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Synthesis of Deuterio-Labelled Brassinosteroids, [26,28-$^2H_6$]Brassinolide, [26,28-$^2H_6$]Castasterone, [26,28-$^2H_6$]Typhasterol, and [26,28-$^2H_6$]Teasterone

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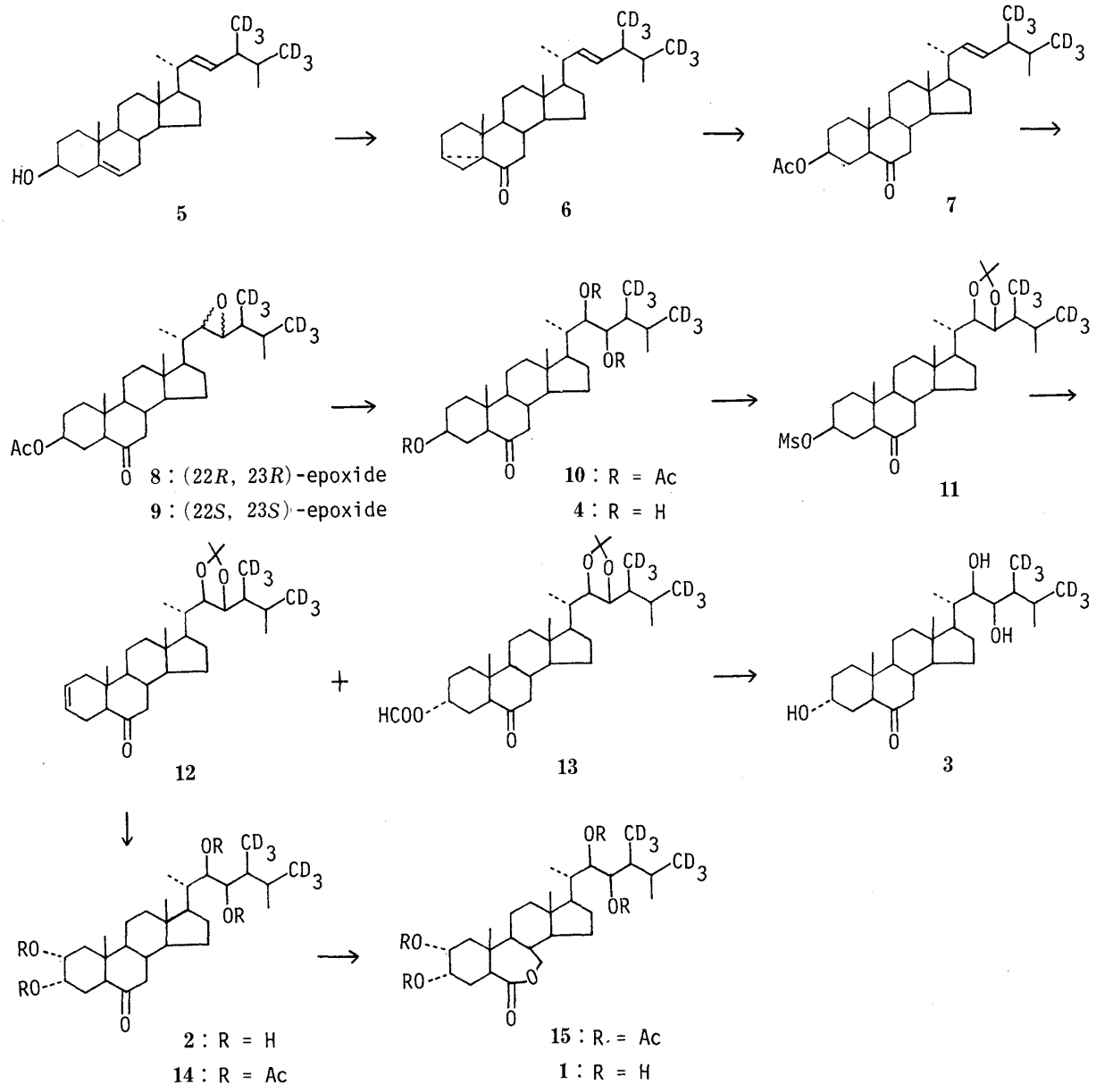
Several deuterio-labelled brassinosteroids, [26,28-$^2H_6$]brassinolide (**1**), [26,28-$^2H_6$]castasterone (**2**), [26,28-$^2H_6$]typhasterol (**3**), and [26,28-$^2H_6$]teasterone (**4**), were synthesized from [26,28-$^2H_6$]-crinosterol (**5**).

Keywords——brassinolide; brassinosteroid; deuterio-labelled brassinosteroid; plant growth promoter; [26,28-$^2H_6$]brassinolide; [26,28-$^2H_6$]castasterone; [26,28-$^2H_6$]typhasterol; [26,28-$^2H_6$]-teasterone

Brassinolide and related C-28 steroids, castasterone, typhasterol, and teasterone, have been found to occur in a wide variety of higher plants as a new class of plant growth promoter.[1] These and other related compounds (brassinosteroids) should play an important role in plant growth and development. We have developed a microanalytical method for quantitating these brassinosteroids as bismethaneboronate or methaneboronate-trimethylsilyl derivatives by gas chromatography-mass spectrometry (GC-MS).[2] We have now synthesized [26,28-$^2H_6$]brassinolide (**1**), [26,28-$^2H_6$]castasterone (**2**), [26,28-$^2H_6$]typhasterol (**3**), and [26,28-$^2H_6$]teasterone (**4**) as deuterio-labelled internal standards, as already reported in a preliminary form.[3] In this paper we describe the details of the synthesis of these four deuterio-labelled brassinosteroids from [26,28-$^2H_6$]crinosterol (**5**).[4]

Solvolysis of the methanesulfonate of [26,28-$^2H_6$]crinosterol (**5**) in aqueous acetone with potassium bicarbonate under reflux gave the corresponding 3,5-cycloalcohol, which, when oxidized with Jones reagent, provided the cyclopropyl ketone **6** in 85% yield. Acid rearrangement of the ketone **6** by refluxing in acetic acid–5 M sulfuric acid followed by acetylation of the resulting product gave the 3β-acetoxy-5α-ergost-22-en-6-one **7** in 93% yield. Epoxidation of **7** in dichloromethane with one equivalent of *m*-chloroperbenzoic acid afforded, after chromatographic separation, the less polar (22*R*,23*R*)-epoxide **8** [64%, δ ($CDCl_3$) 2.49 (1H, dd, J=6.1 and 2.1 Hz) and 2.71 (1H, dd, J=6.1 and 2.1 Hz)] and the more polar (22*S*,23*S*)-epoxide **9** [31%, δ ($CDCl_3$) 2.42—2.51 (2H, m)], whose stereochemistry was assigned by comparison of the proton nuclear magnetic resonance (^{1}H-NMR) signals of the epoxidic protons with those of the known (22*R*,23*R*)- and (22*S*,23*S*)-3β-acetoxy-22,23-epoxy-5α-stigmastan-6-one.[5] The less polar (22*R*,23*R*)-epoxide **8** was submitted to the reported procedure[5,6] for introduction of a (22*R*,23*R*)-vicinal diol functionality as follows. Treatment of **8** with 30% hydrobromic acid in acetic acid at room temperature gave the corresponding mixture of bromoacetates, which was heated with 80% aqueous acetic acid at 100 °C for 24 h. Acetylation of the crude products followed by chromatography on silica gel provided the triacetate **10** in 66% yield. Saponification of **10** with 5% KOH/MeOH under reflux afforded [26,28-$^2H_6$]teasterone (**4**), quantitatively, which exhibited the coupling constant $J_{C\text{-}22,23}$=



Chart

8.6 Hz (doublet) in its ^{1}H-NMR spectrum. This is diagnostic for the (22*R*,23*R*)-stereochemistry.[6,7]

[26,28-$^2H_6$]Teasterone (**4**) was submitted to acetonide formation with acetone containing a catalytic amount of *p*-toluenesulfonic acid and then methanesulfonation with methanesulfonyl chloride in pyridine. The sulfonate **11** thus obtained was heated with lithium carbonate and dimethylformamide[7] at reflux temperature. The 2-ene **12** and the 3α-formate **13** were obtained in 50 and 25% yields, respectively, from **4**. Refluxing of **13** with 80% aqueous acetic acid followed by saponification with 5% KOH/MeOH gave [26,28-$^2H_6$]typhasterol (**3**) in 90% yield. Stereospecific α-face hydroxylation of the 2-ene **12** was carried out with a catalytic amount of osmium tetroxide and an excess of *N*-methylmorpholine *N*-oxide in aqueous tetrahydrofuran. The resulting 2α,3α-diol was refluxed with 80% aqueous acetic acid and then purified by column chromatography to provide [26,28-$^2H_6$]castasterone (**2**) in 87% yield. The tetraacetoxy-6-oxo steroid **14** derived from **2** by acetylation was submitted to Baeyer–Villiger oxidation[8] in dichloromethane with trifluoroperacetic acid in the presence of disodium

hydrogen phosphate at 0 °C to give the 7-oxalactone **15** in 83% yield after chromatographic purification. Saponification of **15** with 5% KOH/MeOH under reflux followed by re-lactonization with 6 M HCl afforded [26,28-$^2H_6$]brassinolide (**1**) in 93% yield.

The deuterium contents of these labelled brassinosteroids **1**, **2**, **3**, and **4** were found to be more than 99% by mass spectrometry. Quantitative analysis of brassinosteroids in higher plants will be the next stage of our research.

Experimental

Melting points were determined on a Yazawa BY-1 hot-stage microscope and are uncorrected. ^{1}H-NMR spectra were recorded on a Hitachi R-24B (60 MHz) or JEOL FX 200 (200 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were taken with a Shimadzu GC-MS 9020 gas chromatograph-mass spectrometer or a JEOL DX 303 gas chromatograph-mass spectrometer. Electron impact mass spectra (EI-MS) were obtained at 70 eV and fast atom bombardment mass spectra (FAB-MS) at 6 kV (Xe beam). For GC-MS analysis, a glass column packed with 1.5% OV-1 (0.26 mm i.d. × 1.0 m) was used at 270 °C and the carrier gas (He) flow-rate was 40 ml/min. Column chromatography was carried out with Kieselgel 60 F_{254} (70—230 mesh, E. Merck) and analytical thin layer chromatography (TLC) was done on precoated silica gel (Kieselgel 60 F_{254}, 0.25 mm thickness, E. Merck). Work-up refers to dilution with water, extraction with the organic solvent indicated in parenthesis, washing of the extract to neutrality, drying over anhydrous magnesium sulfate, filtration, and removal of the solvent under reduced pressure.

[26,28-$^2H_6$]-(22*E*,24*S*)-3α,5-Cyclo-5α-ergost-22-en-6-one (6)——The 3β-ol **5** (3.85 g, 9.53 mmol) in pyridine (25 ml) was treated with methanesulfonyl chloride (2.0 ml) at room temperature for 1 h. Work-up (ethyl acetate) gave the corresponding mesylate, which was refluxed with a mixture of acetone (400 ml) and water (80 ml) in the presence of potassium bicarbonate (7.0 g) for 16 h. Removal of acetone by distillation gave an oily substance. Work-up (ether) and chromatography on silica gel (3.5 cm i.d. × 30 cm) with benzene gave [26,28-$^2H_6$]-(22*E*,24*S*)-3α,5-cyclo-5α-ergost-22-en-6β-ol (3.27 g). This product was dissolved in acetone (100 ml) and the solution was treated with Jones reagent (1.2 eq) at 0 °C for 15 min. Work-up (ether) gave the cyclopropyl ketone **6** (3.25 g, 85% from **5**), mp 105—106.5 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.729 (3H, s, 18-H_3), 0.814 (3H, d, J=6.8 Hz, 27-H_3), 1.006 (3H, s, 19-H_3), 1.013 (3H, d, J=6.4 Hz, 21-H_3), 5.16 (2H, m, 22-H and 23-H). EI-MS m/z: 402 (M^+, 36%), 387 (8), 356 (14), 312 (8), 298 (33), 283 (12), 271 (58), 269 (32), 257 (13), 253 (10), 245 (24), 243 (22), 229 (38), 215 (11), 175 (30), 161 (42), 149 (38), 147 (38), 137 (52), 135 (78), 123 (92), 121 (100).

[26,28-$^2H_6$]-(22*E*,24*S*)-3β-Acetoxy-5α-ergost-22-en-6-one (7)——The cyclopropyl ketone **6** (3.14 g, 7.81 mmol) was treated with 5 M sulfuric acid (5 ml) and acetic acid (25 ml) under reflux for 1.5 h. The reaction mixture was cooled and diluted with crushed ice and water. The resulting precipitate was collected, washed with water and then dried in a desiccator (P_2O_5). The dried material was acetylated with acetic anhydride (10 ml) and pyridine (15 ml) at room temperature overnight. Work-up (ethyl acetate) gave a crude product, which was applied to a column of silica gel (3.5 cm i.d. × 30 cm). Elution with benzene–ethyl acetate (50 : 1) provided the 3β-acetate **7** (3.35 g, 93%), mp 140—141 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.674 (3H, s, 18-H_3), 0.770 (3H, s, 19-H_3), 0.808 (3H, d, J=6.6 Hz, 27-H_3), 1.007 (3H, d, J=6.6 Hz, 21-H_3), 2.029 (3H, s, acetyl), 4.67 (1H, m, 3-H), 5.16 (2H, m, 22-H and 23-H). EI-MS m/z: 462 (M^+, 5%), 402 (2), 387 (1), 372 (3), 358 (11), 343 (5), 329 (15), 316 (5), 303 (4), 271 (17), 253 (10), 177 (18), 149 (100).

[26,28-$^2H_6$]-(22*R*,23*S*,24*S*)- and (22*S*,23*S*,24*S*)-3β-Acetoxy-22,23-epoxy-5α-ergostan-6-one (8 and 9)——The 22-ene **7** (3.17 g, 6.86 mmol) in dichloromethane (35 ml) was treated with *m*-chloroperbenzoic acid (1.19 g, 6.88 mmol) at room temperature in the dark overnight. Powdered calcium hydroxide (3.0 g) was added to the reaction mixture and the whole was stirred at room temperature for 1 h. Filtration and removal of the solvent gave the two products, which were separated by chromatography on silica gel (3.5 cm i.d. × 34 cm) with benzene–ethyl acetate (30 : 1) to provide the less polar (22*R*,23*R*)-epoxide **8** (2.11 g, 64%) as an oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.658 (3H, s, 18-H_3), 0.769 (3H, s, 19-H_3), 0.930 (3H, d, J=6.6 Hz, 27-H_3), 1.052 (3H, d, J=5.6 Hz, 21-H_3), 2.030 (3H, s, acetyl), 2.49 (1H, dd, J=6.1, 2.1 Hz, 22-H), 2.71 (1H, dd, J=6.1, 2.1 Hz, 23-H), 4.67 (1H, m, 3-H).

Further elution with benzene–ethyl acetate (25 : 1) gave the more polar (22*S*,23*S*)-epoxide **9** (1.02 g, 31%), mp 156—158 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.656 (3H, s, 18-H_3), 0.769 (3H, s, 19-H_3), 0.964 (3H, d, J=6.8 Hz, 27-H_3), 0.984 (3H, d, J=6.1 Hz, 21-H_3), 2.031 (3H, s, acetyl), 2.42—2.51 (2H, m, 22-H and 23-H), 4.67 (1H, m, 3-H).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-3β,22,23-Triacetoxy-5α-ergostan-6-one (10)——The (22*R*,23*R*)-epoxide **8** (2.0 g, 4.18 mmol) was treated with 30% hydrogen bromide in acetic acid (5 ml) at room temperature for 3 h. The reaction mixture was diluted with water, neutralized with sodium hydrogen carbonate (powder), and extracted with ether. Removal of ether gave the corresponding bromoacetates, which was then treated with acetic acid (60 ml) and water (15 ml) at 100 °C for 24 h. The reaction mixture was poured into chilled aqueous sodium hydrogen carbonate. Work-

up (ethyl acetate) gave a crude product, which was acetylated with acetic anhydride (7.0 ml) and pyridine (10 ml) at 70 °C overnight. Work-up (ethyl acetate) and chromatography on silica gel (2.5 cm i.d. × 30 cm) with benzene–ethyl acetate (10 : 1) provided the (22*R*,23*R*)-triacetate **10** (1.60 g, 66%), mp 218—220 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.693 (3H, s, 18-H_3), 0.770 (3H, s, 19-H_3), 0.938 (3H, d, J=6.3 Hz, 27-H_3), 1.016 (3H, d, J=6.8 Hz, 21-H_3), 1.994 (3H, s, acetyl), 2.022 (3H, s, acetyl), 2.028 (3H, s, acetyl), 4.67 (1H, m, 3-H), 5.16 (1H, d, J=7.8 Hz, 22-H), 5.32 (1H, d, J=7.8 Hz, 23-H). EI-MS m/z: 520 ($M^+ - 60$, 4%), 505 (1), 460 (14), 443 (4), 432 (5), 414 (4), 400 (6), 388 (7), 383 (8), 371 (37), 358 (7), 329 (100), 311 (42), 299 (22), 293 (14), 283 (8), 271 (46), 253 (14), 243 (10), 229 (18), 149 (70).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-3β,22,23-Trihydroxy-5α-ergostan-6-one (4)——The triacetate **10** (1.51 g, 2.60 mmol) was treated with 5% KOH/MeOH (80 ml) under reflux for 45 min. Work-up (ethyl acetate) gave [26,28-$^2H_6$]teasterone (**4**) (1.18 g, 99%), mp 202—204 °C (MeOH–EtOAc). ^{1}H-NMR (200 MHz, C_5D_5N) δ: 0.751 (3H, s, 18-H_3), 0.785 (3H, s, 19-H_3), 1.113 (3H, d, J=6.8 Hz, 27-H_3), 1.271 (3H, d, J=6.4 Hz, 21-H_3), 3.85 (1H, m, 3-H), 3.99 (1H, d, J=8.6 Hz, 22-H), 4.16 (1H, d, J=8.6 Hz, 23-H). FAB-MS m/z: 455 ($M^+ + 1$). EI-MS m/z: 454 (M^+, 0.3%), 436 (0.6), 420 (1), 403 (0.4), 377 (2), 368 (1), 359 (1), 348 (100), 329 (35), 318 (18), 311 (15), 300 (5), 289 (10), 287 (10), 271 (13), 262 (10), 247 (16), 229 (6), 201 (4), 189 (8), 175 (12), 161 (11), 149 (20), 107 (25), 95 (37), 83 (28). EI-MS (as the methaneboronate-TMS derivative) m/z: 550 (M^+, 43%), 535 (83), 521 (100), 460 (12), 445 (5), 407 (2), 360 (2), 300 (6), 271 (3), 211 (6), 161 (27), 143 (15), 121 (13), 107 (14), 95 (25), 85 (34), 75 (31), 73 (35).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-22,23-Isopropylidenedioxy-5α-ergost-2-en-6-one (12) and [26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-3α-Formyloxy-22,23-isopropylidenedioxy-5α-ergostan-6-one (13)——The triol **4** (1.07 g, 2.36 mmol) in acetone (100 ml) was treated with *p*-toluenesulfonic acid (50 mg) at room temperature for 2 h. Work-up (ether) gave the acetonide, which was then treated with methanesulfonyl chloride (2 ml) and pyridine (10 ml) at room temperature for 1 h. Work-up (ethyl acetate) gave the sulfonate **11** (1.35 g). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 0.66 (3H, s, 18-H_3), 0.78 (3H, s, 19-H_3), 1.35 and 1.36 (6H, s × 2, acetonide), 3.00 (3H, s, mesyl), 3.70 (1H, dd, J=8, 4 Hz, 22-H), 3.94 (1H, d, J=8 Hz, 23-H), 4.60 (1H, m, 3-H). The sulfonate **11** (1.35 g) was refluxed with dimethylformamide (20 ml) and lithium carbonate (1.5 g) for 1 h. Work-up (ethyl acetate) and chromatography on silica gel (2.5 cm i.d. × 30 cm) with benzene–ethyl acetate (15 : 1) provided the 2-ene **12** (560 mg, 50% from **4**), mp 233—235 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.679 (3H, s, 18-H_3), 0.714 (3H, s, 19-H_3), 0.937 (3H, d, J=6.6 Hz, 27-H_3), 0.989 (3H, d, J=6.4 Hz, 21-H_3), 1.345 and 1.371 (6H, s × 2, acetonide), 3.73 (1H, dd, J=8.3, 4.3 Hz, 22-H), 3.83 (1H, d, J=8.3 Hz, 23-H), 5.50—5.74 (2H, m, 2-H and 3-H). EI-MS m/z: 461 ($M^+ - 15$, 4%), 399 (2), 255 (4), 312 (2), 296 (7), 284 (2), 271 (5), 269 (5), 177 (50), 148 (66), 102 (100).

Further elution with the same solvent gave the 3α-formate **13** (290 mg, 25% from **4**), mp 172—173 °C (MeOH). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 0.68 (3H, s, 18-H_3), 1.35 and 1.36 (6H, s × 2, acetonide), 3.70 (1H, dd, J=8, 4 Hz, 22-H), 3.94 (1H, d, J=8 Hz, 23-H), 5.23 (1H, m, $W_{1/2}$=7 Hz, 3-H), 8.00 (1H, s, formyl).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)3α,22,23-Trihydroxy-5α-ergostan-6-one (3)——The 3α-formate **13** (150 mg, 0.304 mmol) was treated with 80% aqueous acetic acid (20 ml) under reflux for 4 h. Removal of the solvent under reduced pressure gave the residue, which was treated with 5% KOH/MeOH (10 ml) at room temperature for 2 h. Work-up (ethyl acetate) and chromatography on silica gel (1.5 cm i.d. × 20 cm) with benzene–ethyl acetate (1 : 2) gave [26,28-$^2H_6$]typhasterol (**3**) (124 mg, 90%), mp 191—193 °C (EtOAc). ^{1}H-NMR (200 MHz, C_5D_5N) δ: 0.758 (3H, s, 18-H_3), 0.802 (3H, s, 19-H_3), 1.112 (3H, d, J=6.3 Hz, 27-H_3), 1.265 (3H, d, J=6.8 Hz, 21-H_3), 3.15 (1H, dd, J=12.2, 2.4 Hz, 5α-H), 3.99 (1H, d, J=8.6 Hz, 22-H), 4.15 (1H, d, J=8.6 Hz, 23-H), 4.37 (1H, m, 3β-H). FAB-MS m/z: 455 ($M^+ - 1$). EI-MS m/z: 454 (M^+, 0.3%), 436 (1), 420 (1), 403 (0.7), 377 (2), 368 (1), 359 (2), 348 (100), 329 (64), 318 (15), 311 (22), 300 (10), 289 (8), 287 (8), 271 (16), 262 (10), 247 (15), 229 (15), 215 (6), 201 (5), 189 (6), 175 (16), 161 (18), 149 (14), 123 (18), 107 (24), 95 (36), 81 (29). EI-MS (as the methaneboronate-TMS derivative) m/z: 550 (M^+, 86%), 535 (100), 532 (26), 521 (66), 460 (63), 445 (22), 421 (5), 383 (4), 319 (7), 300 (12), 271 (9), 229 (23), 211 (9), 161 (42), 121 (26), 95 (36), 85 (48), 75 (36), 73 (40).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (2)——The 2-ene **12** (555 mg, 1.17 mmol) in tetrahydrofuran (10 ml) and water (0.5 ml) was treated with osmium tetroxide (20 mg) and *N*-methylmorpholine *N*-oxide (500 mg, 4.27 mmol) at room temperature overnight. Work-up (dichloromethane) gave a crude product, which was then refluxed with 80% aqueous acetic acid (50 ml) for 3 h. Removal of the solvent gave the residue, which was applied to a column of silica gel (2.5 cm i.d. × 20 cm). Elution with benzene–ethyl acetate (1 : 4) gave [26,28-$^2H_6$]castasterone (**2**) (476 mg, 87%), mp 252—253 °C (EtOAc). ^{1}H-NMR (200 MHz, C_5D_5N) δ: 0.738 (3H, s, 18-H_3), 0.851 (3H, s, 19-H_3), 1.109 (3H, d, J=6.3 Hz, 27-H_3), 1.249 (3H, d, J=6.7 Hz, 21-H_3), 3.14 (1H, dd, J=12.9, 2.9 Hz, 5α-H), 3.99 (1H, d, J=8.6 Hz, 22-H), 4.06 (1H, m, 2β-H), 4.15 (1H, d, J=8.6 Hz, 23-H), 4.45 (1H, m, 3β-H). FAB-MS m/z: 471 ($M^+ + 1$). EI-MS m/z: 470 (M^+, 0.3%), 452 (1), 436 (3), 421 (1), 419 (1), 403 (1), 393 (3), 364 (100), 349 (12), 345 (64), 334 (13), 327 (38), 316 (13), 309 (10), 287 (21), 269 (13), 263 (15), 245 (14), 227 (9), 189 (12), 173 (17), 163 (11), 149 (17), 135 (17), 121 (18), 107 (28), 95 (30), 93 (28), 81 (39), 69 (31). EI-MS (as the bismethaneboronate derivative) m/z: 518 (M^+, 64%), 503 (3), 458 (3), 441 (7), 399 (8), 358 (26), 341 (5), 328 (8), 303 (9), 287 (22), 228 (9), 161 (100), 85 (55).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-2α,3α-22,23-Tetraacetoxy-*B*-homo-7-oxa-5α-ergostan-6-one (15)——The tetraol **2** (361 mg, 0.768 mmol) in pyridine (10 ml) was treated with acetic anhydride (6 ml) at 70 °C for 24 h. Work-up (ethyl

acetate) and chromatography on silica gel (2.5 cm i.d. × 27 cm) with benzene–ethyl acetate (5 : 1) gave the tetraacetate **14** (480 mg, 98%), mp 217—218 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.698 (3H, s, 18-H_3), 0.830 (3H, s, 19-H_3), 0.940 (3H, d, J=6.1 Hz, 27-H_3), 1.020 (3H, d, J=6.6 Hz, 21-H_3), 1.996 (6H, s × 2, two acetyls), 2.024 (3H, s, acetyl), 2.087 (3H, s, acetyl), 4.95 (1H, m, 2β-H), 5.17 (1H, d, J=8.8 Hz, 22-H), 5.32 (1H, d, J=8.8 Hz, 23-H), 5.38 (1H, m, 3β-H).

Trifluoroperacetic acid reagent was prepared by adding trifluoroacetic anhydride (6.7 ml) to a suspension of 30% hydrogen peroxide (1.2 ml) in dichloromethane (7.5 ml) at 0 °C. The reagent solution was added to a solution of the tetraacetate **14** (280 mg, 0.439 mmol) in dichloromethane (5 ml) containing disodium hydrogen phosphate (1.0 g). The mixture was stirred at room temperature for 1 h. Work-up (ethyl acetate) gave a crude product, which was applied to a column of silica gel (2 cm i.d. × 33 cm). Elution with benzene–ethyl acetate (3 : 1) provided the 7-oxalactone **15** (238 mg, 83%), mp 230—232 °C (MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.736 (3H, s, 18-H_3), 0.939 (3H, d, J=6.3 Hz, 27-H_3), 0.987 (3H, s, 19-H_3), 1.017 (3H, d, J=6.6 Hz, 21-H_3), 1.996 (3H, s, acetyl), 2.001 (3H, s, acetyl), 2.015 (3H, s, acetyl), 2.111 (3H, s, acetyl), 3.00 (1H, dd, J=12.0, 4.5 Hz, 5α-H), 4.10 (2H, m, 7-H_2), 4.88 (1H, m, 2β-H), 5.16 (1H, d, J=8.8 Hz, 22-H), 5.32 (1H, d, J=8.8 Hz, 23-H), 5.37 (1H, m, 3β-H).

[26,28-$^2H_6$]-(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (1)——The 7-oxalactone tetraacetate **15** (210 mg, 0.321 mmol) was saponified with 5% KOH/MeOH (15 ml) under reflux for 1 h. The cooled reaction mixture was treated with 6 M HCl (15 ml) at room temperature for 1 h. Work-up (ethyl acetate) and chromatography on silica gel (1.5 cm i.d. × 17 cm) with ethyl acetate provided [26,28-$^2H_6$]brassinolide (1) (145 mg, 93%), mp 285—287 °C (EtOAc). ^{1}H-NMR (200 MHz, C_5D_5N) δ: 0.721 (3H, s, 18-H_3), 1.052 (3H, s, 19-H_3), 1.217 (3H, d, J=6.4 Hz, 21-H_3), 3.61 (1H, dd, J=12.0, 4.2 Hz, 5α-H), 3.96 (1H, d, J=8.6 Hz, 22-H), 4.05 (4H, m, 7-H_2, 2β-H, and 22-H), 4.45 (1H, m, 3β-H). FAB-MS *m/z*: 487 (M^+ + 1). EI-MS *m/z*: 468 (M^+ − 18, 0.7%), 452 (9), 435 (1), 418 (1), 409 (6), 380 (87), 362 (77), 350 (57), 343 (37), 335 (23), 325 (22), 303 (27), 285 (28), 208 (32), 189 (45), 177 (41), 163 (27), 155 (40), 147 (51), 137 (43), 135 (46), 133 (46), 123 (34), 121 (50), 119 (48), 109 (63), 107 (97), 105 (46), 95 (84), 93 (84), 81 (100), 79 (47), 67 (45). EI-MS (as the bismethaneboronate derivative) *m/z*: 534 (M^+, 7%), 457 (7), 397 (6), 374 (47), 346 (18), 338 (31), 319 (6), 305 (6), 277 (5), 207 (8), 177 (66), 161 (100), 95 (36), 85 (79), 81 (70).

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