Pergamon

Tetrahedron 55 (1999) 8341–8352

TETRAHEDRON

Synthesis and Biological Evaluation of Extra-Hydroxylated Brassinolide Analogs

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Received 15 April 1999; accepted 17 May 1999

Keywords: Brassinolide, Dioxirane, Hydroxylation, Rice lamina inclination test, Structure-activity relationships, Molecular probe.

Abstract: Brassinolide (BL) analogs with an extra hydroxyl (OH) group(s), 14α-OH-BL (**2**), 25-OH-BL (**4**) and 14α,25-di-OH-BL (**7**) were synthesized from BL (**1**) *via* direct hydroxylation of the C-14 and/or -25 positions with methyl(trifluoromethyl)dioxirane, and (25*S*)-26-OH-BL (**5**) from a known lactone (**16**). The biological evaluation of these compounds together with (20*R*)-20-OH-BL (**3**), 28-OH-BL (**6**) and (20*R*)-20,28-di-OH-BL (**8**) by the rice lamina inclination test suggested that hydroxylations at C-14, -20, -25 and -26 are inactivation steps in BL metabolism, and that C-28 position of **1** is a promising accessory site for assembling BL-based molecular probes to investigate brassinosteroid-receptors.

INTRODUCTION

Brassinosteroid (BR), a new class of plant hormone with a steroidal skeleton, is a subject of current importance in plant physiology due to its diverse phytohormonal activities and significant potential in agricultural use.[1] Since the first isolation of a highly oxy-functionalized BR, brassinolide (BL, **1**), from rape pollen (*Brassica napus* L.) by Grove *et al.* in 1979,[2] over forty steroids have been enrolled as natural BRs,[3] but BL analogs possessing additional hydroxyl (OH) functions have not yet been identified in plants. However, extra-hydroxylated BL analogs should be of particular interest from the viewpoint of metabolism of **1** and the design of BL-based molecular probes useful for investigation of BR-receptors. Recently, Adam *et al.* identified 25-β-D-glucopyranosyloxy-24-*epi*-BL (**21**) and 25ξ-26-β-D-glucopyranosyloxy-24-*epi*-BL (**23**) or (20*R*)-20-OH-3,24-di-*epi*-BL as metabolites of exogenously applied 24-*epi*-BL (**20**) in cell suspension cultures of *Lycopersicon esculentum* or *Ornithopus sativus*,[4] suggesting the presence of extra-hydroxylated BL analogs in plants. On the other hand, the studies on the structure-activity relationship and biosynthesis of BRs reported so far[5,6] show that **1** is the most active natural BR and a terminus in BR biosynthesis. This leads to a strong assumption that **1** should be an actual ligand molecule to bind to BR-receptors in plants, triggering a signal transduction to induce a

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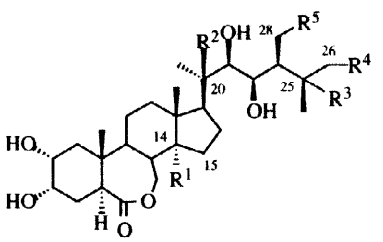
PII: S0040-4020(99)00427-5

variety of BR activities. In designing BL-based molecular probes, the OH groups at C-2, -3, -22 and -23 positions on **1** should be respectively anticipated to serve as the convenient site to attach various functional domains; however, Luo *et al.* recently reported that blocking these positions resulted in considerable loss of activity.[5c] Therefore, another OH function on extra-hydroxylated BL analogs is expected to be a good candidate for the accessory site, but little is known about the effects of the extra hydroxylation of **1** on the BR activities.

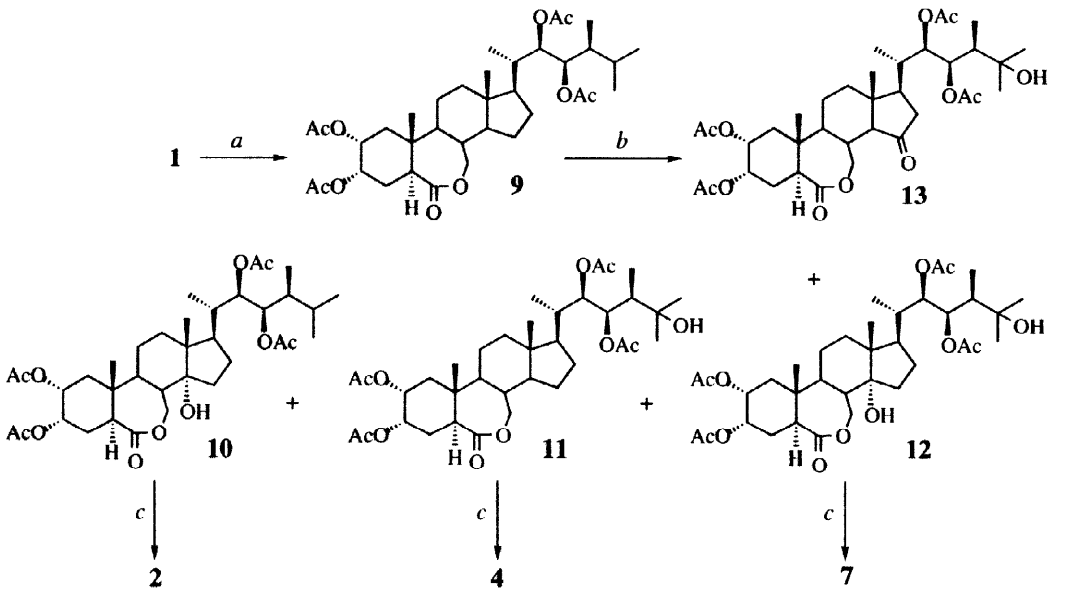
In this paper, we describe the synthesis of 14α-OH-BL (**2**), 25-OH-BL (**4**) and 14α,25-di-OH-BL (**7**) from BL (**1**) *via* direct hydroxylation of the C-14 and/or -25 positions with methyl(trifluoromethyl)dioxirane (TFD),[7] and (25*S*)-26-OH-BL (**5**) from a lactone (**16**), a key synthetic intermediate to **1** and 25-methyl-BL previously reported by us.[8] Also reported are the results of the rice lamina inclination test, a typical bioassay employed for testing BR activity,[9] of these compounds together with **1**, (20*R*)-20-OH-BL (**3**), 28-OH-BL (**6**) and (20*R*)-20,28-di-OH-BL (**8**) previously synthesized by us.[10] A set of these compounds constructs a reference compound library for identification of extra-hydroxylated BL analogs in plants, and the knowledge gained from the bioassay should provide insight into the accessory site suitable for assembling molecular probes to investigate BR-receptors: high biological activity comparable to that of the original ligand **1** is an essential criterion for an effective molecular probe.



1 R^1=R^2=R^3=R^4=R^5=H
2 R^1=OH, R^2=R^3=R^4=R^5=H
3 R^1=R^3=R^4=R^5=H, R^2=OH
4 R^1=R^2=R^4=R^5=H, R^3=OH
5 R^1=R^2=R^3=R^5=H, R^4=OH
6 R^1=R^2=R^3=R^4=H, R^5=OH
7 R^1=R^3=OH, R^2=R^4=R^5=H
8 R^1=R^3=R^4=H, R^2=R^5=OH

RESULTS AND DISCUSSION

Synthesis.—The synthesis of 14α-OH-BL (**2**), 25-OH-BL (**4**) and 14α,25-di-OH-BL (**7**) from BL (**1**) *via* direct hydroxylation with TFD is summarized in Scheme **1**. The direct oxidation of nonactivated alkane C-H bonds has been a continuing challenge to organic chemists.[11] Among the reagents explored for this purpose, TFD developed by Mello *et al.*[12] seems to be remarkably superior due to its efficacy, mildness, and chemo- and site-selectivities, which was well verified by Bovicelli *et al.* in the site-selective C-25 hydroxylation of some cholestane derivatives and Vitamin D_3 Windaus-Grundmann ketone.[13] Later, Voigt *et al.* applied this reagent to 2,3,22,23-tetra-*O*-acetylBL (**9**) to obtain 25-OH derivative **11**.[14] In the course of our recent study on the synthesis of [25,26,27-$^2H_7$]BL,[6] we re-investigated the reactivity of TFD on **9** and found that this reagent oxidized not only the C-25 but also the C-14 and -15. Tetrahydroxyl compound **1** was peracetylated with acetic anhydride in pyridine at 60°C for 20 h to give tetraacetate (**9**) in 95% yield. When **9** was treated with 3 equiv. of TFD, prepared from 1,1,1-trifluoro-2-propanone and OXONE® by following Mello's procedure,[12b] in a mixed solution of trifluoroacetone and CH_2Cl_2, 1:2, at 0°C for 3 h, after flash chromatography, four oxygenated products, **10**, **11**, **12** and **13**, were obtained in 6.5, 48, 18 and 3.6% yields, respectively, along with recovery of the starting material **9** (19%). As expected from the product distribution suggesting that the reactivities of hydrogens on **9** lie in the order, 25-H>14-H>15-H, the temperature dependence of the site-selectivity was observed in this reaction. At -30°C the same treatment of **9** for 5 h led to the nearly exclusive formation of **11** (61%: 85% based on the amount of **9** consumed) and recovery of **9** (28%), while at room temperature (rt) a large

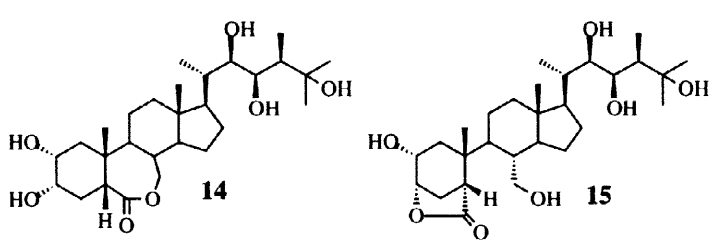


Reagents and conditions: *a*: Ac_2O, pyridine, 60°C, 20 h. *b*: 3 equiv. of TFD, trifluoroacetone-CH_2Cl_2, 1:2, 0°C, 3 h. *c*: i. 5% KOH-90% *aq.* MeOH, rt, 1 h, then reflux, 1.5 h; ii. Dowex-50W (H^+form), pH 3-4, MeOH-H_2O (4:1), rt, 3 h.

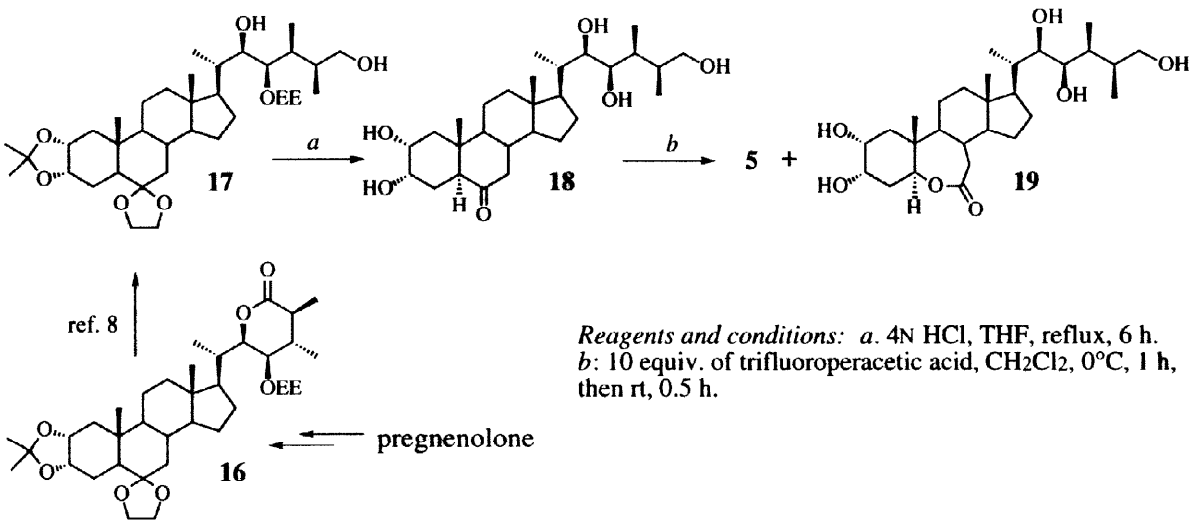
Scheme 1 Synthesis of 14-OH-BL (**2**), 15-OH-BL (**4**) and 14,25-di-OH-BL (**7**) from BL (**1**)

amount of TFD (6 equiv.) and prolonged reaction time (24 h) achieved the predominant formation of dihydroxylated compound **12** in 78% yield.

Removal of the tetraacetyl groups on **10-12** was achieved by an alkali hydrolysis procedure recently established by us to prevent the C-5 epimerization.[5b] Tetraacetates (**10-12**) were treated with 5% KOH in 90% MeOH at rt for 1-2 h until the lactone ring completely opened to the carboxylate, monitored by TLC, and then at refluxing temperature for 1.5 h; and the subsequent re-lactonization with Dowex-50W-2X resin (H^+ form) at pH 3-4 in MeOH-H_2O (4:1) at rt for 3 h afforded 14-OH-BL (**2**), 25-OH-BL (**4**) and 14,25-di-OH-BL (**7**) in 82, 86 and 79% yields, respectively. On the contrary, the conventional procedure[15] led to the formation of considerable amounts of by-products resulted from C-5 epimerization through intermediary methyl esters: *e.g.*, when **11** was treated with 5% KOH in MeOH at refluxing temperature for 3 h, followed by re-lactonization, the desired **4** was obtained in 56% yield along with 5-*epi*-25-OH-BL (**14**) (22%) and 6(6a→3a)*abeo*-5-*epi*-25-OH-BL (**15**) (12%).



The synthesis of (25*S*)-26-OH-BL (**6**) from lactone (**16**), a common synthetic intermediate of BL (**1**) and 25-methyl-BL from pregnenolone is shown in Scheme 2. After reduction of the lactone function of **16** with $LiAlH_4$ in Et_2O,[8] the resulting acetal (**17**) was treated with 4N HCl in refluxing THF for 6 h to give (25*S*)-26-OH-castasterone (**18**) in 74% yield. The Baeyer-Villiger oxidation of **18** with 10 equiv. of trifluoroperacetic



Reagents and conditions: a. 4N HCl, THF, reflux, 6 h. *b*: 10 equiv. of trifluoroperacetic acid, CH_2Cl_2, 0°C, 1 h, then rt, 0.5 h.

Scheme 2. Synthesis of (25*S*)-26-OH-BL (**5**) *via* lactone (**16**).

acid, generated from 30% H_2O_2 and trifluoroacetic anhydride, in CH_2Cl_2 at 0°C for 1 h and then at rt for 0.5 h gave the desired **5** in 14 % yield along with 2% of the isomeric lactone (**19**), after HPLC purification.

The structures of the new compounds, **2**, **5**, **7**, **10**, **12-15**, **18** and **19**, were verified by MS and NMR spectroscopy. NMR experiments on these compounds, except for **19**, as well as the known **9**, **4** and **11** for reference were carried out with 600 MHz by PFG-DQFCOSY, PFG-HMQC and PFG-HMBC,[16] by which all resonances were completely assigned (Experimental section). The stereochemistry of 14-OH on **2**, **7**, **10** and **12** was determined on the basis of the downfield shifts of the 9-H and 17-H compared with those of the 14-deoxy counterparts: *e.g.*, the 9-H and 17-H of 2,3,22,23-tetra-*O*-acetyl-14-OH-BL (**10**) resonanced at δ 1.95 and δ 1.68, respectively, while those of 2,3,22,23-tetra-*O*-acetylBL (**9**) resonanced at δ 1.26 and δ 1.16. This indicates the 1,3-diaxial relationship between 14-OH and 9-H/16-H, confirming the α-configuration of the 14-OH. The retention of the C/D *trans* ring junction of 15-oxo compound **13** was proved by a large coupling constant of 14-H (δ 1.82) with 8-H (δ 2.07), *i.e.*, $J_{14,8}$=11.7 Hz, showing the *anti*-relationship. The resonance without a large coupling constant was characteristic of 5β-H of 25-OH-5-*epi*-BL (**14**) [δ 2.96 (d, $J_{5,4\beta}$=6.3 Hz)] or its lactone isomer (**15**) [δ 3.02 (br d, $J_{5,4\alpha}$=6.4 Hz)] which has no vicinal *anti*-proton at C-4, and this is clearly distinguishable from that of 25-OH-BL (**4**) with 5α-H [δ 3.19 (dd, $J_{5,4\beta}$=12.7 and $J_{5,4\alpha}$=4.4 Hz)].

Bioassay.—Biological activities of **2**, **4**, **5** and **7** synthesized here and (20*R*)-20-OH-BL (**3**), 28-OH-BL (**6**) and (20*R*)-20,28-di-OH-BL (**8**) previously synthesized[10] were examined by using the rice lamina inclination test (*Oryza sativa* L., cv. Tan-ginbozu), and their activities were compared with those of BL (**1**), as shown in Tables 1 and 2. Two assay methods were employed: single application of the test sample, and the co-application with 5 μg of indole-3-acetic acid (IAA), the latter of which was recently developed by us and found to be much more sensitive than the former.[9] By single application (Table 1), the inclination activities of extra-hydroxylated BL analogs are in the following order: BL (**1**) (100%) > 28-OH-BL (**6**) (18) > 25-OH-BL (**4**) (0.2) > 20-OH-BL (**3**) (0.1) and 26-OH-BL (**5**) (0.1) > 14-OH-BL (**2**) (0) and 14,25-di-OH-BL (**7**) (0). The figures in parentheses, except for that of **6**, show the relative activities (a%) estimated by the dosage (b ng) of the reference compound **1** exhibiting the same response of the test compound at 1000 ng, *i.e.*, a=b/1000 x 100. Meanwhile, the relative activity of **6** was estimated by the dosages, b ng of **1** and c ng of **6**, at the midpoint of the maximum

response of **1**, *i.e.*, a=b/c x 100. By co-application with IAA (Table 2), the activity order is as follows: BL (**1**) (100%) > 28-OH-BL (**6**) (25) > 25-OH-BL (**4**) (3) > 26-OH-BL (**5**) (1) > 20-OH-BL (**3**) (0.4) > 20,28-di-OH-BL (**8**) (0.03). In this case, the same method as for **6** described above was adopted to estimate the relative activities. These results indicate that, except for C-28 hydroxylation, hydroxylations at C-14, -20, -25 and -26 of **1** drastically decrease the biological activity, suggesting that these hydroxylations are inactivation steps in BL metabolism, if any, in plants.

Table. 1 Rice lamina inclination test of hydroxylated BL analogs, **2-7**, by single application of the test sample.[a]

Run[b]	Compounds	Dosages (ng)				
		0	1	10	100	1000
1	**1** BL	15±1	39±5	88±5	108±4	111±4
	2 14-OH-BL	–	15±1	15±1	16±1	15±1
	7 14,25-di-OH-BL	–	15±1	15±1	15±1	15±1
2	**1**	16±1	38±4	85±5	106±4	117±5
	3 (20*R*)-20-OH-BL	–	16±1	17±1	21±2	39±3
	4 25-OH-BL	–	15±1	19±1	31±3	57±6
	5 (24*S*)-26-OH-BL	–	15±1	18±1	22±1	39±4
3	**1**	13±1	28±4	78±7	108±4	114±3
	6 28-OH-BL	–	18±2	30±4	90±6	107±5

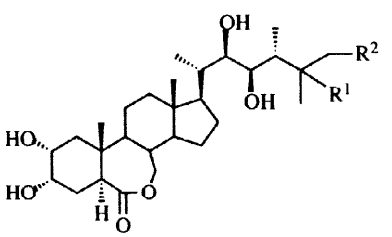
[a]Values are angles (°) between the lamina and sheath, representing the means of 30 replicates ± SE.
[b]In each run, BL (**1**) was tested for comparison.

Table. 2 Rice lamina inclination test of hydroxylated BL analogs, **3-6** and **8**, by co-application of the test sample with IAA (5 μg/plant).[a]

Run[b]	Compounds	Dosages (ng)						
		0	0.01	0.1	1	10	100	1000
1	**1** BL	26±4	53±7	100±6	119±3	125±2	126±1	–
	3 (20*R*)-20-OH-BL	–	25±2	25±3	40±6	81±7	120±1	–
2	**1**	22±3	56±8	103±7	119±3	121±2	124±4	123±3
	4 25-OH-BL	–	22±3	38±7	78±9	104±4	110±5	123±2
3	**1**	23±1	58±7	105±6	120±3	123±1	122±3	–
	5 (24*S*)-26-OH-BL	–	28±3	32±5	62±7	92±6	119±2	–
4	**1**	20±1	50±7	112±1	125±3	121±2	119±4	120±2
	6 28-OH-BL	–	43±7	65±9	106±6	120±5	116±7	117±5
	8 (20*R*)-20,28-di-OH-BL	–	20±2	22±3	22±3	24±2	73±9	98±9

[a]Values are angles (°) between the lamina and sheath, representing the means of 30 replicates ± SE.
[b]In each run, BL (**1**) was tested for comparison.

In this connection, Adam *et al.* recently reported a rice lamina inclination test of 25-OH-24-*epi*-BL (**22**) and 25ξ-26-OH-24-*epi*-BL (**24**), which were enzymatically prepared from their glucosides **21** and **23**, metabolites of exogenously applied 24-*epi*-BL (**20**) in cell suspension cultures of *Lycopersicon esculentum*.[4a] Although the



20 $R^1=R^2=H$
21 R^1=β-D-glucopyranosyloxy, $R^2=H$
22 R^1=OH, $R^2=H$
23 R=H, R^2=β-D-glucopyranosyloxy
24 $R^1=H$, R^2=OH

cultivar (*Oryza sativa* L., cv. Nap IR-415) and assay system (floating leaf segment method) were different from those employed here, their result described that **22** was about ten times more active than the parent compound **20**, while **24** was a little less active than **22**. This led to their conclusion that the hydroxylation at C-25 was an activation step in BR metabolism, which is contrary to our finding described above. However, taking the ubiquitous distribution in the plant kingdom and the high biological activity into account, we propose that BL (**1**) should be a key BR; in contrast, **22** has been detected in only a few plants and its activity in lamina test is less than 1/10 of that of **1**. It is, therefore, more reasonably concluded that hydroxylations at C-14, -20, -25 and -26 of BRs, including **1**, are inactivation steps in BR metabolism.

CONCLUSION

Extra-hydroxylated BL analogs, 14-OH-BL (**2**), 25-OH-BL (**4**) and 14α,25-di-OH-BL (**7**) were successfully synthesized from BL (**1**) *via* direct hydroxylation of the C-14 and/or -25 positions with TFD, and (25*S*)-26-OH-BL (**5**) from lactone (**16**). From a synthetic point of view, the finding that TFD oxy-functionalizes C-14 and -15 on the ring of **9** as well as the side chain C-25 should be important because, although the direct oxy-functionalization of unactivated ring C-H bonds on steroid has been an attractive target for synthetic chemists, the previously known methods, except for microbial oxidation,[17] always resulted in low chemical yields. The biological evaluation of these compounds together with (20*R*)-20-OH-BL (**3**), 28-OH-BL (**6**) and (20*R*)-20,28-di-OH-BL (**8**) by the rice lamina inclination test suggested that hydroxylations at C-14, -20, -25 and -26 are inactivation steps in BL metabolism. Among the hydroxylated BL analogs assayed, only **6** retained sufficient activity, indicating that the C-28 position of **1** should be a promising accessory site for assembling BL-based molecular probes useful for investigation of BR-receptors. With the reference compound library of extra-hydroxylated BL analogs and the knowledge of their activities gained here, further studies on the synthesis of a BL-based molecular probe and identification of hydroxylated BLs in plants are being undertaken.

EXPERIMENTAL SECTION

General. Melting points (mp) were determined on Yanagimoto micromelting point apparatus and are uncorrected. NMR measurements were performed on JEOL JNM-A600, JEOL JNM-ECP500 or Bruker AC-300 spectrometer. All spectra were recorded using standard pulse sequences. Chemical shifts were recorded as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0 ppm) for ^1H and the solvent (δ 77.0 ppm) for ^{13}C as an internal reference in $CDCl_3$ solution; or relative to CD_3OD: δ 3.30 ppm for ^1H and 49.0 ppm for ^{13}C, in CD_3OD or a mixed solution of CD_3OD and $CDCl_3$. All *J*-values are given in Hz. FAB-MS was obtained with a JEOL HX-110 mass spectrometer. All reactions were carried out under a nitrogen atmosphere. Column chromatography was conducted using silica gel FL-60D [Fuji Silysia Chemical Ltd.] as the adsorbent. High-performance liquid chromatography (HPLC) was conducted with Senshu Pak ODS-1151-D (4.6 mm i.d. x 15 cm; Senshu Scientific Co.) at a flow rate of 1 mL/min; the peaks were detected by a photometric detector at 205 nm. The ratios of mixed solvents were v/v.

2,3,22,23-Tetra-*O*-acetylBL (9). A mixture of BL (**1**) (190 mg, 0.40 mmol), acetic anhydride (2.0 mL) and dry pyridine (4.0 mL) was reacted at 60°C for 20 h. After removal of volatile materials *in vacuo*, the residue was extracted with AcOEt. The extracts were successively washed with 2N HCl, water and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was subjected to column chromatography using hexane-AcOEt (3:1) as the eluent, affording 2,3,22,23-tetra-*O*-acetylBL (**9**) (242 mg, 95%): colorless needles, mp 206-209°C (hexane-AcOEt) [lit.,[15] mp 231-233°C (MeOH)]; ^1H NMR (600 MHz; $CDCl_3$) δ 0.74 (3H, s, 18-

H_3), 0.91 (3H, d, *J*=7.3 Hz, 26-H_3), 0.94 (3H, d, *J*=6.8 Hz, 27-H_3), 0.96 (3H, d, *J*=6.8 Hz, 28-H_3), 0.99 (3H, s, 19-H_3), 1.01 (3H, d, *J*=6.8 Hz, 21-H_3), 1.16 (1H, m, 17-H), 1.18 (1H, m, 14-H), 1.19 (1H, m, 12α-H), 1.26 (1H, m, 9-H), 1.28 and 1.68 (each 1H, each m, 15-H_2), 1.31 and 2.09 (each 1H, each m, 16-H_2), 1.33 (1H, m, 24-H), 1.41 (1H, m, 11β-H), 1.42 (1H, m, 25-H), 1.61 (1H, m, 20-H), 1.62 (1H, dd, *J*=12.7 and 12.7 Hz, 1α-H), 1.73 (1H, m, 11α-H), 1.76 (1H, m, 8-H), 1.92 (1H, dd, *J*=12.7 and 4.4 Hz, 1β-H), 1.93 (1H, m, 4α-H), 1.98 (1H, m, 12β-H), 1.99 (3H, s, 23-OAc), 2.00 (3H, s, 2-OAc), 2.01 (3H, s, 22-OAc), 2.11 (3H, s, 3-OAc), 2.29 (1H, ddd, *J*=14.7, 12.2 and 2.0 Hz, 4β-H), 2.99 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 4.04 (1H, dd, *J*=12.7 and 9.3 Hz, 7α-H), 4.04 (1H, dd, *J*=12.7 and 9.3 Hz, 7α-H), 4.12 (1H, br d, *J*=12.7 Hz, 7β-H), 4.87 (1H, ddd, *J*=12.7, 4.4 and 2.9 Hz, 2-H), 5.14 (1H, br d, *J*=8.8 Hz, 22-H), 5.32 (1H, dd, *J*=8.8 and 1.5 Hz, 23-H), 5.36 (1H, m, 3-H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 11.05 (C-28), 11.61 (C-18), 12.75 (C-21), 15.46 (C-19), 20.29 (C-26), 20.86 (C-27), 20.86 (22-OCO<u>C</u>H_3), 20.94 (23-OCO<u>C</u>H_3), 21.04 (2-OCO<u>C</u>H_3), 21.14 (3-OCO<u>C</u>H_3), 22.25 (C-11), 24.69 (C-15), 27.98 (C-16), 29.29 (C-4), 30.38 (C-25), 36.98 (C-20), 38.38 (C-10), 38.87 (C-1), 39.15 (C-8), 39.45 (C-12), 39.82 (C-24), 42.00 (C-5), 42.47 (C-13), 51.31 (C-14), 52.34 (C-17), 58.35 (C-9), 67.90 (C-3), 68.91 (C-2), 70.38 (C-7), 74.06 (C-23), 75.66 (C-22), 169.93 (3-O<u>C</u>OCH$_3$), 170.23 (2-O<u>C</u>OCH$_3$), 170.48 (22-O<u>C</u>OCH$_3$), 170.55 (23-O<u>C</u>OCH$_3$), 175.01 (C-6).

Oxidation of 9 with Methyl(trifluoromethyl)dioxirane (TFD). A 1,1,1-trifluoroacetone solution of TFD was prepared from trifluoroacetone and OXONE® by following Mello's procedure[12b] and used immediately as a 0.5 M solution without titration. During the reaction, the reaction vessel was wrapped with aluminum foil, and light was eliminated as far as possible. To a stirred solution of **9** (110 mg, 0.17 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise a 0.5 M solution of TFD in trifluoroacetone (1 mL, 0.5 mmol) at 0°C, and the mixture was stirred at the same temperature for 3 h. After removal of volatile materials *in vacuo*, the residue was subjected to flash chromatography. Elution with hexane-AcOEt (2:1) gave the starting material **9** (21 mg, 19%), 25-OH compound (**11**) (54 mg, 48%), 14-OH compound (**10**) (7.3 mg, 6.5%) and then 25-OH-15-oxo compound (**13**) (4.1 mg, 3.6%). Further elution with hexane-AcOEt (1:1) gave 14,25-di-OH compound (**12**) (21 mg, 18%).

The same reaction was carried out using a 0.5 M solution of TFD in trifluoroacetone (0.2 mL, 0.1 mmol) in CH_2Cl_2 (0.4.mL) at -30°C for 5 h, **9** (20 mg, 0.03 mmol) afforded **9** (5.6 mg, 28%) and **11** (12.5 mg, 61%).

The same reaction was carried out using a 0.5 M solution of TFD in trifluoroacetone (0.2 mL, 0.1 mmol) in CH_2Cl_2 (0.2 mL) at rt for 24 h, **9** (10 mg, 0.015 mmol) afforded **12** (8.2 mg, 78%).

2,3,22,23-Tetra-*O*-acetyl-14α-OH-BL (10): colorless needles, mp 186-188°C (hexane-AcOEt); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.83 (3H, s, 18-H_3), 0.91 (3H, d, *J*=6.8 Hz, 26-H_3), 0.95 (3H, d, *J*=6.4 Hz, 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), 1.00 (3H, s, 19-H_3), 1.33 (1H, m, 24-H), 1.43 (1H, m, 25-H), 1.43 and 1.70 (each 1H, each m, 11-H_2), 1.43 and 2.21 (each 1H, each m, 16-H_2), 1.65 and 1.77 (each 1H, each m, 12-H_2), 1.65 and 1.77 (each 1H, each m, 15-H_2), 1.67 (1H, m, 20-H), 1.68 (1H, m, 17-H), 1.70 (1H, m, 1α-H), 1.90 (1H, m, 1β-H), 1.92 (1H, m, 4α-H), 1.95 (1H, m, 8-H), 1.95 (1H, m, 9-H), 2.00 (6H, s, 2- and 23-OAc), 2.03 (3H, s, 22-OAc), 2.12 (3H, s, 3-OAc), 2.29 (1H, ddd, *J*=15.6, 12.2 and 0.4 Hz, 4β-H), 3.04 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 4.26 (1H, d, *J*=13.2 Hz, 7β-H), 4.33 (1H, dd, *J*=13.2 and 7.8 Hz, 7α-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); ^{13}C NMR (150 MHz; $CDCl_3$) δ 11.09 (C-28), 12.82 (C-21), 15.08 (C-19), 15.35 (C-18), 20.25 (C-26), 20.92 (C-27), 20.87 (C-11), 20.87 and 21.07 (2- and 23-OCO<u>C</u>H_3), 20.92 (22-OCO<u>C</u>H_3), 21.20 (3-OCO<u>C</u>H_3), 26.45 (C-16), 29.37 (C-4), 30.39 (C-25), 31.71 (C-12), 33.60 (C-15), 36.79 (C-20), 38.40 (C-10), 38.76 (C-1), 39.88 (C-24), 41.65 (C-8), 41.95 (C-5), 46.21 (C-13), 46.82 (C-17), 51.12 (C-9), 67.83 (C-3), 68.93 (C-2), 69.91 (C-7), 73.96 (C-23), 75.73 (C-22), 86.09 (C-14), 170.05 (3-O<u>C</u>OCH$_3$), 170.30 (2-O<u>C</u>OCH$_3$), 170.45 (23-O<u>C</u>OCH$_3$), 170.81 (22-O<u>C</u>OCH$_3$), 175.13 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 665.3906. Calcd. for $C_{36}H_{57}O_{11}$, 665.3901.

2,3,22,23-Tetra-*O*-acetyl-25-OH-BL (11): colorless needles, mp 226-229°C (hexane-AcOEt) [lit.,[14] 238-241°C]; ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.73 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 1.036 (3H, d, *J*=6.8 Hz, 21-H_3), 1.044 (3H, d, *J*=7.3 Hz, 28-H_3), 1.15 (3H, s, 26-H_3), 1.16 (1H, m, 17-H), 1.18 (1H, m, 14-H), 1.18 (1H, m, 12α-H), 1.22 (3H, s, 27-H_3), 1.25 and 2.09 (each 1H, each m, 16-H_2), 1.27 and 1.68 (each 1H, each m, 15-H_2), 1.28 (1H, m, 9-H), 1.41 (1H, m, 11β-H), 1.62 (1H, m, 1α-H), 1.62 (1H, m, 20-H), 1.66 (1H, dq, *J*=1.0 and 7.3 Hz, 24-H), 1.73 (1H, m, 11α-H), 1.75 (1H, s, 25-OH), 1.76 (1H, m, 8-H), 1.92 (1H, m, 1β-H), 1.93 (1H, m, 4α-H), 1.98 (1H, m, 12β-H), 2.00 (6H, s, 2- and 23-OAc), 2.02 (3H, s, 22-OAc), 2.11 (3H, s, 3-OAc), 2.29 (1H, ddd, *J*=15.1, 12.2 and 2.4 Hz, 4β-H), 2.99 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 4.04 (1H, dd, *J*=12.2 and 9.3 Hz, 7α-H), 4.12 (1H, dd, *J*=12.2 and 1.0 Hz, 7β-H), 4.87 (1H, ddd, *J*=12.2, 4.4 and 2.4 Hz, 2-H), 5.12 (1H, dd, *J*=9.3 and 1.0 Hz, 22-H), 5.37 (1H, m, 3-H), 5.49 (1H, dd, *J*=9.3 and 1.0 Hz, 23-H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 9.10 (C-28), 11.65 (C-18), 12.70 (C-21), 15.46 (C-19), 20.84 (22-OCO$\underline{C}H_3$), 21.05 (2-OCO$\underline{C}H_3$), 21.13 (23-OCO$\underline{C}H_3$), 21.17 (3-OCO$\underline{C}H_3$), 22.24 (C-11), 24.69 (C-15), 26.51 (C-26), 28.12 (C-16), 28.63 (C-27), 29.29 (C-4), 37.12 (C-20), 38.40 (C-10), 38.87 (C-1), 39.15 (C-8), 39.42 (C-12), 42.01 (C-5), 42.49 (C-13), 43.36 (C-24), 51.34 (C-14), 52.36 (C-17), 58.36 (C-9), 67.92 (C-3), 68.91 (C-2), 70.39 (C-7), 72.31 (C-23), 72.40 (C-25), 75.47 (C-22), 169.96 (3-O$\underline{C}$OCH$_3$), 170.23 (2-O$\underline{C}$OCH$_3$), 170.48 (22-O$\underline{C}$OCH$_3$), 171.04 (23-O$\underline{C}$OCH$_3$), 175.02 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 665.3900. Calcd. for $C_{36}H_{57}O_{11}$, 665.3901.

2,3,22,23-Tetra-*O*-acetyl-14α,25-di-OH-BL (12): colorless granules, mp 187-191°C (hexane-AcOEt); ^{1}H NMR (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 (3H, s, 26-H_3), 1.22 (3H, s, 27-H_3), 1.27 (1H, s, 14-OH), 1.40 and 2.21 (each 1H, each m, 16-H_2), 1.42 and 1.70 (each 1H, each m, 11-H_2), 1.64 and 1.77 (each 1H, each m, 12-H_2), 1.65 and 1.78 (each 1H, each m, 15-H_2), 1.66 (1H, br q, *J*=7.3 Hz, 24-H), 1.68 (1H, m, 17-H), 1.68 (1H, m, 20-H), 1.70 (1H, m, 1α-H), 1.76 (1H, s, 25-OH), 1.90 (1H, m, 1β-H), 1.91 (1H, m, 4α-H), 1.94 (1H, m, 8-H), 1.94 (1H, m, 9-H), 2.00 (3H, s, 2-OAc), 2.01 (3H, s, 23-OAc), 2.04 (3H, s, 22-OAc), 2.11 (3H, s, 3-OAc), 2.29 (1H, ddd, *J*=15.1, 12.2 and 2.4 Hz, 4β-H), 3.04 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 4.26 (1H, d, *J*=12.7 Hz, 7β-H), 4.33 (1H, dd, *J*=12.7 and 7.3 Hz, 7α-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); ^{13}C NMR (150 MHz; $CDCl_3$) δ 9.15 (C-28), 12.77 (C-21), 15.07 (C-19), 15.36 (C-18), 20.86 (22-OCO$\underline{C}H_3$), 20.89 (C-11), 21.05 (2-OCO$\underline{C}H_3$), 21.13 (23-OCO$\underline{C}H_3$), 21.18 (3-OCO$\underline{C}H_3$), 26.43 (C-26), 26.59 (C-16), 28.65 (C-27), 29.36 (C-4), 31.64 (C-12), 33.56 (C-15), 36.90 (C-20), 38.40 (C-10), 38.73 (C-1), 41.64 (C-8), 41.95 (C-5), 43.43 (C-24), 46.21 (C-13), 46.82 (C-17), 51.11 (C-9), 67.81 (C-3), 68.91 (C-2), 69.91 (C-7), 72.12 (C-23), 72.40 (C-25), 75.59 (C-22), 86.08 (C-14), 170.05 (3-O$\underline{C}$OCH$_3$), 170.30 (2-O$\underline{C}$OCH$_3$), 170.74 (22-O$\underline{C}$OCH$_3$), 170.96 (23-O$\underline{C}$OCH$_3$), 175.13 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 681.3831. Calcd. for $C_{36}H_{57}O_{12}$, 681.3850.

2,3,22,23-Tetra-*O*-acetyl-25-OH-15-oxo-BL (13): colorless needles, mp 274-276°C decomp. (hexane-$CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.80 (3H, s, 18-H_3), 0.99 (3H, s, 19-H_3), 1.02 (3H, d, *J*=6.8 Hz, 28-H_3), 1.11 (3H, d, *J*=6.8 Hz, 21-H_3), 1.14 (3H, s, 26-H_3), 1.22 (3H, s, 27-H_3), 1.26 (1H, m, 9-H), 1.40 and 1.81 (each 1H, each m, 11-H_2), 1.42 and 2.13 (each 1H, each m, 12-H_2), 1.59 (1H, m, 17-H), 1.60 (1H, br q, *J*=6.8 Hz, 24-H), 1.61 (1H, dd, *J*=12.7 and 12.7 Hz, 1α-H), 1.72 (1H, m, 20-H), 1.82 (1H, d, *J*=11.7 Hz, 14-H), 1.85 (1H, dd, *J*=19.0 and 9.8 Hz, 16-H), 1.92 (1H, dd, *J*=12.7 and 4.9 Hz, 1β-H), 1.93 (1H, ddd, *J*=15.1, 4.4 and 4.2 Hz, 4α-H), 2.00 (3H, s, 2-OAc), 2.01 (3H, s, 23-OAc), 2.05 (3H, s, 22-OAc), 2.07 (1H, m, 8-H), 2.10 (3H, s, 3-OAc), 2.29 (1H, ddd, *J*=15.1, 12.2 and 2.4 Hz, 4β-H), 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, 7α-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, 7β-H), 5.37 (1H, ddd, *J*=4.4, 2.4 and 2.4 Hz, 3-H), 5.53 (1H, br d, *J*=9.3 Hz, 23-H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 9.22 (C-28), 12.47 (C-18), 13.19 (C-21), 15.32 (C-19), 20.77 (22-OCO$\underline{C}H_3$), 21.04 (2-

OCOCH$_3$),21.12 (3-OCOCH$_3$), 21.12 (23-OCOCH$_3$), 21.81 (C-11), 26.20 (C-26), 28.86 (C-27), 29.31 (C-4), 37.12 (C-20), 35.36 (C-8), 38.36 (C-10), 38.86 (C-1), 39.38 (C-12), 41.88 (C-5), 42.05 (C-13), 42.10 (C-16), 43.44 (C-24), 47.72 (C-17), 58.01 (C-9), 59.85 (C-14), 67.83 (C-3), 67.92 (C-7), 68.81 (C-2), 71.69 (C-23), 72.36 (C-25), 75.18 (C-22), 169.84 (3-OCOCH$_3$), 170.19 (2-OCOCH$_3$), 170.56 (22-OCOCH$_3$), 170.86 (23-OCOCH$_3$), 174.81 (C-6), 211.94 (C-15); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 679.3698. Calcd. for $C_{36}H_{55}O_{12}$, 679.3694.

General Procedure for Removal of Tetraacetate Protecting Groups of 10-12. A solution of tetraacetates **10-12** (5.0 mg) in a 90% aqueous MeOH containing 5% KOH (0.5 mL) was stirred at rt until the lactone completely opened to the carboxylate (*ca.* 1 h), which was monitored by TLC. The mixture was then stirred at refluxing temperature for 1.5 h, to which MeOH (1.5 mL) and H_2O (0.5 mL) was added and acidified with Dowex 50W resin (H^+ form) to pH 3-4 at 0°C. After stirring at rt for 3 h, the resin was filtered off through a glass filter and the filtrate was evaporated. The products were purified by column chromatography to give **2** (3.0 mg, 82%), **4** (3.2 mg, 86%) and **7** (3.0 mg, 79%), respectively.

14-OH-BL (2): CH_3Cl-MeOH (20:1) as eluent; colorless needles, mp 250-253°C decomp. (AcOEt-MeOH); ^{1}H NMR (600 MHz; CD_3OD) δ 0.84 (3H, s, 18-H$_3$), 0.85 (3H, d, *J*=6.8 Hz, 28-H$_3$), 0.86 (3H, d, *J*=6.3 Hz, 21-H$_3$), 0.90 (3H, s, 19-H$_3$), 0.93 (3H, d, *J*=6.8 Hz, 26-H$_3$), 0.96 (3H, d, *J*=6.8 Hz, 27-H$_3$), 1.18 (1H, m, 24-H), 1.37 and 2.08 (each 1H, each m, 16-H$_2$), 1.47 and 1.75 (each 1H, each m, 11-H$_2$), 1.53 (1H, m, 20-H), 1.63 (1H, m, 12α-H), 1.63 (1H, m, 25-H), 1.65 (1H, m, 1α-H), 1.68 (2 H, m, 15-H$_2$), 1.78 (1H, m, 4α-H), 1.81 (1H, m, 1β-H), 1.87 (1H, m, 12β-H), 1.88 (1H, m, 8-H), 1.97 (1H, ddd, *J*=12.2, 12.2 and 4.9 Hz, 9-H), 2.06 (1H, m, 4β-H), 2.20 (1H, m, 17-H), 3.20 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 3.52 (1H, dd, *J*=8.3 and 1.5 Hz, 22-H), 3.59 (1H, ddd, *J*=12.2, 4.4 and 2.4 Hz, 2-H), 3.68 (1H, dd, *J*=8.3 and 2.0 Hz, 23-H), 3.91 (1H, m, 3-H), 4.23 (1H, d, *J*=12.7 Hz, 7β-H), 4.35 (1H, dd, *J*=12.7 and 8.3 Hz, 7α-H); ^{13}C NMR (125 MHz; CD_3OD) δ 10.74 (C-28), 12.59 (C-21), 15.51 (C-19), 16.04 (C-18), 21.17 (C-26), 21.40 (C-27), 22.11 (C-11), 27.46 (C-16), 31.70 (C-25), 32.85 (C-4), 33.07 (C-12), 38.22 (C-20), 39.20 (C-10), 39.20 (C-15), 41.93 (C-24), 42.28 (C-5), 42.44 (C-1), 43.34 (C-8), 47.41 (C-13), 47.91 (C-17), 52.35 (C-9), 69.01 (C-2), 69.15 (C-3), 71.65 (C-7), 74.40 (C-23), 75.80 (C-22), 86.29 (C-14), 179.36 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 497.3480. Calcd. for $C_{28}H_{49}O_7$, 497.3478.

25-OH-BL (4): CH_3Cl-MeOH (20:1) as eluent; colorless granules, mp 279-282°C decomp. (MeOH) [lit.,[14] mp 309-311°C (MeOH)]; ^{1}H NMR (600 MHz; CD_3OD) δ 0.75 (3H, s, 18-H$_3$), 0.89 (3H, s, 19-H$_3$), 0.90 (3H, d, *J*=6.4 Hz, 21-H$_3$), 0.93 (3H, d, *J*=6.8 Hz, 28-H$_3$), 1.21 (3H, s, 26-H$_3$), 1.26 (1H, m, 14-H), 1.26 (1H, m, 12α-H), 1.26 and 1.73 (each 1H, each m, 15-H$_2$), 1.27 (3H, s, 27-H$_3$), 1.33 (1H, m, 9-H), 1.33 and 2.01 (each 1H, each m, 16-H$_2$), 1.44 (1H, m, 11β-H), 1.48 (1H, dq, *J*=1.5 and 6.8 Hz, 24-H), 1.51 (1H, m, 20-H), 1.59 (1H, m, 1α-H), 1.60 (1H, m, 17-H), 1.70 (1H, m, 8-H), 1.79 (1H, ddd, *J*=14.7, 4.4 and 3.9 Hz, 4α-H), 1.80 (1H, m, 11α-H), 1.81 (1H, m, 1β-H), 2.01 (1H, m, 12β-H), 2.05 (1H, ddd, *J*=14.7, 12.7 and 2.0 Hz, 4β-H), 3.19 (1H, dd, *J*=12.7 and 4.4 Hz, 5-H), 3.49 (1H, dd, *J*=8.8 and 1.5 Hz, 22-H), 3.59 (1H, ddd, *J*=12.2, 4.4 and 2.5 Hz, 2-H), 3.91 (1H, ddd, *J*=3.9, 2.5 and 2.0 Hz, 3-H), 3.96 (1H, dd, *J*=8.8 and 1.5 Hz, 23-H), 4.08 (1H, br d, *J*=12.7 Hz, 7β-H), 4.17 (1H, dd, *J*=12.7 and 9.3 Hz, 7α-H); ^{13}C NMR (150 MHz; CD_3OD) δ 7.93 (C-28), 12.13 (C-18), 12.48 (C-21), 15.86 (C-19), 23.32 (C-11), 25.64 (C-15), 27.98 (C-26), 28.57 (C-16), 28.64 (C-27), 32.74 (C-4), 38.27 (C-20), 39.19 (C-10), 40.47 (C-8), 41.02 (C-12), 42.16 (C-5), 42.29 (C-1), 43.53 (C-13), 43.63 (C-24), 52.46 (C-14), 53.52 (C-17), 59.34 (C-9), 68.90 (C-2), 69.11 (C-3), 71.76 (C-7), 73.54 (C-23), 74.30 (C-25), 75.61 (C-22), 179.22 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 497.3480. Calcd. for $C_{28}H_{49}O_7$, 497.3478.

14,25-di-OH-BL (7): CH_3Cl-MeOH (5:1) as eluent; a colorless amorphous powder; ^{1}H NMR (600 MHz; CD_3OD) δ 0.84 (3H, s, 18-H$_3$), 0.87 (3H, d, *J*=6.8 Hz, 21-H$_3$), 0.90 (3H, s, 19-H$_3$), 0.94 (3H, d, *J*=7.3 Hz, 28-H$_3$), 1.21 (3H, s, 26-H$_3$), 1.27 (3H, s, 27-H$_3$), 1.37 and 2.09 (each 1H, each m, 16-H$_2$), 1.47

(1H, m, 11β-H), 1.51 (1H, dq, *J*=1.5 and 7.3 Hz, 24-H), 1.55 (1H, m, 20-H), 1.63 (1H, m, 12β-H), 1.66 (1H, m, 1α-H), 1.69 (2 H, m, 15-H_2), 1.76 (1H, m, 11α-H), 1.78 (1H, ddd, *J*=14.7, 4.4 and 4.4 Hz, 4α-H), 1.83 (1H, m, 1β-H), 1.87 (1H, m, 12α-H), 1.88 (1H, dd, *J*=11.2 and 8.3 Hz, 8-H), 1.97 (1H, ddd, *J*=12.2, 11.2 and 4.9 Hz, 9-H), 2.06 (1H, ddd, *J*=14.7, 12.2 and 2.0 Hz, 4β-H), 2.20 (1H, br ddd, *J*=10.8, 8.8 and 8.8 Hz, 17-H), 3.20 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 3.50 (1H, dd, *J*=8.3 and 1.0 Hz, 22-H), 3.59 (1H, ddd, *J*=12.2, 4.4 and 2.9 Hz, 2-H), 3.91 (1H, m, 3-H), 3.97 (1H, dd, *J*=8.3 and 1.5 Hz, 23-H), 4.23 (1H, d, *J*=12.7 Hz, 7β-H), 4.35 (1H, dd, *J*=12.7 and 8.3 Hz, 7α-H); ^{13}C NMR (150 MHz; CD_3OD) δ 7.99 (C-28), 12.62 (C-21), 15.49 (C-19), 16.06 (C-18), 22.11 (C-11), 27.48 (C-16), 27.96 (C-26), 28.60 (C-27), 32.86 (C-4), 33.09 (C-12), 33.89 (C-15), 38.06 (C-20), 39.20 (C-10), 42.29 (C-5), 42.46 (C-1), 43.35 (C-8), 43.86 (C-24), 47.44 (C-13), 47.90 (C-17), 52.35 (C-9), 69.03 (C-2), 69.17 (C-3), 71.64 (C-7), 73.60 (C-23), 74.34 (C-25), 75.91 (C-22), 86.30 (C-14), 179.35 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 513.3427. Calcd. for $C_{28}H_{49}O_8$, 513.3427.

25-OH-5-*epi*-BL (14) and 25-OH-6(6a→3a)*abeo*-5-*epi*-BL (15). A mixture of **11** (25 mg, 0.038 mmol) and 5% KOH solution of MeOH (2 mL) was stirred at refluxing temperature for 3 h. After the same acid treatment and work-up procedure, the product mixture was subjected to column chromatography. The elution with CH_2Cl_2-MeOH (30:1) gave 25-OH-5-*epi*-BL (**14**) (4.1 mg, 22%), followed by 25-OH-6(6a→3a)*abeo*-5-*epi*-BL (**15**) (2.2 mg, 12%). Further elution with CH_2Cl_2-MeOH (20:1) gave 25-OH-BL (**4**) (10.5 mg, 56%).

14: colorless needles, mp 236-238°C (hexane-AcOEt)); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.70 (3H, s, 18-H_3), 0.90 (3H, d, *J*=6.8 Hz, 21-H_3), 0.98 (3H, d, *J*=7.3 Hz, 28-H_3), 1.11 (3H, s, 19-H_3), 1.14 and 1.83 (each 1H, each m, 15-H_2), 1.20 (1H, m, 9-H), 1.23 (1H, m, 12α-H), 1.26 (3H, s, 26-H_3), 1.27 (1H, m, 1α-H), 1.31 and 2.02 (each 1H, each m, 16-H_2), 1.37 (3H, s, 27-H_3), 1.43 (1H, m, 24-H), 1.45 (1H, m, 20-H), 1.45 (1H, m, 11β-H), 1.58 (1H, m, 14-H), 1.60 (1H, m, 17-H), 1.78 (1H, m, 8-H), 1.79 (1H, m, 11α-H), 1.83 (1H, m, 1β-H), 1.97 (1H, ddd, *J*=12.7, 3.2 and 3.2 Hz, 12β-H), 2.02 (1H, m, 4β-H), 2.14 (1H, br s, 22-OH), 2.23 (1H, br d, *J*=15.6 Hz, 4α-H), 2.51 (1H, s, 26-OH), 2.68 (1H, br d, *J*=9.8 Hz, 2-OH), 2.96 (1H, d, *J*=6.3 Hz, 5-H), 3.13 (1H, br s, 23-OH), 3.55 (1H, m, 2-H), 3.57 (1H, br d, *J*=8.3 Hz, 22-H), 3.90 (1H, m, 3-H), 4.06 (1H, br d, *J*=8.3 Hz, 23-H), 4.23 (1H, d, *J*=13.2 Hz, 7α-H), 4.40 (1H, dd, *J*=13.2 and 6.8 Hz, 7β-H), 5.63 (1H, br d, *J*=7.8 Hz, 3-OH); ^{13}C NMR (150 MHz; $CDCl_3$) δ 7.28 (C-28), 11.48 (C-18), 11.93 (C-21), 18.93 (C-19), 21.87 (C-11), 24.85 (C-15), 27.20 (C-16), 28.37 (C-4), 28.70 (C-26), 29.14 (C-27), 35.70 (C-8), 36.72 (C-1), 36.72 (C-20), 38.92 (C-10), 39.15 (C-12), 41.37 (C-24), 42.67 (C-13), 43.72 (C-5), 48.43 (C-9), 52.14 (C-17), 53.69 (C-14), 66.02 (C-3), 67.74 (C-2), 69.50 (C-7), 72.81 (C-23), 73.65 (C-25), 74.53 (C-22), 179.28 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 497.3462. Calcd. for $C_{28}H_{49}O_7$, 497.3478.

15: colorless needles, mp 239-243°C decomp. (hexane-AcOEt-MeOH); ^{1}H NMR (600 MHz; CD_3OD) δ 0.73 (3H, s, 18-H_3), 0.90 (3H, d, *J*=6.8 Hz, 21-H_3), 0.93 (3H, d, *J*=7.3 Hz, 28-H_3), 1.12 (3H, s, 19-H_3), 1.20 (1H, m, 12α-H), 1.20 and 1.82 (each 1H, each m, 15-H_2), 1.21 (3H, s, 26-H_3), 1.26 (1H, dd, *J*=14.2 and 10.3 Hz, 1α-H), 1.27 (3H, s, 27-H_3), 1.28 and 2.02 (each 1H, each m, 16-H_2), 1.40 and 1.64 (each 1H, each m, 11-H_2), 1.49 (1H, dq, *J*=1.0 and 7.3 Hz, 24-H), 1.50 (1H, m, 8-H), 1.50 (1H, m, 20-H), 1.58 (1H, m, 14-H), 1.59 (1H, m, 17-H), 1.73 (1H, m, 9-H), 1.88 (1H, br dd, *J*=14.2 and 6.4 Hz, 1β-H), 1.97 (1H, ddd, *J*=12.7, 2.9 and 2.9 Hz, 12β-H), 2.16 (1H, d, *J*=12.7 Hz, 4β-H), 2.26 (1H, ddd, *J*=12.7, 6.4 and 6.4 Hz, 4α-H), 3.02 (1H, br d, *J*=6.4 Hz, 5-H), 3.50 (1H, br d, *J*=8.3 Hz, 22-H), 3.62 (1H, dd, *J*=12.2 and 2.4 Hz, 7-H), 3.87 (1H, dd, *J*=10.3 and 6.4 Hz, 2-H), 3.91 (1H, dd, *J*=12.2 and 1.5 Hz, 7-H), 3.95 (1H, dd, *J*=8.3 and 1.0 Hz, 23-H), 4.62 (1H, d, *J*=6.4 Hz, 3-H); ^{13}C NMR (125 MHz; CD_3OD) δ 7.93 (C-28), 12.08 (C-18), 12.48 (C-21), 17.59 (C-19), 24.02 (C-11), 25.73 (C-15), 28.02 (C-26), 28.52 (C-16), 28.60 (C-27), 31.63 (C-4), 38.29 (C-10), 38.53 (C-20), 41.38 (C-12), 41.86 (C-8), 43.02 (C-1), 43.02 (C-13), 43.73 (C-

24), 44.05 (C-9), 47.27 (C-5), 52.94 (C-14), 53.90 (C-17), 63.22 (C-7), 68.87 (C-2), 73.60 (C-23), 74.36 (C-25), 75.69 (C-22), 84.04 (C-3), 181.60 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 497.3476. Calcd. for $C_{28}H_{49}O_7$, 497.3478.

(25*R*)-26-OH-Castasterone (18). A solution of the acetal **17** (17.5 mg, 0.027 mmol) and 4N HCl (1 mL, 4 mmol) in THF (2 mL) was refluxed for 6 h. After neutralization with 3N NaOH, the solvent was evaporated off, and the resulting solution was extracted with *n*-butanol. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with MeOH-CH_2Cl_2 (1:9) as eluent to afford (25*R*)-26-OH-castasterone (**18**) (9.8 mg, 74%): colorless granules, mp 258-260°C decomp. (MeOH); ^{1}H NMR (600 MHz; $CDCl_3$-CD_3OD, 1:10) δ 0.72 (3H, s, 18-H_3), 0.75 (3H, s, 19-H_3), 0.85 (3H, d, *J*=7.3 Hz, 28-H_3), 0.92 (3H, d, *J*=6.4 Hz, 21-H_3), 0.98 (3H, d, *J*=6.8 Hz, 27-H_3), 1.13 and 1.60 (each 1H, each m, 15-H_2), 1.30 (1H, m, 12α-H), 1.32 and 2.02 (each 1H, each m, 16-H_2), 1.38 (1H, m, 14-H), 1.42 (1H, m, 9-H), 1.42 and 1.68 (each 1H, each m, 11-H_2), 1.53 (1H, m, 20-H), 1.57 (1H, m, 1α-H), 1.62 (1H, m, 24-H), 1.62 (1H, m, 17-H), 1.67 (1H, m, 4β-H), 1.68 (1H, m, 25-H), 1.69 (1H, m, 1β-H), 1.75 (1H, ddd, *J*=15.1, 2.9 and 2.9 Hz, 4α-H), 1.79 (1H, m, 8-H), 2.06 (1H, m, 12β-H), 2.09 (1H, dd, *J*=13.2 and 12.7 Hz, 7α-H), 2.20 (1H, dd, *J*=13.2 and 4.4 Hz, 7β-H), 2.72 (1H, dd, *J*=12.7 and 2.9 Hz, 5-H), 3.53 (1H, m, 22-H), 3.53 (2H, m, 26-H_2), 3.64 (1H, m, 2-H), 3.64 (1H, m, 23-H), 3.94 (1H, m, 3-H); ^{13}C NMR (150 MHz; $CDCl_3$-CD_3OD, 1:10) δ 10.45 (C-28), 12.33 (C-18), 12.61 (C-21), 13.89 (C-19), 15.31 (C-27), 22.34 (C-11), 24.84 (C-15), 27.79 (C-4), 28.62 (C-16), 36.99 (C-24), 38.68 (C-20), 39.18 (C-8), 40.15 (C-25), 40.85 (C-12), 40.96 (C-1), 43.61 (C-10), 43.90 (C-13), 47.50 (C-7), 52.06 (C-5), 53.64 (C-17), 55.03 (C-9), 57.85 (C-14), 65.58 (C-26), 69.10 (C-2), 69.43 (C-3), 74.39 (C-23), 75.38 (C-22), 215.01 (C-6); FAB-MS *m/z* 481 ([M+1]$^+$: positive ion, glycerol).

(25*R*)-26-OH-BL (5). To a stirred solution of 30% H_2O_2 (14 μL, 0.12 mmol) in $CHCl_3$ (0.5 mL) was added trifluoroacetic anhydride (8 μL, 0.62 mmol) and stirring was continued for 15 min at 0°C. A solution of **18** (6.0 mg, 0.012 mmol) in $CHCl_3$ (0.5 mL) was added dropwise to a stirred solution of the trifluoroperacetic anhydride at 0°C, and the reaction mixture was stirred for 1h at 0°C and for 0.5 h at rt. The resulting solution was extracted with $CHCl_3$, and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography with MeOH-CH_2Cl_2 (1:9) as eluent to afford a mixture of (25*R*)-26-OH-BL (**5**) and its lactone isomer **19**, which was then separated by HPLC using H_2O-CH_3CN (3 : 1) as the mobile phase. Elution at t_R of 16.1 mins gave **19** (0.2 mg, 2%) and elution at t_R of 20.4 mins gave **5** (0.9 mg, 14%).

5: a colorless amorphous powder; ^{1}H NMR (600 MHz; CD_3OD) δ 0.75 (3H, s, 18-H_3), 0.85 (3H, d, *J*=6.8 Hz, 28-H_3), 0.89 (3H, s, 19-H_3), 0.91 (3H, d, *J*=6.8 Hz, 21-H_3), 0.98 (3H, d, *J*=6.8 Hz, 27-H_3), 1.25 (1H, m, 14-H), 1.26 and 1.73 (each 1H, each m, 15-H_2), 1.26 (1H, m, 12α-H), 1.28 and 2.02 (each 1H, each m, 16-H_2), 1.34 (1H, m, 9-H), 1.44 (1H, m, 11β-H), 1.54 (1H, m, 20-H), 1.60 (1H, m, 1α-H), 1.60 (1H, m, 17-H), 1.62 (1H, m, 24-H), 1.68 (1H, m, 25-H), 1.70 (1H, m, 8-H), 1.78 (1H, m, 4α-H), 1.80 (1H, m, 11α-H), 1.81 (1H, m, 1β-H), 2.01 (1H, m, 12β-H), 2.06 (1H, m, 4β-H), 3.20 (1H, dd, *J*=12.2 and 4.4 Hz, 5-H), 3.52 (1H, m, 22-H), 3.52 (2H, m, 26-H_2), 3.59 (1H, ddd, *J*=11.7, 4.4 and 2.4 Hz, 2-H), 3.64 (1H, dd, *J*=7.8 and 1.5 Hz, 23-H), 3.92 (1H, m, 3-H), 4.08 (1H, br d, *J*=12.7 Hz, 7β-H), 4.18 (1H, dd, *J*=12.7 and 9.8 Hz, 7α-H); ^{13}C NMR (125 MHz; CD_3OD) δ 10.40 (C-28), 12.10 (C-18), 12.57 (C-21), 15.31 (C-27), 15.87 (C-19), 23.37 (C-11), 25.67 (C-15), 28.64 (C-16), 32.81 (C-4), 36.90 (C-24), 38.77 (C-20), 39.22 (C-10), 40.17 (C-25), 40.53 (C-8), 41.10 (C-12), 42.21 (C-5), 42.34 (C-1), 43.58 (C-13), 52.52 (C-14), 53.63 (C-17), 59.39 (C-9), 65.42 (C-26), 68.95 (C-2), 69.17 (C-3), 71.62 (C-7), 74.50 (C-23), 75.39 (C-22), 179.32 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 497.3486. Calcd. for $C_{28}H_{49}O_7$, 497.3478.

19: a colorless amorphous powder; ^{1}H NMR (300 MHz; CD_3OD) δ 0.74 (3H, s, 18-H_3), 0.85 (3H, d, *J*=6.8 Hz, 28-H_3), 0.91 (3H, d, *J*=6.8 Hz, 21-H_3), 0.93 (3H, s, 19-H_3), 0.98 (3H, d, *J*=6.7 Hz, 27-H_3), 2.42

(1H, d, J=13.7 Hz, 7-H), 2.62 (1H, dd, J=13.7 and 12.2 Hz, 7-H), 3.48-3.56 (3H, 22-H and 26-H_2), 3.64 (br d, J=7.4 Hz, 23-H), 3.67 (1H, m, 2-H), 3.94 (1H, m, 3-H), 4.69 (1H, dd, J=11.2 and 5.4 Hz, 5-H); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 497.3486. Calcd. for $C_{28}H_{49}O_7$, 497.3478.

Bioassay. The rice lamina inclination test was carried out as described previously.[9]

Acknowledgment: This work was supported in part by Grant-in-Aid for Scientific Research (C) to H. Seto (No. 09672183) and Scientific Research (B) to S. Fujioka (No. 10460050) from the Ministry of Education, Science, Sports and Culture of Japan.

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