Research Article

A new synthesis of [26,28-^{2}H$_6$]brassinolide and [26,28-^{2}H$_6$]castasterone via an unusual methyl migration

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Dedicated to Professor Dr Peter Welzel on the occasion of his 65th birthday

Summary

Deuterium-labelled brassinosteroids, namely [26, 28-^{2}H$_6$]castasterone, **8**, and [26, 28-^{2}H$_6$]brassinolide, **9**, were synthesized starting from 6, 6-ethylenedioxy-20-formyl-2α, 3α-isopropylidenedioxy-5α-pregnane, **1**, and 3-[^{2}H$_3$]methyl-but-1-yne-[4, 4, 4-^{2}H$_3$], **11**. Upon alkylating cleavage of the epoxide **6** with trimethylaluminium-*n*-butyllithium an unusual migration of a neighbouring [^{2}H$_3$]methyl group takes place to afford deuteriation at positions 26 and 28. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: brassinosteroids; [26, 28-^{2}H$_6$]castasterone; [26, 28-^{2}H$_6$]brassinolide; 3-[^{2}H$_3$]methyl-but-1-yne-[4, 4, 4-^{2}H$_3$]

Introduction

The brassinosteroids are steroidal phytohormones of ubiquitous occurrence in the plant kingdom showing high growth promoting and antistress activity. Up until now more than 40 native brassinosteroids have been isolated and characterized from a large variety of plants.[1−3] For biosynthetic and metabolic studies[4] of brassinosteroids in plants deuterium-labelled versions[5, 6] are needed. Thus, Takatsuto and Ikekawa[7] synthesized a series of [26, 28-^{2}H$_6$]brassinosteroids starting

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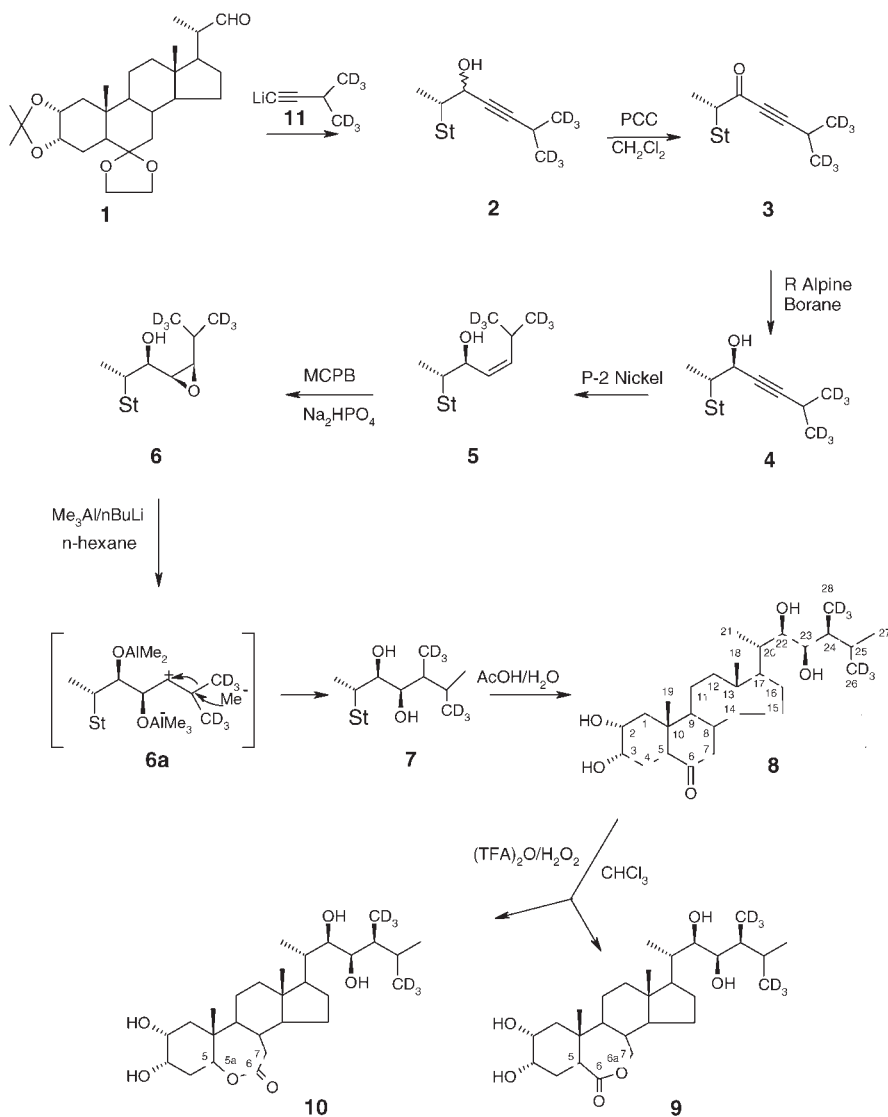
Figure 1. Syntheses of $[26, 28\text{-}^2H_6]$castasterone, 8, and $[26, 28\text{-}^2H_6]$brassinolide, 9

from a $3, 5$-cyclo-22-aldehyde and $[26, 28\text{-}^2H_6]$crinosterol, respectively, by stepwise introduction of the label. More recently, the synthesis of $[25, 26, 27\text{-}^2H_7]$brassinolide from the parent brassinolide has been published.[8] In this paper we describe a new synthesis of $[26, 28\text{-}^2H_6]$ castasterone, **8**, and $[26, 28\text{-}^2H_6]$brassinolide, **9**, via side-chain construction starting from 6,6-ethylenedioxy-20-formyl-$2\alpha, 3\alpha$-isopropylidenedioxy-5α-pregnane, **1**, and 3-$[^2H_3]$methyl-but-1-yne-$[4, 4, 4\text{-}^2H_3]$, **11**, involving an unusual methyl migration (Figure 1).

Results and discussion

The side-chain unit 3-[^{2}H$_3$]methyl-but-1-yne-[4, 4, 4-^{2}H$_3$],[9] **11**, was synthesized in three steps starting from sodium acetylide and [^{2}H$_6$]acetone in a similar manner to that described for the unlabelled compound.[10, 11, 12] Because acetylene in the steel flask is dissolved in acetone, the isotopic incorporation of the 3-[^{2}H$_3$]methyl-but-1-yne-[4, 4, 4-^{2}H$_3$] was somewhat reduced (88%). The lithium salt of this deuteriated side-chain synthon was coupled with the protected steroidal 22-aldehyde **1**[12] which had been produced in eight steps starting from stigmasterol. The obtained diastereomeric mixture of the acetylenic 22-alcohols **2** were oxidized by pyridinium chlorochromate to give the acetylenic 22-ketosteroid **3**. Subsequent reduction with R-Alpine-borane ((+)-α-pinene) as a chiral reagent[13] led stereoselectively to the desired 22R alcohol **4**. Partial hydrogenation of **4** over P-2 nickel catalyst in the presence of ethylenediamine afforded the allylic 22-alcohol **5**. Subsequent epoxidation of the allyl alcohol with *m*-chloroperbenzoic acid in the presence of sodium hydrogen phosphate gave the 23,24 epoxide **6**.[12,14]

For the alkylating fission of the epoxy ring[15] compound **6** was dissolved in a mixture of *n*-hexane and cyclohexane (7.5:2) and cooled to −78°C under an argon atmosphere. To the stirred solution trimethylaluminium (2 M in *n*-heptane) and *n*-butyllithium (1.6 M in *n*-hexane) were added to give, after SiO$_2$-chromatography, 48% of the 22R, 23R-diol **7**. As discussed below the NMR data of this castasterone derivative **7** indicated deuteriation at the C-26 and C-28 positions instead of that the expected C-26 and C-27 positions. Thus, during the cleavage of the epoxy ring migration of one [^{2}H$_3$]methyl group from position 25 to 24 must have taken place probably via an intermediate **6a**. Removal of the protective groups with 80% acetic acid afforded the desired labelled castasterone, **8**.

Some of **8** was purified over HPLC whereas a second part was transformed directly via Baeyer–Villiger oxidation with peroxytrifluoroacetic acid to afford [26, 28-^{2}H$_6$]brassinolide, **9**, and the isomeric 5a-oxa, 6-oxo-lactone **10** in a 6:1 ratio. Additionally in this procedure, small amounts of a monotrifluoroacetic acid ester of [26, 28-^{2}H$_6$]brassinolide were obtained indicating side-chain esterification at C-22 or C-23. Pure [26, 28-^{2}H$_6$]brassinolide, **9**, was obtained

Table 1. ^{1}H NMR data (δ, multiplicity, coupling constants (Hz)) for protons and ^{13}C chemical shifts and coupling constants J_{CF} (Hz) for carbons of compounds 6, 8 and 9 (values without multiplicity are chemical shifts of the HSQC-peaks). In [ ] data of the undeuteriated compounds are given.

Position	6 $\delta_{1H}\,\alpha/\beta$	8 $\delta_{1H}\,\alpha/\beta$	9 $\delta_{1H}\,\alpha/\beta$	6 δ_{13C}	8 δ_{13C}	9 δ_{13C}
1	1.06/1.91	1.56/1.67	1.53/1.852 dd (12.8/4.7)	42.8	41.0	41.0
2	4.098 m	3.656 ddd (11.9/4.9/3.1)	3.631 ddd (12.1/4.5/2.7)	73.0	69.1	67.7
3	4.273 m	3.945 m	3.958 br s	72.9	69.5	67.7
4	2.15^a/1.79^a	1.756 ddd (15.0/3.6/3.6)/1.66	1.901 ddd (15.3/4.3/4.3)/2.097 ddd (15.3/12.2/2.3)	22.1	27.8	30.9
5	1.81	—	3.136 dd (12.2/4.4)	45.6	52.1	40.8
6				109.6	215.1	177.2
7	1.03/1.752 dd (13.2/3.7)	2.108 dd (13.3/12.1)/2.205 dd (13.3/4.9)	4.10/4.10	41.1	47.5	70.4
8	1.51 ddd (12.1/11.1/4.2)	1.80	1.72	33.0	39.2	38.9
9	0.783 ddd	1.43	1.28	52.9	55.1	57.9
10	—	—	—	38.1	43.6	38.0
11	1.55/1.30	1.69/1.40	1.78/1.39	20.9	22.4	22.0
12	1.21/1.977 ddd (12.3/3.3/2.8)	1.31/2.06	1.24/1.98	39.7	40.9	39.4
13	—	—	—	42.4	43.9	42.2
14	1.11	1.38	1.20	55.8	57.9	51.1
15	1.59/1.07	1.59/1.13	1.68/1.24	24.2	24.8	24.5
16	1.92/1.20	2.01/1.29	1.98/1.30	27.8	28.6	27.3

17	1.55	1.61	1.56	52.1	53.7	52.0
18	0.677 s	0.724 s	0.720 s	12.0	12.3	11.5
19	0.837 s	0.762 s	0.912 s	13.5	13.8	15.2
20	1.53	1.50	1.47	40.3	38.5	36.7
21	1.059 d (6.3)	0.908 d (6.7)	0.888 d (6.7)	12.7	12.5	11.6
22	3.603 brdd (6.4/4.3)	3.510 dd (8.3/1.3)	3.509 dd (8.5/1.5)	70.5	75.7	74.2
23	3.059 dd (6.4/4.3)	3.676 dd (8.3/2.0)	3.687 dd (8.5/1.9)	60.1	74.4	73.0
24	2.669 dd (9.7/4.3)	1.14	1.15	63.3	41.6	39.7[40.0]
25	1.54	1.61	1.60	26.6	31.4	30.1[30.4]
26	–[1.09 a]	0.959 d (6.7) [0.964 d (6.7)] proR	0.958 d (6.7) [0.963 d (6.7)] pro R	[20.3]	21.3	20.5[20.6] proR
27	–[0.974 d (6.7 a)]	–[0.933 d (6.8)] ProS –[0.838 d (6.9)]	–[0.943 d (6.7)] proS –[0.839 d (6.9)]	[18.8]	[31.1 b]	19.7[20.5] proS
28	—				[10.7 b]	9.2[9.9]
Me	1.479 s			28.7		
Me	1.327 s			26.7		
Sch	—			107.5		
6 OCH2 a	3.95/3.91			65.5		
6 OCH2 a	3.91/3.75			64.2		

a Exchangeable.
b From the HMBC.

by HPLC separation. The deuterium incorporation of the labelled castasterone and brassinolide at the specified sites was found to be 86.6%.

^{1}H and ^{13}C NMR chemical shifts of (22R, 23S, 24R)-2α, 3α-isopro−pylidenedioxy-6, 6-ethylendioxy-22-hydroxy-23, 24 epoxy-5α-cholestane [26,27-[2]H$_6$], **6**, [26, 28-[2]H$_6$]castasterone, **8**, and [26, 28-[2]H$_6$]brassinolide, **9**, (Table 1) were unambiguously assigned, based on one- and two-dimensional experiments, including homo- and heteronulear shift correlation spectra.

The positions of the deuteriated methyl groups of castasterone were verified by detailed investigation. The high-field region of the proton spectrum showed, besides the two singlets of Me-18 (δ 0.724) and Me-19 (δ 0.762), two intense doublets at δ 0.908 ($J = 6.7$ Hz) and δ 0.959 ($J = 6.7$ Hz) and three weak doublets (δ 0.964, $J = 6.7$ Hz; 0.933, $J = 6.8$ and δ 0.838, $J = 6.9$ Hz). The signal at δ 0.908 showed HMBC correlations with C-17 (δ 53.7), C-20 (δ 38.5) and C-22 (δ 75.7). A correlation with the ^{13}C signal at δ 53.7 was also found for Me-18 (δ 0.724) and therefore this carbon signal was assigned to C-17 and the doublet at δ 0.908 to Me-21. The ^{1}H signal at δ 0.838 had a relative intensity of about 15% compared with the signal of Me-21 and showed HMBC correlations with C-23 (δ 74.4), C-24 (δ 41.6) and C-25 (δ 31.4). Consequently, it had to be assigned to Me-28 in the non-deuteriated portion of castasterone. This finding verified unequivocally the migration of one [[2]H$_3$]methyl group from C-25 to C-24 during the methylation step **6** to **7**. The 2 weak doublets at δ 0.964 and δ 0.933 were assigned to Me-26[proR] and Me-27[proS], respectively, in the undeuteriated portion of the NMR spectrum of castasterone.[16] The intense doublet at δ 0.959 was assigned to Me-26[proR] of the main compound by its HMBC correlations with C-24 (δ 41.6) and C-25 (δ 31.4). No correlation with C-27[proS] could be detected in the routine HMBC spectrum. However, an HMBC experiment with deuterium decoupling showed the cross peak between H$_3$-26 and C-27. In the [13]C{[1]H} spectrum the fully deuteriated carbons C-27[proS] and C-28 displayed weak multiplets at δ 19.9 and δ 9.5, respectively. In each case the upfield shift of 1.2 ppm by comparison with the corresponding signals of the undeuteriated spectrum is due to the deuterium isotope effect, which is known to be about 0.4 ppm per deuterium atom in an α-position.[17]

Conclusions

[26, 28-^{2}H$_6$]Castasterone, **8**, and [26, 28-^{2}H$_6$]brassinolide, **9**, were synthesized starting from 6, 6-ethylenedioxy-20-formyl-2α, 3α-isopropylidenedioxy-5α-pregnane, **1**, and 3-[^{2}H$_3$]methyl-but-1-yne-[4, 4, 4-^{2}H$_3$], **11**. In the alkylating opening of the epoxy ring in **6** with trimethylaluminium a migration of one [^{2}H$_3$]methyl group from positions 25 to 24 takes place, leading to the corresponding 26,28-deuteriated compound **7** instead of the initially expected [26, 27-^{2}H$_6$] labelled brassinosteroids. It can also be assumed that during the corresponding synthesis of unlabelled brassinosteroids[12] such a positional change of one methyl-group takes place.

Experimental

Materials and methods

For column chromatography we used Merck silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM). Melting points (uncorrected) were determined on a Boetius heating table. Optical rotations [α]$_D$ were measured at room temperature on a Jasco Digital Polarimeter DIP-1000.

EIMS (DIS): 70 eV, AMD 402 (AMD Intectra.) GCMS: MD-800 (Fisons Instruments), EI 70 eV; source temperature 200°C; column DB-5MS (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), injection temp. 260°C, interface temp. 300°C, carrier gas He, flow rate 1.3 ml/min. splitless injection, column temperature program: 170°C for 1 min, then raised to 290°C at a rate of 30 grd/min and held on this temperature for 20 min; positive ion ESI mass spectra

NMR: 1D: VARIAN GEMINI spectrometer 300, 300.24 MHz and 75.5 MHz, solvent CD$_3$OD and CDCl$_3$, 2D: (^{1}H-^{1}H-COSY, GHSQC and GHMBC) NMR VARIAN UNITY 500 spectrometer, 499.83 MHz, solvent CD$_3$OD and CDCl$_3$, TMS as internal standard.

6, 6-*Ethylendioxy-22-hydroxy-2α, 3α-isopropylidenedioxy-5α -cholest-23-yne*[26, 27-^{2}H$_6$], 2

A solution of 3-[^{2}H$_3$]methyl-but-1-yne-[4, 4, 4-^{2}H$_3$][9], **11**, (1.78 g) in dry THF (8 ml) was stirred and cooled to −78°C under an argon

atmosphere. *n*-BuLi in heptane (1.6 M; 12 ml) was added dropwise to this solution for 30 min. The solution was stirred for 1 h at $-70°C$, 30 min at $-60°C$ and cooled again to $-70°C$. A solution of 6, 6-ethylendioxy-20-formyl-2α, 3α-isopropylidenedioxy-5α-pregnane, **1**.[12] (2.07 g) in dry THF (30 ml) was added dropwise at $-70°C$ during 30 min. The resulting solution was stirred for 1 h and then warmed up to $-10°C$, before the solution was quenched by the addition of a saturated aqueous solution of ammonium chloride (20 ml). Two phases separated at room temperature and the water layer was extracted twice with EtOAc. The organic phase was dried with Na_2SO_4 and concentrated *in vacuo* to give 2.38 g (98.7%) **2**. This product was not further purified.

6, 6-*Ethylendioxy-2α, 3α-isopropylidenedioxy-5α-cholest-23-yn-22-one*[26, 27-²*H₆*]*, **3**

A solution of **2** (2.38 g) in dry CH_2Cl_2 (15 ml) was added to a stirred suspension of pyridinium chlorochromate (PCC, 1.624 g) and natrium acetate (132 mg) in dry CH_2Cl_2 (15 ml). The mixture was stirred for 20 h at room temperature at the end of which the supernatant was removed and the residue washed twice with CH_2Cl_2 and as well as with diethylether. The supernatant and washings were filtered through florisil (26 g) and concentrated *in vacuo* to give 2.09 g of crude product which was purified by SiO_2 column chromatography. Elution with hexane/ EtOAc (8:2 v/v) gave 1.58 g (66.6%) of amorphous **3**. EI MS *m/z* (%) 518 (M^+, 26), 503 (49), 307 (100), 254 (9), 239 (32), 99 (40), 87 (14), 73 (28).

6, 6-*Ethylendioxy* -22*R-hydroxy-2α, 3α-isopropylidenedioxy-5α-cholest-23-yne*[26, 27-²*H₆*]*, **4**

For the stereoselective reduction of **3**, R-Alpine-Borane (R-B-3-pinanyl-9-borabicyclo[3.3.1]nonane) was used. 14.5 ml of R-Alpine-Borane solution (0.5 M in dry THF) was added dropwise under argon to a stirred solution of the 22-keto-23-acetylenic steroids (1.56 g) in dry THF (29 ml). This mixture was stirred under an argon atmosphere at room temperature for another 6 days. After evaporation of the solvent the crude residue was chromatographed on silica gel. Elution with hexane/ EtOAc (8:2 v/v) gave 1.45 g (92.4%) **4**, mp 173–176°C ($CHCl_3$) EI MS

m/z (%) 520 (M$^+$, 15), 505 (67), 417 (16), 359 (34), 309 (88), 254 (25), 239 (64), 99 (100), 81 (38), 73 (48).

(22*R*, 23*Z*)-6, 6-*Ethylenedioxy*-22-*hydroxy*-2α, 3α-*isopropylidenedioxy*-5α-*cholest*-23-*ene* [26, 27-$^2H_6$], **5**

1.17 ml of an NaBH$_4$ solution (201 mg NaBH$_4$ in 4.75 ml 95% EtOH and 0.25 ml 2N NaOH aq.) was added to a strongly stirred solution of nickel acetate (Ni(OAc)$_2 \cdot$ 4H$_2$O) (147.6 mg) in 95% EtOH (7.9 ml) under a strong current of nitrogen. After the addition of ethylenedia-mine (78.7 μml) in the next step compound **4** (1.21 g) dissolved in EtOH (8.34 ml) was added and the mixture was stirred under a hydrogen atmosphere for 20 h. Diethylether (15 ml) and Celite (1 g) were added before the mixture was filtered. The filtrate was concentrated *in vacuo*. The crude product was purified by SiO$_2$-chromatography. Elution with *n*-hexane/EtOAc (8:2 v/v) gave 1.055 g (86.9%) of **5**.

EI MS: *m/z* (%) 522 (M$^+$, 4), 507 (25), 403 (28), 359 (100), 341 (19), 239 (26), 207 (45), 99 (62).

(22*R*, 23*S*, 24*R*)-2α, 3α -*Isopropylidenedioxy*-6, 6 -*ethylendioxy*-22 -*hydroxy*-23, 24*epoxy*-5α-*cholestane*[26, 27-$^2H_6$], **6**

Compound **5** (1.055 g) was dissolved in dry CH$_2$Cl$_2$ (50 ml). Anhydrous powdered Na$_2$HPO$_4$ (852 mg) was added. The mixture was stirred and cooled down to 5°C. Then *m*-chloroperbenzoic acid (MCPBA, 85%, 852 mg) was added. After stirring at 5°C for 6 h 0.5 N NaOH aq. (27 ml) was added to the reaction mixture. The organic layer was separated and the water layer extracted with CH$_2$Cl$_2$. The organic layer was dried with Na$_2$SO$_4$, concentrated *in vacuo* and chromatographed over SiO$_2$ with hexane/EtOAc (9:1–7 : 3 v/v) as the eluant to give 852.6 mg (78.4%) of **6** mp 154–157°C (from hexane). LC MS Rt 5.18 EI MS: *m/z* (%) 539 ([M + 1]$^+$, 100), 522 (8), 499 (20), 460 (4). ^{1}H and ^{13}C NMR data are displayed in Table 1.

(22*R*, 23*R*, 24*S*)-2α, 3α - *Isopropylidenedioxy*-6, 6- *ethylendioxy*-22, 23-*dihydroxy*-24-*methyl*-5α-*cholestane*[26, 28-$^2H_6$], **7**

To a stirred solution of the epoxy compound **6** (753 mg) in a mixture of dry cyclohexane (20 ml) and dry hexane (75 ml) under an argon atmosphere, cooled down to −78°C, two solutions were added, one of

Me$_3$Al in *n*-heptane (2 N; 4.5 ml) and a second one of BuLi in *n*-hexane (1.6 M; 0.6 ml). The mixture was stirred for 1.5 h at -70°C and then it was slowly warmed up to room temperature. After 20 h the mixture was again cooled down to -40°C and the reaction was quenched by the addition of 1 N HCl (80 ml). The layers were separated and the water layer extracted with EtOAc. The collected organic layers were washed successively with NaHCO$_3$ aq. and brine and after this dried with Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified by chromatography over SiO$_2$ with *n*-hexane/EtOAc (3:2–1 : 1 v/v) as the eluant to give 370 mg (47.7%) of **7**. mp 88–89°C (from EtOAc), $[\alpha]_D + 44.4$ (c = 1, 0; CH$_3$OH); EI-MS: *m/z* (%) 554 (M$^+$, 26), 539 (70), 447 (27), 433 (29), 389 (62), 371 (24), 343 (100), 254 (26), 239 (46), 99 (71).

[26, 28-^{2}H$_6$]Castasterone, 8

Compound **7** (340.8 mg) was dissolved in acetic acid/water (4:1; 20 ml) and then warmed up at 50°C for 1.5 h. After cooling to room temperature the solution was neutralized with Na$_2$CO$_3$ and extracted with CHCl$_3$. The CHCl$_3$ layer was dried with Na$_2$SO$_4$ and concentrated *in vacuo* to give [26, 28-^{2}H$_6$]castasterone, **8**, (289 mg; 99.9%) mp 250–2°C (from methanol). GC MS as boranate Rt 10.38 518 (M$^+$, 27), 399 (10), 358 (18), 287 (32), 161 (78), 85 (100). Separation by HPLC: Merck, LiChrospher 100 RP 18, 10 μm, 250 $\times$ 10 mm, MeCN : H$_2$O (9 : 1 v/v), 5 ml min^{-1}, 210 nm. Rt 22.7 min. ^{1}H and ^{13}C NMR data are displayed in Table 1.

[26, 28-^{2}H$_6$]Brassinolide, 9

Peroxytrifluoroacetic acid was generated *in situ* by slow addition of H$_2$O$_2$ aq. (30%; 0.48 ml) to stirred trifluoroacetic anhydride (2.96 ml) at 0°C under nitrogen. The stirred mixture was allowed to react for 0.5 h. Then a solution of [26, 28-^{2}H$_6$]castasterone (204.6 mg) in CH$_2$Cl$_2$ (25 ml) was added dropwise. The solution was continuously stirred for 2 h at room temperature. The mixture was diluted with CH$_2$Cl$_2$ (20 ml) and washed successively with water (10 ml), with saturated NaHCO$_3$ aq. (2 $\times$ 10 ml), with 10% aq. NaHSO$_3$ (2 $\times$ 10 ml) and with brine (10 ml). The CH$_2$Cl$_2$ extract was dried with Na$_2$SO$_4$ and concentrated *in vacuo* to give 146 mg of crude product. Then the water layer was extracted with ethyl acetate. The ethyl acetate was dried and evaporated off to

give 51.7 mg of crude product. Both crude products (146 mg, 51.7 mg) were purified by chromatography over SiO_2 with $CHCl_3/CH_3OH$ (1:0–9 : 1 v/v) as the eluant to give firstly 12.7 mg (5.0%) of the not-required monotrifluoroacetic ester of [26, 28-$^2H_6$]brassinolide (R_f 0.42 Silufol $CHCl_3/CH_3OH$ 9 : 1 v/v) and 138.3 mg (65.37%) of a mixture of **9** and **10**. Separation by HPLC: Merck, LiChrospher 100 RP 18, 10 μm, 250 × 10 mm, $MeCN : H_2O$ (9 : 1 v/v), 5 ml/min^{-1}, 210 nm gave at Rt 16.5 min. 15 mg (7.1%) the isomer [26, 28-$^2H_6$]brassinolide (the B-homo-5a-oxa-6-lacton), **10**, and at Rt 18.3 min. 93 mg (43.96%) pure [26, 28-$^2H_6$]brassinolide, **9**, mp 285–7°C (from CH_3OH), $[\alpha]_D$ + 46.4 (c = 1, 0; CH_3OH), GC MS as boranate Rt 11.70 min. 534 (M$^+$, 1) 374 (13), 345 (11), 177 (63), 161 (46), 85 (100).

^{1}H and ^{13}C NMR data are displayed in Table 1.

Acknowledgements

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