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Regioselective Synthesis of 24-*epi*-Pterosterone

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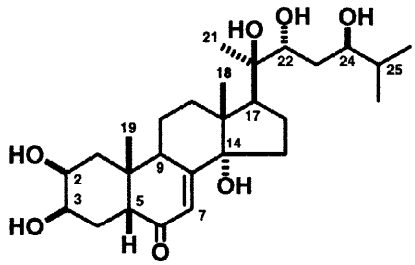
Abstract: In order to have compounds available for structure-activity relationship studies, the ecdysteroid 24-*epi*-pterosterone was synthesized from 20-hydroxyecdysone and pterosterone was obtained from *Vitex glabrata* stem bark. The former was approximately 7-fold less active than the latter in the *Musca* bioassay for moulting hormone activity.

24-*epi*-Pterosterone (**1**) is a rare ecdysteroid isolated recently as a minor constituent of *Athyrium yokoscense* roots.[1] Its C-24 epimer, pterosterone (**2**), was isolated from a number of plant species,[2,3] including *Vitex megapotamica*.[4] Compound **2** exhibited high moulting hormone activity in the *Sarcophaga* bioassay.[5] In



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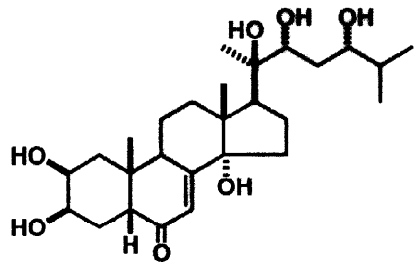

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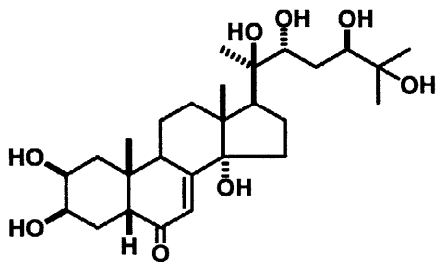
the course of our studies on structure of ecdysteroids and their moulting hormone activity, we have shown that 24-*epi*-abutasterone (**3**) and abutasterone (**4**) exhibited relatively low activity compared to the parent ecdysteroid, 20-hydroxyecdysone (**5**).[6] From this finding, it was logical to conclude that the presence of a C-24 hydroxyl group, both in the 24*R* and 24*S* configurations, might have contributed to such a decrease in activity. However, the reported high activity of compound **2**,[5] the deoxy analogue of **4**, has prompted us to study the moulting hormone activity of the ecdysteroids **1** and **2** using the *Musca* bioassay.



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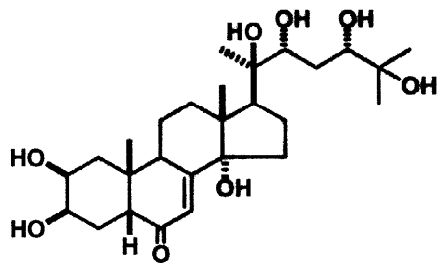

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RESULTS AND DISCUSSION

As the occurrence of pterosterone (**2**) in a *Vitex* species has been reported,[4] we then investigated some *Vitex* plants for ecdysteroids and compound **2** was obtained as a minor constituent of *V. glabrata* stem bark (see

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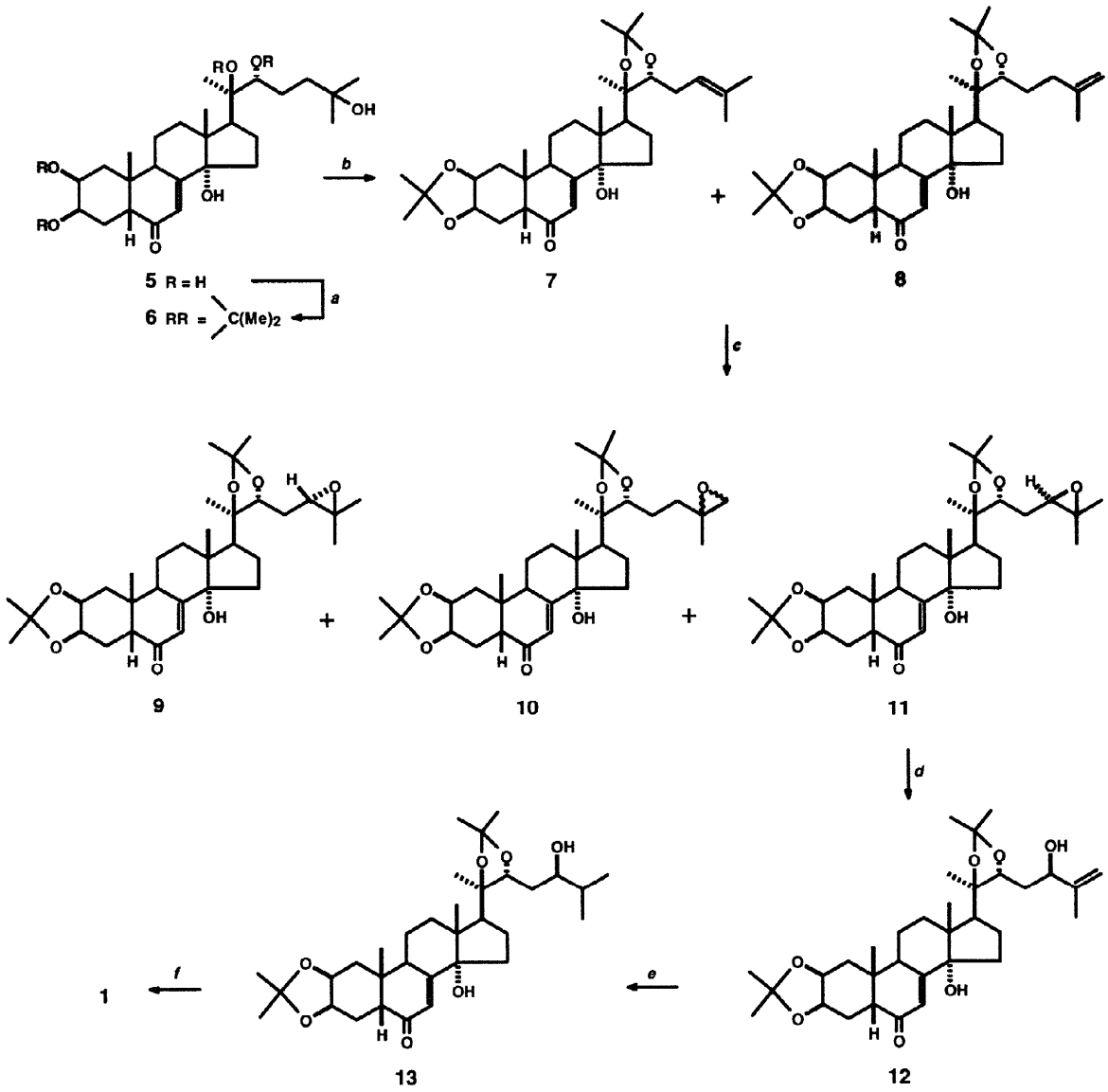
Experimental). ^{1}H NMR spectral data of **2** was consistent with that reported previously.[7] However, the extract did not reveal the presence of 24-*epi*-pterosterone (**1**), and we therefore decided to synthesize this ecdysteroid. Acetonation of the readily available ecdysteroid **5**[8] afforded the corresponding diacetonide **6**,[9] (Scheme) which was treated with MsCl in pyridine in the presence of DMAP to afford the inseparable olefin mixture **7** and **8** in a ratio of 3:2.[6] Reaction of **7** and **8** mixture with *m*-CPBA in $CHCl_3$ gave a mixture of the epoxides **9**, **10** and **11** which were separated by column chromatography. The ratio of **9** to **11** was *ca.* 1:2 and compound **10** was obtained as a *ca.* 1:1 mixture of two 25,26-epoxides, of unknown epoxide stereochemistry. In our experience, a C-24 oxygenated ecdysteroid in a 24*R* configuration was more polar than that in a 24*S* configuration,[6] the more polar isomer **11** was therefore expected to be the required intermediate epoxide for the synthesis of **1**. Regioselective epoxide-ring opening of **11** was achieved by treatment with LiBr in MeCN to give the hydroxy olefin **12** in 50% yield. This key step involved abstraction of a proton from the C-26 methyl group followed by epoxide-ring opening with aid of the lithium ion chelating at the epoxide oxygen. The structure of **12** was



Scheme *Reagents and conditions*: *a*, CH_3COCH_3, *p*-TsOH (85%); *b*, Reaction from **6**: MsCl, pyridine, DMAP, 5 °C to ambient temp. (87%); *c*, *m*-CPBA, $CHCl_3$ (75%); *d*, LiBr, MeCN (50%); *e*, H_2/Pd-C, EtOH (95%); *f*, 70% AcOH (74%)

deduced mainly from ^{1}H NMR spectral data (Table). The presence of the C-25 olefinic function was evident from the two broad doublets attributed to H-26 at δ4.83 and 4.99. The presence of the C-24 hydroxyl group was evident from the presence of the H-24 signal at δ4.21. It should be noted that LiI and LiOH have been used instead of LiBr, but both of them gave less satisfactory results. Hydrogenation of **12**, with Pd-C as a catalyst, afforded the corresponding dihydro compound **13** in 95% yield. Deacetonation of **13** with 70% AcOH gave 24-*epi*-pterosterone (**1**) in 74% yield. ^{1}H NMR spectral data of this compound was consistent with the reported[1] values. The overall yield of **1** from the epoxide **11** was 35%.

Biological activity. 24-*epi*-Pterosterone (**1**) was approximately 7-fold less active than pterosterone (**2**) in the *Musca* bioassay. It was thus concluded that the stereochemical arrangement of the C-24 hydroxyl group of 25-deoxy ecdysteroids is very important for biological activity.

EXPERIMENTAL

General experimental details have been described previously.[10] *Vitex glabrata* stem bark was obtained from Nakornsawan district and a voucher specimen is deposited at the Plant Collection Centre, Faculty of Science, Ramkhamhaeng University.

Isolation of pterosterone from *Vitex glabrata*

Pulverized, dry bark (4.5 kg) of *V. glabrata* was extracted successively with *n*-hexane and EtOH in a Soxhlet extraction apparatus. The EtOH extract was subjected to continuous liquid-liquid extraction, using $CHCl_3$ as a solvent, to afford 41 g of the $CHCl_3$ extract, which was subjected to a series of column chromatography purifications to yield pterosterone (**2**, 21 mg) as fine needles, mp 227-228 °C (from MeOH-$CHCl_3$) (lit.[5] 229-230 °C). ^{1}H NMR (see Table) and IR spectral data were consistent with the reported values.[7] Compound **5** (860 mg) was also obtained as the major component of this extract and was identical (^{1}H NMR and TLC comparisons) to the authentic sample.[11]

Epoxidation of olefins 7 and 8

A mixture of the olefins **7** and **8** (3:2, 40 mg, 0.074 mmol), prepared from the diacetonide **6**, which in turn was prepared from the ecdysteroid **5**[6] (see scheme), was dissolved in $CHCl_3$ (1 ml) and *m*-CPBA (70% 30 mg, 0.248 mmol) was added. The reaction mixture was kept stirring for 20 min and 1% $NaHSO_3$ was added. The mixture was stirred for 30 min and extracted with $CHCl_3$. The residue was chromatographed, using $CHCl_3$-MeOH to give, respectively, the epoxides **9** (8 mg, 19%), **10** (9 mg, 22%) and **11** (14 mg, 34%). Compound **10** was obtained as two isomeric 25,26-epoxides.

9: Amorphous; IR: ν_{max} 3472, 2980, 1666, 1455, 1376, 1243, 1217, 1169, 1105, 1057, 1005, 904, 876, 753 cm^{-1}; ^{1}H NMR data is given in Table; FABMS (+ve): 559.3630 $[M+H]^+$. $C_{33}H_{51}O_7$ requires 559.3634.

10: Amorphous; IR: ν_{max} 3474, 2976, 1661, 1452, 1374, 1244, 1215, 1167, 1105, 1057 cm^{-1}; ^{1}H NMR data is given in Table; FABMS (+ve): m/z 559.3636 $[M+H]^+$. $C_{33}H_{51}O_7$ requires 559.3634.

11: Aggregated needles from $CHCl_3$-hexane, mp 205-206 °C; IR: ν_{max} 3474, 2930, 1661, 1454, 1377, 1243, 1218, 1169, 1105, 1057, 1008, 876 cm^{-1}; ^{1}H NMR data is given in Table; Anal. Calcd. for $C_{33}H_{50}O_7 \cdot 1/2H_2O$: C, 69.81; H, 9.05. Found: C, 69.51; H, 8.69.

Table ^{1}H NMR data of Ecdysteroids (J values in parentheses)

H	1	2	9	10		11	12	13
				Isomer 1*	Isomer 2*			
	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
2	4.16 (*m*)	4.16 (*m*)	4.20 (*m*)	4.25 (*m*)	4.25 (*m*)	4.19 (*m*)	4.16 (*m*)	4.20 (*m*)
3	4.22 (br *s*)	4.22 (br *s*)	4.25 (br *s*)	4.25 (br *s*)	4.25 (br *s*)	4.24 (br *s*)	4.21[≠]	4.25 (br *s*)
5	2.97 (*dd*, *ca* 13, 3.6)	3.00 (*dd*, 13.1, 3.6)	2.33 (*dd*, 12.6, 4.7)	2.34 (*dd*, 12.5, 4.5)	2.34 (*dd*, 12.5, 4.5)	2.33 (*dd*, 12.5, 4.5)	2.29 (*dd*, 12.6, 4.7)	2.33 (*dd*, 12.5, 4.8)
7	6.22 (*d*, 2.4)	6.25 (*d*, 2.1)	5. 80 (*d*, 2.1)	5. 80 (*d*, 2.1)	5. 80 (*d*, 2.1)	5.80 (*d*, 2.1)	5. 76 (*d*, 2.4)	5. 80 (*d*, 2.4)
9	3.58 (*m*)	3.58 (*m*)	2.78 (*m*)	2.78 (*m*)	2.78 (*m*)	2.78 (*m*)	2.73 (*m*)	2.78 (*m*)
17	3.02 (*t*, 9.1)	2.92 (*t*, 9.1)	2.20 (*dd*, 9.7, 7.6)	2.19[#]	2.19[#]	2.22 (*dd*, 9.7, 9.4)	2.12 (*dd*, 9.4, 6.4)	2.18 (*dd*, 9.4, 7.9)
22	4.10 (*m*)	4.12 (br *d*, 10)	3.78 (*dd*, 8.5, 3.9)	3.60 (*dd*, 9.1, 2.3)	3.59 (*dd*, *ca* 9, 2.1)	3. 87 (*dd*, 9.7, 2.7)	3.86 (*dd*, 10.5, 1.6)	3.96 (*dd*, 10.3, 1.5)
24	4.47 (br *d*, 9.7)	3.94 (*m*)	2.86 (*t*, 6.2)			2.91 (*dd*, 7.9, 3.3)	4.21[≠]	3.54 (*m*)
26	-	-	-	2.55 (*d*, 4.8); 2.67 (*d*, 4.8)	2.58 (*d*, 4.7); 2.62 (*d*, 4.7)	-	4.83 (br *s*); 4.99 (br *s*)	-
18-Me	1.21 (*s*)	1.20 (*s*)	0.76 (*s*)	0.76 (*s*)	0.77 (*s*)	0.77 (*s*)	0.72 (*s*)	0.77 (*s*)
19-Me	1.05 (*s*)	1.06 (*s*)	0.96 (*s*)	0.96 (*s*)	0.96 (*s*)	0.95 (*s*)	0.91 (*s*)	0.96 (*s*)
21-Me	1.62 (*s*)	1.58 (*s*)	1.11 (*s*)	1.15 (*s*)	1.13 (*s*)	1.12 (*s*)	1.07 (*s*)	1.12 (*s*)
26-Me	0.98 (*d*, 6.7)	1.01 (*d*, 6.7)	1.27 (*s*)	-	-	1.26 (*s*)	-	0.91 (*d*, 6.7)
27-Me	1.08 (*d*, 6.7)	1.01 (*d*, 6.7)	1.30[a] (*s*)	1.27[b] (*s*)	1.29[c] (*s*)	1.30 (*s*)	1.67 (*s*)	0.93 (*d*, 6.7)
	-	-	1.31[a], 1.32, 1.37, 1.47 (each *s*)	1.31[b], 1.32, 1.37, 1.47 (each *s*)	1.32[c], 1.33, 1.41, 1.47 (each *s*)	1.30, 1.32, 1.39, 1.46 (each *s*)	1.25, 1.26, 1.34, 1.42 (each *s*)	1.30, 1.31, 1.38, 1.46 (each *s*)

* Assigned from a mixture of two isomeric 25,26-epoxides

[#,≠] Partially superimposed signal

[a,b,c] Assignment may be reversed for signals with the same superscript

Reaction of the epoxide 11 with lithium bromide. Synthesis of the hydroxy olefin 12

A mixture of the epoxide **11** (50 mg, 0.089 mmol) and LiBr (200 mg, 2.303 mmol) in MeCN (4 ml) was stirred at ambient temperature for 2 weeks. Water was added and the mixture extracted with $CHCl_3$ (3×25 ml). The product was purified by column chromatography ($CHCl_3$-MeOH, 98:2) and the hydroxy olefin **12** (25 mg, 50%) was obtained as amorphous solid: IR: ν_{max} 3442, 2916, 2848, 1659, 1454, 1370, 1242, 1215, 1166, 1093, 1056 cm^{-1}; ^1H NMR data is given in Table; FABMS (-ve): m/z 557.3485 $[M-H]^-$. $C_{33}H_{49}O_7$ requires 557.3478.

Catalytic hydrogenation of the hydroxy olefin 12

The hydroxy olefin **12** (41 mg, 0.073 mmol) was hydrogenated, using 5% Pd-C (50 mg) as a catalyst; the mixture was filtered through a short Celite column and the solvent evaporated. Column chromatography ($CHCl_3$-MeOH, 98:2) afforded the noncrystalline 24-*epi*-pterosterone 2,3:20,22-diacetonide (**13**) (39 mg, 95%). IR: ν_{max} 3472, 2960, 2874, 1658, 1463, 1372, 1243, 1216, 1168, 1107, 1090, 1057, 1000, 877 cm^{-1}; ^1H NMR data is given in Table; FABMS (-ve): m/z 559.3634 $[M-H]^-$. $C_{33}H_{51}O_7$ requires 559.3634.

Acetonide deprotection of compound 13

To an ethanolic solution of compound **13** (19 mg, 0.034 mmol) was added 70% AcOH (1.5ml, excess) and the mixture stirred for 4 days. Water was added and the mixture was repeatedly extracted with *n*-BuOH until no product was detected in the aqueous phase; the combined organic phase was evaporated by co-distillation with water. The product was chromatographed to give 24-*epi*-pterosterone (**1**, 12 mg, 74%) as prisms, mp 164-165 °C from MeOH-$CHCl_3$ (lit.[1] 151-152 °C). IR: ν_{max} 3382, 2964, 1656, 1469, 1383, 1334, 1052 cm^{-1}; ^1H NMR data is given in Table; Anal. Calcd. for $C_{27}H_{44}O_7 \cdot 3H_2O$: C, 60.65; H, 9.43. Found: C, 60.40; H, 9.73.

Biological activity testing. The *Musca* bioassay was performed by the established method.[12,13]

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