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Synthesis of hexadeuterated 23-dehydroxybrassinosteroids

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Abstract

Two hexadeuterated brassinosteroids (BS) ([26,27-$^2H_6$]-23-dehydroxycastasterone and [26,27-$^2H_6$]-cathasterone) containing a hydroxy group at C_{22} instead of the 22*R*,23*R*-diol function characteristic for most compounds of this class were prepared for biochemical studies. The corresponding non-deuterated compounds are considered intermediates in brassinolide biosynthesis. The carbon skeleton of the side chain with proper stereochemistry at C_{24} was prepared from commercially available (2*R*)-3-hydroxy-2-methylpropanoate. This low molecular fragment was coupled to the tetracyclic steroidal fragment through the reaction of the appropriate sulfone with C_{22} aldehyde. Formation of the necessary configuration of the 22-hydroxy group was achieved by hydride reduction of the corresponding ketone. Deuterium atoms at C_{26} and C_{27} originated from [$^2H_3$]methyl iodide used for alkylation of the intermediate sulfone.

Keywords: Brassinosteroids; Labeled compounds; Brassinolide; Plant growth hormone

1. Introduction

The presence of many functional groups in the brassinolide molecule implies that its biosynthesis is a multistep process. Detailed knowledge of the biosynthetic pathway is very important for a better understanding of the physiological processes and subtle mechanisms of the action of brassinosteroids (BS) in plants. Most BS known to date contain a 22*R*,23*R*-diol function, which is essential for their biological activity [1,2]. Identification of cathasterone lacking one of the hydroxy groups and application of its labeled analog in feeding experiments [3] showed that brassinolide biosynthesis proceeds via initial hydroxylation at C_{22} followed by introduction of the hydroxy group at C_{23} (Scheme 1). This finding has been confirmed by identification of the *CPD* gene as a microsomal cytochrome P450-dependent 23α-hydroxylase [4]. However, in addition to the established biosynthetic sequences, hitherto unknown alternative subpathways or species-specific biosynthetic routes may occur. It is known, for example, that even within one biological species, different biosynthetic routes to brassinolide exist. Thus, in *Catharanthus roseus*, the so-called 'early C_6-oxidation pathway' and the 'late C_6-oxidation pathway' operate independently [5,6]. In this respect, the main objective of the present work was the elaboration of a synthetic approach to and preparation of deuterated 23-dehydroxybrassinosteroids. Such compounds containing six deuterium atoms in the side chain are essential for biosynthetic studies of relevant brassinolide precursors [7].

2. Experimental

2.1. General

Melting points were recorded on a Boetius micromelting point apparatus and are uncorrected. IR spectra were recorded on an UR-20 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz for ^1H, 50 MHz for ^{13}C) spectrometer using TMS as an internal standard in $CDCl_3$. Accurate mass measurements were carried out on a Micromass MasSpec mass spectrometer operating in the 70 eV EI mode. Samples were introduced by direct probe for accurate mass measurement by peak matching. Chemicals were purchased from Aldrich, Fluka, and Steraloids Chemical Companies and were used as received. [$^2H_3$]Methyl iodide (99.5%) was supplied by Deutero Gmbh. Reactions were monitored by TLC using

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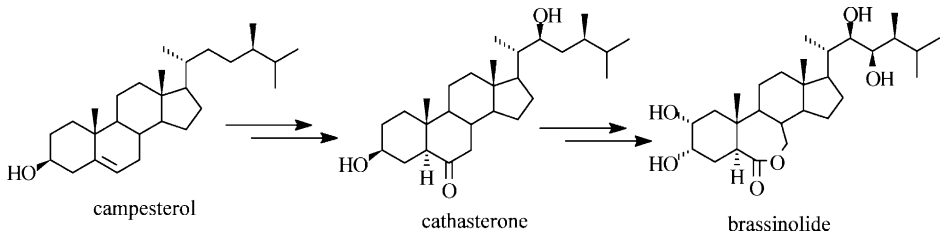


Scheme 1.

aluminum or plastic sheets precoated with silica gel 60 F_{254} (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

2.2. 2-[(2R)-2-Methyl-3-(4-methylphenylsulfonyloxy) propyloxy]tetrahydro-2H-pyran (5)

HCl (36%, 2.5 ml) was added to a solution of (2*R*)-3-hydroxy-2-methylpropanoate (**2**) (10 ml, 90 mmol) in dihydropyrane (25 ml). After incubation at ambient temperature for 24 h, pyridine (25 ml) was added. The solvent was partially evaporated in vacuo, and the residue was dissolved in $CHCl_3$ and filtered through a plug of SiO_2 with the aid of EtOAc. The filtrate was concentrated under reduced pressure to give crude (2*R*)-2-methyl-3-tetrahydro-2*H*-2-pyranyloxypropanoate, which was dissolved in Et_2O (200 ml). $LiAlH_4$ (13.5 g, 0.35 mol) was added portionwise over 1 h, and the reaction mixture was stirred for an additional 1 h and then treated with NaOH solution (13.5 ml, 15%) and water (50.4 ml). The suspension was filtered, and the filtrate was dried over Na_2SO_4 and evaporated to give (2*S*)-2-methyl-3-tetrahydro-2*H*-2-pyranyloxypropan-1-ol. The crude oil was dissolved in pyridine (140 ml), and TsCl (36 g, 0.19 mol) was added. The mixture was left to stand at room temperature for 2 h, diluted with water (400 ml), and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 5:1) to give the tosylate 5 (19.0 g, 64%) as an oil. IR (cm^{-1}): 2950, 2875, 1610, 1465, 1370, 1195, 1370, 1195, 1185, 1045, 980, 820. ^1H NMR δ: 0.93 (s, 3H, 2-Me), 2.46 (s, 3H, Ph-CH_3), 7.36 (d, 2H, J = 8 Hz, Ph), 7.80 (d, 2H, J = 8 Hz, Ph). ^{13}C NMR δ: 13.58, 13.66, 19.26, 19.40, 19.72, 19.79, 21.60, 25.41, 25.47, 25.70, 30.44, 30.68, 30.94, 33.49, 33.64, 61.92, 62.13, 62.86, 63.33, 67.95, 68.39, 72.21, 94.57, 98.48, 98.87, 99.10, 127.94, 129.80, 133.12, 144.65. HRMS calc. for $C_{16}H_{24}O_5S$: 328.134445. Found: 328.124615. EI-MS *m*/*z* (%): 85 (100), 91 (82), 101 (72), 155 (62), 173 (72), 227 (12), 245 (5), 310 (2) $[M - H_2O]^{\bullet+}$, 328 (1) $[M]^{\bullet+}$.

2.3. 2-[(2R)-2-Methyl-3-phenylsulfonylpropyloxy] tetrahydro-2H-pyran (6)

Thiophenol (30.6 ml, 0.3 mol) was added to a solution of sodium methoxide prepared from sodium (18 g, 0.78 mol) and MeOH (300 ml). A solution of the tosylate **5** (19.0 g, 58 mmol) in MeOH (150 ml) was then added, and the mixture was left to stand at ambient temperature for 14 h, diluted with water, and extracted with petroleum ether. The organic layer was dried over Na_2SO_4 and evaporated to give crude (2*R*)-2-methyl-1-phenylsulfanyl-3-tetrahydro-2*H*-2-pyranyloxypropane, which was dissolved in $CHCl_3$ (400 ml), and *m*-chloroperbenzoic acid (57 g, 0.33 mol) was added. The mixture was stirred at room temperature for 3 h, washed with 25% aqueous NH_4OH, water, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 3:1) to give the sulfone **6** (13.2 g, 76%) as an oil. IR (cm^{-1}): 2940, 2880, 1600, 1460, 1325, 1155, 980. ^1H NMR δ: 1.12 (dd, 3H, J = 8.5, 2.5 Hz, 2-Me), 2.24–2.46 (m, 1H, C_2–H), 2.86–3.16 (m, 2H, CH_2–S), 3.30–3.84 (m, 4H, CH_2–O), 4.42–4.56 (m, 1H, O–CH–O), 7.50–7.98 (m, 5H, Ph). ^{13}C NMR δ: 17.03, 17.25, 19.36, 19.46, 25.35, 29.34, 29.62, 29.65, 30.45, 59.17, 59.47, 62.18, 62.31, 70.43, 71.17, 127.86, 129.25, 133.54, 140.05. HRMS calc. for $C_{15}H_{20}O_3S$: 280.113317. Found: 280.113152. EI-MS *m*/*z* (%): 85 (100), 101 (45), 143 (40), 197 (52), 215 (14), 240 (0.5), 280 (0.3) $[M - H_2O]^{\bullet+}$.

2.4. 2-[(2R)-2-Methyl-3-[$^2H_3$]methyl-3-phenylsulfonyl-[4-$^2H_3$]butyloxy]tetrahydro-2H-pyran (7)

A solution of BuLi (2.5 M in hexane, 40 ml, 0.1 mol) was added at −30 °C to a solution of sulfone **6** (10.0 g, 30.5 mmol) in THF (250 ml). The mixture was stirred for 15 min, after which a solution of CD_3I (3.4 ml, 55 mmol) in THF (10 ml) was added, and the temperature was gradually increased to 10 °C over 30 min. The mixture was recooled to −30 °C, and a solution of BuLi (2.5 M in hexane, 20 ml, 50 mmol) was added. The mixture was maintained at this temperature for 15 min, and a second portion of CD_3I (3.4 ml, 55 mmol) in THF (10 ml) was introduced. After 15 min, the mixture was allowed to warm to room temperature, after which it was diluted with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4, evaporated, and the residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 3:1) to give the deuterated derivative **7** (5.5 g, 50%) as an oil. IR (cm^{-1}): 2950, 2875, 2245, 1450, 1390, 1360, 1305, 1155, 1085, 1040, 1030, 1010, 980. ^1H NMR δ: 1.12 (dd, 3H, J = 7, 5 Hz, 2-Me), 2.14–2.30 (m, 1H, C_2–H), 3.14–3.98 (m, 4H, CH_2–O),

4.78 (s, 1H, O–CH–O), 7.42–7.86 (m, 5H, Ph). ^{13}C NMR δ: 13.91, 14.05, 19.40, 25.32, 30.56, 30.59, 36.40, 36.65, 62.12, 62.20, 65.26, 69.62, 69.94, 98.98, 99.07, 128.62, 130.30, 133.36, 136.26. HRMS calc. for $C_{12}H_{11}D_6O_3S$: 247.127502. Found: 247.127529. EI-MS *m/z* (%): 85 (100), 89 (58), 125 (38), 144 (50), 191 (21), 226 (12), 231 (23), 247 (23).

2.5. (2R)-2-Methyl-3-[$^2H_3$]methyl-3-phenylsulfonyl-[4-$^2H_3$]butan-1-ol (8)

A mixture of **7** (5.5 g, 16.5 mmol) and HCl (36%, 1 ml) in MeOH (250 ml) was stirred at room temperature for 40 min. Pyridine (1 ml) was added, and the solvent was evaporated under reduced pressure. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (15:1 ⇒ 1:1) to give the sulfone **8** (4.1 g, 100%) as an oil. IR (cm^{-1}): 2990, 2900, 2250, 1590, 1450, 1290, 1150, 1090, 1055, 1025. ^{1}H NMR δ: 1.16 (d, 3H, J = 7 Hz, 2-Me), 2.12–2.28 (m, 1H, C_2–H), 2.66 (s, 1H, OH), 3.71 (dd, 1H, J = 12, 6 Hz, C_1–H), 3.98 (dd, 1H, J = 12, 4 Hz, C_1–H), 7.52–7.94 (m, 5H, Ph). ^{13}C NMR δ: 13.60, 39.50, 64.63, 65.44, 128.83, 130.43, 133.66, 136.17. HRMS calc. for $C_{12}H_{12}D_6O_3S$: 248.135327. Found: 248.135130. EI-MS *m/z* (%): 88 (88), 107 (100), 143 (65), 144 (62), 190 (9), 218 (4), 248 (0.2) $[M]^{\bullet+}$.

2.6. (2R)-2-Methyl-3-[$^2H_3$]methyl-3-phenylsulfonyl [4-$^2H_3$]butyl 4-methyl-1-benzenesulfonate (9)

TsCl (19.0 g, 0.1 mol) was added to a solution of **8** (4.1 g, 16.5 mmol) in pyridine (100 ml). The mixture was left to stand at ambient temperature for 3 h, diluted with water, and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, evaporated, and the residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 5:1) to give the tosylate **9** (6.1 g, 92%) as an oil. IR (cm^{-1}): 2990, 2250, 1610, 1455, 1370, 1300, 1195, 1185, 1155, 1105, 970. ^{1}H NMR δ: 1.12 (d, 3H, J = 7 Hz, 2-Me), 2.26–2.46 (m, 1H, C_2–H), 2.46 (s, 3H, OTs–CH_3), 4.08 (dd, 1H, J = 10, 7.5 Hz, C_1–H), 4.54 (dd, 1H, J = 10, 3.5 Hz, C_1–H), 7.32–7.88 (m, 9H, Ph and OTs). ^{13}C NMR δ: 13.12, 21.54, 36.47, 64.43, 65.20, 72.16, 127.60, 129.80, 129.84, 130.22, 132.59, 133.74, 135.45, 144.88. HRMS calc. for $C_{13}H_{13}D_6O_3S$: 261.143152. Found: 261.143053. EI-MS *m/z* (%): 89 (100), 125 (9), 144 (9), 155 (36), 174 (6), 247 (6) $[M - Ts]^+$, 261 (13) $[M - PhSO_2]^+$, 402 (0.2) $[M]^{\bullet+}$.

2.7. (2R)-2-Methyl-3-[$^2H_3$]methyl-1-phenylsulfanyl-3-phenylsulfonyl[4-$^2H_3$]butane (10)

Thiophenol (17.5 ml, 0.17 mol) was added to a solution of MeONa prepared from sodium (10 g, 0.44 mol) and MeOH (150 ml). A solution of tosylate **9** (6.1 g, 15.2 mmol) in MeOH (100 ml) was added, and the mixture was left to stand at room temperature for 40 h after which it was extracted with petroleum ether. The combined organic extacts were dried over Na_2SO_4 and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 5:1) to give the starting tosylate **9** (0.8 g, 15% recovery) and the sulfide **10** (3.5 g, 68%) as an oil. IR (cm^{-1}): 2940, 1730, 1590, 1485, 1450, 1385, 1300, 1250, 1150, 1150, 1090, 1030. ^{1}H NMR δ: 1.10 (d, 3H, J = 7 Hz, 2-Me), 2.08–2.26 (m, 1H, C_2–H), 2.60 (dd, 1H, J = 13, 11 Hz, C_1–H), 3.90 (dd, 1H, J = 13, 1.5 Hz, C_1–H), 7.14–7.88 (m, 10H, Ph). ^{13}C NMR δ: 14.88, 37.19, 37.45, 65.86, 126.30, 128.74, 128.90, 130.14, 130.24, 133.51, 136.07. HRMS calc. for $C_{18}H_{16}D_6O_2S_2$: 340.143784. Found: 340.143641. EI-MS *m/z* (%): 77 (9), 89 (16), 123 (100), 180 (5), 199 (17) $[M - PhSO_2]^+$, 340 (21) $[M]^{\bullet+}$.

2.8. (2S)-2-Methyl-3-[$^2H_3$]methyl[4-$^2H_3$]butyl phenyl sulfide (11)

Mg (2 g, 82 mmol) was added to a vigorously stirred solution of **10** (3.3 g, 9.7 mmol) in MeOH (200 ml). The mixture was stirred for 2 h, the resultant precipitate was filtered, and the filtrate was neutralized with 3N HCl. The acidified aqueous layer was extracted with petroleum ether, and the combined organic extracts were dried over Na_2SO_4, evaporated, and chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 5:1) to give the sulfide **11** (1.7 g, 88%) as an oil. IR (cm^{-1}): 2970, 2940, 2885, 2220, 2080, 1600, 1490, 1450, 1390, 1100, 1065, 1030. ^{1}H NMR δ: 0.96 (d, 3H, J = 7 Hz, 2-Me), 1.52–1.72 (m, 1H, C_2–H), 2.72 (dd, 1H, J = 12.5, 8.5 Hz, C_1–H), 3.02 (dd, 1H, J = 13, 5 Hz, C_1–H), 7.08–7.42 (m, 5H, Ph). ^{13}C NMR δ: 15.12, 18.99, 30.95, 38.31, 38.88, 40.39, 125.49, 125.67, 128.74, 128.97, 137.52. HRMS calc. for $C_{12}H_{12}D_6S$: 200.150583. Found: 200.149872.

2.9. (2S)-2-Methyl-3-[$^2H_3$]methyl[4-$^2H_3$]butyl phenyl sulfone (12)

MCPBA (5.8 g, 34 mmol) was added to an ice-bath cooled solution of sulfide **11** (1.7 g, 8.5 mmol) in $CHCl_3$ (100 ml). The mixture was stirred for 3 h at room temperature and then, washed with NH_4OH (25% solution) and water. The organic layer was extracted with petroleum ether. The combined organic phases were washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (15:1 ⇒ 3:1) to give the sulfone **12** (1.2 g, 61%) as an oil. ^{1}H NMR δ: 1.02 (d, 3H, J = 7 Hz, 2-Me), 1.90–2.10 (m, 1H, C_2–H), 2.88 (dd, 1H, J = 14, 8.5 Hz, C_1–H), 3.10 (dd, 1H, J = 14, 3.5 Hz, C_1–H), 7.45–7.96 (m, 5H, Ph). ^{13}C NMR δ: 15.95, 31.95, 33.72, 60.50, 127.88, 129.27, 133.54, 140.15. HRMS calc. for $C_{12}H_{12}D_6O_2S$: 232.140412. Found: 232.140400. EI-MS *m/z* (%): 77 (67), 90 (100), 125 (13), 143 (97), 156 (7), 184 (5), 232 (3) $[M]^{\bullet+}$.

2.10. (24S)-[26,27-$^2H_6$]23-Phenylsulfonyl-6,6-ethylenedioxy-24-methyl-3α,5-cyclo-5α-cholestan- 22-one (14)

A solution of BuLi in hexane (2.5 M, 5 ml, 12.5 mmol) was added to a stirred solution of sulfone **12** (0.9 g, 3.9 mmol) in THF (15 ml) under an argon atmosphere at −40 °C). The mixture was stirred for 30 min at −40 °C, cooled to −80 °C, and a solution of (20*S*)-6,6-ethylenedioxy-20-formyl-3α,5-cyclo-5α-pregnane (**1**) (2.0 g, 5.7 mmol) in THF (20 ml) was added. After stirring at −70 °C for 1 h, the mixture was allowed to warm to room temperature. NH_4Cl (2 g) was added, and the mixture was diluted with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 3:1) to give 1.4 g (62% based on **12**) of (24*S*)-[26,27-$^2H_6$]22-hydroxy-23-phenylsulfonyl-6,6-ethylenedioxy-24-methyl-3α,5-cyclo-5α-cholestane (**13**) as an oil.

A two-necked flask equipped with a magnetic stirrer was charged with $(COCl)_2$ (12 ml, 0.14 mol) in CH_2Cl_2 (40 ml) and cooled to −70 °C. DMSO (22 ml, 0.3 mol) was added, and stirring was continued for 30 min at −70 °C. A solution of hydroxy sulfone **13** (1.0 g, 1.8 mmol) in CH_2Cl_2 (20 ml) was added, and the mixture was stirred at −70 °C for 1 h. Et_3N (80 ml, 0.58 mol) was added dropwise, and the cooling bath was removed. After warming to room temperature, the mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was evaporated, and the residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 5:1) to give the oxo sulfone **14** (0.7 g, 70%) as an oil. IR (cm^{-1}): 2950, 2880, 2225, 1720, 1690, 1660, 1455, 1390, 1310, 1150, 1080. ^1H NMR δ: 0.72 (s, 3H, 18-Me), 1.00 (s, 3H, 19-Me), 3.52–4.10 (m, 4H, –CH_2–O–), 7.46–7.96 (m, 5H, Ph). ^{13}C NMR δ: 11.41, 11.68, 12.04, 12.19, 13.55, 18.98, 19.68, 22.60, 22.87, 23.06, 23.93, 24.05, 24.91, 25.91, 27.72, 29.82, 33.25, 33.48, 34.21, 34.86, 35.35, 37.62, 39.25, 39.65, 40.17, 41.12, 42.61, 44.70, 45.59, 46.00, 46.33, 46.73, 47.36, 51.86, 56.85, 63.72, 64.66, 64.68, 70.93, 71.06, 109.67, 127.68, 128.09, 128.97, 133.08, 143.90, 209.53. HRMS calc. for $C_{36}H_{46}D_6O_5S$: 602.391208. Found: 602.390976. EI-MS *m*/*z* (%): 87 (79), 165 (74), 343 (100), 358 (17), 407 (16), 462 (35), 465 (37), 527 (10), 547 (38), 587 (9) $[M - CH_3]^+$, 602 (54) $[M]^{\bullet+}$.

2.11. (24R)-[26,27-$^2H_6$]6,6-Ethylenedioxy-24-methyl-3α, 5-cyclo-5α-cholestan-22-one (15)

Strips of aluminum foil (2.5 g) were treated with NaOH solution (15%) for 15 min, and the alkali was decanted off. The foil was washed consecutively with water, ethanol, a solution of $HgCl_2$ (0.5%), and ethanol. The mercury-activated aluminum was added to a solution of oxo sulfone **14** (700 mg, 1.16 mmol) in EtOH (150 ml). The mixture was stirred at room temperature for 14 h and filtered through SiO_2. The filtrate was evaporated, and the residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 5:1) to give **15** (350 mg, 65%) as an oil. IR (cm^{-1}): 2270, 2265, 2220, 1720, 1470, 1385, 1170, 1110, 1080. ^1H NMR δ: 0.73 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me), 3.68–4.08 (m, 4H, –CH_2–O–).

2.12. (22S,24R)-[26,27-$^2H_6$]22-Hydroxy-24-methyl-3α, 5-cyclo-5α-cholestan-6-one (17)

$LiAlH_4$ (200 mg, 5.3 mmol) was added to a stirred solution of ketone **15** (350 mg, 0.76 mmol) in THF). Stirring was maintained for 30 min, and then, excess $LiAlH_4$ was destroyed by careful addition of water (0.8 ml) and NaOH solution (15%, 0.2 ml). The precipitate was filtered, and the filtrate was dried over Na_2SO_4 and evaporated to give (22*S*,24*R*)-[26,27-$^2H_6$]22-hydroxy-24-methyl-6,6-ethylenedioxy-3α,5-cyclo-5α-cholestane **16** (330 mg, 94%) as an oil, which was used without further purification for the next step.

Alcohol **16** (270 mg, 0.58 mmol) was dissolved in acetone (137 ml), and a solution of TsOH (16.5 mg) in water (14 ml) was added. The mixture was stirred at room temperature for 2 h before pyridine (2 ml) was added, and the solvent was evaporated under reduced pressure. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 6:1) to give the alcohol **17** (180 mg, 73%) as an oil. ^1H NMR δ: 0. 73 (s, 3H, 18-Me), 0.84 (d, 3H, $J = 6.5$ Hz, 21- or 28-Me), 0.92 (d, 3H, $J = 6.5$ Hz, 28- or 21-Me), 1.02 (s, 3H, 19-Me), 4.72–4.84 (m, 1H, C_{22}–H). ^{13}C NMR δ: 11.27, 11.68, 12.00, 15.83, 19.70, 22.91, 24.02, 25.92, 27.78, 31.57, 33.49, 34.88, 35.35, 39.41, 39.76, 42.66, 44.79, 46.08, 46.35, 46.77, 52.50, 56.92, 71.62, 209.68. HRMS calc. for $C_{28}H_{40}D_6O_2$: 420.387442. Found: 420.388802. EI-MS *m*/*z* (%): 121 (32), 136 (41), 161 (29), 229 (12), 285 (27), 300 (100), 329 (9), 402 (8) $[M - H_2O]^{\bullet+}$, 420 (50) $[M]^{\bullet+}$.

2.13. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-24-methyl-3α, 5-cyclo-5α-cholestan-6-one (18)

A mixture of alcohol **17** (140 mg, 0.33 mmol), Ac_2O (0.5 ml) and pyridine (1 ml) was stirred at room temperature for 50 h, after which it was diluted with water, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 10:1) to give the acetate **18** (154 mg, 100%) as an oil. ^1H NMR δ: 0.70 (s, 3H, 18-Me), 0.82 (d, 3H, $J = 6.5$ Hz, 21- or 28-Me), 0.95 (d, 3H, $J = 6.5$ Hz, 28- or 21-Me), 0.98 (s, 3H, 19-Me), 2.00 (s, 3H, OAc), 4.96–5.08 (m, 1H, C_{22}–H). ^{13}C NMR δ: 11.59, 11.83, 12.60, 15.49, 19.64, 21.23, 22.87, 23.99, 25.88, 28.07, 31.64, 33.47, 34.81, 34.99, 35.21, 35.73, 38.20, 39.69, 42.62, 44.69, 46.05, 46.26, 46.71, 52.48, 56.86, 74.84, 170.69, 209.44.

2.14. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-3β-bromo-24-methyl-5α-cholestan-6-one (**19**)

HBr (48%, 0.24 ml) was added to a solution of **18** (75 mg, 0.16 mmol) in AcOH (2 ml). The mixture was stirred for 30 min, diluted with water, and extracted with $CHCl_3$. The organic layer was washed consecutively with water, saturated $NaHCO_3$, and brine, and then, it was dried over Na_2SO_4 and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 10:1) to give the bromide **19** (88 mg, 100%) as an oil. ^{1}H NMR δ: 0.66 (s, 3H, 18-Me), 0.80 (s, 3H, 19-Me), 2.04 (s, 3H, OAc), 3.94 (m, 1H, C_3–H), 5.00–5.12 (m, 1H, C_{22}–H). ^{13}C NMR δ: 11.80, 12.56, 13.08, 15.50, 21.30, 23.89, 27.95, 31.63, 32.34, 33.40, 35.00, 35.78, 37.80, 38.22, 39.19, 39.38, 40.64, 42.89, 46.50, 50.47, 52.53, 53.84, 56.58, 58.96, 74.82, 170.72, 209.50. HRMS calc. for $C_{28}H_{39}{}^{81}BrD_6O$: 484.300992. Found: 484.301857. Calc. for $C_{28}H_{39}{}^{79}BrD_6O$: 482.303038. Found: 482.303368. EI-MS *m/z* (%): 95 (64), 103 (63), 149 (28), 270 (19), 336 (16), 350 (100), 352 (98), 377 (63), 379 (67), 380 (75), 382 (58), 462 (15), 482 (35), 484 (42), 542 (1) $[M]^{\bullet+}$, 544 (1) $[M]^{\bullet+}$.

2.15. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-24-methyl-5α-cholest-2-en-6-one (**20**)

A mixture of bromide **19** (88 mg, 0.16 mmol), Li_2CO_3 (300 mg) and DMF (3 ml) was refluxed for 1 h, allowed to cool to ambient temperature, diluted with water, and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 12:1) to give: (a) (22*S*,24*R*)-[26,27-$^2H_6$]22-acetoxy-24-methyl-5α-cholest-2-en-6-one (**20**) (40 mg, 53%) as an oil. ^{1}H NMR δ: 0.68 (s, 3H, 18-Me), 0.72 (s, 3H, 19-Me), 0.84 (d, 3H, $J = 6.5$ Hz, 21- or 28-Me), 0.96 (d, 3H, $J = 6.5$ Hz, 28- or 21-Me), 2.05 (s, 3H, OAc), 5.00–5.12 (m, 1H, C_{22}–H), 5.64 (m, 2H, C_2– and C_3–H). ^{13}C NMR δ: 11.75, 12.58, 13.52, 15.51, 21.14, 21.30, 21.71, 23.90, 27.96, 31.66, 35.00, 35.75, 37.74, 38.19, 39.40, 39.48, 40.02, 42.74, 46.92, 52.53, 53.42, 53.83, 56.65, 63.16, 74.88, 124.48, 125.01, 170.77, 211.90. HRMS calc. for $C_{30}H_{42}D_6O_3$: 462.398006. Found: 462.397206. EI-MS *m/z* (%): 93 (21), 107 (19), 121 (17), 229 (7), 243 (5), 270 (17), 297 (16), 300 (18), 402 (12), 434 (17), 447 (68) $[M - CH_3]^+$, 462 (100) $[M]^{\bullet+}$; (b) (22*S*,24*R*)-[26,27-$^2H_6$]22-acetoxy-24-methyl-3α,5-cyclo-5α-cholestan-6-one (**18**) (20 mg, 27%) as an oil.

2.16. (22S,24R)-[26,27-$^2H_6$]2α,3α,22-Trihydroxy-24-methyl-5α-cholestan-6-one (**22**)

OsO_4 (33 mg, 13 mmol) was added to a solution of enone 20 (40 mg, 0.086 mmol) in pyridine (0.8 ml). The mixture was stirred at room temperature for 1 h and then, treated with a solution of Na_2SO_3 (0.2 g), H_2SO_4 (0.04 ml), and water (2 ml) at 30–40 °C for 30 min. The resulting mixture was extracted with $CHCl_3$, the extract was dried over Na_2SO_4, and the solvent was evaporated. The resultant crude product **21** (46 mg) was treated with a solution of KOH in MeOH (5%, 2 ml) and heated under reflux for 1 h. After neutralization with AcOH, the solvent was evaporated under reduced pressure. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (5:1 ⇒ 0:1) to give the triol **22** (27 mg, 69%); mp: 243–246 °C ($CHCl_3$). ^{1}H NMR δ: 0.72 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.84 (d, 3H, $J = 6.5$ Hz, 21- or 28-Me), 0.90 (d, 3H, $J = 6$ Hz, 28- or 21-Me), 2.68 (d, 1H, $J = 12$ Hz, C_5–H), 3.70–3.86 (m, 2H, C_{22}– and C_2–H), 4.08 (m, 1H, C_3–H). ^{13}C NMR δ: 11.26, 11.97, 13.55, 15.81, 21.26, 23.91, 26.31, 27.66, 31.58, 35.28, 37.75, 39.43, 40.17, 42.60, 42.89, 46.74, 50.73, 52.48, 53.75, 56.62, 68.43, 71.64, 212.06. HRMS calc. for $C_{28}H_{42}D_6O_4$: 454.392921. Found: 454.391823. Calc. for $C_{28}H_{40}D_6O_3$: 436.382356. Found: 436.383017. EI-MS *m/z* (%): 81 (18), 93 (16), 121 (17), 163 (10), 247 (11), 289 (15), 316 (57), 334 (100), 345 (5), 336 (3) $[M - H_2O]^+$, 454 (3) $[M]^{\bullet+}$.

2.17. (22S,24R)-[26,27-$^2H_6$]3β,22-Diacetoxy-24-methyl-5α-cholestan-6-one (**23**)

A solution of H_2SO_4 (3N, 0.075 ml) was added to a solution of compound **18** (59 mg, 0.13 mmol) in AcOH (1.5 ml). The mixture was stirred at 115 °C for 1 h, cooled, diluted with water, and extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (20:1 ⇒ 10:1) to give the diacetate **23** (45 mg, 68%) as an oil. ^{1}H NMR δ: 0.70 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.84 (d, 3H, $J = 6$ Hz, 21- or 28-Me), 0.98 (d, 3H, $J = 6.5$ Hz, 28- or 21-Me), 2.02 (s, 6H, AcO), 4.66 (m, 1H, C_3–H), 4.98–5.12 (m, 1H, C_{22}–H). ^{13}C NMR δ: 11.83, 12.57, 13.04, 15.49, 21.32, 21.47, 23.90, 26.10, 26.83, 27.95, 31.65, 34.98, 35.71, 36.42, 37.93, 38.15, 39.40, 40.91, 42.89, 46.56, 52.50, 53.81, 56.47, 56.53, 72.82, 74.84, 170.62, 170.77, 210.26. HRMS calc. for $C_{30}H_{42}D_6O_3$: 462.398006. Found: 462.397766. EI-MS *m/z* (%): 95 (43), 103 (38), 107 (26), 121 (23), 149 (15), 177 (11), 229 (11), 271 (14), 300 (47), 330 (100), 357 (72), 402 (15), 447 (10), 462 (78) $[M - AcOH]^+$, 522 (1.5) $[M]^{\bullet+}$.

2.18. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-3β-hydroxy-24-methyl-5α-cholestan-6-one (**24**)

Diacetate **23** (43 mg, 0.082 mmol) was treated with a solution of KOH in MeOH (2%, 8 ml) at room temperature for 50 min. AcOH was added to neutralize the mixture, and the solvent was evaporated under reduced pressure. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (10:1 ⇒ 1:1) to give monoacetate **24** (37 mg, 94%) as an oil. ^{1}H NMR δ: 0.66 (s, 3H, 18-Me), 0.76 (s, 3H, 19-Me), 0.84 (d, 3H, $J = 6.5$ Hz, 21- or 28-Me), 0.96 (d, 3H, $J = 6.5$ Hz,

28- or 21-Me), 2.04 (s, 3H, –CH_3–CO–), 3.60 (m, 1H, C_3–H), 3.92 (br.s, 1H, –OH), 5.00–5.12 (m, 1H, C_{22}–H). ^{13}C NMR δ: 11.82, 12.54, 13.13, 15.49, 20.68, 21.30, 21.53, 23.93, 27.97, 29.91, 30.58, 31.65, 34.89, 35.74, 36.68, 37.93, 38.19, 39.46, 40.94, 42.89, 46.62, 52.53, 53.90, 56.62, 56.77, 70.64, 74.92, 170.69, 210.98. HRMS calc. for $C_{28}H_{40}D_6O_2$: 420.387442. Found: 420.388942. EI-MS *m/z* (%): 95 (53), 247 (12), 261 (16), 288 (100), 315 (80), 330 (7), 360 (5), 405 (10), 420 (68) $[M - AcOH]^+$, 462 (4) $[M - H_2O]^+$, 480 (2) $[M]^{\bullet+}$.

*2.19. (22S,24R)-[26,27-$^2H_6$]3β,22-Dihydroxy-24-methyl-5α-cholestan-6-one (**25**)*

Monoacetate **24** (8 mg, 16.6 μmol) was treated with a solution of KOH in MeOH (5%, 5 ml) and heated at 45 °C for 5 h. The mixture was neutralized by the addition of AcOH, and the solvent was evaporated. The residue was chromatographed on SiO_2 with petroleum ether–EtOAc (5:1 ⇒ 1:1) to give the diol **25** (6 mg, 82%) as an oil. ^{1}H NMR δ: 0.72 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.84 (d, 3H, $J = 6$ Hz, 21- or 28-Me), 0.92 (d, 3H, $J = 6.5$ Hz, 28- or 21-Me), 3.62 (m, 1H, C_3–H), 3.72–3.86 (m, 1H, C_{22}–H). ^{13}C NMR δ: 11.28, 12.02, 13.19, 15.91, 21.63, 23.98, 27.70, 29.72, 30.13, 30.81, 31.66, 35.34, 36.79, 38.04, 39.49, 39.64, 40.97, 42.99, 46.73, 52.67, 54.04, 56.78, 56.88, 70.73, 71.76, 210.63. HRMS calc. for $C_{28}H_{40}D_6O_2$: 420.387442. Found: 420.386767. EI-MS *m/z* (%): 95 (33), 139 (29), 248 (21), 285 (21), 300 (24), 318 (100), 347 (6), 420 (10) $[M - H_2O]^+$, 438 (3) $[M]^{\bullet+}$.

3. Results and discussion

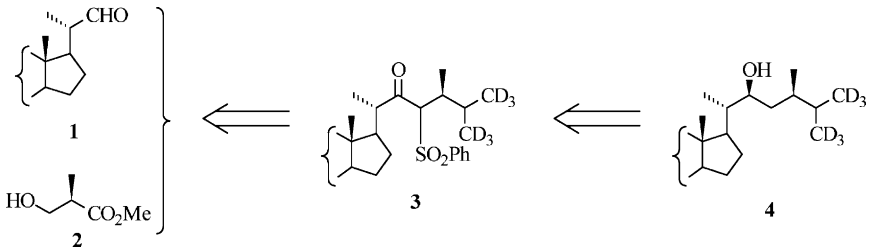
Preparation of BS analogs containing a hydroxy group only at C_{22} in the side chain instead of the 22*R*,23*R*-diol function has been described earlier. These experiments were aimed at the synthesis of more simple derivatives than natural BS for practical purposes [8], for structure–activity relationship [9–11] and biosynthetic [12,13] studies, and to develop synthetic methodologies for steroidal side chain synthesis [14]. However, control over the substituent at C_{24} was exercised [15] only in one study. It is known that most natural BS contain a 24α-methyl group that is essential for biological activity. Synthesis of compounds with a 22α-hydroxy group and carbon side chain characteristic of brassinolide has not been reported before.

In this paper, we describe preparation of [26,27-$^2H_6$]23-dehydroxybrassinosteroids as depicted in the retrosynthetic Scheme 2. Synthesis of related [26,27-$^2H_6$] sterols has been reported [16,17], but none of these methods provided a facile entry into ^{2}H-labeled BS. The standard approach to 22α-alcohols is reaction of organometallic reagents with 22C-aldehydes (**1**) [18,19]. However, it suffers from the formation of a considerable amount (up to 40%) of isomeric 22β-alcohol. In this respect, better results were anticipated by the hydride reduction of 22-ketones [18]. Formation of the desired stereochemistry at C_{24} required the use of a chiral synthetic building block. Our approach made use of (2*R*)-3-hydroxy-2-methylpropanoate (**2**) [20].

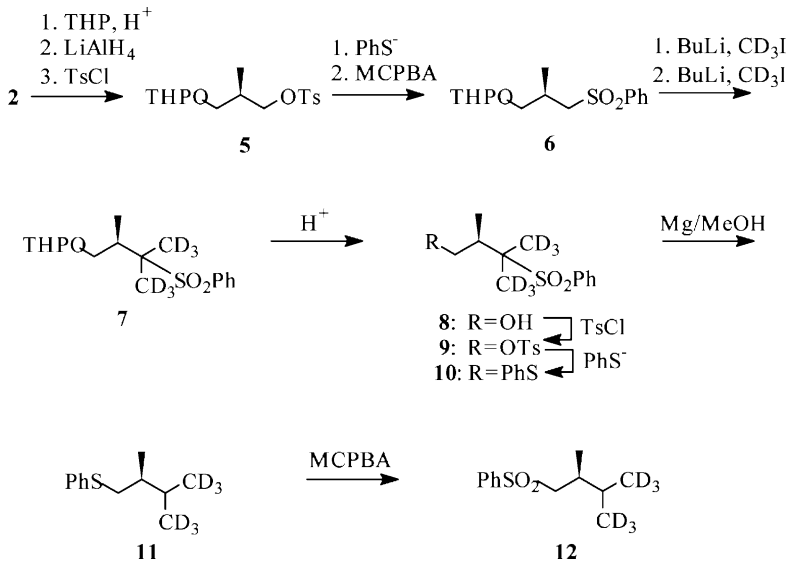
Synthesis of the C_{23}–C_{28} fragment of the side chain was performed as depicted in Scheme 3. The hydroxy group in **2** was protected as a tetrahydropyranyl ether, and via a number of steps, the carbomethoxy group was transformed into a methylene phenylsulfone moiety. Introduction of deuterium was achieved by twice repeated treatment of the sulfone **6** with butyl lithium and [$^2H_3$]methyl iodide. At this stage, the necessary carbon skeleton was prepared, and efforts were directed toward formation of functionality, which was required for the coupling of the low-molecular fragment to the steroidal aldehyde.

Sulfones are known to be of great synthetic utility in carbon–carbon bond forming reactions in the construction of steroidal side chains [19], including those characteristic of BS [20–22]. The same procedure was used for introduction of another phenylsulfone group. However, prior to the oxidation of the sulfide **11**, the phenylsulfone group at C_{25} had to be removed. This was achieved by treatment of **10** with magnesium in methanol.

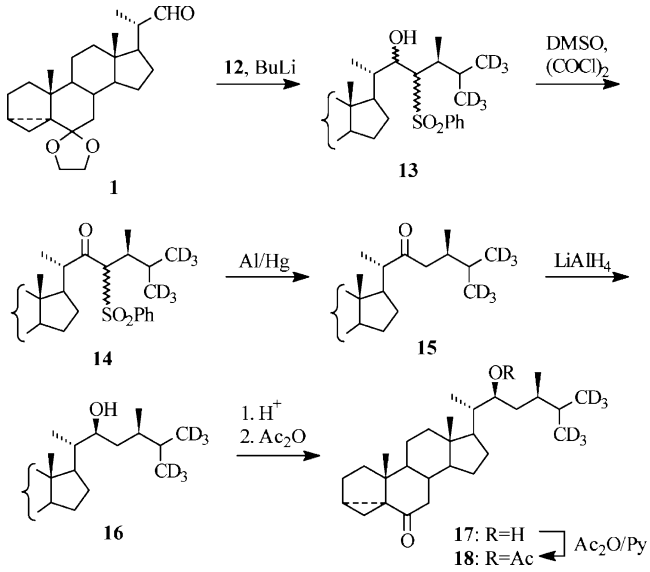
Construction of the necessary side chain was performed as depicted in Scheme 4. Addition of the lithium salt of sulfone **12** to the aldehyde **1**, prepared in five steps from stigmasterol [23,24], gave a mixture of hydroxy sulfones **13**. It has been shown [25] that a similar reaction produced a mixture of all possible isomers at C_{22} and C_{23}. Taking into account that the total amount of 22α-alcohols was rather substantial, removal of phenylsulfonyl group at this stage seemed to be reasonable. However, all attempts of desulfurization of **13** failed, and the only isolated product was the Δ^{22}-derivative, corresponding to a Julia olefination. Conversely, a similar



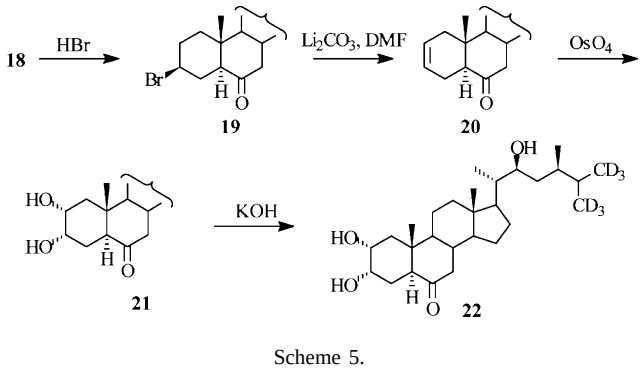
Scheme 2.



Scheme 3.



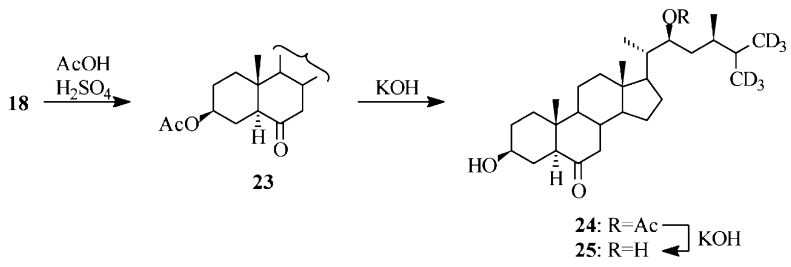
Scheme 4.



Scheme 5.

reaction is well known for keto sulfones [26,27]. The initial attempt to oxidize **13** with CrO_3 in pyridine into the corresponding ketone gave poor results; the reaction proceeded slowly (2–3 days), and the yield of **14** was low. The problem was solved using Swern oxidation. Desulfurization of **14** with aluminum amalgam gave the desired 22-ketone **15** without epimerization of the adjacent chiral center. Hydride reduction of the ketone **15** led to the 22α-alcohol **16**. This gave the required side chain, and for further transformations in the cyclic skeleton, the hydroxy group was protected as the acetate.

Construction of the required cyclic moiety was accomplished according to previously reported procedures [28] for the synthesis of [26-$^2H_3$]BS (Scheme 5). Hydrobromic acid assisted cyclopropane ring opening in **18** followed by dehydrobromination of **19** gave the Δ^2-olefin **20**, which was



Scheme 6.

further subjected to hydroxylation to give the 2α,3α-diol **21**. Upon treatment with base, compound **21** gave the deuterated 23-dehydroxy analog of castasterone **22**.

Treatment of **18** with acetic acid in the presence of sulfuric acid gave the diacetate **23** (Scheme 6). Stepwise saponification gave the monoacetate **24** and then the 23-dehydroxy analog of teasterone **25**.

In conclusion, a new method for preparation of cathasterone side chain have been developed. Two new brassinolide biosynthetic intermediates (as hexadeuterated derivatives **22** and **25**) have been synthesized. The obtained compounds are considered as effective tools for elucidation of alternative subpathways in brassinolide biosynthesis.

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