Communications to the Editor

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SYNTHESIS OF [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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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 (20S)-3α,5-cyclo-20-formyl-6β-methoxy-5α-pregnane (5).

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

Since the discovery of brassinolide, its related C-28 steroids, castasterone, typhasterol, and teasterone, have been isolated from several plant sources.[1] These steroids and a number of their related compounds have been found in a wide variety of higher plants and they (brassinosteroids) constitute a new class of plant growth promoter.[2] We have developed the microanalytical technique of brassinosteroids as bismethaneboronate or methaneboronate-trimethylsilyl derivatives by gas chromatography-mass spectrometry and we have identified several new steroids.[3] In our continuous interest in the microanalysis, we need deuterio-labelled brassinosteroids, in which more than five deuterium atoms are incorporated because of the molecular ion clusters resulting from the isotope of the boron atom. In this communication we describe the first synthesis of the 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).

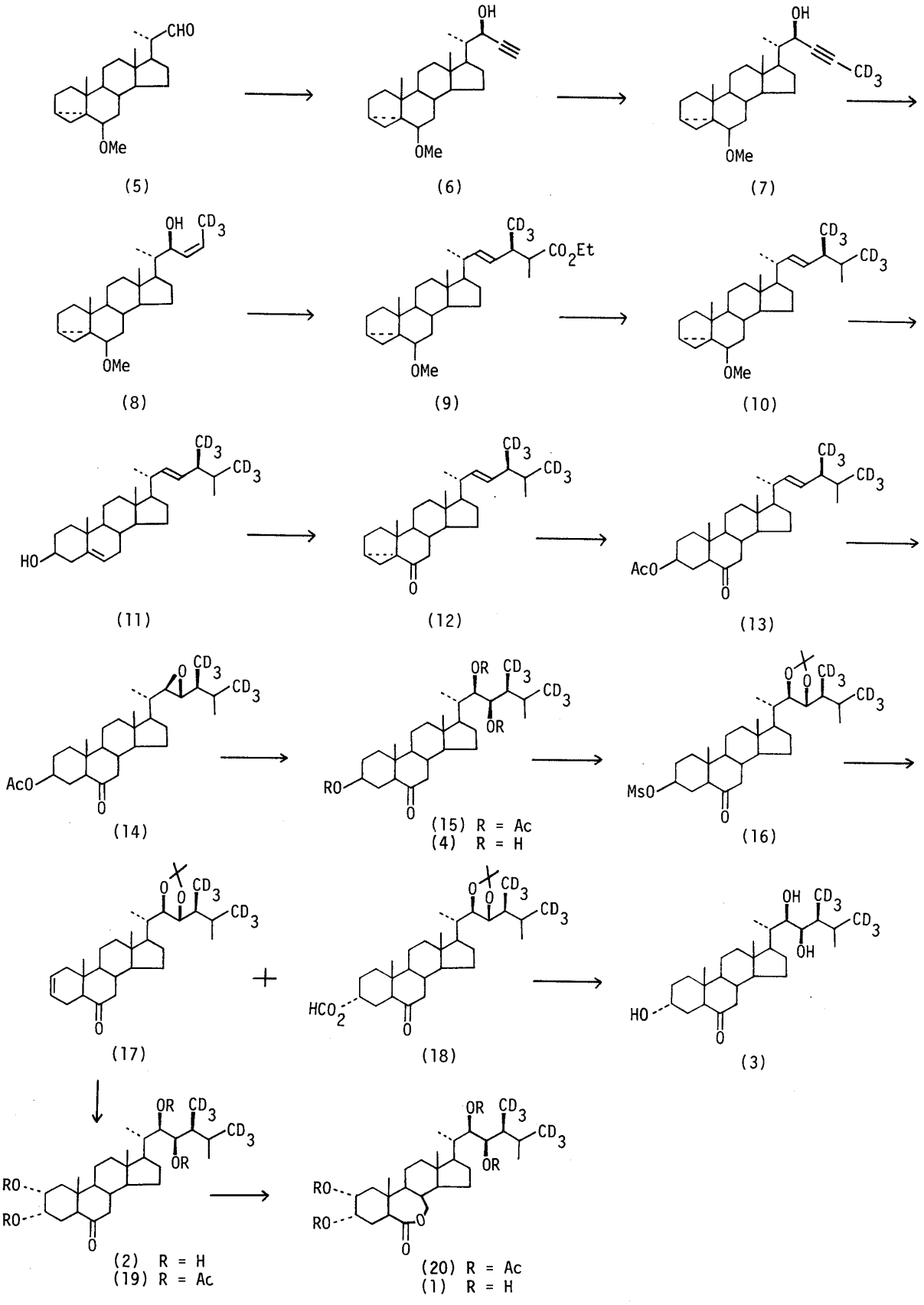
We employed [26,28-$^2H_6$]crinosterol (11) as a key intermediate for their synthesis because it provides the appropriate functionalities for modification to the desired system. Synthesis of 11 started with (20S)-3α,5-cyclo-20-formyl-6β-methoxy-5α-pregnane (5).[4] Coupling of 5 with lithium acetylide in THF at -78°C provided the less polar (22R)-alcohol (6) [61%, mp 131-132°C (hexane), δ ($CDCl_3$) 2.41 (1H, d, J 2 Hz, 24-H)] and the more polar (22S)-isomer [34%, mp 107-109°C (hexane), δ ($CDCl_3$) 2.35 (1H, d, J 2 Hz, 24-H)], whose C-22 configurations were determined as such by converting them into the known (22R)- and (22S)-26,27-dinor-3α,5-cyclo-6β-methoxy-5α-cholest-23-yn-22-ol,[5a] respectively. The (22R)-alcohol (6) was treated with triethylsilyl chloride and pyridine and the resulting product was reacted with n-butyl lithium at -78°C and then with iodomethane-d_3 to afford, after deprotection with tetra-n-butylammonium fluoride, the d_3-acetylenic alcohol (7) [96%, mp 132-133°C (hexane), m/z 387 (M^+)]. The d_3-steroid (7) was partially hydrogenated (H_2/Lindlar catalyst) and the resulting cis-allylic alcohol (8) was reacted with triethylorthopropionate in the presence of propionic acid in refluxing xylene. The orthoester Claisen rearrangement proceeded stereospecifically and the (22E,24R)-26-ester (9) [96%, mp 123-125°C (MeOH), m/z 473 (M^+)] was obtained as an inseparable, epimeric mixture at C-25 position according to the precedents in this steroidal system[5] and the mixture was used as such in the ensuing reactions. Ethoxycarbonyl group of 9 was reduced to d_3-methyl group by successive treatment with i) $LiAlD_4$, ii) MsCl/Py, and iii) $LiAlD_4$. The

resulting d_6-i-ether (10) was hydrolyzed with a catalytic amount of p-TsOH in refluxing aqueous dioxane to give [26,28-$^2H_6$]crinosterol (11) [88% from 9, mp 154-155°C (MeOH); $\delta(CDCl_3)$ 0.69 (3H, s, 18-H_3), 0.81 (3H, d, J 6.8 Hz, 27-H_3), 1.00 (3H, d, J 6.8 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 3.52 (1H, m, 3-H), 5.16 (2H, m, 22-H and 23-H), and 5.35 (1H, m, 6-H); m/z 404 (M^+)], whose d_6-content was found to be more than 98% by mass spectrometry.

The methanesulfonate of crinosterol-d_6 (11) was solvolyzed with aqueous acetone in the presence of $KHCO_3$ under reflux and the resulting i-alcohol was oxidized with Jones reagent to give the cyclopropyl ketone (12) [85%, mp 105-106.5°C (MeOH); m/z 402 (M^+)]. Treatment of 12 with 5M H_2SO_4 in refluxing AcOH followed by acetylation provided the acetate (13) [93%, mp 140-141°C (MeOH); m/z 462 (M^+)]. For the introduction of the desired (22R,23R)-diol function into the side chain of 13, the method developed by Mori et al.[6] was applied. Epoxidation of 13 with one equivalent of m-chloroperbenzoic acid in CH_2Cl_2 at room temperature gave, after chromatographic separation, the less polar (22R,23R)-epoxide (14) [64%, oil, $\delta(CDCl_3)$ 2.49 (1H, dd, J 6.1 and 2.1 Hz, 22-H) and 2.71 (1H, dd, J 6.1 and 2.1 Hz, 23-H)] and the more polar (22S,23S)-isomer [31%, mp 156-158°C (MeOH); $\delta(CDCl_3)$ 2.42-2.51 (2H, m, 22-H and 23-H)]. Their stereochemical assignment was made by comparison of the epoxidic protons of their ^{1}H-NMR data with those of the known (22R,23R)- and (22S,23S)-3β-acetoxy-22,23-epoxy-5α-stigmastan-6-one.[7] The less polar epoxide (14) was treated with 30% HBr/AcOH at room temperature and the resulting mixture of the bromoacetates was heated with 80% aqueous AcOH at 100°C. Acetylation of the crude product followed by purification by chromatography gave the triacetate (15) [66% from 14, mp 218-220°C (MeOH); $\delta(CDCl_3)$ 5.16 (1H, d, J 7.8 Hz, 22-H) and 5.32 (1H, d, J 7.8 Hz, 23-H)], whose ^{1}H-NMR data were in good agreement with those of brassinolide tetraacetate.[6,8] Thus, the (22R,23R)-configuration was confirmed. Saponification of 15 with 5% KOH/MeOH under reflux provided [26,28-$^2H_6$]teasterone (4) in quantitative yield; mp 202-204°C (MeOH-EtOAc); $\delta(C_5D_5N)$ 0.75 (3H, s, 18-H_3), 0.79 (3H, s, 19-H_3), 1.11 (3H, d, J 6.8 Hz, 27-H_3), 1.27 (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 (as methaneboronate-TMS derivative) m/z (70 eV) 550 (M^+, 43%), 535 (83), 521 (100), 460 (12), 445 (5), 407 (2), 360 (2), 300 (6), 271 (3), 221 (6), 161 (27), 143 (15), 121 (13), 107 (14), 95 (25), 85 (34), 75 (31), and (73 (35).

Teasterone-d_6 (4) was submitted to acetonide formation (p-TsOH/acetone) and then methanesulfonation (MsCl/Py). The sulfonate (16) was refluxed with lithium carbonate and dimethylformamide and the 2-ene (17) [50%, mp 233-235°C (MeOH)] and the 3α-formate (18) [25%, mp 172-173°C (MeOH)] were obtained. Refluxing of 18 with 80% aqueous AcOH followed by saponification with 5% KOH/MeOH provided [26,28-$^2H_6$]typhasterol (3) [90%, mp 191-193°C (EtOAc); $\delta(C_5D_5N)$ 0.76 (3H, s, 18-H_3), 0.80 (3H, s, 19-H_3), 1.11 (3H, d, J 6.3 Hz, 27-H_3), 1.27 (3H, d, J 6.8 Hz, 21-H_3), 3.15 (1H, dd, J 12.2 and 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), and 4.37 (1H, m, 3-H); FAB-MS m/z 455 (M^+ + 1); EI-MS (as methaneboronate-TMS derivative) m/z (70 eV) 550 (M^+, 86%), 535 (100), 532 (26), 521 (66), 460 (63), 445 (22), 421 (5), 384 (4), 319 (7), 300 (12), 271 (9), 229 (23), 211 (9), 161 (42), 121 (26), 95 (36), 85 (48), 75 (36), and 73 (40)].

Transformation of the 2-ene (17) into [26,28-$^2H_6$]castasterone (2) and [26,28-$^2H_6$]brassinolide (1) was carried out according to our synthesis of brassinolide[8] as follows. Stereospecific α-face cis-hydroxylation of the 2-ene (17) was effected with a catalytic amount of osmium tetroxide and an excess of N-methylmorpholine N-oxide in aqueous THF at room temperature. The resulting 2α,3α-diol compound was refluxed with 80% aqueous AcOH and then chromatographed on silica gel to provide [26,28-$^2H_6$]castasterone (2) [87%, mp 252-253°C (EtOAc); $\delta(C_5D_5N)$ 0.74 (3H, s, 18-H_3), 0.85 (3H, s, 19-H_3), 1.11 (3H, d, J 6.3 Hz, 27-H_3), 1.25 (3H, d, J 6.7 Hz, 21-H_3), 3.14 (1H, dd, J 12.9 and 2.9 Hz, 5α-H), 3.99 (1H, d, J 8.6 Hz, 22-H), 4.06 (1H, m, 2-H), 4.15 (1H, d, J 8.6 Hz, 23-H), and 4.45 (1H, m, 3-H); FAB-MS m/z 471 (M^+ + 1); EI-MS (as bismethaneboronate) m/z (70 eV) 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), and 85 (55)]. The tetraacetate (19), mp 217-218°C (MeOH), derived from 2, was submitted to Baeyer-Villiger oxidation[9] with trifluoroperacetic acid in CH_2Cl_2 at 0°C in the presence of Na_2HPO_4 and the product was purified by chromatography on silica gel to provide the 7-oxalactone (20) [83%, mp 230-232°C (MeOH); δ($CDCl_3$) 3.00 (1H, dd, J 12.0 and 4.5 Hz, 5α-H) and 4.10 (2H, m, 7-H_2)]. Saponification of 20 with 5% KOH/MeOH under reflux followed by relactonization with 6M HCl afforded [26,28-$^2H_6$]brassinolide (1) [93%, mp 285-287°C (EtOAc); δ(C_5D_5N) 0.72 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 1.22 (3H, d, J 6.4 Hz, 21-H_3), 3.61 (1H, dd, J 12.0 and 4.2 Hz, 5α-H), 3.96 (1H, d, J 8.6 Hz, 22-H), 4.05 (4H, m, 7-H_2, 2-H, and 23-H), and 4.45 (1H, m, 3-H); FAB-MS m/z 487 (M^+ + 1); EI-MS (as bismethaneboronate) m/z (70 eV) 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), and 81 (70)].

In conclusion, we were able to synthesize the 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), whose d_6-contents were determined to be more than 99% by mass spectrometry.

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