Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 1 November 2010, Revised 28 December 2010, Accepted 9 January 2011 Published online 17 February 2011 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1874

Synthesis of deuterium-labeled (24*R*)-methyl brassinosteroids

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Synthesis of labeled brassinosteroids (24-epibrassinolide and its biosynthetic precursors) containing three deuterium atoms in the terminal part of the side chain is reported. Labeling was achieved by three-step reductive transformation of carbethoxy group into methyl using lithium aluminium deuteride. The proposed method ensured high isotopic purity of (24*R*)-methyl brassinosteroids containing a label in a position which is not subjected to its loss.

Keywords: brassinosteroids; epibrassinolide; epicastasterone; episecasterone; episecasterol

Introduction

24-Epibrassinolide holds a special place among natural brassinosteroids (BS).[1] It belongs to the most active representatives of this group of phytohormones, and at the same time it can be relatively easily prepared from available sterols.[2–4] As a result, now it is the most practically useful compound of this group; for a number of years preparations with 24-epibrassinolide (Figure 1) as an active ingredient have been used in agriculture.[5,6] Research in the recent years has revealed its potential usefulness in medicine also.[7–9] Further studies, both in agricultural and medicinal directions, require the availability of labeled 24-epibrassinolide. Recently, we have published synthesis of [7-$^2H_2$]epibrassinolide.[10] This compound proved to be useful for biochemical and physiological investigations, but because of its relatively low isotopic purity (82%) it could not be used in some experiments. Based on the experience we have gained in brassinolide biosynthetic studies,[11–15] labeling the terminal part of the steroidal side chain was considered as an appropriate solution of the problems connected with low isotopic purity. The present work is an extension of our studies[16] toward synthesis of labeled brassinosteroids for biochemical and other biological applications and deals with the preparation of [26-$^2H_3$]epibrassinolide and its biosynthetic precursors.

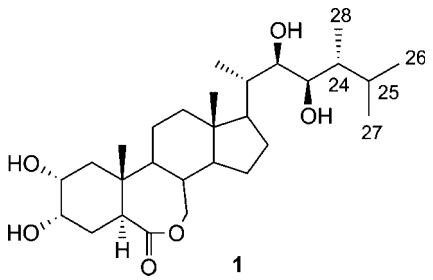

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Figure 1. The Structure of 24-epibrassinolide.

Experimental

Melting points were recorded on a Boetius micro-melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 500 (Bruker Biospin, Rheinstetten, Germany) spectrometer in $CDCl_3$ operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Assignment of ^1H and ^{13}C resonances was performed by the combined use of 1D and 2D experiments, including COSY, HSQC, HMBC, TOCSY, and NOESY methods using standard pulse sequences supplied in the instrument manufacturer's software package. Mass spectra were performed on a LCQ Fleet mass spectrometer (Thermo Electron Corporation, USA) with an APCI source. Spectra were collected in the positive ion mode and analyzed by the Xcalibur software. Chemicals were purchased from Aldrich and Fluka and used as received. Reactions were monitored by TLC using aluminum or plastic sheets, silica gel 60 F_{254} precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

[26–$^2H_3$](22*E*,24*R*)-6β-Methoxy-3α,5-cyclo-24-methyl-5α-cholest-22-ene (4)

To a solution of (22*E*,24*R*)-6β-methoxy-3α,5-cyclo-24-methyl-5α-cholest-22-en-26-oic acid ethyl ester (**3**)[17] (1.22 g, 2.58 mmol) in THF-diethyl ether (7:1, 80 ml), $LiAlD_4$ (217 mg, 5.16 mmol) was added portionwise. The reaction was stirred at an ambient temperature for 1.5 h. Then water (0.22 ml), 15% NaOH (0.22 ml) and again water (0.65 ml) were consecutively added to the mixture. The precipitate was filtered off and the filtrate was evaporated to dryness. Purification of the residue by SiO_2

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chromatography using petroleum ether/EtOAc (10:1 ⇒ 20:1) provided alcohol **4** (930 mg) as an oil.

Triethylamine (0.74 ml, 5.3 mmol) and MsCl (0.25 ml, 3.1 mmol) were added to a stirred solution of alcohol **4** from the previous stage (930 mg, 2.17 mmol) in 25 ml of CH_2Cl_2 at 0°C. The reaction mixture was kept at these conditions for 4 h, and then diluted with water. The layers were separated in a separatory funnel and the aqueous portion was extracted with $CHCl_3$. The combined organic portions were dried over Na_2SO_4 and evaporated. The residue was purified by SiO_2 chromatography using petroleum ether/EtOAc (40:1 ⇒ 15:1) to give crude mesylate **4a** (850 mg) which was used in the next step without additional purification.

To a vigorously stirred solution of mesylate **4a** (850 mg, 1.67 mmol) in THF (18 ml) and diethyl ether (7 ml), $LiAlD_4$ (212 mg, 5.05 mmol) was added. The mixture was stirred at room temperature for 2 h, then another portion of $LiAlD_4$ (212 mg, 5.05 mmol) was added. After stirring for additional 2 h, water (0.43 ml), 15% NaOH (0.43 ml) and again water (1.3 ml) were added. The precipitate was filtered off and the filtrate was evaporated. Column chromatography of the residue using petroleum ether/EtOAc (25:1) as eluent gave olefin **5** (600 mg, 56% from **3**) as an oil. ^{1}H-NMR: δ 0.44 (dd, J = 7.5, 5.3 Hz, 1 H), 0.66 (dd, J = 4.2, 4.2 Hz, 1 H), 0.74 (s, 3 H, 18-Me), 0.82 (d, J = 6.7 Hz, 3 H), 0.92 (d, J = 6.7 Hz, 3 H), 1.01 (d, J = 6.7 Hz, 3 H), 1.03 (s, 3 H, 19-Me), 2.77 (br. s., 1 H, C_6-H), 3.33 (s, 3 H, OMe), 5.12–5.25 (m, 2 H). ^{13}C-NMR: δ 12.45, 13.07, 17.62, 19.29, 19.57, 19.88, 20.94, 21.47, 22.76, 24.17, 24.96, 28.64, 30.47, 32.85, 33.34, 35.06, 35.27, 40.19, 42.67, 42.76, 43.39, 48.06, 56.17, 56.56, 56.60, 82.42, 131.69, 135.88.

[26−^{2}H$_3$](22*R*,23*R*,24*R*)-6β-Methoxy-3α,5-cyclo-24-methyl-5α-cholestan-22,23-diol (6a)

A mixture of olefin **5** (690 mg, 1.66 mmol), $K_3Fe(CN)_6$ (1.64 g, 4.99 mmol), K_2CO_3 (690 mg, 4.99 mmol), methanesulfonamide (475 mg, 4.99 mmol), $K_2OsO_4.2H_2O$ (12 mg, 0.033 mmol), hydroquinidine 1,4-phthalazinediyl diether (129 mg, 0.166 mmol), *tert*-butyl alcohol (20 ml) and water (20 ml) was stirred at room temperature for 14 days. Then Na_2SO_3 (1.5 g) was added and stirring was continued for 24 h. The layers were separated in a separatory funnel and the aqueous part was extracted with $CHCl_3$. The combined organic portions were dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on SiO_2 using petroleum ether/EtOAc (20:1 ⇒ 5:1) to give:

(a)starting olefin **5** (55 mg);
(b)diol **6a** (505 mg, 73% based on consumed olefin **5**). Mp 127–130°C (petroleum ether/EtOAc). ^{1}H-NMR: δ 0.44 (dd, J = 8.0, 5.1 Hz, 1 H), 0.65 (m, 1 H), 0.73 (s, 3 H), 0.85 (d, J = 6.7 Hz, 3 H), 0.92 (d, J = 6.7 Hz, 3 H), 0.97 (d, J = 6.7 Hz, 3 H), 1.03 (s, 3 H 19-Me), 2.78 (t, J = 2.6 Hz, 1 H, C_6-H), 3.33 (s, 3 H, OMe), 3.41 (q, J = 5.1 Hz, 1 H, C_{23}-H), 3.71 (t, J = 4.5 Hz, 1 H, C_{22}-H). ^{13}C-NMR: δ 10.87, 12.07, 12.45, 13.07, 19.26, 21.45, 22.05, 22.77, 24.10, 24.94, 26.75, 28.00, 30.55, 33.34, 35.06, 35.20, 40.20, 40.25, 41.37, 42.65, 43.35, 47.94, 52.86, 56.35, 56.56, 72.84, 76.39, 82.38.

[26−^{2}H$_3$](22*R*,23*R*,24*R*)-6β-Methoxy-3α,5-cyclo-24-methyl-5α-cholestan-22,23-diol diacetate (6)

A mixture of diol **6a** (463 mg, 1.03 mmol), pyridine (3.5 ml), acetic anhydride (0.37 ml, 3.92 mmol) and 4-*N*,*N*-dimethylaminopyridine (11 mg, 0.09 mmol) was kept at 45°C for 4.5 h. Then water was added and the mixture was extracted with $CHCl_3$. The organic fraction was dried over Na_2SO_4 and evaporated. The residue was purified by SiO_2 chromatography using petroleum ether/EtOAc (15:1 ⇒ 10:1) to afford diacetate **6** (510 mg, 93%) as an oil. ^{1}H-NMR: δ 0.43 (dd, J = 8.0, 5.1 Hz, 1 H), 0.65 (dd, J = 4.5, 4.5 Hz, 1 H), 0.72 (s, 3 H, 18-Me), 0.83 (d, J = 7.1 Hz, 3 H), 0.93 (d, J = 6.7 Hz, 3 H), 1.00 (d, J = 6.7 Hz, 3 H), 1.02 (s, 3 H, 19-Me), 2.03 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.77 (t, J = 2.7 Hz, 1 H, C_6-H), 3.32 (s, 3 H, OMe), 5.08 (dd, J = 7.5, 4.6 Hz, 1 H, C_{23}-H), 5.26 (d, J = 7.7 Hz, 1 H, C_{22}-H). ^{13}C-NMR: δ 10.82, 11.90, 13.03, 13.37, 19.26, 20.89, 20.96, 21.46, 22.41, 22.74, 24.05, 24.92, 26.57, 28.16, 30.49, 33.33, 34.87, 35.22, 37.72, 38.63, 40.18, 42.63, 43.30, 47.90, 53.06, 56.36, 56.56, 74.80, 77.60, 82.31, 170.55.

[26−^{2}H$_3$](22*R*,23*R*,24*S*)-24-Methycholest-5-en-3β,22,23-triol 22,23-diacetate (7)

A solution of diacetate **6** (443 mg, 0.83 mmol) and TsOH (94 mg, 0.55 mmol) in dioxane (21 ml) and water (6 ml) was stirred under argon at 80°C for 3 h. Then Et_3N (0.91 ml, 6.5 mmol) was added and solvents were evaporated to dryness. Column chromatography of the residue using $CHCl_3$/MeOH (20:1) as eluent gave diacetate **7** (429 mg, 99%). Mp 203–205°C (petroleum ether/EtOAc). ^{1}H-NMR: δ 0.69 (s, 3 H, 18-Me), 0.83 (d, J = 6.7 Hz, 3 H), 0.93 (d, J = 6.7 Hz, 3 H), 1.01 (d, J = 6.7 Hz, 3 H), 1.02 (s, 3 H, 19-Me), 2.04 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 3.51–3.58 (m, 1 H, C_3-H), 5.08 (dd, J = 7.4, 4.8 Hz, 1 H, C_{23}-H), 5.22–5.30 (m, 1 H, C_{22}-H), 5.35 (d, J = 5.1 Hz, 1 H, C_6-H). ^{13}C-NMR: δ 10.88, 11.57, 13.44, 19.39, 20.88, 20.92, 21.08, 22.39, 24.22, 26.67, 28.11, 29.70, 31.67, 31.80, 31.97, 36.49, 37.27, 37.77, 38.81, 39.75, 42.26, 42.31, 50.08, 53.05, 56.62, 71.77, 74.83, 77.61, 121.63, 140.74, 170.55.

[26−^{2}H$_3$](22*R*,23*R*,24*R*)-24-Methyl-3α,5-cyclo-5α-cholestan-6-on-22,23-diol diacetate (8)

A solution of alcohol **7** (484 mg, 0.93 mmol) and TsCl (644 mg, 3.38 mmol) in pyridine (3 ml) was kept at an ambient temperature overnight. Then the mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness to give crude tosylate **7a** (584 mg).

To a solution of the tosylate **7a** (584 mg) from the previous stage in acetone (62 ml), a solution of AcOK (242 mg) in water (5 ml) was added. The reaction mixture was heated under reflux for 21 h. Then it was cooled down to room temperature and Jones reagent (0.72 ml) was added. The mixture was stirred for 30 min, and then isopropyl alcohol (0.62 ml) was added to remove the excess of oxidizing agent. The reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by SiO_2 chromatography using petroleum ether/EtOAc (20:1 ⇒ 2:1) to afford diacetoxyketone **8** (300 mg, 62%). Mp 146–149°C (petroleum ether/EtOAc). ^{1}H-NMR: δ 0.73 (s, 3 H, 18-Me), 0.83 (d, J = 7.1 Hz, 3 H), 0.93 (d, J = 6.7 Hz, 3 H), 1.00 (s, 3 H, 19-Me), 1.01 (d, J = 6.7 Hz, 3 H), 2.03 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 5.08 (dd, J = 7.4, 4.8 Hz, 1 H, C_{23}-H), 5.26 (d, J = 6.7 Hz, 1 H, C_{22}-H). ^{13}C-NMR: δ 10.81, 11.61, 11.71, 13.41, 19.65, 20.85, 20.93, 22.38, 22.83, 23.95, 25.87, 26.62, 28.00, 33.45, 34.77, 35.28, 37.70, 38.71, 39.63, 42.58, 44.64, 45.98, 46.26, 46.70, 52.92, 56.79, 74.64, 77.48, 170.51, 170.56, 209.47. MS (APCI$^+$) [$C_{32}H_{48}D_3O_5$] *m/z* (MH)$^+$ calcd 518.39, found 518.6.

[26−$^2H_3$](22*R*,23*R*,24*R*)-24-Methy-5α-cholest-2-en-6-on-22,23-diol diacetate (9)

A solution of steroid **8** (154 mg, 0.297 mmol) and pyridinium bromide (158 mg, 0.98 mmol) in dimethylacetamide (3.2 ml) was heated under argon at 165–170°C for 5 h. Then it was cooled and the solvent was evaporated under reduced pressure. The resulting oil was mixed with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by SiO_2 chromatography using petroleum ether/EtOAc (15:1 ⇒ 12:1) to give enone **9** (120 mg, 78%). Mp 180–183°C (petroleum ether). ^{1}H-NMR: δ 0.69 (s, 3 H, 18-Me), 0.71 (s, 3 H, 19-Me), 0.83 (d, *J* = 7.1 Hz, 3 H), 0.93 (d, *J* = 7.1 Hz, 3 H), 1.01 (d, *J* = 6.7 Hz, 3 H), 2.04 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 5.08 (dd, *J* = 7.4, 4.8 Hz, 1 H, C_{23}-H), 5.25 (d, *J* = 7.4 Hz, 1 H, C_{22}-H), 5.57 (dd, *J* = 9.3, 1.6 Hz, 1 H, C_2-H), 5.69 (dd, *J* = 9.9, 2.2 Hz, 1 H, C_3-H). ^{13}C-NMR: δ 10.82, 11.62, 13.41, 13.49, 20.86, 20.93, 21.09, 21.69, 22.38, 23.85, 26.64, 27.90, 37.68, 38.74, 39.35, 39.44, 39.97, 42.70, 46.86, 52.99, 53.34, 53.81, 56.58, 74.67, 77.51, 124.45, 124.99, 170.54, 211.79. MS (APCI$^+$) [$C_{32}H_{48}D_3O_5$] *m/z* $(MH)^+$ calcd 518.39, found 518.2.

[26−$^2H_3$](22*R*,23*R*,24*R*)-24-Methy-5α-cholest-2-en-6-on-22,23-diol (10)

Diacetate **9** (58 mg, 0.112 mmol) was dissolved in a solution of KOH in MeOH (5%, 3 ml). The mixture was heated under reflux for 1 h, then cooled down and acidified by adding a mixture AcOH–MeOH (1:4) to reach pH 7. Solvents were evaporated *in vacuo* and the residue was purified by SiO_2 chromatography using $CHCl_3$/MeOH (25:1) to give diol **10** (40 mg, 83%). Mp 148–150°C (petroleum ether/EtOAc). ^{1}H-NMR: δ 0.69 (s, 3 H, 18-Me), 0.72 (s, 3 H, 19-Me), 0.86 (d, *J* = 7.1 Hz, 3 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.99 (d, *J* = 6.7 Hz, 3 H), 3.42 (q, *J* = 5.2 Hz, 1 H, C_{23}-H), 3.68–3.74 (m, 1 H, C_{22}-H), 5.55–5.61 (m, 1 H, C_2-H), 5.69 (ddd, *J* = 9.9, 5.0, 2.1 Hz, 1 H, C_3-H). ^{13}C-NMR: δ 10.88, 11.77, 12.45, 13.50, 21.14, 21.71, 22.03, 23.88, 26.79, 27.74, 37.78, 39.35, 39.49, 40.04, 40.19, 41.41, 42.69, 46.95, 52.68, 53.34, 53.84, 56.60, 72.71, 76.41, 124.50, 124.95, 211.97. MS (APCI$^+$) [$C_{28}H_{44}D_3O_3$] *m/z* $(MH)^+$ calcd 434.37, found 434.2.

[26−$^2H_3$](22*R*,23*R*,24*R*)-2α,3α-Epoxy-24-methy-5α-cholestan-6-on-22,23-diol ([26−$^2H_3$]secasterol) (11)

A solution of olefin **10** (29 mg, 0.067 mmol) and *m*-chloroperbenzoic acid (70%, 35 mg, 0.14 mmol) in $CHCl_3$ (1 ml) was stirred at room temperature for 1.5 h. Then it was washed successively with 12% NH_4OH and brine and dried over Na_2SO_4. After solvent removal and purification on a silica gel column using $CHCl_3$/MeOH (20:1), epoxide **11** (25 mg, 83%) was obtained as white crystals. Mp 165–167°C (petroleum ether/EtOAc). ^{1}H-NMR: δ 0.67 (s, 3 H, 18-Me), 0.71 (s, 3 H, 19-Me), 0.84 (d, *J* = 6.7 Hz, 3 H), 0.91 (d, *J* = 7.1 Hz, 3 H), 0.98 (d, *J* = 6.7 Hz, 3 H), 3.11–3.15 (m, 1 H, C_2- or C_3-H), 3.26–3.29 (m, 1 H, C_3- or C_2-H), 3.38–3.44 (m, 1 H, C_{23}-H), 3.69 (br. s., 1 H, C_{22}-H). ^{13}C-NMR: δ 10.84, 11.71, 12.43, 15.00, 20.99, 21.04, 22.03, 23.84, 26.75, 27.68, 37.51, 37.81, 38.42, 39.31, 40.18, 41.36, 42.58, 46.84, 49.86, 50.14, 52.37, 52.57, 52.99, 56.36, 72.61, 76.34, 211.46. MS (APCI$^+$) [$C_{28}H_{44}D_3O_4$] *m/z* $(MH)^+$ calcd 450.37, found 450.5.

[26−$^2H_3$](22*R*,23*R*,24*R*)-24-Methy-5α-cholestan-6-on-2α,3α,22,23-tetraol ([26−$^2H_3$]epicastasterone) (12)

A mixture of olefin **10** (38 mg, 0.088 mmol), *N*-methylmorpholine-*N*-oxide (17 mg), osmium tetroxide (0.3 mg), acetone (2 ml) and water (0.1 ml) was stirred at room temperature for 7 h. Then it was diluted with water (3 ml), the precipitate was filtered off, washed with water and dissolved in acetone. The solvent was evaporated *in vacuo* and the residue was purified by SiO_2 chromatography using $CHCl_3$/MeOH (15:1) to give tetraol **12** (21 mg, 51%). Mp 229–231°C (EtOAc). ^{1}H-NMR: 0.70 (s, 3 H, 18-Me), 0.77 (s, 3 H, 19-Me), 0.87 (d, *J* = 7.1 Hz, 3 H), 0.93 (d, *J* = 7.1 Hz, 3 H), 1.00 (d, *J* = 6.7 Hz, 3 H), 2.70 (d, *J* = 12.2 Hz, 1 H, C_5-H), 3.39–3.45 (m, 1 H, C_{23}-H), 3.71 (br. s., 1 H, C_{22}-H), 3.76 (d, *J* = 8.0 Hz, 1 H, C_2-H), 4.05 (br. s., 1 H, C_3-H). ^{13}C-NMR: δ 10.94, 11.90, 12.49, 13.56, 21.30, 22.00, 23.94, 26.45, 26.94, 27.79, 37.80, 39.56, 40.31, 40.40, 41.66, 42.57, 42.92, 46.75, 50.81, 52.84, 53.87, 56.66, 68.38, 68.49, 72.75, 76.46, 211.65. MS (APCI$^+$) [$C_{28}H_{46}D_3O_5$] *m/z* $(MH)^+$ calcd 468.38, found 468.2.

[26−$^2H_3$](22*R*,23*R*,24*R*)-24-Methy-5α-cholestan-6-on-2α,3α,22,23-tetraol 22,23-diacetate (13)

The title compound was obtained from enone **9** in 76% yield according to the procedure described for the preparation of ([26-$^2H_3$]epicastasterone) (**12**). Mp 96–98°C (petroleum ether). ^{1}H-NMR: 0.66 (s, 3 H, 18-Me), 0.75 (s, 3 H, 19-Me), 0.82 (d, *J* = 7.1 Hz, 3 H), 0.92 (d, *J* = 7.1 Hz, 3 H), 1.00 (d, *J* = 6.7 Hz, 3 H), 2.03 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.67 (dd, *J* = 12.7, 2.7 Hz, 1 H, C_5-H), 3.73–3.80 (m, 1 H, C_2-H), 4.05 (d, *J* = 2.2 Hz, 1 H, C_3-H), 5.07 (dd, *J* = 7.4, 4.8 Hz, 1 H, C_{23}-H), 5.24 (d, *J* = 7.7 Hz, 1 H, C_{22}-H). ^{13}C-NMR: δ 10.77, 11.66, 13.37, 13.53, 20.88, 20.93, 21.11, 22.36, 23.82, 26.25, 26.58, 27.86, 37.62, 38.64, 39.28, 40.12, 42.51, 42.77, 46.60, 50.65, 52.87, 53.57, 56.42, 68.22, 68.34, 74.60, 77.52, 170.64, 212.01.

[26−$^2H_3$](22*R*,23*R*,24*S*)-24-Methy-5α-cholestan-6-on-2α,3α,22,23-tetraol tetraacetate (14)

A mixture of the diol **13** (80 mg, 0.145 mmol), Ac_2O (0.1 ml, 1.06 mmol), pyridine (1 ml) and 4-*N*,*N*-dimethylaminopyridine (3 mg) was kept at room temperature for 24 h. The solvents were evaporated under reduced pressure. The residue was purified by SiO_2 chromatography using $CHCl_3$/MeOH (100:1) to give tetraacetate **14** (90 mg, 97%) as an oil. ^{1}H-NMR: δ 0.67 (s, 3 H, 18-Me), 0.81 (d, *J* = 6.7 Hz, 3 H), 0.83 (s, 3 H, 19-Me), 0.92 (d, *J* = 6.7 Hz, 3 H), 1.00 (d, *J* = 6.7 Hz, 3 H), 1.99 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.09 (s, 3 H, OAc), 4.91–4.98 (m, 1 H, C_2-H), 5.07 (dd, *J* = 7.4, 4.8 Hz, 1 H, C_{23}-H), 5.24 (d, *J* = 7.1 Hz, 1 H, C_{22}-H), 5.36–5.41 (m, 1 H, C_3-H). ^{13}C-NMR: δ 10.77, 11.68, 13.40, 13.53, 20.85, 20.92, 21.03, 21.11, 21.19, 22.35, 23.81, 24.79, 26.61, 27.86, 37.51, 37.67, 38.68, 39.24, 42.38, 42.80, 46.40, 51.78, 52.96, 53.61, 56.44, 68.09, 69.08, 74.63, 77.48, 169.98, 170.24, 170.27, 170.57, 210.52.

[26−$^2H_3$](22*R*,23*R*,24*R*)-24-Methy-B-homo-7-oxa-5α-cholestan-6-on-2α,3α,22,23-tetraol tetraacetate (15)

A solution of trifluoroperbenzoic acid was prepared by adding trifluoroacetic anhydride (3.5 ml) to a stirred emulsion of H_2O_2 (30%, 0.4 ml) in CH_2Cl_2 (8.4 ml) at 0°C. An aliquot of the obtained trifluoroacetic acid solution (5.87 ml) was added to a stirred solution of tetraacetate **14** (80 mg, 0.126 mmol) in CH_2Cl_2 (4 ml) at 0°C.

Stirring was continued for 5 h at these conditions, and then the mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by SiO_2 chromatography using petroleum ether/EtOAc (3:1 ⇒ 2:1) to give tetraacetate **15** (45 mg, 55%). Mp 103–105°C (petroleum ether/EtOAc). ^{1}H-NMR: δ 0.72 (s, 3 H, 18-Me), 0.81–0.91 (m, 6 H), 0.94 (d, *J* = 7.1 Hz, 3 H), 0.98 (d, *J* = 6.7 Hz, 3 H), 1.00 (s, 3 H, 19-Me), 2.01 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 3.00 (dd, *J* = 12.2, 4.5 Hz, 1 H, C_5-H), 4.00–4.09 (m, 1 H, C_7-H), 4.09–4.17 (m, 1 H, C_7-H), 4.88 (ddd, *J* = 12.7, 4.6, 2.9 Hz, 1 H, C_2-H), 5.07 (dd, *J* = 7.2, 5.0 Hz, 1 H, C_{23}-H), 5.23 (d, *J* = 6.1 Hz, 1 H, C_{22}-H), 5.37 (m, 1 H, C_3-H). ^{13}C-NMR: δ 10.82, 11.50, 13.37, 15.44, 20.82, 20.90, 21.02, 21.11, 22.28, 22.34, 22.64, 24.73, 26.71, 27.83, 29.34, 31.57, 37.80, 38.41, 38.82, 38.91, 39.20, 39.51, 42.05, 42.53, 51.28, 53.03, 58.42, 67.95, 68.94, 70.41, 74.57, 169.90, 170.18, 170.53, 174.97. MS (APCI$^+$) $[C_{36}H_{54}D_3O_{10}]$ *m/z* $(MH)^+$ calcd 652.41, found 652.3.

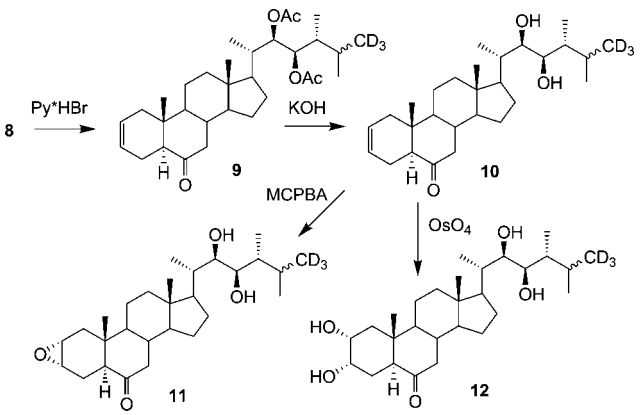
[26−^{2}H$_3$](22*R*,23*R*,24*R*)-24-Methy-B-homo-7-oxa-5α-cholestan-6-on-2α,3α,22,23-tetraol ([26 −^{2}H$_3$]epibrassinolide) (16)

A solution of tetraacetate **15** (40 mg, 0.061 mmol) in 5% KOH in MeOH (4 ml) was heated under reflux for 1.5 h. Then the reaction mixture was cooled down to room temperature and 25% HCl (1 ml) was added. After keeping for 1 h, the mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Crystallization from EtOAc/petroleum ether provided **16** (28 mg, 94%) as white crystals. Mp 230–232°C. ^{1}H-NMR: δ 0. 0.71 (s, 3 H, 18-Me), 0.86 (d, *J* = 7.1 Hz, 3 H), 0.92 (d, *J* = 7.1 Hz, 3 H), 0.93 (s, 3 H, 19-Me), 0.98 (d, *J* = 6.4 Hz, 3 H), 3.13 (dd, *J* = 12.3, 4.0 Hz, 1 H), 3.39–3.45 (m, 1 H, C_{23}-H), 3.67 (m, 1 H, C_{22}-H), 3.67–3.78 (m, 1 H, C_2-H), 4.01–4.16 (m, 3 H, C_2- and C_7-H). ^{13}C-NMR: δ 10.87, 11.64, 12.38, 15.47, 22.03, 22.24, 24.76, 26.80, 27.70, 31.03, 38.32, 39.23, 39.57, 40.21, 40.89, 41.38, 41.50, 42.49, 50.89, 51.24, 52.58, 58.17, 68.07, 68.15, 70.44, 72.57, 76.41, 176.16. MS (APCI$^+$) $[C_{28}H_{46}D_3O_6]$ *m/z* $(MH)^+$ calcd 484.37, found 484.4.

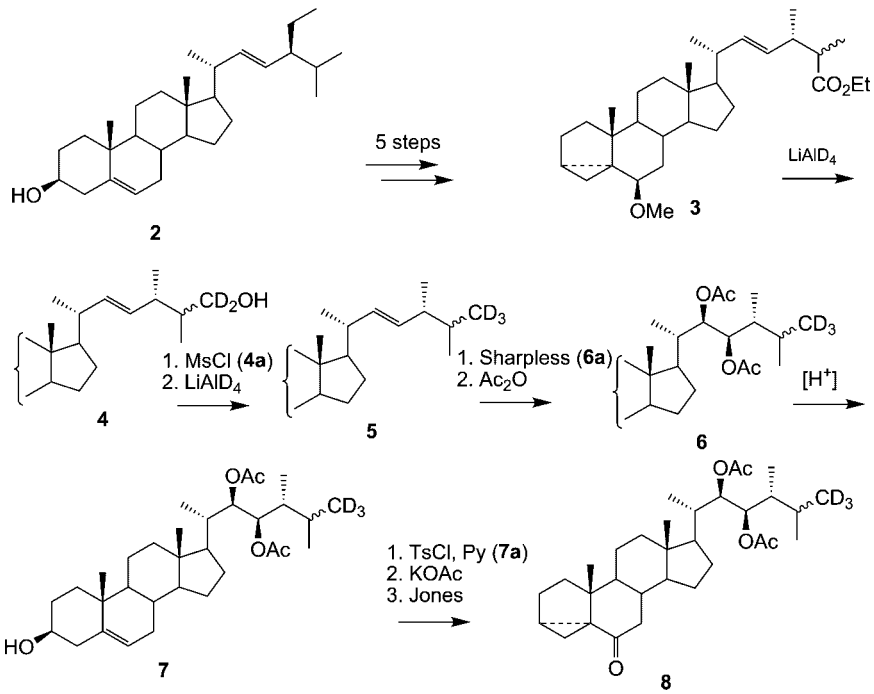
Results and discussion

Synthesis of the target compounds was based on the ester **3** available in five steps from commercial stigmasterol **2**.[17] Labeling with deuterium was achieved at the very beginning of the synthetic sequence. Ester **3** was first submitted to the reduction with lithium aluminum deuteride to give alcohol **4** containing two deuteriums in the molecule (Scheme 1). Its mesylation followed by similar reduction with $LiAlD_4$ afforded the three-deuterio derivative **5** as enantioisotopomeric pair of compounds. Asymmetric dihydroxylation of Δ^{22}-double bond in olefin **5** using Sharpless protocol led, after acetylation, to (22*R*,23*R*)-diacetate **6**. A methoxy at C-6 was replaced by oxo group in four steps to give cycloketone **8** to ensure further construction of brassinosteroid cyclic part.

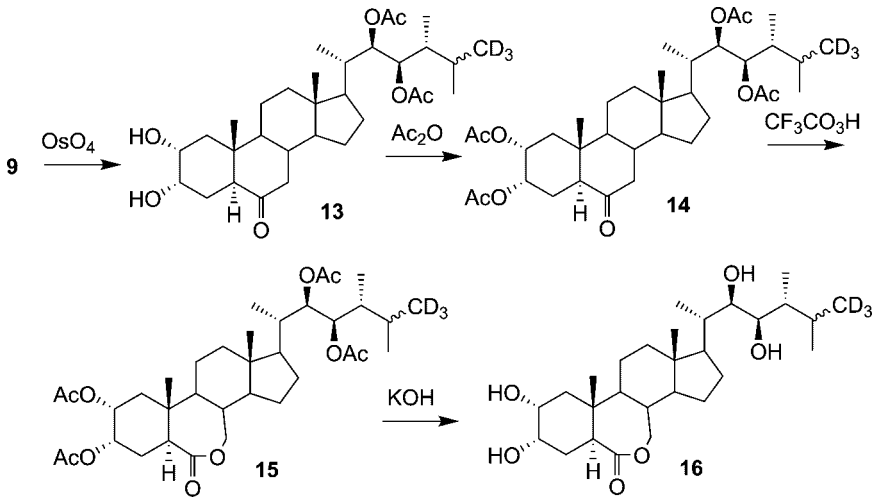
Heating of 3α,5-cyclo-6-ketone **8** in boiling dimethylacetamide in the presence of pyridine hydrobromide led to the rearranged product **9** containing a Δ^2-double bond (Scheme 2).



Scheme 2.



Scheme 1.



Scheme 3.

Removal of acetyl protecting groups in **9** gave diol **10,** which is a deuterated analogue of 24-episecasterol. The latter is expected to be a biosynthetic precursor of 24-epibrassinolide in a pathway similar to that described for secasterol and brassinolide. Recently, non-deuterated 24-episecasterol was prepared and found to be cytotoxic in human breast carcinoma MCF-7 cells.[18] The two electrophilic reactions, epoxidation and dihydroxylation of the olefin **10**, gave epoxide **11** and 24-epicastasterone **12**, correspondingly. Compound **11** is a 2α,3α-epimer of natural brassinosteroid 24-episecasterone found in *Lychnis viscaria* seeds.[19] Although it has not been described as a natural product before, such a possibility cannot be excluded.[13]

Although 24-epibrassinolide **16** could be obtained in one step by the Baeyer–Villiger oxidation of 24-epicastasterone **12**, our previous experience on brassinosteroid synthesis showed that more lengthy route with preliminary protection of hydroxy groups before lactonization gave in general more pure final product. That is why we performed synthesis of 24-epibrassinolide **16** via endiacetate **9**. Its dihydroxylation with OsO_4 afforded dioxydiacetate **13**, which was further acetylated to epicastasterone tetraacetate **14** (Scheme 3). It was subjected to the Baeyer–Villiger oxidation, and after chromatographic purification of the lactone **15** and saponification of the latter, deuterated 24-epibrassinolide **16** was obtained. The isotopic purity of deuterated brassinosteroids was controlled by mass spectra. Thus, for [26-$^2H_3$]epibrassinolide **16** the composition ratio of $^2H_3$:$^2H_2$:$^2H_1$:$^2H_0$ was found to be 99.2:0.3:0.2:0.3.

In conclusion, a number of three-deuterated (24*R*)-methyl brassinosteroids have been prepared. These new labeled compounds are valuable tools for investigating minor biosynthetic pathways and metabolic transformations of 24-epibrassinolide.

Acknowledgement

The work was supported by the Belarusian Republican Foundation for Basic Research (project no. X10P-118).

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