

Purines. XXXV.¹⁾ Synthesis and Cytokinin Activity of Racemic 1'-MethylzeatinTozo FUJII,*^a Taisuke ITAYA,^a Sachiko YOSHIDA,^a and Satoshi MATSUBARA^b^aFaculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan and Laboratory of Applied Biology, Kyoto Prefectural University,^b Shimogamo Hangi-cho, Sakyo-ku, Kyoto 606, Japan. Received May 15, 1989

Racemic 1'-methylzeatin [(±)-2] has been synthesized from (±)-alanine [(±)-3] through a 9-step route proceeding via the intermediates (±)-4–(±)-11. In the tobacco callus bioassay, (±)-2 exhibited a strong cytokinin activity at 0.04–1 μM concentration, a range between the optimum concentrations of both enantiomers (1'R)-2 and (1'S)-2.

Keywords 1'-methylzeatin; racemic synthesis; (±)-alanine; *N*-protected α-amino aldehyde; Wittig reaction; α,β-unsaturated ester; tobacco callus bioassay; cytokinin activity

In quite a recent joint paper²⁾ from our laboratories, we reported that (1'R)-1'-methylzeatin [(1'R)-2], a natural cytokinin isolated from the gall-forming phytopathogenic bacterium *Pseudomonas syringae* pv *savastanoi*³⁾ and later synthesized from D-alanine [(R)-3],^{2,4)} was as active as the known 1'-unsubstituted cytokinin zeatin (1) in the tobacco callus and the lettuce seed germination bioassays. Interestingly, the unnatural enantiomer (1'S)-2 was also active, but less active than (1'R)-2 by a factor of *ca.* 25: the maximal yield of the callus was obtained at 0.04 μM (1'R)-2 and at 1 μM (1'S)-2.^{2,5)} This suggests that racemic 1'-methylzeatin [(±)-2] may exhibit a similar cytokinin activity in a wider range of optimum concentration than does each enantiomer, provided the (1'R)- and (1'S)-enantiomers constituting the racemic modification behave independently of each other *in vivo*. If this is the case, the synthesis and utilization of the racemic modification [(±)-2] will be much more favorable than those of the (1'R)-enantiomer [(1'R)-2] since the racemic synthesis does not require the expensive, optically active

starting material [(R)-3]⁶⁾ and troublesome precautions to avoid racemization in every step. In the present work, we thus investigated the synthesis and cytokinin activity of (±)-2.

The synthetic route to (±)-2 from (±)-alanine [(±)-3], as shown in Chart 1, was essentially the same as that reported by us^{2,4)} for the chiral series. The amino acid (±)-3⁶⁾ was first converted into the *N*-protected amino ester (±)-5 through the amino ester hydrochloride (±)-4 according to the literature procedures.^{7,8)} Reduction of (±)-5 with LiBH₄ and oxidation of the resulting *N*-protected amino alcohol [(±)-6] using Me₂SO and SO₃–pyridine complex in the presence of Et₃N were effected in a manner similar to that employed by Shioiri's group⁹⁾ for the (*S*)-series, producing the aldehyde (±)-7 in 81% overall yield [from (±)-3]. Wittig reaction of (±)-7 with methyl 2-(triphenylphosphoranylidene)propionate¹⁰⁾ in CH₂Cl₂ at 21 °C for 2.5 h gave a mixture of (±)-8 and its (*Z*)-isomer in 94% yield, from which (±)-8 was isolated in 71% yield by recrystallization (from hexane). The ester (±)-8 was then

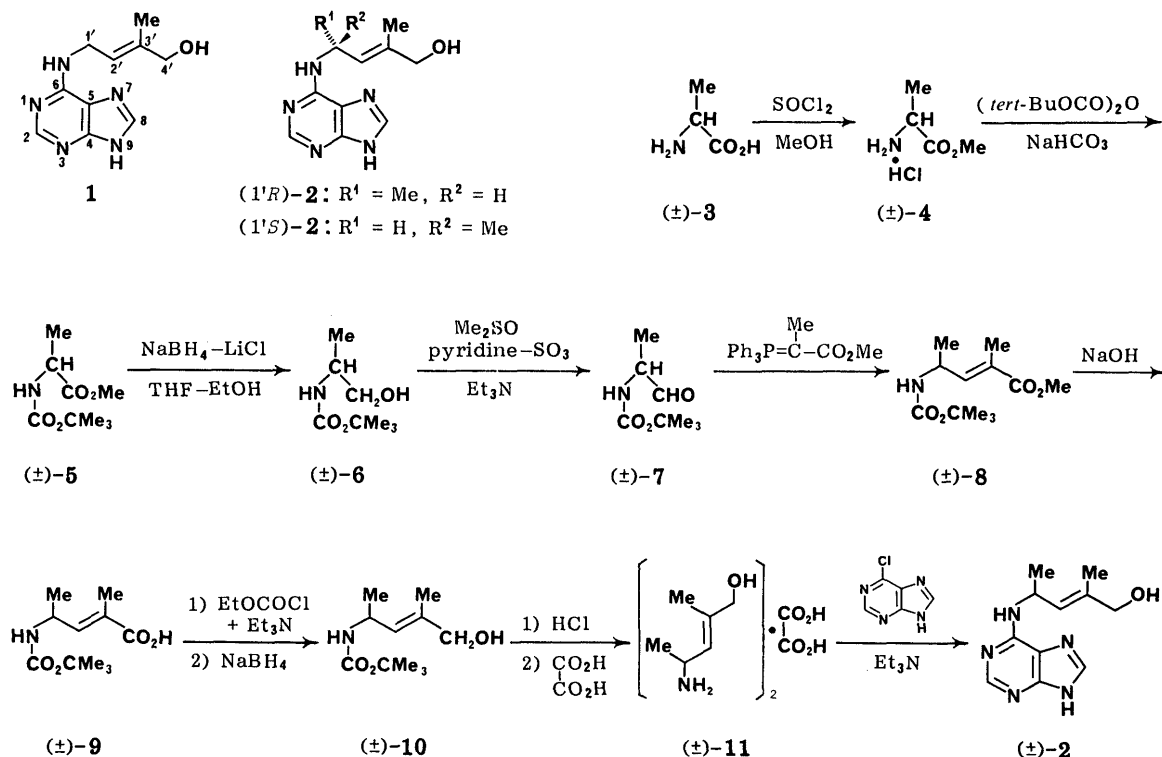


Chart 1

TABLE I. Cytokinin Activity of Racemic and Optically Active 1'-Methylzeatins Tested by the Tobacco Callus Bioassay

Compound	Average fresh weight of tobacco callus (mg)										
	Concentration of test compound (μM)										
	0	0.0001	0.0004	0.001	0.004	0.01	0.04	0.1	0.4	1	4
($\pm$)- 2	21.3	—	22.1	43.1	382.3	1018.5	1624.5	1618.9	1479.7	1038.1	466.3
(1' <i>R</i>)- 2 ^{a)}	17.3	29.2	52.8	120.3	828.1	1064.5	1489.7	1140.2	621.8	556.9	—
(1' <i>S</i>)- 2 ^{a)}	23.3	—	—	—	28.7	61.5	429.5	922.4	1024.5	1512.4	1005.5

a) Taken from reference 2.

hydrolyzed in MeOH with 2N aqueous NaOH at 30 °C for 3 h to furnish the acid ($\pm$)-**9** in 99% yield, and selective reduction of the carboxy group in ($\pm$)-**9** by the method of Yamada and co-workers¹¹⁾ afforded ($\pm$)-**10** in 79% yield. The carbamate ($\pm$)-**10** was hydrolyzed with 10% aqueous HCl at room temperature for 40 min, and the amino alcohol hydrochloride that formed was first converted into the free base [by the use of Amberlite IRA-402 (HCO_3^-)], which was then isolated in the form of the oxalate [($\pm$)-**11**] in 70% yield [from ($\pm$)-**10**]. Purinylation of ($\pm$)-**11** with 6-chloropurine in boiling 1-butanol containing Et_3N for 9.5 h gave the target compound ($\pm$)-**2** in 81% yield.

Table I shows the cytokinin activity of ($\pm$)-**2** as found in the tobacco callus bioassay, together with those reported for (1'*R*)-**2** and (1'*S*)-**2**. It may be seen that racemic 1'-methylzeatin [($\pm$)-**2**] is also active at 0.04–1 μM concentration, a range between the optimum concentrations of both enantiomers. This seems to enhance the practical value of the above racemic synthesis of 1'-methylzeatin.

Experimental

General Notes All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. See reference 2 for details of 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, dd=doublet-of-doublets, dq=doublet-of-quartets, m=multiplet, s=singlet, t=triplet.

($\pm$)-**N-[(1,1-Dimethylethoxy)carbonyl]alanine Methyl Ester [($\pm$)-5]** ($\pm$)-Alanine [($\pm$)-**3**] (13.36 g, 0.15 mol) was first treated with SOCl_2 and MeOH by following the general method⁷⁾ for esterification of α -amino acids, giving ($\pm$)-**4** as a hygroscopic solid (lit.¹²⁾ mp 157 °C). The crude product was then treated with di-*tert*-butyl dicarbonate and NaHCO_3 in a manner similar to that⁹⁾ employed for (*S*)-**4**, and ($\pm$)-**5** was obtained in 95% overall yield [from ($\pm$)-**3**] as a colorless oil. The infrared (IR) (liquid film) and proton nuclear magnetic resonance ($^1\text{H-NMR}$) (CDCl_3) spectra of this sample were superimposable on those of (*R*)- or (*S*)-**5**.²⁾

($\pm$)-**(2-Hydroxy-1-methylethyl)carbamate Acid *tert*-Butyl Ester [($\pm$)-6]** This compound was prepared from ($\pm$)-**5** (14.56 g, 71.6 mmol) in 97% yield by reduction with NaBH_4 -LiCl in a manner similar to that⁹⁾ employed for the (*R*)- or (*S*)-enantiomer. Recrystallization of crude ($\pm$)-**6** (mp 42–44 °C) from hexane and drying over P_2O_5 at 2 mmHg and 30 °C for 21 h afforded colorless needles, mp 50–52 °C; IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3360 (NH), 3240 (OH), 1682 (carbamate CO); $^1\text{H-NMR}$ (CDCl_3) δ : 1.15 (3H, d, $J=6.5$ Hz, CHMe), 1.45 (9H, s, CMe_3), 2.16 (1H, br, OH), 3.50 (dd, $J=11$, 6 Hz) and 3.64 (dd, $J=11$, 3 Hz) (1H each, CHCH_2), 3.80 (1H, m, CHCH_2), 4.66 (1H, br, NH). The routine C, H, N analysis and $^1\text{H-NMR}$ spectrum of this sample suggested contamination with ca. 1/13 eq mol of hexane (small peaks at δ 0.88 and 1.26), which could not be removed by drying of the sample for a further 30 h. Apart from the hexane peaks, however, the $^1\text{H-NMR}$ spectrum was virtually identical with that of (*R*)- or (*S*)-**6**.²⁾

($\pm$)-**(1-Methyl-2-oxoethyl)carbamate Acid *tert*-Butyl Ester [($\pm$)-7]** A solution of SO_2 -pyridine complex (11.94 g, 75 mmol) in dry Me_2SO (75 ml) was added over 4 min to a stirred solution of ($\pm$)-**6** (presumed to contain 1/13 eq mol of hexane as an impurity) (4.38 g, 24.1 mmol) and Et_3N

(7.59 g, 75 mmol) in dry Me_2SO (75 ml) under an atmosphere of N_2 at 21–26 °C with occasional ice-cooling. The mixture was stirred at 22–23 °C for 8 min and then poured onto crushed ice (ca. 600 ml), and the resulting aqueous mixture was extracted with hexane (2 $\times$ 85 ml). The aqueous layer was separated from the hexane layer and extracted with CH_2Cl_2 (10 $\times$ 170 ml). The CH_2Cl_2 extracts were combined, washed successively with 10% aqueous citric acid (2 $\times$ 200 ml), H_2O (2 $\times$ 200 ml), and saturated aqueous NaHCO_3 (200 ml), dried over anhydrous MgSO_4 , and concentrated *in vacuo* to leave ($\pm$)-**7** (3.69 g, 88%) as a colorless solid, mp 76–81 °C. Recrystallization from hexane afforded an analytical sample as colorless plates, mp 83.5–84.5 °C; IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3340 (NH), 1735 (CHO), 1680 (carbamate CO). *Anal.* Calcd for $\text{C}_8\text{H}_{15}\text{NO}_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.10; H, 9.02; N, 8.10. The $^1\text{H-NMR}$ spectrum of this sample in CDCl_3 was superimposable on that of (*R*)- or (*S*)-**7**.²⁾

(*E*)-**($\pm$)-4-[[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid Methyl Ester [($\pm$)-8]** A solution of methyl 2-(triphenylphosphoranylidene)propionate¹⁰⁾ (2.35 g, 6.75 mmol) in CH_2Cl_2 (3 ml) was added to a stirred solution of ($\pm$)-**7** (1.064 g, 6.14 mmol) in CH_2Cl_2 (3 ml) at 15–17 °C with occasional ice-cooling. The resulting mixture was stirred at 21 °C for 2.5 h and then concentrated to dryness *in vacuo*. The oily residue was extracted with hot hexane (7 $\times$ 10 ml), and the combined hexane extracts were concentrated *in vacuo* to leave an oil. Purification of the oil by means of flash chromatography¹³⁾ [column diameter, 20 mm; Silica gel 60 (E. Merck, No. 9385); hexane-AcOEt (3:1, v/v)] afforded a mixture of ($\pm$)-**8** and the (*Z*)-isomer as a colorless solid (1.41 g, 94%). A single recrystallization of the solid from hexane yielded a pure sample of ($\pm$)-**8** (1.06 g, 71%), mp 50–51.5 °C. Further recrystallization from hexane furnished an analytical sample as colorless plates, mp 50.5–52 °C; IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3385 (NH), 1711 (α,β -unsaturated ester CO), 1701 (carbamate CO). *Anal.* Calcd for $\text{C}_{12}\text{H}_{21}\text{NO}_4$: C, 59.24; H, 8.70; N, 5.76. Found: C, 59.00; H, 9.00; N, 5.66. The $^1\text{H-NMR}$ spectrum of this sample in CDCl_3 was identical with that of (*R*)- or (*S*)-**8**.²⁾

(*E*)-**($\pm$)-4-[[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid [($\pm$)-9]** A solution of ($\pm$)-**8** (1.72 g, 7.07 mmol) and 2N aqueous NaOH (7.1 ml) in MeOH (16 ml) was stirred at 30 °C for 3 h. The reaction mixture was concentrated to a volume of ca. 10 ml, brought to pH 1–2 by addition of 2N aqueous HCl, and extracted with CH_2Cl_2 (3 $\times$ 30 ml). The CH_2Cl_2 extracts were dried over anhydrous MgSO_4 and concentrated *in vacuo* to leave a colorless solid (1.61 g, 99%), mp 146–147 °C. Recrystallization of the solid from benzene-hexane (3:2, v/v) yielded an analytical sample of ($\pm$)-**9** as colorless needles, mp 146–147 °C; IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3380 (NH), 1680 (CO_2H and carbamate CO's). *Anal.* Calcd for $\text{C}_{11}\text{H}_{19}\text{NO}_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.55; H, 8.59; N, 5.96. The $^1\text{H-NMR}$ spectrum of this sample in CDCl_3 was virtually identical with that of (*R*)- or (*S*)-**9**.²⁾

(*E*)-**($\pm$)-4-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamate Acid *tert*-Butyl Ester [($\pm$)-10]** A solution of ethyl chloroformate (543 mg, 5 mmol) in dry tetrahydrofuran (THF) (1.5 ml) was added dropwise to a solution of ($\pm$)-**9** (1.146 g, 5 mmol) and Et_3N (506 mg, 5 mmol) in dry THF (6.5 ml) with stirring at –15 °C. The mixture was stirred at temperatures of –10 °C to –5 °C for 40 min, and the precipitate that resulted was filtered off and washed with THF (3 $\times$ 2.5 ml). The filtrate and washings were combined and added dropwise to a stirred mixture of NaBH_4 (473 mg, 12.5 mmol) and H_2O (5 ml) at 5–10 °C over 10 min. The resulting mixture was stirred at room temperature for 3 h, concentrated *in vacuo* to a volume of ca. 6 ml, brought to pH 3–4 with 10% aqueous H_3PO_4 , and extracted with ether (16 ml, then 3 $\times$ 8 ml). The ethereal extracts were washed with saturated aqueous NaHCO_3 , dried over anhydrous MgSO_4 , and concentrated *in vacuo* to leave a colorless oil. The oil was purified by flash chromatography¹³⁾ [column diameter, 30 mm; Silica gel 60 (E. Merck, No. 9385);

AcOEt-hexane (1 : 1, v/v)] to give $(\pm)$ -**10** (847 mg, 79%) as a colorless oil. The IR spectrum (liquid film) of this sample was superimposable on that of (*R*)- or (*S*)-**10**.²⁾

(*E*)- $(\pm)$ -4-Amino-2-methyl-2-penten-1-ol Ethanedioate (2 : 1) (Salt) [($\pm$)-11**]** A mixture of $(\pm)$ -**10** (845 mg, 3.92 mmol) and 10% aqueous HCl (7.8 ml) was shaken at room temperature for 40 min, giving a clear solution. The solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (40 ml), and the column was eluted with H_2O . The eluate (160 ml) was concentrated to dryness *in vacuo* to leave an oil, which was dissolved in EtOH (1 ml). The resulting ethanolic solution was exactly neutralized by addition of a solution of oxalic acid (177 mg, 1.97 mmol) in EtOH (1 ml) and, if necessary, with Et_3N . The precipitate that resulted was collected by filtration, washed with EtOH (10 ml), and dried to give $(\pm)$ -**11**·1/3 H_2O (450 mg, 70%) as a colorless solid. Recrystallization of the solid from EtOH and drying over P_2O_5 at 2 mmHg and 75 °C for 10 h yielded an analytical sample as colorless needles, mp 197–199 °C (dec.); IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3335 (OH), 1582 (COO^- and NH_3^+); $^1\text{H-NMR}$ ($\text{Me}_2\text{SO}-d_6$) (at 25 °C) δ : 1.13 (3H, d, $J=6.5$ Hz, CHMe), 1.58 (3H, br s, $\text{CH}=\text{CMe}$), 3.78 (2H, br s, CH_2OH), 3.85 (1H, dq, $J=9, 6.5$ Hz, CHMe), 5.34 (1H, br d, $J=9$ Hz, $\text{CH}=\text{CMe}$) (overlapped with a broad signal attributable to OH and NH_3^+). *Anal.* Calcd for $\text{C}_{14}\text{H}_{28}\text{N}_2\text{O}_6 \cdot 1/3 \text{H}_2\text{O}$: C, 51.52; H, 8.85; N, 8.58. Found: C, 51.50; H, 9.08; N, 8.45.

(*E*)- $(\pm)$ -2-Methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [($\pm$)-1**]-Methylzeatin [($\pm$)-**2**]** A stirred solution of 6-chloropurine (104 mg, 0.67 mmol) and $(\pm)$ -**11**·1/3 H_2O (128 mg, 0.39 mmol) in 1-butanol (6.7 ml) containing Et_3N (164 mg, 1.62 mmol) was heated under reflux for 9.5 h. The reaction mixture was concentrated *in vacuo* to leave a jelly, which was dissolved in a little H_2O . The resulting aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (7 ml), and the column was eluted with H_2O . The eluate (250 ml) was concentrated *in vacuo*, and the residue was purified by flash chromatography¹³⁾ [column diameter, 20 mm; Silica gel 60 (E. Merck, No. 9385); CHCl_3 -MeOH (20 : 3, v/v)] to give $(\pm)$ -**2** (126 mg, 81%) as a colorless solid. Recrystallization from H_2O produced an analytical sample as colorless prisms, mp 175.5–176.5 °C; $^1\text{H-NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ : 1.25 (3H, d, $J=6.5$ Hz, CHMe), 1.66 (3H, br s, $\text{CH}=\text{CMe}$), 3.77 (2H, m, CH_2OH), 4.72 (1H, t, $J=5$ Hz, OH), 5.30 (1H, m, CHMe), 5.53 (1H, m, $\text{CH}=\text{CMe}$), 7.43 (1H, d, $J=8.5$ Hz, $\text{N}^6\text{-H}$), 8.06

and 8.16 (1H each, s, purine protons), 12.78 (1H, br, NH). *Anal.* Calcd for $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.78; H, 6.62; N, 29.92. The mass, ultraviolet (in 95% aqueous EtOH and in H_2O at pH 1, 7, and 13), and $^1\text{H-NMR}$ (in $\text{Me}_2\text{SO}-d_6$) spectra and thin-layer chromatographic behavior of this sample were identical with those of (*1'R*)-**2** or (*1'S*)-**2**.²⁾

Bioassay Procedure The cytokinin activity of $(\pm)$ -**2** was tested in the tobacco callus bioassay in a manner similar to that described recently²⁾ for (*1'R*)-**2** and (*1'S*)-**2**. The results are shown in Table I.

References and Notes

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