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Synthesis and Biological Activity of 2-Deoxy-20-hydroxyecdysone and Derivatives

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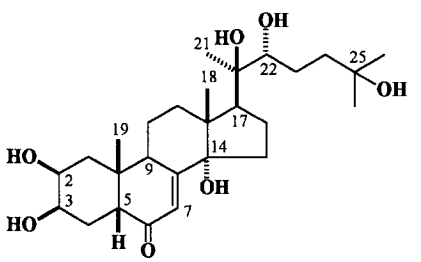
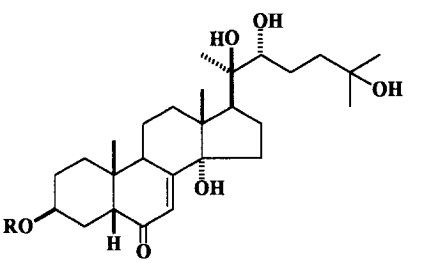
Abstract: The naturally occurring ecdysteroids 2-deoxy-20-hydroxyecdysone (2-D-20-ECD, 2-deoxycrustecdysone), 2-D-20-ECD 3-acetate (2-deoxyecdysterone 3-acetate), 2-D-20-ECD 22-acetate and 2-D-20-ECD 22-benzoate have been partially synthesized from 20-hydroxyecdysone. Moulting hormone activity of these ecdysteroids has been studied using the *Musca* bioassay.

The 2-deoxy (2-D) analogues of the insect moulting hormone 20-hydroxyecdysone (20-ECD, **1**) are a small group of ecdysteroids. Among them are 2-deoxy-20-hydroxyecdysone (2-D-20-ECD, 2-deoxycrustecdysone, **2**), 2-D-20-ECD 3-acetate (2-deoxyecdysterone 3-acetate, **3**), 2-D-20-ECD 22-acetate (**4**) and 2-D-20-ECD 22-benzoate (**5**).[1] Compound **2** has originally been isolated from the marine crayfish *Jasus lalandei* in 1968[2] and then from the fern *Blechnum minus*.[3] This ecdysteroid has also been isolated from some other animal and plant species.[4,5] The acetate and benzoate derivatives of **2**, i.e., compounds **3**, **4** and **5**, were rare phytoecdysteroids isolated from three *Silene* species.[6-8] We wish to report partial synthesis of compounds **2** to **5** from the readily available ecdysteroid **1** and moulting hormone activity of these ecdysteroids has been studied.



1

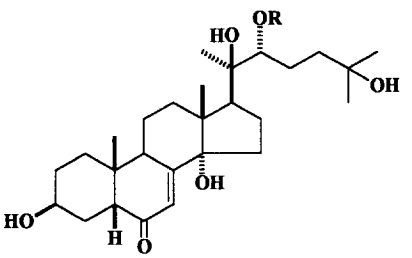


2 R = H
3 R = Ac

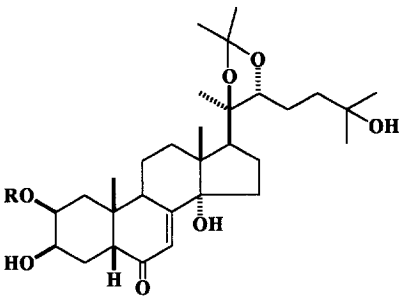
RESULTS AND DISCUSSION

The strategy for the synthesis of ecdysteroid **2** and its derivatives **3-5** involved removal of the extra C2 oxygen function from the readily available ecdysteroid **1**.[9] The synthetic conditions to be employed should not be harmful to the ecdysteroid, e.g., C5 epimerization and dehydration of the 14α-hydroxyl group could be avoided. Obviously, deoxygenation method involving the phosphorane intermediate as described previously[10] could not be used in this case, since it would yield a 3-deoxyecdysteroid analogue.

Deoxygenation of an alcoholic function by metal hydride (especially $LiAlH_4$ or $LiBHEt_3$) reductive elimination of a suitable derivative (tosylate, mesylate, halide, for example) is well-documented.[11] However, it is not possible to effect such reductive elimination of an ecdysteroid without reducing the 6-keto group to the corresponding epimeric alcohols.[12] In our experience, selection of any synthetic route through the reduction of such keto function should not be the first choice. In our case, we found that the C2 oxygen function in **1** could



4 R = Ac
5 R = PhCO



6 R = Ms
7 R = H

be removed by catalytic reductive cleavage of the mesyloxyl group in **6**. The mesylate **6** was prepared as followed. The 20,22-acetonide **7**, prepared by the literature method,[13] was subjected to selective mesylation and the spectroscopic (^{1}H NMR and EIMS) data obtained were consistent with the structure **6**. Thus the downfield (1.11 ppm) shift of the H2 signal of **6** as compared with that of 7 indicated that mesylation took place at the 2-position. As expected, the equatorial 2-hydroxyl group was more readily mesylated than the axial 3-hydroxyl group. The result was in agreement with the reported acetylation rate study.[14] Hydrogenolysis of **6**, with 5% Pd-C as a catalyst, afforded the product **8** together with some unidentified products. The amount of the latter components could be lowered by monitoring the progress of the reaction by TLC. Deoxygenation at C2 position was achieved as evident from the absence of the mesyloxyl and the H2β resonances around δ3 and 4.9, respectively, in the ^{1}H NMR spectrum of **8**. The br s signal of H3 at δ4.08 suggested that the A/B *cis*-ring junction has been preserved under the reaction conditions employed. The chemical shift values of the H5, H7, H9, H17 as well as those of the angular methyl groups were comparable to those of the compound 7. Acetonide deprotection of **8** with 70% AcOH[13] afforded 2-D-20-ECD (**2**). The overall yield of **2** from compound 7 was 22%. The multiplet signal of H5 was the only different feature of the ^{1}H NMR spectrum of **2** as compared with those of most of ecdysteroids, including compound **8**, which normally appear as dd signals with J = *ca* 13 and 4 Hz. However, the characteristic dd spectral feature of H5 was obtained when the ^{1}H NMR spectrum has been recorded in methanol-d_4. In order to confirm the *cis*-A/B nature of the compound **2**, an NOE experiment[10,15] was performed. Thus upon irradiation of the 19-Me signal at δ1.05, enhancement of the H5β signal at δ2.97 was observed. Such NOE enhancement occurs only when the 19-Me and H5 are in *cis*-arrangement. Compound **2** has previously been synthesized by a multi-step transformation from pregnenolone.[16]

Acetylation of **8** to the corresponding acetate **9**, followed by removal of the acetonide protecting group in **9** gave 2-D-20-ECD 3-acetate (**3**) in 56% overall yield from **8** or 19% overall yield from **7**. The main ^{1}H NMR features of **3** were similar to those of **2**, except for the presence of an acetoxyl methyl signal and the relative positions of the H3 and H5 resonances. The H3 resonance appeared at δ5.01, a 0.89 ppm downfield relative to that of **2**. This acetylation shift was of the magnitude expected for such system. The H5 signal appeared at δ2.58, a 0.39 ppm upfield shift as compared with that of the compound **2**. Such diamagnetic shift in going from free C3 hydroxyl function to the corresponding acetate derivative has been observed in the compound **1** series.[13]

The 2,3-acetonide **10** has been chosen as the starting material for the synthesis of the C22 acetate and benzoate derivatives of **2**. This acetonide has been prepared from **1** by the literature procedure.[13] Acetylation of

Table ^{1}H NMR Data of Ecdysteroids and Analogues

H	2		3	4	5	6
	C_5D_5N	CD_3OD	C_5D_5N	C_5D_5N	C_5D_5N	$CDCl_3$
2						4.91 (m)
3	4.12 (br s)	3.98 (m)	5.01 (br s)	4.12 (br s)	4.13 (br s)	4.30 (br s)
5	2.97 (m)	2.41 (dd,12.8, 4.2)	2.58[≠]	2.98 (m)	2.98 (m)	2.52 (dd, 13.4, 3.9)
7	6.25 (d, 2.1)	5.79 (d, 2.1)	6.22 (br s)	6.22 (d, 2.4)	6.24 (d, 1.8)	5.87 (d, 2.4)
9	3.54 (m)	3.20 (m)	3.44 (m)	3.53 (m)	3.55 (m)	3.01 (m)
17	3.03 (t, 9)	2.39 (t, 8)	3.02 (t, 9.1)	3.01 (t, 8.8)	3.11 (t, 9.1)	2.23 (dd, 9.6, 7.5)
22	3.88 (br d, 10.3)	3.32 (dd, *ca* 10, 1.5)[#]	3.88 (br d, 10.1)	5.50 (dd, 10.6, 2.1)	5.79 (dd, 10.5, 1.7)	3.65 (dd, 9.4, 2.4)
18-Me	1.23 (s)	0.88 (s)[a]	1.21 (s)	1.18 (s)	1.21 (s)	0.80 (s)
19-Me	1.05 (s)	0.95 (s)[a]	0.99 (s)	1.04 (s)	1.05 (s)	1.02 (s)
21-Me	1.60 (s)	1.19 (s)[b]	1.59 (s)	1.63 (s)	1.78 (s)	1.16 (s)
26-Me	1.36 (s)	1.18 (s)[b]	1.37 (s)	1.33 (s)	1.30 (s)	1.24 (s)
27-Me	1.36 (s)	1.19 (s)	1.37 (s)	1.34 (s)	1.31 (s)	1.25 (s)
$\rangle$C(Me)$_2$	-	-	-	-	-	1.33, 1.41 (each s)
AcO	-	-	1.98 (s)	2.02 (s)	-	-
MsO	-	-	-	-	-	3.09 (s)
$PhCO_2$	-	-	-	-		-
2', 6'					8.27 (m)	
3', 5'					7.35 (m)	
4'					7.46 (m)	

[#]Partially obscured by water signal.

[≠]Obscured signal.

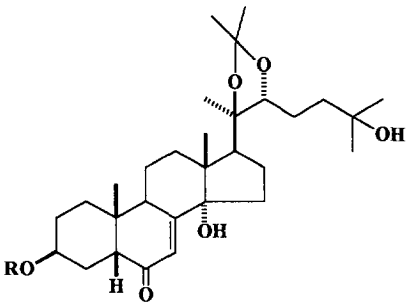
[a,b]Assignments may be reversed for signals with the same superscript.

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

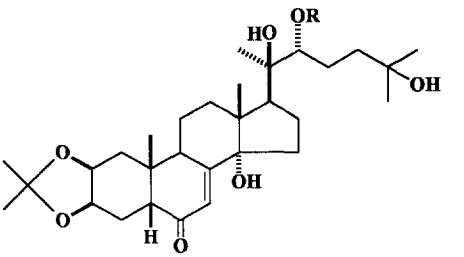
H	7	8	9	13	15	16
	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_5D_5N	$CDCl_3$
2	3.80 (m)			4.88 (m)	4.18 (m)	4.89 (m)
3	4.03 (br s)	4.08 (br s)	5.05 (br s)	4.27 (br s)	4.23 (br s)	4.28 (br s)
5	2.39#	2.48 (dd,11.8, 4.1)	2.35 (dd, 12.5, 4.2)	2.49 (dd, 13.2, 4.1)	3.01 (dd, 13.2, 3.5)	2.50 (dd, 13.4, 3.9)
7	5.84 (d, 2.1)	5.86 (d, 2.1)	5.83 (d, 2.4)	5.84 (d, 2.4)	6.22 (d, 2.4)	5.86 (d, 2.4)
9	2.97 (m)	3.06 (m)	3.07 (m)	3.01 (m)	3.59 (m)	3.02 (m)
17	2.39#	2.24 (dd, 9.1, 7.6)	2.22 (dd, 9.6, 7.7)	2.35 (t, 9.1)	3.08 (t, 9.1)	2.43 (t, 8.9)
22	3.60 (m)	3.66 (dd, 9.4, 2.4)	3.64 (dd, 9.4, 2.1)	4.83 (dd, 10.3, 2.1)	5.78 (br d, 10.3)	5.13 (dd, 10.6, 2.1)
18-Me	0.78 (s)	0.80 (s)	0.78 (s)	0.83 (s)	1.19 (s)	0.85 (s)
19-Me	0.96 (s)	0.99 (s)	0.95 (s)	0.99 (s)	1.06 (s)	1.00 (s)
21-Me	1.15 (s)	1.16 (s)	1.14 (s)	1.24 (s)	1.76 (s)	1.36 (s)
26-Me	1.20 (s)	1.24 (s)	1.21 (s)	1.19 (s)	1.30 (s)	1.19 (s)
27-Me	1.29 (s)	1.25 (s)	1.22 (s)	1.21 (s)	1.30 (s)	1.20 (s)
$\rangle C(Me)_2$	1.32, 1.36 (each s)	1.33, 1.41 (each s)	1.30, 1.39 (each s)	-	-	-
AcO	-	-	2.03 (s)	2.09 (s)	-	-
MsO	-	-	-	3.06 (s)	-	3.07 (s)
$PhCO_2$	-	-	-	-		
2', 6'					8.26 (m)	8.05 (m)
3', 5'					7.35 (m)	7.44 (m)
4'					7.46 (m)	7.57 (m)

#Partially overlapping signals.

10 to the corresponding acetate **11**[13] followed by acetonide deprotection afforded the 22-acetate **12**.[13] Selective mesylation of **12** gave the mesylate **13**. Catalytic hydrogenolysis of **13** gave 2-D-20-ECD 22-acetate (**4**) in 12% overall yield from **10**. The ^{1}H NMR spectral data and features of **4** were very similar to those of **2**, except for the presence of an acetoxyl methyl signal at δ2.02 and the large downfield (1.62 ppm) acetylation shift of the H22 signal. It should be noted that attempt has been made to selectively acetylate the compound **2** in order to obtain only **4**. The results, however, were not promising; the mixture of **3** and **4** were yielded, together with a diacetate.

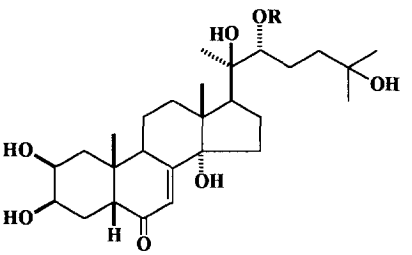


8 R = H
9 R = Ac

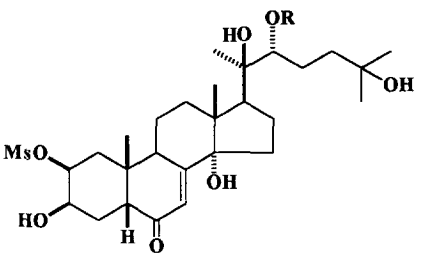


10 R = H
11 R = Ac
14 R = PhCO

The 22-benzoate derivative **5** has been synthesized in similar manner as employed for the synthesis of **4**. Thus benzoylation of **10** followed by acetonide deprotection of the resulting benzoate **14** gave **15**. Selective mesylation of **15** then gave the mesylate **16**. Catalytic hydrogenolysis of **16** furnished 2-D-20-ECD 22-benzoate (**5**) in 36% overall yield from **10**. The ^{1}H NMR spectral data was in agreement with the structure **5**. The presence of a benzoyl group at the C22 position was evident from a large downfield (1.91 ppm) shift of the H22 resonance, as compared with that of **2**. The *cis*-A/B nature of **4** and **5** have also been preserved throughout the transformations as evident from the small $W_{1/2}$ value of H3 and a relatively downfield chemical shifts (δ 3.53 and 3.55, respectively) of the H9 signals.



12 R = Ac
15 R = PhCO



13 R = Ac
16 R = PhCO

Biological activity. The moulting hormone activity of 2-D-20-ECD (**2**) was lower than that of 20-ECD (**1**) in the *Musca* bioassay. 2-D-20-ECD 3-acetate (**3**) was about as active as compound **2**, whereas 2-D-20-ECD 22-acetate (**4**) was less active than **3**. 2-D-20-ECD 22-benzoate (**5**) exhibited very low activity and was the least active ecdysteroid in the series.

Previous reports indicated that the ecdysteroid **2** was as active as **1** in the *Calliphora* assay[2,3] and the result has led to a conclusion that the 2-hydroxyl group is not essential for biological activity.[3,12,17] The relatively

low activity of **2** in the *Musca* assay was quite significant and it raised a hypothesis that the 2-hydroxyl group is also responsible for high activity in the *Musca* assay. The presence of a 2β-hydroxyl group (as in **1**) may be needed for specific binding to the polar centre of the active site. If this is the case in both the *Musca* and *Calliphora* species, it is possible that the *in vivo* C2 hydroxylation in the house fly larvae is not as effective as that occurred in the *Calliphora* species. Alternatively, since the absence of a 2β-stabilizing group caused a *cis*-A/B fused ecdysteroid to epimerize at C5 position to the corresponding *trans*-A/B fused ecdysteroid more readily than the 2,3-dihydroxy analogue,[12] such epimerization may also occur *in vivo* and this resulted in lower activity of the ecdysteroid.

The higher moulting hormone activity of the 3-acetate **3** than the 22-acetate **4** needs some comments. As the activity of **3** was comparable to that of **2**, it was logical to propose that the rate of *in vivo* deacetylation at C3 position was faster than that occurred at the C22 position. The very low activity of the 22-benzoate **5** could then be due to the inefficient *in vivo* debenzoylation. The above interpretations were based on the assumption that difference in the *in vivo* solubility of the ecdysteroids did not effect their biological activity. However, some previous results[17] indicated that such assumption should be made with care.

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. The microanalyses were performed by the Department of Chemistry, Faculty of Science, Mahidol 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 20-ECD 20,22-acetonide 2-mesylate (6)

Compound **7** (254 mg, 0.488 mmol) was dissolved in pyridine (5 ml) and the mixture stirred at 0-5 °C for 10 min. Mesyl chloride (0.3 ml, 3.860 mmol) was added and the reaction mixture left to stir at 5 °C for 1 h and at ambient temperature for 1 h. Water (200 ml) was then added and the mixture extracted with EtOAc (3x50 ml). The combined organic phase was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The crude mixture (434 mg) was purified by column chromatography using $CHCl_3$-MeOH as eluting solvent, with gradually increasing concentration of MeOH to give compound (**6**) (290 mg, quantitative), eluted by $CHCl_3$-MeOH (95:5). IR: ν_{max} 3444, 2966, 1649, 1450, 1427, 1372, 1333, 1254, 1217, 1174, 1103, 1000, 939, 849, 757 cm^{-1}; ^{1}H NMR data is given in Table; EIMS: m/z (% rel. intensity) 345 [M-$C_9H_{17}O_2$-CH_3SO_3H]$^+$ (11), 328 (10), 327 (23), 310 (6), 283 (17), 232 (9), 213 (7), 201 (10), 183 (6), 158 (7), 143 (25), 125 (41), 122 (5), 99 (100). Anal. Calcd. for $C_{31}H_{50}O_9S{\cdot}H_2O$: C, 60.38; H, 8.44. Found: C, 59.95; H, 8.03.

Synthesis of 2-D-20-ECD 20,22-acetonide (8)

Compound **6** (125 mg, 0.209 mmol) in EtOH (1 ml) was subjected to catalytic hydrogenolysis at atmospheric pressure for 4 h, with 5% Pd-C (100 mg) as a catalyst. The mixture was filtered through a short alumina column and the residue washed with EtOH. The filtered solution was evaporated to dryness and the crude product purified by column chromatography to afford a quantity of unidentified products and compound **8** (36 mg, 34 %). IR: ν_{max} 3402, 2960, 1651, 1445, 1374, 1251, 1216, 1172, 1104, 1037, 1000, 952, 895, 871, 754 cm^{-1}; ^{1}H NMR data is given in Table; FABMS (+ve): m/z (% rel. intensity) 505 [M+H]$^+$ (32), 487 (61), 429 (25), 411 (17). Anal. Calcd. for $C_{30}H_{48}O_6{\cdot}2H_2O$: C, 66.66; H, 9.62. Found: C, 66.83; H, 9.37.

Acetonide deprotection of 2-D-20-ECD 20,22-acetonide (8)

The acetonide **8** (10 mg, 0.019 mmol) in 70% AcOH (2 ml) was stirred at ambient temperature for 4 days. The reaction mixture was poured into water and extracted with *n*-BuOH (3x20 ml). The combined organic layer was washed with water, evaporated by co-distillation with water under reduced pressure. The crude product was purified by column chromatography using $CHCl_3$-MeOH, with gradually increasing concentration of MeOH to give 2-D-20-ECD (**2**) (6 mg, 65 %), eluted by $CHCl_3$-MeOH (94:6). IR: ν_{max} 3390, 2962, 1639, 1445, 1378,

1156, 1052, 1032, 949, 895, 872 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 465.3215 $[M+H]^+$. $C_{30}H_{49}O_6$ requires 465.3215.

Acetylation of 2-D-20-ECD 20,22-acetonide (8)

Ac_2O (0.5 ml, 5.294 mmol) was added to a solution of compound **8** (18 mg, 0.035 mmol) in pyridine (2 ml) and the progress of the reaction was monitored by TLC. Water (100 ml) was then added and the mixture extracted with $CHCl_3$ (3x20 ml). The combined $CHCl_3$ extract was washed with water, dried and evaporated. The crude product (22 mg) was purified by column chromatography, using $CHCl_3$-MeOH as eluting solvent, with gradually increasing concentration of MeOH. Fractions eluted by $CHCl_3$-MeOH (98:2) yielded 2-D-20-ECD 3-acetate 20,22-acetonide (**9**) (17 mg, 87 %). IR: ν_{max} 3414, 2972, 1729, 1649, 1465, 1373, 1246, 1155, 1028, 879 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z (% rel. intensity) 547 $[M+H]^+$ (60), 530 (6), 529 (5). Anal. Calcd. for $C_{32}H_{50}O_7 \cdot H_2O$: C, 68.08; H, 9.22. Found: C, 67.85; H, 8.92.

Acetonide Deprotection of 2-D-20-ECD 3-acetate 20,22-acetonide (9)

The acetonide **9** (17 mg, 0.031 mmol) was subjected to acetonide deprotection in the same manner as employed for compound **8** and the crude product chromatographed ($CHCl_3$-MeOH, 94:6) to give 2-D-20-ECD 3-acetate (**3**) (10 mg, 64 %). IR : ν_{max} 3432, 2960, 1733, 1651, 1445, 1378, 1251, 1150, 1026 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 507.3350 $[M+H]^+$. $C_{29}H_{47}O_7$ requires 507.3321.

Synthesis of 20-ECD 22-acetate 2-mesylate (13)

Compound **10** (190 mg, 0.365 mmol) was dissolved in pyridine (4 ml), then Ac_2O (1 ml, 10.588 mmol) was added. The reaction mixture was left to stir at ambient temperature for 5 h. The reaction mixture was poured into water and the mixture extracted with EtOAc (3x20 ml). The combined organic layer was washed with water, dried and evaporated. The product **11** thus obtained was subsequently subjected to acetonide deprotection with 70% AcOH for 1 h. Water was then added to the reaction mixture and the solution extracted with *n*-BuOH (3x25 ml). The combined BuOH layer was washed with water; the solvent was evaporated by co-distillation with water under reduced pressure. The crude product **12** was used in subsequent reaction without purification. Thus to a stirred solution of crude **12** (136 mg) in pyridine (5 ml) at 0-5 °C was added a 1:1 mixture of mesyl chloride and benzene (1 ml, 6.433 mmol) and the mixture stirred for 20 min. The reaction mixture was worked up by addition of cold water and extracted with EtOAc (3x20 ml). The combined organic layer was washed with water, dried and evaporated. The crude product was purified by column chromatography, using $CHCl_3$-MeOH from 100:0 to 97:3, to afford 20-ECD 22-acetate 2-mesylate (**13**) (90 mg, 41% overall yield from **10**). IR: ν_{max} 3444, 2966, 1713, 1656, 1445, 1373, 1337, 1250, 1173, 1149, 1050, 947, 901, 850 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 601.3065 $[M+H]^+$. $C_{30}H_{49}O_{10}S$ requires 601.3046.

Synthesis of 2-D-20-ECD 22-acetate (4)

Compound **13** (60 mg, 0.100 mmol) was subjected to catalytic hydrogenolysis in the same manner as described for the preparation of **8**. The product was subjected to column chromatography to give compound **4** (15 mg, 30%). IR: ν_{max} 3414, 2964, 1715, 1651, 1378, 1249, 1043, 949 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 507.3320 $[M+H]^+$. $C_{29}H_{47}O_7$ requires 507.3321.

Synthesis of 20-ECD 22-benzoate (15)

A solution of compound (**10**) (159 mg, 0.305 mmol) in pyridine (5 ml) was stirred at 5-10 °C for 10 min and a 1:1 mixture of benzoyl chloride and benzene (1 ml, 4.303 mmol) was then added. The reaction mixture was left to stir for 1.5 h. Cold water (100 ml) was then added and the mixture extracted with EtOAc (3x25 ml). The combined organic layer was treated in the usual manner; the crude product **14** was dissolved in MeOH (1 ml) and 70% AcOH (5 ml) was then added. The reaction mixture was stirred at ambient temperature for 5 h, then worked up by water and extracted with EtOAc (3x25 ml). The crude product obtained from the combined organic extract was purified by column chromatography, using $CHCl_3$-MeOH as eluting solvent. Fractions eluted by $CHCl_3$-MeOH (95:5) was identified as 20-ECD 22-benzoate (**15**) (115 mg, 65%). IR: ν_{max} 3420, 2962, 1700, 1651, 1449, 1380, 1280, 1114, 1054, 947, 877, 713 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 585.3427 $[M+H]^+$. $C_{34}H_{49}O_8$ requires 585.3427.

Synthesis of 20-ECD 22-benzoate 2-mesylate (16)

To a solution of compound **15** (107 mg, 0.183 mmol) in pyridine (5 ml) at 5-10 °C was added a 1:1 mixture of mesyl chloride in benzene (1 ml, 6.433 mmol). The reaction mixture was left to stir for 30 min and cold water was then added. The solution was extracted with EtOAc (3x20 ml) and the combined organic layer was washed with water, dried and evaporated. The crude product (120 mg) was chromatographed, using $CHCl_3$-MeOH (96:4) to afford 108 mg (89%) of compound **16**. IR: ν_{max} 3490, 2966, 1710, 1649, 1451, 1317, 1281, 1173, 944, 851, 713 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z (% rel. intensity) 663 $[M+H]^+$ (3), 646 (13), 627 (3). Anal. Calcd. for $C_{35}H_{50}O_{10}S \cdot 1/2H_2O$: C, 62.41; H, 7.65. Found: C, 62.03, H, 8.00.

Synthesis of 2-D-20-ECD 22-benzoate (5)

Compound **16** (90 mg, 0.136 mmol) was subjected to catalytic hydrogenolysis in the same manner as described for the preparation of compound **4**. The product was purified by column chromatography, using $CHCl_3$-MeOH from 100:0 to 97.5:2.5, to give compound **5** (49 mg, 63%). IR: ν_{max} 3404, 2964, 1698, 1649, 1449, 1380, 1281, 1116, 1039, 946, 894, 754, 711 cm^{-1}; ^1H NMR data is given in Table; FABMS (+ve): m/z 569.3479 $[M+H]^+$. $C_{34}H_{49}O_7$ requires 569.3477.

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REFERENCES

1. Lafont, R.; Wilson, I. D. *The Ecdysone Handbook*; The Chromatographic Society: Nottingham. **1992**.
2. Galbraith, M. N.; Horn, D. H. S.; Middleton, E. J.; Hackney, R. J. *Chem. Commun.* **1968**, 83-85.
3. Chong, Y. K.; Galbraith, M. N.; Horn, D. H. S. *Chem. Commun.* **1970**, 1217-1218.
4. Isaac, R. E.; Rees, H. H.; Goodwin, T. W. *J. Chem. Soc., Chem. Commun.* **1981**, 418-420.
5. Saatov, Z.; Usmanov, B. Z.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1979**, 793-797; *Chem. Nat. Comp.* **1979**, *15*, 700-703.
6. Saatov, Z.; Gorovits, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1984**, 60-62.
7. Lafont, R.; Girault, J.-P.; Beynon, P.; Bouthier, A.; Bathori, M.; Varga, E.; Szendrei, K. Isolation and Identification of Phytoecdysteroids using Preparative HPLC and 2D-COSY Proton NMR. In *Insects-Plants*; Labeyrie, V.; Fabres, G.; Lachaise, D. Eds.; Dr. W. Junk Publishers: Dordrecht, Netherlands, **1987**, p. 400.
8. Girault, J.-P.; Bathori, M.; Varga, E.; Szendrei, K.; Lafont, R. *J. Nat. Prod.* **1990**, *53*, 279-293.
9. Suksamrarn, A.; Sommechai, C. *Phytochemistry* **1993**, *32*, 303-306.
10. Suksamrarn, A.; Charoensuk, S.; Yingyongnarongkul, B. *Tetrahedron* **1996**, in press.
11. See, for example, Smith, M. B. *Organic Synthesis*; McGraw-Hill: New York, **1994**; pp. 343-423 and references cited therein.
12. Galbraith, M. N.; Horn, D. H. S.; Middleton, E. J.; Hackney, R. J. *Aust. J. Chem.* **1969**, *22*, 1059-1067.
13. Suksamrarn, A.; Pattanaprateep, P. *Tetrahedron* **1995**, *51*, 10633-10650.
14. Galbraith, M. N.; Horn, D. H. S. *Aust. J. Chem.* **1969**, *22*, 1045-1057.
15. Nicoletti, D.; Ghini, A. A.; Brachet-Cota, A. L.; Burton, G. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1089-1093.
16. Kametani, T.; Tsubuki, M.; Higurashi, K.; Honda, T. *J. Org. Chem.* **1986**, *51*, 2932-2939.
17. 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 Press: Amsterdam, **1980**; pp. 299-324 and references cited therein.

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