2-Fluoroabscisic Acid Analogues: Their Synthesis and Biological Activities

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Fluorine was introduced into the 2-position of the side chain of abscisic acid (ABA) analogues by Wittig reaction of α-ionone derivatives with ethyl triethylphosphono-2-fluoroacetate. The effects of the fluorinated analogues were evaluated on inhibition of cress seed germination and inhibition of gibberellin-inducible α-amylase induction in embryoless barley half-seeds. (2*E*,4*E*)-2-Fluoro-5-(1′-hydroxy-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-3-methyl-2,4-pentadienoic acid (**5b**) showed potent inhibitory activity at the same level as ABA in the cress seed germination test, and **5b** also inhibited gibberellin-inducible α-amylase induction at 4×10^{-6}, 3 times the concentration of ABA (1×10^{-6}) for 50% inhibition of α-amylase production. **5b** also showed dehydrin induction activity. These results indicate that fluorinated ABA analogues mimic ABA action and can be a lead for a plant growth regulator which regulates plant growth or protects plants from environmental stresses.

Keywords: *Inhibition of cress seed germination; inhibition of amylase induction; dehydrin induction; aleurone; 2-fluoroabscisic acid; abscisic acid*

INTRODUCTION

Abscisic acid (ABA, **1**; Figure 1) is a plant hormone that regulates physiological processes (Ohkuma et al., 1963; Zeevaart et al., 1988) such as the acceleration of abscission, induction of dormancy, inhibition of rooting, and stimulation of stomatal closure. In addition to these hormonal activities, ABA has attracted considerable attention due to the role it plays in the response to environmental stress such as drought (Davies et al., 1993) and cold (Prasad et al., 1994; Anderson et al., 1994). Therefore, if ABA analogues mimic ABA action and have a commercial advantage over ABA to field application, they could be developed as plant growth regulators. Since the first synthesis of ABA (Cornforth et al., 1965), varieties of ABA analogues have been reported, but none of them has been developed as a plant growth regulator. A fluorine atom has been increasingly used as a label for obtaining structural information and mechanistic details of biologically active molecules (Welch, 1987; Kim et al., 1995; Todoroki et al., 1995). Substitution of hydrogen with fluorine changes not only the molecule's size and shape slightly but also the electronic nature of the molecule greatly due to the strong electronegativity of fluorine. For example, a pyrethric acid analogue in which fluorine is introduced into the double bond shows strong insecticidal activity (Martel et al., 1984). Kiyota et al. (1996) introduced fluorine at C-2 of epoxy-*β*-ionylideneacetic acid (**2**), which was as active as the parent compound of epoxy-*β*-ionylideneacetic acid. Considering these results, it is likely worthwhile to introduce a fluorine atom into ABA analogues and evaluate their activities. As in some assays ABA analogues, which can be prepared through less expensive methods than ABA, show ABA-like activities (Oritani and Yamashita, 1983; Van der Meulen et al., 1993), we tried to introduce a fluorine atom onto such ABA analogues. In this paper, we describe the synthetic method for obtaining the target molecules **3**, **5**, and the corresponding isomers **4** and **6** and the inhibitory activities of these compounds on germination and α-amylase induction or the dehydrin induction activity of **5b**, which is the most active compound in the former assays.

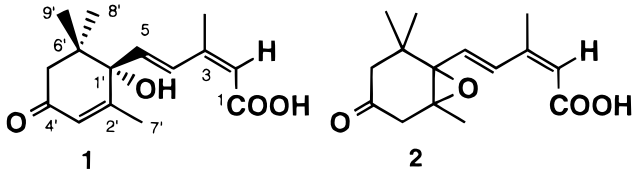

Figure 1. Structures of ABA and its active derivative.

RESULTS AND DISCUSSION

Synthesis. Modification of the cyclohexenone ring system was done by oxidation reaction. Compound **7b** (1′-hydroxy group) was synthesized by the hydrolysis of the 1′,2′-epoxy compound (**8**), which was prepared by epoxidation of *β*-ionone with *m*-chloroperoxybenzoic acid in high yields (Eschenmoser et al., 1981; Kakeya et al., 1991). Compound **7c** (4′-oxo group) was synthesized by Jones oxidation of α-ionone (**7a**) (Widmer et al., 1982).

2-Fluoroabscisic acid analogues **3a**−**c** and **4a**−**c** were prepared by the Wittig−Horner reaction from various ionone derivatives **7a**−**c** with ethyl triethylphosphono-

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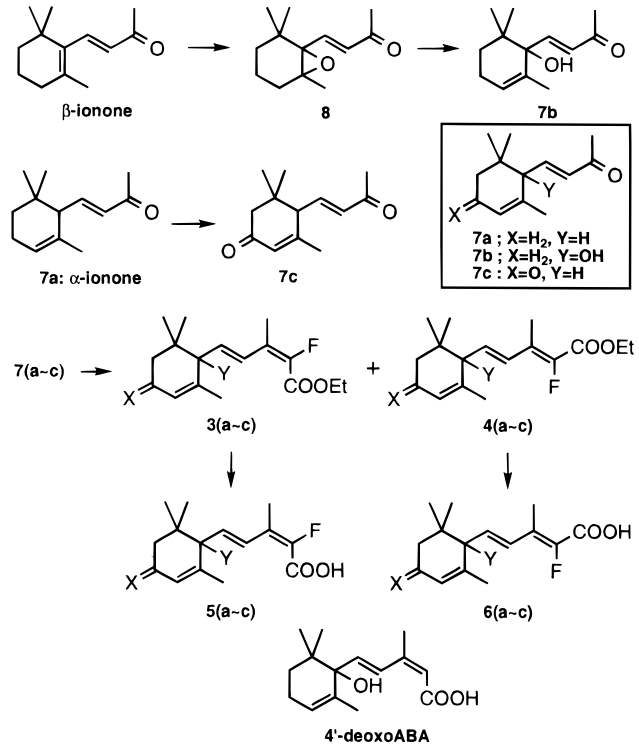

Figure 2. Synthesis of 2-fluoro-ABA analogues.

Table 1. pI_{50} Value of 2-Fluoro-ABA Analogues[a] for Inhibition of Cress Seed Germination

compd	X	Y	R	pI_{50}
3a	H_2	H	Et	4.1
3b	H_2	OH	Et	4.5
3c	O	H	Et	5.1
4a	H_2	H	Et	<3.0
5a	H_2	H	H	5.2
5b	H_2	OH	H	6.0
5c	O	H	H	4.6
6a	H_2	H	H	<3.0

[a] pI_{50} of ABA = 6.1; pI_{50} of 4′-deoxo-ABA = 5.3.

2-fluoroacetate (Pawson et al., 1979), which was prepared by the Arbusov reaction of ethyl bromofluoroacetate with triethyl phosphite. The Wittig−Horner reaction gave a 1:1 isomeric mixture of (2*E*,4*E*)-**3a**−**c** and (2*Z*,4*E*)-**4a**−**c**. These isomers were separated by silica gel column chromatography and hydrolyzed to give the acids (2*E*,4*E*)-**5a**−**c** and (2*Z*,4*E*)-**6a**−**c** (Figure 2).

Biological Activities. The biological activity of the synthesized compounds was examined by cress seed (*Lepidium sativum*) germination (Table 1), which is one of the typical bioassay systems for ABA activity. The geometry (2*Z*,4*E*) of the 2,4-pentadienoic acid moiety of ABA is essential for its hormonal activities, and when the side chain is isomerized to the 2*E*,4*E* isomer, ABA becomes inactive (Millborrow, 1970). The same relationship between the side-chain geometry of 2-F-ABA and the activity was observed in the cress seed assay; that is, the 2*E*,4*E* isomers (**3**, **5**) showed potent activity, whereas the 2*Z*,4*E* isomers (**4a**, **6a**) were inactive. This result suggests that the geometrical isomers (**4a**−**c**, **6a**−**c**) will be inactive in other assays. Therefore, the biological activities of these isomers were not examined in other assays. Ethyl ester derivatives (**3**) inhibited germination, but they were less active than acid derivatives (**5**). Among the acid derivatives, **5b** showed the highest inhibitory activity, and it was as active as ABA.

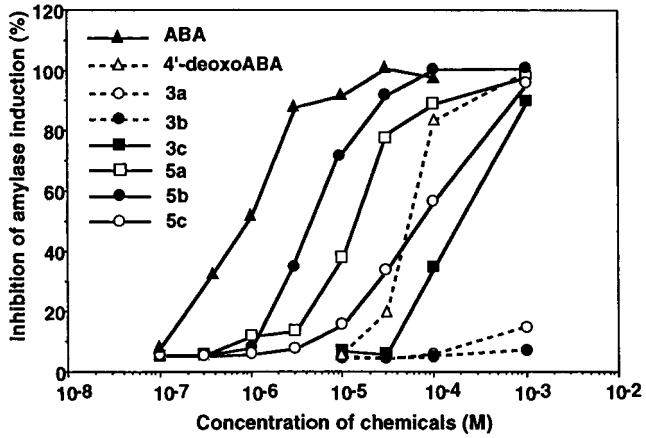

Figure 3. Effects of 2-fluoro-ABA analogues on gibberellin-inducible amylase induction.

Oritani and Yamashita (1974) reported that the parent compound of **5b**, 4′-deoxo-ABA, was far less active than ABA in inhibition of lettuce seed germination and azuki bean seedlings. Actually, in the cress test reported here, the activity of 4′-deoxo-ABA was about one-sixth that of ABA. The lesser effectiveness of 4′-deoxo-ABA compared with that of **5b** was also demonstrated in the α-amylase induction test (see results below and Figure 3). Taking these results into consideration, the reason for the potent activity of **5b** could be due to the introduction of the fluorine atom onto the 2-position of 4′-deoxo-ABA. Because a replacement of hydrogen by fluorine changes not only the electronic properties in its environment but also the lipophilicity and geometry, at present, the main reason for the effect of fluorine on this activity enhancement is not clear. No enhancement of the activity was found by the introduction of fluorine atom onto the 2-position of the side chain of other derivatives (**3a**−**c**, **5a**,**c**).

Inhibition of gibberellin-inducible α-amylase production is also a typical activity of ABA and has been used to determine whether chemicals have ABA-like activity. 2-Fluoroabscisic acid analogues (**3a**−**c**, **5a**−**c**) showed inhibition of the gibberellin-inducible α-amylase production in embryoless barley (*Hordeum vulgare* L. cv. Senbonhadaka) half-seeds (Figure 3). Ester derivatives were less active than acid derivatives, and **5b** also showed the highest inhibitory activity in the compounds tested here. The concentration of **5b** required for 50% inhibition (4×10^{-6}) of α-amylase induction in aleurone layers was ∼4 times higher than that of ABA (1×10^{-6}). The fact that the ethyl esters **3a** and **3b** are inactive and the 1′-deoxy acid ethyl ester **3c** shows some activity, at least at high concentrations, may point to the importance of lipophilicity for the activity of esters. Here, **5b** was far more active than 4′-deoxo-ABA, as was shown in the cress seed germination assay.

ABA is known to mediate rapid physiological responses to water stress such as stomatal closure (Zeevaart and Creelman, 1988) and to be involved in slower responses such as dehydrin synthesis (Chandler and Robertson, 1994). To determine whether **5b** has an ABA-like activity in plants, a "positive" biological response induced by ABA should be included. Alternatively, **5b** may inhibit steps in signal transduction between GA perception and the expression of α-amylase gene by a mechanism different from ABA. For example, okadaic acid, a protein phosphatase inhibitor, blocks GA_3-induced α-amylase production and also greatly

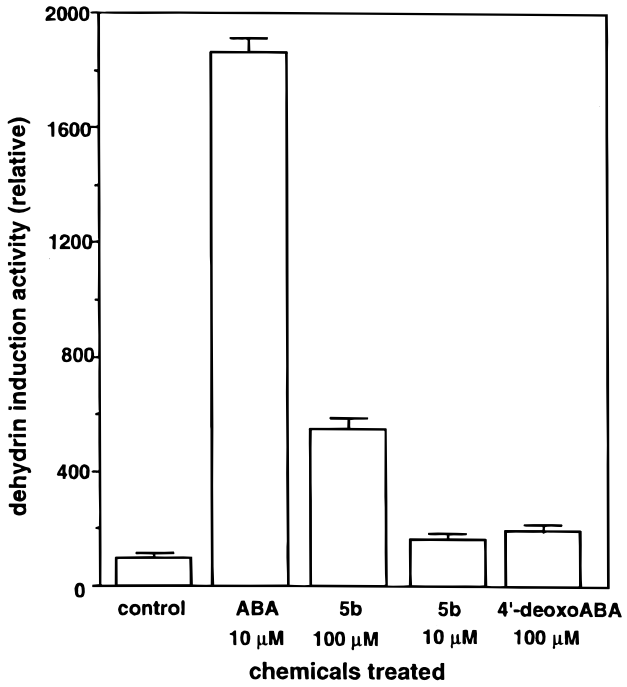

Figure 4. Activation of the dehydrin promoter by (+)-ABA and analogues. Promoter activity was measured by GUS reporter gene activity in barley aleurone protoplast transient assays. Protoplasts were treated with 10 μM (+)-ABA and **5b** and 100 μM **5b**.

reduces the accumulation of α-amylase mRNA but does not lead to the accumulation of ABA-inducible mRNA (Kuo et al., 1996). Therefore, we measured the expression of dehydrin genes (Close et al., 1989; Robertson et al., 1995) because it is up-regulated by ABA. The effect of **5b** on dehydrin gene expression was tested in aleurone protoplasts by measuring the dehydrin promoter activity in transient assays (Figure 4). ABA increased the GUS activity by ~30-fold over the control when protoplasts were treated with 10 μM ABA. **5b** also increased the GUS activity significantly but was less active than ABA even at the concentration 10 times higher than ABA. 4′-Deoxo-ABA was almost inactive at the concentration of 100 μM, suggesting that the introduction of fluorine was also effective in enhancing the activity of 4′-deoxo-ABA.

In this study, it was found that 2-F-ABA analogues mimicked ABA action in three assay systems, and one of the analogues, **5b**, showed the most potent activities. A fluorine atom at C-2 enhanced activity as shown in the cress seed germination assay, α-amylase induction assay, and dehydrin induction assay, when compared with its nonfluorinated compound, 4′-deoxo-ABA. Considering the more potent activity of **5b** relative to that of 4′-deoxo-ABA in these assay systems, the introduction of fluorine onto the side chain seems to be effective for activity exhibition. The differences in activities between ABA and **5b** vary in the assay systems: in the germination test **5b** is as active as ABA; in the α-amylase induction test the activity of **5b** is about one-fourth that of ABA; and in the dehydrin induction test the activity of **5b** is far less than one-tenth that of ABA. It can be thought that ABA receptors in different tissues could have different structural requirements for their ligand; therefore, it is worthwhile to test ABA analogues reported here in other assays for ABA activities. This would be helpful for finding a potency of these analogues for a new plant growth regulator that regulates plant growth or protects plants from environmental stresses.

MATERIALS AND METHODS

Cress Seed Germination Assay. Cress (*L. sativum*) seeds were purchased locally. The germination test following the method of Taylor and Burden (1973) was carried out on triplicates of 25 seeds for each concentration of the samples. The test seeds were placed on Toyo No. 2 filter papers (5 cm) in plastic Petri dishes (5 cm i.d., Eizai Co., Japan) and kept in the dark at 25 °C. The germination rate was counted 48 h after sowing.

α-Amylase Induction Assay. Ten embryoless barley (*H. vulgare* L. cv. Senbonhadaka) half-seeds were incubated in 4 mL of buffer (2 mM acetate and 2 mM $CaCl_2$, pH 5.4), with GA_3 (100 nM) plus ABA analogues (1, 10, or 100 μM) for 48 h at 25 °C. The activity of α-amylase secreted under these conditions was measured according to the procedure of Jones and Varner (1967).

Isolation of Barley Aleurone Protoplasts and Transient Assay. Barley seeds, *H. vulgare* cv. Himalaya, harvested in 1994 in Canberra, Australia, were used. The procedure used for the isolation of barley aleurone protoplasts was based on the previous procedure described by Lin et al. (1996) with minor modifications. Here, we incubated 10 aleurone layers in a flask with 1.5 mL of enzyme solution. After protoplasts had been isolated, pelleted protoplasts were resuspended in 1 mL of IM (0.5 M mannitol, 0.11 M glucose, 0.055 M sucrose, 14 mM L-arginine, 10 mM MES, 0.32% B5-Gamborg salt, pH 5.5) and mixed gently with 100 μg of salmon sperm DNA and 100 μg of Hv41(−935)-IGN plasmid DNA (Robertson et al., 1995). The suspension was left undisturbed for 1 min. Protoplast transfection medium (PTM) consisting of 17.3% (w/v) polyethylene glycol 6000 (PEG), 10 mM Tris-HCl, pH 9.0, 0.67 M mannitol, and 0.133 M $Ca(NO_3)_2 \cdot 4H_2O$ was filter sterilized. Three volumes of PTM was added to the protoplast suspension, mixed, and left at room temperature for 20 min with occasional swirling. Forty milliliters of IM was then added in 10 mL aliquots with 2 min between additions. The protoplasts were collected by centrifugation for 2 min at 50*g* and washed twice in 30 mL of IM. Pelleted protoplasts were resuspended in an appropriate volume (generally 15 mL from 150 grains) of IM containing 50 units/mL nystatin, 150 μg/mL cefotaxime, 20 mM $CaCl_2$, 1.5 μg/mL aprotinin, and 1.5 μg/mL leupeptin. One milliliter of the protoplast preparation was aliquoted into flasks. These samples were incubated in the dark at 25 °C for 24 h in the presence of 0 or 10 μM ABA or 100 μM **5b**, in triplicate for each sample. Fluorometric assays of GUS activity were performed in triplicate for each sample, as described by Jefferson (1988).

Chemicals. ^{1}H NMR spectra were recorded with a JEOL PMX 60 SI (60 MHz) or a Varian Gemini 200 (200 MHz), with tetramethylsilane as an internal standard. Mass spectra were recorded with a Shimadzu GC-MS QP-1000 spectrometer and HR-MS with a JEOL AX-505 mass spectrometer.

*4-(1′,2′-Epoxy-2′,6′,6′-trimethyl-2′-cyclohex-1′-yl)-3-buten-2-one (**8**).* To a solution of 9.62 g (0.05 mol) of β-ionone in 100 mL of methylene chloride being cooled in an ice bath was added 17.26 g (0.05 mol, 50% purity) of *m*-chloroperoxybenzoic acid. The reaction mixture was stirred at room temperature for 20 h and then filtered and washed with aqueous $NaHCO_3$ and then 10% sodium bisulfite. The organic phase was dried with magnesium sulfate and evaporated under reduced pressure. The residue was purified on column chromatography (silica gel, ethyl acetate/*n*-hexane = 1:4) to give 8.84 g of **8** (yield = 85%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (s, 3H), 1.14 (s, 6H), 1.38−1.95 (m, 6H), 2.28 (s, 3H), 6.28 (d, 1H, J = 15.7 Hz), 7.02 (d, 1H, J = 15.7 Hz); MS, *m/z* 208 (M^+, 5), 193 (18), 165 (13), 135 (100), 123 (100), 107 (51), 95 (52), 79 (25), 69 (30), 43 (100). Anal. Calcd (%) for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68; O, 15.36. Found: C, 74.90; H, 9.70; O, 15.40.

*4-(1′-Hydroxy-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-3-buten-2-one (**7b**).* Under N_2 gas, 25 mL (0.0625 mol of 2.5 M solution)

of *n*-butyllithium was added to a solution of 4.39 g (0.06 mol) of diethylamine in 100 mL of dry diethyl ether at −78 °C. To the reaction mixture was added 4.16 g (0.02 mol) of **8**, and the mixture was stirred for 2 h. The reaction mixture was poured into ice and extracted with 150 mL of diethyl ether, and then the organic phase was dried with magnesium sulfate and evaporated under reduced pressure. The purification of the residue on column chromatography (silica gel, ethyl acetate/*n*-hexane = 1:2) gave 0.975 g of **7b** (yield = 23%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91 (s, 3H), 1.01 (s, 3H), 1.45−1.68 (m, 2H), 1.59 (s, 3H), 2.00−2.15 (m, 3H), 2.29 (s, 3H), 5.56 (bs, 1H), 6.35 (d, 1H), 6.81 (d, 1H); MS, *m*/*z* 208 (M^+, 4), 193 (9), 181 (5), 165 (21), 152 (11), 123 (20), 109 (100), 81 (22), 43 (39). Anal. Calcd (%) for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68; O, 15.36. Found: C, 74.93; H, 9.69; O, 15.38.

*4-(4′-Oxo-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-3-buten-2-one (**7c**).* To a solution of 1.5 g (7.8 mmol) of α-ionone in 40 mL of acetone was added dropwise 265 mL (0.75 mol solution) of Jones reagent (chromic acid in diluted sulfuric acid). The reaction mixture was stirred at room temperature for 10 h and then filtered, and the solvent was evaporated under reduced pressure. The purification of the residue on column chromatography (silica gel, ethyl acetate/*n*-hexane = 1:2) gave 0.45 g of **7c** (yield = 28%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (s, 3H), 1.08 (s, 3H), 1.90 (d, 3H, J = 1.0 Hz), 2.29 (s, 3H), 2.15−2.41 (m, 2H), 2.72 (d, 1H, J = 9.5 Hz), 5.98 (s, 1H), 6.19 (d, 1H, J = 15.8 Hz), 6.68 (dd, 1H, J = 15.8 Hz, 9.5 Hz); MS, *m*/*z* 206 (M^+, 2), 191 (2), 164 (2), 150 (44), 135 (6), 108 (100), 43 (100). Anal. Calcd (%) for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79; O, 15.51. Found: C, 75.73; H, 8.80; O, 15.46.

General Procedure for Wittig−Horner Reaction and Separation of Isomers. To a solution of 1.33 g (5.5 mmol) of ethyl triethylphosphono-2-fluoroacetate in 50 mL of anhydrous tetrahydrofuran was added 165 mg (5.5 mmol of 80% dispersion in mineral oil) of sodium hydride under a flow of nitrogen. After the mixture had been stirred at room temperature for 1 h, 5 mmol of **7a**−**c** was added and then refluxed for 12 h. The reaction mixture was poured into crushed ice and extracted with ether, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. Column chromatography (silica gel, ethyl acetate/*n*-hexane = 1:4) of the residue gave **3a**−**c** (2*E*,4*E*) and **4a**−**c** (2*Z*,4*E*).

*(2E,4E)-Ethyl 2-fluoro-3-methyl-5-(2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-2,4-pentadienoate (**3a**):* yield = 37%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.92 (s, 3H), 1.15−1.45 (m, 2H), 1.35 (t, 3H), 1.58 (d, 3H), 1.90−2.10 (m, 2H), 1.98 (d, 3H), 2.29 (d, 1H), 4.31 (q, 2H), 5.45 (s, 1H), 5.89 (dd, 1H), 7.29 (d, 1H); MS, *m*/*z* (relative intensity) 280 (M^+, 5), 224 (15), 175 (5), 157 (100), 144 (40), 131 (30), 107 (5); HR-MS, *m*/*z* 280.1903, calcd for $C_{17}H_{25}FO_2$, 280.1838.

*(2E,4E)-Ethyl 2-fluoro-5-(1′-Hydroxy-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-3-methyl-2,4-pentadienoate (**3b**):* yield = 38%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (s, 3H), 0.99 (s, 3H), 1.35 (t, 3H), 1.61 (s, 3H), 1.41−1.71 (m, 2H), 1.98 (d, 3H, J = 4.4 Hz), 1.80−2.08 (m, 3H), 4.29 (q, 2H), 5.55 (bs, 1H), 6.07 (d, 1H, J = 16 Hz), 7.45 (d, 1H, J = 16 Hz); MS, *m*/*z* (relative intensity) 296 (M^+, 15), 279 (100), 233 (14), 181 (11), 165 (25), 147 (85), 139 (23), 123 (13), 109 (17), 91 (12), 55 (13), 43 (25); HR-MS, *m*/*z* 296.1763, calcd for $C_{17}H_{25}FO_3$, 296.1788.

*(2E,4E)-Ethyl 2-fluoro-3-methyl-5-(4′-oxo-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-2,4-pentadienoate (**3c**):* yield = 36%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.02 (s, 3H), 1.33 (t, 3H), 1.95 (s, 3H), 1.97 (s, 3H), 2.05−2.40 (m, 2H), 2.67 (d, 1H), 4.32 (q, 2H), 5.94−6.12 (m, 2H), 7.50 (d, 1H); MS, *m*/*z* (relative intensity) 294 (M^+, 13) 296 (M^+, 15), 277 (100), 165 (25), 147 (85), 139 (23), 123 (17), 109 (21), 91 (15), 55 (17), 43 (35); HR-MS, *m*/*z* 294.1675, calcd for $C_{17}H_{23}FO_3$, 294.1631.

*(2Z,4E)-Ethyl 2-fluoro-3-methyl-5-(2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-2,4-pentadienoate (**4a**):* yield = 39%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.92 (s, 3H), 1.15−1.45 (m, 2H), 1.35 (t, 3H), 1.58 (d, 3H), 1.90−2.10 (m, 2H), 2.23 (d, 3H, J = 3.2 Hz), 2.30 (d, 1H), 4.31 (q, 2H), 5.45 (s, 1H), 5.89 (dd, 1H, J = 15.5, 9.5 Hz), 6.59 (d, 1H, J = 15.7 Hz); MS, *m*/*z* (relative intensity) 280 (M^+, 12), 224 (37), 175 (15), 157 (100); HR-MS, *m*/*z* 296.1773, calcd for $C_{17}H_{25}FO_2$, 280.1838.

General Procedure of Hydrolysis. A mixture of 0.75 mmol of ethyl ester **3a**−**c** or **4a**−**c** and 0.41 g of potassium hydroxide in 5 mL of ethanol and 5 mL of water was refluxed for 1 h. Ethanol was then evaporated under reduced pressure. The residue was acidified with 2 N HCl solution and then extracted with ethyl acetate three times. The organic phase was washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. Column chromatography (silica gel, ethyl acetate/*n*-hexane = 1:4) of the residue gave **5a**−**c** (2*E*,4*E*) and **6a**−**c** (2*Z*,4*E*).

*(2E,4E)-2-Fluoro-3-methyl-5-(2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-2,4-pentadienoic acid (**5a**):* yield = 78%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.91 (s, 3H), 1.15−1.50 (m, 2H), 1.57 (d, 3H, J = 1.5 Hz), 1.90−2.10 (m, 2H), 2.00 (d, 3H, J = 3 Hz), 2.29 (d, 1H, J = 9.5 Hz), 5.44 (s, 1H), 5.93 (dd, 1H, J = 15.6, 9.5 Hz), 7.27 (d, 1H), 9.2 (bs, 1H); MS, *m*/*z* (relative intensity) 252 (M^+, 4), 237 (3), 196 (50), 176 (34), 163 (18), 151 (48), 129 (100), 122 (29), 117 (10), 107 (30); HR-MS, *m*/*z* 252.1557, calcd for $C_{15}H_{21}FO_2$, 252.1525.

*(2E,4E)-2-Fluoro-5-(1′-hydroxy-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-3-methyl-2,4-pentadienoic acid (**5b**):* yield = 79%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (s, 3H), 1.01 (s, 3H), 1.41−1.72 (m, 2H), 1.68 (s, 3H), 1.99 (d, 3H), 1.80−2.10 (m, 3H), 5.61 (bs, 1H), 6.05 (d, 1H), 7.55 (d, 1H), 9.8 (bs, 1H); MS, *m*/*z* (relative intensity) 268 (M^+, 5), 251 (12), 194 (5), 169 (3), 149 (5), 135 (9), 122 (100), 107 (60), 45 (20); HR-MS, *m*/*z* 268.1522, calcd for $C_{15}H_{21}FO_3$, 268.1475.

*(2E,4E)-2-Fluoro-3-methyl-5-(4′-oxo-2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-2,4-pentadienoic acid (**5c**):* yield = 81%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (s, 3H), 1.07 (s, 3H), 1.93 (d, 3H, J = 1.2 Hz), 2.02 (d, 3H, J = 4.4 Hz), 2.37−2.45 (m, 2H), 2.75 (d, 1H, J = 9.5 Hz), 5.87−6.05 (m, 2H), 7.48 (d, 1H, J = 15.7 Hz), 9.9 (bs, 1H); MS, *m*/*z* (relative intensity) 266 (M^+, 28), 210 (17), 190 (100), 164 (19), 138 (42), 124 (21); HR-MS, *m*/*z* 266.1357, calcd for $C_{15}H_{19}FO_3$, 266.1318.

*(2Z,4E)-2-Fluoro-3-methyl-5-(2′,6′,6′-trimethyl-2′-cyclohexen-1′-yl)-2,4-pentadienoic acid (**6a**):* yield = 75%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.83 (s, 3H), 0.93 (s, 3H), 1.20−1.51 (m, 2H), 1.58 (d, 3H, J = 1.5 Hz), 1.87−2.10 (m, 2H), 2.23 (d, 3H, J = 3 Hz), 2.31 (d, 1H, J = 9.6 Hz), 5.47 (s, 1H), 5.99 (dd, 1H, J = 15.7 Hz, 9.7 Hz), 6.63 (d, 1H, J = 15.6 Hz), 9.80 (bs, 1H); MS, *m*/*z* (relative intensity) 252 (M^+, 13), 237 (8), 196 (72), 176 (58), 151 (100), 131 (55), 129 (41), 109 (30), 91 (58), 77 (44); HR-MS, *m*/*z* 252.1491, calcd for $C_{15}H_{21}FO_2$, 252.1525.

ABBREVIATIONS USED

ABA, abscisic acid; GA, gibberellin; GUS, β-glucuronidase.

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