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# SYNTHESIS OF [26-$^2H_3$]-CAMPESTERIN AND [26-$^2H_3$]-CAMPESTANOL, DEUTERATED ANALOGS OF BIOSYNTHETIC PRECURSORS OF 28C-BRASSINOSTEROIDS

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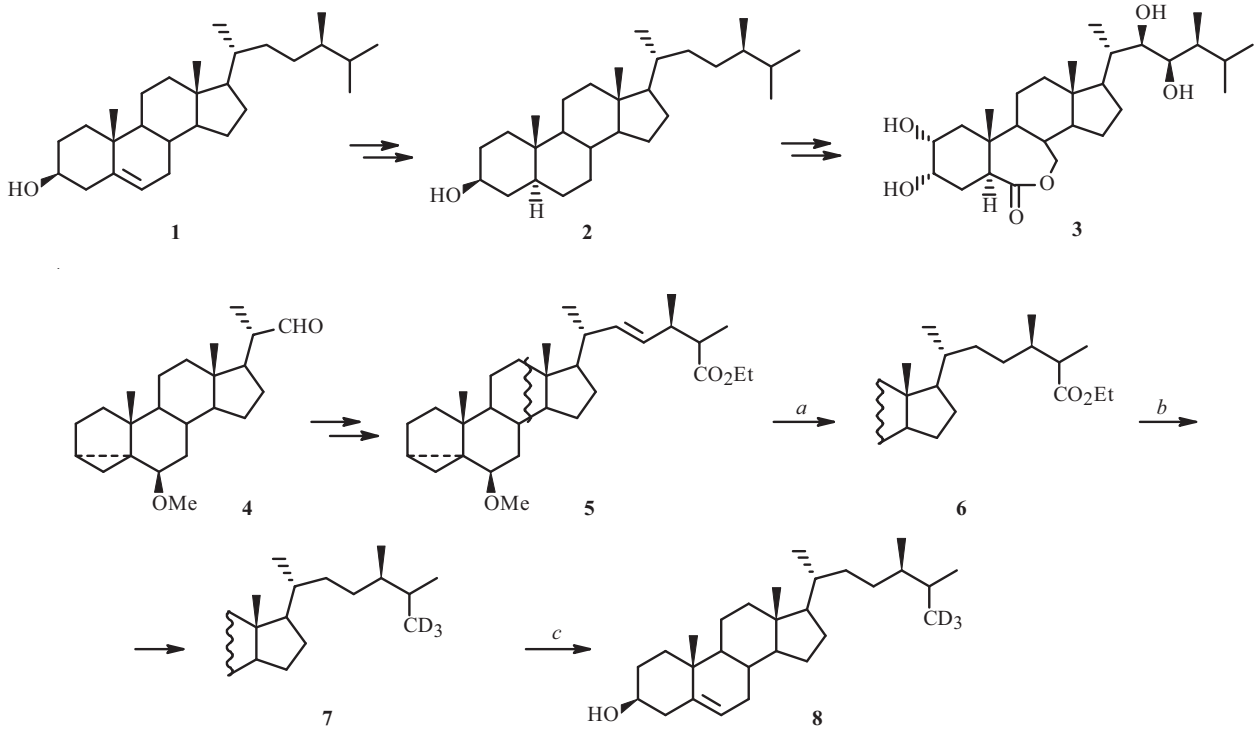
*[26-$^2H_3$]-Campesterin and [26-$^2H_3$]-campestanol were synthesized as standards for studying brassinolide biosynthesis.*

**Keywords:** brassinosteroids, brassinolide, campesterin, campestanol, labeled compounds.

Over 30 years have passed since the discovery of brassinosteroids [1]. However, many questions about the biosynthesis of these compounds in plants [2, 3], especially in the early stages, currently remain open. This is applicable even to such well studied compounds as 28C-brassinosteroids and, in particular, brassinolide [4]. The starting compound in the biosynthesis of 28C-brassinosteroids is considered to be campesterin (**1**), which is transformed into brassinolide **3** or its analogs as a result of a combination of biochemical transformations including intermediate formation of campestanol (**2**) [5].




*a*. $H_2$, $PtO_2$; *b*. 1,3. $LiAlD_4$, 2. TsCl; *c*. TsOH

One of the principal methods for establishing biosynthetic sequences is incubation of isotopically labeled analogs of the studied compounds with plant culture and subsequent study of their metabolic products by GC–MS [6].

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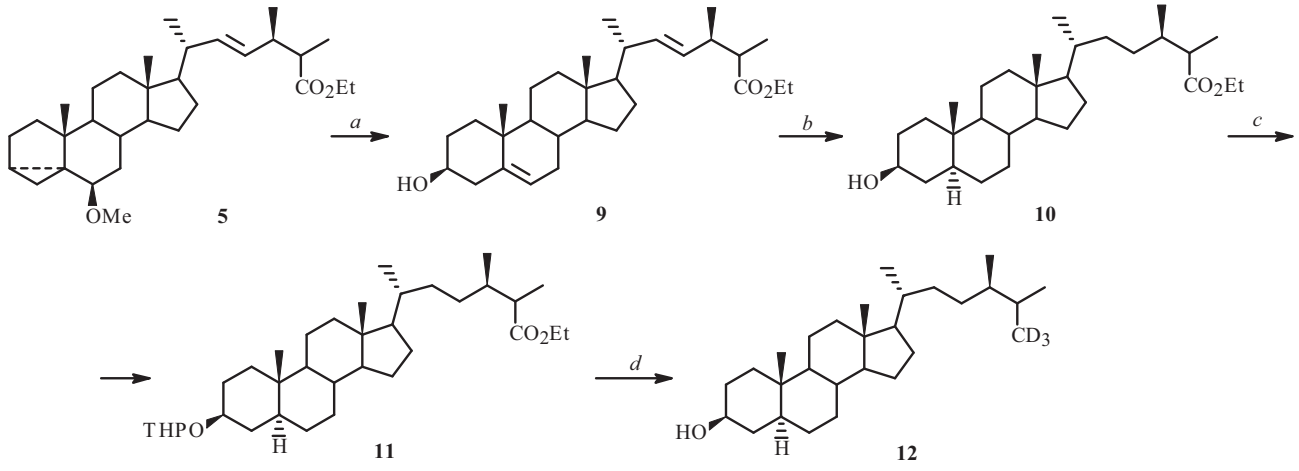
Herein the synthesis of analogs of campesterin and campestanol, which are starting compounds in the initial stage of brassinolide biosynthesis, labeled with three deuterium (D) atoms on C-26 is examined. The method selected for introducing the label was tested during the synthesis of several deuterated biosynthetic precursors of this phytohormone [7] and combined a relatively simple preparation of them with the ability to branch out to compounds with a set of required properties [8].

[26-$^2H_3$]Campesterin (**8**) was synthesized in quantitative yield by hydrogenation over platinum oxide of the double bond in **5** (prepared from aldehyde **4** in three steps in 61% yield [9]).

The isotopic label was introduced through successive reduction steps of ester **6** by $LiAlD_4$, tosylation, and desulfurization of the intermediate tosylate. Regeneration of the 3$\beta$-hydroxy-$\Delta^5$ system using *p*-toluenesulfonic acid gave labeled campesterin **8**.

Compound **8** could be transformed into the required [26-$^2H_3$]campestanol **12** in one step by catalytic hydrogenation of the $\Delta^5$-bond. However, partial loss of the label was possible because of simultaneous occurrence of hydrogenation and dehydrogenation processes. The isotopic purity of the target product could be reduced by storing the labeled derivatives over catalyst. The problem was solved by carrying out the hydrogenation of both double bonds of **9** that did not contain the isotopic label. The three D atoms were introduced on C-26 like in the synthesis of [26-$^2H_3$]campesterin **8**. The C-3 hydroxyl was protected beforehand in order to avoid side reactions during tosylation.




*a*. TsOH; *b*. $H_2$, $PtO_2$; *c*. DHP, PPTS; *d*. 1,3. $LiAlD_4$, 2. TsCl, 4. TsOH

Thus, we synthesized deuterated analogs of two precursors of the natural phytohormone brassinolide, [26-$^2H_3$]campesterin and [26-$^2H_3$]campestanol, as labeled standards for performing biosynthetic investigations.

## EXPERIMENTAL

NMR spectra were recorded on a Bruker Avance DRX-500 (Germany) instrument. Chemical shifts were determined relative to residual solvent resonances ($CHCl_3$, δ 7.26 ppm for $^1H$; δ 77.00 ppm for $^{13}C$). Mass spectra were measured in an LCQ Fleet mass spectrometer (Thermo Electron Corp., USA) in positive-ion mode with chemical ionization at atmospheric pressure (APCI). Melting points were measured on a Kofler block. TLC used Kieselgel 60 $F_{254}$ chromatographic plates. Column chromatography was carried out over Kieselgel 60 silica gel (VWR, Art. 7734).

**Ethyl (24*R*)-6$\beta$-Methoxy-24-methyl-3$\alpha$,5-cyclo-5$\alpha$-cholestan-26-oate (6).** A two-necked flask was charged with EtOH (2 mL) and $PtO_2$ (10 mg, 0.044 mmol) and evacuated and filled with $H_2$ three times in succession. The suspension was stirred in the $H_2$ atmosphere for 5 min until the $PtO_2$ was reduced (black flakes), treated with a solution of ethyl (22*E*,24*R*)-6$\beta$-methoxy-24-methyl-3$\alpha$,5-cyclo-5$\alpha$-cholest-22-en-26-oate (**5**, 198 mg, 0.421 mmol) in THF (2 mL) and more EtOH (2 mL). The mixture was hydrogenated and stirred for 2 h (TLC monitoring). The catalyst was filtered off through a layer of silica gel. The filtrate was evaporated in vacuo to afford **6** (199 mg, 100%) as an oil.

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.42 (1H, dd, J = 8, 5, H-4), 0.64 (1H, t, J = 5, H-4), 0.70 (3H, s, H-18), 1.01 (3H, s, H-19), 1.09 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.25 (3H, t, J = 7, -$O_2CH_2C\underline{H}_3$), 2.31 (1H, m, H-25), 2.76 (1H, t, J = 3, H-6), 3.31 (3H, s, OMe), 4.11 (2H, m, -$CO_2C\underline{H}_2CH_3$).

$^{13}$C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.23, 13.04, 13.82, 14.30, 17.15, 18.54, 19.27, 21.48, 22.74, 24.15, 24.94, 28.30, 29.48, 29.68, 30.45, 32.94, 33.33, 35.02, 35.27, 35.67, 36.03, 40.26, 42.76, 43.36, 44.95, 47.99, 56.24, 56.52, 59.91, 82.41, 176.37.

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 441 (100) [M – MeOH + H]$^+$.

**(24*R*)-[26-$^2H_3$]-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestane (7).** A solution of **6** (189 mg, 0.400 mmol) in $Et_2O$ (4 mL) was stirred, treated in portions with $LiAlD_4$ (34 mg, 0.810 mmol), stirred for 15 min, and treated successively with $H_2O$ (34 μL), NaOH solution (34 μL, 15%), and $H_2O$ (102 μL). The resulting precipitate was filtered off. The filtrate was evaporated to dryness. The solid (66 mg) was dissolved in Py (4 mL), treated with TsCl (152 mg, 0.797 mmol), held at room temperature for 12 h, diluted with $H_2O$, and extracted with $CHCl_3$ (3 × 15 mL). The combined organic extracts were dried over $Na_2SO_4$ and evaporated in vacuo to afford a solid (267 mg) that was dissolved in $Et_2O$ (6 mL), stirred, treated with $LiAlD_4$ (77 mg, 1.8 mmol), stirred at room temperature for 6 h, and treated successively with $H_2O$ (77 μL), NaOH solution (77 μL, 15%), and $H_2O$ (231 μL). The precipitate was filtered off. The filtrate was evaporated in vacuo. The solid was placed on a $SiO_2$ column and eluted with toluene to afford **7** (150 mg, 90% for three steps) as an oil.

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.42 (1H, dd, J = 8, 5, H-4), 0.64 (1H, t, J = 5, H-4), 0.71 (3H, s, H-18), 0.77 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.84 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.90 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.02 (3H, s, H-19), 2.77 (1H, t, J = 3, H-6), 3.32 (3H, s, OMe).

$^{13}$C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.25, 13.05, 15.37, 18.66, 19.29, 20.13, 21.51, 22.77, 24.18, 24.96, 28.32, 29.70, 30.32, 30.47, 32.17, 33.34, 33.67, 35.03, 35.29, 35.89, 38.77, 40.29, 42.76, 43.38, 48.02, 56.29, 56.52, 56.54, 82.44.

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 386 (100) [M – MeOH + H]$^+$.

**(24*R*)-[26-$^2H_3$]-24-Methylcholest-5-en-3β-ol 8/[26-$^2H_3$]-campesterin (8).** A solution of **7** (141 mg, 0.338 mmol) in dioxane (4.5 mL) was treated with $H_2O$ (1.5 mL) and $TsOH \cdot H_2O$ (9.6 mg, 0.051 mmol), held for 3 h at 75°C, treated with Py (0.5 mL), and evaporated to dryness. The dry solid was placed on a $SiO_2$ column and eluted by petroleum ether:EtOAc (10:1→5:1) to afford deuterated **8** (115 mg, 85%) as white crystals, mp 156–158°C (hexane).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/ Hz): 0.67 (3H, s, H-18), 0.76 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.84 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.91 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.00 (3H, s, H-19), 3.52 (1H, tt, J = 11.5, H-3), 5.35 (1H, m, H-6).

$^{13}$C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 11.86, 15.37, 18.19, 18.70, 19.38, 20.12, 21.09, 24.29, 28.22, 29.69, 30.32, 31.67, 31.91, 32.17, 33.71, 35.87, 36.51, 37.27, 38.80, 39.80, 42.32, 50.16, 56.13, 56.78, 71.80, 121.69, 140.77.

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 445 (100) [M + MeCN + H]$^+$, 386 (49) [M – $H_2O$ + H]$^+$.

**Ethyl (24*R*)-24-Methylcholest-5,22-dien-26-oate (9).** Ester **5** (195 mg, 0.414 mmol) was dissolved in dioxane (5.5 mL) and treated with $H_2O$ (1.8 mL) and $TsOH \cdot H_2O$ (11.8 mg, 0.062 mmol). The mixture was heated at 75°C for 1.5 h, treated with Py (0.5 mL), and evaporated in vacuo. The dry solid was placed on a $SiO_2$ column and eluted with petroleum ether:EtOAc (5:1) to afford **9** (172 mg, 91%) as white crystals, mp 115–118°C (EtOAc:hexane).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, H-18), 0.96 (3H, d, J = 7, CHC$\underline{H}_3$), 0.98–1.01 (6H, m, H-19 and >CHC$\underline{H}_3$), 1.06 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.25 (3H, t, J = 7, -$CO_2CH_2C\underline{H}_3$), 3.52 (1H, m, H-3), 4.12 (2H, m, -$CO_2C\underline{H}_2CH_3$), 5.05–5.28 (2H, m, H-22 and H-23), 5.34 (1H, m, H-6).

$^{13}$C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.02, 14.27, 15.03, 19.31, 19.38, 20.79, 21.05, 24.31, 28.72, 31.64, 31.87, 36.50, 37.25, 39.65, 39.97, 40.14, 40.27, 42.28, 45.54, 50.13, 55.75, 56.81, 60.03, 71.78, 121.65, 129.98, 137.74, 140.75, 176.36.

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 457 (100) [M + H]$^+$, 439 (66) [M – $H_2O$ + H]$^+$.

**Ethyl (24*R*)-24-Methylcholestan-3β-ol-26-oate (10).** A two-necked flask was charged successively with EtOH (3 mL), $PtO_2$ (3.3 mg, 14 μmol), and a solution of **9** (66 mg, 0.14 mmol) in THF (1 mL). The flask was evacuated and filled with $H_2$ three times in succession. The suspension was stirred vigorously under the $H_2$ atmosphere for 1 d. When the reaction was finished (TLC monitoring) the catalyst was filtered off through a layer of $SiO_2$. The filtrate was evaporated in vacuo to isolate saturated steroid **10** (63 mg, 94%) as white crystals, mp 93–94°C (hexane).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.64 (3H, s, H-18), 0.79 (3H, s, H-19), 0.84–0.89 (6H, m, >CHC$\underline{H}_3$), 1.09 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.24 (3H, t, J = 7, -$CO_2CH_2C\underline{H}_3$), 2.30 (1H, m, H-25), 3.52 (1H, m, H-3), 4.12 (2H, m, -$CO_2C\underline{H}_2CH_3$).

$^{13}$C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.05, 12.29, 12.62, 13.80, 14.29, 15.60, 17.15, 18.51, 21.22, 24.18, 28.23, 28.70, 29.44, 29.68, 31.04, 31.47, 32.05, 32.94, 33.27, 35.43, 35.47, 35.65, 36.01, 36.97, 38.16, 40.00, 42.58, 44.69, 44.82, 44.94, 54.31, 56.17, 56.46, 59.93, 71.35, 176.40.

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 443 (100) [M – $H_2O$ + H]$^+$, 397 (23) [M – $H_2O$ – EtOH]$^+$.

**Ethyl (24*R*)-3$\beta$-Tetrahydropyranyloxy-24-methylcholestan-26-oate (11).** A solution of **10** (58 mg, 0.13 mmol) and dihydropyran (23 μL, 0.25 mmol) in anhydrous $CH_2Cl_2$ (1 mL) was treated with pyridinium *p*-toluenesulfonate (3.2 mg, 13 μmol), stirred at room temperature for 8 h, and evaporated in vacuo. The solid was placed on a $SiO_2$ column and eluted with toluene to afford **11** (64 mg, 93%) as an oil.

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.63 (3H, s, H-18), 0.79 (3H, s, H-19), 0.85–0.89 (6H, m, >CHC$\underline{H}_3$), 1.09 (3H, d, J = 7, >CHC$\underline{H}_3$), 2.30 (1H, m, H-25), 3.43–3.95 (3H, m, H-3 and OTHP), 4.11 (2H, m, -$CO_2$C$\underline{H}_2$$CH_3$), 4.67–4.73 (1H, m, OTHP).

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 443 (100) [M – THPOH + H]$^+$, 397 (20) [M – THPOH – EtOH]$^+$, 341 (9) [$C_{25}H_{41}$ (cleavage at C-24/C-25) – THPOH]$^+$.

**(24*R*)-[26-$^2H_3$]-24-Methylcholestan-3$\beta$-ol 12/[26-$^2H_3$]campestanol (12).** A solution of **11** (64 mg, 0.117 mmol) in $Et_2O$ (1.5 mL) was stirred, treated with $LiAlD_4$ (13 mg, 0.310 mmol), stirred for 30 min at room temperature, and treated successively with $H_2O$ (64 μL), NaOH solution (64 μL, 15%), and $H_2O$ (192 μL). The resulting precipitate was filtered off. The filtrate was evaporated to dryness. The crude solid (70 mg) was dissolved in Py (1 mL), treated with TsCl (45 mg, 0.236 mmol), held at room temperature for 12 h, diluted with $H_2O$, and extracted with $CHCl_3$ (3 × 10 mL). The combined organic extracts were dried over $Na_2SO_4$ and evaporated in vacuo. The solid (80 mg) was dissolved in $Et_2O$ (2 mL), stirred, treated with $LiAlD_4$ (25 mg, 0.595 mmol), stirred at room temperature for 6 h, and worked up successively with $H_2O$ (13 μL), NaOH solution (13 μL, 15%), and $H_2O$ (39 μL). The precipitate was filtered off. The filtrate was evaporated in vacuo. The dry solid (63 mg) was dissolved in EtOH (1 mL) and THF (0.5 mL), treated with TsOH·$H_2O$ (2.2 mg, 11.6 μmol), stirred at 55°C for 2 h, treated with Py (0.05 mL), and evaporated to dryness. The solid was placed on a $SiO_2$ column and eluted with petroleum ether:EtOAc (7:1→5:1) to afford **12** (48 mg, 67%) as white crystals, mp 138–140°C (EtOH).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.64 (3H, s, H-18), 0.76 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.80 (3H, s, H-19), 0.84 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.89 (3H, d, J = 7, >CHC$\underline{H}_3$), 3.58 (1H, m, H-3).

$^{13}$C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.07, 12.31, 15.36, 18.64, 20.12, 21.25, 24.21, 28.24, 28.73, 29.70, 30.30, 31.52, 32.08, 32.17, 33.67, 35.45, 35.51, 35.88, 37.00, 38.21, 38.77, 40.04, 42.59, 44.85, 54.36, 56.24, 56.50, 71.38.

Mass spectrum (APCI$^+$, *m*/*z*, $I_{rel}$, %): 388 (100) [M – $H_2O$ + H]$^+$, 341 (34) [$C_{25}H_{41}$ (cleavage at C-24/C-25) – $H_2O$]$^+$, 313 (30) [$C_{23}H_{37}$ (cleavage at C-23/C-24) – $H_2O$]$^+$.

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