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Synthesis and Biological Activity of Methyl 3-Demethyl-Abscisate and Its Related Analogs†

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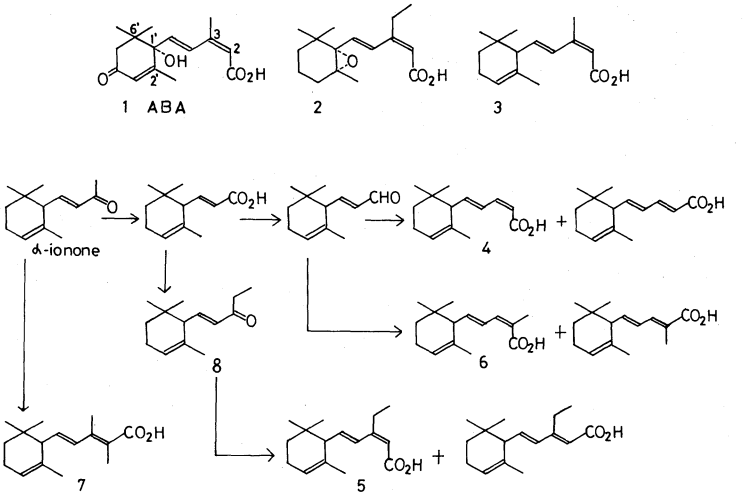
To elucidate the role of the methyl substituent on the side chain of abscisic acid (ABA), we synthesized (2*Z*,4*E*)-3-demethyl-α-ionylideneacetic acid (**4**) and its related analogs, methyl (2*Z*)-3-demethyl-β-ionylideneacetate 1′,2′-epoxide (**9**) and methyl (2*Z*) and (2*E*)-3-demethyl-abscisate (**12**) and (**13**). The biological assay of these compounds suggested that the 3-methyl group on the side chain of ABA was indispensable to biological activity.

Many investigations on the structure-activity relationship in the analogs of abscisic acid (ABA) (**1**) have been reported,[1] and analogs of (2*Z*)-α-ionylideneacetic acid having the same skeleton as ABA possessed strong growth inhibitory activities. Recently the authors reported that 6′-demethyl ABA showed strong growth inhibition on rice seedlings,[2,3] but 2′-demethyl ABA showed no activity.[2] But the effect of the methyl substituent on the side chain of ABA on the biological activity is still obscure. Tamura *et al.* reported the weak activity of 3-ethyl-epoxy-analog (**2**)[4] and we showed the inactivity of (2*E*+2*Z*)-3-demethyl-α-ionylideneacetic acid.[5] In order to examine the effect of the 3-methyl group of the side chain on physiological activity, we synthesized 3-demethyl-abscisate and some analogs.



SCHEME 1.

† Studies on Abscisic Acid. Part XVI. For Part XV, see *Agric. Biol. Chem.*, **46**, 817 (1982). This work was supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture of Japan.

Firstly we synthesized the α-ionylideneacetic acid analogs. As (2*Z*,4*E*)-α-ionylideneacetic acid (**3**) showed an activity comparable to ABA,[6] we synthesized (2*Z*,4*E*)-3-demethyl-α-ionylideneacetic acid (**4**), the (2*Z*,4*E*)-3-ethyl analog (**5**), the (2*Z*,4*E*)-3-demethyl-2-methyl analog (**6**) and 2-methyl-α-ionylideneacetic acid (**7**).

Methyl α-cyclocitrylideneacetate,[7] obtained by hypohalite oxidation of α-ionone and subsequent esterification, was reduced to the corresponding alcohol with lithium aluminum hydride. Oxidation of the alcohol to α-cyclocitrylideneacetaldehyde with active manganese dioxide followed by condensation with ethoxycarbonylmethylenetriphenylphosphorane afforded 3-demethyl-α-ionylideneacetate[5] as a mixture of the (2*Z*) and (2*E*) isomers. Chromatographic separation of the (2*Z*) and (2*E*) isomers and saponification with dilute alkali yielded (2*Z*,4*E*)-3-demethyl-α-ionylideneacetic acid (**4**) and (2*E*,4*E*)-acid.

The 3-ethyl analog (**5**) was prepared from homo-α-ionone (**8**). α-Cyclocitrylideneacetyl chloride was reacted with diethyl cadmium to yield homo-α-ionone (**8**), which was then reacted with ethoxycarbonylmethylenetriphenylphosphorane to afford the 3-ethyl esters as (2*Z*) and (2*E*) mixtures. Chromatographic separation and alkaline hydrolysis afforded (2*Z*,4*E*)-acid (**5**) and (2*E*,4*E*)-acid.

The 3-demethyl-2-methyl analog (**6**) was synthesized from α-cyclocitrylideneacetaldehyde by a Wittig reaction with 1-(ethoxycarbonyl)ethylidenetriphenylphosphorane and subsequent saponification.

2-Methyl-α-ionylideneacetic acid (**7**) was synthesized from α-ionone by a Wittig reaction.

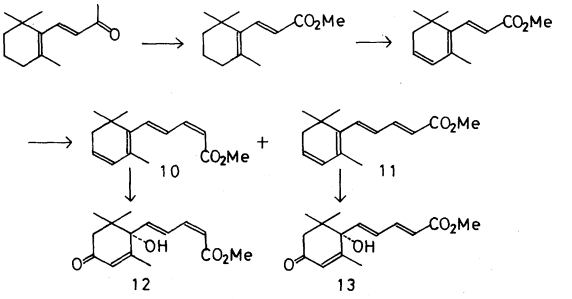
The growth inhibitory activity of the above synthesized compounds was tested on rice seedlings with the results shown in Table I. (2*Z*,4*E*)-3-Demethyl-α-ionylideneacetic acid (**4**) showed weak inhibition at a concentration of 2×10^{-4} mol/liter, but none of the compounds showed any remarkable inhibition at 10^{-4} mol/liter. The corresponding esters and (2*E*) isomers also did not inhibit the growth of rice seedlings.

Next, we synthesized methyl (2*Z*)-3-demethyl-β-ionylideneacetate 1′,2′-epoxide (**9**). Methyl β-cyclocitrylideneacetic acid obtained by the hypohalite oxidation of β-ionone was reduced to the corresponding alcohol with lithium aluminum hydride. Oxidation of the alcohol with active manganese dioxide gave β-cyclocitrylideneacetaldehyde which was reacted with methoxycarbonylmethylenetri-

TABLE I. GROWTH INHIBITORY ACTIVITY OF α-IONYLIDENE-ACETIC ACID ANALOGS ON RICE SEEDLINGS (*Oryza sativa* L. cv. Sasaminori)

Compounds	Concentration (mol/liter)			
	10^{-5}	10^{-4}	2×10^{-4}	10^{-3}
4	103%[a]	80	55	47
5	102	93	67	49
6	100	86	75	75
7	101	100	98	74
3	53	0	0	0

[a] Percentage of control growth. After growing for 5 days at 30°C (5000 lux), the lengths of the second leaf sheaths of the seedlings were measured.



SCHEME 2.

phenylphosphorane to afford methyl 3-demethyl-β-ionylideneacetate[5] as a mixture of the (2*Z*) and (2*E*) isomers in the ratio of 1 : 10. After separation of the isomers on a silica gel column, pure (2*Z*)-ester was oxidized with monoperoxyphthalic acid to give 1′,2′-epoxide (**9**). This epoxide showed 50% inhibition of the growth of rice seedlings at a concentration of 10^{-4} mol/liter (Fig. 1).

Finally we synthesized the (2*Z*) and (2*E*)-3-demethyl ABA esters (**12**) and (**13**). Methyl dehydro-β-cyclocitrylideneacetate, obtained by bromination of methyl β-cyclocitrylideneacetate with N-bromosuccinimide and followed by dehydrobromination with quinoline, was reduced to the corresponding alcohol with lithium aluminum hydride in ether. Oxidation of the alcohol with manganese dioxide followed by condensation with methoxycarbonylmethylenetriphenylphosphorane gave a mixture of (2*Z*) and (2*E*)-tetraenoate (**10** and **11**) in the ratio of *ca.* 1 : 10, which was separable by column chromatography on silica gel. The photosensitized oxygenation of **10** and **11** followed by rearrangement with basic alumina afforded (2*Z*) and (2*E*)-methyl 3-demethyl-abscisate (**12**) and (**13**), respectively. The inhibitory activity of both esters was tested on rice seedlings with the results shown in Fig. 1. Both esters showed no appreciable activity at a concentration of 10^{-4} mol/liter. (2*E*)-Ester (**13**) only showed a weak closing activity on the stomatal openings of isolated epidermis of *Commelina communis* compared with that of ABA. These results suggest that the 3-methyl group on the side chain of ABA is essential for the biological activity of ABA.

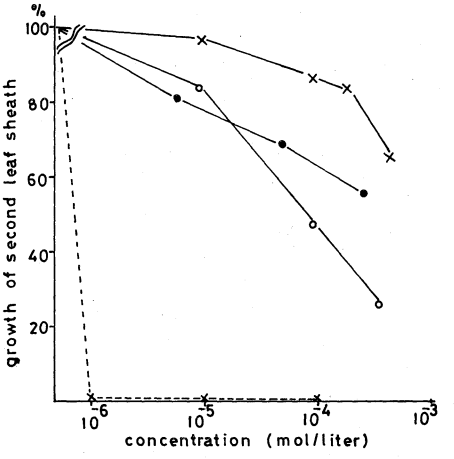


FIG. 1. The Growth Inhibitory Activity of Demethyl ABA Analogs on Rice Seedlings.

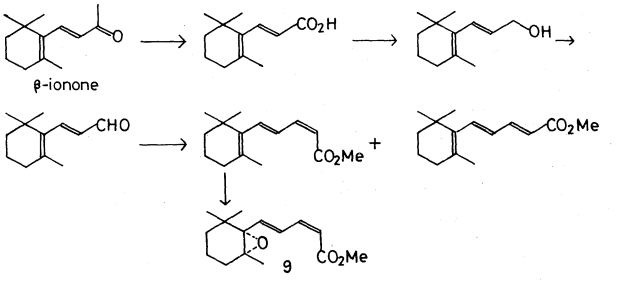
○—○, **9**; ×—×, **13**; ●—●, **12**; ×---×, ABA.

The lengths of the second leaf sheaths of Sasaminori (an ordinary species of *Oryza sativa*) were measured after growing for 4.5 days at 25°C (5000 lux) in a 0.4% agar solution.

EXPERIMENTAL

All mp's and bp's were uncorrected. IR spectra were recorded on a JASCO IRA-I spectrophotometer. NMR spectra were recorded on a JEOL-JNM-MH-60-II and JEOL-JNM-FX-100 with TMS as an internal standard. Gas chromatography was carried out on a JEOL-JGC-1100.

1) 3-Demethyl-α-ionylideneacetic acid[1] (**4**). A mixture of 2.5 g of α-cyclocitrylideneacetaldehyde[5] and 4.75 g of ethoxycarbonylmethylenetriphenylphosphorane was stirred at 170°C for 100 min under nitrogen. After cooling to room temperature, 100 ml of hexane–ether (1 : 1) was added and the deposited triphenylphosphine oxide was



SCHEME 3.

removed by filtration. The filtrate was concentrated and the residue (3.5 g) was chromatographed on 60 g of silica gel. (2*E*)-Ester (2.1 g) was obtained after elution of the (2*Z*)-ester (0.20 g) and (2*E*+2*Z*)-ester fractions (0.35 g). A solution of 1.3 g of (2*E*)-ester in 300 ml of methanol was irradiated by UV light from a 100 W high pressure mercury lamp for 100 min at room temperature. The isomeric composition of the equilibrated solution was checked by TLC as about 1:1. [Kiesel gel 60-H, benzene-hexane (1:1), *Rf* 0.45 (*Z*), 0.28 (*E*)]. The isomer mixture was chromatographed on silica gel with benzene–hexane (1:1) to give (2*Z*)-ester (0.60 g), (2*Z*+2*E*)-ester (0.20 g), and (2*E*)-ester (0.40 g). Methyl (2*Z*)-3-demethyl-α-ionylideneacetate: IR ν_{max}^{film} cm^{-1}: 3050, 1720, 1630, 970. NMR (CCl_4). δ: 5.40 (1H, d, J=10 Hz, C-2-H), 7.25 (1H, dd, J=16, 10 Hz, C-4-H). (2*E*)-Ester: IR ν_{max}^{film} cm^{-1}: 3040, 1715, 1635, 1610, 1000. NMR (CCl_4) δ: 5.75 (1H, d, J=15.5 Hz, C-2-H), 7.22 (1H, dd, J=16, 10 Hz, C-3-H).

(2*Z*)-Ester (0.30 g) was saponified with 5% methanolic potash at room temperature overnight to yield 0.20 g of oily (2*Z*)-acid (**4**). IR ν_{max}^{film} cm^{-1}: 1690, 1620, 1590, 960. NMR (CCl_4) δ: 7.13 (1H, dd, J=16, 10 Hz, C-4-H). The (2*E*)-ester was saponified in the same way to (2*E*)-acid. IR ν_{max}^{film} cm^{-1}: 1695, 1630, 1005.

*2) 5-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-ethyl-2,4-pentadienoic acid (**5**).* α-Cyclocitrylideneacetic acid (9.0 g) was dissolved in 10 ml of methylene chloride and 11 g of $SOCl_2$ was added under cooling. After the evolution of SO_2 gas has ceased, the contents were concentrated to give an oily chloride. Grignard reagent was prepared from 1.67 g of Mg and 7.6 g of ethyl bromide in ether, and 6.6 g of cadmium chloride in ether was added to the reagent at 0°C with stirring for an hour. The acid chloride obtained above was dissolved in 50 ml of ether, added to the cold diethyl cadmium solution and stirred at room temperature for several hours. The reaction mixture was pured into ice-cold dil. H_2SO_4, and the product was extracted with ether. The extract was washed with dil. NaOH, H_2O and dried over $MgSO_4$. After removal of the ether, the oily product (8.9 g) was distilled to give 5.8 g of homo-α-ionone (**8**), bp 95~98°C (3 mmHg). GLC (10% SE-30 on Chromosorb W, 3 mm × 2 m, 170°C, N_2 flow rate 20 ml/min, FID): t_R 12.7 min. IR ν_{max}^{film} cm^{-1}: 1680, 1620. NMR (CCl_4) δ: 0.81 (3H, s), 0.86 (3H, s), 1.00 (3H, t, J=7 Hz), 1.27 (2H, d), 1.51 (3H, s), 1.93 (2H, m), 2.12 (1H, d, J=8 Hz), 2.38 (2H, q, J=7 Hz), 5.24 (1H, br. s), 5.69 (1H, d, J=15 Hz), 6.26 (1H, dd, J=14, 8 Hz).

A mixture of homo-α-ionone (2.6 g) and 4.55 g of ethoxycarbonylmethylenetriphenylphosphorane was heated at 170°C for 2 hr under nitrogen. After cooling, 100 ml of hexane–ether (1:1) was added and the precipitate was filtered off. The filtrate was concentrated and the residual oil (4.2 g) was chromatographed on silica gel (60 g) with benzene–hexane (1:1) to afford (2*Z*+2*E*)-ester (1.55 g). GLC (5% SE-30 on Chromosorb W, 3 mm × 1 m, 165°C, N_2 flow rate 20 ml/min, FID), t_R: 8.3 min for (2*Z*)-ester and 9.5 min for (2*E*)-ester. The mixed ester (0.50 g) was rechromatographed on 60 g of silica gel with benzene–hexane (1:1) to yield 0.30 g of (2*Z*)-ester, 0.05 g of (2*Z*+2*E*)-ester and 0.10 g of (2*E*)-ester. (2*Z*)-Ester: IR ν_{max}^{film} cm^{-1}: 1715, 1630, 1600, 980. NMR (CCl_4) δ: 1.22 (6H, t, J=7.0 Hz), 1.54 (3H, d, J=1.5 Hz), 2.28 (2H, q, J=7 Hz), 3.98 (2H, q, J=7.0 Hz), 7.25 (1H, d, J=16 Hz, C-4-H). (2*E*)-Ester: IR ν_{max}^{film} cm^{-1}: 1725, 1640, 985.

(2*Z*)-Ester was saponified with methanolic potash to yield (2*Z*)-acid (**5**) as an oil. IR ν_{max}^{film} cm^{-1}: 1690, 1625, 1600, 985. NMR δ: 7.26 (1H, d, J=16 Hz, C-4-H). (2*E*)-Ester was also saponified to yield crystalline (2*E*)-acid, mp 95~97°C, IR ν_{max}^{nujol} cm^{-1}: 1690, 1625, 1605. NMR (CCl_4) δ: 1.08 (3H, t, J=8 Hz), 2.77 (2H, q, J=8 Hz), 5.35 (1H, br. s, C-3′-H), 5.90 (1H, s, C-2-H). *Anal.* Found: C, 76.77; H, 9.85, Calcd. for $C_{16}H_{24}O_2$: C, 77.37; H, 9.74%.

*3) 2-Methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentadienoic acid (**6**).* α-Cyclocitrylideneacetaldehyde (0.5 g) was condensed with 1-(ethoxycarbonyl)ethylidenetriphenylphosphorane (1.1 g) in xylene at refluxing temperature for 3 hr. The usual work up afforded about 0.40 g of (2*E*)-ester. UV irradiation of the (2*E*)-ester in methanol afforded the equilibrated mixture composed of a 1:1 mixture of (2*Z*) and (2*E*)-ester checked by GLC. GLC analysis (3 mm × 2 m column packed with 5% SE-30 on Chromosorb W, 178°C isothermal, He flow rate 20 ml/min) showed retention times of 10.0 min for (2*Z*)-ester and 13.7 min for (2*E*)-ester. Separation of each isomer on a silica gel column afforded pure (2*Z*)-ester (0.20 g) and (2*E*)-ester (0.20 g). (2*Z*)-Ester: NMR (CCl_4): 7.01 (1H, dd, J=15, 10 Hz, C-4-H), 6.25 (1H, d, J=10 Hz, C-3-H), IR ν_{max}^{film} cm^{-1}: 1720, 1635, 1600, 985. (2*E*)-Ester: NMR (CCl_4) δ: 7.01 (1H, d, J=10 Hz, C-3-H), 5.75 (1H, dd, J=15, 10 Hz, C-4-H), IR ν_{max}^{film} cm^{-1}: 1715, 1635, 1605, 975.

Each ester was saponified with methanolic potash at room temperature to afford free acid. (2*Z*)-Acid (**6**): IR ν_{max}^{film} cm^{-1}: 1685, 1630, 1595, 980. (2*E*)-Acid: IR ν_{max}^{film} cm^{-1}: 1685, 1630, 1600, 980.

*4) 2,3-Dimethyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentadienoic acid (**7**).* A mixture of 1.8 g of α-ionone and 3.35 g of 1-(ethoxycarbonyl)ethylidenetriphenylphosphorane was heated at 180°C for 15 hr under nitrogen. The usual work up and chromatographic purification afforded 1.05 g of (2*E*)-ester. By UV irradiation only a trace amount of (2*E*)-ester could be converted to (2*Z*)-ester checked by TLC. (2*E*)-Ester: IR ν_{max}^{film} cm^{-1}: 1715, 1625, 1595, 970. NMR (CCl_4) δ: 1.95 (3H, s, C-2-Me), 2.01 (3H, s, C-3-Me), 5.64 (1H, dd, J=9 Hz, C-5-H), 6.33 (1H, d, J=16 Hz, C-4-H). (2*E*)-Ester was saponified with methanolic potash to yield (2*E*)-acid (**7**) as an oil. IR ν_{max}^{film} cm^{-1}: 1720, 1630, 970.

*5) 5-(1,2-Epoxy-2,6,6-trimethyl-1-cyclohexyl)-2,4-pentadienoic acid (**9**).* A mixture of 3.0 g of α-cyclocitryl-

ideneacetaldehyde and 5.6 g of methoxycarbonylmethylenetriphenylphosphorane was heated at 170°C for 60 min. After cooling, 100 ml of hexane–ether (1 : 1) was added and the deposited precipitate was filtered off. The filtrate was concentrated and the residual oil (3.1 g) was chromatographed on silica gel (55 g) with benzene–hexane (1 : 1) to afford 0.40 g of (2*Z*)-ester, 1.6 g of (2*Z*+2*E*)-ester fraction and 1.4 g of (2*E*)-ester. (2*Z*)-Ester: IR ν_{max}^{film} cm^{-1}: 1730, 1620, 1000. NMR (CCl_4) δ: 5.54 (1H, d, J=11 Hz, C-2-H), 7.44 (1H, dd, J=16, 10 Hz, C-4-H). (2*E*)-Ester (1.05 g) was isomerized by UV irradiation to afford an equilibrated mixture of (2*Z*) and (2*E*)-ester in the ratio of *ca.* 1 : 6.

(2*Z*)-Ester (0.414 g) was dissolved in 30 ml of dry ether, ethereal monoperoxyphthalic acid (3.2 mM, 12 ml) was added and the mixture stirred overnight at room temperature. The filtered reaction mixture was diluted with ether, washed with aq. sodium hydrogen sulfite, sodium hydrogen carbonate and water, and dried over $MgSO_4$. Concentration and chromatographic purification afforded 0.125 g of epoxide. IR ν_{max}^{film} cm^{-1}: 1730, 1635, 1600, 1440, 1005, 970. NMR [$(CD_3)_2CO$, 100 MHz] δ: 0.909 (3H, s), 1.119 (6H, s), 3.647 (3H, s), 5.673 (1H, d, J=11.2 Hz, C-2-H), 7.488 (1H, dd, J=15.1, 11.7 Hz, C-4-H). *Anal.* Found: C, 72.31; H, 9.15, Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86%.

*6) Methyl 3-Demethyl-abscisate (***12***) and (***13***).*

a) 3-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-propenal. To a stirred solution of 0.80 g of lithium aluminum hydride in 200 ml of dry ether was added dropwise 4.0 g of methyl dehydro-β-cyclocitrylideneacetate[7] in 20 ml of ether at 0~4°C. After refluxing for an hour the mixture was poured into aq. NH_4Cl and extracted with ether. The ether layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated. Distillation of the residue gave 2.9 g of alcohol at 104°C (4 mmHg). IR ν_{max}^{film} cm^{-1}: 3340, 3040, 970. NMR (CCl_4) δ: 0.99 (6H, s), 1.82 (3H, s), 2.06 (2H, d), 3.50 (1H, br. s, OH), 4.17 (2H, d, J=5.0 Hz), 5.67~6.25 (4H, m). A mixture of 3.0 g of the alcohol, 17 g of active manganese dioxide and 100 ml of CCl_4 was shaken at room temperature for 40 hr. Concentration of the filtrate afforded 2.7 g of trienal as an oil. IR ν_{max}^{film} cm^{-1}: 3040, 2720, 1680, 1605, 975.

*b) Methyl 5-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2,4-pentadienoate (***10***) and (***11***).* A mixture of 3.5 g of the trienal and 7.0 g of methoxycarbonylmethylenetriphenylphosphorane in 40 ml of dry benzene was refluxed under nitrogen for 4 hr. After removal of the benzene, 50 ml of ether was added and the deposited triphenylphosphine oxide was separated by filtration. The filtrate was concentrated and the residue (2.3 g) was chromatographed on 230 g of silica gel. Elution with benzene afforded 184 mg of (2*Z*)-ester and 2.20 g of (2*E*)-ester. (2*Z*)-Ester (**10**): IR ν_{max}^{film} cm^{-1}: 3040, 1715, 1615, 1000. NMR (CCl_4) δ: 1.034 (3H, s), 1.078 (3H, s), 1.28 (3H, s), 1.914 (3H, s), 4.12 (2H, q), 2.08 (2H, br. d), 5.63~5.88 (3H, m), 6.13~6.40 (2H), 7.03~7.45 (1H). (2*E*)-Ester (**11**): IR ν_{max}^{film} cm^{-1}: 3035, 1720, 1620, 1000.

*c) Methyl 3-Demethyl-abscisate (***12***) and (***13***).* A solution of 0.184 g of (2*Z*)-methyl dehydro-β-ionylideneacetate (**10**) and 0.040 g of Rose Bengal in 100 ml of methanol was shaken with oxygen at room temperature under the illumination of an ordinary 40 W fluorescent lamp for 16 hr. After evaporation of the methanol, a residue was absorbed on 10 g of alumina and left to stand for 2 hr. After washing with benzene, the product was eluted with benzene–ethyl acetate (1 : 1) to afford 0.07 g of 3-demethyl abscisate. Purification by preparative TLC on silica gel afforded 0.0105 g of crystalline (2*Z*)-methyl 3-demethyl-abscisate (**12**), mp 109~114°C, IR ν_{max}^{KBr} cm^{-1}: 3435, 1725, 1645, 1598. NMR [$(CD_3)_2CO$, 100 MHz] δ: 1.034 (3H, s), 1.078 (3H, s), 1.914 (3H, d, J=1.5 Hz), 2.24~2.63 (2H), 3.676 (3H, s), 4.480 (1H, s, OH), 5.703 (1H, d, J=11.2 Hz, C-2-H), 5.838 (1H, br. s, C-3′-H), 6.429 (1H, d, J=15.2 Hz, C-5-H), 6.809 (1H, dd, J=11.3, 10 Hz, C-3-H), 7.748 (1H, dd, J=15.2, 10 Hz). *Anal.* Found: C, 68.22; H, 7.60. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63%.

Similarly (2*E*)-tetraenoate (**11**) (1.0 g) was photooxidized to afford 0.30 g of an oily ketol-ester fraction, which solidified in benzene to yield 0.20 g of crystalline (2*E*)-ester (**13**), mp 147~147.5°C. IR ν_{max}^{KBr} cm^{-1}: 3440, 1720, 1650, 1615. NMR ($CDCl_3$, 100 MHz) δ: 1.017 (3H, s), 1.098 (3H, s), 1.617 (1H, s, OH), 1.898 (3H, d, J=1.2 Hz), 2.25~2.57 (2H), 3.757 (3H, s), 5.941 (1H, br. s), 5.952 (1H, d, J=15.4 Hz), 6.159 (1H, d, J=15.2 Hz), 6.505 (1H, dd, J=15.2, 10.5 Hz), 7.296 (1H, dd, J=15.3, 10.5 Hz). *Anal.* Found: C, 68.30; H, 7.75. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63%.

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