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SYNTHESIS AND PLANT GROWTH INHIBITORY ACTIVITIES OF (+)- AND (−)-(2*Z*,4*E*)-5-(1′,2′-EPOXY-2′,6′,6′-TRIMETHYLCYCLOHEXYL)-3-METHYL-2,4-PENTADIENOIC ACID*

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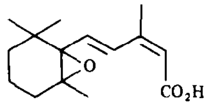
Key Word Index—Plant growth inhibitor; absolute configuration; relationship between enantiomers and biological activity; rice seedling growth; lettuce seed germination; abscisic acid analogue; (2*Z*,4*E*)-5-(1′,2′-epoxy-2′,6′,6′-trimethylcyclohexyl)-3-methyl-2,4-pentadienoic acid.

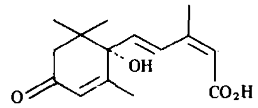
Abstract—Chiral (+)- and (−)-enantiomers of (2*Z*,4*E*)-5-(1′,2′-epoxy-2′,6′,6′-trimethylcyclohexyl)-3-methyl-2,4-pentadienoic acid have been synthesized from the chiral epoxy alcohols (+)- and (−)-1′,2′-dihydro-1′,2′-epoxy-*β*-ionone, which were prepared by Katsuki-Sharpless' asymmetric epoxidation of *β*-cyclogeraniol. The (+)-enantiomer showed strong inhibitory activity in a rice seedling and lettuce germination assay, whereas the (−)-enantiomer was 10^3-times less active.

INTRODUCTION

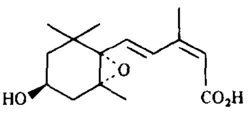
(2*Z*,4*E*)-Epoxy acid, **1**, has strong plant growth inhibitory activity [1, 2], like abscisic acid (ABA), **2**, and can be converted into ABA via xanthoxin acid, **3**, in plants [3]. Research so far on the biological activity of ABA analogues [4, 5] has shown relatively small differences between enantiomers. But, Burden *et al.* [6] reported that (±)-*O*-methylxanthoxin, **4b**, has half the activity of the natural enantiomer, **4b**. It was of interest, therefore, to examine whether the enantiomers of (2*Z*,4*E*)-epoxy acid (**1**), which is structurally related to xanthoxin, **4a** [7], and showed the same differences in biological activity. In this paper, we describe a successful synthesis of (+)- and (−)-**1** and the inhibitory activity of both enantiomers.



(±)-**1**



(±)-**2**



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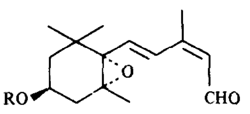
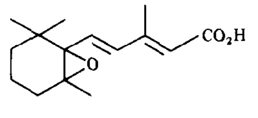


4a R=H
4b R=Me



(±)-**5**

RESULTS AND DISCUSSION

Although the optical resolution of (±)-(2*E*,4*E*) and (2*Z*,4*E*)-epoxy acids, **1** and **5**, could give partially resolved **1** and **5**, an asymmetric synthesis was carried out to obtain the optically pure enantiomers and to establish the absolute configuration of the chiral epoxy acid, **1**. This was achieved by application of Katsuki-Sharpless' asymmetric epoxidation [8].

β-Cyclocitral, prepared by cyclization of citral [9], was reduced with lithium aluminum hydride in ether to give *β*-cyclogeraniol, **6**. Asymmetric epoxidation of **6** with *tert*-butylhydroperoxide, titanium tetraisopropoxide and (+)-diethyl tartrate (natural) gave (−)-(1*S*,2*S*)-1,2-epoxy-2,6,6-trimethylcyclohexane-1-methanol, (−)-**7**, [$[\alpha]_D$ −25.8° ($CHCl_3$) (95% enantiomeric excess, e.e.)], in 80% yield. The absolute configuration of (−)-**7** was assumed to be (1*S*,2*S*) from the steric course of the reaction [8]. Similarly, the asymmetric epoxidation of **6** using (−)-diethyl tartrate (unnatural) gave (+)-(1*R*,2*R*)-epoxy alcohol, (+)-**7** [$[\alpha]_D$ +25.0° ($CHCl_3$)]. Oxidation of (−)- and (+)-**7** with dimethyl sulfoxide 'activated' by oxalyl chloride [10] gave the chiral aldehydes, (+)-**8**, $[\alpha]_D$ +48.8° ($CHCl_3$) and (−)-**8**, $[\alpha]_D$ −58.0° ($CHCl_3$), respectively, in 89% yields. Wittig reaction of (+)- and (−)-**8** with the yield derived from chloroacetone [11] gave (+)-**9**, mp 48.5–49.0°, $[\alpha]_D$ +104.7° ($CHCl_3$), and its enantiomer, (−)-**9**, mp 49.5–50.0°, $[\alpha]_D$ −103.0° ($CHCl_3$), respectively in 85% yields.

Acemoglu *et al.* [12] had synthesized (−)-**9** by using Sharpless' epoxidation of (−)-(3′*R*)-3′-hydroxy-*β*-ionone, **12**, derived from (−)-(*S*)-*α*-ionone (**11**). As our product, (−)-**9**, had a higher optical rotation, $[\alpha]_D$ −103.0° ($CHCl_3$), than the value, $[\alpha]_D$ −96° ($CHCl_3$), reported by Acemoglu *et al.* [12] it was considered optically almost

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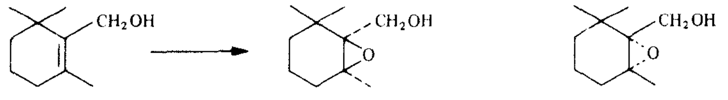
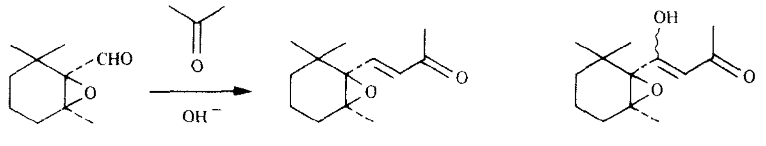
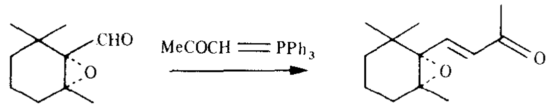
*Part 17 in the series "Studies on Abscisic Acid". For Part XVI see (1982) *Agric. Biol. Chem.* **46**, 3069.



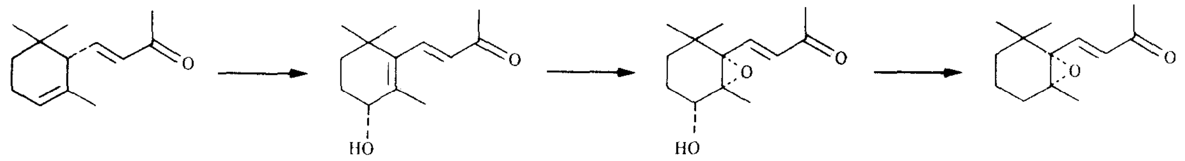
(−)-7 (+)-7



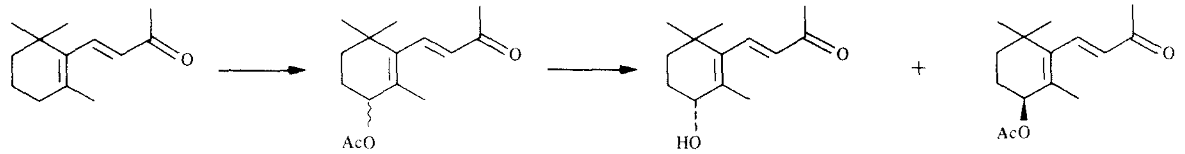
(+)-8 (+)-9 10



(−)-8 (−)-9



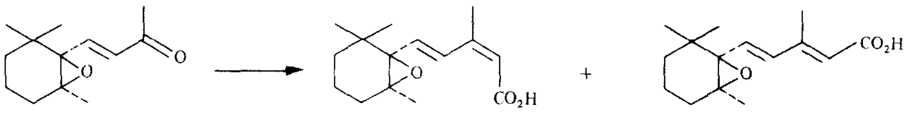
(−)-11 (R)-12 13 (−)-9



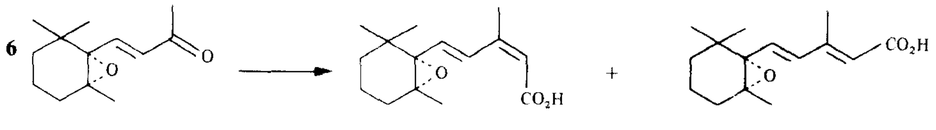
14 (±)-15 (R)-12 (S)-15

pure. In addition, the enzymatically asymmetric hydrolysis [13], developed by us, was applied to the preparation of the chiral intermediate, **12**. (+)-3′-acetoxy-β-ionone, **15**, prepared from β-ionone, **14**, was enzymatically hydrolysed with the culture broth of *Bacillus subtilis* var. *niger* [13] to give (−)-**12** (14% optical purity and (−)-(3′*S*)-3′-acetoxy-β-ionone, **15**, $[\alpha]_D$ −54.5° (EtOH) (70% optical purity) [14] after 68.7% hydrolysis.

The reaction of (+)-**9** with methoxycarbonylmethylenetriphenylphosphorane gave a mixture of (−)-(2*Z*,4*E*)- and (+)-(2*E*,4*E*)-epoxymethyl esters (97% yield), which was hydrolysed with methanolic sodium hydroxide to give



(+)-9 (−)-1 (+)-5



(−)-9 (+)-1 (−)-5

(−)-(1′R,2′S)-(2Z,4E)-epoxy acid, (−)-**1**, mp 121–122°, $[\alpha]_D$ −19.5° ($CHCl_3$), and (+)-(1′R,2′S)-(2E,4E)-epoxy acid, (+)-**5**, mp 84–85°, $[\alpha]_D$ +72.5° ($CHCl_3$). Similarly, the reaction of (−)-**9** with the phosphorane, followed by alkaline hydrolysis, gave (+)-(1′S,2′R)-(2Z,4E)-epoxy acid, (+)-**1**, mp 120–121°, $[\alpha]_D$ +20.0° ($CHCl_3$), and (−)-(1′S,2′R)-(2E,4E)-epoxy acid (−)-**5**, mp 86°, $[\alpha]_D$ −74.5° ($CHCl_3$).

The inhibitory activities of the chiral epoxy acids (+)-**1** and (−)-**1** were assessed in the rice seedling growth and the lettuce germination tests. In the rice seedling test only the (+)-enantiomer showed strong growth inhibitory activity (Fig. 1). Also, in the germination assay of lettuce seeds only the (+)-enantiomer showed a strong inhibitory effect at 0.5×10^{-5}–1×10^{-6} M concentrations. The (−)-enantiomer was *ca* 10^3 times less active in the above two bioassays. The above results show that the (+)-(1′S,2′R)-enantiomer, (+)-**1**, with the same configuration of the epoxy ring as the natural epoxy carotenoids and their degraded product, (1′S,2′R)-xanthoxin, **4a**, has strong biological activity. Therefore, it is considered that the difference of the activity between the enantiomers may be caused by the easy conversion of the natural form, (+)-**1**, into (+)-ABA in plants [3].

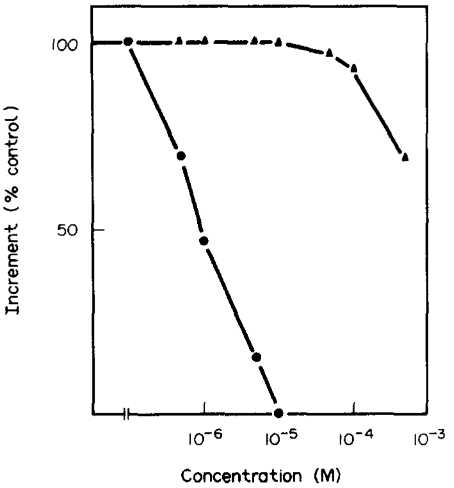


Fig. 1. The growth inhibitory activity of the epoxy acids (+)-**1** and (−)-**1** on rice seedlings (*O. sativa* var. Koshiziwase). After growing at 28° (2000 lx) for 5 days, the length of the second leaf sheath was measured. (●) The (2Z,4E)-epoxy acid, (+)-**1**. (▲) The (2Z,4E)-epoxy acid, (−)-**1**.

EXPERIMENTAL

All bps and mps are uncorr. TLC: 0.25 mm Si gel 60-H (Merck) with C_6H_6–EtOAc (4:1).

Optical resolution of (±)-(2E,4E)- *and* (2Z,4E)-*epoxy acids*, **1** *and* **5**. A salt of (±)-**5**, mp 151–152°, with quinine was recrystallized (×3) from MeOH to give a salt, mp 199–200°, $[\alpha]_D^{19}$ −43.3° (CH_2Cl_2; *c* 0.6) which was treated with dil. HCl to give (+)-**5**, mp 95–97° (from hexane), $[\alpha]_D^{19}$+7.9° (EtOH; *c* 0.4) 15.8% optical purity. The salt, mp 179–180°, $[\alpha]_D^{19}$ −91.3° (CH_2Cl_2; *c* 0.8), separated from the mother liquor, gave (−)-**5**, mp 99–100°, $[\alpha]_D^{19}$ −2.7° (EtOH; *c* 0.3), 5.4% optical purity. A salt of (±)-**1**, mp 146–147°, with (−)-α-methylbenzylamine was recrystallized (×3) from Me_2CO to give the salt, mp 153–154°,$[\alpha]_D^{19}$ −26.0° (CH_2Cl_2; *c* 1.0), which gave (−)-**1**, mp 124–127°, $[\alpha]_D^{19}$ −10.6° (EtOH; *c* 0.5), 59.6% optical purity on treatment with dil. HCl.

Preparation of β-cyclogeraniol, **6**. Reduction of β-cyclocitral, bp 120–125°/25 mmHg (23.0 g), with $LiAlH_4$ in dry Et_2O for 4 hr under reflux gave β-cyclogeraniol, **6**, bp 115–117°/25 mmHg (22.5 g), mp 40° (from hexane). IR ν_{max}^{film} cm^{-1}: 3350, 1655 (w), 1015 and 995.

Preparation of chiral 1,2-*epoxy*-2,6,6-*trimethylcyclohexane*-1-*methanols*, (−)-**7** *and* (+)-**7**. To a stirred soln of titanium isopropoxide (5.68 g, 20 mmol), 4.12 g (20 mmol) L-(+)-diethyl tartrate and 3.08 g (20 mmol) β-cyclogeraniol, **6**, in 200 ml dry CH_2Cl_2, was added drop-wise 40 mmol dry *tert*-butyl hydroperoxide in 10 ml CH_2Cl_2 at −25°. The reaction mixture was stirred overnight at −20°. Dimethyl sulfide (5.0 g) was added and the mixture was stirred for 40 min at −20°. The cold reaction mixture was added to stirred 5% aq. NaF at room temp. Stirring was continued for several hr after which the aq. phase was satd with NaCl. After filtration, the filtrate was extracted (×3) with CH_2Cl_2. The combined organic extract was dried ($MgSO_4$) and concd to give an oily mixture of (−)-**7** (R_f 0.48) and the recovered diethyl tartrate (R_f 0.34). The mixture was chromatographed on a column of Si gel. Elution with hexane–EtOAc (4:1) afforded (−)-1,2-epoxy-2,6,6-trimethylcyclohexane-1-methanol, **7**, as a colourless liquid (2.72 g), $[\alpha]_D^{20}$ −25.8° ($CHCl_3$; *c* 0.8), IR ν_{max}^{film} cm^{-1}: 3460, 1035 and 910. Treatment of (−)-**7** with (−)-MTPA chloride in dry pyridine overnight at room temp. afforded the (−)-MTPA ester of (−)-**7**, ^1H NMR (100 MHz, $CDCl_3$, TMS as int. standard): δ 0.80 (3H, *s*), 1.02 (3H, *s*), 1.34 (3H, *s*), 3.56 (3H, OMe), 4.14 (1H, *d*, J = 12 Hz), 4.75 (1H, *d*, J = 12 Hz) and 7.42 (5H, aromatic). ^1H NMR analysis of the (−)-MTPA ester of (−)-**7** in the presence of Eu $(fod)_3$ (0.1 mol equivalent to the MTPA ester in $CDCl_3$) gave an enantiomeric excess (e.e.) of 95%. Similarly, the asymmetric epoxidation of **6** using unnatural (−)-diethyl tartrate, by the same treatment as described above, gave (+)-**7**, $[\alpha]_D^{20}$ +25.0° ($CHCl_3$; *c* 0.8), and the 3,5-dinitrobenzoate of (+)-**7**, mp 37°.

Preparation of chiral 1,2-*epoxy*-2,6,6-*trimethylcyclohexane*-1-*carbaldehydes*, (+)-**8** *and* (−)-**8**. To a soln of oxalyl chloride (1.0 ml) in 50 ml dry CH_2Cl_2 was added 1.7 ml DMSO in 3 ml CH_2Cl_2 at −50°. After stirring for 2 min, the alcohol (−)-**7** (1.7 g) was added drop-wise within 5 min. Et_3N (7.0 ml) was added and the reaction mixture was stirred for 4 hr and then allowed to warm to room temp. for 2 hr. H_2O (50 ml) was added and the aq. layer was extracted with CH_2Cl_2. The combined organic layer was washed with dil. HCl, aq. $NaHCO_3$ and dried ($MgSO_4$). After evaporation of the solvent, the obtained aldehyde (+)-**8** (1.6 g) was almost pure (R_f 0.80). Further purification was performed by Si gel CC. Elution with hexane–Et_2O (9:1) gave (+)-**6** (1.5 g) as a colourless oil. $[\alpha]_D^{20}$ +58.8° ($CHCl_3$; *c* 4.0), IR ν_{max}^{film} cm^{-1}: 2800, 1730 and 1070. Also, oxidation of (−)-**7** with CrO_3 in pyridine overnight at room temp. gave (+)-**8** (*ca* 50% yield). Similarly, the oxidation of (+)-**7** with dimethyl sulfoxide gave (−)-**8**, $[\alpha]_D^{20}$ −58.0° ($CHCl_3$; *c* 4.0).

Preparation of chiral 1′,2′-*dihydro*-1′,2′-*epoxy*-β-*ionones*, (+)-**9** *and* (−)-**9**. A mixture of (+)-**8** (3.36 g) and 9.50 g acetonylidenetriphenylphosphorane in 50 ml dry toluene was refluxed under N_2. After evaporation of the solvent, the residual oil was treated with hexane–Et_2O to separate triphenylphosphine oxide as crystals. The mother liquor was evaporated and then distilled to give (+)-**9**, bp 123–125°/5 mmHg, mp 48.5–49.0° (from C_6H_6) (3.52 g), $[\alpha]_D^{20}$ +104.7° ($CHCl_3$; *c* 0.7). Similarly, the reaction of (−)-**8** with the phosphorane gave (−)-**9**, mp 49.5–50.0°, $[\alpha]_D^{20}$ −103.0° ($CHCl_3$; *c* 0.7). ^1H NMR (100 MHz, $CDCl_3$, TMS as int. standard): δ0.94 (3H, *s*), 1.16 (6H, *s*), 2.28 (3H, *s*), 6.30

(1H, *d*, $J = 16$ Hz) and 7.02 (1H, *d*, $J = 16$ Hz).

Preparation of (±)-*3′-acetoxy-β-ionone*, (±)-**15**. Reaction of β-ionone (**14**) (19.2 g) with *N*-bromosuccinimide (19.0 g) in 40 ml CCl_4 under reflux for 30 min followed by treatment with 9.5 g NaOAc in 50 ml HOAc for 4 hr at 90–100°, gave (±)-15, bp 136–145°/4 mmHg (13.0 g), R_f 0.69, IR ν_{max}^{film} cm^{-1}: 3040, 1740, 1670, 1610, 1240 and 1020.

Asymmetric hydrolysis of (±)-*3′-acetoxy-β-ionone* (**15**). To a cultured broth (100 ml) of *B. subtilis* var. *niger* [13] was added 1.5 g (±)-**15** and the mixture shaken for 24 hr at 28°. The cultured broth was extracted with EtOAc and the organic extract was dried ($MgSO_4$). Evaporation of the solvent gave an oily residue, which was chromatographed on Si gel. Elution with C_6H_6–EtOAc (4:1) gave 470 mg (*S*)-3′-acetoxy-β-ionone (**15**), $[\alpha]_D^{20}$ −54.5° (EtOH; *c* 5.0) (70% optical purity) and then (*R*)-(−)-3′-hydroxy-β-ionone (**12**), $[\alpha]_D^{20}$ −1.0° (EtOH; *c* 5.0) (14% optical purity). ^1H NMR (100 MHz, $CDCl_3$, TMS as int. standard): δ1.04 (3H, *s*), 1.07 (3H, *s*), 1.85 (3H, *s*), 2.31 (3H, *s*), 4.00 (1H), 4.70 (1H, *s*, OH), 6.11 (1H, *d*, $J = 16$ Hz), and 7.18 (1H, *d*, $J = 16$ Hz). Similarly, 1.0 g (±)-15 was shaken with the cultured broth (200 ml) of *Brevibacterium ammoniagenes* IAM 1645 [13] for 48 hr at 28° to give 600 mg of the acetate, (*S*)-**15**, $[\alpha]_D^{20}$ −30.8° (EtOH; *c* 2.0) (40% optical purity), and the alcohol (*R*)-**12**, (325 mg), $[\alpha]_D^{20}$ −4.2° (EtOH; *c* 3.0) (60% optical purity).

Preparation of chiral (2Z,4E)-*and* (2*E*,4*E*)-5-(*1′,2′-epoxy-2′,6′,6′-trimethylcyclohexyl*)-3-*methyl*-2,4-*pentadienoic acids*, (−)-**1** *and* (+)-**5**, (+)-**1** *and* (−)-**5**. A mixture of the ketone, (+)-**9** (820 mg), and methoxycarbonylmethylenetriphenyl-phosphorane (2.50 g) in 12 ml dry xylene was refluxed for 24 hr under N_2. After evaporation of the solvent, the residual oil was treated with hexane–Et_2O to separate triphenylphosphine oxide as crystals. The mother liquor was chromatographed on Si gel (30 g). Elution with C_6H_6 gave 600 mg (+)-**5** methyl ester, which was hydrolysed with 10% ethanolic NaOH to give the (2*E*,4*E*)-epoxy acid, (+)-**5**, mp 84–85° (from hexane), $[\alpha]_D^{20}$ +73.0° ($CHCl_3$; *c* 2.0). ^1H NMR (100 MHz, $CDCl_3$, TMS as int. standard): δ0.94 (3H, *s*), 1.12 (3H, *s*), 1.15 (3H, *s*), 2.31 (3H, *s*), 5.82 (1H, *s*), 6.34 (2H, *s*), 9.92 (1H). Further elution with C_6H_6–EtOAc (5:1) afforded the (−)-**1** methyl ester (410 mg). ^1H NMR (100 MHz, $CDCl_3$, TMS as int. standard): δ0.97 (3H, *s*), 1.10 (3H, *s*), 1.18 (3H, *s*), 2.02 (3H), 3.70 (3H, *s*), 6.26 (1H, *d*, $J = 16$ Hz) and 7.58 (1H, *d*, $J = 16$ Hz). The (−)-**1** methyl ester was hydrolysed to the (2*Z*,4*E*)-epoxy acid, (−)-**1**, mp 121–122° (from C_6H_6–hexane), $[\alpha]_D^{20}$ −19.5° ($CHCl_3$; *c* 1.0). Similarly, the ketone, (−)-**9** (1.042 g), was reacted with 3.507 g of the phosphorane in 15 ml xylene for 22 hr under N_2 to give (−)-**5** methyl ester and (+)-**1** methyl ester, which were hydrolysed with 10% ethanolic NaOH to give the (2*E*,4*E*)-epoxy acid, (−)-**5** (706 mg), mp 86°, $[\alpha]_D^{20}$ −75.0° ($CHCl_3$; *c* 1.0), $[\alpha]_D^{20}$ −50.0° (EtOH; *c* 2.0) and the (2*Z*,4*E*)-epoxy acid, (+)-**1** (500 mg), mp 121°, $[\alpha]_D^{20}$ +20.0° ($CHCl_3$; *c* 1.0), $[\alpha]_D^{20}$ +17.8° (EtOH; *c* 1.4), respectively.

Bioassay procedure. The rice seedling test was carried out by using rice seedlings (*Oryza sativa* L. var. Koshiziwase) as previously described [2]. After growing at 28° (2000 lx) for 5 days, the mean increment of the second leaf sheath, measured on two replicas, was expressed as a percentage of the mean increment of the control, as shown in Fig. 1. Lettuce seeds (Great Lakes) were germinated on 0.7% agar medium at 25° (2000 lx) for 3 days as previously described [2].

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