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SYNTHESIS, CRYSTAL STRUCTURE AND CYTOKININ ACTIVITIES OF β-SUBSTITUTED 6-STYRYLPURINES

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Key Word Index—6-Styrylpurines; crystal structure; cytokinin activity; tobacco callus assay; betacyanin assay.

Abstract—Conformationally restricted (*Z*)- and (*E*)-β-substituted 6-styrylpurines were synthesized by addition reaction to 6-phenylethynylpurine and photoisomerization. The crystalline (*Z*)- and (*E*)-β-chloro-substituted compounds exist in the *anti*- and *syn*-forms, respectively. While the (*Z*)-β-chloro- and bromo-substituted compounds were almost as active as (*E*)-6-styrylpurine and N^6-benzyladenine (BA) in the *Amaranthus* betacyanin assay, they were more active than the latter in the tobacco callus assay. On the other hand, their (*E*)-isomers showed activities almost comparable to that of kinetin in both assays. The crystal structure, as well as the high cytokinin activity of the conformationally restricted (*Z*)-isomers strongly suggest that the *anti-transoid* form is most probably the active conformation of purine cytokinins.

INTRODUCTION

Natural and synthetic cytokinins, most of which are N^6-alkyl- and N^6-acyladenines, exert significant promoting effects on the growth of tobacco callus and betacyanin biosynthesis in *Amaranthus*. Although their structure–activity relationships have been well documented [1], there have been few studies on 6-carbon-substituted purines [2–6], which have a carbon atom instead of a nitrogen atom at the 6-position. Such synthetic cytokinins have an advantage of being resistant to the breakage of their side-chains during bioassay, as shown in the high cytokinin activity of (*E*)-6-styrylpurine (**1**) and its related N^6-(Δ^2-isopentenyl) adenine analogue [4]. Furthermore, conformationally restricted β-substituted 6-styrylpurines are useful model compounds for investigation of the active conformations of purine cytokinins. Thus, additional synthetic and biological studies on purine derivatives are necessary to comprehensively understand the structure–activity relationships of cytokinins. In recent years, our efforts have been directed toward the development of carbon-substituted cytokinin analogues involving purine, pyrimidine and other azaheterocycles [7]. This paper describes convenient synthesis and cytokinin activities in tobacco callus and betacyanin assays of some 6-β-substituted styrylpurines. Additionally, conformations related to biological activities are discussed based on their cytokinin activities in the assays and their crystal structures.

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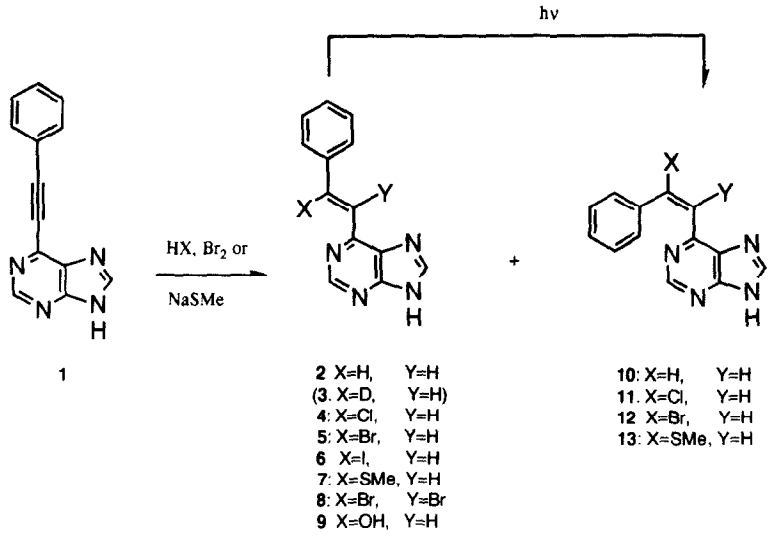
RESULTS AND DISCUSSION

Synthesis

Addition of HCl and HBr to 6-phenylethynylpurine (**1**) in dry MeOH [6] gave (*Z*)-isomers **4** and **5** (Scheme 1). Incomplete drying of the solvent resulted in formation of 6-phenacylpurine (**9**) as a by-product which was obtained in the reaction of **1** with 0.1N H_2SO_4 in MeOH and existed predominantly in the enol-form in the solid state and in solution. In contrast, a similar reaction with aqueous HI proceeded successfully to give HI adduct **6**. Although the position of the halogen is somewhat uncertain, it is believed to be β by comparing the ^{1}H NMR spectra for **2**, **4** and deuterated **3**. The chemical shift of the vinyl proton of **4** (7.9 ppm) in CD_3OD was similar to the α-proton (7.68 ppm) rather than the β-proton (8.28 ppm) of **2**. The signal of the β-proton disappeared in deuterated **3**. These findings were consistent with the result of X-ray analysis described later. Bromine added to **1** in $CHCl_3$ gave the α,β-dibromo compound **8**, which exhibited a multiplet of phenyl protons at 7.4–7.8 ppm, characteristic of (*Z*)-β-substituted 6-styrylpurines. Addition to **1** under basic conditions was confined to mercaptan. Treating **1** with aqueous NaSMe in EtOH furnished (*Z*)-**7** and its (*E*)-isomer **13** in a ratio of 3:1.



Scheme 1. Synthesis of β-substituted 6-styrylpurines.

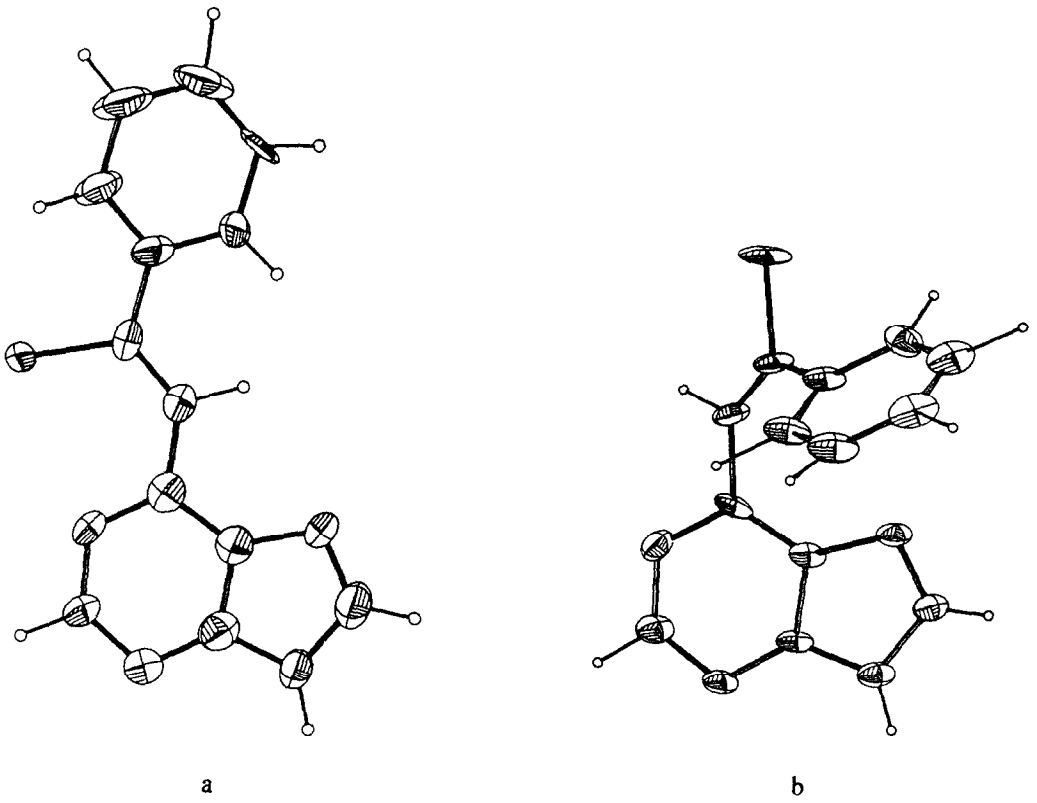


Fig. 1. Crystal structures of (*Z*)-**4** (a) and (*E*)-β-chloro-substituted 6-styrylpurine **11** (b).

Since the addition of hydrogen halides was only possible with the (*Z*)-isomers, the corresponding (*E*)-isomers were synthesized by *cis–trans* photoisomerization [5]. Exposure of **4** and **5** in MeOH directly to sunlight gave the (*E*)-isomers **11** and **12**, respectively. However, similar isomerization of iodide **6** was unsuccessful because of degradation. All styrylpurines were labile in the presence of light.

Crystal structure

Two 6-β-substituted styrylpurines, (*Z*)-**4** and (*E*)-chloride **11**, were submitted to X-ray crystallography

(Fig. 1). Compound **4** exists in an *anti*-conformation, in which the phenyl group is distal to the imidazole moiety of the purine ring, a geometry similar to N^6-adenines involving kinetin (K) [8] and BA [9]. However, the phenyl group and purine ring of **4** are almost in the same plane, unlike N^6-adenines and 6-phenethylpurine [10]. The β-chlorine atom appears to diminish the coplanarity of the purine ring and the vinyl group only slightly. On the other hand, the (*E*)-isomer **11** exists in the *syn*-form, that is, the phenyl group is above the imidazole moiety of the purine ring. In this conformation, the purine ring, the vinyl group and the phenyl group are neither coplanar nor parallel with each other.

Cytokinin activity

In the *Amaranthus* betacyanin assay (Table 1), the (*Z*)-chloride **4** and the bromide **5** were as active as (*E*)-6-styrylpurine (**2**) and BA. (*Z*)-Iodide **6** and the (*Z*)-methylthio derivative **7**, which has a larger substituent, were slightly less active. Phenacyl derivative **9** exhibited the same activity as that of **6**. Among the (*E*)-isomers, unsubstituted **10** was the most active. Substitution of its β-hydrogen with a halogen or a methylthio group decreased its activity. Although they were 5–10 times less active than the corresponding (*Z*)-isomers, the (*E*)-isomers, except **13**, still retained a moderate activity, the activity being comparable to that of K. Dibromo compound **8** was slightly less active than 6-alkynylpurine **1**. Substitution at both the α- and β-positions with bromine caused a significant decrease in activity.

Table 1. Cytokinin activity of β-substituted 6-styrylpurines and N^6-adenines

Compound	Betacyanin biosynthesis Concentration (M)*	Tobacco callus Concentration (M)†
1	4.5×10^{-6}	3.7×10^{-7}
2	1.2×10^{-7}‡	1.8×10^{-8}
4	1.5×10^{-7}	2.8×10^{-10}
5	1.7×10^{-7}	3.2×10^{-10}
6	3.0×10^{-7}	4.0×10^{-8}
7	7.1×10^{-7}	6.0×10^{-8}
8	8.7×10^{-7}	2.9×10^{-7}
9	3.0×10^{-7}	1.8×10^{-7}
10	6.9×10^{-7}‡	1.5×10^{-8}
11	1.3×10^{-6}	1.9×10^{-8}
12	2.0×10^{-6}	1.1×10^{-8}
13	7.2×10^{-6}	n.d.
N^6-Benzyladenine	1.0×10^{-7}	5.0×10^{-9}
Kinetin	2.8×10^{-6}	1.3×10^{-8}

*Concentration at which the sample gives the same amount of betacyanin (differential absorbance between 542 nm and 620 nm) as that produced by 0.1 μM of N^6-benzyladenine.

†Concentration at which the sample gives the same callus yield (fresh weight) as half of the maximum yield induced by kinetin.

‡Reported data [17].

n.d. not determined.

In contrast, (*Z*)-chloride **4** and (*Z*)-bromide **5** were *ca* 15 times stronger than BA in the tobacco callus assay. Compound **4** showed optimal growth at 1×10^{-9} M and its activity was detectable at 1×10^{-10} M (Fig. 2). Their (*E*)-isomers **11** and **12** were almost as active as K. The activity lower than expected of unsubstituted **2**, which is very sensitive to light, may arise from incomplete shielding from light during the assay.

Relationship between conformation and activity

For β-substituted 6-styrylpurines, only rotations about the C6–Cα bond and the Cβ–C (phenyl) bond are allowed. Freedom of these rotations are further restricted by steric or electronic repulsion due to the β-substituent. The preferred *anti*-conformation in crystals of the (*Z*)-chloride **4** is predicted by CPK modelling studies. The findings of strong activities in both assays and the preferred conformation in crystals of this compound suggest that the most probable active conformation of purine cytokinin is *anti-transoid*. Since the dihedral angle of the C6–Cα–Cβ–C (phenyl) chain of **4** is 180° due to an α-double bond, a dihedral angle of *ca* 90° for C6–N6–Cα–Cβ or C (phenyl) chain of N^6-substituted adenines [11] is not necessarily required for having an active conformation of purine cytokinins. In addition, moderate activity of the (*E*)-isomer **11** reveals that the activity is partially caused by a *cisoid* conformation [5]. Although the *syn*-form is preferred in crystals of **11**, CPK modelling studies suggest the possibility of an energetically stable *anti*-form. For (*E*)-isomers, there is still an unresolved active conformation, either *syn* or *anti*. X-ray analysis of other β-substituted purines and molecular mechanical calculations will be helpful for resolving active conformations of purine cytokinins more precisely.

EXPERIMENTAL

General. Mps uncorr.; ^{1}H NMR spectra were measured at 90 MHz with TMS as int. standard. EIMS spectra were

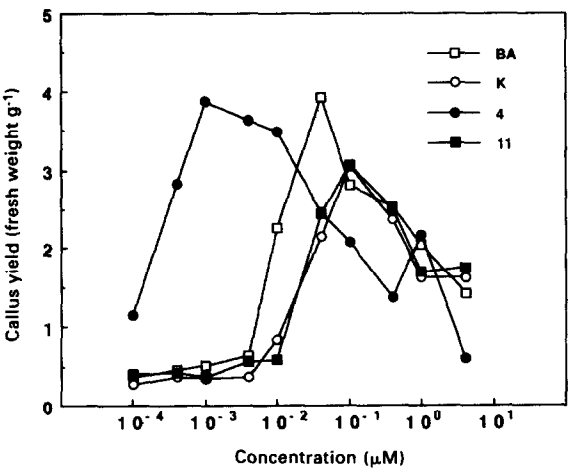


Fig. 2. Effect of β-substituted 6-styrylpurines and N^6-adenines on the growth of tobacco callus.

recorded at 70 eV. For purification, silica gel was used. Synthesis of **1** [12], **2** and **10** [5] has been reported previously.

6-Phenylethynylpurine (**1**). According to the method of ref. [13], a mixt. of 6-chloropurine (155 mg, 1 mmol), phenylacetylene (124 mg, 1.2 mmol), $(PPh_3)_2PdCl_2$ (10 mg), CuI (3 mg) and Et_3N (0.5 ml) in DMSO (0.5 ml) was heated under N_2 at 90° for 1.5 hr. After removing Et_3N under red. pres., crushed ice was added to the mixt. The crude crystalline ppts were purified by chromatography by eluting with EtOAc–MeOH (5:1). Faintly yellow needles (145 mg, 65%) were recrystallized from the same solvent. Mp 198–199°. This compound was strongly fluorescent when irradiated at 254 nm. IR ν_{KBr}^{max} cm^{-1}: 3450, 3050, 2250 (C≡C), 1605, 760, 690. ^1H NMR (CD_3OD): 7.4–7.9 (5H, *m*, phenyl), 8.62 (1H, *s*, 8-H), 8.92 (1H, *s*, 2-H). Found: C, 69.82; H, 3.68; N, 23.69. $C_{13}H_8N_4$ requires C, 70.90; H, 3.66; N, 25.44%.

(E)-6-(*2-d-2-Phenylethenyl*)*purine* (**3**). In a similar manner to **2** [2], 6-methylpurine hydrochloride (85 mg, 0.5 mmol) was heated in α-deuterobenzaldehyde [14] (402 mg, 3.8 mmol) at 170° for 10 min. Neutralization with conc. NH_3 and chromatographic elution with EtOAc gave crystals (15 mg, 13%). All operations were performed under low light conditions. Mp 255–257° is consistent with that of **2**. ^1H NMR (CD_3OD): 7.4–7.6 and 7.7–7.9 (5H, *m*, phenyl), 7.69 (1H, *s*, CH=CPh), 8.46 (1H, *s*, H-8), 8.80 (1H, *s*, H-2).

(Z)-6-(*2-Chloro-2-phenylethenyl*)*purine* (**4**). A soln of **1** (220 mg, 1 mmol) in dry MeOH (10 ml) satd with HCl was refluxed for 10 min. The mixt. was cooled, neutralized with conc. NH_3, dried under red. pres. and chromatographed. Successive elutions with EtOAc and then with EtOAc–MeOH (10:1) gave **4** (180 mg, 70%) as pale yellow needles. Mp 232–233°. IR ν_{KBr}^{max} cm^{-1}: 3000, 1620 (CH=CPh), 1590, 770, 690. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 337 (4.20), $\lambda_{max}^{H_2O}$ nm (log ε): 308 (4.21), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 310 (4.14). ^1H NMR (DMSO-d_6): 7.4–7.7 and 7.8–8.1 (5H, *m*, phenyl), 7.88 (1H, *s*, CH=CPh), 8.67 (1H, *s*, 8-H), 9.00 (1H, *s*, 2-H). Found: C, 58.79; H, 3.89; N, 20.83. $C_{13}H_9N_4Cl{\cdot}0.5H_2O$ requires C, 58.76; H, 3.79; N, 21.09%. For X-ray analysis the sample was recrystallized from EtOH.

(Z)-6-(*2-Bromo-2-phenylethenyl*)*purine* (**5**). Similarly, **5** was synthesized by refluxing in HBr–MeOH and recrystallized from EtOH. Yield: 89%. IR ν_{KBr}^{max} cm^{-1}: 3000, 1620 (CH=CPh), 1590, 760, 630. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 337 (4.20), $\lambda_{max}^{H_2O}$ nm (log ε): 308 (4.21), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 310 (4.14). ^1H NMR (DMSO-d_6): 7.3–7.6 and 7.7–7.9 (5H, *m*, phenyl), 8.01 (1H, *s*, CH=CPh), 8.66 (1H, *s*, 8-H), 8.99 (1H, *s*, 2-H). Found: C, 53.30; H, 3.12; N, 18.99. $C_{13}H_9N_4Br$ requires C, 51.85; H, 3.01; N, 18.60%.

(Z)-6-(*2-Iodo-2-phenylethenyl*)*purine* (**6**). Compound **1** (111 mg, 0.5 mmol) dissolved in MeOH containing 47% HI (0.54 ml, 3.4 mmol) was stirred for 5 min at room temp. The mixt. was cautiously neutralized with conc. NH_3 and dried under red. pres. to give a solid residue. This was purified by chromatography using EtOAc–MeOH (20:1) as eluent. Recrystallization from EtOH gave pale yellow needles (101 mg, 58%). Mp 204–208°. IR ν_{KBr}^{max} cm^{-1}: 3000, 1610 (CH=CPh), 1590, 760, 690. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 328 (3.93), $\lambda_{max}^{H_2O}$ nm (log ε): 303 (4.00). UV spectrum of **6** in 0.1N NaOH identical to that of **1**. ^1H NMR (DMSO-d_6): 7.3–7.6 and 7.7–7.8 (5H, *m*, phenyl), 7.88 (1H, *s*, CH=CPh), 8.65 (1H, *s*, 8-H), 8.99 (1H, *s*, 2-H). Found: C, 45.83; H, 2.65; N, 16.34. $C_{13}H_9N_4I$ requires C, 44.85; H, 2.61; N, 16.09%.

(Z)-(**7**) *and* (E)-6-(*2-Methylthio-2-phenylethenyl*)*purine* (**13**). A mixt. of **1** (220 mg, 1 mmol) and 15% aq. MeSNa (700 mg, 1.5 mmol) in EtOH was refluxed for 1 hr. Drying under red. pres. followed by chromatographic sepn using EtOAc as eluent furnished **7** (153 mg, 57%) and its (*E*)-isomer **13** (48 mg, 18%) together with recovered **1** (33 mg, 15%). Recrystallization from EtOAc gave pale yellow needles of **7**. Mp 195–198°. IR ν_{KBr}^{max} cm^{-1}: 3350, 3250, 1620 (CH=C), 1585, 1330 (S-Me), 765, 705. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 383 (4.26), $\lambda_{max}^{H_2O}$ nm (log ε): 351 (4.26), $\lambda_{max}^{1N\ NaOH}$ nm (log ε): 343 (4.22). ^1H NMR (CD_3OD): 1.97 (3H, *s*, SMe), 7.14 (1H, *s*, CH=CPh), 7.48 (5H, *br s*, phenyl), 8.51 (1H, *s*, 8-H), 8.91 (1H, *s*, 2-H). Found: C, 62.44; H, 4.50; N, 20.92. $C_{14}H_{12}N_4S$ requires C, 62.66; H, 4.51; N, 20.88%. Recrystallization of **13** from EtOAc–MeOH afforded pale yellow needles. Mp 187–195°. IR ν_{KBr}^{max} cm^{-1}: 3450, 3100, 1620 (CH=C), 1585, 1330 (S-Me), 760, 700. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 384 (4.28), $\lambda_{max}^{H_2O}$ nm (log ε): 336 (4.16), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 332 (4.18). ^1H NMR (CD_3OD): 2.56 (3H, *s*, SMe), 6.83 (1H, *s*, CH=CPh), 7.26 (5H, *s*, phenyl), 8.37 and 8.47 (2H, each *s*, 8-H and 2-H). Found: C, 62.08; H, 4.82; N, 20.87. $C_{14}H_{12}N_4S$ requires C, 62.66; H, 4.51; N, 20.88%. Both isomers photoisomerized in CD_3OD and DMSO-d_6 on weak irradiation.

(E)-6-(*1,2-Dibromo-2-phenylethenyl*)*purine* (**8**). A mixt. of **1** (222 mg, 1 mmol) and Br_2 (188 mg, 1.2 mmol) in $CHCl_3$ (50 ml) was refluxed for 2 hr. Chromatography eluting with EtOAc afforded needles (209 mg, 55%), which were recrystallized from aq. MeOH. Mp 259–261°. IR ν_{KBr}^{max} cm^{-1}: 3050, 1635 (CH=C), 1590, 1480, 770, 710. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 273 (4.07), $\lambda_{max}^{H_2O}$ nm (log ε): 275 (4.05), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 288 (4.04). ^1H NMR (DMSO-d_6): 7.4–7.8 (5H, *m*, phenyl), 8.77 (1H, *s*, 8-H), 9.02 (1H, *s*, 2-H). Found: C, 41.36; H, 2.08; N, 14.95. $C_{13}H_8N_4Br_2$ requires C, 41.09; H, 2.12; N, 14.74%.

6-Phenacylpurine (**9**). Details of the synthesis of this compound have not been reported previously. Alkyne **1** (100 mg, 0.45 mmol) was refluxed in MeOH (10 ml) containing 2N H_2SO_4 (0.5 ml) for 24 hr. Neutralization with conc. NH_3, followed by chromatographic sepn using EtOAc as eluent gave yellow needles (30 mg, 28%), which were recrystallized from MeOH. Mp 256–258°. IR ν_{KBr}^{max} cm^{-1}: 3100, 1635 (CH=CPh), 1590, 1240, 750, 690. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 380 (4.35), $\lambda_{max}^{H_2O}$ nm (log ε): 386 (4.46), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 390 (4.49). ^1H NMR (DMSO-d_6): 6.63 (1H, *s*, CH=CPh, exchangeable with D_2O), 7.4–7.7 and 7.8–8.1 (5H, *m*, phenyl), 8.49 (1H, *s*, 2-H), 8.60 (1H, *s*, 8-H). Assignment of 2-H and 8-H was based on H–D exchange in refluxing CD_3OD–D_2O (1:2). The chemical shift of 8-H shifted to high field on addition of

D_2O. EIMS: *m/z* 238 ($[M]^+$, 80), 161 ($[Pu-CH_2CO]^+$, 46), 105 ($[Ph-CO]^+$, 100). Found: C, 65.29; H, 3.91; N, 23.28. $C_{13}H_{10}N_4O$ requires C, 65.53; H, 4.23; N, 23.52%.

(E)-6-(2-*Chloro*-(2-*phenylethenyl*)*purine* (**11**). A soln of the (*Z*)-isomer **4** (96 mg, 0.36 mmol) in MeOH (70 ml) in a Pyrex flask was exposed directly to sunlight for 4 hr. Evapn and subsequent chromatographic sepn using EtOAc as eluent followed by EtOAc–MeOH (10:1) gave the crude product. It was recrystallized from aq. MeOH to yield needles (61 mg, 63%). Analytical samples were recrystallized from EtOAc. Mp 208–210° (sint.). IR ν_{KBr}^{max} cm^{-1}: 3100, 1620 (CH=CPh), 1600, 1580, 1320, 770, 720. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 337 (4.20), $\lambda_{max}^{H_2O}$ nm (log ε): 308 (4.21), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 307 (4.13). ^{1}H NMR (DMSO-d_6): 7.40 (5H, *br s*, phenyl), 7.53 (1H, *s*, CH=CPh), 8.56 (1H, *s*, 8-H), 8.59 (1H, *s*, 2-H). Assignment of 2-H and 8-H was unambiguously established by similar H–D exchange by refluxing in D_2O–DMSO-d_6 (3:1). Found: C, 60.98; H, 3.39; N, 21.60. $C_{13}H_9N_4Cl$ requires C, 60.83; H, 3.53; N, 21.83%.

(E)-6-(2-*Bromo*-2-*phenylethenyl*)*purine* (**12**). Similarly, direct exposure to sunlight of the (*Z*)-isomer **5** (50 mg, 0.17 mmol) in MeOH afforded needles (28 mg, 55%) after recrystallization from aq. MeOH. Mp 195–196° (sint.). Analytical samples were recrystallized from EtOAC. IR ν_{KBr}^{max} cm^{-1}: 3000, 1620 (CH=CPh), 1590, 1320, 770, 710. UV $\lambda_{max}^{0.1N\ HCl}$ nm (log ε): 320 (3.91), $\lambda_{max}^{H_2O}$ nm (log ε): 302 (3.96), $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε): 305 (3.96). ^{1}H NMR (DMSO-d_6): 7.38 (5H, *br s*, phenyl), 7.80 (1H, *s*, CH=CPh), 8.56 (2H, *s*, 8-H and 2-H). Found: C, 51.05; H, 3.19; N, 17.17. $C_{13}H_9N_4Br$·0.25EtOAc requires C, 52.03; H, 3.43; N, 17.34%.

X-Ray analysis. X-Ray analysis was carried out with a RIGAKU AFC-5R automated four-circle diffractometer equipped with CuKα radiation ($\lambda = 1.5418$A). Structures were solved by direct methods using the program MULTAN84 [15] and refined by the block-diagonal least square procedure. Calcn was performed on PANAFACOM U-1200.

Bioassays. Betacyanin and tobacco callus assays were conducted using *Amaranthus caudatus* and *Nicotiana tabacum* var. Wisconsin No. 38, respectively, as reported in ref. [16]. Cytokinin activity of each sample is expressed in terms of a defined concn (see footnote Table 1).

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