Rapid Paper

Isolation of (2Z,4E)- γ -Ionylideneethanol from *Cercospora cruenta*, a Fungus Producing (+)-Absciscic AcidTakayuki ORITANI, Munetaka NIITSU,
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(2Z,4E)- γ -Ionylideneethanol was separated from the mycelium of *Cercospora cruenta*, and its structure was confirmed by comparing the spectral data with those of an authentic specimen synthesized from γ -ionone. Gas chromatographic analysis of the extract showed the presence of a trace amount of (2Z,4E)- γ -ionylideneacetic acid.

In the previous paper,¹⁾ the authors presented a new biosynthetic pathway of (+)-absciscic acid (ABA, **1**), a plant hormone, in *Cercospora cruenta*,²⁾ which could produce (-)-(2Z,4E)-4'-hydroxy- γ -ionylideneacetic acid (**2**) as a biosynthetic precursor of (+)-ABA. On this biosynthetic route, γ -ionylideneacetic acid (**3**) and γ -ionylideneethanol (**4**) were assumed to be present as biosynthetic intermediates from farnesyl pyrophosphate to **2**. Here, we describe the isolation of (2Z,4E)- γ -ionylideneethanol (**4**) from *C. cruenta* and the synthesis of ($\pm$)-**4** from ($\pm$)- γ -ionone (**5**).

Isolation of (2Z,4E)- γ -ionylideneethanol from *Cercospora cruenta*

The mycelium, separated from the culture broth of *C. cruenta*, was extracted with

methanol-chloroform (1:1) to give a metabolite mixture. The separation of the metabolites by silica gel chromatography with benzene afforded a small amount of a carotene pigment and a glyceride as the main product. Further elution with the benzene-ethyl acetate gave a small amount of a crude unknown alcohol, fatty acids and ergosterol. Alkaline hydrolysis of the glyceride gave fatty acids, which consisted of palmitic, stearic, oleic and linolenic acids by GLC analysis. The unknown alcohol was purified twice by thin layer chromatography (TLC) and then HPLC to give the relatively pure component, whose IR spectrum indicated the presence of a hydroxyl group at 3320 cm^{-1} and olefinic double bonds at 3080, 1640 and 970 cm^{-1} . The GC-MS exhibited peaks at m/z 220 (M^+ , molecular formula $\text{C}_{15}\text{H}_{24}\text{O}_1$), 202 ($\text{M}^+ - \text{H}_2\text{O}$), 187, 131, 105 and 92. The $^1\text{H-NMR}$ spectrum showed the presence of a (2Z,4E)-3-methyl-2,4-pentadien-1-ol moiety at δ 1.89 (3-methyl), 4.28 (CH_2O , d), 5.52 (2-CH=, t), 5.88 (5-CH=, dd, $J=9$ and 16 Hz) and 6.41 (4-CH=, d, $J=16$ Hz), a methylene group at 4.55 (1H) and 4.77 (1H), and a geminal dimethyl group at δ 0.82 (3H) and 0.89 (3H). The alcohol was converted to 3,5-dinitrobenzoate, mp 98.5°C, $[\alpha]_D +18^\circ$ (CHCl_3). The MS of the latter showed peaks at m/z 415 ($\text{M}^+ + 1$, 16%), 414 (M^+ , $\text{C}_{22}\text{H}_{26}\text{O}_6\text{N}_2$, 36%), 202 (100%) and 133 (53%). Thus, it was deduced that the alcohol was (2Z,4E)-5-(2',2'-dimethyl-6'-methylene-1'-cyclohexyl-3-methyl-2,4-pentadien-1-ol) (**4**), that is, (2Z,4E)- γ -ionylideneethanol, whose IR and $^1\text{H-NMR}$ spectra were identical with those of a synthetic specimen described later. The accumulation of **4** in the mycelium of *C. cruenta* was a maximum in 5-day cultures at the middle stage of fermentation (about 1 mg in 4.2 liters of the culture broth) and decreased to less than one fifth in 9-day cultures at the pellet forming stage.

Identification of (2Z,4E)- γ -ionylideneacetic acid (**3a**) in the acidic metabolites of *Cercospora cruenta*

The acidic metabolites, separated from the

mycelium extract with aqueous sodium bicarbonate, were methylated with diazomethane to give the methyl ester fraction. The fraction showing the same *R_f* value as that of synthetic ($\pm$)-(2*Z*,4*E*)-methyl γ -ionylideneacetate was separated by TLC and analyzed by GLC using two columns packed with 2% silicone OV-1 and 10% silicone SE-30. The result showed that a trace amount of (2*Z*,4*E*)- γ -ionylideneacetic acid (**3a**, 25 μ g) was contained in the mycelium of the culture broth (2.1 liters).

Synthesis of ($\pm$)-(2*Z*,4*E*)- γ -ionylideneethanol (**4**)

Thermal decomposition of 2'-acetoxy-2',3'-dihydro- α -ionone, which was prepared from ($\pm$)- α -ionone (**5**) by the method of Ohloff *et al.*,³⁾ resulted in a mixture of α -ionone (**5**) and γ -ionone (**6**). The mixture was separated by chromatography with silica gel containing 2.5% of silver nitrate to give pure ($\pm$)- γ -ionone (**6**). The Wittig reaction of ($\pm$)-**6** with

ethoxycarbonylmethylenetriphenylphosphorane gave a mixture of ($\pm$)-(2*Z*,4*E*)- and (2*E*,4*E*)-ethyl γ -ionylideneacetate (**3b** and **7b**), (2*Z*:2*E*=1:2), which was separated by silica gel chromatography and followed by alkaline hydrolysis, to give ($\pm$)-(2*Z*,4*E*)- γ -ionylideneacetic acid (**3a**), mp 108~111°C, and ($\pm$)-(2*E*,4*E*)- γ -acid (**7a**), mp 92~95°C. Reduction of **3b** and **7b** with sodium bis-(2-methoxyethoxy)aluminum hydride (Red-AL) in benzene afforded ($\pm$)-(2*Z*,4*E*) and (2*E*,4*E*)- γ -ionylideneethanols (**4** and **8**), respectively.

The growth inhibitory activities of ($\pm$)-

(2*Z*,4*E*)- γ -acid (**3a**), ($\pm$)-(2*E*,4*E*)- γ -acid (**7a**) and natural (2*Z*,4*E*)- γ -alcohol (**4**) were assessed in rice seedlings. The (2*Z*,4*E*)-isomer (**3a**) showed stronger inhibitory activity than that of the (2*E*,4*E*)-isomer (**7a**) (the activity was 58% for **3a** and 77% for **7a** against the control growth of 100%), and natural **4** showed the strongest activity (40%) at 5×10^{-4} M concentration. The activities of the compounds **3a**, **7a** and **4** were low, compared with the activities of ABA and (2*Z*,4*E*)- α -ionylideneacetic acid (each 0% at 10^{-4} M).⁴⁾ This suggested the possibility that the predictable precursors **3a** and **4** may exhibit the activity after conversion to ABA in plants.

EXPERIMENTAL

Melting points were determined with a micro-stage apparatus (Mitamura Riken Kogyo Inc.) and are uncorrected. IR spectra were recorded on a JASCO IR-810 spectrometer. Mass spectra were measured with a Hitachi M-52 spectrometer. ¹H-NMR (100 MHz) spectra were measured on a JEOL JNM-FX 100 spectrometer with TMS as an internal standard. UV spectra were measured on a Hitachi Model 124 spectrometer. Thin layer chromatography (TLC) was performed on silica gel (Merck 60 H, 0.25 mm thickness). Gas chromatographic (GLC) analyses were performed by a JEOL JGC-20K instrument with a flame ionization detector, using two 2 m $\times$ 3 mm stainless steel columns packed with 2% silicone OV-1 and 10% silicone SE-30, respectively.

*1) Isolation of (2*Z*,3*E*)- γ -ionylideneethanol (**4**) from *C. cruenta*.* *C. cruenta* IFO 6164 was cultured in 4.2 liters of a potato-yeast medium¹⁾ under shaking and lighting (*ca.* 1500 lux) for 5 days at 28°C. The mycelium, separated by filtering the culture broth with a gauze, was sufficiently ground with quartz sand and extracted twice with methanol-chloroform (1:1) to give the metabolites (4.27 g), which were chromatographed with silica gel. Elution with benzene gave about 3 mg of an orange-colored carotene: *R_f* (benzene), 0.92 on TLC; *R_f* (hexane-benzene=9:1), 0.42 on TLC; UV $\lambda_{\text{max}}^{\text{hexane}}$ nm: 418 (a shoulder), 450 and 477 (similar to that of β -carotene with 450 and 477), and then 3.50 g of a glyceride: *R_f* (benzene), 0.25~0.30 on TLC; IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3010, 1745, 1650, 1240, 1160, 1120, 720 and 680; ¹H-NMR (CDCl₃) δ : 0.88 (CH₃), 1.26 (CH₂)_n, 2.31 (CH₂CO), 4.23 (CH₂O-, CHO-) and 5.35 (CH=). The glyceride was hydrolyzed with 5% ethanolic NaOH to give fatty acids, which consisted of 30% of palmitic acid and 70% of C₁₈-fatty acids by GLC analyses of the corresponding methyl esters. Further elution with benzene-ethyl acetate (4:1-1:1) gave a fraction

(100 mg) containing γ -ionylideneethanol (**4**), 350 mg of fatty acids and 250 mg of ergosterol, mp 168°C, whose $^1\text{H-NMR}$ spectrum was identical with that of an authentic sample. The fatty acids were methylated with diazomethane to yield the methyl esters, which consisted of methyl palmitate (t_R , 1.8 min; 3%), methyl stearate (t_R , 2.8 min; 10%), methyl oleate (t_R , 3.1 min; 56%) and methyl linolenate (t_R , 3.1 min; 31%) by TLC-MS analysis with 2% silicone OV-1 (2 m; column temp., 220°C; N_2 , 1.10 kg/cm 2). The alcohol fraction (R_f : 0.50) was twice separated by TLC with benzene-ethyl acetate and further purified by HPLC (0.45 mm $\times$ 250 mm; Fine SIL-5; solvent, CH_2Cl_2 ; flow rate, 2 ml/min; t_R , 8.5 min for **4**) to give 1.0 mg of (2*Z*,4*E*)- γ -ionylideneethanol (**4**) as an oil. GLC with 10% silicone SE-30 (2 m; column temp., 190°C; N_2 , 1.61 kg/cm 2) t_R , 8.4 min. GC-MS m/z (rel. int.): 220 (M^+ , 0.8), 202 ($\text{M}^+ - \text{H}_2\text{O}$, 61), 187 (46), 164 (9), 159 (74), 133 (36), (36), 131 (57), 107 (37), 105 (52), 92 (100) and 91 (75). IR $\nu_{\text{max}}^{\text{film}}$ cm $^{-1}$: 3320, 3080, 3040, 1640, 1460, 1440, 1380, 1365, 1020, 1000 and 970. $^1\text{H-NMR}$ (CDCl_3) δ : 0.82 (3H, s), 0.89 (3H, s), 1.89 (3H, q, $J=1$ Hz), 2.51 (1H, d, $J=9$ Hz), 4.28 (2H, d, $J=7$ Hz), 4.55 (1H, s), 4.74 (1H, m), 5.52 (1H, t, $J=7$ Hz), 5.88 (1H, dd, $J=9$ and 16 Hz) and 6.41 (1H, d, $J=16$ Hz). The alcohol (**4**) was reacted with 3,5-dinitrobenzoyl chloride in pyridine to yield the 3,5-dinitrobenzoate of **4**, mp 98.5°C (from hexane-ether), $[\alpha]_{\text{D}}^{21} + 18^\circ$ ($c=0.1$, CHCl_3); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 238 ($\epsilon=39600$); CD (EtOH): $[\theta]$ (nm) $+2.1 \times 10^4$ (238). MS (12.5 eV) m/z (rel. int.): 415 ($\text{M}^+ + 1$, 16), 414 (M^+ , 36), 202 (100), 133 (53). $^1\text{H-NMR}$ (CDCl_3) δ : 0.83 (3H, s), 0.91 (3H, s), 1.97 (3H), 4.56 (1H, s), 4.75 (1H, s), 5.08 (2H, d, $J=7$ Hz), 5.58 (1H, t, $J=7$ Hz), 6.01 (1H, dd, $J=10$ and 16 Hz), 9.17 (3H, aromatic).

2) *Identification of (2*Z*,4*E*)- γ -ionylideneacetic acid (**3a**) in *C. cruenta*.* The fraction of the mycelium metabolites, which was separated from the culture broth (2.1 liters), was extracted with 3% aqueous NaHCO_3 to give an acidic fraction (120 mg). The latter was methylated with diazomethane and then chromatographed on silica gel with benzene to yield a methyl ester fraction. Separation of the fraction by TLC with hexane-isopropyl ether (5:1) twice gave the fraction (0.4 mg) containing (2*Z*,4*E*)-methyl γ -ionylideneacetate (**3c**, R_f : 0.67). The standard sample ($\pm$)-**3c** was prepared by methylation of ($\pm$)-**3a** with diazomethane. GLC analysis with 2% silicone OV-1 on Uniport HP (2 m; column temp., 200°C; N_2 , 0.75 kg/cm 2) showed that the latter fraction (0.4 mg) contained 26.5 μg of **3c** (t_R , 2.5 min). Similarly, an acidic fraction (51 mg), separated from the filtrate of the culture broth (2.1 liters), contained about 0.6 μg of **3a** by GLC analysis of the corresponding methyl ester fraction. GLC with 10% silicone SE-30 on chromosorb W (2 m; column temp., 200°C; N_2 , 1.05 kg/cm 2) t_R : 10.4 min for **3c**.

3) *Preparation of ($\pm$)- γ -ionone (**6**).* A solution of ($\pm$)-2'-acetoxy-2',3'-dihydro- α -ionone 31 (20 g), mp 111.5~

112.5°C, in 15 ml of diethyl carbitol was gradually passed into a quartz tube heated at 470~480°C and the trapped product was extracted with ether to give a mixture of an α -ionone (**5**) and γ -ionone (**6**), bp 97~102°C/3 mmHg (0.8 g, 53% yield). The mixture consisted of 65% of **5** (t_R : 5.6 min) and 35% of **6** (t_R : 6.5 min) by GLC analysis with 5% Lac-2R-446 on chromosorb W (AW; 2 m; column temp., 150°C; N_2 , 0.7 kg/cm 2). The mixture (500 mg) was chromatographed on silica gel containing 2.5% of AgNO_3 and eluted with hexane-isopropyl ether (9:1~4:1) to give 164 mg of pure ($\pm$)- γ -ionone (**6**) 31 as an oil. IR $\nu_{\text{max}}^{\text{film}}$ cm $^{-1}$: 3080, 2930, 1675, 1640, 1620, 1250, 990, 890. $^1\text{H-NMR}$ (CDCl_3) δ : 0.87 (3H, s), 0.91 (3H, s), 2.27 (3H, s), 1.26~2.23 (6H, m), 2.59 (1H, d, $J=10$ Hz), 4.55 (1H, s), 4.80 (1H, s), 6.09 (1H, d, $J=16$ Hz), 6.95 (1H, dd, $J=10$ and 16 Hz).

4) *Synthesis of ($\pm$)-(2*Z*,4*E*) and (2*E*,4*E*)- γ -ionylideneacetic acids (**3a**) and (**7a**).* A mixture of ($\pm$)- γ -ionone (**6**, 163 mg) and 590 mg of ethoxycarbonylmethylenetriphenylphosphorane in 0.4 ml of xylene was heated on an oil bath at 150~160°C under nitrogen for 25 hr. After cooling, the reaction mixture was chromatographed on silica gel. Elution with benzene afforded a mixture of crude ($\pm$)-(2*Z*,4*E*) and (2*E*,4*E*)-ethyl γ -ionylideneacetates (**3b** and **7b**) as an oil (218 mg, 98% yield). The mixture consisted of 30% of ($\pm$)-**3b** (t_R : 7.5 min), 3% of (2*Z*,4*E*)-ethyl β -ionylideneacetate 41 (t_R : 8.1 min), 60% of ($\pm$)-**7b** (t_R : 9.1 min) and 7% of (2*E*,4*E*)-ethyl β -ionylideneacetate 41 (t_R : 9.9 min) by GLC analysis with 4% silicone SE-30 on chromosorb G (2 m; column temp., 185°C; He, 1.0 kg/cm 2). Further, silica gel chromatography with hexane-benzene (2:1~1:1), followed by purification with TLC using 2.5% of AgNO_3 -silica gel, gave pure ($\pm$)-(2*Z*,4*E*)-ethyl γ -ionylideneacetate (**3b**, t_R : 7.5 min) as an oil. IR $\nu_{\text{max}}^{\text{film}}$ cm $^{-1}$: 3080, 2930, 1710, 1625, 1600, 980, 890. The (2*Z*,4*E*)-ester (**3b**) was hydrolyzed with 5% ethanolic KOH at room temperature to give ($\pm$)-(2*Z*,4*E*)- γ -acid (**3a**), mp 108~111°C (from acetonitrile). MS m/z (rel. int.): 235 ($\text{M}^+ + 1$, 4), 234 (M^+ , 27), 219 (32), 201 (17), 191 (25), 189 (76), 173 (21), 165 (32), 147 (59), 135 (36), 124 (57), 111 (100), 107 (59), 105 (50). IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 3000~2600, 3080, 1680, 1630, 1600, 980, 890. $^1\text{H-NMR}$ (CDCl_3) δ : 0.83 (3H, s), 0.91 (3H, s), 2.07 (3H, s), 1.21~2.23 (6H, m), 2.65 (1H, d, $J=10$ Hz), 4.54 (1H, s), 4.76 (1H, s), 5.66 (1H, s), 6.30 (1H, dd, $J=10$ and 16 Hz), 7.55 (1H, d, $J=16$ Hz). The fraction of the (2*E*,4*E*)-ester (**7b**), separated by silica gel chromatography, was hydrolyzed with 5% ethanolic KOH to yield ($\pm$)-(2*E*,4*E*)- γ -ionylideneacetic acid (**7a**), mp 96~97°C (from hexane); MS m/z (rel. int.): 234 (M^+ , 99), 189 (100), 111 (77). IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 3080, 3000~2500, 1680, 1630, 1600, 980, 890. $^1\text{H-NMR}$ (CDCl_3) δ : 0.84 (3H, s), 0.90 (3H, s), 2.32 (3H, d, $J=1$ Hz), 1.21~2.24 (6H, m), 2.55 (1H, d, $J=8$ Hz), 4.53 (1H, s), 4.75 (1H, s), 5.75 (1H, s), 6.20 (1H, m), 6.26 (1H, dd, $J=8$ and 15 Hz).

5) *Synthesis of ($\pm$)-(2Z,4E) and (2E,4E)- γ -ionylideneethanols (4) and (8).* To a stirred solution of ($\pm$)-(2Z,4E)-ethyl ester (**3b**, 32 mg) in 1 ml of dry benzene was added 0.25 ml of a 15% solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene at 3°C. After stirring at 20°C for 1 hr, 10% ethereal acetic acid (5 ml) was added to the reaction mixture, which was then extracted with ether. The ethereal extract was washed with water and dried over MgSO_4 . Evaporation of the solvent, followed by separation with TLC (benzene-ethyl acetate = 4:1), afforded ($\pm$)-(2Z,4E)- γ -ionylideneethanol (**4**) as an oil (19 mg, 71%). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3320, 3080, 1640, 1000, 970, 890. The IR and ^1H -NMR spectra of ($\pm$)-**4** were identical with those of natural (+)-**4**. MS m/z : 220 (M^+), 202 ($\text{M}^+ - \text{H}_2\text{O}$), 187, 159, 133, 131. Similarly, (+)-(2E,4E)-ethyl ester (**7b**) was reduced with sodium bis(2-methoxyethoxy)aluminum hydride in benzene to give ($\pm$)-(2E,4E)- γ -alcohol (**8**, 70% yield) as an oil. MS m/z : 220 (M^+), 202 ($\text{M}^+ - \text{H}_2\text{O}$). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3330, 3080, 3040, 1640, 1000, 970, 890. ^1H -NMR (CDCl_3) δ : 0.81 (3H, s), 0.89 (3H, s), 1.82 (3H, s), 1.00~2.28 (6H, m), 2.47 (1H, d, $J=9$ Hz), 4.26 (2H, d, $J=7$ Hz), 4.56 (1H, s), 4.73 (1H, d,

$J=1$ Hz), 5.59 (1H, t, $J=7$ Hz), 5.87 (1H, dd, $J=9$ and 16 Hz), 6.07 (1H, d, $J=16$ Hz).

6) *Bioassay procedure.* The rice seedling test was carried out by using seedlings (*Oryzae sativa* L. var. Koshijiwase) as previously described.⁴⁾ After growing at 28°C under 5000 lux for 5 days, the mean increment of the second leaf sheath was measured as a percentage of that of the control.

REFERENCES

- 1) T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **49**, 245 (1985).
- 2) T. Oritani, M. Ichimura and K. Yamashita, *Agric. Biol. Chem.*, **46**, 1959 (1982).
- 3) G. Ohloff and S. Mignat, *Liebigs Ann. Chem.*, **652**, 115 (1962).
- 4) T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **34**, 108 (1970).