Convenient Synthesis and Cytokinin Activity of β-Substituted 4-Styrylpyridines, the Simplest Cytokinin Analogs with a Moderate Cell Division-Promoting Activity

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Designed synthesis of *Z*- and *E*-isomers of β-substituted 4-styrylpyridines as cytokinin analogs was conveniently achieved by nucleophilic and electrophilic addition of ethanol, methyl mercaptan, and hydrogen halides (HCl, HBr, and HI) to 4-(phenylethynyl)pyridine prepared by palladium-catalyzed coupling. They easily underwent *E*−*Z* photoisomerization under monochromatic UV light or sunlight. A tobacco callus assay revealed that the *Z*-isomers were more active than their *E*-isomers and that the *Z*-ethoxy derivative, which showed the highest activity among the β-substituted 4-styrylpyridines, was one-fifth as potent as kinetin. The *Z*-ethoxy derivative also promoted betacyanin biosynthesis of *Amaranthus* seedlings at 4−100 μM.

Keywords: *Synthetic cytokinin; 4-styrylpyridines; palladium-catalyzed coupling; addition reaction; photoisomerization; tobacco callus bioassay; betacyanin bioassay*

INTRODUCTION

Cytokinins promote cell division of plant tissues. Recent studies on their analogs revealed that the purine ring is not necessarily required for strong cytokinin activity and is replaceable with other nitrogen-containing heterocycles. For example, modification of N^6-benzyladenine (BA, **1**) on the basis of analysis of structural correlation led to the finding of *N*-(2-chloro-4-pyridyl)-*N*′-phenylurea (**3**), the strongest synthetic cytokinin ever known, via pyrimidine derivative **2** (Figure 1; Okamoto et al., 1981; Shudo, 1994). Most natural and synthetic cytokinins are *N*-alkyl and *N*-acyl derivatives. In contrast, there are few carbon-substituted cytokinin analogs, although they give insight into the mode of the cytokinin−receptor interaction that has not been fully studied. For these *N*-alkyl and *N*-acyl derivatives, analogous modification is also available. In fact, 6-*trans*-styrylpurine derivative **4** (Nishikawa et al., 1985, 1994) and 4-styrylpyrimidine derivative **5** (Nishikawa et al., 1989) promoted significantly the growth of tobacco callus tissues as well as the betacyanin biosynthesis of *Amaranthus* seedlings. These results indicate that the exocyclic nitrogen atoms of N^6-substituted adenines can be replaced with a sp^2 carbon atom without lowering their cytokinin activity. On the other hand, however, little has been known about the function of nitrogen atoms of the heterocyclic rings, particularly the purine ring of N^6-substituted adenines on cytokinin action at the molecular level, in spite of the fact that there have been some systematic studies on deazaadenines (Rogozinska et al., 1973; Sugiyama et al., 1975).

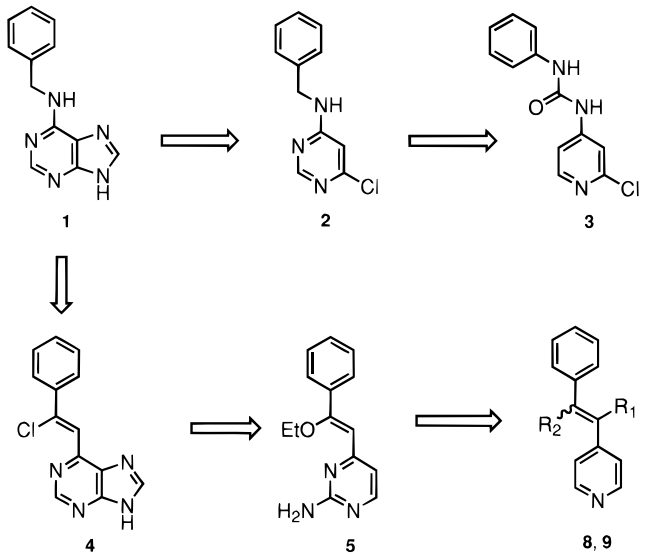

Figure 1. Structural relationships of *N*- and carbon-substituted cytokinin analogs.

Our main concern is to specify that the nitrogen atom of the purine ring participated in cytokinin−receptor binding and to disclose the role of other nitrogen atoms in the binding by comparative studies on purine and non-purine cytokinins. Pyridine derivatives which have a minimal structure required for the activity are ideal for this purpose. Our success in the development of the carbon-substituted derivatives **4** and **5** prompted us to develop 4-styrylpyridine analogs **8** and **9** as a logical extension (Scheme 1), although (*E*)-4-styrylpyridine, known as stilbazole, was completely inactive in a betacyanin bioassay (Nishikawa et al., 1989). For the synthesis of β-substituted 4-styrylpyridines, 4-(phenylethynyl)pyridine (**7**) is a key intermediate. Palladium-catalyzed coupling using CuI as cocatalyst (Sonogashira et al., 1975) has been widely used to introduce an alkynyl group into pyridine (Kanesho Co. Ltd., 1981; Sakamoto et al., 1986) because of its convenience and

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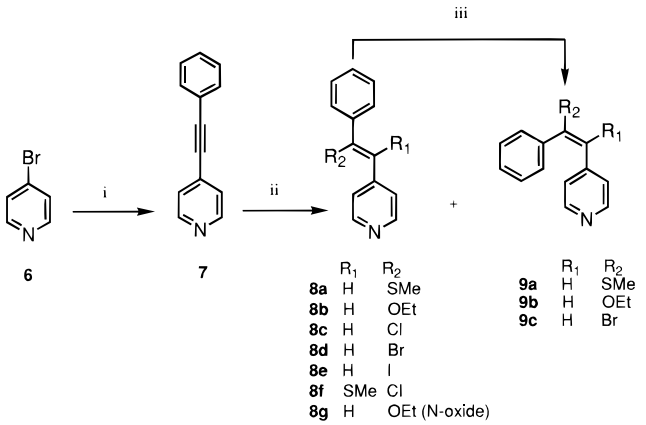
Scheme 1. Synthesis of β-Substituted 4-Styrylpyridines[a]



[a] (i) PhC≡CH, $(PPh_3)_2PdCl_2$, CuI, Et_3N/MeCN; (ii) HX or Me_3SiX (X = Cl, Br, I)/$CHCl_3$, NaOEt/EtOH or MeSNa/EtOH; (iii) *hν*/AcOEt.

high efficiency. Simple addition of small molecules including hydrogen halide, alcohol, and mercaptan to **7** together with *E*−*Z*-photoisomerization would provide β-substituted (*Z*)- and (*E*)-4-styrylpyridines, as in the cases of 6-styrylpurines (Nishikawa et al., 1985, 1994) and 4-styrylpyrimidines (Nishikawa et al., 1989). Thus we have synthesized some β-substituted 4-styrylpyridines conveniently from commercially available 4-bromopyridine (**6**) via alkynyl compound **7** prepared by the palladium-catalyzed coupling and tested their cytokinin activity in both betacyanin and tobacco callus bioassays. Eventually, we found *Z*-β-ethoxy derivative **8b** which exhibited activity at the same order of concentration as kinetin in the tobacco callus bioassay. To our knowledge, there has been no report on cytokinin activity of carbon-substituted pyridine derivatives with a moderate cytokinin activity.

This paper describes convenient synthesis of the β-substituted 4-styrylpyridines and their cytokinin activity in both bioassays. In addition, a possible role of nitrogen atoms of N^6-substituted adenines is discussed based on their structural similarity to the new cytokinin analogs.

MATERIALS AND METHODS

Melting points were measured with a Yanagimoto hot stage apparatus and are uncorrected. UV and IR spectra were recorded on a Hitachi UV 200-10 or Shimazu UV 300 spectrometer and a Shimazu IR 470 spectrophotometer, respectively. EI and HR mass spectra were taken with a Hitachi M80-B and a JEOL JMS-DX-303 spectrometer, respectively. ^{1}H NMR spectra were measured with a Hitachi R-90H spectrometer (90 MHz) using tetramethylsilane as an internal standard. For measurement of HMBC spectra, a JEOL JNM-A500 spectrometer (500 MHz) of the Mie University Cooperative Research Center was used. Microanalyses were performed with a Yanagimoto MT-3 CHN apparatus. For photoisomerization at a given wavelength, a JASCO CT-10s monochrometer equipped with a 150 W xenon lamp was used. For TLC and column chromatography, Merck precoated silica gel plates 60_{F254} and Wakogel C-200 or Fuji Silisia BW-200 were used, respectively. Unless otherwise noted, all operations including syntheses and bioassays were carried out with shielding from light or under light as weak as possible because of lability to light of the β-substituted 4-styrylpyridines synthesized.

Synthesis of Compounds. *6-(Phenylethynyl)pyridine (**7**).* In a manner similar to the reported methods (Sonogashira et al, 1975; Kanesho Co., 1981), a mixture of 4-bromopyridine hydrochloride (**6**; 7.00 g, 36.0 mmol; Aldrich), phenylacetylene (4.04 g, 39.6 mmol), $(PPh_3)_2PdCl_2$ (253 mg, 0.36 mmol), and CuI (70 mg, 0.36 mmol) in triethylamine (18 mL) in acetonitrile (50 mL) was stirred under a nitrogen atmosphere at room temperature for 44 h. The resultant crystals of triethylamine hydrochloride were filtered off, and the filtrate was evaporated under reduced pressure to give the residue. Purification of the residue by column chromatography (*n*-hexane−ethyl acetate, 3:1, v/v) afforded **7** as yellow crystals (5.50 g, 85%), mp 87−91 °C. Recrystallization from *n*-hexane−ethyl acetate yielded plates of mp 93 °C (lit. mp 95−95.5 °C, Smith, Jr., et al., 1948; lit. mp 102−104 °C, Lukes and Ernest, 1949; lit. mp 92−93 °C, Beccalli et al., 1985).

*(Z)- (**8a**) and (E)-4-[2-(methylthio)-2-phenylethenyl]pyridine (**9a**).* A mixture of alkyne **7** (897 mg, 5.00 mmol) and 15% aqueous methyl mercaptan sodium salt (5.0 mL, 8.6 mmol) in EtOH (10 mL) was refluxed for 1 h. TLC analysis showed completion of the reaction. Without neutralization, the mixture was dried under reduced pressure and chromatographed on silica gel to give the *Z*-isomer **8a** (853 mg, 75%), mp 44−51 °C. Recrystallization from *n*-hexane−ethyl acetate furnished yellow needles, mp 52−54 °C: UV (MeOH) λ_{max} 319 nm (7820), λ_{min} 269 nm (4890); IR (KBr) ν_{max} 3050 (aromatic CH), 2900 (CH_3), 1580 (C═C), 1530, 1480, 1400, 760, 700 (phenyl) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.01 (3H, s, SCH_3), 6.60 (1H, s, CH═C), 7.2−7.6 (7H, m, 3-H, 5-H, phenyl), 8.60 (2H, d, J = 5.1 Hz, 2-H, 6-H). Anal. Calcd for $C_{14}H_{13}NS$: H, 5.76; C, 73.97; N, 6.16. Found: H, 5.77; C, 74.00; N, 6.10. Picrate recrystallized from EtOH was of mp 174−179 °C.

Further elution with the same solvent mixture followed by evaporation gave the *E*-isomer **9a** as a yellow oil (220 mg, 19%): UV (MeOH) λ_{max} 313 nm (7660), λ_{min} 270 nm (3000); IR (KBr) ν_{max} 3050 (aromatic CH), 2900 (CH_3), 1670 (CH═C), 1580 (C═C), 1530, 1480, 1430, 1400, 760, 710, 700 (phenyl) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.31 (3H, s, SCH_3), 6.28 (1H, s, CH═C), 6.75 (2H, d, J = 5.7 Hz, 3-H, 5-H), 7.33 (5H, s, phenyl), 8.23 (2H, d, J = 5.7 Hz, 2-H, 6-H). Picrate, mp 156−161 °C (with sublimation), was similarly prepared. Anal. Calcd for $C_{20}H_{16}N_4O_7S$: H, 3.53; C, 52.63; N, 12.27. Found: H, 3.43; C, 51.63; N, 12.06.

*(Z)- (**8b**) and (E)-4-(2-Ethoxy-2-phenylethenyl)pyridine (**9b**).* After sodium (162 mg, 7.0 mg atom) dissolved completely in dry EtOH, **7** (896 mg, 5.00 mmol) was added to the solution and refluxed for 4 days. The mixture was dried under reduced pressure and submitted to column chromatography. Elution with *n*-hexane−ethyl acetate (1:1, v/v) afforded the *Z*-isomer **8b** as a colorless oil (552 mg, 49%) after evaporating the solvents: UV (MeOH) λ_{max} 298 nm (27 700), λ_{min} 244 nm (2230); IR (KBr) ν_{max} 3050 (aromatic CH), 2950 (CH_3), 1620, 1580 (C═C), 1530, 1480, 1400, 760, 700 (phenyl) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.0 Hz, CH_2CH_3), 5.82 (1H, s, CH═C), 7.2−7.5 (7H, m, 3-H, 5-H, phenyl), 8.46 (2H, d, J = 5.9 Hz, 2-H, 6-H). Picrate, mp 165−170 °C (with sublimation), was prepared as described above. Anal. Calcd for $C_{21}H_{18}N_4O_8$: H, 3.99; C, 55.51; N, 12.33. Found: H, 3.94; C, 54.36; N, 11.98.

Further elution with the same solvents separated the *E*-isomer **9b** from **8b**. Evaporating the solvents yielded **9b** as colorless needles (161 mg, 14%), mp 82.5−87 °C, which were recrystallized from *n*-hexane−ethyl acetate to afford pure product of the same melting point: UV (MeOH) λ_{max} 292 nm (10 800), λ_{min} 257 nm (4710); IR (KBr) ν_{max} 3050 (aromatic CH), 2950 (CH_3), 1620, 1580 (C═C), 1530, 1480, 1400, 1260, 760, 700 (phenyl) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (3H, t, J = 7.0 Hz, CH_2CH_3), 4.03 (2H, q, J = 7.0 Hz, CH_2CH_3), 5.68 (1H, s, CH═C), 6.75 (2H, d, J = 6.2 Hz, 3-H, 5-H), 7.34 (5H, s, phenyl), 8.25 (2H, d, J = 6.2 Hz, 2-H, 6-H). Picrate, mp 146−147 °C. Anal. Calcd for $C_{21}H_{18}N_4O_8$: H, 3.99; C, 55.51; N, 12.33. Found: H, 3.91; C, 54.24; N, 11.97.

*(Z)-4-(2-Chloro-2-phenylethenyl)pyridine (**8c**).* HCl gas, generated from NaCl and H_2SO_4, was passed through a solution of **7** (537 mg, 2.99 mmol) in dry $CHCl_3$ (20 mL) for 40 min, and the mixture was refluxed. After reflux for 2 days, the mixture was saturated with HCl again and refluxed for an additional 2 days. The mixture was diluted with MeOH, neutralized with 28% ammonia, dried under reduced pressure, and purified by column chromatography (*n*-hexane−ethyl acetate, 1:1, v/v) to afford colored crystalline powders (169 mg,

26%): mp 56−59 °C; UV (MeOH) λ_{max} 287 nm (18 800), λ_{min} 240 nm (6130); IR (KBr) ν_{max} 3000 (aromatic CH), 1575 (C═C), 760, 680 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.98 (1H, s, CH═C), 7.4−7.7 (7H, m, 3-H, 5-H, phenyl), 8.64 (2H, d, J = 5.9 Hz, 2-H, 6-H). Anal. Calcd for $C_{13}H_{10}ClN$: H, 4.67; C, 72.40; N, 6.49. Found: H, 4.60; C, 71.07; N, 6.61.

(*Z*)-*4-(2-Bromo-2-phenylethenyl)pyridine* (***8d***). Similarly, **7** (538 mg, 3.00 mmol) was dissolved in $CHCl_3$ (20 mL) saturated with HBr and refluxed for 8 days, during which HBr gas was passed through the solution three times after 8 h, 2 days, and 3 days. The mixture contained unreacted **7** (by TLC). It was dried under reduced pressure and purified by column chromatography (*n*-hexane−ethyl acetate, 1:1, v/v) to yield **8d** as a colorless crystalline mass (289 mg, 26%): mp 54−57 °C; UV (MeOH) λ_{max} 284 nm (19 600), λ_{min} 241 nm (5240); IR (KBr) ν_{max} 3000 (aromatic CH), 1610 (CH═C), 1590 (C═C), 1410, 760, 690 (phenyl), 570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.14 (1H, s, CH═C), 7.3−7.7 (7H, m, 3-H, 5-H, phenyl), 8.65 (2H, d, J = 5.9 Hz, 2-H, 6-H). Anal. Calcd for $C_{13}H_{10}BrN$: H, 3.87; C, 60.02; N, 5.38. Found: H, 4.27; C, 57.37; N, 5.07.

(*Z*)-*4-(2-Iodo-2-phenylethenyl)pyridine* (***8e***). A mixture of **7** (176 mg, 1.00 mmol) and iodotrimethylsilane (0.80 mL, 4.9 mmol) in distilled $CHCl_3$ (15 mL) was refluxed for 24 h. TLC analysis showed completion of the reaction. Similar chromatographic purification (*n*-hexane−ethyl acetate, 1:1, v/v) provided **8e** as a colorless crystalline powder (176 mg, 57%): mp 49−54 °C; UV (MeOH) λ_{max} 277 nm (12 200), λ_{min} 242 nm (8400); IR (KBr) ν_{max} 3050, 3000 (aromatic CH), 1580 (C═C), 1430, 1395, 755, 690 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.98 (1H, s, CH═C), 7.3−7.7 (7H, m, 3-H, 5-H, phenyl), 8.65 (2H, d, J = 5.9 Hz, 2-H, 6-H). Anal. Calcd for $C_{13}H_{10}IN$: H, 3.28; C, 50.84; N, 4.56. Found: H, 3.26; C, 50.41; N, 4.47.

(*Z*)-*4-[1-Chloro-2-(methylthio)-2-phenylethenyl]pyridine* (***8f***). Alkyne **7** (180 mg, 1.00 mmol) was reacted with chlorotrimethylsilane (1.7 mL, 13 mmol) in DMSO (4.0 mL) at 100 °C for 4 h. After completion of the reaction (by TLC), the mixture was concentrated under reduced pressure below 40 °C and submitted to column chromatography. Elution with *n*-hexane−ethyl acetate (1:1, v/v) and then with MeOH removed the DMSO from the reaction mixture. The eluates containing **8f** were concentrated, neutralized with 28% ammonia, dried under reduced pressure, and purified by column chromatography (*n*-hexane−ethyl acetate, 1:1, v/v) to yield colorless needles (190 mg, 73%): mp 84−89 °C; IR (KBr) ν_{max} 2900 (CH_3), 1620 (CH═C), 1580 (C═C), 1395, 1300, 1260, 750, 700 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.84 (3H, s, SCH_3), 7.4−7.6 (7H, m, 3-H, 5-H, phenyl), 8.71 (2H, d, J = 6.0 Hz, 2-H, 6-H); EIMS (70 eV) m/z (rel intensity) 261 (M^+, 100), 211 (70), 208 (14), 152 (19), 77 (76); HRMS found m/z 261.0244, calcd for $C_{14}H_{12}ClNS$ 261.0351.

NMR spectrum of its *E*-isomer in a mixture obtained in photoisomerization by using a monochrometer described later is as follows: ^1H NMR ($CDCl_3$) δ 1.94 (3H, s, SCH_3), 7.4−7.6 (7H, m, 3-H, 5-H, phenyl), 8.54 (2H, d, J = 6.0 Hz, 2-H, 6-H).

(*Z*)-*4-(2-Ethoxy-2-phenylethenyl)pyridine N-Oxide* (***8g***). A mixture of compound **8b** (224 mg, 1.00 mmol) and *m*-chloroperoxybenzoic acid (70% purity, 259 mg, 1.05 mmol; Nacalai Tesque) in distilled $CHCl_3$ (10 mL) was stirred at room temperature overnight. The mixture was dried and chromatographed on silica gel (*n*-hexane−ethyl acetate, 1:1, v/v) to give **8g** as a colorless oil (158 mg, 65%): IR (KBr) ν_{max} 3060 (aromatic CH), 2920, 2890 (aliphatic CH), 1630 (CH═C), 1480, 1240 (*N*-oxide), 770, 700 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$−CD_3OD) δ 1.33 (3H, t, J = 7.0 Hz, CH_2CH_3), 3.89 (2H, q, J = 7.0 Hz, CH_2CH_3), 5.83 (1H, s, CH═C), 7.2−7.5 (5H, m, phenyl),7.61 (2H, dd, J = 5.2, 2.1 Hz, 3-H, 5-H), 8.12 (2H, dd, J = 5.2, 2.1 Hz, 2-H, 6-H). Picrate, mp 126−130 °C. Anal. Calcd for $C_{21}H_{18}N_4O_9$: H, 3.86; C, 53.62; N, 11.91. Found: H, 3.78; C, 52.87; N, 11.96.

Photoisomerization Using a Monochrometer. Compound **8a** dissolved in $CDCl_3$ (10 mg/mL, 0.50 mL) in a Pyrex NMR tube was kept at a distance of 11 cm from the shutter of a monochrometer and irradiated at 280, 320, or 380 nm. NMR spectra were taken at a given time to determine the *Z*/*E* ratio by integrating the signals of 2-H and 6-H protons of **8a** and its isomer **9a**.

Preparation of *E*-Methylthio Derivative 9a by Photoisomerization. A mixture of **8a** (252 mg, 1.11 mmol) dissolved in ethyl acetate (50 mL) in a 100 mL Pyrex flask was exposed directly to sunlight for 3.5 h on a fine day. The *Z*/*E* ratio of the mixture was 57/43 (by ^1H NMR). Evaporation of the solvent and subsequent chromatographic separation (*n*-hexane−ethyl acetate, 3:1, v/v) yielded the *E*-isomer **9a** as a yellow oil (57 mg, 23%), which in all respects was identical with the product obtained by chemical synthesis described above.

Preparation of *E*-Bromide 9c by Photoisomerization. The *Z*-isomer **8d** (119 mg) in ethyl acetate was similarly photoisomerized indoors with indirect exposure to sunlight for 6 h to avoid decomposition. Purification by column chromatography (*n*-hexane−ethyl acetate, 1:1, v/v) followed by evaporation afforded a brown crystalline solid (41 mg, 35%), mp 25−30 °C, positive to Beilstein's test: UV (MeOH) λ_{max} 273 nm (10 600), λ_{min} 248 nm (7700); IR (KBr) ν_{max} 3030, 3000 (aromatic CH), 2970 (aliphatic CH), 1620 (CH═C), 1590 (C═C), 1410, 710, 690 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.83 (2H, m, 3-H, 5-H), 7.20 (1H, s, CH═C), 7.34 (5H, m, phenyl), 8.38 (2H, d, J = 5.5 Hz, 2-H, 6-H); EIMS (70 eV) m/z (rel intensity) 259 (M^+, 24), 217 (37), 215 (79), 180 (55), 152 (60), 77 (61). Anal. Calcd for $C_{13}H_{10}BrN$: H, 3.87; C, 60.02; N, 5.38. Found: H, 4.27; C, 63.41; N, 5.65.

Tobacco Callus Assay. Callus tissues of *Nicotiana tabacum* var. Wisconsin No. 38 grown on agar medium (Linsmaier and Skoog, 1965) for 40 days were weighed, as reported previously (Nishikawa et al., 1986). The assay was done in four replicas for each concentration, and fresh weights of callus tissues were averaged. Cytokinin activity of each compound was determined from its concentration−response curve and expressed in terms of a concentration at which it gives the half-maximum yield (fresh weight) induced by kinetin.

Betacyanin Assay. As reported (Biddington and Thomas, 1973; Nishikawa et al., 1986), 10 derooted seedlings of *Amaranthus caudatus* L. were incubated in a phosphate buffer (pH 6.3) containing varying amount of test compound at 28 °C for 24 h in the dark, and the amount of betacyanin was measured photometrically as differential absorbance between 542 and 620 nm.

X-ray Analysis. X-ray diffraction data were measured on a Rigaku AFC-5R automated four-circle diffractometer equipped with Cu Kα radiation (λ = 1.5418 Å) at 20 °C. A total of 1648 independent reflections ($2\theta \leq 122°$, $\theta - 2\theta$ scan technique) were observed ($|F_o| \geq 3\sigma(F_o)$). The structure was solved by the program MULTAN (Main et al., 1984) and refined in the usual way (final R = 0.046, R_w = 0.049, residual electron density 0.29 $eÅ^{-3}$). The crystal structure model was drawn by ORTEP (Johnson, 1976).

Crystal data of **8f** are as follows: formula, $C_{14}H_{12}ClNS$; crystal morphology, prismatic; crystal size, 0.1 × 0.1 × 0.04 mm; Z, 8; D_c, 1.367; space group, orthorhombic, *Pbca*; cell dimensions, a = 12.447(1), b = 17.011(1), c = 12.064(1) with estimated standard deviations in parentheses.

RESULTS AND DISCUSSION

Synthesis of 4-Styrylpyridines. Compared to α-substituted 4-styrylpyridines, there have been few β-substituted 4-styrylpyridines, some of which were synthesized by the condensation of substituted acetophenone and 4-methylpyridine (Terada et al., 1989) or 4-pyridinecarboxaldehyde and substituted benzylphosphonate (Koeppel et al., 1975; Terada et al., 1986). Palladium-catalyzed alkynylation by using $(PPh_3)_2PdCl_2$−CuI (Sonogashira et al., 1975) is a method of choice to obtain 4-(phenylethynyl)pyridine (**7**) (Kanesho Co., Ltd., 1981), a key intermediate for the synthesis of β-substituted 4-styrylpyridines **8** and **9** (Scheme 1). Thus 4-bromopyridine (**6**) was reacted with phenylacetylene in the presence of the catalysts in a solvent mixture of MeCN−Et_3N at room temperature to yield alkyne **7** in a high yield. While electrophilic addition of HCl to **7** using

chlorotrimethylsilane in boiling MeOH did not proceed at all, the reaction with HCl in $CHCl_3$ at reflux afforded *Z*-chloride **8c** in a low yield (26%). The position of the chlorine atom was determined by HMBC NMR. The compound gave a cross-peak due to a long range $^1H-^{13}C$ *J* coupling between its olefinic proton of singlet and the carbons at the 3- and 5-positions of the pyridine ring, not the *ortho*-carbons of the phenyl ring. This indicated that the chlorine atom is attached at the β-position. Compound **8c** was assumed to have *Z*-configuration by comparing chemical shifts in ^1H NMR between a pair of geometrical isomers which were interchangable by photoisomerization, as described later.

Similar reaction of **7** with bromotrimethylsilane in the presence of *t*-BuOH in $CHCl_3$ gave *Z*-bromide **8d** in 14% yield after a prolonged reflux (16 days). Alternatively the reaction with HBr in boiling $CHCl_3$ improved the yield of **8d** to 26%. HCl and HBr additions by refluxing for a long period accompanied serious degradation that led to the low yields of **8c**,**d** which were isolated as a sole product. HI generated from iodotrimethylsilane in $CHCl_3$ was added to **7**, giving *Z*-iodide **8e** in a better yield (57%).

The reaction of **7** with chlorotrimethylsilane in DMSO to obtain **8c** proceeded smoothly to provide an unexpected α,β-disubstituted product in a satisfactory yield. Its molecular weight determined by HRMS coincided with that of MeSCl adduct **8f**. Similar to other *Z*-β-substituted 4-styrylpyridines **8**, the adduct showed ^1H NMR signals ($CDCl_3$) of 2-H and 6-H protons at lower field (8.71 ppm) than those of its photoisomerized one (8.54 ppm), suggesting the *E*-configuration. In addition, methylthio group and chlorine atom were assumed to be at α- and β-positions of the vinyl group, respectively, on the basis of the following mechanistic consideration. Methanesulfenyl chloride generated via chlorosulfonium ion (Knipe, 1981) in the reaction of DMSO with chlorotrimethylsilane as a source of hydrogen chloride probably attacks the triple bond of **7** by Ad_E2 mechanism (Capozzi et al., 1977). Similar methylthiation using DMSO and chloromethyl methyl ether or acetyl chloride (Hocker et al., 1975; Anzai, 1979) has been reported. Thus, we assigned the structure of **8f** to the adduct. The geometry of **8f** was finally confirmed by X-ray crystallographic study as the *E*-configuration (Figure 2). In crystals, **8f** exists in a conformation in which the phenyl and pyridine rings are largely slant against the vinyl group due to bulky α- and β-substituents, and the resultant interplanar angle between the two rings is 71.0°.

Electrophilic additions of HI and MeSCl under acidic conditions afforded only the *trans*-isomers **8e**,**f**, respectively, with high regio- and stereoselectivity, whereas similar additions of HCl and HBr were not selective due to byproduct formation.

On the other hand, nucleophilic addition of **7** under basic conditions yielded a mixture of *Z*- and *E*-isomers. Treatment of **7** with methyl mercaptan sodium salt in EtOH at reflux proceeded easily to yield a mixture of *Z*-**8a** and its *E*-isomer **9a**, the ratio of *Z*/*E* being 86/14 (based on isolated yield). In contrast, similar addition of EtOH in the presence of NaOEt required reflux for a longer period to provide a mixture of *Z*-**8b** and *E*-isomer **9b** with the *Z*/*E* ratio of 78/22. Similarly a cross-peak arising from the coupling between the olefinic proton and the 3- and 5-carbons in HMBC NMR spectra proved that the methylthio and ethoxy groups of these isomers are at the β-position. In addition, their configuration

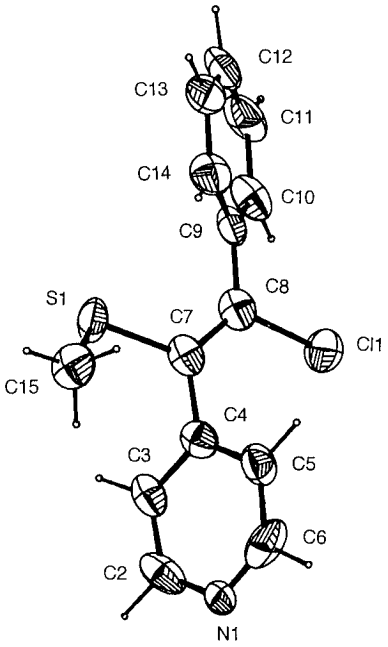


Figure 2. Crystal structure and atomic numbering system of *E*-methanesulfenyl chloride adduct **8f**.

was determined on the basis of different chemical shifts in ^1H NMR of the olefinic protons, phenyl protons, and those of the pyridine ring. The *E*-isomers showed signals of the protons of the pyridine ring at higher field than the *Z*-isomers, as expected from the shielding effect of the phenyl ring of the former.

In case of the nucleophilic additions, the formation of the *E*-isomers diminished stereoselectivity in the reactions, but regioselectivity was still high. Compound **8b** was oxidized with *m*-chloroperoxybenzoic acid to its *N*-oxide **8g** in order to assess the effect of *N*-oxidation on cytokinin activity.

Photoisomerization. 4-Styrylpyridines undergo *E*−*Z* photoisomerization under UV and visible light (Galiazzo et al., 1969). In order to study the effect of wavelength on the *Z*/*E* ratio at a photostationary state, the *Z*-methylthio derivative **8a**, which photoisomerized to its *E*-isomer **9a** without byproduct formation, was employed for an experiment by using a monochrometer. Absorption maxima of **8a** and **9a** in MeOH were 319 and 313 nm, respectively. Irradiation of **8a** in $CDCl_3$ at 280 nm for 3 h and at 320 nm for 1 h afforded a mixture of almost the same *Z*/*E* ratio of 57/43 that was determined by ^1H NMR (Figure 3). Photoequilibrium was achieved more rapidly by irradiation at 320 nm than by irradiation at 280 nm. On the other hand, exposure of **8a** to UV light at 380 nm for 3 h gave a mixture of the *Z*/*E* ratio of 69/31 in a new photoequilibrium in which the amount of **8a** increased. This indicates that photostationary *Z*/*E* ratio of β-substituted 4-styrylpyridines can be altered in order to enrich one of the geometrical isomers by irradiating at different wavelength, as observed for α-substituted 4-styrylpyridines (Galiazzo et al., 1969). Further irradiation of the mixture at 320 nm for 1 h led to the former photoequilibrium. These results confirm that the *E*−*Z* isomerization under the conditions is reversible. For other β-substituted styrylpyridines, particularly β-halogenated derivatives, degradation hampered quantitation by ^1H NMR.

For preparative purpose, similar photoisomerization was carried out by exposing directly or indirectly to

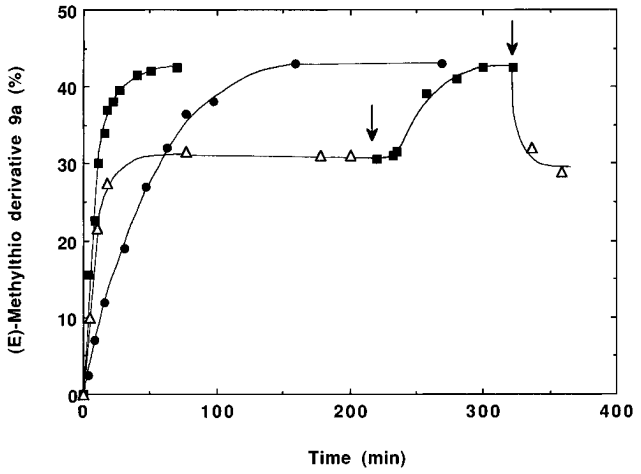


Figure 3. *Cis*−*trans* photoisomerization of *Z*-methylthio derivative **8a** to its *E*-isomer **9a** at different wavelengths. Compound **8a** in $CDCl_3$ in a Pyrex NMR tube was irradiated at 280 (●), 320 (■), and 380 (△) nm, and *Z*/*E* ratios were determined by ^{1}H NMR at a given time. Arrows indicate the time at which the wavelength was changed.

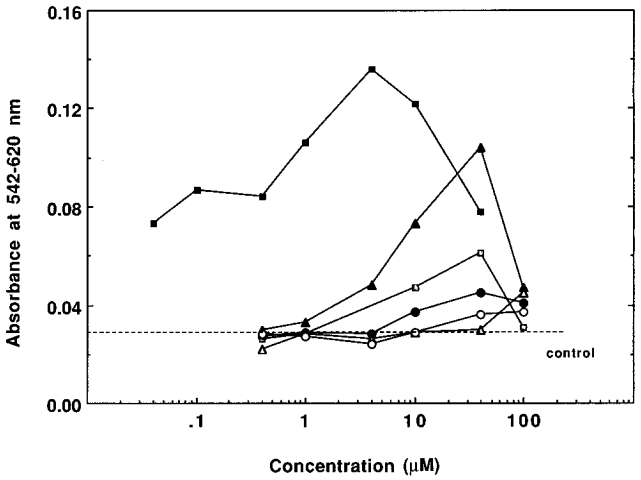


Figure 4. Effects of *β*-substituted 4-styrylpyridines and N^6-benzyladenine on betacyanin biosynthesis by *Amaranthus* seedlings: *Z*-methylthio **8a** (●), *Z*-ethoxy (▲), *Z*-ethoxy *N*-oxide **8g** (□), *E*-methylthio **9a** (○), *E*-ethoxy (△), and N^6-benzyladenine (■).

sunlight. Direct exposure of **8a** in AcOEt to sunlight for 3.5 h yielded a mixture of **8a** and its *E*-isomer **9a** with the *Z*/*E* ratio of 57/43 (by ^{1}H NMR); the value was identical with that obtained in the photoisomerization at 280 and 320 nm. Chromatographic separation gave **9a** in 23% yield. Similarly, indirect exposure of *Z*-bromo derivative **8d** to sunlight for 6 h yielded its *E*-isomer **9c** in 35% yield. However, the photoisomerization was accompanied with serious degradation, even under weak sunlight conditions.

Biological Activity. In an *Amaranthus* betacyanin assay, *Z*-ethoxy derivative **8b** promoted betacyanin biosynthesis at 4−100 μM and produced almost the same amount of betacyanin as that induced by 0.1 μM BA at 10 μM; thus it was about 100 times weaker than BA (Figure 4). *Z*-Methylthio derivative **8a** was less active than **8b**, and the activity of *N*-oxide **8g** was intermediate between them. *E*-Isomers **9a**,**b** were weaker than their *Z*-isomers **8a**,**b**, respectively, and exhibited weak or no activity at concentrations above 40 μM.

Since cytokinin activities of the 4-styrylpyridines detected in the *Amaranthus* assay were relatively low,

Table 1. Cytokinin Activity of *β*-Substituted 4-Styrylpyridines for the Growth of Tobacco Callus of *N. tabacum* Var. Wisconsin No. 38[a]

compound				cytokinin activity
	R_1	R_2	config	(μM)
8a	H	SMe	*Z*	0.29
8b	H	OEt	*Z*	0.052
8c	H	Cl	*Z*	3.6
8d	H	Br	*Z*	0.53
8e	H	I	*Z*	1.8
8f	SMe	Cl	*E*	ND
8g	H	OEt	*E*	0.38
9a	H	SMe	*E*	inactive
9b	H	OEt	*E*	1.6
9c	H	Br	*E*	ND
stilbazole	H	H	*E*	inactive
kinetin				0.011

[a] ND, not determined. Tobacco callus assay was done in four replicas for each concentration, and the fresh weights of callus tissues after ca. 40 days incubation were averaged. Cytokinin activity was determined in terms of a defined concentration ($C_{1/2maxK}$) from the dose−response curve of each compound, as described in Materials and Methods.

the more sensitive tobacco callus assay was used to assess their substituent effects on the activity. The 4-styrylpyridines except *E*-methylthio derivative **9a**, which was inactive at 100 μM, were weakly or moderately active, whereas *E*-4-styrylpyridine, stilbazole, was inactive (Table 1). The most active among the derivatives was the *Z*-ethoxy derivative **8b**, the activity of which was approximately one-fifth that of kinetin. Ethoxy group was the most favorable *β*-substituent, as in the case of 4-styrylpyrimidines (Nishikawa et al., 1989). Its *N*-oxide **8g** showed lower activity than the parent **8b**. Thus, there was no enhancement in the activity due to *N*-oxidation of the pyridine ring, in contrast to the results of *N*-(2-chloro-4-pyridryl)-*N*′-phenylurea *N*-oxides (Henrie et al., 1988). Additionally, *Z*-bromide **8d** was as active as *Z*-methylthio derivative **8a**. Among the *Z*-halides, the bromide **8d** exceeded iodide **8e** and chloride **8c** in activity. Thus, the observed activity order of the *Z*-isomers was EtO > MeS, Br > Cl, I ≫ H (inactive). Since stilbazole was completely inactive, the presence of *β*-substituents is essential for the activity. We cannot explain the order in terms of electronic or steric parameters of the *β*-substituent at present. Restriction of their conformation by the *β*-substituent might be involved.

It is important that the activity of **8b** became close to that of kinetin in the tobacco callus bioassay because the pyridine derivative is the simplest cytokinin-active analog ever developed. The compound is composed of two planar rings attached to a vinyl moiety and has only one nitrogen atom and no hydrogen donor, different from BA and *N*-(2-chloro-4-pyridryl)-*N*′-phenylurea. Structural similarity of the heteroaromatic rings among 6-styrylpurines, 4-styrylpyrimidines, 4-styrylpyridines, and BA implies that the 3-nitrogen atom of the N^6-substituted adenines plays a crucial role in cytokinin−receptor interaction. This is supported by the fact that N^6-substituted 1-deazaadenines retained high activity in contrast to the corresponding 3-deazaadenines (Rogozinska et al., 1973; Sugiyama et al., 1975). It is reasonably conceivable that the exocyclic nitrogen atom at the 6-position acts as a substituent-directing linkage atom (Nishikawa et al., 1986) due to its sp^2 nature (Soriano-Garcia and Parthasarathy, 1975), when we take into consideration that the nitrogen atom is replaceable with a sp^2 carbon.

In this study we have shown that *Z*-β-ethoxy derivative **8b**, which has the simplest structure among cytokinins, exhibited a moderate activity in promoting tobacco callus tissues. Electronic property of the nitrogen atom of pyridine significantly depends on the presence of 2-substituents. Chemical modification at the 2-position of *N*-phenyl-*N′*-(4-pyridyl)urea led to the finding of highly potent urea cytokinins, the activities of which were reinforced by electron-withdrawing groups (Okamoto et al., 1981). If electron density of the pyridine ring of β-substituted 4-styrylpyridines is optimized by introducing an appropriate substituent into the 2-position, their activity may increase further. We are continuing efforts to synthesize 2-substituted 4-styrylpyridines.

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Supporting Information Available: Table listing fractional coordinates and equivalent isotropic thermal parameters, bond distances, and selected bond angles for **8f** (2 pages). Ordering information is given on any current masthead page.

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