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Research Article

Synthesis of [7,7-$^2H_2$]epibrassinolide

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Abstract: Synthesis of labelled epibrassinolide containing two deuterium atoms in a position which is not subjected to isotopic exchange is reported. Key transformations include preparation of 6,7-seco steroidal diacid, its cyclization to a cyclic anhydride followed by a regioselective reduction with $NaBD_4$. The obtained [7,7-$^2H_2$]epibrassinolide can be used in biochemical experiments when the loss of isotopic label should be avoided. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: brassinosteroids; epibrassinolide; lactone; seco steroids

Introduction

Isotopically labelled brassinosteroids (BS) play an essential role as analytical tools in the study of the biosynthetic and regulatory pathways of these compounds.[1,2] A number of deuterated[3–9] and tritiated BS[10] have been prepared with isotopic labels adjacent to the C-6 carbonyl group or in the terminal part of the side chain. Compounds belonging to the first group can be easily prepared by $^2H(^3H)/^1H$ exchange of the corresponding 6-ketones. Unfortunately, such labelling is inappropriate for studies that attempt to avoid loss of the label. BS-containing deuterium or tritium atoms in the side chain (at carbon atom) are not subject to isotopic exchange. These derivatives are more convenient for biosynthetic studies. However, some metabolic transformations of BS can result in loss of the side chain.[11–13] In this respect, synthesis of BS bearing a label at a stable position in the cyclic part seems to be the preferred option.

The present study investigated the synthesis of C-7 deuterium-labelled BS for biosynthetic studies. In lactones this position is resistant to isotopic exchange under physiological conditions and loss of the side chain will not be a problem for using these derivatives in metabolic studies.

The proposed approach is illustrated in Scheme 1. The lactone **1** should be transformed into the cyclic anhydride **2**, which should be regioselectively reduced to give the labelled lactone **3**. This last reduction has been demonstrated for similar compounds.[14]

Results and discussion

It was desirable to start the preparation of the labelled compound from an easily available derivative, and the best choice was epibrassinolide **4**. The intended transformations of the lactone group in **4** could be carried out after protecting both diol functions. Two routes were investigated to obtain 6,7-seco derivatives from diacetonide **5** (Scheme 2).

The first route started with saponification of **5** to its sodium salt **6**. The latter is a rather unstable compound that tends to form lactone **5** again when it is kept in a solution at room temperature. However, Dess–Martin oxidation could be carried out with **6** to the corresponding aldehydoacid **9**, which was obtained in a reasonable (58%) yield.

The second route to 6,7-seco steroid **9** implied hydride reduction of **5**. The reaction proceeded with the formation of the expected 6,7-diol **8** along with a small amount of lactol **7**. A clear correlation between C_6–H and the C_{19}–H was present in the NOESY

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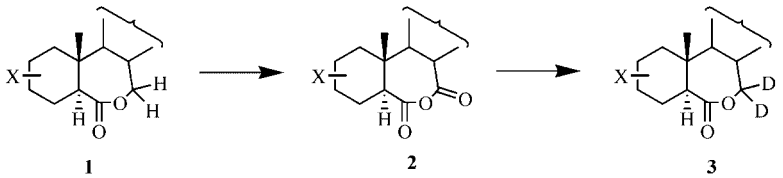
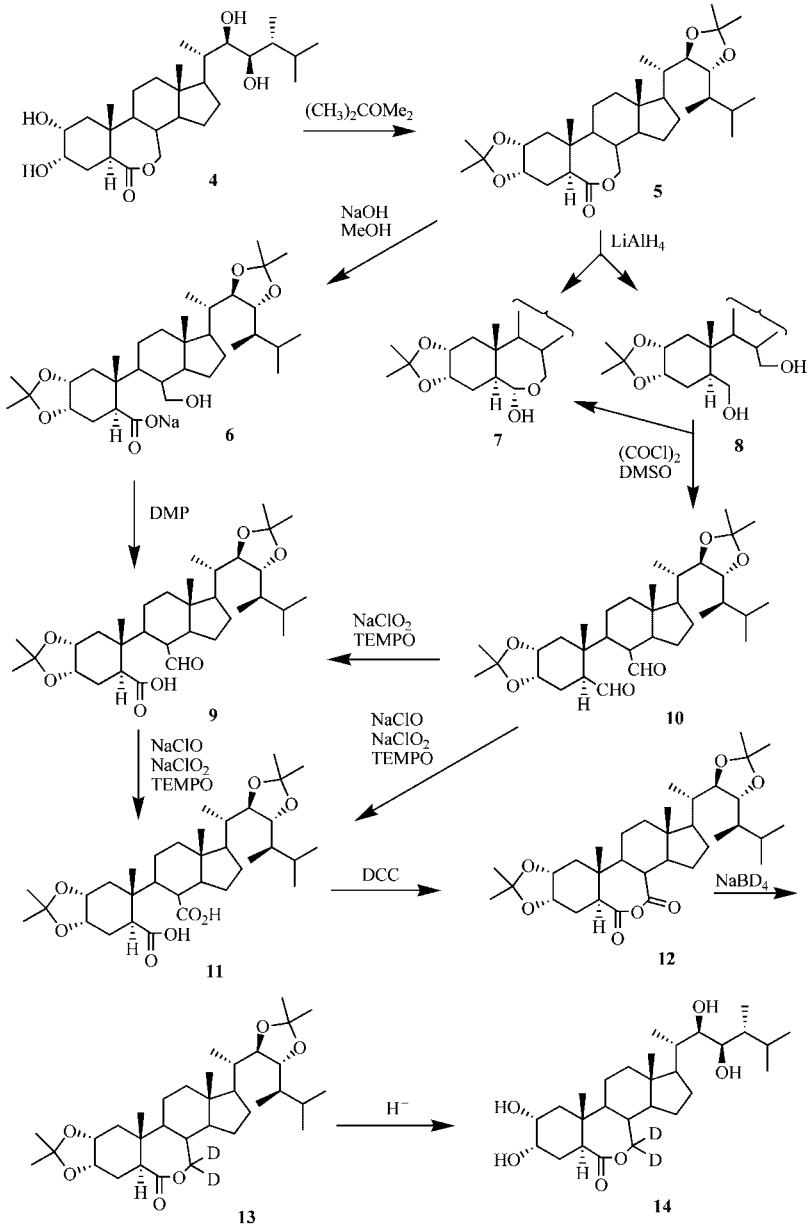


Scheme 1



Scheme 2

spectrum of compound **7**, confirming the β-orientation of the hydrogen atom at C_6.

Compound **7** was also obtained as a minor by-product in the Swern oxidation of diol **8** to dialdehyde **10**. The diacid **11** was obtained directly from dialdehyde **10** or from dialdehyde **9** by sodium chlorite oxidation catalyzed by TEMPO using phosphate buffer (pH = 6.88).[15] The buffer was used to avoid possible

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racemization at C-5 and/or C-8. Treatment of the diacid **11** with dicyclohexylcarbodiimide gave cyclic anhydride **12**.

In a small-scale reduction of **12** with $NaBH_4$, the product was identical to diacetonide **5**, confirming the potential success of this approach. Indeed, the reduction of **12** with $NaBD_4$ gave deuterated derivative **13**. The content of deuterium in compound **13** was estimated from its ^1H NMR spectrum using the integral intensity of the multiplet at δ 4.1 (2H, C_7–H). This signal had 18% of the intensity of the same signal for compound **5**, which corresponds to 82% deuterium content in compound **13**. Removal of the acetonide protecting groups in **13** at the last step provided [7,7-$^2H_2$]epibrassinolide **14**. The use of this compound in biochemical experiments will be reported elsewhere.

Experimental

Melting points were recorded on a Boetius micromelting 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 done by the combined use of 1D and 2D experiments, including COSY, HSQC, HMBC, TOCSY, and NOESY methods. All experiments were carried out using standard pulse sequences supplied with the spectrometer. The exact mass measurements were carried out on a Finnigan MAT 95 mass spectrometer, operating in the 70 eV-EI mode. Chemicals were purchased from Aldrich and Fluka and used as received. Reactions were monitored by TLC using aluminium or plastic sheets, silica gel 60 F_{254} precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

(22R,23R,24R)-2α,3α,22,23-Diisopropylidenedioxy-B-homo-7-oxa-5α-ergostan-6-one (5)

2,2-Dimethoxypropane (2 ml, 16 mmol) and TsOH (20 mg, 0.116 mmol) were added to a suspension of epibrassinolide **2** (500 mg, 1.04 mmol) in CH_2Cl_2 (20 ml). After the addition was complete, the reaction mixture turned dark brown and the resulting solution was stirred for 1.5 h. Then it was diluted with $CHCl_3$, washed with brine, water, dried over Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by SiO_2 chromatography using EtOAc/petroleum ether (1:5) provided **5** (525 mg, 90%) as an oil. ^1H NMR: δ 0.69 (d, $J = 6.7$ Hz, 3H, 28-Me), 0.70 (s, 3H, 18-Me), 0.81 (d, $J = 7.0$ Hz, 3H, 26-Me), 0.89 (s, 3H, 19-Me), 0.90 (d, $J = 7.0$ Hz, 3H, 27-Me), 0.96 (d, $J = 5.8$ Hz, 3H, 21-Me), 1.30 (s, 3H), 1.33 (s, 3H), 1.35 (s, 3H), 1.49 (s, 3H), 2.31 (dd, 1H, $J_1 = 16.0$ Hz, $J_2 = 3.2$ Hz, C_4–H), 3.28 (dd, 1H, $J_1 = 10.6$ Hz, $J_2 = 2.9$ Hz, C_5–H), 3.55 (m, 1H, C_{23}–H), 3.92 (m, 1H, C_{22}–H), 4.1 (m, 2H, C_7–H), 4.4 (m, 2H, C_2– and C_3–H). ^{13}C NMR: δ 9.84, 11.93, 12.53, 16.00, 19.66, 21.12, 22.97, 23.59, 24.45, 26.42, 27.15, 27.35, 27.73, 30.07, 33.43, 35.87, 38.00, 39.36, 39.54, 40.16, 43.03, 43.77, 51.68, 53.34, 54.69, 71.20, 72.45, 73.05, 80.25, 82.27, 107.58, 107.92, 176.64. EIMS: *m/z* 560 $[M]^+$ (1), 545 $[M–CH_3]^+$ (91), 489 (16), 445 (12), 171 (62), 142 (100), 99 (44); HRMS (*m/z*): calcd for $C_{33}H_{53}O_6$, 545.3842 $[M–CH_3]^+$, found, 545.3849.

(22R,23R,24R)-2α,3α,22,23-Diisopropylidenedioxy-6,7-seco-5α-ergostan-7-ol-6-oic acid sodium salt (6)

A mixture of lactone **5** (525 mg, 0.94 mmol) and a 20% solution of NaOH in MeOH (35 ml) was stirred at 35–40°C for 1 h. Then it was partly evaporated *in vacuo*, diluted with water, and extracted with $CHCl_3$ (20 × 10 ml). Organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give 600 mg of crude **6** which was used in the next step without additional purification.

(22R,23R,24R)-2α,3α,22,23-Diisopropylidenedioxy-7-oxo-6,7-seco-5α-ergostan-6-oic acid (9)

Sodium salt **6** (600 mg, ~1.17 mmol) from the previous step was dissolved in dry CH_2Cl_2 (50 ml) and Dess–Martin periodinane (746 mg, 2.4 mmol) was added. After 1 h of stirring at room temperature, an additional amount of Dess–Martin periodinane (200 mg, 0.81 mmol) was added. Stirring was continued for another 45 min, and then the reaction mixture was diluted with saturated solution of $Na_2S_2O_3$ and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by SiO_2 chromatography using EtOAc/petroleum ether (1:3, 1:1) to give:

(a) lactone **5** (160 mg, 30 %);
(b) aldehyde **9** (316 mg, 58%). ^1H NMR: δ 0.69 (s, 3H, 18-Me), 0.70 (d, $J = 6.7$ Hz, 3H, 28-Me), 0.81 (d, $J = 6.8$ Hz, 3H, 26-Me), 0.90 (d, $J = 7.0$ Hz, 3H, 27-Me), 0.95 (s, 3H, 19-Me), 0.98 (d, $J = 6.1$ Hz, 3H, 21-Me), 1.33 (s, 3H), 1.34 (s, 3H), 1.38 (s, 3H), 1.51 (s, 3H), 2.96 (dd, $J_1 = 11.8$ Hz, $J_2 = 4.6$ Hz, 1H, C_5–H), 3.56 (dd, $J_1 = 9.2$ Hz, $J_2 = 7.1$ Hz, 1H, C_{22}–H), 3.92 (d, $J = 7.0$ Hz, 1H, C_{23}–H), 4.16 (m, 1H, C_2–H), 4.26 (m, 1H, C_3–H), 9.38 (d, $J = 5.7$ Hz, 1H, C_7–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.86, 11.47, 12.58, 15.98, 20.20, 20.91, 21.10, 24.63, 26.35, 27.12,

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27.18, 27.33, 27.39, 27.71, 28.60, 35.44, 38.20, 38.86, 39.92, 41.58, 42.29, 43.76, 45.69, 48.35, 52.60, 53.00, 71.46, 72.51, 80.29, 82.26, 107.88, 108.05, 178.16, 204.93.

(c) a mixture of **5** and **9** (110 mg).

Reduction of lactone (5) with $LiAlH_4$

$LiAlH_4$ (40 mg, 1.05 mmol) was added portionwise to a stirred solution of diacetonide **5** (300 mg, 0.53 mmol) in ether (20 ml) at room temperature and stirring was continued for 2 h. After the usual work up, column chromatography of the residue using $CHCl_3$/EtOAc (2:1, 1:1) as eluent gave:

(a) (*22R,23R,24R*)-*2α,3α,22,23-Diisopropylidenedioxy-B-homo-7-oxa-5α-ergostan-6α-ol* **7** (24 mg, 8%). ^{1}H NMR: δ 0.69 (s, 3H, 18-Me), 0.70 (d, J = 6.7 Hz, 3H, 28-Me), 0.81 (d, J = 6.7 Hz, 3H, 26-Me), 0.90 (d, J = 7.0 Hz, 3H, 27-Me), 0.97 (d, J = 6.4 Hz, 3H, 21-Me), 0.98 (s, 3H, 19-Me), 1.30 (s, 3H), 1.34 (s, 3H), 1.38 (s, 3H), 1.51 (s, 3H), 3.48 (dd, 1H, J_1= 12.2 Hz, J_2= 2.2 Hz, C_7–H), 3.56 (dd, 1H, J_1 = 6.7 Hz, J_2= 8.9 Hz, C_{23}–H), 3.75 (dd, 1H, J_1 = 12.2 Hz, J_2= 10.9 Hz, C_7–H), 3.93 (d, 1H, J = 6.7 Hz, C_{22}–H), 4.28 (m, 1H, C_3–H), 4.32 (m, 1H, C_2–H), 4.77 (d, 1H, J = 8.7 Hz, C_6–H). ^{13}C NMR: δ 9.84, 12.01, 12.49, 16.02, 17.79, 21.14, 23.44, 24.32, 26.80, 27.16, 27.36, 27.73, 28.07, 28.50, 35.88, 37.24, 38.03, 40.11, 40.72, 42.60, 43.02, 43.77, 52.18, 53.32, 56.18, 64.43, 72.97, 73.17, 76.74, 77.00, 77.25, 80.29, 82.38, 97.63, 107.68, 107.91.

(b) (*22R,23R,24R*)-*2α,3α,22,23-Diisopropylidenedioxy-6,7-seco-5α-ergostane-6,7-diol* **8** (243 mg, 81%). ^{1}H NMR: δ 0.70 (s, 3H, 18-Me), 0.73 (d, J = 6.7 Hz, 3H, 28-Me), 0.83 (d, J = 6.7 Hz, 3H, 26-Me), 0.93 (d, J = 6.7 Hz, 3H, 27-Me), 0.99 (d, J = 6.0 Hz, 3H, 21-Me), 1.06 (s, 3H, 19-Me), 1.35 (s, 3H), 1.40 (s, 3H), 1.44 (s, 3H), 1,55 (s, 3H), 3.29 (t, 1H, J = 10.2 Hz, C_6–H), 3.59 (dd, 1H, J = 9.3, 7.2 Hz, C_{23}–H), 3.64 (d, 1H, J = 12.0 Hz, C_7–H), 3.84 (dd, 1H, J_1= 10.2 Hz, J_2= 2.0 Hz, C_6–H), 3.96 (d, 1H, J = 7.2 Hz, C_{22}–H), 3.99 (d, 1H, J = 12.0 Hz, C_7–H), 4.18 (m, 1H, C_2–H), 4.28 (m, 1H, C_3–H). ^{13}C NMR: δ 9.84, 11.53, 12.47, 16.02, 19.32, 21.14, 23.20, 24.55, 26.42, 26.91, 27.12, 27.32, 27.49, 27.71, 28.70, 35.53, 38.02, 38.05, 38.51, 39.80, 39.89, 42.01, 43.70, 44.94, 51.51, 53.62, 62.19, 62.97, 72.29, 73.34, 80.23, 82.24, 107.61, 107.88. EIMS: m/z 564 $[M]^+$ (8), 562 $[M–2]^+$ (9), 549 $[M–CH_3]^+$ (21), 171 (77), 142 (100), 99 (58); HRMS (m/z): calcd for $C_{33}H_{57}O_6$, 549.4155 $[M–CH_3]^+$, found, 549.4147.

Swern oxidation of diol (8)

To a solution of $(COCl)_2$ (0.6 ml) in CH_2Cl_2 (2 ml), a solution of DMSO (1.2 ml) in CH_2Cl_2 (2 ml) was added dropwise at −70°C under argon. After the mixture was stirred at the same temperature for 30 min, a solution of diol **8** (200 mg, 0.35 mmol) in CH_2Cl_2 (2 ml) was added. The mixture was stirred for an additional 30 min and treated with Et_3N (4 ml) at −60°C. The cooling bath was removed, the temperature of the mixture was allowed to come to room temperature, and water (10 ml) was added. The organic layer was separated, the water layer was twice washed with CH_2Cl_2, and the combined organic solutions were dried (Na_2SO_4) and evaporated *in vacuo*. Column chromatography of the residue using EtOAc/petroleum ether (1:10, 1:5) as eluent gave:

(a) lactol **7** (21 mg, 10%).

(b) (*22R,23R,24R*)-*2α,3α,22,23-Diisopropylidenedioxy-6,7-dioxo-6,7-seco-5α-ergostane* **10** (130 mg, 65%). ^{1}H NMR: δ 0.66 (s, 3H, 18-Me), 0.67 (d, J = 7.0 Hz, 3H, 28-Me), 0.78 (d, J = 6.8 Hz, 3H, 26-Me), 0.87 (d, J = 7.0 Hz, 3H, 27-Me), 0.90 (s, 3H, 19-Me), 0.95 (d, J = 6.4 Hz, 3H, 21-Me), 1.30 (s, 3H), 1.32 (s, 3H), 1.34 (s, 3H), 1,47 (s, 3H), 2.36 (m, 1H, C_8–H), 2.88 (m, 1H, C_5–H), 3.52 (dd, 1H, J_1 = 7.1 Hz, J_2= 9.2 Hz, C_{22}–H), 3.88 (d, J = 7.3 Hz, 1H, C_{23}–H), 4.10 (m, 1H, C_2–H), 4.24 (m, 1H, C_3–H), 9.38 (d, J = 5.6 Hz, 1H, C_7–H), 9.76 (d, J = 2.1 Hz, 1H, C_6–H). ^{13}C NMR: δ 9.9.81, 11.39, 12.54, 15.93, 20.13, 21.06, 21.35, 23.91, 24.51, 26.31, 27.09, 27.30, 27.33, 27.64, 28.55, 35.43, 37.97, 38.65, 40.27, 41.48, 43.70, 45.10, 47.93, 48.18, 52.48, 52.89, 71.08, 72.40, 80.23, 82.17, 107.90, 107.95, 203.86, 204.54. EIMS: m/z 560 $[M]^+$ (1), 545 $[M–CH_3]^+$ (51), 489 (12), 445 (11), 171 (87), 142 (100), 99 (64); HRMS (m/z): calcd for $C_{34}H_{56}O_6$, 560.4077 $[M]^+$, found, 560.4085; calcd for $C_{33}H_{53}O_6$, 545.3842 $[M–CH_3]^+$, found, 545.3837.

(22R,23R,24R)-2α,3α,22,23-Diisopropylidenedioxy-6,7-seco-5α-ergostan-6,7-dioic acid (11)

TEMPO (8.8 mg, 0.06 mmol) and phosphate buffer (2.74 ml, pH = 6.88) were added to a solution of aldehyde **9** (316 mg, 0.55 mmol) in a mixture of THF (3 ml) and CH_3CN (1.5 ml). Then solutions of $NaClO_2$ (95 mg in 0.7 ml of H_2O) and NaClO (1.7 ml of water solution with 13% of active Cl) were added simultaneously to the mixture at 35°C. The reaction mixture was stirred at this temperature for 5 h, after which solutions of $NaClO_2$ (40 mg in 0.3 ml of H_2O) and NaClO

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(0.8 ml) were added and the mixture was stirred at 40–45°C for an additional 2.5 h. Then it was cooled to room temperature and mixed with a cold saturated solution of Na_2SO_3. After 15 min, an additional amount of phosphate buffer was added and the reaction mixture was extracted with EtOAc. The combined organic solutions were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by column chromatography on SiO_2 using EtOAc/petroleum ether (3:1) to give diacid **11** (84 mg, 26%). ^{1}H NMR: δ 0.59 (s, 3H, 18-Me), 0.63 (d, J = 6.9 Hz, 3H), 0.74 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 7.0 Hz, 3H), 1.27–1.28 (m, 6H), 1.32 (s, 3H), 1.44 (s, 3H), 2.22 (t, J = 10.9 Hz, 1H, C_8–H) 2.88 (t, J = 8.4 Hz, 1H, C_5–H), 3.50 (dd, J_1= 9.3 Hz, J_2= 7.0 Hz, 1H, C_{22}–H), 3.87 (d, J = 6.7 Hz, 1H, C_{23}–H), 4.13 (m, 1H, C_2–H), 4.20 (m, 1H, C_3–H). ^{13}C NMR: δ 9.84, 11.02, 12.48, 14.09, 15.93, 18.25, 21.08, 21.16, 24.48, 26.33, 26.91, 27.04, 27.28, 27.64, 28.64, 29.66, 34.22, 38.21, 38.79, 40.20, 41.43, 42.68, 43.73, 44.78, 46.45, 52.78, 71.53, 72.70, 80.33, 82.37, 107.87, 108.24, 178.74, 181.69. EIMS: m/z 592 $[M]^+$ (1), 577 $[M–CH_3]^+$ (59), 559 (17), 521 (11), 477 (12), 171 (89), 142 (100), 99 (60); HRMS (m/z): calcd for $C_{34}H_{56}O_8$, 592.3975 $[M]^+$, found, 592.3995; calcd for $C_{33}H_{53}O_8$, 577.3740 $[M–CH_3]^+$, found, 577.3722.

Using the same procedure, diacid **11** was prepared from dialdehyde **10** in 32% yield.

(22R,23R,24R)-2α,3α,22,23-Diisopropylidenedioxy-B-homo-7-oxa-5α-ergostan-6,7a-dione (12)

Dicyclohexylcarbodiimide (25 mg, 0.12 mmol) was added to a solution of diacid **11** (48 mg, 0.08 mmol) in dry CH_2Cl_2 (12 ml) under argon. After being stirred for 2 h at room temperature, the mixture was cooled to 0°C, and the white precipitate was filtered and washed with CH_2Cl_2. The filtrate was washed with a 5% solution of HCl, brine, and water. The combined organic solutions were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by column chromatography on SiO_2 to give anhydride **12** (43 mg, 94%). ^{1}H NMR: δ 0.67 (s, 3H, 18-Me), 0.70 (d, J = 6.9 Hz, 3H, 28-Me), 0.81 (d, J = 6.8 Hz, 3H, 26-Me), 0.91 (d, J = 6.9 Hz, 3H, 27-Me), 0.97 (d, J = 6.5 Hz, 3H, 21-Me), 1.06 (s, 3H, 19-Me), 1.29 (s, 3H), 1.34 (s, 3H), 1.38 (s, 3H), 1.43 (s, 3H), 2.60 (t, J = 11 Hz, 1H, C_8–H), 3.04 (dd, J_1 = 10.9 Hz, J_2= 3.7 Hz, 1H, C_5–H), 3.55 (dd, J_1 = 9.4 Hz, J_2= 7.1 Hz, 1H, C_{22}–H), 3.93 (d, J = 6.9 Hz, 1H, C_{23}–H), 4.33 (m, 2H, C_2– and C_3–H). ^{13}C NMR: δ 9.83, 11.56, 12.61, 15.97, 18.19, 21.11, 22.36, 23.92, 24.16, 26.22, 27.15, 27.31, 27.38, 27.71, 27.76, 33.84, 37.74, 37.95, 38.43, 41.74, 43.44, 43.76, 46.35, 50.54, 52.79, 53.15, 71.98, 72.53, 80.23, 82.15, 107.94, 108.04, 168.76, 173.17. EIMS: m/z 559 $[M–CH_3]^+$ (94), 503 (15), 459 (9), 401 (11), 171 (100), 142 (100), 99 (84); HRMS (m/z): calcd for $C_{33}H_{51}O_7$, 559.3635 $[M–CH_3]^+$, found, 559.3650.

[7,7-$^2H_2$](22R,23R,24R)-2α,3α,22,23-Diisopropylidenedioxy-B-homo-7-oxa-5α-ergostan-6-one (13)

A solution of anhydride **12** (40 mg, 0.07 mmol) in THF (4 ml) was treated with $NaBD_4$ (5 mg, 0.12 mmol), and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated *in vacuo*, Ac_2O (0.2 ml) and pyridine (0.4 ml) were added, and the resulting mixture was kept at 80°C for 2 h. The usual work up followed by column chromatography (SiO_2; EtOAc/petroleum ether (1:10) gave lactone **13** (34 mg, 85%). Its ^{1}H NMR spectrum was identical to that of non-deuterated lactone **5**, except for the intensity of signals at δ 4.1 belonging to protons at C-7. The ^{13}C NMR spectrum of **13** was fully identical with that of **5**. EIMS: m/z 562 $[M]^+$ (0.2), 547 $[M–CH_3]^+$ (45), 491 (9), 447 (8), 361 (9), 171 (78), 142 (100), 99 (59); HRMS (m/z): calcd for $C_{33}H_{51}D_2O_6$, 547.3968 $[M–CH_3]^+$, found, 547.3988.

[7,7-$^2H_2$](22R,23R,24R)-2α,3α,22,23-Dihydroxy-B-homo-7-oxa-5α-ergostan-6-one ([7,7-$^2H_2$]epibrassinolide) (14)

A solution of lactone **13** (30 mg) in methanol (3 ml) with conc. HCl (one drop) was refluxed for 1 h. Then acid was neutralized with pyridine, and the solvents were evaporated *in vacuo*. Column chromatography (SiO_2; $CHCl_3$/MeOH (25:1)) of the residue gave compound **14** (21 mg, 82%). Its ^{1}H and ^{13}C NMR spectra were similar (or identical) with the corresponding data obtained for non-deuterated epibrassinolide **4**. HRMS (m/z): calcd for $C_{28}H_{46}D_2O_6$, 482.3576 $[M]^+$, found, 482.3575.

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