Purines. LV.[1] Syntheses and Cytokinin Activities of Some Adenine and Adenosine Derivatives Related to 1′-Methylzeatin

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Received February 3, 1993

(1′*S*)-1′-Methyl-*cis*-zeatin [(1′*S*)-2] and its 9-β-D-ribofuranoside [(1″*S*)-4] were synthesized from L-alanine through [*S*-(*Z*)]-4-amino-2-methyl-2-penten-1-ol ethanedioate [(*S*)-3]. Condensations of 2-hydroxy-6-methylthiopurine (16) with the *trans*-isomeric amine salt [(*S*)-15], its enantiomer [(*R*)-15], and the racemic modification [(±)-15] furnished (1′*S*)-, (1′*R*)-, and (±)-2-hydroxy-1′-methyl-*trans*-zeatins (6), respectively. A similar condensation of 16 with methylamine yielded 2-hydroxy-N^6-methyladenine (7). These adenine derivatives were tested for cytokinin activity in the tobacco callus bioassay, and the order of their activity was (1′*R*)-6 > (±)-6 > (1′*S*)-2 > 7; on the other hand, (1″*S*)-4 and (1′*S*)-6 were completely inactive at 0.1—100 μM and 0.01–10 μM concentrations, respectively. As a result of the above syntheses of (1′*R*)-6, (1′*S*)-6, (±)-6, and 7, the gross structures of a marine green alga cytokinin and of a blue coral cytokinin were established to be 6 and 7, respectively.

Keywords 1′-methyl-*cis*-zeatin; 1′-methyl-*cis*-zeatin riboside; 2-hydroxy-1′-methylzeatin chiral synthesis; Horner–Wadsworth–Emmons reaction; diisobutylaluminum hydride reduction; cytokinin activity

The isolation of (1″*R*)-1″-methyl-*trans*-zeatin 9-β-D-ribofuranoside [(1″*R*)-**5**][2] in 1985 and its aglycone, (1′*R*)-1′-methyl-*trans*-zeatin [(1′*R*)-**1**],[3] in 1986 from the culture filtrate of the gall-forming phytopathogenic bacterium *Pseudomonas syringae* pv *savastanoi* and the establishment of their structures by means of chemical synthesis[4] exemplify the recent additions of new members to the natural cytokinin group.[5] The new members are characterized by their N^6-substituents consisting of a branched allyl alcoholic C_6-unit with an asymmetric center adjacent to the N^6 atom. This uniqueness led us to investigate syntheses and structure–activity relationships of various analogues, such as the (1′*S*)-enantiomer [(1′*S*)-**1**],[4] and its 9-β-D-riboside [(1″*S*)-**5**][4]; the 4′-*O*-acetyl, 4″-*O*-acetyl, and dihydro derivatives[6] of (1′*R*)-**1** and (1″*R*)-**5**; the racemic aglycone [(±)-**1**][7]; the *cis* isomers [(1′*R*)-**2** and

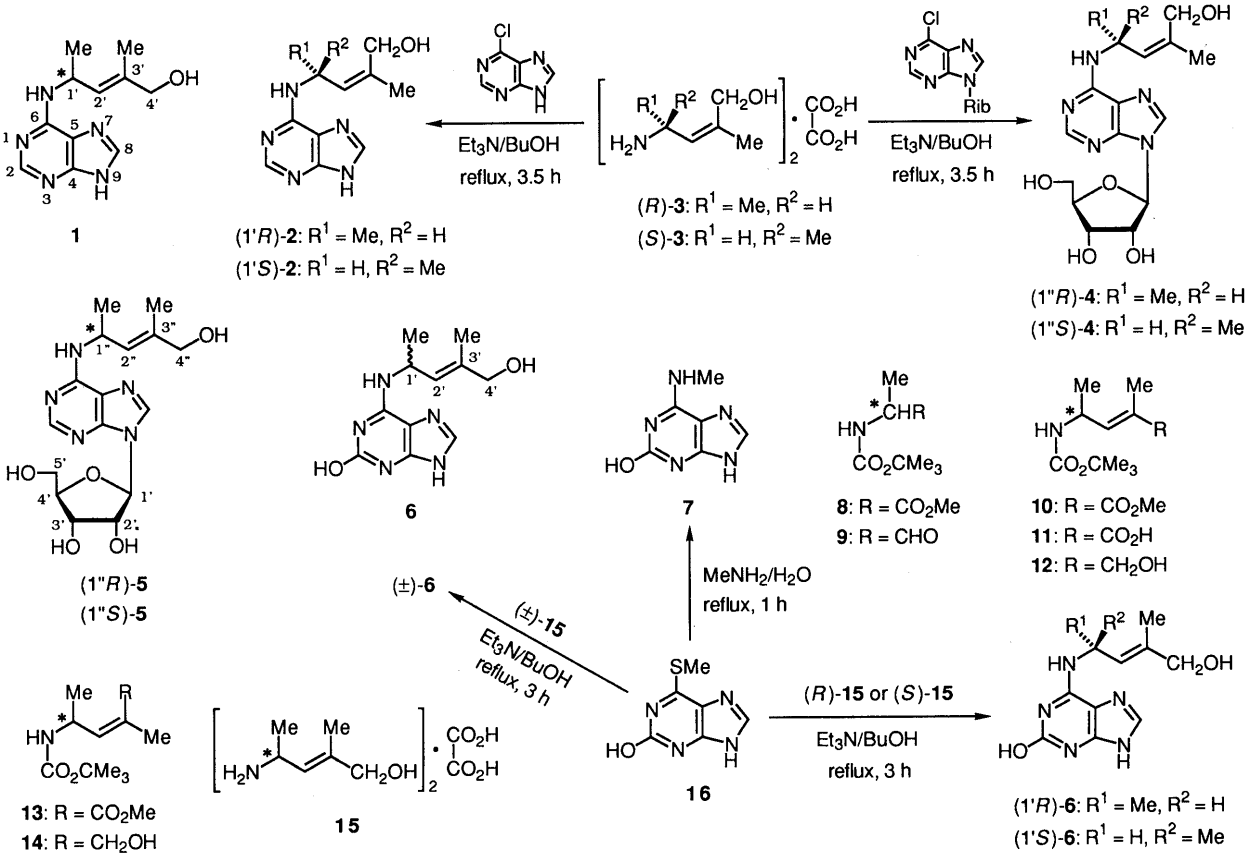

Chart 1

(1″*R*)-**4**][8] of (1′*R*)-**1** and (1″*R*)-**5**; and so on. However, the (1′*S*)-enantiomer [(1′*S*)-**2**] and the (1″*S*)-diastereomer [(1″*S*)-**4**] in the *cis* series remained to be synthesized. In 1990, Farooqi *et al.*[9] reported the isolation of a new cytokinin from methanolic extracts of a marine green alga (code No. NIO-143) and another new cytokinin from blue coral (NIO-156). They proposed the gross structure **6** for the former and structure **7** for the latter on the basis of spectroscopic data.[9] However, the absolute stereochemistry at the asymmetric center remained unknown for the former cytokinin when the present study was undertaken.

Our continuing interest[4,6-8,10] in synthesizing N^6-substituted adenine derivatives possessing cytokinin activity thus led us to synthesize both enantiomers [(1′*R*)-**6** and (1′*S*)-**6**] of 2-hydroxy-1′-methyl-*trans*-zeatin (**6**) in order to determine the absolute configuration of the natural sample by comparison. We also synthesized racemic 2-hydroxy-1′-methyl-*trans*-zeatin [(±)-**6**], 2-hydroxy-N^6-methyladenine (**7**), (1′*S*)-1′-methyl-*cis*-zeatin [(1′*S*)-**2**] and its 9-β-D-ribofuranoside [(1″*S*)-**4**] in this connection and tested the synthetic samples for cytokinin activity in the tobacco callus bioassay. A brief account of a part of the results recorded here has been published in a preliminary form.[11]

The key intermediates selected for the syntheses of both enantiomers and the racemic modification of **6** were the amine oxalates (*R*)-**15**, (*S*)-**15**, and (±)-**15**. They were prepared from D-, L-, and (±)-alanines *via* the previously reported synthetic route,[4,7,8] proceeding through the α,β-unsaturated esters [(*R*)-, (*S*)-, and (±)-**10**] and the allyl alcohols [(*R*)-, (*S*)-, and (±)-**12**],[4,7] but with some modification. In these three series, the conversion of **10** into **12** had previously been effected in two steps consisting of alkaline hydrolysis of **10** and $NaBH_4$ reduction of the resulting carboxylic acid (**11**) by the mixed anhydride method.[4,7] In the present work, however, reduction of (*R*)-**10** with diisobutylaluminum hydride (DIBAH) in CH_2Cl_2–hexane at −78 °C for 75 min was found to give (*R*)-**12** in one step in 96% yield. This result paralleled that obtained with the corresponding *cis* isomer [(*R*)-**13**→(*R*)-**14**]. Similar reductions of (*S*)-**10** and of (±)-**10** furnished (*S*)-**12** and (±)-**12** in 96% and 89% yields, respectively. Previously, the aldehyde (*S*)-**9**, an early intermediate for the synthesis of (*S*)-**10**, had been prepared from the ester (*S*)-**8** by $LiBH_4$ reduction to the corresponding alcohol, followed by Me_2SO oxidation using SO_3–pyridine complex in the presence of Et_3N. In the present work, this two-step sequence was replaced by DIBAH reduction (CH_2Cl_2–hexane, −78 °C, 30 min) to afford (*S*)-**9** in one step in 66% yield, paralleling the previously reported result[8] in the (*R*)-series [(*R*)-**8**→(*R*)-**9**].

Separate purinylations of (*R*)-**15**, (*S*)-**15**, and (±)-**15** with 2-hydroxy-6-methylthiopurine monohydrate (**16**·H_2O)[12] in boiling 1-butanol containing Et_3N for 3 h provided (1′*R*)-**6**, (1′*S*)-**6**, and (±)-**6** in 90%, 84%, and 90% yields, respectively. The UV, ^{1}H-NMR, and mass spectra of synthetic (*R*)-**6**, (*S*)-**6**, or (±)-**6** were found to be virtually identical with those of the marine green alga cytokinin,[9] establishing that the natural cytokinin is indeed 2-hydroxy-1′-methyl-*trans*-zeatin. However, we were unable to establish the chiroptical identity on account of paucity of the natural cytokinin, thus leaving its absolute stereochemistry unknown.

The candidate structure **7** for the new cytokinin from blue coral[9] has been synthetically known since 1968.[13] Treatment of **16**·H_2O with boiling aqueous methylamine for 1 h, a procedure slightly modified from that[13] reported, gave **7** in 85% yield. The synthetic **7** was found to be identical (by comparison of the UV, ^{1}H-NMR, and mass spectra) with a natural sample.[14]

For the syntheses of the remaining targets [(1′*S*)-**2** and (1″*S*)-**4**] in the *cis* series, the previously reported synthetic route to (1′*R*)-**2** and to (1″*R*)-**4** from (*R*)-**9** was repeated, but starting with (*S*)-**9** instead of (*R*)-**9**. Thus, application of the Still–Gennari modification[15] [$(CF_3CH_2O)_2P(O)$-$CH(Me)CO_2Me$, $KN(SiMe_3)_2$, 18-crown-6/MeCN, tetrahydrofuran, −78 °C, 75 min] of the Horner–Wadsworth–Emmons reaction[16] to (*S*)-**9** gave the (*Z*)-ester [(*S*)-**13**] in 65% yield. On reduction with DIBAH in CH_2Cl_2–hexane at −78 °C for 50 min, (*S*)-**13** furnished the allylic alcohol (*S*)-**14** in 93% yield. Treatment of (*S*)-**14** with 10% aqueous HCl at room temperature for 1 h and conversion of the resulting amine hydrochloride into the oxalate gave (*S*)-**3** in 81% yield [from (*S*)-**14**]. Purinylation of (*S*)-**3** with 6-chloropurine in boiling 1-butanol containing Et_3N for 3.5 h provided (1′*S*)-**2** in 78% yield. A similar condensation of (*S*)-**3** with 6-chloro-9-β-D-ribofuranosylpurine[17] afforded (1″*S*)-**4** in 83% yield.

Table I shows the cytokinin activities of the above target

TABLE I. Cytokinin Activity of 1′-Methylzeatin Analogues Tested by the Tobacco Callus Bioassay

Compound	Average fresh weight of tobacco callus (mg)												
	Concentration of test compound (μM)												
	0	0.001	0.01	0.04	0.1	0.4	1	4	10	40	100	400	1000
(1′*R*)-**1**[a]	17	120	1065	1490	1140	622	557	—	—	—	—	—	—
(1′*S*)-**1**[a]	23	—	62	430	922	1025	1512	1006	—	—	—	—	—
(1′*R*)-**2**[b]	16	—	19	26	32	153	835	1869	1252	567	—	—	—
(1′*S*)-**2**	24	—	—	—	22	21	23	94	875	1438	161	—	—
(1″*R*)-**4**[b]	28	—	—	—	29	37	84	186	219	657	1067	—	—
(1″*S*)-**4**	26	—	—	—	30	23	27	21	16	18	17	—	—
(1′*R*)-**6**	31	—	52	84	280	1118	1458	948	569	—	—	—	—
(1′*S*)-**6**	25	—	27	27	22	28	30	32	25	—	—	—	—
(±)-**6**	29	—	31	40	64	476	1432	1529	1013	—	—	—	—
7	18	—	—	—	—	—	23	55	75	189	539	222	106

a) Taken from ref. 4*b*. *b*) Taken from ref. 8*b*.

compounds as found in the tobacco callus bioassay, together with those reported previously for (1′*R*)-**1**,[4b] (1′*S*)-**1**,[4b] (1′*R*)-**2**,[8b] and (1″*R*)-**4**.[8b] It may be seen that the maximal yield of the callus was obtained at 0.04 μM (1′*R*)-**1**; 1 μM (1′*S*)-**1**; 1 μM (1′*R*)-**6**; 1—4 μM (±)-**6**; 4 μM (1′*R*)-**2**; 40 μM (1′*S*)-**2**; 100 (or >100) μM (1″*R*)-**4**. 2-Hydroxy-N^6-methyladenine (**7**) was very weakly active at 100 μM concentration, and both (1″*S*)-**4** and (1′*S*)-**6** were completely inactive at 0.1—100 μM and at 0.01—10 μM concentrations, respectively. Interestingly, in all cases the *R* configuration at the 1′- or 1″-position seems to be more important than the *S* configuration in determining cytokinin activity.[4b] It is also apparent that introduction of a hydroxy group into **1** at the 2-position in both the (1′*R*)- and (1′*S*)-series reduces the activity to a considerable extent. The activities of both **7** and N^6-methyladenine itself (for which the concentration required for detectable response has been reported[18] to be 100 μM) were too weak to check the validity of this structure–activity relationship. As expected,[4b,7,8b] the nucleoside (1″*S*)-**4** was less active than the aglycone (1′*S*)-**2**; and (±)-**6** was slightly less active than (1′*R*)-**6**, but much more active than (1′*S*)-**6**.

In conclusion, the present results confirm that the gross structure of the new, marine green alga cytokinin is represented by formula **6**. The natural cytokinin at the crude extract level has been shown to be active in the cucumber cotyledon greening bioassay,[9] and in the tobacco callus bioassay the above synthetic (1′*R*)-**6** was active at 1 μM concentration, whereas the synthetic (1′*S*)-**6** was completely inactive at 0.01—10 μM concentration. Therefore, it seems likely that the formula (1′*R*)-**6** is a complete expression of the green alga cytokinin, unless it is racemic. Interestingly, a plant growth factor produced by the fungus *Alternaria brassicae* has recently been assigned the gross structure **6**.[19] It is hoped that the synthetic (1′*R*)-**6**, (1′*S*)-**6**, and (±)-**6** will aid further isolation and identification of this unique cytokinin from natural sources.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. See ref. 10 for details of chromatographies, instrumentation, and measurements. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, m = multiplet, s = singlet.

(*S*)-(1-Methyl-2-oxoethyl)carbamic Acid *tert*-Butyl Ester [(*S*)-9] A stirred solution of (*S*)-**8**[4] (2.05 g, 10 mmol) in dry CH_2Cl_2 (50 ml) was cooled to −78 °C in an atmosphere of argon, and a 1.0 M solution (20 ml, 20 mmol) of DIBAH in hexane was added dropwise over 35 min. After the mixture had been stirred at −78 °C for 30 min, the reaction was quenched by adding 2 N aqueous HCl (10 ml). The resulting mixture was brought to pH 5 by addition of saturated aqueous $NaHCO_3$ and then stirred at room temperature for 40 min. After addition of appropriate amounts of CH_2Cl_2 and H_2O for easy separation of the aqueous and organic layers, the former layer was separated from the latter and extracted with CH_2Cl_2. The CH_2Cl_2 extracts and the above organic layer were combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a colorless solid (1.61 g). Recrystallization of the solid from hexane (12 ml) gave (*S*)-**9** (1.16 g, 66%) as colorless plates, mp 84—87 °C; $[\alpha]_D^{18}$ −33.3° (*c* = 1.01, MeOH). This sample was identical (by comparison of the IR spectrum) with authentic (*S*)-**9** [mp 89—90 °C; $[\alpha]_D^{16}$ −34.3° (*c* = 1.00, MeOH)].[4b]

[*R*-(*E*)]-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*R*)-12] A stirred solution of (*R*)-**10**[4] (1.95 g, 8 mmol) in dry CH_2Cl_2 (20 ml) was cooled to −78 °C in an atmosphere of N_2, and a 0.92 M solution (26 ml, 24 mmol) of DIBAH in hexane was added dropwise over 15 min. After the mixture had been stirred at −78 °C for 75 min, the reaction was quenched by adding a 5 M solution (15 ml) of AcOH in CH_2Cl_2 at −78 °C. The resulting mixture was then stirred at room temperature, and 10% aqueous tartaric acid (50 ml) and H_2O (50 ml) were added in that order. The aqueous layer was separated from the organic layer and extracted with CH_2Cl_2. The CH_2Cl_2 extracts and the above organic layer were combined, washed successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a colorless oil (1.81 g). Purification of the oil by means of flash chromatography[20] [silica gel, hexane–AcOEt (1 : 1, v/v)] afforded (*R*)-**12** (1.66 g, 96%) as a colorless oil, $[\alpha]_{365}^{23}$ −8.0° (*c* = 1.00, MeOH). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic (*R*)-**12** [$[\alpha]_{365}^{16}$ −8.4° (*c* = 1.00, MeOH)].[4b]

[*S*-(*E*)]-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*S*)-12] Reduction of (*S*)-**10**[4] (317 mg, 1.3 mmol) with DIBAH (3 mmol) at −78 °C for 45 min and work-up of the reaction mixture were effected in a manner similar to that described above for (*R*)-**12**, giving (*S*)-**12** (269 mg, 96%) as a colorless oil, $[\alpha]_{365}^{25}$ +8.2° (*c* = 1.01, MeOH). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic (*S*)-**12** [$[\alpha]_{365}^{17}$ +7.9° (*c* = 1.00, MeOH)].[4b]

(*E*)-(±)-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(±)-12] Reduction of (±)-**10**[7] (487 mg, 2 mmol) with DIBAH (6 mmol) at −78 °C for 45 min and work-up of the reaction mixture were carried out in a manner similar to that described above for (*R*)-**12**, giving (±)-**12** (382 mg, 89%) as a colorless oil. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic (±)-**12**.[7]

[*S*-(*Z*)]-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid Methyl Ester [(*S*)-13] Condensation of (*S*)-**9** (*vide supra*) (660 mg, 3.8 mmol) with methyl 2-[bis(2,2,2-trifluoroethoxy)phosphinyl]propionate [$(CF_3CH_2O)_2P(O)CH(Me)CO_2Me$][15] for 75 min and work-up of the reaction mixture were performed as reported previously[8b] for the synthesis of (*R*)-**13**. Recrystallization of the crude product (791 mg), mp 53—53.5 °C, from hexane gave a pure sample of (*S*)-**13** (607 mg, 65%) as colorless prisms, mp 54—54.5 °C; $[\alpha]_D^{21}$ +73.4° (*c* = 1.01, MeOH). *Anal.* Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.76. Found: C, 59.36; H, 8.95; N, 5.86. The IR, ^{1}H-NMR, and mass spectra of this sample were identical with those of (*R*)-**13**.[8]

[*S*-(*Z*)]-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*S*)-14] Reduction of (*S*)-**13** (452 mg, 1.86 mmol) with DIBAH (5.6 mmol) and work-up of the reaction mixture were carried out as reported previously[8b] for the synthesis of (*R*)-**14**, yielding (*S*)-**14** (372 mg, 93%) as a colorless solid, mp 67—67.5 °C. Recrystallization of the solid from hexane furnished an analytical sample as colorless scales, mp 67.5—68 °C; $[\alpha]_D^{21}$ +3.2° (*c* = 1.00, MeOH); $[\alpha]_{365}^{22}$ +22.4° (*c* = 1.00, MeOH). *Anal.* Calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.15; H, 10.12; N, 6.42. The IR, ^{1}H-NMR, and mass spectra of this sample were identical with those of (*R*)-**14**.[8]

[*S*-(*Z*)]-4-Amino-2-methyl-2-penten-1-ol Ethanedioate (2 : 1) (Salt) [(*S*)-3] Hydrolysis of (*S*)-**14** (538 mg, 2.5 mmol) with 10% aqueous HCl (5 ml) and work-up of the reaction mixture were effected as reported previously[8b] for the synthesis of (*R*)-**3**, affording (*S*)-**3** (324 mg, 81%) as a colorless solid, mp 199.5—200 °C (dec.). Recrystallization of the solid from 95% aqueous EtOH provided an analytical sample as colorless filaments, mp 199.5—200.5 °C (dec.); $[\alpha]_D^{21.5}$ +9.0° (*c* = 0.50, MeOH). *Anal.* Calcd for $C_{14}H_{28}N_2O_6$: C, 52.48; H, 8.81; N, 8.74. Found: C, 52.29; H, 8.99; N, 8.60. The IR and ^{1}H-NMR spectra of this sample were identical with those of (*R*)-**3**.[8]

[*S*-(*Z*)]-2-Methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [(1′*S*)-1′-Methyl-*cis*-zeatin] [(1′*S*)-2] Purinylation of (*S*)-**3** (96.1 mg, 0.3 mmol) with 6-chloropurine (92.7 mg, 0.6 mmol) and work-up of the reaction mixture were performed as described previously[8b] for the synthesis of (1′*R*)-**2**, giving (1′*S*)-**2** (109 mg, 78%) as a slightly yellowish solid. Recrystallization of the solid from MeCN gave an analytical sample as colorless, minute prisms, mp 179.5—181 °C; $[\alpha]_D^{20}$ +131° (*c* = 0.148, EtOH); CD (*c* = 6.02 × 10^{-5} M, MeOH) $[\theta]^{18.5}$ (nm): +24900 (274) (pos. max.), −53800 (217) (neg. max.). *Anal.* Calcd for $C_{11}H_{15}N_5O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.43; H, 6.41; N, 29.93. The UV, IR, ^{1}H-NMR, and mass spectra of this sample were identical with those of (1′*R*)-**2**.[8]

[*S*-(*Z*)]-*N*-(4-Hydroxy-1,3-dimethyl-2-butenyl)adenosine [(1″*S*)-1″-Methyl-*cis*-zeatin 9-β-D-Ribofuranoside] [(1″*S*)-4] A stirred solution of (*S*)-**3** (106 mg, 0.33 mmol) and 6-chloro-9-β-D-ribofuranosylpurine[17] (172 mg, 0.6 mmol) in 1-butanol (6 ml) containing Et_3N (0.6 ml) was heated under reflux for 3.5 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was dissolved in a little H_2O. The resulting

aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (4 ml), and the column was eluted with H_2O (50 ml). The eluate was concentrated *in vacuo*, and the residual solid was purified by means of flash chromatography[20] [silica gel, AcOEt–EtOH (7 : 2, v/v)] to obtain (1″*S*)-**4** (183 mg, 83%), mp 146.5—147 °C. Recrystallizations from MeCN and drying over P_2O_5 at 1 mmHg and 50 °C for 7 h yielded an analytical sample as colorless, minute needles, mp 134—136.5 °C; $[\alpha]_D^{24.5}$ +13.1° (c=0.161, MeOH); $[\alpha]_{365}^{25}$ +245° (c=0.161, MeOH); CD ($c=3.45\times10^{-5}$ M, MeOH) $[\theta]^{18.5}$ (nm): +19700 (276) (pos. max.), −45500 (218) (neg. max.); MS *m/z*: 365 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 270 nm (ε 18000); $\lambda_{max}^{H_2O}$ (pH 1) 265 (19600); $\lambda_{max}^{H_2O}$ (pH 7) 269 (19800); $\lambda_{max}^{H_2O}$ (pH 13) 269 (19700); ^{1}H-NMR (Me_2SO-d_6) δ: 1.24 [3H, d, J=6.5 Hz, C(1″)-Me], 1.68 [3H, s, C(3″)-Me], 3.5—3.7 [2H, m, C(5′)-H's], 3.85—4.25 [4H, m, C(3′)-H, C(4′)-H, and C(4″)-H's], 4.45—4.8 [2H, m, C(2′)-H and OH], 5.05—5.5 [5H, m, C(1″)-H, C(2″)-H, and three OH's], 5.87 [1H, d, J=6 Hz, C(1′)-H], 7.74 (1H, dull d, J=8 Hz, NH), 8.17 and 8.33 (1H each, s, purine protons). *Anal.* Calcd for $C_{16}H_{23}N_5O_5$: C, 52.60; H, 6.34; N, 19.17. Found: C, 52.55; H, 6.53; N, 19.07.

[*R*-(*E*)]-4-(2-Hydroxy-9*H*-purin-6-ylamino)-2-methyl-2-penten-1-ol [(1′*R*)-2-Hydroxy-1′-methyl-*trans*-zeatin] [(1′*R*)-6] A stirred solution of 2-hydroxy-6-methylthiopurine monohydrate (**16**·H_2O)[12] (328 mg, 1.64 mmol) and (*R*)-**15**[4] (577 mg, 1.8 mmol) in 1-butanol (20 ml) containing Et_3N (3.5 ml) was heated under reflux for 3 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was triturated with H_2O (10 ml). The insoluble solid that resulted was filtered off, washed with a little H_2O, and dried to give a first crop (335 mg) of (1′*R*)-**6**·1/5H_2O as a faintly yellowish solid, mp > 300 °C (darkened at 275 °C). The aqueous filtrate and washings were combined and concentrated to dryness *in vacuo*, and the residue was purified by flash chromatography[20] [silica gel, $CHCl_3$–MeOH (4 : 1, v/v)] to furnish a second crop (39 mg). The total yield of (1′*R*)-**6**·1/5H_2O was 374 mg (90%). Recrystallization of the crude product from EtOH gave an analytical sample as a colorless, microcrystalline solid, mp > 300 °C (darkened at 275 °C) (after having been dried over P_2O_5 at 2 mmHg successively at room temperature for 18 h; at 50 °C for 6 h; and at 75 °C for 6 h, and then allowed to stand at room temperature for 4 d in a closed vessel saturated with H_2O); $[\alpha]_D^{19}$ +41.6° (c=0.288, MeOH); CD ($c=8.82\times10^{-5}$ M, MeOH) $[\theta]^{19}$ (nm): −5900 (278) (neg. max.), +16300 (248) (pos. max.), +11400 (236) (neg. max.), +19000 (226) (pos. max.); MS *m/z*: 249 (M^+); UV $\lambda_{max}^{H_2O}$ (pH 1) 289 nm (ε 16900); $\lambda_{max}^{H_2O}$ (pH 7) 244 (10400), 283 (13300); $\lambda_{max}^{H_2O}$ (pH 12)[21] 287 (16400); ^{1}H-NMR (CD_3OD) δ: 1.33 [3H, d, J=6.5 Hz, C(1′)-Me], 1.77 [3H, s, C(3′)-Me], 3.95 [2H, s, C(4′)-H's], 5.25 [1H, m, C(1′)-H], 5.50 [1H, br d, J=9 Hz, C(2′)-H], 7.80 [1H, s, C(8)-H]. *Anal.* Calcd for $C_{11}H_{15}N_5O_2\cdot1/5H_2O$: C, 52.25; H, 6.14; N, 27.69. Found: C, 52.32; H, 6.18; N, 27.66. The UV, ^{1}H-NMR, and mass spectra of this sample were found to be virtually identical with those of the natural cytokinin (**6**).[9,11]

[*S*-(*E*)]-4-(2-Hydroxy-9*H*-purin-6-ylamino)-2-methyl-2-penten-1-ol [(1′*S*)-2-Hydroxy-1′-methyl-*trans*-zeatin] [(1′*S*)-6] A stirred solution of **16**·H_2O[12] (328 mg, 1.64 mmol) and (*S*)-**15**[4] (577 mg, 1.8 mmol) in 1-butanol (20 ml) containing Et_3N (3.5 ml) was heated under reflux for 3 h. The reaction mixture was worked up as described above for (1′*R*)-**6**, yielding (1′*S*)-**6**·1/5H_2O (350 mg, 84%) as a colorless solid, mp > 300 °C (darkened at 275 °C). Recrystallization and drying in the same manner as described above for (1′*R*)-**6** provided an analytical sample of (1′*S*)-**6**·1/5H_2O as a colorless, microcrystalline solid, mp > 300 °C (darkened at 275 °C); $[\alpha]_D^{17}$ −38.1° (c=0.267, MeOH); CD ($c=8.15\times10^{-5}$ M, MeOH) $[\theta]^{19}$ (nm): +6260 (278) (pos. max.), −16000 (248) (neg. max.), −10600 (236) (pos. max.), −17700 (226) (neg. max.). *Anal.* Calcd for $C_{11}H_{15}N_5O_2\cdot1/5H_2O$: C, 52.25; H, 6.14; N, 27.69. Found: C, 52.46; H, 6.28; N, 27.69. The UV, IR, ^{1}H-NMR, and mass spectra of this sample were identical with those of (1′*R*)-**6**·1/5H_2O.

(*E*)-(±)-4-(2-Hydroxy-9*H*-purin-6-ylamino)-2-methyl-2-penten-1-ol [(±)-2-Hydroxy-1′-methyl-*trans*-zeatin] [(±)-6] A stirred solution of **16**·H_2O[12] (65 mg, 0.32 mmol) and (±)-**15**·1/3H_2O[7] (116 mg, 0.36 mmol) in 1-butanol (4 ml) containing Et_3N (0.7 ml) was heated under reflux for 3 h. The reaction mixture was worked up in a manner similar to that described above for (1′*R*)-**6**, giving (±)-**6**·1/5H_2O (74 mg, 90%) as a colorless solid, mp > 300 °C (darkened at 270 °C). Recrystallization from EtOH and drying over P_2O_5 at 2 mmHg and room temperature for 24 h yielded an analytical sample of (±)-**6**·1/5H_2O as a colorless, microcrystalline solid, mp > 300 °C (darkened at 270 °C). *Anal.* Calcd for $C_{11}H_{15}N_5O_2\cdot1/5H_2O$: C, 52.25; H, 6.14; N, 27.69. Found: C, 52.54; H, 6.05; N, 27.56. Except for the IR spectrum in the solid state, the spectral characteristics (UV, ^{1}H-NMR, and mass spectra) of this sample were identical with those of (1′*R*)-**6**·1/5H_2O or of (1′*S*)-**6**·1/5H_2O.

2-Hydroxy-N^6-methyladenine (7) A stirred mixture of **16**·H_2O[12] (300 mg, 1.5 mmol) and 40% aqueous $MeNH_2$ (20 ml) was heated under reflux for 1 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residual solid was recrystallized from H_2O to obtain a first crop (189 mg) of **7**·1/10H_2O as a colorless solid, mp > 300 °C. Usual work-up of the mother liquor from the above recrystallization afforded a second crop (23 mg). The total yield of **7**·1/10H_2O was 212 mg (85%). Recrystallization from H_2O and drying over P_2O_5 at 2 mmHg and 75 °C for 6 h gave an analytical sample as a colorless, microcrystalline solid, mp > 300 °C [lit.[13] mp > 260 °C (for anhydrous **7**)]; MS *m/z*: 165 (M^+); UV $\lambda_{max}^{H_2O}$ (pH 1) 285 nm (ε 14500); $\lambda_{max}^{H_2O}$ (pH 7) 241 (9500), 282 (11700); $\lambda_{max}^{H_2O}$ (pH 12)[21] 284 (15000); ^{1}H-NMR (Me_2SO-d_6) δ: 2.90 (3H, s, NMe), 7.74 [1H, s, C(8)-H]. *Anal.* Calcd for $C_6H_7N_5O\cdot1/10H_2O$: C, 43.16; H, 4.35; N, 41.95. Found: C, 43.18; H, 4.05; N, 42.20. The UV, ^{1}H-NMR, and mass spectra of this sample were shown to be virtually identical with those of a natural sample isolated from blue coral (code No. NIO-156).[9,14]

Bioassay Procedure The cytokinin activities of (1′*S*)-**2**, (1″*S*)-**4**, (1′*R*)-**6**, (1′*S*)-**6**, (±)-**6**, and **7** were tested in the tobacco callus bioassay in a manner similar to that described recently[4b] for (1′*R*)-**1** and (1′*S*)-**1**. The results are shown in Table I.

Acknowledgment This work was supported by a Grant-in-Aid for Scientific Research (B) (No. 04453152) from the Ministry of Education, Science and Culture, Japan. We are also grateful to Dr. A. H. Abad Farooqi of the Central Institute of Medicinal and Aromatic Plants (Lucknow, India) for his invaluable help in making a comparison between his natural and our synthetic cytokinins.

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