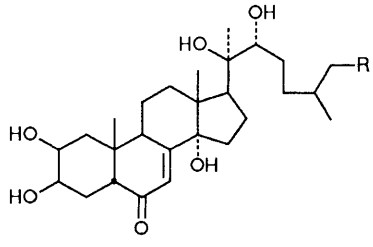
Inokosterone was isolated from the Chinese herb *Achyranthus fauriei* following published procedures.[7] Treatment of inokosterone (**1**, 53 mg) with dry acetone (3 ml) and anhydrous *p*-TsOH (5 mg) yielded 45 mg of the diacetonide (**2**, 87% yield) after work-up and purification through a silica gel column (5 g), elution with 5% EtOH–$CHCl_3$. The product **2** shows the following NMR signals: δ ($CDCl_3$, 250 MHz) 5.80 (d, *J* 2.1 Hz, 7-H), 4.24 (m, 3-H), 4.20 (m, 2-H), 3.47 (d, *J* 5.2 Hz, 26-H_2), 2.78 (dt, *J* 8.1, 8.1 and 2.1 Hz, 9-H), 2.33 (dd, *J* 12.5 and 3.7 Hz, 5-H), 1.47 and 1.30 (2,3-acetonide-Me), 1.38 and 1.30 (20, 22-acetonide-Me), 1.12 (20-Me), 0.96 (s, 10 Me), 0.93 and 0.91 (d, *J* 6.5 Hz, 25-Me) and 0.77 (13-Me). The 26-hydroxy of the diacetonide **2** (25 mg) was mesylated by dropwise treatment with mesyl chloride (0.1 ml) in dry pyridine (2 ml) at 0 °C for 0.5 h and at ambient temperature for 1 h. After addition of pyr-H_2O (2 : 1, 1 ml), the solution was partitioned between H_2O and Et_2O. The residue of the organic layer (24 mg) was purified by silica gel (5 g) chromatography, elution with 1 and 2.5% EtOH–$CHCl_3$, to give 19 mg of mesylate **3**; Me of mesyl group at δ 3.02, and 26-H_2 at δ 4.10.

The iodo derivative **4** was prepared by treating 5 mg mesylate **3** with 28 mg Bu_4NI in 2 ml toluene at 75 °C under argon for 19 h.[8] The cooled reaction mixture was then partitioned three times[8] between 30 ml H_2O and 30 ml ether, and the residue of the ether layer (7 mg) was purified with silica gel column (3 g), elution with 1% EtOH–$CHCl_3$ to give 6 mg of the iodo product; NMR δ ($CDCl_3$): 5.83 (d, *J* 2.0 Hz), 4.27 (m, 3-H), 4.23 (m, 2-H), 3.25 (m, 26-H_2), 2.80 (ddd, *J* 8.5, 6.4 and 2.0 Hz, 9-H), 2.36 (dd, *J*, 12.5 and 4.6 Hz, 5-H), 1.50 and 1.33 (s, 2,3-acetonide-Me), 1.41 and 1.33 (s, 20,22-acetonide-Me), 1.13 (s, 20-Me), 1.02 (d, *J* 6.3 Hz, 25-Me), 0.99 (s, 10-Me), and 0.79 (s, 13-Me). The 20,22-acetonide, which is relatively stable toward HCl–THF or HOAc–THF–H_2O pairs, can be removed readily treating with 10% $HClO_4$–MeOH (1 : 1). 26-Mesylinkosterone **5** or 26-iodoponasterone A **6** was obtained in 70% yield by overnight treatment of mesyldiacetonide **3** or iododiacetonide **4** with 10% $HClO_4$–MeOH (1 : 1), work-up (neutralization with $NaHCO_3$ then extraction with EtOAc) and purification (silica gel, eluent: 10, 15 and 20% EtOH–$CHCl_3$).

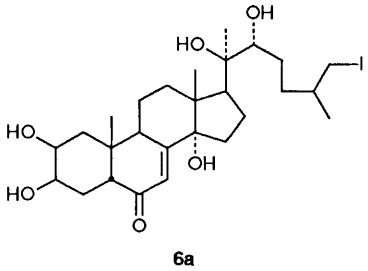
Mesylate **5** shows the following NMR signals: δ (CD_3OD) 5.80 (d, *J* 2.2 Hz, 7-H), 4.09 (m, 26-H_2), 3.94 (m, 3-H), 3.83 (dt, *J* 11.4, 3.9 and 3.9 Hz, 2-H), ~3.40 (overlapping with MeOH, 22-H), 3.13(m, 9-H), 3.06 (s, mesyl-Me), 2.36 (dd, *J* 12.5 and 4.6 Hz, 5-H). 1.18 (s, 20-Me), 1.10 and 1.08 (d, *J* 6.6 Hz, 25-Me), 0.96 (s, 10-Me) and 0.88 (s, 13-Me). Spectral data of iodoponasterone **6** are as follows: desorption chemical ionization (DCI) (NH_3 reactant gas) $C_{27}H_{43}O_6I$, *m/z* 590 (M^+), λ_{max} (MeOH) 244 nm (ε 12 000); δ (CD_3OD) 5.80 (d, *J* 2.2 Hz, 7-H), 3.94 (m, 3-H), 3.38 (m, 2-H), 3.40 (overlapping with MeOH, 22-H), 3.22 (m, 26-H_2), 3.14 (m, 9-H), 2.36 (dd, *J* 12.5 and 4.6 Hz, 5-H), 1.14 (s, 20-Me), 1.00 and 0.98 (d, *J* 5.6 Hz, 25-Me), 0.96 (s, 10-Me) and 0.88 (s, 13-Me). As summarized in **6a**, the DCI–MS of **6** showed peaks at *m/z* 462 (37%), 444 (40%) and 427 (37%) resulting from the loss of HI, HI+H_2O and H+H_2O+OH, respectively. The chemical shifts of 26-H_2, δ 3.47 (in **2**, CH_2OH), 4.10 (in **3** and **5**, CH_2OMs), and 3.22 (in **4** and **6**, CH_2I) can be accounted for by the electronegativity differences of substituents on C-26; the mesylate NMR signals at δ 3.02 also agree with **3** and **5**.



2 R = OH
3 R = OMs
4 R = I



1 R = OH
5 R = OMs
6 R = I
7 R = H



6a

DCI–MS (NH_3): *m/z*

590 (M^+, 37%)
572 (M^+ -H2O, 95%)
554 (M^+ -2H2O, 100%)
536 (M^+ -3H2O, 91%)
462 (M^+ -HI, 37%)
444 (M^+ -HI-H2O, 40%)
427 (M^+ -HI-H2O-OH, 37%)

The HPLC retention times for **5** and **6** are 3.42 and 10 min, respectively, under the following conditions: 5 μm analytical Ultrasphere ODS column, MeOH–H_2O (60 : 40) as eluent, 1 ml/min (flow rate) and detection at 242 nm.

We have examined the structure–activity relationships among inkosterone **1**, mesylate **5**, iodoponasterone A **6**, ponasterone A **7**, and 20-hydroxyecdysone (20-HE), using the standard Drosophila Kc cell assay, in which previous results have correlated well with known receptor affinities.[6,9] Relative to 20-HE, the activities were **1** 0.1, **5** 0.1, **6** 16, and **7** 8. Thus, in the Kc cell bioassay, 26-iodoponasterone **6** is more potent than 20-HE by at least an order of magnitude, and is in fact more active than any previously described ecdysone, except the synthetic analogue 14-desoxymuristerone A.[10] Procedures for the preparation of [^{125}I]26-iodoponasterone A of high specific acitivity from the mesylate **5** have been described previously.[4]

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