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2-Fluoro and 2-Methoxycarbonyl Epoxy-β-ionylideneacetic Acids as Abscisic Acid Analogs

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Abscisic acid (ABA) is easily isomerized to inactive *trans*-ABA by light. To solve this problem, two variations of epoxy-β-ionylideneacetic acid were synthesized as ABA analogs, each of them having a methoxycarbonyl or a fluoric substituent at the 2-position. The 2*E*-, and 2*Z*-fluorinated analogs showed moderate growth inhibitory activity toward rice seedlings and lettuce seeds, whereas the methoxycarbonyl analog was inactive toward rice seedling growth and only partially active toward lettuce germination. The 2*E*-fluorinated analog was extensively isomerized to the 2*Z*-isomer by UV irradiation. We think that a steric requisite for the 2*E*-position was high, and that the fluorine substituent was not effective for fixing the 2-double bond in the *E*-configuration.

Key words: abscisic acid; epoxy-β-ionylidenefluoroacetate; epoxy-β-ionylidenemalonate; photoisomerization; seed germination

Abscisic acid (ABA; **1**, Fig. 1) is a plant hormone with particular activity for promoting senescence and inhibiting growth.[1] However, ready isomerization of the 2*Z*-double bond by light into *trans*-ABA (**2**) results in a significant loss of activity.[2] So far, only a few analogs have been synthesized to solve this problem. Ohkuma[3] has reported the synthesis of a lactonic analog (**3**) which was inactive. Chen and MacTaggart,[4] and Kim *et al.*[5] introduced a benzene ring with a variety of substituents to the side chain, producing such compounds as **4**[4] and **5**[5] which were moderately active.

Tamura and Nagao,[6] and Oritani and Yamashita[7,8] have synthesized an effective and facile ABA analog, epoxy-β-ionylideneacetic acid [(+)-**6**], which was metabolized to give ABA *via* xanthoxin acid (**7**) in plants[9] and the fungus, *Cercospora cruenta*.[10] To develop more effective ABA analogs, we designed modified racemic epoxy-β-ionylideneacetic acids with different substituents at the 2-position (Fig. 2): one with geminal carboxylic groups (**8**) and its dimethyl ester (**9**), and two others with a fluorine atom (**10** and **11**). As the former two compounds (**8** and **9**) both had a geminal structure, the 2*Z*-carboxylic moiety essential to ABA activity was retained in case of isomerization of the 2-double bond. We designed the latter compounds (**10**, with **11** given as a byproduct) to study the effects of the fluoric substituent on biological activity and photostability. Fluorine seemed to act as a hydrogen mimic, the covalent bond length with carbon and the van der Waals radius of fluorine being very similar to those of hydrogen; however, the bond energy with carbon and the electronegativity of fluorine are much larger than those of hydrogen.[11] So far, many vinyl fluorides have been synthesized to modify biological activity.[12–19] In particular, Martel *et al.* have reported that pyrethric acid analog **12** was very photostable and had strong insecticidal, acaricidal, and nematocidal activity.[19] In addition, thermodynamic and photochemical studies on stilbene and α,β-difluorostilbene revealed that the thermal equilibrium constant (in a cyclohexane solution at 27°C, $K=[Z]/[E]$) for α,β-difluorostilbene ($K=0.067$) was considerably larger than that of stilbene ($K=0.002$), while the Z/E ratio in the photostationary state ($\lambda_{irr}=313$ nm) for α,β-difluorostilbene (80/20) was little different from that of stilbene (93/7).[20] From these results, we thought that the 2-double bond of **10** could be stabilized.

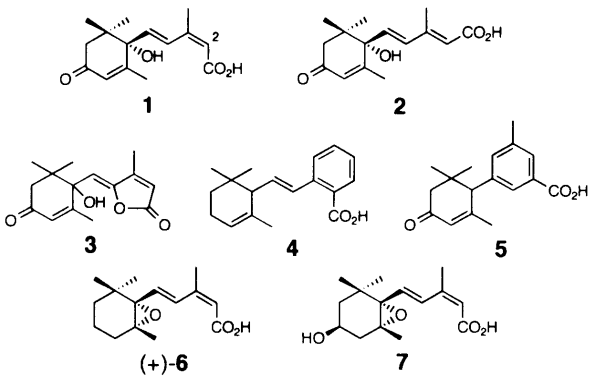

Fig. 1. Abscisic Acid-related Compounds.

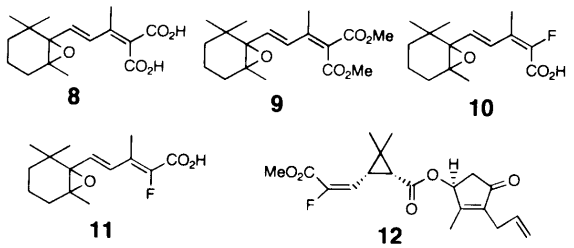

Fig. 2. Synthetic Targets (**8**–**11**) and Reported Photostable Vinyl Fluoride **12**.

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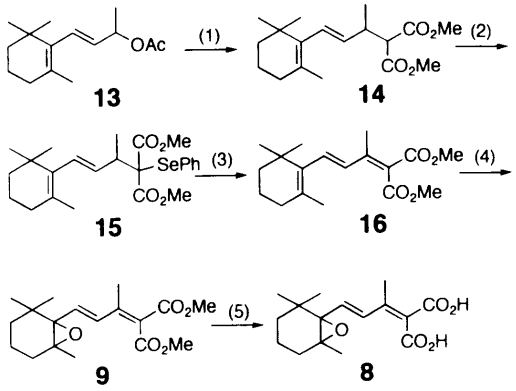
In this paper, the synthesis and plant growth inhibitory activity (toward rice seedlings and lettuce seeds) of the title compounds are described. The results of a photostability test on the fluorinated analogs are also reported.

Synthesis

As shown in Scheme 1, our synthesis of **8** and **9** started from β-ionyl acetate (**13**). A palladium-catalyzed coupling reaction[21] with dimethyl 2-sodiomalonate was quite successful to give diester **14**. Dehydrogenation of **14** was not effective: oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) only resulted in the recovery of the starting material, and bromination-dehydrobromination gave a complex mixture. On the other hand, treatment of the lithium enolate of **14** with phenylselenenyl bromide gave corresponding phenylselenenide **15** in a 90.1% yield. Conjugated trienediester **16** was obtained by an oxidation-elimination reaction on **15**. Treatment of **16** with *m*-chloroperbenzoic acid (MCPBA) resulted in selective epoxidation of the double bond in the cyclohexene ring[6] to give **9** in a 95.0% yield. Alkaline hydrolysis of **9** by using refluxing KOH–MeOH conditions gave a mixture of dicarboxylic acid **8** and decarboxylated monocarboxylic acids. Our final choice of the conditions for this step was to use $Ba(OH)_2$ in boiling water.[22] The monobarium salt was precipitated during the reaction, and dicarboxylic acid **8** was released by acidification. The overall yield of **8** from β-ionyl acetate (**13**) was 75.2% in 6 steps. However, compound **8** gradually decomposed at ambient temperature, even under nitrogen, so the biological activities were evaluated only for diester **9**.

The fluorinated analogs were synthesized as shown in Scheme 2. The starting material was a known compound (**18**),[23] which had been prepared by the Wittig–Horner reaction of diethyl (ethoxycarbonylfluoromethyl)phosphonate with β-ionone (**17**). The *E*/*Z* ratio of **18** was 6/4, which is equal to that in the literature.[23] These geometric isomers were separated after epoxidation of the double bond in the cyclohexene ring. The geometry of the 2-position was determined by the chemical shifts of the ^1H- and ^{19}F-NMR spectra: the 3-Me signals of **20** were shifted downfield ($\delta = 2.23$ ppm), while those of **19** were at 2.00 ppm[12–14]; the ^{19}F signal of **20** was at -126.1 ppm, and that of **19** was at -122.7 ppm.[13] Each of esters **19** and **20** was hydrolyzed to respectively give corresponding acids **10** and **11**.

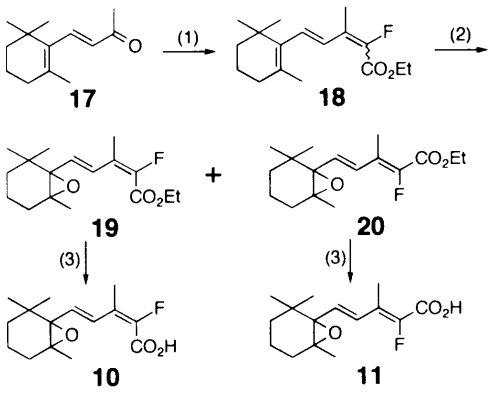


Scheme 1. Syntheses of Geminal Disubstituted Analogs **8** and **9**.

Reagents: 1) PPh_3, $Pd(PPh_3)_4$, THF, $NaCH(CO_2Me)_2$ (85.8%; 98.8%, taking into account recovered **13**). 2) a. $LiN(iso\text{-}Pr)_2$, THF; b. PhSeBr (90.1%). 3) MCPBA, $NaHCO_3$, CH_2Cl_2 (98.3%). 4) MCPBA, $NaHCO_3$, CH_2Cl_2 (95.0%). 5) a. $Ba(OH)_2$, H_2O, reflux; b. 2 M aq. HCl (90.5%).

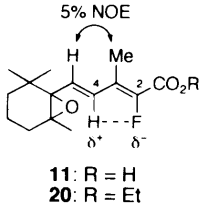
Photostability

The 2-double bonds of analogs **10** and **11** were not isomerized under fluorescent light during the biological tests; this was confirmed by analyzing samples which had been recovered from the test media. Therefore, we investigated the effect of the fluoric substituent on the photostability of the double bond under stronger conditions.[7] Three compounds [**10**, **11**, and (±)-**6**] were respectively dissolved in ethyl acetate and irradiated by a high-pressure mercury lamp for 6 h. The *E*/*Z* ratio of each product was determined by an HPLC analysis of the corresponding methyl ester. Parent compound **6** was partially isomerized: the *Z*/*E* ratio was 65/35. It has been reported that irradiating 2*E*(*trans*)-**6** gave a 50/50 mixture of 2*EZ*-**6**.[7] On the other hand, **10** (*E*-form) was extensively isomerized (*E*/*Z* = 5/95, 4.4% of an unidentified compound was formed; the 2*E*-configuration of **10** corresponded to the 2*Z*-configuration of ABA), while **11** (*Z*-form) was only slightly isomerized (*E*/*Z* = 0.5/99.5). Our result indicates that the fluorine substituent strongly enhanced the *Z*-orientation of the 2-double bond and did not fix the *E*(*cis*)-structure that is essential to ABA activity. We assume that the reason for the high stability and high orientation of **11** (*Z*-form) was hydrogen bonding between the electronegative fluorine and the slightly electropositive hydrogen at the 4-position (Fig. 3). This assumption is supported by the unusual result for the conformational analysis of 1-fluoropropane[24]: the *gauche*-form was more stable than the *anti*-form because of attractive electrostatic interaction between the negative fluorine and the rather positive carbon chain. The *s*-*trans*-conformation of this compound was demon-



Scheme. 2. Syntheses of Fluorinated Analogs **10** and **11**.

Reagents: 1) NaH, $(EtO)_2P(O)CHFCO_2Et$, Et_2O (38.3%; 56.6%, taking into account recovered **17**). 2) MCPBA, $NaHCO_3$, CH_2Cl_2 (54.0% of **19** and 36.4% of **20**). 3) KOH, MeOH (73.0% for **10**, 78.4% for **11**).



11: R = H
20: R = Et

Fig. 3. Presumed Electron-attracting Effect between F-2 and H-4 of Compound **11**.

strated by $\{^1H\}$-^1H NOE difference spectroscopy of corresponding ester **20**. NOE (5%) was observed between 3-Me and 5-H. The contribution of the vinylic fluorine substituent to the stability of the double bond is not clear from our result.

Biological activities

The biological activities of synthetic compounds **9**, **10**, and **11** were assessed on rice seedling growth and lettuce germination, parent compound (±)-**6** being used as a reference.[6,7] The activity of the methyl ester of (±)-**6** was the same as that of (±)-**6** on lettuce,[7] and stronger than that of (±)-**6** on rice.[6] As already mentioned, the 2-double bonds of analogs **10** and **11** were not isomerized in the media during the biological tests.

Table I shows the results of the test on lettuce germination. 2*E*-Fluorinated analog **10** was comparatively as active as parent compound (±)-**6**. Interestingly, 2*Z*-fluorinated analog **11** and geminal diester analog **9** showed moderate activity. We think that the electronegative fluorine atom acted as a carboxyl group mimic. We also think that the 2*E*-carboxyl (or methoxycarbonyl) group did not disturb very much the interaction with the receptor region of lettuce. The results for the rice seedling test are shown in Table II. Geminal diester analog **9** was inactive; thus, the 2*E*-methoxycarbonyl substituent strongly inhibited the spatial or electrical interaction with the receptor region of rice. The activity of both of fluorinated analogs **10** and **11** was comparable; however, both were about 10 times less potent than (±)-**6**. We think that, as in the lettuce assay, the electronegative fluorine atom partly acted as a carboxyl group mimic, and that the 2*E*-carboxyl group disturbed the interaction less potently than the 2*E*-methoxycarbonyl group did. Another possibility is that these analogs were not converted to ABA-type metabolites in rice seedlings. It remains unknown whether the reduced activity was due to any delay of metabolism to ABA-type or to the unacceptable nature of the corresponding ABA-type metabolites to the ABA receptor in both plants.

Table I. Growth Inhibitory Activity of the Epoxy-β-ionylideneacetic Acid Analogs toward Lettuce Germination (*Lactuca sativa* L. cv. Green Lake 2B61)

Compounds	Percentage of control germination ratio				
	10^{-3}	5×10^{-4}	10^{-4}	5×10^{-5}	10^{-5} (mol/liter)
9	11	22	85	86	100
10	0	0	13	36	67
11	0	3	60	73	87
(±)-**6**			7	30	

The germination ratio was counted (control = 80%) after the lettuce seeds had been incubated for 6 days at 27 °C (1500 lux).

Table II. Growth Inhibitory Activity of the Epoxy-β-ionylideneacetic Acid Analogs toward Rice Seedlings (*Oryza sativa* L. cv. Satohonami)

Compounds	Percentage of control growth				
	10^{-3}	5×10^{-4}	10^{-4}	5×10^{-5}	10^{-5} (mol/liter)
9	105	94	97	107	100
10	46	56	79	95	88
11	48	74	90	99	86
(±)-**6**			41		72

The length of the second leaf sheath of the rice seedlings was measured (control = 28.5 mm) after they had been incubated for 4 days at 27 °C (1700 lux).

Experimental

IR spectra were recorded with a JASCO IR-810 spectrometer. ^1H- and ^{13}C-NMR spectra were recorded with a JEOL JNM GSX-270 spectrometer (270 MHz for ^1H and 68 MHz for ^{13}C) in $CDCl_3$ unless otherwise noted, using tetramethylsilane as an internal standard. $\{^1H\}$-^1H NOE difference spectrum was recorded with a JEOL JNM GSX-400 spectrometer (400 MHz) in $CDCl_3$, while ^{19}F-NMR spectra were recorded with the same instrument (376 MHz) in $CDCl_3$ at 27 °C, using $CFCl_3$ as an internal standard. Refractive indices were measured by a Hitachi PRA-B refractometer, and HPLC analyses were performed with a Hitachi L-6000 pump and an L-4200 UV-VIS detector. Melting point (mp) and boiling point (bp) values are uncorrected.

Dimethyl (±)-(E)-2-methyl-4-(2',6',6'-trimethyl-1'-cyclohexenyl)-3-butene-1,1-dicarboxylate (**14**). A solution of (±)-β-ionyl acetate (**13**, 1.00 g, 4.23 mmol), triphenylphosphine (0.10 g, 0.38 mmol) and tetrakis(triphenylphosphine)palladium (0.16 g, 0.14 mmol) in dry tetrahydrofuran (THF, 8 ml) was stirred for 15 min at room temperature under nitrogen. To this was added a solution of dimethyl 2-sodiomalonate, which had been prepared from sodium hydride (*ca.* 60% oil dispersion, 0.56 g, *ca.* 14 mmol) and dimethyl malonate (1.94 g, 14.7 mmol), in dry THF (24 ml). The resulting dark yellow suspension was refluxed for 24 h. The reaction mixture was pured into water, and the aqueous layer was separated and extracted with diethyl ether. The combined organic layers were successively washed with a satd. aq. $NaHCO_3$ solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (50 g, hexane/EtOAc = 40 : 1 10 : 1) to give 0.130 g (13.0%) of unreacted **13** and 1.12 g of **14** (85.8%; 98.8%, taking into account recovered **13**). An analytical sample was distilled to give a colorless oil, bp 121 125 °C at 0.3 mmHg and $n_D^{31}=1.4823$. IR ν_{max} (film) cm^{-1}: 1760 (s, C=O), 1740 (s, C=O), 1435 (s), 1240 (s), 1020 (m), 970 (m, *E*-HC=CH). ^1H-NMR δ: 0.93 (3H, s, 6'-Me), 0.95 (3H, s, 6'-Me), 1.13 (3H, d, J=6.6 Hz, 2-Me), 1.38 1.45 (2H, m), 1.52 1.63 (2H, m), 1.61 (3H, s, 2'-Me), 1.94 (2H, pseudo t, J=6.1 Hz, 3'-H), 2.95 3.10 (1H, m, 2-H), 3.35 (1H, d, J=9.3 Hz, 1-H), 3.70 (3H, s, OMe), 3.74 (3H, s, OMe), 5.28 (1H, dd, J=8.5, 15.9 Hz, 3-H), 5.94 (1H, pseudo d, J=15.9 Hz, 4-H). *Anal.* Found: C, 69.85; H, 9.01%. Calcd. for $C_{18}H_{28}O_4$: C, 70.09; H, 9.15%.

Dimethyl (±)-(E)-2-methyl-4-(2',6',6'-trimethyl-1'-cyclohexenyl)-1-phenylselenenyl-3-butene-1,1-dicarboxylate (**15**). A solution of **14** (0.480 g, 1.56 mmol) in dry THF (5 ml) was added dropwise to a stirred solution of lithium diisopropylamide [prepared from diisopropylamine (0.19 g, 1.8 mmol) and *n*-butyllithium in hexane (1.6 M, 1.2 ml, 1.0 mmol)] in dry THF (10 ml) at −78 °C under nitrogen, stirring being continued for 1 h. To this was added phenylselenenyl bromide (0.55 g, 2.30 mmol) in dry THF (5 ml), and the reaction mixture was stirred for 12 h, during which time it was allowed to warm to room temperature. The reaction was quenched with a satd. aq. NH_4Cl solution. The aqueous layer was separated and extracted with diethyl ether, while the combined organic layers were successively washed with a satd. aq. $NaHCO_3$ solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (30 g, hexane/diethyl ether = 30 : 1 8 : 1) to give 0.650 g of **15** (90.1%) as a pale yellow oil, $n_D^{20}=1.5538$. IR ν_{max} (film) cm^{-1}: 1730 (s, C=O), 1440 (m), 1250 (s), 1240 (s), 975 (w, *E*-HC=CH), 740 (m, Ph), 695 (m, Ph). ^1H-NMR δ: 0.96 (3H, s, 6'-Me), 0.98 (3H, s, 6'-Me), 1.29 (3H, d, J=7.1 Hz, 2-Me), 1.4 1.5 (2H, m), 1.5 1.65 (2H, m), 1.66 (3H, s, 2'-Me), 1.96 (2H, t, J=6.1 Hz, 3'-H), 2.97 (1H, d of quartet, J=8.6, 7.1 Hz, 2-H), 3.66 (3H, s, OMe), 3.67 (3H, s, OMe), 5.50 (1H, dd, J=8.6, 15.9 Hz, 3-H), 5.92 (1H, pseudo d, J=15.9 Hz, 4-H), 7.3 7.6 (3H, m, arom. H), 7.6 7.7 (2H, m, arom. H). *Anal.* Found: C, 61.99; H, 7.02%. Calcd. for $C_{24}H_{32}O_4Se$: C, 62.19; H, 6.96%.

Dimethyl (E)-2-methyl-4-(2',6',6'-trimethyl-1'-cyclohexenyl)-1,3-butadiene-1,1-dicarboxylate (**16**). To a solution of **15** (0.770 g, 1.66 mmol) and $NaHCO_3$ (210 mg, 2.5 mmol) in CH_2Cl_2 (8 ml) was added MCPBA (*ca.*

70%, 420 mg, *ca.* 1.7 mmol) at 0 °C, and the mixture was stirred for 3 h at this temperature. The reaction mixture was filtered through a Celite pad, the filtrate being successively washed with a satd. aq. $Na_2S_2O_3$ solution, water, satd. aq. $NaHCO_3$ solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed twice on SiO_2 (50 g, hexane/diethyl ether = 60 : 1–10 : 1) to give 0.500 g of **16** (98.3%). An analytical sample was distilled to give a pale yellow oil, bp 135–138 °C at 2.0 mmHg and $n_D^{25} = 1.5437$. IR ν_{max} (film) cm^{-1}: 1720 (s, C=O), 1585 (m, C=C), 1220 (s, C–O–C), 1060 (m, C–O–C). ^{1}H-NMR δ: 1.05 (6H, s, 6′-Me × 2), 1.4–1.5 (2H, m), 1.55–1.7 (2H, m), 1.74 (3H, d, J = 0.7 Hz, 2′-Me), 2.05 (2H, pseudo t, J = 6.0 Hz, 3′-H), 2.23 (3H, s, 2-Me), 3.79 (3H, s, OMe), 3.80 (3H, s, OMe), 6.73 (1H, pseudo dd, J = 0.7, 16.1 Hz), 6.84 (1H, d, J = 16.1 Hz). *Anal.* Found: C, 69.97; H, 8.62%. Calcd. for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55%.

Dimethyl (±)-*(E)-4-(1′,2′-epoxy-2′,6′,6′-trimethylcyclohexyl)-2-methyl-1,3-butadiene-1,1-dicarboxylate* (**9**). To a solution of **16** (0.130 g, 0.424 mmol) and $NaHCO_3$ (100 mg, 1.19 mmol) in CH_2Cl_2 (2 ml) was added MCPBA (*ca.* 80%, 130 mg, *ca.* 0.60 mmol) at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was then filtered through a Celite pad, the filtrate being washed with a satd. aq. $Na_2S_2O_3$ solution, water, satd. aq. $NaHCO_3$ solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (20 g, hexane/EtOAc = 20 : 1) to give 130 mg of **9** (95.0%) as a colorless oil, $n_D^{29} = 1.5130$. IR ν_{max} (film) cm^{-1}: 1740 (s, C=O), 1725 (m, C=O), 1630 (m), 1590 (m), 1220 (s, C–O–C), 1060 (m, C–O–C). ^{1}H-NMR δ: 0.94 (3H, s, 2′-Me), 1.0–1.1 (1H, m), 1.11 (3H, s, 6′-Me), 1.15 (3H, s, 6′-Me), 1.35–1.55 (3H, m), 1.7–1.8 (1H, m), 1.85–2.0 (1H, m), 2.21 (3H, s, 2-Me), 3.79 (3H, s, OMe), 3.80 (3H, s, OMe), 6.43 (1H, d, J = 15.5 Hz, 4-H), 6.79 (1H, d, J = 15.5 Hz, 3-H). *Anal.* Found: C, 66.76; H, 8.27%. Calcd. for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13%.

(±)-(E)-4-(1′,2′-Epoxy-2′,6′,6′-trimethylcyclohexyl)-2-methyl-1,3-butadiene-1,1-dicarboxylic acid (**8**). A suspension of **9** (506 mg, 1.57 mmol) and $Ba(OH)_2 \cdot 8H_2O$ (630 mg, 2.00 mmol) in water (6 ml) was stirred for 6 h at refluxing temperature. The monobarium salt of this dicarboxylic acid was formed as a white precipitate during the reaction. This precipitate was collected by filtration, and washed twice with hot water and three times with hexane–diethyl ether (1 : 1) to give barium dicarboxylate dihydrate (675 mg, 1.45 mmol, 92.3%). *Anal.* Found: C, 41.73; H, 5.08%. Calcd. for $C_{16}H_{24}O_7Ba$: C, 41.27; H, 5.19%.

The foregoing barium salt (245 mg, 0.526 mmol) was dissolved in diethyl ether (4 ml), and the solution acidified with 2 M aq. HCl (2 ml). The organic layer was separated, and the aqueous layer was extracted twice with diethyl ether and once with EtOAc. The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give **8** (152 mg, 0.516 mmol, 98.1%) as a pale yellow viscous oil. IR ν_{max} (nujol) cm^{-1}: 3700–2200 (s, O–H), 1700 (s, C=O), 1685 (s, C=O), 1590 (m, C=C), 1240 (s), 935 (w), 915 (w). ^{1}H-NMR (acetone-d_6) δ: 0.84 (3H, s, 2′-Me), 1.1–1.6 (3H, m), 1.13 (3H, s, 6′-Me), 1.25 (3H, s, 6′-Me), 1.65–2.05 (3H, m), 2.24 (3H, s, 2-Me), 3.4–3.7 (2H, br. s, COOH), 6.93 (1H, d, J = 16.0 Hz, 4-H), 7.12 (1H, d, J = 16.0 Hz, 3-H).

Ethyl (2EZ,4E)-2-fluoro-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexenyl)-2,4-pentadienoate (**18**). To a suspension of sodium hydride (*ca.* 60% oil dispersion, 495 mg, *ca.* 12.4 mmol) in dry diethyl ether (12 ml) was added diethyl (ethoxycarbonylfluoromethyl)phosphonate (3.00 g, 12.4 mmol) at 0 °C, and the mixture was stirred for 4 h at this temperature. After the evolution of hydrogen had subsided, β-ionone (**17**, 2.38 g, 12.4 mmol) was added, and the mixture was stirred for more 5 h at refluxing temperature. The reaction mixture was then poured into water, the organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layers were successively washed with 2 M aq. HCl, satd. aq. $NaHCO_3$ solution, water and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (50 g, hexane/EtOAc = 30 : 1–10 : 1) to give **18** and β-ionone (780 mg, 32.3%). Compound **18** was further distilled to give 1.33 g (38.3%) of a yellow oil, bp 120–128 °C at 1.0 mmHg. IR ν_{max} (film) cm^{-1}: 1720 (s, C=O), 1620 (m, C=C), 1245 (s, C–O–C), 1060 (m, C–O–C), 970 (m), 765 (m). ^{1}H-NMR δ: 1.03 (3H, s, 6′-Me), 1.05 (3H, s, 6′-Me), 1.346 (1.8H, t, J = 7.1 Hz, *E*-CH_2CH_3), 1.352 (1.2H, t, J = 7.1 Hz, *Z*-CH_2CH_3), 1.4–1.5 (2H, m), 1.55–1.7 (2H, m), 1.72 (1.2H, d, J = 0.7 Hz, *Z*-2′-Me), 1.75 (1.8H, d, J = 0.5 Hz, *E*-2′-Me), 2.0–2.05 (2H, m), 2.03 (1.8H, d, $^4J_{CF}$ = 4.4 Hz, *E*-3′-Me), 2.26 (1.2H, d, $^4J_{CF}$ = 3.2 Hz, *Z*-3′-Me), 4.30 (3H, quartet, CH_2CH_3), 6.52 (2H, br. d, J = 16.3 Hz, *Z*-4-H and *E*-5-H), 6.63 (0.4H, dd, J = 16.3, 1.5 Hz, *Z*-5-H), 7.36 (0.6H, dd, J = 16.3, 1.7 Hz, *E*-4-H). The *E*/*Z* ratio (6/4) was equal to that quoted in the literature.[23]

Ethyl (±)-*(2E,4E)-5-(1′,2′-epoxy-2′,6′,6′-trimethyl-1′-cyclohexenyl)-2-fluoro-3-methyl-2,4-pentadienoate* (**19**) *and its* (±)-*(2Z,4E)-isomer* (**20**). To a solution of **18** (0.700 g, 2.50 mmol) and $NaHCO_3$ (400 mg, 4.8 mmol) in CH_2Cl_2 (8 ml) was added MCPBA (*ca.* 80%, 650 mg, *ca.* 3.0 mmol) at 0 °C, and this mixture was stirred for 5 h at room temperature. The reaction mixture was filtered through a Celite pad. The filtrate was diluted with diethyl ether, successively washed with a satd. aq. $Na_2S_2O_3$ solution, water, satd. aq. $NaHCO_3$ solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (80 g, hexane/diethyl ether = 60 : 1–30 : 1), the first elution giving 270 mg of **20** (36.4%) as colorless prisms, mp 56–58 °C (*N,N*-dimethylformamide/water). IR ν_{max} (nujol) cm^{-1}: 1715 (s, C=O), 1625 (m, C=C), 1610 (m, C=C), 1320 (s), 1300 (s), 1240 (s, C–O–C), 1125 (m), 1055 (m, C–O–C), 985 (w), 895 (m), 765 (m). ^{1}H-NMR δ: 0.95 (3H, s, 2′-Me), 1.07 (1H, m), 1.11 (3H, s, 6′-Me), 1.15 (3H, s, 6′-Me), 1.35 (3H, t, J = 7.2 Hz, CH_3CH_2), 1.4–1.48 (2H, m), 1.53 (1H, m), 1.75 (1H, dt, J = 15.4, 5.6 Hz), 1.92 (1H, m), 2.23 (3H, d, $^4J_{HF}$ = 3.2 Hz, 3-Me), 4.30 (2H, quartet, J = 7.2 Hz, CH_3CH_2), 6.29 (1H, dd, J = 15.9 Hz, $^5J_{HF}$ = 0.5 Hz, 5-H), 6.79 (1H, dd, J = 15.9 Hz, $^4J_{HF}$ = 2.0 Hz, 4-H). $\{^1H\}$-^{1}H NOE difference: the 5-H signals (6.29 ppm) were increased by 5% with irradiation of the 3-Me signals (2.23 ppm), while the 4-H signals (6.79 ppm) were increased by only 0.5%. ^{19}F-NMR δ: −126.1. *Anal.* Found: C, 68.90; H, 8.47%. Calcd. for $C_{17}H_{25}O_3F$: C, 68.89; H, 8.50%.

Further elution gave 400 mg of **19** (54.0%) as a colorless oil, $n_D^{23} = 1.5010$. IR ν_{max} (film) cm^{-1}: 3080 (w, =C–H), 1720 (s, C=O), 1640 (w, C=C), 1610 (w, C=C), 1315 (s), 1250 (s, C–O–C), 1140 (m), 1060 (m, C–O–C), 980 (w, C=C), 770 (m). ^{1}H-NMR δ: 0.96 (3H, s, 2′-Me), 1.15 (1H, m), 1.11 (3H, s, 6′-Me), 1.17 (3H, s, 6′-Me), 1.36 (3H, t, J = 7.2 Hz, CH_3CH_2), 1.4–1.48 (2H, m), 1.53 (1H, m), 1.75 (1H, dt, J = 15.1, 5.6 Hz), 1.90 (1H, m), 2.00 (3H, d, $^4J_{HF}$ = 4.2 Hz, 3-Me), 4.31 (2H, quartet, J = 7.2 Hz, CH_3CH_2), 6.22 (1H, d, J = 15.9 Hz, 5-H), 7.37 (1H, d, J = 15.9 Hz, $^4J_{HF}$ = 1.7 Hz, 4-H). ^{13}C-NMR δ: 12.0 (d, J = 9 Hz), 14.2 (CH_3CH_2), 17.1, 21.3, 25.9 (d, J = 7 Hz), 30.0, 33.7, 35.4, 35.6, 61.3 (CH_3CH_2), 65.5, 71.1, 127.5 (d, $^2J_{CF}$ = 18 Hz, C-3), 128.5 (d, $^4J_{CF}$ = 2 Hz, C-5), 131.9 (d, $^3J_{CF}$ = 11.5 Hz, C-4), 145.3 (d, $^1J_{CF}$ = 253 Hz, 2-C), 160.9 (d, $^2J_{CF}$ = 35 Hz, 1-C). ^{19}F-NMR δ: −122.7. *Anal.* Found: C, 68.60; H, 8.47%. Calcd. for $C_{17}H_{25}O_3F$: C, 68.89; H, 8.50%.

(±)-(2E,4E)-5-(1′,2′-Epoxy-2′,6′,6′-trimethylcyclohexyl)-2-fluoro-3-methyl-2,4-pentadienoic acid (**10**) *and its* (±)-*(2Z,4E)-isomer* (**11**). A solution of **19** (91.0 mg, 0.307 mmol) in 0.5 M KOH–MeOH (2 ml) was refluxed for 2 h. The reaction mixture was then cooled and MeOH was evaporated *in vacuo*. The residue was diluted with water and extracted with diethyl ether. The aqueous layer was acidified with 2 M aq. HCl, saturated with $(NH_4)_2SO_4$, and extracted with diethyl ether and EtOAc. The combined extracts were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give **10** (60.0 mg, 73.0%) as colorless prisms, mp 89.5–90 °C (hexane–diethyl ether). IR ν_{max} (nujol) cm^{-1}: 3600–2200 (m, O–H), 1690 (s, C=O), 1625 (w, C=C), 1600 (m, C=C), 1425 (m), 1260 (m), 1150 (m), 980 (m), 740 (m), 635 (m). ^{1}H-NMR δ: 0.96 (3H, s, 2′-Me), 1.06 (1H, m), 1.11 (3H, s, 6′-Me), 1.17 (3H, s, 6′-Me), 1.35–1.55 (3H, m), 1.76 (1H, dt, J = 15.4, 5.7 Hz), 1.94 (1H, m), 2.03 (3H, d, $^4J_{HF}$ = 4.4 Hz, 3-Me), 6.29 (1H, d, J = 15.8 Hz, 5-H), 7.38 (1H, dd, J = 15.8 Hz, $^4J_{HF}$ = 1.7 Hz, 4-H). ^{13}C-NMR δ: 12.2 (d, J = 9 Hz), 17.0, 21.2, 25.9 (d, J = 5 Hz), 29.9, 33.7, 35.6, 42.2 (6′-C), 66.0, 71.6, 128.3 (d, $^4J_{CF}$ = 3 Hz, 5-C), 129.6 (d, $^2J_{CF}$ = 17 Hz, 3-C), 132.7 (d, $^3J_{CF}$ = 11 Hz, 4-C), 144.6 (d, $^1J_{CF}$ = 251 Hz, 2-C), 163.7 (d, $^2J_{CF}$ = 36 Hz, 1-C). *Anal.* Found: C, 66.75; H, 7.72%. Calcd. for $C_{15}H_{21}O_3F$: C, 67.14; H, 7.89%.

A solution of **20** (151 mg, 0.509 mmol) was treated in the same manner as that described for **10** to give **11** (107 mg, 78.4%) as colorless needles, mp 103.5–104.5 °C (hexane). IR ν_{max} (nujol) cm^{-1}: 3600–2400 (m, O–H), 1690 (s, C=O), 1630 (w, C=C), 1610 (m, C=C), 1420 (m), 1290 (m), 1255 (s, C–O–C), 1150 (m), 980 (w), 630 (w). ^{1}H-NMR δ: 0.95 (3H, s, 2′-Me), 1.06 (1H, m), 1.12 (3H, s, 6′-Me), 1.15 (3H, s, 6′-Me), 1.35–1.55 (3H, m), 1.75 (1H, dt, J = 15.0, 5.7 Hz), 1.92 (1H, m), 2.25 (3H, d, $^4J_{HF}$ = 3.3 Hz, 3-Me), 6.37 (1H, dd, J = 15.8 Hz, $^5J_{HF}$ = 0.5 Hz, 5-H), 6.81 (1H, dd, J = 15.8 Hz, $^4J_{HF}$ = 1.8 Hz, 4-H). ^{13}C-NMR δ: 12.4, 17.0, 21.0, 25.9, 29.9, 33.7, 35.6, 40.5 (6′-C), 65.8, 71.4, 127.1 (d, J_{CF} = 11 Hz), 129.3 (d, J_{CF} = 8 Hz), 134.0 (d, J_{CF} = 6 Hz), 143.3 (d, $^1J_{CF}$ = 255 Hz, 2-C), 166.3 (d, $^2J_{CF}$ = 35 Hz, 1-C). *Anal.* Found: C, 67.15; H, 7.95%. Calcd. for $C_{15}H_{21}O_3F$: C, 67.14; H, 7.89%.

Irradiation experiment. Ten milligrams of sample **6**, **10**, or **11** was dissolved in EtOAc (5 ml), and the solution irradiated with a high-pressure mercury lamp {Riko UVL-100HA lamp, Pyrex® filter, irradiated at 279.9–577.0 nm [nm (mW/cm^2): 312.6 (23.4), 365.0 (60.7), 404.7 (25.8), 435.8 (42.2), 546.1 (49.8), 577.0 (42.2)]} for 6 h at 20°C. The reaction mixture was concentrated *in vacuo*, and each residual crude carboxylic acid was treated with diazomethane to give the corresponding methyl ester. The crude product was analyzed by HPLC [Erma ERC-Silica-1181 (6 × 250 mm) column; hexane/2-propanol = 20 : 1 (1.0 ml/min), detected at 254 nm, **6**-Me indicates the methyl ester of **6**]. For **6**: t_R = 5.5 min (34.7%, **6**-Me) and 5.8 min [65.3%, (2*E*)-**6**-Me]. For **10**: t_R = 5.6 min (87.4%, **11**-Me), 8.4 min (4.3%, **10**-Me) and 8.7 min (4.4%, structure undetermined). For **11**: t_R = 5.6 min (97.6%, **11**-Me) and 8.3 min (0.55%, **10**-Me).

Lettuce germination assay. The inhibitory effect on lettuce germination was assayed in the same manner as that described.[7] A group of twenty lettuce seeds (*Lactuca sativa* L. cv. Green Lake 2B61) was placed in a 5-cm Petri dish with a test solution of a 0.7% agar medium. After being incubated at 27°C under fluorescent light (1500 lux) for 6 days, the germination ratio was measured for two replicates.

Rice seedling assay. The inhibitory effect on rice seedling growth was assayed in the same manner as that described peviously[7] under non-sterile conditions. A group of ten rice seedlings (*Oryza sativa* L. cv. Satohonami) that had been germinated in water at 30°C for 3 days was transplanted to a test tube (36ϕ × 100 mm) with 7 ml of a 0.7% agar medium, and this was incubated for 4 days at 27°C under fluorescent light (1700 lux). The length of the second leaf sheath was measured for two replicates.

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