25-Deoxyecdysteroids: Synthesis and Moulting Hormone Activity of Two C-25 Epimers of Inokosterone

Boon-ek Yingyongnarongkul and Apichart Suksamrarn*
Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand.
* Corresponding author.

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ABSTRACT A convenient synthesis of inokosterone, pterosterone and 24-*epi*-pterosterone has been accomplished. The former ecdysteroid exists as two C-25 epimers and could be separated from each other through their diacetonide derivatives. Each of the C-25 epimers has been subjected to moulting hormone activity testing using the *Musca* bioassay and it was found that the less polar isomer, inokosterone "epimer 1", was approximately two-fold more active than the more polar component, inokosterone "epimer 2"

KEYWORDS: ecdysteroid, inokosterone, synthesis, C-25 epimers, moulting hormone activity.

INTRODUCTION

Ecdysteroids are polyhydroxysteroids with a *cis*-fused A/B ring junction and 14α-hydroxy-7-en-6-one system. These compounds exhibit physiological activities in insects and have been found in both invertebrates and plant species.[1-3] The established function of ecdysteroids in insects is regulation of moulting. Other hormonal functions include regeneration, metamorphosis, reproduction and differentiation.[1,2] 20-Hydroxyecdysone (**1**) is the representative of this class of compounds.[3] Essential features contributing to moulting hormone activity include an A/B *cis*-ring junction, a 6-keto-7-ene function, a free 14α-hydroxyl group and a full sterol side chain.[4] It has also been found that unsubstituted 9-position is additional essential feature for moulting hormone activity.[5] Number, location and stereochemistry of hydroxyl groups in the molecule are also responsible for high activity of ecdysteroids.

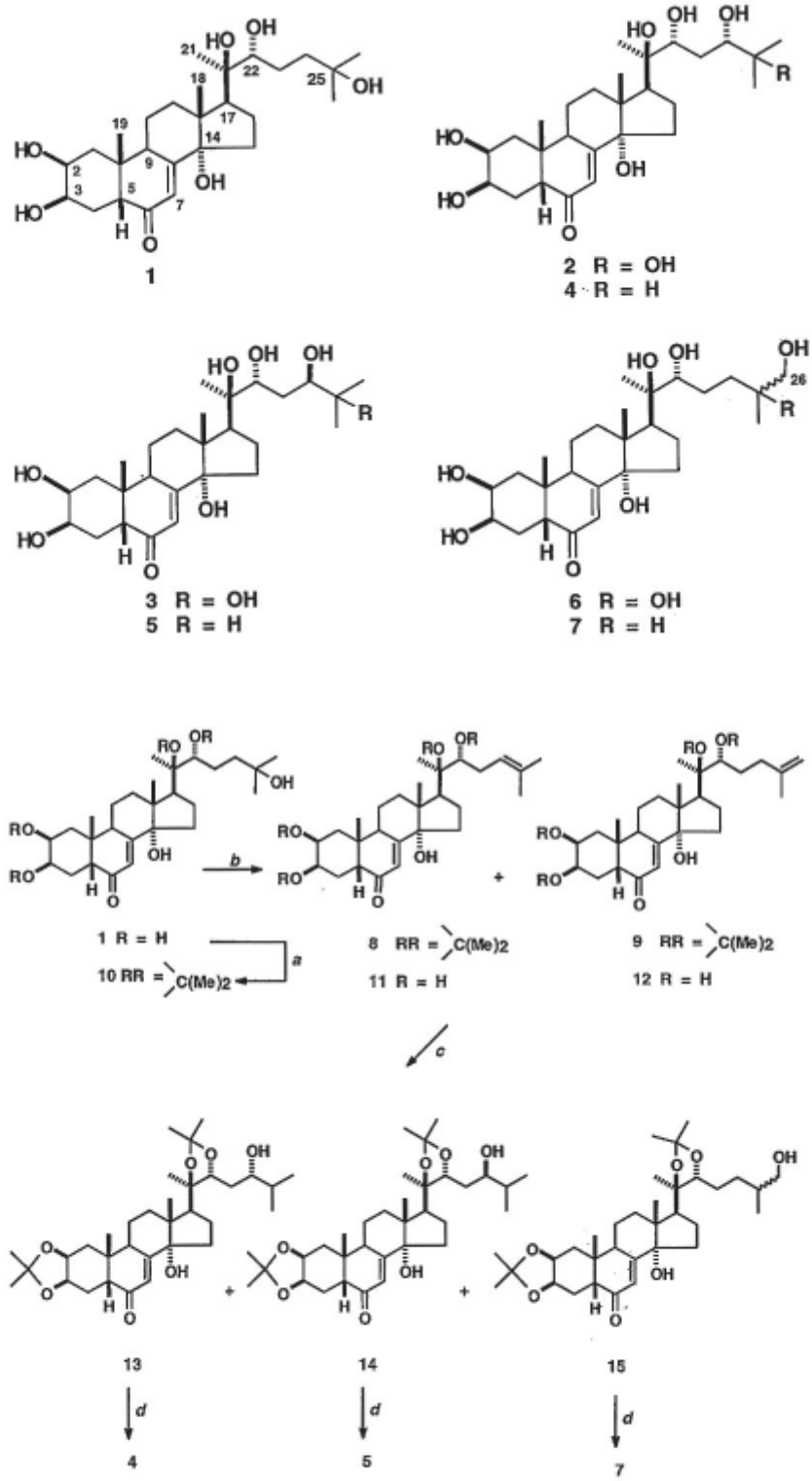
In our studies on structure-activity relationships of ecdysteroids, we have recently demonstrated that while abutasterone (**2**) and its C-24 epimer, 24-*epi*-abutasterone (**3**), exhibited comparable activity, they were less active than compound **1**.[6] On the other hand, pterosterone (**4**), the C-25 deoxy analogue of **2**, was more active than compound **3**, while 24-*epi*-pterosterone (**5**) was much less active than its C-24 epimer, *ie* compound **4**.[7] Also, in our recent study, we discovered that 20,26-dihydroxyecdysone (**6**) isolated from a number of *Vitex* plants existed as two C-25 epimers and the two compounds could be separated from each other by HPLC. The shorter retention time component in reversed-phase HPLC was approximately two-fold less active than its epimer.[8] It was therefore logical to study moulting hormone activity of the C-25 deoxy analogue of **6**, *ie* inokosterone (**7**). Compound **7** was a minor ecdysteroid isolated from the plant *Achyranthes fauriei*.[9] This ecdysteroid existed as a 1:2 mixture of two C-25 epimers, but separation of these epimers has not been achieved.[10]

In this paper we report on a convenient, partial synthesis and separation of two C-25 epimers of inokosterone (**7**). Biological evaluation of these two epimeric ecdysteroids has also been undertaken. The ecdysteroids **4** and **5** have also been synthesized in this study.

RESULTS AND DISCUSSION

Synthesis

The key intermediate for the synthesis of the ecdysteroids **4** and **5** was the olefin **8** and that for the ecdysteroid **7** was the olefin **9**. It has been reported that the two olefins, previously synthesized from the diacetonide **10**, could not be separated from each other by column chromatography. Separation of the two compounds was effected only through their deacetonide products **11** and **12**, which could be partially separated after careful column chromatography.[6] We therefore considered using the olefin diacetonide mixture **8** and **9** in our synthesis. Hydroboration reaction was chosen as the key step



Scheme. *Reagents and Conditions*: *a*, MeCOMe/TsOH; *b*, reaction from **10**: MsCl/pyridine/DMAP; *c*, (i) BH_3·THF, (ii) H_2O_2/NaOH; *d*, 70% AcOH.

to generate a hydroxyl function at either 24- or 26-position to yield the required compounds. Compound **4** has recently been isolated as a minor ecdysteroid from the bark of *Vitex glabrata* and compound **5** was available for biological evaluation by regioselective synthesis.[7] It was found that the two ecdysteroids were well separated on TLC. Thus, it was envisaged that the two compounds, if present as a mixture, could be separated from each other without any difficulty.

Starting from the readily available ecdysteroid **1** isolated from *V. glabrata*,[11] a 3:2 mixture of the olefin diacetonides **8** and **9** was obtained from the diacetonide **10**[12] (see scheme). Compounds **8** and **9** mixture was subjected to hydroboration using diborane-THF complex, followed by treatment with alkaline hydrogen peroxide to afford, after careful column chromatography, pterosterone 2,3:20,22-diacetonide (**13**), 24-*epi*-pterosterone 2,3:20:22-diacetonide (**14**) and two C-25 epimeric inokosterone diacetonide (**15**), *ie* inokosterone 2,3:20,22-diacetonide "epimer 1" and inokosterone 2,3:20,22-diacetonide "epimer 2", in 21, 22, 20 and 20%, respectively. ^{1}H NMR data (table 1) were in agreement with the structures. Deacetonation using aqueous acetic acid gave pterosterone (**4**), 24-*epi*-pterosterone (**5**), and each of C-25 epimeric inokosterone (**7**). The ^{1}H NMR (table 2) and other spectroscopic data were consistent with the reported values[7] and agreed well with structures. It should be noted that this is the first report of separation of inokosterone (**7**) into two C-25 epimers. Both epimers (*ie* compounds **7** "epimer 1" and **7** "epimer 2") exhibited almost identical ^{1}H NMR spectral data (see table 2). The corresponding diacetonides (*ie* compounds **15** "epimer 1" and **15** "epimer 2") also showed very similar ^{1}H NMR spectral data (see table 1). The ^{1}H NMR signals (table 2) at δ 3.65 (*dd, J* = 10.3 and 6.4 Hz) and 3.77 (*dd, J* = 10.3 and 5.5 Hz) of the "epimer 1" and at δ 3.64 (*dd, J* = 10.3 and 6.1 Hz) and 3.72 (*dd, J* = 10.3 and 5.8 Hz) of the "epimer 2" are characteristic signals of the methylene protons at the 26-position of 25-deoxy-26-hydroxyecdysteroid. The existing data, however, did not permit the assignment of the absolute configuration at C-25 of each of the epimers of compound **7**.

Moulting hormone activity

Inokosterone (**7**) "epimer 1" exhibited approximately two-fold higher moulting hormone activity than its C-25 epimer, inokosterone (**7**) "epimer 2", in the *Musca* bioassay. The finding suggested that stereochemical arrangement at the 25-position of compound **7** effected the binding of ecdysteroid to insect receptor. It is noteworthy that it will be beneficial if the stereochemistry of each of the two isomers has been solved, in order to have a better understanding of the receptor-ligand binding.

Experimental

IR spectra were recorded in KBr on a Jasco IR-700 spectrophotometer. ^{1}H NMR spectra were recorded on a Jeol JNM-A500 spectrometer. Mass spectra were measured on a Finnigan MAT 90 instrument. Column chromatography and TLC were carried out using Merck's silica gel 60 (>230 mesh) 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.

20-Hydroxyecdysone 2,3:20,22-diacetonide (10)

Compound **10** was prepared from compound **1** by the literature procedure[12] with modification. Thus, a mixture of compound **1** (160 mg) and *p*-TsOH (25 mg) in dry acetone (1 ml) was stirred at ambient temperature for 3 hrs. Water was added and the solution extracted with $CHCl_3$ (3x30 ml). The organic phase was washed with water, dried over anhydrous sodium sulphate, evaporated to dryness and chromatographed ($CHCl_3$-MeOH, 99:1) to afford compound **10** (146 mg, 78 %), mp 232-234°C from acetone-hexane (lit.[12] 233-234 °C). Spectroscopic (IR, ^{1}H NMR) data of this compound were identical to the reported values.[12]

Preparation of the olefin diacetonides 8 and 9.

The diacetonide **10** (1.4388 g) was dissolved in pyridine (10 ml) and the solution was kept at about 5 °C for 5 min. Mesyl chloride (2 ml) was added and the reaction mixture stirred for 20 min. 4-Dimethylaminopyridine (DMAP) (50 mg) was added and stirring continued at 5°C for 20 min., then slowly allowed to warm up to ambient temperature, during which time the progress of the reaction was monitored by TLC. 1% Aqueous $NaHCO_3$ solution was added and the mixture extracted with $CHCl_3$ (3x40 ml). The combined $CHCl_3$ layer was washed with water, dried and evaporated to dryness. The crude mixture was chromatographed using $CHCl_3$-MeOH (99:1) to afford a 3:2 mixture of compounds **8** and **9** (925 mg, 67 %). IR ν_{max} 3470, 2975, 1658, 1445, 1373, 1243, 1215, 1057, 884 cm^{-1}; ^{1}H NMR data were consistent with those reported previously;[6] FAB MS (-ve) *m/z* (% relative intensity) 541 [M-H]$^-$

Table 1. ^{1}H NMR data of compounds **13**, **14**, **15** "epimer 1" and **15** "epimer 2".

H	**13**	**14**	**15** "epimer 1"	**15** "epimer 2"
2	4.20 (*m*)	4.20 (*m*)	4.20 (*m*)	4.20 (*m*)
3	4.25 (*br s*)	4.25 (*br s*)	4.25 (*br s*)	4.25 (*br s*)
5	2.34 (*dd*, 12.6, 4.7)	2.33 (*dd*, 12.5, 4.8)	2.33 (*dd*, 12.5, 4.5)	2.33 (*dd*, 12.6, 4.7)
7	5.81 (*d*, 2.1)	5.80 (*d*, 2.4)	5.80 (*d*, 2.1)	5.80 (*d*, 2.1)
9	2.78 (*m*)	2.78 (*m*)	2.78 (*m*)	2.78 (*m*)
17	2.17 (*dd*, 8.5, 7.9)	2.18 (*dd*, 9.4, 7.9)	2.19 (*dd*, 9.7, 9.4)	2.18 (*dd*, 9.4, 8.5)
22	3.80 (*dd*, 9.7, 2.7)	3.96 (*dd*, 10.3, 1.5)	3.61 (*dd*, 6.7, 5.8)	3.62 (*br d*, 9.1)
24	3.53 (*m*)	3.54 (*m*)		
26	-	-	3.45 (*dd*, 10.6, 5.8); 3.48 (*dd*, 10.6, 5.7)	3.44 (*dd*, 10.3, 6.1); 3.48 (*dd*, 10.3, 6.4)
18-Me	0.77 (*s*)	0.77 (*s*)	0.769 (*s*)	0.767 (*s*)
19-Me	0.96 (*s*)	0.96 (*s*)	0.960 (*s*)	0.959 (*s*)
21-Me	1.14 (*s*)	1.12 (*s*)	1.119 (*s*)	1.115 (*s*)
26-Me	0.93 (*d*, 6.7)	0.91 (*d*, 6.7)	-	-
27-Me	0.93 (*d*, 6.7)	0.93 (*d*, 6.7)	0.924 (*d*, 6.7)	0.912 (*d*, 6.7)
>C(Me)$_2$	1.31, 1.32, 1.39, 1.47 (each *s*)	1.30, 1.31, 1.38, 1.46) (each *s*)	1.295, 1.307, 1.381, 1.468) (each *s*)	1.297, 1.307, 1.384, 1.468) (each *s*)

Recorded in $CDCl_3$.

Table 2. ^{1}H NMR data of compounds **4**, **5**, **7** "epimer 1" and **7** "epimer 2".

H	**4**	**5**	**7** "epimer 1"	**7** "epimer 2"
2	4.16 (*m*)	4.16 (*m*)	4.17 (*m*)	4.17 (*m*)
3	4.22 (*br s*)	4.22 (*br s*)	4.23 (*m*)	4.23 (*m*)
5	3.00 (*dd*, 13.1, 3.6)	2.97 (*dd*, *ca* 13, 3.6)	3.01 (*dd*, 13.2, 3.8)	3.01 (*dd*, 13.2, 3.8)
7	6.25 (*d*, 2.1)	6.22 (*d*, 2.4)	6.25 (*d*, 2.4)	6.25 (*d*, 2.4)
9	3.58 (*m*)	3.58 (*m*)	3.59 (*m*)	3.59 (*m*)
17	2.92 (*t*, 9.1)	3.02 (*t*, 9.1)	2.95 (*t*, 9.1)	2.94 (*t*, 9.1)
22	4.12 (*br d*, 10)	4.10 (*m*)	3.85 (*br d*, *ca* 9)	3.86 (*br d*, 9.7)
24	3.94 (*m*)	4.47 (*br d*, 9.7)		
26	-	-	3.65 (*dd*, 10.3, 6.4); 3.77 (*dd*, 10.3, 5.5)	3.64 (*dd*, 10.3, 6.1); 3.72 (*dd*, 10.3, 5.8)
18-Me	1.20 (*s*)	1.21 (*s*)	1.215 (*s*)	1.217 (*s*)
19-Me	1.06 (*s*)	1.05 (*s*)	1.064 (*s*)	1.067 (*s*)
21-Me	1.58 (*s*)	1.62 (*s*)	1.563 (*s*)	1.577 (*s*)
26-Me	1.01 (*d*, 6.7)	0.98 (*d*, 6.7)	-	-
27-Me	1.01 (*d*, 6.7)	1.08 (*d*, 6.7)	1.036 (*d*, 6.7)	1.040 (*d*, 6.1)

Recorded in pyridine-d_5.

(20), 501(29), 485(10), 339(17), 325 (30), 183(100).

Hydroboration of the olefin diacetonides 8 and 9.

To a solution of compounds **8** and **9** mixture (ratio 3:2) (74 mg) in THF (2 ml) was added portionwise, with stirring, BH_3·THF complex (*ca* 1M in THF, 0.15 ml) during a period of 2 hrs. Hydrogen peroxide solution (35 %, 0.1 ml) and 1N NaOH (0.15 ml) was then added and stirring continued for 5 min. Water (100 ml) was added and the mixture extracted with EtOAc (3x30 ml). The solvent was evaporated and the residue carefully chromatographed, with $CHCl_3$-MeOH as eluting solvent, to yield compound **13** (16 mg, 21%). Compounds **14**, **15** "epimer 1" and **15** "epimer 2" were respectively eluted but found to be contaminated by each other. Repeated column chromatography yielded pure compounds **14** (17 mg, 22%), **15** "epimer 1" (15 mg, 20%) and **15** "epimer 2" (15 mg, 20%).

Compound **13**: IR ν_{max} 3535, 3458, 2960, 1642, 1373, 1245, 1225, 1170, 1060, 881 cm^{-1}; ^1H NMR data are given in table 1; HR FABMS (-ve) *m/z* 559.3639 $[M-H]^-$. $C_{33}H_{52}O_7$-H requires 559.3634.

Compound **14**: IR ν_{max} 3468, 2961, 2870, 1660, 1466, 1371, 1244, 1217, 1169, 1058, 878 cm^{-1}; ^1H NMR data are given in table 1; HR FABMS (-ve) *m/z* 559.3634 $[M-H]^-$. $C_{33}H_{52}O_7$-H requires 559.3634.

Compound **15** "**epimer 1**": IR ν_{max} 3451, 2938, 1658, 1647, 1371, 1244, 1217, 1170, 1108, 1059, 883 cm^{-1}; ^1H NMR data are given in table 1; HR FABMS (-ve) *m/z* 559.3632 $[M-H]^-$. $C_{33}H_{52}O_7$-H requires 559.3634.

Compound **15** "**epimer 2**": IR ν_{max} 3436, 2937, 2875, 1658, 1451, 1371, 1244, 1217, 1170, 1108, 1058, 883 cm^{-1}; ^1H NMR data are given in table 1; HR FABMS (-ve) *m/z* 559.3632 $[M-H]^-$. $C_{33}H_{52}O_7$-H requires 559.3634.

Acetonide deprotection of compounds 13, 14 and 15

To a solution of compound **13** (18 mg) in dioxane (0.1 ml) was added 70% aqueous AcOH (1 ml) and the mixture was stirred for 3 days. Water (50 ml) was added; the mixture was thoroughly extracted with *n*-butanol until no product was detected in the aqueous phase and the solvent was evaporated by co-distillation with water. Column chromatography yielded pterosterone (**4**) (11 mg, 71%). IR ν_{max} 3368, 2958, 1641, 1444, 1384, 1306, 1072, 877 cm^{-1}; ^1H NMR data are given in table 2 and are consistent with the reported values.[7]

Compound **14** (19 mg) was subjected to deacetonation in the same manner described for compound **13** to afford 24-*epi*-pterosterone (**5**) (12 mg, 74%). IR ν_{max} 3390, 2965, 2870, 1658, 1465, 1439, 1385, 1333, 1108, 1054, 1012, 875 cm^{-1}; ^1H NMR data (table 2) are consistent with the reported values.[7]

Similarly, compounds **7** "epimer 1" and **7** "epimer 2" were obtained respectively from compounds **15** "epimer 1" and **15** "epimer 2" in 75 and 77% yields.

Compound **7** "**epimer 1**": IR ν_{max} 3386, 2937, 1640, 1383, 1049, 872 cm^{-1}; ^1H NMR data are given in table 2; HR FABMS (-ve) *m/z* 479.3004 $[M-H]^-$. $C_{27}H_{44}O_7$-H requires 479.3008.

Compound **7** "**epimer 2**": IR ν_{max} 3387, 2943, 1641, 1384, 1052 cm^{-1}; ^1H NMR data are given in table 2; HR FABMS *m/z* 479.3001 $[M-H]^-$. $C_{27}H_{44}O_7$-H requires 479.3008.

Biological activity testing

Mature *Musca domestica* larvae were ligated on the seventh or eighth segment and those had formed puparia at their anterior ends, whereas the abdomonal compartments remained larval stage, were used in the established *Musca* bioassay.[13] A 1 mg portion of ecdysteroid was dissolved in 48 µl of absolute ethanol and the solution was subsequently diluted with distilled water to a volume of 300 µl. The resulting ecdysteroid solution, the initial concentration of which was 10 µg/3 µl, was diluted with distilled water to appropriate concentrations, *ie*, 0.50, 0.25, 0.175, 0.10, 0.05 and 0.025 µg/3 µl. Ten ligated larvae were used in each ecdysteroid concentration. The results were scored according to Ohtaki *et al.*[14] The EC_{50} for inokosterone (**7**) "epimer 1" and inokosterone (**7**) "epimer 2" were $3.39x10^{-5}$ and $7.37x10^{-5}$ M, respectively.

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