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Synthesis of 2-Dehydro-3-*epi*-20-hydroxyecdysone

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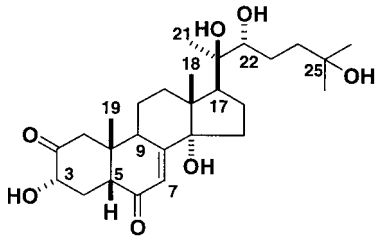
Abstract—2-Dehydro-3-*epi*-20-hydroxyecdysone, a novel ecdysteroid isolated from *Froelichia floridana*, has been partially synthesized from the readily available ecdysteroid, 20-hydroxyecdysone.

Introduction

2-Dehydro-3-*epi*-20-hydroxyecdysone (**1**) is a minor ecdysteroid isolated recently from the seeds of *Froelichia floridana* (Nutt.) Moq.[1] This compound is the first naturally occurring ecdysteroid with a keto function at the 2-position[2] and hence has attracted attention in view of biological activity. Since edysteroid **1** was obtained in only 0.15 mg from the plant material, it was therefore interesting to obtain this compound by synthesis. 20-Hydroxyecdysone (**2**) was chosen as the readily available starting material[3] in our synthesis.



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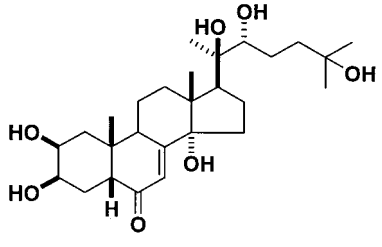

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Results and Discussion

Initially, our synthetic strategy was to selectively oxidise the 3β-hydroxyl group of compound **2** to the keto function, which would then be reduced to the corresponding 3α-hydroxyl group. Oxidation of the 2-hydroxyl group to the keto function would then give compound **1**. It has been reported that oxidation of **2** with $NaIO_4$,[4] Jones reagent,[5] CrO_3–pyridine[6] or pyridinium chlorochromate[7] afforded the same product, poststerone (**3**). Compound **3** together with its 3-dehydro analogue were obtained when **2** was subjected to Jones oxidation, but with some modification.[8] We found that reaction of **2** with other well-known oxidising agents also produced compound **3**.[9] It was therefore obvious that the 20,22-dihydroxyl system had to be protected at early stage of the synthesis. Thus, starting from compound **2**, the 20,22-acetonide **4** was prepared by the literature procedure.[10] In order to selectively oxidise the 3-hydroxyl group, the more active 2-hydroxyl group was protected as the acetate derivative **5**.[10] We then turned to the oxidising agent. It has been reported[11] that upon oxidation of **5** with Jones reagent, an inseparable 3:1 mixture of the corresponding ketone **6** and its isomeric ketone **7** were yielded. The latter product was formed from compound **8**, which in turn was derived from **5** by C-2→C-3 acetyl migration. Acetyl migration is known in ecdysteriod field, especially in acidic condition.[10,11] We therefore would like to consider using other oxidising agents. DMSO/Ac_2O has been employed in the oxidation of compound of type **5**.[12,13] However, in our hands reaction of compound **5** with this reagent gave at least seven spots on TLC, including the starting material. Eventually CrO_3–pyridine has been chosen by our group to oxidise **5** to the corresponding ketone **6**, together with the hemiketal **9** in 43 and 23% yields, respectively. The structure of the major oxidation product was identified to be **6** by spectroscopic (IR, ^1H NMR and mass spectral) data and by comparison of ^1H NMR data with those of the reported data.[12] The double doublet (J=11 and 7 Hz) signal of H-2 at δ 5.07 and the

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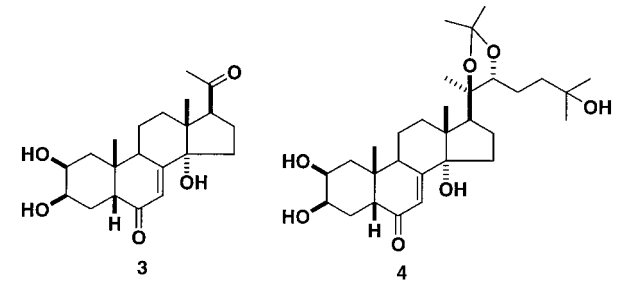
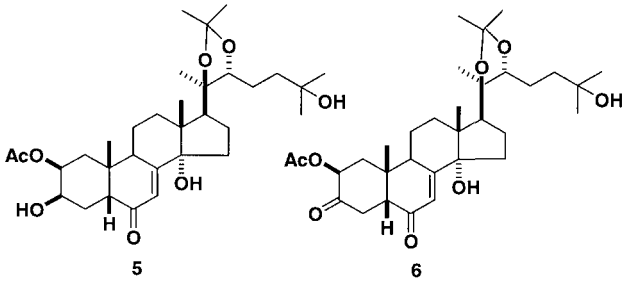
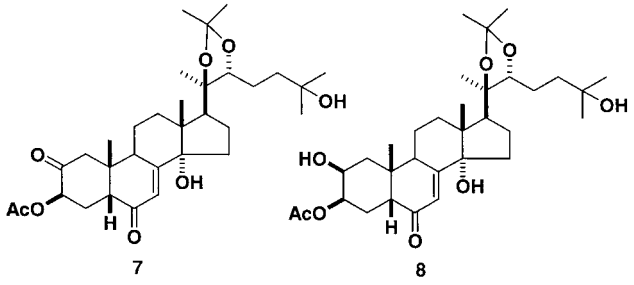
absence of H-3 signal agreed well with structure **6**.



3 **4**

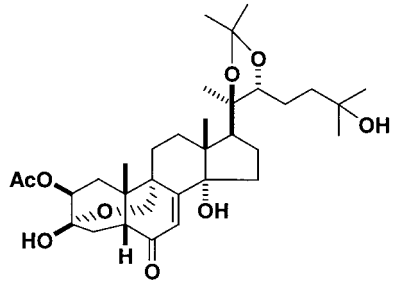


5 **6**



7 **8**

The structure of the minor product was concluded to be **9** on the basis of spectroscopic data, especially those of ^{1}H NMR and ^{13}C NMR data. The sharp singlet nature of H-7 at δ 5.91 and the absence of the H-9 signal in the ^{1}H NMR spectrum, together with the presence of the C-3 hemiketal signal at δ 96.6 in the ^{13}C NMR data agreed very well with structure **9**.



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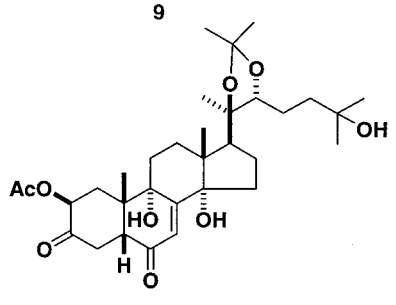

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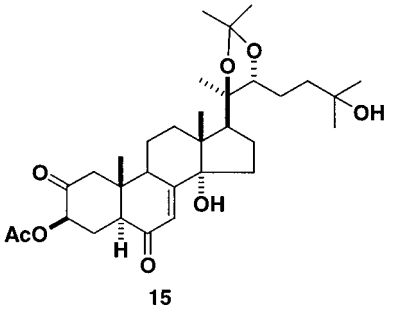
This compound was supposed to derive from **6** by allylic oxidation at the 9-position[9] to give **10**, followed by intramolecular nucleophilic attack of the 9-hydroxyl group at the 3-keto function to yield **9**. It should be noted that the quantity of the product **9** could be lowered by reducing reaction time and quantity of the oxidising agent, but the products were accompanied by the starting material.

The next step was to convert compound **6** to the required ecdysteroid **1**. It was known that α-keto alcohols and α-keto acetates could undergo keto-enol tautomerization. In our case, if compound **6** could enolize to the intermediate **11** and if C-2→C-3 acetyl migration could take place as that occurred in saturated system[10] to the isomeric intermediate **12**, the keto acetates **13** and/or **14** would be obtained (see Scheme 1). Investigations of molecular models of **13** and **14** indicated that the former, which has the required stereochemical arrangement at C-3, would be a preferred structure since the acetate group at the 3-position of the latter suffered from di-axial interaction. From these reasons, we then decided to choose synthetic strategy according to Scheme 1.

Compound **6** was subjected to acid-catalyzed isomerization, but preliminary results were discouraging. Dilute mineral acids caused decomposition of compound **6**. Dilute AcOH did not yield **13**; initial reaction was deacetonation of **6**. Dilute *p*-TsOH gave similar result and more concentrated *p*-TsOH caused dehydration of the starting material. However, when isomerization was performed in MeOH–$CHCl_3$ in the presence of silica gel, compound **6** slowly transformed to the isomeric keto acetate **13**. In practice, when approximately half of compound **6** was changed to compound **13**, the mixture was subjected to separation and isomerization of compound **6** was repeated. The structure of **13** was established on the basis of spectroscopic (IR, ^{1}H NMR and mass spectral) data. The saturated keto function was located at the 2-position as evident from the presence of two doublet signals of two H-1, with J=13.9 Hz, at δ 2.22 and 2.59. A large $W_{1/2}$ value (21 Hz) of H-3 resonance revealed its axial orientation. The alternative 3β-acetoxy-5α- configuration (i.e. compound **15**), which would result in a large $W_{1/2}$ value as well as that of compound **13**, was ruled



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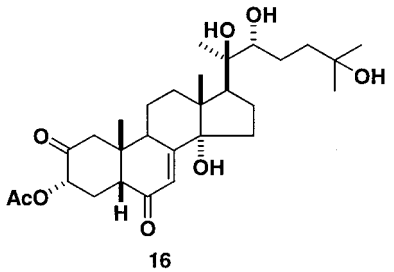

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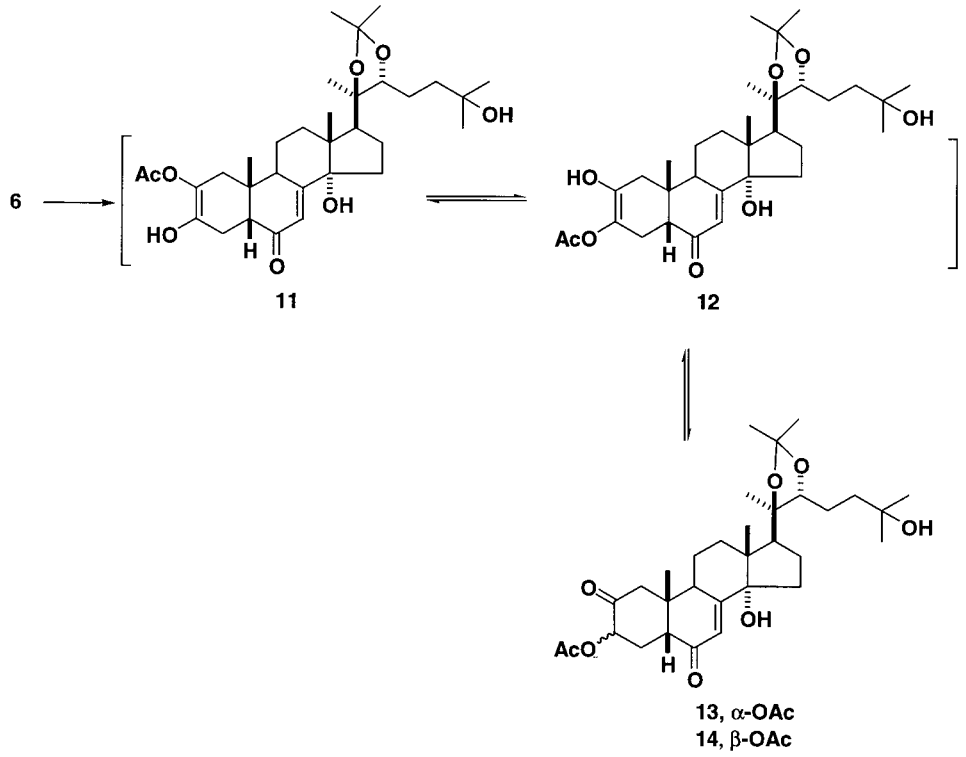

Scheme 1.

out from NOE experiment. Thus irradiation of the 19-Me signal at δ 1.03 resulted in enhancement of the H-5 signal at δ 2.58. Such NOE enhancement occurred only when ecdysteroid adopted a *cis*-A/B ring fusion.[14,15]

It should be noted that trace of a minor compound was noticed during purification of compound **6** by column chromatography. It was later shown that this compound was identical to compound **13** obtained from silica gel-catalyzed isomerization of compound **6** by TLC and ^{1}H NMR comparisons.

Compound **13** was subjected to acetonide deprotection using 70% AcOH to give the acetate **16**, which was subsequently deacetylated with 10% aq guanidine acetate to yield the target ecdysteroid **1** in 35% from compound **13**. The overall yield from compound **6** was 21%. ^{1}H NMR and ^{13}C NMR data of compound **1** were consistent with the reported[1] values; most of the chemical shift values of our synthetic compound **1** were about 0.01 to 0.03 ppm higher than those of the reported natural ecdysteroid **1**. It is noteworthy that care must be taken in the deacetylation step, especially when bases other than the guanidine class are employed, in order to avoid undesired side reactions including epimerization at the 5-position and decomposition of starting material or the product. Despite its relatively slow hydrolysis rate, base of this type has successfully been employed in ecdysteroid reactions.[15,16]

Experimental

Melting points were determined on an Electrothermal melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Jeol JNM-A500 spectrometer. Mass spectra were measured on a Bruker Esquire LC and a Finnigan MAT 90 instruments. Unless indicated otherwise, column chromatography and TLC were carried out using Merck's silica gel 60 (<0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were visualized under UV light and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating.

Chromium trioxide–pyridine oxidation of compound 5

Compound **5** (30 mg, 0.053 mmol) was dissolved in dry CH_2Cl_2 (1.5 mL) and a solution of CrO_3 (267 mg, 2.67 mmol) in dry pyridine (0.5 mL) and dry CH_2Cl_2 (5 mL) was added. The reaction mixture was kept stirring at ambient temperature for 3 min and the solvent evaporated at ambient temperature in vacuo. EtOAc (60 mL) was added and the precipitate filtered off. 10% $NaHSO_3$ solution was added to the stirred EtOAc solution until light greenish aqueous layer was apparent. The organic phase was washed with water (3×30 mL); the moisture was removed by anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The residue thus obtained was chromatographed, using $CHCl_3$–MeOH as eluent, to yield 3-dehydro-20-hydroxyecdysone 2-acetate 20,22-acetonide (**6**) (13 mg, 43%) and 20-hydroxyecdysone 2-acetate 20,22-acetonide 3α, 9α-epoxide (**9**) (7 mg, 23%). A small quantity (ca 0.5 mg) of the least polar component was also obtained in the synthesis.

6. Fine needles (from EtOAc-hexane), mp 235–237°C; ν_{max} 3440, 2970, 1731, 1678, 1452, 1372, 1324, 1244, 1170, 1105, 1042, 1000, 923, 756 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.76 (s, 18-Me), 1.07 (s, 19-Me), 1.11 (s, 21-Me), 1.22 (s,

26-Me), 1.23 (s, 27-Me), 1.30 and 1.39 (each s, acetonide Me), 2.12 (s, MeCOO), 2.28 (t, J=8.7 Hz, H-17), 2.73 (dd, J=12.5, 5.5 Hz, H-5), 3.64 (dd, J=9.4, 2.1 Hz, H-22), 5.07 (dd, J=11, 7 Hz, H-2), 5.91 (br s, H-7); HRMS (FAB (−ve): $[M-H]^-$, found 559.3271. $C_{32}H_{48}O_8$–H requires 559.3270.

9. Fine needles (from CH_2Cl_2–hexane), mp 236–238°C; [Found: C, 66.82; H, 8.33. $C_{32}H_{48}O_9$ requires C, 66.67; H, 8.33%]; ν_{max} 3468, 2972, 1738, 1678, 1445, 1372, 1323, 1243, 1169, 1105, 1042, 998, 922, 871 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.77 (s, 18-Me), 0.99 (s, 19-Me), 1.15 (s, 21-Me), 1.22 (s, 26-Me), 1.24 (s, 27-Me), 1.33 and 1.41 (each s, acetonide Me), 2.10 (s, MeCOO), 2.22 (t, J=9 Hz, H-17), 3.66 (br d, J=ca 8 Hz, H-22), 4.86 (br d, J=ca 9 Hz, H-2), 5.91 (s, H-7); δ_C (125.65 MHz, $CDCl_3$) 17.6 (C-18), 20.8 (acetate Me), 21.1 (C-11), 21.2 (C-19)[a], 21.9 (C-21)[a], 23.5 (C-16), 26.8 (acetonide Me), 27.9, 28.0, 28.2, 30.5 (C-4, C-12, C-15, C-23), 28.9 (acetonide Me)[b], 29.2 (C-26)[b], 29.3 (C-27)[b], 36.4 (C-10), 39.0 (C-1), 41.2 (C-24), 46.9 (C-13), 48.6 (C-17), 50.5 (C-5), 70.3 (C-25), 70.7 (C-2), 78.4 (C-20), 82.0 (C-22), 84.3 (C-14), 86.0 (C-9), 96.6 (C-3), 106.9 (acetonide C), 122.1 (C-7), 157.9 (C-8), 171.3 (acetate CO), 200.6 (C-6),[a,b] Assignments may be reversed for signals with the same superscript; m/z (ES, +ve) 599 $[M+Na]^+$; m/z (ES, −ve) 575 $[M-H]^-$.

Conversion of compound 6 to compound 13

To a solution of compound **6** (20 mg, 0.036 mmol) in MeOH–$CHCl_3$ (1:10, 4 mL) was added SiO_2 (Merck's silica gel 60, 0.063–0.200 mm, 500 mg) and the contents stirred for 10 h. The mixture was filtered, the solvents was evaporated and the residue chromatographed to yield compound **13** (9 mg) and the starting material **6** (7 mg). Compound **6** was subjected to another isomerization and column chromatography of the mixture gave compound **13** (2 mg) and compound **6** (2 mg). This give a total yield of compound **13** of 61% based on the unrecovered starting material.

13. Amorphous; ν_{max} 3478, 2972, 1727 (br), 1663, 1461, 1372, 1312, 1233, 1169, 1136, 1106, 1084, 1057, 1000, 870 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.72 (s, 18-Me), 1.03 (s, 19-Me), 1.08 (s, 21-Me), 1.16 (s, 26-Me), 1.17 (s, 27-Me), 1.25 and 1.34 (each s, acetonide Me), 2.08 (s, MeCOO), 2.22 (d, J=13.9 Hz, H-1α), 2.58 (dd, J=13.2, 4.1 Hz, H-5), 2.59 (d, J=13.9 Hz, H-1β), 2.72 (m, H-9), 3.58 (dd, J=9.4, 2.1 Hz, H-22), 5.18 (dd, J=12.5, 7 Hz, H-3, $W_{1/2}$=21 Hz), 5.82 (d, J=2.4 Hz, H-7); HRMS (FAB, −ve): $[M-H]^-$, found 559.3269. $C_{32}H_{48}O_8$–H requires 559.3270.

Acetonide deprotection of compound 13

To a solution of compound **13** (55 mg, 0.098 mmol) in EtOH (0.5 mL) was added 70% AcOH (3 mL) and the mixture stirred at 45°C for 5 h. Water (100 mL) was added and the mixture extracted with EtOAc (3×50 mL). The combined EtOAc extract was washed with water, dried and evaporated to dryness to give 46 mg of compound **16**, part of which was used directly in subsequent reaction.

Deacetylation of compound 16

A solution of compound **16** (25 mg, 0.048 mmol) in EtOH (1 mL) and 10% aq guanidine acetate (1 ml, 0.840 mmol) was stirred at 50°C for 2 days. Water (50 mL) was added and the mixture extracted with n-BuOH (3×40 mL). The combined organic phase was washed with water and evaporated by co-distillation with water under reduced pressure. The residue was subjected to reversed-phase column chromatography (Merck's silica gel 60 RP-18 (40–63 μm) for preparative HPLC) using H_2O–MeOH as eluting solvent and fractions eluted by H_2O–MeOH (7:3) gave pure compound **1** (9 mg, 35% overall from the acetate acetonide **13**); Amorphous; ν_{max} 3400, 2964, 2830, 1718, 1662, 1552, 1453, 1383, 1315, 1208, 1072 cm^{-1}; δ_H (500 MHz, C_5D_5N): 1.07 (s, 19-Me), 1.16 (s, 18-Me), 1.38 (s, 26-Me and 27-Me), 1.53 (s, 21-Me), 2.38 (d, J=13.7 Hz, H-1α), 2.73 (d, J=13.7 Hz, H-1β), 2.86 (dd, J=13.2, 4.1 Hz, H-5), 2.92 (t, J=9 Hz, H-17), 3.27 (m, H-9), 3.84 (br d, J=9.6 Hz, H-22), 4.58 (dd, J=11.9, 7 Hz, H-3, $W_{1/2}$ 21 Hz), 6.20 (d, J=2.1 Hz, H-7); δ_C (125.65 MHz, C_5D_5N) 17.7 (C-18), 20.8 (C-11), 21.3 (C-16), 21.5 (C-21), 22.9 (C-19), 27.4 (C-23), 29.8 (C-26), 30.1 (C-27), 31.4 (C-15), 31.7 (C-12), 36.1 (C-4 and C-9), 42.5 (C-24), 42.9 (C-10), 48.0 (C-13), 49.1 (C-1), 49.9 (C-17), 55.7 (C-5), 69.5 (C-25), 74.8 (C-3), 76.7 (C-20), 77.4 (C-22), 83.9 (C-14), 121.1 (C-7), 165.1 (C-8), 200.3 (C-6), 209.9 (C-2); m/z (ES, +ve) 501 $[M+Na]^+$; HRMS (FAB (−ve): $[M-H]^-$, found 447.2855. $C_{27}H_{42}O_7$–H requires 477.2852.

Acknowledgements

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