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Synthesis and Biological Activity of 3-Deoxyecdysteroid Analogues

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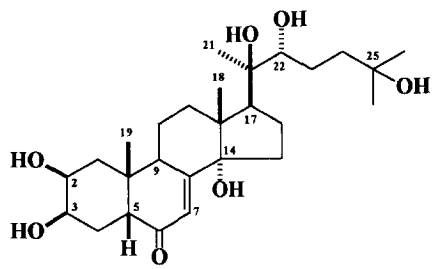
Abstract: 2-Dehydro-3-deoxy-20-hydroxyecdysone, 3-deoxy-20-hydroxyecdysone and 2-*epi*-3-deoxy-5α-20-hydroxyecdysone have been partially synthesized from 20-hydroxyecdysone. Moulting hormone activity of these compounds has been evaluated using the *Musca* bioassay.

Ecdysteroids are insect moulting hormones found in both invertebrates and plant species.[1-3] 20-Hydroxyecdysone (1) is a well-known representative of this class of compounds. Some attention has been paid to the relationships between structure and biological activity of these compounds and their analogues in order to provide some insight into the binding of ecdysteroids to the receptor. Essential features contributing to high moulting hormone activity include an A/B *cis*-ring junction, a 6-keto-7-ene grouping, a full sterol-side-chain and a free 14α-hydroxyl group.[4] Number and location of hydroxyl groups in the molecule are also responsible for high activity of ecdysteroids. Many works indicated that the 3-hydroxyl group is required for high activity of ecdysteroids and that the 2-hydroxyl group is not essential for such activity.[4-6] However, most of the ecdysteroids and their analogues used in moulting hormone bioassay were those with the 3-oxygenated and 2,3-dioxygenated functional groups in the A-ring, or those without any functionality in this ring. To date, no work has been focussed on the biological activity of ecdysteroids with only 2-oxygenated function in the A-ring. It is therefore of interest to synthesize and study biological activity of ecdysteroids with only one oxygen function at the 2-position of the A-ring.



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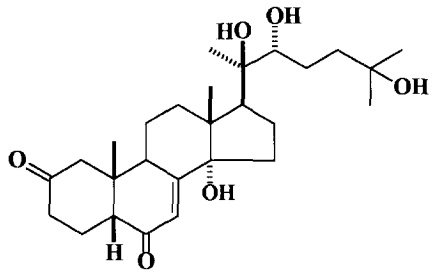
RESULTS AND DISCUSSION

In connection with our studies on structure-activity relationships of ecdysteroids and ecdysteroid analogues using the *Musca* bioassay, we would like to investigate the activity of 2-oxygenated 3-deoxyecdysteroids, particularly 2-dehydro-3-deoxy-20-hydroxyecdysone (**2**) and 3-deoxy-20-hydroxyecdysone (**3**). To our knowledge, there is no ecdysteroid, both naturally occurring and synthetic, with only one oxygen function at the 2-position of ring A.[7] To synthesize such compound, in principle if compound **2** has been synthesized first , the corresponding alcohols **3** and **4** should then be obtained by selective reduction of the C2 keto group of **2**. Alternatively, if **3** or **4** has been synthesized, the compound **2** should then be obtained by selective oxidation of either of these compounds.



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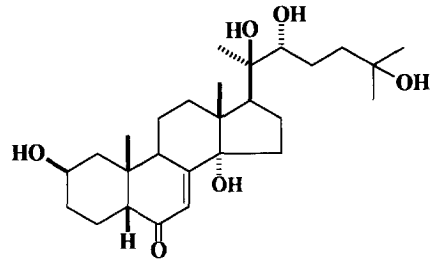

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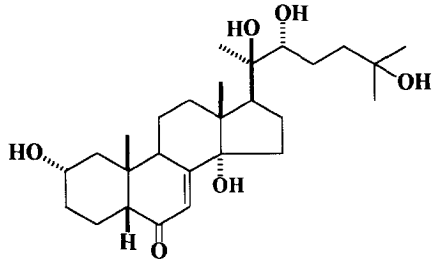
The starting material used in our synthesis was the readily available ecdysteroid **1**.[8,9] The synthetic strategy was removal of the C3 oxygen function and subsequent manipulation at C2 to give the required compounds. Despite the availability of many reactions for such first synthetic step, the structure of the starting compound **1** itself limited selection of reactions. It was found that, for example, under basic condition (aq. NaOH or NH_4OH) compound **1** was autoxidized to calonysterone and 9,20-dihydroxyecdysone.[10] Some bases (e.g., K_2CO_3 and $KHCO_3$) caused epimerization at C5 of ecdysteroids.[11-13] It was well-known that the C14 hydroxyl group of ecdysteroids subjected to dehydration rather easily, especially in certain acidic condition.[14-16]



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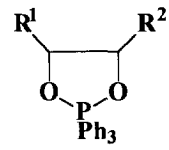

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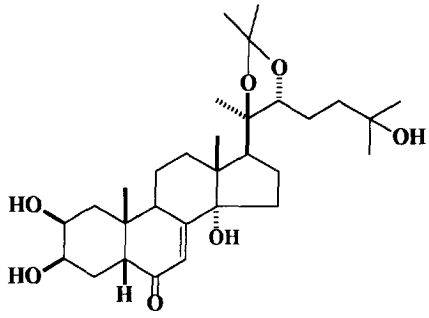
Triphenylphosphine (TPP)-diethyl azodicarboxylate (DEAD) has been employed in the Mitsunobu reaction.[17] However, it has been reported that in some cases TPP-DEAD reacted with 1,2-diols to give the corresponding phosphoranes **5**.[18-20] To us, this type of organophosphorus compound was a very interesting intermediate: expulsion of triphenylphosphine oxide from **5** should be highly possible, since in principle a large gain in energy of forming a P=O bond should be resulted.[21] It was therefore possible to remove one of the oxygen functions in the A-ring of **1**. At this stage we did not look further whether deoxygenation would take

place at the desired position. Our initial aim was to see if the phosphorane of the type **5** would be obtained. If deoxygenation happened to take place at the C2 position it was not quite a useless attempt, since it would provide a method of synthesizing 3-oxygenated ecdysteroids from 2,3-dihydroxyl analogues. To avoid any undesired site of reaction, the C20 and C22 dihydroxyl groups have been protected as the acetonide group by the literature procedure [22] to give **6**. Reaction of **6** with TPP-DEAD in refluxing THF did not give promising



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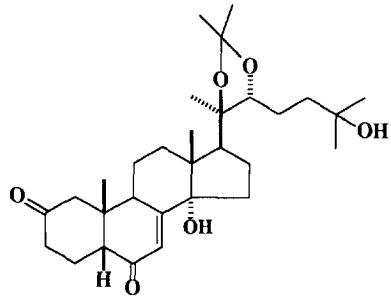
result. When the reaction was performed in the presence of formic acid, no phosphorane was isolated. In stead, the target ketone **7** was obtained in 53% yield, together with a small quantity (2%) of a *ca* 2:1 mixture of 20-hydroxyecdysone 20,22-acetonide 2-formate (**8**) and the corresponding 3-formate **9**. The spectroscopic data of **8** and **9** were consistent with the structures. The structure of the product **7** was established on the basis of



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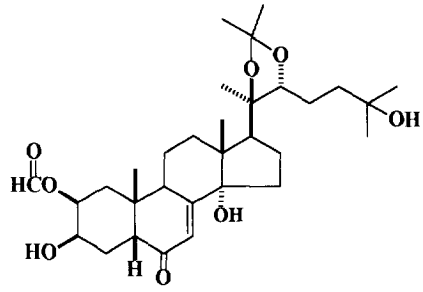

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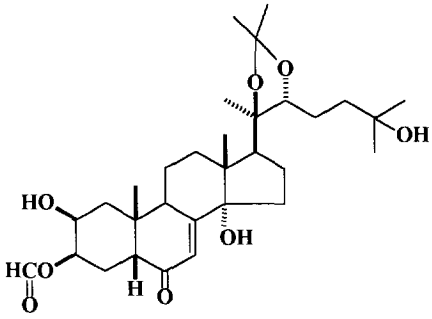

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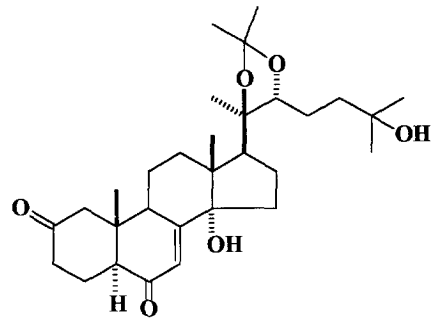
spectroscopic data. The IR spectrum of 7 showed carbonyl absorption bands of a saturated six-membered-ring ketone and an α,β-unsaturated ketone at 1710 and 1662 cm^{-1}, respectively. The ^{13}C NMR resonances (pyridine-d_5) at δ209.5 and 200.3 confirmed the presence of those two keto functions. A striking difference in the ^1H NMR spectrum of 7 as compared with that of the starting material **6** was the absence of the C2 and C3 carbinol protons. Conclusive evidence for placing the saturated keto group at the 2-position was the presence of two doublets (J = 13.8 Hz) of $H1_{ax}$ and $H1_{eq}$ at δ2.07 and 2.54, respectively. The rest of the ^1H NMR and ^{13}C NMR spectral data agreed well with the structure 7. The only point we were unhappy with was the relatively upfield shift of the H9 resonance of 7 (δ3.08 in pyridine-d_5 and δ2.71 in $CDCl_3$) as compared with that of the starting acetonide **6** (δ3.54 in pyridine-d_5[22] and δ2.97 in $CDCl_3$), since it has been observed that C5 epimerization from *cis*-A/B to *trans*-A/B ring fusion in some ecdysteroids caused upfield shift of the H9 signal.[13] It was therefore probable that the product was the compound **10**, instead of 7. However, NOE experiments[23] of the product in $CDCl_3$ led to a conclusion that the product is 7. Thus irradiation at the H5 frequency caused enhancement of the 19-Me and $H1_{ax}$, whereas irradiating at the H9 frequency did not effect the H5 signal. These results could be observed only when the ecdysteroid assumed the *cis*-A/B arrangement. Comparison of the H9 chemical shift values of ecdysteroids should therefore be made among those with the same or similar environment around the A-ring.



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The acetonide protecting group in 7 was removed by stirring with 70% AcOH[22] to yield the compound **2**. The ^1H NMR pattern and coupling constants of **2** were very similar to those of 7, except for the absence of two acetonide methyl resonances and relative positions of some proton signals. The ^{13}C NMR data were also in agreement with the structure. Selective reduction at the C2 keto group of 7 with $NaBH_4$ afforded two isomeric alcohols in a 1:3 ratio. The minor, more polar component has been shown to be **11** on the basis of ^1H NMR spectral data. Thus the carbinol proton (H2) signal of **11** at δ3.90 in $CDCl_3$ appeared as a broad multiplet, with the peak-width at half-height ($W_{1/2}$) value of 23 Hz, indicating the axial nature of H2 (i.e., the C2 hydroxyl group was in β-orientation).[13,24] The relatively downfield H9 signal (appeared at δ3.46 in pyridine-d_5 and at δ 2.98 in $CDCl_3$) suggested the A/B ring junction in **11** to be *cis*.[13] The *cis* nature of this ring fusion was further confirmed by NOE experiment. Upon irradiation at the 19-Me frequency resulted in enhancement of the H5 signal.

The major, less polar component was initially thought to be **12**, the C2 epimer of **11**. However, careful analysis of the ^1H NMR spectrum revealed this major component to be **13**, not **12**. The relatively upfield H9 resonance (chemical shift value was less than δ2.6 and δ2.3 in pyridine-d_5 and $CDCl_3$, respectively) suggesting the A/B-ring fusion to be *trans*.[13] NOE experiment also confirmed the *trans*-A/B ring fusion of **13**. Thus irradiation of the 19-Me of **13** resulted in enhancement of the H2β signal at δ4.03 ($CDCl_3$). The α-orientation of the 2-hydroxyl group was evident from the large $W_{1/2}$ value of the axial H2 signal (20 Hz in $CDCl_3$). Generation of **13** could be explained as followed. In the borohydride reduction step, the reagent preferentially approached

Table ^{1}H NMR Data of Ecdysteroids

H	1*	2	3	6*	7	
	C_5D_5N	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	C_5D_5N
1_{ax}		2.09 (d, 13.8)			2.04 (d, 14.1)	2.07 (d, 13.8)
1_{eq}		2.58 (d, 13.8)			2.54 (dd, 14.1, 1.4)	2.56 (d, 13.8)
2	4.15 (m)$^{\neq}$	-	4.12 (m)	3.80 (m)	-	-
3	4.20 (br s)$^{\neq}$			4.03 (br s)		
5	2.97 (m)$^{\#}$	2.57 (dd, 9.4, 4.3)	2.17 (dd, 12.5, 4)	2.39 (m)$^{\neq}$	2.46 (dd, 9.9, 4.4)	2.54 (dd, 9, 4.3)
7	6.22 (d, 2.1)	6.19 (d, 1.9)	6.20 (d, 2.5)	5.84 (d, 2.1)	5.86 (d, 2.1)	6.20 (d, 1.8)
9	3.55 (m)	3.12 (ddd, 12, 6.3, 1.9)	3.50 (m)	2.97 (m)	2.71 (m)	3.08 (ddd, 12.1, 5.9, 1.8)
17	2.96 (m)$^{\#}$	2.97 (t, 9.1)	2.98 (t, 9)	2.39 (m)$^{\neq}$	2.22 (dd, 9.4, 7.9)	2.73 (t, 8.8)
22	3.84 (br d, 8.9)	3.85 (br d, 9)	3.87 (br d, 10)	3.60 (m)	3.63 (dd, 9.5, 2.5)	3.92 (dd, 9.7, 2.5)
18-Me	1.18 (s)	1.17 (s)	1.20 (s)	0.78 (s)	0.77 (s)	0.96 (s)
19-Me	1.02 (s)	1.00 (s)	1.02 (s)	0.96 (s)	1.04 (s)	0.96 (s)
21-Me	1.56 (s)	1.53 (s)	1.57 (s)	1.15 (s)	1.12 (s)	1.52 (s)
26-Me	1.34 (s)	1.36 (s)	1.36 (s)	1.20 (s)	1.21 (s)	1.34 (s)
27-Me	1.34 (s)	1.36 (s)	1.36 (s)	1.29 (s)	1.22 (s)	1.35 (s)
$\rangle C(Me)_2$	-	-	-	1.32, 1.36 (each s)	1.30, 1.38 (each s)	1.28, 1.44 (each s)

*Data taken from ref. 22.

$^{\neq,\#}$Signal within the same column denotes partially overlapping signal.

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

H	8*	9*	11		13		14
	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	C_5D_5N
2	5.14 (m)	4.03 (m)	3.90 (br m, $W_{1/2}$ = 23)	4.12 (br m, $W_{1/2}$ = 24)	4.03 (br, $W_{1/2}$ = 20)	4.24 (br s, $W_{1/2}$ = *ca*18)	4.26 (br s)
3	4.14 (br s)	5.30 (br s)					
5	2.51 (dd, 13.2, 4.1)	2.30 (dd, 13.3, 3.8)	1.96 (dd, 12.9, 4.1)	2.18 (dd, 12.5, 4)	b	a	a
7	5.85 (d, 2.8)	5.85≠	5.82 (d, 2.4)	6.22 (d, 2.4)	5.81 (br s)	6.20 (br s)	6.20 (br s)
9	3.06 (m)	3.01 (m)	2.98 (m)	3.46 (m)	b	2.44 ? (m)#	2.54 ? (m)#
17	a	a	2.21 (dd, 9.6, 7.8)	2.77 (t, 8.6)	2.25 (dd, 8.9, 8.2)	2.79 (t, 8.6)	3.03 (t, 9.2)
22	3.63 (dd, 9.3, 2)	3.63 (dd, 9.3, 2)	3.64 (dd, *ca* 8, 2)	3.96 (dd, 10.1, 2.7)	3.64 (dd, 9.1, 1.8)	3.94 (dd, 9.5, 2.8)	2.87 (d, 9.2)
18-Me	0.78 (s)	0.78 (s)	0.78 (s)	1.02 (s)	0.74 (s)	1.02 (s)	1.22 (s)
19-Me	0.99 (s)	0.99 (s)	0.95 (s)	1.02 (s)	0.97 (s)	1.02 (s)	1.04 (s)
21-Me	1.14 (s)	1.14 (s)	1.14 (s)	1.56 (s)	1.11 (s)	1.52 (s)	1.54 (s)
26-Me	1.21 (s)	1.21 (s)	1.21 (s)	1.37 (s)	1.20 (s)	1.35 (s)	1.38 (s)
27-Me	1.23 (s)	1.23 (s)	1.22 (s)	1.38 (s)	1.21 (s)	1.36 (s)	1.38 (s)
$\diagdown C(Me)_2$	1.30, 1.39 (each s)	1.30, 1.39 (each s)	1.31, 1.39 (each s)	1.34, 1.47 (each s)	1.30, 1.38 (each s)	1.29, 1.45 (each s)	-
HCO_2	8.06 (s)	8.14 (s)	-	-	-	-	-

*Signals were assigned from a mixture of compounds **8** and **9**.

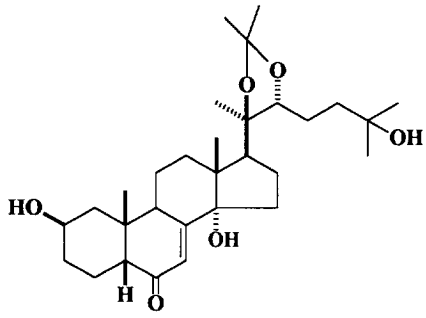
≠Partially superimposed signal.

[a]Obscured signal.

[b]Obscured by other signals. However, no signal of this proton appeared at lower field than δ2.3.

#Obscured signal or assignment was not confirmed. However, no signal of this proton appeared at lower field than δ2.6.

the C2 keto group from the less hindered, β-face of 7 to give **12** as initial major product. Steric interaction between the 2α-hydroxyl group and H9 resulted in C5 epimerization of **12** to the corresponding *trans*-A/B fused compound **13**.



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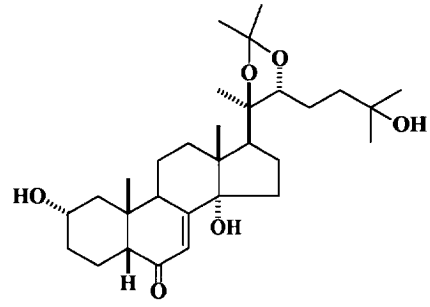

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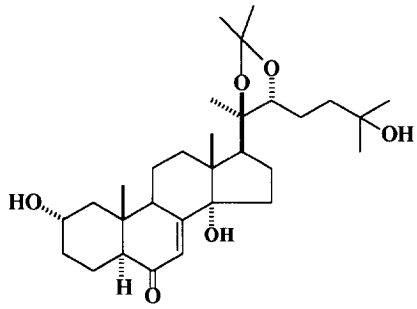

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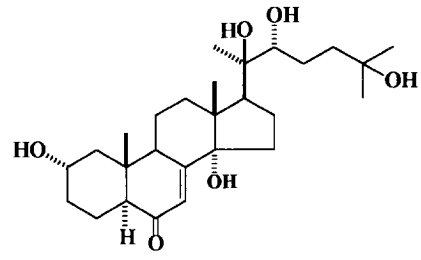

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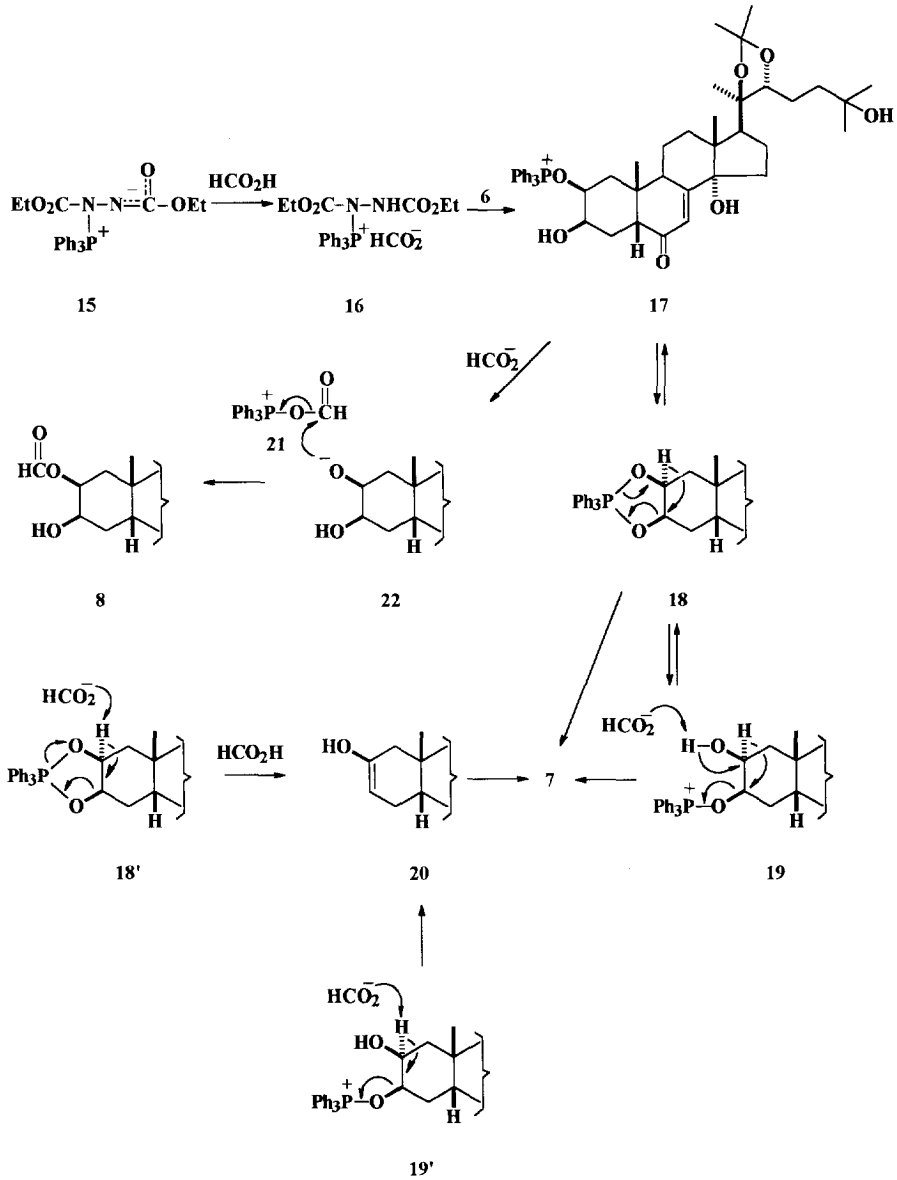
The acetonide protecting groups in **11** and **13** were removed by treating **11** and **13** with 70% AcOH[22] to yield the alcohols **3** and **14**. Alternatively, deprotection of the acetonide grouping in **7** and subsequent reduction of **2** to corresponding alcohols **3** and **14** were also investigated, but separation of the two isomeric alcohols became complicated.

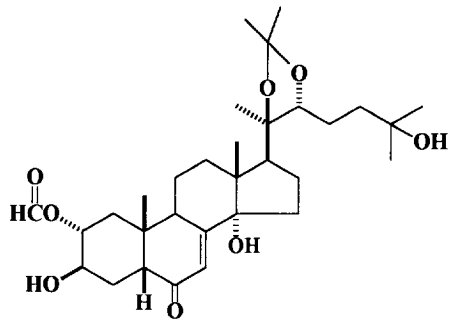
A possible explanation for the formation of the compound **7** and the formate **8** is summarized in **Scheme**. Initial reaction involved addition of TPP to DEAD to give the intermediate **15**, which would then abstract a proton from formic acid to yield the phosphonium salt **16**.[17] Reaction of **6** with **16** then gave **17**, which would possibly exist in the form of the phosphorane **18**. Under experimental condition employed, elimination of triphenylphosphine oxide took place and the compound **7** was obtained. Also, the product **7** could possibly derive from the phosphonium salt **19**, which in turn could probably derived from **17** through **18**. In principle, the salt **19** could obtained from the reaction at C3 hydroxyl group of **6** with **16**, but in practice this was less likely since the acetylation rate of the secondary hydroxyl groups in **1** was in the order 2>22>3.[15] Generation of **7** from **18** or **19** possibly involved C2→C3 hydride transfer as shown in the Scheme. Alternative possible mechanism involved proton abstraction at C2 to yield **7** through the enol **20** (i.e. **18'**or **19'**→**20** →**7**). Whether the reaction would proceed *via* the above mechanisms or some other mechanisms, we were lucky enough that deoxygenation occurred at the 3-position.

The presence of the second product, the formate **8**, could be due to attack of the formate anion at the positively-charged phosphorus in **17** (or **16**) to give **21** and **22**. Subsequent attack of **22** (or just simply **6**) at the formate carbonyl group in **21** then afforded **8** and triphenylphosphine oxide. Alternative attack of the

Scheme



formate anion at the C2 carbon of **17** did not occur since in this case inversion of configuration at C2 should result, i.e., the Mitsunobu product 2-*epi*-formate **23** should be obtained. It is quite well-known that the α-side of ring A of ecdysteroid is severely hindered and usual S_N2 displacement at C2 is expected to be difficult.[24] The isomeric formate **9** could arise from formyl migration of the 2-formate **8**, or, alternatively, be derived from **19** by a similar route to that of **8**. However, we believe that the former route is more likely, since C-2 to C-3 acetyl migration has been observed in ecdysteroids.[25-27]



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Biological activity

Compound **2** was moderately active in the *Musca* bioassay, based on the activity of **1**. Compound **3** was more active than **2**, but still less active than **1**. Compound **14,** on the other hand, exhibited very low moulting hormone activity. Since it has been established that the presence of 3β-hydroxyl group is essential for an ecdysteroid to exhibited high moulting hormone activity,[4-6] it is very likely that compounds **2** and **3** metabolized to **1** during the *in vivo* assay. The lower activity of **3** than that of **2** was probably because the former required an additional step to be transformed to **1**. The very low activity of **14** was in agreement with previous[4,5] observations for the very low activity or inactivity of 5α-ecdysteroids.

EXPERIMENTAL

IR spectra were recorded in KBr on a Jasco IR-700 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AM300 and a Jeol JNM-A500 spectrometers. ^{13}C NMR spectra were recorded on the former NMR spectrometer operating at 75.5 MHz. EIMS were measured on a Hewlett Packard 5896 instrument operating at 70 eV. 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. Spot on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

Synthesis of 2-dehydro-3-deoxy-20-hydroxyecdysone 20,22-acetonide (7)

A solution of compound **6** (415 mg, 0.798 mmol) and triphenylphosphine (1 g, 3.816 mmol) in dry THF (8 ml) was stirred at ambient temperature. A solution of 10% diethyl azodicarboxylate in THF (3.5 ml, 2.252 mmol) was added to the mixture and the contents stirred at 50 °C for 1 h. Formic acid (0.15 ml, 3.898 mmol) was then added dropwise and the mixture stirred at the same temperature for 6 days. The reaction mixture was poured into water and extracted with EtOAc (3x100 ml). The combined EtOAc extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (1.5 g) was subjected to column chromatography using $CHCl_3$ and $CHCl_3$-MeOH as eluting solvents, with a gradually increasing concentration of MeOH. The first fraction (212 mg, 53%) eluted by $CHCl_3$ was identified as 2-dehydro-3-deoxy-20-hydroxyecdysone 20,22-acetonide (**7**) and the second fraction (9 mg, 2%), eluted by $CHCl_3$-MeOH (99:1), was found to be a *ca* 2:1 mixture of 20-hydroxyecdysone 20,22-acetonide 2-formate (**8**) and 20-hydroxyecdysone 20,22-acetonide 3-formate (**9**).

7: IR: ν_{max} 3472, 2962, 1710, 1662, 1463, 1371, 1312, 1217, 1169, 1139, 1102, 1000, 922, 869, 754 cm^{-1}; ^{1}H NMR data is given in Table; ^{13}C NMR (pyridine-d_5): δ17.2 (C18), 22.1 (C16), 22.2 (C21), 22.3 (C11)[a], 23.6 (C19), 24.2 (C4)[a], 24.3 (C23)[a], 27.1 (acetonide Me), 29.4, 29.8 and 30.0 (C26, C27 and acetonide Me),

30.9 (C15)[b], 32.1 (C12)[b], 39.1 (C9), 39.5 (C3), 41.5 (C10), 42.1 (C24), 49.3 (C13), 49.8 (C17), 50.6 (C1), 53.0 (C5), 69.1 (C25), 82.4 (C22), 84.4 (C14), 84.9 (C20), 106.9 (acetonide C), 120.7 (C7), 164.9 (C8), 200.3 (C6), 209.5 (C2), [a,b]Assignments may be reversed for signals with the same superscript; EIMS: m/z (% rel. intensity) 409 $[M+H-CH_3COCH_3-2H_2O]^+$ (1), 391 (1), 345 (16), 327 (8), 309 (2), 201 (5), 158 (3), 143 (19), 125 (19), 102 (100), 99 (73), 81 (13), 59 (93).

8+**9**: IR: ν_{max} 3428, 2962, 1724, 1658, 1449, 1376, 1250, 1173, 1103, 1078, 1000, 878 cm^{-1}; ^1H NMR data is given in Table.

Acetonide deprotection of 2-dehydro-3-deoxy-20-hydroxyecdysone 20,22-acetonide (7)

Compound 7 (50 mg, 0.099 mmol) in 70% AcOH (3ml) was stirred at ambient temperature for 3 days. Water (100 ml) was added and the mixture extracted with EtOAc (3x50 ml). The combined EtOAc extract was washed with water, dried and evaporated to dryness. The crude mixture was purified by column chromatography using $CHCl_3$ and $CHCl_3$-MeOH, with a gradually increasing concentration of MeOH, to give the starting acetonide 7 (10 mg), eluted by $CHCl_3$, and 2-dehydro-3-deoxy-20-hydroxyecdysone (**2**), eluted by $CHCl_3$-MeOH (98:2) (34 mg, 93% based on un-recovered starting material 7).

2: mp 116-118 °C, IR: ν_{max} 3428, 2962, 1705, 1659, 1459, 1383, 1316, 1227, 1193, 1144, 1074, 952, 923, 872, 752 cm^{-1}; ^1H NMR data is given in Table; ^{13}C NMR (pyridine-d_5): δ17.8 (C18), 21.5 (C16), 21.5 (C21), 22.3 (C11)[c], 23.6 (C19), 24.3 (C4)[c], 27.4 (C23), 29.9 (C26), 30.1 (C27), 31.1 (C15)[d], 32.3 (C12)[d], 39.0 (C9), 39.6 (C3), 41.6 (C10), 42.6 (C24), 49.5 (C13), 49.9 (C17), 50.7 (C1), 53.2 (C5), 69.4 (C25), 76.6 (C20), 77.4 (C22), 84.4 (C14), 120.6 (C7), 165.4 (C8), 200.4 (C6), 209.5 (C2), [c,d]Assignments may be reversed for signals with the same superscript; EIMS: m/z (% rel. intensity) 345 $[M-C_6H_{13}O_2]^+$ (15), 328 (32), 327 (32), 309 (19), 301 (2), 283 (5), 269 (100), 191 (49), 161 (14), 143 (14), 125 (19), 99 (43), 81 (33). Anal. Calcd for $C_{27}H_{42}O_6 \cdot 2H_2O$: C, 65.06; H, 9.23. Found: C, 64.67; H, 9.54.

Reduction of 2-dehydro-3-deoxy-20-hydroxyecdysone 20,22-acetonide (7)

To a solution of compound 7 (30 mg, 0.059 mmol) in dry THF (1 ml) was added a 1 ml portion of the freshly prepared solution of $NaBH_4$ (26 mg in 10 ml AR MeOH). After stirring for 1 min at ambient temperature, the reaction was stopped by addition of one drop of AcOH. Water (30 ml) was then added and the solution extracted with EtOAc (3 x 50 ml). The combined EtOAc layer was washed with water, dried and evaporated. The crude products were separated by column chromatography, using $CHCl_3$-MeOH (99:1 to 93:7) as eluting solvent to afford 2-*epi*-3-deoxy-5α-20-hydroxyecdysone 20,22-acetonide (**13**) (23 mg, 76%) and 3-deoxy-20-hydroxyecdysone 20,22-acetonide (**11**) (7 mg, 23%).

11: IR: ν_{max} 3426, 2926, 1649, 1453, 1371, 1254, 1215, 1168, 1103, 1065, 998, 877 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 347 $[M-C_9H_{17}O_2]^+$ (25), 330 (7), 329 (13), 312 (6), 311 (5), 201(6), 143 (16), 125 (27), 99 (45), 81 (22), 59 (100). Anal. Calcd for $C_{30}H_{46}O_6 \cdot H_2O$: C, 69.23; H, 9.23. Found: C, 68.90; H, 9.42.

13: IR: ν_{max} 3426, 2956, 1649, 1454, 1371, 1249, 1215, 1171, 1099, 1051, 1000, 960, 879 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 347 $[M-C_9H_{17}O_2]^+$ (14), 330 (11), 329 (23), 312 (17), 311(14), 201 (9), 158 (12), 157 (10), 143 (43), 125 (74), 99 (51), 81 (46), 59 (100). Anal. Calcd for $C_{30}H_{46}O_6 \cdot 2H_2O$: C, 66.91; H, 9.29. Found: C, 67.24; H, 9.27.

Acetonide deprotection of 3-deoxy-20-hydroxyecdysone 20,22-acetonide (11)

Compound **11** (6 mg, 0.011 mmol) was treated with 70% AcOH (1.5 ml) at ambient temperature for 3 days and water (50 ml) was then added. The mixture was extracted three times (30 ml each) with hexane-EtOAc (1:1) and with EtOAc (3x30 ml). Each combined organic layer was washed with water, dried and evaporated to dryness. The hexane-EtOAc extract gave the starting material **11** (1.5 mg) and the EtOAc extract gave 3-deoxy-20-hydroxyecdysone (**3**) (3 mg, 76% based on un-recovered starting material **11**); IR: ν_{max} 3402, 2962, 2930, 1650, 1455, 1381, 1317, 1260, 1223, 1141, 1108, 1057, 991, 951, 929, 876 cm^{-1}; ^1H NMR data is given in Table. Anal. Calcd for $C_{27}H_{44}O_6 \cdot H_2O$: C, 67.21; H, 9.54. Found: C, 67.04; H, 9.21.

Acetonide deprotection of 2-*epi*-3-deoxy-5α-20-hydroxyecdysone 20,22-acetonide (13)

Compound **13** (8 mg, 0.015 mmol) was subjected to acetonide deprotection in similar manner as employed for **7**. After the workup the crude product (8 mg) was purified by column chromatography using $CHCl_3$-MeOH (99:1 to 96:4) as eluting solvent to afford 2-*epi*-3-deoxy-5α-20-hydroxyecdysone (**14**) (7 mg, 96%); IR: ν_{max} 3412, 2956, 1650, 1459, 1381, 1329, 1223, 1147, 1068, 950, 875 cm^{-1}; ^1H NMR data is given in Table; EIMS: m/z (% rel. intensity) 446 $[M-H_2O]^+$ (9), 330 (31), 329 (38), 312 (39), 311 (76), 117 (20), 99 (72), 81 (88), 55 (100). Anal. Calcd for $C_{27}H_{44}O_6 \cdot H_2O$: C, 67.21; H, 9.54. Found: C, 67.27; H, 9.46.

Biological activity testing. The *Musca* bioassay has been performed by the method referred to previously.[9]

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