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Note

Syntheses of (±)-Methyl 6′α-Demethyl-6′α-cyanoabscisate and (±)-Methyl 6′α-Demethyl-6′α-methoxycarbonylabscisate

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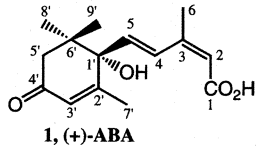
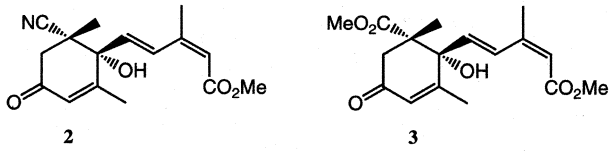
New abscisic acid analogs possessing a cyano or methoxycarbonyl group at the 6′α-position of methyl abscisate were synthesized by regioselective hydrocyanation. These compounds had weak activity in the rice second leaf sheath elongation test.

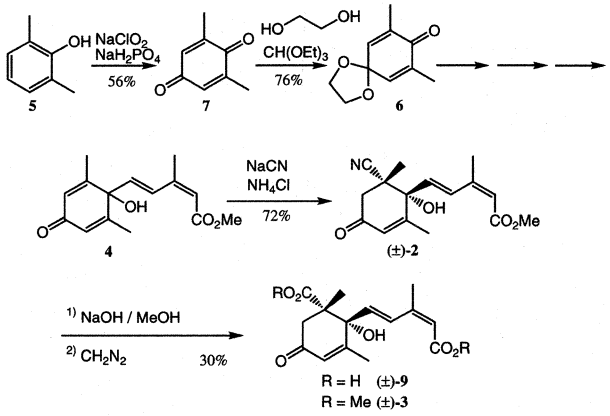
Key words: 6′α-cyano ABA; 6′α-methoxycarbonyl ABA; Michael reaction; rice seedling test

Abscisic acid (ABA **1**) is one of the plant hormones, regulating dormancy, tolerance to drought, stomatal closing of plants and so on.[1] It is believed to lose these activities by oxidation at the 8′-methyl group to form 8′-hydroxy ABA and its cyclized product, phaseic acid.[2,3] Much attention has therefore been given to inhibiting the oxidation at the 8′-methyl group of ABA and the cyclization reaction to form phaseic acid, and many 8′-substituted ABA analogs have been reported,[4,5] some of which had much stronger activity than that of ABA itself.[6–9] We aimed to produce more active ABA analogs and synthesized (±)-methyl 6′α-demethyl-6′α-cyanoabscisate (**2**) and (±)-methyl 6′α-demethyl-6′α-methoxycarbonylabscisate (**3**) which would not cyclize to form phaseic acid-type compounds. These compounds were examined for their biological activity by using the rice seedling test. We report here the synthesis of these compounds and their biological activities.





Scheme 1. ABA and new ABA Analogs.



Scheme 2. Syntheses of ABA Analogs.

We planned to produce these compounds by the stereoselective Michael addition of a cyano group[10] to the quinol compound (**4**) which had been reported by B. Lei *et al.*[11] Compound **4** was synthesized by the modified procedure of Lei *et al.* In their procedure, 2,6-dimethylphenol (**5**) was oxidized to form compound **6** by using iodobenzene diacetate. We tried to oxidize **5** by using cheaper reagents, and succeeded in oxidizing **5** to benzoquinone **7** by using $NaClO_2$. **7** was then acetalized with ethylene glycol to give compound **6**, which was converted to quinol **4** according to Lei's procedure.[11] Quinol **4** was then heated with NaCN and NH_4Cl in DMF for 2 days to give (±)-**2** in a 72% yield. The other target, (±)-**3**, was obtained by hydrolyzing (±)-**2** and then methylating with CH_2N_2 in a 30% yield (Scheme 2). The ^1H- and ^{13}C-NMR spectra of **2** and **3** showed single compounds, and no regioisomers were detected.

The relative configurations of **2** and **3** were determined after their conversion to **8** by comparing the ^1H-NMR spectra with that of methyl phaseate (**8**). Thus, (±)-**3** was reduced with $LiAlH_4$ and then oxidized with MnO_2 to afford **8**. The ^1H-NMR spectrum of this compound matched that of the authentic spectrum of methyl phaseate[12] but not of methyl epiphaseate[12] (Scheme 3).

The plant growth regulatory activities of (±)-**2** and

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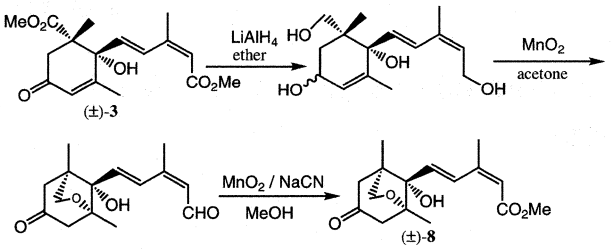

Scheme 3. Determination of Relative Configuration.

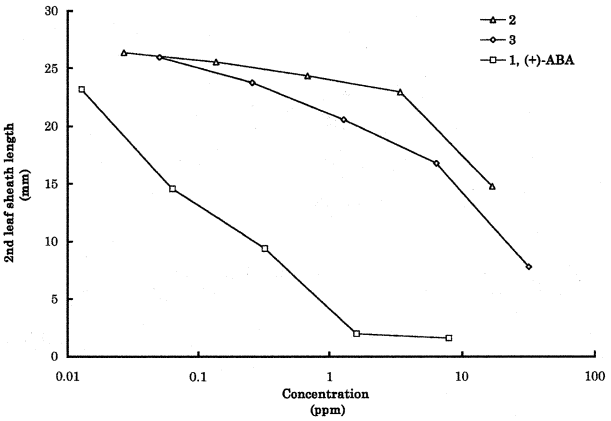

Fig. Effect of **1**–**3** on the Growth of Rice Seedlings.

(±)-**3** were examined by using the rice second leaf sheath elongation test. (±)-**3** showed about 100 times weaker activity than that of (+)-ABA, while (±)-**2** exhibited weaker activity than that of (±)-**3** (Figure). While it is possible that the enzymatic hydrolysis of (±)-**2** and (±)-**3** in a plant would give 6′α-hydroxycarbonyl ABA (**9**), the preparation of methyl ester (±)-**3** from nitrile (±)-**2** *via* free acid **9** indicates that **9** didn't naturally cyclize to a phaseic acid-type of compound. (±)-**9** did not show effective growth inhibition in the rice seedling test. These results indicate (±)-**2**, (±)-**3** and (±)-**9** had no biological activities like those of ABA.

Experimental

Melting point (mp) data were determined with a Yanaco MP J 3 instrument and are uncorrected. IR spectra were recorded with JASCO IR Report-100 apparatus, and only strong or structurally important peaks are listed. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian GEMINI 2000/300 (300 MHz for 1H and 75 MHz for ^{13}C), Varian Unity INOVA 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) or Varian Unity INOVA 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) instrument, using $CDCl_3$ as the solvent. Analytical TLC was performed on Merck silica gel 60 F_{254} plates visualized with UV (254 nm) light and by phosphomolybdic acid (5% in EtOH) or by 2,4-dinitrophenylhydrazine (in *aq.* H_2SO_4 and EtOH). Column chromatography was performed with Merck silica gel 60 of 0.063–0.200 mm mesh size, and preparative TLC plates were made from Merck silica gel 60 PF_{254}.

2,6-Dimethyl-1,4-benzoquinone (**7**). To a solution of 2,6-dimethylphenol (10 g, 82 mmol) and NaH_2PO_4 (20 g, 170 mmol) in 150 ml of hexane and 50 ml of ethylene glycol was gradually added $NaClO_2$ (22 g, 240 mmol) while vigorously stirring at rt for 1 h. A large-scale experiment needs careful control of the reaction to avoid an explosion. To the resulting mixture was added water. The organic layer was separated, and the aqueous layer was extracted with ether (100 ml × 2). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and condensed *in vacuo* below 30°C. Purification of the residue by silica gel column chromatography (hexane:ethyl acetate = 7:1) afforded 6.3 g (46 mmol, 56%) of **7** as yellow crystals. An analytical sample was obtained by recrystallization from hexane/diethyl ether as yellow needles, mp 70.0–70.9°C (lit.[13] 72–73°C). IR ν_{max} (KBr) cm^{-1}: 3030, 1650, 1610, 1380, 1310, 1290, 1180, 920. ^{1}H-NMR (300 MHz) δ: 2.06 (6H, s, -C*H*$_3$), 6.56 (2H, s, H-3, 5). ^{13}C-NMR (75 MHz) δ: 16 (*-CH$_3$), 133 (C-3, 5), 146 (C-2, 6), 187.7, 188.3.*

2,6-Dimethyl-4,4-ethylenedioxycyclohexa-2,5-dienone (**6**). To a solution of triethyl orthoformate (7.0 ml, 42 mmol), quinone **7** (2.9 g, 21 mmol) and ethylene glycol (10 ml, 180 mmol) in 30 ml dry ether was added *p*-TsOH·1H_2O (150 mg, 0.77 mmol), and the mixture was stirred at rt for 2 d. The resulting mixture was poured into sat. *aq.* $NaHCO_3$ and extracted with ether (50 ml × 3). The organic layer was washed with brine, dried over $MgSO_4$, filtered and then condensed *in vacuo*. Purification of the residue by silica gel column chromatography (hexane:ethyl acetate = 5:1) gave 2.9 g (16 mmol, 76%) of **6** as white crystals. mp 45.9–46.9°C (lit.[11] 47–49°C). IR ν_{max} (KBr) cm^{-1}: 2960, 2900, 1680, 1640, 1350, 1220, 1090, 1030, 970, 950, 760. ^{1}H-NMR (300 MHz) δ: 1.88 (6H, s, -C*H*$_3$), 4.12 (4H, s, -O-C*H*$_2$-C*H*$_2$-O-), 6.42 (2H, s, H-3, 5). ^{13}C-NMR (125 MHz) δ: 16 (-*C*H$_3$), 65 (-O-*C*H$_2$-*C*H$_2$-O-), 99 (C-4), 136 (C-2, 6), 138 (C-3, 5), 187 (C-1).

(±)-Methyl 6′α-demethyl-6′α-cyanoabscisate (**2**). A mixture of quinol **4** (260 mg, 1.0 mmol), NaCN (54 mg, 1.1 mmol) and NH_4Cl (57 mg, 1.1 mmol) in 6 ml of DMF and 1 ml of water was heated in an oil bath (90–100°C) for 31 h. To the resulting mixture was added water and ether, and the solution was extracted with ether (40 ml × 3). The organic layer was washed with brine, dried over $MgSO_4$, filtered and condensed *in vacuo*. The crude product was carefully purified by preparative TLC (hexane:ethyl acetate = 3:4) to

afford 210 mg (0.72 mmol, 72%) of monocyanide **2** as a colorless oil. The dicyanide (31 mg, 0.10 mmol, 10%) was also obtained in a less-polar field. An analytical sample was obtained by recrystallization (hexane/diisopropyl ether) as white crystals, mp 128.0–130.4°C. IR ν_{max} (film) cm^{-1}: 3440, 2990, 2950, 2950, 2240, 1710, 1670, 1240, 1160. ^{1}H-NMR (300 MHz) δ: 1.48 (3H, s, H-9′), 2.01 (3H, d, J=1.4 Hz), 2.04 (3H, d, J=1.4 Hz), 2.55 (1H, d, J=18 Hz, H-5′), 2.81 (1H, dd, J=1.1, 18 Hz, H-5′), 3.31 (1H, br. -OH), 3.71 (3H, s, $-OCH_3$), 5.82 (1H, d, J=0.5 Hz), 5.88 (1H, d, J=16 Hz, H-5), 6.13 (1H, t, J=1.2 Hz), 7.91 (1H, d, J=16 Hz, H-4). ^{13}C-NMR (150 MHz) δ: 18.9, 20.5, 21.0, 45.3, 45.5, 51.4, 77.6, 119.7, 121.9, 128.0, 130.3, 131.4, 148.2, 162.4, 166.2, 192.9 (C-4′). HRFABMS (NOBA): $[M+H]^+$ at m/z 290.1391 (calcd. for $C_{16}H_{20}O_4N$, m/z 290.1392). *Anal.* Calcd. for $C_{16}H_{19}O_4N$: C, 66.42; H, 6.62; N, 4.84%. Found: C, 66.12; H, 6.56; N, 4.71%.

(±)-Methyl 6′α-demethyl-6′α-methoxycarbonylabscisate (**3**). A solution of (±)-**2** (89 mg, 0.31 mmol) in 3 ml of MeOH was added to 10% NaOH *aq.* (10 ml) at an ice-bath temperature and stirred at rt for 13 h. The resulting mixture was acidified by 1 M HCl *aq.* to pH 1 and then extracted with ether (50 ml ×3). The organic layer was washed with brine, dried over $MgSO_4$, filtered and then condensed *in vacuo*. The crude dicarboxylic acid was dissolved in 30 ml of $CHCl_3$ and then treated with CH_2N_2 in the usual manner. The resulting mixture was concentrated *in vacuo*, and the residue was purified by preparative TLC (hexane:ethyl acetate=2:1) to give 30 mg (0.093 mmol, 30%) of diester **3** as a colorless oil. An analytical sample was obtained by recrystallization from diisopropyl ether as white cubic crystals, mp 121.0–121.6°C. IR ν_{max} (film) cm^{-1}: 3440, 2950, 1700, 1660, 1600, 1450, 1440, 1240, 1200, 1160. ^{1}H-NMR (500 MHz) δ: 1.35 (3H, s, H-9′), 1.99 (3H, d, J =1.5 Hz), 2.00 (3H, d, J=1.2 Hz), 2.50 (1H, d, J= 18 Hz, H-5′), 2.94 (1H, dd, J=1.3, 18 Hz, H-5′), 3.70 (3H, s), 3.72 (3H, s), 5.32 (1H, d, J=1.2 Hz, -OH), 5.76 (1H, s), 5.94 (1H, d, J=16 Hz, H-5), 5.95 (1H, s), 7.90 (1H, dd, J=0.7, 16 Hz, H-4). ^{13}C-NMR (125 MHz) δ: 19.3, 19.9, 21.0, 45.2, 51.2, 52.3, 53.1, 78.2, 118.9, 127.1, 130.9, 132.5, 148.9, 166.2, 167.4, 178.4, 195.3 (C-4′). HREIMS: $[M]^+$ at m/z 322.1414 (calcd. for $C_{17}H_{22}O_6$, m/z 322.1416).

(±)-Methyl phaseate (**8**). To a suspension of $LiAlH_4$ (21 mg, 0.55 mmol) in dry ether (2 ml) was added **3** (7 mg, 0.02 mmol) in dry ether (2 ml), and the mixture was stirred for 15 min. To the resulting mixture was added a small amount of water, before acidifying with a small amount of 1 M HCl *aq.* to pH 4. To this mixture were then added EtOAc (100 ml) and $MgSO_4$, before stirring vigorously for 20 min. The mixture was then filtered through a pad of Celite, condensed *in vacuo* and dissolved in 10 ml of acetone. To this solution was added MnO_2 (570 mg, 6.6 mmol), before stirring at rt for 12 h. This mixture was then filtered through a pad of Celite, condensed *in vacuo* and dissolved to MeOH (15 ml). To this solution were added MnO_2 (1.0 g, 12 mmol), NaCN (50 mg, 1.0 mmol) and AcOH (60 μl, 1.0 mmol), and the mixture was stirred at rt for 1 d. The resulting mixture was then filtered through a pad of Celite and condensed *in vacuo*. The residue was purified twice by preparative TLC (hexane:ethyl acetate=1:1) to give 1 mg (0.003 mmol, 15%) of (±)-methyl phaseate (**8**). The ^{1}H-NMR spectrum of this compound matched that of the authentic specimen.[12]

Bioassay. In a 0.75% agar medium (8 ml) containing (±)-**2**, (±)-**3** or (+)-ABA were sown 5 seeds of rice (*Oryza sativa* L. cv. *Kokoromachi*), which had been preincubated until the germ length was about 2 mm, and the sown seeds were incubated in continuous light at 27°C for 6 d. The length of second leaf sheath was measured and the average value from the 5 seeds was calculated.

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