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Synthesis of new Δ^5-7-oxygenated and $\Delta^{5,7}$-unsaturated brassinosteroid analogs

Bernhard Hellrung, Brunhilde Voigt, Jürgen Schmidt, and Günter Adam

Department of Natural Products Chemistry, Institute of Plant Biochemistry, Halle/Saale, Germany

*We report on the synthesis of the brassinosteroid analogs (22R,23R)-3β,7β,22,23-tetrahydroxy-stigmast-5-ene (**13**), (22R,23R)-3β,7α,22,23-tetrahydroxy-stigmast-5-ene (**15**), and (22R,23R)-3β,22,23-trihydroxy-stigmast-5,7-diene (**18**) by means of the osmium-catalyzed asymmetric dihydroxylation of intermediate **1**, available from stigmasterol. This reaction sequence produced the expected (22S,23S)- and (22R,23R)-triols **6** and **7** as well as the 22,23-diketo derivatives **2** and **3**. The phytohormone activity of the new brassinosteroid analogs is discussed.*
(*Steroids* **62**:415–421, 1997)

Keywords: synthesis; brassinosteroid; phytohormones; osmium-catalyzed asymmetric dihydroxylation; (22R,23R)-3β,7β,22,23-tetrahydroxy-stigmast-5-ene; (22R,23R)-3β,7α,22,23-tetrahydroxy-stigmast-5-ene and (22R,23R)-3β,22,23-trihydroxy-stigmast-5,7-diene; mass spectrometry

Introduction

The brassinosteroids are a new class of naturally occurring phytohormones whose original structure as well as interesting biological properties stimulated a broad range of research activity.[1,2] In the course of our studies on the occurrence of native brassinosteroids in European cultivated plants, Δ^5-7-oxygenated and $\Delta^{5,7}$-unsaturated brassinosteroids have also been suggested.[3,4] As part of a program to prepare such structures as analytical standards and as analogs for biological examination, we developed a suitable reaction sequence starting from isostigmasterol (**1**). Specifically, the brassinosteroid analogs (22R,23R)-stigmast-5-ene-3β,22,23-triol (**8**), (22R,23R)-3β,22,23-trihydroxy-stigmast-5-en-7-one (**11**), (22R,23R)-3β,7β,22,23-tetrahydroxy-stigmast-5-ene (**13**), (22R,23R)-3β,7α,22,23-tetrahydroxy-stigmast-5-ene (**15**), and (22R,23R)-3β,22,23-trihydroxy-stigmast-5,7-diene (**18**) were synthesized, and their phytohormone activity was tested.

Experimental

Instrumental methods

All reagents and solvents were used as supplied without further purification, unless otherwise noted. Ether and methylene chloride were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA) in Sure-Seal bottles and transferred under an inert atmosphere. Analytical thin-layer chromatography (TLC) was conducted using Merck silica gel plates (200 microns) containing a fluorescent indicator (silica gel 60 F_{254}). Detection was performed by spraying with sulfuric acid (85%) at 110°C and by observation under ultraviolet light. Flash column chromatography was performed using Merck silica gel 60, 230–400 mesh, and elution was performed with n-hexane/ethyl acetate. Melting points were determined on a micromelting point apparatus PHMK-05 (Wägetechnik Rapido) with digital thermometer DTM 2110 (Thermometerwerk Geraberg) and are uncorrected. Optical rotations were recorded on a JASCO DIP-1000 in methanol, the circular dichroism on a JASCO J-710 in methanol, the IR-spectra on a Bruker IFS 28, and the ultraviolet spectra on an Uvikon 941 (Kontron Instruments) in methanol. ^1H and ^{13}C NMR spectra were recorded on a Varian unity 500 at 500 MHz and 125.7 MHz, respectively in $CDCl_3$ and with TMS as the internal standard. Chemical shifts (δ) are given in ppm downfield from TMS and observed coupling constants (J) are given in Hertz (Hz). Spin multiplicities are indicated by symbols: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The 70 eV electron impact mass spectra (EIMS) and the accurate measurements (resolution ca 7500) were obtained with an AMD-402 mass spectrometer (AMD Intectra GmbH, Harpstedt). The electrospray mass spectra (ESIMS) were recorded on a Finnigan TSQ 7000 instrument. The glass chromatography mass spectroscopy (GCMS) data of the methylboronate-TMS derivatives of compounds **8, 11, 13, 15,** and **18** were obtained using a MD-800 (Fisons Instruments) under the following conditions: DB5MS-column (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), temperature program: 170°C for 1 min then elevated to 290°C within 30 grd min^{-1}. The methylboronation was carried out with pyridine containing methylboronic acid at 70°C for 30 min. Further trimethylsilylation was done using N,O-

Dedicated to Prof. Dr. h. c. Klaus Schreiber on the occasion of his 70th birthday.

Address reprint requests to Prof. Günter Adam, Department of Natural Products Chemistry, Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany.

Received August 19, 1996; accepted December 2, 1996.

655 Avenue of the Americas, New York, NY 10010

0039-128X/97/$17.00
PII S0039-128X(97)00008-1

bistrimethylsilylacetamide. Elemental analyses were performed on a Hereus Vario EL instrument.

Bioassay

The phytohormone activity of the new brassinosteroid analogs was tested using the rice lamina inclination bioassay according to the method of Arima et al.[5]: Rice seedlings (*Oryza sativa* L. cv Koshihikari), germinated in water for 3 days in the light, were planted on 1% agar and grown for 6 days in the dark. From the second leaves of the etiolated seedlings, explants including the lamina joint were cut out 2 cm below the joint and incubated while floating on water for 1 day in the dark. The explants, bent about 15°, were selected, and 10 pieces of them were placed on a Petri dish (9 cm diameter) containing 20 mL aquous solution of a test sample. After 2-day incubation in the dark, the internal angles between the laminae and sheaths were measured. All operations were carried out at 28°C. 24-Epibrassinolide was used as standard.

Compounds

3α,5-Cyclo-stigmastan-6,22,23-trione (2): A mixture of isostigmasterol (**1**) (3.00 g, 6.7 mmol), $K_3Fe(CN)_6$ (14.97 g), K_2CO_3 (anhydr., 6.29 g), methanesulfonamide (1.44 g), dihydroquinidine-4-chlorobenzoate (DHQD, 0.71 g), and OsO_4 (0.075 g) in t-BuOH/H_2O 1:1 (300 mL) was stirred at room temperature for 6 days in the dark (TLC control). Solid Na_2SO_3 (6 g) was added, and the mixture was stirred for 18 h. The solvent was evaporated and the residue extracted with ethyl acetate. The organic layer was washed with H_2O (50 mL), three times with 0.25 M H_2SO_4, once with brine (50 mL), and dried over Na_2SO_4. Removal of the solvent and separation by flash chromatography (eluent: n-hexane/ethyl acetate 98:2 (v/v)) gave 0.10 g (3%) yellow crystals of **2**, mp 84–87°C, $[\alpha]_D^{24}$ = +29.5° (c = 3.30). CD: $\Delta\varepsilon_{288}$ −2.13. IR (nujol): ν = 1689 cm^{-1} (C = O). UV-VIS: λ_{max} (lg ε) = 452 nm (0.122), 230 nm (3.253), 224 nm (3.182). ^1H NMR: δ = 0.77 (t, *J* = 7.3 Hz, 3H, 29-H_3), 0.79 (s, 3H, 18-H_3), 0.86 (d, *J* = 1.5 Hz, 3H, 27*-H_3), 0.87 (d, *J* = 1.2 Hz, 3H, 26*-H_3), 1.01 (s, 3H, 19-H_3), 1.09 (d, *J* = 7.0 Hz, 3H, 21-H_3), 3.22–3.18 (m, 1H, 24-H), 3.51–3.45 (m, 1H, 20-H). ^{13}C NMR: 201.93, 203.08 (C-22, C-23), 209.28 (C-6). EIMS: *m/z* (%) = 440 (M^+, 1), 412 (8), 299 (100), 175 (9), 161 (19), 113 (39), 85 (83). Anal. calcd. for $C_{29}H_{44}O_3$ (440.6): C, 79.05; H, 10.07. Found: C, 79.35; H, 10.28.

3α,5-Cyclo-6β-hydroxy-stigmastan-22,23-dione (3): Further elution with n-hexane/ethyl acetate 90:10 (v/v) yielded 0.75 g (25%) yellow crystals of **3**, mp 122–125°C, $[\alpha]_D^{25}$ = +36.2 (c = 1.40). CD: $\Delta\varepsilon_{276}$ −0.06. IR (nujol): ν = 3571 cm^{-1} (OH), 1700 (C = O). UV-VIS: λ_{max} (lg ε) = 454 nm (0.143), 218 nm (2.975). ^1H NMR: δ = 0.29 (dd, *J* = 8.2 and 4.9 Hz, 1H, 3-H), 0.53 (dd, *J* = 4.3 and 4.3 Hz, 1H, 4-H), 0.76 (t, *J* = 7.3 Hz, 3H, 29-H_3), 0.80 (s, 3H, 18-H_3), 0.86 (d, *J* = 2.4 Hz, 3H, 27*-H_3), 0.87 (d, *J* = 2.4 Hz, 3H, 26-H_3), 1.06 (s, 3H, 19-H_3), 1.08 (d, *J* = 6.7 Hz, 3H, 21-H_3), 3.22–3.18 (m, 1H, 24-H), 3.26 (t, *J* = 2.5 Hz, 1H, 6α-H), 3.49–3.46 (m, 1H, 20-H). ^{13}C NMR: 73.66 (C-6), 202.24, 203.17 (C-22,C-23). EIMS: *m/z* (%) = 442 (M^+, 1), 329 (8), 283 (100), 213 (8), 159 (9), 121 (17), 113 (9), 85 (44). Anal. calcd. for $C_{29}H_{46}O_3$ (442.6): C, 78.65; H, 10.47. Found: C, 78.36; H, 10.43.

(22S,23S)-3α,5-Cyclo-22,23-dihydroxy-stigmastan-6-one (4): Further elution with n-hexane/ethyl acetate 80:20 (v/v) gave 40 mg (1%) of **4**, mp 131–135°C, $[\alpha]_D^{20}$ = +26.9° (c = 0.95). CD: $\Delta\varepsilon_{288}$ −1.83. IR (nujol): ν = 3424 cm^{-1} (OH), 1690/1682 (C = O). ^1H NMR: δ = 0.76 (s, 3H, 18-H_3), 0.88 (d, *J* = 6.8 Hz, 3H, 27*-H_3), 0.95 (d, *J* = 6.8 Hz, 3H, 26-H_3), 0.96 (t, *J* = 7.5 Hz, 3H, 29-H_3), 1.01 (s, 3H, 19-H_3), 1.04 (d, *J* = 6.8 Hz, 3H, 21-H_3), 3.64–3.60 (m, 2H, H-22, H-23). ^{13}C NMR: 70.59, 72.15 (C-22, C-23), 209.7 (C-6). EIMS: *m/z* (%) = 444 (M^+,3), 426 (1), 330 (100), 329 (60), 312 (12), 300 (21), 271 (7), 175 (6), 161 (9), 121 (5), 81 (3). Anal. calcd. for $C_{29}H_{48}O_3$ (444.64): C, 78.33; H, 10.88; Found: C, 78.30; H, 10.78.

(22R,23R)-3α,5-Cyclo-22,23-dihydroxy-stigmastan-6-one (5): Further elution with n-hexane/ethyl acetate 75:25 (v/v) gave 70 mg (2%) of 5, mp 144–149°C, $[\alpha]_D^{26}$ = +23.3° (c = 1.85). CD: $\Delta\varepsilon_{287}$ −1.95. IR (nujol): ν = 3512 cm^{-1} (OH), 1671 (C = O). ^1H NMR: δ = 0.76 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 3.60 (d, *J* = 7.0 Hz, 1H, 22-H), 3.72 (dd, *J* = 8.5 and 2.4 Hz, 1H, 23-H). ^{13}C NMR: 72.72, 74.55 (C-22,C-23), 209.6 (C-6). EIMS: *m/z* (%) = 444 (M^+,2), 426 (2), 410 (6), 330 (100), 329 (88), 253 (6), 161 (26), 109 (10), 97 (16). Anal. calcd. for $C_{29}H_{48}O_3$ (444.64): C, 78.33; H, 10.88. Found: C, 78.04; H, 10.88.

(22S,23S)-3α,5-Cyclo-stigmastane-6β,22,23-triol (6): Further elution with n-hexane/ethyl acetate 75:25 (v/v) gave 0.5 g (17%) of **6**, mp 104–106°C, $[\alpha]_D^{20}$ = +25.2° (c = 1.55). IR (nujol): ν = 3388 cm^{-1} (OH). ^1H NMR: δ = 0.29 (dd, *J* = 8.2 and 4.9 Hz, 1H, 3-H), 0.53 (dd, *J* = 4.5 and 4.5 Hz, 1H, 4-H), 0.76 (s, 3H, 18-H_3), 0.88 (d, *J* = 7.0 Hz, 3H, 27*-H_3), 0.95 (t, *J* = 7.0 Hz, 3H, 29-H_3), 0.97 (d, *J* = 7.6 Hz, 3H, 26*-H_3), 1.03 (d, *J* = 7.0 Hz, 3H, 21-H_3), 1.06 (s, 3H, 19-H_3), 3.26 (t, *J* = 2.7 Hz, 1H, 6α-H), 3.62 (m, w/2 = 20 Hz, 2H, H-22, H-23). EIMS: *m/z* (%) = 446 (M^+, 1), 428 (6), 332 (36), 313 (100), 312 (70), 295 (56), 255 (56), 213 (56), 159 (26), 145 (25), 121 (23), 81 (18). Anal. calcd. for $C_{29}H_{50}O_3$ × ½ H_2O (455.66): C, 76.54; H, 11.28. Found: C, 76.39; H, 11.51.

(22R,23R)-3α,5-Cyclo-stigmastane-6β,22,23-triol (7): Further elution with n-hexane/ethyl acetate 70:30 v/v gave 1.25 g (40%) of **7**, mp 143–146°C and $[\alpha]_D^{22}$ = +36.6° (c = 3.35). IR (KBr): ν = 3421 cm^{-1} (OH). ^1H NMR: δ = 0.29 (dd, *J* = 8.2 and 4.9 Hz, 1H, 3-H), 0.53 (dd, *J* = 4.5 and 4.5 Hz, 1H,4-H), 0.74 (s, 3H, 18-H_3), 0.91 (s, *J* = 6.4 Hz, 3H, 21-H_3), 0.95 (d, *J* = 7.0 Hz, 3H, 26*-H_3), 0.96 (t, *J* = 7.3 Hz, 3H, 29-H_3), 0.97 (d, *J* = 6.7 Hz, 3H, 27*-H_3), 1.06 (s, 3H, 19-H_3), 3.27 (d, *J* = 2.4 Hz, 1H, 6α-H), 3.61 (dd, *J* = 8.8 and 2.7 Hz, 1H, 22-H), 3.72 (dd, *J* = 8.8 and 3.7 Hz, 1H, 23-H)-EIMS: *m/z* (%) = 446 (M^+, 1.5),330 (56), 313 (100), 295 (56), 277 (33), 255 (48), 227 (30), 213 (55), 161 (38), 95 (40), 81 (33). Anal. calcd. for $C_{29}H_{50}O_3$ × ½ H_2O (455.66): C, 76.54; H, 11.28. Found: C,76.84; H,11.16.

(22R,23R)-Stigmast-5-ene-3β,22,23-triol (8): (22R,23R)-triol **7** (24 mg) in THF-H_2O (9:1) (2 mL) was treated with 0.05 mL of 5 N H_2SO_4 at rt for 3 h (TLC-control). The solvent was evaporated, and the residue was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ and brine and subsequently dried over Na_2SO_4. Removal of the solvent and flash chromatography [eluent: n-hexane/ethyl acetate 60:40 (v/v)] afforded 19 mg (79%) of **8**. Recrystallization from ethyl acetate/n-hexane yielded colorless needles, mp 157–160°C, $[\alpha]_D^{18}$ = −22.5° (c = 0.55). IR (KBr): ν = 3415 cm^{-1} (OH). ^1H NMR: δ = 0.71 (s, 3H, 18-H_3), 0.90 (d, *J* = 6.1 Hz, 3H, 21-H_3), 0.94 (d, *J* = 6.4 Hz, 3H, 26*-H_3), 0.96 (t, *J* = 7.0 Hz, 3H, 29-H_3), 0.97 (d, *J* = 6.9 Hz, 3H, 27*-H_3), 1.02 (s, 3H, 19-H_3), 3.53 (septet, *J* = 5.2 Hz, 1H, 3α-H), 3.74 (d, *J* = 1.2 Hz, 1H, 22-H), 3.76 (d, *J* = 1.0 Hz, 1H, 23-H), 5.35 (d, *J* = 4.9 Hz, 1H, 6-H). HRMS: 361.2759 (calcd. 361.2743 for $C_{23}H_{37}O_3$), 332.2697 (calcd. 332.2716 for $C_{22}H_{36}O_2$), 313.2535 (calcd. 313.2531 for $C_{22}H_{33}O$), 295.2438 (calcd. 295.2426 for $C_{22}H_{31}$). Anal. calcd. for $C_{29}H_{50}O_3$ × ½ H_2O (455.66): C, 76.54; H, 11.28. Found: C, 76.36; H, 11.51.

(22R,23R)-3β,22,23-Triacetoxy-stigmast-5-ene (9): Acetanhydride (10 mL) and DMAP (0.1 g) were added to a solution of **8** (930 mg, 2.08 mmol) in pyridine (10 mL). The mixture was stirred for 17 h, poured into ice-diluted HCl, and extracted with ethyl acetate. The organic layer was washed with water, aq. $NaHCO_3$, and brine and subsequently dried over Na_2SO_4. Evaporation and chromatography (eluent: n-hexane/ethyl acetate 90:10 (v/v)) gave 980 mg (82%) of **9**, mp 139–143°C and $[\alpha]_D^{20} = -16.4°$ (c = 1.15). IR (nujol): ν = 1746 cm^{-1} (OAc). ^1H NMR: δ = 0.70 (s, 3H, 18-H_3), 0.84 (d, J = 6.7 Hz, 3H, 26*-H_3), 0.96 (d, J = 6.7 Hz, 3H, 27*-H_3), 0.98 (t, J = 7.3 Hz, 3H, 29-H_3), 1.02 (s, 3H, 19-H_3), 1.03 (d, J = 8.2 Hz, 3H, 21-H_3), 1.96, 2.02 (2s, 6H, OAc (C-22, C-23)), 2.04 (s, 3H, 3β-OAc), 4.61 (septet, J = 5.1 Hz, 1H, 3α-H), 5.19 (d, J = 9.5 Hz, 1H, 22-H), 5.31 (d, J = 9.2 Hz, 1H, 23-H), 5.38 (d, J = 4.6 Hz, 1H, 6-H). EIMS: *m/z* (%) = 512 (100), 392 (18), 295 (14), 253 (19), 213 (11), 146 (22), 97 (34), 81 (19), 57 (23). Anal. calcd. for $C_{35}H_{56}O_6$ (572.74): C, 73.35; H, 9.86. Found: C, 73.05; H, 9.84.

(22R,23R)-3β,22,23-Triacetoxy-stigmast-5-en-7-one (10): A solution of CrO_3 (1 g) in CH_2Cl_2 (20 mL) was stirred with pyridine (0.8 ml) at −30°C. After 10 min, **9** (200 mg, 0.35 mmol) was added, and the mixture was allowed to warm up to rt with stirring for 3 h. After addition of saturated aq. $NaHSO_3$ and acidification with HCl (10%, 5 mL), the organic phase was separated, and the aqueous layer was extracted with ethyl acetate. The combined layers were washed with water and dried over Na_2SO_4. Removal of solvent and separation of the crude product by flash chromatography followed by elution with n-hexane/ethyl acetate 80:20 (v/v) gave 120 mg (59%) of **10**, mp 176–179°C and $[\alpha]_D^{20}$ = −77.8° (c = 1.10). IR (KBr): ν = 1745 cm^{-1} (OAc), 1664 (C = O). UV: λ_{max} (lg ε) = 286 nm (0.515). ^1H NMR: δ = 0.70 (s, 3H, 18-H_3), 0.84 (d, J = 6.7 Hz, 3H, 26*-H_3), 0.97 (t, J = 6.7 Hz, 3H, 29-H_3), 0.98 (d, J = 7.3 Hz, 3H, 27*-H_3), 1.04 (d, J = 6.7 Hz, 3H, 21-H_3), 1.21 (s, 3H, 19-H_3), 1.97, 2.00, 2.05 (3s, 9H, OAc), 4.72 (septet, J = 4.8 Hz, 1H, 3α-H), 5.20 (d, J = 9.2 Hz, 1H, 22-H), 5.30 (d, J = 9.2 Hz, 1H, 23-H), 5.70 (s, 1H, 6-H). EIMS: *m/z* (%) = 526 (54), 466 (10), 406 (11), 369 (16), 327 (100), 269 (17), 187 (15), 174 (19), 161 (12). Anal. calcd. for $C_{35}H_{54}O_7$ (586.71): C, 71.65; H, 9.28. Found: C, 71.56; H, 9.38.

(22R,23R)-3β,22,23-Trihydroxy-stigmast-5-en-7-one (11): A solution of **10** (150 mg, 0.256 mmol) in THF (10 mL) was treated with KOH/MeOH (1.8 mL, 5%) at rt for 4 h. The solvent was removed, and the residue was extracted with ethyl acetate, washed with water, dried over Na_2SO_4, and crystallized from ethyl acetate/n-hexane to give 85 mg (72%) of **11**, mp 186–188°C and $[\alpha]_D^{20}$ = −109.3° (c = 0.71). CD: $\Delta\varepsilon_{329}$ +1.46, $\Delta\varepsilon_{264}$ −0.22. IR (KBr): ν = 3386 cm^{-1} (OH), 1672 (C = O). UV: λ_{max} (lg ε) = 330 nm (0.65). ^1H NMR: δ = 0.70 (s, 3H, 18-H_3), 0.92 (d, J = 6.1 Hz, 3H, 21-H_3), 0.95 (d, J = 7.3 Hz, 3H, 26*-H_3), 0.96 (t, J = 6.7 Hz, 3H, 29-H_3), 0.97 (d, J = 6.1 Hz, 3H, 27*-H_3), 1.20 (s, 3H, 19-H_3), 3.63 (d, J = 8.6 Hz, 1H, 22-H), 3.68 (septet, J = 4.5 Hz, 1H, 3α-H), 3.72 (d, J = 8.9 Hz, 1H, 23-H), 5.70 (d, J = 1.8 Hz, 1H, 6-H). EIMS: *m/z* (%) = 460 (M^+, 1), 375 (3), 346 (100), 345 (53), 327 (41), 316 (25), 287 (13), 259 (7), 205 (9), 192 (30), 175 (9), 161 (13), 135 (11). HRMS: 346.2492 (calcd. 346.2508 for $C_{22}H_{34}O_3$), 316.2377 (calcd. 316.2402 for $C_{21}H_{32}O_2$), 287.2019 (calcd. 287.2011 for $C_{19}H_{27}O_2$), 205.1226 (calcd. 205.1229 for $C_{13}H_{17}O_2$), 192.1163 (192.1150 for $C_{12}H_{16}O_2$). Positive ESIMS: *m/z* = 483 ($[M + Na]^+$, 100%).

(22R,23R)-3β,22,23-Triacetoxy-stigmast-5-ene-7β-ol (12): Ketone **10** (207 mg, 0.353 mmol) and $CeCl_3 \times 7H_2O$ (154 mg, 0.413 mmol) were dissolved in THF/MeOH 2:1 (3 mL). Sodium borohydride (30 mg, 1 mmol) was added slowly with stirring. After 5 min, the reaction was quenched with 5% HCl (1 mL), and the solution was extracted with ethyl acetate (2 × 5 mL) and dried over Na_2SO_4. Evaporization followed by recrystallization from ethyl acetate/n-hexane gave 92% alcohol **12**, mp 104–106°C and $[\alpha]_D^{23}$ = +0.5° (c = 1.01). IR (KBr): ν = 3528 cm^{-1} (OH), 1734/1723 (OAc), 1669 (C = O), 1632 (C = C). ^1H NMR: δ = 0.72 (s, 3H, 18-H_3), 0.84 (d, J = 6.7 Hz, 3H, 26*-H_3), 0.97 (d, J = 6.7 Hz, 3H, 27*-H_3), 0.98 (t, J = 7.3 Hz, 3H, 29-H_3), 1.04 (d, J = 6.7 Hz, 3H, 21-H_3), 1.06 (s, 3H, 19-H_3), 1.97, 2.02, 2.04 (3s, 9H, OAc), 3.84 (d, J = 7.9 Hz, 1H, 7α-H), 4.62 (septet, J = 5.2 Hz, 1H, 3α-H), 5.19 (d, J = 9.1 Hz, 1H, 22-H), 5.29 (d, J = 1.2 Hz, 1H, 23-H), 5.31 (d, J = 1.5 Hz, 1H, 6-H). EIMS: *m/z* (%) = 588 (M^+, 3), 528 (100), 468 (6), 426 (29), 329 (11), 269 (34), 253 (6), 161 (9), 109 (9), 97 (18). Anal. calcd. for $C_{35}H_{56}O_7$ (588.73): C, 71.40; H, 9.59. Found: C, 71.24; H, 9.64.

(22R,23R)-3β,7β,22,23-Tetrahydroxy-stigmast-5-ene (13): Alkaline hydrolysis of **12** (150 mg, 0.263 mmol) and work-up as described for **11** followed by crystallization from ethyl acetate/n-hexane gave **13** (94 mg, 80%) as needles, mp 106–108°C and $[\alpha]_D^{22}$ = +5.8° (c = 1.10). IR (KBr): ν = 3418 cm^{-1} (OH). ^1H NMR: δ = 0.72 (s, 3H, 18-H_3), 1.06 (s, 3H, 19-H_3), 3.61 (d, J = 8.6 Hz, 1H, 22-H), 3.56 (septet, J = 4.6 Hz, 1H, 3α-H), 3.72 (d, J = 8.2 Hz, 1H, 23-H), 3.85 (d, J = 7.9 Hz, 1H, 7α-H), 5.29 (t, J = 2.1 Hz, 1H, 6-H). EIMS: *m/z* (%) = 462 (M^+, 14), 444 (88), 426 (9), 377 (2), 347 (17), 330 (100), 329 (58), 311 (42), 293 (14), 271 (18), 253 (18), 229 (10), 211 (8), 176 (29), 158 (33), 145 (22), 109 (22). HRMS: 462.3691 (M^+, calcd. 462.3710 for $C_{29}H_{50}O_4$), 444.3628 ($[M-H_2O]^+$, calcd. 444.3603 for $C_{29}H_{48}O_3$), 330.2538 (calcd. 330.2517 for $C_{22}H_{34}O_2$), 271.2033 (calcd. 271.2062 for $C_{19}H_{27}O$), 176.1204 (calcd. 176.1201 for $C_{12}H_{16}O$), 158.1063 (calcd. 158.1095 for $C_{12}H_{14}$). Positive ESIMS: *m/z* (%) = 485 ($[M+Na]^+$, 100).

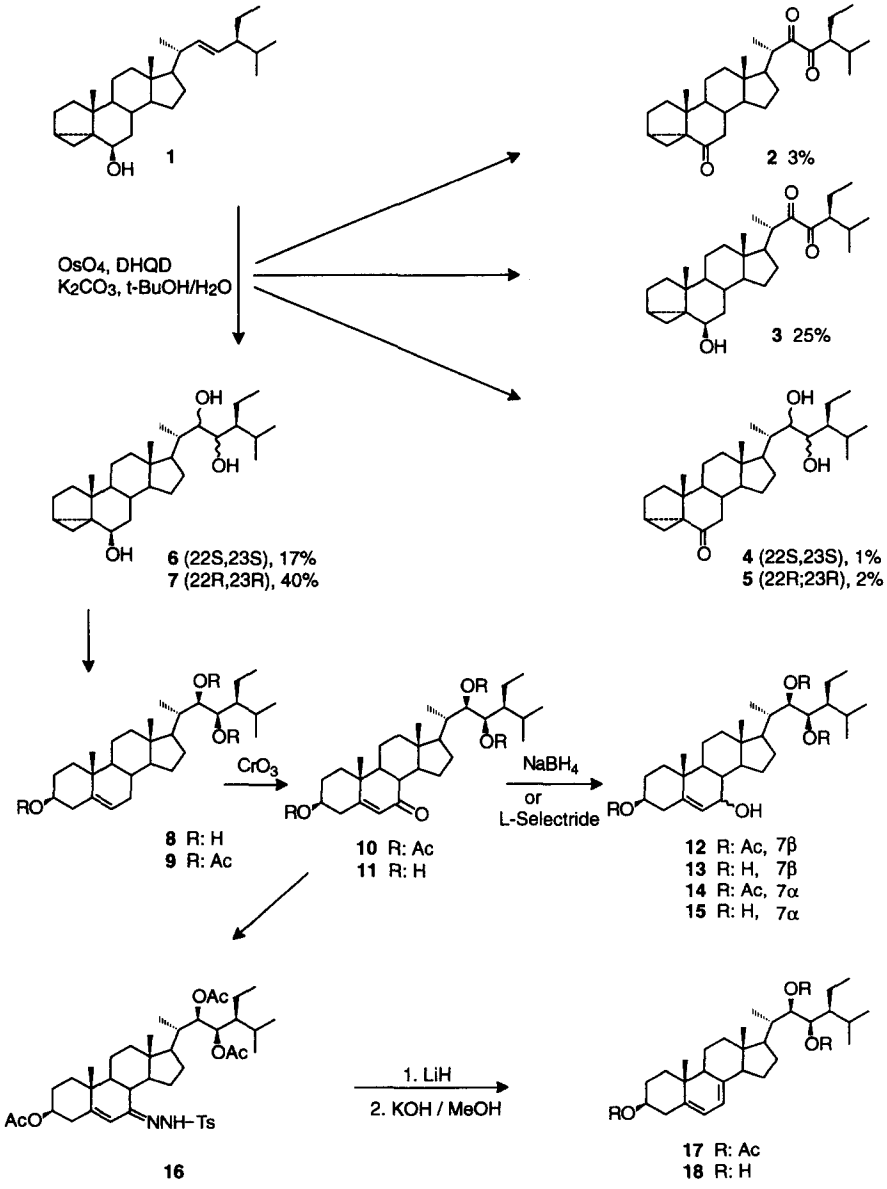
(22R,23R)-3β,22,23-Triacetoxy-stigmast-5-ene-7α-ol (14): Ketone **10** (160 mg, 0.273 mmol) in THF (6 mL) was stirred under argon at −78°C with a 1 M solution of L-Selectride in THF (1 mL). After 15 min (TLC-control), acetone (1 mL) was added. The solvent was evaporated, and the residue was treated with 5% HCl (5 mL) and ethyl acetate (10 mL). The organic phase was washed with water (3 × 5 mL), dried over (Na_2SO_4), and evaporated. Chromatography with n-hexane/ethyl acetate 80:20 (v/v) as the eluent gave the 7α-alcohol **14** with a yield of 70%, mp 77–80°C and $[\alpha]_D^{23}$ = −57.7° (c = 1.05). IR (KBr): ν = 3389 cm^{-1} (OH), 1745 (OAc). ^1H NMR: δ = 0.71 (s, 3H, 18-H_3), 0.84 (d, J = 6.7 Hz, 3H, 26*-H_3), 0.97 (d, J = 6.7 Hz, 3H, 27*-H_3), 0.98 (t, J = 7.6 Hz, 3H, 29-H_3), 1.01 (s, 3H, 19-H_3), 1.04 (d, J = 6.7 Hz, 3H, 21-H_3), 1.96, 2.01, 2.04 (3s, 9H, OAc), 4.65 (septet, J = 5.5 Hz, 1H, 3α-H), 3.84 (broad s, 1H, 7β-H), 5.19 (d, J = 9.2 Hz, 1H, 22-H), 5.31 (d, J = 9.2 Hz, 1H, 23-H), 5.62 (d, J = 5.2 Hz, 1H, 6-H). EIMS: *m/z* (%) = 546 (M^+, 6), 528 (100), 426 (9), 329 (6), 287 (20), 269 (17), 253 (8), 156 (15), 109 (16), 97 (31), 57 (28). Anal. calcd. for $C_{35}H_{56}O_7$ (588.73): C, 71.40; H, 9.59. Found: C, 71.33; H, 9.55.

(22R,23R)-3β,7α,22,23-Tetrahydroxy-stigmast-5-ene (15): Alkaline hydrolysis of **14** (150 mg, 0.255 mmol) and workup as described for **11** followed by crystallization from ethyl acetate/n-hexane yielded **15** (90 mg, 76%), mp 249–252°C and $[\alpha]_D^{23}$ = −46.0° (c = 0.50). IR (KBr): ν = 3384 cm^{-1} (OH). ^1H NMR (pyridine): δ = 0.91 (s, 3H, 18-H_3), 1.07 (s, 3H, 19-H_3), 1.08 (d, J = 7.0 Hz, 3H, 21-H_3), 1.11 (t, J = 7.6 Hz, 3H, 29-H_3), 1.16 (d, J = 6.9 Hz, 3H, 26*-H_3), 1.31 (d, J = 7.0 Hz, 3H, 27*-H_3), 3.81 (septet, J = 5.8 Hz, 1H, 3α-H), 4.05 (d, J = 8.4 Hz, 1H, 22-H), 4.11 (br s, 1H, 7β-H), 4.14 (d, J = 8.5 Hz, 1H, 23-H), 5.91 (d, J = 2.0 Hz, 1H, 6-H). EIMS: *m/z* (%) = 462 (M^+, 7), 444 (37), 426

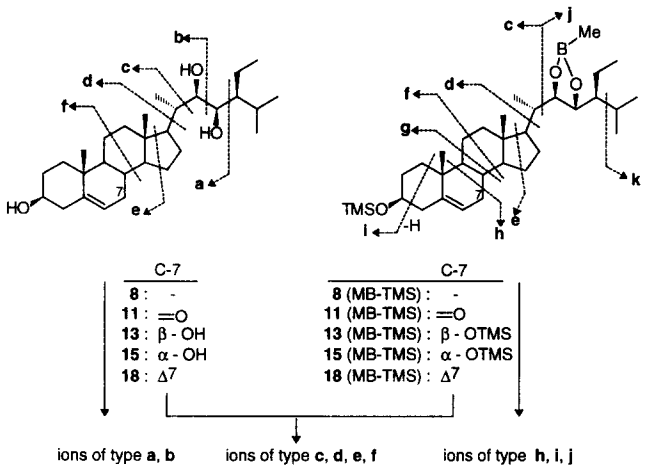
(5), 377 (2), 347 (8), 330 (100), 329 (56), 311 (39), 293 (20), 271 (20), 253 (17), 229 (9), 211 (8), 176 (36), 158 (32), 145 (22). HRMS: 444.3600 ([M-H_2O]$^+$, calcd. 444.3603 for $C_{29}H_{48}O_3$), 330.2573 (calcd. 330.2559 for $C_{22}H_{34}O_2$), 311.2340 (calcd. 311.2375 for $C_{22}H_{31}O$), 271.2052 (calcd. 271.2062 for $C_{19}H_{27}O$), 176.1200 (calcd. 176.1201 for $C_{12}H_{16}O$), 158.1065 (calcd. 158.1096 for $C_{12}H_{14}$). Positive ESIMS: *m/z* (%) = 485 ([M + Na]$^+$, 100). Anal. calcd. for $C_{29}H_{50}O_4 \times \frac{1}{2}H_2O$ (471.65): C, 73.80; H, 10.89. Found: C, 73.51; H, 10.59.

Hydrazone 16: Enone **10** (0.199 g, 0.339 mmol) was dissolved in dry THF (4 mL) under argon and light-exclusion, and toluene-4-sulfono-hydrazine (0.17 g) was subsequently added. The mixture was stirred with reflux for 18 h at rt (TLC-control). Flash chromatography in the dark yielded p-tosylhydrazone **16** (0.25 g, 97%), mp 122–124°C, $[\alpha]_D^{21} = -130.0°$ (c = 1.30) IR (nujol): ν = 1738 cm^{-1} (OAc), 1634/1598 (C = C). Ultraviolet: λ_{max} (lg ε) = 275 nm (1.340), 226 nm (2.278). ^{1}H NMR: δ = 0.68 (s, 3H, 18-H_3), 0.85 (d, *J* = 6.7 Hz, 3H, 26*-H_3), 0.97 (d, *J* = 6.7 Hz, 3H, 27*-H_3), 1.01 (t, *J* = 7.3 Hz, 3H, 29-H_3), 1.03 (d, *J* = 6.4 Hz, 3H, 21-H_3), 1.10 (s, 3H, 19-H_3), 1.99, 2.05, 2.06 (3s, 9H, OAc), 4.60 (septet, *J* = 5.5 Hz, 1H, 3α-H), 5.24 (d, *J* = 9.1 Hz, 1H, 22-H), 5.31 (d, *J* = 9.1 Hz, 1H, 23-H), 5.96 (s, 1H, 6-H), 7.33 (d, *J* = 8.2 Hz, 2H, p-Ts), 7.83 (d, *J* = 8.2 Hz, 2H, p-Ts). EIMS: *m/z* (%) = 755 (M$^+$, 1), 510 (100), 375 (17), 253 (25), 158 (33), 145 (15). Anal. calcd. for $C_{42}H_{62}O_8N_2S$ (754.91): C, 66.82; H, 8.28; N, 3.71; S, 4.25. Found: C, 66.96; H, 8.26; N, 3.73; S, 4.26.

(22R,23R)-3β,22,23-Triacetoxy-stigmast-5,7-diene (17): In a 25-mL three-necked flask, **16** (0.10 g), was dissolved in dry toluene (1.5 mL) distilled from LiH. The mixture was treated with LiH (0.10 g) in the dark, and the rt was increased to 100°C. After 60 min, the solution was cooled to 0°C and slowly poured into ice water. The organic phase was separated, washed, dried over Na_2SO_4, and evaporated. Flash chromatography followed by elution with n-hexane/ethyl acetate 80:20 (v/v) gave 45 mg (60%) of amorphous **17**, $[\alpha]_D^{28} = -15.3°$ (c = 0.81). IR (nujol): ν = 1748 cm^{-1} (OAc). Ultraviolet: λ_{max} (lg ε) = 293 nm (0.429), 282 nm



Scheme 1



Scheme 2 Mass spectral fragmentation of compounds **8, 11, 13, 15, 18,** and their methylboronate-trimethylsilyl (MB-TMS) derivatives.

(0.759), 270 nm (0.727), 260 nm (0.55). ^{1}H NMR: δ = 0.64 (s, 3H, 18-H_3), 0.84 (d, *J* = 3.4 Hz, 3H, 26*-H_3), 0.85 (d, *J* = 3.4 Hz, 3H, 27-H_3), 0.95 (s, 3H, 19-H_3), 0.97 (t, *J* = 6.7 Hz, 3H, 29-H_3), 0.98 (d, *J* = 7.6 Hz, 3H, 21-H_3), 1.97, 2.03, 2.04 (3s, 9H, OAc), 4.70 (septet, *J* = 4.6 Hz, 1H, 3α-H), 5.20 (d, *J* = 8.2 Hz, 1H, 22-H), 5.31 (d, *J* = 8.8 Hz, 1H, 23-H), 5.39 (t, *J* = 2.7 Hz, 1H, 6*-H), 5.57 (dd, *J* = 5.6 and 2.1 Hz, 1H, 7*-H). EIMS: *m/z* (%) = 570 (M^+, 1), 510 (100), 495 (9), 450 (4), 375 (9), 253 (13), 211 (6), 158 (21), 145 (9). Anal. calcd. for $C_{35}H_{54}O_6$ (570.68): C, 73.66; H, 9.54. Found: C, 73.64; H, 9.64.

(22R,23R)-3β,22,23-Trihydroxy-stigmast-5,7-diene (18). Hydrolysis of **17** with 5% KOH/MeOH gave 87 mg (74%) of **18**, mp 169–172°C and $[\alpha]_D^{20}$ = −32.7° (c = 0.53). IR (nujol): ν = 3352 cm^{-1} (OH). Ultraviolet: λ_{max} (lg ε) = 293 nm (1.043), 282 nm (1.801), 271 nm (1.727), 260 nm (1.20). ^{1}H NMR: δ = 0.64 (s, 3H, 18-H_3), 0.94 (d, *J* = 6.7 Hz, 3H, 21-H_3), 0.95 (s, 3H, 19-H_3), 0.96 (t, *J* = 7.3 Hz, 3H, 29-H_3), 0.97 (d, *J* = 4.0 Hz, 3H, 26*-H_3), 0.98 (d, *J* = 6.7 Hz, 3H, 27*-H_3), 3.67–3.62 (m, 2H, 22-H, 3α-H), 3.73 (d, *J* = 6.7 Hz, 1H, 23-H), 5.41–5.39 (m, 1H, 6*-H), 5.59–5.57 (m, 1H, 7*-H). EIMS: *m/z* (%) = 444 (M^+, 100), 426 (6), 411 (87), 385 (10), 329 (4), 311 (10), 293 (8), 271 (17), 253 (6), 237 (6), 211 (12), 199 (8), 159 (10), 143 (13), 109 (6). HRMS: 444.3618 (M^+, calcd. 444.3603 for $C_{29}H_{48}O_3$), 411.3252 (calcd. 411.3263 for $C_{28}H_{43}O_2$), 271.2067 (calcd. 271.2062 for $C_{19}H_{27}O$), 253.1966 (calcd. 253.1956 for $C_{19}H_{25}$).

Results and Discussion

Isostigmasterol (**1**), which is readily available via its tosylate from stigmasterol, was used as the starting material for the preparation of the desired brassinosteroid analogs. For the asymmetric dihydroxylation[6] of **1** (Scheme 1), we use the chiral ligand dihydroquinidine p-chlorobenzoate (DHQD) to afford, as found also for its 6-methoxy derivative,[7] the preferred (22R,23R)-diol configuration, which is essential for a high bioactivity.[8] The expected (22S,23S)- and (22R,23R)-triols **6** and **7** were isolated by flash chromatography on silica gel in 17 and 40% yield, respectively. The configurations of both epimers at C-22 and C-23 are confirmed also by the chemical shifts of the corresponding protons, which are in agreement with published NMR data.[9] The synthesis of (22S,23S)-diol **6** has been reported earlier and involves a mixture of phytosterols from rice bran oil[10] as starting reactants.

As further products of the asymmetric dihydroxylation of **1**, we surprisingly obtained the yellow 6,22,23-triketone **2** and the 22,23-diketo derivative **3** in 3 and 25% yield,

Table 1 70 eV EIMS of the brassinosteroid analogs **8, 11, 13, 15,** and **18**

Ion	8	11	13	15	18
M^+	446 (1)	460 (0.7)	462 (14)	462 (7)	444 (100)
$[M-H_2O]^+$	428 (3)	442 (1)	444 (88)	444 (37)	426 (6)
$[M-H_2O-Me]^+$		—	—	—	411 (87)
$[M-2H_2O]^+$	410 (1)	424 (1)	426 (9)	426 (5)	408 (2)
a	361 (4)	375 (3)	377 (2)	377 (2)	359 (1)
(**b**+H)	332 (100)	346 (100)	NS	NS	330 (3)
b	331 (44)	345 (53)	347 (16)	347 (8)	329 (4)
(**b**+H-H_2O)	314 (46)	328 (17)	330 (100)	330 (100)	312 (8)
(**b**-H_2O)	313 (68)	327 (36)	329 (58)	329 (56)	311 (10)
(**b**-2H_2O)	295 (35)	309 (7)	311 (42)	311 (39)	293 (8)
(**b**-3H_2O)	—	NS	293 (14)	293 (20)	—
c	302 (6)[a]	NS	NS	NS	299 (4)
(**c**-H)	—	316 (25)	—	—	—
(**c**-H_2O)	284 (5)[b]	NS	—	—	281 (4)
(**c**-H-H_2O)	—	298 (5)	300 (10)	300 (10)	—
d	273 (16)	287 (13)	NS	NS	271 (17)
(**d**-H_2O)	255 (36)	269 (5)	271 (18)	271 (20)	253 (11)
(**d**-2H_2O)	—	—	253 (19)	253 (17)	
e	231 (7)	245 (NS)	247 (NS)	247 (NS)	229 (NS)
(**e**-H_2O)	213 (23)	227 (NS)	229 (10)	229 (9)	211 (12)
(**e**-2H_2O)		—	211 (13)	211 (12)	
f	192 (28)	—	—	—	
(**f**-H_2O)	159 (23)[c]	174 (7)	176 (29)	176 (36)	159 (10)[c]
(**f**-2H_2O)		—	158 (33)	158 (32)	—

NS = not significant; [a] (**c**+H); [b] (**c**+H-H_2O); [c] (**f**+H-H_2O).

Table 2 GC-MS data of the brassinosteroid analogs **8, 11, 13, 15,** and **18**

Compound	RR_t[a]	Key ions [*m/z* (rel. int.)]
8	1.50	542 (M^+,14), 452 ([M-TMSOH]$^+$,27), 437 (8), 413 ([M-129]$^+$,14), 353 (4), 283 (4), 255 ([**d**-TMSOH], 12), 243 (16), 217 (10), 195 (11), 173 (12), 169 (**j**,13), 161 (18), 145 (17), 129 (**i**, 100), 85 (**k**,36), 73 (39)
11[b]	1.92	556 (M^+, 100), 539 (9), 466 ([M-TMSOH]$^+$, 30), 451 (42), 387 (10), 359 (5), 297 (7), 269 (9), 264 (**f**, 18), 187 (18), 174 [(**f**-TMSOH), 14], 161 (27), 129 (**i**, 43), 85 (**k**, 34), 73 (55)
13[c]	1.57	630 (M^+, 2), 540 ([M-TMSOH]$^+$, 100), 451(1), 247 [(**f**-H-TMSOH), 2], 233 [(**g**-H-TMSOH), 5], 208 [(**h**-TMSOH), 3], 169 (**j**, 4), 157 (4), 129 (**i**, 9), 85 (**k**, 22), 73 (36)
15[c]	1.44	630 (M^+, 3), 540 ([M-TMSOH]$^+$, 100), 451 (2), 247 [(**f**-H-TMSOH), 3], 233 [(**g**-H-TMSOH), 5], 208 [(**h**-TMSOH), 5], 169 (**j**, 6), 157 (7), 129 (**i**, 15), 85 (**k**, 22), 73 (36)
18[b]	1.54	540 (M^+, 20), 450 ([M-TMSOH]$^+$, 30), 435 (100), 409 (66), 253 [(**d**-TMSOH), 15], 237 (10), 211 [(**e**-TMSOH), 21], 169 (**j**, 23), 144 [(**g**-TMSOH), 22], 143 (24), 131 (28), 129 (**i**, 17), 85 (**k**, 39), 73 (43)

[a] Relative retention times with respect to 5α-cholestane (Rt = 5.47 min).
[b] as methylboronate-trimethylsilylether.
[c] as methylboronate-bistrimethylsilylether.

respectively. Traces of the 6-keto-22,23-diols **4** and **5** were isolated as minor products. Whereas in the NMR spectra of the 6β-hydroxy-3α,5-cyclo compounds **3, 6,** and **7**, the expected signal for the 3-proton appears as double doublet at 0.29, the corresponding 6-ketones **2, 4,** and **5** lack such a signal, which must be attributable to a long-range deshielding by the carbonyl function, thereby shifting the 3-proton signal into the methyl/methylene region of the NMR spectrum.

(22R,23R)-22,23-dihydroxyisostigmasterol (**7**) was isomerized with aqueous H_2SO_4 to give the (22R,23R)-Δ^5-triol **8**. Subsequent acetylation followed by allylic oxidation with chromic acid in dichloromethane led to the enone triacetate **10**, which was hydrolyzed to the enone triol **11**. Stereospecific reduction of the carbonyl function of **10** was achieved with sodium borohydride in the presence of cerium trichloride in tetrahydrofuran/methanol and led to the 7β-hydroxylated triacetate **12** in 93% yield. Alkaline hydrolysis of **12** with 5% KOH/MeOH gave tetrol **13** in 80% yield. The 7α-epimer of **12** was prepared by reduction of **10** with *L-Selectride* in tetrahydrofuran at −78°C[11] to give the corresponding allylic alcohol **14**, and upon alkaline hydrolysis, tetrol **15** was produced in 76% yield. The stereochemistry of the 7-epimeric compounds **13** and **15** follows from their ^{1}H NMR spectra[11] with a 7α-H doublet at 3.85 ppm in **13** and a 7β-H singlet at 4.11 ppm for **15**.

For the synthesis of the corresponding $\Delta^{5,7}$-unsaturated brassinosteroid analog **17**, the Δ^5-7-keto derivative **10** was reacted with toluene-4-sulfonohydrazine in dry tetrahydrofuran under anarobic conditions at 75°C to give the corresponding tosylhydrazone **16**. Reductive elimination of the 7-hydrazone function of **16** with lithium hydride in toluene at 100°C[12] yielded (22R,23R)-3β,22,23-triacetoxy-stigmastan-5,7-diene (**17**), which was hydrolyzed to give (22R,23R)-3β,22,23-trihydroxy-stigmast-5,7-diene (**18**) in 74% yield.

The structures of compounds **8–18** are in agreement with their spectroscopic data. Specifically, the EI mass spectral fragmentation behavior of the brassinosteroid analogs **8, 11, 13, 15,** and **18** is characterized by α-cleavages in the side chain (ions of type **a–d**) and by splitting of ring D leading to the key ion type **e** (Scheme 2, Table 1). The ions of type **a,b**, and **c** represent significant key ions in the EI mass spectra of free brassinosteroids.[13] While ions **d** and **e** also appear in the mass spectra of sterols, the ion of type **f**, which arose by splitting of ring C, is typical for both 3-hydroxy-$\Delta^{5,7}$ as well as for 3-hydroxy-7-oxo-Δ^5-steroids.[14,15]

The EI mass spectra of the derivatized brassinosteroids (methylboronate/trimethylsilylether, MB-TMS) show key ions at *m/z* 169 (**j**) and 85 (**k**), characterizing the stigmastane side chain with vicinal hydroxy groups at C-22 and C-23.[13] Comparable ions in the nonderivatized compounds are not significant. Ions of type **g** and **h** only appear in the mass spectra of the 3,7-dihydroxy compounds **13** and **15** (Scheme 2). The ion of type **i** also represents a key ion in the mass spectra of other silylated Δ^5-3β-hydroxysteroids.[16] While the EI mass spectra of the methylboronate/bistrimethylsilylether derivatives of the two C-7-stereoisomers **13** and **15** do not show any difference, these two compounds can be distinguished by their retention data in the GC (Table 2).

For the determination of the phytohormone activity of the brassinosteroid analogs **8, 11, 13, 15,** and **18**, the highly sensitive and specific rice lamina inclination test[5] was used. The results showed that the 7β-hydroxylated alcohol **13**, at a concentration of 0.1 ppm, has 91% phytohormone activity, and the 7α-alcohol **15** has 82% activity with respect to 24-epibrassinolide as the standard (100%). The triol **8**, the enone **11**, and the diene **18** showed distinctly lower activities of 57, 35, and 60%, respectively, indicating the importance of a 7-hydroxy function for high bioactivity in this series.

Acknowledgments

We gratefully acknowledge the financial support of this work by the Deutsche Forschungsgemeinschaft. We are also indebted to Dr. A. Porzel for the NMR measurements.

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