Synthesis and biological activity of side-chain analogues of ecdysone and 20-hydroxyecdysone

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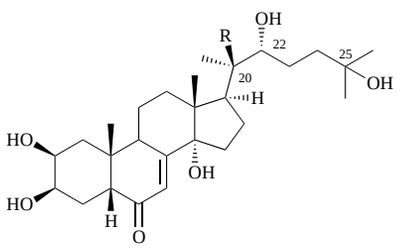
Side-chain analogues of 20-hydroxyecdysone 1 and ecdysone 2 containing a tetrahydrofuran ring, including shidasterone 4, have been prepared and assessed for biological activity using a bioassay derived from a tumorous B_{II} blood cell line of *Drosophila melanogaster*. The synthetic strategy involves selective protection of the 2,3-*cis*-diol of 1 and 2 followed by activation of the C-22 hydroxy group prior to ring-closure.

Introduction

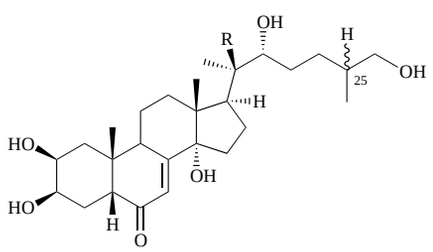
Ecdysteroids are a family of polyhydroxylated steroid hormones which bear a *cis*-fused A/B ring junction, a 7-en-6-one chromophoric group and a 14α-hydroxy group.[1] The two most important members of this family are 20-hydroxyecdysone **1** and ecdysone **2**. 20-Hydroxyecdysone [(20*R*,22*R*)-2β,3β,14α,20,22,25-hexahydroxy-5β-cholest-7-en-6-one] **1**, differs from its biosynthetic precursor ecdysone **2** by the presence of a hydroxy group at C-20 and as a consequence by an opposite configuration at C-20. 20-Hydroxyecdysone is involved in many physiological processes of insects and crustaceans, but is mainly known as their moulting hormone since it regulates moulting and metamorphosis in conjuction with the juvenile hormones. A related steroid inokosterone **3**, which possesses a hydroxy group at C-26 rather than C-25, was first isolated from *Achyranthes fauriei*[2] and shown to be an epimeric mixture at C-25 (C-25*S* **3a**:C-25*R* **3b**, 2:1). So far around 85 ecdysteroids have been isolated from invertebrates and more than 130 from plants, probably as a result of plants building up defence mechanisms against insects through evolution.[3] However, ecdysteroids *per se* cannot be used as pest control agents because of their chemical nature and the fact that some insects possess efficient detoxification pathways.

The chemical synthesis of ecdysteroids is complex and expensive, they have poor penetration properties, through cuticle and gut, as a result of a high polarity and they are unstable under field conditions. However, they might be an invaluable lead towards the development of non-steroidal analogues. The identification of the first non-steroidal agonist was reported in 1988[4] stimulating the search for related compounds with improved biological activity.[5] So far a number of structural features of 20-hydroxyecdysone have been identified as important for maximum bioactivity through various bioassays, among which the β-face of the A/B rings, the presence of a 14α-hydroxy group and the vicinity of the 22-hydroxy group are of importance.[6] A convenient bioassay has been developed in our laboratories, derived from a B_{II} tumorous blood cell line of *Drosophila melanogaster* to assess the structural requirements for maximal bioactivity,[7] with the aim of screening various 20-hydroxyecdysone analogues, phytoecdysteroids as well as chemically synthesised ecdysteroids.

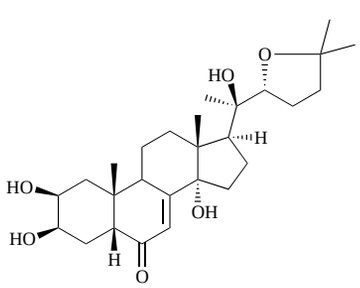
As part of this programme, we decided to investigate the role of the ecdysteroid side-chain by the synthesis of a series of analogues of both 20-hydroxyecdysone **1** and ecdysone **2** in which conformational flexibility of the side-chain was restricted by the introduction of a ring-system. Recently we reported[8] the first chemical synthesis of shidasterone {(20*R*)-2β,3β,14α,20-tetrahydroxy-20-[(2*R*)-5,5-dimethyltetrahydrofuran-2-yl]-5β-pregn-7-en-6-one} **4**, a side-chain analogue of 20-hydroxyecdysone **1** containing a tetrahydrofuran ring. Shidasterone was first isolated in 1969[9] and partially characterised in 1975[10] although the unambiguous determination of its configuration at C-22 was only recently revealed.[8] Herein we describe full



1, R = OH; 20-hydroxyecdysone
2, R = H; ecdysone



3; inokosterone [**3a** = C-25*S*, **3b** = C-25*R*]



4; shidasterone

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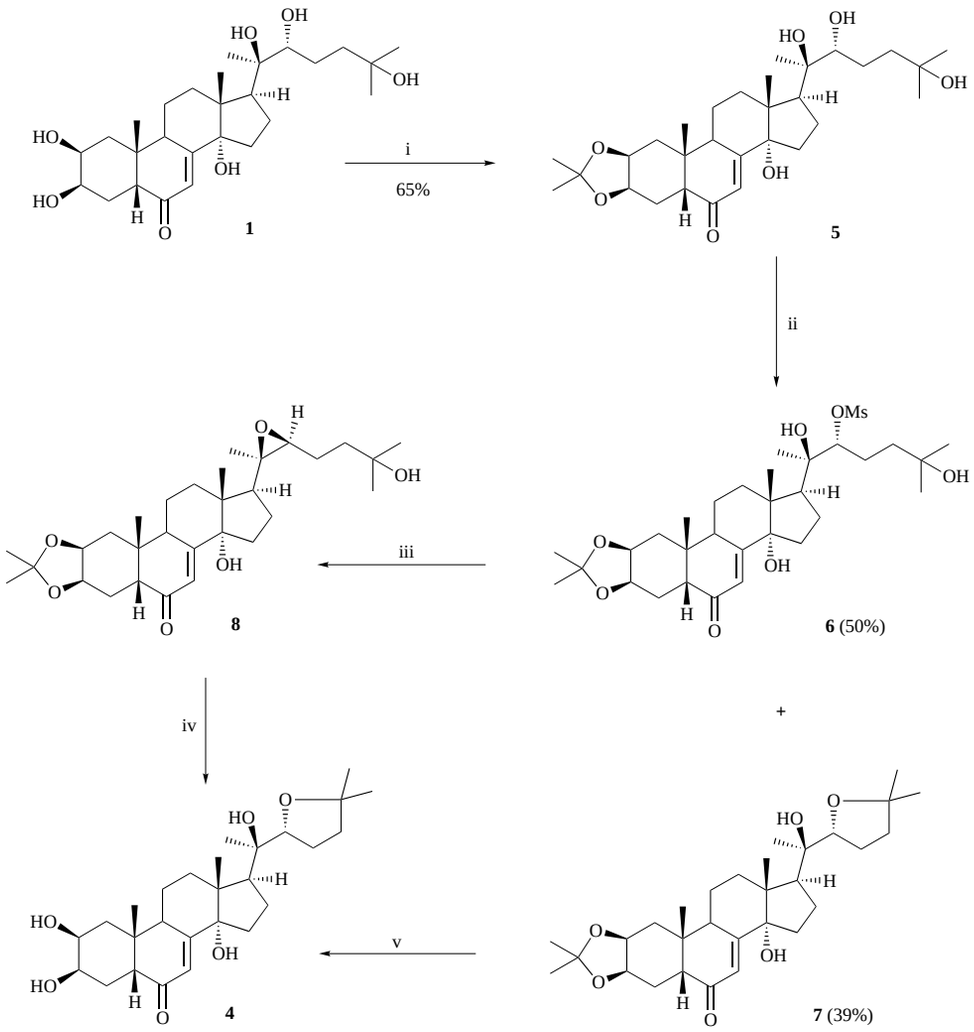


Scheme 1 *Reagents and conditions:* i, (*a*) phenylboronic acid, dry DMF; (*b*) fused *p*-TsOH, dry DMP, dry acetone; (*c*) 30% NaOH–H_2O_2 in THF–water (9:1); ii, CH_3SO_2Cl, $(Pr^i)_2NEt$, dry CH_2Cl_2; iii, spray-dried KF, dry MeCN or 1 M TBAF in THF, dry THF; iv, (*a*) $Et_3N{\cdot}3HF$, 60 °C; (*b*) 0.1 M HCl–dioxane (1:1) or directly 0.1 M HCl–dioxane (1:1); v, 0.1 M HCl–dioxane (1:1)

details of these experiments together with the synthesis of two novel side-chain analogues of ecdysone **2**. The biological activities of the new derivatives have been determined and compared with data for ecdysone **2**, 20-hydroxyecdysone **1** and the individual epimers, **3a** and **3b**, of inokosterone.

Results and discussion

Synthesis of side-chain analogues

In order to selectively manipulate the side-chain of 20-hydroxyecdysone **1**, it was necessary to protect the 2,3-*cis*-diol moiety first. This was achieved by adopting the published route involving initial protection of the 20,22-diol by formation of a 20,22-phenyl boronate, followed by isopropylidene formation at C-2/C-3 and subsequent removal of the phenyl boronate (Scheme 1).[10] Application of these conditions to 20-hydroxyecdysone **1** afforded 2,3-*O*-isopropylidene-20-hydroxyecdysone **5** in 65% overall yield. Treatment of the isopropylidene **5** with mesyl (methanesulfonyl) chloride and diisopropylethylamine (DIPEA) yielded a mixture of the mesylate (methanesulfonate) **6** (50%) and 2,3-*O*-isopropylideneshidasterone **7** (39%). The mesylate **6** was not very stable but showed spectroscopic data in accord with its structure. The structure of 2,3-*O*-isopropylideneshidasterone **7** was assigned on the basis of the NMR data and confirmed by FAB mass spectrometry (found $[M + H]^+$, 503.3366; required $[M + H]^+$, 503.3372).

Treatment of the mesylate **6** with either spray-dried KF in dry MeCN or anhydrous 1 M tetra-*n*-butylammonium fluoride (TBAF) in THF afforded the epoxide **8** in 76 and 57% yields, respectively. The ^1H NMR spectrum of **8** showed that the C-22 proton had a chemical shift of 2.76 ppm, which is in the characteristic range for an epoxide. The epoxide **8** was thought to be a versatile intermediate towards C-20 and/or C-22 analogues of 20-hydroxyecdysone **1** *via* ring opening with nucleophilic agents such as fluoride ion to afford fluoroderivatives of 20-hydroxyecdysone. Attempts to optimise the ratio in favour of the epoxide **8** proved difficult, the best results being obtained using freshly purchased anhydrous TBAF (45% of **8**) (Table 1). TLC monitoring indicated that mesylation occurred rapidly, but after approximately 10 min formation of the furan derivative **7** could be detected, prior to complete consumption of the starting material **5**.

Formation of the epoxide **8** from the mesylate **6** presumably occurs *via* nucleophilic displacement of the mesylate moiety by the C-20 hydroxy group resulting in a 20*R*,22*S* configuration for the epoxide. Formation of the tetrahydrofuran ring in **7** results from subsequent ring-opening of the epoxide **8** by the 25-OH group leading to a 22*R* configuration in **7**. Evidence for this proposal was derived from the following observations. An attempt to deprotect the epoxide **8** using 0.1 M HCl yielded shidasterone **4** in 91% yield. Furthermore, treatment of the epoxide **8** with $Et_3N{\cdot}3HF$ at 60 °C, followed by deprotection of the 2,3-*cis* diol afforded shidasterone **4** in 48% yield. Finally, shidasterone was also obtained by removal of the 2,3-*O*-isopropylidene moiety of compound **7**. These three samples of shidasterone **4** were shown to be identical by TLC [$CHCl_3$–EtOH (1:1), R_f 0.40] and HPLC [MeOH–H_2O (60:40) t_r 34 min] analysis suggesting that formation of the 20,22-epoxide

Table 1 The effect of various parameters on the conversion of 2,3-*O*-isopropylidene-20-hydroxyecdysone **5** to epoxide **8** and protected shidasterone **7**. *Reagents and conditions:* i, mesyl chloride (5 equiv.), DIPEA (5 equiv.), 10% DMAP, room temp., 20 min; ii (a) spray-dried KF (25 equiv.) (further dried under high vacuum prior to use), dry MeCN, reflux, 2 h; (b) anhydrous 1 M TBAF in THF (20 equiv.), dry THF, room temp., 30 min; or (c) as in (b) but with freshly purchased anhydrous 1 M TBAF in THF.

Procedure	Recovered starting material **5** (%)	20,22-Epoxide **8** (%)	2,3-*O*-Isopropylidene shidasterone **7** (%)	Overall yield (%)
i and ii (a)	8	3.5	42.5	54
i and ii (b)	17	27	31	75
i and ii (c)	Not recovered	46	10	56
i and ii (c)	6	45	10	61
i and ii (c)	15	33	6	54

Table 2 Evidence for the assignment at C-22 of the ecdysone derivatives **9**, **11**, **13** and **14**

	Compound					
	2 (22*R*)	(22*S*)	**9** (22*R*)	**13** (22*S*)	**14** (22*R*)	**11** (22*S*)
R_f	0.47 (7:3)[a]	0.26 (7:3)[a]	0.48 (7:1)[a]	0.42 (7:1)[a]	0.40 (7:1)[a]	0.28 (7:1)[a]
t_r[b]	13 min (60:40)[c]	32 min (60:40)[c]	22 min (70:30)[c]	38 min (70:30)[c]	12 min (80:20)[c]	22 min (80:20)[c]
$[\alpha]_D$	+64.7 (EtOH)	+40.9 (MeOH)	+36.2 ($CHCl_3$)	+28.9 ($CHCl_3$)	+133.4 ($CHCl_3$)	+60.4 ($CHCl_3$)
22-H (^{1}H NMR), overlapping dd appearing as	br d-like; *J* 10 Hz	t-like; *J* 6 Hz	br d-like; *J* 10 Hz	t-like; *J* 6 Hz	overlapping with 3-H	t-like; *J* 7 Hz
C-23 (ppm) (^{13}C NMR)	25.5	31.0	24.6	30.3	25.1	29.5

[a] Ratio of $CHCl_3$:EtOH. [b] C_{18} column. [c] Ratio of MeOH:H_2O in solvent system.

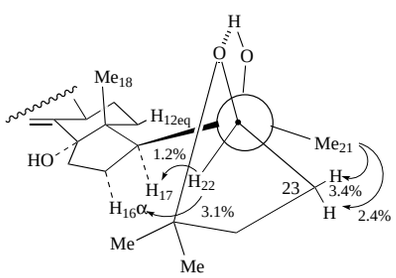


Fig. 1 NOE experiment for 2,3-isopropylidene shidasterone **7**

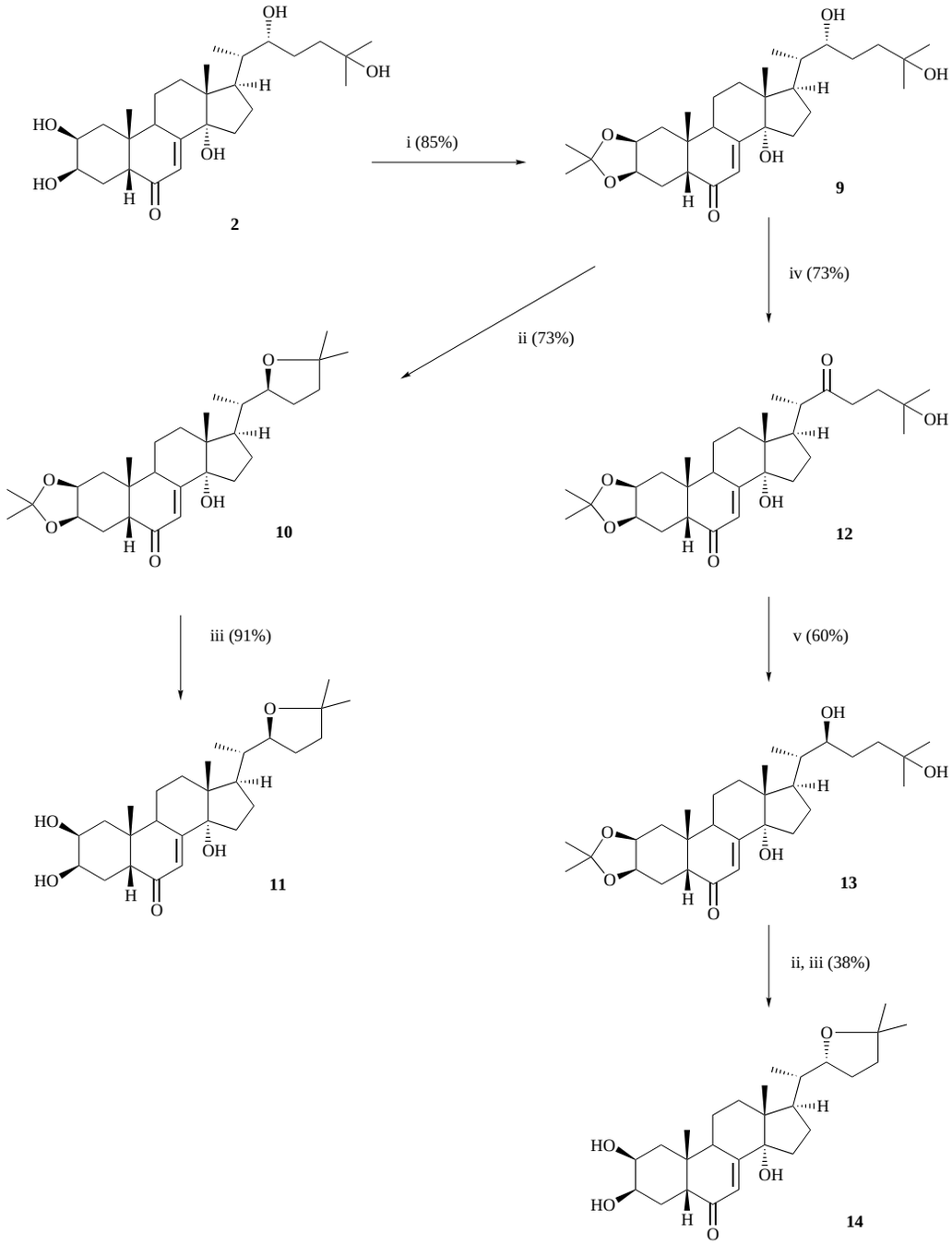
moiety is the initial step towards the intramolecular cyclisation leading to the formation of the furan ring of compounds **4** and **7**. Supporting spectroscopic data were derived from NOE experiments on 2,3-*O*-isopropylideneshidasterone **6** (Fig. 1).

The absence of a 20-hydroxy group in ecdysone **2** simplifies the preparation of the corresponding 2,3-*O*-isopropylidene protected compound **9**. Thus treatment of ecdysone **2** with fused toluene-*p*-sulfonic acid (*p*-TsOH), dimethoxypropane (DMP) and acetone in DMF afforded 2,3-*O*-isopropylideneecdysone **9** in 85% yield after purification by reversed-phase preparative HPLC (Scheme 2).[11] Failure to implement strictly anhydrous conditions has been reported to result in a reduction of the yield[12] and the joint use of *p*-TsOH and DMP in DMF was found to afford the best results. Extensive ^{1}H and ^{13}C NMR data are available in the literature for both 20-hydroxyecdysone **1** and ecdysone **2**[13] and were used to assign the spectra of all new compounds reported here.

Treatment of 2,3-*O*-isopropylideneecdysone **9** with trifluoromethanesulfonic anhydride (triflic anhydride) in dry pyridine resulted in a rapid conversion to the tetrahydrofuran containing compound **10** in 73% yield. In contrast to the 20-hydroxyecdysone series (Scheme 1, **5** to **7**), it is presumed that this reaction proceeds *via* direct nucleophilic attack of the C-25 hydroxy group on to the C-22 carbon *via* an S_N2 process, resulting in the inversion of configuration at C-22 and hence a 22*S* configuration for the cyclised derivative **10**. The driving force for the reaction is presumably the formation of the thermodynamically favoured tetrahydrofuran five-membered ring. The proposed structure for **10** is in full agreement with spectroscopic data (such cyclised side-chain ecdysteroid derivatives have already been isolated as natural products in the 20-hydroxyecdysone series[9,10] but none in the ecdysone series has been reported yet). Deprotection of **10** with 0.1 M HCl in dioxane afforded furan derivative **11** in 91% yield.

The C-22 epimer of compound **11** was also prepared *via* the following sequence. In order to invert the 22*R*-hydroxy group of **9**, it was decided to employ an oxidation–reduction sequence. The 22-hydroxy group of alcohol **9** was oxidised to the ketone **12** *via* treatment with tetra-*n*-propylammonium perruthenate (TPAP, 10 mol%) and *N*-methylmorpholine *N*-oxide (NMO) as a co-oxidant affording the desired 2,3-*O*-isopropylidene-22,22-*O*-didehydroecdysone **12** in a 73% yield. Characteristically, the IR spectrum showed a new band at 1702 cm^{-1} for the saturated ketone and in the ^{1}H NMR spectrum, the signal of H-22 disappeared while in the ^{13}C NMR spectrum, the signal due to C-22 moved downfield from 74.4 to 214.9 ppm. Reduction of the ketone **12** with $LiAlH(OBu^t)_3$ in THF afforded 2,3-*O*-isopropylidene-22-*epi*-ecdysone **13**, in 60% yield. The reaction was monitored by TLC analysis and was quenched immediately non-UV-active polar material began to appear (resulting from the reduction of the unsaturated C-6 ketone). Some starting ketone **12** (10%) and the 22*R*-alcohol **9** (9%) were also recovered. $LiAlH(OBu^t)_3$ had been favoured over $NaBH_4$ to maximise the ratio of the 22*S*-derivative.[14] Data for the 22*S*-alcohol **13** are in full agreement with the proposed structure (Table 2). The ^{1}H NMR spectra for the alcohols **9** and **13** are almost identical, apart from a smaller coupling constant for the 22-H signal of compound **13**. As a result the 22-H appears respectively as a doublet of doublets and as a triplet for the 22*R*- and the 22*S*-derivatives.

Treatment of 2,3-*O*-isopropylidene-22-*epi*-ecdysone **13** with triflic anhydride in pyridine followed by deprotection of the crude product with 0.1 M HCl in dioxane afforded the furan derivative **14** in an overall yield of 38%. The C-22 configuration was assigned *R* on the basis of the presumed S_N2 mechanism (see above). However, this mechanism-based assignment of configuration is also supported by a series of other observations. It was reported by Barton *et al*. that 22*R*-steroids are less



Scheme 2 *Reagents and conditions:* i, fused *p*-TsOH, dry DMP, dry acetone, dry DMF; ii, triflic anhydride, dry pyridine; iii, 0.1 M HCl–dioxane (1:1); iv, TPAP, NMO, dry CH_2Cl_2; v, $LiAlH(OBu^t)_3$, THF; vi, (*a*) triflic anhydride, dry pyridine, (*b*) 0.1 M HCl–dioxane (1:1)

polar than 22*S*-steroids and possess a higher $[\alpha]_D$ value than their 22*S*-counterparts.[15] This has been shown to be the case with all the 22*R*- and 22*S*-derivatives described herein, including the tetrahydrofuran derivatives **11** and **14** (Tables 2, 3 and 4). Moreover, it was stated[15] while purifying various 22*R*/22*S*-derivatives on a C_{18} reversed-phase HPLC column that surprisingly, 22*S*-derivatives did not elute before the less polar, by TLC analysis, 22*R*-derivatives as expected. This chromatographic behaviour was consistent for all 22*R*/22*S*-derivative pairs which have been tested, including the compounds **10** and **11** (Table 2). It was also stated[15] that 22-H appears as a triplet and as a broad doublet, respectively, for a 22*S*- and 22*R*-configuration and that the ^{13}C chemical shift of C-23 is deshielded (around 30 ppm) for 22*S* and shielded (around 25 ppm) for 22*R*. It appears that the 22*R* and 22*S* assignments for **11** and **14** agree with all the above five criteria (Table 2). Thus, the circumstantial evidence obtained is consistent with the assignment of the respective 22*S* and 22*R* configurations of **11** and **14**.

Biological activity

We have examined the biological activity of the cyclised side-chain analogues of 20-hydroxyecdysone **1** and ecdysone **2** using a microplate-based bioassay which has been developed using a tumorous B_{II} blood cell line from *Drosophila melanogaster*.[9] This bioassay has been shown to be reproducible, sensitive, specific to ecdysteroids and quantitative. Table 5 gives the effective dose of the ecdysteroid required for a 50% biological response (ED_{50}). Also included are the data for the individual isomers of inokosterone **3a** and **3b** which were separated on a small scale by repeated reversed-phase HPLC (44% methanol in water; 2 ml min^{-1}; t_r 24.5 min for **3b** and 25.8 min for **3a**). All compounds tested showed only agonistic activity, no antagonistic activity was found at any concentration. Examination of the data suggests that the biological activity is differently affected by cyclised side-chain derivatives. The ecdysone analogues **11** and **14** are six- to eight-fold less active than ecdysone **2** while shidasterone **4** has a drastically reduced activ-

Table 3 ^{1}H NMR chemical shifts of derivatives of 20-hydroxyecdysone **1** and ecdysone **2** (ppm)[a]

	Ecdysone **2** D_2O	(22*R*)-Furan derivative **14** $CDCl_3$	(22*S*)-Furan derivative **11** $CDCl_3$	20-Hydroxy-ecdysone **1** CD_3OD	2,3-*O*-Isopropyl-idene 20-HE **5** CD_3OD	2,3-*O*-Isopropyl-idene 20-HE **5** $[^2H]_6DMSO$	2,3-*O*-Isopropyl-idene 20-HE **5** $CDCl_3$	Shidasterone **4** $CDCl_3$	Shidasterone **3** CD_3OD
1_{ax}-H	1.38 (ov. dd; *J* 14, 13)	1.39 (ov. dd; *J* 15, 13)	1.35 (ov. dd; *J* 15, 13)	1.41	—	1.12	—	1.40	1.42 (ov. dd; *J* 13, 12)
1_{eq}-H	1.87	1.84	1.75	1.78	—	1.87	—	1.86	—
2_{ax}-H	3.99 (ov. ddd, *J* 12, 3, 3)	3.89 (ov. ddd; *J* 12, 3, 3)	4.02 (ov. ddd; *J* 12, 3, 3)	3.83 (ov. ddd; *J* 12, 4, 4)	4.24 (m; $w_{1/2}$ 23)	4.20 (m; $w_{1/2}$ 25)	4.25 (m; $w_{1/2}$ 22)	3.89 (m; $w_{1/2}$ 17)	3.84 (ov. ddd; *J* 12, 3, 3)
3_{eq}-H	4.07 (m; $w_{1/2}$ 8)	4.03 (m; $w_{1/2}$ 14)	3.94 (m; $w_{1/2}$ 10)	3.95 (m; $w_{1/2}$ 8)	4.24 (m; $w_{1/2}$ 23)	4.20 (m; $w_{1/2}$ 25)	4.25 (m; $w_{1/2}$ 22)	4.04 (app. br s)	3.94 (m, $w_{1/2}$ 20)
4_{ax}-H	1.75	1.64	1.56	1.65	—	1.50	—	1.64	—
4_{eq}-H	1.75	1.85	1.68	1.75	—	1.82	—	1.84	—
5-H	2.34 (ov. dd; *J* 10, 9)	2.43 (ov. dd; *J* 13, 4)	2.34 (ov. dd; *J* 13, 5)	2.38 (m; $w_{1/2}$ 20)	2.25 (ov. dd; *J* 9, 7)	2.11 (dd; *J* 12, 5)	2.32 (m; $w_{1/2}$ 32)	2.43 (dd; *J* 13, 4)	2.36 (m, $w_{1/2}$ 25)
7-H	5.98 (d; *J* 2.5)	5.83 (d; *J* 2.5)	5.78 (d; 2.5)	5.81 (d; *J* 2.5)	5.81 (d; *J* 2.5)	5.64 (app. br s)	5.83 (d; *J* 2.5)	5.85 (d; *J* 2.5)	5.81 (d; *J* 2.5)
9-H	3.10 (m; $w_{1/2}$ 24)	2.98 (ov. ddd; *J* 12, 8, 2.5)	3.06 (ov. ddd; *J* 12, 7, 2.5)	3.15 (ov. ddd; *J* 12, 8, 2.5)	2.95 (ov. ddd; *J* 11.5, 8, 2.5)	2.81 (app. ov. dd; *J* 12, 8)	2.84 (ov. ddd; *J* 12, 9, 2.5)	3.02 (ov. ddd; *J* 12, 7, 2.5)	3.15 (ov. ddd; *J* 12, 7, 2.5)
11_{ax}-H	1.75	1.64	1.62	1.65	—	1.48	—	1.62	—
11_{eq}-H	1.85	1.77	1.77	1.78	—	1.65	—	1.78	—
12_{ax}-H	1.97	2.04	2.13	2.14 (ov. ddd; *J* 13, 13, 4)	2.13 (ov. ddd; *J* 13, 13, 4)	2.03 (ov. ddd; *J* 13, 13, 4)	—	2.06	2.15 (ov. ddd; *J* 13, 13, 4)
12_{eq}-H	1.63	1.78	1.74	1.85	—	1.75	—	1.85	—
15_{α}-H	1.38	2.02	1.88	1.60	—	1.48	—	1.52	—
15_{β}-H	1.84	1.87	2.13	1.95	—	1.80	—	2.02	—
16_{α}-H	1.65	1.48	1.45	1.75	—	1.65	—	1.87	—
16_{β}-H	1.95	1.58	1.74	1.95	—	1.85	—	2.04	—
17-H	2.04 (m, $w_{1/2}$ 32)	2.00	2.13	2.38 (m, $w_{1/2}$ 20)	2.40 (ov. dd; *J* 10, 8)	2.27 (ov. dd; *J* 10, 8.5)	2.32 (m, $w_{1/2}$ 32)	2.30 (ov. dd; *J* 10, 8)	2.36 (m, $w_{1/2}$ 25)
18-Me	0.74 (s)	0.68 (s)	0.64 (s)	0.90 (s)	0.87 (s)	0.78 (s)	0.87 (s)	0.83 (s)	0.84 (s)
19-Me	1.00 (s)	0.97 (s)	0.93 (s)	0.98 (s)	0.96 (s)	0.88 (s)	1.00 (s)	0.98 (s)	0.96 (s)
20-H	1.83	2.15	1.46						
21-Me	0.94 (d; *J* 6.6)	0.90 (d; *J* 6.6)	0.89 (d; *J* 6.6)	1.19 (s)	1.29 (s)	1.07 (s)	1.22 (s)	1.19 (s)	1.21 (s)
22-H	3.70 (br d-like; 10, $w_{1/2}$ 16)	4.03 (ov. m with 3-H, $w_{1/2}$ 22)	4.12 (ov. dd; *J* 8.7; appearing as a t-like, *J* 6, $w_{1/2}$ 18)	3.33 (app. d; *J* 10)	3.34 (app. d; *J* 10)	3.13 (app. d; *J* 10)	3.48 (app. d; *J* 10)	3.89 (m, $w_{1/2}$ 17)	3.94 (m, $w_{1/2}$ 20)
23_a-H	1.35	1.70	1.70	1.28	—	1.12	—	1.72	—
23_b-H	1.58	1.85	1.90	1.62	—	1.47	—	1.86	—
24_a-H	1.48	1.60	1.54	1.78	—	1.65	—	—	—
24_b-H	1.75	1.77	1.64	1.45	—	1.28	—	—	—
26-Me	1.22 (s)	1.22 (s)	1.19 (s)	1.19 (s)	1.21 (s)	1.07 (s)	1.27 (s)	1.24 (s)	1.24 (s)
27-Me	1.23 (s)	1.23 (s)	1.23 (s)	1.19 (s)	1.21 (s)	1.08 (s)	1.27 (s)	1.25 (s)	1.25 (s)
29-Me	—	—	—	—	1.33 (s)	1.28 (s)	1.33 (s)	—	—
30-Me	—	—	—	—	1.47 (s)	1.41 (s)	1.50 (s)	—	—

[a] ov. = overlapping.

Table 4 ^{13}C chemical shifts of derivatives of 20-hydroxyecdysone **1** and ecdysone **2** (ppm)

	Ecdysone **2** CD_3OD	(22*R*)-Furan derivative **14** $CDCl_3$	(22*S*)-Furan derivative **8** $CDCl_3$	20-Hydroxy-ecdysone **1** CD_3OD	2,3-*O*-Isopropyl-idene 20-HE **5** CD_3OD	2,3-*O*-Isopropyl-idene 20-HE **5** $[^2H]_6DMSO$	2,3-*O*-Isopropyl-idene-20-HE **5** $CDCl_3$	Shidasterone **4** CD_3OD
C-1	37.5	36.9	36.7	37.3	38.8	37.3	37.6	37.3
C-2	68.7	67.8	67.4	68.6	73.5	71.4	72.2	68.6
C-3	68.5	67.4	67.1	68.5	73.2	71.2	71.7	68.4
C-4	32.8	31.4	31.7	32.7	27.7	26.0	26.7	32.7
C-5	51.8	50.0	50.1	51.7	52.5	50.5	50.8	51.7
C-6	206.4	203.4	204.3	206.6	205.6	201.6	202.8	206.4
C-7	122.0	121.6	121.6	122.1	121.6	120.1	121.5	122.0
C-8	167.5	164.0	164.9	168.1	167.2	164.8	163.3	167.9
C-9	35.3	34.0	33.9	35.0	35.7	33.9	34.6	35.0
C-10	39.2	38.3	38.3	39.3	38.9	37.2	37.8	39.2
C-11	21.6	20.5	20.5	21.5	21.5*	20.1	20.6	21.4*
C-12	32.1	32.2	30.9	32.4	32.5	30.9	31.2	32.2
C-13	48.2	47.2	46.7	48.6	48.6	47.1	47.7	48.6
C-14	85.1	84.7	84.8	85.2	85.2	82.9	84.8	85.2
C-15	32.1	30.7	30.7	31.7	31.7	30.2	31.6	31.6
C-16	27.0	25.8	26.1	21.5	21.6*	20.4	20.5	21.6*
C-17	48.8	48.1	48.1	50.5	50.5	48.7	49.1	51.7
C-18	16.2	15.8	15.5	18.1	16.0	17.1	17.4	18.0
C-19	24.4	23.9	23.9	24.4	24.0	23.2	23.6	24.3
C-20	43.4	37.9	39.5	78.0	77.7	75.7	76.9	76.9
C-21	13.3	12.6	12.9	21.1	21.1	21.9	20.7	20.6
C-22	75.3	80.3	79.9	78.4	78.4	76.2	76.7	85.4
C-23	25.5	25.1	29.5	27.3	27.4	26.1	26.1	28.4
C-24	42.2	38.8	38.5	42.3	42.4	41.4	40.8	39.5
C-25	71.4	79.9	81.1	71.4	71.3	68.7	70.6	81.7
C-26	29.3	28.0	27.3	29.1	29.0	29.0	29.3	28.3
C-27	29.5	28.6	28.3	29.7	29.7	29.9	30.0	28.9
C-28	—	—	—	—	109.5	107.3	108.3	—
C-29	—	—	—	—	26.6	26.4	26.4	—
C-30	—	—	—	—	28.8	28.4	28.5	—

* Assignments may be reversed.

Table 5 ED_{50} of the ecdysteroid derivatives tested using the B_{II} cell line-based bioassay

Ecdysteroid	ED_{50}/M
20-Hydroxyecdysone **1**	7.5×10^{-9}
Ecdysone **2**	1.1×10^{-6}
Shidasterone **4**	1.5×10^{-6}
(25*S*)-Inokosterone **3a**	2.7×10^{-7}
(25*R*)-Inokosterone **3b**	1.5×10^{-7}
(22*R*)-Furan derivative **14**	1.0×10^{-5}
(22*S*)-Furan derivative **11**	7.3×10^{-6}

ity (200-fold less active than 20-hydroxyecdysone **1**). The similar activities found for the 22*S*-furanyl **11** and 22*R*-furanyl **14** suggest that the spatial orientation of the furan ring is such that there is little steric difference between the two epimers. The C-25*R* isomer of inokosterone **3b** was found to be twice as active as the C-25*S* isomer **3a** but both were considerably less active (20- to 40-fold) than 20-hydroxyecdysone **1**.

Experimental

General

Ecdysone (250 mg) was obtained mainly from Simes, Milan, although a small sample (50 mg) was kindly donated by Professor R. Lafont, École Normale Supérieure, Paris. 20-Hydroxyecdysone was purchased from Simes and from Sci-Tech, Prague. Inokosterone was obtained from Rhoto Pharmaceutical Co., Japan. All reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were distilled prior to use from sodium–benzophenone and phosphorus pentoxide (P_2O_5), respectively. All other anhydrous solvents were distilled from the stated drying agents and stored under an atmosphere of argon or nitrogen over activated 4 Å molecular sieves: acetone ($CaSO_4$), acetonitrile (CaH_2), dimethylformamide (CaH_2), ethanol (magnesium methoxide), methanol (magnesium methoxide), pyridine (KOH), triethylamine (KOH). DMF was also purchased anhydrous from Aldrich. Petrol refers to the light fraction of boiling point 40–60 °C.

High-performance liquid chromatography was performed on a Gilson HPLC system, comprising two model 303 pumps, a model 803 B manometric module, a 811 C mixing unit, a Holochrome variable wavelength detector and a Rheodyne 7125 injector unit, with sample loops of 1, 2 and 5 cm^3 as well as 50 μl for analytical purposes. The flow-rate delivered by the pumps and the composition of the mobile phase were controlled by a PC computer with a Gilson gradient manager GME 712 program. A Phillips PM8251 single pen recorder plotted the elution profiles. The eluent was collected either manually or with a Gilson model 203 fraction collector. Samples were injected using Hamilton HPLC syringes. Separation and purification were carried out at room temperature. Water for HPLC was deionised and double glass-distilled, while MeOH, CH_3CN and CH_2Cl_2 were HPLC grade. All solvents were degassed immediately prior to use by filtration under suction through 0.45 μm (or 0.5 μm for CH_3CN and CH_2Cl_2) Millipore filters. HPLC was used analytically as well as in semi-preparative and preparative modes, with the respective mobile phase flow-rate [1, 2 and 10 (or 9.9) $cm^3\ min^{-1}$] and monitored for absorption at 242 nm. Three types of column were used. (1) Reversed-phase Spherisorb octadecyl (ODS or C_{18}) columns, with a 5 μm particle size (analytical: 250 × 4.6 mm; semi-preparative: 250 × 10 mm and preparative: 250 × 25 mm) purchased from Jones Chromatography. (2) Normal-phase Apex II DIOL column with a 5 μm particle size (semi-preparative: 150 × 10 mm) purchased from Jones Chromatography. (3) Normal-phase Zorbax SIL column (semi-preparative: 250 × 9.4 mm) purchased from Dupont.

Thin-layer chromatography was performed on Merck 60F-254 (0.25 mm, art. 5715) glass-backed silica gel plates. Visualisation was by UV fluorescence quenching as well as treatment with an acidic solution of ethanolic *p*-anisaldehyde.

The SEP-PAK cartridge pre-purification was conducted as follows: activation of the cartridge by MeOH (5 cm^3) and then water (10 cm^3), loading of the cartridge with the sample solubilised in water containing 10% MeOH (typically 5 cm^3), elution of the salts with water (20 cm^3), elution of the compound with MeOH (typically 20 cm^3) and concentration of solvents.

IR spectra were recorded on a Perkin-Elmer 881 grating infra-red spectrometer and the peaks are quoted in wavenumbers (cm^{-1}) relative to a polystyrene standard (1601 cm^{-1}). IR bands are qualified as weak (wk), medium (md), strong (st) and, when appropriate, broad (br).

Optical rotations were performed on a Thorn NPL automatic polarimeter type 243 and are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded on Bruker AM250 and AC300 spectrometers. Some NMR spectra of ecdysone derivatives were also recorded by Professor J.-P. Girault, Université René Descartes, Paris, on a Bruker 500 MHz spectrometer. Chemical shifts are expressed in parts per million (ppm) and are reported as: δ_H, δ_C position, number of equivalent nuclei (by integration), multiplicity (s singlet, d doublet, t triplet, q quartet, m multiplet), coupling constants (*J* in Hz), width at half-height for multiplets ($w_{1/2}$ in Hz) and when necessary broad (br). Assignments were achieved with the aid of published NMR data on ecdysteroids.[54] The values of 26- and 27-methyls are interchangeable, but they are always reported with the 27-methyl having the more deshielded chemical shift as in the literature.[54]

High-resolution mass spectra were recorded at the SERC Mass Spectrometry Centre, Swansea on a VG ZAB-E high-resolution instrument. Unless otherwise indicated, they were measured by fast atom bombardment (FAB), using xenon gas and an *m*-nitrobenzyl alcohol matrix.

Note: It proved difficult to recrystallise a number of the reported compounds and thus we were not able to obtain satisfactory microanalyses. In addition, in two cases mass spectral data could not be obtained under either FAB or EI conditions.

Treatment of 2,3-*O*-isopropylidene-20-hydroxyecdysone 5 with methanesulfonyl (mesyl) chloride

Diisopropylethylamine (DIPEA) (50 μl, 0.29 mmol, 7.5 equiv.) was added to a solution of 2,3-*O*-isopropylidene-20-hydroxyecdysone **5** (20 mg, 38.5 μmol) in dry CH_2Cl_2 (5 cm^3) under an atmosphere of N_2. The reaction mixture was cooled to 0 °C and stirred for 10 min. Mesyl chloride (23 μl, 0.29 mmol, 7.5 equiv.) was added and the reaction mixture was stirred for 5 min at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for a further 25 min. After quenching with ice (approx. 2 g) and dilution with CH_2Cl_2, the reaction mixture was washed with water (3 × 10 cm^3). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification was carried out by HPLC on a semi-preparative Apex DIOL normal-phase column (flow rate 2 cm^3 min^{-1}, gradient of MeOH in CH_2Cl_2 from 0 to 5% in 30 min) and yielded 2,3-O-*isopropylidene*-20-*hydroxyecdysone* 22-*mesylate* **6** (11.5 mg, 19.3 μmol, 50%) (t_r 20 min) and 2,3-O-*isopropylideneshidasterone* **7** (7.5 mg, 15 μmol, 39%) (t_r 15 min) as gums in an overall yield of 89%. The reaction was repeated at room temperature and yielded (7.2 mg, 12 μmol, 63%) and **7** (1.5 mg, 3 μmol, 16%) in an overall yield of 79%.

2,3-*O*-Isopropylidene-20-hydroxyecdysone 22-mesylate 6. R_f ($CHCl_3$–EtOH, 7:1) 0.60, olive green spot; $[\alpha]_D^{25}$ +77.4 (*c* 0.12 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 1671wk (cyclohexenone); λ_{max}(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(300 MHz, $CDCl_3$) 5.77 (1H, d, *J* 2.5, 7-H), 4.56 (1H, d, *J* 8, 22-H), 4.20 (2H, d, $w_{1/2}$ 25, 2-H and 3-H), 3.10 (3H, s, $MeSO_2O$), 2.81 (1H, overlapping ddd, *J* 12, 8 and 2.5, 9-H), 2.29 (2H, m, $w_{1/2}$ 23, 5-H and 17-H), 1.45 (3H, s, 30-CH_3), 1.29 (3H, s, 29-CH_3), 1.26 (3H, s, 21-CH_3), 1.21 (3H, s, C-CH_3), 1.17 (3H, s, 26-CH_3), 0.94 (3H, s, 19-CH_3), 0.84 (3H, s, 18-CH_3).

The mesylate **6** was too unstable to allow accumulation of a ^{13}C NMR spectrum overnight: no ^{13}C NMR data could be obtained. For the same reason, no mass spectrum was recorded.

2,3-*O*-Isopropylideneshidasterone 7. R_f ($CHCl_3$–EtOH, 7:1) 0.65, olive green spot; $[\alpha]_D^{25}$ +65.4 (*c* 0.37 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3600 sharp wk (free OH), 3400br wk (bonded OH), 1664st (cyclohexenone); λ_{max}(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(300 MHz, $CDCl_3$) 5.81 (1H, d, *J* 2.5, 7-H), 4.23 (2H, m, $w_{1/2}$ 20, 2-H and 3-H), 3.87 (1H, overlapping dd, *J* 9 and 8, 22-H), 2.81 (1H, overlapping ddd, *J* 12, 7 and 2.5, 9-H), 2.33 (1H, dd, *J* 12, 5, 5-H), 2.29 (2H, overlapping dd, *J* 10, 8, 17-H), 1.48 (3H, s, 30-CH_3), 1.32 (3H, s, 29-CH_3), 1.23 (3H, s, 27-CH_3), 1.22 (3H, s, 26-CH_3), 1.18 (3H, s, 21-CH_3), 0.97 (3H, s, 19-CH_3), 0.81 (3H, s, 18-CH_3); δ_C(62.7 MHz, $CDCl_3$) 202.7 (C-6), 163.4 (C-8), 121.4 (C-7), 108.3 (C-28), 84.9 (C-14), 83.9 (C-22), 80.8 (C-25), 75.2 (C-20), 72.2 (C-2), 71.7 (C-3), 51.0 (C-17), 50.8 (C-17 and C-5), 47.4 (C-13), 38.7 (C-24), 37.8 (C-10), 37.7 (C-1), 34.6 (C-9), 31.7 (C-15), 31.0 (C-12), 28.6 (C-27), 28.5 (C-30), 28.1 (C-26), 27.2 (C-23), 26.7 (C-4), 26.4 (C-29), 23.6 (C-19), 20.9 and 20.6 (C-16 and/or C-11), 20.8 (C-21), 17.4 (C-18); *m/z* 525 ($[M + Na]^+$, 39.6%), 503 ($[M + H]^+$, 90), 485 ($[M + H - H_2O]^+$, 100), 467 ($[M + H - 2H_2O]^+$, 15.7), 427 ($[M + H - H_2O - Me_2CO]^+$, 9.5) (Found: $[M + H]^+$, 503.3366. $C_{30}H_{47}O_6$ requires $[M + H]^+$, 503.3372).

2,3-*O*-Isopropylidene-20-hydroxy-20,22-anhydroecdysone 8

Method A. Tetra-*n*-butylammonium fluoride (TBAF) (1 M in THF, 0.05 cm^3, 50 μmol) was added to a solution of 2,3-*O*-isopropylidene-20-hydroxyecdysone 22-mesylate **6** (2 mg, 3.48 μmol) in dry THF (1 cm^3) under an atmosphere of N_2. The reaction mixture was stirred for 5 min at room temperature, quenched with water (2 cm^3) and stirred for a further 10 min, after which the THF was removed under reduced pressure. The residue, in water (2 cm^3), was pre-purified through a C_{18} SEP-PAK cartridge and further purified by HPLC through a C_{18} semi-preparative reversed-phase column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (80:20)] yielding 2,3-O-*isopropylidene*-20-*hydroxy*-20,22-*anhydroecdysone* **8** (1 mg, 1.96 μmol, 57%) (t_r 14 min) as a gum.

Method B. A solution of 2,3-*O*-isopropylidene-20-hydroxyecdysone 22-mesylate **6** (6.2 mg, 10.4 μmol) and spray-dried KF (15 mg, 260 μmol, 25 equiv.) in dry MeCN (3 cm^3) was heated under reflux for 2 h and then cooled, filtered and the solvent concentrated *in vacuo*. Purification was carried out by HPLC through a C_{18} semi-preparative reversed-phase column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (75:25)] to yield epoxide **8** (3.9 mg, 7.9 μmol, 76%) (t_r 7 min) as a gum.

Method C. Diisopropylethylamine (0.04 cm^3, 0.23 mmol, 5 equiv.) was added to a solution of 2,3-*O*-isopropylidene-20-hydroxyecdysone **5** (30 mg, 57.5 μmol) in dry CH_2Cl_2 (5 cm^3) under an atmosphere of N_2. The reaction mixture was stirred for 5 min at room temperature before the addition of mesyl chloride (18 μl, 0.23 mmol, 5 equiv.) was added and then stirred for a further 30 min. TBAF (1 M in THF, 1.5 cm^3) was added and the reaction mixture was stirred for 40 min before being quenched with ice (approx. 2 g) and water (5 cm^3). THF (5 cm^3) was added and the organic solvents were removed under reduced pressure. The compounds were pre-purified through a preparative C_{18} SEP-PAK cartridge. Purification was carried out by HPLC through a semi-preparative Apex DIOL normal-phase column (flow rate 2 cm^3 min^{-1}, gradient of MeOH in CH_2Cl_2 from 0 to 5% in 30 min) and yielded 2,3-*O*-isopropylideneshidasterone **7** (2.8 mg, 5.6 μmol, 9.5%, t_r 17 min), epoxide **8** (13 mg, 25.9 μmol, 43%, t_r 23 min) and the starting material (1.7 mg, 3.3

μmol, 5.5%, t_r 29 min) as colourless gums with a 60% overall yield.

2,3-*O*-Isopropylidene-20-hydroxy-20,22-anhydroecdysone 8. R_f ($CHCl_3$–EtOH, 7:1) 0.60, brown–blue spot; $[\alpha]_D^{25}$ −0.42 (*c* 0.25 in $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3608 sharp md (free OH), 3377br md (bonded OH), 1667st (cyclohexenone); λ_{max}(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(300 MHz, $CDCl_3$) 5.83 (1H, d, *J* 2.5, 7-H), 4.24 (2H, m, $w_{1/2}$ 12, 2-H and 3-H), 2.83 (1H, overlapping ddd, *J* 11, 7.5 and 2.5, 9-H), 2.76 (1H, m, $w_{1/2}$ 14, 22-H), 2.42 (1H, overlapping dd, *J* 10 and 9, 17-H), 2.34 (1H, dd, *J* 12.5 and 5, 5-H), 1.48 (3H, s, 30-CH_3), 1.32 (6H, 2 overlapping s, 21-CH_3 and 29-CH_3), 1.24 (6H, s, 27-CH_3 and 26-CH_3), 0.97 (3H, s, 19-CH_3), 0.75 (3H, s, 18-CH_3); δ_C(75.5 MHz, $CDCl_3$) 202.7 (C-6), 162.8 (C-8), 121.6 (C-7), 108.3 (C-28), 83.9 (C-14), 72.1 (C-2), 71.6 (C-3), 70.5 (C-25), 66.2 (C-22), 61.3 (C-20), 50.9 (C-5), 46.8 (C-17), 46.7 (C-13), 40.7 (C-24), 37.9 (C-10), 37.7 (C-1), 34.4 (C-9), 31.8 (C-12), 29.8 (C-15), 29.6 (C-27), 29.3 (C-26), 28.5 (C-30), 26.7 (C-4), 26.4 (C-29), 23.6 (C-23), 23.5 (C-19), 22.3 (C-21), 21.3 (C-16), 20.2 (C-11), 18.5 (C-18). Despite two attempts no consistent mass spectroscopic data for **8** could be obtained.

Shidasterone 4

Method A: deprotection of 2,3-*O*-isopropylideneshidasterone 7. Aqueous HCl (0.1 M, 2 cm^3) was added dropwise to a solution of 2,3-*O*-isopropylideneshidasterone **7** (15 mg, 29.8 μmol) in MeOH (2 cm^3). The reaction mixture was stirred at room temperature for 3 h, then diluted with water (5 cm^3) and neutralised with aqueous NaOH (0.1 M, 2 cm^3). MeOH was removed under reduced pressure and the product was pre-purified through a C_{18} semi-preparative SEP-PAK cartridge. Purification was carried out by HPLC on a C_{18} semi-preparative reversed-phase column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (60:40)] and yielded *shidasterone* **4** (8.8 mg, 19 μmol, 92%) (t_r 34 min) as a gum.

Method B: deprotection and acidic ring-opening of the epoxide 8. Aqueous HCl (0.1 M, 1 cm^3) was added dropwise to a solution of epoxide **8** (6 mg, 12 μmol) in dioxane (1 cm^3) at room temperature. The reaction mixture was stirred for 3 h, then diluted with water (5 cm^3) and neutralised with aqueous NaOH (0.1 M, 1 cm^3). After removal of the solvents under reduced pressure, the product was pre-purified through a C_{18} semi-preparative SEP-PAK cartridge. Purification was carried out by HPLC on a C_{18} semi-preparative reversed-phase column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (70:30)] and yielded shidasterone **4** (3.5 mg, 7.6 μmol, 64%) (t_r 16 min) as a gum. Using the same HPLC system, but with MeOH–H_2O (60:40), the recorded retention time (t_r) was 34 min.

Method C: treatment of the epoxide 8 with $Et_3N{\cdot}HF$. A solution of epoxide **8** (8 mg, 15.9 μmol) in $Et_3N{\cdot}3HF$ (1.5 cm^3) was heated at 60 °C overnight. The reaction mixture was worked-up by the addition of ice (approx. 2 g), diluted with water (10 cm^3) and neutralised by the addition of aqueous NH_3. The products were extracted with ethyl acetate (3 × 10 cm^3). The organic phases were combined, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Aqueous HCl (0.1 M, 1 cm^3) was added to the concentrated solution which had been previously diluted by dioxane (1 cm^3). The reaction was stirred for 2.5 h, then diluted with water (5 cm^3) and neutralised with aqueous NaOH (0.1 M, 1 cm^3). After concentration of the solvents under reduced pressure, the product was pre-purified through a C_{18} semi-preparative SEP-PAK cartridge. Purification was carried out by HPLC on a C_{18} semi-preparative reversed-phase column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (70:30)] and yielded shidasterone **4** (3.7 mg, 7.8 μmol, 49%) (t_r 16 min) as a gum.

Shidasterone 4. R_f ($CHCl_3$–EtOH, 7:1) 0.40, olive green spot; $[\alpha]_D^{25}$ +65.0 (*c* 0.18 in $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3600 sharp m (free OH), 3400br md (bonded OH), 1664st (cyclohexenone); λ_{max}(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(300 MHz, CD_3OD) 5.81 (1H, d, *J* 2.5, 7-H), 3.94 (2H, m, $w_{1/2}$ 20, 3-H and 22-H), 3.84 (1H, overlapping ddd, *J* 12, 3 and 3, 2-H), 3.15 (1H, overlapping ddd, *J* 12, 7.5 and 2.5, 9-H), 2.36 (2H, m, $w_{1/2}$ 25, 5-H and 17-H), 2.15 (1H, overlapping ddd, *J* 13, 13 and 4, 12_{ax}-H), 1.42 (1H, overlapping dd, *J* 13 and 12, 1_{ax}-H), 1.25 (3H, s, 27-CH_3), 1.24 (3H, s, 26-CH_3), 1.21 (3H, s, 21-CH_3), 0.96 (3H, s, 19-CH_3), 0.84 (3H, s, 18-CH_3); δ_C(75.5 MHz, CD_3OD) 206.4 (C-6), 167.9 (C-8), 122.0 (C-7), 85.4 (C-22), 85.2 (C-14), 81.7 (C-25), 76.9 (C-20), 68.6 (C-2), 68.4 (C-3), 51.7 (C-5 and C-17), 48.6 (C-13), 39.5 (C-24), 39.2 (C-10), 37.3 (C-1), 35.0 (C-9), 32.7 (C-4), 32.2 (C-12), 31.6 (C-15), 28.9 (C-27), 28.4 (C-23), 28.3 (C-26), 24.3 (C-19), 21.6 and 21.4 (C-11 and/or C-16), 20.6 (C-21), 18.0 (C-18); *m/z* 485 ($[M + Na]^+$, 13.7%), 463 ($[M + H]^+$, 55.5), 445 ($[M + H - H_2O]^+$, 24.2), 123 (100) (Found: $[M + H]^+$, 463.3028. $C_{27}H_{42}O_6$ requires $[M + H]^+$, 463.3059).

2,3-*O*-Isopropylideneecdysone 9

To a solution of ecdysone **2** (35 mg, 75 μmol) in dry DMF (2 cm^3) under an atmosphere of N_2, was added a solution of fused toluene-*p*-sulfonic acid (*p*-TsOH) (10 mg, 0.5 equiv., 37.5 μmol) in a mixture of dry 2,2-dimethoxypropane (DMP) (1 cm^3) and dry acetone (1 cm^3). The reaction mixture was stirred at room temperature for 3 h until the conversion was found to be complete by TLC analysis ($CHCl_3$–EtOH, 7:1) and then quenched with water (5 cm^3). DMP and acetone were removed under reduced pressure. The residue was diluted with ethyl acetate (10 cm^3) and washed with saturated aqueous NaCl (6 × 15 cm^3). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification was carried out by HPLC on a preparative reversed-phase C_{18} column [flow rate 9.9 cm^3 min^{-1}, MeOH–H_2O (80:20)] and yielded 2,3-O-*isopropylideneecdysone* **9** (33 mg, 65.5 μmol, 86%) (t_r 11 min) as a gum; R_f ($CHCl_3$–EtOH, 7:1) 0.48, dark blue spots; $[\alpha]_D^{25}$ +36.2 (*c* 0.3, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3433br wk (OH), 1664st (cyclohexenone); λ_{max}(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(250 MHz, $CDCl_3$) 5.81 (1H, d, *J* 2.5, 7-H), 4.16–4.28 (2H, m, $w_{1/2}$ 19, 3-H and 2-H), 3.65 (1H, overlapping dd, *J* 10 and 2.5, 22-H), 2.82 (1H, overlapping ddd, *J* 11.5, 7.5 and 2.5, 9-H), 2.34 (1H, dd, *J* 12.5 and 4.5, 5-H), 1.48 (3H, s, 30-CH_3), 1.32 (3H, s, 29-CH_3), 1.24 (3H, s, 27-CH_3), 1.23 (3H, s, 26-CH_3), 0.98 (3H, s, 19-CH_3), 0.94 (3H, d, *J* 8, 21-CH_3), 0.68 (3H, s, 18-CH_3); δ_C(62.7 MHz, $CDCl_3$) 202.8 (C-6), 183.2 (C-8), 121.3 (C-7), 108.3 (C-28), 84.6 (C-14), 74.4 (C-22), 72.2 (C-2), 71.7 (C-3), 70.7 (C-25), 50.8 (C-5), 47.6 (C-17), 47.3 (C-13), 42.0 (C-20), 40.1 (C-24), 37.8 (C-10), 37.6 (C-1), 34.8 (C-9), 31.9 (C-12), 30.8 (C-15), 30.2 (C-27), 29.2 (C-26), 28.5 (C-30), 26.8 (C-4), 26.4 (C-29), 25.9 (C-16), 24.6 (C-23), 23.6 (C-19), 20.7 (C-11), 15.8 (C-18), 12.8 (C-21); *m/z* 527 ($[M + Na]^+$, 48%), 505 ($[M + H]^+$, 30), 487 ($[M + H - H_2O]^+$, 100), 469 ($[M + H - 2H_2O]^+$, 61), 411 ($[M + H - 2H_2O - Me_2CO]^+$, 27), 329 ($[M + H - \text{side-chain} - Me_2CO]^+$, 29) (Found: $[M + H]^+$, 505.3529. $C_{30}H_{48}O_6$ requires $[M + H]^+$, 505.3529).

2,3-*O*-Isopropylidene-22,22-*O*-didehydroecdysone 12

A mixture of 2,3-*O*-isopropylideneecdysone **9** (30 mg, 60 μmol), *N*-methylmorpholine *N*-oxide (20 mg, 170 μmol, 2.89 equiv.) and powdered 4 Å molecular sieves (100 mg), were stirred at room temperature for 15 min in dry CH_2Cl_2 (4 cm^3). Tetra-*n*-propylammonium perruthenate (TPAP) (2.1 mg, 6 μmol, 0.1 equiv.) was added and the mixture stirred under a positive pressure of argon for 3 h. The reaction mixture was filtered through a short pad of silica–Celite (1:1), which was then washed with ethyl acetate (10 cm^3). After concentrating the filtrate *in vacuo* the product was purified by HPLC through a semi-preparative reversed-phase C_{18} column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (85:15)], yielding 2,3-*isopropylidene*-22,22-O-*didehydroecdysone* **12** (22 mg, 43.8 μmol, 73%) (t_r 10 min); R_f ($CHCl_3$–EtOH, 7:1) 0.70, yellow spot; $[\alpha]_D^{25}$ +28.4 (*c* 0.51, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3431br wk (OH), 1702st (saturated ketone), 1664st (cyclohexenone); λ_{max}(MeOH)/nm 242

(γ-OH cyclohexenone); δ_H(250 MHz, $CDCl_3$) 5.81 (1H, d, *J* 2.5, 7-H), 4.22 (2H, m, $w_{1/2}$ 19, 3-H and 2-H), 2.85 (1H, overlapping ddd, *J* 12, 10 and 2.5, 9-H), 2.60 (4H, m, 20-H, 23_a-H, 23_b-H and 17-H), 2.34 (1H, dd, *J* 13 and 5, 5-H), 1.46 (3H, s, 30-CH_3), 1.30 (3H, s, 27-CH_3), 1.21 (6H, 2 overlapping s, 27-CH_3 and 26-CH_3), 1.09 (3H, d, *J* 6.5, 21-CH_3), 0.98 (3H, s, 19-CH_3), 0.67 (3H, s, 18-CH_3); δ_C(62.7 MHz, $CDCl_3$) 214.9 (C-22), 202.7 (C-6), 162.7 (C-8), 12.3 (C-7), 108.3 (C-28), 84.4 (C-14), 72.2 (C-2), 71.6 (C-3), 70.1 (C-25), 50.8 (C-5), 49.3 (C-20), 47.1 (C-13), 37.8 (C-10), 37.7 (C-1), 34.8 (C-9), 31.9 (C-12), 30.7 (C-13), 29.5 (C-27), 29.4 (C-26), 28.5 (C-30), 26.7 (C-4), 26.4 (C-29), 25.9 (C-16), 23.6 (C-19), 20.6 (C-11), 16.9 (C-18), 16.0 (C-21); *m/z* 525 ($[M + Na]^+$, 43%), 503 ($[M + H]^+$, 18), 485 ($[M + H - H_2O]^+$, 100), 467 ($[M + H - 2H_2O]^+$, 87), 427 ($[M + H - H_2O - Me_2CO]^+$, 18), 409 ($[M + H - 2H_2O - Me_2CO]^+$, 16) (Found: $[M + H]^+$, 503.3373. $C_{30}H_{46}O_6$ requires $[M + H]^+$, 503.3372).

2,3-*O*-Isopropylidene-22-*epi*-ecdysone 13

Keto ecdysone **12** (15 mg, 30 μmol) was dissolved in dry THF (3 cm^3) under an argon atmosphere and the flask was then cooled to 0 °C. A solution of $LiAlH(OBu^t)_3$ in THF (1 M, 1.5 ml, 1.5 mmol, 50 equiv.) was added dropwise to the reaction mixture. The solution was cooled to −78 °C and stirred for 2 h before being allowed to warm to room temperature. The reaction was monitored by TLC analysis ($CHCl_3$–EtOH, 7:1) and after 2 h quenched with ethanol (5 cm^3) and water (10 cm^3) to avoid any further decomposition. The organic solvents were removed *in vacuo* leaving the products in an aqueous solution, which was extracted with ethyl acetate (3 × 20 cm^3). The organic phases were combined and dried over anhydrous $MgSO_4$. The mixture was filtered and concentrated under reduced pressure. The components were separated by HPLC through a semi-preparative reversed-phase C_{18} column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (70:30)] to give 2,3-*O*-isopropylideneecdysone **9** (1.35 mg, 2.7 μmol, 9%) (t_r 22 min), starting material **12** (1.5 mg, 3 μmol, 10%) (t_r 28 min) and 2,3-O-*isopropylidene*-22-epi-*ecdysone* **13** (9 mg, 18 μmol, 60%) (t_r 38 min); R_f ($CHCl_3$–EtOH, 7:1) 0.42, dark blue spot; $[\alpha]_D^{27}$ +28.9 (*c* 0.13, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3420br wk (OH), 1658md (cyclohexenone); λ_{max}(MeOH)/nm 243 (γ-OH cyclohexenone); δ_H(250 MHz, $CDCl_3$) 5.83 (1H, d, *J* 2.5, 7-H), 4.22 (2H, m, $w_{1/2}$ 19, 3-H and 2-H), 3.68 (1H, overlapping dd, *J* 8.5 and 2, 22-H), 2.84 (1H, overlapping ddd, *J* 11, 9 and 2.5, 9-H), 2.37 (1H, dd, *J* 13 and 5, 5-H), 2.25 (1H, overlapping dd, *J* 9 and 9, 17-H), 1.50 (3H, s, 30-CH_3), 1.33 (3H, s, 29-CH_3), 1.26 (3H, s, 27-CH_3), 1.24 (3H, s, 26-CH_3), 1.00 (3H, s, 19-CH_3), 0.95 (3H, d, *J* 6.5, 21-CH_3), 0.70 (3H, s, 18-CH_3); δ_C(62.7 MHz, $CDCl_3$), 202.9 (C-6), 163 (C-8), 121.3 (C-7), 108.3 (C-28), 85.0 (C-14), 74.2 (C-22), 72.2 (C-2), 71.7 (C-3), 70.8 (C-25), 50.6 (C-5), 47.2 (C-17), 47.0 (C-13), 40.8 (C-20 and C-24), 37.7 (C-10), 37.6 (C-1), 34.8 (C-9), 31.6 (C-12), 31.0 (C-15), 30.3 (C-23), 30.2 (C-27), 28.9 (C-26), 28.5 (C-30), 26.7 (C-4), 26.4 (C-29), 26.0 (C-16), 23.7 (C-19), 20.7 (C-11), 15.7 (C-18), 12.0 (C-21); *m/z* 527 ($[M + Na]^+$, 45%), 505 ($[M + H]^+$, 95), 487 ($[M + H - H_2O]^+$, 100), 469 ($[M + H - 2H_2O]^+$, 39) (Found: $[M + H]^+$, 505.3529. $C_{30}H_{48}O_6$ requires $[M + H]^+$, 505.3529).

2,3-*O*-Isopropylidene-22,25-anhydroecdysone 10

Trifluoromethanesulfonic anhydride (0.1 cm^3, 0.6 mmol, 18 equiv.) was added dropwise to a solution of 2,3-*O*-isopropylideneecdysone **9** (26.4 mg, 53.4 μmol) in dry pyridine (2 cm^3) under an atmosphere of N_2, while maintaining a temperature of 0 °C. Effervescence took place for 3 min and the reaction mixture became dark orange. The reaction mixture was allowed to warm to room temperature, stirred for 1 h and then quenched by the addition of ice (approx. 2 g) and saturated aqueous $CuSO_4$ (10 cm^3), with stirring for 10 min. The products were then extracted with CH_2Cl_2 (3 × 20 cm^3). The organic phases were combined and washed with saturated aqueous $CuSO_4$ (2 × 20 cm^3), then with water (20 cm^3) and finally dried over anhydrous $MgSO_4$. After filtration, the solvents were removed from the combined organic phases under reduced pressure. Purification was carried out by HPLC on a preparative reversed-phase C_{18} column [flow rate 9.9 cm^3 min^{-1}, MeOH–H_2O (90:10)] to give 2,3-O-*isopropylidene*-22,25-*anhydroecdysone* **10** (18.4 mg, 37.9 μmol, 72%) (t_r 30 min) as a gum; R_f ($CHCl_3$–EtOH, 7:1) 0.64, dark blue spot; $[\alpha]_D^{25}$ +36.3 (*c* 0.16, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1660md (cyclohexenone); λ_{max}(MeOH)/nm 243 (γ-OH cyclohexenone); δ_H(250 MHz, $CDCl_3$) 5.81 (1H, d, *J* 2.5, 7-H), 4.24 (2H, m, $w_{1/2}$ 19, 3-H and 2-H), 4.06 (1H, overlapping ddd, *J* 7.5, 7 and 2, 22-H), 2.61 (1H, overlapping ddd, *J* 11, 7.5 and 2.5, 9-H), 2.33 (1H, dd, *J* 12.5 and 5, 5-H), 1.49 (3H, s, 30-CH_3), 1.32 (3H, s, 29-CH_3), 1.23 (3H, s, 27-CH_3), 1.19 (3H, s, 26-CH_3), 0.98 (3H, s, 19-CH_3), 0.90 (3H, d, *J* 6.5, 21-CH_3), 0.66 (3H, s, 18-CH_3); δ_C(62.7 MHz, $CDCl_3$) 202.7 (C-6), 163.1 (C-8), 121.2 (C-7), 108.2 (C-28), 85.1 (C-14), 80.2 (C-25), 80.0 (C-22), 72.2 (C-2), 71.7 (C-3), 50.8 (C-5), 47.8 (C-17), 47.3 (C-13), 39.3 (C-20), 38.7 (C-24), 37.7 (C-10), 37.6 (C-1), 31.7 (C-12), 30.9 (C-15), 29.5 (C-23), 28.5 (C-27 and C-30), 27.5 (C-26), 26.7 (C-4), 26.4 (C-29), 26.1 (C-16), 23.7 (C-19), 15.6 (C-18), 13.1 (C-21); *m/z* 509 ($[M + Na]^+$, 26%), 487 ($[M + H]^+$, 100), 469 ($[M + H - H_2O]^+$, 51), 429 ($[M + H - Me_2CO]^+$, 13.7), 411 ($[M + H - H_2O - Me_2CO]^+$, 12.5) (Found: $[M + H]^+$, 487.3424. $C_{30}H_{46}O_5$ requires $[M + H]^+$, 487.3423).

22,25-Anhydroecdysone 11

Aqueous HCl (0.1 M, 2 cm^3) was added dropwise to a solution of furanylpregnenone **10** (10 mg, 20.4 μmol) in dioxane (2 cm^3) at room temperature. The reaction mixture was then stirred for 2 h, after which time the reaction appeared to be complete by TLC analysis. The reaction mixture was diluted with water (5 cm^3) and neutralised with aqueous NaOH (0.1 M, 2 cm^3). After evaporation of the solvents under reduced pressure, the salts were separated from the expected product using a semi-preparative C_{18} SEP-PAK cartridge. Purification was then carried out by HPLC on a semi-preparative C_{18} column [flow rate 2 cm^3 min^{-1}, MeOH–H_2O (80:20)] to give 22,25-*anhydroecdysone* **11** (8.3 mg, 18.6 μmol, 91%) (t_r 21 min) as a gum; R_f ($CHCl_3$–EtOH 7:1) 0.28, dark blue spot; $[\alpha]_D^{25}$ +60.4 (*c* 0.08, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1660md (cyclohexenone); λ_{max}(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(500 MHz, $CDCl_3$) 5.78 (1H, d, *J* 2.5, 7-H), 4.12 (1H, overlapping dd, *J* 8 and 7, 22-H), 4.02 (1H, overlapping ddd, *J* 12, 3 and 5, 2-H), 3.94 (1H, m, $w_{1/2}$ 10, 3-H), 3.06 (1H, overlapping ddd, *J* 12, 7 and 2.5, 9-H), 2.34 (1H, dd, *J* 13 and 3, 5-H), 1.35 (1H, overlapping dd, *J* 15 and 13, 1_{ax}-H), 1.23 (3H, s, 27-CH_3), 1.19 (3H, s, 26-CH_3), 0.93 (3H, s, 19-CH_3), 0.89 (3H, d, *J* 6.6, 21-CH_3), 0.64 (3H, s, 18-CH_3); δ_C(125.8 MHz, $CDCl_3$) 204.3 (C-6), 164.9 (C-8), 121.6 (C-7), 84.8 (C-14), 81.1 (C-25), 79.9 (C-22), 67.4 (C-2), 60.9 (C-3), 50.1 (C-5), 48.1 (C-17), 46.7 (C-13), 39.5 (C-20), 38.5 (C-24), 38.3 (C-10), 36.7 (C-1), 33.7 (C-9), 31.7 (C-4), 30.9 (C-12), 30.7 (C-15), 29.5 (C-23), 28.3 (C-27), 27.3 (C-26), 26.1 (C-16), 23.9 (C-19), 20.5 (C-11), 15.5 (C-18), 12.9 (C-21); *m/z* 469 ($[M + Na]^+$, 71%), 447 ($[M + H]^+$, 100), 430 ($[M - H_2O]^+$, 72), 411 ($[M + H - 2H_2O]^+$, 16.7) (Found: $[M + H]^+$, 447.3110. $C_{27}H_{42}O_5$ requires $[M + H]^+$, 447.3110).

22,25-Anhydro-22-*epi*-ecdysone 14

Trifluoromethanesulfonic anhydride (0.05 cm^3, 0.3 mmol, 15 equiv.) was added dropwise to a solution of 2,3-*O*-isopropylidene-22-*epi*-ecdysone **12** (10 mg, 19.8 μmol) in dry pyridine (2 cm^3) under an atmosphere of N_2 while maintaining a temperature of 0 °C. The colour of the reaction mixture became dark orange after 3 min. The reaction mixture was allowed to warm to room temperature and stirred for a further 90 min, after which the reaction appeared to be complete by TLC analysis ($CHCl_3$–EtOH, 7:1). The reaction mixture was diluted with CH_2Cl_2 (5 cm^3) and quenched by the addition of ice (approx.

2 g) and saturated aqueous $CuSO_4$ (10 cm^3). The product was extracted with CH_2Cl_2 (2 × 10 cm^3). The organic phases were combined and washed first with saturated aqueous $CuSO_4$ (2 × 20 cm^3), then with water (20 cm^3), and finally dried over anhydrous $MgSO_4$. After filtration the solvents were removed from the combined organic phases under reduced pressure, and the product was dissolved in dioxane (2 cm^3). HCl (0.1 M, 2 cm^3) was added dropwise to the solution of the crude product and the reaction mixture was stirred for 3 h until TLC analysis showed that the reaction was complete. The reaction mixture was diluted with water (10 cm^3) and neutralised with aqueous NaOH (0.1 M, 2 cm^3). After evaporation of the solvents under reduced pressure, the product was separated from the salts using a semi-preparative C_{18} SEP-PAK cartridge and further purified by HPLC on a semi-preparative C_{18} column [flow 2 cm^3 min^{-1}, MeOH–H_2O (80 : 20)]. 22,25-*Anhydro*-22-epi-*ecdysone* **14** (3.4 mg, 7.7 μmol, 38.5%) (t_r 12 min) was isolated as a gum; R_f ($CHCl_3$–EtOH, 7 : 1) 0.40, dark blue spot; $[\alpha]_D^{24}$ +133.4 (*c* 0.07, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1660wk (cyclohexenone); λ_{max}-(MeOH)/nm 242 (γ-OH cyclohexenone); δ_H(250 MHz, $CDCl_3$) 5.83 (1H, d, *J* 2.5, 7-H), 4.03 (2H, m, $w_{1/2}$ 14, 3-H and 22-H), 3.89 (1H, overlapping ddd, *J* 12, 3 and 3, 2-H), 2.98 (1H, overlapping ddd, *J* 12, 8 and 2.5, 9-H), 2.43 (1H, dd, *J* 13 and 4, 5-H), 1.39 (1H, overlapping dd, *J* 15 and 13, 1_{ax}-H), 1.23 (3H, s, 27-CH_3), 1.22 (3H, s, 26-CH_3), 0.97 (3H, s, 19-CH_3), 0.90 (3H, d, *J* 6.6, 21-CH_3), 0.68 (3H, s, 18-CH_3); δ_C(62.7 MHz, $CDCl_3$) 203.4 (C-6), 164.0 (C-8), 121.6 (C-7), 84.7 (C-14), 80.3 (C-22), 79.9 (C-25), 67.8 (C-2), 67.4 (C-3), 50.0 (C-5), 48.1 (C-17), 47.2 (C-13), 38.8 (C-24), 38.3 (C-10), 37.9 (C-20), 36.9 (C-1), 34.0 (C-9), 32.2 (C-12), 31.4 (C-4), 30.7 (C-15), 28.6 (C-27), 28.0 (C-26), 25.8 (C-16), 25.1 (C-23), 23.9 (C-19), 20.5 (C-11), 15.8 (C-18), 12.6 (C-21); *m/z* 447 ($[M + H]^+$, 12.7%), 429 ($[M + H - H_2O]^+$, 16.9), 411 ($[M + H - 2H_2O]^+$, 4.9), 99 (100) (Found: $[M + H]^+$, 447.3110. $C_{27}H_{42}O_5$ requires $[M + H]^+$, 447.3110).

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