Synthesis of [26,28-$^2H_6$]Crinosterol, a Synthetic Intermediate of [26,28-$^2H_6$]Brassinolide and [26,28-$^2H_6$]Castasterone

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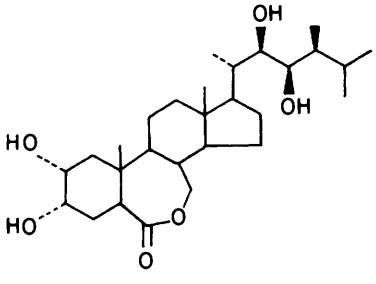
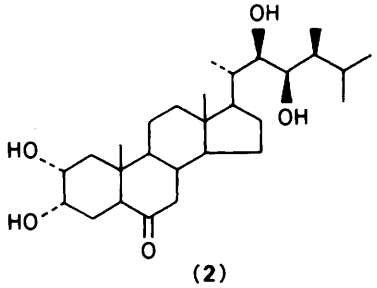
Stereoselective synthesis of [26,28-$^2H_6$]crinosterol, [26,28-$^2H_6$]-(22*E*,24*S*)-ergosta-5,22-dien-3β-ol (**4**), was achieved in *ca.* 50% overall yield from (20*S*)-20-formyl-6β-methoxy-3α,5-cyclo-5α-pregnane (**5**). Coupling of (**5**) with lithium acetylide gave a 2:1 mixture of separable products (**6**) and (**7**), whose configurations at C-22 were determined by chemical correlation with the known compounds (**8**) and (**9**), respectively. The triethylsilyl ether of the 22*R* isomer (**6**) was lithiated and then treated with [$^2H_3$]iodomethane, to provide, after desilylation, the [$^2H_3$]acetylenic alcohol (**10**). Partial hydrogenation of (**10**) with Lindlar catalyst followed by orthoester Claisen rearrangement yielded the [$^2H_3$]ester (**12**), whose ester group was reduced to a trideuteriomethyl group. Acid treatment of the resulting [$^2H_6$]ether (**13**) afforded [26,28-$^2H_6$]crinosterol (**4**), the deuterium content of which was 98%.

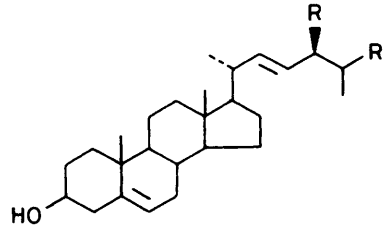
Brassinolide (**1**) and castasterone (**2**) are naturally occurring steroidal hormones with plant growth-promoting activity.[1,2] These, and related steroids, have recently been found to occur in a wide variety of higher plants. We have reported that these steroidal hormones could be analysed as their bismethaneboronate derivatives by g.c.-m.s. and we have identified several new steroids in this way.[3] As part of our continuing interest in the microanalysis of thes brassino-steroids, we need to obtain the deuterio-labelled compounds, in which more than five deuterium atoms must be incorporated because of the molecular ion clusters resulting from the isotope of the boron atom.



(**1**)



(**2**)



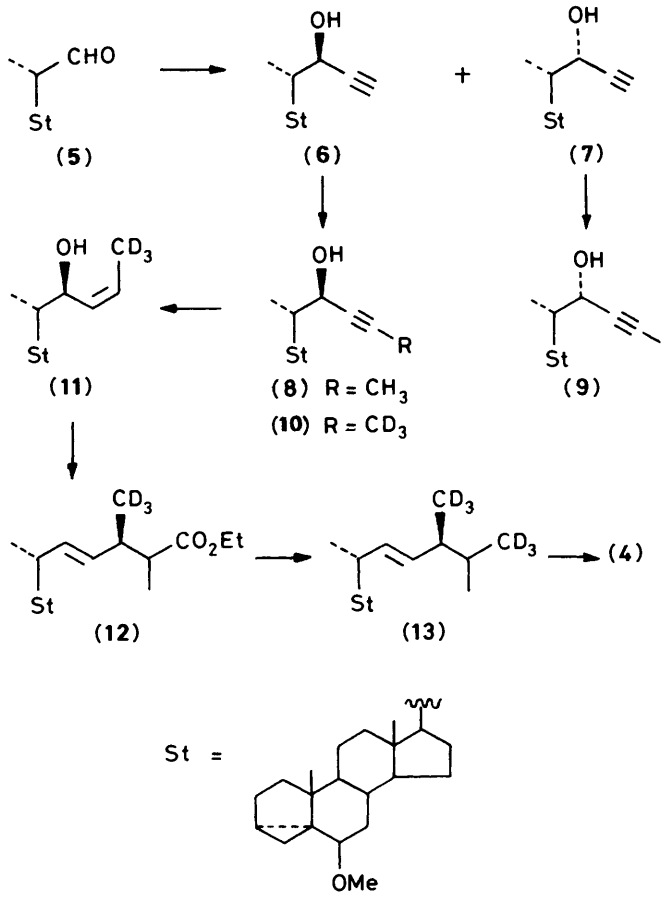
(**3**) R = CH_3
(**4**) R = CD_3

For the convenient synthesis of brassinolide (**1**) and castasterone (**2**), crinosterol (**3**),[4] a 24-epimer of brassicasterol, is an ideal starting material because it possesses the appropriate groups for modification to the desired system, and has the correct stereochemistry. On the basis of our requirement for deuterium incorporation, and the previous synthesis of brassinolide (**1**) and castasterone (**2**) from crinosterol (**3**),[5] we chose [26,28-$^2H_6$]crinosterol (**4**) as the most suitable intermediate for the labelled brassinolide and castasterone. In this paper, we describe the stereoselective synthesis of [26,28-$^2H_6$]crinosterol (**4**), which can be easily transformed into [26,28-$^2H_6$]brassinolide and [26,28-$^2H_6$]castasterone, according to reported methods.[5]

Our plan for the synthesis of [26,28-$^2H_6$]crinosterol (**4**) was as follows; first, introduction of the [$^2H_3$]-methyl group into the (22*R*)-acetylenic alcohol (**6**); then, orthoester Claisen rearrangement of the *cis*-allylic alcohol (**11**) obtained by partial hydrogenation of (**10**); and finally, reduction of the ethoxycarbonyl group of the resulting [28-$^2H_3$]ester (**12**) to the [$^2H_3$]methyl group.

A coupling reaction of (20*S*)-20-formyl-6β-methoxy-3α,5-cyclo-5α-pregnane (**5**), derived from stigmasterol as described in the literature,[6] with lithium acetylide[7] in THF at −78 °C provided, after chromatographic separation, the less polar alcohol (**6**) [60.7%; δ($CDCl_3$) 2.41 (1 H, d, *J* 2 Hz, 24-H)] and the more polar alcohol (**7**) [34.2%; δ($CDCl_3$) 2.35 (1 H, d, *J* 2 Hz, 24-H)]. Their configurations at C-22 were determined as follows. Protection of the 22-hydroxy group of (**6**) and (**7**) as the triethylsilyl ether, followed by lithiation with butyl-lithium at —78 °C and reaction with methyl iodide afforded, after deprotection with tetrabutylammonium fluoride, the known (22*R*)- and (22*S*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ols (**8**) and (**9**),[8] respectively. It should be noted that the chemical shifts of the acetylenic protons of (**6**) and (**7**) are different enough to be used to distinguish the compounds from each other.

Having established the stereochemistry of (**6**) and (**7**), we transformed the (22*R*)-22-alcohol (**6**) into [25-$^2H_3$]-(22*R*)-6β-methoxy-3α,5-cyclo-26,27-dinor-6α-cholest-23-yn-22-ol (**10**) in 95.6% overall yield using [$^2H_3$]-iodomethane in place of methyl iodide in the above-described reaction sequence. The [$^2H_3$]-acetylenic alcohol (**10**) was partially hydrogenated with Lindlar catalyst under hydrogen to give the *cis*-allylic alcohol (**11**),



which was then submitted to an orthoester Claisen rearrangement reaction. Reaction of (**11**) with an excess of triethyl orthopropionate in the presence of propionic acid in refluxing xylene proceeded stereoselectively and the (22*E*,24*R*)-26-ester (**12**) was obtained in 95.5% yield from (**10**), according to the accepted mechanism and to precedents in this steroidal system.[4,8] Transformation of the ethoxycarbonyl group of (**12**) into a [$^2H_3$]methyl group was achieved (96.2%) by the following successive reactions: reduction with lithium aluminium deuteride; methanesulphonation; and reduction with lithium aluminium deuteride. The resulting [$^2H_6$]ether (**13**) was then hydrolysed to give [24,28-$^2H_6$]crinosterol (**4**), m.p. 154—155 °C, in 91% yield. The deuterium content of (**4**) was determined to be 98% by m.s.

Experimental

M.p.s were determined by a hot-stage microscope apparatus and were uncorrected. ^{1}H N.m.r. spectra were recorded on a Hitachi R-24A spectrometer (60 MHz) in deuteriochloroform solution with tetramethylsilane as an internal standard, unless otherwise stated. I.r. spectra were taken with a Hitachi Model 260-10 spectrometer. Electron impact mass spectra were taken with a Shimadzu LKB 9000S mass spectrometer at 20 eV. Analytical t.l.c. was carried out on precoated plates of silica gel (Merck, Kieselgel 60 F_{254}, 0.25 mm thickness). Column chromatography was effected with silica gel (Merck, Kieselgel 60 F_{254}, 70—230 mesh). Work-up refers to dilution with water, extraction with an organic solvent indicated in parenthesis, washing of the extract to neutrality, drying over anhydrous magnesium sulphate, filtration, and removal of the solvent under reduced pressure.

(22R)- *and* (22S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*cholest*-23-*yn*-22-*ol* (**6**) *and* (**7**).—A solution of butyl-lithium in hexane (1.56M; 21.2 ml, 33.08 mmol) was added dropwise to a solution of acetylene (2.0 l) in tetrahydrofuran (THF) (80 ml) at −78 °C under argon. The solution was stirred at −78 °C for 20 min, and a solution of the 22-aldehyde (**5**) (9.5 g, 27.62 mmol) in THF (40 ml) was then added to the resulting lithium acetylide reagent at −78 °C. Stirring was continued for 20 min, after which the cooling bath was removed, and the mixture was stirred for a further 10 min. Work-up (ether) gave two separable products (11 g) with R_F values of 0.35 and 0.25 (hexane–ethyl acetate, 5:1, developed once). The crude products were applied to a column of silica gel (4.5 cm i.d. × 30 cm). Elution with hexane–ethyl acetate (15:1) provided the *less polar alcohol* (**6**) (22*R* isomer) (6.2 g, 60.7%), m.p. 131—132 °C (from hexane); δ($CDCl_3$) 0.73 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.10 (3 H, d, *J* 6 Hz, 21-H_3), 2.41 (1 H, d, *J* 2 Hz, 24-H), 2.73 (1 H, m, 6-H), 3.30 (3 H, s, Me), and 4.43 (1 H, m, 22-H) (Found: C, 80.95; H, 12.8. $C_{25}H_{38}O_2$ requires C, 81.03; H, 12.74%).

Further elution with the same solvent gave the *more polar alcohol* (**7**) (22*S* isomer) (3.5 g, 34.2%), m.p. 107—109 °C (from hexane); δ($CDCl_3$) 0.74 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.05 (3 H, d, *J* 6 Hz, 21-H_3), 2.35 (1 H, d, *J* 2 Hz, 24-H), 2.73 (1 H, m, 6-H), 3.30 (3 H, s, Me), and 4.40 (1 H, m, 22-H) (Found: C, 81.1; H, 12.5%).

(22R)-6β-*Methoxy*-3α,5-*cyclo*-26,27-*dinor*-5α-*cholest*-23-*yn*-22-*ol* (**8**).—The alcohol (**6**) (100 mg, 0.27 mmol) in pyridine (2 ml) was treated with chlorotriethylsilane (0.1 ml, 0.597 mmol) at room temperature for 1 h. Work-up (ether) and chromatography on silica gel (1.5 cm i.d. × 10 cm) with hexane–ethyl acetate (30:1) gave the triethylsilyl ether of (**6**) (130 mg). This was added to THF (2.5 ml) and then treated with butyl-lithium in hexane (1.56M; 0.2 ml, 0.312 mmol) at −78 °C under argon for 30 min. Methyl iodide (0.1 ml) was then added and stirring was continued for 30 min at −78 °C. Work-up (ether) gave a crude product, which in THF (2 ml) was treated with a solution of tetrabutylammonium fluoride in THF (0.4 ml, 0.4 mmol) at room temperature for 30 min. Work-up (ether) and chromatography on silica gel (1.5 cm i.d. × 10 cm) with hexane–ethyl acetate (10:1) afforded the known (22R)-22 alcohol (**8**) (99.5 mg, 96%), m.p. 129—130 °C (from ethyl acetate) (lit.,[8] m.p. 129—130 °C); δ($CDCl_3$) 0.73 (3 H, s, 18-H), 1.02 (3 H, s, 19-H_3), 1.10 (3 H, d, *J* 6 Hz, 21-H_3), 1.85 (3 H, *J* 2 Hz, 25-H_3), 2.74 (1 H, m, 6-H), 3.32 (3 H, s, OMe), and 4.44 (1 H, m, 22-H). The physicochemical properties of compound (**8**) were in agreement with those of the authentic sample, prepared according to the published method.[8]

(22S)-6β-*Methoxy*-3α,5-*cyclo*-26,27-*dinor*-5α-*cholest*-23-*yn*-22-*ol* (**9**).—The more polar alcohol (**7**) (130 mg, 0.351 mmol) was transformed, as described for (**8**), into the known (22*S*)-22-alcohol (**9**) (131 mg, 97%), m.p. 133—134 °C (from hexane) (lit.,[8] m.p. 133—134.5 °C); δ($CDCl_3$) 0.74 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.04 (3 H, d, *J* 6 Hz, 21-H_3), 1.85 (3 H, d, *J* 2 Hz, 25-H_3), 2.77 (1 H, m, 6-H), 3.34 (3 H, s, OMe), and 4.42 (1 H, m, 22-H). The physicochemical properties of synthetic compound (**9**) were identical with those of the authentic sample, which was obtained according to the reported method.[8]

[25-$^2H_3$]-(22R)-6β-*Methoxy*-3α,5-*cyclo*-26,27-*dinor*-5α-*cholest*-23-*yn*-22-*ol* (**10**).—The (22*R*)-22-alcohol (**6**) (6.1 g, 16.49 mmol) in pyridine (16 ml) was treated with chlorotriethylsilane (6 ml, 35.8 mmol) at room temperature for 1 h. After work-up (ether), the resulting triethylsilyl ether (7.88 g) in THF (90 ml) was treated with butyl-lithium in hexane (1.56M; 13 ml, 20.28 mmol) at −78 °C under argon. Then, [$^2H_3$]iodomethane (2 ml) was added at −78 °C. The mixture was stirred at −78 °C

for 30 min after which it was warmed to room temperature. Stirring was continued for a further 30 min. Work-up (ether) gave the product (8.0 g); δ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.00 (3 H, s, 19-H_3), 2.73 (1 H, m, 6-H), 3.28 (3 H, s, Me), and 4.37 (1 H, br s, $W_{\frac{1}{2}}$ 2.8 Hz, 22-H). The product, in THF (100 ml), was desilylated with a solution of tetrabutylammonium fluoride in THF (26 ml, 26 mmol) at room temperature for 30 min. Work-up (ether) gave a crude product, which was applied to a column of silica gel (4.5 cm × 18 cm). Elution with hexane–ethyl acetate (10:1) afforded the deuterio-labelled *acetylenic alcohol* (**10**) [6.1 g, 95.6% from (**6**)], m.p. 132—133 °C (from hexane); δ($CDCl_3$) 0.73 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.08 (3 H, d, *J* 6 Hz, 21-H_3), 2.75 (1 H, m, 6-H), 3.32 (3 H, s, OMe), and 4.43 (1 H, m, 22-H); $\nu_{max.}$($CHCl_3$) 3 600w, 3 000m, 2 940s, 2 870s, 2 360w, 2 330w, 2 250w, 1 458m, 1 380m, 1 350w, 1 328w, 1 300w, 1 272w, 1 210s, 1 185w, 1 150w, 1 125m, 1 100s, 1 080s, 1 022s, 980m, 973m, 958m, 950w, 920w, 900w, 862w, 843w, 820w, and 730s cm^{-1}; *m/z* 387 (M^+, 25%), 372 (24), 355 (28), 332 (54), 329 (23), 315 (14), 312 (10), 289 (15), and 283 (100).

[25-$^2H_3$]-(22S,23Z)-6β-*Methoxy*-3α,5-*cyclo*-26,27-*dinor*-5α-*cholest*-23-*en*-22-*ol* (**11**).—The 22*R*-acetylenic alcohol (**10**) (6.0 g, 15.5 mmol) in ethyl acetate (120 ml) and quinoline (4.4 ml) was treated with 5% Pd–$CaCO_3$ (Lindlar type, 1.08 g) at room temperature under hydrogen for 2 h. Filtration and work-up (ethyl acetate) afforded the *allyl alcohol* (**11**) (6.0 g), m.p. 84—86 °C (from acetone–methanol); δ($CDCl_3$) 0.71 (3 H, s, 18-H_3), 1.00 (3 H, s, 19-H_3), 2.73 (1 H, m, 6-H), 3.27 (3 H, s, OMe), 4.50 (1 H, br d, *J* 6 Hz, 22-H), and 5.44 (2 H, m, 23-H and 24-H); *m/z* 389 (M^+, 5%), 374 (5), 357 (7), 334 (15), 315 (6), and 283 (100).

[28-$^2H_3$]-(22E,24S)-*Ethyl*-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*oate* (**12**).—The mixture of the allyl alcohol (**11**) (6.0 g, 15.5 mmol), triethyl orthopropionate (9 ml, 44.7 mmol), propionic acid (10 drops), and xylene (80 ml) was refluxed under argon for 1 h. Methanol was added to the reaction mixture and the solvent was evaporated under reduced pressure to leave the residue, which was applied to a column of silica gel (4.5 cm i.d. × 25 cm). Elution with hexane–ethyl acetate (30:1) provided the *ester* (**12**) [7.0 g, 95.5% from (**10**)], m.p. 123—125 °C (from methanol); δ($CDCl_3$) 0.72 (3 H, s, 18-H_3), 0.99 (3 H, d, *J* 6 Hz, 21-H_3), 1.00 (3 H, s, 19-H_3), 2.71 (1 H, m, 6-H), 3.25 (3 H, s, OMe), 4.05 (2 H, q, *J* 7 Hz, CO_2CH_2Me), and 5.10 (2 H, m, 22-H and 23-H); $\nu_{max.}$($CHCl_3$) 2 930s, 2 868s, 2 352w, 2 325w, 2 230w, 1 722s, 1 455m, 1 375m, 1 345w, 1 328w, 1 298w, 1 267m, 1 210s, 1 185s, 1 160s, 1 098s, 1 080s, 1 020s, 975m, 945w, 920w, 895w, 865w, and 725s cm^{-1}; *m/z* 473 (M^+, 26%), 458 (40), 441 (99), 426 (21), 418 (77), 415 (12), 340 (18), 320 (10), 313 (19), 285 (21), 283 (34), 255 (93), 253 (75), 240 (10), 227 (49), 233 (69), and 186 (100).

[26,28-$^2H_6$]-(22E,24S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*ene* (**13**).—The ester (**12**) (6.0 g, 12.68 mmol) in THF (200 ml) was refluxed with lithium aluminium deuteride (1.5 g, 35.71 mmol) under argon for 4 h. Work-up (ether) gave the 26-ol (5.5 g) as an oil; δ($CDCl_3$) 0.76 (3 H, s, 18-H_3), 0.99 (3 H, d, *J* 6 Hz, 21-H_3), 1.02 (3 H, s, 19-H_3), 2.72 (1 H, m, 6-H), 3.28 (3 H, s, OMe), and 5.13 (2 H, m, 22-H and 23-H). The 26-ol in pyridine (50 ml) was treated with methanesulphonyl chloride (1.5 ml, 19.39 mmol) at room temperature for 1.5 h. Work-up (ethyl acetate) gave the mesylate (6.5 g); δ($CDCl_3$) 0.71 (3 H, s, 18-H_3), 0.98 (3 H, d, *J* 6 Hz, 21-H), 1.00 (3 H, s, 19-H_3), 2.70 (1 H, m, 6-H), 2.89 (3 H, s, mesyl), 3.24 (3 H, s, OMe), and 5.12 (2 H, m, 22-H and 23-H). The mesylate in THF (150 ml) was refluxed with lithium aluminium deuteride under argon atmosphere for 5 h. Work-up (ether) gave a crude product, which was applied to a column of silica gel (4.5 cm i.d. × 20 cm). Elution with hexane–ethyl acetate (25:1) afforded the [$^2H_6$]steroid (**13**) [5.1 g, 96.2% from the ester (**12**)], m.p. 145—147 °C (from methanol); δ($CDCl_3$) 0.72 (3 H, s, 18-H_3), 0.98 (3 H, d, *J* 6 Hz, 21-H_3), 1.01 (3 H, s, 19-H_3), 2.72 (1 H, m, 6-H), 3.28 (3 H, s, −OMe), and 5.10 (2 H, m, 22-H and 23-H); *m/z* 418 (M^+, 51%), 403 (41), 386 (59), 371 (8), 363 (90), 860 (21), 340 (14), 314 (13), 299 (4), 285 (16), 283 (17), 255 (100), 253 (28), 229 (22), and 131 (44).

[26,28-$^2H_6$]-(22E,24S)-*Ergosta*-5,22-*dien*-3β-*ol* (**4**).—The mixture of the ether (**13**) (5.0 g, 11.96 mmol), toluene-*p*-sulphonic acid (20 mg), water (15 ml), and dioxane (100 ml) was refluxed for 3.5 h. Work-up (dichloromethane) and chromatography on silica gel (4.5 cm i.d. × 23 cm) eluting with benzene–ethyl acetate (20:1) gave [26,28-$^2H_6$]*crinosterol* (**4**) (4.4 g, 91%), m.p. 154—155 °C (from methanol); δ($CDCl_3$, 200 MHz) 0.691 (3 H, s, 18-H_3), 0.811 (3 H, d, *J* 6.8 Hz, 27-H_3), 0.997 (3 H, d, *J* 6.8 Hz, 21-H_3), 1.008 (3 H, s, 19-H_3), 3.52 (1 H, m, 3-H), 5.16 (2 H, m, 22-H and 23-H), and 5.35 (1 H, m, 6-H); $\nu_{max.}$($CHCl_3$) 3 600w, 2 840s, 2 770s, 2 330w, 2 210w, 1 458m, 1 375m, 1 210m, 1 050s, 1 022s, 977m, 962m, 842w, and 725s cm^{-1}; *m/z* 404 (M^+, 75%), 389 (8), 396 (27), 371 (10), 358 (9), 340 (16), 319 (7), 314 (9), 300 (64), 285 (13), 282 (10), 271 (65), 255 (100), 229 (16), 213 (23, 159 (42), 147 (39), 145 (33), 133 (42), 131 (64), and 112 (31). The deuterium content of the synthetic [$^2H_6$]-sterol (**4**) was found to be 98%.

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