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A General Approach to Synthesis of Labeled Brassinosteroids: Preparation of [25,26,27-$^2H_7$]Brassinolide with 60% Isotopic Purity from the Parent Brassinolide

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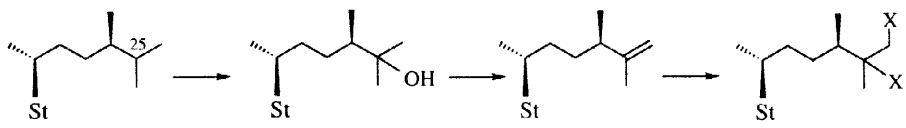
Abstract: From brassinolide (BL) **1**, [25,26,27-^2H_n]BL **10** was synthesized in 5 steps including C-25 hydroxylation, dehydration and catalytic deuteriogenation. In direct oxy-functionalization of tetra-*O*-acetyl BL **2** with methyl(trifluoromethyl)dioxirane leading to 25-hydroxyl compound **3**, 14-hydroxyl, 25-hydroxy-15-oxo and 14,25-dihydroxyl derivatives, **4**, **5** and **6**, were newly identified; the catalytic deuteriogenation of $\Delta^{25(26)}$-BL **8** using 5% palladium-on-charcoal resulted in abundant incorporation of deuterium atoms to give **10** with 60% isotopic purity of [25,26,27-$^2H_7$]BL.

Brassinosteroids (BRs) are of current importance in plant physiology, which are considered as a new class of phytohormones due to their various physiological activities and ubiquitous distribution in the plant kingdom.[1] In the investigation of BRs, the isotopically labeled BRs are powerful tools to elucidate the biosynthesis, metabolism, mode of action at the molecular level, and thus several strategies for synthesis of labeled BRs have been developed so far.[2] However, these all need tedious multi-step processes, except for one special case in which dolichosterone and dolicholide, hardly available rare natural $\Delta^{24(28)}$-BRs, were used as a precursor for tritiation.[3] Since nowadays some natural BRs are commercially available, we planned a general approach to labeled BRs from the parent BRs, which included three basic steps, C-25 hydroxylation, dehydration to introduce a $\Delta^{25(26)}$-double bond and catalytic deuteriogenation or tritiation of the double bond, as illustrated in Scheme 1. In this paper we report the synthesis of [25,26,27-^2H_n]brassinolide **10** from brassinolide (BL) **1**, a highly oxy-functionalised and the most active natural BR, following this strategy. In this context, the intriguing findings observed in the direct oxy-functionalization at C-25 and in catalytic deuteriogenation will be described.



St=steroidal rings X=^2H or ^3H

Scheme 1

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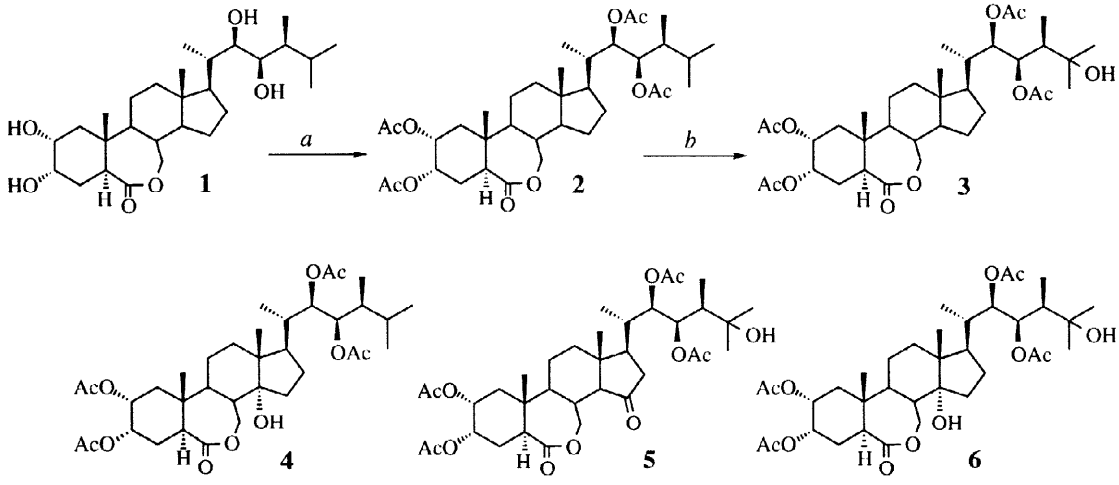
The site selective oxy-functionalization at side chain C-25 of steroids has been an important subject to organic chemists due to the biological significance of 25-hydroxyl steroids, and thus a variety of oxidation reagents have been tested for this purpose.[4] Among them, the efficacy of methyl(trifluoromethyl)dioxirane (TFD) developed by Mello *et al.*[5] is particularly outstanding, which was recently verified by Bovicelli *et al.* in C-25 hydroxylation of some cholestane derivatives and Vitamin D_3 Windaus-Grundmann ketone.[6] More recently, Voigt *et al.* applied this reagent to 2,3,22,23-tetra-*O*-acetylBL **2** and obtained 25-hydroxyl derivative **3** in good yield.[7] Thus, we essentially followed their method to introduce the C-25 hydroxyl function on the BL molecule. As a result, we found that TFD oxy-functionalized not only C-25, but also C-14 and C-15 on **2**. Upon treatment of **2**, derived from **1** [Ac_2O, pyridine, 60°C, 20 h, 95%, mp 206-209°C: lit.,[8] 231-233°C], with 3 equiv. of TFD in trifluoroacetone and CH_2Cl_2, 1:2, at 0°C for 3 h, the desired 25-hydroxyl derivative **3** (mp 226-229°C: lit.,[7] 238-241°C) was obtained in 48% yield as the major product, along with 14-hydroxyl, 25-hydroxy-15-oxo and 14,25-dihydroxyl compounds, **4** (6.5%, mp 186-188°C), **5** (3.6 %, mp 274-276°C decomp.) and **6** (18%, mp 187-191°C),[9] and 19% of the starting material **1** was recovered (Scheme 2). The product distribution suggested that the reactivities of hydrogens on **2** lay in the order, 25-H>14-H>15-H. As expected, the low reaction temperature reduced the formation of **4**, **5** and **6**, *i.e.*, the same treatment of **1** at -30°C for 5 h led to the nearly exclusive formation of **3** (61%: 85% based on the amount of **1** consumed) and recovery of **1** (28%). Meanwhile, a large amount of TFD (6 equiv.) and prolonged reaction time (24 h) at room temperature (rt) resulted in the predominant formation of dihydroxylated compound **6** in 78% yield.



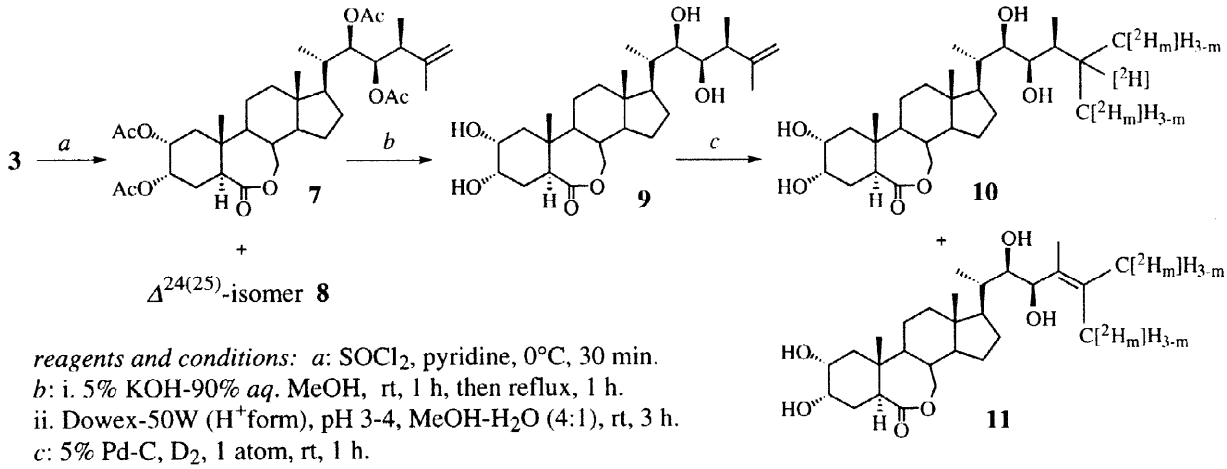
Scheme 2

Reagents and conditions: *a*: Ac_2O, pyridine, 60°C, 20 h.
b: 3 equiv. of TFD, trifluoroacetone-CH_2Cl_2, 1:2, -30°C, 5 h.

Dehydration of the tertiary alcohol **3** was performed with thionyl chloride and pyridine at 0°C, giving rise to a 65:35 mixture of the desired $\Delta^{25(26)}$-olefin **7** and its double bond isomer, $\Delta^{24(25)}$-olefin **8**, in 86% yield. Since these isomers could not be separated by column chromatography, the mixture was then subjected to deprotection reaction of the tetra-*O*-acetyl groups. The mixture was treated with 5% potassium hydroxide in 90% aqueous methanol at rt for 1 h until the lactone ring completely opened to the carboxylate and then at refluxing temperature for 2 h.[10] After re-lactonization with a cationic exchange resin [Dowex-50W-X2 (H^+ form)] at pH 3-4 in MeOH-H_2O (4:1) at rt for 3 h, $\Delta^{25(26)}$-BL **9** (mp 232-233°C)[9] was obtained in 51% yield from **3**, while $\Delta^{24(25)}$-BL **12** expected from **8** could not be detected at all. A mixture of by-products less polar than **9** was suspected to be originated from **8**, but these structures have not yet been identified.

With a suitable precursor, $\Delta^{25(26)}$-BL **9**, for deuteriogenation or tritiation, in hand catalytic deuteriogenation of **9** was finally attempted over both platinum oxide (PtO_2) or 5% palladium-on-charcoal (5% Pd-C) catalyst using deuterium gas (≥99.5 D-mol%) at rt and at atmospheric pressure in a tetrahydrofuran solution. In the case of PtO_2, deuteriogenated BL **10** was obtained quantitatively, but the low incorporation rate calculated from the MS spectra, *i.e.*, the composition ratio of $^2H_0$:$^2H_1$:$^2H_2$=18:46:36, was disappointing. In contrast, in the case of 5% Pd-C, although a certain amount of double bond migration of $\Delta^{25(26)}$ to $\Delta^{24(25)}$ took place, deuterium atoms were incorporated at a surprisingly high rate. When **9** was deuteriogenated over 5% Pd-C (two-fold weight of substrate) for 1 h, a 75:25 mixture of **10** and deuteriogenated $\Delta^{24(25)}$-BL **11** was obtained in quantitative yield, which were separated by HPLC [Senshu Pak ODS-1151D, 150 x 4.6 mm i.d., Senshu Scientific Co., Ltd.; mobile phase: H_2O–acetonitrile, 55:45; flow rate: 1.0 ml/min; retention times: **10** at 7.5 min; **11** at 4.0 min].[9]

The incorporation rate of deuterium atoms of **10** was calculated on the basis of MS data of **10** and BL **1**,[11] which showed that the rate was remarkably high, and **10** was a cluster of [25,26,27-^2H_n]BLs. The composition ratio of the cluster, $^2H_2$:$^2H_3$:$^2H_4$:$^2H_6$:$^2H_7$=3:8:14:15:60, indicated that the obtained **10** was 60% isotopically pure [25,26,27-$^2H_7$]BL which should be completely deuteriogenated at C-25, -26 and -27. With 60% isotopic purity, [25,26,27-$^2H_7$]BL **10** fully deserves an internal standard for quantitative analysis of endogenous BL in plants, and the high incorporation rate should promise the preparation of [25,26,27-^3H_n]BL with high specific activity. Similarly, the incorporation rate of the by-product **11** was also high. The calculated composition ratio of the cluster, $^2H_2$:$^2H_3$:$^2H_5$:$^2H_6$=7:13:12:68, showed that **11** was [26,27-$^2H_6$]-$\Delta^{24(25)}$-BL with 68% isotopic purity. The observed high incorporation of deuterium atoms to both C-26 and -27 as well as C-25 of **10** should be explained as the result of the repeated deuteriogenation-dehydrogenation process on the catalyst surface participated by π-bonded and half-deuteriogenated intermediates or π-allyl intermediate: the intervention of such intermediates in the usual catalytic hydrogenation reactions is generally accepted.[12]



reagents and conditions: a: $SOCl_2$, pyridine, 0°C, 30 min.
b: i. 5% KOH-90% *aq.* MeOH, rt, 1 h, then reflux, 1 h.
ii. Dowex-50W (H^+form), pH 3-4, MeOH-H_2O (4:1), rt, 3 h.
c: 5% Pd-C, D_2, 1 atom, rt, 1 h.

Scheme 3

As described, we have achieved the synthesis of abundantly deuteriogenated [25,26,27-^2H_n]BL **10** from BL **1**. Since **1** is one of the most labile steroids, this basic strategy should provide a general protocol for efficient labeling of a variety of steroids including BRs by deuterium or tritium atoms. In addition, we found that TFD efficiently oxy-functionalized C-14 as well as C-25 on the BL molecule. With a view to elucidating the synthetic utility of TFD for functionalization of steroidal rings, studies on the reactivities of TFD on other steroids and the synthesis of [14,15-^2H_n]BL from **6** are under way.

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References and Notes

1. For a review, see Fujioka, S.; Sakurai, A. *Nat. Prod. Rep.* **1997**, *14*, 1-10.
2. Marquardt, V.; Adam, G. in *Chemistry of Plant Protection* Ebing, W. Ed.-in-Chief; Springer-Verlag: New York, 1991; vol. 7, pp. 103-139, and references cited therein.
3. Yokota, T.; Watanabe, S.; Ogino, Y.; Yamaguchi, I.; Takahashi, N. *J. Plant Growth Regul.* **1990**, *9*, 151-159.
4. For examples see, Cohen, Z.; Keinan, E.; Mazur, Y.; Ulman, A. *J. Org. Chem.*, **1976**, *41*, 2651-2652; Cohen, Z.; Mazur, Y. *J. Org. Chem.*, **1979**, *44*, 2318-2320; Kiegiel, J.; Wovkulich, P. M.; Groves, J. T.; Neumann, R. *J. Org. Chem.* **1988**, *53*, 3891-3893; Uskokovic, M. R. *Tetrahedron Lett.* **1991**, *32*, 6057-6060.
5. Mello, R.; Fiorentino, M.; Sciacovelli, O.; Curci, R. *J. Org. Chem.* **1988**, *53*, 3890-3891; Mello, R.; Fiorentino, M.; Fusco, C.; Curci, R. *J. Am. Chem. Soc.* **1989**, *111*, 6749-6757.
6. Bovicelli, P.; Lupattelli, P.; Mincione, E.; Prencipe, T.; Curci, R. *J. Org. Chem.* **1992**, *57*, 5052-5054.
7. Voigt, B.; Porzel, A.; Golsch, D.; Adam, W.; Adam, G. *Tetrahedron* **1996**, *52*, 10653-10658.
8. Takatsuto, S.; Yazawa, N.; Ishiguro, M.; Morisaki, M.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1984**, 139-146.
9. All new compounds were fully characterized by ^{1}H and ^{13}C NMR and MS spectra. Selected ^{1}H NMR data of compounds **4**, **5**, **6**, **9**, **10** and **11** are shown here. **4** (600 MHz, $CDCl_3$): δ 0.83 (3H, s, 18-H_3), 0.89 (3H, s, 19-H_3), 0.91 (3H, d, *J*=6.8 Hz, 26- or 27-H_3), 0.95 (3H, d, *J*=6.4 Hz, 26- or 27-H_3) 0.97 (3H, d, *J*=6.8 Hz, 28-H_3), 0.98 (3H, d, *J*=6.4 Hz, 21-H_3), 2.00 (6H, s, 2 x Ac), 2.03 and 2.12 (each 3H, each s, 2 x Ac), 3.04 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 4.26 (1H, d, *J*=13.2 Hz, 7-H), 4.33 (1H, dd, *J*=13.2 and 7.8 Hz, 7-H), 4.87 (1H, ddd, *J*=12.7, 4.4 and 2.9 Hz, 2-H), 5.16 (1H, d, *J*=8.8 Hz, 22-H), 5.34 (1H, dd, *J*=8.8 and 2.0 Hz, 23-H), 5.37 (1H, m, 3-H); **5** (600 MHz, $CDCl_3$): δ 0.80 (3H, s, 18-H_3), 0.99 (3H, s, 19-H_3), 1.14 and 1.22 (each 3H, each s, 26- and 27-H_3), 1.02 (3H, d, *J*=6.8 Hz, 28-H_3), 1.11 (3H, d, *J*=6.8 Hz, 21-H_3), 2.00, 2.01, 2.05 and 2.10 (each 3H, each s, 4 x Ac), 2.80 (1H, dd, *J*=19.0 and 9.3 Hz, 16-H), 3.02 (1H, dd, *J*=12.2 and 4.2 Hz, 5-H), 3.91 (1H, dd, *J*=12.2 and 8.8 Hz, 7-H), 4.88 (1H, ddd, *J*=12.7, 4.9 and 2.4 Hz, 2-H), 5.00 (1H, br d, *J*=9.3 Hz, 22-H), 5.20 (1H, br d, *J*=12.2 Hz, 7-H), 5.37 (1H, dt, *J*=4.4 and 2.4 Hz, 3-H), 5.53 (1H, br d, *J*=9.3 Hz, 23-H); **6** (600 MHz, $CDCl_3$): δ 0.83 (3H, s, 18-H_3), 1.00 (3H, s, 19-H_3), 1.01 (3H, d, *J*=6.4 Hz, 21-H_3), 1.05 (3H, d, *J*=7.3 Hz, 28-H_3), 1.15 and 1.22 (each 3H, each s, 26- and 27-H_3), 2.00, 2.01, 2.04 and 2.11 (each 3H, each s, 4 x Ac), 3.04 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 4.26 (1H, d, *J*=12.7 Hz, 7-H), 4.33 (1H, dd, *J*=12.7 and 7.3 Hz, 7-H), 4.87 (1H, ddd, *J*=12.7, 4.4 and 2.4 Hz, 2-H), 5.13 (1H, d, *J*=9.3 Hz, 22-H), 5.37 (1H, m, 3-H), 5.51 (1H, br d, *J*=9.3 Hz, 23-H); **9** (300 MHz, $CDCl_3$-CD_3OD, 10:1): δ 0.72 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 0.95 (3H, d, *J*=6.4 Hz, 21- or 28-H_3), 1.02 (3H, d, *J*=7.0 Hz, 21- or 28-H_3), 1.78 (3H, s, 27-H_3), 3.13 (1H, dd, *J*=12.1 and 4.5 Hz, 5-H), 3.56 and 3.58 (each 1H, br ABq, *J*=6.9 Hz, 22- and 23-H), 3.63 (1H, ddd, *J*=12.2, 4.6 and 2.8 Hz, 2-H), 3.97 (1H, m, 3-H), 4.10 (2H, d-like, *J*=5.3 Hz, 7-H_2), 4.81 and 4.91 (each 1H, each br s, 26-H_2); **10** (300 MHz, $CDCl_3$-CD_3OD, 10:1): δ 0.72 (3H, s, 18-H_3), 0.84 (3H, d, *J*=6.9 Hz, 21- or 28-H_3), 0.89 (3H, d, *J*=0.65 Hz, 21- or 28-H_3), 0.92 (3H, s, 19-H_3), 3.12 (1H, dd, *J*=12.2 and 4.7 Hz, 5-H), 3.53 and 3.69 (each 1H, br ABq, *J*=8.5 Hz, 22- and 23-H), 3.67 (1H, m, 2-H), 3.99 (1H, m, 3-H), 4.06-4.12 (2H, 7-H_2); this spectrum was broadly identical to that of BL **1** except for the absence of resonances based on 26- and 27-H_3; **11** (300 MHz, $CDCl_3$-CD_3OD, 10:1): δ 0.65 (3H, s, 18-H_3), 0.89 (3H, d, *J*=6.8 Hz, 21-H_3), 1.59 (3H, s, 28-H_3), 3.13 (1H, dd, *J*=11.8 and 4.3 Hz, 5-H), 3.61 (1H, d, *J*=9.1 Hz, 22-H), 3.63 (1H, m, 2-H), 3.96 (1H, m, 3-H), 4.09 (2H, d-like, 7-H_2), 4.48 (1H, d, *J*=9.1 Hz, 23-H).
10. This procedure was necessary to eliminate a side-reaction, C-5 epimerization, which was observed to a considerable extent when the conventional conditions for removal of the tetra-*O*-acetyl protecting groups of the BL congeners [5% methanolic potassium hydroxide, reflux: for an example, see ref. 8] were used.
11. FAB-MS data around $[M+1]^+$ (positive ion) of BL **1**, [25,26,27-$^{2}H_7$]BL **10** and [26,27-$^{2}H_6$]-$\Delta^{24(25)}$-BL **11** are as follows: **1** *m/z* 479 (24%), 480 (13), 481 ($[M+1]^+$, 100), 482 (33); **10** *m/z* 481 (2%), 482 (4), 483 (12), 484 (16), 485 (30), 486 (24), 487 (36), 488 ($[M+1]^+$, 100), 489 (34); **11** *m/z* 479 (1%), 480 (5), 481 (10), 482 (25), 483 (20), 484 (25), 485 ($[M+1]^+$, 100), 486 (30).
12. House, O. H. *Modern Synthetic Reactions*; 2nd edn, W. A. Benjamin, Inc., Menlo Park, CA, 1972, pp. 1-44; Siegel, S. in *Comprehensive Organic Syntheses*, ed. Trost, B. M.; Fleming, I., Pergamon Press, Oxford, 1991, vol. 8, pp. 417-441, and references cited therein.