Purines. XXXV.[1] Synthesis and Cytokinin Activity of Racemic 1′-Methylzeatin

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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 paper[2] 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 (±)-**3**[6] 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 $LiBH_4$ and oxidation of the resulting *N*-protected amino alcohol [(±)-**6**] using Me_2SO and SO_3–pyridine complex in the presence of Et_3N were effected in a manner similar to that employed by Shioiri's group[9] for the (*S*)-series, producing the aldehyde (±)-**7** in 81% overall yield [from (±)-**3**]. Wittig reaction of (±)-**7** with methyl 2-(triphenylphosphoranylidene)propionate[10] in CH_2Cl_2 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

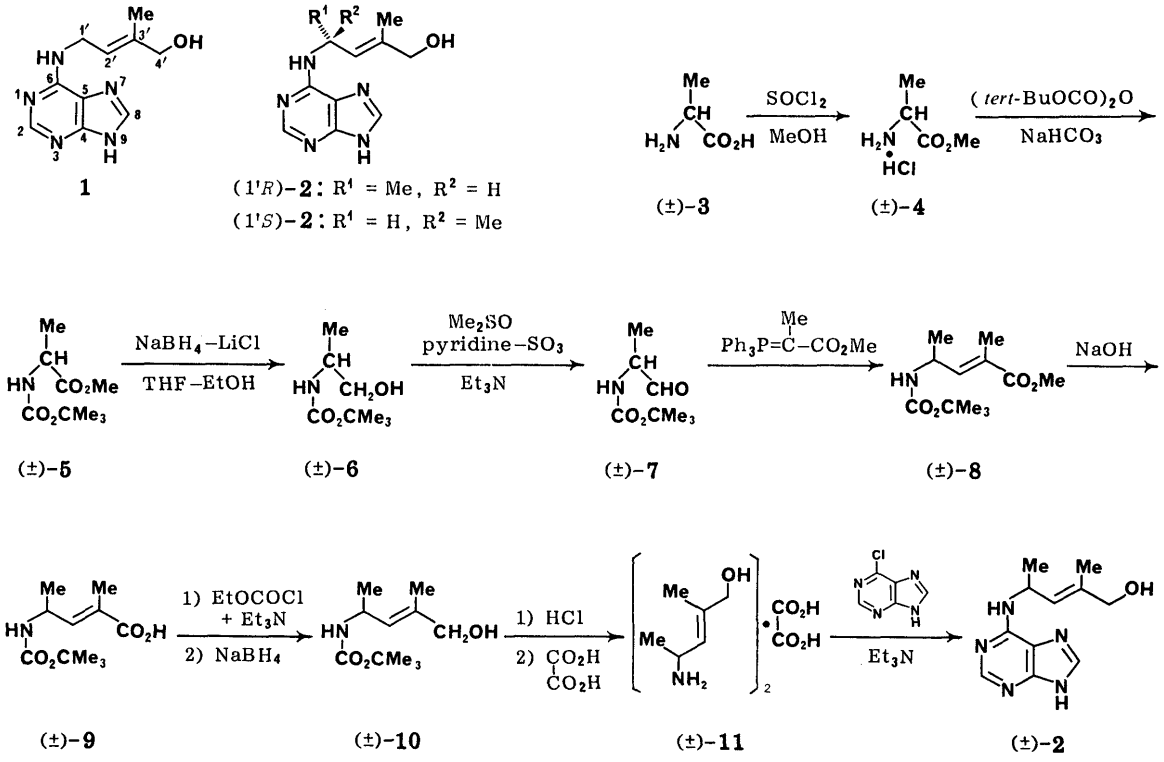

Chart 1

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
(±)-**2**	21.3	—	22.1	43.1	382.3	1018.5	1624.5	1618.9	1479.7	1038.1	466.3
(1′*R*)-**2**[a]	17.3	29.2	52.8	120.3	828.1	1064.5	1489.7	1140.2	621.8	556.9	—
(1′*S*)-**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 2 N aqueous NaOH at 30 °C for 3 h to furnish the acid (±)-**9** in 99% yield, and selective reduction of the carboxy group in (±)-**9** by the method of Yamada and co-workers[11] afforded (±)-**10** in 79% yield. The carbamate (±)-**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 [(±)-**11**] in 70% yield [from (±)-**10**]. Purinylation of (±)-**11** with 6-chloropurine in boiling 1-butanol containing Et_3N for 9.5 h gave the target compound (±)-**2** in 81% yield.

Table I shows the cytokinin activity of (±)-**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 [(±)-**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.

(±)-*N*-[(1,1-Dimethylethoxy)carbonyl]alanine Methyl Ester [(±)-5] (±)-Alanine [(±)-**3**] (13.36 g. 0.15 mol) was first treated with $SOCl_2$ and MeOH by following the general method[7] for esterification of α-amino acids, giving (±)-**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 that[8] employed for (*S*)-**4**, and (±)-**5** was obtained in 95% overall yield [from (±)-**3**] as a colorless oil. The infrared (IR) (liquid film) and proton nuclear magnetic resonance (^{1}H-NMR) ($CDCl_3$) spectra of this sample were superimposable on those of (*R*)- or (*S*)-**5**.[2]

(±)-(2-Hydroxy-1-methylethyl)carbamic Acid *tert*-Butyl Ester [(±)-6] This compound was prepared from (±)-**5** (14.56 g, 71.6 mmol) in 97% yield by reduction with $NaBH_4$–LiCl in a manner similar to that[9] employed for the (*R*)- or (*S*)-enantiomer. Recrystallization of crude (±)-**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 ν_{max}^{Nujol} cm^{-1}: 3360 (NH), 3240 (OH), 1682 (carbamate CO); ^{1}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}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}H-NMR spectrum was virtually identical with that of (*R*)- or (*S*)-**6**.[2]

(±)-(1-Methyl-2-oxoethyl)carbamic Acid *tert*-Butyl Ester [(±)-7] A solution of SO_3–pyridine complex (11.94 g, 75 mmol) in dry Me_2SO (75 ml) was added over 4 min to a stirred solution of (±)-**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 (2×85 ml). The aqueous layer was separated from the hexane layer and extracted with CH_2Cl_2 (10×170 ml). The CH_2Cl_2 extracts were combined, washed successively with 10% aqueous citric acid (2×200 ml), H_2O (2×200 ml), and saturated aqueous $NaHCO_3$ (200 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave (±)-**7** (3.69 g, 88%) as a colorless solid, mp 76—81 °C. Recrystallization from hexane afforded an analytical smaple as colorless plates, mp 83.5—84.5 °C; IR ν_{max}^{Nujol} cm^{-1}: 3340 (NH), 1735 (CHO), 1680 (carbamate CO). *Anal.* Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.10; H, 9.02; N, 8.10. The ^{1}H-NMR spectrum of this sample in $CDCl_3$ was superimposable on that of (*R*)- or (*S*)-**7**.[2]

(*E*)-(±)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid Methyl Ester [(±)-8] A solution of methyl 2-(triphenylphosphoranylidene)propionate[10] (2.35 g, 6.75 mmol) in CH_2Cl_2 (3 ml) was added to a stirred solution of (±)-**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 (7×10 ml), and the combined hexane extracts were concentrated *in vacuo* to leave an oil. Purification of the oil by means of flash chromatography[13] [column diameter, 20 mm; Silica gel 60 (E. Merck, No. 9385); hexane–AcOEt (3:1, v/v)] afforded a mixture of (±)-**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 (±)-**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 ν_{max}^{Nujol} cm^{-1}: 3385 (NH), 1711 (α,β-unsaturated ester CO), 1701 (carbamate CO). *Anal.* Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.76. Found: C, 59.00; H, 9.00; N, 5.66. The ^{1}H-NMR spectrum of this sample in $CDCl_3$ was identical with that of (*R*)- or (*S*)-**8**.[2]

(*E*)-(±)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid [(±)-9] A solution of (±)-**8** (1.72 g, 7.07 mmol) and 2 N 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 2 N aqueous HCl, and extracted with CH_2Cl_2 (3×30 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 (±)-**9** as colorless needles, mp 146—147 °C; IR ν_{max}^{Nujol} cm^{-1}: 3380 (NH), 1680 (CO_2H and carbamate CO's). *Anal.* Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.55; H, 8.59; N, 5.96. The ^{1}H-NMR spectrum of this sample in $CDCl_3$ was virtually identical with that of (*R*)- or (*S*)-**9**.[2]

(*E*)-(±)-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(±)-10] A solution of ethyl chloroformate (543 mg, 5 mmol) in dry tetrahydrofuran (THF) (1.5 ml) was added dropwise to a solution of (±)-**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 (3×2.5 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 3×8 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 chromatography[13] [column diameter, 30 mm; Silica gel 60 (E. Merck, No 9385);

AcOEt–hexane (1 : 1, v/v)] to give (±)-**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*)-(±)-4-Amino-2-methyl-2-penten-1-ol Ethanedioate (2 : 1) (Salt) [(±)-11] A mixture of (±)-**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 (±)-**11**·1/3H_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 ν_{max}^{Nujol} cm^{-1}: 3335 (OH), 1582 (COO^- and NH_3^+); ^{1}H-NMR (Me_2SO-d_6) (at 25 °C) δ: 1.13 (3H, d, J=6.5 Hz, CHMe), 1.58 (3H, br s, CH=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, CH=CMe) (overlapped with a broad signal attributable to OH and NH_3^+). *Anal.* Calcd for $C_{14}H_{28}N_2O_6 \cdot 1/3\ H_2O$: C, 51.52; H, 8.85; N, 8.58. Found: C, 51.50; H, 9.08; N, 8.45.

(*E*)-(±)-2-Methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [(±)-1′-Methylzeatin] [(±)-2] A stirred solution of 6-chloropurine (104 mg, 0.67 mmol) and (±)-**11**·1/3H_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 chromatography[13)] [column diameter, 20 mm; Silica gel 60 (E. Merck, No. 9385); $CHCl_3$–MeOH (20 : 3, v/v)] to give (±)-**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}H-NMR (Me_2SO-d_6) δ: 1.25 (3H, d, J=6.5 Hz, CHMe), 1.66 (3H, br s, CH=CMe), 3.77 (2H, m, CH_2OH), 4.72 (1H, t, J=5 Hz, OH), 5.30 (1H, m, CHMe), 5.53 (1H, m, CH=CMe), 7.43 (1H, d, J=8.5 Hz, N^6-H), 8.06 and 8.16 (1H each, s, purine protons), 12.78 (1H, br, NH). *Anal.* Calcd for $C_{11}H_{15}N_5O$: 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}H-NMR (in Me_2SO-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 (±)-**2** was tested in the tobacco callus bioassay in a manner similar to that described recently[2)] for (1′*R*)-**2** and (1′*S*)-**2**. The results are shown in Table I.

References and Notes

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