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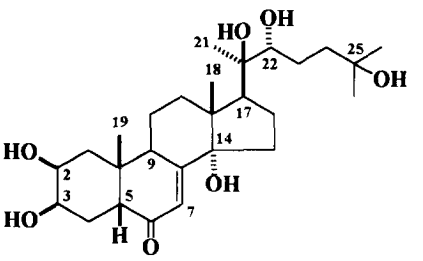
Synthesis and Moulting Hormone Activity of 3-*epi*-2-Deoxy-20-hydroxyecdysone and Analogues

Apichart Suksamrarn* and Boon-ek Yingyongnarongkul

Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

Abstract: The minor ecdysteroid 3-*epi*-2-deoxy-20-hydroxyecdysone (3-*epi*-2-D-20-ECD) has been partially synthesized from 20-hydroxyecdysone. 2-D-5α-20-ECD and the 3-dehydro analogues, 3-dehydro-2-D-20-ECD and 3-dehydro-2-D-5α-20-ECD, have also been synthesized. Moulting hormone activity of these compounds has been studied using the *Musca* bioassay.

20-Hydroxyecdysone (20-ECD, **1**) is a representative ecdysteroid with a 2,3-dihydroxyl function. Its 2-deoxy analogues are minor ecdysteroids isolated from some plants and invertebrates.[1] Apart from 2-deoxy-20-hydroxyecdysone (2-D-20-ECD, **2**), 2-D-20-ECD 3-acetate (**3**), 2-D-20-ECD 22-acetate (**4**) and 2-D-20-ECD 22-benzoate (**5**) which have been mentioned previously,[2] 3-*epi*-2-D-20-ECD (**6**) is another member of this small group of ecdysteroids.[1] This compound has been isolated from the leech *Hinido medicinalis* as a metabolite of exogenous 2-deoxyecdysone (**7**)[3] and was a constituent of the plant *Tinospora capillipes*.[4] We wish to report partial synthesis of this rare ecdysteroid from the readily available ecdysteroid **1**.[5,6] 2-D-5α-20-ECD (**8**) and the 3-dehydro analogues, 3-dehydro-2-D-20-ECD (**9**) and 3-dehydro-2-D-5α-20-ECD (**10**), have also been synthesized from **1**. Moulting hormone activity of these ecdysteroids has been studied.



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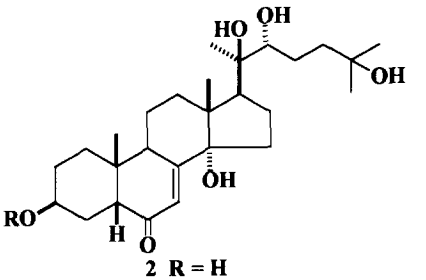

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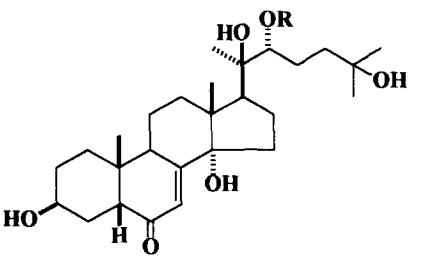

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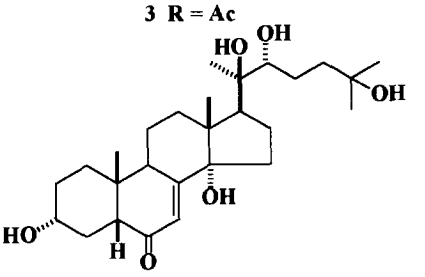

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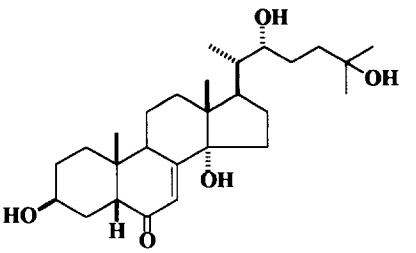

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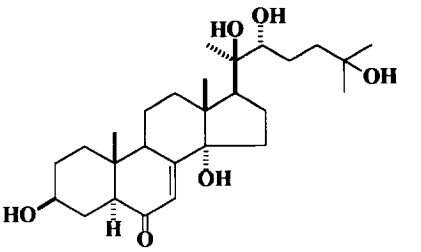

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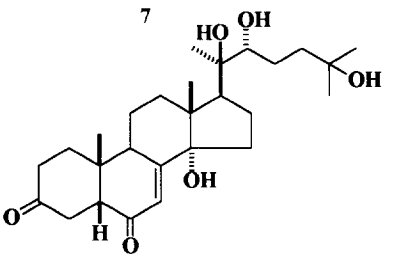

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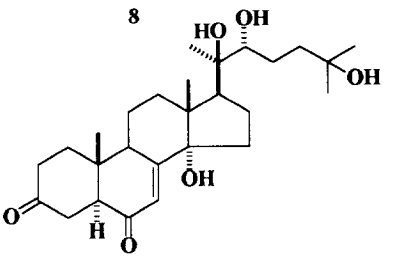

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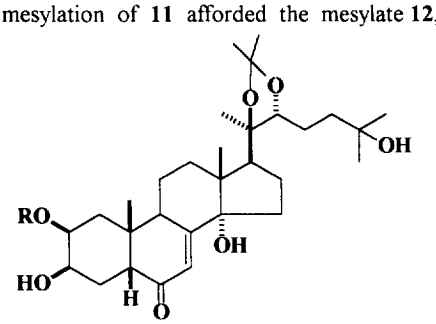
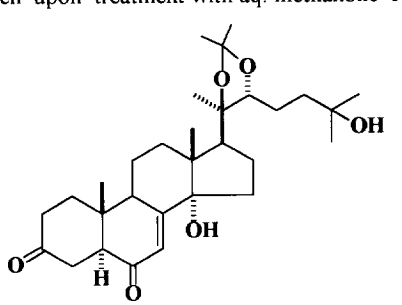
RESULTS AND DISCUSSION

In order to synthesize the ecdysteroid **6**, we initially planned to oxidize the ecdysteroid **2**, synthesized previously by our group,[2] to the corresponding keto compound **9**, which would then be selectively reduced to **6**. However, a more concise route for the synthesis of **9** was devised and this was accomplished by a key step involving C3 dehydrogenation and C2 deoxygenation. From compound **9**, the target ecdysteroid **6** has been synthesized. The keto compound **10** and the ecdysteroid analogue **8** have also been synthesized.

The C20 and C22 hydroxyl groups in **1** were protected as the acetonide **11** by the literature method.[7] Selective mesylation of **11** afforded the mesylate **12**,[2] which upon treatment with aq. methanolic NaOH the

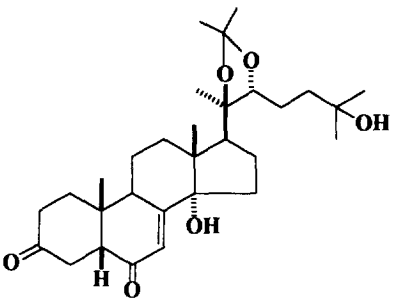


11 R = H
12 R = Ms



13

keto compound **13** was obtained. The structure of **13** has been established as followed. The presence of a new saturated keto group was evident from the IR absorption band at 1710 cm^{-1}. The absence of carbinol protons at the C2 and C3 positions in the ^1H NMR spectrum was consistent with the structure **13**. Placement of the saturated keto function at the C3 position of the A-ring, instead of the C2 position, was based on the mechanistic consideration as well as the absence of two sets of doublets, with a relatively large coupling constant, of two geminal protons at C1 position of the latter possible structure. The large upfield shift of H9 signal in $CDCl_3$ at δ2.78 (as compared with δ3.01 in **12**) suggested the *trans*-A/B ring fusion[8] in **13**. Furthermore, NOE experiment[2] also confirmed the α-orientation of H5. Thus irradiation of the H5 signal at δ2.65 gave rise to enhancement of H9 signal at δ2.78.



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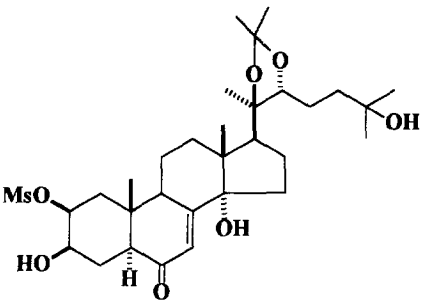


15

The results indicated that C5 epimerization has taken place at some stage in the reactions leading to the product **13**. Epimerization at this position is known in ecdysteroid field.[8,9] Under the basic condition employed, H5β epimerized to H5α. Two possible pathways leading to the product **13** could be proposed. The first possibility was the elimination of MsOH to give **14**, followed by base-catalyzed epimerization of **14** to **13**. The second possibility involved C5 epimerization of the *cis*-A/B mesylate **12** to the corresponding *trans*-A/B isomer **15**, followed by elimination of MsOH to the ketone **13**. We have re-investigated the reaction of **12** with aq. methanolic NaOH and, after careful TLC examination of the progress of the reaction, two almost completely overlapping spots were visualized, the less polar of which corresponded to compound **12**. Just after the appearance of the more polar component, the product **13** which was the least polar component was detected. The new component was then isolated and identified as the mesylate **15**. The ^{1}H NMR data was consistent with the structure **15**, especially the relatively large upfield shift (0.34 ppm) of the H9 signal (as compared with the epimeric mesylate **12**) which indicated that the A/B ring fusion was *trans*.[8] NOE enhancement of the H5α signal at δ2.31 was observed when the H9 signal at δ2.67 was irradiated, thus confirming the α-orientation of the H5. It was therefore most likely that the ketone **13** was produced through the intermediate mesylate **15**.

Sodium borohydride reduction of **13** yielded an inseparable mixture of the alcohols **16** and **17** in a ratio of 5:1 (from ^{1}H NMR). The mixture was subjected to acetylation and the resulting acetylated mixture was chromatographed to afford individual compounds **18** and **19**. The relatively upfield resonance of the ^{1}H NMR spectrum of H9 (δ2.68, $CDCl_3$) of **18** suggested a *trans*-A/B ring system. A large peak-width at half-height ($W_{1/2}$, 23 Hz) of H3 signal at δ4.69 indicated that the C3 hydroxyl group was in β-orientation[10] (i.e., H3 was in the axial configuration). Compound **19** was initially expected to be compound **20**, the C3 epimer of **18**. However, careful ^{1}H NMR analysis of this product has shown that it was compound **19**, instead of **20**. Thus a relatively downfield signal of H9 (δ3.15, $CDCl_3$) suggested a *cis*-A/B ring fusion. Consequently, the C3 hydroxyl group should be in the α-orientation since the $W_{1/2}$ value of H3 at δ4.70 was large[10] (23 Hz). In principle, the ecdysteroid **6** should be obtained from **19** by acetonide deprotection followed by deacetylation. However, it was obvious that the yield of **6** would be very low, since compound **19** was a minor component. We therefore would like to explore another alternative route.

It was evident that the major alcohol **16** resulted from the approach of the borohydride from the α-face, the less hindered side of an ecdysteroid with a *trans*-A/B ring system. The minor alcohol **17** could arise from one of a number of possible pathways. The first possibility might involve epimerization of the initially formed alcohol **21** to the more stable C5 epimer **17**, since the α-orientation of the C3 hydroxyl group in **21** resulted in 1,3-diaxial interaction of this group with the C1 and C5 axial hydrogens. The second possibility would involve C5 epimerization of the ketone **13** under the the reduction condition to the corresponding *cis*-A/B epimer **14**, followed by borohydride reduction. In this case the reducing agent would approach the C3 keto function of **14** from the β-face, the less hindered side of an ecdysteroid with a *cis*-A/B ring junction.

In order to improve method of synthesis of **6** in higher yield, we then turn our interest to the synthesis of the ketone **14**. To avoid epimerization at the C5 position of the mesylate **12** to the mesylate **15**, or possibly the expected ketone **14** to the ketone **13**, we have replaced $NaHCO_3$ for NaOH. This base, however, could also epimerize **12** to **15**. Ecdysteroid without a 2β-hydroxyl group was known to epimerize to the corresponding

Table ^{1}H NMR Data of Ecdysteroids

H	6	8	9	10	12	13	14	15
	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
2					4.91 (m)			4.93 (br s)
3	3.83 (br m, W1/2 = *ca* 25)	3.78 (br m, W1/2 = *ca* 21)	-	-	4.30 (br s)	-	-	3.69 (m)
5	2.29 (dd, 13.4, 3.6)	≠	2.59 (dd, 11.4, 4.1)	≠	2.52 (dd, 13.4, 3.9)	2.65 (dd, 12.5, 4.9)	2.48*	2.31 (dd, 12, 3.2)
7	6.23 (d, 2.4)	6.19 (d, 2.7)	6.19 (d, 2.1)	6.18 (d, 2.4)	5.87 (d, 2.4)	5.94 (d, 2.7)	5.88 (d,2.1)	5.90 (d, 2.7)
9	3.70 (m)	3.02 (m)	3.62 (m)	3.09 (m)	3.01 (m)	2.78 (m)	3.28 (m)	2.67 (m)
17	3.02 (t, 9)	3.01#	3.04 (t, 9.3)	≠	2.23 (dd, 9.6, 7.5)	2.22 (m)	2.28 (dd, 9.7, 7.6)	2.20 (dd, 9.1, 7.9)
22	3.88 (br d, 10.1)	3.89 (br d, 10)	3.88 (br d, 10)	3.88 (br d, 9.4)	3.65 (dd, 9.4, 2.4)	3.64 (dd, 9.5, 2.1)	3.66 (dd, 9.1, 2.4)	3.63 (br d, 8.8)
18-Me	1.22 (s)	1.21 (s)	1.22 (s)	1.21 (s)	0.80 (s)	0.81 (s)	0.83 (s)	0.77 (s)
19-Me	0.98 (s)	0.91 (s)	1.04 (s)	1.03 (s)	1.02 (s)	1.04 (s)	1.08 (s)	0.98 (s)
21-Me	1.59 (s)	1.59 (s)	1.60 (s)	1.59 (s)	1.16 (s)	1.15 (s)	1.17 (s)	1.13 (s)
26-Me	1.37 (s)	1.38 (s)	1.36 (s)	1.38 (s)	1.24 (s)	1.21 (s)	1.24 (s)	1.20 (s)
27-Me	1.37 (s)	1.38 (s)	1.36 (s)	1.38 (s)	1.25 (s)	1.22 (s)	1.25 (s)	1.21 (s)
>C(Me)$_2$	-	-	-	-	1.33, 1.41 (each s)	1.31, 1.40 (each s)	1.34, 1.42 (each s)	1.30, 1.39 (each s)
AcO	-	-	-	-	-	-	-	-
MsO	-	-	-	-	3.09 (s)	-	-	3.08 (s)

≠Obscured signal.

#Obscured by H9.

*Partially superimposed signal.

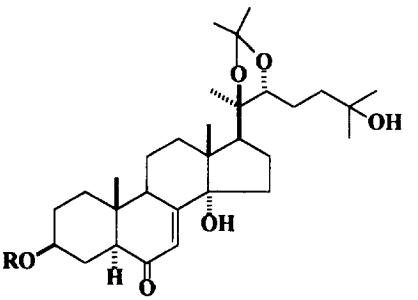
Table ^{1}H NMR Data of Ecdysteroids (continued)

H	**16***	**17***	**18**	**19**	**23**	**24**	**25**
	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_5D_5N	C_5D_5N
2							
3	3.62 (br m)	3.62 (br m)	4.69 (br m, $W_{1/2}$ = 23)	4.70 (br m, $W_{1/2}$ = 23)	5.05 (br s)	4.78 (br m)	4.78 (br m)
5	2.27 (dd, 12.3, 3.7)	≠	2.30 (dd, 12.2, 3.6)	2.09 (dd, 13.3, 3.8)	2.35 (dd, 12.5, 4.2)	≠	≠
7	5.91 (d, 2.7)	5.86 (d, *ca* 2)	5.88 (d, 2.7)	5.84 (d, 2.4)	5.83 (d, 2.4)	6.22 (d, 2.1)	6.16 (d, 2.7)
9	2.69 (m)	3.17 (m)	2.68 (m)	3.15 (m)	3.07 (m)	3.52 (m)	2.99 (m)[b]
17	*ca* 2.22[a]	*ca* 2.22[a]	≠	2.23 (dd, 9.7, 7.9)	2.22 (dd, 9.6, 7.7)	3.01 (t, 9.1)	3.00 (m)[b]
22	3.66 (br d, 9.2)	3.66 (br d, 9.2)	3.64 (dd, 9.2, 2.1)	3.64 (br d, 9.2)	3.64 (dd, 9.4, 2.1)	3.88 (br d, 9.4)	3.90 (d, 9.4)
18-Me	0.80 (s)	0.80 (s)	0.78 (s)	0.78 (s)	0.78 (s)	1.20 (s)	1.19 (s)
19-Me	0.86 (s)	0.92 (s)	0.85 (s)	0.91 (s)	0.95 (s)	0.91 (s)	0.86 (s)
21-Me	1.16 (s)	1.16 (s)	1.14 (s)	1.14 (s)	1.14 (s)	1.59 (s)	1.58 (s)
26-Me	1.23 (s)	1.23 (s)	1.21 (s)	1.22 (s)	1.21 (s)	1.36 (s)	1.38 (s)
27-Me	1.24 (s)	1.24 (s)	1.22 (s)	1.23 (s)	1.22 (s)	1.36 (s)	1.38 (s)
>C(Me)$_2$	1.33, 1.41 (each s)	1.33, 1.41 (each s)	1.31, 1.39 (each s)	1.31, 1.39 (each s)	1.30, 1.39 (each s)	-	-
AcO	-	-	2.01 (s)	1.98 (s)	2.03 (s)	1.91 (s)	2.00 (s)

*Assigned from a mixture of compounds **16** and **17**.

≠Obscured signal.

[a,b]Signals with the same superscript denote partially superimposed signals.



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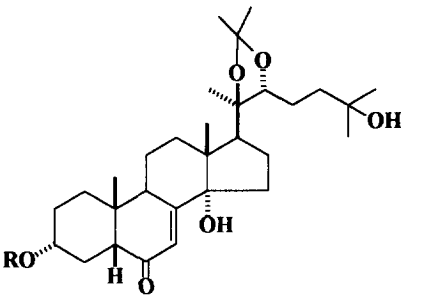
18 R = Ac



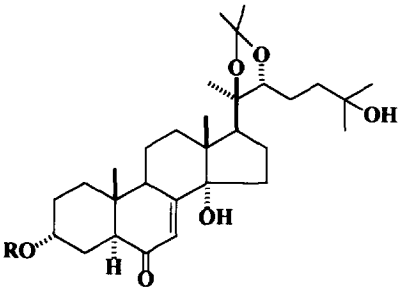
17 R = H
19 R = Ac

trans-A/B fused epimer more readily than the one with such stabilizing group.[10] We then considered to use guanidine in place of the above inorganic bases. We have found that this organic base is a relatively milder base, but it was sufficiently strong for the hydrolysis of the ester group in an ecdysteroid molecule, even an ester which was difficult to hydrolyze.[6] In our experience, this base is suitable for working with ecdysteroids, since it did not cause epimerization at C5 position and did not catalyze autoxidation as some other bases did.[11] Moreover, use of base of this type with different basic strength can also be made by selection of various salts of this base (guanidine acetate, guanidine hydrochloride and guanidine nitrate, for example). In our case, guanidine hydrochloride-$KHCO_3$ (both 4:1 and 2:1 mol. ratio) still caused epimerization of the mesylate **12**, while guanidine itself had the problem concerning solubility in the solvent system used in the reaction. However, we found that guanidine acetate was a suitable base; the mesylate **12** has slowly been converted to the ketone **14** without detectable amount of the mesylate **15** as well as the epimeric ketone **13**. The *cis*-A/B nature of **14** was evident from a relatively lowfield resonance (δ3.28) of the H9 signal, as compared with that of compound **13** (δ2.78). The stereochemistry of the A/B ring junction was further confirmed by the following NOE experiments. Irradiation of the 19-Me signal at δ1.08 caused enhancement in the signal of H5 at δ2.48. Enhancement of the 19-Me signal was also observed when the H5 signal was subjected to irradiation.

Sodium borohydride reduction of the ketone **14** furnished a mixture of products showing only one homogeneous spot on TLC. The ^{1}H NMR spectrum revealed that it was in fact a mixture of one major product and minor product(s), the latter being present in quantity of less than *ca* 10%. The mixture was then subjected to acetylation and TLC of the crude products indicated one major and one minor acetates. ^{1}H NMR spectrum of the isolated major component was identical to **19**, the minor acetylated product of the borohydride reduction of **13**. The isolated minor component, on the other hand, turned out to be a mixture of two acetate derivatives. ^{1}H NMR spectrum of the first component of the minor acetate was identical to **18**, the major acetylated product of the reduction product of **13**. The second component was a new acetate of the series. This unknown reduction product, from which the new acetylated product was obtained, could be the compound **21** or **22**. However, ^{1}H NMR spectrum of the acetylated product suggested the compound to be **23** (the acetylated product of **22**), not **20** (the acetylated product of **21**), from the following observations. The relatively downfield signal of H9 at δ3.07, as compared with δ2.68 of its C5 epimer **18**, suggested the A/B ring junction to be *cis*. The broad singlet signal of H3 at δ5.05 indicated the β-orientation of the C3 hydroxyl group. The ratio of the major acetate **19** and the minor acetates **18** and **23** was 10:1, while that of **18** and **23** was *ca* 10:7, the latter being estimated from ^{1}H NMR spectrum.

Sodium borohydride reduction of the *cis*-A/B fused ketone **14** thus provided a better method of synthesizing compound **17** (isolated as its acetate **19**), which was then subjected to acetonide deprotection with 70% AcOH[7] to the acetate **24**. Deacetylation of **24** was effected by treatment with guanidine acetate to yield 3-*epi*-2-D-20-ECD (**6**).The overall yield of **6** from the mesylate **12** was 21%. The spectroscopic (^{1}H NMR, MS) data was consistent with the structure **6** and with the reported data of **6** synthesized previously by a different route.[10] In order to prove that deacetylation step of **24** to **6** with guanidine acetate did not cause C5

epimerization, NOE experiment of the product **6** has been performed. Thus irradiation of the 19-Me signal at δ 0.98 resulted in enhancement of the H5 signal at δ2.29.



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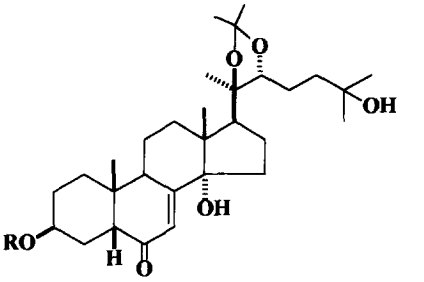
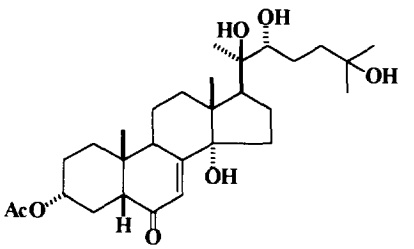
21 R = H



22 R = H
23 R = Ac

In order to prepare ecdysteroid analogues for further studies, the compounds **13**, **14** and **18** were subjected to deacetonation[7] and the compounds **10**, **9** and **25** were respectively obtained. Compound **25** was deacetylated with guanidine acetate to yield compound **8**. The spectroscopic data of these ecdysteroid analogues were consistent with their structures.



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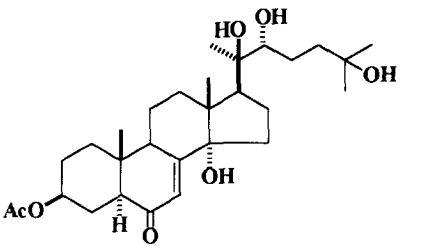

25

Biological activity. We have reported[2] that the moulting hormone activity of 2-D-20-ECD (**2**) was lower than that of 20-ECD (**1**) in the *Musca* bioassay. The activity of 2-D-20-ECD 3-acetate (**3**) was comparable to that of **2**, but was higher than that of 2-D-20-ECD 22-acetate (**4**). 2-D-20-ECD 22-benzoate (**5**) was the least active ecdysteroid in the series. In the present work, we found that 3-*epi*-2-D-20-ECD (**6**) was much less active than **2**, but it was nevertheless more active than **4**. The reported[10] activity of the ecdysteroid **6** was 1/3 of that of **2** in the *Calliphora* assay; the activities of the two assays were therefore in the same direction.

Interestingly, the activity of 2-D-5α-20-ECD (**8**), though relatively low, was comparable to that of **4**. Since it was established that a *cis*-A/B ring junction is an essential feature for an ecdysteroid to exhibit moulting hormone activity,[12] the bioassay results suggested that the *in vivo* C5 epimerization of **8** to the corresponding *cis*-A/B fused ecdysteroid was possible.

Although the activity of **6** was also lower than that of **2** in our *Musca* assay, it was significantly higher than those of other 3α-hydroxy analogues.[12] One possible explanation for this rather unusually high activity was that the ecdysteroid **6** could be transformed to its C3 epimer, the ecdysteroid **2**, possibly through the intermediate **9**, 3-dehydro-2-D-20-ECD. We therefore determined the moulting hormone activity of **9** and it turned out that the activity of this compound was comparable to that of compound **4**, thus suggesting that such *in vivo* transformation was possible. The activity of 3-dehydro-2-D-5α-20-ECD (**10**) and 2-D-5α-20-ECD (**8**), the C5 epimers of **9** and **2**, respectively, have also been evaluated and, as expected, it was found that these compounds were less active than their C5 epimers. However, the positive bioassay results, though relatively less active, suggested that there might be mechanism(s) for these compounds to be converted to their C5 epimers. It worth to note that compound **10** was less active than **8** and possible explanation was that **10** required more step(s) in going to **2** (and then to **1**) than that required in **8**.

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. EI mass spectra were measured on a Hewlett Packard 5896 instrument operating at 70 eV. FAB mass spectra were measured on a Finnigan MAT 90 instrument. The microanalyses were performed by the Department of Chemistry, Faculty of Science, Mahidol University and the Scientific and Technological Research Equipment Centre, Chulalongkorn University. 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.

Synthesis of 3-dehydro-2-D-5α-20-ECD 20,22-acetonide (13)

To a solution of the mesylate **12**[2] (172 mg, 0.287 mmol) in MeOH (4 ml) was added 5% NaOH (5 ml, 6.250 mmol) and the reaction mixture stirred at ambient temperature for 50 min. The mixture was acidified with 5% AcOH and extracted with $CHCl_3$ (3x40 ml). The combined $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The crude mixture (126 mg) was purified by column chromatography using $CHCl_3$-MeOH as eluting solvent, with increasing amount of the more polar component. Fractions eluted by $CHCl_3$-MeOH (97.5:2.5) afforded **13** as gums (85 mg, 59%); IR: ν_{max} 3450, 2968, 1710, 1662, 1468, 1375, 1250, 1215, 1105, 1002, 745 cm^{-1}; ^{1}H NMR data is given in Table; FABMS (-ve) : m/z 501.3217 $[M-H]^-$. $C_{30}H_{45}O_6$ requires 501.3216.

Synthesis of 3-dehydro-2-D-5α-20-ECD 20,22-acetonide (13) and 5α-20-ECD 20,22-acetonide 2-mesylate (15)

The mesylate **12** (550 mg, 0.919 mmol) was reacted with 5% NaOH in the same manner as described in the above reaction, except that the reaction mixture was stirred for 30 min. The crude products were separated by column chromatography, using $CHCl_3$-MeOH as eluting solvent. Fractions eluted by $CHCl_3$-MeOH (97.5:2.5) afforded compound **13** (210 mg, 45%). The second combined fraction eluted by $CHCl_3$-MeOH (95.5:4.5) gave the starting material **12** (95 mg). The last combined fraction, eluted by $CHCl_3$-MeOH (95:5), was identified to be compound **15** and was obtained non-crystalline. (38 mg, 7%).

15: IR: ν_{max} 3438, 2970, 1667, 1458, 1374, 1340, 1253, 1216, 1172, 1093, 977, 919 cm^{-1}; ^{1}H NMR data is given in Table; EIMS: m/z (% rel. intensity) 327 $[M-C_9H_{17}O_2-H_2O-CH_3SO_3H]^+$ (6), 309 (9), 283 (26), 265 (10), 231 (6), 213 (9), 201 (10), 195 (7), 183 (12), 158 (9), 143 (22), 140 (6), 125 (41), 122 (6); FABMS (-ve): m/z 597.3097 $[M-H]^-$. $C_{31}H_{49}O_9S$ requires 597.3097.

Sodium borohydride reduction of 3-dehydro-2-D-5α-20-ECD 20,22-acetonide (13)

To a solution of the ketone **13** (43 mg, 0.085 mmol) in THF (1 ml) was added 2 ml (0.229 mmol) portion of the freshly prepared solution of $NaBH_4$ (13 mg in 3 ml absolute EtOH). After stirring for 5 min at ambient temperature, the reaction was stopped by addition of one drop of AcOH and water (60 ml) was then added. The resulting solution was extracted with $CHCl_3$ (3x15 ml); the combined $CHCl_3$ layer was washed with water, dried and evaporated. The crude mixture (53 mg) was subjected to column chromatography, using $CHCl_3$-MeOH as eluting solvent. Fractions eluted by $CHCl_3$-MeOH (97:3) gave an inseparable mixture, the ^{1}H NMR data of which (see Table) indicated a 5:1 mixture of 2-D-5α-20-ECD 20,22-acetonide (**16**) and 3-*epi*-2-D-20-ECD 20,22-acetonide (**17**) (41 mg, 95%).

Acetylation of a mixture 2-D-5α-20-ECD 20,22-acetonide (16) and 3-*epi*-2-D-20-ECD 20,22-acetonide (17)

The mixture of compounds **16** and **17** (41 mg, 0.081 mmol) was dissolved in pyridine (2 ml), then Ac_2O (0.5 ml, 5.294 mmol) was added. The reaction mixture was left to stir at ambient temperature for 2 h and worked up in the usual manner. The crude products were separated by column chromatography ($CHCl_3$-MeOH from 99:1 to 98.5:1.5) to afford 3-*epi*-2-D-20-ECD 3-acetate 20,22-acetonide (**19**) (22 mg) and 2-D-5α-20-ECD 3-acetate 20,22-acetonide (**18**) (19 mg).

18: Amorphous, mp 245-247 °C (from $CHCl_3$-MeOH); IR: ν_{max} 3466, 2968, 1733, 1670, 1468, 1375, 1239, 1113, 1035, 1001, 927, 896, 870 cm^{-1}; ^{1}H NMR data is given in Table; EIMS: m/z (% rel. intensity) 389

$[M-C_9H_{17}O_2]^+$ (11), 371 (8), 329 (14), 327 (15), 311 (6), 267 (12), 201 (9), 183 (6), 158 (6), 143 (25), 125 (40); FABMS (+ve): m/z 547.3643 $[M+H]^+$. $C_{32}H_{51}O_7$ requires 547.3634.

19: Amorphous (from $CHCl_3$-MeOH); IR: ν_{max} 3420, 2964, 1742, 1655, 1456, 1373, 1238, 1138, 1029 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 389 $[M-C_9H_{17}O_2]^+$ (7), 371 (16), 329 (41), 327 (15), 311 (20), 267 (26), 201 (11), 183 (10), 158 (8), 143 (38), 125 (63). Anal. Calcd. for $C_{32}H_{50}O_7$: C, 70.32; H, 9.15. Found: C, 70.70; H, 8.72.

Synthesis of 3-dehydro-2-D-20-ECD 20,22-acetonide (14)

To a solution of the mesylate **12** (137 mg, 0.229 mmol) in MeOH (1 ml) was added 5% guanidine acetate (10 ml, 4.201 mmol) and the reaction mixture left to stir at 50 °C for 16 days. Water (100 ml) was then added and the mixture extracted with EtOAc (3x20 ml). The combined EtOAc layer was treated in the usual manner and the crude product (114 mg) was chromatographed using $CHCl_3$-MeOH as eluting solvent. Fractions eluted by $CHCl_3$-MeOH (97:3) afforded compound **14** as foams (96 mg, 84%); IR: ν_{max} 3438, 2966, 1711, 1662, 1453, 1373, 1251, 1216, 1172, 1103, 1001, 754 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 474 $[M-CO]^+$ (1), 456 (2), 438 (5), 396 (15), 314 (24), 298 (49), 271 (100), 91 (48); FABMS (-ve) : m/z 501.3219 $[M-H]^-$. $C_{30}H_{45}O_6$ requires 501.3216.

Sodium borohydride reduction of 3-dehydro-2-D-20-ECD 20,22-acetonide (14)

Compound **14** (86 mg, 0.171 mmol) was subjected to sodium borohydride reduction in similar manner to that employed for compound **13**, except that the reaction mixture was stirred at 5-10 °C in place of ambient temperature. The product was purified by column chromatography, using $CHCl_3$-MeOH as eluting solvent, and fraction eluted by $CHCl_3$-MeOH (97:3) gave a mixture of products (75 mg). ^1H NMR data indicated that the mixture consisted of the major product **17** and a small quantity of minor product(s). This mixture has been used in subsequent experiment.

Acetylation of a mixture of 3-*epi*-2-D-20-ECD 20,22-acetonide (17) and minor product(s)

The mixture of **17** and minor component(s) (29 mg) was subjected to acetylation in the same manner as described in case of the mixture of **16** and **17**. The products were separated by column chromatography using $CHCl_3$-MeOH as eluting solvent to give 3-*epi*-2-D-20-ECD 3-acetate 20,22-acetonide (**19**) (20 mg, 64%), eluted by $CHCl_3$-MeOH (99.5:0.5). Fractions eluted by $CHCl_3$-MeOH (99:1) afforded a *ca* 10:7 mixture of 2-D-20-ECD 3-acetate 20,22-acetonide (**23**) and 2-D-5α-20-ECD 3-acetate 20,22-acetonide (**18**) (3 mg, 10%).

Acetonide deprotection of 3-*epi*-2-D-20-ECD 3-acetate 20,22-acetonide (19)

Compound **19** (16 mg, 0.029 mmol) was subjected to acetonide deprotection in the same manner as described for **18**. The products were purified in the usual fashion to afford 3-*epi*-2-D-20-ECD 3-acetate (**24**) as gums (10 mg, 67%); IR: ν_{max} 3420, 2964, 1742, 1655, 1456, 1373, 1307, 1238, 1176, 1138, 1103, 1081, 1029 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 389 $[M-C_6H_{13}O_2]^+$ (5), 371 (22), 329 (19), 311 (100), 293 (8), 267 (15), 161 (16), 143 (19), 125 (24), 117 (14), 107 (20), 99 (47), 81 (59); FABMS (-ve) : m/z 505.3164 $[M-H]^-$. $C_{29}H_{45}O_7$ requires 505.3165.

Deacetylation of 3-*epi*-2-D-20-ECD 3-acetate (24)

To a solution of the acetate **24** (9 mg, 0.017 mmol) in MeOH (1 ml) was added 5% guanidine acetate (2 ml, 0.840 mmol) and the mixture stirred at 50 °C for 2 weeks. Water (40 ml) was then added and the mixture extracted with *n*-BuOH (3x20 ml). The combined organic layer was washed with water and evaporated by co-distillation with water under reduced pressure to afford 3-*epi*-2-D-20-ECD (**6**) (6 mg, 75%); IR: ν_{max} 3340, 2960, 1640, 1558, 1408, 1381, 1299, 1054, 963, 926, 874, 654 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 465.3217 $[M+H]^+$. $C_{27}H_{45}O_6$ requires 465.3216.

Acetonide deprotection of 3-dehydro-2-D-5α-20-ECD 20,22-acetonide (13)

The acetonide **13** (57 mg, 0.113 mmol) in 70% AcOH (2 ml) was stirred at ambient temperature for 4 days. Water (100 ml) was then added and the mixture extracted with *n*-BuOH (3x30 ml). The combined organic layer was washed with water and evaporated by co-distillation with water under reduced pressure. The crude mixture (40 mg) was subjected to column chromatography using $CHCl_3$-MeOH as eluting solvent. Fractions eluted by $CHCl_3$-MeOH (96:4) afforded the starting material **13** (5 mg) and fractions eluted by $CHCl_3$-MeOH (95:5) gave 3-dehydro-2-D-5α-20-ECD (**10**) as needles (from MeOH-$CHCl_3$), mp 255-256 °C (31 mg, 60%

based on unrecovered starting material); IR: ν_{max} 3416, 2960, 1711, 1657, 1461, 1377, 1184, 1152, 1125, 1067, 945, 919, 904, 865 cm^{-1}; ^1H NMR data is given in Table; Anal. Calcd. for $C_{27}H_{42}O_6 \cdot 1/2H_2O$: C, 68.78; H, 9.12. Found: C, 68.53; H, 9.14.

Acetonide deprotection of 3-dehydro-2-D-20-ECD 20,22-acetonide (14)

The mesylate **14** (21 mg, 0.041 mmol) was subjected to acetonide deprotection in the same manner as described in case of preparation of **10** from **13**. The product was purified by column chromatography to afford 3-dehydro-2-D-20-ECD (**9**) as powders (from MeOH-$CHCl_3$), mp 225-226 °C (10 mg, 53%); IR: ν_{max} 3392, 2958, 1696, 1672, 1383, 1146, 919 cm^{-1}; ^1H NMR data is given in Table; Anal. Calcd. for $C_{27}H_{42}O_6 \cdot 1/2H_2O$: C, 68.78; H, 9.12. Found: C, 68.78; H, 8.80.

Acetonide deprotection of 2-D-5α-20-ECD 3-acetate 20,22-acetonide (18)

Compound **18** (9 mg, 0.016 mmol) was treated with 70% AcOH (1 ml) and the mixture stirred at ambient temperature for 5 days. Water (30 ml) was then added and the mixture extracted with EtOAc (3x15 ml). The combined EtOAc extract was washed with water, dried and evaporated. The crude product (8 mg) was chromatographed using $CHCl_3$-MeOH as eluting solvent. Fractions eluted by $CHCl_3$-MeOH (97:3) gave 2-D-5α-20-ECD 3-acetate (**25**) as needles (from MeOH-$CHCl_3$), mp 257-259 °C (6 mg, 75%); IR: ν_{max} 3420, 2958, 1735, 1712, 1656, 1464, 1381, 1237, 1135, 1029, 960, 902, 874, 835 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 389 $[M-C_6H_{13}O_2]^+$ (6), 371 (29), 329 (16), 327 (27), 311 (70), 293 (12), 285 (9), 267 (30), 161 (22), 143 (43), 125 (21), 117 (27), 107 (40), 99 (84), 81 (100); FABMS (-ve) : m/z 505.3162 $[M-H]^-$. $C_{29}H_{45}O_7$ requires 505.3165.

Deacetylation of 2-D-5α-20-ECD 3-acetate (25)

The acetate **25** (5 mg, 0.009 mmol) was subjected to deacetylation in the same manner as described for compound **24** to give 2-D-5α-20-ECD (**8**) (3 mg, 66%); IR: ν_{max} 3408, 2930, 1647, 1556, 1441, 1382, 1156, 1066, 900, 869, 848 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 465.3218 $[M+H]^+$. $C_{27}H_{45}O_6$ requires 465.3216.

Biological activity testing. The *Musca* bioassay has been performed by the method referred to previously.[5]

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