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Synthesis and biological activity of 3′-chloro, -bromo, and -iodoabscisic acids, and biological activity of 3′-fluoro-8′-hydroxyabscisic acid

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Abstract

(+)-3′-Chloro, -bromo and -iodoabscisic acids were synthesized, and their biological activities and metabolism were examined, as well as that of (+)-3′-fluoroabscisic acid. This was in order to estimate the effects of altered electron density at the C-2′ position of abscisic acid on both biological activity and metabolic stability. The biological activities of (+)-3′-chloro and -bromoabscisic acids were similar to, or higher than, those of (+)-abscisic acid in four bioassays, while (+)-3′-iodoabscisic acid was more potent in two bioassays and less potent than (+)-abscisic acid in the other two bioassays. The biological activities of 3′-haloabscisic acids seem to be dependent on the 3′-halogens, but not on the electron density at C-2′. The metabolism of (+)-3′-haloabscisic acids to ca. 50% of their original levels in the media of rice cell suspension culture was similar to that of (+)-abscisic acid. Metabolites from (+)-3′-chloro, -bromo and -iodoabscisic acids were not found in the culture media and cells, while (+)-3′-fluoroabscisic acid gave (+)-3′-fluoro-8′-hydroxyabscisic acid as a stable metabolite. (+)-3′-fluoro-8′-hydroxyabscisic acid showed biological activities lower than (+)-3′-fluoroabscisic acid, but higher than (−)-3′α-Fluorophaseic acid. These findings suggested that the biological activity of (+)-8′-hydroxyabscisic acid is intermediate between those of (+)-abscisic acid and (−)-phaseic acid. Abscisic acid may be inactivated in a stepwise manner by metabolism in plants.

Keywords: Abscisic acid; 3′-Bromoabscisic acid; 3′-Chloroabscisic acid; 3′-Fluoroabscisic acid; 3′-Fluoro-8′-hydroxyabscisic acid; 8′-Hydroxyabscisic acid; 3′-Iodoabscisic acid; Phaseic acid

1. Introduction

The plant hormone (+)-abscisic acid (ABA, **1**) induces adaptive reactions to protect plants from environmental stresses such as desiccation and low temperature (Davies & Jones, 1991). Plants metabolize **1** to (+)-8′-hydroxy-ABA (**2**) which cyclizes to (−)-phaseic acid (**3**) which has a low biological activity, by nucleophilic attack of the 8′-hydroxyl group on C-2′, numbered as in **1**, and **3** is metabolized to inactive (−)-dihydrophaseic acid (**4**) (Fig. 1) (Hirai, 1999). Although any direct measurement of biological activity of **2** is difficult due to its spontaneous cyclization to **3**, 8′-hydoxy-ABA (**2**) showed a biological activity similar to that of **1** in stimulating biosynthesis of very long chain fatty acids in *Brassica napus* embryos (Zou, Abrams, Barton, Taylor, Pomeroy & Abrams, 1995). On the other hand,it displayed only weak biological activity in inhibition of germination of wheat embryos (Walker-Simmons, Holappa, Abrams & Abrams, 1997). This difference in observed responses in the two bioassays might be due to a differential decrease in concentrations of **2** in the assay media by cyclization of **2** to **3** during the assays. In other studies, more stable analogs of **2**, namely, 8′-methoxy- and 8′-fluoro-

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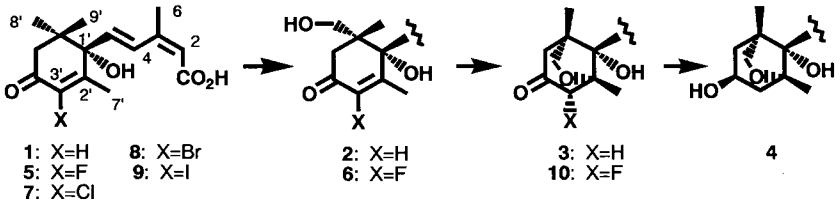


Fig. 1. Metabolic pathway of **1** and **5**, and structures of **7**–**9**.

ABAs, were synthesized in attempts to estimate the probable biological efects of **2** (Todoroki, Hirai & Koshimizu, 1994, 1995b); the cyclization of 8′-methoxy-ABA is blocked by replacement of the 8′-hydroxyl group with a methoxy group. Additionallly, fluorine is electronically equivalent to the oxygen of the hydroxyl group; the C–F bond is physicochemically similar to the C–OH bond, with the distinct difference between the monofluoro and hydroxyl groups being the lack of a dissociable hydrogen, i.e. these properties of fluorine make 8′-fluoro-ABA a stable mimic of **2** which cannot cyclize to **3**. Interestingly, 8′-methoxy- and 8′-fluoro-ABAs were as active as **1** in inhibition of lettuce seed germination (Todoroki et al., 1994; 1995b), perhaps implying that **2** might also be as active as **1**. In any case, an ABA analog incapable of cyclization after 8′-hydroxylation could show high biological activity.

We previously synthesized (+)-3′-fluoro-ABA (**5**) which has an increased electron density at C-2′ due to repulsion between the π-electron at C-2′ and the electrons of the outermost shells of the fluorine atom, and reported its bioactivity and metabolism, and the stability of (+)-3′-fluoro-8′-hydroxy-ABA (**6**) (Todoroki, Hirai & Ohigashi, 1995). In contrast to fluorine, other halogens, i.e., chlorine, bromine and iodine, would be expected to have very little effect on the electron density at C-2′ (Breitmaier & Voetler, 1987). To compare the effects of the different halogens on the electron densities at C-2′, on the bioactivities and on metabolism with those of fluorine, (+)-3′-chloro-, bromo- and iodo-ABAs (**7**, **8** and **9**, respectively) were synthesized.

A previous study showed that the effect of **5** in a rice seedling assay was similar to that of **1**, although **6** was more stable than **2** (Todoroki et al., 1995). This finding, however, also suggested that the bioactivity of **6** was lower than that expected. We, therefore, tested the bioactivity of **6** to examine the effects of 8′-hydroxylation on the bioactivity of **5**, and to further compare the bioactivity of **2** with that of **6**.

In this paper, we describe the synthesis and biological activity of (+)-3′-chloro-, bromo- and iodo-ABAs (**7**, **8** and **9**, respectively), metabolism of **5** and **7**–**9** in a rice cell suspension culture, and biological activities of **6** and (−)-3′α-fluorophaseic acid (**10**) (Fig. 1).

2. Results and discussion

2.1. Synthesis

(+)-2′,3′-Dihydro-2′α,3′α-epoxy-ABA (**11**) was synthesized from **1**, and the chloro analog **7** was synthesized from **11** via (+)-chlorohydrin (**12**) by two treatments with hydrogen chloride (Fig. 2). The position of the chlorine in **12** could not be assigned from its ^{1}H-NMR spectrum since the signal of the H-3′ proton in the other possible product (i.e. 2′-chloro-3′-hydroxy derivative) would be expected to have a similar chemical shift to that of **12**; however, conversion of **12** to **7** indicated that the chlorine was bound to C-3′, with both the existence and the position of chlorine in **7** being confirmed by the molecular formula from HRMS and by the disappearance of H-3′ in the ^{1}H-NMR spectrum, respectively. This indicated that the chloride ion attacked the β-face of C-3′ of **11** which is less sterically hindered than C-2′ to open the 2′α,3′α-epoxy ring. The low yield of **7** from **12** would be caused by the *cis* relationship between the 2′-hydroxyl group and H-3′.

The bromo analog **8** was synthesized from **11** via **13** by the same method as used for **7** with hydrogen bromide. However, this method could not be applied to synthesis of the iodo analog **9**. Treatment of (±)-**11** with hydrogen iodide gave (±)-**1**, suggesting that iod-

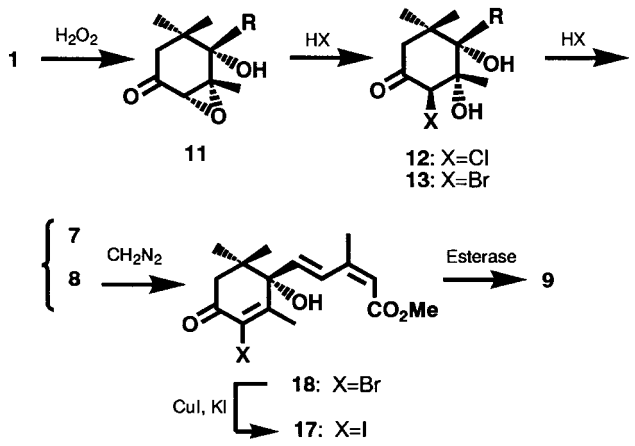
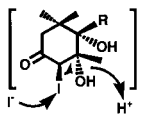


Fig. 2. Synthesis of (+)-3′-halo-ABAs, **7**–**9**. R is the side chain of **1**.

ide ion attacked the 3′-iodine of iodohydrin (**14**), a putative intermediate, to eliminate the 2′-hydroxyl group. Reaction of (±)-**11** with sodium iodide also gave (±)-**1**. Direct iodination of methyl ester of (±)-**1** with iodine azide gave 2*Z*-2-iodo-ABA (±)-**15** (McIntosh, 1971). 2-Iodo-4-oxoisophorone (**16**) was synthesized from 4-oxoisophorone with iodine azide, but the 4-carbonyl group of **16** could not be protected before introduction of the side chain. The ethylene ketal of 4-oxoisophorone did not react with iodine azide (data not shown). Methyl ester (**17**) of **9** was finally synthesized from the methyl ester (**18**) of **8** by copper iodide and potassium iodide (Suzuki, Kondo & Ogawa, 1985), and hydrolysis of **17** with esterase gave **9** (Fig. 2). The CD spectra of **7**–**9** showed the same Cotton effects as that of **1**, confirming that the absolute configuration at C-1′ of the analogs was *S*.



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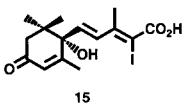

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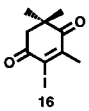

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The electron densities of **7**–**9** were estimated by ^{13}C-NMR analyses (Breitmaier & Voetler, 1997) and calculations, and Table 1 summarizes the chemical shifts and Mulliken charges of C-2′ and C-3′ of **1**, **5** and **7**–**9**. C-2′ and C-3′ signals of **7**–**9** were assigned by their HMQC and HMBC spectra. The effects of 3′-halogens on chemical shifts at C-2′ and C-3′ were as expected (Breitmaier & Voetler, 1987). The up-field shift of C-2′ of **5** was the highest among the compounds examined, confirming the effect of the 3′-fluorine on the increase in electron density at C-2′. On the other hand, the chemical shift at C-2′ of **7** suggested that the 3′-chlorine had a smaller effect on π-electron at C-2′. The effects of 3′-bromine on the electron density at C-2′ were very small since the difference between the chemical shifts of C-2′ of **1** and **8** was only 1.4 ppm. The chemical shift of C-2′ of **9** showed that the 3′-iodine slightly decreased the electron density at C-2′. As expected, the effects of the halogens on the chemical shifts of C-3′ in **5** and **7**–**9** were correlated with their electronegativities (Breitmaier & Voetler, 1987; Downs & Adams, 1973). Changes in Mulliken charges at C-2′ and C-3′ of **1**, **5** and **7**–**9** paralleled with those of the chemical shifts.

2.2. Biological activity

The biological activities of **7**–**9** were evaluated with four bioassays along with **1**, **3**, **5**, **6** and **10**: elongation of the second leaf sheath of rice seedlings; α-amylase induction by gibberellin A_3 in barley half-seeds without embryos; lettuce seed germination; and stomatal opening of the epidermal strips of spiderwort. The results are summarized in Table 2.

In the rice seedling assay, the bioactivities of **7**–**9** were similar to those of **1** and **5**. The bioactivities of the 3′-halo-ABAs were not affected by the electron density at C-2′ or by the 3′-halogens themselves, indicating that this assay is tolerant of steric size and hydrophobicity of the 3′-halogens. The bioactivity in this long term assay could be affected by metabolism of analogs in addition to their affinity to carriers and receptors, suggesting that the 3′-halogens did not affect the metabolic rate of the analogs. The results of the α-amylase assay resembled those of the rice seedling assay. The bioactivities of **5** and **7**–**9** were similar to that of **1**. This assay seemed not to be affected by steric size and hydrophobicity of the 3′-halogens. The bioactivities of **7**–**9** in the lettuce seed assay were higher than those of **1** and **5**. This result suggested that the bioactivities of **1**, **5** and **7**–**9** were correlated not with the electron density at C-2′ but with steric size and hydrophobicity of 3′-substituents (Downs & Adams, 1973; Moriguchi, Kanada & Komatsu, 1976). ABA-binding components, such as receptors, of lettuce seeds might prefer large and hydrophobic substituents at C-3′.

In the stomatal opening assay, the bioactivities of **5**, **7** and **8** were similar to that of **1**, but **9** showed 1/9 bioactivity of **1**. This result showed that bioactivity was not correlated with electron density at C-2′. Bioactivity in this assay most reflected affinity to recep-

Table 1
Chemical Shifts (δ ppm) at C-2′ and C-3′ in **1**, **5** and **7**–**9** in the ^{13}C-NMR spectra (125 MHz, acetone-d_6) and Mulliken charges[a]

Compound	Chemical shifts		Mulliken charges	
	C-2′	C-3′	C-2′	C-3′
1	163.0	127.1	−0.0222	−0.2357
5	138.3 (−24.7)	151.0 (+23.9)	−0.0662 (−0.0440)	−0.0611 (+0.1746)
7	158.2 (−4.8)	130.6 (+3.5)	−0.0241 (−0.0019)	−0.2675 (−0.0318)
8	161.6 (−1.4)	124.8 (−2.3)	−0.0095 (+0.0127)	−0.2591 (−0.0234)
9	167.5 (+4.5)	119.4 (−7.7)	−0.0032 (+0.0190)	−0.2787 (−0.0430)

[a] Shifts relative to **1** are shown in parentheses.

Table 2
IC_{50} values for **1**, **3** and **5**–**10** in bioassays

Compound	Rice elongation (μM)	Barley α-amylase (μM)	Lettuce germination (μM)	Stomatal opening (nM)
1	3.4	4.2	16.0	2.2
3	70	9.2	> 100	285
5	4.2	9.4	6.5	4.4
6	17	–[a]	64	16.0
7	3.4	3.0	2.9	0.8
8	2.4	1.3	2.5	1.2
9	3.9	2.7	2.2	20.4
10	100	–[a]	> 100	190

[a] Not tested.

tors, and the effect of metabolism on the bioactivity was small due to the short assay time, which is in agreement with the lack of a correlation between the bioactivity and the electron density at C-2′. The low bioactivity of **9** seemed to be caused by conformational changes due to the bulky 3′-iodine, but no such changes were observed in its NOESY spectrum which indicated a cross peak between H-5 and H-5′β as well as that of **1** (Milborrow, 1984). It appears that the large size of the iodine may be limiting bioactivity otherwise the other halides are all similarly active. More bulky groups at C-3′ than bromine, the van der Waals radius of which is 0.198 nm (Downs & Adams, 1973) may decrease affinity of receptors in guard cells for analogs. The bioactivity of a 3′-thioether analog was also 1/10 that of **1** in the cress seed germination assay (Balsevich, Bishop & Banowetz, 1997). The possibility that the electronic characteristics of iodine rather than steric size affects bioactivity in this assay could not be excluded. The slightly higher bioactivities of **7** and **8** than that of **1** might have been due to the hydrophobicity of the 3′-halogens with adequate steric size.

Changes in the electron density at C-2′ in the 3′-halo-ABAs did not affect the bioactivities in the four assays. The bioactivities of 3′-halo-ABAs seemed to be dependent on the physicochemical properties of the 3′-halogens, i.e., electronegativity, steric size and hydrophobicity, rather than the effect on 2′-electron density. However, it is unclear at present what characteristics of chlorine and bromine contribute to increases in the bioactivity in the lettuce seed and stomatal assays. Analog (±)-**15** was inactive in the rice seedling and lettuce seed assays probably due to its having the same geometry of the side chain as 2*E*-**1**.

*2.3. Metabolism of 3′-halo-ABAs and biological activity of (+)-3′-fluoro-8′-hydroxy-ABA (**6**)*

3′-Halo-ABAs, **5** and **7**–**9**, were added at 20 μM to suspension cultures of rice cells to examine whether they were metabolized or not. Fig. 3 showed changes in their concentrations in the media. Concentrations of **5** and **7** decreased at rates similar to that of **1**, while **8** and **9** concentrations decreased faster than that of **1**, with in each case about 50% of each of the four analogs being metabolized in 0.6–0.8 days. 3′-Halo-8′-hydroxy-ABAs and 3′-halophaseic acids derived from **7**–**9** were not found in the media and cells while **6** formed from **5** was detected in the media, suggesting that **7**–**9** were metabolized by a different pathway from 8′-hydroxylation, or their 8′-hydroxylated derivatives are unstable and decomposed to unknown metabolites. This finding suggested that the biological activities of **7**–**9** were dependent not on the metabolic stability but on the nature of the 3′-substituents themselves. The concentration of **6** increased to 7 μM after 2 days, and then decreased gradually thereafter (Fig. 3b). Formation of **10** and 3′β-fluorophaseic acid was not observed. If **6** is as active as **5**, this metabolite could contribute to the increase in bioactivity of **5** in long term assays.

Inhibitory activities of **6** are summarized in Table 2 along with those of **10** prepared from **6**. Bioactivities of **6** were higher than those of **10**, but lower than those of **5** in rice seedling, lettuce seed, and stomata assays. The bioactivities of **10** were low similarly to **3**. After incubation of an aqueous solution of **6** under the same conditions as used for the rice seedling assay for 7 days, the ratio of **6**, its 2*E* isomer, **10** and its 2*E* isomer in the solution was 64 : 31 : 4 : 1, showing that cyclization of **6** to **10** was small and the amount of 2-

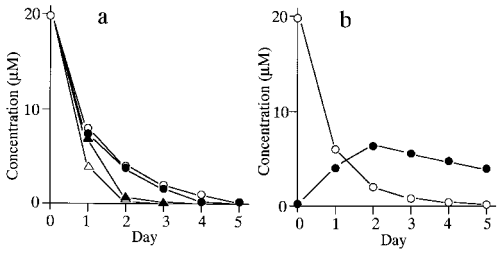

Fig. 3. Changes in concentrations of **1** and **7**–**9** (a) and of **5** and **6** (b) in the culture media. (a) ○, **1**; ●, **7**; △, **8**; ▲, **9**. (b) ○, **5**; ●, **6**.

E-**6** formed was similar to that of 2*E*-**1** formed from **1** (data not shown). These observations confirmed that the bioactivities of **6** were almost entirely due to the analog itself. The low bioactivity of **6** would explain why the bioactivity of **5** was similar to that of **1** in the long term assay although **6** was stable. C-8′ and C-9′ of **1** seemed to interact with a hydrophobic site on ABA-binding proteins since (+)-8′ and (+)-9′-alkylated analogs showed bioactivities similar to that of **1** (Nakano, Todoroki, Hirai & Ohigashi, 1995). If this was the case, the low bioactivity of **6** may have been due to a negative effect of the 8′-hydroxyl group on the hydrophobic interaction. A hydrogen bonding in which the 8′-hydroxyl group acts as a proton donor may cause the negative effect. Methoxy and fluorine groups cannot participate in hydrogen bonding in which these groups act as proton donor (Todoroki, Hirai & Koshimizu, 1995a, 1995b), which may explain the difference in the bioactivities between **6**, and 8′-methoxy- and 8′-fluoro-ABAs. This presumably means that **6** more reflects the effect of the 8′-hydroxyl group on the bioactivities than 8′-methoxy- and 8′-fluoro-ABAs.

Thus, bioactivity of the unstable metabolite **2** can be estimated from the bioactivity of **6**. Bioactivities of **5** and **10** were similar to those of **1** and of **3**, respectively, indicating that the effect of the 3′-fluorine on the bioactivities was small. This suggests that bioactivity of **2** must be similar to that of **6**. These observations indicated that the biological activity of **2** is probably lower than that of **1**, and higher than that of **3**. Consequently, **1** would be inactivated in a stepwise manner by metabolism in plants: in the first step, **1** with high bioactivity is converted to **2** with medium bioactivity by 8′-hydroxylation; then, **2** is cyclized to **3** with low bioactivity; and in the third step, **3** is metabolized to inactive **4** by reduction of the 4′-carbonyl group. Inactivation of **1** is completed probably at **4**, since **3** can isomerize to **2** again unless the 4′-carbonyl group of **3** is reduced. Low bioactivities of **3** could be partially caused by a small amount of **2** formed from **3** by isomerization, suggesting that bioactivity of **3** itself is lower than that observed. The stepwise inactivation of **1** suggests that an analog resistant to 8′-hydroxylation such as (+)-8′,8′,8′-trifluoro-ABA would show long lasting bioactivity as was found by Todoroki et al. (1995a).

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR, NOESY, and HMQC and HMBC spectra were recorded with TMS as an internal standard using Brüker AC300 (300 MHz) and ARX500 (500 MHz) spectrometers. ^{13}C-NMR spectra were recorded reference to the acetone-d_6 as 30.2 ppm. For clarity, the conventional ABA numbering system was used to assign peaks in the ^{1}H- and ^{13}C-NMR spectra. Mass spectra were obtained using a Jeol JMS-DX300/DA5000 mass spectrometer, whereas GC–MS was conducted with a 1% OV-17 column (1 m × 3 mm) at a He flow rate of 60 ml/min, and the oven temperature was increased from 90° to 200° at a rate of 32°/min. Optical rotations were measured with a Jasco DIP-1000 digital polarimeter, and CD spectra were recorded with a Jasco J-720W spectropolarimeter.

3.2. (+)-2′,3′-Dihydro-2′α,3′α-epoxy-ABA (*11*) and (+)-3′-fluoro-ABA (*5*)

These compounds were synthesized as described (Todoroki et al., 1995). ^{1}H-NMR (500 MHz, $CDCl_3$) of **11**: δ 0.94 (3H, *s*, H-8′), 0.99 (3H, *s*, H-9′), 1.39 (3H, *s*, H-7′), 1.84 (1H, *dd*, J = 14.6 Hz, H-5′), 2.07 (3H, *s*, H-6′), 2.84 (1H, *d*, J = 14.6 Hz, H-5′), 3.30 (1H, *s*, H-3′), 5.79 (1H, *s*, H-2), 6.11 (1H, *d*, J = 15.8 Hz, H-5), 8.02 (1H, *d*, J = 15.8 Hz, H-4). ^{1}H-NMR (500 MHz, $CDCl_3$) of **5**: δ 1.05 (3H, *s*, H-9′), 1.14 (3H, *s*, H-8′), 1.87 (3H, *d*, J = 3.2 Hz, H-7′), 2.05 (3H, *s*, H-6), 2.44 (1H, *br. d*, J = 17.1 Hz, H-5′), 2.49 (1H, *d*, J = 17.1 Hz, H-5′), 5.79 (1H, *br. s*, H-2), 6.11 (1H, *d*, J = 16.1 Hz, H-5), 7.81 (1H, *d*, J = 16.1 Hz, H-4).

3.3. (+)-2Z,4E-5-[(1S,2S,3R)-3-Chloro-1,2-dihydroxy-2,6,6-trimethyl-4-oxocyclohexan-1-yl]-3-methyl-2,4-pentadienoic acid [2′,3′-dihydro-3′β-chloro-2′α-hydroxy-ABA (*12*)]

To a stirred solution of **11** (89 mg) in MeOH (4.5 ml) was added 10% HCl (4.5 ml), and the mixture was stirred at 18° for 2.4 h. Then, the reaction mixture was diluted with H_2O to 40 ml and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was next subjected to silica gel chromatography using *n*-hexane–EtOAc–HOAc (80 : 20 : 1) as eluant to afford **12** (33 mg, 33% yield). $[\alpha]_D^{30}$ +67° (MeOH; *c* 0.049); UV λ_{max}^{MeOH} nm (log ε): 260 (4.19); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.94 (3H, *s*, H-8′ or H-9′), 0.99 (3H, *s*, H-9′ or H-8′), 1.39 (3H, *s*, H-7′), 1.83 (1H, *dd*, J =14.6 and 0.9 Hz, H-5′), 2.06 (3H, *d*, J =1.1 Hz, H-6), 2.83 (1H, *d*, J =14.6 Hz, H-5′), 3.30 (1H, *d*, J = 0.6 Hz, H-3′), 5.79 (1H, *s*, H-2), 6.11 (1H, *d*, J = 15.7 Hz, H-5), 8.02 (1H, *d*, J = 15.7 Hz, H-4). Compound **12** (1 mg) was methylated with CH_2N_2 to give the methyl ester (1 mg). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 332 $[M + 2]^+$ (1.3), 330 $[M]^+$ (4), 312 (1.5), 295 $[M - Cl]^+$ (10), 280 (14), 277 (23), 263 (18), 245 (20), 238 (26), 224 (76), 196 (39), 170 (38), 161 (63),

153 (34), 125 (100), 121 (78); HRMS: $[M]^+$ at *m/z* 330.1230 ($C_{16}H_{23}O_5Cl$ requires 330.1234); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1740, 1730, 1710, 1690, 1600.

3.4. (+)-2Z,4E-5-[(1S)-3-Chloro-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-2,4-pentadienoic acid [3′-chloro-ABA (7)]

To a stirred solution of **12** (17 mg) in MeOH (1.0 ml) was added 10% HCl (1.0 ml), and the mixture was stirred at room temperature for 6.6 h. The reaction mixture was diluted with H_2O to 40 ml and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was subjected to silica gel chromatography using *n*-hexane–EtOAc–HOAc (80 : 20 : 1) as eluant to afford **7** (6.5 mg, 42% yield). $[\alpha]_D^{26}$ +221° (MeOH; *c* 0.175); CD: $\Delta\varepsilon_{240}$ −18.4, $\Delta\varepsilon_{268}$ +27.2,$\Delta\varepsilon_{329}$ −1.9 (MeOH; *c* 0.001); UV λ_{max}^{MeOH} nm (log ε): 260 (4.28); ^{1}H-NMR (300 MHz, CD_3OD): δ 1.03 (3H, *s*, H-8′ or H-9′), 1.05 (3H, *s*, H-9′ or H-8′), 2.03 (3H, *d*, *J* = 1.1 Hz, H-6), 2.07 (3H, *s*, H-7′), 2.40 (1H, *d*, *J* = 16.7 Hz, H-5′), 2.64 (1H, *d*, *J* = 16.7 Hz, H-5′), 5.75 (1H, *s*, H-2), 6.22 (1H, *d*, *J* = 16.1 Hz, H-5), 7.76 (1H, *d*, *J* = 16.1 Hz, H-4); ^{13}C-NMR (125 MHz, acetone-d_6): δ 17.90 (C-7′), 21.56 (C-6), 23.77 (C-8′), 24.86 (C-9′), 42.26 (C-6′), 50.13 (C-5′), 81.73 (C-1′), 119.45 (C-2), 129.76 (C-4), 130.55 (C-3′), 137.47 (C-5), 151.12 (C-3), 158.15 (C-2′), 167.51 (C-1), 189.83 (C-4′). Compound **7** (1 mg) was methylated with CH_2N_2 to give the methyl ester (1 mg). EIMS (probe) 70 eV, *m/z* (rel. int.): 314 $[M + 2]^+$ (0.6), 312 $[M]^+$ (1.2), 294 (7), 280 (10), 269 (4), 245 (31), 239 (5), 224 (76), 217 (9), 196 (30), 181 (7), 168 (20), 161 (42), 141 (5), 133 (23), 125 (100), 112 (26); HRMS: $[M]^+$ at *m/z* 312.1134 ($C_{16}H_{21}O_4Cl$ requires 312.1128); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1720, 1700, 1690, 1630, 1600.

*3.5. (+)-2Z,4E-5-[(1S,2S,3R)-3-Bromo-1,2-dihydroxy-2,6,6-trimethyl-4-oxocyclohexan-1-yl]-3-methyl-2,4-pentadienoic acid [2′,3′-dihydro-3′β-bromo-2′α-hydroxy-ABA (**13**)]*

To a stirred solution of **11** (680 mg) in MeOH (50 ml) was added 10% HBr (50 ml), and the mixture was stirred at 18° for 1.3 h. The reaction mixture was diluted with H_2O to 400 ml and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was subjected to silica gel chromatography using *n*-hexane–EtOAc–HOAc (76 : 24 : 1) as eluant to afford **13** (314 mg, 36% yield). $[\alpha]_D^{30}$ +100° (MeOH; *c* 0.075); UV λ_{max}^{MeOH} nm (log ε): 261 (4.35); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3300–2500, 2950, 1725, 1685, 1635, 1600, 1450, 1385, 1245, 1180, 1120; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.06 (3H, *s*, H-8′ or H-9′), 1.10 (3H, *s*, H-9′ or H-8′), 1.42 (3H, *s*, H-7′), 2.09 (3H, *d*, *J* = 1.2 Hz, H-6), 2.34 (1H, *d*, *J* = 12.8 Hz, H-5′), 3.04 (1H, *d*, *J* = 12.8 Hz, H-5′), 5.33 (1H, *s*, H-3′), 5.80 (1H, *s*, H-2), 6.47 (1H, *d*, *J* = 15.6 Hz, H-5), 7.88 (1H, *d*, *J* = 15.6 Hz, H-4); FABMS (matrix, 3-nitrobenzyl alcohol), *m/z* (rel. int.): 385 $[M + Na + 2]^+$ (12), 383 $[M + Na]^+$ (14), 362 $[M + 2]^+$ (13), 360 $[M]^+$ (14), 345 (25), 343 (30), 327 (34), 303 (11), 281 $[M - Br]^+$ (40), 263 (51), 245 (26), 235 (15), 223 (58), 207 (100), 182 (32), 163 (75).

*3.6. (+)-2Z,4E-5-[(1S)-3-Bromo-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-2,4-pentadienoic acid and its methyl ester [3′-bromo-ABA (**8**) and its methyl ester(**18**), respectively]*

To a stirred solution of **13** (396 mg) in MeOH (30 ml) was added 30% HBr (6.0 ml), and the mixture was stirred at room temperature 3.7 h. The reaction mixture was diluted with H_2O to 300 ml and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was subjected to silica gel chromatography with *n*-hexane–EtOAc–HOAc (80 : 20 : 1) as eluant to afford **8** (86 mg, 23% yield). $[\alpha]_D^{26}$ +259° (MeOH; *c* 0.062); CD: $\Delta\varepsilon_{244}$ −20.9, $\Delta\varepsilon_{272}$ +38.1, $\Delta\varepsilon_{325}$ −3.0 (MeOH; *c* 0.001); UV λ_{max}^{MeOH} nm (log ε): 262 (4.38); ^{1}H-NMR (300 MHz, CD_3OD): δ 1.03 (3H, *s*, H-8′ or H-9′), 1.04 (3H, *s*, H-9′ or H-8′), 2.04 (3H, *s*, H-6), 2.12 (3H, *s*, H-7′), 2.44 (1H, d, *J* = 16.7 Hz, H-5′), 2.67 (1H, *d*, *J* = 16.7 Hz, H-5′), 5.75 (1H, *s*, H-2), 6.22 (1H, *d*, *J* = 16.1 Hz, H-5), 7.77 (1H, *d*, *J* = 16.1 Hz, H-4); ^{13}C NMR (75 MHz, acetone-d_6): δ 21.43 (C-7′), 21.55 (C-6), 23.78 (C-8′), 24.80 (C-9′), 42.25 (C-6′), 49.99 (C-5′), 82.38 (C-1′), 119.57 (C-2), 124.78 (C-3′), 129.83 (C-4), 137.30 (C-5), 151.02 (C-3), 161.60 (C-2′), 167.63 (C-1), 189.84 (C-4′). Compound **8** (56 mg) was methylated with CH_2N_2 to give methyl ester (**18**) (57 mg). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.03 (3H, *s*, H-8′ or H-9′), 1.11 (3H, *s*, H-9′ or H-8′), 2.01 (3H, *d*, *J* = 1.2 Hz, H-6), 2.14 (3H, s, H-7′), 2.58 (1H, *d*, *J* = 17.1 Hz, H-5′), 2.59 (1H, *d*, *J* = 17.1 Hz, H-5′), 3.72 (3H, *s*, 1-CO_2CH_3), 5.78 (1H, *s*, H-2), 6.10 (1H, *d*, *J* = 16.0 Hz, H-5), 7.87 (1H, *d*, *J* = 16.0 Hz, H-4); EIMS (probe) 70 eV, *m/z* (rel. int.): 358 $[M + 2]^+$ (0.1), 356 $[M]^+$ (0.1), 340 (0.2), 338 (0.2), 326 (0.5), 324 (0.5), 315 (0.2), 313 (0.2), 300 (0.1), 277 $[M - Br]^+$ (10), 270 (42), 268 (44), 259 (11), 245 (42), 217 (11), 189 (8), 161 (47), 133 (27), 125 (100), 112 (23); HRMS: $[M]^+$ at *m/z* 356.0663 ($C_{16}H_{21}O_4Br$ requires 356.0623); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1785, 1715, 1635, 1600, 1450, 1435, 1370, 1240, 1160.

*3.7. Treatment of **11** with HI and NaI*

To a stirred solution of (±)-**11** (178 mg) in MeOH

(9 ml) was added 30% HI (1.8 ml), and the mixture was stirred at room temperature for 3.9 h. The reaction mixture was diluted with H_2O to 100 ml and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was subjected to silica gel chromatography using *n*-hexane–EtOAc–HOAc (85 : 15 : 1) as eluant to afford (±)-**1** (40 mg, 24% yield). ^{1}H-NMR (300 MHz, CD_3OD): δ 1.03 (3H, *s*, H-9′), 1.06 (3H, *s*, H-8′), 1.93 (3H, *d*, J = 1.3 Hz, H-7′), 2.04 (3H, *d*, J = 1.1 Hz, H-6′), 2.17 (1H, *d*, J = 16.9 Hz, H-5′), 2.52 (1H, *d*, J = 16.9 Hz, H-5′), 5.74 (1H, *br. s*, H-2), 5.92 (1H, *br. s*, H-3′), 6.24 (1H, *d*, J = 16.2 Hz, H-5), 7.77 (1H, *d*, J = 16.2 Hz, H-4). To a stirred solution of (±)-**11** (172 mg) in HOAc (0.5 ml) and propionic acid (1 ml) was added NaOAc (23 mg) and NaI (187 mg), and the mixture was stirred at −20° for 1.6 h. The reaction mixture was diluted with 5% $NaHCO_3$ aqueous solution to 45 ml, acidified with 3 M HCl to pH 3, and extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ aqueous solution and H_2O, dried (Na_2SO_4), filtered and concentrated to give (±)-**1** as the major product.

3.8. (±)-2Z,4E-5-(1-Hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-2-iodo-3-methyl-2,4-pentadienoic acid [2Z-2-iodo-ABA (**15**)]

To a stirred solution of sodium azide (194 mg, 2.98 mmol) in CH_3CN (0.76 ml) was added iodine monochloride (73 mg, 0.45 mmol) in 0.14 ml of CH_3CN at −20°C. The mixture was stirred for 35 min at −20°C, then the methyl ester of (±)-**1** (115 mg, 0.41 mmol) in 1.5 ml of CH_3CN was added to the mixture. The mixture was stirred at 0°C for 4.1 h and at room temperature for 18.9 h, then diluted with H_2O to 45 ml, acidified with 3 M HCl to pH 3, and extracted with EtOAc. The organic layer was washed with 10% Na_2SO_3 aqueous solution and H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was subjected to silica gel chromatography using *n*-hexane–EtOAc–HOAc (80 : 20 : 1) as eluant to afford (±)-**15** (16 mg, 9% yield). UV λ_{max}^{MeOH} nm (log ε): 237 (4.38); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.03 (3H, *s*, H-8′ or H-9′), 1.11 (3H, *s*, H-9′ or H-8′), 1.89 (3H, *d*, J = 1.4 Hz, H-7′), 2.31 (3H, *s*, H-6), 2.34 (1H, *dd*, J = 17.2 and 0.9 Hz, H-5′), 2.49 (1H, *dd*, J = 17.2 and 0.5 Hz, H-5′), 5.96 (1H, *m*, H-3′), 6.47 (1H, *d*, J = 15.7 Hz, H-5), 6.83 (1H, *d*, J = 15.7 Hz, H-4); FABMS (matrix, 3-nitrobenzyl alcohol), *m/z* (rel. int.): 391 $[M + H]^+$ (9), 358 (5), 341 (13), 316 (9), 265 $[M - I]^+$ (77), 245 (33), 237 (50), 223 (100), 205 (100), 191 (31), 177 (56), 189 (8), 161 (43), 125 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3603, 3411, 3300–2500, 3017, 2971, 2114, 1667, 1628, 1424, 1366, 1254, 1177, 1123, 984.

3.9. 3-Iodo-2,6,6-trimethyl-2-cyclohexen-1,4-dione [2-iodo-4-oxoisophorone (**16**)]

To a stirred solution of sodium azide (23 g, 346 mmol) in 90 μl of CH_3CN was added dropwise iodine monochloride (8.6 g, 53 mmol) in 17 ml of CH_3CN at −20°C over a period of 15 min. Then 4-oxoisophorone (7.6 g) was added to the mixture, followed by stirring at room temperature for 28.5 h. The mixture was diluted with H_2O to 170 ml, and extracted with EtOAc. The organic layer was washed with 10% Na_2SO_3 aqueous solution and H_2O, dried (Na_2SO_4), filtered and concentrated. The residual oil was subjected to silica gel chromatography with toluene–EtOAc (97 : 3) as eluant to afford **16** (5.8 g, 42% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.25 (6H, *s*, 5-CH_3 × 2), 2.32 (3H, *s*, 3-CH_3), 2.90 (2H, *s*, H-6); GC-EIMS 70 eV, *m/z* (rel. int.): 278 $[M]^+$ (24), 263 (11), 250 (4), 235 (4), 222 (12), 194 (5), 237 (50), 151 $[M - I]^+$ (6), 127 (3), 108 (2), 67 (100).

3.10. (+)-Methyl 2Z,4E-5-[(1S)-1-hydroxy-3-iodo-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-2,4-pentadienoate [methyl 3′-iodoabscisate (**17**)]

A mixture of **18** (57 mg), potassium iodide (400 mg), copper(I) iodide (156 mg), and hexamethylphosphoramide (0.48 ml) was stirred at 150 for 0.7 h under N_2. After the reaction was quenched by H_2O, the mixture was diluted with 10% Na_2SO_3 aqueous solution to 45 ml and extracted with EtOAc. The organic layer was washed with 10% aqueous Na_2SO_3 and H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residual oil was subjected to silica gel chromatography using toluene–EtOAc (9 : 1) as eluant to afford **17** (29 mg, 44% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.03 (3H, *s*, H-8′ or H-9′), 1.09 (3H, *s*, H-9′ or H-8′), 2.01 (3H, *d*, J = 1.3 Hz, H-6), 2.25 (3H, *s*, H-7′), 2.63 (2H, s, H-5′), 3.72 (3H, *s*, 1-CO_2CH_3), 5.78 (1H, *d*, J = 0.4 Hz, H-2), 6.09 (1H, *dd*, J = 16.1 and 0.3 Hz, H-5), 7.86 (1H, *dd*, J = 16.1 and 0.5 Hz, H-4); EIMS (probe) 70 eV, *m/z* (rel. int.): 404 $[M]^+$ (4), 386 (3), 372 (9), 316 (46), 288 (12), 277 $[M - I]^+$ (12), 259 (13), 245 (36), 227 (5), 217 (11), 203 (5), 189 (8), 161 (43), 125 (100); HRMS: $[M]^+$ at *m/z* 404.0477 ($C_{16}H_{21}O_4I$ requires 404.0483); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1700, 1680, 1635, 1600, 1370, 1165, 985.

3.11. (+)-2Z,4E-5-[(1S)-1-Hydroxy-3-iodo-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-2,4-pentadienoic acid [3′-iodo-ABA (**9**)]

To a solution of **17** (29 mg) in a mixture of MeOH (0.1 ml) and 0.1 M KH_2PO_4–K_2HPO_4 buffer (pH 8.0, 4 ml) was added porcine liver esterase (EC 3.1.1.1, 2000 units). The mixture was shaken at 30°C for 5.8 h,

then diluted with H_2O, acidified with 3 M HCl to pH 3 and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated. The residual oil was subjected to silica gel chromatography using *n*-hexane–EtOAc–HOAc (82 : 18 : 1) as eluant to afford **9** (21 mg, 77% yield). $[\alpha]_D^{26}$ +224° (MeOH; *c* 0.021); CD: $\Delta\varepsilon_{243}$ −5.2, $\Delta\varepsilon_{283}$ +23.7, $\Delta\varepsilon_{329}$ −2.2 (MeOH; *c* 0.001); UV λ_{max}^{MeOH} nm (log ε): 258 (4.37); ^{1}H-NMR (300 MHz, CD_3OD): δ 1.03 (6H, *s*, H-8′ and H-9′), 2.03 (3H, *d*, *J* = 1.2 Hz, H-6), 2.22 (3H, *s*, H-7′), 2.49 (1H, *d*, *J* =16.6 Hz, H-5′), 2.70 (1H, *d*, *J* = 16.6 Hz, H-5′), 5.75 (1H, *s*, H-2), 6.20 (1H, *d*, *J* = 16.1 Hz, H-5), 7.75 (1H, *d*, *J* = 16.1 Hz, H-4); ^{13}C-NMR (75 MHz, acetone-d_6): δ 21.56 (C-6), 23.81 (C-8′), 24.65 (C-9′), 27.63 (C-7′), 42.31 (C-6′), 48.96 (C-5′), 82.43 (C-1′), 109.66 (C-3′), 119.40 (C-2), 129.74 (C-4), 137.42 (C-5), 151.16 (C-3), 167.47 (C-2′), 167.62 (C-1), 191.16 (C-4′).

3.12. Calculation

Mulliken charges at C-2′ and C-3′ of **1**, **5** and **7**–**9** were calculated by the PM3 method using CAChe 3.1., Oxford Molecular Ltd., Oxford, 1997.

3.13. Bioassays

The four bioassays were performed as previously reported (Todoroki *et al.,* 1994). For the elongation assays, the length of the second leaf sheath of rice (*Oryza sativa* L. cv. Nihonbare) seedlings was measured after incubation with the test solution in continuous light at 30°C for 7 days. For the germination assay, the number of germinated lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds was counted after incubation with the test solution at 25°C for 48 h. For the α-amylase assay, after incubating barley (*Hordeum vulgare* L. cv. Himalaya) half-seeds without embryos in the test solution at 30°C for 48 h in the dark, the absorbance of the test solution at 660 nm was measured by the Somogyi–Nelson method. For the stomatal opening assay, the width of the stomatal aperture on the epidermal strip of spiderwort (*Tradescantia reflexa* Rafin) was measured after incubation with the test solution in continuous light at 25°C for 3 h.

3.14. Maintenance of rice cells

Suspension cultures of rice (*O. sativa* L. cv. Nipponkai) were maintained at 24° in continuous light on a rotary shaker at 90 rpm in Linsmaier and Skoog medium. Half of the cells were transferred into new medium once a week.

3.15. Quantification of *1*, *5*, and *7*–*9* in culture media

Cultured cells (4 g fresh weight) were transferred into 70 ml of fresh medium in a 300 ml flask, and a solution of the compound (0.37 mg) in 0.5 ml of H_2O was added to yield a final concentration of 20 μM. The cultures were incubated at 24° for 8 days with shaking. One-ml samples were collected from flasks for HPLC analysis after 0, 1, 2, 3, 4 and 5 days. The samples were kept at −20° until HPLC analysis on an ODS column (YMC AQ-311, 6 i.d. × 100 mm). The column was eluted at 1.0 ml min^{-1} with 40–60% MeOH in 0.1% aqueous HOAc, and the eluent was monitored at 254 nm. Peaks in the chromatograms were identified by comparing their retention times with those of authentic standards, and the amounts of compounds were calculated with reference to calibration curves of the authentic samples between peak area and amount. Experiments were performed at least in duplicate.

3.16. Searching metabolites of *7*–*9* in culture media and cells

Four flasks containing **7** and cell suspension were prepared by the same method as that for the quantification. The cultures were incubated at 24° on a rotary shaker at 90 rpm for 3 days, and filtrated. The pH of the filtrate was adjusted to pH 3 with 1 N HCl, and the filtrate was extracted four times with 200 ml of EtOAc. The combined EtOAc layer was washed with water, dried (Na_2SO_4), filtered and concentrated. The residue (21 mg) was subjected to silica gel chromatography (5 g) using a mixture of toluene and EtOAc as eluant. 3′-Chloro-8′-hydroxy-ABA and 3′-chlorophaseic acid of **7** were not found in the eluted fractions. Recovered cells (22.1 g fresh weight) was soaked in 50 ml of MeOH for three days, and removed by filtration. The filtrate was concentrated, and the residue was analyzed with HPLC in the same condition as that for the quantification, and silica gel TLC developed with toluene–EtOAc–HOAc (14 : 6 : 1). 3′-Chloro-8′-hydroxy-ABA and 3′-chlorophaseic acid of **7** were not detected with HPLC and TLC analyses. Compounds **8** and **9** were treated and analyzed by the same manner as that for **7**.

3.17. Preparation of *6* and *10*

A solution of **5** (0.4 mg) in 0.5 ml of H_2O was added to 70 ml of a suspension of 10 g of cells in a flask so that the final concentration was 20 μM. Twelve flasks containing **5** and cell suspension were prepared. The cultures were incubated at 24° on a rotary shaker at 90 rpm for 24 h, and the cells were removed by filtration. The pH of the filtrate was

adjusted to 3 with 1 N HCl, and the filtrate was extracted four times with 500 ml of EtOAc. The combined EtOAc layer was washed with water, dried (Na_2SO_4), filtered and concentrated. The residue (52 mg) was chromatographed on silica gel (5.2 g) with a mixture of toluene–EtOAc as the eluant. Materials eluted with 50% EtOAc were purified with an ODS HPLC column (YMC AQ-313, 6 i.d. × 100 mm) by eluting with 45% MeOH in 0.1% aqueous HOAc at 1.0 ml min^{-1} to give 0.61 mg of **6**. $[\alpha]_D^{25}$ +273° (MeOH; *c* 0.046); UV λ_{max}^{MeOH} nm (log ε): 255 (4.34); ^{1}H-NMR (500 MHz, CD_3OD): δ 1.05 (3H, *s*, H-9′), 1.85 (3H, *d*, *J* = 3.4 Hz, H-7′), 2.02 (3H, *s*, H-6), 2.43 (1H, *d*, *J* = 17.3 Hz, H-5′), 2.60 (1H, *d*, *J* = 17.3 and 4.7 Hz, H-5′), 3.62 (1H, *d*, *J* = 11.3 Hz, H-8′), 3.65 (1H, *d*, *J* = 11.3 Hz, H-8′), 5.79 (1H, *br*. *s*, H-2), 6.11 (1H, *d*, *J* = 16.1 Hz, H-5), 7.73 (1H, *d*, *J* = 16.1 Hz, H-4). Compound **6** (0.32 mg) was dissolved in 0.7 ml of 0.1% NH_3 aqueous soln, and left at 25° for 1 h. The solution was concentrated, and the residue was purified with an ODS column under the same conditions as used for **6** to afford 0.2 mg of **10**. $[\alpha]_D^{26}$ −90° (MeOH; *c* 0.021); UV λ_{max}^{MeOH} nm (log ε): 255 (4.37); ^{1}H-NMR (500 MHz, CD_3OD): δ 1.03 (3H, *s*, H-9′), 1.32 (3H, *d*, *J* = 1.8 Hz, H-7′), 2.01 (3H, *s*, H-6), 2.38 (1H, *dd*, *J* = 16.8 and 6.6 Hz, H-5′), 2.79 (1H, *dd*, *J* = 16.8 and 2.8 Hz, H-5′), 3.55 (1H, *d*, *J* = 7.6 Hz, H-8′), 3.94 (1H, *dd*, *J* = 7.6 and 2.8 Hz, H-8′), 5.02 (1H, *d*, *J* = 47.0 Hz, H-3′), 5.85 (1H, *br*. *s*, H-2), 6.29 (1H, *d*, *J* = 15.9 Hz, H-5), 8.00 (1H, *d*, *J* = 15.9 Hz, H-4).

*3.18. Incubation conditions of **6** in the rice seedling assay*

An aqueous solution (2 ml) of **6** at 0.1 mM was incubated under continuous light at 30°C for 7 days. Aliquots of 5 μl of the solution were analyzed every day with an ODS column under the same conditions as used for the preparation of **6**. Compound **6** and its 2*E*-isomer, and **10** and its 2*E*-isomer were eluted at R_t 8.9, 6.5, 4.8, and 4.3 min, respectively.

*3.19. Preparation of **3***

Three mg of **3** was prepared by hydrolysis of the β-hydroxy-β-methylglutaryl ester of 8′-hydroxy-ABA isolated from immature seeds of *Robinia pseudacacia* (Hirai, Fukui & Koshimizu, 1978). ^{1}H-NMR (500 MHz, CD_3OD) of **3**: δ 1.01 (3H, *s*, H-9′), 1.21 (3H, *s*, H-7′), 2.06 (3H, *d*, *J* = 0.9 Hz, H-6), 2.39 (1H, *dd*, *J* = 18.0 and 2.5 Hz, H-5′), 2.47 (1H, *dd*, *J* = 18.0 and 2.4 Hz, H-5′), 2.70 (1H, *dd*, *J* = 18.0 and 2.5 Hz, H-3′), 2.80 (1H, *d*, *J* = 18.0 Hz, H-3′), 3.66 (1H, *d*, *J* = 7.6 Hz, H-8′), 3.94 (1H, *dd*, *J* = 7.6 and 2.9 Hz, H-8′), 5.79 (1H, *br*. *s*, H-2), 6.45 (1H, *d*, *J* = 15.9 Hz, H-5), 8.10 (1H, *d*, *J* = 15.9 Hz, H-4).

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