Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 1093–1099

Stereoselective synthesis and moulting activity of integristerone A and analogues

Saowanee Kumpun,[a] Boon-ek Yingyongnarongkul,[a] René Lafont,[b] Jean-Pierre Girault[c] and Apichart Suksamrarn[a,*]

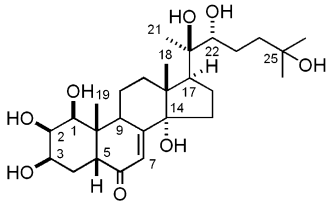
[a]*Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand*
[b]*Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Université Pierre et Marie Curie, CNRS FRE 2852, 7 Quai St. Bernard, 75252 Paris 05, France*
[c]*Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris V-René Descartes, CNRS UMR 8601, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France*

Received 19 September 2006; revised 8 November 2006; accepted 23 November 2006

Abstract—Integristerone A, a rare ecdysteroid of plant origin, has been synthesized from 20-hydroxyecdysone with 2-deoxy-1,2-didehydro-20-hydroxyecdysone as the key intermediate, followed by stereoselective asymmetric dihydroxylation. The analogues 1,2-di-*epi*-integristerone A and 1,2-di-*epi*-5α-integristerone A have also been synthesized. Integristerone A exhibited approximately 9-fold lower moulting activity than the parent 20-hydroxyecdysone in the *Musca domestica* bioassay, indicating that the presence of a 1β-hydroxyl group resulted in a decrease in activity. As expected, the 1,2-di-*epi*-5α-analogue was inactive in this assay.

1. Introduction

Integristerone A (**1**) is a member of the 1,2,3-trihydroxysteroids, a rare and relatively small group of ecdysteroids, the arthropod moulting hormones.[1] This compound was first isolated from the plant *Rhaponticum integrifolium*.[2] No synthesis of 1,2,3-trihydroxy ecdysteroids has been reported to date. This paper deals with a concise, stereoselective synthesis of compound **1** and its analogues from 20-hydroxyecdysone (**2**).



1

2. Results and discussion

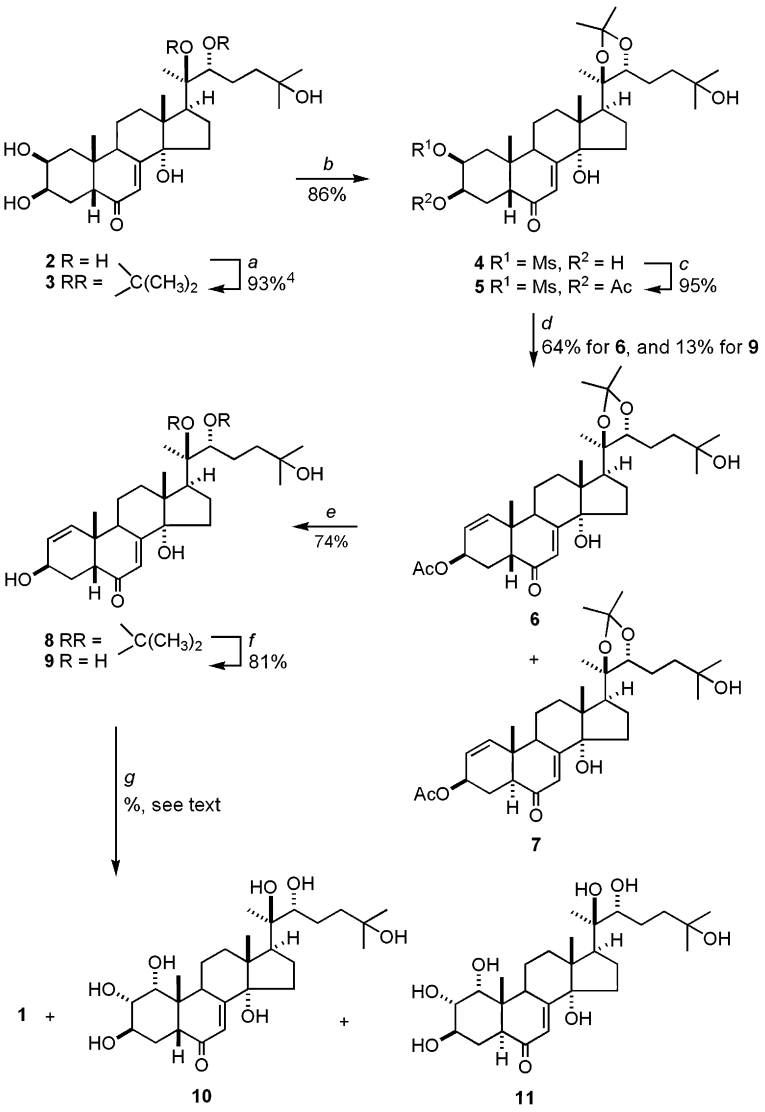
2.1. Synthesis of integristerone A (1) and analogues

The synthesis of compound **1** is outlined in Scheme 1. The readily available starting material 20-hydroxyecdysone (**2**)[3] was subjected to selective 20,22-acetonidation to the corresponding 20,22-acetonide **3** using acetone and *p*-TsOH.[4] Selective mesylation by treatment of a pyridine solution of **3** with MsCl afforded the mesylate **4** in 86% yield, which was acetylated to the corresponding acetate **5** in 95% yield. Elimination of MsOH from **5** to yield the olefin **6** using a number of bases was attempted, but unsatisfactory results were obtained. It was not surprising that elimination of MsOH was not a facile step, since a molecular model indicated that the C-1 hydrogen and the C-2 MsO group could assume an antiperiplanar conformation only when the A-ring adopted the less preferred boat conformation. However, we eventually found that DBU effected the required elimination reaction of **5** in DMF solution at 150 °C to give the required olefin **6** in 64% yield together with the minor C-5 epimer **7** in 13% yield. The absence of the MsO signal and the presence of H-1 (d, J=10 Hz) and H-2 (dd, J=10, 4.4 Hz) signals at δ 5.95 and 5.73 in the ^{1}H NMR spectrum of **6** established its structure. The stereochemistry at C-3 was preserved as evident from the small $W_{1/2}$ value (10 Hz) of H-3. Other spectroscopic (IR and MS) spectra were consistent with structure **6**. Compound **6** was deacetylated with 10% K_2CO_3 in MeOH to yield compound **8** in 74% yield, which was subsequently subjected to acetonide deprotection using 70% aq AcOH in the presence of the phase-transfer catalyst, benzyltrimethylammonium chloride, to give compound **9** in 81% yield.

The less polar olefin **7**, the C-5 epimer of **6**, was obtained as the minor component. This implied that C-5 epimerization had taken place at some stage in the reaction leading to

Keywords: Ecdysteroid; Integristerone A; Synthesis; Moulting activity.
* Corresponding author. Tel.: +662 3190931; fax: +662 3191900; e-mail: apichart@ram1.ru.ac.th

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.11.063



Scheme 1. Syntheses of the ecdysteroids **1** and **10**. Reagents and conditions: (a) CH_3COCH_3, *p*-TsOH; (b) MsCl, pyridine, 0–4 °C to ambient temp; (c) Ac_2O, pyridine; (d) DBU, DMF, 150 °C; (e) 10% aq K_2CO_3, MeOH; (f) 70% AcOH, $PhCH_2N^+Me_3Cl^-$; and (g) OsO_4, ligand, solvent (see text).

product **7**. Epimerization at this position is known in ecdysteroid field.[5,6] Under the basic condition employed, H-5β epimerized to H-5α. The ^1H and ^{13}C NMR data were consistent with structure **7**, especially the relatively large $W_{1/2}$ value (20 Hz) of H-3 at δ 5.36 and the splitting pattern of H-2 and H-1 at δ 5.54 and 5.88, respectively. NOE experiments were employed. Thus, irradiation at H-5's frequency resulted in NOE enhancement of the H-9 and H-3 signals, and irradiation at the H-9 frequency caused NOE enhancement of the H-5 signal. Irradiation at the H-3 frequency resulted in NOE enhancement of the H-5 and H-4eq signals. The NOE experiments thus confirmed the α-orientation of H-5.

The next step was to perform dihydroxylation of the olefin **9**. Since the A/B-ring junction of **9** is cis, it was therefore expected that dihydroxyaltion using OsO_4 should result in the required 1,2-di-β-hydroxyl analogue, i.e. integristerone A (**1**), as a major, if not the sole, product. Thus, treatment of **9** in pyridine with a pyridine solution of OsO_4 followed by addition of 5% $NaHSO_3$ solution afforded, after column chromatography and HPLC separations, compound **1** (56%) and minor products **10** (27%) and **11** (7%) (Scheme 1). The ^1H NMR spectral data of **1** were consistent with the reported values.[2]

Dihydroxylation using OsO_4 would give two diastereomers, thus either compound **10** or **11** could possibly be the 1,2-diepimer of **1**. The ^1H NMR spectra of compound **10** were unusual since two broad proton signals and one missing signal were observed. Thus, high temperature ^1H NMR was performed and the results are shown in Figure 1. The proton signal at 3.19 ppm was observed at 353 K and was concluded to be H-9 according to 2D NMR techniques (HSQC and HMBC). The broadening of these signals are the consequence of a slow conformational exchange of ring-A, on the NMR chemical shift time scale at 300 K and 500 MHz moving to intermediate time scale at higher temperature (353 K). It should be noted that heating of **10** resulted in slow C-5 epimerization to **11**. From the molecular model (Fig. 2), H-9 would be shifted downfield due to the close proximity to the 2α-hydroxyl group, which has been observed previously.[7] Two possibilities to reduce the steric

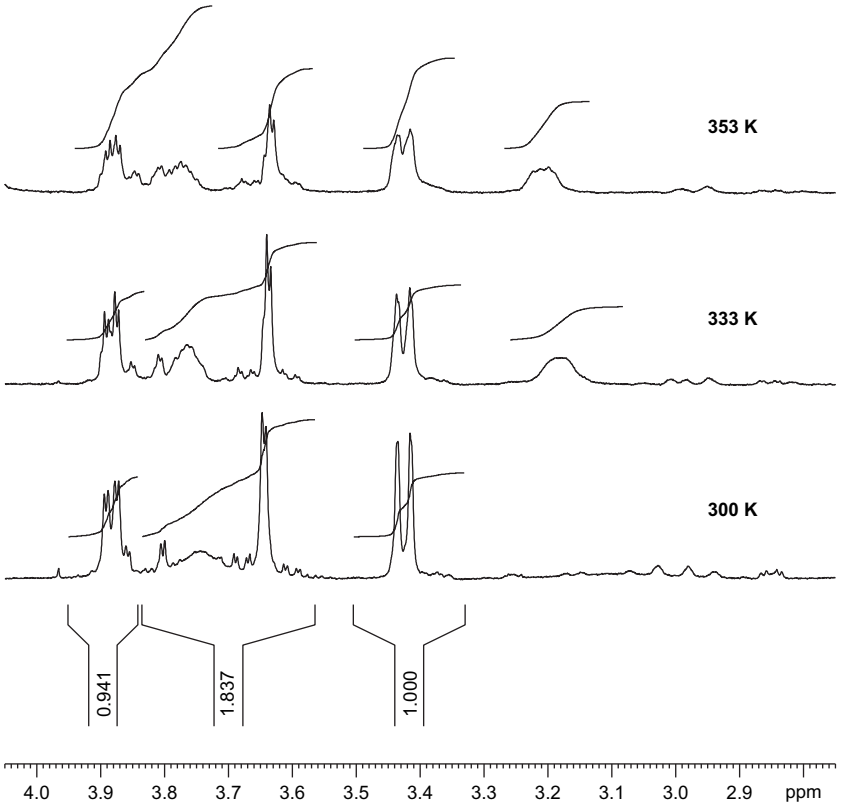


Figure 1. Partial ^{1}H NMR spectrum of compound **10** under various temperatures.

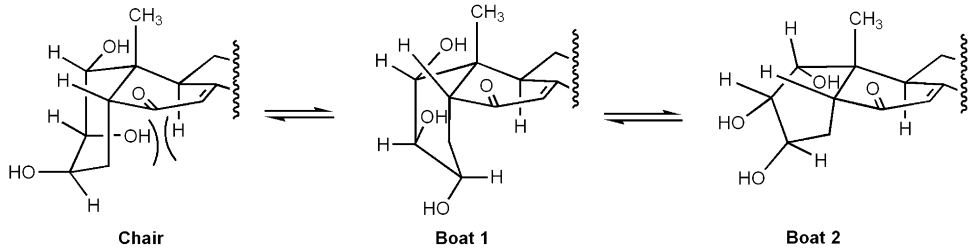


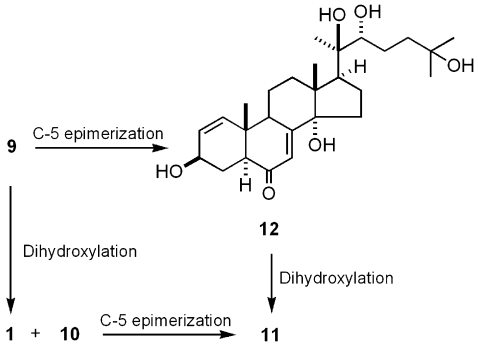
Figure 2. Ring-A geometry of compound **10**.

interaction between H-9 and 2-OH were C-5 epimerization or boat conformation of ring-A. 2D NOESY experiment was thus used to confirm the A/B-ring junction. The H-5 signal presents NOESY correlations with H-1 and 19-Me, and the 19-Me signal presents NOESY correlations with H-1 and H-5. The 2D NOESY experiments thus confirmed the β-orientation of H-5. The more likely possibility was a boat (or half-chair) conformation of ring-A. The boat conformation (either conformation 1 or 2) had less steric interaction when the cis-A/B ring fusion is formed (Fig. 2). The evidence for a boat conformation of ring-A was the large $W_{1/2}$ value of H-3 (37 Hz), which implies an axial position. Compound **10** was thus confirmed to be 1,2-di-*epi*-integristerone A.

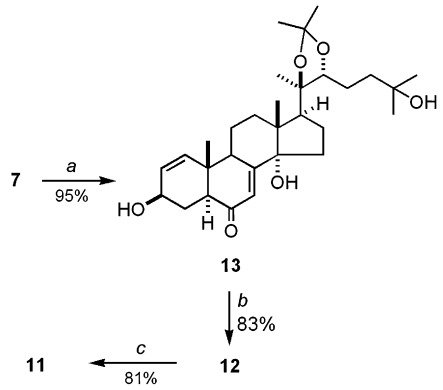
The ^{1}H NMR spectroscopic features of compound **11** were also found to be much different from those of **1**. The striking difference was the unusual downfield shift of H-9 signal (in C_5D_5N) at δ 4.30, whereas that of **1** appeared at δ 3.54. A molecular model of **11** indicated the close proximity of H-9 and the 1α-hydroxyl group and this resulted in a large downfield shift of the H-9 signal. 2D NMR techniques were employed to assign the proton signals, which in turn led to the confirmation that the minor product was **11**. Thus, the H-3 signal of **11** appeared as a multiplet signal at δ 4.49 ppm, with the $W_{1/2}$ value of 22 Hz, indicating the axial nature of H-3. The relatively large $W_{1/2}$ of H-3 suggested the A/B-ring junction in **11** to be trans.[7,8] NOE experiments also confirmed the trans-A/B ring fusion of **11**. Thus, irradiation at the H-5 frequency of **11** resulted in enhancement of H-9 and H-3 signals. Irradiation at the H-9 frequency caused NOE enhancement of the H-5 signal, and irradiation at the 19-Me frequency resulted in NOE enhancement of the H-1eq and H-2ax signals, not the H-5 signal. NOE experiments thus confirmed the α-orientation of H-5 of compound **11**.

There are two possibilities which could lead to the product **11** from the olefin **9**. The first possibility is outlined in Scheme 2. Since the dihydroxylation of compound **9** has taken place over a relatively longer time than that occurring at an olefinic function in the side chain,[9] C-5 epimerization

could possibly have occurred at some stage to give **12**, which was eventually dihydroxylated from the less hindered α-face to yield **11**. To prove whether **12** could be dihydroxylated to **11**, compound **7** was deacetylated to give compound **13**, which was subjected to deacetonidation to obtain the olefin **12**. Dihydroxylation of **12** was performed using the same condition as that of **9** to yield **11** in 81% yield, or 64% overall yield from **7** (Scheme 3). This method turned out to provide the best synthesis of **11**, the analogue of **1**. Dihydroxylation of **12** occurred more readily than **9**. To prove whether C-5 epimerization of **9** had occurred under the dihydroxylation conditions employed, the olefin **9** was stirred under dihydroxylation condition, but in the absence of OsO_4. After stirring with 5% $NaHSO_3$ followed by the usual work-up, the residue was analyzed by HPLC, but no compound **12** was detected. This led us to conclude that C-5 epimerization of **9** did not take place under the dihydroxylation conditions.



Scheme 2. Possible pathway for the generation of compound **11**.



Scheme 3. Synthesis of compound **11** from the 5α-olefin **7**. Reagents and conditions: (a) 10% aq K_2CO_3, MeOH; (b) 70% AcOH, $PhCH_2N^+Me_3Cl^-$; and (c) OsO_4, THF, pyridine.

The second possibility leading to **11** is also outlined in Scheme 2. The olefin **9** was dihydroxylated to give **1** and its C-1 and C-2 epimers, **10**, followed by C-5 epimerization of **10** to yield **11**. This possibility is more likely, since the molecular model indicated that the C-2 hydroxyl group of **10** has a strong steric interaction with H-9, C-5 epimerization has therefore taken place under the reaction/work-up conditions to form the more preferred *trans*-A/B ring fusion, compound **11**. In the presence of 2% Na_2CO_3, compound **10** readily epimerized to **11**. A similar observation has been reported previously.[5]

Table 1. Asymmetric dihydroxylation of the olefin **9**[a]

Entry	Ligand	Ratio of products **1**:**10**:**11**[b]
1	DHQ–MQE	92:7:1
2	DHQ–PE	97:3:0
3	$(DHQ)_2$–PHAL	92:6:2
4	$(DHQ)_2$–PYR	88:11:1
5	DHQD–MQE	18:51:18
6	DHQD–PE	15:46:19
7	$(DHQD)_2$–PHAL	50:30:19
8	$(DHQD)_2$–PYR	48:30:3[c]

[a] The ratio of the ligand–OsO_4–olefin **9** was 4:4:1, with *t*-BuOH–THF–H_2O (7:4:1) as a solvent.
[b] Determined by HPLC.
[c] The reaction was terminated before the starting material **9** was used up.

In order to study the effect of the chiral ligands on the ratio of the products **1**, **10**, and **11**, four chiral ligands in each of the dihydroquinine (DHQ) and dihydroquinidine (DHQD) series were used in place of pyridine, the achiral ligand. The DHQ and DHQD series were those of the 4-methyl-2-quinolyl ether (MQE), phenanthryl ether (PE), 1,4-phthalzinediyl diether (PHAL), and 2,5-diphenyl-4,6-pyrimidinediyl ether (PYR).[7] The reaction was performed in *t*-BuOH–THF–H_2O (7:4:1 v/v/v) solvent system and the ratio of the ligand–OsO_4–olefin was 4:4:1. It should be noted that, despite the relatively large excess of OsO_4, the asymmetric dihydroxylation proceeded very slowly. The DHQ chiral ligand series gave rise to **1** as the major product ranging from 88 to 97% (Table 1, entries 1–4) whereas the DHQD chiral ligand series yielded **1** in 15–50% (Table 1, entries 5–8). In the case of DHQD series, though the percentage of the product **1** was significantly less than that in the DHQ series, C-5 epimerization of **10** to **11** occurred more readily than the dihydroxylation in pyridine. It is worth noting that, in the case of $(DHQD)_2$–PYR, the dihydroxylation reaction was terminated before all the starting olefin **9** was used up, since decomposition of the product was observed.

2.2. Biological activity

The moulting activity of integristerone A (**1**) was approximately 9-fold lower than that of 20-hydroxyecdysone (**2**) in the *Musca* bioassay.[10] It was thus evident that introduction of an additional 1β-hydroxyl group to the parent ecdysteroid **2** resulted in a significant decrease in activity. As expected for a 3β-hydroxy-5α-ecdysteroid,[11] 1,2-di-*epi*-5α-integristerone A (**11**) was inactive in the same assay.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on KBr discs on a Perkin–Elmer FT-IR Spectrum BX spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 and AVANCE 500 spectrometers. Mass spectra were measured on Finnigan MAT 90 and LC-Q instruments. Column chromatography and TLC were carried out using Merck 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.

3.2. Synthesis of integristerone A

3.2.1. Mesylation of compound 3. The acetonide **3** (130 mg, 0.25 mmol), prepared from **2** according to the procedure reported previously,[4] was dissolved in dry pyridine (5 mL) and the solution was stirred at 0–4 °C for 10 min. Mesyl chloride (0.15 mL, 1.930 mmol) was slowly added to the solution; the mixture was stirred for 1 h and stirring continued at ambient temperature for 30 min. Cold water (200 mL) was added and the mixture extracted with EtOAc (3×300 mL); the combined organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and the solvent removed in vacuo at temperature 35–40 °C. Column chromatography of the crude product afforded the mesylate **4** (129 mg, 86%) as a white solid. The spectroscopic (IR, ^{1}H NMR) data of **4** were consistent with those of reported values.[8]

3.2.2. Acetylation of the mesylate 4. A mixture of the mesylate **4** (60 mg, 0.125 mmol) in pyridine (1.2 mL) was stirred at 0–4 °C for 5 min and Ac_2O (1 mL) was added. The reaction mixture was allowed to warm up to ambient temperature and stirring continued for 2 h. Water (60 mL) was added and the mixture extracted with EtOAc (3×50 mL). The combined organic phase was washed with H_2O, dried over anhydrous Na_2SO_4; the solvent was evaporated and the residue chromatographed using $CHCl_3$–MeOH (98:2) to yield compound **5** (61 mg, 95%) as a white solid, mp 235–237 °C; IR ν_{max} 3455, 2972, 1745, 1662, 1449, 1376, 1247, 1177, 1145, 1107, 1039, 1001, 985, 883, 861 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me), 1.15 (s, 3H, 21-Me), 1.22 (s, 3H, 26-Me), 1.23 (s, 3H, 27-Me), 1.31, 1.40 (each s, 2×3H, acetonide Me), 2.12 (s, 3H, AcO), 2.22 (t, J=8.5 Hz, 1H, H-17), 2.33 (dd, J=13.2, 3.6 Hz, 1H, H-5), 2.99 (obscured signal, m, 1H, H-9), 3.02 (obscured signal, s, 3H, OMs), 3.64 (br d, J=7.5 Hz, 1H, H-22), 4.93 (m, 1H, H-2), 5.42 (m, 1H, H-3), 5.86 (d, J=2.0 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0 (C-18), 20.3 (C-11), 21.1 (acetate Me), 21.8 (C-21), 23.5 (C-23), 23.6 (C-19), 26.8 (C-26, acetonide Me), 28.9 (acetonide Me), 29.6 (C-27), 30.7 (C-15), 31.6 (C-12), 33.5 (C-9), 35.5 (C-1), 38.8 (mesylate Me), 41.3 (C-24), 47.1 (C-13), 49.0 (C-17), 50.4 (C-5), 67.3 (C-3), 70.3 (C-25), 75.5 (C-2), 82.0 (C-22), 84.4 (C-20), 84.7 (C-14), 106.9 (acetonide C), 121.5 (C-7), 164.6 (C-8), 170.1 (acetate CO), 201.5 (C-6); ESMS (+ve), *m/z* (% rel intensity): 663 [M+Na]$^+$ (25), 1303 [2M+Na]$^+$ (100).

3.2.3. Base-catalyzed elimination of the mesylate 5. Preparation of the olefin 6. To a solution of compound **5** (32 mg, 0.050 mmol) in DMF (1.5 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.8 mL, 5.350 mmol) and the mixture was stirred at 150 °C under nitrogen for 6 h. After the usual work-up, the crude product was purified by column chromatography, using $CHCl_3$–MeOH (98.5:1.5) as an eluent, to yield the less polar product **7** (3.5 mg, 13%) and the olefin **6** (17.3 mg, 64%).

Compound ***6***. Foams; IR ν_{max} 3447, 2971, 2934, 1735, 1716, 1654, 1374, 1249, 1020, 1002, 955, 879 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (s, 3H, 18-Me), 1.08 (s, 3H, 19-Me), 1.13 (s, 3H, 21-Me), 1.20 (s, 3H, 26-Me), 1.21 (s, 3H, 27-Me), 1.30, 1.38 (each s, 2×3H, acetonide Me), 2.02 (s, 3H, AcO), 2.20 (dd, J=9.2, 7.8 Hz, 1H, H-17), 2.37 (dd, J=13.3, 3.2 Hz, 1H, H-5), 2.85 (m, 1H, H-9), 3.63 (br d, J=8.2 Hz, 1H, H-22), 5.22 (m, $W_{1/2}$= 10 Hz, 1H, H-3), 5.73 (dd, J=10, 4.4 Hz, 1H, H-2), 5.87 (d, J=2.3 Hz, 1H, H-7), 5.95 (d, J=10 Hz, 1H, H-1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (C-18), 21.1 (acetate Me), 21.2 (C-21), 21.9 (C-19), 23.6 (C-23), 26.8 (acetonide Me), 28.3 (C-27), 28.9 (acetonide Me), 29.6 (C-26), 31.1 (C-12), 31.8 (C-15), 37.9 (C-10), 39.1 (C-9), 41.4 (C-24), 47.3 (C-13), 49.1 (C-17), 50.0 (C-5), 65.1 (C-3), 70.3 (C-25), 82.0 (C-22), 84.4 (C-20), 85.0 (C-14), 107.0 (acetonide C), 121.9 (C-7), 123.7 (C-2), 140.9 (C-1), 164.3 (C-8), 170.4 (acetate CO), 202.1 (C-6); HR-FABMS (positive-ion mode) *m/z* 545.3476 [M+H]$^+$ (calcd for $C_{32}H_{48}O_7$+H, 545.3478).

Compound ***7***. Foams; IR ν_{max} 3448, 2970, 2934, 1736, 1716, 1676, 1376, 1249, 1031, 1002, 872 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-Me), 0.93 (s, 3H, 19-Me), 1.14 (s, 3H, 21-Me), 1.21 (s, 3H, 26-Me), 1.22 (s, 3H, 27-Me), 1.31, 1.39 (each s, 2×3H, acetonide Me), 1.53 (obscured signal, 1H, H-4ax), 2.05 (s, 3H, AcO), 2.22 (dd, J=7.9, 7.8 Hz, 1H, H-17), 2.45 (br dd, J=12.7, 6.7 Hz, 1H, H-4eq), 2.57 (dd, J=12.7, 2.1 Hz, 1H, H-5), 2.82 (m, 1H, H-9), 3.64 (br d, J=8.4 Hz, 1H, H-22), 5.36 (m, $W_{1/2}$=20 Hz, 1H, H-3), 5.54 (br d, J=10.1 Hz, 1H, H-2), 5.88 (dd, J=10.1, 1.8 Hz, 1H, H-1), 5.93 (d, J=2.5 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5 (C-19), 17.0 (C-18), 20.4 (C-11), 21.0 (C-16), 21.2 (acetate Me), 21.8 (C-21), 22.9 (C-4), 23.5 (C-23), 26.8 (acetonide Me), 28.9 (C-27), 29.0 (acetonide Me), 29.6 (C-26), 30.8 (C-15), 31.6 (C-12), 39.8 (C-10), 41.3 (C-24), 43.4 (C-9), 47.1 (C-13), 49.0 (C-17), 51.5 (C-5), 70.1 (C-3), 70.3 (C-25), 81.9 (C-22), 84.4 (C-20), 84.8 (C-14), 106.9 (acetonide C), 123.1 (C-7), 126.1 (C-2), 135.9 (C-1), 162.5 (C-8), 170.7 (acetate CO), 198.9 (C-6); HMBC correlations ($CDCl_3$): H-1 (C-3, C-5, C-9, C-10), H-2 (C-3, C-4, C-10), H-3 (C-1, C-2, CO acetate), H-4eq (C-2, C-3, C-5, C-10), H-5 (C-3, C-6, C-10, C-19), H-7 (C-5, C-9, C-14), H-9 (C-7, C-8, C-10), H-17 (C-13, C-16, C-18), 18-Me (C-12, C-13, C-14, C-17), 19-Me (C-1, C-9, C-10), 21-Me (C-17, C-20, C-22), H-22 (C-24), 26-Me (C-24, C-25, C-27), 27-Me (C-24, C-25, C-26), Me acetonide (acetonide C), Me acetate (C-3, CO acetate); HR-FABMS (positive-ion mode) *m/z* 545.3477 [M+H]$^+$ (calcd for $C_{32}H_{48}O_7$+H, 545.3478).

3.2.4. Deacetylation of compound 6. To a solution of compound **6** (19 mg, 0.035 mmol) in MeOH (1.0 mL) was added 10% aq K_2CO_3 (0.3 mL) and the mixture stirred for 30 min. After the usual work-up, the product was purified by column chromatography to give compound **8** (13 mg, 74%) as a white amorphous solid, mp 205–208 °C (decomposed); IR ν_{max} 3422, 2967, 2934, 1654, 1458, 1375, 1252, 1215, 1172, 1103, 1024, 1002, 876 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (s, 3H, 18-Me), 1.07 (s, 3H, 19-Me), 1.13 (s, 3H, 21-Me), 1.21 (s, 3H, 26-Me), 1.22 (s, 3H, 27-Me), 1.30, 1.39 (each s, 2×3H, acetonide Me), 2.19 (dd, J=8.5, 8.4 Hz, 1H, H-17), 2.39 (dd, J=13.2, 2.8 Hz, 1H, H-5), 2.84 (m, 1H, H-9), 3.63 (br d, J=8.1 Hz, 1H, H-22), 4.22 (br s, $W_{1/2}$=10 Hz, 1H, H-3), 5.79 (dd, J=10, 4 Hz, 1H, H-2), 5.84 (d, J=10 Hz, 1H, H-1), 5.89 (d, J=1.9 Hz, 1H,

H-7); HR-FABMS (negative-ion mode) *m/z* 501.3216 $[M-H]^-$ (calcd for $C_{30}H_{46}O_6-H$, 501.3216).

3.2.5. Acetonide deprotection of compound 8. A mixture of compound **8** (12 mg, 0.024 mmol) and $PhCH_2N^+Me_3Cl^-$ (3 mg) in MeOH (0.2 mL) and 70% AcOH (0.5 mL) was stirred at ambient temperature for 4 days and worked up in normal fashion to give, after column chromatography, the olefin **9** (9 mg, 81%) as an amorphous solid, mp 280–282 °C (decomposed); IR ν_{max} 3420, 2971, 2934, 1653, 1378, 1031, 959, 901, 876 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$+3 drops of CD_3OD) δ 0.91 (s, 3H, 18-Me), 1.13 (s, 3H, 19-Me), 1.24 (s, 3H, 21-Me), 1.27 (s, 3H, 26-Me), 1.28 (s, 3H, 27-Me), 2.37 (t, *J*=8 Hz, 1H, H-17), 2.45 (dd, *J*=13.1, 2.9 Hz, 1H, H-5), 2.91 (m, 1H, H-9), 3.45 (obscured by solvent signal, H-22), 4.25 (m, 1H, H-3), 5.84 (dd, *J*=9.9, 3.9 Hz, 1H, H-2), 5.89 (d, *J*=9.9 Hz, 1H, H-1), 5.93 (d, *J*=2.3 Hz, 1H, H-7); HR-FABMS (negative-ion mode) *m/z* 461.2904 $[M-H]^-$ (calcd for $C_{27}H_{42}O_6-H$, 461.2903).

3.2.6. Dihydroxylation of olefin 9. A solution of OsO_4 (1 g) in pyridine (5 mL) was prepared and a portion (62 μL, 2.31 equiv) was added to a solution of the olefin **9** (10 mg, 0.021 mmol) in pyridine (1 mL) and the solution stirred for 1 h. Another portion of the OsO_4 solution (62 μL, 2.31 equiv) was then added and stirring continued for 3 h. $NaHSO_3$ 5% (1 mL) was added and the mixture was stirred for 20 min. The mixture was repeatedly extracted with *n*-BuOH until no products were detected in the aqueous phase. The combined organic phases were evaporated by co-distillation with H_2O and the residue was chromatographed to give **10** (2.8 mg, 27%), **11** (1 mg, 7%), and **1** (5.8 mg, 56%).

*Compound **1***. Colorless needles from CH_2Cl_2–MeOH, mp 244–246 °C (lit.[2] 244–246 °C); IR ν_{max} 3442, 2971, 2934, 1654, 1385 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.89 (s, 3H, 18-Me), 1.07 (s, 3H, 19-Me), 1.18 (s, 2×3H), 1.19 (s, 3H) (21-Me, 26-Me, 27-Me), 2.39 (dd, *J*=9, 8.5 Hz, 1H, H-17), 2.61 (dd, *J*=12.5, 4.3 Hz, 1H, H-5), 3.08 (m, 1H, H-9), 3.32 (obscured by solvent signal, H-22), 3.83 (br s, $W_{1/2}$=8 Hz, 1H, H-1), 3.89 (br t, $W_{1/2}$=8 Hz, 1H, H-2), 4.04 (m, $W_{1/2}$=11 Hz, 1H, H-3), 5.83 (d, *J*=2.3 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 1.20 (s, 3H, 18-Me), 1.36 (s, 2×3H, 26-Me, 27-Me), 1.41 (s, 3H, 19-Me), 1.55 (s, 3H, 21-Me), 2.96 (t, *J*=9.1 Hz, 1H, H-17), 3.27 (m, 1H, H-5), 3.54 (m, 1H, H-9), 3.86 (br d, *J*=8.9 Hz, H-22), 4.27 (br s, 1H, H-2), 4.31 (br s, 2H, H-1, H-3), 6.25 (br s, 1H, H-7); HR-FABMS (negative-ion mode) *m/z* 495.2954 $[M-H]^-$ (calcd for $C_{27}H_{44}O_8-H$, 495.2957).

*Compound **10***. White solid, mp 244–247 °C; IR ν_{max} 3449, 2963, 2920, 1656, 1454, 1382, 1064, 1017 cm^{-1}; ^1H NMR (500 MHz, D_2O, 353 K) δ 0.86 (s, 3H, 18-Me), 1.16 (s, 3H, 19-Me), 1.21 (s, 3H, 21-Me), 1.23 (s, 3H, 26-Me), 1.24 (s, 3H, 27-Me), 1.79 (1H, H-16b), 1.87 (1H, H-16a), 1.96 (2H, H-12), 2.05 (1H, H-15a), 2.36 (t, *J*=9.5 Hz, 1H, H-17), 2.66 (br s, 1H, H-5), 3.19 (very broad, 1H, H-9), 3.42 (d, *J*=10.6 Hz, 1H, H-22), 3.64 (d, *J*=2.9 Hz, 1H, H-1), 3.74 (br s, $W_{1/2}$=37 Hz, 1H, H-3), 3.88 (dd, *J*=8.2, 3.1 Hz, 1H, H-2), 5.92 (br s, $W_{1/2}$=3 Hz, 1H, H-7); ^{13}C NMR (125 MHz, D_2O) δ 19.5 (C-18), 21.9 (C-21), 23.6 (C-19), 22.8 (C-16), 28.4 (C-23), 30.0 (C-26), 30.5 (C-27), 32.3 (C-15), 35.3 (C-12), 43.1 (C-24), 45.9 (C-9), 50.5 (C-13), 51.7 (C-17), 52.8 (C-5), 70.5 (C-3), 74.3 (C-25), 75.0 (C-1), 76.0 (C-2), 79.8 (C-22), 81.4 (C-20), 89.8 (C-14), 124.1 (C-7); HR-FABMS (negative-ion mode) *m/z* 495.2952 $[M-H]^-$ (calcd for $C_{27}H_{44}O_8-H$, 495.2958).

*Compound **11***. White solid, mp 241–243 °C; IR ν_{max} 3419, 2966, 2922, 2848, 1654, 1384, 1063 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N): δ 1.02 (s, 3H, 19-Me), 1.23 (s, 3H, 18-Me), 1.38 (s, 2×3H, 26-Me, 27-Me), 1.55 (s, 3H, 21-Me), 3.01 (dd, *J*=9.2, 8.5 Hz, 1H, H-17), 3.08 (dd, *J*=12.2, 3.6 Hz, 1H, H-5), 3.89 (br d, *J*=8.7 Hz, 1H, H-22), 4.09 (dd, *J*=9.3, 2.1 Hz, 1H, H-2), 4.23 (br s, 1H, H-1), 4.30 (m, 1H, H-9), 4.49 (m, $W_{1/2}$=22 Hz, 1H, H-3), 6.20 (d, *J*=2.4 Hz, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 14.6 (C-19), 19.2 (C-18), 21.7 (C-11), 22.7 (C-16), 22.9 (C-21), 28.7 (C-23), 31.2 (C-4, C-26), 31.4 (C-27), 33.1 (C-12),[a] 33.2 (C-15),[a] 40.6 (C-9), 43.9 (C-24), 46.1 (C-10), 49.2 (C-13), 49.6 (C-5), 51.4 (C-17), 70.8 (C-25), 71.5 (C-3), 75.9 (C-2), 76.9 (C-1), 78.1 (C-20), 78.8 (22), 85.3 (C-14), 123.4 (C-7), 167.4 (C-8), 202.3 (C-6), 'a' stands for assignments, which may be reversed for signals with the same superscript; HMBC correlations (C_5D_5N): H-1 (C-2, C-3, C-5), H-2 (C-3), H-5 (C-4, C-9, C-10, C-19), H-7 (C-5, C-9, C-14), H-17 (C-13, C-16, C-18), 18-Me (C-12, C-14, C-17), 19-Me (C-1, C-5, C-9), 21-Me (C-17, C-22), H-22 (C-24), 26-Me (C-24, C-25, C-27), 27-Me (C-24, C-25, C-26); HR-FABMS (negative-ion mode) *m/z* 495.2958 $[M-H]^-$ (calcd for $C_{27}H_{44}O_8-H$, 495.2957).

3.3. Asymmetric dihydroxylation of olefin 9

General procedure. To a solution of the chiral ligand (0.016 nmol) in *t*-BuOH–THF–H_2O (7:4:1, 1.8 mL) was added a THF solution of OsO_4 (24 μL, 0.016 mmol) and the mixture stirred for 10 min. A solution of the olefin **9** (2 mg, 0.004 mmol) in *tert*-BuOH–THF–H_2O (7:4:1, 0.4 mL) was then added and the mixture stirred for 5 h. The ratio of the ligand, OsO_4, and olefin was 4:4:1. A 5% solution of $NaHSO_3$ (2 mL) was added and stirring continued for another 30 min. The mixture was repeatedly extracted with *n*-BuOH until no products were detected in the aqueous phase. The combined organic phases were evaporated by co-distillation with H_2O and the residue was chromatographed to separate a mixture of the products **1**, **10**, and **11** from the ligand. The ratio of the ecdysteroids was determined by HPLC analysis (column: Spherisorb ODS2, 5 μm, 250×4.6 mm, mobile phase: MeOH–H_2O (40:60); flow-rate: 0.50 mL min^{-1}; detector: 254 nm). The results are shown in Table 1, entries 1–8.

3.3.1. Deacetylation of olefin 7. A 10% aq K_2CO_3 solution (0.3 mL) was added to a stirred solution of **7** (50 mg, 0.09 mmol) in MeOH (2.0 mL) and the mixture was kept stirring for 30 min. After the usual work-up, the crude product was purified by column chromatography to afford **13** (44 mg, 95%) as a white amorphous powder, mp 223–225 °C; IR ν_{max} 3419, 2971, 2927, 1674, 1667, 1371, 1252, 1215, 1172, 1111, 1093, 1056, 999, 872 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-Me), 0.91 (s, 3H, 19-Me), 1.14 (s, 3H, 21-Me), 1.21 (s, 3H, 26-Me), 1.22 (s, 3H, 27-Me), 1.31, 1.39 (each s, 2×3H, acetonide Me), 2.22 (m, 1H, H-17), 2.44 (br dd, *J*=13.2, 6.4 Hz, 1H, H-4eq), 2.51 (dd, *J*=12.6, 1.9 Hz, 1H, H-5), 2.80 (m, 1H,

H-9), 3.64 (br d, J=8.6 Hz, 1H, H-22), 4.30 (m, 1H, H-3), 5.62 (br d, J=10.1 Hz, 1H, H-2), 5.79 (dd, J=10.1, 1.6 Hz, 1H, H-1), 5.93 (d, J=2.6 Hz, 1H, H-7); HR-FABMS (negative-ion mode) m/z 501.3218 $[M-H]^-$ (calcd for $C_{30}H_{46}O_6-H$, 501.3216).

3.3.2. Acetonide deprotection of compound 13. Compound **13** (44 mg, 0.08 mmol) was subjected to acetonide deprotection in the same manner as described for the preparation of compound **9**. The product was purified by column chromatography to give olefin **12** (30 mg, 83%); colorless needles (from CH_2Cl_2–MeOH), mp 224–226 °C; IR ν_{max} 3421, 2971, 2934, 1654, 1385, 1035 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (s, 3H, 18-Me), 0.92 (s, 3H, 19-Me), 1.192 (s, 3H, 26-Me), 1.198 (s, 3H, 27-Me), 1.28 (s, 3H, 21-Me), 2.38 (m, 1H, H-17), 2.59 (br d, J=12.4 Hz, 1H, H-5), 2.85 (m, 1H, H-9), 3.31 (obscured signal, 1H, H-22), 4.23 (m, 1H, H-3), 5.61 (br d, J=10.1 Hz, 1H, H-2), 5.84 (br d, J=10.2 Hz, 1H, H-1), 5.88 (br s, 1H, H-7); HR-FABMS (negative-ion mode) m/z 461.2906 $[M-H]^-$ (calcd for $C_{27}H_{42}O_6-H$, 461.2903).

3.3.3. Dihydroxylation of olefin 12. To a stirred solution of olefin **12** (16 mg, 0.03 mmol) in pyridine (1 mL) was added a solution of OsO_4 (70 μL, 2.0 equiv) in pyridine and the mixture stirred for 1 h. $NaHSO_3$ 5% (1 mL) was added and the mixture was stirred for 10 min. The mixture was repeatedly extracted with *n*-BuOH until no products were detected in the aqueous phase. The combined organic phases were evaporated and the residue was chromatographed to give **11** (13 mg, 81%).

3.4. Moulting bioassay

Compounds **1** and **11** were subjected to the *Musca* bioassay using *Musca domestica* larvae.[10] A 3 μL portion of ecdysteroid aqueous solution was injected into each larva. The bioassay results were scored[12] and EC_{50}, the molar concentration of each compound required to effect puparium formation of 50% effectiveness, of each compound was determined by plotting concentrations against % effectiveness of puparium formation.[13] The EC_{50} value of compound **1** was 1.53×10^{-4} M, whereas that of the reference compound **2** was 1.65×10^{-5} M. Compound **11** was inactive in the test.

Acknowledgements

This work was supported by the Thailand Research Fund (TRF). One of us (S.K.) acknowledges financial support from the Royal Golden Jubilee Ph.D. Program of TRF. We are grateful to Mr. Nitirat Chimnoi, Chulabhorn Research Institute, for recording the mass spectra and Dr. Gildas Bertho, CNRS UMR 8601, Université Paris V-René Descartes, for recording the NMR spectra at 500 MHz. Critical reading of the manuscript by Dr. L. Dinan is also deeply acknowledged.

References and notes

1. Lafont, R.; Harmatha, J.; Marion-Poll, F.; Dinan, L.; Wilson, I. D. *Ecdybase, a free ecdysteroid database*; 2002. Available at <http://ecdybase.org>.
2. Baltaev, U.; Gorovitz, M. B.; Rashkes, Y. V.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1977**, 813–819.
3. Werawattanametin, K.; Podimuang, V.; Suksamrarn, A. *J. Nat. Prod.* **1986**, *49*, 365–366.
4. Suksamrarn, A.; Pattanaprateep, P. *Tetrahedron* **1995**, *51*, 10633–10650.
5. Suksamrarn, A.; Charoensuk, S.; Yingyongnarongkul, B. *Tetrahedron* **1996**, *52*, 10673–10684.
6. Suksamrarn, A.; Yingyongnarongkul, B. *Tetrahedron* **1997**, *53*, 3145–3154.
7. Homvisasevongsa, S.; Chuaynugul, A.; Chimnoi, N.; Suksamrarn, A. *Tetrahedron* **2004**, *60*, 3433–3438.
8. Suksamrarn, A.; Yingyongnarongkul, B. *Tetrahedron* **1996**, *52*, 12623–12630.
9. Yingyongnarongkul, B.; Suksamrarn, A. *Tetrahedron* **1998**, *54*, 2795–2800.
10. Kaplanis, J. N.; Tabor, L. A.; Thompson, M. J.; Robbins, W. E.; Shortino, T. J. *Steroids* **1966**, *8*, 625–631.
11. Bergamasco, R.; Horn, D. H. S. The Biological Activities of Ecdysteroids and Analogues. In *Progress in Ecdysone Research*; Hoffmann, J. A., Ed.; Elsevier/North-Holland Biomedical: Amsterdam, 1980; pp 299–324 and references cited therein.
12. Ohtaki, T.; Milkman, R. D.; Williams, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 981–984.
13. Suksamrarn, A.; Pattanaprateep, P.; Tanachatchairatana, T.; Haritakun, W.; Yingyongnarongkul, B.; Chimnoi, N. *Insect Biochem. Mol. Biol.* **2002**, *32*, 193–197.