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Dihydrozeatin: An Improved Synthesis and Resolution of Both Isomers

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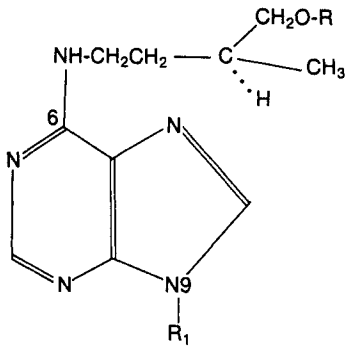
Abstract. (±)-Dihydrozeatin was synthesized in a 3-step synthesis by: (1) a Michael condensation of methyl methacrylate with nitromethane to give (±) - methyl 2 - methyl - 4 - nitrobutyrate, which was (2) reduced to (±) - 4 - amino - 2 - methylbutan - 1 - ol and (3) reaction of the aminoalcohol with 6-chloropurine. Hydrolysis of racemic nitroester gave (±) - 2 - methyl - 4 - nitrobutyric acid, which was resolved by means of (+) - and (−) - α - methylbenzylamine salts. Conversion of the salts to the corresponding methyl esters and subsequent reductions yielded optically active 4 - amino-2 - methylbutan - 1 - ols. Examination of the NMR spectra of the resolved methyl 2 - methyl - 4 - nitrobutyrates in the presence of a chiral shift reagent established their optical purities to be greater than 98%. The specific rotations at 589 nm of the *S*-(−) and *R*-(+)-dihydrozeatins derived from optically active butanols were appreciably lower than previously reported. Application of the Drude equation to ORD values from 320 to 589 nm verified the low 589 nm rotations of the dihydrozeatin enantiomers. The biological activities of (*R*), (*S*) and (*R,S*) dihydrozeatins in the betacyanin stimulation assay with *Amaranthus* parallel the activities found in other cytokinin bioassays.

S - (−) - Dihydrozeatin, **1**, an active intermediate in the biosynthetic pathway of the natural cytokinins, has been reported to be widely dispersed in plants (Koshimizu et al. 1967, Shindy and Smith 1975, Purse et al. 1976). Recently the 9-riboside, **2** (Wang and Horgan 1978, Duke et al. 1979), the O - β - D -



1 R = R_1 = H
2 R = H; R_1 = β-D-ribofuranose
3 R = β-D-glucofuranose; R_1 = H
4 R = β-D-glucofuranose; R_1 = β-D-ribofuranose

glucoside, **3**, and the O - β - D - glucoside of dihydrozeatin riboside, **4**, have been isolated (Wareing et al. 1977, Wang et al. 1977, Duke et al. 1979), and also occur ubiquitously. The 9-riboside, **2**, is of special interest because it has not been observed as a constituent of tRNA, as have other 5-carbon chain cytokinins such as N^6 - (Δ^2 - isopentenyl) adenosine and the ribosides of *E*- and *Z*-zeatins. The question of whether cytokinin bases are derived from tRNA breakdown is still moot (Chen et al. 1976, Burrows 1978, Stuchbury et al. 1979, Barnes et al. 1980, Nishinari and Syōno 1980, Maass and Klämbt 1981). Thus, the origin of dihydrozeatin is related to the biosynthesis of cytokinins and the control of their cellular concentrations (Palmer et al. 1981).

The O-β -D - glucoside, **3**, is directly involved in concentration control, since it can be hydrolysed to dihydrozeatin (Smith and van Staden 1978, van Staden 1979) or alternatively be translocated to a nonrecoverable location and completely inactivated (van Staden and Smith 1978).

(±)-Dihydrozeatin has been synthesized by several methods involving reduction or cleavage of purine derivatives (Koshimizu et al. 1967, Leonard et al. 1969, Matsubara et al. 1977, Letham et al. 1969) and by reaction of (±) - 4 - amino - 2 - methylbutan - 1 - ol, **6**, with 6 - chloropurine (Letham et al. 1969, Fujii and Ogawa 1972).

Enantiomers of dihydrozeatin have been prepared by hydrolysis of the ribosides (Matsubara et al. 1977) and from reactions of the resolved 4 - amino - 2 - methylbutan - 1 - ols with 6-chloropurine (Fujii and Ogawa 1972, Matsubara et al. 1977). The overall yields from reaction sequences used to prepare the chiral aminoalcohols were low, and the optical purities of the aminoalcohols and of the derived dihydrozeatins are not known. In any study relating biological activity to optical isomerism, it is imperative that the optical purity or difference in enantiomeric mole fraction, e.e. (enantiomeric excess) (Morrison and Mosher 1971), be known with certainty. This is of special concern, since we have prepared *S* - (−) - and *R* - (±) - dihydrozeatins possessing optical rotations at 589 nm appreciably different from those previously reported.

Matsubara et al. (1977) reported biological activities of *S* - (−) - , *R* - (+) - , and (±)-dihydrozeatins in 4 different standard assays: (1) growth stimulation in cytokinin-dependent tobacco callus, (2) increase in germination of lettuce seed, (3) increase in weight of radish cotyledons, and (4) retardation of chlorophyll loss in radish cotyledons. Our preparations have been tested in a fifth commonly used bioassay which measures the stimulation of betacyanin production in *Amaranthus*.

Materials and Methods

Chemicals

Chemicals were obtained from commercial sources or synthesized by conventional means. The enantiomers of α-methylbenzylamine were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, USA. Silicar 7 is a product of the Mallinckrodt Chemical Works, St. Louis, Missouri, USA.

Physical Measurements

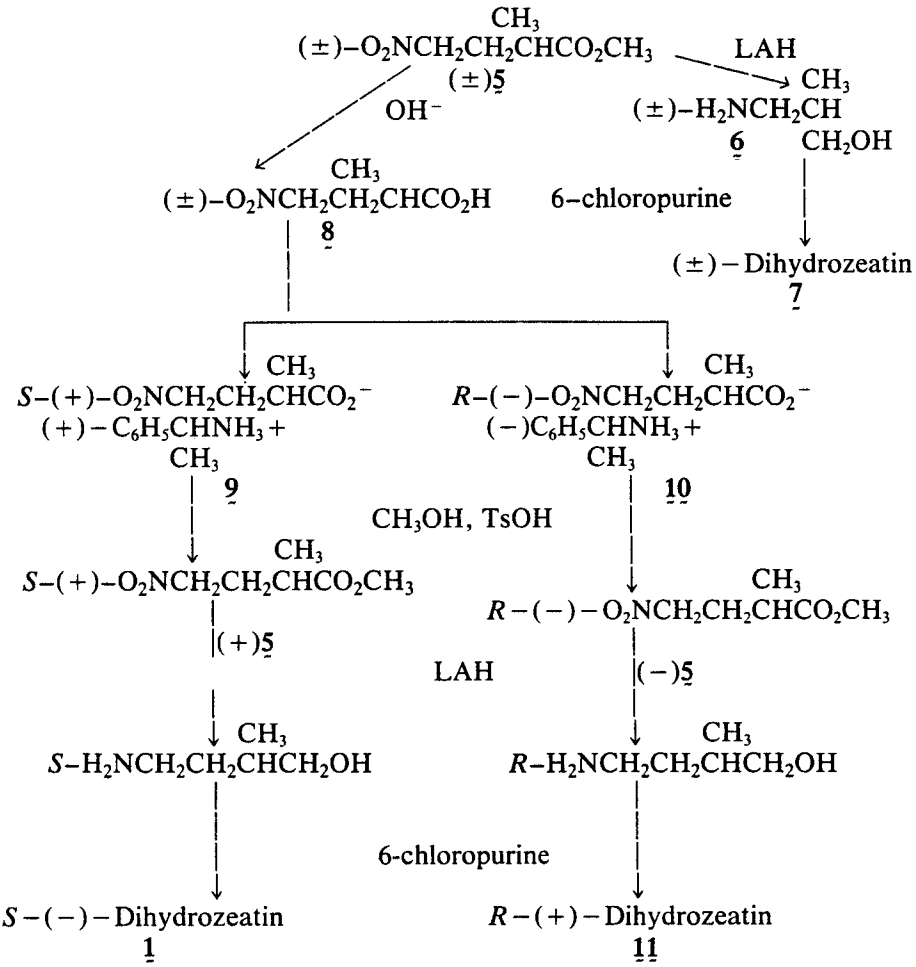
Optical rotation data was taken with a Cary model 60 spectropolarimeter, a Bendix polarimeter, Model 143A, and a Perkin-Elmer polarimeter, Model 241. Proton NMR spectra were taken at room temperature on Varian HA-100 (100 MHz) and Varian EM-390 (90MHz) spectrometers. Melting points were taken on a Kofler hot stage and were uncorrected.

Betacyanin Assay

The detailed method of Elliott (1979) was followed, using the variation of 46-h germination at 37°C. Seeds of *Amaranthus caudatus* L. var. Early Splendor were obtained from Northrup King Co., Minneapolis, Minnesota, USA. Half-seedlings (40) minus the root were homogenized with a glass-Teflon homogenizer. All treatments were assayed in triplicate. Standard deviations were less than ±15%.

Synthesis

(±) - Methyl 2 - methyl - 4 - nitrobutyrate **5.** This was prepared according to Leonard and Shoemaker (1949); NMR (CCl_4) δ4.37 (t,2H), 3.66 (s,3H), 2.0–2.7 (m, 3H), 1.22 (d, 3H, J = 7.5Hz). On the addition of 17.6 mg of $Eu(tfc)_3$ to the CCl_4 solution in the NMR tube (16.7 mg **5**), the methyl doublet shifted to δ1.54, and each peak split into a doublet with a separation of 1.2 Hz. This splitting in the presence of the chiral shift reagent enabled the enantiomeric purity of samples of resolved methyl 2 - methyl - 4 - nitrobutyrates, (+)-**5** and (−)-**5**, to be estimated to ±2%.



(±) - 2 - Methyl - 4 - nitrobutyric acid **8.** One mol (161 g) of (±)-**5** was added dropwise with stirring to a soln of 88 g of KOH in 500 ml H_2O at room temp (water bath). Stirring was continued for 3 h, and the resulting homogeneous soln was extracted 2× with 200 ml of ether. The aqueous layer was cooled and acidified (pH 2) with cold dil H_2SO_4. The precipitated salts were removed by filtration and washed with ethyl acetate. The aqueous filtrate was extracted 3× with 200 ml portions of ethyl acetate. The washings and extracts were combined, washed 2× with 100-ml portions of H_2O, and then dried with Na_2SO_4. The ethyl acetate was mostly removed on a rotary evaporator (40°) and the remainder by pumping at 0.001/mm overnight; yield, 119 g (81%) of a thick yellowish oil which crystallized in the refrigerator. On recrystallization from methyl acetate-petroleum ether, **8** had a mp 30–32°C; NMR, Me_2SO-d_6, δ4.42 (dd, complex 2H), 2.68 (m,1H), 2.43 (m,2H), 1.10 (d,3H). Anal. calcd for $C_5H_9NO_4$: C,40.81; H,6.17, N,9.52. Found: C,40.3; H,6.17; N,9.17. A partially resolved sample $[\alpha]_D^{28}$ +13.08 (c5, EtOH) had the analysis: C,41.0; H,6.36.

(±) - 2 - Methyl - 4 - nitrobutyramide. Five grams of **8** was allowed to stand overnight with 15 ml of $SOCl_2$. The excess $SOCl_2$ was removed *in vacuo,* and the residue was poured into conc NH_4OH. The yield of amide was essentially quantitative; recrystallized from methyl acetate-petroleum ether; mp 65–66°C; NMR (Me_2SO-d_6) δ 5.80 (b,2H), 4.40 (t,2H), 2.27 (m, complex, 3H), 1.22 (d,3H). Anal. calcd for $C_5H_{10}N_2O_3$: C, 41.09; H,6.9; N,19.17. Found: C,41.3; H,6.86; N,19.0.

(+) - α - Methylbenzylammonium (+) - 2 - methyl - 4 - nitrobutyrate **9.** A soln of 50.8 g (0.35 mol) of **8** in 250 ml of anhyd ether was added to 20.2 g (21.5 ml; 0.17 mol) of d - (+) - α - methylbenzylamine, 98%, in 250 ml of anhyd ether. The soln was allowed to stand at room temp for 2 h and then overnight in a refrigerator. The crystals were collected, washed with ether, and air dried; yield 24.7 g, $[\alpha]_D^{25}$ +10.3° (c5, EtOH). On being recrystallized from 200 ml of ethyl acetate, 11.1 g were recovered, $[\alpha]_D^{25}$ +11.06° (c5, EtOH). The above 11.1 g of salt was mixed with 5 g of a different batch $[\alpha]_D^{25}$ + 11.18° (c5, EtOH) and recrystallized from 150 ml of ethyl acetate; mp 121–123°C; yield 7.4 g (11% based on amine); $[\alpha]_{365}^{25}$ + 27.4°, $[\alpha]_{436}^{25}$ + 22.2°, $[\alpha]_{546}^{25}$ + 13.82°, $[\alpha]_{578}^{25}$ + 12.24°, $[\alpha]_{589}^{25}$ + 11.76° (c5, EtOH). Anal. calcd for $C_{13}H_{20}N_2O_4$:C, 58.19; H,7.51; N,10.44. Found: C,58.6; H,7.55; N,10.3.

(−)-α-Methylbenzylammonium (−) - 2 - methyl - 4 - nitrobutyrate **10.** This was prepared as was **9** described above, but from 1 - (−) - α - methylbenzylamine. The first crop weighed 29.8 g. An additional 8 g from another lot was added, and the 37.8 g was recrystallized from 275 ml of ethyl acetate (22.09 g). The second recrystallization (160 ml of ethyl acetate) gave 14.77 g, and a third recrystallization gave 9.79 g (21% based on amine); mp 119–121°C; $[\alpha]_{365}^{25}$ − 27.88°, $[\alpha]_{436}^{25}$ − 22.24°, $[\alpha]_{546}^{25}$ − 13.84°, $[\alpha]_{578}^{25}$ − 12.32°, $[\alpha]_{589}^{25}$ − 11.84° (c5, EtOH). Anal. calcd for $C_{13}H_{20}N_2O_4$: C,58.19; H,7.51; N,10.44. Found: C,58.5; H,7.37; N,10.3.

S - (+) - Methyl 2 - methyl - 4 - nitrobutyrate (+) **5.** A soln of 5.36 g (20 mmol) of **9** and 4.1 g of 4-toluenesulfonic acid monohydrate in 100 ml of CH_3OH was allowed to stand overnight at room temperature and then refluxed for 1 h. The excess MeOH was removed *in vacuo,* and 100 ml of H_2O and 100 ml of ether were added to the residue. The ether layer was separated, washed with H_2O, dil $KHCO_3$ soln, again with H_2O and dried over Na_2SO_4. Distillation gave 2.9 g (90%) of **11;** bp 70–73°C (0.1 mm); $[\alpha]_{365}^{25}$ + 64.1°, $[\alpha]_{436}^{25}$ + 49.98°, $[\alpha]_{546}^{25}$ + 27.63°, $[\alpha]_{578}^{25}$ + 24.46°, $[\alpha]_{589}^{25}$ + 23.47° (c5, EtOH); NMR (CCl_4) δ4.36 (t,2H), 3.70 (s,3H), 2.0–2.70 (m,3H), 1.24 (d,3H). Anal. calcd for $C_6H_{11}NO_4$: C,44.71; H,6.88. Found: C,45.0; H,7.04. On the addition of 24 mg of Eu(tfc)$_3$ to the sample of ester (17 mg) in the NMR tube, the methyl doublet centered on δ1.24 shifted to δ1.65. There was no splitting of the shifted doublet; estimated e.e. > 98%.

R-(−)-Methyl 2-methyl-4-nitrobutyrate (−)-**5**. This was prepared similarly to (+)-**5**. A yield of 4.85 g (83%) was obtained from 9.75 g (36 mmol) of **10**; bp ≈ 72° (0.1 mm); $[\alpha]^{25}_{365}$ − 63.62°, $[\alpha]^{25}_{436}$ − 44.58°, $[\alpha]^{25}_{546}$ − 27.24°, $[\alpha]^{25}_{578}$ − 24.11°, $[\alpha]^{25}_{589}$ − 23.15° (c5, EtOH); NMR (CCl_4) δ4.37(t,2H), 3.70 (s,3H), 2.0–2.70 (m,3H), 1.22(d,3H). Anal. calcd for $C_6H_{11}NO_4$: C,44.71; H,6.88. Found: C,44.8; H,6.98. The addition of 23.4 mg of $Eu(tfc)_3$ to 16.35 mg of (−)-**5** in the NMR tube caused the doublet centered at δ1.22 to shift to δ1.65, with no observable splitting, estimated e.e. > 98%.

(±)-4-Amino-2-methylbutan-1-ol **6**. A soln of 34.8 g (0.216 mol) of (±)-**5** in 100 ml of anhyd ether was added, under N_2 with stirring and cooling (ice) to 25 g of LAH in 500 ml of anhyd ether. After the addition was complete, the mixture was refluxed for 2 h and then cooled. A soln of 50 ml of EtOH in 100 ml of ether was cautiously added; when the vigorous reaction had abated, another 100 ml of EtOH was added, followed by 50 ml of satd NaCl soln in water. The organic layer was decanted and the semisolid residue was extracted 2× with a mixture of 100 ml EtOH: ether (1:1). The combined organic solvent extracts were evaporated *in vacuo*, dried with K_2CO_3, and distilled; 15.5 g (69%), bp 73–75°C (0.2 mm); NMR (pyridine-d_5)δ3.55(d,2H), 2.76(m,2H), 1.80(m,1H), 1.42(m,2H), 0.95 (d,3H). Anal. calcd for $C_5H_{13}NO$: C,58.21; H,12.7. Found: C,57.9; H,12.5.

(±)-O,N-Bis-(trifluoroacetyl)-4-amino-2-methylbutan-1-ol. A mixture of 7 g (9.7 mmol) of **6** and 8.1 g (39 mmol) of trifluoroacetic anhydride was refluxed for 3 h. Distillation gave 2.1 g (60%) of product: bp 108–110°C (0.25 mm); NMR($CDCl_3$) δ4.22(d,2H), 3.45(dd,2H), 1.6(m, complex, 4H), 1.05(d,3H). Anal. calcd for $C_9H_{11}F_6NO_3$: N,4.75. Found: N,4.63. The addition of $Eu(tfc)_3$ to the NMR sample resulted in spectral shifts, but no splitting of the CH_3 doublet occurred.

(±)-N-(4-Hydroxy-3-methylbutyl)phthalimide. A mixture of 7.0 g (68 mmol) of **6** and 10.0 g of phthalic anhydride were refluxed in 50 ml of 1,2-dimethoxyethane for 3 h. The solvent was removed *in vacuo*, and the residue was distilled at 0.05 mm (bath temp 205°C); yield 12.2 g (77%); mp 53–54°C; NMR ($CDCl_3$) δ7.74 (m,4H), 3.71(t,2H), 3.44(m,3H), 1.67(m, complex, 3H), 0.98(d,3H). Anal. calcd for $C_{13}H_{15}NO_3$: C,66.93; H,6.48 N,6.01. Found: C,66.9; H,6.54; N,6.00.

(±)-Dihydrozeatin **7**. A mixture of 7.5 g (49 mmol) of 6-chloropurine and 5.1 g (50 mmol) of **6** in 50 ml of 1-BuOH and 8 ml of Et_3N was heated on the steam bath overnight. The BuOH was removed *in vacuo*, a solution of 6 g of $KHCO_3$ was added to the residue, and the H_2O removed *in vacuo*. The dark residue was extracted 4× with 50 ml portions of hot EtOH and the extracts combined, decolorized with carbon, and evaporated to dryness *in vacuo*. Recrystallization from EtOH-MeCN gave 7.6 g (70%) of **7**, mp 167–168°C; NMR(Me_2SO-d_6) δ12.82 (s,1H), 8.20(s,1H), 8.06(s,1H), 7.47(t,1H), 4.45(b,1H),

3.1–3.8(bm,4H), 1.1–1.9 (m, complex, 3H), 0.87(d,3H). Anal. calcd for $C_{10}H_{15}N_5O$: C,54.28; H,6.83; N,31.66. Found: C,54.2; H,6.75; N,31.8.

S - (−) - Dihydrozeatin **1.** A soln of 2.7 g (16.7 mmol) of (+)-**5** in 20 ml of ether was added to a stirred mixture of 2.35 g of LAH (under N_2) in such a way as to maintain refluxing. Another 10 ml of ether was added, and stirring was continued for 2 h. The reaction mixture was cooled with ice, and 15 ml of EtOH was slowly added, followed by 5 ml of satd NaCl soln. The liquid was decanted and the semisolid mass was extracted 2× with 40-ml portions of ether-EtOH(9:1) and 2× with 50-ml portions of ether-EtOH(4:1). The extracts were combined and the solvents removed *in vacuo*. The residual oil was added to a mixture of 1.9 g (12 mmol) of 6-chloropurine, 3 ml of Et_3N, and 30 ml of 1-BuOH and heated on the steam bath for 3 h. Ten ml of satd $KHCO_3$ soln and 20 ml of H_2O were added and the solvents removed *in vacuo*. The residue was extracted with hot EtOH (3 × 30 ml). The EtOH extracts were combined, filtered, and evaporated *in vacuo*. The solid residue was chromatographed on a Silicar-7 (200–325 mesh) column (25 mm × 40 cm) using a linear gradient of ethyl acetate vs. 25% EtOH in ethyl acetate. The main fraction absorbing at 254 nm was collected and recrystallized from EtOH-ethyl acetate, yield 0.85 g (23% from (+)-**5**); mp 154–155°C. The NMR spectrum was identical to that of **7.** The optical data is given in Table 1 (c 2.5, EtOH). Anal. calcd for $C_{10}H_{15}N_5O$: C,54.28; H,6.83; N,31.66. Found: C,54.0; H,6.81; N,31.5.

R - (+) - Dihydrozeatin **11.** This was prepared exactly in detail as **1** from 4.28 g (16 mmol) of (−)-**5** and 3.4 g LAH. There was obtained 1.1 g (31%) of **11** after chromatography; mp 154–155°C; NMR identical to **7;** the optical data is reported in Table 1 (c5, EtOH). Anal. calcd for $C_{10}H_{15}N_5O$: C,54.28; H,6.83; N,31.66. Found: C,54.3; H,6.79; N,31.2

Results and Discussion

Methyl (±) - 2 - methyl - 4 - nitrobutyrate, **5,** was readily formed upon the reaction of methyl methacrylate with nitromethane in about 20% yield (Leonard and Shoemaker 1949). LAH reduction of (±)-**5** simultaneously reduced the nitro group to an amine (Nystrom and Brown 1948) and the ester to an alcohol to yield (±)- **6** (80 %). Reaction of (±)- **6** with 6-chloropurine gave (±)-dihydrozeatin, **7** (70%) (Scheme 1).

Hydrolysis of (±)-**5,** gave (±) - 2 - methyl - 4 - nitrobutyric acid, **8,** which formed salts with several optically active bases such as d-(+)- and 1 - (−) - α - methylbenzylamine. The salts were then converted directly into the optically active methyl esters, (+)-**5** and (−)-**5,** which were examined by NMR spectroscopy using the chiral shift reagent, tris - [3 - (trifluoromethylhydroxymethylene) - d - camphorato], europium III, (Eu $(tfc)_3$). The NMR spectrum of (±)-**5** in carbon tetrachloride gave a doublet for the C-2 methyl at δ1.22 (3H, J = 7.5 Hz), each line of which, in the presence of Eu $(tfc)_3$ (1:1 w/w) was split into 2 lines of equal intensity with a separation of 1.2 Hz. The e.e. of the resolved nitro esters was determined from the relative line heights (Goering et al. 1971). A sample of (+)-**5** with an e.e. of 72% gave *S* - (−) -

dihydrozeatin, **1**, $[\alpha]_D^{25}$ − 6.75° (calculated for pure isomer, $[\alpha]_D^{25}$ − 9.38°). The 2 enantiomeric dihydrozeatins prepared from (+)- and (−)-**5** of >98% e.e. had rotations of $[\alpha]_D^{25}$ + 9.48° and $[\alpha]_D^{25}$ − 9.48°. These rotations are significantly lower than the values of $[\alpha]_D^{21}$ − 12.1°, $[\alpha]_D^{21}$ + 11.7° reported by Fujii and Ogawa (1972), and the value of $[\alpha]_D^{20}$ + 13.1° ± 0.5° reported by Matsubara et al. (1977).

It is highly unlikely that racemization of the methyl 2 - methyl - 4 - nitrobutyrates occurs during LAH reduction. Amino acid esters completely retain chiral integrity during LAH reductions (Poindexter and Meyers 1977), and there is no reason to believe that 2-methylsubstituted esters (instead of 2-amino) would behave differently. Furthermore, analogous LAH reductions of 4 - amino - 2 - methylbutyric acids were used by Adams and Flĕs (1959) and Fujii and Ogawa (1972) to prepare optically active 4 - amino - 2 - methylbutan - 1 - ols, **6**.

Koshimizu et al. (1967) and Fujii and Ogawa (1972) described the ORD curve of (−)-dihydrozeatin as a negative plain curve, which was confirmed in the present work. It has been instructive to apply Heller's modification (Heller 1958) of the Drude equation (Brewster 1967) to the 3 sets of rotational data on isomeric dihydrozeatins. Table 1 lists the specific rotations for four preparations from the present work in addition to those previously reported by others. In the Drude equation plot used, $1/\lambda^2$ is plotted against $1/\lambda^2[\alpha]$ using the data in Table 1. The linear least squares fit of data (Fig. 1) from our preparations attests to the validity of the Drude equation over the observed spectral range from 320 to 589 nm. Except for the specific rotations at 589 nm reported by Fujii and Ogawa (1972) and by Matsubara et al. (1977), their data also yield a satisfactory least squares line of differing slope in a Drude plot. As a result, the measurements at 589 nm of Fujii and Ogawa (1972) and of Matsubara et al. (1977) appear to be in error. If the ORD measurements of these workers are valid, the specific rotation of their dihydrozeatins will be very low, $[\alpha]_D \sim 6°$. However, confirmation of the slopes of the data of our samples utilizing two spectropolarimeters and a polarimeter for 589 nm rotations has given mutually consistent results in which we have complete confidence.

Adams and Flĕs (1959) and later Fujii and Ogawa (1972) used the 4 - amino - 2 - methylbutan - 1 - ol oxalate salts as crystalline standards to measure the rotation of the amine. We synthesized (±) - bis - (O,N - trifluoroacetyl) - 4 - amino - 2 - methylbutan - 1 - ol in the hope that we could use NMR to observe the splitting of the methyl doublet with $Eu(tfc)_3$ similar to that found for (±)-methyl 2 - methyl - 4 - nitrobutyrate which would allow determination of optical purity of the aminoalcohol samples and their oxalate salts. Unfortunately, no shifts were observed under the conditions employed.

The regular displacement of the Drude curves to the left (Fig. 1) with increasing optical purity enables us to estimate that sample VII has an e.e. of about 92% (the e.e. of the parent ester was not determined).

In the 4 bioassays reported by Matsubara et al. (1977), the *R* - (+) dihydrozeatin was more active at lower concentrations than the *S* - (−) - isomer, whereas in the tobacco callus and radish cotyledon weight assay (both concerned with growth), both isomers showed the same activity at higher concentrations. In the lettuce seed germination and retardation of chlorophyll loss assays, however, the *S* - (−) - isomer was only half as active as the *R* - (+) - isomer at the highest concentrations used. *R* - (+) - Dihydrozeatin had the

Table 1. Specific rotations of various dihydrozeatin samples.

λ(nm)	I[a]	II[a]	III[b]	IV[c,d]	V[e]	VI[e]	VII[d,f]
320	+46.7°	−48°		−30.8°			−43.0°
350	+33.2°	−33.7°		−24°			−31.4°
400	+20.7°	−19.9°		−16.2°			−21.8°
436					−19.7°	+19.7°	−16.2°
450	+13.1°	−14.2°		−12.4°			
546					−11.3°	+11.3°	
578					−9.88°	+9.92°	
589	+11.7°	−12.1°	+13.1° (±0.5°)	−6.75°	−9.48°	+9.48°	−8.7° (−8.9°)

[a] Fujii and Ogawa, (1972).
[b] Matsubara et al (1977).
[c] 72% e.e. (nitroester).
[d] rotations measured on Cary 60; $[\alpha]_D$ checked on Bendix 143A.
[e] e.e. > 98% (nitroester); rotations taken on Perkin-Elmer 241.
[f] e.e. % of nitroester not known; e.e. of dihydrozeatin estimated 92%; see text.

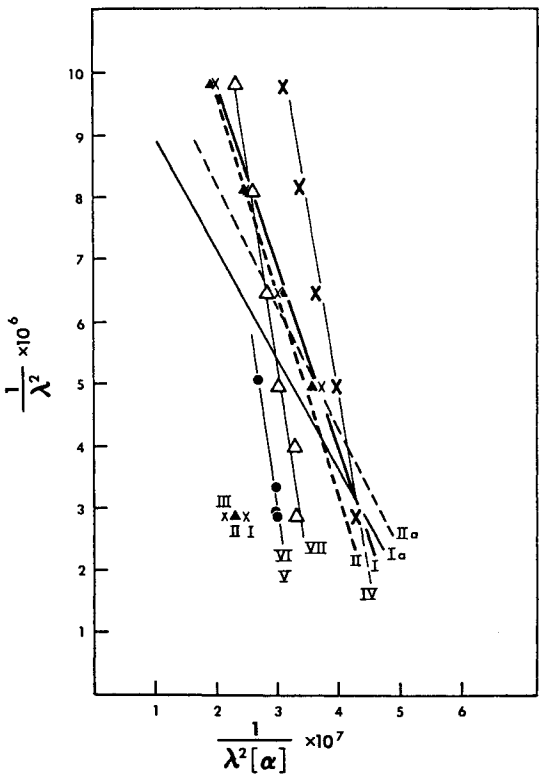


Fig. 1. Drude equation plot (Heller 1958) relating wavelength with optical rotatory power. Curves I(x———x) and II (▲---▲) were calculated from the data of Fujii and Ogawa (1972) including the 589 nm rotations. Curves Ia(-----) and IIa(------) were calculated omitting the 589 nm rotations. The isolated points I, II, and III were 589 nm rotations from Fujii and Ogawa (I and II) and Matsubara (1977) (III). Curves IV(-x———x), V, and VI-(-●--●--) and VII(-△—△-) are from the present work and represent different preparations.

same activity as *E*-zeatin in the callus assay and lower activity in the lettuce seed germination test.

In the betacyanin stimulation assay used by us (Fig. 2), *R* - (+) - dihydrozeatin was more active than the *S* - (−) - enantiomer. The racemic mixture had the same activity as the *R* - (+) - form, but reached a greater maximum activity. *E*-Zeatin was more active at lower concentrations than the dihydrozeatins, but at 10^2 μM was very similar to (±)- and *S* - (−) - dihydrozeatins.

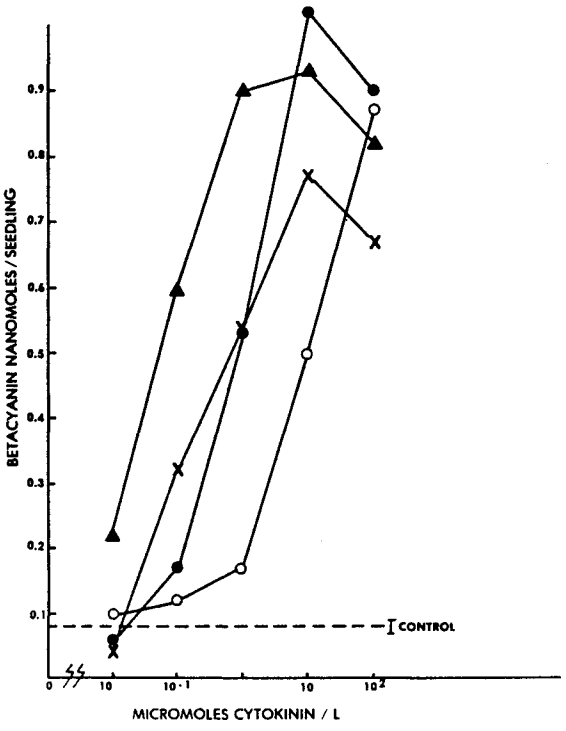


Fig. 2. Stimulation of betacyanin synthesis in *Amaranthus caudatus* L. var. Early Splendor; ▲ - *E* - zeatin; x- *R* - (+) - dihydrozeatin; ● - (±) - dihydrozeatin; O - *S*(−) - dihydrozeatin. Assay method followed was that of Elliott (1979). Each point represents 3 expts of 40 seedlings each; standard deviation less than ±15%.

It is not possible with our present knowledge to propose any mechanism and certainly not a common mode of action for the 5 diverse bioassays. The general structure-activity relationships hold throughout, however. In addition, the linear portions of curves in any one assay seem to have the same slopes. This would indicate that the rate determining step(s) is/are structure dependent in that assay. This may be reasonable if the several cytokinins have different affinities for the same critical reaction site or if there was a characteristic specific biological loss for each of the different cytokinins (different lag periods) and similar intrinsic biological activities.

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