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Regioselective Oxyfunctionalization of Brassinosteroids by Methyl(trifluoromethyl)dioxirane: Synthesis of 25-Hydroxy-brassinolide and 25-Hydroxy-24-epibrassinolide by Direct C-H Insertion

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Abstract: The direct oxyfunctionalization of suitable brassinosteroid derivatives at position 25 using methyl(trifluoromethyl)dioxirane is reported. Whereas reaction with tetraacetyl brassinolide led after deprotection directly to the desired 25-hydroxy-brassinolide, in the (24R) series the 22,23-isopropylidenedioxy derivative has been shown to be a suitable precursor for the 25-hydroxylation leading to 25-hydroxy-24-epicastasterone and upon Baeyer-Villiger oxidation to 25-hydroxy-24-epibrassinolide, respectively. Copyright © 1996 Elsevier Science Ltd

The brassinosteroids represent a new class of steroidal phytohormones of an ubiquitous occurrence in the plant kingdom with high growth promoting and antistress activity.[1] The biological significance of 25-hydroxylated members recently found as metabolites in plant cell cultures[2,3] as well as their potential native occurrence, stimulated our efforts to develope effective methods for a direct oxyfunctionalization of brassinosteroids at this position. Especially we were interested in the 25-hydroxylation of brassinolide and 24-epibrassinolide, which are the most important native members used also for practical application.[4] Herewith we report on the regioselective oxyfunctionalization of suitable derivatives of both phytohormones with methyl(trifluoromethyl)dioxirane (TFD)[5] to afford the desired 25-hydroxylated brassinolide, 24-epibrassinolide as well as 24-epicastasterone.

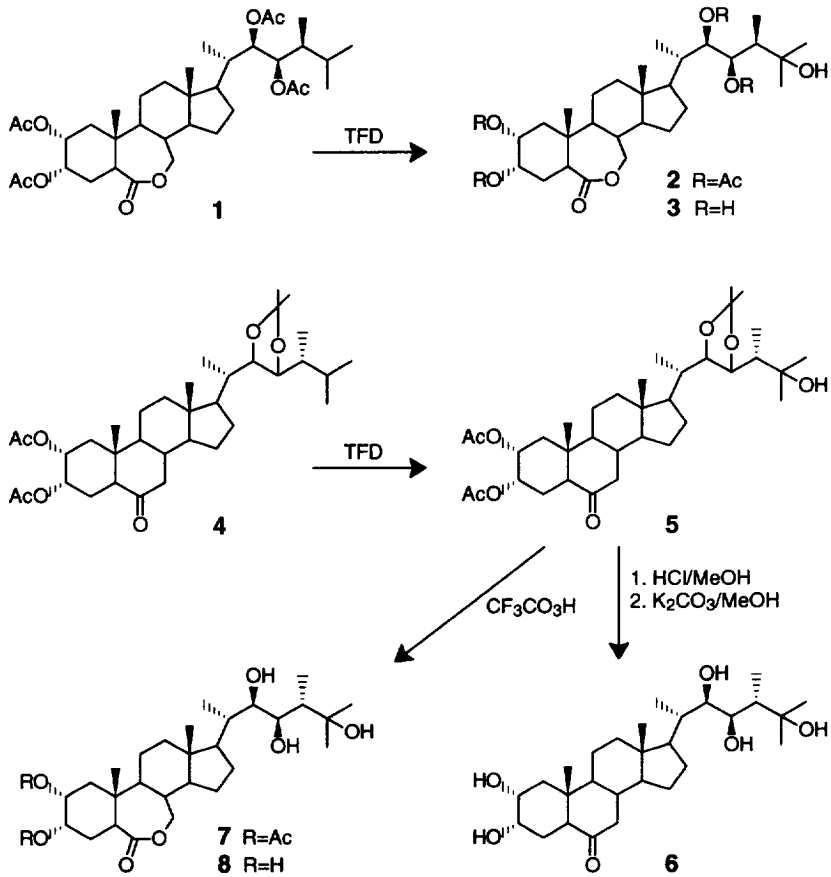
RESULTS AND DISCUSSION

Tetraacetyl-brassinolide (**1**) was reacted with 1.2 equiv. of TFD in methylene chloride during two days at room temp. (Scheme 1). Separation of the crude reaction product by flash chromatography on silica gel gave the 25-hydroxy-brassinolide derivative **2** in 71% (relative to consumed **1**). Hydrolysis of **2** with 5% methanolic KOH led to 25-hydroxy-brassinolide **3**. The 25-hydroxylation of tetraacetyl brassinolide (**1**) with the (24S)-configuration is in agreement with results obtained by Bovicelli et al.[6], who showed the same preferred regioselectivity upon reaction of TFD with steroids bearing an unsubstituted cholestane side chain moiety.

A striking different regioselectivity was observed upon reaction of TFD with the tetraacetyl derivative of the (24R)-configurated 24-epibrassinolide in which 14α-hydroxylation took place as the main process.[7] This unexpected regioselectivity could be circumvented by using the 22,23-isopropylenedioxy precursor **4**[8] instead of the corresponding tetraacetate (Scheme 1). In that case, derivative **4** of the likewise native brassinosteroid

24-epicastasterone[1c] afforded with TFD the desired 25-hydroxy derivative **5** in 62% yield. Deprotection of the 22,23-isopropylidenedioxy derivative **5** with methanolic HCl, followed by hydrolysis with K_2CO_3 in methanol, gave the new 25-hydroxy-24-epicastasterone (**6**). Baeyer-Villiger oxidation of **5** with trifluoroperoxyacetic acid and preparative HPLC separation led to 2α,3α-diacetoxy-25-hydroxy-24-epibrassinolide (**7**) and its 5a-oxa-6-oxo regioisomer in a 1 : 0.3 ratio.[9] Hydrolysis of **7** with K_2CO_3 in methanol afforded the desired 25-hydroxy-24-epibrassinolide (**8**). The 25-β-D-glucopyranosyl derivative of **8** was recently detected as a metabolite of exogenously applied 24-epibrassinolide in cell suspension cultures of *Lycopersicon esculentum* .[2]

Scheme 1



The structures of the 25-hydroxylated compounds **2**, **3**, **5**, **6**, **7** and **8** were verified by MS and NMR spectroscopy. Combined use of 1D and 2D NMR experiments, in particular direct (HMQC) and long-range heteronuclear ^{1}H, ^{13}C correlation spectra (HMBC) result in a complete unambiguously assignment of the ^{1}H and ^{13}C NMR signals. Relevant ^{1}H NMR and full ^{13}C NMR data are given in Tables 1 and 2 (for further NMR data of brassinosteroids see ref. 1c).

Table 1: ^{1}H NMR data (δ, multiplicity, coupling constants in Hz) for the side chain protons of **2**, **3**, **5**, **6**, **7** and **8**

	2[a]	**3**[b]	**5**[a]	**6**[b]	**7**[a]	**8**[b]
H-20	*1.62*	*1.44*	*1.56*	*1.39*	*1.40*	*1.40*
Me-21	1.037, s, 6.7	0.892, d, 7.0	0.991, d, 5.8	1.003, d, 6.7	1.000, d, 6.7	0.979, d, 6.7
H-22	5.116, d, 9.4	3.538, d, 8.0	4.015, d, 7.1	3.641, br s	3.633, br s	3.602, br s
H-23	5.490, d, 9.4	4.038, d, 8.0	3.753, dd,10.3/7.1	3.447, dd, 9.9/1.5	3.458, d, 9.2	3.403, br d, 9.9
H-24	*1.64*	*1.42*	*1.77*	*1.65*	*1.73*	*1.66*
Me-26[c]	1.211, s	1.347, s	1.174, s	1.222, s	1.276, s	1.224, s
Me-27[c]	1.150, s	1.237, s	1.199, s	1.254, s	1.293, s	1.256, s
Me-28	1.044, d, 7.1	0.959, d, 7.0	0.783, d, 7.0	0.810, d, 6.9	0.815, d, 6.7	0.782, d, 6.9

a) in $CDCl_3$; b) in ca. 6:1 $CDCl_3$: CD_3OD; c) may be reversed;
values in *italic* face are chemical shifts of HMQC cross peaks

Our results indicate a remarkable directing influence of the side chain stereochemistry of brassinosteroids on the regioselectivity of the C-H insertion reaction with TFD. Investigations for a deeper understanding of such steric effects by molecular modeling are under way.

The phytohormone activity of the synthesized 25-hydroxylated brassinosteroid analogues was studied using the highly sensitive and specific rice lamina bioassay according to the method of Arima et al.[10] The obtained results showed that 25-hydroxy-brassinolide (**3**) and 25-hydroxy-24-epibrassinolide (**8**) at 0.1 ppm exhibit the same activity as the parent phytohormones brassinolide and 24-epibrassinolide, respectively.

EXPERIMENTAL SECTION

***General*:** Melting points (m.p.) were determined on a Boetius hot stage microscope and are uncorrected. IR spectra were recorded on a Bruker IFS 28 instrument. Optical rotations were measured on a DIP 1000-polarimeter, UV spectra on an Uvikon 941 Kontron instrument. CD spectra were recorded with a Jasco J 710 spectrometer. Mass spectra (EI-MS, 70 eV) were obtained with a AMD 402 spectrometer, Electro spray ionisation (ESI) mass spectra with a Finnigan TSQ 7000 instrument. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 500 spectrometer at 499.84 as well as 125.7 MHz in $CDCl_3$ with TMS as internal standard. For TLC plates precoated with silica gel 60 PF_{254} 0.2 mm (Merck) and for column chromatography silica gel 60, 0.04 - 0.063 mm (Merck), were used. The preparative HPLC was carried out on a Knauer instrument, with a LiChrospher 100 RP 18, 10 μm, 10 - 250 mm column, MeOH/H_2O 7:3 v/v as eluent and UV detection at 210 nm.

Table 2: ^{13}C chemical shifts of **2**, **3**, **5**, **6**, **7** and **8**

C	2[a]	3[b]	5[a]	6[b]	7[a]	8[b]
1	38.8	41.1	37.5	39.8	38.8	41.3
2	68.9	67.9	69.1	67.9	68.9	68.0
3	67.9	67.8	68.1	68.0	67.9	68.0
4	29.2	31.0	24.8	23.8	29.3	31.3
5	42.0	40.8	51.8	50.7	42.0	41.2
6	n.d.	n.d.	210.5	n.d.	175.2	n.d.
7	70.4	70.4	46.5	46.6	70.6	70.9
8	39.1	39.0	37.6	37.7	39.2	39.3
9	58.3	58.0	53.6	53.6	58.3	58.2
10	38.3	40.7	42.5	42.5	38.4	38.3
11	22.2	22.0	21.3	21.0	22.2	22.3
12	39.4	39.4	39.1	39.2	39.4	39.6
13	42.4	42.2	42.8	42.6	42.5	42.6
14	51.3	51.1	56.3	56.4	51.2	51.3
15	24.6	24.4	23.8	26.2	24.8	24.9
16	28.0	27.2	27.8	27.8	28.0	28.0
17	52.4	52.0	53.3	52.5	52.8	52.8
18	11.6	11.5	11.8	11.6	11.6	11.6
19	15.4	15.2	13.6	13.3	15.4	15.4
20	37.0	36.6	38.2	42.6	42.9	42.9
21	12.6	11.6	12.6	12.2	12.4	12.4
22	75.5	74.4	83.5	71.8	71.9	71.9
23	72.3	72.8	81.0	76.8	77.1	77.1
24	43.3	41.2	48.2	45.5	45.5	45.6
25	72.4	73.3	73.7	75.0	76.1	75.3
26[c]	28.5	28.7	29.0	29.8	31.1	29.9
27[c]	26.4	28.0	23.8	22.2	22.6	22.4
28	9.0	7.0	13.2	13.6	14.0	13.7
OAc	n.d./21.0		170.2[d]/21.1		170.3[d]/21.1	
OAc	n.d./21.0		170.0[d]/21.2		170.1[d]/21.0	
OAc	n.d./21.0					
OAc	n.d./20.8					
$C_q/CH_3/CH_3$			108.9/27.2/27.1			

a) $CDCl_3$; b) ca. 6:1 $CDCl_3$: CD_3OD; c), d) may be reversed in the column; n.d. : not detected (HMBC spectrum)

C-H insertion reaction with TFD - General procedure. To a solution of 0.5 mmol of brassinosteroid derivative in 10 ml of dry methylene chloride were added 1.2 equiv. of a 0.35 molar solution of TFD in methylene chloride at 0° C under nitrogen in the darkness. The reaction was monitored by peroxide test with potassium iodide and by TLC. Upon stirring for 40 hr at rt, the solvent was evaporated under reduced pressure and the residue purified using flash chromatography on silica gel by elution with n-hexane/ethyl acetate.

Tetraacetyl derivative of 25-hydroxy-brassinolide (2). Synthesized from **1** upon reaction with TFD followed by SiO_2 chromatography. With n-hexane/ethyl acetate 1:1 v/v were eluated 71 % **2**, m.p. 238-241° C; EI-MS: *m/z* (relative intensities) 665 (M^++1, 2), 586 (M^+-78, 9), 577 (M^+-87, 32), 544 (M^+-120, 41), 535 (34%), 526 (26), 504 (33), 463 (100).

25-Hydroxy-brassinolide (3). A solution of **2** (17 mg, 0.025 mmol) in MeOH (2 ml) and methanolic KOH (0.1 ml, 5 %) was stirred under N_2 at rt for 20 hr. After dilution with MeOH (2 ml) HCl (1 ml, 6 N) was added and the reaction mixture stirred for 30 min. MeOH was removed and the residue purified by extraction with chloroform followed by crystallization from MeOH to give **3** (9.9 mg, 78 %) with m.p. 309-311° C; EI-MS: *m/z* 497 (M^++1, 1), 463 (M^+-15-H_2O, 2), 409 (M^+-87, 9), 393 (409-16, 13), 379 (M^+-117, 68), 361 (379-H_2O, 15); ESI-MS: *m/z* 497 ($[M+H]^+$, 100), 479 ($[M+H-H_2O]^+$, 20), 450 (8), 409 ($[M-C_5H_{11}O]^+$, 76); HR-MS: *m/z* 409.2569 (calcd. for $C_{23}H_{37}O_6$ 409.2590), 379.2487 (calcd. for $C_{22}H_{35}O_5$ 379.2489), 350.2462 (calcd. for $C_{21}H_{34}O_4$ 350.2467).

(22R,23R,24R)-2α,3α-Diacetoxy-22,23-isopropylidenedioxy-24-methyl-5α-cholestan-6-one (4). Synthesized from (22R,23R,24R)-22,23-isopropylidenedioxy-24-methyl-5α-cholest-2-en-6-one[11] by OsO_4 catalyzed dihydroxylation and subsequent acetylation. Flash chromatography and elution with n-hexane/ethyl acetate 75:25 v/v afforded **4** (79 %) with m.p. 210-211° C (needles from ethyl acetate/n-hexane); $[\alpha]_D^{26}$ +2.9° (c 1.29, MeOH); IR (nujol): ν_{max} 1748 (OAc), 1706 cm^{-1} (CO); UV (c 1.29, MeOH) λ_{max} 283 nm, ε_M 50; CD (c 5.74, $CHCl_3$) $\Delta\varepsilon_{298}$ -1.67; EI-MS: *m/z* 573 (M^+-15, 35), 517 (M^+-71, 14), 473 (573-100, 18%), 171 (52), 142 (100). ^1H NMR (300 MHz, $CDCl_3$) δ: 0.67 (3H, s, 18-H_3), 0.71 (3H, d, 7.0 Hz, 28-H_3), 0.82 (3H, d, 7.0 Hz, 26-H_3), 0.83 (3H, s, 19-H_3), 0.91 (3H, d, 7.0 Hz, 27-H_3), 0.99 (3H, d, 6.1 Hz, 21-H_3), 1.35 and 1.40 (acetonide-H_3), 1.99 and 2.10 (OAc), 2.33 (1H, dd, 13.4/4.6 Hz, 7α-H), 2.59 (1H, dd, 13.6/4.6 Hz, 5α-H), 3.57 (1H, dd, 9.5/7.0 Hz, 23-H), 3.95 (1H, d, 6.7 Hz, 22-H), 4.95 (1H, m, 2β-H), 5.39 (1H, d, 3.0 Hz, 3β-H). Anal. calcd. for $C_{35}H_{56}O_7$: C, 71.39; H, 9.59. Found: C, 71.28; H, 9.43.

25-Hydroxy-compound 5. Synthesized from **4** upon reaction with TFD followed by flash chromatography. Elution with n-hexane/ethyl acetate 65:35 v/v gave **5** (62 %), m. p. 229-232° C; $[\alpha]_D^{24}$ - 8.6° (c 1.06, MeOH); IR (nujol): ν_{max} 3486 (OH), 1746 (OAc), 1705 cm^{-1} (CO); UV (c 1.06, MeOH) λ_{max} 286 nm, ε_M 50; CD (c 9.20, $CHCl_3$) $\Delta\varepsilon_{295}$ - 2.73; EI-MS: *m/z* 589 (M^+-15, 20), 571 (589-H_2O, 7), 517 (M^+-87, 100). Anal. calcd. for $C_{35}H_{56}O_8$: C, 69.51; H, 9.26. Found: C, 69.38; H, 9.10.

25-Hydroxy-24-epicastasterone (6). A solution of 5 (60 mg, 0.1 mmol) in MeOH (10 ml) was treated with HCl (1 ml, 4 N) for 2 hr at 50° C under argon. MeOH was removed and the residue purified by extraction with ethyl acetate. The crude product was dissolved in MeOH (5 ml) and hydrolyzed with K_2CO_3 (10 mg) for 5 hr at rt. The solvent was removed and the residue purified by preparative HPLC to give **6** (37 mg, 77 %), m.p. 280-282° C; $[\alpha]_D^{25}$ - 6.7° (c 0.89, MeOH). EI-MS: *m/z* 447 (M^+-15-H_2O, 4), 393 (M^+-87, 10), 375 (393-H_2O, 16), 363 (M^+-117, 58), 345 (363-H_2O, 69); ESI-MS: *m/z* 481 ($[M+H]^+$, 18), 463 ($[M+H-H_2O]^+$, 62), 393 ($[M-C_5H_{11}O]^+$, 100); HR-MS: m/z 375.2514 (calcd. for $C_{23}H_{35}O_4$ 375.2535), 363.2495 (calcd. for $C_{22}H_{35}O_4$ 363.2536), 345.2406 (calcd. for $C_{22}H_{33}O_3$ 345.2430).

2α,3α-Diacetyl-25-hydroxy-24-epibrassinolide (7). To a solution of **5** (150 mg, 0.25 mmol) in dry chloroform (10 ml) at 0° C a mixture of hydrogen peroxide (0.4 ml, 30 %) and trifluoroacetic anhydride (2.5 ml) in chloroform (3 ml) was dropped and the mixture was stirred under argon for 2 hr. After addition of chloroform (10 ml) the organic layer was washed with water, aqueous Na_2CO_3 and brine, dried over Na_2SO_4 and evaporated to give a crude product. SiO_2 chromatography and elution with n-hexane/ethyl acetate 4:6 v/v yielded 99 mg (69 %) of lactone **7** and its 5a-oxa-6-oxo regioisomer in 1:0.3 ratio (measured from ^1H NMR integrals of 5α-protons), separated by preparative HPLC: **7**, m.p. 225-226° C; $[\alpha]_D^{22}$ + 17.4° (c 1.47, MeOH); IR (nujol) ν_{max} 3373 (OH), 1738 (lactone), 1713 cm^{-1} (CO); EI-MS: *m/z* 581 (M^++1, 7), 547 (M^+-15-H_2O, 11), 463 (M^+-117, 84), 403 (463-AcOH, 28), 361 (463-98, 100).

25-Hydroxy-24-epibrassinolide (8). A solution of **7** (6 mg, 0.01 mmol) in MeOH (2 ml) was stirred with K_2CO_3 (5 mg) for 5 h at rt. The solvent was removed and the residue extracted with chloroform to give **8** (4.5 mg, 90 %), m.p. 284-287° C; $[\alpha]_D^{22}$ + 15.2° (c 0.87, MeOH); EI-MS: *m/z* 497 (M^++1, 3), 479 (M^+-H_2O, 2), 463 (M^+-15-H_2O, 6), 409 (M^+-87, 33), 379 (M^+-117, 100); ESI-MS: *m/z* 497 ($[M+H]^+$, 45), 479 ($[M+H-H_2O]^+$, 38), 450 (36), 409 ($[M-C_5H_{11}O]^+$, 100); HR-MS: m/z 409.2589 (calcd. for $C_{23}H_{37}O_6$ 409.2591), 379.2481 (calcd. for $C_{22}H_{35}O_5$ 379.2484), 350.2461 (calcd. for $C_{21}H_{34}O_4$ 350.2465).

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