Synthesis and Physiological Activity of Methyl 6',6'-Didemethyl Abscisate and New Chiral Analogs*

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Methyl 6', 6'-didemethyl abscisate (**5**) was synthesized and assayed to elucidate the physiological activity of methyl substituents on the cyclohexene ring of abscisic acid (ABA). During this study two new chiral stereoisomeric analogs **6** and **7** were synthesized from *l*- and *d*-carvone. The rice seedling assay and germination assay of garden radish showed that 6'-methyl groups of ABA were not important in biological activity and that 5'-isopropenyl analogs **6** and **7** were inactive.

There are many investigations concerning synthesis and structure-activity relationship in the analogs of abscisic acid (**1**).¹⁾ In order to examine the effect of methyl substituents on the cyclohexene ring on the activity, three monodemethyl ABA (**2**), (**3**) and (**4**) were previously

synthesized.²⁾ Also, it was reported that both **2** and **3** showed the strong growth inhibition, but **4** showed weak activity on rice seedlings. From these results, it was suggested that 2'-methyl group of ABA was indispensable for the strong growth inhibitory activity.

For further elucidation of the effect of 6'-methyl groups on the cyclohexene ring on physiological activity, we synthesized methyl 6', 6'-didemethyl abscisate (**5**). In addition, two new chiral stereoisomeric analogs **6** and **7** were synthesized from readily accessible *l*- and *d*-carvone.

At first, we synthesized methyl 6', 6'-didemethyl abscisate (**5**) from 2-methyl-4, 4-ethylenedioxy-1-cyclohexenecarbaldehyde (**9**)³⁾ which was prepared from Hageman's ester (**8**). The aldol condensation of **9** with acetone afforded the ketone **10**. The Wittig reaction of **10** with methoxycarbonylmethyl-enetriphenylphosphorane gave the ester **11** as

a stereoisomeric mixture of (2 *Z*) and (2 *E*) isomers (*ca.* 1:1 by GLC), which was converted to the epoxide **12** by epoxidation with *m*-chloroperbenzoic acid. Although drastic hydrolysis of acetal **12** with several acids at high temperature gave many unseparable products, treatment of **12** with *p*-toluenesulfonic acid in 1, 2-dimethoxyethane-water at room temperature gave the crystalline glycol **13** of mp 79~80°C. Treatment of this acetal **13** with oxalic acid in tetrahydrofuran-water (1:1) under reflux afforded a stereoisomeric mixture of (2 *Z*)-ester **5** and (2 *E*)-ester **14**, which was separated into each component by preparative TLC.

Next, two chiral analogs **6** and **7**, structurally related to ABA, were synthesized as shown in

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Scheme I

Scheme II

Scheme II. The reaction of *l*-carvone with the Grignard reagent prepared from (*Z*)-3-methyl-2-penten-4-yn-1-ol in methylene chloride⁴⁾ gave a diastereomeric mixture of diol **15** which was unseparable by HPLC (HP-01). The diastereomeric mixture **15** was reduced with sodium bis-(2-methoxyethoxy)-aluminum hydride to give the diene-diol **16**. Oxidation of the diol **16** with active manganese dioxide in methylene chloride, followed by treatment with active manganese dioxide in methanol containing sodium cyanide and acetic acid, afforded a diastereomeric mixture of esters. The *trans* isomer concerning 5'-isopropenyl group and 1'-side chain was obtained by recrystallization, but *cis* isomer was not isolated. The stereochemistry of this *trans* isomer **6** was deduced by CD spectra. Comparison of the CD spectrum of **6** with that of (*R*)-ethyl 1'-hydroxy- α -ionylideneacetate⁵⁾ showed that **6** had (1' *S*) configuration. The optical antipode **7** was prepared from *d*-carvone following the same method.

Growth inhibitory activity of esters **5**, **6** and **7** were tested on rice seedling assay. Compounds **5** and **6** were also tested on germination assay of garden radish seeds. Methyl

TABLE I. GROWTH RESPONSE OF ABA ANALOGS ON RICE SEEDLINGS (*Oryza sativa* L. cv. Koshizi-wase)

Compounds	Concentration (M)	Plant height (%)	Second leaf sheath (%)	Root (%)
5	5×10^{-4}	5.6 ^a	1.5 ^a	60.4 ^a
	2×10^{-4}	8.95	5.8	100
	1×10^{-4}	12.0	10.0	106
	2×10^{-5}	68.3	60.0	106
6	1×10^{-4}	105	102	104
	5×10^{-5}	106	106	138
7	1×10^{-3}	103	97.0	93.0
	1×10^{-4}	103	103	129
ABA	2×10^{-5}	6.3	3.4	14.9

^a Percentage of control growth after growing at 28°C (2000 lux) for 5 days.

TABLE II. GERMINATION INHIBITORY ACTIVITY OF ABA ANALOGS ON GARDEN RADISH SEEDS (*Raphanus sativus* L. cv. Natsuminowase No.2)

Compounds	Concentration (M)	Germination
5	1×10^{-5}	0 ^a
6	3×10^{-3}	70
	1×10^{-3}	70
Control		70

^a Percentage of germination after incubating at 28°C (2000 lux) for 6 days.

6',6'-didemethyl abscisate (**5**) showed more than 5% inhibition compared with the activity of ABA at the concentration of 2×10^{-8} M. On the other hand, two optically active analogs **6** and **7** were inactive.

These results suggest that 6'-geminal dimethyl groups are not essential for the appearance of the biological activity of ABA.

EXPERIMENTAL

All mp's and bp's were uncorrected. IR spectra were recorded on JASCO IRA-1 spectrophotometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a JEOL JM-MH-60-II and 100 MHz on a JEOL PS-100 spectrometer. Gas-liquid chromatography was carried out on a JEOL JGC-1100 and Hitachi gas chromatograph 163 (glass capillary column). High performance liquid chromatography was carried out on a JASCO TRI ROTAR. Mass spectra were obtained at 70 eV and 25 eV utilizing a Hitachi M-52 mass spectrometer by direct injection. CD spectra were recorded on Union Giken Dichrograph Mark III-J.

1) (*E*)-4-(4',4'-ethylenedioxy-2'-methyl-1'-cyclohexenyl)-3-buten-2-one (**10**). A mixture of 10 g of 4,4-ethylenedioxy-2-methyl-1-cyclohexenecarbaldehyde (**9**), 77.5 ml of acetone, 100 ml of 5% aq. NaOH and 0.5 g of NaCl was stirred at room temperature for 14 hr. The reaction mixture was poured into water and extracted twice with ether. The combined extracts were washed with saturated NaCl solution, dried over anhydrous K_2CO_3 and evaporated. The residue was distilled under reduced pressure to give 6.3 g of oily **10**, bp 159~162°C/3 mmHg. IR $\nu_{\max}^{\text{film}}$ cm^{-1} : 1665, 1620, 970. NMR (CCl_4) δ : 1.90 (3H, bs), 2.19 (3H, s), 3.68 (4H, s), 5.90 (1H, d, $J=16$ Hz), 7.40 (1H, d, $J=16$ Hz). UV $\lambda_{\max}^{\text{EtOH}}$ nm: 292.

2) Methyl (2*Z* and *E*, 4*E*)-5-(4',4'-ethylenedioxy-2'-methyl-1'-cyclohexenyl)-3-methyl-2,4-pentadienoate (**11**). A mixture of 11.3 g of **10** and 45.5 g of methoxycarbonylmethylenetriphenylphosphorane was stirred for 2 hr at 165°C under nitrogen. After cooling to room temperature, ether was added and deposited triphenylphosphine oxide was separated by filtration. The filtrate was condensed and the residue was fractionated by distillation under reduced pressure. A fraction boiling at 186~187°C/4.0~4.2 mm was chromatographed on 120 g of florisil to give 3.9 g of **11** as an oil. IR $\nu_{\max}^{\text{film}}$ cm^{-1} : 1705, 1600. NMR (CCl_4) δ : 1.85 (3H, bs), 2.05 (3H, s), 3.63 (3H, s), 3.87 (4H, s), 5.55 (1H, m), 6.93 (1H, d, $J=16$ Hz), 7.77 (1H, d, $J=16$ Hz), 5.7 (1H, bs), 6.1 (1H, d, $J=16$ Hz). UV $\lambda_{\max}^{\text{EtOH}}$ nm: 312. Gas

liquid chromatographic analysis using a 1 m $\times$ 3 mm column packed with 10% SE-30 on Chromosorb W, (column temperature: 220°C, FID, N_2 flow rate 20 ml/min) showed two peaks at $t_R=8.7$ min for (2*Z*) ester and 7.7 min for (2*E*) ester in the ratio of 1:1.

3) Methyl (2*Z* and *E*, 4*E*)-5-(4',4'-ethylenedioxy-2'-methyl-1',2'-epoxycyclohexyl)-3-methyl-2,4-pentadienoate (**12**). *m*-Chloroperbenzoic acid (123.4 mg) was slowly added in small portions to a stirred mixture of **11** (200 mg) in methylene chloride (7.2 ml) and aq. $NaHCO_3$ (2.2 ml, pH 8.0). The mixture was stirred at room temperature for 1 hr. The reaction mixture was poured into saturated $NaHCO_3$ solution, extracted with methylene chloride. The combined extracts were washed with saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4 . Evaporation gave a viscous oil **12**. As the product was unstable to alumina, silica gel, and florisil, it was used for next reaction without purification. IR $\nu_{\max}^{\text{film}}$ cm^{-1} : 1720, 1640, 1610. NMR (CCl_4) δ : 1.15 (3H, s), 1.20 (3H, s), 1.96 (3H, bs), 3.62 (3H, s), 3.82 (4H, s), 5.73 (1H, bs), 6.02 (1H, d, $J=16.5$ Hz), 7.62 (1H, d, $J=16$ Hz), 2.22 (3H, s), 5.6 (1H, bs), 6.32 (1H, d, $J=16$ Hz).

4) Methyl (2*Z* and *E*, 4*E*)-5-(4',4'-ethylenedioxy-1',2'-dihydroxy-2'-methylcyclohexyl)-3-methyl-2,4-pentadienoate (**13**). A mixture of 200 mg of **12**, 6.0 mg of *p*-toluenesulfonic acid, 1.35 ml of DME, and 0.2 ml of water was stirred at room temperature for 4 hr. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with ether three times. Combined extracts were washed with water, dried over anhydrous Na_2SO_4 . After evaporation, the residue was purified by preparative TLC on silica gel (Merck PF₂₅₄) using chloroform-acetone (6:1) to give **13** as a crystalline mass. Recrystallization from benzene-hexane gave 87 mg of pure glycol **13**, mp 79~80°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3420, 1720, 1710, 1630, 1600. NMR ($CDCl_3$) δ : 1.09 (3H, s), 2.05 (3H, s), 3.71 (3H, s), 3.99 (4H, s), 5.69 (1H, bs), 6.61 (1H, d, $J=16$ Hz), 7.81 (1H, d, $J=16$ Hz), 2.2 (3H, s), 5.79 (1H, bs), 6.36 (1H, d, $J=16$ Hz). UV $\lambda_{\max}^{\text{EtOH}}$ nm: 265. Gas liquid chromatographic analysis using a 11.7 m glass capillary column coated with OV-101 (column temperature: 220°C, FID, N_2 flow rate: 1.7 ml/min) showed two peaks at $t_R=2.5$ min for (2*Z*)-ester and 3.2 min for (2*E*)-ester in the ratio of 1:1.24. Anal. Found: C, 61.89; H, 7.92%. Calcd. for $C_{16}H_{24}O_8$: 61.52; H, 7.75.

5) Methyl (2*Z*, 4*E*)-5-(1'-hydroxy-2'-methyl-4'-oxo-2'-cyclohexenyl)-3-methyl-2,4-pentadienoate (**5**). A mixture of 87 mg of **13**, 900 mg of oxalic acid, 8.6 ml of THF-water (1:1) was refluxed for 2.5 hr. After cooling to room temperature, reaction mixture was saturated with NaCl and extracted with methylene chloride. The extract was washed with saturated

NaHCO₃ solution, saturated NaCl solution, dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by preparative TLC on silica gel using chloroform-acetone (4:1) to give 5 mg of **5** and 30 mg of (2*E*) isomer **14** as a crystal. Recrystallization from benzene-hexane gave 3.5 mg of **5**, mp 102.5~103°C. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3430, 1715, 1660, 1600, 1440, 1240, 1160, 980, 925. NMR (CCl₄) δ : 1.93 (3H, s), 2.01 (3H, s), 2.80 (1H, m), 3.64 (3H, d, *J*=1 Hz), 5.64 (1H, m), 5.79 (1H, m), 6.04 (1H, d, *J*=16 Hz), 7.73 (1H, d, *J*=16 Hz). MS *m/e* 232 (M-18), 218 (M-32), 125 (100). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 262 (ϵ =13400), 238 (sh).

Methyl (2*E*, 4*E*)-5-(1'-hydroxy-2'-methyl-4'-oxo-2'-cyclohexenyl)-3-methyl-2, 4-pentadienoate (**14**). IR $\nu_{\text{max}}^{\text{CCl}_4}$ cm⁻¹: 3440, 1725, 1660, 1610, 1440, 1240, 1160, 980, 925. NMR (CCl₄) δ : 1.89 (3H, s), 2.25 (3H, s), 2.73 (1H, m), 3.64 (3H, s), 5.76 (2H, m), 6.02 (1H, d, *J*=16 Hz), 6.53 (1H, d, *J*=16 Hz).

6) (Z)-5-[(1'*S* and R, 5'*S*)-1'-Hydroxy-5'-isopropenyl-2'-methyl-2'-cyclohexenyl]-2-penten-4-yn-1-ol (**15**). To a solution of ethyl magnesium bromide (from 1.3 g of magnesium and 9.5 g of ethyl bromide) in ether (40 ml), (Z)-3-methyl-2-penten-4-yn-1-ol (2.8 g) in methylene chloride (20 ml) was added dropwise at 20°C and the mixture was stirred for further 1 hr. A solution of 1-carvone (2.19 g) in methylene chloride (20 ml) was added dropwise and the mixture was stirred under reflux overnight and then cooled. Saturated NH₄Cl solution was added to decompose the Grignard complex. The reaction mixture was extracted with ether. The extract was washed with saturated NaCl solution and dried over anhydrous Na₂SO₄. After evaporation, the residue was chromatographed on 100 g of silica gel to give 2.7 g of **15** as a viscous oil. IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3360, 3100, 3040, 1645. NMR (CDCl₃) δ : 1.74 (3H, s), 1.84 (6H, bs), 4.22 (2H, d, *J*=6 Hz), 4.5 (2H, m), 4.69 (2H, bs), 5.38 (1H, m), 5.73 (1H, t, *J*=6 Hz). Liquid chromatographic analysis using HP-01 column [solvent methanol-water (96:1), UV detector (240 nm)] showed a single peak, *t_R*=2.8 min.

7) (2*Z*, 4*E*)-5-[(1'*S* and R, 5'*S*)-1'-Hydroxy-5'-isopropenyl-2'-methyl-2'-cyclohexenyl]-3-methyl-2, 4-pentadien-1-ol (**16**). A 70% solution (5.4 ml) of sodium bis-(2-methoxyethoxy)-aluminum hydride in toluene was diluted with THF (5.4 ml) and then added dropwise to a solution of **15** (900 mg) in THF (10 ml). The mixture was stirred at room temperature for 1.5 hr. and then an excess of hydride was decomposed by a cautious addition of saturated NH₄Cl solution (20 ml). The products were extracted with ethyl acetate. The combined extracts were washed with saturated NaCl solution, dried over anhydrous Na₂SO₄ and evaporated. The residue was chromatographed on 34 g of silica gel

to give 700 mg of **16** as an oil. IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3360, 3100, 3040, 1645, 970. NMR (CDCl₃) δ : 1.7 (6H, s), 1.90 (3H, s), 3.28 (2H, m), 4.23 (2H, d, *J*=4 Hz), 4.7 (2H, m), 5.74 (1H, d, *J*=16 Hz), 6.56 (1H, d, *J*=16 Hz).

8) Methyl (2*Z*, 4*E*)-5-[(1'*S*, 5'*R*)-1'-hydroxy-5'-isopropenyl-2'-methyl-2'-cyclohexenyl]-3-methyl-2, 4-pentadienoate (**6**). Manganese dioxide (6 g) was added to a solution of **16** in methylene chloride (50 ml), and the mixture was shaken at room temperature for an hour and then filtered. The filtrate was evaporated and the residue was shaken with manganese dioxide (6.4 g), NaCN (640 mg) and acetic acid (0.15 ml) in methanol (50 ml) at room temperature overnight. After filtration, methanol was removed from the filtrate under reduced pressure. The residue was purified by preparative TLC on silica gel to give 310 mg of diastereomeric mixture of **6**. Crystallization from hexane-ethyl acetate gave 25 mg of **6**, mp 63.5~64°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3440, 3090, 1720, 1635, 1600, 1160, 890. NMR (CDCl₃) δ : 1.73 (6H, s), 2.03 (3H, s), 2.78 (1H, s), 3.67 (3H, s), 4.7 (2H, s), 5.82 (1H, m), 6.08 (1H, d, *J*=16 Hz), 7.54 (1H, d, *J*=16 Hz). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 265 (ϵ =1800). MS *m/e*: 276 (M⁺), 258 (M-18), 244 (M-32), 125 (100). CD $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 262 [θ]=53800. Anal. Found: C, 73.87; H, 8.80%. Calcd. for C₁₇H₂₄O₃: C, 73.88; H, 8.75.

Methyl (2*Z*, 4*E*)-5-[(1'*R*, 5'*S*)-1'-hydroxy-5'-isopropenyl-2'-methyl-2'-cyclohexenyl]-3-methyl-2, 4-pentadienoate (**7**). CD $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 262, [θ]=+44000.

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REFERENCES

- 1) B. V. Milborrow, *Ann. Rev. Plant Physiol.*, **25**, 259 (1974).
- 2) M. Nanzyo, T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **41**, 1711 (1977).
- 3) E. Baggiolini, H. P. Hamlow and K. Schaffner, *J. Am. Chem. Soc.*, **92**, 4906 (1970).
- 4) M. Akhtar, A. E. Faruk, C. J. Harris, G. P. Moss, S. W. Russell and B. C. L. Weedon, *J. Chem. Soc. Perkin I*, **1978**, 1511.
- 5) T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **37**, 1115 (1973).