Synthesis of (22R,23R)-2α,3α,22,23-diepoxy-5α-hydroxy-stigmastan-6-one from stigmasterol

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A new (22R,23R)-2α,3α,22,23-diepoxy-5α-hydroxy-stigmastan-6-one diepisecasterone analogue have been synthesised from stigmasterol.

Keywords: phytohormones, secasterone, brassinosteroids, epoxides, steroids

Brassinosteroids (BS) are polyhydroxylated steroid hormones which play a very important role in regulating the growth, differentiation and stress responses of plants throughout their life cycle.[1,2] In recent years, many of the proteins required for steroid response in plants have been identified. The process begins when the brassinosteroid binds directly to the extracellular domain of protein BRI1 and then initiates a signalling cascade.[3,4]

Sixty-one naturally occurring brassinosteroids have now been discovered.[5,6] Among them, there are two 2β,3β-epoxybrassinosteroids, secasterone[7] and 24-episecasterone[8] and one with the 2α,3α epoxy ring, 2,3-diepisecasterone.[6] There are no natural BS with epoxy function in the side chain or with a 5α-hydroxyl group. Instead many BS analogues with 22,23 epoxy ring or 5α-hydroxy-6-ketone functions have been synthesised and tested as plant growth regulators.[9-19]

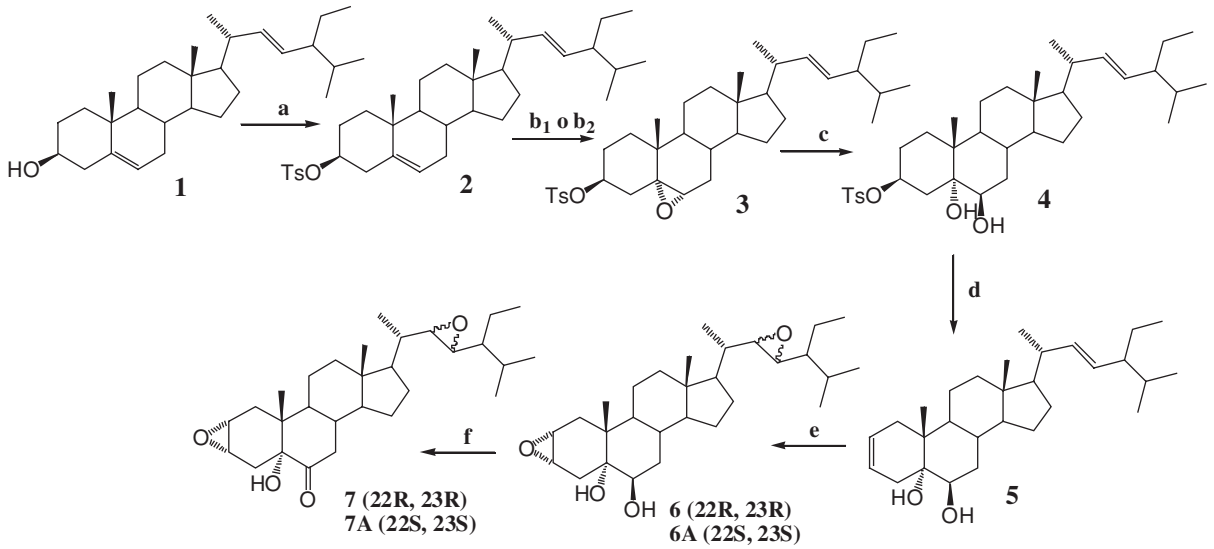
Here we describe the synthesis and characterisation of a new 5α-hydroxy-6-ketone brassinosteroid analogue containing a 2α,3α epoxy ring as in 2,3-diepisecasterone[6] and also of a 22R, 23R epoxy group in the side chain. The procedure to obtain the (22R,23R)-2α,3α,22,23-diepoxy-5α-hydroxy-stigmastan-6-one involves six steps as shown in Scheme 1.

The synthesis of the compound **3** was achieved by selective epoxidation of the double bond in ring B using two different methods. The first method used magnesium monoperoxyphthalate hexahydrate (MMPP),[11,20,21] in a mixture of dichloromethane and water, using triethyl-*t*-butylamonium (TEBA), as the phase transfer catalyst. The second used peroxyacetic acid generated *in situ*[17] from acetic anhydride and 32% hydrogen peroxide in chloroform with sodium acetate as catalyst. Epoxidation of stigmasterol tosylate **2** using both procedures gives a mixture in which the 5α,6α-epoxide **3** predominated over the 5β,6β-epoxide by about 4:1. No compound with epoxide function at C_{22},C_{23} was detected.

Nucleophilic *trans*-diaxial opening using perchloric acid (aq) of a mixture of **3** and its isomer afforded the 5α,6β-dihydroxylated steroid **4**. Elimination of the 3β-tosylate with LiBr and Li_2CO_3 in dimethylformamide (DMF) to give the diene **5**. In this procedure no compound with a Δ^3-double bound was detected.

Treatment of the diene **5** with m-chloroperbenzoic acid (MCPBA) at room temperature for 24 hours yielded a mixture of 22R,23R–diepoxide **6** and 22S,23S–diepoxide **6A**. Epoxidation of the Δ^2-double bond took place on the less hindered face giving the 2α,3α epoxide stereoselectively. However epoxidation of the Δ^{22} double bond produced a diasteroisomeric mixture (R,R and S,S) in which the 22R,23R-epoxide predominated over the 22S, 23S-epoxide by about 4:3. The crude mixture containing compounds **6** and **6A** was purified by column chromatography. However the isomers were not separated. The mixture's ^{13}C NMR spectrum was recorded and the signals of both isomers were then assigned.

This mixture of diasteroisomers **6** and **6A** was oxidised with Jones reagent at 5°C to give a mixture of (22R, 23R)-2α,3α,22,23-diepoxy-5α-hydroxy-stigmastan-6-one **7** and its 22S, 23S isomer **7A**. Successive chromatographic columns, led to the isolation of the less polar compound **7** from the mixture of **7** and **7A**, albeit in a very low yield. As before, the ^{13}C NMR spectrum of **7** and that of the mixture of the two isomers were recorded and all signals were assigned.



Scheme 1 Reagents and conditions: (a) TsCl/Pyr; (b_1) MMPP/TEBA/CH_2Cl_2-H_2O; (b_2) $(CH_3CO)_2O$/NaAc/H_2O_2/ $CHCl_3$; (c) $HClO_4$/H_2O/acetone; (d) LiBr/Li_2CO_3/DMF; (e) MCPB/ $CHCl_3$; (f) Jones R. /acetone.

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Table 1 IR (ν in cm^{-1}) and ^1H NMR (δ in ppm) spectral data of pure compounds **3**, **4**, **5** and **7**

	IR (KBr)	^1H NMR ($CDCl_3$)
3	2953, 2870, 1463, 1373, 1036, 968	7.76, 7.31 (m, 4H-Ph), 4.57 (m, $H_{3\alpha}$), 2.80 (m, $H_{6\alpha}$), 5.10 (m, H_{23}, H_{22}), 1.00 (s, Ch_3-19), 0.95 (d, CH_3-21, ^3J = 6.20Hz), 0.78 (m, CH_3-29), 0.81 (d, CH_3-26, CH_3-27, ^3J = 6.4Hz), 0.60 (m, CH_3-18)
4	3500, 2953, 2870, 1463, 1373	7.75, 7.32 (m, 4H-Ph), 4.90 (m, $H_{3\alpha}$), 5.10 (m, H_{22}, H_{23}), 1.16 (s, CH_3-19), 1.01 (d, CH_3-21, ^{3} J= 6.6 Hz), 0.80 (t, CH_3-29), 0.83 (d, CH_3-26, CH_3-27, ^{3} J = 6.5 Hz) 0.65 (s, CH_3-18)
5	3400, 2950, 2900, 1460, 1380	5.60, 5.70 (m; $H_{2\alpha}$, $H_{3\alpha}$), 2.82, 2.78 (m; $H_{4\alpha}$, $H_{4\beta}$), 3.70 (m; H_6), 0.70 (m; CH_3-18), 1.05 (s; CH_3-19), 1.02 (d, CH_3-21, ^3J = 6.6 Hz), 5.15 (m, H_{22}, $^3J_{20\text{-}22}$ = 8.4 Hz, $^3J_{22\text{-}23}$ = 15.2 Hz), 5.01 (m, H_{23}, $^3J_{23\text{-}24}$ = 8.4 Hz, $^3J_{22\text{-}23}$ = 15.2 Hz), 0.82 (d; CH_3-26, 27, ^3J = 66.6 Hz), 0.81 (t; CH_3-29)
7	3480, 2957, 2870, 1720, 1450, 1383, 1029	3.50 (m, $H_{3\alpha}$), 2.60 (m, H_{7ax}, ^2J = $^3J_{aa}$ = 12.4 HZ), 2.48 (dd, H_{23}, ^2J = 2.5 Hz, ^3J = 7.1 Hz), 2.71 (m, H_{22}), 2.38 (dd, H_{7ec}, ^2J = $^3J_{aa}$ = 12.6 Hz, $^3J_{ae}$ = 4.0 Hz), 0.70 (s, CH_3-19), 0.97 (d, ^3J = 6.2 Hz, CH_3-21), 0.94 (t, CH_3-29), 0.91, 0.89 (d, CH_3-26, CH_3-27, ^3J = 6.9 Hz), 0.61 (s, CH_3-18)

The ^{13}C NMR spectra allowed us to determine the stereochemistry of the oxirane ring in the side chain.[22] The 22R,23R epoxy steroids **6** and **7** differ from 22S, 23S epoxy compounds **6A** and **7A** by the chemical shifts of C_{17}, C_{22} and C_{23} (Table 2) and the values correspond with the previous report.[22] In the R,R-epoxide, the C_{22} and C_{23} show a very similar chemical shift (Δδ≈ 0.1–0.3 ppm); whereas in the S,S-epoxide those carbons are clearly distinguishable (Δδ≈ 4.5 ppm).

Experimental

Melting points (m.p.) were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra in $CDCl_3$ were recorded on a Bruker ACF 250 MHz spectrometer. Chemical shifts are given in ppm, with TMS as internal reference. Coupling constant values are given in Hz. IR spectra were obtained on a Phillips Analytical PV 9600 FTIR. Elemental analyses were performed on Carlo Erba-1106, a Carlo Erba EA-1108 and Perkin Elmer CHN 2400 instruments. Unless otherwise indicated, all solvents and reagents used were of commercial grade. Reactions were monitored by thin layer chromatography (TLC) on plates pre-coated with silica gel F254 0.2 mm (Merck). Column chromatography was carried out on silica gel 60 (Merck) using *n*-hexane/EtOAc of crescent polarity as the eluent. The stigmasterol tosylate was synthesised by the usual technique described in the literature[21]. Melting points and ^1H NMR and ^{13}C NMR chemical shifts of compounds **3** and **4** agree with previous reported values.[13] "Usual work-up" refers to extracting with an organic solvent, washing the extract with water and brine, drying over anhydrous Na_2SO_4 and concentrating under reduced pressure. Table 1 shows IR and ^1H NMR spectral data of pure compounds **3**, **4**, **5** and **7**. Table 2 contains the complete ^{13}C NMR assignments for all synthesised compounds.

(22E)-5,6α-Epoxy-3β-tosyloxystigmast-22-ene (**3**):
Method 1: 2.0 g (3.5 mmol) of stigmasterol tosylate **2**, 12 ml of dichloromethane and 0.05 g of TEBA were mixed and stirred under reflux for 1 h; The solution of MMPP (2, 3 g) in water (13 ml) was added dropwise. The mixture was then cooled to room temperature, stirred for 3 h. Usual work-up yielding 1.7 g (82 %) of crude. A small portion (0.1 g) was purified by flash chromatography to obtain 0.045 g (45 %) of **3**. *Method 2:* A mixture of tosylate **2** (2.0 g, 3.5 mmol), chloroform (10 ml) and sodium acetate (0.5 g) was cooled to a temperature ranging between 0 and 5 °C. Acetic anhydride (1.5 ml) and H_2O_2 (2 ml) were further added dropwise. The reaction was stirred and cooled for 5 h. Usual work-up yielded 1.6 g (75.7%) of crude. A small portion of (0.1 g) was purified by flash chromatography to obtain 0.037 g (37% yield) of **3**. m.p. 171–173 °C (acetone). EA: calc: C 74.2%; H 9.3%; Found: C 74.3%; H 9.2%.

Table 2 ^{13}C NMR chemical shifts (δ in ppm) of all synthesised compounds

	3	**4**	**5**	**6**	**6[a]**	**7**	**7[a]**
C-1	32.2	32.2	35.2	35.6	35.6	33.4	33.4
C-2	28.2	27.6	126.7	52.3	52.3	51.6	51.6
C-3	79.8	80.4	123.1	54.9	54.9	54.0	54.0
C-4	37.0	38.1	37.4	29.6	29.6	26.4	26.4
C-5	65.1	76.1	73.2	73.1	73.1	79.1	79.1
C-6	59.2	75.8	74.0	73.9	73.9	209.7	209.7
C-7	28.7	34.5	34.1	34.4	34.4	41.9	41.9
C-8	29.8	31.0	29.9	30.1	30.1	37.9	37.9
C-9	42.4	45.6	46.1	46.0	46.0	45.6	45.6
C-10	34.7	38.0	38.1	37.8	37.8	41.8	41.8
C-11	20.5	21.0	20.8	20.5	20.5	20.7	20.7
C-12	39.2	39.7	39.8	39.5	39.5	39.2	39.2
C-13	42.2	42.4	42.4	42.8	42.8	43.1	43.1
C-14	56.9	55.9	56.0	55.1	55.1	55.8	55.8
C-15	24.1	24.2	24.2	24.4	24.4	24.1	24.2
C-16	28.6	28.9	28.9	27.8	26.9	27.7	26.8
C-17	55.6	55.9	55.97	53.4	56.0	53.3	55.9
C-18	12.0	12.3	12.2	11.9	12.1	11.8	12.0
C-19	15.7	16.6	16.3	16.9	16.9	15.9	15.9
C-20	40.4	40.5	40.6	38.7	38.8	38.6	38.7
C-21	21.6	21.2	21.2	16.2	16.2	16.1	16.2
C-22	138.1	138.2	138.3	62.2	63.1	62.2	62.9
C-23	129.4	129.3	129.3	62.1	58.6	61.9	58.5
C-24	51.2	51.2	51.2	48.2	48.7	48.2	48.7
C-25	31.8	31.9	31.9	29.1	29.2	29.1	29.3
C-26	21.1	21.2	19.0	20.2	19.3	20.2	19.4
C-27	21.0	19.0	19.0	19.5	19.4	19.5	19.4
C-28	25.4	25.4	25.4	20.7	20.9	20.8	20.9
C-29	12.2	12.3	12.2	12.3	12.3	12.4	12.3
	144.8	144.6					
	134.2	134.4					
TsO	129.8	129.8					
	127.7	127.6					

(22E)-3β-Tosyloxystigmast-22-en-5α,6β-diol (**4**): Perchloric acid (1 ml) was added to a solution of crude **3** (3 g) in acetone (70 ml) and water (6 ml). The solution was stirred for 5 h. The mixture was cooled and poured onto ice water. Usual work-up yielded 2.9 g (95%) of crude mixture. A small portion of this crude (0.1 g) was purified by flash chromatography, resulting in 0.037 g (37 %) of the pure steroid **4**. M.p. 142–143 °C (acetone). EA: calc: C72.0%; H 9.4%; Found: C 71.9%; H9.3%.

(22E)-Stigmast-2,22-dien-5α,6β-diol (**5**): A solution of 2.8 g of crude **4** in 40 ml of DMF was treated with 4.3 g LiBr and 3.3 gr Li_2CO_3, then refluxed for 3–4 h in the dark; cooled to room temperature and poured onto a solution of HCl (10%). Usual work-up yielding a solid, which was purified by column chromatography resulting in 1.6 g (3.7 mmol, 80%) of compound **5**. M.p. 152.9–153.4°C (acetone). EA: calc.: 81.2% C, 11.3% H; found: 81.1% C, 11.4% H.

(22R,23R)-2α,3α,22,23-diepoxy-stigmast-5α,6β-diol and (22S, 23S)-2α,3α,22,23-diepoxy-stigmast-5α,6β-diol (**6,6A**): Diene **5** (1.5 g, 3.5 mmol) in chloroform (20 ml) was treated with MCPBA (0.7 g, 4 mmol) at room temperature and stirred in the dark for 24 h. Usual work-up yielded a crude product which was purified by flash chromatography to give 1.1 g (2.2 mmol, 52%) of a mixture of isomers **6** and **6A**.

(22R,23R)-2α,3α-22,23-diepoxy-5α-hydroxy-stigmastan-6-one (**7**): Jones reagent (1 ml) was added dropwise to a solution of a mixture of **6** and **6A** (1g, 2.2 mmol) in acetone (12 ml). After 1 h, 10 ml of isopropyl alcohol was added to the stirred reaction and the resulting mixture was concentrated to half volume and then poured onto 54 ml of cold water. Usual work-up produced a crude product which was purified by successive chromatographic column affording 0.27 g (0.6 mmol, 31 %) of the final product **7** and the remainder of the **7** and **7A** mixture. M.p. 99.3–99.6 °C (acetone). EA: calc.: C 75.9%; H 10.1%; Found: C 76.0%; H 10.2 %.

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