Synthesis of Shidasterone and the Unambiguous Determination of its Configuration at C-22

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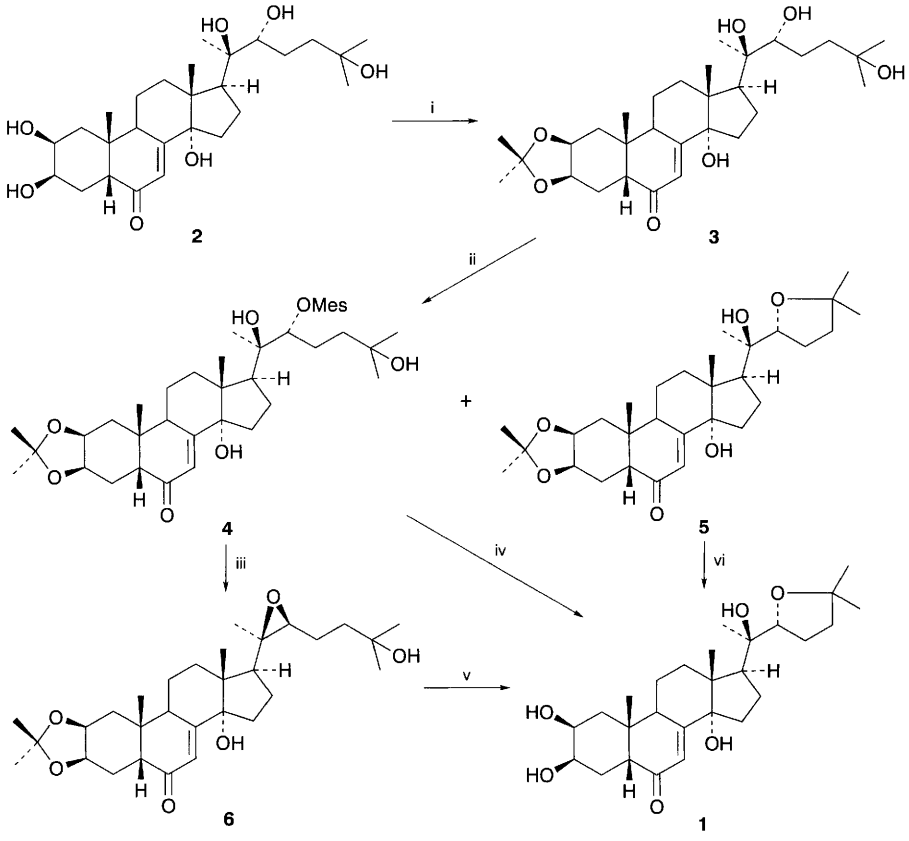
Shidasterone **1** is prepared from 2,3-isopropylidene-22-mesylate 20-hydroxyecdysone **4** by three different synthetic sequences; mechanistic considerations together with independent NOE studies reveal a (22*R*) configuration for shidasterone **1**.

Ecdysteroids control the moulting and metamorphosis processes of insects and crustaceans. An understanding of the structural requirements for maximum activity of these hormones is of great significance since there is a growing interest in ecdysteroid agonists and antagonists as possible invertebrate pest control agents.[1] Shidasterone **1** is an ecdysteroid which was first isolated from the plant *Blechnum niponicum* in 1969 and initially identified as a C-20 and/or C-22 epimer of 20-hydroxyecdysone **2**.[2] In 1970, an ecdysteroid named stachysterone D was isolated from *Stachyurus praecox* and, on the basis of mass spectrometry, was postulated to have an ether linkage between C-22 and C-25.[3] In 1975, the structure of shidasterone was investigated by Hikino *et al.*[4] By an analysis of the downfield shifts of C-22 and C-25 in the ^{13}C NMR spectrum, the side-chain of shidasterone **1** was postulated to contain an ether linkage between C-22 and C-25. This was confirmed by careful re-examination of the mass spectrum for shidasterone. The authors deduced that shidasterone was either identical to stachysterone D or its C-22 epimer. The C-22 configuration of either shidasterone of stachysterone D has not yet been elucidated. We wish here to report the first chemical synthesis of shidasterone **1** and the unambiguous determination of its configuration at C-22 as (*R*).

The 2,3-*cis* diol of 20-hydroxyecdysone **2** was first protected in a three-step sequence,[5] yielding 2,3-isopropylidene 20-hydroxyecdysone **3** which was purified by reversed-phase HPLC [Spherisorb octadecyl ODS2 5 μm; MeOH–H_2O (70 : 30), flow rate = 2 cm^3 min^{-1}, retention time = 16 min; yield = 65%] (Scheme 1). After treatment of the acetonide **3** with mesyl chloride and diisopropylethylamine (DIPEA), a mixture of the mesylate **4** (50%) and 2,3-isopropylidene shidasterone **5** (39%) was obtained.†‡ The mesylate **4** was not very stable but exhibited spectroscopic data in accord with its structure; the ^1H NMR spectrum of **4** recorded in $CDCl_3$ showed a characteristic downfield shift of the C-22 proton to δ 3.48 (from δ 4.51 for **3**) as well as the methyl group of the mesyl moiety at δ 3.10. The structure of **5** was assigned on the basis of the NMR data and confirmed by FAB mass spectrometry (found $[M + H]^+$, *m/z* 503.3366; required $[M + H]^+$, *m/z* 503.3372). Treatment of the mesylate **4** with either spray-dried KF in dry MeCN or



Scheme 1 *Reagents and conditions*: i, (*a*) phenylboronic acid, dry DMF, room temp., 1 h; (*b*) dry DMP, dry acetone, fused *p*-TsOH, 3 h; (*c*) 30% NaOH–H_2O_2 in THF–water (9 : 1), overall yield 65%; ii, CH_3SO_2Cl (7.5 equiv.), DIPEA (7.5 equiv.), dry CH_2Cl_2, 0 °C then room temp., overall yield 89%; iii, spray-dried KF, dry MeCN, 2 h, reflux, yield 76% or 1 mol dm^{-3} TBAF in THF, dry THF, 5 min, yield 57%; iv, (*a*) $Et_3N{\cdot}3HF$, 60 °C, overnight, (*b*) 0.1 mol dm^{-3} HCl–dioxane (1 : 1), room temp., overall yield 48% from **4**; v, 0.1 mol dm^{-3} HCl–dioxane (1 : 1), room temp., yield 91%; vi, 0.1 mol dm^{-3} HCl–dioxane (1 : 1), room temp., yield 92%

anhydrous 1 mol dm^{-3} tetrabutylammonium fluoride (TBAF) in THF afforded the epoxide **6** in 76 and 57% yield, respectively. The ^1H NMR spectrum of **6** showed that the H-22 proton had a chemical shift of δ 2.76, which is in the characteristic range for an epoxide. An attempt to deprotect the 2,3-isopropylidene epoxide **6** by treatment with 0.1 mol dm^{-3} HCl unexpectedly yielded shidasterone **1** in 91% yield.§ Treatment of the mesylate **4** with $Et_3N{\cdot}3HF$ at 60 °C, followed by deprotection of the 2,3-*cis* diol, afforded compound **1** in a 48% yield. Shidasterone was also obtained by removal of the 2,3-isopropylidene moiety of compound **5**. All these three samples of shidasterone were shown to be *identical* by a variety of spectroscopic§ and analytical methods {TLC [$CHCl_3$–EtOH (1 : 1), R_f = 0.40] and HPLC [MeOH–H_2O (60 : 40), retention time = 34 min]}. The fact that the three samples of shidasterone were identical suggests that in all the reactions the formation of the 20,22-epoxide moiety is the initial step followed by intramolecular cyclisation leading to the formation of the tetrahydrofuranyl ring of compounds **1** and **5**. The configuration of the latter compounds therefore has to be (22*R*) since the epoxide **4** is constrained to have a (22*S*) configuration; the formation of the furanyl ring from the epoxide **4** would then occur with inversion of configuration according to an S_N2 mechanism.

Supporting independent evidence for a (22*R*) configuration was obtained from a series of NOE experiments on 2,3-isopropylidene shidasterone **5**. Irradiation of 17-H resulted in an enhancement of 22-H (1.2%) and similarly irradiation of 22-H led to an enhancement of 17-H (1.2%) and 16α-H (3.1%). These results indicate that the molecule adopts a conformation in which 22-H is spatially close to 17-H and 16α-H. This conformation may be stabilised by hydrogen bonding between the proton borne by the C-20 hydroxy group and the oxygen atom linking C-22 and C-25. In a separate experiment, irradiation of the C-21 methyl group enhanced signals at δ 1.72 and 1.86, by 2.4 and 3.4%, respectively. These signals were assigned to the 23α- and 23β-protons, respectively. The proximity in space of the C-21 methyl group to H-23α and H-23β is only possible if C-22 has a (22*R*) configuration. Taken together with the mechanistic considerations outlined above this assigns the configuration of 2,3-isopropylidene shidasterone **5**, and hence shidasterone **1**, at C-22.

The rearrangement of the epoxide group of **6** into the furanyl ring of **1** may be viewed as a variation of the Payne rearrangement, the reaction being driven by the greater thermodynamic stability of the furanyl ring. It is possible that the isolations of stachysterone D and shidasterone in 1969 and 1970 were artefacts of the conditions used for the extraction of the plant samples. If the formation of the furanyl ring of the isolated compounds is chemically based, then the work described herein provides further evidence for stachysterone D and shidasterone being identical rather than epimeric since formation of the furanyl ring for the isolated compounds probably also goes through initial formation of the 20,22-epoxide.

We have examined the biological activity of shidasterone **1** using a microplate-based bioassay which has been developed using the B_{II} cell line by Dinan and coworkers. This bioassay has been shown to be reproducible, sensitive, specific to ecdysteroids and quantitative.[6] Shidasterone was shown to be 200-fold less active than 20-hydroxyecdysone **2**, the effective dose for a 50% response being 1.6×10^{-6} mol dm^{-3} as opposed to 7.5×10^{-9} mol dm^{-3} for 20-hydroxyecdysone.

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Footnotes

† All new compounds gave satisfactory analytical and/or spectroscopic data.

‡ 2,3-Isopropylidene shidasterone **5**, R_f ($CHCl_3$–EtOH 7 : 1) 0.65, olive green spot; $[\alpha]_D^{25}$ + 65.4 (*c* 0.37, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3600w (sharp) (free OH), 3400w (br) (bonded OH), 1664 st. (cyclohexenone); λ_{max} (MeOH)/nm 242 (γ-OH cyclohexenone); δ_H (300 MHz, $CDCl_3$) 5.81 (1H, d, *J* 2.5 Hz, 7-H), 4.23 (2H, m, $w_{1/2}$ = 20 Hz, 2-H and 3-H), 3.87 (1H, overlapping ddd, *J* 12, 7 and 2.5 Hz, 9-H), 2.33 (1H, dd, *J* 12 and 5 Hz, 5-H), 2.29 (2H, overlapping dd, *J* 10 and 8 Hz, 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 − H_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).

§ Shidasterone **1**; R_f ($CHCl_3$–EtOH 7 : 1) 0.40, olive green spot; $[\alpha]_D^{25}$ + 65.0 (*c* 0.18, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3600m (sharp) (free OH), 3400m (br) (bonded OH), 1664 st. (cyclohexenone); λ_{max} (MeOH)/nm 242 (γ-OH cyclohexenone); δ_H (300 MHz, CD_3OD) 5.81 (1H, d, *J* 2.5 Hz, 7-H), 3.94 (2H, m, $w_{1/2}$ = 20 Hz, 3-H and 22-H), 3.84 (1H, overlapping ddd, *J* 12, 3 and 3 Hz, 2-H), 3.15 (1H, overlapping ddd, *J* 12, 7.5 and 2.5, 9-H), 2.36 (2H, m, $w_{1/2}$ = 25 Hz, 5-H and 17-H), 2.15 (1H, overlapping ddd, *J* 13, 13 and 4 Hz, 12_{ax}-H), 1.42 (1H, overlapping dd, *J* 13 and 12 Hz, 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).

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