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SYNTHESIS OF [26-$^2H_3$]-6-DEOXO-24-EPICASTASTERONE

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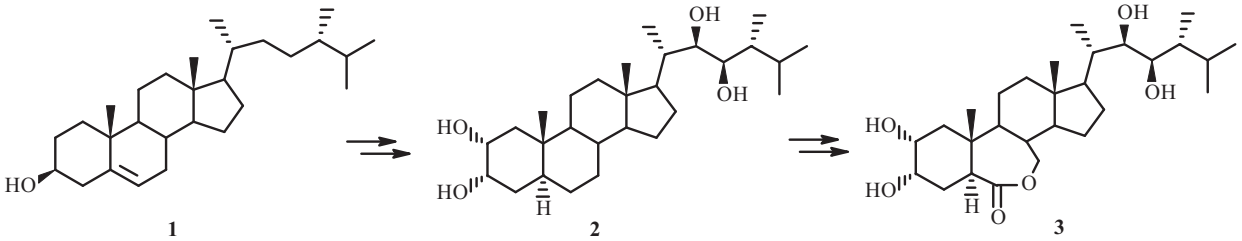
Deuterium-labeled 6-deoxo-24-epicastasterone was synthesized as a tool for studying the biosynthesis of 24-epibrassinolide in plants. The carbon skeleton of the side chain with the required C-24 methyl stereochemistry was constructed through a Claisen rearrangement. Deuterium atoms were introduced by reduction with $LiAlD_4$ of the obtained γ,δ-unsaturated ester. Both diols were introduced as a result of Sharpless asymmetric dihydroxylation of the intermediate $\Delta^{2,22}$-diene.

Keywords: brassinosteroids, epibrassinolide, 6-deoxo-24-epicastasterone, deoxygenation, labeled compounds.

Biosynthesis of brassinosteroids [1] in plants is a multi-step process that includes up to 10 biochemical steps for transformation of starting sterol into the target phytohormone [2–4]. The process is studied most completely for the progenitor of this class of plant hormones, brassinolide. Investigations showed that several simultaneously functioning pathways exist for biosynthesis of campesterin, a biosynthetic precursor of brassinolide [5, 6]. Obviously, an analogous situation may occur for other phytohormones of this class, including the most important with respect to practical use, 24-epibrassinolide [7]. Unfortunately, the biosynthesis of such hormones is studied in less detail. One reason for this is the unavailability of the required labeled derivatives of actual or proposed brassinosteroids for use as internal analytical standards [8, 9]. Such compounds should satisfy several requirements [10]. In addition to chemical and isotopic purity, compounds used as standards should contain at least three deuterium (D) atoms in order to avoid overlap with [M + 1] and [M + 2] peaks that belong to ^{13}C and ^{18}O isotopic isomers. An important condition is the presence of a label in a position that is not subject to isotopic exchange. We synthesized earlier several labeled derivatives of brassinolide that contained three [11] or six [12] D atoms in addition to trideuterated derivatives of 24-epibrassinolide and several of its biosynthetic precursors [13] with a functional group in ring B.

A study of brassinolide biosynthesis showed that one of its most important biosynthetic pathways includes initial introduction of hydroxyl groups in the side chain with subsequent functionalization of ring B (late C-6 oxidation biosynthetic pathway) [5]. The observation in plants of 6-deoxo-24-epicastasterone **2** [14] argues in favor of the existence of an analogous biosynthetic pathway for 24-epibrassinolide **3** from 22,23-dihydrobrassicasterin **1**. More detailed biosynthetic investigations, a required condition of which is the availability of a labeled standard, could provide a final answer. Therefore, the main goal of the present work was to synthesize [26-$^2H_3$]-6-deoxo-24-epicastasterone as an internal standard for biochemical investigations.



1 **2** **3**

The position for introducing the label was selected based on previous experience with the chemical synthesis of deuterated brassinosteroids for biochemical investigations. The best results on the usefulness of the target compounds for resolving biochemical problems and the effectiveness of their chemical synthesis were obtained for derivatives containing three D atoms in the side chain terminal position.

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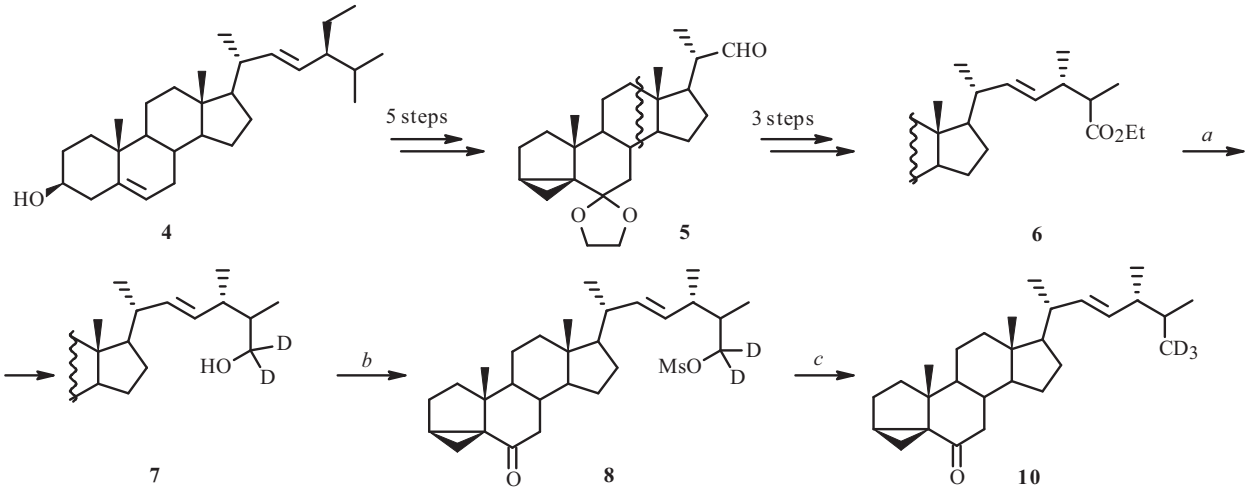
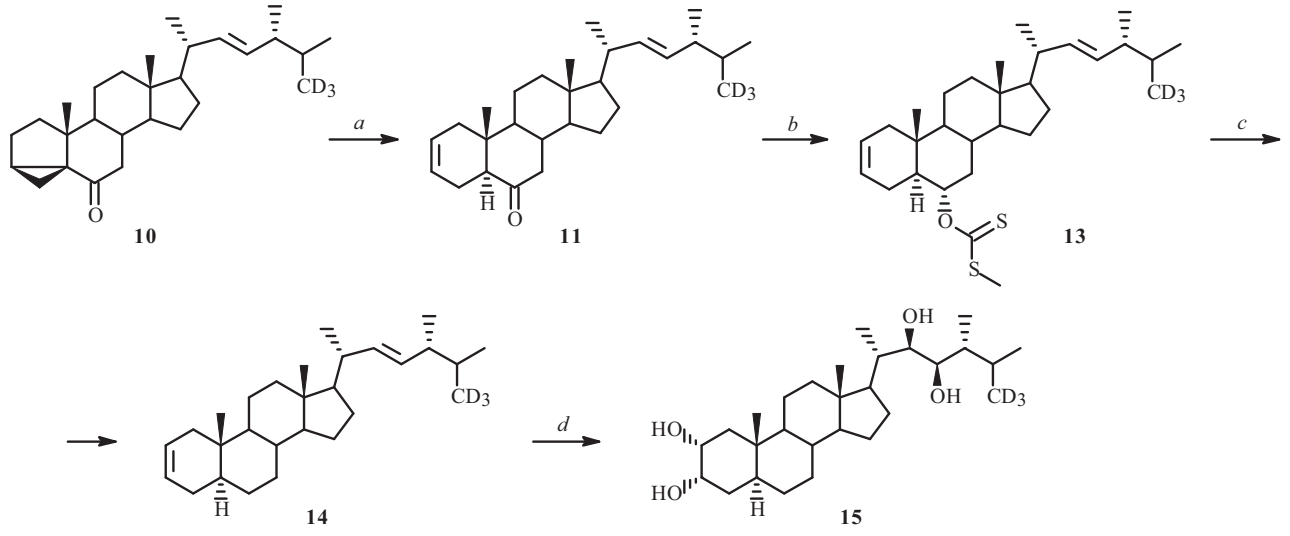
Convenient intermediates for their synthesis were γ,δ-unsaturated esters, Claisen rearrangement products of Δ^{23}-22-allyl alcohols. We used ester **6**, which was obtained from stigmasterol **4** through formation of 22-aldehyde **5** [15] according to the reported methods [16, 17], to prepare [26-$^2H_3$]-6-deoxo-24-epicastasterone **15**. Compound **6** already contained the side-chain carbon skeleton with the required C-24 methyl stereochemistry in addition to the Δ^{22}-bond and ester as a prerequisite for introducing the diol and D atoms, respectively.



a. $LiAlD_4$; *b*. 1. MsCl, 2. SiO_2; *c*. 1. $LiAlD_4$, **9**, 2. $H_2Cr_2O_7$



a. Py·HBr; *b*. 1. $LiAlH_4$, **12**, 2. CS_2, DBU, 3. MeI; *c*. Bu_3SnH, AIBN; *d*. K_2OsO_4, $K_3Fe(CN)_6$

Reduction of **6** with $LiAlD_4$ gave the corresponding alcohol **7** with two D atoms on C-26. Introduction of the last D atom was proposed by mesylation of **7** and reduction of the resulting mesylate with $LiAlD_4$. However, reduction product **8** was isolated after chromatographic purification instead of the expected derivative with a protected 6-ketone. Obviously, in this instance traces of acetic acid in the EtOAc used for elution were sufficient to remove the dioxolane protecting group on silica gel. Despite the fact that this removal occurred contrary to initial plans, compound **8** turned out to be suitable for further transformations. Treating **8** with $LiAlD_4$ introduced the third D atom on C-26 and simultaneously reduced the 6-ketone. The latter was regenerated by oxidation of the resulting alcohol **9** by Jones reagent.

Cyclopropylketone **10** was transformed into $\Delta^{2,22}$-dienone **11** in one step by refluxing in dimethylacetamide in the presence of pyridinium bromide. Huang–Minlon reduction of hydrazones or desulfurization of dithioketals are the most obvious and commonly used methods in the chemistry of brassinosteroids [14, 18–20] for transforming 6-ketones into derivatives without a functional group in ring B. An important drawback of these approaches is the formation of side products (possibly, C-5 isomers) that are difficult to separate by chromatography. This hinders the isolation of target compounds of the required purity.

The method used in the present work to remove the 6-ketone should resolve this issue. Hydride reduction of dienone **11** gave alcohol **12**, the chromatographic purification of which caused no problems. Defunctionalization of ring B was achieved by radical deoxygenation of xanthate **13**. This process did not affect the neighboring asymmetric atom, thereby guaranteeing the absence of the difficultly separated C-5 isomer in this step.

Both diols were introduced in one step by Sharpless dihydroxylation of diene **14** in the presence of dihydroquinidinium chlorobenzoate. The use of quinidine catalysts was a prerequisite for forming 22*R*,23*R*-diols as the main products [21].

Thus, the described sequence of 10 steps enabled [26-$^2H_3$]-6-deoxo-24-epicastasterone **15** to be prepared in overall yield 18% from ester **6**. It can be used as a tool to study the biosynthesis of 24-epibrassinolide.

EXPERIMENTAL

NMR spectra were recorded on a Bruker Avance DRX-500 instrument (Germany). Chemical shifts were determined relative to residual solvent resonances ($CHCl_3$, δ 7.26 ppm for ^1H; δ 77.00 ppm for ^{13}C; C_5H_5N, δ 135.91 ppm for ^{13}C). Mass spectra were taken in an LCQ Fleet mass spectrometer (Thermo Electron Corp., USA) in positive-ion mode with chemical ionization at atmospheric pressure (APCI). Optical rotation angles were measured on a Jasco-20 spectropolarimeter (Japan). Melting points were measured on a Kofler block. TLC was performed on Kieselgel 60 F_{254} chromatographic plates; column chromatography, over Kieselgel 60 silica gel (VWR, Art. 7734).

(22*E*,24*S*)-[26-$^2H_2$]-6-(1,3-Dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholest-22-en-26-ol (7). A solution of ethyl (22*E*,24*S*)-6-(1,3-dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholest-22-en-26-oate (**6**, 1.6 g, 3.2 mmol, obtained by the literature methods [6, 17]) in THF:Et_2O (3:2, 25 mL) was stirred vigorously, treated with $LiAlD_4$ (0.35 g, 8.3 mmol), and stirred at room temperature for 3.5 h. The excess of $LiAlD_4$ was decomposed by successive addition to the mixture of H_2O (0.35 mL), NaOH solution (15%, 0.35 mL), and H_2O again (1.05 mL). The resulting precipitate was filtered off. The filtrate was evaporated in vacuo. The solid was placed on a SiO_2 column and eluted by petroleum ether:EtOAc (20:1→11:1) to afford **7** (1.23 g, 83%) as an oil.

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.32 (1H, t, J = 4, H-4), 0.60 (1H, dd, J = 8, 4, H-4), 0.716, 0.724 (3H, s, H-18), 0.84 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.98 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.00 (3H, s, H-19), 1.23 (3H, d, J = 6, >CHC$\underline{H}_3$), 3.74 (1H, q, J = 7, -OCH_2CH_2O-), 3.81–3.86 (1H, m, -OCH_2CH_2O-), 3.90 (1H, dt, J = 8, 6, -OCH_2-CH_2O-), 4.02 (1H, dt, J = 8, 6, -OCH_2CH_2O-), 5.19–5.27 (1H, m, H-22 or H-23), 5.38–5.51 (1H, m, H-23 or H-22).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 7.25, 12.35, 12.64, 18.34, 18.96, 20.26, 20.86, 22.57, 23.01, 23.41, 24.10, 24.14, 24.89, 28.42, 28.46, 28.56, 33.20, 34.08, 38.03, 39.22, 39.51, 39.97, 40.17, 40.55, 42.73, 45.58, 47.40, 55.72, 55.93, 56.23, 56.28, 64.60, 64.85, 109.85, 109.89, 130.12, 131.59, 136.91, 137.17.

(22*E*,24*S*)-[26-$^2H_2$]-26-Methanesulfonyloxy-24-methyl-3α,5-cyclo-5α-cholest-22-en-6-one (8). A solution of **7** (1.77 g, 3.87 mmol) in CH_2Cl_2 (40 mL) was stirred, cooled to 0°C, treated with Et_3N (1.04 mL, 7.5 mmol) and MsCl (0.35 mL, 4.5 mmol), left at room temperature overnight, and worked up with saturated $NaHCO_3$ solution (20 mL). The aqueous layer was separated from the organic layer and extracted with $CHCl_3$. The combined organic phases were dried over Na_2SO_4 and evaporated. The solid was separated over a SiO_2 column with elution by petroleum ether:EtOAc (30:1→10:1) to afford **8** (1.57 g, 82%) as an oil.

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, H-18), 0.90 (3H, d, J = 7.1, >CHC$\underline{H}_3$), 0.98–1.04 (6H, m, 2CHC$\underline{H}_3$), 1.00 (3H, s, H-19), 3.00 (3H, s, OMs), 5.16 (1H, dd, J = 15, 8, H-22 or H-23), 5.25 (1H, dt, J = 15, 8, H-23 or H-22).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 11.64, 12.23, 12.41, 18.35, 19.67, 20.87, 22.64, 22.81, 24.00, 25.87, 28.47, 33.43, 34.73, 35.32, 37.22, 37.48, 37.71, 39.56, 40.18, 42.61, 44.75, 46.03, 46.29, 46.74, 55.60, 55.64, 56.99, 128.71, 130.72, 137.92, 209.75.

(22*E*,24*R*)-[26-$^2H_3$]-24-Methyl-3α,5-cyclo-5α-cholest-22-en-6-one (10). A solution of **8** (0.47 g, 0.96 mmol) in THF:Et_2O (7:1, 25 mL) was stirred, treated in portions with $LiAlD_4$ (81 mg, 1.92 mmol), stirred at room temperature for 2 h, and treated successively with H_2O (0.08 mL), NaOH solution (15%, 0.08 mL), and H_2O again (0.24 mL). The resulting precipitate was filtered off. The filtrate was evaporated. The resulting (22*E*,24*S*)-[6,26,26,26-$^2H_4$]-24-methyl-3α,5-cyclo-5α-cholest-22-en-6α-ol (**9**, 0.46 g) was dissolved in Me_2CO:H_2O (10:1, 55 mL), stirred, treated with Jones reagent (0.82 mL), stirred at room temperature for 30 min, and treated with *i*-PrOH (0.8 mL). The resulting dark-green precipitate was filtered off, dissolved in H_2O (10 mL), and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4.

The solvent was evaporated. The solid was chromatographed over a SiO_2 column with elution by petroleum ether and then petroleum ether:EtOAc (20:1) to afford **10** (0.43 g, 94%), mp 104–106°C (petroleum ether).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, H-18), 0.81 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.91 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.00 (3H, s, H-19), 1.02 (3H, d, J = 7, >CHC$\underline{H}_3$), 5.15 (1H, dd, J = 15, 8, H-22 or H-23), 5.21 (1H, dd, J = 15, 7, H-23 or H-22).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 11.64, 12.24, 17.57, 19.55, 19.68, 20.93, 22.84, 24.02, 25.89, 28.46, 32.82, 33.45, 34.76, 35.30, 39.60, 40.11, 42.59, 42.73, 44.80, 46.09, 46.31, 46.76, 55.92, 57.06, 131.99, 135.53, 209.80.

Mass spectrum (APCI$^+$, *m*/*z*, I_{rel}, %): 400 (100) $[M + H]^+$.

(22*E*,24*R*)-[26-$^2H_3$]-24-Methyl-5α-cholesta-2,22-dien-6-one (11). A solution of **10** (0.60 g, 1.5 mmol) and pyridinium bromide (0.72 g, 4.5 mmol) in dimethylacetamide (16.5 mL) was heated at 165°C under Ar for 3 h. The solvent was evaporated in vacuo. The solid was dissolved in H_2O and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The solid was chromatographed over a SiO_2 column with elution by petroleum ether:EtOAc (50:1→30:1) to afford **11** (0.38 g, 63%), mp 112–115°C (petroleum ether).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, H-18), 0.71 (3H, s, H-19), 0.81 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.90 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.01 (3H, d, J = 7, >CHC$\underline{H}_3$), 5.15 (1H, dd, J = 15, 8, H-22 or H-23), 5.21 (1H, dd, J = 15, 7, H-23 or H-22), 5.57 (1H, dq, J = 10, 3, H-2 or H-3), 5.68 (1H, ddt, J = 10, 4, 2, H-3 or H-2).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.14, 13.51, 17.58, 19.56, 20.92, 21.10, 21.72, 23.93, 28.33, 32.83, 37.71, 39.37, 40.06, 40.09, 42.73, 47.01, 53.44, 53.85, 55.95, 56.84, 124.53, 124.95, 132.01, 135.50, 212.10.

Mass spectrum (APCI$^+$, *m*/*z*, I_{rel}, %): 400 (100) $[M + H]^+$.

(22*E*,24*R*)-[26-$^2H_3$]-24-Methyl-5α-cholesta-2,22-dien-6-ol (12). A solution of **11** (0.38 g, 0.95 mmol) in THF:Et_2O (3:1, 15 mL) was stirred, treated in portions with $LiAlH_4$ (0.072 g, 1.9 mmol), stirred at room temperature for 3 h, and treated successively with H_2O (0.1 mL), NaOH solution (15%, 0.1 mL), and H_2O again (0.3 mL) to decompose the excess of reductant. The resulting precipitate was filtered off. The organic phase was dried over Na_2SO_4 and evaporated. The solid was chromatographed over a SiO_2 column with elution by petroleum ether:EtOAc (50:1) to afford **12** (0.33 g, 86%), mp 105–106°C (EtOAc:petroleum ether).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, H-18), 0.81 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.91 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.96 (3H, s, H-19), 1.01 (3H, d, J = 7, >CHC$\underline{H}_3$), 3.84 (1H, q, J = 3, H-6), 5.10–5.25 (2H, m, H-22 and H-23), 5.58 (1H, dd, J = 10, 5, H-2 or H-3), 5.63–5.72 (1H, m, H-3 or H-2).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.24, 15.20, 17.61, 19.57, 20.78, 20.95, 24.19, 26.42, 28.51, 30.09, 32.84, 34.58, 39.63, 39.82, 40.20, 41.70, 42.46, 42.74, 44.50, 54.20, 56.10, 56.23, 70.53, 125.62, 125.71, 131.72, 135.82.

Mass spectrum (APCI$^+$, *m*/*z*, I_{rel}, %): 384 (100) $[M - H_2O + H]^+$.

***O*-[(22*E*,24*R*)-[26-$^2H_3$]-24-Methyl-5α-cholesta-2,22-dien-6α-yl] *S*-methylcarbonodithionate (13).** A solution of **12** (273 mg, 0.68 mmol) in DMF (3.5 mL) was treated with CS_2 (0.36 mL, 6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.61 mL, 4.1 mmol), held at room temperature for 48 h, treated with CH_3I (0.36 mL, 5.78 mmol), stirred at room temperature for 30 min, treated with HCl (1 N, 1.5 mL) to decompose the excess of reagents in the mixture, diluted with H_2O, and extracted with $CHCl_3$. The combined organic extracts were evaporated. The solid was placed on a SiO_2 column and eluted by petroleum ether to afford **13** (272 mg, 81%), mp 132–134°C (Et_2O:MeOH).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, H-18), 0.81 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.91 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.98 (3H, s, H-19), 1.01 (3H, d, J = 7, >CHC$\underline{H}_3$), 2.57 (3H, s, SMe), 5.11–5.24 (2H, m, H-22 and H-23), 5.54–5.65 (2H, m, H-2 and H-3), 5.89 (1H, q, J = 3, C$\underline{H}$-OCS$_2$Me).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 12.25, 14.81, 17.62, 18.96, 19.57, 20.72, 20.94, 24.12, 26.10, 28.39, 31.05, 32.84, 34.51, 35.49, 39.70, 40.11, 41.26, 42.44, 42.77, 44.41, 53.75, 56.04, 56.14, 83.17, 125.32, 125.44, 131.82, 135.72, 216.12.

(22*E*,24*R*)-[26-$^2H_3$]-24-Methyl-5α-cholesta-2,22-diene (14). A solution of **13** (270 mg, 0.55 mmol) in anhydrous benzene (10 mL) under a stream of Ar was treated with azobisisobutyronitrile (2.7 mg), heated to 100°C, treated under a stream of Ar with Bu_3SnH (0.48 mL, 1.82 mmol), stirred at 100°C for 1 h, and evaporated to dryness. The solid was placed on a SiO_2 column and eluted by petroleum ether to afford **14** (210 mg, 98%) as an oil.

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.67 (3H, s, H-18), 0.75 (3H, s, H-19), 0.81 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.91 (3H, d, J = 7, >CHC$\underline{H}_3$), 1.00 (3H, d, J = 7, >CHC$\underline{H}_3$), 5.11–5.23 (2H, m, H-22 and H-23), 5.59 (2H, m, H-2 and H-3).

^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 11.67, 12.19, 17.61, 19.56, 20.88, 20.93, 24.19, 28.54, 28.75, 29.70, 30.31, 31.81, 32.85, 34.60, 35.60, 39.77, 39.91, 40.20, 41.45, 42.38, 42.74, 54.08, 56.11, 56.55, 125.85, 125.95, 131.64, 135.90.

(22*R*,23*R*,24*R*)-[26-$^2H_3$]-2α,3α,22,23-Tetrahydroxy-24-methyl-5α-cholestane (15). A suspension of **14** (210 mg, 0.54 mmol) in *t*-BuOH (11.5 mL) and H_2O (8.5 mL) was treated with $K_3Fe(CN)_6$ (1.076 g, 3.27 mmol), K_2CO_3 (452 mg, 3.27 mmol), $CH_3SO_2NH_2$ (107 mg, 1.09 mmol), $K_2OsO_4 \cdot 2H_2O$ (4 mg, 0.011 mmol), hydroquinidinium 4-chlorobenzoate (76 mg, 0.16 mmol), stirred at room temperature for 12 d, treated with Na_2SO_3 (1.0 g), and stirred for another 24 h. The aqueous layer was separated from the organic layer and extracted with $CHCl_3$. The combined organic phases were evaporated. The solid was chromatographed over a SiO_2 column with elution by $CHCl_3$:MeOH (50:1) to afford **15**, (172 mg, 69%), $[\alpha]_D^{20}$+20.37° (0.0081 g/mL, MeOH), mp 243–245°C (MeOH).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.66 (3H, s, H-18), 0.79 (3H, s, H-19), 0.84 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.91 (3H, d, J = 7, >CHC$\underline{H}_3$), 0.96 (3H, d, J = 6, >CHC$\underline{H}_3$), 3.36–3.43 (1H, m, H-23), 3.69 (1H, br.s, H-22), 3.76 (1H, dt, J = 12, 4, H-2), 3.96 (1H, br.s, H-3).

^{13}C NMR spectrum (125 MHz, C_5D_5N, δ, ppm): 11.80, 12.56, 13.17, 13.67, 17.85, 21.67, 22.80, 24.86, 27.37, 28.66, 28.86, 32.75, 35.48, 36.00, 37.50, 39.09, 40.76, 41.70, 42.30, 42.42, 43.07, 53.86, 55.03, 57.05, 69.50, 70.22, 72.84, 76.74.

Mass spectrum (APCI$^+$, *m*/*z*, I_{rel}, %): 436 (6) $[M - H_2O + H]^+$, 418 (100) $[M - 2H_2O + H]^+$, 400 (59) $[M - 3H_2O + H]^+$, 382 (10) $[M - 4H_2O + H]^+$.

REFERENCES

1. V. A. Khripach, V. N. Zhabinskii, and A. de Groot, *Brassinosteroids. A New Class of Plant Hormones*, Academic Press, San Diego, 1999.
2. A. Sakurai, *Plant Physiol. Biochem.*, **37**, 351 (1999).
3. B. Schneider, *Prog. Bot.*, **63**, 286 (2002).
4. S. Fujioka and T. Yokota, *Annu. Rev. Plant Biol.*, **54**, 137 (2003).
5. Y. H. Choi, S. Fujioka, T. Nomura, A. Harada, T .Yokota, S. Takatsuto, and A. Sakurai, *Phytochemistry*, **44**, 609 (1997).
6. S. Fujioka, T. Noguchi, T. Yokota, S. Takatsuto, and S. Yoshida, *Phytochemistry*, **48**, 595 (1998).
7. V. A. Khripach, V. N. Zhabinskii, and N. B. Khripach, in: *Brassinosteroids*, S. Hayat and A. Ahmad (eds.), Kluwer Academic Publishers, Dordrecht, 2003, p. 189.
8. S. Takatsuto, *J. Chromatogr. A*, **658**, 3 (1994).
9. V. A. Khripach and V. N. Zhabinskii, *Chem. Rec.*, **7**, 265 (2007).
10. P. Traldi, F. Magno, I. Lavagnini, and R. Seraglia, *Quantitative Applications of Mass Spectrometry*, Wiley, 2006.
11. V. A. Khripach, V. N. Zhabinskii, O. V. Konstantinova, A. P. Antonchick, and B. Schneider, *Steroids*, **67**, 587 (2002).
12. A. P. Antonchick, B. Schneider, V. N. Zhabinskii, and V. A. Khripach, *Steroids*, **69**, 617 (2004).
13. V. A. Khripach, V. N. Zhabinskii, O. V. Gulyakevich, Y. V. Ermolovich, and O. V. Konstantinova, *J. Labelled Comp. Radiopharm.*, **54**, 332 (2011).
14. B. Spengler, J. Schmidt, B. Voigt, and G. Adam, *Phytochemistry*, **40**, 907 (1995).
15. K. Okada and K. Mori, *Agric. Biol. Chem.*, **47**, 89 (1983).
16. M. Anastasia, P. Allevi, P. Ciuffreda, and A. Fiecchi, *J. Chem. Soc., Perkin Trans. 1*, 2365 (1983).
17. V. A. Khripach, V. N. Zhabinskii, O. V. Konstantinova, N. B. Khripach, A. P. Antonchick, and B. Schneider, *Steroids*, **67**, 597 (2002).
18. K. Mori, M. Sakakibara, and K. Okada, *Tetrahedron*, **40**, 1767 (1984).
19. T. Watanabe, T. Noguchi, T. Yokota, K. Shibata, H. Koshino, H. Seto, S. K. Kim, and S. Takatsuto, *Phytochemistry*, **58**, 343 (2001).
20. J. A. Ramirez, C. Brosa, and L. R. Galagovsky, *Phytochemistry*, **66**, 581 (2005).
21. V. N. Zhabinskii, V. A. Khripach, and V. K. Ol′khovik, *Zh. Org. Khim.*, **32**, 327 (1996).