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Preparation, conformational analysis, and biological evaluation of 6a-carbabrassinolide and related compounds

Hideharu Seto,[a,b,*] Sayoko Hiranuma,[b] Shozo Fujioka,[a,b] Hiroyuki Koshino,[c] Toshiro Suenaga[d] and Shigeo Yoshida[a,b]

[a]*Plant Functions Laboratory, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-0198, Japan*
[b]*Plant Science Center, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-0198, Japan*
[c]*Molecular Characterization Division, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-0198, Japan*
[d]*Synthetic Organic Chemistry Laboratory, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-0198, Japan*

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Abstract—In order to probe the ligand recognition sites of brassinosteroid (BR)-receptors, B-ring modified brassinolides (BLs), i.e. 6a-carbaBL (**3**), 6a-carba-B-homoBL (**4**), the C-5 epimers of compounds **3** and **4** (**6** and **7**, respectively), and 6-deoxo-6a-oxo-6a-carbaBL (**8**), were prepared from castasterone (**2**) via homologation with trimethylsilyldiazomethane and boron trifluoride etherate. Biological evaluation of these compounds using the rice lamina inclination (RLI) test gave the following order of potency (percent activity): BL (**1**) (100%)>**2** (13%)>**3** (0.3%)≫**4**, **6**, **7**, **8** (0%). Using the rescue test of hypocotyl length of the BR deficient mutant, *Arabidopsis det2*, the corresponding order was: **1** (100%)>**8** (44%)>**2** (28%)>**3** (4%)>**6** (0.5%)>**4** (0.1%), and **7** (inhibition). The markedly different activities of **8** in the two assay systems provide an evident experimental proof that the BR-receptors involved in RLI and in *Arabidopsis* hypocotyl elongation recognize BR molecules in different structure manners, at least in the B-ring region.

1. Introduction

Brassinosteroids (BRs) are considered to be phytohormones due to their physiological activities and ubiquitous distribution throughout the plant kingdom.[1] As a result of increased interest in BR receptors and the mode of action of BRs at the physiological and molecular levels, as well as a greater awareness of the practical application of BRs in agriculture, the structure–activity relationships of BRs have been widely studied using natural BRs and their synthetic analogs.[2–7] The results of these studies indicate that the optimal structural features, i.e. the 2α,3α,22*R*,23*R*-tetrahydroxyl groups, the A/B *trans* ring junction, and the seven-membered lactone moiety of the B-ring, which are required for high-level BR activity, are reflected in those of brassinolide (BL, **1**), a terminal molecule in BR biosynthesis. Such understanding of the structure–activity relationships of BRs provides insight into how BR-receptors[8] perceive the ligand molecule at the recognition site. To date, this information has been furnished mainly by activity data from the rice lamina inclination (RLI) test, which has the advantages of facility, high sensitivity, and high reproducibility over other BR-activity assay systems.[2,9,10] However, it is conceivable that BR receptors related to different physiological phenomena or different plant species may not be identical to each other in terms of ligand recognition. Therefore, it seems likely that some BR analogs can be distinguished by differences in BR-receptors. However, BR analogs that have specific activities in particular bioassay systems has not been reported, although several papers have reported that the relative BR activities vary in some extent with the type of bioassay employed.[2,11]

In an extension of our preliminary work on synthesis and biological activity of 6a-carbaBL (carbaBL; **3**),[4] we found that **3** and its related compounds gave different activity profiles in the RLI and *Arabidopsis det2* hypocotyl elongation (DHE) bioassays. In particular, a ketone isomer of **3**, 6-deoxo-6a-oxo-6a-carbaBL (iso-carbaBL; **8**), was inactive in the RLI assay but had high-level activity comparable to **1** in the DHE assay. This was the first experimental evidence of differences in structural recognition of BR receptors implicated in various physiological phenomena and plant species.

In this paper, we describe the synthesis of compound **3** and 6a-carba-*B*-homoBL (carba-homoBL; **4**), their respective C-5 epimers [5-*epi*-6a-carbaBL (5-*epi*-carbaBL; **6**) and 5-*epi*-6a-carba-*B*-homoBL (5-*epi*-carba-homoBL; **7**), respectively], and compound **8** from castasterone (CS; **2**), via homologation with trimethylsilyldiazomethane ($TMSCHN_2$) and boron trifluoride etherate ($BF_3{\cdot}Et_2O$).

Keywords: brassinosteroids; trimethylsilyldiazomethane; homologation reaction; structure–activity relationship; rice lamina inclination test; *Arabidopsis det2* mutant; *det2* hypocotyl elongation test.

* Corresponding author. Tel.: +81-48-4674192; fax: +81-48-4674324; e-mail: hseto@postman.riken.go.jp

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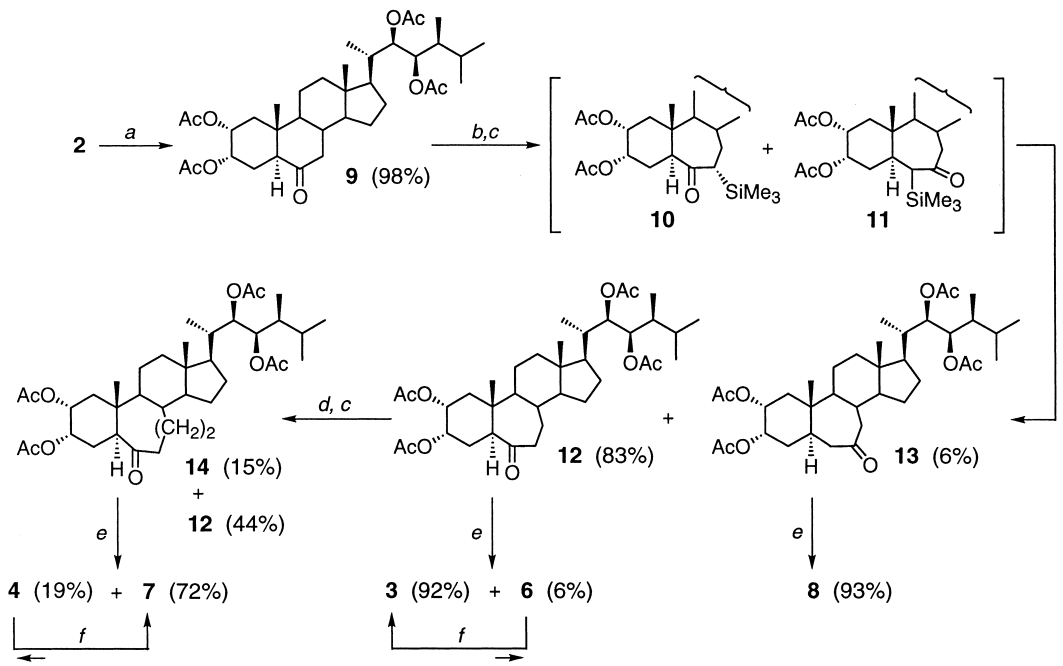
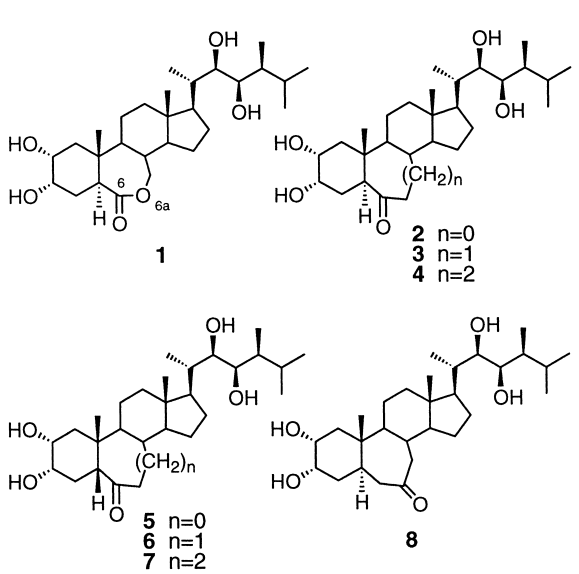


Scheme 1. *Reagents and conditions*: (a) Ac_2O, 4-DMAP, pyridine, rt, 15 h. (b) 2.8 equiv. of $TMSCHN_2$, 4.2 equiv. of $BF_3{\cdot}EtO_2$, CH_2Cl_2, −15°C, 2.5 h. (c) a small amount of 2N HCl, SiO_2, Et_2O, rt, 3 h. (d) 4.0 equiv. of $TMSCHN_2$, 6.0 equiv. of $BF_3{\cdot}EtO_2$, CH_2Cl_2, −10°C, 5 h. (e) 5% KOH–90% aq. MeOH, reflux, 2 h. (f) 5% KOH–90% aq. MeOH, reflux, 5 h.

We also report the biological activities of these compounds both in the RLI assay using *Oryza sativa* L. cv. Tan-ginbozu and in the DHE assay. The BR-deficient *Arabidopsis* mutant *det2* (*de-etiolated2*) manifests dwarfism, and its short hypocotyl is rescued to the wild-type length by the exogenous application of active BRs.[12,13] In this work, a modified version of the DHE test[12] was employed. In addition, we discuss the difference between the recognition sites of two receptors that are involved in RLI and DHE, on the basis of the bioassay results and the conformations of the test compounds which were deduced by NMR experiments and molecular calculations.



2. Results and discussion

2.1. Synthesis

The synthesis of carbaBL (**3**), and the related compounds **4** and **6**–**8**, from CS (**2**) via homologation with $TMSCHN_2$ and $BF_3{\cdot}Et_2O$[14,15] is summarized in Scheme 1. Tetraacetate (**9**), derived from **2** in 98% yield by peracetylation with Ac_2O and a catalytic amount of 4-dimethylaminopyridine in pyridine at room temperature (rt) for 15 h, was treated with 2.8 equiv. of $TMSCHN_2$ and 4.2 equiv. of $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at −15°C for 2.5 h. After aqueous work-up, the resulting mixture, which consisted of α-trimethylsilyl ketones (**10** and **11**)[16] and ketones (**12** and **13**), was treated with a small amount of 2N HCl in the presence of silica gel in ether at rt for 3 h, thereby producing the 6-oxo-*B*-homosteroid (**12**) and the 7-oxo-*B*-homosteroid (**13**) in 83 and 6% isolated yields, respectively. The major product **12** was further homologated under similar reaction conditions. Although the reaction rate was very slow, when **12** was treated with 4.0 equiv. of $TMSCHN_2$ and 6.0 equiv. of $BF_3{\cdot}Et_2O$ at −10°C for 5 h, followed by acid treatment, a 6-oxo-*B*-dihomosteroid (**14**) was obtained in 15% isolated yield along with 44% recovery of the starting material (**12**). In this reaction, the ketone isomer, 7-oxo-*B*-dihomosteroid (**15**), could not be identified. Attempts to increase the yield by prolongation of the reaction time, elevation of the reaction temperature, and the utilization of much larger amounts of reagents, resulted in the occurrence of unidentified side-reactions. The slow reaction rate of **12** may be attributed partially to the low reactivity of seven-membered cyclic ketones to nucleophilic attack.[15] In addition, steric hindrance at the β-face of the carbonyl function attacked by $TMSCHN_2$ may be more severe in **12** than **9**,[16] which is anticipated from the conformations of

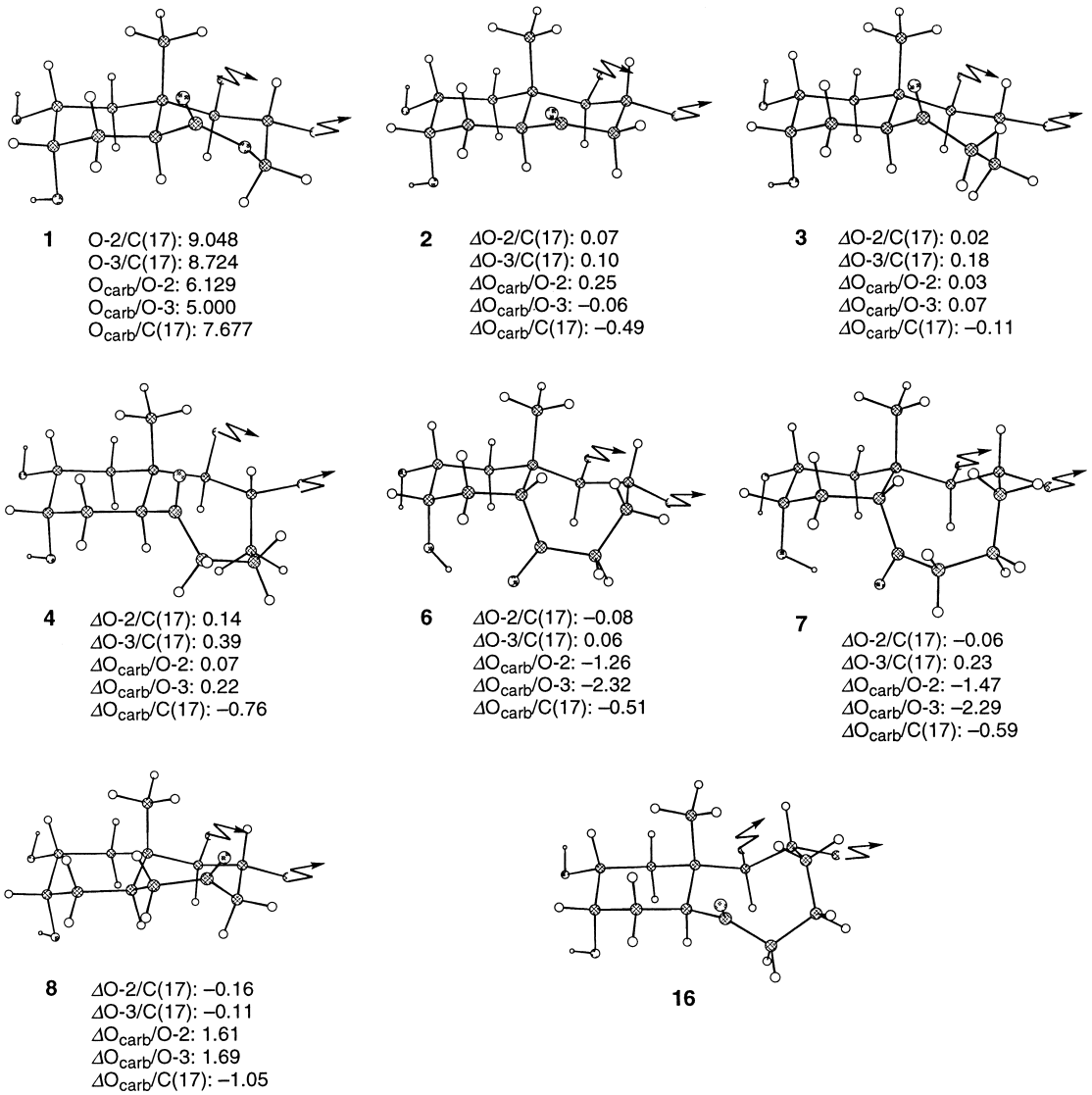
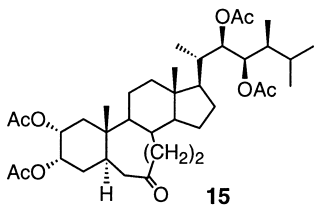


Figure 1. A/B ring moieties of the stable conformers of compounds **1**–**8** in a solution. Conformer **16** is another possible conformer of **4** deduced from molecular calculation, having energy difference of 0.69 kJ/mol higher than **4**. The values *x*/*y* (Å) of **1** show the spatial distances between atoms x and y, and the values Δ*x*/*y* of others are deviations from *x*/*y* of **1**.

the corresponding two compounds, **2** and **3**, respectively (Fig. 1).



Compounds **12**–**14** were then subjected to deprotection of the tetra-*O*-acetyl groups. On treatment with 5% potassium hydroxide in 90% aqueous methanol at refluxing temperature for 2 h, **12** furnished carbaBL (**3**) and 5-*epi*-carbaBL (**6**) in 92 and 6% yields, respectively, **13** gave iso-carbaBL (**8**) in 93% yield, and **14** gave carba-homoBL (**4**) and 5-*epi*-carba-homoBL (**7**) in 19 and 72% isolated yields, respectively. In order to determine the equilibrium ratio and obtain the additional amount of the minor C-5 epimer (**6**), the isolated compounds **3** and **6** were respectively treated under the same alkali conditions for 5 h, thus affording a mixture of **3** and **6** in a ratio of ca. 92:8. The same treatment of **4** and **7** for 5 h gave a ca. 22:78 mixture of **4** and **7**, respectively. The ratios were estimated by ^{1}H NMR spectra. C-5 epimerization of CS (**2**) under alkali conditions was also attempted, in order to prepare 5-*epi*-CS (**5**) for the bioassay. However, this reaction gave quantitative recovery of **2**, and compound **5** was not detected in the ^{1}H NMR spectrum of the crude product mixture.

The structures of compounds **3**, **4** and **6**–**8** were verified by MS and NMR spectroscopy. NMR experiments were carried out at 600 MHz by PFG-DQFCOSY, PFG-HMQC, and PFG-HMBC,[17] and the NOE differential experiments were conducted at 400 MHz, by which all of the resonances were unequivocally assigned (Section 3).

2.2. Conformational analysis

Conformational searches were carried out on the reduced structures of **3**, **4**, and **6**–**8** (and **1** and **2** as references), i.e.

Table 1. RLI test (*O. sativa* cv. Tan-ginbozu) of 6a-carbaBL analogs, **3**, **4** and **6**–**8**, and CS (**2**)

Run[a]	Compounds	Dosages (ng)					Activities[b] (%)
		0	1	10	100	1000	
1	**1** BL	13±1	30±5	73±6	114±4	103±6	100
	2 CS	–	16±1	33±4	77±7	94±7	13
	3 carbaBL	–	11±1	13±1	30±4	51±6	0.3
2	**1**	15±1	31±2	69±7	83±7	91±7	
	4 caba-homoBL	–	–	16±1	18±1	21±2	0
	6 5-*epi*-carbaBL	–	–	17±1	17±1	17±1	0
	7 5-*epi*-carba-homoBL	–	–	13±1	14±1	15±1	0
3	**1**	14±1	29±4	85±7	104±5	105±5	
	8 iso-carbaBL	–	16±1	15±1	16±1	19±2	0

Values are angles (°) between the lamina and sheath, representing the means of 30 replicates ±SE.
[a] In each run, BL (**1**) was tested for comparison.
[b] The relative activities, except for **2**, were estimated by the dosage of the references compound **1** exhibiting the same response of the test compound at 1000 ng: that of **2** was estimated by the dosages of **1** and **2** at the midpoint of the maximum response of **1**.

the C-17 side-chain was replaced with an iso-propyl group to save time for a full conformational search. Potential conformers were explored by a Low-Mode conformational search[18] with 10000 initial structures using the MM2* force field in the Macromodel 6.0.[19]

Fig. 1 illustrates A/B ring moieties of the stable conformers of **1**–**4** and **6**–**8** in solution. The ^{1}H NMR data on α-methylene protons adjacent to the carbonyl function of each compound (except for **1**) were in a good accordance with those expected for the calculated stable conformers: δ 2.02 (1H, dd, *J*=13.2, 12.7 Hz, 7α-H) and 2.29 (1H, dd, *J*=13.2, 4.4 Hz, 7β-H) of **2**;[20] δ 2.29 (1H, ddd, *J*=17.6, 12.7, 4.9 Hz, 6aβ-H) and 2.46 (1H, ddd, *J*=17.6, 4.4, 2.9 Hz, 6aα-H) of **3**; δ 2.25 (1H, ddd, *J*=11.2, 6.8, 2.9 Hz, 6aβ-H) and 2.76 (1H, ddd, *J*=11.2, 11.2, 7.3 Hz, 6aα-H) of **4**; δ 2.24 (1H, ddd, *J*=19.5, 3.7, 3.4 Hz, 6aβ-H) and 2.38 (1H, ddd, *J*=19.5, 14.0, 3.0 Hz, 6aα-H) of **6**; δ 2.40 (1H, ddd, *J*=11.7, 3.9, 3.9 Hz, 6aα-H) and 2.48 (1H, ddd, *J*=12.3, 11.7, 6.8 Hz, 6aβ-H) of **7**; δ 2.20 and 2.31 (each 1H, each m, 6-H_2), 2.36 (1H, d, *J*=12.2 Hz, 7β-H) and 2.53 (1H, dd, *J*=12.2, 12.2 Hz, 7α-H) of **8**. In the case of **4**, the conformational search gave another possible stable conformer (**16**) that was only 0.69 kJ/mol higher in energy. In addition, the observed coupling patterns of α-methylene protons adjacent to the carbonyl function of **4** (described above) were also noted for the conformer **16**. However, in PFG 1D selective ROESY spectra,[21] ROEs were observed from 5-H at δ 3.16 to 6aα-H at δ 2.76 and 7-H at δ 1.63, and from 6aα-H to 5-H and 7-H. This confirms that the conformer **4** has α-, or down-oriented 7-H_2. Thus, the stable conformers of **2**–**4** and **6**–**8** in solution were eventually assigned. The A/B ring moiety of the stable conformer of BL (**1**) has been elucidated previously.[5]

The spatial positions of the oxygen atoms of the tetrahydroxyl and carbonyl groups of BL (**1**) are considered to be important for high-level BR activity.[6,22] Therefore, deviations (*Δ*) from the distances between the oxygen atoms of the C-2 and C-3 hydroxyl groups (O-2 and O-3, respectively), the oxygen atom of the B-ring carbonyl group (Ocarb), and the C-17 carbon atom [C(17)] on **1** were calculated (Fig. 1), where the spatial relationships between C(17) and other specified atoms substitute for those of the C-22 and C-23 hydroxyl oxygens (O-22 and O-23, respectively). The results indicate that the relative positions of O-2, 3, 22 and 23 on **2**–**4** and **6**–**8** are similar to those on **1**. Consequently, changes of the BR activities of these compounds (described below) may be attributed to structural changes in their B-rings.

It is worth mentioning that the equilibrium ratios of **2**/**5**: 100/0, **3**/**6**: 92/8, and **4**/**7**: 22/78, which were observed after C-5 epimerization under alkali conditions (vide supra), were broadly consistent with the calculated values: 96/4, 93/7, and 10/90 at 25°C, respectively. Their calculations were carried out according to a Boltzmann distribution of conformers within 25 kJ/mol of the minimum: 4 and 9 unique conformers were found for **2** and **5**, respectively; 10 and 14 for **3** and **6**, respectively; 16 and 13 for **4** and **7**, respectively. This exemplifies that calculations based on molecular mechanics are useful in predicting the product ratios of equilibrium reactions.[23]

2.3. Bioassay

The BR activities of the synthesized compounds and CS (**2**) were examined using the RLI and DHE bioassays, and compared with those of BL (**1**). The RLI test is employed typically for testing BR activity,[9,10] where the leaf lamina angles induced by test samples reflect the levels of BR activity. This test was carried out using *O. sativa*, cv. Tan-ginbozu, as described previously.[10] On the other hand, the DHE test was conducted according to our original protocol, with some modifications (see Section 3). In our earlier work,[13] the dwarf phenotype of the *Arabidopsis det2* mutant, which is deficient in the 5α-reductase needed for BR biosynthesis, was found to be rescued to the wild-type phenotype by exogenous application of BR biosynthetic intermediates that lay downstream of the defective step, e.g. CS (**2**) and BL (**1**). In particular, hypocotyl elongation of the etiolated seedlings of *det2* was rescued. Thus, the modified DHE test was evaluated as an assay of BR activity, and found to be similar to the RLI test in terms of facility, sensitivity, and reproducibility.

The results of the RLI and DHE tests are shown in Tables 1 and 2, respectively. The BR activity values of the test

Table 2. *det2* hypocotyl elongation test (*Arabidopsis det2* mutant) of 6a-carbaBL analogs, **3**, **4** and **6**–**8**, and CS (**2**)

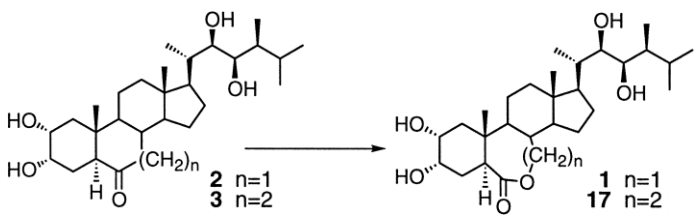
Run[a]	Compounds	Dosages (nM)						Activities[b] (%)
		0	1	10	100	1000	10000	
1	**1** BL	4.2±0.2	7.2±0.2	12.7±0.3	15.0±0.5	14.1±0.4	–	100
	2 CS	–	5.9±0.3	9.7±0.4	12.9±0.6	14.5±0.4	–	28
	3 carbaBL	–	4.9±0.1	6.2±0.2	10.1±0.4	13.2±0.4	–	4
2	**3**	4.6±0.2	–	5.6±0.2	11.6±0.7	13.8±0.7	13.6±0.5	
	4 carba-homoBL	–	–	4.7±0.2	4.9±0.3	9.3±0.6	12.5±0.4	0.1
	6 5-*epi*-carbaBL	–	–	4.5±0.2	5.4±0.2	12.9±0.8	14.4±0.7	0.5
	7 5-*epi*-carba-homoBL	–	–	4.6±0.1	4.9±0.3	4.9±0.2	2.5±0.3	0[c]
3	**1**	3.6±0.2	6.3±0.2	10.5±0.8	15.1±0.4	13.2±0.4	–	
	8 iso-carbaBL	–	5.3±0.2	8.7±0.4	14.1±0.6	14.5±0.6	–	44

Values are hypocotyls lengths (mm), representing the means of 15 replicates ±SE.
[a] For comparison, BL (**1**) was tested in runs 1 and 3, and carbaBL (**3**) was tested in run 2.
[b] The relative activities of compounds in runs 1 and 3 were estimated by the dosages of the test compound and **1** at the midpoint of the maximum response of **1**: those of compounds in run 2 were calculated by comparison with 4% activity of **3**.
[c] At 10000 nM the elongation was obviously inhibited.

compounds are shown as percentages of BL (**1**) activity; footnotes b in Tables 1 and 2 describe the calculation method. The BR activities from RLI assay had the following order of magnitude: **1** (100%)>**2** (13%)>**3** (0.3%)[24]≫**4**, **8**, **6**, **7** (0%), where **4** and **8** exhibited slight (≪0.1%) activities. On the other hand, the BR activities from the DHE assay had the order of magnitude: **1** (100%)>**8** (44%)>**2** (28%)>**3** (4%)>**6** (0.5%)>**4** (0.1%), and **7** was inhibitory. All of the compounds, except for **7** which inhibited DHE at a concentration of 10 μM, showed DHE activities that were higher than the activities measured in the RLI assay. In particular, compounds, **4**, **6**, and **8** had DHE activities that were more than two orders of magnitude higher than the corresponding samples in the RLI assay. This suggests that the structural recognition of B-ring moiety by the receptor implicated in DHE is not so strict as that by the receptor implicated in RLI. The most surprising result was obtained for iso-carbaBL (**8**). Compound **8** was practically inactive in the RLI assay but showed a high level of activity (comparable to that of BL) in the DHE assay. The large differences between **8** and **1** with respect to the distances separating the carbonyl and tetrahydroxyl oxygens (Fig. 1) indicate that these two compounds have significantly different spatial arrangements of their B-ring carbonyls. This suggests that the receptors involved in RLI and DHE are drastically different in terms of their binding sites on the B-ring of BR.

The activity order of **1**>**2**>**3**, which was observed in both assay systems, is also noteworthy. Since the spatial arrangements of the A/B ring moieties of **1** and **3** are more closely related than those of **1** and **2** (Fig. 1), an order of **1**>**3**>**2** might have been expected. This unexpected result may be explained by assuming that biosynthetic transformation via the Baeyer–Villiger oxidation of 6-oxo-BRs (**2** and **3**) to the corresponding lactones (**1** and **17**) proceeds quickly in the two assay systems (Scheme 2), as previously suggested by Baron et al.[7] Thus, the activities of **2** and **3** may be those of their metabolites, **1** and **17**, rather than their own, which means that compound **2** is not as active as it would appear to be from the assay results. Although further experimental proof is needed, we speculate that CS (**2**) is merely a biosynthetic precursor of the authentic, active BR, i.e. BL (**1**). The alternative view that compound **2**, as well as **1**, functions as a ligand that binds to BR receptor in plants is based primarily on the high BR activity shown by compound **2**.

In summary, the B-ring-modified BLs: i.e. carbaBL (**3**), carba-homoBL (**4**), the C-5 epimers of **3** and **4** (**6** and **7**, respectively), and iso-carbaBL (**8**), were prepared from CS (**2**) via homologation with $TMSCHN_2$ and $BF_3{\cdot}Et_2O$. Biological evaluation of these compounds, together with BL (**1**) and CS (**2**), by the RLI and DHE tests clearly demonstrated that the recognition of BR receptors involved in RLI and DHE was unambiguously different, at least with respect to the B-ring region of BRs. To date, the structure–activity relationships of BRs have been based mainly on results from the RLI test. Our current findings suggest that systematic diverse assays are needed to arrive at a precise and detailed understanding of the structure–activity relationships of BRs.



Scheme 2. Speculative conversion of 6-oxo-BRs: CS (**2**)/carbaBL (**3**), to the lactones: BL (**1**)/**17** in assay systems.

3. Experimental

3.1. Syntheses

General. Melting points (mp) were determined using a Yanagimoto micromelting point apparatus and are uncorrected. NMR measurements were performed on a Bruker AC-300, JEOL JNM-A400, or JEOL JNM-A600 spectrometer. All of the 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 ^{1}H, or to the solvent (δ 77.0 ppm) for ^{13}C as internal references. All *J*-values are given in Hz. High resolution mass spectra (HR-EI-MS and HR-FAB-MS) were obtained with a JEOL-SX-102 or JEOL-HX-110 mass spectrometer. Analytical thin layer chromatography (TLC) was conducted on micro-slides that were coated with Merck Kieselgel KG60F-254; the developed plates were stained with 10% (w/v) vanillin in concentrated sulfuric acid at 180°C. All of the reactions were carried out under a nitrogen atmosphere. Column chromatography was conducted using silica gel FL-60D [Fuji Silysia Chemical Co.] as the adsorbent. The ratios of mixed solvents are shown as v/v.

3.1.1. 2,3,22,23-Tetra-*O*-acetylcastasterone (9). Castasterone (**2**) (102 mg, 0.22 mmol) was acetylated with acetic anhydride (1.0 mL, 9.8 mmol), 4-(*N*,*N*-dimethylamino)pyridine (50 mg, 0.41 mmol), and dry pyridine (5.0 mL) for 15 h at rt. The reaction mixture was poured into ice-water, and the resulting solution was extracted with AcOEt. The extract was washed successively with a saturated aqueous $NaHCO_3$ solution, a saturated aqueous $KHSO_4$ solution, and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a yellow solid, which was purified by flash chromatography using hexane–EtOAc (4:1) as eluent, thereby giving 2,3,22,23-tetra-*O*-acetylcastasterone (**9**) (136 mg, 98%): colourless needles, mp 209–212°C (hexane–AcOEt) [lit.,[25] mp 215–217°C (MeOH)].

3.1.2. 2,3,22,23-Tetra-*O*-acetyl-6a-carbaBL (12) and 2,3,22,23-tetra-*O*-acetyl-6-deoxo-6a-oxo-6a-carbaBL (13). A 10% solution of $TMSCHN_2$ in hexane (1.5 mL, 1.31 mmol; Tokyo Kasei Co.) was added dropwise at −20°C to a stirred solution of tetra-*O*-acetylcastasterone (**9**) (300 mg, 0.474 mmol) and $BF_3{\cdot}Et_2O$ (250 μL, 1.98 mmol) in dry CH_2Cl_2 (20 mL). The mixture was stirred at −15°C for 2.5 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and evaporated, thus giving a pale yellow gum (321 mg). The gum was dissolved into Et_2O (70 mL), to which silica gel (1.2 g of Wakogel C-300; Wako Pure Chemical Industries) and 2N HCl (0.3 mL) was added. The heterogeneous solution was stirred at rt for 3 h, and Na_2SO_4 (500 mg) was added. After filtration through a glass-filter and evaporation, the residual solid was subjected to flash chromatography. Elution with hexane–AcOEt (3:1) gave 2,3,22,23-tetra-*O*-acetyl-6a-carbaBL (**12**) (255 mg, 83%): colourless needles, mp 188–189°C (hexane–AcOEt); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.66 (3H, s, 18-H_3), 0.83 (3H, s, 19-H_3), 0.91 and 0.94 (each 3H, each d, *J*=6.3 Hz, 26- and 27-H_3), 0.96 (3H, d, *J*=6.8 Hz, 28-H_3), 1.00 (3H, d, *J*=6.8 Hz, 21-H_3), 1.12 (1H, m, 15β-H), 1.16 (1H, m, 17-H), 1.16 (1H, m, 12α-H), 1.16 (1H, m, 8-H), 1.23 (1H, m, 16β-H), 1.24 (1H, m, 14-H), 1.25 (1H, m, 9-H), 1.33 (1H, m, 24-H), 1.40 (1H, m, 11β-H), 1.42 (1H, m, 25-H), 1.60 (1H, m, 20-H), 1.61 (1H, m, 4α-H), 1.63 (1H, m, 7α-H), 1.65 (1H, dd, *J*=12.7, 12.7 Hz, 1α-H), 1.69 (1H, m, 11α-H), 1.80 (1H, m, 15α-H), 1.89 (1H, m, 7β-H), 1.90 (1H, dd, *J*=12.7, 4.8 Hz, 1β-H), 1.93 (1H, ddd, *J*=12.7, 2.9, 2.9 Hz, 12β-H), 2.00 (2×3H, s, 2- and 23-OAc), 2.03 (3H, s, 22-OAc), 2.05 (1H, m, 16α-H), 2.11 (3H, s, 3-OAc), 2.26 (1H, ddd, *J*=15.6, 12.7, 2.4 Hz, 4β-H), 2.32 (1H, ddd, *J*=18.1, 12.7, 4.9 Hz, 6aβ-H), 2.47 (1H, ddd, *J*=18.1, 5.4, 3.9 Hz, 6aα-H), 3.13 (1H, dd, *J*=12.7, 4.4 Hz, 5-H), 4.84 (1H, ddd, *J*=12.7, 4.8, 2.7 Hz, 2-H), 5.16 (1H, dd, *J*=9.3, 1.0 Hz, 22-H), 5.33 (1H, m, 3-H), 5.33 (1H, dd, *J*=9.3, 2.0 Hz, 23-H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 11.04 (C-28), 11.39 (C-18), 12.77 (C-21), 15.58 (C-19), 20.29 and 20.85 (C-26 and −27), 20.90 (22-OCO*C*H_3), 20.94 (23-OCO*C*H_3), 21.08 (2-OCO*C*H_3), 21.19 (3-OCO*C*H_3), 22.75 (C-11), 25.62 (C-15), 27.64 (C-16), 27.83 (C-7), 27.93 (C-4), 30.39 (C-25), 37.01 (C-20), 39.15 (C-8), 39.52 (C-12), 39.61 (C-1), 39.80 (C-24), 40.76 (C-10), 41.75 (C-13), 43.57 (C-6a), 48.03 (C-5), 52.51 (C-17), 55.85 (C-14), 58.41 (C-9), 68.36 (C-3), 69.27 (C-2), 74.16 (C-23), 75.74 (C-22), 170.19 (3-O*C*OCH$_3$), 170.29 (2-O*C*OCH$_3$), 170.51 (23-O*C*OCH$_3$), 170.62 (22-O*C*OCH$_3$), 213.56 (C-6); HR-FAB-MS *m*/*z* ([M+1]$^+$: positive ion, *m*-nitrobenzyl alcohol): found, 647.4163; calcd for $C_{37}H_{59}O_9$, 647.4159. Elution with hexane–AcOEt (2:1) gave 2,3,22,23-tetra-*O*-acetyl-6-deoxo-6a-oxo-6a-carbaBL (**13**) (18 mg, 6%): colourless granules, mp 231–234°C decomp. (hexane–AcOEt); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.70 (3H, s, 18-H_3), 0.88 (3H, s, 19-H_3), 0.90 and 0.94 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 0.96 (3H, d, *J*=6.8 Hz, 28-H_3), 1.00 (3H, d, *J*=6.8 Hz, 21-H_3), 1.16 (1H, m, 17-H), 1.17 (1H, m, 12α-H), 1.18 (1H, m, 9-H), 1.23 (1H, m, 14-H), 1.23 and 1.73 (each 1H, each m, 15-H_2), 1.25 and 2.05 (each 1H, each m, 16-H_2), 1.32 (1H, m, 24-H), 1.37 (1H, m, 11β-H), 1.42 (1H, m, 25-H), 1.51 (1H, m, 8-H), 1.57 (1H, m, 4β-H), 1.60 (1H, m, 20-H), 1.62 (1H, dd, *J*=12.7, 12.7 Hz, 1α-H), 1.75 (1H, m, 4α-H), 1.78 (1H, m, 11αH), 1.85 (1H, dd, *J*=12.7, 4.4 Hz, 1β-H), 1.95 (1H, ddd, *J*=12.7, 3.4, 3.4 Hz, 12β-H), 1.99 (3H, s, 23-OAc), 2.00 (3H, s, 2-OAc), 2.01 (3H, s, 22-OAc), 2.12 (3H, s, 3-OAc), 2.18 (1H, m, 5-H), 2.20 (1H, dd, *J*=18.1, 3.4 Hz, 6α-H), 2.29 (1H, dd, *J*=18.1, 11.7 Hz, 6β-H), 2.39 (1H, d, *J*=12.7 Hz, 7β-H), 2.48 (1H, dd, *J*=12.7, 12.7 Hz, 7α-H), 4.82 (1H, ddd, *J*=12.7, 4.4, 2.9 Hz, 2-H), 5.15 (1H, dd, *J*=8.8, 1.0 Hz, 22-H), 5.23 (1H, m, 3-H), 5.32 (1H, dd, *J*=8.8, 1.5 Hz, 23-H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 11.05 (C-28), 11.54 (C-18), 12.72 (C-21), 13.32 (C-19), 20.30 and 20.85 (C-26 and −27), 20.87 (22-OCO*C*H_3), 20.94 (23-OCO*C*H_3), 21.09 (2-OCO*C*H_3), 21.18 (3-OCO*C*H_3), 23.01 (C-11), 25.69 (C-15), 27.60 (C-16), 30.40 (C-25), 34.60 (C-4), 34.68 (C-5), 35.98 (C-8), 37.01 (C-20), 38.86 (C-1), 39.75 (C-12), 39.82 (C-24), 40.91 (C-10), 42.20 (C-13), 47.62 (C-7), 47.66 (C-6), 52.66 (C-17), 55.94 (C-14), 58.40 (C-9), 68.43 (C-3), 69.62 (C-2), 74.11 (C-23), 75.63 (C-22), 170.27 (3-O*C*OCH$_3$), 170.38 (2-O*C*OCH$_3$), 170.51 (23-O*C*OCH$_3$), 170.54 (22-O*C*OCH$_3$), 212.45 (C-6a); HR-FAB-MS *m*/*z* ([M+1]$^+$: positive ion, *m*-nitro-benzyl alcohol): found, 647.4150; calcd for $C_{37}H_{59}O_9$, 647.4159.

3.1.3. 2,3,22,23-Tetra-*O*-acetyl-6a-carba-*B*-homoBL (14). A 10% solution of $TMSCHN_2$ in hexane (1.42 mL,

1.24 mmol) was added dropwise at −15°C to a stirred solution of tetra-*O*-acetyl-6a-carbaBL (**12**) (200 mg, 0.309 mmol) and $BF_3{\cdot}Et_2O$ (234 μL, 1.86 mmol) in dry CH_2Cl_2 (20 mL), and the mixture was stirred at −10°C for 5 h. After the same work-up procedure as described above, a yellow gum (220 mg) was obtained. The gum was dissolved into Et_2O (50 mL), and silica gel (0.8 g) and 2N HCl (0.2 mL) was added. The heterogeneous solution was stirred at rt for 3 h, and Na_2SO_4 (500 mg) was added. After filtration through a glass-filter and evaporation, the residue was subjected to flash chromatography using hexane–AcOEt (3:1) as the eluent. The fractions eluted at R_f 0.22 were collected (137 mg) and subjected to HPLC (Pegasil-ODS, 20 mm i.d. ×25 cm; Senshu Scientific Co.; peak detection with a UV detector at 205 nm) using CH_3CN–H_2O (85:15) as the mobile phase at a flow rate of 10 mL/min. Elution at R_t 22.8 min gave the starting material (**12**) (94 mg, 44%), and elution at R_t 28.0 min gave 2,3,22,23-tetra-*O*-acetyl-6a-carba-*B*-homoBL (**14**) (31 mg, 15%): a colourless foam; ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.66 (3H, s, 18-H_3), 0.90 (3H, d, *J*=6.4 Hz, 26- or 27-H_3), 0.94 (3H, d, *J*=6.8 Hz, 26- or 27-H_3), 0.96 (3H, d, *J*=6.8 Hz, 28-H_3), 1.00 (3H, d, *J*=6.8 Hz, 21-H_3), 1.10 (3H, s, 19-H_3), 1.13 (1H, m, 17-H), 1.20 (1H, m, 12α-H), 1.23 and 2.03 (each 1H, each m, 16-H_2), 1.25 (1H, m, 8-H), 1.25 (1H, m, 14-H), 1.33 (1H, m, 24-H), 1.42 (1H, m, 11β-H), 1.42 (1H, m, 25-H), 1.56 and 1.65 (each 1H, each m, 7a-H_2), 1.58 (1H, m, 20-H), 1.64 (1H, m, 9-H), 1.64 (1H, m, 1α-H), ca. 1.65 (2H, 15-H_2), 1.68 (1H, m, 1β-H), 1.70 (1H, m, 4α-H), 1.72 (1H, m, 7α-H), 1.73 (1H, m, 11αH), 1.88 (1H, m, 7β-H), 1.91 (1H, m, 12β-H), 1.988 (3H, s, 2-OAc), 1.99 (1H, m, 4β-H), 1.992 (3H, s, 23-OAc), 2.02 (3H, s, 22-OAc), 2.11 (3H, s, 3-OAc), 2.29 (1H, ddd, *J*=11.2, 7.3, 2.9 Hz, 6aβ-H), 2.70 (1H, ddd, *J*=11.2, 11.2, 7.3 Hz, 6a110α-H), 2.99 (1H, dd, *J*=12.7, 2.9 Hz, 5-H), 4.97 (1H, ddd, *J*=12.2, 4.9, 3.4 Hz, 2-H), 5.15 (1H, dd, *J*=8.8, 1.0 Hz, 22-H), 5.32 (1H, m, 3-H), 5.32 (1H, dd, *J*=8.8, 2.0 Hz, 23-H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 11.07 (C-28), 11.80 (C-18), 12.75 (C-21), 14.69 (C-19), 20.31 and 20.86 (C-26 and -27), 20.86 (23-OCO*C*H_3), 20.95 (22-OCO*C*H_3), 21.06 (2-OCO*C*H_3), 21.18 (3-OCO*C*H_3), 23.35 (C-7), 24.07 (C-11), 25.22 (C-15), 27.80 (C-16), 29.22 (C-4), 30.39 (C-25), 31.68 (C-7a), 37.10 (C-20), 37.95 (C-8), 39.68 (C-1), 39.80 (C-12 and −24), 41.56 (C-13), 43.09 (C-10), 43.81 (C-6a), 52.09 (C-9), 52.31 (C-17), 52.82 (C-5), 56.25 (C-14), 68.02 (C-3), 69.48 (C-2), 74.18 (C-23), 75.77 (C-22), 170.10 (3-O*C*OCH$_3$), 170.26 (2-O*C*OCH$_3$), 170.50 (23-O*C*OCH$_3$), 170.62 (22-O*C*OCH$_3$), 217.17 (C-6); HR-EI-MS *m/z* [M]$^+$: found, 660.4222; calcd for $C_{38}H_{60}O_9$, 660.4237.

3.1.4. 6a-CarbaBL (carbaBL, 3) and 5-*epi*-6a-carbaBL (5-*epi*-carbaBL, 6). A solution of tetraacetate (**12**) (52 mg, 0.080 mmol) in 90% aqueous MeOH (5.2 mL) that contained 5% KOH was stirred at refluxing temperature for 2 h, and then MeOH (10 mL) was added. The solution was acidified with Dowex 50W resin (H^+ form) to pH 3–4 at rt and filtered through a glass filter. The filtrate was evaporated and the residue was subjected to flash chromatography. Elution with $CHCl_3$–MeOH (30:1) gave 5-*epi*-carbaBL (**6**) (2.2 mg, 6%): a colourless amorphous powder; ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.72 (3H, s, 18-H_3), 0.85 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.8 Hz, 21-H_3), 0.92 (1H, m, 9-H), 0.95 (3H, d, *J*=6.3 Hz, 26- or 27-H_3), 0.97 (3H, d, *J*=6.8 Hz, 26- or 27-H_3), 1.07 (1H, dd, *J*=12.7, 12.7 Hz, 1α-H), 1.12 (3H, s, 19-H_3), 1.15 and 1.72 (each 1H, each m, 15-H_2), 1.17 (1H, m, 12α-H), 1.21 (1H, m, 24-H), 1.27 and 1.96 (each 1H, each m, 16-H_2), 1.37 (1H, m, 11β-H), 1.37 (1H, m, 14-H), 1.50 (1H, m, 20-H), 1.57 (1H, m, 17-H), 1.63 (1H, m, 25-H), 1.72 (1H, m, 11α-H), 1.74 (1H, dd, *J*=12.7, 4.4 Hz, 1β-H), 1.81 (1H, ddd, *J*=15.6, 3.4, 3.0 Hz, 7α-H), 1.85–2.00 (2H, 4-H_2), 1.86 (1H, m, 8-H), 1.93 (1H, m, 12β-H), 2.18 (1H, m, 7β-H), 2.24 (1H, ddd, *J*=19.5, 3.7, 3.4 Hz, 6aβ-H), 2.38 (1H, ddd, *J*=19.5, 14.0, 3.0 Hz, 6aα-H), 2.56 (1H, br d, *J*=8.3 Hz, 2-OH), 3.38 (1H, br d, *J*=5.9 Hz, 5-H), 3.50 (1H, m, 2-H), 3.54 (1H, br d, *J*=8.3 Hz, 22-H), 3.71 (1H, dd, *J*=8.3, 1.0 Hz, 23-H), 3.82 (1H, m, 3-H), 5.69 (1H, d, *J*=7.3 Hz, 3-OH); ^{13}C NMR (150 MHz; $CDCl_3$) δ 10.10 (C-28), 11.81 (C-18 and 21), 19.12 (C-19), 20.74 and 20.87 (C-26 and -27), 22.36 (C-11), 24.58 (C-7), 25.11 (C-15), 27.25 (C-16), 27.93 (C-4), 30.77 (C-25), 34.95 (C-8), 36.71 (C-1), 36.80 (C-20), 37.58 (C-6a), 39.75 (C-12), 40.05 (C-24), 41.53 (C-10), 42.52 (C-13), 50.10 (C-9), 50.29 (br, C-5), 52.68 (C-17), 53.92 (C-14), 65.78 (C-3), 68.02 (C-2), 73.49 (C-23), 74.65 (C-22), 219.92 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): found, 479.3732; calcd for $C_{29}H_{51}O_5$, 479.3737. Elution with $CHCl_3$–MeOH (20:1) gave carbaBL (**3**) (35 mg, 92%): colourless needles, mp 230–231°C (MeOH–AcOEt); ^{1}H NMR (600 MHz; $CDCl_3$–CD_3OD, 10:1) δ 0.65 (3H, s, 18-H_3), 0.77 (3H, s, 19-H_3), 0.84 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.8 Hz, 21-H_3), 0.94 and 0.96 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 1.12 and 1.80 (each 1H, each m, 15-H_2), 1.13 (1H, m, 8-H), 1.18 (1H, m, 24-H), 1.24 (1H, m, 9-H), 1.24 (1H, m, 12α-H), 1.25 and 1.95 (each 1H, each m, 16-H_2), 1.28 (1H, m, 14-H), 1.40 (1H, m, 11β-H), 1.47 (1H, m, 20-H), 1.55 (1H, m, 1α-H), 1.57 (1H, m, 17-H), 1.60 (1H, m, 4α-H), 1.63 (1H, m, 25-H), 1.68 (1H, m, 7α-H), 1.75 (1H, m, 11α-H), 1.85 (1H, m, 1β-H), 1.88 (1H, m, 7β-H), 1.94 (1H, m, 12β-H), 2.09 (1H, ddd, *J*=14.7, 12.2, 1.5 Hz, 4β-H), 2.29 (1H, ddd, *J*=17.6, 12.7, 4.9 Hz, 6aβ-H), 2.46 (1H, ddd, *J*=17.6, 4.4, 2.9 Hz, 6aα-H), 3.26 (1H, dd, *J*=12.2, 3.4 Hz, 5-H), 3.52 (1H, br d, *J*=8.3 Hz, 22-H), 3.59 (1H, m, 2-H), 3.68 (1H, dd, *J*=8.3, 1.0 Hz, 23-H), 3.94 (1H, m, 3-H); ^{13}C NMR (150 MHz; $CDCl_3$–CD_3OD, 10:1) δ 9.87 (C-28), 11.35 (C-18), 11.58 (C-21), 15.33 (C-19), 20.47 and 20.60 (C-26 and −27), 22.56 (C-11), 25.43 (C-15), 26.99 (C-16), 27.52 (C-7), 29.52 (C-4), 30.42 (C-25), 36.72 (C-20), 38.93 (C-8), 39.52 (C-12), 39.99 (C-24), 40.57 (C-10), 41.53 (C-13), 41.73 (C-1), 43.69 (C-6a), 46.93 (C-5), 52.27 (C-17), 55.69 (C-14), 58.16 (C-9), 67.91 (C-2 and -3), 72.99 (C-23), 74.22 (C-22), 216.32 (C-6); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): found, 479.3737; calcd for $C_{29}H_{51}O_5$, 479.3737.

3.1.5. 6-Deoxo-6a-oxo-6a-carbaBL (iso-carbaBL, 8). The same procedure as was used to **12** was applied to tetraacetate (**13**) (14 mg, 0.022 mmol). Flash chromatography using $CHCl_3$–MeOH (20:1) as the eluent gave iso-carbaBL (**8**) (9.6 mg, 93%): colourless needles, mp 236–238°C (MeOH–AcOEt); ^{1}H NMR (600 MHz; $CDCl_3$–CD_3OD, 10:1) δ 0.69 (3H, s, 18-H_3), 0.81 (3H, s, 19-H_3), 0.84 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.8 Hz, 21-H_3), 0.94 and 0.96 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 1.18 (1H, m, 24-H), 1.19 (1H, m, 9-H), 1.23 (1H, m, 12α-H), 1.23 and 1.74 (each 1H, each m, 15-H_2),

1.25 and 1.95 (each 1H, each m, 16-H_2), 1.28 (1H, m, 14-H), 1.37 (1H, m, 11β-H), 1.45 (1H, m, 4α-H), 1.47 (1H, m, 20-H), 1.48 (1H, m, 8-H), 1.50 (1H, dd, *J*=13.2, 12.2 Hz, 1α-H), 1.57 (1H, m, 17-H), 1.63 (1H, m, 25-H), 1.73 (1H, m, 4β-H), 1.81 (1H, dd, *J*=13.2, 4.4 Hz, 1β-H), 1.85 (1H, m, 11α-H), 1.95 (1H, m, 12β-H), 2.20 −2.31 (2H, 6-H_2), 2.27 (1H, m, 5-H), 2.36 (1H, d, *J*=12.2 Hz, 7β-H), 2.53 (1H, dd, *J*=12.2, 12.2 Hz, 7α-H), 3.52 (1H, br d, *J*=8.3 Hz, 22-H), 3.59 (1H, ddd, *J*=12.2, 4.4, 3.4 Hz, 2-H), 3.68 (1H, dd, *J*=8.3, 1.5 Hz, 23-H), 3.88 (1H, m, 3-H); ^{13}C NMR (150 MHz; $CDCl_3$–CD_3OD, 10:1) δ 9.98 (C-28), 11.50 (C-18), 11.65 (C-21), 13.15 (C-19), 20.58 and 20.72 (C-26 and 27), 22.85 (C-11), 25.59 (C-15), 27.05 (C-16), 30.56 (C-25), 33.29 (C-5), 35.96 (C-8), 36.72 (C-4), 36.81 (C-20), 39.82 (C-12), 40.03 (C-24), 40.60 (C-10), 41.19 (C-1), 42.07 (C-13), 47.56 (C-6), 47.84 (C-7), 52.45 (C-17), 55.83 (C-14), 58.21 (C-9), 68.12 (C-3), 68.48 (C-2), 73.10 (C-23), 74.22 (C-22), 214.51 (C-6a); HR-FAB-MS *m/z* ($[M+1]^+$: positive ion, glycerol): found, 479.3740; calcd for $C_{29}H_{51}O_5$, 479.3737.

3.1.6. 6a-Carba-*B*-homoBL (carba-homoBL, 4) and 5-*epi*-6a-carba-*B*-homoBL (5-*epi*-carba-homoBL, 7). The same procedure as was used to **12** was applied to tetraacetate (**14**) (17 mg, 0.027 mmol). Flash chromatography using $CHCl_3$–MeOH (30:1) as the eluent gave 5-*epi*-carba-homoBL (**7**) (9.1 mg, 72%): colourless needles, mp 205–206°C (AcOEt–hexane); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.69 (3H, s, 18-H_3), 0.84 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.4 Hz, 21-H_3), 0.95 and 0.97 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 1.07 (3H, s, 19-H_3), 1.07 (1H, m, 9-H), 1.13 and 1.58 (each 1H, each m, 15-H_2), 1.14 (1H, m, 12α-H), 1.21 (1H, m, 24-H), 1.24 and 1.92 (each 1H, each m, 16-H_2), 1.30 (1H, m, 11β-H), 1.36 (1H, dd, *J*=12.7, 11.7 Hz, 1α-H), 1.36 (1H, m, 14-H), 1.48 (1H, m, 20-H), 1.53 (1H, m, 17-H), 1.64 (1H, m, 25-H), 1.64 and 1.82 (each 1H, each m, 7-H_2), 1.72 (1H, m, 1β-H), 1.73 (1H, m, 8-H), 1.73 (1H, m, 11α-H), 1.85 (1H, m, 7aα-H), ca. 1.86 (2H, 4-H_2), 1.92 (1H, m, 12β-H), 2.05 (1H, m, 7aβ-H), 2.40 (1H, ddd, *J*=11.7, 3.9, 3.9 Hz, 6aα-H), 2.48 (1H, ddd, *J*=12.3, 11.7, 6.8 Hz, 6aβ-H), 3.33 (1H, br d, *J*=5.9 Hz, 5-H), 3.54 (1H, dd, *J*=8.3, 1.5 Hz, 22-H), 3.60 (1H, ddd, *J*=11.7, 3.9, 3.9 Hz, 2-H), 3.71 (1H, dd, *J*=8.3, 2.0 Hz, 23-H), 3.77 (1H, m, 3-H), 5.10 (1H, d, *J*=10.3 Hz, 3-OH); ^{13}C NMR (150 MHz; $CDCl_3$) δ 10.10 (C-28), 11.65 (C-18), 11.75 (C-21), 17.72 (C-7), 18.62 (C-19), 20.74 and 20.86 (C-26 and 27), 23.06 (C-11), 25.20 (C-15), 26.56 (C-7a), 27.25 (C-16), 27.30 (C-4), 30.77 (C-25), 35.87 (C-8), 36.49 (C-1), 36.84 (C-20), 39.89 (C-12), 40.01 (C-24), 41.80 (C-13), 42.00 (C-10), 45.68 (C-9), 47.06 (C-6a), 52.88 (C-14 and -17), 53.28 (C-5), 66.74 (C-3), 68.15 (C-2), 73.42 (C-23), 74.68 (C-22), 223.39 (C-6); HR-FAB-MS *m/z* ($[M+1]^+$: positive ion, glycerol): found, 493.3886; calcd for $C_{30}H_{53}O_5$, 493.3893. Elusion with $CHCl_3$–MeOH (20:1) gave carba-homoBL (**4**) (2.4 mg, 19%): colourless needles, mp 234–236°C (MeOH–H_2O); ^{1}H NMR (600 MHz; $CDCl_3$–CD_3OD, 10:1) δ 0.65 (3H, s, 18-H_3), 0.84 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.8 Hz, 21-H_3), 0.94 (3H, d, *J*=6.8 Hz, 26- or 27-H_3), 0.96 (3H, d, *J*=6.3 Hz, 26- or 27-H_3), 1.04 (3H, s, 19-H_3), 1.12 and 1.67 (each 1H, each m, 15-H_2), 1.18 (1H, m, 8-H), 1.18 (1H, m, 24-H), 1.23 and 1.95 (each 1H, each m, 16-H_2), 1.24 (1H, m, 12α-H), 1.30 (1H, m, 14-H), 1.45 (1H, m, 11β-H), 1.46 (1H, m, 20-H), 1.53 (1H, m, 17-H), 1.54 (1H, dd, *J*=12.2, 12.2 Hz, 1α-H), 1.59 (1H, m, 9-H), 1.63 (1H, m, 25-H), 1.63 (2H, 7a-H_2), 1.68 (1H, dd, *J*=12.2, 4.4 Hz, 1β-H), 1.69 (1H, m, 7β-H), 1.72 (1H, ddd, *J*=15.1, 3.4, 3.4 Hz, 4α-H), 1.75 (1H, m, 11α-H), 1.82 (1H, ddd, *J*=15.1, 12.7, 2.4 Hz, 4β-H), 1.91 (1H, m, 7α-H), 1.92 (1H, m, 12β-H), 2.25 (1H, ddd, *J*=11.2, 6.8, 2.9 Hz, 6aβ-H), 2.76 (1H, ddd, *J*=11.2, 11.2, 7.3 Hz, 6aα-H), 3.16 (1H, dd, *J*=12.7, 3.4 Hz, 5-H), 3.52 (1H, dd, *J*=8.3, 1.5 Hz, 22-H), 3.68 (1H, dd, *J*=8.3, 2.0 Hz, 23-H), 3.71 (1H, m, 2-H), 3.92 (1H, m, 3-H); ^{13}C NMR (150 MHz; $CDCl_3$–CD_3OD, 10:1) δ 9.87 (C-28), 11.58 (C-18 and -21), 14.64 (C-19), 20.48 and 20.61 (C-26 and -27), 23.62 (C-11), 23.83 (C-7), 25.08 (C-15), 27.09 (C-16), 30.42 (C-25), 30.90 (C-4), 32.23 (C-7a), 36.85 (C-20), 37.92 (C-8), 39.68 (C-12), 39.98 (C-24), 41.24 (C-13), 41.83 (C-1), 43.05 (C-10), 43.72 (C-6a), 51.22 (C-5), 52.03 (C-9 and -17), 56.45 (C-14), 67.56 (C-3), 68.09 (C-2), 73.00 (C-23), 74.22 (C-22), 220.15 (C-6); HR-FAB-MS *m/z* ($[M+1]^+$: positive ion, glycerol); found, 493.3886; calcd for $C_{30}H_{53}O_5$, 493.3893.

3.2. Treatment of compounds 2–4, 6, and 7 with 5% potassium hydroxide in 90% aqueous methanol

Solutions of compounds **2**–**4**, **6**, and **7** (0.5–2 mg each) in 90% aqueous MeOH (0.4 mL) containing 5% KOH were stirred at refluxing temperature for 5 h. After the same work-up procedure as described above and a short-pad flash chromatography with $CHCl_3$–MeOH (10:1), the products were analysed by ^{1}H NMR spectroscopy (300 MHz). In summary, CS (**2**) gave compound **2**, both **3** and **6** gave a 92:8 mixture of **3** and **6**, and both **4** and **7** gave a 22:78 mixture of **4** and **7**.

3.3. Conformational analysis

See the main text.

3.4. Bioassays

3.4.1. Rice lamina inclination (RLI) test. The RLI test was carried out as described previously.[10]

3.4.2. *Arabidopsis det2* hypocotyl elongation (DHE) test. The DHE test was performed on agar-solidified half-strength Murashige and Skoog medium (8 mL) that contained 1% sucrose and a defined concentration of the test sample in a tissue culture dish (Falcon, 60×15 mm, Becton Dickinson Labware). For each test, approximately 30 seeds were sown on the medium and grown in the dark at 22°C. After 8 days, 15 seedlings were chosen randomly, and their hypocotyl lengths were measured. The average lengths are shown in Table 2.

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