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TETRAHEDRON

Asymmetric Dihydroxylation of Stachysterone C: Stereoselective Synthesis of 24-*epi*-Abutasterone

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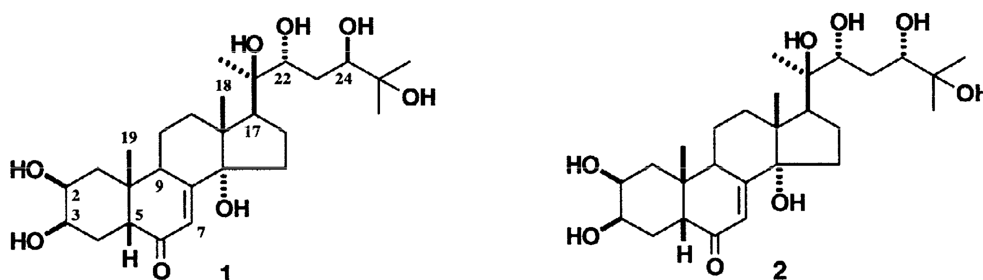
Abstract: Stachysterone C was synthesized from 20-hydroxyecdysone (20-ECD). Sharpless asymmetric dihydroxylation of this rare ecdysteroid using osmium tetroxide and a chiral ligand afforded 24-*epi*-abutasterone, another rare ecdysteroid, and its C-24 epimer, abutasterone. High diastereomeric excess of the former ecdysteroid was obtained when the chiral ligands dihydroquinidine 1,4-phthalazinediyl diether and dihydroquinidine 2,5-diphenyl-4,6-pyrimidinediyl diether were employed. The two C-24 epimeric ecdysteroids exhibited similar moulting hormone activity in the *Musca* bioassay. However, they were significantly less active than 20-ECD. © 1998 Elsevier Science Ltd. All rights reserved.

24-*epi*-Abutasterone (**1**) is a rare ecdysteroid isolated recently in a small quantity (3 mg) from a complex mixture of the methanolic extract of 2.5 kg of *Vitex canescens* root bark.[1] The structure of **1** was deduced mainly from spectroscopic (^{1}H and ^{13}C NMR) spectral comparisons with its C-24 epimer, abutasterone (**2**). The ecdysteroid **2** was found in a number of plant species.[2-4] In continuation of our study on structure-activity relationships of ecdysteroids with different oxygenation at the side chain, we would like to see whether introduction of an extra hydroxyl group to the 24-position of the parent ecdysteroid, 20-hydroxyecdysone (**3**),



will result in any change in moulting hormone activity of **3**. Also, we would like to compare the activity of ecdysteroids with a 24*S*-hydroxyl group (e.g., **2**) with those with the 24*R*-hydroxyl group (e.g., **1**). The rationale behind this study was initiated by our discovery of pinnatasterone (**4**)[5] and canescensterone (**5**)[6] from the stem barks of *V. pinnata* and *V. canescens*, respectively. Compound **4** exhibited very low moulting hormone activity, whereas compound **5** showed very high activity in the *Musca domestica* assay. Though it was obvious that the pyrrole 2-carboxylate moiety was responsible for such high activity of compound **5**, it was logical to study biological activity related to stereochemical arrangement of the C-24 hydroxyl group. It was therefore advantageous to have enough of compound **1** for biological activity testing. In this paper we describe a concise, stereoselective synthesis of **1** using the ecdysteroid **3** as the starting material.

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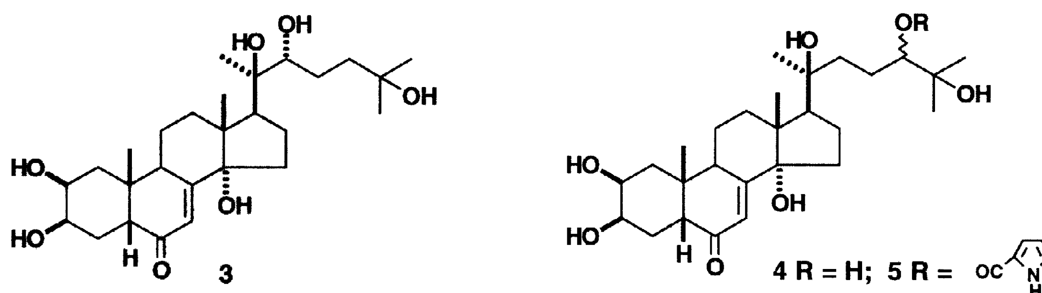
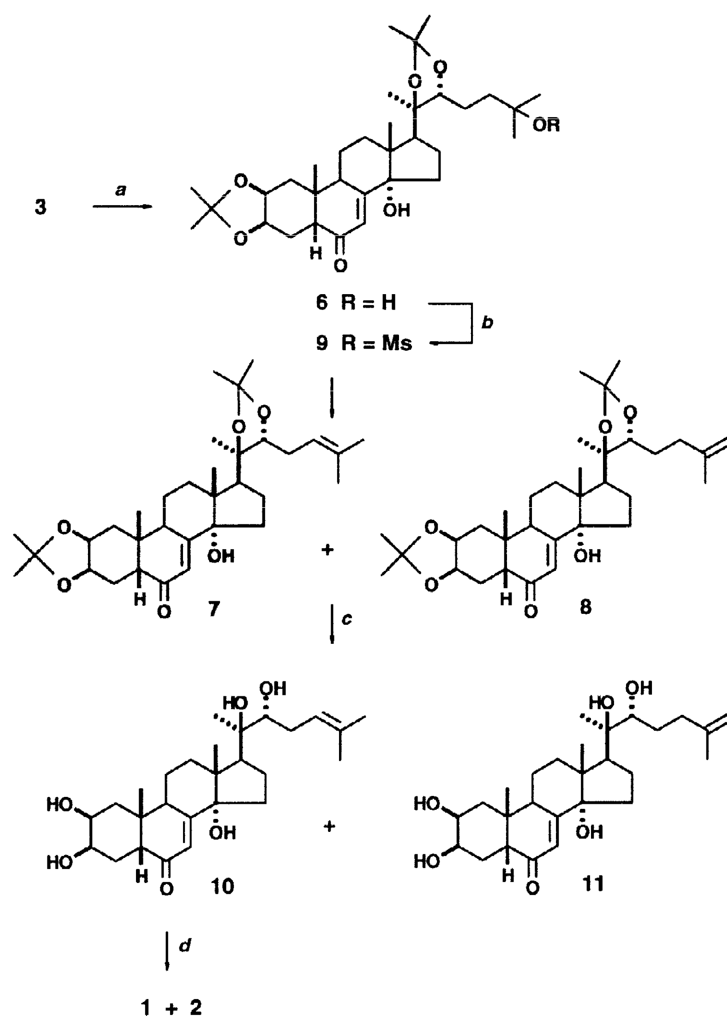
RESULTS AND DISCUSSION

Starting from the readily available ecdysteroid **3**,[6] the diacetonide **6** was prepared by the literature method.[7] Treatment of the pyridine solution of **6** with mesyl chloride in the presence of DMAP furnished an inseparable 3:2 mixture of the olefin diacetonide **7** and **8**, presumably through the mesylate **9** (Scheme).



The acetonide protecting groups in **7** and **8** were removed by treatment with 70% AcOH[7] to the corresponding olefin mixture, which was separated by repeated column chromatography to stachysterone C (**10**) and 25,26-didehydroponasterone A (**11**). It should be noted that compound **10** is a rare ecdysteroid isolated from *Stachyurus praecox* in 1970.[8] The key step of the synthesis of **1** was the stereoselective dihydroxylation of **10** using OsO_4 in the presence of a suitable chiral ligand. According to the Sharpless asymmetric dihydroxylation of olefins,[9] the cinchona alkaloid derivatives we decided to use as the chiral ligands were dihydroquinidine 4-methyl-2-quinolyl ether (DHQD-MQE) and dihydroquinidine 9-phenanthryl ether (DHQD-PE).[9] Before investigating the selectivity of the osmylation of the olefin **10** with these chiral ligands, we chose to examine the dihydroxylation of **10** without a chiral ligand and pyridine was used in this case, using a stoichiometric quantity of OsO_4. The reaction was performed in *tert*-BuOH-THF-H_2O (7:4:1) solvent system and the olefin **10** was transformed to 24-*epi*-abutasterone (**1**) and abutasterone (**2**) in high yield, with the products **1** and **2** in a ratio of 2:1 (Table 2, entry 1). The resulting ecdysteroids could be separated by careful column chromatography. The experiment performed in the absence of a chiral ligand, therefore, revealed that the diastereomer **1** was intrinsically favoured. We then investigated the stereoselectivity of the two chiral ligands, DHQD-MQE and DHQD-PE. The ratio of the ligand:OsO_4:olefin was 1.5:1.3:1. Both of these chiral ligands gave rise to the products **1** and **2** in a ratio of 4:1 (Table 2, entries 2 and 3). We then turned to the more recent "second generation" chiral ligands, dihydroquinidine 1,4-phthalazinediyl diether, $(DHQD)_2$-PHAL, and dihydroquinidine 2,5-diphenyl-4,6-pyrimidinediyl diether, $(DHQD)_2$-PYR.[9] Under the same conditions employed in the case of the foregoing "first generation" chiral ligands, the reaction in the presence of $(DHQD)_2$-PHAL was found to be 7:1 selective in favour of compound **1** (Table 2, entry 4), whereas that in the presence of $(DHQD)_2$-PYR was 6:1 (Table 2, entry 5). The high diastereoselectivity of both of the latter ligands therefore allowed stereoselective synthesis of the ecdysteroid **1** in high diastereomeric excess. In the latter two cases, the overall yield of **1** from the diacetonide **6** was 22 - 23 %. It should be noted that asymmetric dihydroxylation of the olefin **10** with a catalytic quantity of OsO_4 was also investigated, but the reaction proceeded very sluggishly, as has been reported in a different system.[10] It is worth mentioning that synthesis of **1** also provides indirect evidence for structural confirmation of the naturally occurring 24-*epi*-abutasterone. Also, the synthesized abutasterone (**2**), especially in the case of using the non-chiral ligand, could be used in the bioassay.



Scheme *Reagents and conditions*: *a*, CH_3COCH_3, *p*-TsOH; *b*, MsCl, pyridine, DMAP, 5 °C to ambient temp.; *c*, 70% AcOH, EtOH; *d*, OsO_4, ligand (see Table 2), *t*-BuOH-THF-H_2O (7:4:1)

In order to obtain further information of the osmylation of the olefin **10** to the ecdysteroids **1** and **2**, the chiral ligands of the dihydroquinine (DHQ) series, which were expected to effect asymmetric dihydroxylation of the olefin **10** in favour of compound **2**, the ligands DHQ-MQE, DHQ-PE, $(DHQ)_2$-PHAL and $(DHQ)_2$-PYR were selected. The dihydroxylation of **10** using DHQ-MQE and DHQ-PE gave, in both cases, the ecdysteroid **1** as the major product of a 10:7 diastereomer mixture (Table 2, entries 6 and 7). The diastereomer ratio changed to 4:1 in favour of the ecdysteroid **1** by performing the reaction both with $(DHQ)_2$-PHAL and $(DHQ)_2$-PYR (Table 2, entries 8 and 9). It was evident that intrinsic factor of the dihydroxylation of **10** became more significant in the latter two cases.

Biological activity. Both of the ecdysteroids **1** and its C-24 epimer, the ecdysteroid **2**, were less active than 20-hydroxyecdysone (**3**) in the *Musca* bioassay. It was thus evident that introduction of an additional C-24 hydroxyl group to the parent ecdysteroid **3** resulted in a significant decrease in activity. As the moulting hormone activity of the two epimeric ecdysteroids **1** and **2** was not significantly different, this led to the conclusion that the presence of a C-24 hydroxyl group, either with the *R* or *S* configuration, resulted in a decrease in activity.

Table 1 ^{1}H NMR Data of Ecdysteroids

H	**1**	**2**	**7**[*]	**8**[*]	**10**	**11**
	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	C_5D_5N	C_5D_5N
2	4.18 (m)	4.19 (m)	4.23 (m)	4.23 (m)	4.17 (m)	4.17 (m)
3	4.23 (br s)	4.23 (br s)	4.27 (br s)	4.27 (br s)	4.24 (br s)	4.23 (br s)
5	2.99 (dd, 12.9, 3.5)	3.00 (dd, 12, 3.6)	2.36 (dd, 12.6, 4.7)	2.36 (dd, 12.6, 4.7)	3.02 (dd, 13.2, 3.5)	3.02 (dd, 13.1, 3.6)
7	6.22 (d, 2.1)	6.24 (d, 2.1)	5.83 (d, *ca* 2)	5.83 (d, *ca* 2)	6.25 (d, 2.1)	6.26 (d, 2.1)
9	3.57 (m)	3.58 (m)	2.81 (m)	2.81 (m)	3.60 (m)	3.59 (m)
17	3.08 (t, 9.1)	2.99 (t, 8.2)	*ca* 2.27[≠]	*ca* 2.27[≠]	2.95 (t, 9.3)	2.93 (t, 9.1)
22	4.51 (dd, 9.6, 2.3)[a]	4.08 (dd, 9.7, 1.8)[b]	3.70 (dd, 8.2, 4.8)	3.66 (dd 9.7, 2.7)	3.89 (dd, 9.7, 1.5)	3.84 (br d, 10.9)
24	4.37 (dd, 9, 2.7)[a]	4.24 (dd, 10.1, 1.5)[b]	5.18 (m)		5.55 (br t, 7)	
26	-	-	-	4.72, 4.75 (each br s)	-	4.75, 4.79 (each br s)
18-Me	1.22 (s)	1.19 (s)	0.79 (s)	0.79 (s)	1.21 (s)	1.22 (s)
19-Me	1.06 (s)	1.06 (s)	0.98 (s)	0.98 (s)	1.07 (s)	1.07 (s)
21-Me	1.63 (s)	1.59 (s)	1.17 (s)[c]	1.15 (s)[c]	1.59 (s)[f]	1.57 (s)[g]
26-Me	1.46 (s)	1.45 (s)	1.64 (s)	-	1.59 (s)	-
27-Me	1.47 (s)	1.50 (s)	1.71 (s)[d]	1.74 (s)[d]	1.63 (s)[f]	1.67 (s)[g]
>C(Me)$_2$	-	-	1.33[e], 1.33, 1.41, 1.49 (each s)	1.32[e], 1.33, 1.41, 1.49 (each s)	-	-

[*] Assigned from a mixture of compounds **7** and **8**.
[a–g] Assignments may be reversed for signals with the same superscript.
[≠] Obscured signal.

Table 2 Dihydroxylation of the olefin **10**

Entry	Ligand	Ratio of Products **1**:**2**	% Yield
1	pyridine	2:1[a]	88
2	DHQD-MQE	4:1[a]	89
3	DHQD-PE	4:1[a]	88
4	(DHQD)$_2$-PHAL	7:1[a,b]	84
5	(DHQD)$_2$-PYR	6:1[a,b]	82
6	DHQ-MQE	10:7[a]	80
7	DHQ-PE	10:7[a]	80
8	(DHQ)$_2$-PHAL	4:1[a]	76
9	(DHQ)$_2$-PYR	4:1[a]	76

[a] Determined by ^{1}H NMR spectral analysis.
[b] Determined by HPLC analysis.

EXPERIMENTAL

General experimental details have been described previously.[11]

Reaction of 20-hydroxyecdysone 2,3:20,22-diacetonide (6) with mesyl chloride

Compound **6**[7] (1.5565 g, 2.779 mmol) was dissolved in pyridine (5 ml) and the solution stirred at 0-5 °C for 10 min. Mesyl chloride (1 ml, 12.867 mmol) and 4-dimethylaminopyridine (DMAP, 50 mg) were added and the reaction mixture was left to stir at 5 °C for 20 min, then slowly allowed to warm up to ambient temperature during the period of 4 h. Water was added to the reaction mixture and the solution extracted with $CHCl_3$ (3x35 ml). The combined $CHCl_3$ layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude mixture was subjected to column chromatography using $CHCl_3$-MeOH as eluting solvent, with an increasing amount of the more polar component. Fractions eluted by $CHCl_3$-MeOH (99:1) afforded a 3:2 mixture of stachysterone C 2,3:20,22-diacetonide (**7**) and 25,26-didehydroponasterone A 2,3:20,22-diacetonide (**8**) (1.3160 g, 87%).

7+8: IR: ν_{max} 3474, 2976, 1658, 1445, 1373, 1243, 1215, 1057, 884, 756 cm^{-1}; ^1H NMR data of compounds **7** and **8** are given in Table 1; EIMS: m/z (% rel. intensity) 542 $[M]^{+\cdot}$ (2), 527 (9), 509 (12), 484 (8), 467 (7), 466 (11), 403 (34), 385 (13), 327 (7), 183 (16), 140 (9), 125 (100), 107 (35). Anal. Calcd for $C_{33}H_{50}O_6{\cdot}H_2O$: C, 70.71; H, 9.28. Found: C, 70.93; H, 9.39.

Acetonide deprotection of 7 and 8

To a solution of the acetonides **7** and **8** (147 mg, 0.271 mmol) in MeOH (1 ml) was added 70% AcOH (5 ml) and the mixture was stirred at ambient temperature for 4 days. The reaction mixture was poured into water and the solution 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 which contained stachysterone C (**10**) and 25,26-didehydroponasterone A (**11**) in a ratio of 3:2 (from ^1H NMR) was chromatographed using $CHCl_3$-MeOH with increasing concentration of MeOH to afford pure compound **10**, a mixture of compounds **10** and **11**, and pure compound **11**. The compounds **10** and **11** mixture was chromatographed to yield more of pure compounds **10** and **11** together with a mixture of **10** and **11**, the latter of which was subjected to another column chromatography. This resulted in the separation of a total of 68 mg (54%) of compound **10**, 28 mg of compound **11** and a mixture (7 mg) consisting mainly of the compound **11** and some of the compound **10**.

10: Amorphous, mp 225-227 °C (from MeOH-$CHCl_3$); IR: ν_{max} 3414, 2926, 1647, 1445, 1382, 1116, 1056, 875 cm^{-1}; ^1H NMR data is given in Table 1; FABMS (+ve): m/z (% rel. intensity) 463 $[M+H]^+$ (54), 445 (29), 427 (6). Anal. Calcd for $C_{27}H_{42}O_6{\cdot}3/2\ H_2O$: C, 66.25; H, 9.20. Found: C, 66.21; H, 8.84.

11: Needles, mp 263-265 °C (from MeOH-$CHCl_3$); IR: ν_{max} 3347, 2938, 1641, 1443, 1378, 1355, 1316, 1262, 1161, 1122, 1061, 997, 949, 891, 874 cm^{-1} ; ^1H NMR data is given in Table 1; FABMS (+ve): m/z 463.3057 $[M+H]^+$. $C_{27}H_{42}O_6$ requires 463.3059.

Dihydroxylation of stachysterone C (10) with OsO_4 and pyridine

A solution of OsO_4 (250 mg) in THF (5 ml) was prepared and a portion (288 μl, 0.056 mmol) was added to a solution of pyridine (0.8 ml) in *tert*-BuOH-THF-H_2O (7:4:1, 4 ml). Parts of the remaining OsO_4 solution were used in subsequent asymmetric dihydroxylation of the olefin **10**. After 10 min stirring, a solution of the olefin **10** (20 mg, 0.043 mmol) in *tert*-BuOH-THF-H_2O (7:4:1, 1.6 ml) was then added and stirring continued for 10 min. A 1% solution of $NaHSO_3$ (8 ml) was added and stirring continued for 30 min. Saturated brine (80 ml) was added; the mixture was repeatedly extracted with *n*-BuOH until no products were detected in the

aqueous phase and the combined organic phase was evaporated by co-distillation with water. The residue, the ^{1}H NMR spectrum of which indicated a 2:1 mixture of 24-*epi*-abutasterone (**1**) and abutasterone (**2**), was subjected to column chromatography, using $CHCl_3$-MeOH as eluting solvents, to give compound **2** (7 mg) and compound **1** (12 mg). TLC and spectroscopic (^{1}H NMR and IR) comparisons of **1** with 24-*epi*-abutasterone isolated from *V. canescens* root bark[1] revealed the identity of the compounds. Compound **1** crystallized as needles from MeOH-EtOAc, mp 257-259 °C; FABMS (+ve): m/z 497.3118 $[M+H]^+$. $C_{27}H_{45}O_8$ requires 497.3114.

2: Needles, mp 258-260 °C from MeOH-EtOAc (lit.[2] 257-259 °C); IR: ν_{max} 3376, 2944, 1639, 1461, 1382, 1183, 1090, 1072, 922, 874 cm^{-1} ; ^{1}H NMR spectral data of 2 (Table 1) were consistent with the reported abutasterone.[4] FABMS (+ve): m/z (% rel. intensity) 497 $[M+H]^+$ (7), 479 (3), 461 (3), 443 (3).

Asymmetric dihydroxylation of 10 with OsO_4 and chiral ligands

General procedure. To a solution of 0.016 mmol of the chiral ligand in *tert*-BuOH-THF-H_2O (7:4:1, 1.8 ml) was added the previously prepared THF solution of OsO_4 (72 µl, 0.014 mmol) and the mixture stirred for 10 min. A solution of the olefin **10** (5 mg, 0.011 mmol) in *tert*-BuOH-THF-H_2O (7:4:1, 0.4 ml) was then added and stirring continued for 10 min. The ratio of the ligand, OsO_4 and olefin was 1.5:1.3:1. A 1% solution of $NaHSO_3$ (2 ml) was added and stirring continued for another 30 min. Saturated brine (20 ml) was then added and the mixture repeatedly extracted with *n*-BuOH until no products were detected in the aqueous phase. The combined organic phase was evaporated and the residue was chromatographed to separate a mixture of compounds **1** and **2** from the ligand. The ratio of the two ecdysteroids was determined by ^{1}H NMR spectral analysis and, in the case of the dihydroxylation using $(DHQD)_2$-PHAL and $(DHQD)_2$-PYR where the product ratio could not accurately be determined by the ^{1}H NMR method, HPLC analysis (column: Spherisorb ODS2, 5 µm, 250x4.6 mm; mobile phase: MeOH-H_2O (1:3); flow rate: 1 ml min^{-1} ; detector: 254 nm). The results are shown in Table 2, entries 2-9.

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

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